



**Programa de Doctorado en Recursos y Tecnologías Agrarias,
Agroambientales y Alimentarias**

**FORMULACIÓN DE EMULSIONES
GELIFICADAS CON ACEITES SALUDABLES
Y HARINAS DE PSEUDOCEREALES PARA
LA SUSTITUCIÓN DE GRASA EN
PRODUCTOS CÁRNICOS**

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La presente Tesis Doctoral, titulada **“FORMULACIÓN DE EMULSIONES GELIFICADAS CON ACEITES SALUDABLES Y HARINAS DE PSEUDOCEREALES PARA LA SUSTITUCIÓN DE GRASA EN PRODUCTOS CÁRNICOS”**, se presenta bajo la modalidad de **tesis por compendio** de las siguientes **publicaciones**:

- “Healthier Oils: A new Scope in the Development of Functional Meat and Dairy Products: A Review” publicado en la revista Biomolecules <https://doi.org/10.3390/biom13050778>
- “Assessment of Chemical, Physicochemical, and Lipid Stability Properties of Gelled Emulsions Elaborated with Different Oils Chia (*Salvia hispanica* L.) or Hemp (*Cannabis sativa* L.) and Pseudocereals” publicado en la revista Foods. <https://doi.org/10.3390/foods10071463>
- “Improving the lipid profile of beef burgers added with chia oil (*Salvia hispanica* L.) or hemp oil (*Cannabis sativa* L.) gelled emulsions as partial animal fat replacers” publicado en la revista LWT-Food Science and Technology. <https://doi.org/10.1016/j.lwt.2022.113416>
- “Chia and hemp oils-based gelled emulsions as replacers of pork backfat in burgers: effect on lipid profile, technological attributes and oxidation stability during frozen storage” publicado en la revista International Journal of Food Science and Technology <https://doi.org/10.1111/ijfs.15907>
- “Total and Partial Fat Replacement by Gelled Emulsion (Hemp Oil and Buckwheat Flour) and Its Impact on the Chemical, Technological and Sensory Properties of Frankfurters” publicado en la revista Foods. <https://doi.org/10.3390/foods10081681>
- “Innovative formulation in pâté using a gelled emulsion of hemp oil (*Cannabis Sativa* L.) as fat replacer” publicado en la revista LWT-Food Science and Technology. <https://doi.org/10.1016/j.lwt.2024.116630>
- “Alheiras with animal fat replacement: application of a gelled emulsion based on hemp oil (*Cannabis sativa* L.) and buckwheat” publicado en la revista European Food Research and Technology. <https://doi.org/10.1007/s00217-023-04295-w>
- “Development of plant-based burgers using gelled emulsions as fat source and beetroot juice as colorant: Effects on chemical, physicochemical, appearance and sensory characteristics” publicado en la revista LWT-Food Science and Technology. <https://doi.org/10.1016/j.lwt.2022.114193>

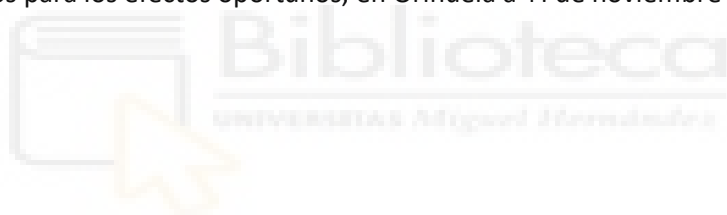


El Dr. D. *Manuel Viuda Martos*, director, y la Dra. Dña. *Juana Fernández López*, codirectora de la tesis doctoral titulada **“Formulación de emulsiones gelificadas con aceites saludables y harinas de pseudocereales para la sustitución de grasa en productos cárnicos”**

INFORMAN:

Que Dña. *Carmen María Botella Martínez* ha realizado bajo nuestra supervisión el trabajo titulado **“Formulación de emulsiones gelificadas con aceites saludables y harinas de pseudocereales para la sustitución de grasa en productos cárnicos”** conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

Lo que firmamos para los efectos oportunos, en Orihuela a 14 de noviembre de 2024.



Director de la tesis

Dr. D. *Manuel Viuda Martos*

Codirectora de la tesis

la Dra. Dña. *Juana Fernández López*

Dr. Dña. Juana Fernández López, Catedrática de Universidad y Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias (ReTos-AAA) de la Universidad Miguel Hernández de Elche (UMH),

CERTIFICA:

Que la Tesis Doctoral titulada “**Formulación de emulsiones gelificadas con aceites saludables y harinas de pseudocereales para la sustitución de grasa en productos cárnicos**“ de la que es autora la graduada en Ciencia y Tecnología de los Alimentos, **Dña. Carmen María Botella Martínez**, ha sido realizada bajo la dirección del **Dr. Manuel Viuda Martos** y la codirección de la **Dra. Juana Fernández López**, actuando como tutor de la misma el Dr. José Ángel Pérez Álvarez. Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA, siendo por tanto apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a 14 de noviembre de 2024.

Dra. Dña. Juana Fernández López
Coordinadora del Programa Doctorado ReTos-AAA



Esta Tesis Doctoral se ha desarrollado en el Instituto de Investigación e Innovación Agroalimentaria y Agroambiental de la Universidad Miguel Hernández (CIAGRO-UMH), dentro del Grupo de Investigación de Innovaciones en Productos Alimentarios (IPOA) con financiación por parte de la Generalitat Valenciana a través de la concesión de la Ayuda (ref: CIAICO/2023/004) para grupos de investigación consolidados AICO 2024 (CIAICO 2023) “Desarrollo de emulsiones gelificadas a partir de coproductos de la industria agroalimentaria Valenciana y aceites vegetales con perfil lipídico saludable”.

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*A mis padres por
darme todo lo que
no tuvieron*

*A mi hermana
por hacerme volver*



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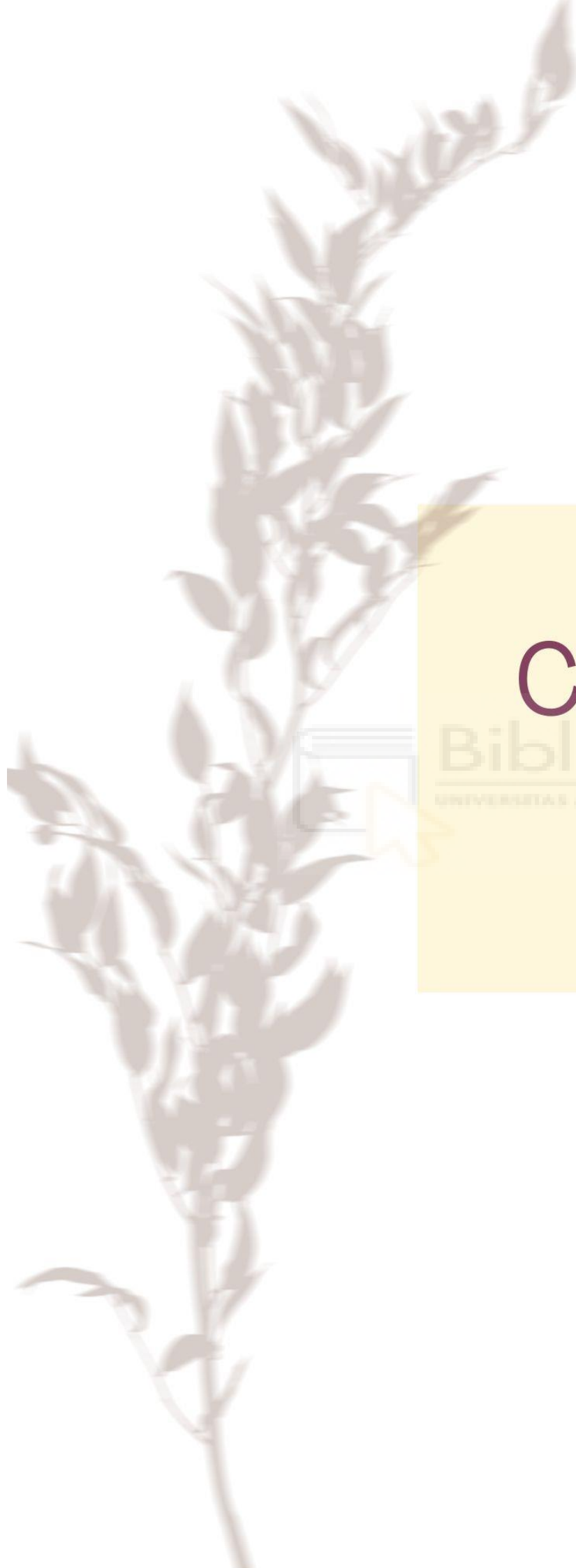
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CAPÍTULO 1

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ESTRUCTURA DE LA TESIS



1. ESTRUCTURA DE LA TESIS

Para la realización de la presente Tesis Doctoral se ha seguido una metodología por compendio de publicaciones científicas, tanto de investigación como de revisión, en concreto consta de 8 publicaciones (6Q1 y 2Q2). Por lo tanto, su estructura sigue la Normativa de estudios de doctorado de la Universidad Miguel Hernández de Elche para la presentación de “Tesis por compendio de publicaciones”. Además, con esta Tesis se pretende obtener la mención “Doctor Internacional” (art. 15, RD 99/2011 modificado por RD 576/2023).

PUBLICACIÓN 1

Botella-Martínez, C., Pérez-Álvarez, J.A., Sayas-Barberá, E., Navarro-Rodríguez de Vera, C., Fernández-López, J. & Viuda-Martos, M. (2023). Healthier oils: a new scope in the development of functional meat and dairy products: a review. *Biomolecules*, 13(5), 778. doi.org/10.3390/biom13050778.

Categoría JCR: Biochemistry & Molecular Biology

Quartil: Q1

Rango: 67/313

Factor de impacto: 4,8 (2023).

PUBLICACIÓN 2

Botella-Martínez, C., Pérez-Álvarez, J.A., Sayas-Barberá, E., Fernández-López, J. & Viuda-Martos, M. (2021). Assessment of chemical, physicochemical, and lipid stability properties of gelled emulsions elaborated with different oils chia (*Salvia hispanica* L.) or hemp (*Cannabis sativa* L.) and pseudocereals. *Foods*, 10(7), 1463. doi.org/10.3390/foods10071463.

Categoría JCR: Food Science & Technology

Quartil: Q1

Rango: 35/144

Factor de impacto: 5,561 (2021).

PUBLICACIÓN 3

Botella-Martínez, C., Gea-Quesada, A., Sayas-Barberá, E., Pérez-Álvarez, J. Á., Fernández-López, J., & Viuda-Martos, M. (2022). Improving the lipid profile of beef burgers added with chia oil (*Salvia hispanica L.*) or hemp oil (*Cannabis sativa L.*) gelled emulsions as partial animal fat replacers. *LWT*, 161, 113416. doi.org/ 10.1016/j.lwt.2022.113416.

Categoría JCR: Food Science & Technology

Quartil: Q1

Rango: 24/142

Factor de impacto: 6,0 (2022).

PUBLICACIÓN 4

Botella-Martínez, C., Sayas-Barberá, E., Pérez-Álvarez, J. Á., Viuda-Martos, M., & Fernández-López, J. (2023). Chia and hemp oils-based gelled emulsions as replacers of pork backfat in burgers: effect on lipid profile, technological attributes and oxidation stability during frozen storage. *International Journal of Food Science & Technology*, 58(6), 3234-3243. doi.org/10.1111/ijfs.15907.

Categoría JCR: Food Science & Technology

Quartil: Q2

Rango: 86/173

Factor de impacto: 2,6 (2023).

PUBLICACIÓN 5

Botella-Martínez, C., Viuda-Martos, M., Pérez-Álvarez, J. A., & Fernández-López, J. (2021). Total and partial fat replacement by gelled emulsion (hemp oil and buckwheat flour) and its impact on the chemical, technological and sensory properties of frankfurters. *Foods*, 10(8), 1681. doi.org/10.3390/foods10081681.

Categoría JCR: Food Science & Technology

Quartil: Q1

Rango: 35/144

Factor de impacto: 5,561 (2021).

PUBLICACIÓN 6

Botella-Martínez, C., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2024). Innovative formulation in pâté using a gelled emulsion of hemp oil (*Cannabis Sativa L.*) as fat replacer. *LWT*, 206, 116630. doi.org/10.1016/j.lwt.2024.116630.

Categoría JCR: Food Science & Technology

Quartil: Q1

Rango: 17/173

Factor de impacto: 6,0 (2023).

PUBLICACIÓN 7

Botella-Martínez, C., Fernández-López, J., Ferreira, I., Leite, A., Vasconcelos, L., Domínguez, R., Pérez-Álvarez, J.A., Teixeira, A., & Viuda-Martos, M. (2023). Alheiras with animal fat replacement: application of a gelled emulsion based on hemp oil (*Cannabis sativa L.*) and buckwheat. *European Food Research and Technology*, 249(9), 2273-2285. doi.org/10.1007/s00217-023-04295-w.

Categoría JCR: Food Science & Technology

Quartil: Q2

Rango: 73/173

Factor de impacto: 3,0 (2023).

PUBLICACIÓN 8

Botella-Martínez, C., Viuda-Martos, M., Fernández-López, J. A., Pérez-Álvarez, J. A., & Fernández-López, J. (2022). Development of plant-based burgers using gelled emulsions as fat source and beetroot juice as colorant: Effects on chemical, physicochemical, appearance and sensory characteristics. *LWT*, 172, 114193. doi.org/10.1016/j.lwt.2022.114193.

Categoría JCR: Food Science & Technology

Quartil: Q1

Rango: 24/142

Factor de impacto: 6,0 (2022).

La estructura de la Tesis consta de 8 capítulos:

Capítulo 1. Resumen/Abstract

En este primer capítulo se presenta un resumen, tanto en inglés como en castellano de la memoria de Tesis Doctoral.

Capítulo 2. Introducción

Este capítulo consta de tres apartados, en los que se incluye una revisión bibliográfica sobre alimentación y salud, incluyendo información acerca de los alimentos funcionales e implicación del consumo de grasas en la salud. Se profundiza en los productos cárnicos funcionales, donde se expone y destaca la función tecnológica de la grasa, los desafíos relacionados con la sustitución de grasa en productos cárnicos y las estrategias para mejorar su perfil lipídico. El capítulo termina profundizando en las emulsiones gelificadas, los ingredientes usados y los procesos de gelificación; también se revisan los emulsionantes y gelificantes más utilizados en la elaboración de emulsiones gelificadas aplicadas en alimentación; finalmente se comentan diversos estudios relevantes en los cuales se han aplicado emulsiones gelificadas en el desarrollo de productos cárnicos.

Capítulo 3. Objetivos

En este capítulo se describe el objetivo general y los objetivos específicos de la presente Tesis Doctoral.

Capítulo 4. Materiales y Métodos

En este capítulo se especifican todos los materiales, métodos, procesos y análisis aplicados durante la elaboración de la presente Tesis Doctoral. Está dividido en 8 apartados, un apartado sobre materias primas

empleadas tanto en la elaboración de las emulsiones gelificadas como en los productos cárnicos y análogos, en los cuales posteriormente se incorporaron las emulsiones gelificadas. Otro apartado sobre la elaboración de las distintas emulsiones gelificadas. Un apartado sobre la determinación del perfil lipídico de los aceites empleados para generar las emulsiones gelificadas. Otro sobre la caracterización de las emulsiones gelificadas elaboradas. Y por último, cuatro apartados sobre la aplicación de las emulsiones gelificadas seleccionadas en diferentes productos cárnicos: frescos (hamburguesa), productos cocidos (salchichas tipo Frankfurt y paté), producto tradicional portugués (alheira) y un análogo tipo hamburguesa.

Capítulo 5. Resultados y Discusión

En este capítulo se muestran los resultados más destacables obtenidos en las publicaciones que componen la presente Tesis Doctoral, así como la discusión de dichos resultados. El capítulo se divide en ocho apartados, uno relativo a la caracterización y evaluación de las harinas de pseudocereales y los aceites vegetales utilizados para la generación de las emulsiones gelificadas. Otro sobre la caracterización de las emulsiones como tal y su estabilidad a las condiciones de conservación. Los otros cinco apartados se dedican a la funcionalidad y estabilidad de los distintos tipos de productos o análogos cárnicos desarrollados, en los cuales se han incorporado las emulsiones gelificadas.

Capítulo 6. Conclusiones/Conclusions

En este capítulo se detallan las conclusiones de esta Tesis Doctoral, las cuales se presentan también en inglés.

Capítulo 7. Referencias

Toda la bibliografía consultada para elaborar la presente Tesis Doctoral se recopila en este capítulo.

Capítulo 8. Publicaciones

En el último capítulo de la presente Tesis se incluyen todas las publicaciones científicas que componen la base de esta, presentadas en el idioma original de publicación. Está dividida en 8 apartados, uno por cada publicación presentada.

La primera publicación es una revisión bibliográfica donde se analiza el estado del arte de los aceites y métodos para estructurar aceites y su incorporación en productos de origen animal, que ha servido de guía para la introducción de la presente Tesis Doctoral:

1. **Botella-Martínez, C.,** Pérez-Álvarez, J.A., Sayas-Barberá, E., Navarro-Rodríguez de Vera, C., Fernández-López, J. & Viuda-Martos, M. (2023). Healthier oils: a new scope in the development of functional meat and dairy products: a review. *Biomolecules*, 13(5), 778. doi.org/10.3390/biom13050778.

La segunda publicación hace referencia a la caracterización de varias de las emulsiones gelificadas desarrolladas en la presente Tesis Doctoral, analizando su composición química, físico-química, estabilidad de la emulsión y la evolución de la oxidación lipídica durante su conservación en congelación. Dichas emulsiones fueron las generadas con la combinación de aceite de chía, aceite de cáñamo, así como la mezcla 1:1 de ambos aceites

con cada una de las harinas de pseudocereales (amaranto, trigo sarraceno, teff y quinoa blanca) usadas en esta Tesis:

2. **Botella-Martínez, C.,** Pérez-Álvarez, J.A., Sayas-Barberá, E., Fernández-López, J. & Viuda-Martos, M. (2021). Assessment of chemical, physicochemical, and lipid stability properties of gelled emulsions elaborated with different oils chia (*Salvia hispanica* L.) or hemp (*Cannabis sativa* L.) and pseudocereals. *Foods*, 10(7), 1463. doi.org/10.3390/foods10071463.

Las siguientes publicaciones (tercera y cuarta publicación) incluyen la elaboración y caracterización de un producto cárnico fresco (hamburguesa) con sustitución de la grasa animal con dos emulsiones gelificadas, así como la evaluación de la vida útil del producto desarrollado:

3. **Botella-Martínez, C.,** Gea-Quesada, A., Sayas-Barberá, E., Pérez-Álvarez, J. Á., Fernández-López, J., & Viuda-Martos, M. (2022). Improving the lipid profile of beef burgers added with chia oil (*Salvia hispanica* L.) or hemp oil (*Cannabis sativa* L.) gelled emulsions as partial animal fat replacers. *LWT*, 161, 113416. doi.org/ 10.1016/j.lwt.2022.113416.
4. **Botella-Martínez, C.,** Sayas-Barberá, E., Pérez-Álvarez, J. Á., Viuda-Martos, M., & Fernández-López, J. (2023). Chia and hemp oils-based gelled emulsions as replacers of pork backfat in burgers: effect on lipid profile, technological attributes and oxidation stability during frozen storage. *International Journal of Food Science & Technology*, 58(6), 3234-3243. doi.org/10.1111/ijfs.15907.

Las siguientes publicaciones (*quinta y sexta publicación*) incluyen la elaboración y caracterización de dos productos cárnicos cocidos (salchichas tipo Frankfurt y paté) con sustitución de la grasa animal con una emulsión gelificada elaborada con harina de trigo sarraceno y aceite de cáñamo:

5. **Botella-Martínez, C.,** Viuda-Martos, M., Pérez-Álvarez, J. A., & Fernández-López, J. (2021). Total and partial fat replacement by gelled emulsion (hemp oil and buckwheat flour) and its impact on the chemical, technological and sensory properties of frankfurters. *Foods*, *10*(8), 1681. doi.org/10.3390/foods10081681.
6. **Botella-Martínez, C.,** Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2024). Innovative formulation in pâté using a gelled emulsion of hemp oil (*Cannabis Sativa L.*) and buckwheat flour as fat replacer. *LWT*, *206*, 116630. doi.org/10.106/j.lwt.2024.116630.

La *séptima publicación* incluye la elaboración y caracterización de un producto cárnico tradicional portugués (alheira) con sustitución de grasa animal con una emulsión gelificada elaborada con harina de trigo sarraceno y aceite de cáñamo:

7. **Botella-Martínez, C.,** Fernández-López, J., Ferreira, I., Leite, A., Vasconcelos, L., Domínguez, R., Pérez-Álvarez, J.A., Teixeira, A., & Viuda-Martos, M. (2023). Alheiras with animal fat replacement: application of a gelled emulsion based on hemp oil (*Cannabis sativa L.*) and buckwheat. *European Food Research and Technology*, *249*(9), 2273-2285. doi.org/10.1007/s00217-023-04295-w.

Finalmente, la *octava publicación*, hace referencia a la elaboración y caracterización de un análogo cárnico (hamburguesa vegana) con adición de dos emulsiones gelificadas elaboradas con harina de trigo sarraceno y aceite de cáñamo o de chía, como fuente de grasa:

8. **Botella-Martínez, C.,** Viuda-Martos, M., Fernández-López, J. A., Pérez-Álvarez, J. A., & Fernández-López, J. (2022). Development of plant-based burgers using gelled emulsions as fat source and beetroot juice as colorant: Effects on chemical, physicochemical, appearance and sensory characteristics. *LWT*, 172, 114193. doi.org/10.1016/j.lwt.2022.114193.

RESUMEN

El futuro del sector cárnico depende de la adopción de prácticas más sostenibles y respetuosas con el medioambiente, alineadas con las recomendaciones institucionales y los retos derivados del cambio climático. Esta transición beneficiará al medioambiente y reflejará una industria capaz de responder a las demandas actuales de los consumidores, quienes valoran cada vez más la calidad, seguridad y valor nutricional de los alimentos, junto con una mayor conciencia medioambiental. No obstante, las formulaciones tradicionales de productos cárnicos, que incluyen grasa animal, presentan connotaciones negativas desde el punto de vista nutricional, ya que el consumo de grasas saturadas y trans está asociado a un mayor riesgo de enfermedades cardiovasculares, obesidad, hipertensión y cáncer. En respuesta, la industria alimentaria está investigando alternativas como la reducción o sustitución de las grasas animales por grasas vegetales para mejorar el perfil lipídico de estos productos. Entre las opciones más estudiadas se encuentran las emulsiones gelificadas, estructuras que

combinan propiedades de líquidos y sólidos, generando materiales coloidales con propiedades reológicas similares a las de la grasa animal.

El objetivo principal de esta tesis fue analizar el impacto de la sustitución de grasa animal por emulsiones gelificadas elaboradas a partir de aceites vegetales (cáñamo, chía, lino y sésamo) y harinas de pseudocereales (amaranto, teff, trigo sarraceno y quinoa blanca) sobre las propiedades químicas, físico-químicas, nutricionales y sensoriales de diversos productos cárnicos y análogos de carne. En la primera etapa del estudio, se desarrollaron 40 emulsiones gelificadas combinando cada harina con cada aceite, así como con mezclas 1:1 de los aceites, para luego caracterizarlas y evaluar su estabilidad tras 15 días de congelación a -18 °C. Las emulsiones más prometedoras, principalmente las combinaciones de harina de amaranto y trigo sarraceno con aceites de chía y cáñamo se utilizaron en productos cárnicos como hamburguesas de ternera, salchichas tipo Frankfurt, paté y embutidos tradicionales portugueses (alheiras), reemplazando diferentes proporciones de grasa animal (hasta un 100% en algunos casos). Además, se desarrolló una hamburguesa vegana, utilizando emulsiones gelificadas como fuente de grasa para mejorar sus propiedades tecnológicas y sensoriales.

La incorporación de emulsiones gelificadas se mostró viable desde el punto de vista tecnológico, permitiendo una reducción significativa del contenido graso en los productos reformulados en comparación con los tradicionales. Asimismo, se observó una mejora en el perfil lipídico, con una disminución de los ácidos grasos saturados y un aumento de los poliinsaturados, destacando los ácidos α -linolénico y linoleico, lo que permitió etiquetar algunas hamburguesas de ternera como "altas en ácidos grasos omega 3". Esta mejora del perfil lipídico se tradujo en una reducción de índices nutricionales como los índices trombogénico y aterogénico, así

como en una mejora en la relación entre ácidos grasos omega 3 y omega 6. Aunque las emulsiones a base de aceites vegetales son más susceptibles a la oxidación, en productos como las salchichas tipo Frankfurt y las alheiras no se observaron diferencias significativas en comparación con los productos control. En otros productos, la oxidación aumentó a medida que aumentaba el porcentaje de sustitución de grasa animal, pero sin superar el umbral de 2 mg de MDA/kg de muestra, lo que hubiera implicado una percepción de rancidez por parte de los consumidores. En cuanto a la evaluación sensorial, los productos reformulados obtuvieron una alta aceptación general. La principal diferencia respecto a las muestras control se observó en el color de algunos productos con altos niveles de sustitución de grasa, particularmente aquellos que contenían aceite de cáñamo, debido a su tonalidad verdosa.

En conclusión, el uso de emulsiones gelificadas representa una innovación que contribuye a la salud de los consumidores y al cuidado del medio ambiente al reducir el uso de grasa animal, logrando productos más saludables sin comprometer sus propiedades tecnológicas, texturales, sensoriales o de seguridad alimentaria.

ABSTRACT

The future of the meat sector depends on adopting more sustainable and environmentally friendly practices, in line with institutional recommendations and the challenges posed by climate change. This transition will not only benefit the environment but will also demonstrate an industry capable of meeting the demands of today's consumers, who increasingly prioritize food quality, safety, and nutritional value, alongside greater environmental consciousness. However, traditional formulations of meat products, which often include animal fat, carry negative nutritional

implications, as the consumption of saturated and trans fats is linked to a higher risk of cardiovascular disease, obesity, hypertension, and cancer. In response to these concerns, the food industry is exploring alternatives, such as reducing or replacing animal fats with vegetable fats to improve the lipid profile of these products. Among the most studied options are gelled emulsions, structures that combine properties of liquids and solids, generating colloidal materials with rheological properties like those of animal fat.

The main objective of this thesis was to evaluate the impact of replacing animal fat with gelled emulsions made from vegetable oils (hemp, chia, flaxseed, and sesame) and pseudocereal flours (amaranth, teff, buckwheat, and white quinoa) on the chemical, physicochemical, nutritional, and sensory properties of several meat products and meat analogs. In the initial phase of the study, 40 gelled emulsions were formulated by combining each flour with each oil, as well as with 1:1 mixtures of the oils. These emulsions were then characterized and assessed for their stability after 15 days of freezing at -18°C . The most promising emulsions, particularly the combinations of amaranth and buckwheat flours with chia or hemp oils, were incorporated into meat products such as beef burgers, Frankfurters, pâté, and traditional Portuguese sausages (alheiras), replacing different proportions of animal fat (up to 100% in some cases). Additionally, a plant-based burger was developed using gelled emulsions as a fat source to enhance its technological and sensory properties.

The incorporation of gelled emulsions proved to be technologically feasible, allowing a significant reduction of fat content in the reformulated products compared to traditional ones. An improvement in the lipid profile was also observed, with a decrease in saturated fatty acids and an increase in polyunsaturated fatty acids, especially α -linolenic and linoleic acids, which

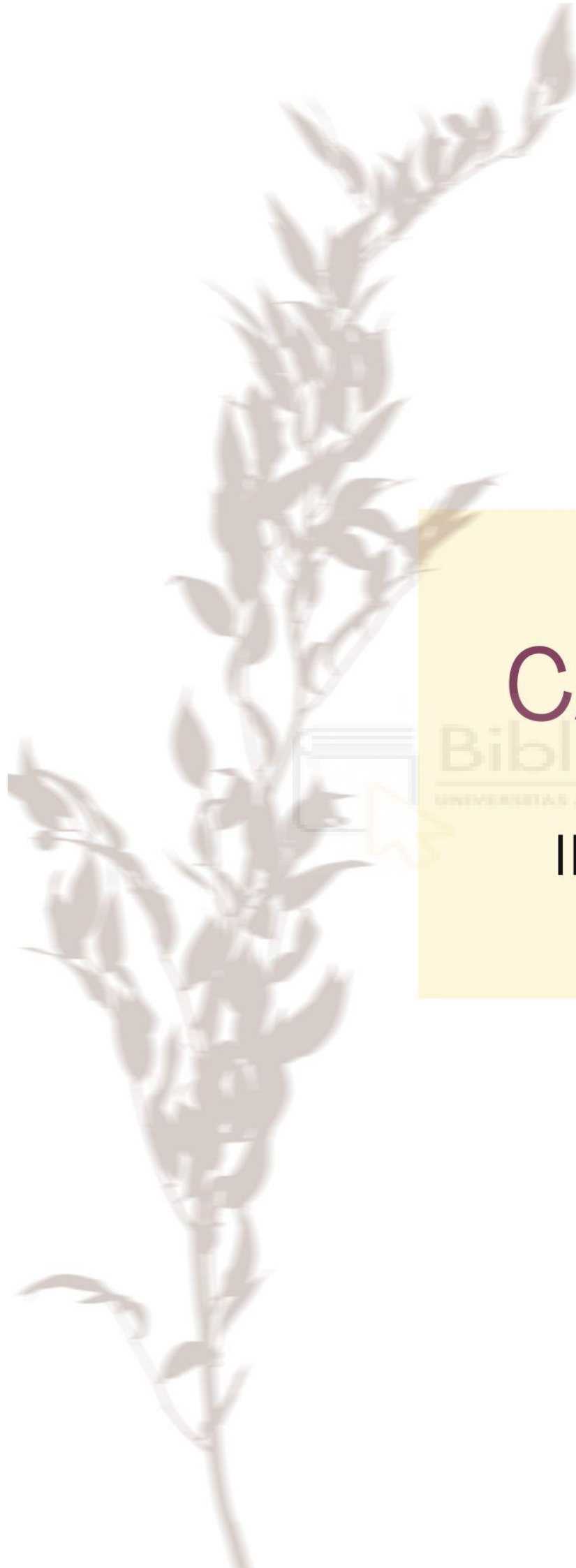
allowed some beef patties to be labeled as 'high in omega 3 fatty acids'. The enhanced lipid profile also contributed to a decrease in nutritional indices, such as thrombogenic and atherogenic indices, and improved the omega 3 to omega 6 fatty acid ratio. While vegetable oil-based emulsions are generally more susceptible to oxidation, no significant differences were observed in products like Frankfurters and alheiras compared to control samples. In other products, oxidation increased as the percentage of animal fat substitution increased, but without exceeding the threshold of 2 mg MDA/kg sample, which would have implied a perception of rancidity by consumers. Sensory evaluations revealed a high level of overall acceptance for the reformulated products. The most notable difference compared to control samples was a slight alteration in the color of some products with higher fat replacement levels, especially those containing hemp oil, due to their greenish hue.

In conclusion, the use of gelled emulsions represents a significant innovation that promotes consumer health and environmental sustainability by reducing the reliance on animal fats. This approach enables the production of healthier meat products without compromising their technological, textural, sensory, or food safety characteristics.

CAPÍTULO 2

Biblioteca
UNIVERSIDAD Miguel Hernández

INTRODUCCIÓN



2. INTRODUCCIÓN

2.1. ALIMENTACIÓN Y SALUD

Según la Organización Mundial de la Salud (OMS) y la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO), una dieta saludable es una de las bases para la salud, el bienestar, el crecimiento óptimo y el desarrollo. Es cierto que la consideración de “dieta saludable” puede variar en función de las necesidades individuales, los alimentos disponibles, los hábitos alimentarios, las normas culturales y otras consideraciones, pero los principios básicos de una dieta saludable son los mismos para todo el mundo. En este sentido, la OMS y la FAO recomiendan (Aldaya et al., 2021; FAO, 2023; FAO & OMS, 2020):

- Dieta variada, buscando el equilibrio entre ingesta y gasto calórico
- Los carbohidratos como la principal fuente de calorías
- Reducir las grasas totales y sustituir las grasas saturadas por grasas insaturadas y eliminar las grasas *trans* industriales
- Reducir los azúcares libres
- Limitar la ingesta de sodio
- Consumir verduras y frutas

Todas estas recomendaciones han sido adoptadas por los organismos oficiales de nuestro país y se han desarrollado diversas campañas de comunicación (Figura 1) con el objetivo principal de sensibilizar a la población de las consecuencias que tiene para la salud no seguir dichas recomendaciones. Así por ejemplo, la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN), en el marco de la Estrategia para la Nutrición, Actividad Física y Prevención de la Obesidad (NAOS), ha

desarrollado la campaña "*Plan Cuídate+ menos sal es más salud*", que se lanzó con el objetivo de informar a los consumidores sobre los problemas derivados de un consumo elevado de sal; también se incluyó la campaña "*Plan cuídate+ 2012*" en la cual se incorporaron nuevos contenidos sobre las grasas de los alimentos y la importancia de reducir su ingesta, en especial de las grasas saturadas, así como consejos y referencias para promover la realización de actividad física por parte de la población; la campaña "*5 al día*" que se lanzó para estimular el consumo de, al menos 5 raciones de frutas y hortalizas al día; la campaña "*#Azúcar, TeDejo*" lanzada para concienciar y sensibilizar al consumidor sobre la importancia de reducir el consumo de alimentos ricos en azúcares añadidos; o la campaña "*Come sano, muévete y cuida tu planeta*" en donde, y como se discutirá más adelante, ya se incorpora el término de sostenibilidad, al de alimentación saludable. Estas campañas (o similares) también se han desarrollado a nivel internacional, como, por ejemplo, la campaña que lanzó la *American Heart Association* en USA "*The facts on fat*" en la que se recomendaba el reemplazo en la dieta de grasas saturadas (*bad fats*) por grasas insaturadas (*good fats*).

Todas ellas son solo un ejemplo de la estrecha relación entre alimentación y salud que lleva a las políticas sanitarias (mundiales, europeas y nacionales) a establecer recomendaciones en las que se insta a los países a emprender acciones de prevención de enfermedades no transmisibles relacionadas con la alimentación (obesidad, hipertensión, diabetes, etc.). Pero, además, es de vital importancia que todas estas recomendaciones no sean asumidas solo por la administración, sino que sea una tarea de corresponsabilidad que debe implicar también a la industria alimentaria, organizaciones de consumidores y expertos científicos.



Figura 1. Estrategias adoptadas por organismos nacionales e internacionales para adoptar las recomendaciones de una dieta saludable de la OMS y la FAO.

Todas estas recomendaciones y campañas han calado sin duda alguna en nuestra sociedad, porque como menciona Mintel, (2024) cuando se analizan las tendencias actuales de consumo de alimentos, los alimentos saludables están en cabeza, aunque ahora también se exige que estos alimentos sean sostenibles. Cuando se habla de alimentos saludables, se incluyen alimentos bajos en calorías, grasas saturadas, azúcares añadidos y sodio. El término sostenible se refiere a que los productos sean producidos de manera sostenible, utilizando ingredientes de origen local y respetuosos con el medio ambiente (Betoret et al., 2022; Schroën et al., 2020).

2.1.1. ALIMENTOS FUNCIONALES

Es evidente que, tras las demandas de los consumidores en la alimentación, la cual se ha visto fomentada por los organismos oficiales y apoyadas por campañas establecidas por instituciones nacionales, la industria alimentaria debe adaptarse y ofrecer alimentos que consigan cubrir

las necesidades y expectativas del consumidor actual. Muchos de estos alimentos se encuadran dentro de los llamados alimentos fortificados y funcionales.

La Autoridad Europea de Seguridad Alimentaria (EFSA) define un alimento funcional como: "un alimento que afecta beneficiosamente a una o más funciones objetivo en el cuerpo, más allá de los efectos nutricionales, de una manera relevante para mejorar el estado de salud y bienestar y/o reducir el riesgo de enfermedades. Un alimento funcional puede ser un alimento natural o un alimento al que se le ha añadido o eliminado un componente mediante medios tecnológicos o biotecnológicos, y debe demostrar sus efectos en cantidades que normalmente se esperan consumir en la dieta" (Parlamento Europeo, 2006).

Así pues, un alimento funcional es un alimento que aporta beneficios a la salud más allá del valor nutricional del mismo (Hammoudi Halat et al., 2023). En la última década, esta categoría de alimentos ha experimentado un crecimiento notable. Como resultado de ello, la industria alimentaria ha mostrado un interés creciente en su comercialización, enfocándose significativamente en la fortificación con nutrientes y compuestos bioactivos, así como en la recuperación y mejora de alimentos para incrementar sus beneficios para la salud (Sorrenti et al., 2023).

Existe una gran diversidad de compuestos bioactivos añadidos a las formulaciones de los alimentos funcionales que hacen que se distingan de los alimentos tradicionales (Akhtar et al., 2024). Estos compuestos van desde vitaminas y minerales, antioxidantes (ácidos fenólicos y flavonoides, tocoferoles, etc.), proteínas (péptidos bioactivos), probióticos, prebióticos o fibra dietética (ligninas, fructooligosacáridos, beta-glucanos, inulina, etc.), hasta ácidos grasos esenciales omega 3 y omega 6, proporcionando a los alimentos a los que se incorporan unas propiedades que van más allá de la

nutrición básica, operando a niveles celulares y moleculares y ayudando a modular funciones fisiológicas (Cloninger et al., 2019).

Es en el Reglamento (CE) 1924/2006 del Parlamento Europeo y del Consejo de 20 de diciembre de 2006, donde se recogen las declaraciones nutricionales y de propiedades saludables de los alimentos, las cuales se corresponden en muchos casos, con algunos de los beneficios saludables atribuidos a los llamados alimentos funcionales. Este reglamento decretó una lista positiva de declaraciones nutricionales, que sufrió distintas ampliaciones tanto en el año 2010 como en el 2012 (116/2010 y 1047/2012). En la Tabla 1, se muestra un resumen de las declaraciones y su correspondiente condición de uso, así como la cantidad a partir o por debajo de la cual se pueden atribuir dichas declaraciones.

Tabla 1. Declaraciones nutricionales autorizadas en el anexo del Reglamento (CE) N.º 1924/2006.

DECLARACIÓN	CONDICIONES DE USO
Bajo valor energético	Si el producto no contiene más de 40 kcal (170 kJ) /100 g en el caso de los sólidos o más de 20 kcal (80 kJ) /100 ml en el caso de los líquidos. Para los edulcorantes de mesa se aplicará un límite de 4 kcal (17 kJ) por porción, con propiedades edulcorantes equivalentes a 6 g de sacarosa (una cucharadita de sacarosa aproximadamente).
Valor energético reducido	Si el valor energético se reduce, como mínimo, en un 30 %, con una indicación de la característica o características que provocan la reducción del valor energético total del alimento.
Sin aporte energético	Si el producto no contiene más de 4 kcal (17 kJ) /100 ml. Para los edulcorantes de mesa se aplicará un límite de 0,4 kcal (1,7 kJ) por porción, con propiedades edulcorantes equivalentes a 6 g de sacarosa.
Bajo contenido en grasa	Si el producto no contiene más de 3 g de grasa por 100 g en el caso de los sólidos o 1,5 g de grasa por 100 ml en el caso de los líquidos (1,8 g de grasa por 100 ml para la leche semidesnatada).
Sin grasa	Si el producto no contiene más de 0,5 g de grasa por 100 g o 100 ml. No obstante, se prohibirán las declaraciones expresadas como «X % sin grasa».
Bajo contenido de grasas saturadas	Si la suma de ácidos grasos saturados y de ácidos grasos trans en el producto no es superior a 1,5 g/100

	g para los productos sólidos y a 0,75 g/100 ml para los productos líquidos, y en cualquier caso la suma de ácidos grasos saturados y de ácidos grasos trans no deberá aportar más del 10 % del valor energético.
Sin grasas saturadas	Si la suma de grasas saturadas y de ácidos grasos trans no es superior a 0,1 g por 100 g o 100 ml.
Bajo contenido de azúcares	Si el producto no contiene más de 5 g de azúcares por 100 g en el caso de los sólidos o 2,5 g de azúcares por 100 ml en el caso de los líquidos.
Sin azúcares	Si producto no contiene más de 0,5 g de azúcares por 100 g o 100 ml.
Sin azúcares añadidos	Si no se ha añadido al producto ningún monosacárido ni disacárido, ni ningún alimento utilizado por sus propiedades edulcorantes. Si los azúcares están naturalmente presentes en los alimentos, en el etiquetado deberá figurar asimismo la siguiente indicación: «CONTIENE AZÚCARES NATURALMENTE PRESENTES».
Bajo contenido de sodio/sal	Si el producto no contiene más de 0,12 g de sodio, o el valor equivalente de sal, por 100 g o por 100 ml. Por lo que respecta a las aguas distintas de las aguas minerales naturales cuya composición se ajuste a las disposiciones de la Directiva 80/777/CEE, este valor no deberá ser superior a 2 mg de sodio por 100 ml.
Muy bajo contenido de sodio/sal	Si el producto no contiene más de 0,04 g de sodio, o valor equivalente de sal, por 100 g o por 100 ml. Esta declaración no se utilizará para las aguas minerales naturales y otras aguas.
Sin sodio o sin sal	Si el producto no contiene más de 0,005 g de sodio, o el valor equivalente de sal, por 100 g.
Sin sodio o sin sal añadidos	Si no se ha añadido al producto sodio o sal, ni ingrediente alguno con sodio o sal añadidos, y siempre que el producto no contenga más de 0,12 g de sodio, o su valor equivalente de sal, por 100 g o por 100 ml.».
Fuente de fibra	Si el producto contiene como mínimo 3 g de fibra por 100 g o, como mínimo, 1,5 g de fibra por 100 kcal.
Alto contenido en fibra	Si el producto contiene como mínimo 6 g de fibra por 100 g o 3 g de fibra por 100 kcal.
Fuente de proteínas	Si las proteínas aportan como mínimo el 12 % del valor energético del alimento.
Alto contenido de proteínas	Si las proteínas aportan como mínimo el 20 % del valor energético del alimento.
Fuente de (nombre de las vitaminas) y/o (nombre de los minerales)	Si el producto contiene como mínimo una cantidad significativa tal como se define en el Anexo de la Directiva 90/496/CEE o una cantidad establecida por las excepciones concedidas en virtud del artículo 6 del Reglamento (CE) no 1925/2006 del Parlamento Europeo y del Consejo, de 20 de diciembre de 2006, [sobre la adición de vitaminas, minerales y otras determinadas sustancias a los alimentos].
Alto contenido de (nombre de las vitaminas) y/o (nombre de los minerales)	Si el producto contiene como mínimo dos veces el valor de la «fuente de [NOMBRE DE LAS VITAMINAS] y/o [NOMBRE DE LOS MINERALES]».
Contiene (nombre del nutriente u otra sustancia)	Si el producto cumple todas las disposiciones aplicables previstas en el presente Reglamento, y en

	particular en el artículo 5. Por lo que respecta a las vitaminas y minerales, se aplicarán las condiciones correspondientes a la declaración «fuente de».
Mayor contenido de (nombre del nutriente)	Si el producto cumple las condiciones previstas para la declaración «fuente de» y el incremento de su contenido es de, como mínimo, el 30 % en comparación con un producto similar.
Contenido reducido de (nombre del nutriente)	<p>Solamente podrá declararse que se ha reducido el contenido de uno o más nutrientes, así como efectuarse cualquier otra declaración que pueda tener el mismo significado para el consumidor, si la reducción del contenido es de, como mínimo, el 30 % en comparación con un producto similar, excepto para micronutrientes, en los que será admisible una diferencia del 10 % en los valores de referencia establecidos en la Directiva 90/496/CEE, así como para el sodio, o el valor equivalente para la sal, en que será admisible una diferencia del 25 %.</p> <p>«Solamente podrá declararse “contenido reducido de grasas saturadas”, así como efectuarse cualquier otra declaración que pueda tener el mismo significado para el consumidor, si:</p> <p>a) la suma de ácidos grasos saturados y de ácidos grasos trans en el producto objeto de la declaración es, como mínimo, un 30 % inferior a la de un producto similar, y</p> <p>b) el contenido de ácidos grasos trans en el producto objeto de la declaración es igual o inferior al de un producto similar.</p> <p>Solamente podrá declararse “contenido reducido de azúcares”, así como efectuarse cualquier otra declaración que pueda tener el mismo significado para el consumidor, si el aporte energético del producto objeto de la declaración es igual o inferior al de un producto similar.».</p>
Sin sodio o sin sal añadidos	Si no se ha añadido al producto sodio o sal, ni ingrediente alguno con sodio o sal añadidos, y siempre que el producto no contenga más de 0,12 g de sodio, o su valor equivalente de sal, por 100 g o por 100 ml.».
Light/lite (ligero)	Las declaraciones en las que se afirme que un producto es «light» o «lite» (ligero), y cualquier otra declaración que pueda tener el mismo significado para el consumidor, deberán cumplir las mismas condiciones que las establecidas para el término «contenido reducido»; asimismo, la declaración deberá estar acompañada por una indicación de la característica o características que hacen que el alimento sea «light» o «lite» (ligero).
Naturalmente/ natural	Cuando un alimento reúna de forma natural la condición o las condiciones establecidas en el presente Anexo para el uso de una declaración nutricional, podrá utilizarse el término «naturalmente/natural» antepuesto a la declaración.
Fuente de ácidos grasos omega 3	Si el producto contiene al menos 0,3 g de ácido alfa-linolénico por 100 g y por 100 kcal, o al menos 40 mg

	de la suma de ácido eicosapentaenoico y ácido docosahexaenoico por 100 g y por 100 kcal.
Alto contenido de ácidos grasos omega 3	Si el producto contiene al menos 0,6 g de ácido alfa-linolénico por 100 g y por 100 kcal, o al menos 80 mg de la suma de ácido eicosapentaenoico y ácido docosahexaenoico por 100 g y por 100 kcal.
Alto contenido de grasas monoinsaturadas	Si al menos un 45 % de los ácidos grasos presentes en el producto proceden de grasas monoinsaturadas y las grasas monoinsaturadas aportan más del 20 % del valor energético del producto.
Alto contenido de grasas poliinsaturadas	Si al menos un 45 % de los ácidos grasos presentes en el producto proceden de grasas poliinsaturadas y las grasas poliinsaturadas aportan más del 20 % del valor energético del producto.
Alto contenido de grasas insaturadas	Si al menos un 70 % de los ácidos grasos presentes en el producto proceden de grasas insaturadas y las grasas insaturadas aportan más del 20 % del valor energético del producto.».

(Fuente: Reglamento (CE) N.º 1924/2006).

La función antioxidante tiene un papel crucial minimizando el impacto del daño que generan los radicales libres en el cuerpo humano, disminuyendo la inflamación y previniendo enfermedades crónicas (Stephen et al., 2023; Vignesh et al., 2022; Wang & Kang, 2020). Esta función antioxidante por parte de los alimentos funcionales se consigue al fortificar a los alimentos tradicionales con ácidos fenólicos, flavonoides, lignanos, ácido ascórbico, retinol, tocoferoles, péptidos bioactivos, etc., los cuales proceden de diversas fuentes como frutas, vegetales, frutos secos, leche, miel, etc., así como de sus coproductos (Vignesh et al., 2024).

Otra forma de enriquecer los alimentos tradicionales y hacerlos por tanto alimentos funcionales, es mediante la adición de probióticos y prebióticos, que favorecen el crecimiento de la microbiota gastrointestinal. Entre los compuestos que se pueden adicionar para hacer la función de prebiótico, se encuentran fructooligosacáridos, beta-glucanos e inulina, los cuales se obtienen de multitud de frutas y vegetales, así como de sus coproductos (Castro-López et al., 2023; Gomaa, 2020; Vignesh et al., 2023). En cuanto a los probióticos, son bacterias vivas encargadas de mantener el balance y la diversidad del microbioma intestinal (Maftei et al., 2024). Los

alimentos que pueden proporcionar dichos probióticos son los alimentos lácteos y los alimentos fermentados, como por ejemplo los yogures, el kéfir o el chucrut (Patarata et al., 2023; Plaza-Diaz et al., 2019). Una forma efectiva de obtener alimentos funcionales, bastante beneficiosos para la salud es mediante la combinación de los pre y probióticos en un mismo alimento, dando como resultado la mejora de la microbiota la cual a su vez se ve implicada en multitud de sistemas (Davani-Davari et al., 2019).

Sin embargo, la función de mayor interés para el presente estudio es la obtención de alimentos funcionales enriquecidos con ácidos grasos poliinsaturados (AGPI), específicamente omega 3 y omega 6. Los AGPI omega 3 más importantes son el ácido α -linolénico (ALA), ácido docosahexaenoico (DHA) y el ácido eicosapentaenoico (EPA), mientras que los AGPI omega 6 son el ácido linoleico (AL), el ácido γ -linolénico (GLA) y el ácido araquidónico (AA) (Djuricic & Calder, 2021; Łęska & Czyżak-Runowska, 2017). Los ácidos grasos omega 3 y omega 6, presentes en abundancia en pescados grasos, semillas y frutos secos, son ampliamente reconocidos por su impacto positivo en la salud cardiovascular y cognitiva, además de tener un efecto beneficioso en trastornos neurológicos, en ciertos cánceres, diabetes tipo 2, en la coagulación sanguínea y en los niveles de fibrinógeno (Fekete et al., 2023; Łęska & Czyżak-Runowska, 2017; Mukhametov et al., 2022). Estos ácidos grasos se consideran esenciales, debido a que no pueden ser sintetizados por el cuerpo humano y deben ser ingeridos a través de la dieta o mediante suplementos en caso de deficiencias. Además, la ingesta diaria recomendada para adultos es de 250 mg de EPA o DHA, 2 g de ALA y 10 g de AL (EFSA, 2009), por lo que el enriquecimiento de alimentos con estos ácidos grasos podría contribuir a aumentar la ingesta dietética y alcanzar los valores recomendados. Los ácidos grasos omega 3 y 6, presentan propiedades antiinflamatorias, en concreto el ALA y el EPA, inhiben la acción de la enzima

ciclooxigenasa (COX) la cual, al entrar en contacto con el AA lo convierte en eicosanoides proinflamatorios, siendo la acción del ALA de inhibición directa sobre la enzima COX y del EPA actuando como competidor del AA (Ye & Ghosh, 2018). Otra acción del EPA a nivel antiinflamatorio es por la modulación de la actividad del factor nuclear kappa B, factor que desempeña un papel importante en la expresión de genes implicados en la respuesta inflamatoria (Palanisamy et al., 2015). Tanto el AL como el AA son precursores de los eicosanoides, los cuales incluyen en la inflamación y las respuestas inmunitarias (Sienko et al., 2023). Esta acción antiinflamatoria es especialmente relevante en el contexto de la salud cardiovascular, dado que la inflamación crónica es un factor que contribuye al desarrollo de enfermedades cardíacas (Vignesh et al., 2023, Marangoni et al., 2019). La ingesta regular de alimentos ricos en omega 3 y 6 se ha asociado con un menor riesgo de trastornos cardiovasculares, tales como la enfermedad de las arterias coronarias y la hipertensión. A parte de los beneficios cardiovasculares, los ácidos grasos omega 3 y 6 desempeñan un papel vital en la función cognitiva. El AA y el DHA, en particular son componentes implicados en el cerebro y está asociado a un mayor rendimiento cognitivo (Sherzai et al., 2023, Brenna, 2016). El AA desempeña un papel crucial en el desarrollo cerebral y en la función inmunitaria de los infantes y se encuentra comúnmente en la leche materna, así como ayuda a mitigar la acumulación de grasas en el hígado y a mantener la masa corporal magra previendo el hígado graso (Hadley et al., 2016). Por ello, incorporar fuentes de ácidos grasos omega 3 y omega 6 en la dieta sirve como medicina proactiva para promover el bienestar cardiovascular y mantener la agudeza cognitiva a lo largo de la vida, aparte de apoyar a la salud en general.

2.1.2. IMPLICACIONES DEL CONSUMO DE GRASAS EN LA SALUD

Según lo visto en el apartado anterior, existen varias declaraciones relacionadas con el contenido de grasa saturada, insaturada, monoinsaturada, poliinsaturada, rico o con alto contenido en omega 3. Por ello es importante conocer las implicaciones de la grasa en la salud del consumidor, ya que se recogen hasta 9 declaraciones nutricionales autorizadas relacionadas con la grasa.

Las grasas, o lípidos, son compuestos orgánicos de naturaleza hidrofóbica, fáciles de disolver en compuestos orgánicos de baja polaridad (Domínguez et al., 2022). Aunque las hay de muy diversos tipos, las más importantes en nutrición humana son los triglicéridos, los ácidos grasos, los fosfolípidos y los esteroides (Bowen-Forbes & Goldson-Barnaby, 2023). Los triglicéridos son los mayoritarios en la dieta y se componen de una molécula de glicerol unida a tres ácidos grasos (Jadhav & Annapure, 2023). Los ácidos grasos (AG) pueden variar en la longitud de su cadena y grado de insaturación (ácidos grasos saturados (AGS) e insaturados (AGI) y a su vez estos últimos se clasifican según el número de insaturaciones que presenten en ácidos grasos monoinsaturados (AGMI: una sola insaturación o doble enlace) y ácidos grasos poliinsaturados (AGPI: varias insaturaciones) (Agregán et al., 2022). En función de la distancia del primer doble enlace con respecto al grupo metil terminal del AG se distinguen dos familias de AG: AG omega 3 y omega 6 (Kousparou et al., 2023). Así mismo, los AG pueden clasificarse en esenciales (deben ingerirse con la dieta, como el ácido graso α -linolénico y linoleico) y no esenciales (los cuales pueden sintetizarse en el organismo a partir de otros) (Kapoor et al., 2021). Los ácidos grasos trans (AGT) son isómeros de los AGI. Los cuales, según la posición del hidrógeno en los carbonos adyacentes al carbono del doble enlace, se pueden clasificar los AG en *cis* o *trans*. Los AG *trans* presentan los hidrógenos del doble enlace

en oposición y los isómeros *cis* los presentan en la misma posición. Los cuales, a pesar de ser insaturados, se comportan como si fueran AGS debido a que forman cadenas lineales alrededor del doble enlace, quedando con configuración empaquetada (Pipoyan et al., 2021). A estos últimos, se los ha asociado con enfermedades coronarias (OMS, 2023). Los fosfolípidos son componentes fundamentales de las membranas celulares (de bicapa lipídica) e intervienen en la solubilización de los ácidos biliares, favoreciendo la digestión de las grasas. Por último, los esteroides como el colesterol son también un componente importante en las membranas de las células animales y precursor de muchas moléculas en nuestro organismo (Bowen-Forbes & Goldson-Barnaby, 2023).

La grasa es un nutriente de gran importancia en el organismo humano ya que presenta diversas funciones tanto estructurales (contribuye a la integridad de las membranas celulares y la formación de tejidos), como de transporte de vitaminas liposolubles, fuente de energía (9 kcal/g, más del doble de los carbohidratos), así como su papel como precursor de moléculas biológicas con funciones metabólicas críticas (prostaglandinas, tromboxanos, leucotrienos, lipoxinas, etc.). La grasa también tiene funciones aislantes y protectoras. En los niños, las grasas no solo son una fuente principal de energía, sino que también proporcionan ácidos grasos esenciales y ácidos grasos poliinsaturados, que son fundamentales para un adecuado crecimiento, desarrollo cognitivo, y en general, para la prevención de enfermedades crónicas no transmisibles (ECNT) (Billingsley et al., 2018; Djuricic & Calder, 2021; Hadley et al., 2016). En la Figura 2A, se presenta una asociación del tipo de lípido o lipoproteína con su función en el organismo humano. Por otra parte, existen diversos estudios que asocian efectos negativos del consumo de grasa, como la inflamación y enfermedades crónicas. Estos estudios relacionan el consumo de grasas saturadas y grasas

trans con un aumento del riesgo cardiovascular, debido al aumento de la cantidad de colesterol total en sangre (colesterol de lipoproteína de baja densidad (LDL) y de colesterol de lipoproteína de alta densidad (HDL)), posiblemente debido a cambios en el metabolismo de los hepatocitos, promoviendo la inflamación sistemática en el cuerpo, lo cual se ha relacionado con el desarrollo de enfermedades cardiovasculares, obesidad, diabetes tipo II, hipertensión y algunos tipos de cáncer (Bhandari et al., 2020; Bhargava et al., 2022). En la Figura 2B puede verse el tipo de lípido asociado al desarrollo de enfermedades coronarias de una forma directa o condicionada. La FAO y la OMS recomiendan una ingesta total de grasas *trans* inferior al 1% en la cantidad energética diaria ingerida (OMS, 2023). Por lo tanto, tanto una ingesta alta como deficiente en grasas puede tener repercusiones en la salud a corto, medio y largo plazo (Chianese et al., 2017; Chilton et al., 2017; Ros et al., 2015).

Por ello, existen recomendaciones de ingesta de grasa en la dieta por parte de la OMS, la cual recomienda que las kilocalorías provenientes de la grasa se encuentren entre el 20-35% del total de kilocalorías de la dieta, así como que la ingesta de AGS ha de ser menor del 10% de la energía total ingerida diariamente con la dieta (OMS, 2023). Quedando por lo tanto una ingesta entre el 6-11% para los AGPI y AGMI, la diferencia.

Como se deduce de estas recomendaciones de ingesta de grasa, no solo es importante la cantidad de grasa ingerida sino, y quizás más importante, el tipo de grasa. Por ello se han definido diferentes índices para medir la calidad de la fracción lipídica ingerida en la dieta (Tabla 2). De entre ellos, los más usuales son: la relación entre los ácidos grasos saturados y los ácidos grasos poliinsaturados (AGPI/AGS), la relación omega 6/omega 3 (n-6/n-3), el índice aterogénico (IA), el índice trombogénico (IT), la relación hipocolesterolémica/Hipercolesterolémica (h/H), y el valor nutricional de las

grasas. Además, se ha establecido una relación entre estos índices nutricionales y el desarrollo de enfermedades de diversa índole (Chen & Liu, 2020).

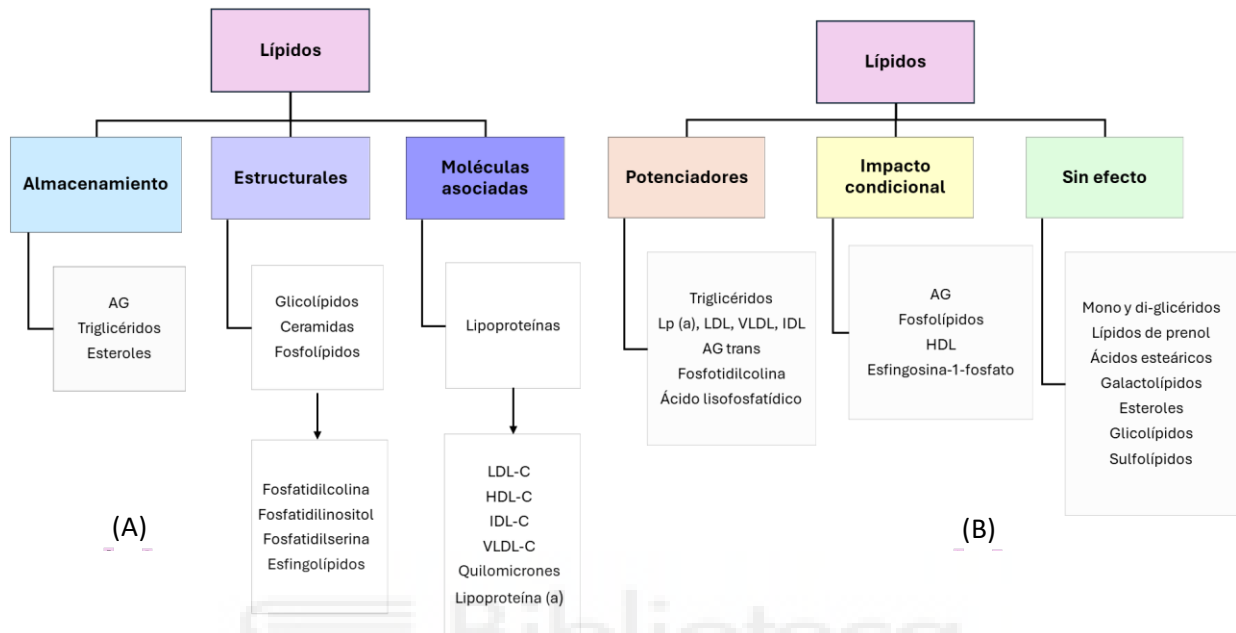


Figura 2. (A) Clasificación de los lípidos según su función. (B) Clasificación de los lípidos en función de su papel en el desarrollo de enfermedades cardiovasculares. (Adaptada de (Bhargava et al., 2022)).

La relación entre AGPI y AGS en la dieta se considera muy importante porque se ha observado que el consumo de AGS de cadena larga (de 12 a 16 carbonos) está asociado con el aumento de los niveles de colesterol total y de colesterol LDL en el plasma sanguíneo (Rimm et al., 2018; Wu et al., 2020). Por otro lado, un mayor consumo de AGPI en la dieta ha demostrado reducir la incidencia de enfermedades cardiovasculares (Langley et al., 2020; Vissers et al., 2019). Es por ello por lo que, si la relación AGPI/ AGS supera valores de 0,4, es un indicativo de una dieta con una relación adecuada entre los AGPI y los AGS, siendo un factor determinante en el bienestar humano (Pretorius & Schönfeldt, 2021; Realini et al., 2021). Se ha observado que la ingesta de alimentos que presentan una relación entre AGPI y AGS superior a 0,4,

contribuye a una disminución en los niveles de colesterol y por ende a una protección cardiovascular (Wu et al., 2020).

Como se comentó con anterioridad (apartado 2.1.1) la ingesta de ácidos grasos omega 3 y omega 6 tienen un importante efecto positivo en la salud, por ello otra relación a tener en cuenta desde el punto de vista nutricional en las grasas ingeridas en la dieta es la proporción de omega 6/omega 3, la cual debería ser inferior a 4 (Li et al., 2020; Patel et al., 2022) debido a que un incremento de esta relación se ha asociado a un riesgo mayor de sufrir obesidad y diabetes tipo II (Monnard & Dulloo, 2021; Urlic et al., 2020).

El índice de aterogenicidad (IA) refleja la relación entre la suma de los ácidos grasos saturados y la suma de los ácidos grasos insaturados. Los AGS C12:0, C14:0 y C16:0, con la excepción del C18:0, son considerados pro-aterogénicos, ya que favorecen la adhesión de lípidos a las células de los sistemas circulatorio e inmunológico. Por otro lado, los AGPIs son considerados anti-aterogénicos, dado que inhiben la acumulación de placa y disminuyen los niveles de fosfolípidos, colesterol y ácidos grasos esterificados. En consecuencia, el consumo de alimentos con un IA bajo reduciría los niveles de colesterol total y LDL-C en el plasma sanguíneo humano (Monteiro et al., 2018; Omri et al., 2019; Yurchenko et al., 2018).

El índice de trombogenicidad (IT) evalúa el potencial trombogénico de los ácidos grasos, indicando su tendencia a formar coágulos en los vasos sanguíneos. Este índice refleja la relación entre los AG pro-trombóticos (C12:0, C14:0 y C16:0) y los AG anti-trombóticos (AGMI y las familias omega 3 y omega 6). Por consiguiente, el consumo de alimentos con un IT bajo es beneficioso para la salud cardiovascular (Chen & Liu, 2020).

El índice hipocolesterolémico/hipercolesterolémico (h/H) es un indicador empleado en el análisis del perfil de ácidos grasos en la carne de cordero,

introducido por Santos-Silva et al. (2002) y que se emplea como indicador de calidad en la carne de este animal (de Castro et al., 2023). La elevada proporción de AGS en los corderos resulta en un bajo índice AGPI/AGS, lo que motivó la creación del índice h/H para valorar el impacto de la composición de ácidos grasos en los niveles de colesterol. En comparación con el índice AGPI/AGS, el h/H ofrece una evaluación más precisa del impacto de la composición de ácidos grasos en las enfermedades cardiovasculares. No obstante, el índice h/H presenta ciertas limitaciones, ya que podría beneficiarse de la inclusión de una mayor variedad de ácidos grasos y la asignación de distintos pesos a las diversas especies moleculares de estos ácidos (Chen & Liu, 2020).

Tabla 2. Índices nutricionales empleados en la evaluación de alimentos para medir la calidad de su grasa.

Índice	Nombre completo	Fórmula para su cálculo
AGPI/AGS	Ácidos grasos poliinsaturados/ácidos grasos saturados	$\sum \text{AGPI/AGS}$
IA	Índice Aterogénico	$[\text{C12:0} + (4 * \text{C14:0}) + \text{C16:0}] / \sum \text{AGI}$
IT	Índice trombogénico	$(\text{C14:0} + \text{C16:0} + \text{C18:0}) / [(0.5 * \text{AGMI}) + (0.5 * \sum n-6 \text{AGPI}) + (3 * \sum n-3 \text{AGPI}) + (n-3/n-6)]$
h/H	Ratio hipocolesterolémico/hipercolesterolémico	$(\text{cis-C18:1} + \sum \text{AGPI}) / (\text{C12:0} + \text{C14:0} + \text{C16:0})$
HPI	Índice promotor de salud	$\sum \text{AGI} / [\text{C12:0} + (4 * \text{C14:0}) + \text{C16:0}]$
IU	Índice de insaturación	$1 * (\% \text{ monoenoicos}) + 2 * (\% \text{ dienoicos}) + 3 * (\% \text{ trienoicos}) + 4 * (\% \text{ tetraenoicos}) + 5 * (\% \text{ pentaenoicos}) + 6 * (\% \text{ hexaenoicos})$
EPA+DHA	Sumatorio del ácido eicosapentaenoico y ácido docosahexaenoico	$\text{C22:6 n-3} + \text{C20:5 n-3}$
FLQ	Calidad lipídica de pescado/calidad lipídica de la carne	$100 * (\text{C22:6 n-3} + \text{C20:5 n-3}) / \sum \text{AG}$
LA/ALA	Ratio ácido linoleico/alfa-linolénico	$\text{C18:2 n-6} / \text{C18:3 n-3}$
TFA	Ácidos grasos <i>trans</i>	$\sum \text{TAG}$

(Adaptada de Chen & Liu, (2020)).

2.2. PRODUCTOS CÁRNICOS FUNCIONALES

La carne es uno de los alimentos básicos en la dieta. El consumo de carne ha ido aumentando a lo largo de las décadas, debido al aumento del poder adquisitivo de la población y al abaratamiento de los costes de producción tras la revolución industrial. En el último año (2023), los españoles destinaron a la compra de carne y derivados un 6,4% más que en el año 2022, destinando un 19,8% del presupuesto, situándose su consumo en 41,11 kg/persona/año, lo que supuso un incremento del 5,2% respecto al 2022. Pero en comparación con el año 2019 supuso una contracción del consumo de carne por parte de los hogares españoles del 7,8% (MAPAMA, 2023a). Este consumo se realizó principalmente en forma de carne fresca, que supuso un 72,1% de la carne total, seguido de la carne transformada (25,1%) y únicamente un 2,8% se destinó al consumo de carne congelada. De entre las carnes frescas, la más consumida por las familias españolas fue la de pollo (40,4%), seguida de la carne de cerdo (30,8%), vacuno (13%) y por último la carne de conejo (2%) por detrás de carne de despojos (2,2%) y de ovino y caprino (2,9%).

En la categoría de carne transformada se encuentran incluidos los productos cárnicos como, jamón curado y paleta, lomo embuchado normal e ibérico, chorizos, salchichón y salami, fuet y longanizas, jamón cocido, paleta cocida y fiambres. El consumo *per capita* de estos productos cárnicos transformados durante el año 2023, fue de 10,34 kg/persona/año, suponiendo una reducción ligera (0,8%) respecto al 2022. Los fiambres fueron los productos más consumidos en esta categoría (2,12 kg/persona/año) seguidos del jamón y la paleta curada normal (1,35 kg/persona/año) y entre los productos menos consumidos el tocino y manteca (0,38 kg/persona/año), salchichón/salami (0,37 kg/persona/año) y lomo embuchado (0,23 kg/persona/año) (Figura 3) (MAPAMA 2023b).

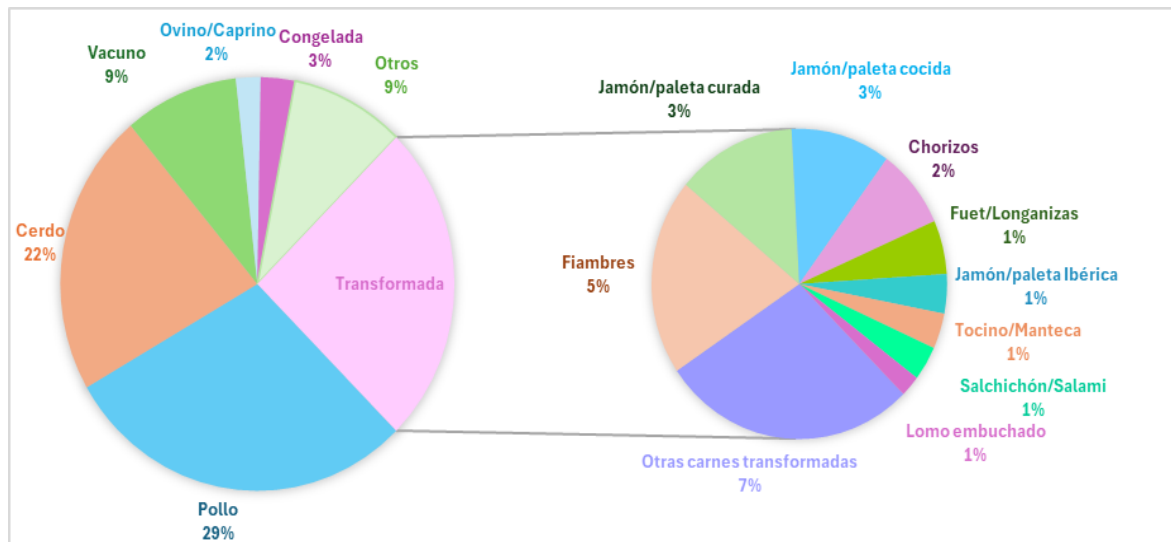


Figura 3. Consumo (%) en los hogares españoles de los diferentes tipos de carne.

(Fuente: MAPAMA (2023b).

Si se comparan las cifras y se ve la evolución del consumo de carne y carnes transformadas en la última década (Figura 4), se puede observar que desde el año 2014 hasta el 2019 hubo un descenso de consumo de carne y de carne transformada, con un repunte en el año 2020 debido a la crisis sanitaria por el Covid-19 donde el crecimiento fue del 12,9% en la carne y un 10,8% específicamente en la carne transformada respecto al año anterior (2019). Tras este repunte, de nuevo el consumo de carne total y carne transformada siguió descendiendo (21% y 15% respectivamente) hasta el año 2022. Aunque en la actualidad parece que se va recuperando levemente dicho consumo, tanto el de carne como el de carne transformada, si se comparan con los valores de hace una década (2014) es evidente que la tendencia general de consumo de este tipo de alimentos es a la baja.

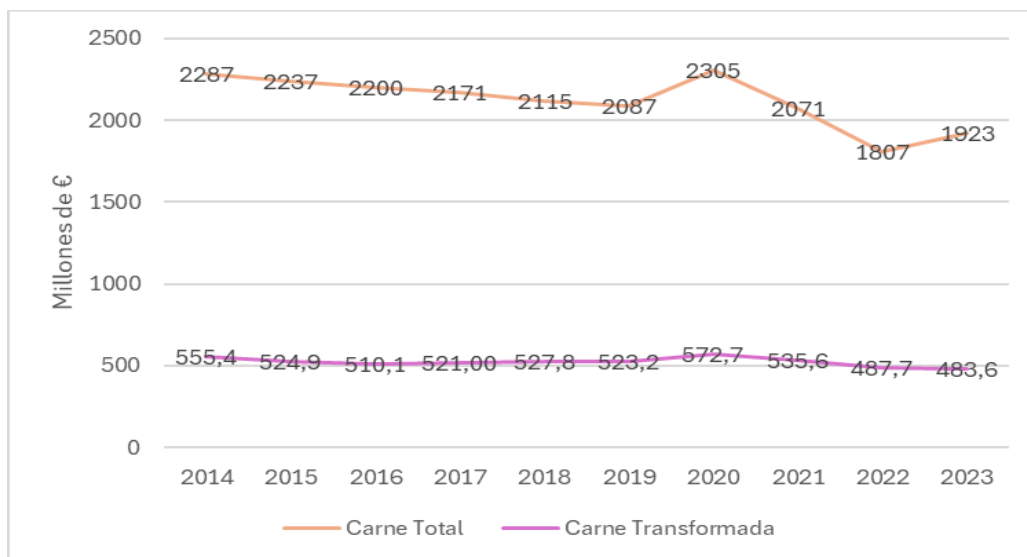


Figura 4. Consumo (gasto) en los hogares españoles de carne y carne transformada a lo largo de los últimos 10 años (2014-2023). (Fuente: MAPAMA (2023a,b).

Por ello, para contrarrestar o frenar este descenso en el consumo de carne y derivados cárnicos, la industria cárnica debe adaptarse a las demandas del consumidor actual, innovando continuamente y desarrollando nuevos productos. Como ya se ha comentado previamente, los consumidores demandan alimentos más sanos, nutritivos, seguros, sabrosos, y sostenibles (por la preocupación global y creciente por el medio ambiente). Por ello, también la industria cárnica ha de adaptarse a estas demandas y debería enfocarse hacia el desarrollo de productos cárnicos funcionales. Las estrategias usadas en la actualidad para conseguir productos cárnicos funcionales son diversas, entre las cuales destacan (Figura 5):

- La reducción de sodio
- La fortificación con vitaminas y minerales,
- La incorporación de ingredientes funcionales
- Adición de antioxidantes naturales
- Reducción de grasa saturada
- Enriquecimiento con ácidos grasos poliinsaturados y omega 3

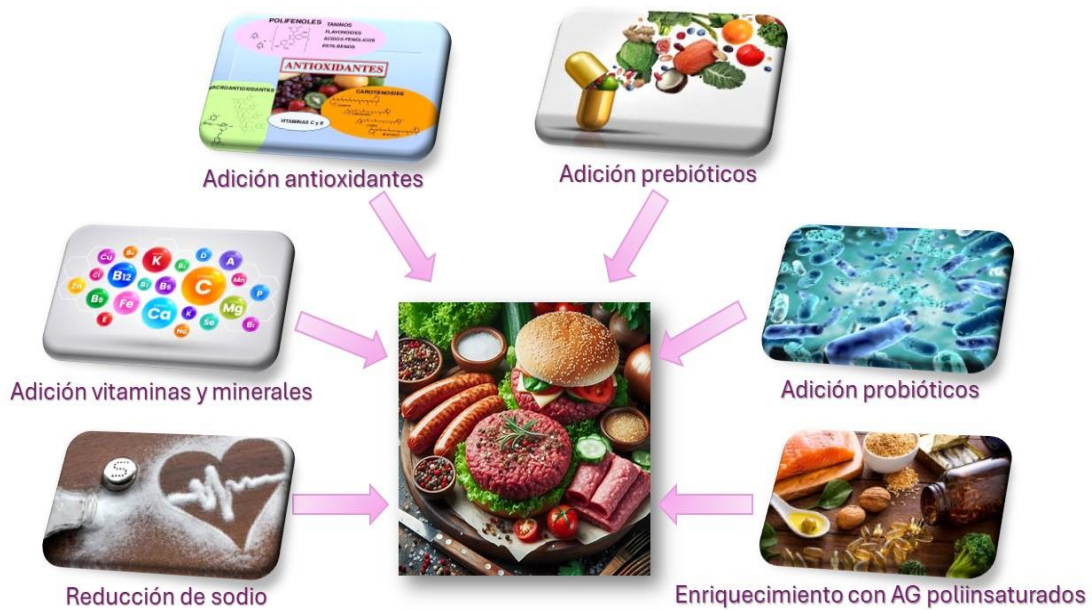


Figura 5. Tendencia de las estrategias empleadas para obtener alimentos cárnicos funcionales.

Como se puede observar, las tendencias en el desarrollo de productos cárnicos funcionales no difieren de las comentadas para el resto de los alimentos funcionales y están en línea con las recomendaciones para la elaboración de alimentos más saludables.

2.2.1. FUNCIÓN TECNOLÓGICA DE LAS GRASAS

Referente a las dos últimas estrategias de mejora en los productos cárnicos (reducción de grasa saturada y enriquecimiento con ácidos grasos poliinsaturados), es necesario en primer lugar, entender la importancia de las grasas desde un punto de vista tecnológico, para así comprender los desafíos a los que se enfrenta la industria y la comunidad científica a la hora de reducir y/o sustituir la grasa en los productos cárnicos.

La utilización de la grasa como ingrediente en la elaboración de productos cárnicos, cumple múltiples funciones complejas que varían en función del

tipo de producto que se esté fabricando. Por ejemplo, la grasa influye en la textura del producto (Domínguez et al., 2021; Dreher et al., 2020; López-Pedrouso et al., 2021), lo que se manifiesta en un endurecimiento o una textura más gomosa cuando se reduce su contenido. También tiene un papel importante en la estabilidad de las emulsiones cárnicas, reduciendo las pérdidas por cocción y mejorando la capacidad de retención de agua (Domínguez et al., 2021; Ferdous et al., 2024; Li et al., 2020; Szymańko et al., 2021). Asimismo, afecta el color del producto al aportar brillo (Domínguez et al., 2022a; Paglarini et al., 2022). En cuanto al sabor, la grasa actúa como fuente y vehículo de compuestos volátiles, influyendo en el gusto, la apariencia, la sensación en boca, el aroma y la jugosidad de los productos (Fu et al., 2022; Kumar, 2021; Nieto & Lorenzo, 2021; Owusu-Ansah et al., 2022). Además, desempeña un papel en la transferencia de calor durante la fritura y en proporcionar una sensación de saciedad (Pehlivanoglu et al., 2018).

La grasa ideal (tecnológicamente) que se utiliza en la elaboración de los productos cárnicos, es la grasa dorsal de cerdo. La grasa dorsal de cerdo, proveniente del tejido adiposo de la región dorsolumbar del animal y localizada entre la piel y el músculo *Longissimus dorsi*, se caracteriza por ser una grasa "dura" (Ospina-E et al., 2010). Esta grasa es muy apreciada en la elaboración de productos cárnicos por su excelente rendimiento, y tecnológicamente se prefiere sobre las grasas de bovinos y aves (Heck et al., 2019; Simunovic et al., 2022). Tanto en la carne como en la grasa de cerdo, existe una gran presencia de AGS cuya cantidad varían de acuerdo con el tipo y el contenido de grasa presente en la alimentación del animal (Vicente & Pereira, 2024; Yi et al., 2023).

Desde un punto de vista tecnológico, también se han de tener en cuenta las características físicas de la grasa dorsal de cerdo, las cuales dependen de la temperatura y de los ácidos grasos que lo conforman, dando

a los productos cárnicos atributos texturales como la dureza, la masticabilidad, la jugosidad y la gomosidad (Franco et al., 2019; Rodrigues et al., 2023). Los atributos de calidad asociados con la grasa dorsal de cerdo están relacionados con el color, el porcentaje de grasa extraíble, la consistencia, el índice de iodo, el contenido del ácido graso C18:2, la relación entre ácidos grasos C18:0/C18:2, la firmeza, el comportamiento de fusión, la cantidad y relación de ácidos grasos, el índice de dobles enlaces, el contenido de sólidos de las grasas (SFC), el índice de saponificación y el tiempo de cristalización, entre otros atributos (Kucha et al., 2018; Lebret & Čandek-Potokar, 2022). De entre los atributos mencionados anteriormente, los tres más destacables para poder seleccionar una buena grasa desde el punto de vista físico son, el punto de deslizamiento, el tiempo de cristalización y el punto de fusión, las cuales forman parte del SFC (Jimenez-Colmenero et al., 2015; Ospina-E et al., 2010; Perța-Crișan et al., 2023).

Desde un punto de vista químico, el grado de insaturaciones que presenta la grasa dorsal de cerdo, permite predecir el comportamiento de la firmeza de la grasa, pero no el punto de fusión, debido a que este está influenciado por el contenido de AGS y particularmente por los niveles de AGMI y AGPI (Franco et al., 2019; Pewan et al., 2020). El punto de fusión y la consistencia de la grasa de cerdo está relacionada con el contenido de ácido esteárico (C18:0) debiendo superar el 12% de dicho ácido graso para considerarse una grasa de buena calidad (Ambrosio et al., 2021). En la determinación del punto de fusión, intervienen más factores, no solo la composición química de la grasa, como por ejemplo, el contenido de grasa, de agua y de colágeno. Al parecer, la cantidad de grasa superficial y el espesor son más importantes que la composición de la grasa de cerdo respecto a la firmeza (Gläser et al., 2004; Soladoye et al., 2017). La composición química de la grasa de cerdo es muy variable, ya que se ha visto una relación directa








entre la alimentación del animal y dicha composición. En general, alrededor del 47% del total de ácidos grasos en la grasa dorsal de cerdo son AGMI, siendo el ácido graso oleico el mayoritario (constituyendo entorno al 40% del contenido total de grasa) (Lebret & Čandek-Potokar, 2022; Ospina-E et al., 2010). Por otro lado, la fracción de AGS compuesta por ácidos grasos C12:0-C18:0, ha de ser superior al 41% para obtener una grasa de elevada calidad (Gläser et al., 2004; Ospina-E et al., 2010). Es posible aumentar el contenido de AGPI, pero al aumentar estos en la composición de la grasa dorsal de cerdo, se ha visto una correlación con una pérdida de consistencia de este y por ello varios autores han puesto un límite de un 15% como máximo (Hoa et al., 2021; Kušec et al., 2022).

2.2.2. DESAFÍOS RELACIONADOS CON LA SUSTITUCIÓN DE GRASA EN PRODUCTOS CÁRNICOS

La cantidad de grasa en los productos cárnicos varía según varios factores, como el tipo de producto y la formulación empleada, el procesado del producto cárnico, la calidad y por lo tanto la cantidad de grasa que contengan las materias primas, entre otros. En la Tabla 3, se presentan los valores de energía, grasa total, grasas saturadas, hidratos de carbono, contenido de azúcar, proteína y sal de productos cárnicos comerciales, tanto frescos, cocidos como crudo-curados. Los productos cárnicos presentan contenidos de grasa que varían desde un 1,5% (como es el caso del fiambre de pollo) hasta cerca de un 60% en ciertos embutidos, tocino o bacón (Tabla 3).

TABLA 3. Composición de productos cárnicos comerciales, expresada en g por 100 g de producto.

Producto cárnico	Formulación	Valor energético (kcal)	Grasas	Grasas saturadas	Hidratos de carbono	Azúcares	Proteína	Sal
Búrguer meat vacuno 	Vacuno: 80	215	16,3	7,5	1,4	<0,5	16	1,6
Búrguer meat vacuno-cerdo 	Cerdo: 49 Vacuno: 35	201	14	6,14	1,3	0,8	17,5	1,65
Hamburguesas de cerdo 	Cerdo: 78	198	14,9	15,71	1,5	0,5	14,5	2,74
Longaniza fresca 	Cerdo: 69	194	15,48	5,85	0,96	0,75	13,3	1,81
Tocino 	Panceta: 90	548	57,2	21	1	1	8,4	9,2
Salchichas tipo Frankfurt 	Cerdo: 35 Pavo: 19 Pollo: 15	231,2	18,5	6,5	4,5	0,5	12	2,1
Paté de hígado de cerdo 	Tocino: 37 Hígado: 27	296	27	11	1,3	1,1	12	1,5
Fiambre de pechuga de pollo 	Pollo: 57	90	1,5	0,5	0,5	0	14	2,3

Producto cárnico		Formulación	Valor energético (kcal)	Grasas	Grasas saturadas	Hidratos de carbono	Azúcares	Proteína	Sal
Mortadela		Cerdo: 50 Pavo: 15	235	19	6,6	5	0,7	11	2,1
Jamón cocido		Cerdo: 75	101	3	1,2	1,5	1,5	17	2,2
Bacon		Panceta: 95	262,5	22,5	7,8	1	1	14	2,2
Salchichón		Cerdo (no especifica cantidad)	491	44,6	15,9	5,16	3	17,4	4,7
Salchichón de pavo		Pavo: muslos y contra muslos de pavo (125g para 100 g salchichón)	306	22	7,7	4	3	23	3,5
Chorizo		Cerdo (no especifica cantidad)	360	29	11	1,8	1,8	23	3,5
Fuet		Cerdo (no especifica cantidad)	421	34	13,3	1,4	1,4	27,5	3,7

(Fuente: open FOOD facts (<https://acortar.link/GsrhNN>) y Carrefour (<https://acortar.link/MOvcWU>)).

Debido a las importantes funciones tecnológicas de la grasa en los productos cárnicos, no es una tarea fácil reducir su contenido o incluso sustituirlo por otras grasas con perfiles lipídicos diferentes, o incluso por otros ingredientes. Son muchos los estudios realizados con este fin en los que la tarea emprendida ha resultado ser tecnológicamente inviable. Algunos de los ingredientes no grasos utilizados para disminuir el contenido de grasa en los productos cárnicos son carbohidratos, fibra dietética y proteínas, ya que serán los responsables de conferir a los productos cárnicos características similares a las que aporta la grasa animal (Barbut, 2011; Kumar, 2021). Los componentes anteriormente mencionados suelen ser obtenidos de frutas, verduras, cereales y proteínas, como es el caso de las pectinas, gomas y fibras lo cual, al provenir de estas fuentes, los consumidores los perciben como más naturales (Banaś & Harasym, 2021). Los miméticos de grasa, que contienen en su formulación proteínas y carbohidratos, son conocidos por su incapacidad para reproducir completamente la funcionalidad de la grasa en los productos en los que se incorporan (Marangoni et al., 2020). Los sustitutos en base de carbohidratos, se obtiene de granos, cereales y plantas, los cuales al usarse con agua proporcionan una fluidez similar a la de las grasas, formándose redes de carbohidrato-agua. Los carbohidratos más utilizados como sustitutos son los almidones, maltodextrinas, gomas, harinas, salvado, fibras de inulina, carrageninas, polidextrosa e incluso tubérculos (Asyrul-Izhar et al., 2023; Chen et al., 2020; Salcedo-Sandoval et al., 2015; Yang et al., 2022). Entre sus ventajas se encuentra la capacidad de reducir el contenido calórico de los alimentos. No obstante, presentan ciertos desafíos, como que tienen un sabor limitado, ya que carecen de sabores lipídicos, lo que a menudo requiere procesos adicionales de preparación.

En los últimos años se han realizado avances con la adición de distintas fibras dietéticas como fibra de guisante, trigo, avellana, soja, cereales, algarroba, nuez, aceituna, granada incluso algas, los cuales tienen características adecuadas para generar propiedades texturizantes dada su elevada capacidad de retención de agua y grasa (Choi et al., 2016; Han & Bertram, 2017; Pintado et al., 2016a).

Los sustitutos a base de proteína aumentan la retención de agua en los productos, al interactuar con grupos polares en la matriz proteica y forman enlaces de hidrógeno con agua libre. Estos sustitutos con base proteica reemplazan las características de la grasa a la par que reducen el impacto nocivo de las proteínas en los productos alimenticios bajos en grasa (Yashini et al., 2021). Las sustancias más empleadas como sustitutos en base proteica son proteína de huevo, suero y vegetales. Debido a su dualidad, donde presentan zonas hidrofílicas e hidrofóbicas también les confiere propiedades gelificantes y de retención de humedad, haciendo que sean menores las pérdidas por cocción en estos productos (Asyrul-Izhar et al., 2023).

Por el contrario, los sustitutos de grasa con base lipídica, conocidos como "sustitutos de grasa" le confiere una serie de características sensoriales y funcionales a los productos bajos en grasa, brindando a los fabricantes y consumidores una variedad de alternativas para adaptar las características de los alimentos reducidos en grasa a sus preferencias y necesidades. Esta versatilidad permite el desarrollo de opciones alimentarias más saludables sin detrimento del sabor, la textura o la calidad en general.

La variable física más influyente a la hora de elegir sustitutos de grasa es la consistencia, dado que finalmente será la que aporte ciertos atributos texturales característicos a los productos cárnicos. El color, aunque también se ve afectado, no parece ser la variable más crítica en el momento de pensar

en los sustitutos de grasa animal. Diversos estudios revelaron que el uso de aceites vegetales como sustitutos de grasa de cerdo, no presentaron diferencias entre las muestras sustituidas y el producto tradicional (Asyrulzhar et al., 2023; Domínguez et al., 2022a; Saldaña et al., 2015).

En otros casos, el objetivo no es disminuir el contenido de grasa en los productos cárnicos, sino modificar su perfil lipídico para hacerlos más saludables.

2.2.3. ESTRATEGIAS DE MEJORA DEL PERFIL LIPÍDICO EN PRODUCTOS CÁRNICOS

Por todo lo anteriormente expuesto queda claro que la mejora del perfil lipídico de los productos cárnicos es uno de los retos más importantes a los que se enfrenta la industria cárnica. Para conseguir este objetivo, existen diversas estrategias que se podrían englobar en dos grandes grupos: por un lado, la asociada a las prácticas de producción animal con modificaciones tanto genéticas como a nivel nutricional en la alimentación animal, y por otro la reformulación de los derivados cárnicos.

Las prácticas de producción animal relacionadas con la modificación del perfil lipídico se han centrado principalmente en la disminución del contenido total de grasa y en el incremento de las grasas mono y poliinsaturadas a través de la alimentación del animal. En relación con la parte genética, la selección de razas y los entrecruzamientos también han dado como resultado canales con un menor contenido de grasa y un perfil lipídico mejorado (Alfaia et al., 2019; Wood et al., 2008).

En cuanto a las estrategias empleadas a la hora de reformular los productos cárnicos son muy diversas a la par que eficientes dado que inciden directamente sobre la composición final del producto. A la hora de desarrollar productos cárnicos más saludables existen varios factores a tener

en cuenta, ya que estos pueden influir en el éxito del reemplazo de una parte o de la totalidad de la grasa, así como en la modificación del perfil lipídico del producto final. Entre los factores que se deben considerar se encuentran la naturaleza del producto, el proceso de elaboración, los ingredientes empleados y la cantidad de grasa de este (Espinales et al., 2024; Ursachi et al., 2020).

Como se ha mencionado anteriormente, para reducir el contenido de grasa, generalmente se utilizan materias primas cárnicas más magras o bien se sustituye la grasa por otros ingredientes de naturaleza no grasa. Para la modificación del perfil lipídico, una de las principales estrategias que se lleva a cabo es reemplazar la grasa tradicionalmente adicionada (grasa dorsal de cerdo), de forma parcial o total, por otra fuente lipídica con un perfil de ácidos grasos caracterizado por una mayor presencia de AGI en línea con las recomendaciones actuales establecidas por los organismos públicos europeos y mundiales como la FAO y OMS (apartado 2.1). En este sentido, los aceites de origen vegetal o marino representan las opciones más adecuadas para cumplir con dichas recomendaciones ya que se caracterizan por tener una mejor relación AGP/AGS y omega 6/omega 3 que la grasa de origen animal como la grasa dorsal de cerdo (Barros et al., 2021; Jiménez-Colmenero, 2007). También existe una alternativa a las grasas de origen animal de distinto origen al marino o al vegetal, que sería el uso de aceite procedente de insectos comestibles como es el caso de *Tenebrio molitor* y *Acheta domesticus* que actualmente están aceptados como nuevo alimento en la legislación europea (Kolobe et al., 2023; EUR-Lex Reglamento 2015/2283).

La incorporación de aceites con un perfil lipídico más saludable, rico en AGMI y AGPI, se ha realizado en diversos productos cárnicos mediante diferentes procedimientos como son: la incorporación directa, la

encapsulación de estos, generando una preemulsión, mediante la incorporación de un oleogel y mediante la creación de una emulsión gelificada (Figura 6). Para llevar a cabo estos procesos es importante evaluar el efecto que tendrá cada uno de ellos sobre las características físico-químicas del producto final, por ello es crucial determinar la cantidad y la forma en que se añadirán estos aceites ya que también podrían repercutir en la aceptabilidad del producto final en el que se han aplicado.

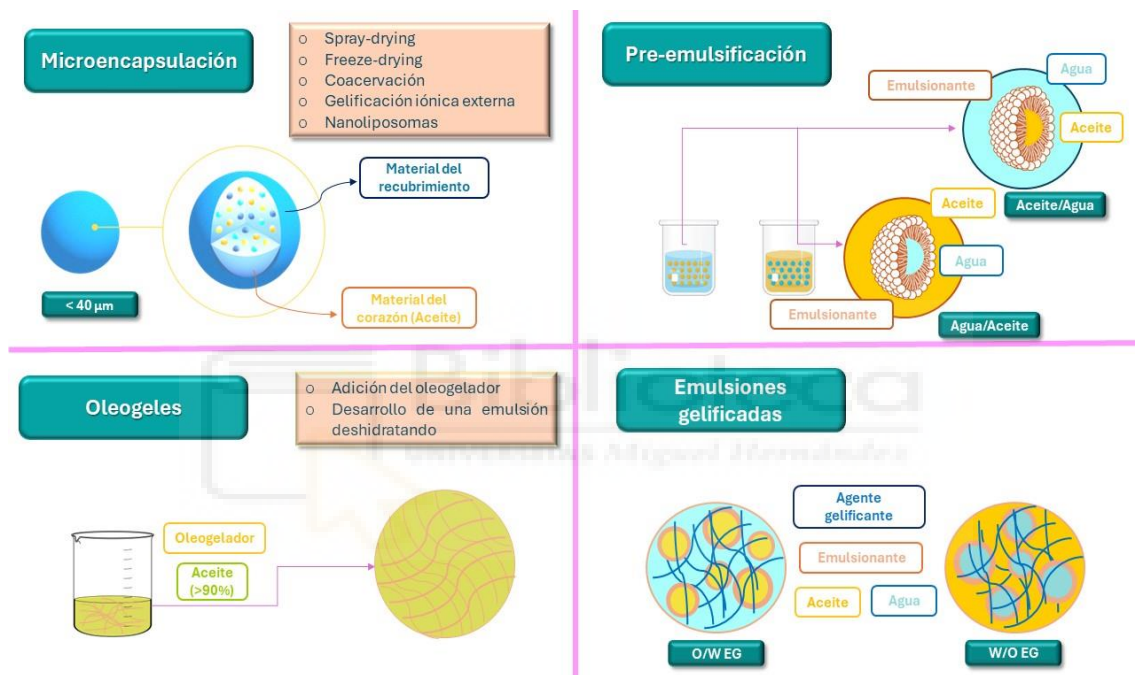


Figura 6. Resumen de las diferentes estrategias empleadas para estructurar aceites.

2.2.3.1. INCORPORACIÓN DIRECTA DE ACEITES

La alternativa de reemplazo más sencilla es la sustitución de la grasa animal, la cual es sólida a temperatura ambiente, por aceites ricos en AGMI y AGPI que están en forma líquida a temperatura ambiente. A pesar de ser la forma más sencilla de reemplazo de una grasa por otra, presenta ciertas dificultades ya que la función tecnológica de la grasa cambiará debido a las diferencias en su estado de agregación entre un tipo de grasa y otra, viéndose principalmente afectada la textura de los productos finales en los

cuales se produzca dicha sustitución, como es el caso de la disminución de la firmeza en productos cárnicos cocidos, en hamburguesas o en embutidos fermentados (López-López et al., 2009; Rodríguez-Carpena et al., 2012). Otro inconveniente que presenta la incorporación directa de aceites en los productos cárnicos está relacionado con la oxidación lipídica, que se ve favorecida por la presencia de AGMI y AGPI en los aceites los cuales, quedan más expuestos y son más propensos a sufrir oxidación lipídica al no quedar completamente retenidos en la matriz (Kim et al., 2020; Shin et al., 2019).

2.2.3.2. INCORPORACIÓN DE ACEITES ENCAPSULADOS

Debido a los inconvenientes mencionados anteriormente que presenta la adición de forma directa de aceites en los productos cárnicos, es necesario dar una matriz y una estructura a dichos aceites para protegerlos y facilitar su integración en la matriz cárnica de los mismos. Para ello una de las estrategias más empleadas es la encapsulación de los aceites para su posterior incorporación en los diferentes productos cárnicos. La encapsulación es un proceso que aporta multitud de ventajas entre las que se incluyen la reducción de la oxidación lipídica de los aceites protegiéndolos frente a factores ambientales (temperatura, luz, oxígeno, etc.), y el enmascaramiento del efecto negativo que puede tener la inclusión directa de aceite en las propiedades sensoriales del producto final como es el caso del el flavor, el color y la textura (Cardoso et al., 2020; da Silva et al., 2022). Por otro lado, la microencapsulación también es favorable respecto a otros métodos para mantener las características funcionales de los compuestos bioactivos presentes en los aceites vegetales, de pescado o de insectos utilizados como sustituto de la grasa animal (de Lima et al., 2022; Munekata et al., 2017).

Existe una amplia variedad de técnicas mediante las cuales se puede llevar a cabo la encapsulación de los aceites, entre las que se incluyen: el *spray-drying*, *freeze-drying*, la coacervación, el uso de gelificación iónica externa y mediante el uso de nanoliposomas (Alemzadeh et al., 2020). La principal diferencia entre las diferentes técnicas mencionadas es el tamaño de cápsula obtenido (micro o nano). Con la técnica de *spray-drying* se obtienen tamaños inferiores a 40 μm y en ella, los aceites se dispersan en el interior de una disolución polimérica para su posterior atomización y deshidratación (Ojagh & Hasani, 2018; Solomando et al., 2020; Ullah et al., 2020). Para llevar a cabo la encapsulación mediante la técnica *freeze-drying*, previamente se realiza una liofilización (deshidratación a vacío posterior a la congelación) para posteriormente formar las micropartículas (Pourashouri et al., 2021; Venturini et al., 2019). La coacervación es el método más usado para encapsular aceites, que consiste en disolver en agua entre el 1 y el 10% del polímero y dispersar el aceite en la disolución a una temperatura de 40-50 $^{\circ}\text{C}$ para posteriormente depositar el recubrimiento y estabilizar la fase grasa (Timilsena et al., 2020). La gelificación iónica representa el proceso de extrusión más empleado llevado a cabo por la interacción entre el alginato y el calcio, dando lugar a una gelificación (Alemzadeh et al., 2020). Por último, para la obtención de los nanoliposomas se emplean fosfolípidos como material para desarrollar la pared de la cápsula y suelen tener un diámetro inferior a 100 nm (Bondu & Yen, 2022).

El uso de la encapsulación de aceites para su posterior incorporación en productos de origen animal con el objetivo de sustituir la grasa animal de forma total o parcial, se ha llevado a cabo en multitud de productos cárnicos, como es el caso del estudio llevado a cabo por Domínguez et al. (2017) en el que se microencapsuló aceite de pescado para su posterior incorporación en salchichas tipo Frankfurt, o el caso de la incorporación de aceite de chufa,

chía o linaza microencapsulados en paté de ciervo llevado a cabo por Vargas-Ramella et al. (2020a). Así como la incorporación en hamburguesas de cerdo de aceite de chía microencapsulado reemplazando un 50% de la grasa de cerdo evaluadas durante 120 días en congelación, llevado a cabo por Heck et al. (2019).

La principal desventaja que presenta el método de la encapsulación de aceites como sustitutos de la grasa animal, frente a otros más usados en la actualidad, es su elevado costo, lo que hace patente la necesidad de explorar y desarrollar alternativas más económicas y fáciles de llevar a cabo y extrapolar a la industria alimentaria.

2.2.3.3. INCORPORACIÓN DEL ACEITE PRE-EMULSIONADO

La pre-emulsión del aceite es uno de los mecanismos empleados para llevar a cabo la reducción del contenido en grasa y la mejora del perfil lipídico en los productos cárnicos. Este proceso consiste en la mezcla de los aceites con diferentes emulsionantes, principalmente de origen proteico como son la proteína de suero de leche, lecitina, proteína de soja y caseinatos (Urgu-Öztürk et al., 2020) y con carbohidratos como los éteres de celulosa (Espert et al., 2020). Los procesos de generación de aceites pre-emulsionados difieren ampliamente tanto en los tipos de emulsionantes como en las proporciones de la parte acuosa y oleosa, proteína o carbohidratos utilizadas y por ello no es un proceso que se pueda estandarizar. Sin embargo, existen diversos estudios que muestran su efecto positivo en productos cárnicos como salchichas de pollo, hamburguesas de cerdo, salchichas de ternera, salchichas cocinadas de cordero salchichas de oveja, entre otros productos (Bolger et al., 2018; Carvalho Barros et al., 2020; Lee et al., 2020; Li et al., 2020; Lima et al., 2021; Urgu-Öztürk et al., 2020). En estos estudios se han empleado una gran variedad de aceites y mezclas de aceites de semillas,

como el de lino, colza, avellana, chía, oliva y soja empleados en múltiples proporciones y combinados con emulsionantes como la lecitina, la proteína de soja, el caseinato de sodio, el asilado de proteína de suero entre otras.

2.2.3.4. INCORPORACIÓN DE UN OLEOGEL

Los oleogeles son aceites que han sido transformados en estructuras sólidas o semisólidas que poseen propiedades viscoelásticas y tienen naturaleza hidrofóbica debido al establecimiento de una red tridimensional y termorreversible aportada por una estructura de gel (oleogelador) (Okuro et al., 2020). La formación de oleogel permite que una alta concentración de aceite líquido (superior al 90%) se estructure en un sistema similar a un gel (Rogers et al., 2009). Los oleogeles se pueden obtener de forma directa mediante la adición del oleogelador al aceite o de forma indirecta elaborando una emulsión y realizando una posterior deshidratación de la estructura para obtener el oleogel (Dent et al., 2022; Malvano et al., 2022). Se pueden emplear tanto oleogeladores de naturaleza polimérica (quitosano, etilcelulosa, hidroxipropilmetilcelulosa, metilcelulosa, etc.), lipídica (cera de abejas, cera de salvado de arroz, cera de candelilla, alcoholes grasos, etc.) u otros compuestos como esfingolípidos, tocoferoles, fitoesteroles y lecitina (Meng et al., 2018). Existen diversos estudios en los cuales se han empleado oleogeles como sustitutos de grasas de origen animal, siendo los aceites vegetales más empleados para la elaboración de los oleogeles los procedentes del sésamo, cacahuete, oliva, chía y girasol (da Silva et al., 2019; Malvano et al., 2022; Martins et al., 2019; Moghtadaei et al., 2018; Oliveira et al., 2023; Pintado et al., 2020; Zbikowska et al., 2022).

2.2.3.5. INCORPORACIÓN DE EMULSIONES GELIFICADAS

Una emulsión es una dispersión generada por un líquido en forma de gotas (fase oleosa o acuosa) distribuido en otra en fase líquida en la cual es insoluble (Zhu et al., 2018). Una emulsión gelificada es una emulsión con una estructura de red similar a un gel que posee propiedades texturales parecidas a las de un sólido (Dickinson, 2006; Guo et al., 2023). En este tipo de estructura, las emulsiones y el gel coexisten cuando las gotas emulsionadas son insertadas en la matriz de gel lo que da lugar a un material coloidal complejo (Abdullah et al., 2022). Dicho material, presenta propiedades reológicas estables debido a su similitud con la grasa animal en términos de propiedades y características (Nasirpour-Tabrizi et al., 2020; Pintado et al., 2015). Otra propiedad importante que cabe destacar es que estas estructuras permiten la inclusión de ingredientes funcionales tanto de naturaleza hidrofóbica como hidrofílica. Para la obtención de emulsiones gelificadas a parte de los constituyentes de la fase oleosa y acuosa, es necesario incorporar otros componentes como son los emulsionantes y los gelificantes.

2.3. EMULSIONES GELIFICADAS

La incorporación de aceites en matrices sólidas, en forma de emulsiones gelificadas, puede dar lugar a sustitutos de grasa (Ren et al., 2022) con características texturales similares a las grasas animales usadas en los productos cárnicos, lo que conlleva una mejora en la retención de grasa. El proceso inicial para la formulación de una emulsión gelificada incluye la estabilización de una emulsión mediante proteínas, seguida de la adición de hidrocoloides u otros ingredientes como polisacáridos o tensoactivos. Posteriormente, la emulsión se transforma en un gel mediante la adición de

gotas de emulsión o por la gelificación de la fase acuosa gracias a las proteínas añadidas (Yiu et al., 2023). La estructura de estas emulsiones gelificadas de aceite en agua (O/W) se caracteriza por una red compuesta de gotas de emulsión agregadas y moléculas de biopolímero reticulado, lo que determina sus propiedades texturales (Dickinson, 2012, 2013). Estas emulsiones no solo combinan las características de la emulsión y del gel, sino que también ofrecen nuevas propiedades funcionales y diversas aplicaciones industriales, incluso permitiendo la incorporación de harinas en la fase acuosa para mejorar aún más sus características (Dickinson, 2011; Mao & Miao, 2015; McClements, 2012; Pintado et al., 2015; Poyato et al., 2015; Wan et al., 2023; Yiu et al., 2023).

2.3.1. INGREDIENTES Y PROCESOS DE GELIFICACIÓN

Existen varios tipos de emulsiones gelificadas, pudiéndose clasificar según la naturaleza de la emulsión que las compone y según la naturaleza del gelificante. Según la naturaleza de la emulsión, existen emulsiones simples (ES) de dos tipos: aceite en agua (O/W, *oil in water*) o agua en aceite (W/O, *water in oil*). Las emulsiones O/W (Figura 7A) son aquellas dónde la fase continua es la acuosa y las gotas de la fase lipídica se encuentran dispersas en su interior, mientras que en las emulsiones W/O (Figura 7B), la fase continua es la fase lipídica y la dispersa es la acuosa. Para mantener la estabilidad de estos sistemas, es necesario del uso de tensoactivos o surfactantes, debido a que las fases son inmiscibles y se crea una elevada tensión interfacial, lo que hace que sean sistemas termodinámicamente inestables (Costa et al., 2019; Tran, 2011).

Por otro lado, estarían las emulsiones dobles (ED) que se caracterizan por la coexistencia de una emulsión O/W y otra W/O, en la que los glóbulos de la fase dispersa contienen en su interior gotas igualmente dispersas de

menor tamaño (Mehrnia et al., 2017; Sangwan et al., 2023). Las ED se pueden clasificar en emulsiones de “aceite en agua en aceite” (O1/W/O2) (Figura 7C), las cuales se corresponden con un sistema disperso líquido-líquido, en el cual la fase interna es una emulsión O1/W con un tensoactivo hidrofílico que se encuentra disperso en una fase lipídica externa O2 con un emulsionante lipofílico en la interfase W/O2. El otro tipo de emulsiones dobles son las de “agua en aceite en agua” (W1/O/W2) (Figura 7D), constituidas por una emulsión W1/O con un emulsionante lipofílico en la interfase contenido en una fase acuosa externa, donde se halla un tensoactivo hidrofílico en la interfase O/W2 (Lamba et al., 2015).

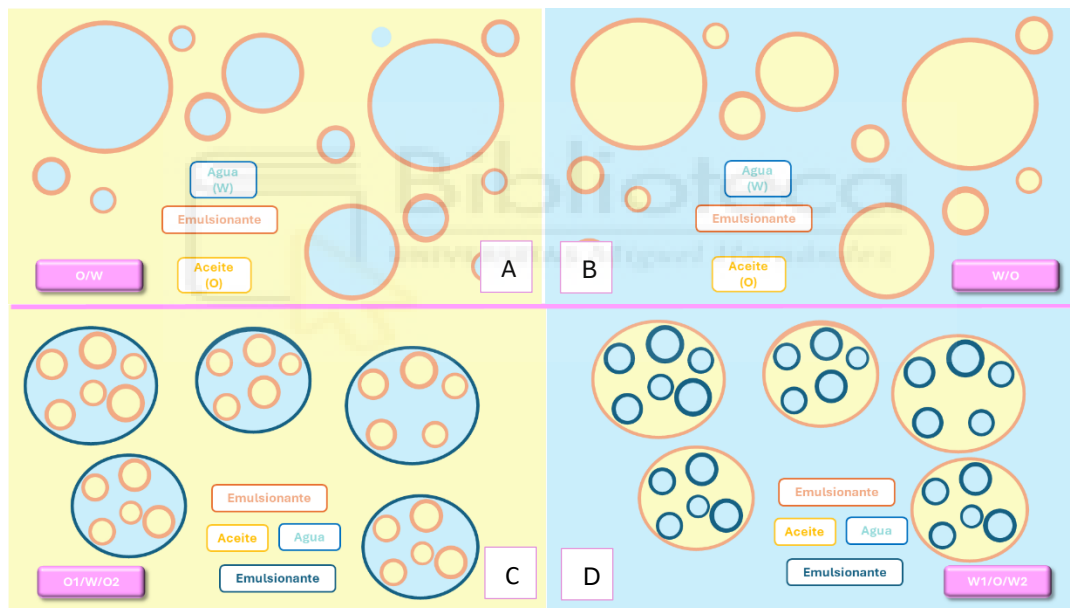


Figura 7. Representación esquemática de diferentes emulsiones simples O/W (A); W/O (B) y dobles O1/W/O2 (C) y W1/O/W2 (D).

El proceso de preparación de las emulsiones gelificadas se realiza principalmente en dos pasos: primero se incorporan los componentes lipídicos y acuosos y tras la adición del emulsionante a la mezcla, se genera la emulsión utilizando un homogeneizador de alta velocidad o de alta presión (Dickinson, 2013). La interfase lípido-agua se consigue estabilizar a medida que se adiciona la cantidad idónea de proteína, ya que si se adiciona una

cantidad insuficiente podría producirse el embebido de las gotas por las moléculas de proteína, obteniéndose una emulsión muy cremosa. Por el contrario, un exceso de proteína podría causar que las moléculas de proteína que cubren la superficie de las gotas sean expulsadas de la unión superficial provocando la coalescencia de estas (Dickinson, 2010). El segundo paso implica la conversión de la emulsión estable en una emulsión gelificada, la cual se puede conseguir mediante el uso de un tratamiento térmico o en frío (Dickinson, 2013; Ren et al., 2022).

2.3.1.1. INGREDIENTES

Tras ver los tipos de emulsiones que existen y el proceso de formación de las emulsiones gelificadas, se va a profundizar en los ingredientes más usados para generarlas. Como ya se mencionó con anterioridad, es necesaria una fase oleosa, pudiéndose emplear aceites de distinta naturaleza para generar emulsiones gelificadas, siempre con el objetivo de disminuir el contenido de AGS y aumentar el de los AGMI y AGPI, obteniendo también una mejor relación de AGS/AGPI y de omega 6/omega 3. Entre los aceites usados para el mencionado propósito, en la actualidad, destacan los de origen vegetal y marino.

2.3.1.1.1. Aceites vegetales

Un aspecto común destacable en los aceites vegetales es su elevado porcentaje de ácidos grasos insaturados y de triacilgliceroles. Dentro de este grupo se puede hacer una diferenciación entre aceites de semillas o de frutos.

Las semillas son unos de los cultivos más producidos ya que contienen un elevado porcentaje de proteínas y ácidos grasos insaturados. Entre las

semillas más usadas para la extracción de aceite se encuentra la soja, la colza, el girasol, el algodón, el maíz y el cacahuete. En concreto las semillas de colza, soja y girasol suponen un 87% de la producción de aceite vegetal (Widmar, 2022). Los aceites de estas semillas son ricos en ácidos grasos omega 3 y omega 6, vitaminas A, D, E y K, y minerales como el zinc, calcio, magnesio, potasio, cobre y hierro. La mayoría de los aceites vegetales se extraen principalmente con disolventes (en concreto n-hexano) o por extracción mecánica, aunque algunos aceites de semillas necesitan un proceso de refinado previo al consumo para mejorar la conservación y las condiciones nutricionales, debido a que contienen ciertas sustancias llamadas antinutrientes que pueden ser tóxicas, como por ejemplo los glucosinolatos, ácido fítico, inhibidores de tripsina, fosfato de inositol, taninos condensados y sinapina (Matthäus et al., 2005; Zhao et al., 2022).

Aceite de soja

De las semillas del cultivo de soja (*Glycine max*) (Figura 8A) se extrae el aceite de soja (Figura 8B) dicho aceite está compuesto por un 14-16% de ácidos grasos saturados (el 10% se corresponde con ácido palmítico (C16:0) y un 4% de ácido esteárico (C18:0)), un 20-24% de ácidos grasos monoinsaturados, un 18-20% de ácido oleico y un 62-66% de ácidos grasos poliinsaturados, de entre los cuales un 54% es ácido linoleico (C18:2) y un 12% ácido linolénico (C18:3) (Chen et al., 2014; Zaaboul et al., 2022). Como sucede con otros aceites vegetales, el aceite de soja también contiene fitoesteroles como el β -sitosterol, campesterol, stigmasteroles etc., tocoferoles (α -tocoferol y β -tocoferol), β -caroteno, luteína y clorofilas (Zaaboul et al., 2022).



Figura 8. Cultivo de *Glycine max* (8A). Semillas y apariencia del aceite extraído de las semillas de soja (8B).

(Fuente: Google imágenes (<https://acortar.link/kD1kwS>; <https://acortar.link/j2lkRI>))

Aceite de colza

Del cultivo de la colza (principalmente *Brassica napus* L.) (Figura 9A) se extrae el aceite de colza (Figura 9B). Dicho aceite se presenta la mejor composición de ácidos grasos respecto a otros aceites más comunes, con una cantidad de ácidos grasos saturados de entre el 7-8%, un 63-66% de ácidos grasos monoinsaturados y una excelente relación entre ácidos poliinsaturados omega 6 (ácido linoleico con 19,0 g/100 g) y omega 3 (ácido α -linolénico (9,1 g/100 g) (Vingering et al., 2010). Esta composición es la responsable de las propiedades saludables asociadas al consumo de aceite de colza. El aceite de colza contiene fitoesteroles principalmente en forma de β -sitosterol y vitaminas E y K (Ghazani & Marangoni, 2016).



Figura 9. Cultivo de *Brassica napus* (9A). Semillas y apariencia del aceite extraído de las semillas de colza (9B).

(Fuente: Google imágenes (<https://acortar.link/pLMscj>; <https://acortar.link/gZ73vq>)).

Aceite de girasol

De las semillas de la flor del girasol (*Helianthus annuus*) (Figura 10A) se extrae el aceite de girasol (Figura 10B), los principales ácidos grasos que componen a este tipo de aceite son ácidos grasos poliinsaturados (44-75% ácido linoleico), seguido de ácidos grasos monoinsaturados (22-24% de ácido oleico) y ácidos grasos saturados (15%, 7% ácido palmítico y un 8% de ácido esteárico), además de ser fuente de vitamina E (Akkaya, 2018). Existen cuatro tipos diferentes de aceites de girasol, dependiendo del procesado industrial y de la mejora vegetal (*plant breeding*): alto linoleico (superior al 69% ácido linoleico), alto oleico (superior al 82% de ácido oleico), medio-oleico (65% de ácido oleico) y alto esteárico combinado con alto oleico (18% esteárico y 72% oleico). Sin embargo, de estos cuatro tipos de aceites de girasol, el más usado es el alto oleico y el medio oleico (NSA, 2018).



Figura 10. Cultivo de *Helianthus annuus* (10A). Semillas y apariencia del aceite extraído de las semillas de girasol (10B).

(Fuente: Google imágenes (<https://acortar.link/PldujH>; <https://acortar.link/aeCrBx>)).

Aceite de algodón

De las semillas de las plantas de algodón de varias especies, principalmente *Gossypium hirsutum* (Figura 11A) y *Gossypium herbaceum*, se extrae el aceite de las semillas de algodón (Figura 11B). El valor nutricional, la composición química y el rendimiento de las semillas se ven afectados por

muchos factores, pero principalmente por la contribución genética (Figueiredo et al., 2008). La fracción principal de ácidos grasos en el aceite de algodón es poliinsaturada lo que supone entre un 52 y un 55%, con el ácido linoleico como principal ácido graso, seguido del palmítico (26%) y del ácido esteárico con un 2% y como ácidos grasos monoinsaturados el principal es el ácido oleico con un 18% (Sharif et al., 2019).



Figura 11. Cultivo de *Gossypium hirsutum* (11A). Semillas y apariencia del aceite extraído de las semillas de algodón (11B).

(Fuente: Google imágenes (<https://acortar.link/OuFpX2>; <https://acortar.link/eLD0Wp>)).

Aceite de maíz

Del germen de semillas de la planta de maíz (*Zea mays L.*) (Figura 12A) se extrae el aceite de maíz (12B), y por esta razón es comúnmente conocido como “aceite de germen de maíz”. El germen representa entre el 9-11% del peso de la semilla y contiene aproximadamente un 80% de lípidos en la semilla completa (Espinosa-Pardo et al., 2020). El aceite de maíz está compuesto por un 60% de ácidos grasos poliinsaturados (principalmente linoleico con un 52%, un 25% de ácidos grasos monoinsaturados (oleico mayoritariamente) y entre un 15 y 17% de ácidos grasos saturados (ácido palmítico predominantemente)) (Moreau et al., 2011; Carrillo et al., 2017). El aceite de maíz también representa una importante fuente de lípidos minoritarios y compuestos bioactivos, como los fitoesteroles (55-67% de β -

sitosterol, 19-24% de campesterol, 4-8% de estigmasterol y del 4-8% de Δ -5-avenasterol), tocoferoles, tocotrienoles y carotenoides (especialmente xantofilas, luteína y zeaxantina) (Li et al., 2019).

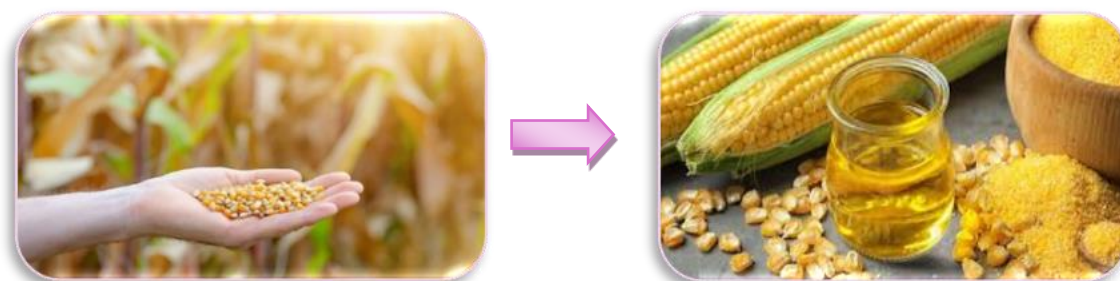


Figura 12. Cultivo de *Zea mays* L. (12A). Semillas y apariencia del aceite extraído de semillas de girasol (12B).

(Fuente: Google imágenes (<https://acortar.link/dSJ714>; <https://acortar.link/WKAXfl>)).

Aceite de cacahuete

De la planta *Arachis hypogaea* (Figura 13A) se extrae el aceite cacahuete (13B). Dicho fruto seco destaca más por su contenido proteico que por su uso como aceite. El ácido graso predominante en el aceite de cacahuete es el ácido oleico, que representa entre el 48 y el 57%, seguido del ácido linoleico entre un 27 y un 38%. La cantidad de ácidos grasos saturados de este aceite está entre el 10-15%, con el palmítico y esteárico como predominantes (8-11% y 2-4%, respectivamente) (Carrín & Carelli, 2010). También podemos encontrar aceite de cacahuete con un contenido medio y alto de ácido oleico con un 66-69% y un 78-80% de ácido oleico, respectivamente (Idrissi et al., 2022; Shin et al., 2009). Así mismo, en su composición se pueden encontrar compuestos de carácter lipídico con función bioactiva en cantidades destacables como los fitoesteroles (207 mg/100 g) (Bonku & Yu, 2020), las vitaminas liposolubles, como los tocoferoles (vitamina E) y el pantotenato (Toomer, 2018).

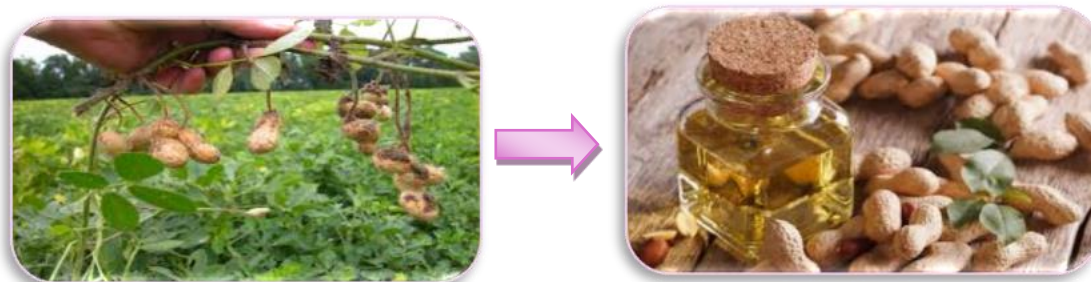


Figura 13. Cultivo de *Arachis hypogaea* L. (13A). Semillas y apariencia del aceite extraído de semillas de cacahuete (13B).

(Fuente: Google imágenes (<https://acortar.link/swSXPt>; <https://acortar.link/uUZW1y>)).

Aceite de nuez

Del fruto del nogal (Figura 14A) (*Juglans regia*) por presión en frío se extrae el aceite de nuez (Figura 14B). Las nueces contienen entre un 52 y un 70% de aceite, dependiendo del cultivo, destacando su contenido de ácido linoleico (48,50-53,24%), ácido linolénico (13,7-15,9%), ácido palmítico (2,4-5,3%) y ácido esteárico (1,4-4,1%) (Cittadini et al., 2020; Elouafy et al., 2022). El aceite de nuez también contiene una mayor cantidad de fitoesteroles (106,5 mg/100 g) que otros tipos de aceite de semillas (Gao et al., 2022).



Figura 14. Nueces con cáscara (14A). Fruto y apariencia del aceite extraído de las nueces (14B).

(Fuente: Google imágenes (<https://acortar.link/2Hxd3a>; <https://acortar.link/XzvKml>)).

Aceite de chía

La chía (*Salvia hispanica* L.) es originaria de América Central y es una hierba anual perteneciente al género *Lamiaceae* (Figura 15A) y cuya floración ocurre durante los meses de verano. Las semillas son de forma ovalada y de colores que van desde el marrón y rojo oscuro hasta el gris (Figura 15B). Son una buena fuente de proteína ya que contienen entre el 15-25%, de fibra dietética (41%) y de vitaminas y minerales (Muñoz et al., 2013; Urbizo-Reyes et al., 2020). El contenido de aceite en las semillas de chía es de aproximadamente un 34%, siendo una excelente fuente de omega 3 ya que presenta el mayor porcentaje de ácido linolénico (ALA) conocido hasta ahora (62-64%), así como el mayor contenido (82,3%) de ácidos grasos esenciales (ácido linolénico y ácido linoleico) (Muñoz-Tébar et al., 2019). El ácido linolénico constituye más del 60% de todos los ácidos grasos totales de las semillas de chía, lo que convierte a este producto en una de las fuentes más importantes de ácido linolénico en nuestra dieta (Oteri et al., 2023). Además, el aceite de chía tiene la ventaja adicional de tener un bajo contenido en ácidos grasos saturados, en concreto el aceite contiene tan solo un 6,9% de ácido palmítico y un 2,8% de ácido esteárico (Muñoz-Tébar et al., 2019). Las semillas de chía contienen tocoferoles y polifenoles a los que se les atribuyen propiedades antioxidantes (Fernández-López, et al., 2020b; López-García et al., 2019; Pellegrini et al., 2018b). Las semillas de chía son ricas en minerales como calcio, cobre, hierro, fósforo, magnesio, potasio y zinc (Pintado et al., 2018). En cuanto a las vitaminas, destaca su contenido en vitamina A y vitaminas del grupo B, con niveles elevados de niacina, tiamina y riboflavina, siendo incluso superiores a los de otros cultivos (de Falco et al., 2017). Además, el aceite presenta niveles de vitamina E considerables en forma de tocoferoles y tocotrienoles, conteniendo mayoritariamente Y-

tocoferol con una cantidad entre 654,86 y 698,32 mg/100 g, así como cantidades importantes de α -tocoferol (70,53-78,13 mg/ 100 g) y δ -tocoferoles (29,76-41,18 mg/100 g) (Ghafoor et al., 2020).



Figura 15. Cultivo de *Salvia hispanica* L. (15A). Semillas y apariencia del aceite extraído de semillas de chía (15B).

(Fuente: Google Imágenes (<https://acortar.link/UWbr7e>; <https://acortar.link/SgeYM4>)).

Aceite de cáñamo

El cáñamo (*Cannabis sativa* L.) (Figura 16A) es un cultivo muy resistente y versátil (Adesina et al., 2020) y su cultivo se realiza a nivel industrial principalmente debido a su elevada cantidad de ácido canabidiólico (CBDA), el ácido precursor del canabidiol (CBD). El CBD tiene reconocidas propiedades beneficiosas como antimicrobiano, anticonvulsivo y antiepiléptico (Spano et al., 2020).

Las semillas del cáñamo contienen entre un 20 y un 25% de proteína, un 25-35% de lípidos y entre un 20-30% de carbohidratos (de los cuales el 10-15% es fibra) y entre un 3-6% de cenizas. Las semillas de cáñamo además son fuente de compuestos minoritarios con elevado valor biológico como las vitaminas (A, C y E) y los minerales (Tura et al., 2022; Zhao et al., 2022).

Las semillas de cáñamo se caracterizan por poseer una interesante composición de ácidos grasos con un contenido elevado (hasta un 75%) de ácidos grasos poliinsaturados. Este aceite también presenta una relación única de omega 6/omega 3 de 3:1 (Zhao et al., 2022) y entre los AGPI que lo

componen destacan los ácidos grasos linoleico y α -linolénico con una cantidad del 50-60% y del 20-25% respectivamente (Abdollahi et al., 2020). Además, este aceite presenta una coloración verdosa (Figura 16B) debido a sus elevadas cantidades de clorofila, por la recolección en gran cantidad de semillas inmaduras (Matthäus & Brühl, 2008). En cuanto a la cantidad de ácidos grasos saturados, el ácido palmítico es el principal, con una cantidad entre el 6,0-9,1%, seguido del AG esteárico con un rango entre el 2,1-4,6% y del behénico (0,2-0,7%) (Zhao et al., 2022). La cantidad de ácidos grasos pueden variar según la variedad, el genotipo intrínseco, las condiciones medioambientales, climáticas o factores agronómicos locales (Faugno et al., 2019). En su fracción insaponificable, el aceite de cáñamo contiene una cantidad de tocoferoles comprendida entre 22,1 a 102,0 mg/100 g (Chen et al., 2010; Matthäus & Brühl, 2008), entre los que destacan el γ -tocoferol (17,2-89,8 mg/100 g) y el α y δ tocoferol den más de 50 especies investigadas (Zhao et al., 2022). También presenta fitoesteroles siendo el dominante el β -sitosterol con una cantidad de 190,5 mg/100 g de aceite seguido del estigmasterol (10,0 mg/100 g de aceite) y el campesterol (50,6 mg/100 g de aceite) (Montserrat-De La Paz et al., 2014; Siano et al., 2019; Vecka et al., 2019). Los fitoesteroles son compuestos orgánicos lipofílicos presentes en las membranas celulares de las plantas y estas moléculas comparten una similitud estructural con el colesterol (Farinon et al., 2020). Existen diversos estudios que han demostrado que la reducción de la solubilidad del colesterol por parte de los fitoesteroles evita su incorporación en la micela lipídica, disminuyendo su absorción intestinal y dando lugar a niveles más bajos en sangre (Gao et al., 2022; Kritchevsky & Chen, 2005; Li et al., 2022).



Figura 16. Cultivo de *Cannabis sativa* L. (16A). Semillas y apariencia del aceite extraído de semillas de cáñamo (16B).

(Fuente: Google imágenes (<https://acortar.link/9aAkqG>; <https://acortar.link/t6IUvz>)).

Aceite de lino

El lino (*Linum usitatissimum*) (Figura 17A) o linaza es una planta nativa del este de Asia y del Mediterráneo, perteneciente a la familia de las *Linaceae*. Su cultivo se remonta a hace más de 6.000 años, lo que lo convierte en uno de los cultivos más antiguos y adaptados, que resulta de gran importancia por sus diversos usos, tanto en textiles como en producción de aceite (Katoch & Bhatia, 2021; Singh et al., 2011). Sus semillas son pequeñas, ovales y planas, de coloración entre el amarillo-dorado y el marrón-rojizo (Figura 17B). Las semillas de lino contienen en torno a un 40% de aceite y también son ricas en fibra dietética y proteínas con una cantidad de un 28% (rica en ligninas) y un 21%, respectivamente. El aceite de lino es un aceite rico en ácidos grasos poliinsaturados, sobre todo es rico en ALA (55-60%) y ácido linoleico (14-18%). Los AGMI que presenta son generalmente el ácido oleico, con una cantidad entre el 18-22%. Su concentración de ácidos grasos saturados (en torno al 9% del total de AG) es relativamente baja, destacando el ácido palmítico y el esteárico (Ahmed et al., 2023; Romanić et al., 2021).



Figura 17. Cultivo de *Linum usitatissimum* L. (17A). Semillas y apariencia del aceite extraído de semillas de lino (17B).

(Fuente: Google imágenes (<https://acortar.link/sqyrK2>; <https://acortar.link/UTIZUL>).

Aceite de sésamo

Sesamum indicum L. (sésamo o ajonjolí) es uno de los cultivos de oleaginosas más producidos de la familia *Pedaliaceae*. Se cultivan alrededor de 10 millones de hectáreas aproximadamente cada año, generando 5,90 millones de toneladas, donde el 93% de la producción se da en Asia y África (Zech-Matterne et al., 2015). Es una planta herbácea de entre 60 y 150 cm de alto (Figura 18A) y sus semillas son ricas en grasa, proteína, minerales, vitaminas y fibra dietética. Su aporte proteico es de entre el 35-50%, siendo ricas en metionina y triptófano (Sharaby & Butovchenko, 2019). Además, las semillas de sésamo tienen propiedades antiinflamatorias y antihipertensivas siendo este efecto atribuido a los lignanos encontrados en el sésamo, incluyendo la sesamina, sesaminol, sesamolina y glucósidos de sesaminol (Eom et al., 2021). El aceite de sésamo (Figura 18B) es rico en ácidos grasos insaturados (80%) y su contenido en grasa representa un 45-57% (Özdemir et al., 2018; Wei et al., 2022). En las semillas de sésamo se han encontrado doce AGI: ácido graso oleico, linoleico, palmítico, esteárico, araquidónico, linolénico, palmitoleico, lignocérico, caproico, behénico, mirístico y margárico (Dar et al., 2019; Wacal et al., 2019). Entre ellos destacan el ácido graso oleico y el linoleico como AGIs, en un rango del 26,0% al 54,9%, y el contenido de

AGS entre 0,5% y 10,6% (Wei et al., 2022). Las semillas de sésamo también contienen algunos antinutrientes como el ácido oxálico y el ácido fítico (Petroski & Minich, 2020).



Figura 18. Cultivo de *Sesamum indicum* L (18A). Semillas y apariencia del aceite extraído de semillas de sésamo (18B).

(Fuente: Google imágenes (<https://acortar.link/RQJD0j>; <https://acortar.link/g8duv7>)).

Aceite de oliva

El aceite de oliva, al contrario que los mencionados con anterioridad, es un aceite extraído del fruto del olivo (*Olea europaea* L.) (Figura 19). El olivo es una de las plantas más cultivadas y de mayor importancia a nivel mundial para la obtención de aceite, siendo su principal ácido graso el oleico, con un contenido entre el 65-85%. Es un aceite con un aporte de AGS de entre el 8 y el 13%. Contiene cantidades variables de ácido linoleico (3-21%) y ácido linolénico (<1%) (Espínola et al., 2021; Vingering et al., 2010). En cuanto a los compuestos bioactivos, son los mismos que en la mayoría de los aceites (tocoferoles y compuestos fenólicos como el hidroxitirosol y la oleuropeína), pero también tiene compuestos provitamina A y clorofilas (Jiménez-López et al., 2020).



Figura 19. Fruto del olivo (*Olea europaea* L) y apariencia del aceite extraído de los frutos del olivo.

(Fuente: thefoodtech.com (<https://acortar.link/zbhIRg>)).

2.3.1.1.2. Aceites de origen marino

Aceites de algas marinas

El aceite de algas marinas (tanto microalgas como macroalgas), ha ganado una atención significativa como una fuente alternativa a los aceites comestibles de origen vegetal. En general, estos productos tienen entre un 0,1 y un 10% de lípidos, pero a pesar de que la fracción mayoritaria de ácidos grasos son los ácidos saturados (45-55%, palmítico mayoritariamente), es un aceite interesante debido a que su contenido en ácidos grasos poliinsaturados ronda el 15-30%, con los ácidos grasos omega 3 α -linolénico, DHA y EPA y el araquidónico (omega 6) como los AGPI mayoritarios (Kendel et al., 2015). El contenido de los AGPI (25-40%) es ligeramente superior al de los AGMI (19-25%) (Caf et al., 2019) y la composición de los ácidos grasos puede variar en cada tipo de alga ya que las especies de alga parda (*Phylum Phaeophyceae*) muestran la mayor cantidad de AGMI de entre todas las especies. Las algas verdes (*Phylum Chlorophyta*) se distinguen por su contenido en ácidos grasos poliinsaturados de la categoría C16:0 y C18:0 además de contener ácidos C16:3 (hexadecatrienoicos) y C16:4

(hexadecatetraenoicos) aunque el ácido graso más abundante es el alinolénico. También presentan una destacable relación de AGPI C18:0/C20:0 y un elevado grado de insaturación. Las algas rojas (*Phylum Rhodophyta*) sin embargo son ricas en ácido araquidónico (C20:4, omega 6) y eicosapentaenoico (C20:5, omega 3) los cuales predominan en su composición y también presentan cantidades elevadas de ácido graso oleico (Rohani-Ghadikolaei et al., 2012). Los aceites de algas además de ser ricos en ácidos grasos omega 3 y omega 6 contienen compuestos como carotenoides, tocoferoles y fitoesteroles en su fracción lipídica confiriendo propiedades antioxidantes al aceite (Caf et al., 2019).

Aceite de pescado

El aceite de pescado se extrae de los tejidos de los peces, ya sea del hígado en los peces magros o de la carne en los peces grasos. Las principales fuentes de este aceite provienen de especies pelágicas capturadas en grandes volúmenes, especialmente aquellos pescados grasos como el salmón, el atún, la caballa y el arenque, así como de peces pequeños como las anchoas y el capellán. Al igual que el aceite de algas, el de pescado se caracteriza por presentar elevadas cantidades de ácidos grasos omega 3 de cadena larga, incluyendo el ácido eicosapentaenoico (C20:5), el ácido docosapentaenoico (C22:5) y el ácido docosahexaenoico (C22:6). El creciente reconocimiento de los beneficios de los ácidos grasos omega 3 para la salud y la nutrición ha propiciado un notable incremento en la demanda de aceite de pescado (Nitesh et al., 2020). El contenido lipídico en las especies marinas fluctúa entre el 0,3 y el 20%, dependiendo de diversos factores como la alimentación, la condición biológica, la edad, el sexo, el estado reproductivo, la madurez, la temperatura, la estación del año y la región geográfica

(Cardona et al., 2015). La cantidad de ácidos grasos saturados varía entre el 28-37%, siendo el AGS predominante el palmítico, seguido del esteárico, mientras que la cantidad de ácidos grasos monoinsaturados varía entre el 18% y el 38% principalmente compuestos por los ácidos grasos palmitoleico y oleico. Por último, los ácidos grasos poliinsaturados representan entre el 11% y el 35% del total de los ácidos grasos y destacan el DHA y el EPA (Durmuş, 2019).

2.3.2. EMULSIONANTES Y GELIFICANTES

2.3.2.1. EMULSIONANTES

Otro de los ingredientes necesarios para generar una emulsión gelificada es el emulsionante, el cual se trata de un compuesto anfipático que posee una parte hidrofílica (afinidad por sustancias acuosas) y otra parte hidrofóbica (afinidad por sustancias de origen lipídico) (Espinoza-Leandro et al., 2023; Freire et al., 2018) con una función tensoactiva, es decir que disminuyen la tensión interfacial entre la fase acuosa y lipídica, facilitando así la formación de pequeñas gotas y permitiendo que ambas fases se mezclen. Tienen un papel importante en la formación de la emulsión dado que son los que determinan cual actuará como fase dispersa y cual como fase continua (Freire et al., 2018). Aunque existen diversas clasificaciones de los emulsionantes, una de las más utilizadas es la basada en el HLB (balance hidrofílico/lipofílico), lo que permite clasificar a los emulsionantes según su afinidad por el agua o por el aceite en una escala arbitraria del 1 al 20. Una mayor numeración indicará que tendrán mayor afinidad hidrofílica o acuosa, denominándose por ello emulsionantes hidrofílicos, dando lugar a emulsiones del tipo O/W. Sin embargo, una menor numeración significará una mayor afinidad por la parte hidrofóbica o lipídica, denominándose por

ello emulsionante lipofílico y generando emulsiones del tipo W/O (Yan et al., 2023). En la Tabla 4, se presentan algunos de los emulsionantes, de ambos tipos, más empleados en la generación de emulsiones gelificadas para la sustitución de grasa en productos cárnicos. Entre los emulsionantes hidrofílicos empleados para el desarrollo de emulsiones gelificadas destacan el caseinato de sodio (SC), el aislado o concentrado de proteína de soja (ISP), el aislado o concentrado de proteína de suero (WPI) y la albúmina de suero bovino. En cuanto a los emulsionantes de naturaleza lipofílica los más empleados son las lecitinas, los mono- y diacilglicéridos (MDAG) o el poliglicerol del ácido polirricinoleico, (PGPR).

Existen estudios donde se ha demostrado que las albúminas del amaranto presentan buenas propiedades emulsionantes en pH cercanos a 5 y que sin embargo sus globulinas presentan una función deficiente en pH inferiores a 5 pero óptima en el rango entre 5 y 7. En cuanto a la quinoa, se ha demostrado que tiene una menor capacidad como emulsionante que la albúmina de suero bovino pero la estabilidad de la emulsión formada era mayor que en las generadas por otros cereales, como el trigo, o la soja (Janssen et al., 2017). En cuanto a las propiedades gelificantes de los pseudocereales no existen muchos estudios que prueben su capacidad gelificante, por ejemplo, Bejosano & Corke (1998) estudiaron la capacidad de gelificación de proteínas de amaranto y trigo sarraceno en emulsiones cárnicas concluyendo que la capacidad gelificante del trigo sarraceno era mayor a la del amaranto. La capacidad de gelificación igual que sucedía con la capacidad de emulsión, están significativamente influenciadas por las condiciones de pH. En el caso de la quinoa, las globulinas que la componen se agregan fuertemente en respuesta a diferentes valores de pH, lo que afecta a la estructura secundaria y la solubilidad de la proteína. Normalmente, se forman geles semisólidos a $\text{pH} < 9$, mientras que no es

posible la gelificación a $\text{pH} > 10$ (Dakhili et al., 2019; Vidaurre-Ruiz et al., 2023). Algunos pseudocereales como el amaranto, el trigo sarraceno, y la quinoa se han empleado en forma de harina para obtener productos sin gluten como pasta, *noodles*, granolas y productos en base de harina, productos de panadería (Anberbir et al., 2024; Bender & Schönlechner, 2021). Las propiedades emulsionantes comentadas anteriormente, hacen adecuadas a estas harinas para su incorporación en productos cárnicos. Así pues, existen estudios del uso de pseudocereales en la elaboración de productos cárnicos como el llevado a cabo por Verma et al. (2019) los cuales incorporaron un 1,5 y 3% de harina de amaranto y quinoa para sustituir la harina de trigo refinada empleada en la elaboración de unos *nuggets* de carne de cabra, los resultados indicaron que la incorporación de este tipo de harina aumentaba la estabilidad de emulsión del producto cárnico con una buena aceptabilidad entre los consumidores. Fernández-López et al. (2020a) elaboraron una mortadela con adición de un 3% de semillas enteras de quinoa negra y un 3% del coproducto procedente de la molienda húmeda de dicha quinoa. Como resultados más destacables en el estudio mencionado anteriormente, la adición de ambos productos (semillas enteras y coproductos de quinoa) mejoró la estabilidad de la emulsión y se obtuvo una menor oxidación lipídica en las mortadelas con adición de fibra.

Bahmanyar et al. (2021) incorporaron semillas de amaranto y trigo sarraceno a hamburguesas de ternera sin obtener grandes diferencias desde el punto de vista físico-químico. A pesar de sus propiedades emulsionantes y gelificantes, las harinas de pseudocereales no se habían utilizado hasta el momento para obtener emulsiones gelificadas.

2.3.2.2. GELIFICANTES

Un gelificante es una sustancia que tiene la capacidad de generar geles, es decir es el responsable de aportar estructura a un sistema, dotándolo de una red tridimensional capaz de retener un líquido en su interior. Por ello, el agente gelificante y el método de estructuración (o proceso de gelificación) se realizará en función de la naturaleza del sistema.

Procesos de gelificación

El proceso de gelificación se puede inducir de diferentes formas:

La *gelificación por inducción térmica* consiste en superar los 65 °C para conseguir la gelificación de las proteínas, ya que con la aplicación de altas temperaturas las proteínas se desnaturalizan permitiendo la formación de enlaces disulfuro intra e intercatenario, que darán lugar a estructuras tridimensionales debido a las interacciones químicas entre los enlaces disulfuro, interacciones hidrofóbicas y los enlaces de hidrógeno entre moléculas (Ren et al., 2022). Estos geles se obtienen por tanto al emplear proteínas, como sucede con el uso de huevo, productos lácteos y la WPI (*whey protein isolate*, aislado de proteína de suero), y el tipo de red formada depende de múltiples variables como la temperatura, el tiempo de exposición a la misma, la cantidad de proteína incorporada, la fuerza iónica y el pH (Dickinson, 2012).

La *gelificación química o por inducción iónica* emplea gelificantes de naturaleza proteica y polisacárida combinados. En este proceso el mecanismo de gelificación se debe a la agregación y las interacciones químicas realizadas entre los polisacáridos y las proteínas. La variación del pH por adición de ácidos como el glucono- δ -lactona o la adición de sales de

Ca^{+2} procedente de CaCl_2 , modifica el punto isoeléctrico de las proteínas provocando su desnaturalización, exponiendo sus enlaces y facilitando así las interacciones con los polisacáridos, dando lugar a estructuras más estables (Cortez-Trejo et al., 2022; Jie et al., 2022).

La *gelificación enzimática* utiliza enzimas con la finalidad de modificar la naturaleza de los enlaces peptídicos, siendo la enzima transglutaminasa (TG) una de las más empleada. Esta enzima actúa potenciando la formación de enlaces covalentes y confiriendo al sistema de propiedades termoestables, elásticas y rígidas (Delgado-Pando et al., 2010; Dickinson, 2012; Herrero & Ruiz-Capillas, 2021).

La *gelificación por adición directa de polisacáridos* es un proceso también muy utilizado para obtener emulsiones gelificadas, debido a que ciertos polisacáridos tienen la propiedad de espesar y gelificar medios acuosos, siendo los más usados el alginato sódico, la goma xantana o las carrageninas. Estos polisacáridos facilitan la interacción de polímeros para formar redes continuas con funcionalidad de emulsiones gelificadas (Pintado et al., 2015).

La información detallada sobre los emulsionantes y gelificantes empleados en la industria cárnica se muestra en la Tabla 4, en la cual se dan distintas combinaciones de emulsionantes y gelificantes, así como sus procesos de gelificación y aceites empleados para conseguir emulsiones gelificadas como sustitutos de grasa animal, total o parcial, y el tipo de producto en el que se ha llevado a cabo dicha sustitución.

Tabla 4. Combinación de emulsionantes y gelificantes para generar EG y productos cárnicos a los que se han aplicado.

Tipo de gelificación	Productos cárnicos	Gelificante	Emulsionante	Aceite empleado	Referencia
GELIFICACIÓN ENZIMÁTICA	Salchichas tipo Frankfurt	Transglutaminasa	Aislado o concentrado de proteína de soja/caseinato de sodio	Oliva	Delgado-Pando et al. (2010)
	Salchichas tipo salami	Transglutaminasa	Aislado o concentrado de proteína de soja	Colza	Dreher et al. (2021)
ADICIÓN DIRECTA DE GELIFICANTE	Salchichas tipo Frankfurt	Agente gelificante basado en alginato	Aislado o concentrado de proteína de soja	Oliva	Pintado et al. (2021)
	Salchichas tipo Frankfurt bajas en grasa	Agente gelificante basado en alginato	Polvo de chía	Oliva	Herrero et al. (2017) Pintado et al. (2016b)
	Salchichas de Bolonia	Agente gelificante basado en alginato	Mucílago de chía	Oliva	Cámara et al. (2020)
	Longanizas secas (fuet)	Agente gelificante basado en alginato	Polvo de chía/ salvado de avena	Oliva	Pintado & Cofrades, (2020)
	Plant-based burgers	κ-carragenato, glucomanano de konjac, metilcelulosa, goma gellan	Aislado de proteína de soja/goma xantana	Palma y colza	Funami et al. (2023)
	Salchichas tipo Frankfurt	Gelatina	Polvo de cascarilla de cacao, gelatina	Nuez	Botella-Martínez et al. (2021)
	Salchicha emulsionada con grasa de vacuno	Gelatina	Polvo de huevo blanco/inulina de achicoria	Cacahuete, lino	Nacak et al. (2021)
INDUCCIÓN IÓNICA	Pate de hígado de cerdo bajo en grasa	Ca(OH) ₂	Glucomanano de konjac, almidón pregelificado, i-carragenato	Oliva, lino y pescado	Delgado-Pando et al. (2011)
	Salchichas de cerdo fermentadas en seco	Ca(OH) ₂	Harina de konjac, almidón pregelificado	Oliva, lino y pescado	Jiménez-Colmenero, (2013)

INDUCCIÓN TÉRMICA	Salchichas de vacuno fermentadas		Albúmina, gelatina/Inulina	Cacahuete, lino	Öztürk-Kerimoğlu et al. (2021b)
	Salchichas de pollo		Caseinato de sodio, gelatina/Inulina	Comino negro, lino	Kavuşan et al. (2020)
	Hamburguesas de ternera		κ -carragenato	Alga Microalga	Alejandre et al. (2017) Alejandre et al. (2019)
	Salchichas secas de cerdo (Harbin)		Polvo de alcachofa de Jerusalén	Oliva pre-emulsionada	Zhu et al. (2020)
	Salchichas secas de cerdo (Harbin)		Harina de konjac, κ -carragenato y β -glucano	Germen de maíz	Chen et al. (2021)
	Hamburguesas de cerdo bajas en grasa		κ -carragenato	Chía, lino	Heck et al. (2019)
	Salchichas de cerdo de Bolonia		Aislado o concentrado de proteína de soja, caseinato de sodio /polvo de chía, inulina, carragenato	Soja	de Souza Paglarini et al. (2019)

(Fuente: Adaptado de Ren et al., (2022)).

2.3.3. APLICACIONES DE EMULSIONES GELIFICADAS EN EL DESARROLLO DE PRODUCTOS CÁRNICOS

Existen diversos estudios donde se han empleado las emulsiones gelificadas como método para la sustitución de grasa animal en productos cárnicos, utilizando aceites vegetales como los de girasol, soja, oliva y chía. El uso de estas emulsiones gelificadas como sustitutos de grasa constituye una estrategia efectiva tanto para disminuir la cantidad de grasa, como para mejorar el perfil lipídico en productos cárnicos frescos, cocidos y curados (Carvalho Barros et al., 2020; de Souza Paglarini et al., 2019; Herrero & Ruiz-Capillas, 2021; Lucas-González et al., 2020). Como ya se mencionó con anterioridad, los aceites vegetales han demostrado capacidad para mejorar los perfiles lipídicos mediante una reducción de los ácidos grasos saturados y el aumento de los ácidos grasos poliinsaturados. Sin embargo, es importante recordar que también pueden acelerar las reacciones de oxidación lipídica, reducir la vida útil del producto y afectar las propiedades sensoriales. Para minimizar estos impactos negativos en la sustitución lipídica en los productos cárnicos, la incorporación de dichos aceites en forma de emulsiones gelificadas es una estrategia muy interesante (de Souza Paglarini et al., 2019; Pintado et al., 2020).

En la Tabla 5, se recopilan diferentes estudios donde se ha llevado a cabo la sustitución de grasa animal en diversos productos cárnicos mediante el uso de emulsiones gelificadas, clasificados según el tipo de producto cárnico en el cual se empleó.

Tabla 5. Resumen de estudios que evalúan los efectos sobre los parámetros de calidad de los productos cárnicos con sustitución total o parcial de grasa animal por emulsiones gelificadas.

Productos cárnicos frescos	Aceite empleado	Sustitución	Respuesta en el producto cárnico	Referencia
Salchichas frescas bajas en grasa (longaniza)	Oliva	90%	<ul style="list-style-type: none"> Mejóro la cantidad de grasa, minerales y aminoácidos. Menores pérdidas por cocinado. Aumentó la fuerza de cizalla de Kramer. Afectó a las propiedades sensoriales, aunque fueron catalogadas como aceptables. 	Pintado et al. (2018)
Hamburguesa de ternera	Nuez	50 y 100%	<ul style="list-style-type: none"> Disminuyó el contenido en grasa. Aumentó el contenido en humedad y cenizas. Disminuyó la cantidad de AGS y aumentó la cantidad de AGPI. Mejóro los índices nutricionales: disminuyó el IA y el IT. Aumentó las pérdidas por cocinado. Disminuyó la dureza y la masticabilidad. 	Botella-Martínez et al. (2021)
Hamburguesas de cerdo bajas en grasa	Chía, lino	20, 40, 60, 80 y 100%	<ul style="list-style-type: none"> Incrementó la proteína. Mejóro el perfil de ácidos grasos en hamburguesas crudas. Aumentó el grado de oxidación lipídica. No afectó a las propiedades tecnológicas. 	Heck et al. (2019)
Hamburguesas de cerdo	Girasol	25, 50, 75 y 100%	<ul style="list-style-type: none"> Disminuyó un 41% el contenido de grasa. Incrementó los AGI (74,5%). Disminuyó el colesterol (47%). Disminuyó el grado de oxidación lipídica. 	Poyato et al. (2015)
Hamburguesas de ternera	Canola, oliva	0, 25, 50, 75 y 100%	<ul style="list-style-type: none"> Aumentó la humedad y las pérdidas por cocinado. No se vio variación en los valores de oxidación lipídica (< 75%). Mejóro los ratios nutricionales: incrementó la relación omega 6/omega 3 y disminuyó el contenido de AGS, el IA y el IT. 	Dias et al. (2022)
Hamburguesas de ternera	Microalga	100%	<ul style="list-style-type: none"> No hubo diferencias en el sensorial entre las muestras. Disminuyó la cantidad de grasa un 51%. Aumentó los AG omega 3. Aumentó la capacidad antioxidante y disminuyó el grado de oxidación lipídica. 	Alejandro et al. (2019)

Productos cárnicos cocidos	Aceite empleado	Sustitución	Respuesta en el producto cárnico	Referencia
Salchichas de Bolonia	Soja	50 y 100%	<ul style="list-style-type: none"> Incrementó la dureza. Redujo la cantidad de grasa (31%). Aumentó el grado de oxidación lipídica. Disminuyó la cantidad de AGS y aumentó la cantidad de AGPI. Mejoró los índices nutricionales: disminuyó el IA y el IT. 	de Souza Paglarini et al. (2021)
Salchicha emulsionada con grasa de vacuno	Cacahuete, lino	Por debajo de 40%	<ul style="list-style-type: none"> Mejoró el perfil lipídico y los ratios nutricionales: disminuyó los AGS y el colesterol e incrementó los AGMI y AGPI. Mejoró la EE y las pérdidas por cocinado. Presentaron alteraciones en el color y de textura: aumentó b* y aumentó la dureza. Aumentó los valores de oxidación lipídica. 	Nacak et al. (2021)
Grasa de vacuno en salchichas funcionales de pollo fresco	Comino negro, lino	50, 75 y 100%	<ul style="list-style-type: none"> Aumentó la humedad y la cantidad de proteína. Disminuyeron las pérdidas por cocinado. Disminuyó L* de las muestras sustituidas. AGS disminuyeron un 52,61%. Disminuyó la dureza de las muestras. La sustitución del 50% y 75% presentó valores de oxidación lipídica a día 0 menores que la muestra control. 	Kavuşan et al. (2020)
Salchichas de cerdo bajas en grasa (Harbin)	Camelia	10, 20, 30, 40, 50%	<ul style="list-style-type: none"> Aumentó la humedad. Variaron los parámetros de color: L* y a*. El grado de oxidación lipídica disminuyó. Disminuyó el contenido de AGS. Aumentó el contenido de AGPI. Disminuyó la dureza de las muestras. 	Wang et al. (2018)
Salchichas de cerdo de Bolonia	Soja	50% y 100%	<ul style="list-style-type: none"> Mejoró el perfil lipídico de las salchichas. Disminuyó la cantidad de grasa. Afectó al color de las salchichas: aumentó L* y redujo a*. Estructura más compacta, aumentó la dureza, gomosidad y fuerza de cizalla. 	de Souza Paglarini et al. (2019)
Salchichas tipo Frankfurt	Olive	100%	<ul style="list-style-type: none"> Aumentó los AGPI y disminuyó los AGS. Presentaron menor aceptabilidad las muestras sustituidas. Los valores de oxidación lipídica aumentaron. 	Pintado et al. (2021)

Productos cárnicos fermentados	Aceite empleado	Sustitución	Respuesta en el producto cárnico	Referencia
Fermentados funcionales (fuet)	Oliva, chía	80%	<ul style="list-style-type: none"> Mejóro el perfil de ácidos grasos. Disminuyó la relación omega 6/omega 3. Se obtuvieron valores de dureza similares al control. 	Pintado & Cofrades. (2020)
Salchichas de cerdo fermentadas en seco	Lino	65%	<ul style="list-style-type: none"> Disminuyó la cantidad de AGS y aumentó la cantidad de AGMI y la relación omega 6/omega 3. La cantidad de AG linolénico y α-linoleico aumentó. Disminuyeron los parámetros de textura. Disminuyó L* y aumentó a*. Procesos de lipólisis e incremento oxidativo. 	Glisic et al. (2019)
Salchichas de vacuno fermentadas	Cacahuete, lino	50% y 100%	<ul style="list-style-type: none"> Mejóro el perfil lipídico. Disminuyó el contenido lipídico total. Disminuyó el colesterol (50%). Aumentaron los AGMI. Mejóro: la relación omega 6/omega 3, relación AGPI/AGS, IA y el IT. 	Öztürk-Kerimoğlu et al. (2021b)
Salchichas tipo salami	Canola	25, 50, 75 y 100%	<ul style="list-style-type: none"> Cambios texturales: aumentó la dureza y disminuyó la cohesividad y elasticidad. Disminuyó la aceptabilidad de los productos sustituidos respecto al control. 	Dreher et al. (2021)
Salchichas de vacuno fermentadas	Oliva	20%	<ul style="list-style-type: none"> Disminuyó las mermas. Cambios texturales: la muestra con EG disminuyó la cohesividad y aumentó la elasticidad. Aumentó la cantidad de AGPI. 	Câmara et al. (2020)
Salchicha fermentada seca	Lino	26,3%; 32,8% y 39,5%	<ul style="list-style-type: none"> Aumentó en un 10,3% los AGPI y redujo la relación omega6/omega 3 No afectó a la oxidación lipídica primaria y secundaria. 	Alejandre et al. (2016)

AG: ácidos grasos, AGS: ácidos grasos saturados; AGI: ácidos grasos insaturados; AGMI: ácidos grasos monoinsaturados, AGPI: ácidos grasos poliinsaturados; IA: índice aterogénico; IT: índice trombogénico; EE: estabilidad de la emulsión; a*: parámetro verde-rojo del espacio CIEL*a*b*; b*: parámetro azul-amarillo del espacio CIEL*a*b*; L*: parámetro que mide la luminosidad en el espacio CIEL*a*b*; EG: emulsión gelificada.

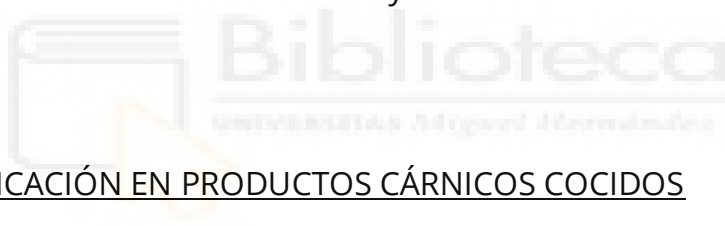
2.3.3.1. APLICACIÓN EN PRODUCTOS CÁRNICOS FRESCOS

El uso de emulsiones gelificadas para la reformulación de productos cárnicos frescos se ha llevado a cabo en multitud de estos productos, como por ejemplo el estudio realizado por Pintado et al. (2018) donde se analizó la sustitución, en salchichas frescas, de la panceta de cerdo por emulsiones gelificadas realizadas a base de harina de chía o salvado de avena con aceite de oliva y alginato como gelificante. Los resultados mostraron que la sustitución de grasa no afectaba a las propiedades sensoriales, pero sí a ciertas propiedades tecnológicas (pérdidas por cocinado y textura), dando lugar a productos reducidos en grasa y con un aumento de fibra. Otro tipo de producto fresco con adición de emulsión gelificada ha sido el chorizo fresco de ciervo. Así, en un estudio llevado a cabo por Martínez et al. (2023) se realizó la sustitución del 50, 75 y 100%, de la grasa por emulsiones gelificadas elaboradas con aceites de semillas, uno comercial que llevaba una mezcla de aceite de chía y amapola y otra mezcla de aceite de semillas de coproductos de la industria agroalimentaria (melón y calabaza). Esta sustitución finalmente provocó una mejora en el perfil de ácidos grasos de los productos sustituidos los cuales sensorialmente fueron valorados positivamente por los consumidores. Kavuşan et al. (2020), elaboraron unas salchichas frescas de pollo a las cuales le sustituyeron un 50, 75 y 100% de la grasa de vacuno por una emulsión gelificada con una proporción 1:1 de aceite de comino negro y lino, estabilizada con caseinato sódico, gelatina e inulina. Como resultados más destacables, los autores informaron de una disminución del contenido de grasa, con una disminución de más del 50% de grasa saturada, con valores de oxidación lipídica aceptables a pesar del aumento en ácidos grasos insaturados. Por otro lado, sí se vio afectado el color del producto, dando lugar a coloraciones más oscuras por parte de las

salchichas con mayor concentración de emulsión gelificada, probablemente debido al comino negro.

Otro de los productos cárnicos susceptibles de sustituir la grasa de origen animal son las hamburguesas, las cuales son uno de los productos cárnicos más estudiados debido a su popularidad de consumo entre los jóvenes. Este tipo de productos contienen una cantidad de grasa comprendida entre el 20% y 30%, por ello los estudios se centran en reducir el contenido total de grasa animal y en su sustitución por grasas con un perfil lipídico más saludable (Carvalho Barros et al., 2020). Por ello existen estudios (Alejandre et al., 2019; Botella-Martínez et al., 2021; Dias et al., 2022; Heck et al., 2019; Poyato et al., 2015) de reformulación de hamburguesas (más saludables) con el uso de emulsiones gelificadas, ya que la inclusión de los aceites vegetales de esta forma les confiere una mayor estabilidad frente a la oxidación lipídica, así como ayuda a mejorar las propiedades sensoriales, frente al uso de otras técnicas de sustitución de grasa animal (Delshadi et al., 2020). Botella-Martínez et al. (2021) llevó a cabo un estudio donde se analizó la sustitución del 50% y 100% de grasa animal, por una emulsión gelificada a base de polvo de cascarilla de cacao y aceite de nuez, en hamburguesas de vacuno. En dicho estudio, las muestras con una sustitución de grasa animal del 50% obtuvieron una mayor aceptabilidad que las muestras con una sustitución de grasa animal del 100%, debido fundamentalmente a las diferencias existentes en los parámetros de color de la muestra más sustituida respecto a la formulación control (sin emulsión gelificada). Las muestras con una sustitución del 50 y 100% de grasas por la emulsión gelificada, presentaron mayores valores de oxidación lipídica que el control. Lucas-González et al. (2020) evaluaron los efectos de un reemplazo parcial de grasa animal (5% y 10%) por una emulsión gelificada elaborada con aceite de chía y harina de castaña en hamburguesas de cerdo. Los resultados

obtenidos fueron favorables, sin impacto destacable sobre la calidad de las hamburguesas de cerdo sustituidas y mejorando sus propiedades saludables derivadas del aumento en los ácidos grasos poliinsaturados, así como la disminución de los índices aterogénico y trombogénico. Únicamente el parámetro de oxidación lipídica se vio afectado por esta sustitución. En un estudio similar, Heck et al. (2019) analizaron la influencia de la sustitución del 20, 40, 60, 80 y 100% en hamburguesas de ternera con una emulsión gelificada que contenía un 12,5% de aceite de chía y un 12,5% de aceite de lino. La reformulación de las hamburguesas logró reducir el contenido de lípidos y mejorar el perfil de ácidos grasos ya desde sustituciones bajas (20%) y sensorialmente fueron aceptadas todas las muestras hasta el 60% de sustitución. La inclusión de la emulsión gelificada produjo cambios texturales, aumentando la dureza y masticabilidad de los productos sustituidos.



2.3.3.2. APLICACIÓN EN PRODUCTOS CÁRNICOS COCIDOS

Según investigaciones recientes, el reemplazo de grasa animal por aceites vegetales en productos cárnicos emulsionados cocidos consigue una mejora del perfil nutricional, una reducción del colesterol, así como la mejora de distintos índices como el índice aterogénico, el índice trombogénico y la relación omega 6/omega 3 (de Souza Paglarini et al., 2019; Lima et al., 2021; Nacak et al., 2021; Nieto & Lorenzo, 2021). La incorporación de aceites vegetales o marinos ricos en ácidos grasos poliinsaturados omega 3 durante el procesamiento térmico de productos cárnicos puede ser difícil debido al aumento de la oxidación, así como a las complicaciones técnicas y sensoriales en el producto final (Heck et al., 2018; Nieto & Lorenzo, 2021; Pintado et al., 2016b). Además, debido a su impacto en la estabilidad de la emulsión, la composición del aceite y la relación de grasa podrían afectar la

calidad de los productos finales (Nieto & Lorenzo, 2021). Aun así, en la literatura científica es posible encontrar numerosos estudios (de Souza Paglarini et al., (2019); Nacak et al., (2021); Pintado et al., (2021); Poyato et al., (2015) donde se analizaron el empleo de emulsiones gelificadas como sustitutos de grasa en productos cocidos y/o emulsionados, categoría de productos muy popular a la hora de aplicar esta estrategia para sustituir grasa. Se han ensayado en diversos productos, desde salchichas tipo Frankfurt, salchichas tipo Bolonia, salchichas tipo Harbin y paté. Existen diversos estudios asociados con la mejora del perfil lipídico en estos productos (Lima et al., 2022; Nacak et al., 2021; Nieto & Lorenzo, 2021), observándose en la mayoría un efecto en la textura, volviéndola más suave al aumentar la cantidad de ácidos grasos insaturados (Vargas-Ramella et al., 2020a), así como una mejora en la dispersión y distribución de los aceites vegetales en los productos cárnicos incorporados (Vargas-Ramella et al., 2020a; Youssef & Barbut, 2010) y un efecto en el color debido a la coloración amarillenta propia de los aceites vegetales (De Souza Paglarini et al., 2019; Franco et al., 2020; Nacak et al., 2021). Se han empleado varios tipos de emulsiones gelificadas elaboradas con aceite de soja, oliva o lino e incorporadas en productos cárnicos cocidos con un resultado similar. Esto ha llevado a una optimización del perfil lipídico, bien a través de un aumento en el contenido de ácidos grasos monoinsaturados, como en el caso del uso de aceite de oliva (Pintado & Cofrades, 2020), o mediante un aumento en los niveles de ácidos grasos poliinsaturados, cuando se usan aceites como el de soja, cacahuete, lino o camelia (de Souza Paglarini et al., 2019; Kavuşan et al., 2020; Nacak et al., 2021; Paglarini et al., 2022; Wang et al., 2018). En varios estudios, se encontró que los aceites vegetales con un alto contenido de ácidos grasos poliinsaturados aumentaban la oxidación lipídica, pero no se encontró ningún efecto con un alto contenido de monoinsaturados (Öztürk-

Kerimoğlu, et al., 2021b; Vargas-Ramella et al., 2020a). Pintado et al. (2016b) elaboraron geles de chía y aceite de oliva como sustitutos de grasa en salchichas tipo Frankfurt con un bajo contenido de grasas. Los resultados demostraron que agregar la emulsión gelificada con polvo de chía disminuyó el nivel de grasa un 40%, pudiéndose denominar el producto final como un producto con un "contenido de grasa reducido". Las salchichas tipo Frankfurt reformuladas contenían mayor cantidad de minerales, como magnesio, manganeso y calcio. Los productos sustituidos demostraron estabilidad oxidativa durante la conservación. De Souza Paglarini et al. (2021) encontraron modificaciones negativas en la dureza y la jugosidad de salchichas elaboradas con emulsiones gelificadas con aislado de proteína de soja y aceite de soja. Aunque se vio, en el producto sustituido, un aumento en la cantidad de fibra, pudiendo considerarse como un producto "fuente de fibra" y también como consecuencia de la sustitución se dio un incremento en la cantidad de lípidos insaturados. En el estudio de Wang et al. (2018), también se observó una disminución del 40% en la dureza y el contenido de grasa de las salchichas cocidas. Esto ocurrió sin tener impacto en características físico-químicas como el pH, la actividad de agua o el color. Más recientemente, Vargas-Ramella et al. (2020b) llevaron a cabo la incorporación de hidrogeles compuestos por una emulsión de alginato con distintos aceites (oliva, colza y soja) en salchichón de ciervo, mejorando tanto la composición como la calidad sensorial, en comparación con las salchichas control.

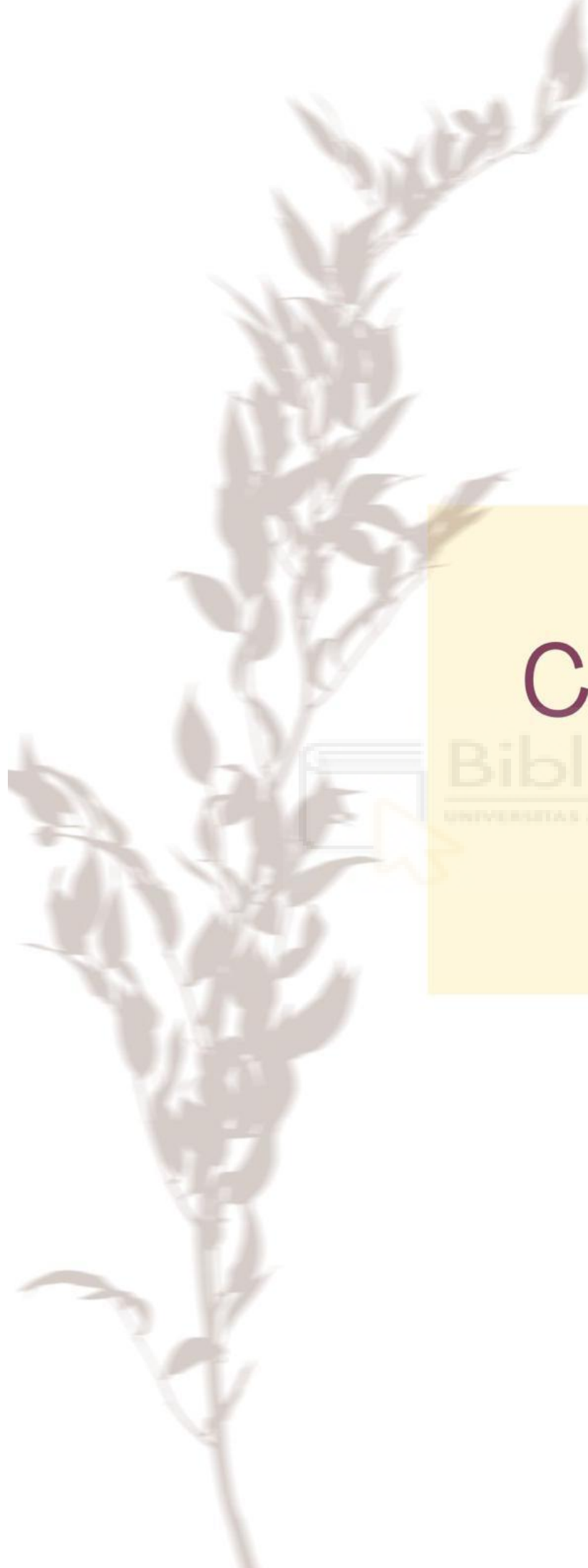
Con todo lo expuesto anteriormente, parece evidente que la utilización de emulsiones gelificadas como estrategia para para la sustitución de grasa animal en los productos cárnicos es muy interesante porque permitiría tanto la reducción del contenido de grasa como la mejora del perfil lipídico en el producto final. Sin embargo, son muchos los factores que pueden afectar a

su viabilidad tecnológica y a el impacto que pueda llegar a tener sobre las propiedades nutritivas, saludables y sensoriales en el producto final. Teniendo en cuenta la gran cantidad de factores implicados tanto en el desarrollo de la propia emulsión gelificada (aceites, emulsionantes, gelificantes, proceso de gelificación, etc.), como el tipo de producto cárnico al que se quiere incorporar (frescos, cocidos, emulsionados, análogos de carne, etc.), el nivel de sustitución, la forma de la incorporación, el momento de incorporación en el proceso de elaboración, etc. Por ello, sería interesante abordar el efecto que tendrían algunos de dichos factores sobre la calidad final de varios de los productos cárnicos de mayor consumo o interés en la población actual. Para este trabajo se seleccionaron aceites vegetales con perfiles lipídicos saludables (aceite de cáñamo, chía, lino y sésamo), las harinas de pseudocereales (amaranto, trigo sarraceno, teff y quinoa blanca) como emulsionantes y los gelificantes (gelatina, goma gellan, carragenato y goma garrofin) con un proceso de gelificación por adición directa de gelificante.

CAPÍTULO 3

Biblioteca
UNIVERSITATIS Aegyptii Helwanensis

OBJETIVOS



3. OBJETIVOS

3.1. OBJETIVO GENERAL

El objetivo general de la presente Tesis Doctoral ha sido analizar el impacto, sobre las propiedades químicas, fisicoquímicas, nutricionales y sensoriales, de la sustitución de grasa animal en productos cárnicos frescos y cocidos, por emulsiones gelificadas elaboradas con aceites vegetales y harinas de pseudocereales.

3.2. OBJETIVOS ESPECÍFICOS

Para abordar este objetivo general, se plantean los siguientes objetivos específicos:

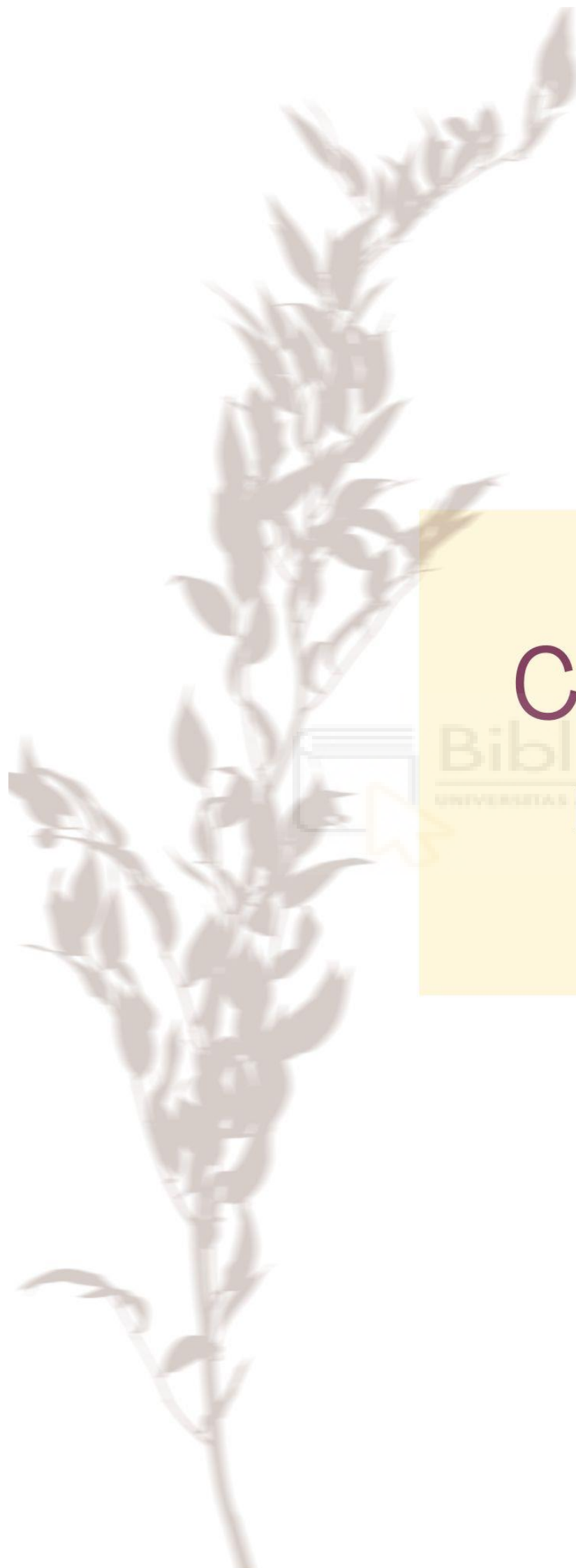
1. Caracterizar química y fisicoquímicamente, así como analizar la estabilidad lipídica de las emulsiones gelificadas elaboradas a partir de aceites vegetales de cáñamo, chía, lino y sésamo (ricos en ácidos grasos mono y poliinsaturados) y harinas de pseudocereales como amaranto, trigo sarraceno, quinoa blanca y teff, empleadas como emulsionantes.
2. Estudiar el efecto sobre las propiedades fisicoquímicas y la estabilidad oxidativa de las emulsiones gelificadas elaboradas a partir de aceites vegetales como cáñamo, chía, lino y sésamo (ricos en ácidos grasos mono y poliinsaturados) y harinas de pseudocereales como amaranto, trigo sarraceno, quinoa blanca y teff, tras el almacenamiento en congelación (-23 °C) durante 15 días.

3. Analizar el efecto de la sustitución de la grasa de origen animal por emulsiones gelificadas elaboradas con aceites vegetales (aceite de chía o cáñamo) y harina de amaranto sobre las propiedades químicas, fisicoquímicas, de cocción y sensoriales, así como la estabilidad oxidativa de un producto cárnico fresco tipo hamburguesa.
4. Evaluar el efecto que dicha sustitución tiene sobre la calidad de las hamburguesas durante su conservación en condiciones de congelación (60 días).
5. Analizar cómo afecta la sustitución de la grasa de origen animal por emulsiones gelificadas elaboradas con aceite de cáñamo y harina de trigo sarraceno, a las propiedades químicas, físico químicas y sensoriales de los productos cárnicos cocidos tipo salchicha Frankfurt y paté.
6. Evaluar cómo afecta la sustitución de grasa de origen animal por emulsiones gelificadas elaboradas con aceite de cáñamo y harina de trigo sarraceno, a las propiedades químicas, físico químicas, de cocinado y estabilidad de un producto cárnico tradicional portugués denominado alheira.
7. Estudiar el efecto sobre las propiedades químicas, físico químicas, de cocinado y sensoriales de un análogo cárnico, tipo hamburguesa, de la adición de grasa en forma de emulsión gelificada elaborada con aceites de chía o aceite de cáñamo y harina de trigo sarraceno.

CAPÍTULO 4

Biblioteca
UNIVERSITARIA

MATERIALES Y MÉTODOS



4. MATERIALES Y MÉTODOS

Para una visión general del trabajo desarrollado, en la Figura 20 se presenta de forma resumida el diseño experimental, así como los materiales y métodos empleados para alcanzar los objetivos planteados.

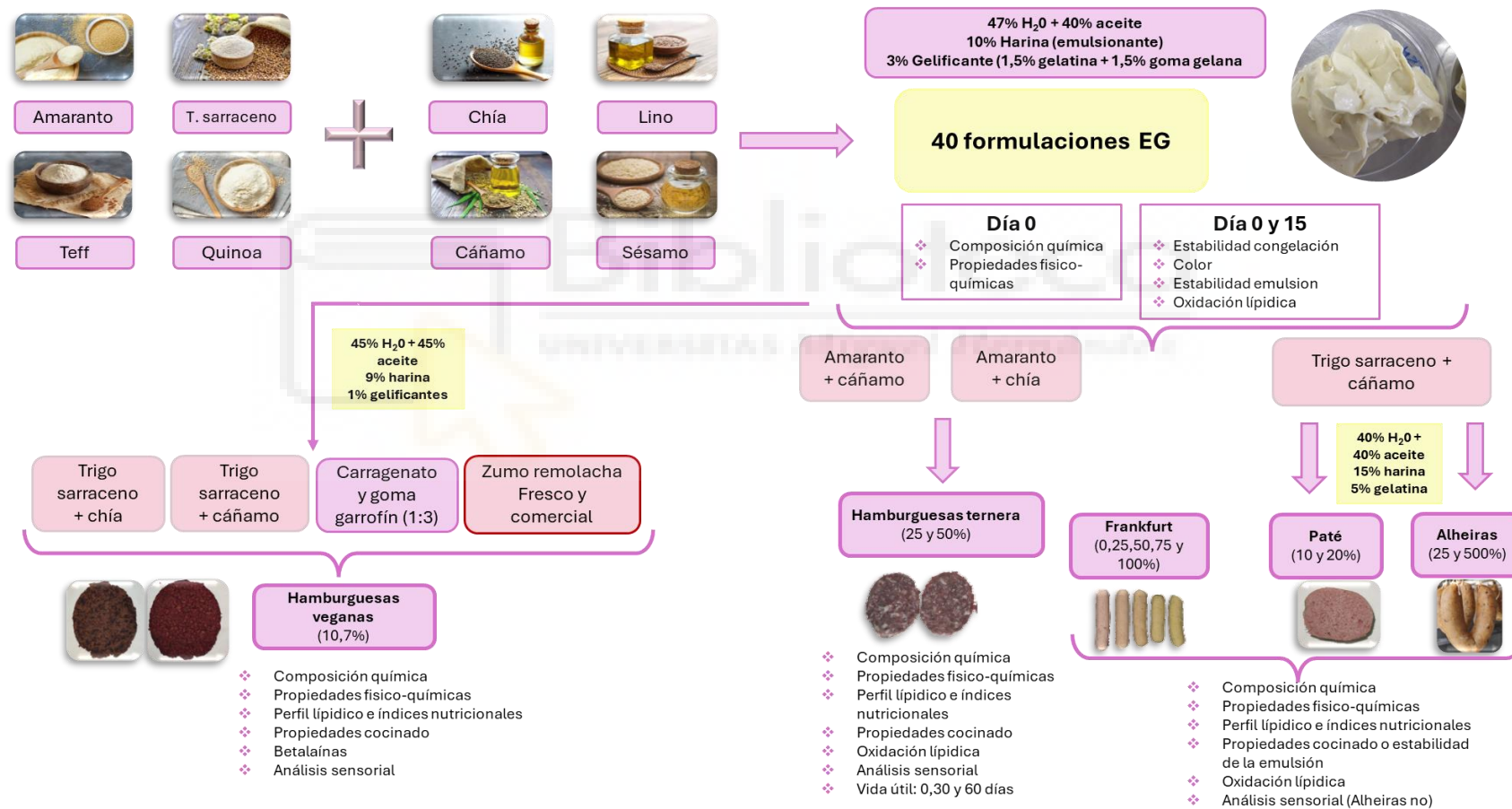


Figura 20. Esquema general del desarrollo experimental y de las propiedades evaluadas.

4.1. EMULSIONES GELIFICADAS

4.1.1. INGREDIENTES

Para la preparación de las emulsiones gelificadas se han utilizado:

- como fase lipídica, cuatro tipos de aceites: aceite de cáñamo, aceite de chía, aceite de lino y aceite de sésamo, todos adquiridos de marcas comerciales. El aceite de cáñamo, lino y sésamo fueron adquiridos de Laboratorios Almond, S.L. (Murcia, España), y el aceite de chía fue adquirido de Herbolarios Navarro (Alicante, España).
- como emulsionantes, harinas de pseudocereales, en concreto, harina de amaranto, suministrada por Tentorium Energy S.L. (Tarragona, España), harina de trigo sarraceno y harina de quinoa blanca, ambas suministradas por Biogran S.L. (Madrid, España) y por último harina de teff suministrada por el Granero Integral, S.L. (Madrid, España).
- como gelificantes, goma gellan (polisacárido extracelular excretado por el microorganismo *Pseudomonas elodea*) y gelatina (colágeno de cerdo), ambas suministradas por Sosa Ingredientes S.L. (Barcelona, España). Goma garrofín (galactomanano vegetal) y carragenato (polisacárido extraído de algas como las especies *Euchema*, *Chondrus crispus* y *Gigartina*) suministrados por Innovative Cooking S.L. (Madrid, España).

4.1.2. PROCESO DE ELABORACIÓN

Se elaboraron distintas emulsiones gelificadas según la naturaleza del estudio las cuales se describen a continuación.

4.1.2.1. EMULSIONES GELIFICADAS CON GOMA GELLAN Y GELATINA

Se elaboraron 40 emulsiones O/W gelificadas diferentes como resultado de (i) la combinación de todas las harinas con todos los aceites y (ii) la combinación de todas las harinas con combinaciones de mezclas 1:1 de los 4 aceites de semillas (cáñamo, chía, lino y sésamo). En la Tabla 6 se muestra la composición de cada una de las emulsiones gelificadas desarrolladas.

Tabla 6. Formulación de emulsiones gelificadas (EG) con goma gellan y gelatina.

Muestra	Agua	Amaranto	Trigo sarraceno	Teff	Quinoa	Gelatina	Goma gellan	Aceite chía	Aceite lino	Aceite cáñamo	Aceite sésamo
ACH	47	10	-	-	-	1,5	1,5	40	-	-	-
AL	47	10	-	-	-	1,5	1,5	-	40	-	-
ACA	47	10	-	-	-	1,5	1,5	-	-	40	-
AS	47	10	-	-	-	1,5	1,5	-	-	-	40
TSCH	47	-	10	-	-	1,5	1,5	40	-	-	-
TSL	47	-	10	-	-	1,5	1,5	-	40	-	-
TSCA	47	-	10	-	-	1,5	1,5	-	-	40	-
TSS	47	-	10	-	-	1,5	1,5	-	-	-	40
TECH	47	-	-	10	-	1,5	1,5	40	-	-	-
TEL	47	-	-	10	-	1,5	1,5	-	40	-	-
TECA	47	-	-	10	-	1,5	1,5	-	-	40	-
TES	47	-	-	10	-	1,5	1,5	-	-	-	40
QBCH	47	-	-	-	10	1,5	1,5	40	-	-	-
QBL	47	-	-	-	10	1,5	1,5	-	40	-	-
QBCA	47	-	-	-	10	1,5	1,5	-	-	40	-
QBS	47	-	-	-	10	1,5	1,5	-	-	-	40
AM1	47	10	-	-	-	1,5	1,5	20	-	-	20
AM2	47	10	-	-	-	1,5	1,5	-	-	20	20
AM3	47	10	-	-	-	1,5	1,5	-	20	-	20
AM4	47	10	-	-	-	1,5	1,5	20	-	20	-
AM5	47	10	-	-	-	1,5	1,5	20	20	-	-
AM6	47	10	-	-	-	1,5	1,5	-	20	20	-
TSM1	47	-	10	-	-	1,5	1,5	20	-	-	20

TSM2	47	-	10	-	-	1,5	1,5	-	-	20	20
TSM3	47	-	10	-	-	1,5	1,5	-	20	-	20
TSM4	47	-	10	-	-	1,5	1,5	20	-	20	-
TSM5	47	-	10	-	-	1,5	1,5	20	20	-	-
TSM6	47	-	10	-	-	1,5	1,5	-	20	20	-
TEM1	47	-	-	10	-	1,5	1,5	20	-	-	20
TEM2	47	-	-	10	-	1,5	1,5	-	-	20	20
TEM3	47	-	-	10	-	1,5	1,5	-	20	-	20
TEM4	47	-	-	10	-	1,5	1,5	20	-	20	-
TEM5	47	-	-	10	-	1,5	1,5	20	20	-	-
TEM6	47	-	-	10	-	1,5	1,5	-	20	20	-
QBM1	47	-	-	-	10	1,5	1,5	20	-	-	20
QBM2	47	-	-	-	10	1,5	1,5	-	-	20	20
QBM3	47	-	-	-	10	1,5	1,5	-	20	-	20
QBM4	47	-	-	-	10	1,5	1,5	20	-	20	-
QBM5	47	-	-	-	10	1,5	1,5	20	20	-	-
QBM6	47	-	-	-	10	1,5	1,5	-	20	20	-

Todas las cantidades vienen expresadas en g /100 g. ACH: harina de amaranto con aceite de chía; AL: harina de amaranto con aceite de lino; ACA: harina de amaranto con aceite de cáñamo; AS: harina de amaranto con aceite de sésamo. TSCH: harina de trigo sarraceno con aceite de chía; TSL: harina de trigo sarraceno con aceite de lino; TSCA: harina de trigo sarraceno con aceite de cáñamo; TS: harina de trigo sarraceno con aceite de sésamo. TECH: harina de teff con aceite de chía; TEL: harina de teff con aceite de lino; TECA: harina de teff con aceite de cáñamo; TES: harina de teff con aceite de sésamo. QBCH: harina de quinoa blanca con aceite de chía; QBL: harina de quinoa blanca con aceite de lino; QBCA: harina de quinoa blanca con aceite de cáñamo; QBS: harina de quinoa blanca con aceite de sésamo. AM1: harina de amaranto con mezcla 1:1 de aceite de chía y sésamo; AM2: harina de amaranto con mezcla 1:1 de aceite de cáñamo y sésamo; AM3: harina de amaranto con mezcla 1:1 de aceite de lino y sésamo; AM4: harina de amaranto con mezcla 1:1 de aceite de chía y cáñamo; AM5: harina de amaranto con mezcla 1:1 de chía y lino; AM6: harina de amaranto con mezcla 1:1 de aceite de lino y cáñamo. TSM1: harina de trigo sarraceno con mezcla 1:1 de aceite de chía y sésamo; TSM2: harina de trigo sarraceno con mezcla 1:1 de aceite de cáñamo y sésamo; TSM3: harina de trigo sarraceno con mezcla 1:1 de aceite de lino y sésamo; TSM4: harina de trigo sarraceno con mezcla 1:1 de aceite de chía y cáñamo; TSM5: harina de trigo sarraceno con mezcla 1:1 de chía y lino; TSM6: harina de trigo sarraceno con mezcla 1:1 de aceite de lino y cáñamo. TEM1: harina de teff con mezcla 1:1 de aceite de chía y sésamo; TEM2: harina de teff con mezcla 1:1 de aceite de cáñamo y sésamo; TEM3: harina de teff con mezcla 1:1 de aceite de lino y sésamo; TEM4: harina de teff con mezcla 1:1 de aceite de chía y cáñamo; TEM5: harina de teff con mezcla 1:1 de chía y lino; TEM6: harina de teff con mezcla 1:1 de aceite de lino y cáñamo. QBM1: harina de quinoa blanca con mezcla 1:1 de aceite de chía y sésamo; QBM2: harina de quinoa blanca con mezcla 1:1 de aceite de cáñamo y sésamo; QBM3: harina de quinoa blanca con mezcla 1:1 de aceite de lino y sésamo; QBM4: harina de quinoa blanca con mezcla 1:1 de aceite de chía y cáñamo; QBM5: harina de quinoa blanca con mezcla 1:1 de chía y lino; QBM6: harina de quinoa blanca con mezcla 1:1 de aceite de lino y cáñamo.

Para la preparación de las emulsiones gelificadas se utilizó un homogeneizador de alimentos (Thermomix 31, Vorwerk-España M.S.L., S.C., España). Se adicionó agua (47 g/100 g de emulsión) y gelatina (1,5 g/100 g de emulsión) y se homogeneizaron durante 2 minutos, a elevada velocidad y a una temperatura de 60 °C. Pasado este tiempo, se le adicionó la harina del pseudocereal que actuó como emulsionante (10 g/100 g de emulsión) y se mezcló durante 1 minuto a velocidad media manteniendo la temperatura a 60 °C (con el objetivo de mantenerla caliente para la adición de la goma gellan, la cual para actuar y gelificar necesita la aplicación de calor). Tras la homogenización del agua con la gelatina y el emulsionante se bajó la temperatura del homogeneizador a 37 °C, y se añadió la goma gellan (1,5 g/100 g de emulsión) y se mezcló durante 2,5 minutos a 250 rpm. Finalmente, a la mezcla anterior se le adicionó el aceite (40 g/ 100 g de emulsión) de forma gradual (15 mL/min) y se mezcló durante 5 minutos a una temperatura de 37 °C y a una velocidad de 1100 rpm. En la Figura 21 se muestra un esquema donde se observa el proceso de elaboración de las emulsiones.

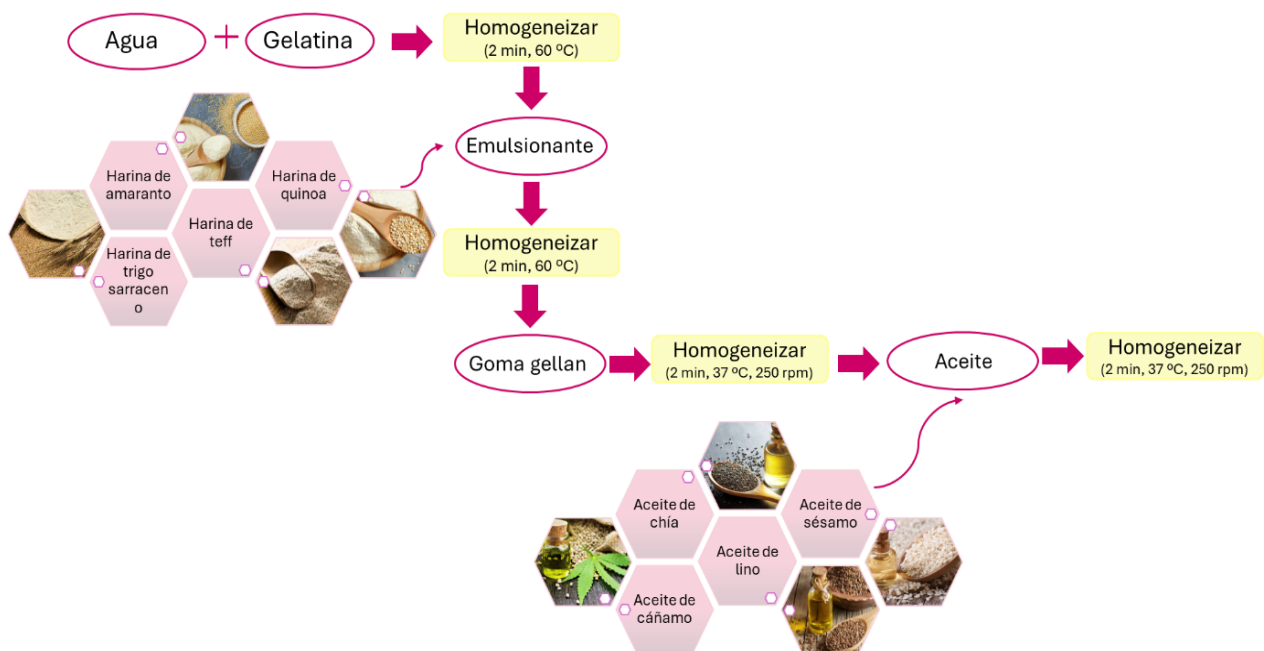


Figura 21. Diagrama de flujo de la elaboración de emulsiones gelificadas con gelatina y goma gellan.

A la hora de incorporar estas emulsiones gelificadas en los diferentes productos cárnicos, se elaboraban 24 horas antes y se mantuvieron en condiciones de congelación (-23 °C), para evitar fenómenos de embarrado y pérdida de estructura al incorporarlas en el producto cárnico final.

4.1.2.2. EMULSIONES GELIFICADAS CON GELATINA ÚNICAMENTE COMO GELIFICANTE

Con el objetivo de conseguir una consistencia mayor, dada la complejidad del producto cárnico al cual se iban a incorporar (alheira y paté), se decidió reformular las emulsiones gelificadas, disminuyendo un 7% la cantidad de agua, prescindiendo del segundo gelificante (goma gellan) empleado en el apartado 4.1.2.1, usando por lo tanto gelatina como único gelificante. Además, al eliminar la goma gellan que era el motivo de alcanzar una temperatura de 60 °C para la elaboración de las emulsiones gelificadas, se procedió a realizar una gelificación en frío, preservando así más la integridad de los aceites empleados y evitando todo lo posible los efectos de oxidación. También se aumentó la cantidad de emulsionante y de gelificante. Estos cambios permitieron obtener una emulsión gelificada con una consistencia mayor, mejores propiedades texturales y más estable al tratamiento de congelación.

La preparación de las emulsiones gelificadas se llevó a cabo en un homogeneizador de alimentos Thermomix 31 (Vorwerk-España M.S.L., Madrid, España). Se adicionó agua (40 g/100 g de emulsión) y la gelatina (5 g/100 g de emulsión) y se homogeneizaron durante 2 minutos, a elevada velocidad y sin aplicar calor. Pasado este tiempo, se le adicionó la harina del pseudocereal que actuó como emulsionante (15 g/100 g de emulsión). Tras la homogenización del agua con la gelatina y el emulsionante se mezcló durante 2,5 minutos a 250 rpm. Finalmente, a la mezcla anterior se le

adicionó el aceite (40 g/100 g de emulsión de forma gradual (15 mL/min) y se mezcló durante 5 minutos más sin temperatura a una velocidad de 1100 rpm. En la Figura 22 se muestra un esquema donde se observa el proceso de elaboración de las emulsiones. Tras la elaboración de las emulsiones, éstas se guardaron durante 24 horas en condiciones de congelación (-23 °C) hasta su incorporación en el correspondiente producto cárnico.

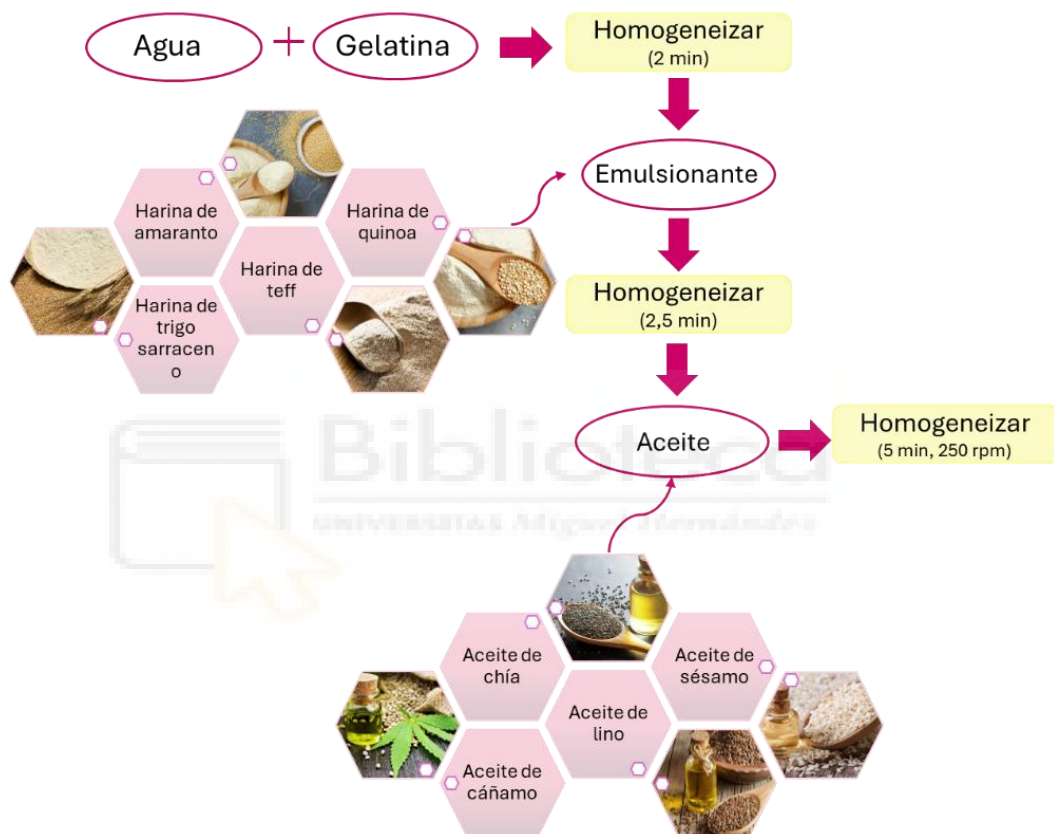


Figura 22. Diagrama de flujo para la elaboración de las emulsiones gelificadas en frío solo con gelatina como gelificante.

4.1.2.3. REFORMULACIÓN DE LAS EMULSIONES GELIFICADAS PARA SU POSTERIOR INCORPORACIÓN EN LAS HAMBURGUESAS VEGANAS

Se elaboró una tercera emulsión gelificada con el objetivo de que pudiese incorporarse en un análogo cárnico, hamburguesa vegana. En este caso, fue necesario sustituir los gelificantes de origen animal y microbiano, por gelificantes de origen vegetal. Así pues, para adaptar la emulsión

gelificada a las necesidades de este tipo de productos, se decidió utilizar carragenato y goma de garrofín por su poder gelificante y su implicación en la generación de la emulsión.

Para la preparación de estas emulsiones gelificadas se incorporaron 45 g/100 g de aceite de chía o de cáñamo, 45 g/100 g de agua, 9 g/100 g de harina de trigo sarraceno y 1 g/100 g de gelificante (mezcla 1:3 de carragenato-goma garrofín). La metodología de preparación fue la siguiente: se calentó el agua por encima de los 90 °C y se le adicionó la mezcla de gelificantes, se batió con una batidora manual quickchef DD1000 (Moulinex, Alençon, Francia) durante 5 minutos a una velocidad aproximada de 5600 rpm. A continuación, se le adicionó el emulsionante, en este caso se empleó la harina de trigo sarraceno, se mezcló de nuevo durante 1 minuto a la misma velocidad (5600 rpm). Por último, se adicionó de forma progresiva el aceite de chía o de cáñamo, según la emulsión que se quisiera elaborar, a un ritmo de aproximadamente 15 mL/min. Tras la incorporación de todo el aceite, se procedió a batir de nuevo durante 1 minuto para terminar de homogeneizar el producto. Se dejó enfriar a temperatura ambiente hasta que se consiguió su gelificación completa y tras esto se guardó en condiciones de congelación a -23 °C durante 24 horas.

4.1.3. CARACTERIZACIÓN DE LOS INGREDIENTES

4.1.3.1. COMPOSICIÓN QUÍMICA DE LAS HARINAS DE PSEUDOCEREALES

El contenido de humedad, proteína, grasa y cenizas se determinaron para las cuatro muestras de harinas utilizadas en la presente Tesis (amaranto, trigo sarraceno, teff y quinoa blanca) siguiendo los métodos oficiales de análisis (AOAC, 2010). La cantidad de hidratos de carbono se calculó para cada harina por diferencia del resto de componentes.

4.1.3.1.1. Humedad

La humedad se determinó por el método correspondiente de la AOAC (2010). Se trata de un método gravimétrico donde los resultados vienen expresados en g de agua/100 g de muestra.

4.1.3.1.2. Proteína

El contenido en proteína de las harinas de pseudocereales se determinó por el método Kjeldahl, con un factor de conversión de nitrógeno de 6,25. La digestión se llevó a cabo en un digestor Büchi modelo 426 (Digestion Unit, Barcelona, España). Se utilizó un destilador automático Kjeltex TM 8400, (Foss Iberia, Barcelona, España) para obtener la cantidad de proteína, expresados los resultados en g de proteína/100 g de muestra.

4.1.3.1.3. Grasa total

La determinación de grasa total de las muestras se determinó siguiendo la metodología correspondiente descrita por la AOAC (2010). Utilizando un extractor automático SOXTERM® SOX 6-place, (Gerhardt GMBH & Co. KG, Königswinter, Alemania). Como extractante se usó éter de petróleo. Los resultados se expresaron como g de grasa/100 g de muestra.

4.1.3.1.4. Cenizas

Mediante la metodología correspondiente recogida en la AOAC (2010) se determinó la cantidad de cenizas de las muestras, cuyo resultado se expresó como g de cenizas/100 g de muestra. En este método, las muestras se llevan a incineración a una temperatura de 525 °C hasta alcanzar un peso constante en una mufla modelo 12 PR/300 SERIE 8B (Fons Hobersal, Barcelona, España).

4.1.3.2. PROPIEDADES TECNOFUNCIONALES DE LAS HARINAS DE PSEUDOCEREALES

4.1.3.2.1. Capacidad de retención de agua (CRA) y capacidad de retención de aceite (CRO)

Para llevar a cabo estos análisis, se utilizó el método de Robertson et al. (2000). Para el cual se pesaron aproximadamente $0,500 \pm 0,005$ g de muestra y 10 mL de agua o aceite de girasol. Los resultados se expresaron como g de agua o aceite/g de muestra. Las determinaciones se realizaron por triplicado para cada una de las muestras.

4.1.3.2.2. Capacidad de hinchamiento (SWC)

La capacidad de hinchamiento de las muestras se determinó siguiendo la metodología descrita por Gómez-Ordóñez et al. (2010). El resultado se expresó como mL de agua/g de muestra. Las determinaciones se realizaron por triplicado para cada una de las muestras.

4.1.3.2.3. Capacidad emulsionante (AE) y estabilidad de la emulsión (EE)

La capacidad de las muestras para formar emulsiones, así como la estabilidad de la emulsión formada se estudiaron siguiendo el método propuesto por Chau et al. (1997). Las determinaciones se realizaron por triplicado y los resultados para ambas propiedades se expresaron en %.

4.1.3.3. ACTIVIDAD ANTIOXIDANTE DE LAS HARINAS DE PSEUDOCEREALES

Para la evaluación de la actividad antioxidante de las harinas de pseudocereales se aplicaron cuatro métodos diferentes. Todas las

determinaciones se realizaron mediante medidas espectrofotométricas, para lo cual se usó un espectrofotómetro HP 8451 (Hewlett Packard, Cambridge, Reino Unido).

4.1.3.3.1. Método del radical 2,2-difenil-1-picrilhidrazilo (DPPH)

La capacidad de las muestras para atrapar el radical DPPH o donar hidrógenos, se evaluó siguiendo la metodología propuesta por Brand-Williams et al. (1995) . Se realizó una curva con Trolox para extrapolar los resultados obtenidos al medir la absorbancia a 517 nm de las muestras. Las medidas se realizaron por triplicado y los resultados se expresaron en mg de Trolox equivalente/g muestra.

4.1.3.3.2. Método del radical 2,2'-azino-bis-(3-etilbenzotiazolin)-6-sulfonato de amonio (ABTS)

La capacidad de las muestras para atrapar el radical catiónico ABTS^{•+} mediante el procedimiento propuesto por Leite et al. (2011). Se utilizó como antioxidante de referencia una curva de Trolox para extrapolar los resultados obtenidos al medir la absorbancia a 734 nm, tanto para las muestras como para la curva de referencia. Las medidas se realizaron por triplicado y los resultados se expresaron en mg de Trolox equivalente/g muestra.

4.1.3.3.3. Método de capacidad de quelación de iones ferrosos (FIC)

Para determinar la capacidad quelante de las muestras, se siguió la metodología de Carter (1971). Se analizaron a 562 nm y como referente se elaboró una recta de calibrado con diferentes concentraciones de ácido etilendiaminotetraacético (EDTA), un conocido quelante. Las

determinaciones se realizaron por triplicado y los resultados se expresaron como μg EDTA equivalente/g muestra.

4.1.3.3.4. Método del poder antioxidante por reducción del ion férrico (FRAP)

Este análisis mide la capacidad de las moléculas para reducir el complejo férrico, basado en la transferencia de electrones. La determinación se realizó a 700 nm siguiendo el método propuesto por Oyaizu (1986). Las determinaciones se realizaron por triplicado, los resultados se expresaron como mg Trolox equivalentes/g muestra y para su determinación se utilizó una curva de calibrado con un referente (Trolox) en las mismas condiciones que se trataron las muestras.

4.1.3.4. PERFIL LIPÍDICO DE LOS ACEITES VEGETALES

Para el análisis del perfil lipídico se identificaron los ácidos grasos que contenían los cuatro aceites empleados (cáñamo, chía, lino y sésamo) así como todas las mezclas de estos en proporción 1:1. Para lograrlo, a los AG se les realizó una transmetilación utilizando el método de Pellegrini et al. (2018) lo que resultó en la transformación en ácidos grasos metil éster (AGME). Estos AGME se analizaron en un cromatógrafo de gases HP 6890 (Tecknokroma, Barcelona, España) usando un detector de ionizador de llama con una columna capilar Suprewax-280 (0,25 mm de diámetro interno, 30 m de longitud de columna y 0,25 μm de *film*; Tecknokroma, Barcelona, España). La temperatura del inyector y del detector fueron de 250 y 270 °C respectivamente. El programa de temperatura fue el siguiente: temperatura inicial de 60 °C, tras 1 minuto se elevó 10 °C por minuto hasta alcanzar los 170 °C. Tras dos minutos de mantenimiento de estas condiciones, se elevó a una razón de 3 °C por minuto hasta 230 °C y se mantuvo esta temperatura

durante 10 minutos. Finalmente se alcanzaron los 260 °C y se mantuvo 1 minuto dicha temperatura tras un aumento de 2 °C por minuto. La presión en la columna interna fue de 11 *psi* y el volumen de inyección fue de 0,2 µL en *split*. El gas de carga fue el helio. Se utilizaron estándares de AGME y se compararon los resultados con los tiempos de retención de estos estándares. Con los datos obtenidos de los cromatogramas se calcularon el total de AGS, el total de AGMI, total de AGPI, la relación AGS/AGI y la relación omega 3/omega 6.

4.1.4. CARACTERIZACIÓN DE LAS EMULSIONES GELIFICADAS

Se analizaron las 40 emulsiones gelificadas cuya elaboración quedó detallada en el apartado 4.1.2.1.

4.1.4.1. COMPOSICIÓN QUÍMICA DE LAS EMULSIONES GELIFICADAS

Se determinó la cantidad de humedad, cenizas, proteína y grasa según el método de la AOAC correspondiente (AOAC, 2010), tal y como ya se describió en los anteriores apartados 4.1.3.1.1; 4.1.3.1.2; 4.1.3.1.3; 4.1.3.1.4. Estas determinaciones se realizaron por triplicado para cada una de las emulsiones gelificadas. Los resultados de todos los parámetros se expresaron en g/100 g de muestra.

4.1.4.2. PROPIEDADES FÍSICO-QUÍMICAS DE LAS EMULSIONES GELIFICADAS

4.1.4.2.1. pH

El pH se determinó en un pH-metro Crison 510 (Crison, Barcelona, España) equipado con un electrodo de punción.

4.1.4.2.2. Análisis de textura

Para el análisis de textura de las emulsiones gelificadas se utilizó un texturómetro TA-XT2i (Stable Micro Systems, Surrey, Reino Unido) equipado con un cabezal cónico HDP/SR, (Stable Microsystem, Surrey, Reino Unido) de medición ejerciendo una fuerza de presión máxima de 5 kg. El análisis implicó un ángulo de 45 grados formado entre la base del cono y la altura de este, moviéndose este cabezal a una velocidad de 3 mm/s. El test de untabilidad constó de dos fases, recogiendo la fuerza a lo largo del tiempo a una velocidad de 3 mm/s realizando unas 200 medidas por segundo. En la primera fase, el cono superior se sumergió en la muestra a ensayar dentro de un recipiente fijado a la base en forma de cono invertido hasta una profundidad de 23 mm. Se midió la fuerza máxima registrada, denominada firmeza (N) y el área bajo la curva que representa la fuerza total de la fase inicial, denominada untabilidad. En la fase siguiente, el cabezal se desplazó en sentido contrario, registrándose el trabajo de cizallamiento (N.s). Las mediciones se realizaron a una temperatura de 20 ± 2 °C y los parámetros de textura se determinaron a partir de cinco réplicas de cada muestra.

4.1.4.3. ESTABILIDAD DE LAS EMULSIONES GELIFICADAS A LA CONGELACIÓN

Debido a que las muestras de las emulsiones gelificadas debían mantenerse en condiciones de congelación hasta su aplicación para preservarlas de los efectos oxidativos, dado que contienen una elevada cantidad de ácidos grasos insaturados, se evaluó la influencia del tiempo de congelación en la estabilidad de la emulsión, el color y la oxidación lipídica de las emulsiones gelificadas. Las medidas se realizaron a tiempo 0 y a los 15 días, siguiendo el mismo procedimiento.

4.1.4.3.1. Estabilidad de la emulsión

La estabilidad de la emulsión se determinó siguiendo el procedimiento de Pintado et al. (2015) con algunas modificaciones. Se pesaron inicialmente tubos de centrífuga de 15 mL vacíos, tras esto se introdujeron aproximadamente 4 g de muestra en dichos tubos y se volvió a pesar. Tras eso, se centrifugaron el tubo con la muestra durante un minuto a 3000 rpm. Los tubos se introdujeron en un baño a 70 °C durante 30 minutos, se dejaron enfriar a temperatura ambiente. Se volvieron a centrifugar durante 3 minutos a 3000 rpm. El sobrenadante se extrajo y se descartó, se pesó el pellet con el tubo de centrífuga. Los resultados se analizaron por triplicado y se expresaron en g de fluido total expulsado (FTE) por 100 g de muestras y se calcularon utilizando la ecuación 1 (Ec. 1):

$$\%FTE = \frac{\text{peso del tubo con la muestra} - \text{peso del tubo con el pellet}}{\text{peso de muestra}} \times 100 \quad (\text{Ec. 1})$$

4.1.4.3.2. Medida instrumental del color

La evaluación del color se realizó mediante el sistema de coordenadas CIEL*a*b*, utilizando un espectrofotómetro Minolta CM-700 (Minolta Camera Co., Osaka, Japón), con un ángulo de observación de 10 ° y un iluminante D65, modo SCI. Para evitar interferencias en la medida del color, se colocó un cristal de baja reflectancia (Minolta CR-A51/1829-752) entre la muestra y el equipo. Las coordenadas CIEL*a*b* determinadas fueron: Luminosidad (L*), coordenada rojo/verde (a*) y coordenada amarillo/azul (b*). A partir de ellas se calcularon las magnitudes psicofísicas tono (h*) y croma (C*) utilizando las ecuaciones 2 y 3, respectivamente (Ec. 2 y Ec. 3).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (\text{Ec. 2})$$

$$h^* = \tan^{-1}(b^*/a^*) \quad (\text{Ec. 3})$$

4.1.4.3.3. Estabilidad oxidativa

La estabilidad oxidativa de las emulsiones gelificadas se evaluó midiendo las sustancias reactivas al ácido tiobarbitúrico (TBARs). La determinación del TBARs para cada muestra se realizó por triplicado utilizando la técnica descrita por Rosmini et al. (1996). Los valores de TBARs se calcularon a partir de una curva estándar de malonaldehído (MDA) y se expresaron en mg MDA/kg de muestra. Las determinaciones se realizaron por triplicado para cada una de las emulsiones gelificadas.

4.1.5. ANÁLISIS ESTADÍSTICO

Cada muestra de las harinas fue analizada por triplicado. Se realizaron tres réplicas del análisis del perfil lipídico de los aceites. El proceso de elaboración de las emulsiones gelificadas se realizó por triplicado, en tres días distintos (tres lotes independientes) y cada lote fue analizado por triplicado. Los resultados tanto de las harinas como de los aceites como de la caracterización de las emulsiones gelificadas vienen expresados como la media más/menos la desviación estándar de los datos obtenidos. Se realizó un análisis ANOVA de un factor para determinar si existieron diferencias estadísticamente significativas entre las determinaciones realizadas en los distintos tipos de harina, aceites. Así como en las emulsiones gelificadas para la determinación de la composición química y las propiedades físico-químicas. Para la determinación de la estabilidad de las emulsiones gelificadas a la congelación se aplicó un ANOVA de dos factores. El programa usado para determinar las diferencias estadísticas fue el SPSS versión 24.0 (SPSS Inc., Chicago, USA) para evaluar la significancia en un nivel de $p > 0,05$ mediante test de Tukey *post hoc*.

4.2. APLICACIÓN DE LAS EMULSIONES GELIFICADAS EN PRODUCTOS CÁRNICOS Y ANÁLOGOS CÁRNICOS

4.2.1. INGREDIENTES

A continuación, se detallan las materias primas cárnicas usadas en la elaboración de los distintos productos cárnicos y en el análogo elaborados para la presente tesis doctoral.

En la elaboración de producto fresco (hamburguesa de ternera), se utilizó carne de ternera y panceta de cerdo ambas adquiridas en un supermercado local (Orihuela, Alicante).

Para la elaboración de los productos cocidos (salchichas tipo Frankfurt y paté), se utilizaron como materias primas carne magra de cerdo y panceta de cerdo para las salchichas tipo Frankfurt e hígado, papada y panceta de cerdo para la elaboración del paté, todas las materias cárnicas de estas elaboraciones se adquirieron durante todo el estudio en carnicerías locales (Orihuela, Alicante).

En el caso de la elaboración alheiras (producto tradicional portugués), se elaboraron con carne de gallina, pato y panceta de cerdo, ingredientes adquiridos de una carnicería local portuguesa (Bragança, Portugal). Se usó un aceite de oliva con Denominación de Origen Protegida de *Tras-os-Montes*.

Para la elaboración de las hamburguesas análogas de carne (hamburguesas veganas), se utilizaron como ingredientes soja texturizada y fibra de guisante adquirida en Suministros River S.L.U. (Alicante, España); harina de cacahuete suministrada por ViperCo Group Ltd (Batley, UK), zumo comercial de remolacha de Juver Alimentación S.L.U (Murcia, España) y zumo fresco de remolacha que fue diluido en una proporción 1:3.

Todos los aditivos y especias usados para la elaboración de los diferentes productos cárnicos fueron adquiridos de Suministros River S.L.U. (Alicante, España) excepto los empleados para la elaboración del producto

tradicional portugués que se elaboraron con especias adquiridas en supermercados locales de Bragança (Portugal).

4.2.2. APLICACIÓN EN PRODUCTOS CÁRNICOS FRESCOS: HAMBURGUESAS

4.2.2.1. FORMULACIÓN Y PROCESO DE ELABORACIÓN DE LAS HAMBURGUESAS

Se realizaron cinco lotes de hamburguesas de ternera de 12 hamburguesas cada lote. Uno de ellos se realizó como control, elaborado con una formulación tradicional, siguiendo lo dado en la Tabla 7. En las otras cuatro formulaciones se reemplazaron diferentes proporciones de grasa de cerdo (25 o 50%) por la emulsión gelificada elaborada con harina de amaranto y aceite de chía o harina de amaranto y aceite de cáñamo.

Tabla 7. Formulación de las hamburguesas de ternera reformuladas con harina de amaranto y aceite de chía o cáñamo

Ingredientes	Control	Burger chía25	Burger chía50	Burger cáñamo25	Burger cáñamo50
Ternera	80	80	80	80	80
Panceta	20	15	10	15	10
Agua	5	5	5	5	5
Sal	1,5	1,5	1,5	1,5	1,5
Pimienta blanca	0,05	0,05	0,05	0,05	0,05
EG	0	5	10	5	10

El porcentaje de los ingredientes no cárnicos vienen referidos sobre el 100% de carne. EG: emulsión gelificada elaborada con aceite de chía y harina de amaranto para el reemplazo de un 25% de grasa animal en las hamburguesas chía25 y de un reemplazo de un 50% de grasa animal en las hamburguesas chía50. Emulsión gelificada elaborada con aceite de cáñamo y harina de amaranto para el reemplazo de un 25% de grasa animal en las hamburguesas cáñamo25 y de un reemplazo de un 50% de grasa animal en las hamburguesas cáñamo50.

Para elaborar las hamburguesas de ternera, en primer lugar, se cortó la carne y la grasa en cubos de aproximadamente 5 cm y se picaron ambas materias usando un platillo de 8 mm en una picadora. Posteriormente, se mezcló en un recipiente la carne picada con el agua, sal y pimienta con un gancho con forma de espiral y se homogeneizó toda la mezcla durante 5 minutos a una velocidad de 80 rpm.

En todas las formulaciones, las proporciones de grasa (20%) se reemplazaron en un 25 y 50% por cada una de las dos emulsiones gelificadas (amaranto/aceite de chía o amaranto/aceite de cáñamo). Una vez reemplazada la grasa por la emulsión, se mezcló de nuevo durante 5 minutos. Para dar forma y obtener las hamburguesas se utilizó una máquina comercial que dio lugar a hamburguesas de 80 g y 1 cm de grosor. Las hamburguesas se almacenaron en bolsas y se llevaron a refrigeración a 4 °C hasta su posterior análisis. Parte de las hamburguesas se cocinaron con una plancha hasta que alcanzaron una temperatura interna de 72 °C, lo que supuso aproximadamente 4 minutos de cocinado por cada lado. El proceso de elaboración se muestra en la Figura 23.

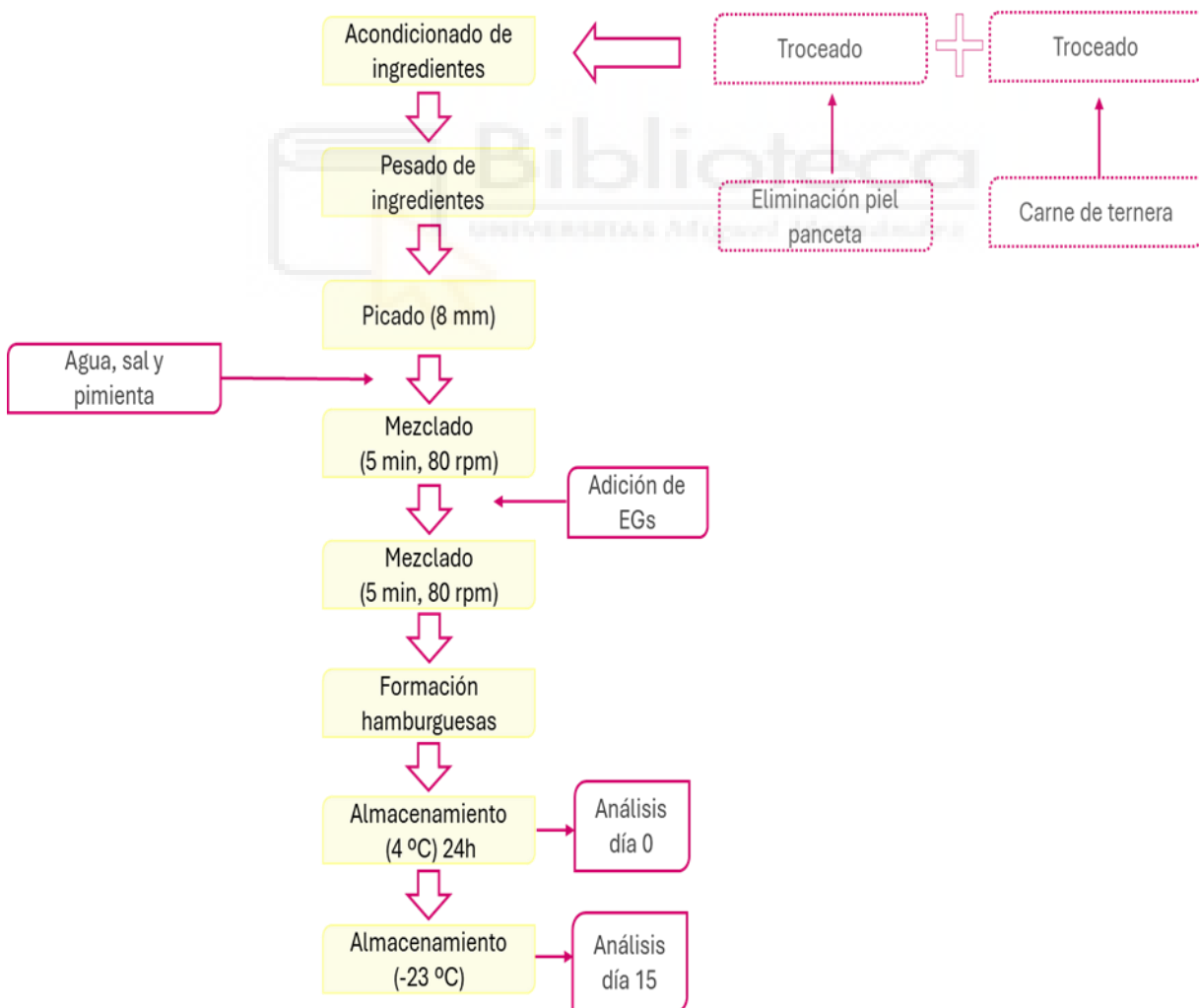


Figura 23. Diagrama de flujo de la elaboración de las hamburguesas de ternera.

4.2.2.2 CARACTERIZACIÓN DE LAS HAMBURGUESAS DE TERNERA

4.2.2.2.1. Composición química

Se determinó la cantidad de humedad, cenizas, proteína y grasa según el método de la AOAC correspondiente (AOAC, 2010), tal y como ya se describió en los anteriores apartados 4.1.3.1.1; 4.1.3.1.2; 4.1.3.1.3; 4.1.3.1.4. Estas determinaciones se realizaron por triplicado tanto para las hamburguesas crudas como cocinadas. Los resultados de todos los parámetros se expresaron en g/100 g de muestra.

4.2.2.2.2. Propiedades físico-químicas

4.2.2.2.2.1. pH

La determinación de pH se realizó por una medida de punción, con el equipamiento y la metodología descrita anteriormente en el apartado 4.1.4.2.1. La determinación se realizó en tres hamburguesas crudas para cada formulación y tres hamburguesas cocinadas por formulación.

4.2.2.2.2.2. Actividad de agua

La actividad de agua (a_w) se midió a 25 °C en un higrómetro Novasina TH-500, (Novasina, Pfaeffikon, Suiza). La determinación se realizó por triplicado para cada uno de los lotes de hamburguesas crudas.

4.2.2.2.2.3. Análisis de textura

La textura de los lotes de salchichas tipo Frankfurt se determinó mediante un análisis de perfil textural (TPA) usando un texturómetro TA-XT2i (Stable Micro Systems, Surrey, Inglaterra). Se aplicaron dos ciclos de compresión del 75% a una velocidad constante de 1 mm/s a una temperatura entre los 15 y los 20 °C, con la sonda cilíndrica (P100) de 100 mm de diámetro

sobre las muestras de hamburguesa cortada en secciones de 1x1x1 cm de lado. Se obtuvieron curvas fuerza-tiempo de deformación de las cuales se calcularon parámetros como la dureza (N) (máximo pico de fuerza durante la primera compresión); la elasticidad (mm) (la altura que el alimento alcanza durante el tiempo entre el final de la primera compresión y el principio de la segunda); cohesividad (adimensional) (la relación entre el área de la fuerza positiva durante la segunda compresión respecto a la primera compresión) y masticabilidad (Nxmm) (como el producto del tiempo de la dureza y el tiempo de la cohesividad y elasticidad). Se realizó siguiendo la metodología de Claus, (1995). Estas determinaciones se realizaron para 5 muestras de cada uno de los 4 lotes de hamburguesas cocinadas.

4.2.2.2.2.4. Parámetros de color

La determinación de los parámetros de color para los distintos lotes de hamburguesas se llevó a cabo de igual forma que en lo descrito anteriormente en el apartado 4.1.4.3.2. Con la misma metodología y el mismo colorímetro. Con los parámetros de coordenadas CIEL*a*b* se determinaron las magnitudes tono y croma (h^* y C^*) (Ec. 2 y Ec. 3). También se calcularon las diferencias de color para cada lote respecto a la formulación control mediante la ecuación 4 (Ec. 4). Las medidas se realizaron a una temperatura aproximada de 25 °C y se realizaron 18 medidas por cada lote de hamburguesas tanto en crudo como en cocinado.

$$\Delta E = \sqrt{(L_S^* - L_{Con}^*)^2 + (a_S^* - a_{Con}^*)^2 + (b_S^* - b_{Con}^*)^2} \quad (\text{Ec. 4})$$

4.2.2.2.3. Perfil lipídico e índices nutricionales

El perfil lipídico de las hamburguesas de ternera tanto crudas como cocinadas se determinó siguiendo las mismas condiciones anteriormente mencionadas en el apartado 4.1.3.4. El contenido graso de las muestras se extrajo por medio del método Folch et al. (1957). Tras la extracción, la fase lipídica se transmetiló según el método de Pellegrini et al. (2018). Los resultados de los ácidos grasos se expresaron en g de ácido graso/100 g de grasa total.

Además, se calcularon una serie de índices nutricionales a partir de la composición lipídica de las muestras de los cinco lotes de hamburguesas, utilizando las ecuaciones desarrolladas por Ulbricht & Southgate. (1991), en concreto se calcularon el índice aterogénico (IA) (Ec. 5), índice trombogénico (IT) (Ec. 6) y relación hipocolesterolémica/hipercolesterolémica (h/H) (Ec. 7) empleando la ecuación dada por Fernández et al. (2007). También se calcularon los sumatorios de ácidos grasos saturados, ácidos grasos monoinsaturados, ácidos grasos poliinsaturados, sumatorio de ácidos omega 3, omega 6 y la ratio de AGPI/AGS y la ratio entre omega 6 y omega 3.

$$IA = \frac{C12:0+(4xC14:0)+C16:0}{\sum AGMI + \sum n6 + \sum n3} \quad (\text{Ec.5})$$

$$IT = \frac{C14:0+C16:0+C18:0}{(0,5x \sum MUFA)+(0,5x \sum n6)+(3x \sum n3)+(\frac{\sum n3}{\sum n6})} \quad (\text{Ec. 6})$$

$$h/H = \frac{C18:1n9+C18:1n7+\sum PUFA}{C14:0+C16:0} \quad (\text{Ec. 7})$$

4.2.2.2.4. Propiedades de cocinado

Para las propiedades de cocinado de las diferentes muestras de hamburguesas, se tomaron medidas acerca del espesor, diámetro y peso tanto antes como después del cocinado.

El peso, el grosor y el diámetro de las hamburguesas de cada lote se midieron a temperatura ambiente (~ 25 °C) antes y después del cocinado. Para estimar los cambios dimensionales, las reducciones en el diámetro y los aumentos de espesor, así como las pérdidas por cocinado, se emplearon las siguientes ecuaciones 8 (Ec. 8), 9 (Ec. 9) y 10 (Ec. 10):

$$\%Encogimiento = \frac{\text{Diámetro crudo} - \text{diámetro cocinado}}{\text{diámetro crudo}} \times 100 \quad (\text{Ec. 8})$$

$$\%Aumento \text{ de espesor} = \frac{\text{espesor cocinado} - \text{espesor crudo}}{\text{espesor crudo}} \times 100 \quad (\text{Ec. 9})$$

$$\%Perdida \text{ de peso} = \frac{\text{peso crudo} - \text{peso cocinado}}{\text{peso crudo}} \times 100 \quad (\text{Ec. 10})$$

Todas las medidas se realizaron por triplicado para cada uno de los 5 lotes de muestras.

4.2.2.2.5. Estabilidad oxidativa

La estabilidad oxidativa de las hamburguesas de ternera se evaluó de igual forma que en las emulsiones gelificadas explicado en el apartado 4.1.4.3.3. Esta medida se realizó tanto en las hamburguesas crudas como cocinadas y se realizó por triplicado para cada uno de los lotes de hamburguesas.

4.2.2.2.6. Evaluación sensorial

El análisis sensorial se realizó en el laboratorio especializado de la Universidad Miguel Hernández en Orihuela, España. El panel de evaluación estuvo integrado por 40 personas, 15 hombres y 25 mujeres entre los 20 y 60 años, incluyendo tanto a personal como a estudiantes. Se les presentaron cinco muestras de cada formulación (codificadas con una numeración aleatoria de 3 dígitos) para evaluar los atributos de las hamburguesas crudas.

Posteriormente, las muestras se cocinaron en una plancha y se mantuvieron en un horno hasta su evaluación sensorial, para ello se sirvieron en trozos de aproximadamente 1,5 x 1,5 cm y se sirvieron a temperatura ambiente. Cada panelista evaluó todas las formulaciones en un orden aleatorio, se utilizó una escala de 9 puntos para medir la intensidad del color (1: extremadamente claro a 9: extremadamente oscuro), el aroma a rancio (1: imperceptible a 9: extremadamente rancio) y el aspecto visual (1: disgusta extremadamente a 9: gusta extremadamente). En cuanto a las muestras cocidas, se evaluaron atributos como aceptabilidad general, jugosidad, masticabilidad, sensación de grasa y granulometría, también utilizando una escala hedónica de 9 puntos, donde 1 indicaba disgusta extremadamente y 9 indicaba gusta extremadamente. Esta evaluación sensorial fue aceptada por la Oficina de Evaluación de Proyectos de la Universidad Miguel Hernández con referencia PLR.DTA.MVM.02.21 (OEP, UMH, Elche, Alicante, España).

4.2.2.3. EVALUACIÓN DE LA VIDA ÚTIL DE LAS HAMBURGUESAS DE TERNERA

Para determinar la vida útil de los cinco lotes de las hamburguesas de ternera (control, hamburguesa con un reemplazo del 25% y 50% de grasa animal por emulsión elaborada con amaranto y aceite de chía, hamburguesa con un reemplazo del 25% y 50% de reemplazo de grasas animal por una emulsión gelificada elaborada con harina de amaranto y aceite de cáñamo), se realizaron a día 0, 30 y 60 las determinaciones de las propiedades de cocinado (descritas en el apartado 4.2.2.2.4.), la evaluación del perfil lipídico y el cálculo de los índices nutricionales (descrito en los apartados 4.1.3.4 y 4.2.2.2.3.) para las muestras crudas y cocinadas y la determinación de la estabilidad oxidativa (descrita en el apartado 4.1.4.3.3) tanto para las muestras crudas como cocinadas. Todas las determinaciones se realizaron por triplicado.

4.2.2.4. ANÁLISIS ESTADÍSTICO DE LAS HAMBURGUESAS DE TERNERA

El proceso de elaboración de las emulsiones gelificadas y de las hamburguesas, se realizó por triplicado, tres lotes independientes elaborados en tres días diferentes. Cada lote fue analizado por triplicado. Los resultados se expresaron como la media y la desviación estándar de los datos obtenidos. Se realizó un análisis ANOVA de un factor para determinar si existieron diferencias estadísticamente significativas entre los distintos tipos lotes de hamburguesas, para todas las determinaciones a excepción de la evaluación de la vida útil y el cocinado que para esos análisis se aplicó un ANOVA de dos factores. El programa usado para determinar las diferencias estadísticas fue el SPSS versión 24.0 (SPSS Inc., Chicago, USA) para evaluar la significancia en un nivel de $p > 0,05$ se realizó mediante test de Tukey *post hoc*. Para la evaluación sensorial los panelistas se consideraron como un factor arbitrario.

4.2.3. APLICACIÓN EN PRODUCTOS COCIDOS

4.2.3.1. SALCHICHAS TIPO FRANKFURT

4.2.3.1.1. Formulación y proceso de elaboración de las salchichas tipo Frankfurt

Las salchichas tipo Frankfurt fueron elaboradas mediante una formulación tradicional (formulación en base cárnica, los ingredientes cárnicos representan el 100% y el porcentaje del resto de ingredientes se calcularon sobre esta base) (Tabla 8). Se elaboraron 4 lotes más a parte del lote control, en los cuales se sustituyó un 25%, 50%, 75% y 100% de grasa animal (panceta) por una emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno (se elaboraron tres réplicas de los lotes en diferentes días.

Tabla 8. Formulación de las salchichas tipo Frankfurt reformuladas con EG de harina de trigo sarraceno y aceite de cáñamo

Ingredientes	Control	Frankfurt25	Frankfurt50	Frankfurt75	Frankfurt100
Magro de cerdo	65	65	65	65	65
Panceta	35	26,25	17,5	8,75	0
EG	0	8,75	17,5	26,25	35
Agua	15	15	15	15	15
Sal	1,5	1,5	1,5	1,5	1,5
Almidón de patata	3	3	3	3	3
Caseinato	1,5	1,5	1,5	1,5	1,5
Polifosfato	300	300	300	300	300
NaNO₃	150	150	150	150	150
Humo líquido	0,2	0,2	0,2	0,2	0,2

Datos expresados en g/100 g, excepto el polifosfato y nitrito sódico que vienen expresados en mg/kg. El humo líquido viene expresado en mL/kg. Los porcentajes de ingredientes no cárnicos se refieren al 100% de los ingredientes cárnicos (magro y panceta). Control: muestra de control de salchicha tipo Frankfurt preparada con una fórmula tradicional; Frankfurt25: muestra que contiene un 25% de emulsión gelificada de cáñamo como sustituto de la grasa; Frankfurt50: muestra que contiene un 50% de emulsión gelificada de cáñamo como sustituto de la grasa. Frankfurt75: muestra que contiene un 75% de emulsión gelificada de cáñamo como sustituto de la grasa. Frankfurt100: muestra que contiene un 100% de emulsión gelificada de cáñamo como sustituto de la grasa.

La elaboración de las salchichas tipo Frankfurt (Figura 24), se llevó a cabo en la planta piloto del grupo de investigación IPOA en la Universidad Miguel Hernández, siguiendo un protocolo de procesamiento industrial. Inicialmente los ingredientes cárnicos fueron picados en una homogeneizadora modelo 1094-Homogeneizer, (Tekator, Höganäs, Suecia) y mezclados con la sal y el resto de los ingredientes durante 2 minutos a una temperatura aproximada de 12 °C. Tras esta homogeneización, la masa cárnica resultante fue embutida en tripa sintética de celulosa de 20 mm de diámetro (Fibran, Girona, España), usando una embudidora de pistón modelo EM-12 (Mainca, Barcelona, España). Las muestras se cocieron en un baño a 80 °C hasta que el centro del producto alcanzó los 72 °C. Tras alcanzar esta temperatura las muestras fueron enfriadas rápidamente en hielo durante 5 minutos, se envasaron y se conservaron a 4 °C en condiciones de oscuridad.

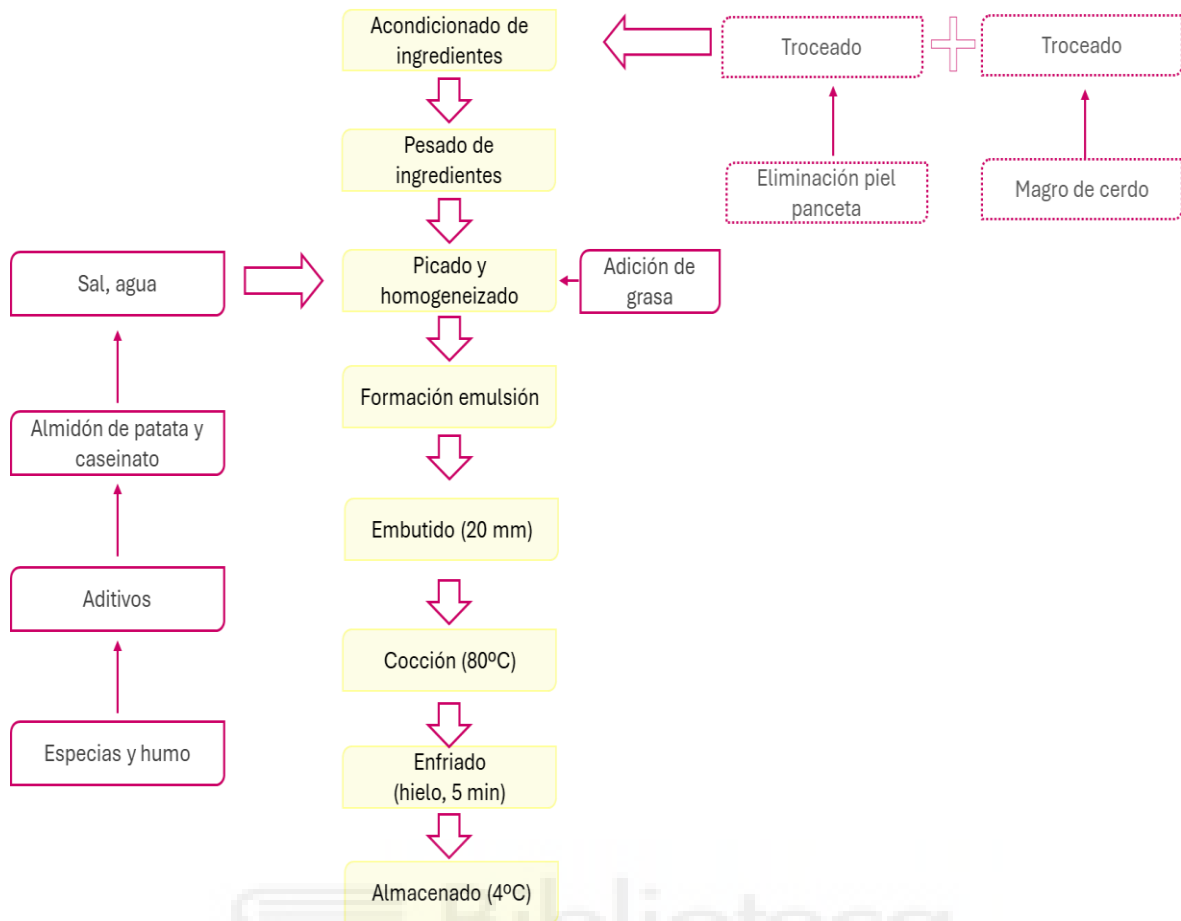


Figura 24. Diagrama de flujo de la elaboración de las salchichas tipo Frankfurt.

4.2.3.1.2. Estabilidad de la emulsión de las salchichas tipo Frankfurt

La estabilidad de la emulsión se determinó para todos los lotes de salchichas tipo Frankfurt previa a la cocción, siguiendo el mismo procedimiento descrito anteriormente en el apartado 4.1.4.3.1. La determinación se realizó por triplicado para cada lote de salchichas tipo Frankfurt.

4.2.3.1.3. Caracterización de las salchichas tipo Frankfurt

4.2.3.1.3.1. Composición química

La composición química de los 5 lotes de salchichas tipo Frankfurt elaborados, se determinó mediante los métodos correspondientes de la AOAC (2010), tal como se describió en los apartados anteriores 4.1.3.1.1.;

4.1.3.1.2.; 4.1.3.1.3.; 4.1.3.1.4. Donde se determinó el contenido en humedad, proteína, grasa y cenizas por triplicado para cada lote de salchichas tipo Frankfurt. Los resultados de todos los parámetros analizados se expresaron en g/100 g de muestra.

4.2.3.1.3.2. Perfil lipídico e índices nutricionales

El perfil lipídico de los lotes de salchichas tipo Frankfurt se determinó siguiendo el mismo método y las mismas condiciones que se detallaron anteriormente en el apartado 4.1.3.4., directamente sobre la grasa de las muestras. Dicha grasa se extrajo de los 5 lotes mediante la aplicación del método descrito por Folch et al. (1957). Como se detalla en el apartado 4.2.2.2.3., dicha grasa se transmetiló siguiendo el método de Pellegrini et al. (2018). Todas las determinaciones se realizaron por triplicado y los resultados de los ácidos grasos se expresaron como g de ácido graso/100 g de grasa.

Con los resultados obtenidos del perfil lipídico se calcularon una serie de índices nutricionales o de calidad de la grasa: los sumatorios de AGS, AGI, AGMI y AGPI, los sumatorios de ácidos grasos omega 3 y omega 6, para posteriormente poder calcular así las relaciones AGPI/AGS y omega 6/omega 3. También se calcularon los índices de aterogeneidad (IA) y el índice de trombogeneidad (IT) mediante las ecuaciones 5 y 6 (apartado 4.2.2.2.3.). Así como, la relación hipocolesterolemica/hipercolesterolemica (h/H) mediante la ecuación 7 (apartado 4.2.2.2.3.).

4.2.3.1.3.3. Propiedades físico-químicas

4.2.3.1.3.3.1. pH

La determinación de pH se realizó con el equipo y la metodología descrita anteriormente en el apartado 4.1.4.2.1. La determinación se realizó por triplicado para cada uno de los cinco lotes de salchichas tipo Frankfurt.

4.2.3.1.3.3.2. Actividad de agua

La determinación de la actividad de agua se realizó a temperatura ambiente (20 °C) con el equipamiento descrito en el apartado 4.2.2.2.2. Esta determinación se realizó por triplicado para cada uno de los lotes de salchichas tipo Frankfurt.

4.2.3.1.3.3.3. Análisis de textura

La textura de las salchichas tipo Frankfurt se determinó mediante un análisis de perfil textural (TPA) usando el mismo equipo y siguiendo las mismas condiciones descritos en el apartado 4.2.2.2.3. Se realizó sobre las muestras cortadas en secciones de 2 cm de altura en horizontal. Y se calcularon parámetros como la dureza (N); la elasticidad (mm); la adhesividad (Nxs) (trabajo negativo entre los dos ciclos de compresión); Cohesividad (adimensional) y masticabilidad (Nxmm). Estas determinaciones se realizaron para 5 muestras de cada uno de los 5 lotes de salchichas tipo Frankfurt.

4.2.3.1.3.3.4. Parámetros de color

La determinación de los parámetros de color para los distintos lotes de salchichas tipo Frankfurt se llevó a cabo de igual forma (equipo y metodología) que lo descrito anteriormente en el apartado 4.1.4.3.2. Para

ello las muestras se cortaron en secciones de 2 cm de altura y sobre ellas se colocó un cristal de baja reflectancia Minolta CR-A51/1829-752. Con los valores de las coordenadas CIEL*a*b* se determinaron las magnitudes tono y croma (h^* y C^*), utilizando las ecuaciones 2 y 3, y también se determinaron las diferencias de color mediante la ecuación 4. Las medidas se realizaron a una temperatura aproximada de 25 °C y se realizaron 18 medidas por cada lote de salchichas tipo Frankfurt.

4.2.3.1.3.4. Estabilidad oxidativa

La estabilidad oxidativa de los distintos lotes de salchichas tipo Frankfurt se midió siguiendo el procedimiento descrito anteriormente en el apartado 4.1.4.3.3. Los resultados se expresaron en mg malonaldehído (MDA)/kg de muestra y las determinaciones se realizaron por triplicado para cada uno de los lotes de salchichas tipo Frankfurt.

4.2.3.1.4. Evaluación sensorial de las salchichas tipo Frankfurt

La evaluación sensorial se llevó a cabo en el laboratorio de evaluación sensorial de la UMH en Orihuela, dotado de cabinas individuales, bajo condiciones de luz blanca. Participaron 17 panelistas no entrenados, miembros del personal y estudiantes de la Universidad Miguel Hernández con edades comprendidas entre 18 y 55 años. El análisis sensorial se realizó con una escala hedónica de 7 puntos (1: disgusta extremadamente a 7: me gusta extremadamente) para evaluar los atributos de color, dureza, jugosidad, olor a cáñamo, sabor a cáñamo, sabor salado y aceptabilidad general. Las muestras de salchicha tipo Frankfurt de cada uno de los lotes se sirvieron cortadas en piezas de 2 cm de espesor, a una temperatura de unos 37 °C (para ello se mantuvieron en un baño a esta temperatura hasta que se sirvieron para su evaluación sensorial). A cada muestra se le asignó una

numeración al azar compuesta por 3 dígitos para identificarlas y a cada panelista se les sirvió en un orden aleatorio.

4.2.3.1.3.6. Análisis estadístico

El proceso de elaboración de la emulsión gelificada y de las salchichas tipo Frankfurt, se realizó por triplicado, tres lotes independientes elaborados en tres días diferentes para las cinco muestras. Cada lote fue analizado por triplicado. Los resultados se expresaron como la media y la desviación estándar de los datos obtenidos. Se realizó un análisis ANOVA de un factor para determinar si existieron diferencias estadísticamente significativas entre los distintos tipos lotes de salchichas tipo Frankfurt. Las diferencias estadísticas se establecieron a un nivel de significancia $p < 0,05$ mediante el test de Tukey-b usando el software SPSS (versión 24.0, SPSS, Chicago, EEUU). Para la evaluación sensorial los panelistas se consideraron como un factor aleatorio.

4.2.3.2. PATÉ

4.2.3.2.1. Formulación y proceso de elaboración del paté

Se elaboraron tres lotes, de 1 kg cada uno, de paté de hígado de cerdo. Los tres lotes de paté se elaboraron en la planta piloto de la Escuela Politécnica Superior de Orihuela siguiendo una formulación tradicional. Se preparó una muestra control, siguiendo una formulación tradicional con una cantidad de ingredientes cárnicos cuya suma fue del 100% (65% papa de cerdo, 25% hígado de cerdo y 10 % panceta). Sobre esta formulación se sustituyó toda la grasa animal (panceta) por una emulsión gelificada (elaborada según la metodología descrita en el apartado 4.1.2.2.) con aceite de cáñamo y con harina de trigo sarraceno como emulsionante (Paté10) y otra formulación en la cual se sustituyó toda la cantidad de panceta y un 10%

de la papada por un 20% de la misma emulsión gelificada (paté20). El resto de los ingredientes añadidos, se especifican en la Tabla 9.

Tabla 9. Formulación de las diversas muestras de paté con trigo sarraceno-aceite de cáñamo EG

Ingredientes	Control	Paté10	Paté20
Papada	65	65	55
Hígado	25	25	25
Panceta	10	0	0
EG	0	10	20
Agua	15	15	15
Sal	2	2	2
Caseinato	1	1	1
Polifosfato (mg/kg)	300	300	300
NaNO₃ (mg/kg)	125	125	125
Pimienta blanca	0,05	0,05	0,05
Nuez moscada	0,03	0,03	0,03
Tomillo	0,03	0,03	0,03

Datos expresados en g/100 g. Los porcentajes de ingredientes no cárnicos se refieren al 100% de los ingredientes cárnicos (papada, hígado y panceta). Control: muestra de control de paté preparado con una fórmula tradicional; Paté10: muestra que contiene un 10% de emulsión gelificada de cáñamo como sustituto de la grasa; Paté20: muestra que contiene un 20% de emulsión gelificada de cáñamo como sustituto de la grasa.

La elaboración del paté se realizó siguiendo la metodología descrita por Lucas-González et al. (2019), como se muestra en la Figura 25. El proceso comienza con el picado de los ingredientes cárnicos. Antes de ello se procedió a la retirada de la piel de la papada y de la panceta, el hígado se dejó durante 10 minutos en agua fría y se coció la papada a 100 °C durante 15 minutos. A continuación, se pesaron y trocearon todos los ingredientes cárnicos en un homogeneizador modelo 1094-Homogeneizer (Tekator, Höganäs, Suecia). Primero se picó la grasa (panceta o emulsión gelificada) y tras ella la papada, se adicionó la sal y tras homogeneizar de nuevo 1 minuto, se adicionó el hígado y todos los aditivos y especias. Para formar la emulsión se adicionó el agua a 80 °C. Tras la homogeneización, la pasta fina se embutió en tripa artificial de 5 cm de diámetro Fibran-Pack (Fibran, Girona, España) usando una embudidora Kenwood MG-510 (Kenwood, Barcelona, España). Se realizaron unidades de 15-20 cm de longitud y se fueron cerrando con clips

(Niedecker, Hattersheim, Alemania). Todos los lotes se cocieron en un baño a una temperatura de 95 °C, hasta que en el interior del producto se alcanzaron los 72 °C. Tras alcanzar esta temperatura las muestras de paté fueron enfriadas rápidamente en un abatidor *air-o-chill* (Elextrolux, Madrid, España). Las muestras se conservaron a 4 °C hasta su análisis.

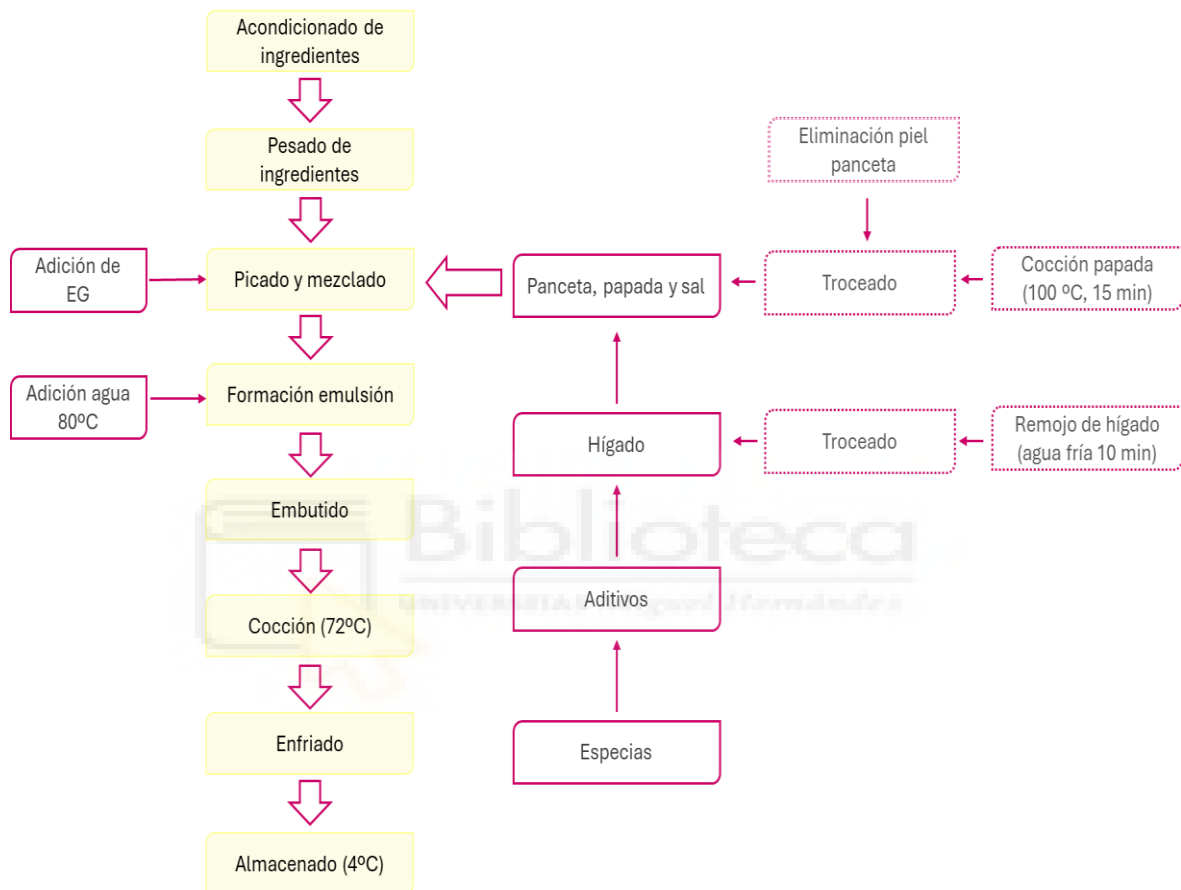


Figura 25. Diagrama de flujo de la elaboración del paté de hígado de cerdo.

4.2.3.2.2. Estabilidad de la emulsión del paté

La estabilidad de la emulsión se determinó para los tres lotes de paté tipo previa a la cocción, siguiendo el mismo procedimiento descrito anteriormente en el apartado 4.1.4.3.1. La determinación se realizó por triplicado para cada lote de paté. Los resultados midieron el fluido total expelido (FTE), calculado mediante la Ec. 1.

4.2.3.2.3. Caracterización del paté

4.2.3.2.3.1. Composición química

La composición química (humedad, proteína, grasa y cenizas) de los 3 lotes de paté elaborados se determinó mediante los métodos correspondientes de la AOAC, (2010), tal como se describió en los apartados anteriores 4.1.3.1.1.; 4.1.3.1.2.; 4.1.3.1.3. y 4.1.3.1.4. Los análisis se realizaron por triplicado y los resultados se expresaron en g/100 g de muestra.

También se le realizó la determinación de colesterol total, mediante cromatografía líquida de alta eficacia (HPLC) usando como fase móvil 2-propanol y hexano (2:98) en modo isocrático con un flujo de 1 mL/min y usando como detector, un detector array (DAD) a 208 nm, siguiendo el procedimiento descrito por Domínguez et al. (2016).

La determinación cuantitativa de los minerales se realizó mediante espectrometría de masas por plasma acoplado inductivamente (ICP-MS) modelo MS-2030 (Shimadzu, Tokyo, Japón), el cual operó en las siguientes condiciones: un flujo de 0,91 L/min del gas nebulizador, a una frecuencia de 1200 W y un voltaje de 1,6 V; el gas frío 12,0 L/min; gas auxiliar a un flujo de 0,70 L/min. Para la cuantificación de minerales, se liofilizaron las muestras previamente y 0,2 g de este liofilizado se digirió junto a ácido nítrico al 67% y peróxido de hidrógeno al 33% en un microondas modelo *Mars one* (CEM, Carolina del Norte, EEUU). Los compuestos estándar utilizados se diluyeron y utilizaron para calibrar el ICP-MS para así medir los minerales de las muestras. Los resultados se obtuvieron tras analizar un triplicado de dos muestras de cada uno de los lotes. El contenido final de minerales se expresó en mg/100 g de muestra.

4.2.3.2.3.2. Perfil lipídico e índices nutricionales

El perfil lipídico de los lotes de paté se determinó tras la extracción de la grasa de los 3 lotes mediante el método descrito por Folch et al. (1957). Los ácidos grasos fueron transesterificados siguiendo el método descrito por Domínguez et al. (2015). Con un patrón interno para la cuantificación de C19:0 en una concentración de 0,3 mg/mL, añadido antes de la metilación de la muestra. Todas las determinaciones se realizaron por triplicado y los resultados de los ácidos grasos se expresaron como g de ácido graso/100 de grasa.

A partir del perfil lipídico se calcularon los índices nutricionales, sumatorios y relaciones descritas en el apartado 4.2.2.3.

4.2.3.2.3.3. Propiedades físico-químicas

4.2.3.2.3.3.1. pH

La determinación de pH se realizó con el equipo y la metodología descrita anteriormente en el apartado 4.1.4.2.1. La determinación se realizó por triplicado para cada uno de los tres lotes de salchichas tipo Frankfurt.

4.2.3.2.3.3.2. Análisis de textura

Para el análisis de textura de los tres lotes de paté, se determinaron los parámetros de firmeza (N) y el trabajo de cizallamiento (N.S), con el mismo equipo, procedimiento y condiciones descritas anteriormente en el apartado 4.1.4.2.2. Las mediciones se realizaron a una temperatura de 15 ± 2 °C y los parámetros de textura se determinaron a partir de cinco réplicas de cada muestra.

4.2.3.2.3.3. Determinación de los parámetros de color

La determinación de los parámetros de color para los distintos lotes de paté se llevó a cabo de igual forma (equipo y procedimiento) que lo descrito anteriormente en el apartado 4.1.4.3.2. Las medidas se realizaron a una temperatura aproximada de 15 °C y se realizaron 18 medidas por cada lote de paté.

4.2.3.2.3.4. Estabilidad oxidativa

La estabilidad oxidativa de los distintos lotes de paté se midió siguiendo el procedimiento descrito anteriormente en el apartado 4.1.4.3.3. Los resultados se expresaron en mg malonaldehído (MDA)/kg de muestra y las determinaciones se realizaron por triplicado par cada uno de los lotes de paté.



4.2.3.2.3.5. Evaluación sensorial

La evaluación sensorial se llevó a cabo en el laboratorio de evaluación sensorial de la Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández, dotado de cabinas individuales, y bajo condiciones de luz blanca. Participaron 70 panelistas no entrenados, miembros del personal y estudiantes de la Universidad Miguel Hernández con edades comprendidas entre 20 y 65 años. El análisis sensorial de las distintas muestras de paté se realizó con una escala hedónica de 7 puntos (1: disgusta extremadamente a 7: me gusta extremadamente) para evaluar los atributos de apariencia general, color, brillo, olor general, rancidez, dureza, jugosidad, untabilidad, cohesividad, sabor general y una pregunta final sobre aceptabilidad general. Las muestras de paté de cada uno de los lotes se sirvieron cortadas en piezas de 1 cm de espesor y se presentó a cada panelista las tres muestras a la vez

a temperatura ambiente, numeradas por asignación al azar compuesta por 3 dígitos para identificarlas; a cada panelista se les sirvió en un orden aleatorio. Esta evaluación sensorial fue aceptada por la Oficina de investigación Responsable de la Universidad Miguel Hernández (POR-Reg. 211019105733, Ref.PLR.DTA.MVM.02.21 (UMH, Elche, España).

4.2.3.2.4. Análisis estadístico de paté

El proceso de elaboración de la emulsión gelificada y de los lotes de paté, se realizó por triplicado, tres lotes independientes elaborados en tres días diferentes para las tres muestras. Cada lote fue analizado por triplicado. Los resultados se expresaron como la media más/menos la desviación estándar de los datos obtenidos. Se realizó un análisis ANOVA de un factor para determinar si existieron diferencias estadísticamente significativas entre los distintos tipos de paté. Las diferencias estadísticas se encontraron con un nivel de significancia $p < 0,05$ mediante el test de Tukey-b usando el software SPSS (versión 24.0, SPSS, Chicago, EEUU). Para la evaluación sensorial los panelistas se consideraron como un factor aleatorio.

4.2.4. APLICACIÓN EN UN PRODUCTO TRADICIONAL PORTUGUÉS: ALHEIRAS

4.2.4.1. FORMULACIÓN Y PROCESO DE ELABORACIÓN DE LAS ALHEIRAS

Se prepararon tres tipos de alheiras (Tabla 10), una alheira control, en la cual como fuente de grasa se usó la panceta de cerdo (12,81%) y los otros dos lotes, se sustituyó un 25% (Alheira25) de la panceta por una emulsión gelificada de trigo sarraceno y aceite de cáñamo (elaborada según la metodología descrita previamente en el apartado 4.1.2.2.) y el último lote fue elabora con una sustitución del 50% de la panceta de cerdo por la misma

emulsión gelificada (Alheira50). La formulación de las diferentes alheiras se muestran en la Tabla 10.

Tabla 10. Formulación de las diversas muestras de alheiras

Ingredientes	Control	Alheira25	Alheira50
Pan	17,08	17,08	17,08
Carne de gallina y pato	21,36	21,36	21,36
Panceta de cerdo	12,81	9,61	6,41
EG	0,00	3,20	6,40
Sal	0,64	0,64	0,64
Ajo	0,17	0,17	0,17
Aceite de oliva	0,78	0,78	0,78
Pimentón	0,17	0,17	0,17
Caldo*	46,98	46,98	46,98

*Caldo de cocción de la carne de gallina y pato. Valores expresados en g/100 g. Control: muestra de control de alheira preparada con panceta de cerdo; Alheira25: muestra que contiene un 25% de emulsión gelificada de cáñamo y harina de trigo sarraceno como sustituto de la grasa; Alheira50: muestra que contiene un 50% de emulsión gelificada de cáñamo y harina de trigo sarraceno como sustituto de la grasa.

Se elaboraron tres lotes de alheiras (Figura 26), de 1 kg cada uno. Los tres lotes de alheiras se elaboraron en el laboratorio de canales y calidad de la carne de la Escuela de Agricultura del Instituto de Bragança, *Cantinho do Alfredo*, (Bragança, Portugal). Para la elaboración de las alheiras, la carne de gallina y de pato se pusieron a hervir a 100 °C durante 45 minutos, con un 2% de sal en una proporción de 2,2:1 (agua:carne). Después de esto, el pan previamente cortado en rebanadas finas se colocó en un recipiente hondo cortado y sobre él se vertió el caldo de la cocción del hervido de las carnes. Cuando el pan estuvo con la textura adecuada, la carne se deshilachó y se le adicionó, también la sal, el ajo, el pimentón y el aceite de oliva. Se homogeneizó la mezcla durante 10 minutos y finalmente se adicionó la grasa: (i) panceta o (ii) panceta y emulsión gelificada (previamente descongelada y a 4 °C). Se volvió a homogeneizar hasta que todos los ingredientes se integraron bien. Esta mezcla se embutió en intestino natural de vacuno y se sometió a un secado, sin ahumado, a 15 °C con una humedad del 75%

durante 10 días. Tres alheiras de cada formulación se cocinaron en un grill a 180 °C hasta que el centro de cada alheira alcanzó los 72 °C.

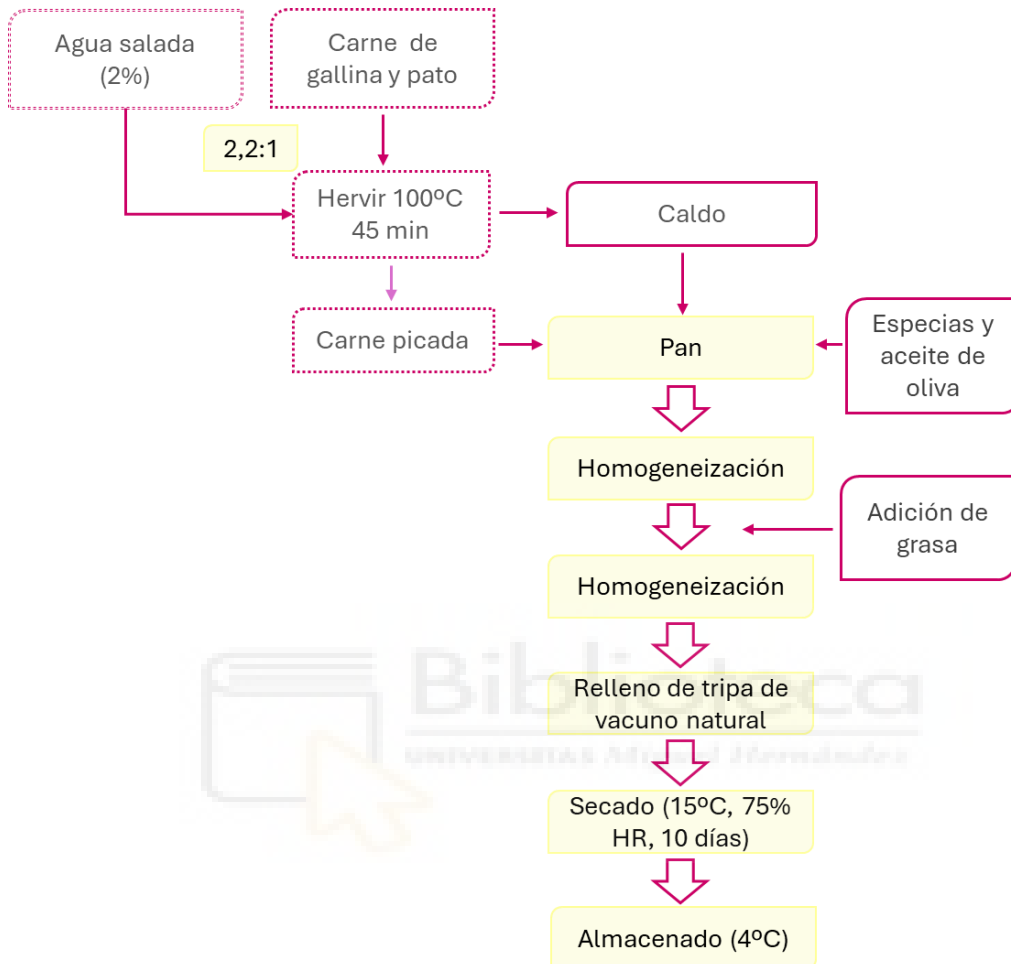


Figura 26. Diagrama de flujo de la elaboración de las alheiras.

4.2.4.2. CARACTERIZACIÓN DE LAS ALHEIRAS

4.2.4.2.1. Composición química

La composición química (humedad, proteína, grasa, cenizas y contenido de cloruro) de los 3 lotes de alheiras elaborados, se determinó mediante normas portuguesas.

4.2.4.2.1.1. *Humedad*

La humedad de las muestras de los tres lotes de alheiras fue analizada mediante la norma portuguesa 1614 (NP-ISO-1614, 2009). Para ello, se añadieron 3 g de muestra con 5 mL de etanol y se calentaron a 70 °C hasta la completa evaporación del etanol, tras esto las muestras se llevaron a 103 °C en un horno hasta alcanzar peso constante. Estas determinaciones se realizaron por triplicado para las muestras de cada lote tanto en alheiras crudas como cocinadas. Los resultados se expresaron en g agua/100g de muestra.

4.2.4.2.1.2. *Proteína*

La determinación de la cantidad de proteína de las muestras se realizó según la norma portuguesa 1612 (NP-ISO-1612, 2002). Se trata de una determinación por medio del método Kjeldahl con un factor de conversión de nitrógeno de 6,25. Utilizando un sistema Kjeldahl valorante Buchi K-415 acoplado al mineralizador Buchi K-446 y una unidad auto Kjeldahl Buchi destilador K-370. Las determinaciones se realizaron de tres alheiras crudas y tres cocinadas de cada lote y los resultados se expresaron en g proteína/100 g de muestra.

4.2.4.2.1.3. *Grasa*

La grasa de las muestras analizadas se determinó por el método Folch et al. (1957). Las determinaciones se realizaron sobre tres alheiras crudas y tres cocinadas de cada lote y los resultados se expresaron en g grasa/100 g de muestra.

4.2.4.2.1.4. Cenizas

El contenido de cenizas de los lotes de alheiras tanto crudas como conocidas se determinó de acuerdo con la norma portuguesa 1615 (NP-ISO-1615, 2002). En ella la determinación se realizó por medio de incineración de las muestras en una mufla a 550 °C hasta alcanzar un peso constante. Los resultados se expresaron como g de cenizas/100 g de muestra.

4.2.4.2.1.5. Contenido en cloruro total

El contenido en cloruro total de las muestras se evaluó utilizando la metodología detallada en la norma portuguesa 1845 (NP-ISO-1845, 1982). Se trata de una valoración del contenido de cloruro con nitrato de plata 0,1 N mediante un valorante automático de la marca Tritino Plus. Los resultados se expresaron como porcentaje en masa. La determinación se realizó por triplicado en las muestras crudas y cocinadas de los tres lotes de alheiras.

4.2.4.2.2. Propiedades físico-químicas

4.2.4.2.2.1. pH

La determinación de pH se realizó según la norma portuguesa 3441 (NP-ISO-3441, 2008) mediante un pH-metro de punción Crison 507 (Crison, Barcelona, España). La determinación se realizó en tres muestras de cada lote crudas y tres muestras de cada lote cocinadas en diversos puntos de las muestras.

4.2.4.2.2.2. Actividad de agua

La actividad de agua se determinó a temperatura ambiente (25 °C) según el método correspondiente de la AOAC (1990) con un equipo HigroPalmAw1 8303 (Rotronic, Bassersdorf, Suiza). Se realizaron las medidas por triplicado para cada lote tanto crudas como cocinadas.

4.2.4.2.2.3. Determinación de los parámetros de color

La determinación de los parámetros de color para los distintos lotes de alheiras se llevó a cabo utilizando un colorímetro CM-2600D (Minolta Camera Co., Osaka, Japón) con ángulo de observación de 10° y un iluminante D65 en modo SCI. Se utilizó un cristal de baja reflectancia (CR-A51: Minolta Co.) entre la muestra y el colorímetro. Cada alheira fue evaluada seis veces por distintos puntos de la superficie, 18 medidas por cada lote de alheiras, tanto en las muestras crudas como cocinadas.

4.2.4.2.3. Pérdidas por cocinado

Las pérdidas por cocinado de los tres lotes de alheiras, se calcularon con la ecuación 10, dada en el apartado 4.2.2.2.4. Las medidas fueron tomadas por triplicado para cada formulación.

4.2.4.2.4. Perfil lipídico e índices nutricionales

El perfil lipídico de los lotes de alheira tanto cruda como cocinada, se determinó de la grasa directa extraída de las muestras mediante el método descrito por Folch et al. (1957). Los ácidos grasos fueron transesterificados siguiendo el método descrito por Teixeira et al. (2020). Con un patrón interno para la cuantificación de C19:0 en una concentración de 0,3 mg/mL. Todas las determinaciones se realizaron por triplicado y los resultados de los ácidos grasos se expresaron como g de ácido graso/100 g de grasa.

Con los resultados obtenidos del perfil lipídico se calcularon una serie de índices nutricionales o de calidad de la grasa, y una serie de sumatorios según lo descrito en el apartado 4.2.2.2.3.

4.2.4.2.5. Estabilidad oxidativa

La estabilidad oxidativa de los distintos lotes de alheiras se midió siguiendo el procedimiento descrito en la norma portuguesa 3356 (NP-ISO-3356, 2009) midiendo las sustancias reactivas al ácido tiobarbitúrico (TBARs). Los valores de TBARs se calcularon a partir de una curva estándar de malonaldehído (MDA) y se expresaron en mg MDA/kg de muestra. y las determinaciones se realizaron por triplicado para cada uno de los lotes de alheiras tanto en muestras crudas como cocinadas.

4.2.4.3. ANÁLISIS ESTADÍSTICO DE LAS ALHEIRAS

El proceso de elaboración de la emulsión gelificada y de las alheiras, se realizó por triplicado. Tres lotes independientes elaborados en tres días diferentes para las tres muestras. Cada lote fue analizado por triplicado. Los resultados se expresaron como la media y la desviación estándar de los datos obtenidos. Se realizó un análisis ANOVA de doble factor para evaluar las propiedades de las alheiras y el cocinado. Las diferencias estadísticas se encontraron con un nivel de significancia $p < 0,05$ mediante el test de Tukey-b usando el software SPSS (versión 27.0, SPSS, Chicago, EEUU).

4.2.5. APLICACIÓN EN ANÁLOGOS DE CARNE: HAMBURGUESAS VEGANAS

4.2.5.1. FORMULACIÓN Y PROCESO DE ELABORACIÓN DE LAS HAMBURGUESAS VEGANAS

Se elaboraron cuatro lotes de hamburguesas veganas sin tratamientos previos siguiendo la formulación recogida en la Tabla 11. De esos cuatro lotes, dos se elaboraron con la emulsión gelificada con harina de trigo sarraceno y aceite de cáñamo, usando como colorante el zumo comercial de

remolacha (PBCCA) o el zumo fresco de remolacha (PBFCA). Los otros dos lotes, proceden de la combinación de ambos zumos con una emulsión gelificada elaborada a base de harina de trigo sarraceno y aceite de chía (PBCCH y PBFCH, respectivamente).

Tabla 11. Formulación de las hamburguesas veganas con harina de trigo sarraceno/aceite de chía y harina de trigo sarraceno/aceite de cáñamo

Ingredientes	PBFCH	PBCCH	PBFCA	PBCCA
Zumo remolacha fresco	52,6	0	52,6	0
Zumo de remolacha comercial	0	52,6	0	52,6
Texturizado de soja	21,4	21,4	21,4	21,4
Harina de cacahuete	11,4	11,4	11,4	11,4
Chía-GE	10,7	10,7	0	0
Cáñamo-GE	0	0	10,7	10,7
Fibra de guisante	1,3	1,3	1,3	1,3
Sal	1,5	1,5	1,5	1,5
Perejil en polvo	0,4	0,4	0,4	0,4
Cebolla en polvo	0,3	0,3	0,3	0,3
Ajo en polvo	0,3	0,3	0,3	0,3
Pimienta negra	0,2	0,2	0,2	0,2

Los ingredientes vienen referidos en g/100 g. PBFCH: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBCCH: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBFCA: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo. PBCCA: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo.

Para la elaboración de los lotes de hamburguesas veganas se siguió el procedimiento recogido en la Figura 27. En primer lugar, la soja texturizada se hidrató durante 30 minutos con el zumo comercial o fresco de remolacha, dependiendo de la formulación. El zumo comercial fue usado de forma directa, sin embargo, el zumo fresco de remolacha se diluyó previamente en una proporción 1:3 (zumo fresco de remolacha: agua). Una vez estuvo la soja hidratada, se le adicionó la harina de cacahuete y la fibra de guisante y se mezclaron. Tras esto, se añadieron las emulsiones gelificadas (harina de trigo sarraceno/aceite de chía o harina de trigo sarraceno/aceite de cáñamo) picadas previamente con un tamaño similar al de un grano de arroz, y se

mezcló todo hasta obtener una masa homogénea (5 minutos). Por último, se añadieron la sal y todas las especias (5 minutos). Para dar forma a las hamburguesas veganas, se utilizó una máquina comercial y se obtuvieron hamburguesas veganas de aproximadamente 1 cm de espesor y unos 80 g de peso. Tras su formación, se envasaron y se mantuvieron en condiciones de refrigeración (4 °C) hasta su análisis. Para obtener las hamburguesas veganas cocinadas necesarias para los análisis, seis hamburguesas de cada formulación se cocinaron en una plancha hasta alcanzar una temperatura interna de 72 °C, lo que aproximadamente fueron 4,5 minutos por cada lado.

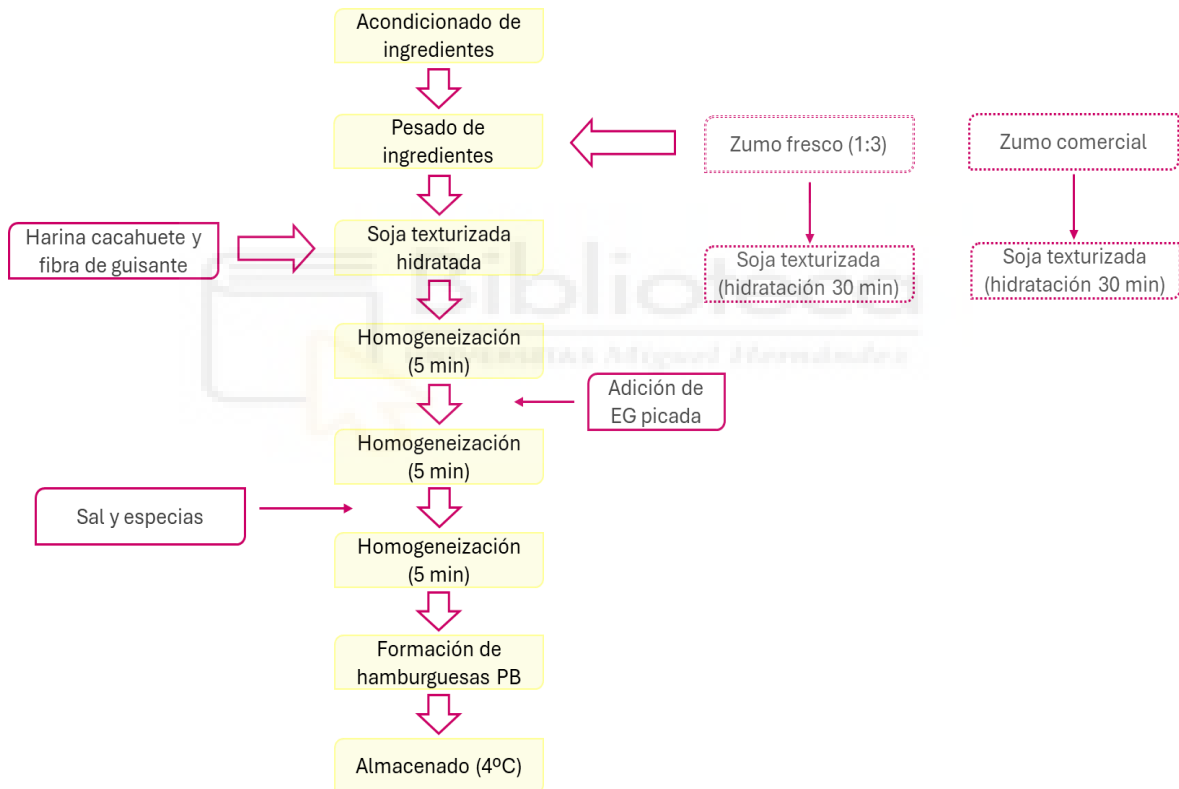


Figura 27. Diagrama de flujo de la elaboración de las hamburguesas veganas

4.2.5.2. CARACTERIZACIÓN DE LAS HAMBURGUESAS VEGANAS

4.2.5.2.1. Composición química

La composición química (húmedad, proteína, grasa y cenizas) de los 4 lotes de hamburguesas veganas se determinó mediante los métodos

correspondientes de la AOAC (2010) descritos anteriormente en los apartados 4.1.3.1.1.; 4.1.3.1.2.; 4.1.3.1.3. y 4.1.3.1.4. Las determinaciones se realizaron por triplicado para cada lote de hamburguesas veganas tanto crudas como cocinadas. Los resultados se expresaron como g/100 g de muestra.

También se determinó el contenido de minerales con la metodología descrita anteriormente en el apartado 4.2.3.2.3.1. El contenido final de minerales se expresa en mg/100 g de muestra. La determinación se hizo por triplicado para las muestras crudas.

La determinación del contenido en betalaínas se realizó tanto en los zumos, fresco y comercial, como en las muestras de hamburguesas veganas crudas. Para la extracción de los pigmentos de las hamburguesas crudas, 1 g de las muestras liofilizadas se disolvieron en 5 mL de etanol-agua 80:20 (v/v) y se mantuvo durante 5 minutos en agitación. Posteriormente se centrifugó a 15000xg durante 10 minutos en una centrífuga Hermle Z383K (Hermle, Wehingen, Alemania), y el sobrenadante se pasó por un filtro de nailon de 0,45 µm. Para la identificación de las betalaínas en los zumos de remolacha, estos se diluyeron previamente y se filtraron. El contenido de betalaína se cuantificó para cada muestra según lo descrito por Fernández-López et al. (2002). La concentración total de betalaínas se cuantificó como la suma de las concentraciones de betaxantinas y betacianinas. El contenido de betaxantinas se determinó como vulgaxantina I a 485 nm. El contenido de betacianinas se determinó como betanina a 535 nm (Wruss et al., 2015). Las muestras se analizaron con la ayuda de un equipo de cromatografía modular Waters HPLC (Waters, Milford, EEUU). Se utilizó una columna Spherisorb ODS-2 (Waters, Milford, EEUU), 5 µm, 250 × 4,6 mm. Se usaron dos fases móviles con un flujo de 1 mL/min. Como fase móvil A se usó 175 mmol/L de

ácido acético en agua y como fase móvil B, 175 mmol/L de ácido acético en acetonitrilo.

4.2.5.2.2. Perfil lipídico e índices nutricionales

El perfil lipídico de los lotes de hamburguesas veganas se determinó siguiendo el mismo método, las mismas condiciones y equipamiento que se detallaron anteriormente en el apartado 4.1.3.4. Todas las determinaciones se realizaron por triplicado y los resultados de los ácidos grasos se expresaron como g de ácido graso/100 de grasa.

Con los resultados obtenidos del perfil lipídico se calcularon una serie de índices nutricionales o de calidad de la grasa y se calcularon una serie de sumatorios descritos en el apartado 4.2.2.2.3.

4.2.5.2.3. Propiedades físico-químicas

4.2.5.2.3.1. Determinación de pH

La determinación de pH se realizó mediante la metodología y usando el equipamiento descrito anteriormente en el apartado 4.1.4.2.1. La determinación se realizó por triplicado, unas 6 medidas por muestra, para cada uno de los cuatro lotes de hamburguesas veganas tanto crudas como cocinadas.

4.2.5.2.3.2. Actividad de agua

La determinación de la actividad de agua se realizó a temperatura ambiente (25 °C) con el equipamiento descrito en el apartado 4.2.2.2.2. Esta determinación se realizó por triplicado para cada uno de los lotes de hamburguesas veganas crudas.

4.2.5.2.3.3. Análisis de textura

La textura de los lotes de hamburguesas veganas se determinó mediante un análisis de perfil textural (TPA) usando el mismo equipo y las mismas condiciones, sonda y parámetros descritos en el apartado 4.2.2.2.3. Se realizó sobre las muestras cortadas en 2 x 2 x 2 cm. Y se calcularon parámetros como la dureza (N); la elasticidad (mm); Cohesividad (adimensional) y masticabilidad (Nxmm). Estas determinaciones se realizaron para 9 muestras de cada uno de los 4 lotes de hamburguesas veganas cocinadas.

4.2.5.2.3.4. Determinación de los parámetros de color

La determinación de los parámetros de color para los distintos lotes de hamburguesas veganas se llevó a cabo con la misma metodología, condiciones y equipamiento descrito con anterioridad en el apartado 4.4.3.2. Las medidas se realizaron a una temperatura aproximada de 25 °C y se realizaron 18 medidas por cada lote de hamburguesas veganas tanto en crudo como en cocinado.

4.2.5.2.4. Propiedades de cocinado

La determinación de las propiedades de cocinado para los distintos lotes de hamburguesas veganas se llevó a cabo de igual forma que en lo descrito anteriormente en el apartado 4.2.2.2.4. Se realizaron las mediciones para tres hamburguesas de cada lote y los resultados de estas propiedades se expresaron en %.

4.2.5.3 ANÁLISIS ESTADÍSTICO DE LAS HAMBURGUESAS VEGANAS

El proceso de elaboración de las emulsiones gelificadas y de las hamburguesas veganas, se realizó por triplicado, tres lotes independientes elaborados en tres días diferentes para las cuatro muestras. Cada lote fue analizado por triplicado. Los resultados se expresaron como la media y la desviación estándar de los datos obtenidos. Se realizó un análisis ANOVA de doble factor para evaluar las propiedades de las hamburguesas y el cocinado. Las diferencias estadísticas se encontraron con un nivel de significancia $p < 0,05$ mediante el test de Tukey-b usando el software SPSS (versión 27.0, SPSS, Chicago, EEUU). Para la evaluación sensorial los panelistas se consideraron como un factor arbitrario.

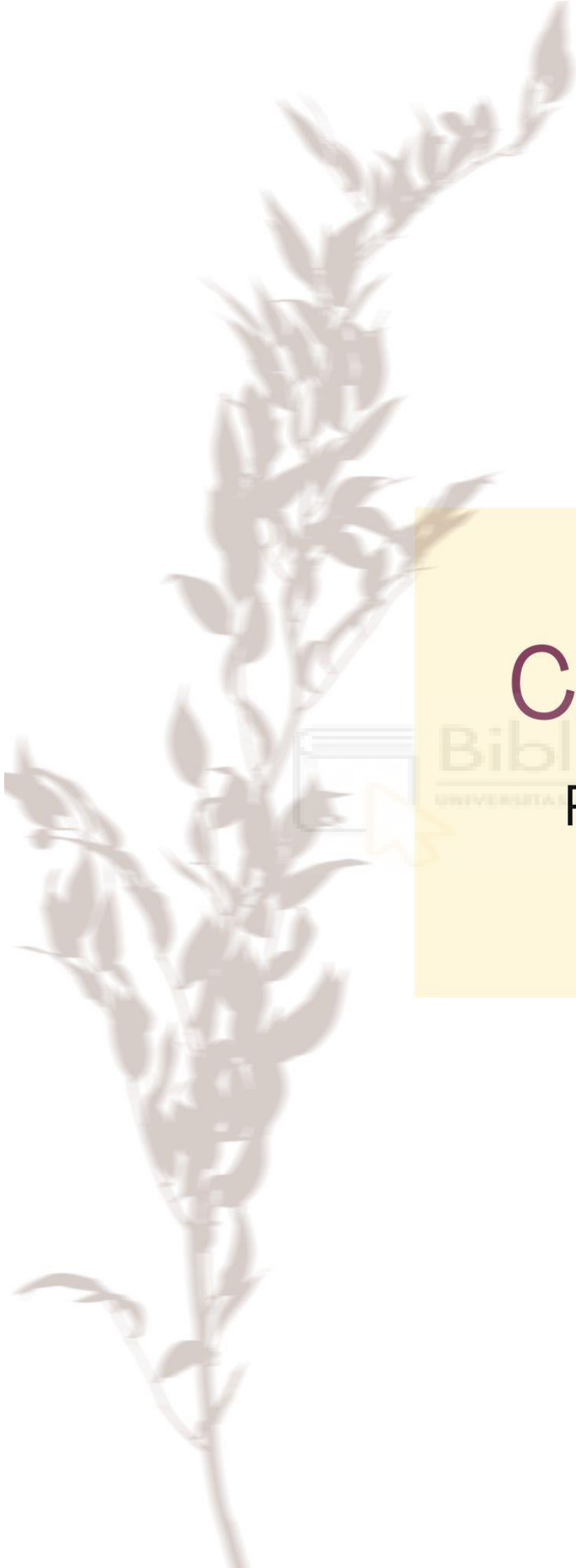


CAPÍTULO 5

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RESULTADOS Y DISCUSIÓN



5. RESULTADOS Y DISCUSIÓN

En el presente capítulo se muestra un resumen de los resultados más destacados obtenidos durante la realización de la presente Tesis. El conjunto completo de resultados, así como su discusión extensa se muestran en los correspondientes artículos recogidos en el capítulo 8.

Como ya se mencionó anteriormente en el apartado 4.1.2., las harinas de pseudocereales usadas para la elaboración de emulsiones gelificadas en la presente Tesis Doctoral fueron la harina de amaranto, la harina de trigo sarraceno, la harina de teff y la harina de quinoa blanca y los aceites fueron el de cáñamo, chía, lino y sésamo, así como las 6 combinaciones en una proporción 1:1 entre ellos. A continuación, se van a mostrar algunos de los resultados más destacables del análisis de dichas harinas y de dichos aceites, ya que teniendo en cuenta estos resultados y los de la caracterización de las 40 emulsiones resultantes de todas las combinaciones mencionadas se realizó la posterior selección para la inclusión de estas en los distintos productos cárnicos y análogo de carne analizados.

5.1. CARACTERIZACIÓN DE LAS HARINAS DE PSEUDOCEREALES Y LOS ACEITES VEGETALES

5.1.1. CARACTERIZACIÓN DE LAS HARINAS DE PSEUDOCEREALES

La Tabla 12 muestra los resultados del análisis de la composición química de las cuatro harinas de pseudocereales usadas para la elaboración de las emulsiones gelificadas.

Tabla 12. Composición centesimal de las harinas de pseudocereales utilizadas en la elaboración de las emulsiones gelificadas.

	Hidratos de carbono	Proteína	Grasa	Cenizas	Humedad
HAM	69,77±0,06 ^d	11,98±0,37 ^a	6,29±0,80 ^a	2,76±0,04 ^a	9,21±0,15 ^a
HQB	72,59±0,22 ^b	13,90±2,37 ^a	5,88±0,15 ^a	2,32±0,06 ^c	9,18±0,43 ^a
HTE	74,09±0,27 ^a	11,48±0,01 ^a	2,34±0,08 ^b	2,48±0,04 ^b	9,61±0,21 ^a
HTS	69,19±0,12 ^c	12,75±0,12 ^a	3,57±0,59 ^b	1,87±0,06 ^d	9,18±0,09 ^a

Valores expresados en g/100 g. HAM: harina de amaranto; HQB: harina de quinoa blanca; HTE: harina de teff; HTS: haría de trigo sarraceno. Los resultados seguidos de distinta letra minúscula (a-d) representan diferencias significativas ($p < 0,05$) para cada parámetro según el test post-hoc de Tukey. Los datos se presentan como media y DS.

En ella, se puede observar como las cuatro harinas no presentaron diferencias significativas ($p > 0,05$) en el contenido de proteína (11,98-13,90 g/100 g de muestra) ni de humedad (9,18-9,61 g/100 g de muestra). En cuanto al contenido de grasa, se obtuvo una variabilidad de esta con valores desde los 2,34 g /100 g de muestra para la harina de teff hasta los 6,29 g/100 g de muestra para la harina de amaranto, sin diferencias significativas ($p > 0,05$) de esta última con la harina de quinoa blanca (5,88 g/100 g de muestra).

A la vista de los resultados de la composición química analizada, como ya se ha mencionado anteriormente no se encontraron diferencias significativas en el contenido de proteínas entre las cuatro muestras de harina de pseudocereales ($p > 0,05$). Aunque la cantidad de proteína podría estar implicada en la formación de emulsiones por parte de los emulsionantes, estos resultados no proporcionan evidencias suficientes para realizar una elección entre una harina u otra, por ello se deberían evaluar otras propiedades.

Ciertos parámetros de mucho interés para tener en cuenta a la hora de generar emulsiones son las propiedades tecnofuncionales, con la información de estas propiedades se podría predecir previamente y obtener información sobre el comportamiento de estas harinas a la hora de generar emulsiones con ellas. En la Tabla 13 se muestran la capacidad de retención de agua (CRA), la capacidad de retención de aceite (CRO), la capacidad de

hinchamiento (SWC), la actividad emulsionante (AE) y la estabilidad de la emulsión (EE) para las harinas de pseudocereales estudiadas. Medir este tipo de propiedades son importantes ya que si un producto tiene una baja CRA y CRO puede presentar pérdidas de humedad y grasa en los productos a los cuales se le incorpora con posterioridad. También son propiedades que ayudan a la mejora del sabor y la sensación en boca de los alimentos, debido a dicha capacidad de retener el aceite o el agua (Ling et al., 2016).

Tabla 13. Propiedades tecnofuncionales de las harinas de pseudocereales utilizadas para la elaboración de emulsiones gelificadas.

	CRA	CRO	SWC	AE	EE
HAM	1,15±0,03 ^a	0,95±0,04 ^a	1,83±0,37 ^{ab}	42,67±2,89 ^b	85,81±11,34 ^a
HQB	1,35±0,03 ^a	1,09±0,09 ^a	2,09±0,60 ^{ab}	54,33±3,06 ^a	95,25±6,60 ^a
HTE	1,25±0,27 ^a	0,68±0,02 ^b	2,67±0,23 ^a	16,17±4,37 ^c	70,83±13,13 ^a
HTS	1,37±0,01 ^a	0,68±0,03 ^b	1,52±0,12 ^b	57,50±2,50 ^a	93,98±2,35 ^a

Valores de CRA, CRO y SWC, expresados en g/g; valores de AE y EE, expresados en g/100 g. HAM: harina de amaranto; HQB: harina de quinoa blanca; HTE: harina de teff; HTS: harina de trigo sarraceno. CRA: capacidad de retención de agua; CRO: capacidad de retención de aceite; SWC: capacidad de hinchamiento; AE: capacidad emulsionante; EE: estabilidad de la emulsión. Los resultados seguidos de distinta letra minúscula (a-c) representan diferencias significativas ($p < 0,05$) para cada parámetro según el test post-hoc de Tukey. Los datos se presentan como media y DS.

En general todas las harinas presentaron una mayor capacidad de retención de agua que de aceite (Tabla 13). La CRA no presentó diferencias significativas ($p > 0,05$) para ninguna de las cuatro harinas. Sin embargo, en la CRO se observa como el orden de capacidad de retención ($p < 0,05$) fue harina de quinoa blanca = harina de amaranto > harina de teff = harina trigo sarraceno. Para la capacidad de hinchamiento solo hubo diferencias entre la harina de teff y la harina de trigo sarraceno, siendo esta última la que menor valor presentó de entre todas las harinas estudiadas. La propiedad que más nos puede interesar de cara al uso de estas harinas en la presente Tesis es su AE y EE, para la segunda propiedad (EE) no existieron diferencias ($p > 0,05$) entre las cuatro harinas. Las harinas de quinoa y de trigo sarraceno fueron las que mayor actividad emulsionante presentaron con diferencias

significativas ($p < 0,05$) con la de amaranto. La harina de teff presentó el menor valor de actividad emulsionante de las cuatro con unos valores de 16,17 g/100 g de harina.

Para completar la información, se analizó la capacidad antioxidante por cuatro métodos diferente, DPPH, ABTS, FIC y FRAP. Los resultados de la capacidad antioxidante se recogen en la Tabla 14, en ella destaca la harina de trigo sarraceno con los valores más altos para todos los métodos antioxidantes ensayados con diferencias significativas ($p < 0,05$) con el resto de las harinas. Por el contrario, la harina de amaranto fue la que menor capacidad antioxidante mostró para el método DPPH y la harina de teff para el método FIC con diferencias significativas con las otras tres harinas ($p < 0,05$).

Tabla 14. Capacidad antioxidante medida por diversos métodos de las harinas pseudocereales utilizadas en la elaboración de las emulsiones gelificadas.

	DPPH	ABTS	FRAP	FIC
HAM	0,07±0,01 ^c	0,24±0,07 ^b	1,02±0,27 ^b	0,12±0,02 ^{bc}
HQB	0,45±0,01 ^b	0,91±0,29 ^b	2,88±0,27 ^b	0,14±0,02 ^b
HTE	0,48±0,09 ^b	0,69±0,80 ^b	2,10±0,27 ^b	0,08±0,00 ^c
HTS	1,91±0,27 ^a	5,12±1,66 ^a	17,78±2,50 ^a	0,28±0,04 ^a

Valores expresados mg de equivalentes de Trolox/g muestra para DPPH, FRAP y ABTS; mg EDTA/g muestra para FIC. HAM: harina de amaranto; HQB: harina de quinoa blanca; HTE: harina de teff; HTS: haría de trigo sarraceno. Los resultados seguidos de distinta letra minúscula (a-c) representan diferencias significativas ($p < 0,05$) para cada parámetro según el test post-hoc de Tukey. Los datos se presentan como media y DS.

5.1.2. CARACTERIZACIÓN DE LOS ACEITES VEGETALES

Parte de los resultados de este trabajo han sido publicados en la revista Foods (2021), 10, 1463 (Open Access).

En la Figura 28, se presentan los ácidos grasos mayoritarios de los cuatro aceites usados para la elaboración de emulsiones gelificadas y de sus mezclas 1:1. En general se puede observar que se caracterizan por tener muy

poca cantidad de ácidos grasos saturados (10-16,88 g/100 g) y que la mayoría de los ácidos grasos son poliinsaturados (41,31-80,38 g/100 g, aceite de sésamo y aceite de cáñamo respectivamente). Referente a los ácidos grasos monoinsaturado, el contenido de estos aceites y sus mezclas fue entre 11,19 g/100 g (aceite de cáñamo) y 43,04 g/100 g (aceite de sésamo). Se puede observar en la Figura 28 como el ácido graso mayoritario para el aceite de cáñamo es el linoleico con un contenido de 54,44 g/100 g y que en el aceite de sésamo también predomina este ácido graso (40,9 g/100 g), aunque su ácido graso mayoritario es el oleico con una cantidad de 41,32 g/100 g. Así pues, la mezcla 1:1 del aceite de cáñamo-sésamo da unos valores importantes de ácido graso linoleico pero menores al del cáñamo solo, siendo de 47,73 g/100 g. Como ácido graso mayoritario el α -linolénico lo presentaron el aceite de chía y de lino con una cantidad de 56,61 y 53,82 g/100 g, respectivamente. Por ello, cuando se mezclan 1:1 estos aceites, siguió obteniéndose una proporción considerable del ácido α -linolénico con una cantidad de 55,17 g/100 g. Para las mezclas de aceite de sésamo y chía se obtienen proporciones muy similares entre sí para los ácidos grasos oleico, linoleico y α -linolénico, lo mismo sucede con la mezcla 1:1 de aceite de sésamo y lino (30,43; 28,31 y 26,96 g/100 g para oleico, linoleico y α -linolénico, respectivamente). Por último, las mezclas 1:1 de chía-cáñamo y 1:1 de cáñamo-lino presentaron un perfil similar entre sí, como ácidos grasos mayoritarios el α -linolénico seguido del linoleico.

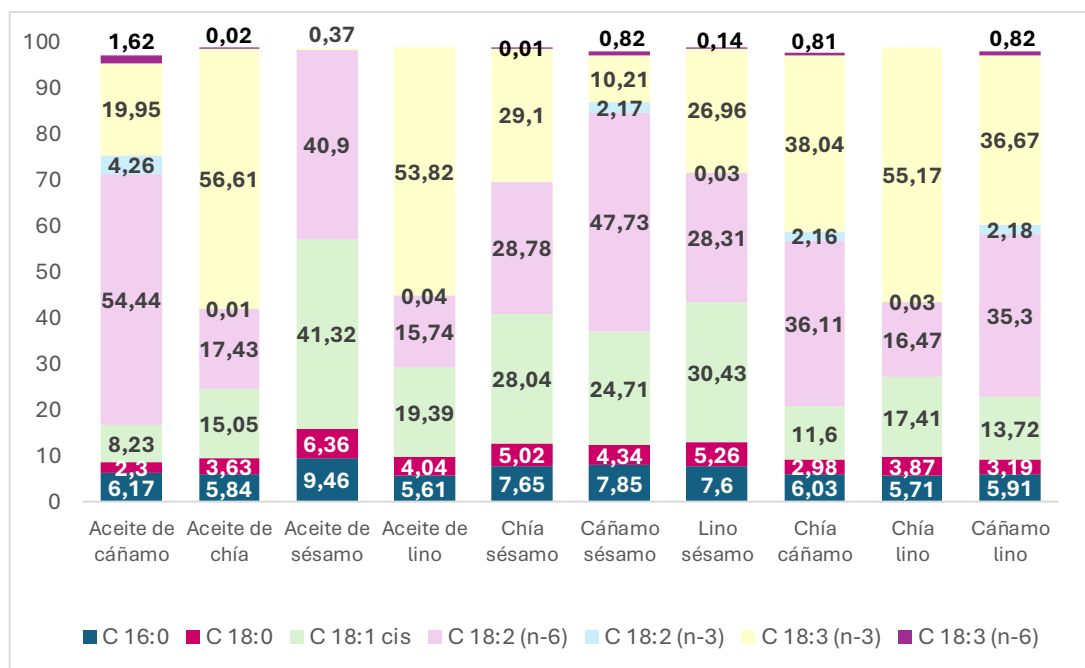


Figura 28. Ácidos grasos mayoritarios tanto saturados como insaturados de los aceites utilizados para elaborar las emulsiones gelificadas y sus mezclas 1:1. Los resultados vienen expresados en g de ácido graso/100 g de grasa.

En la Figura 28, se puede observar como el ácido graso predominante en el aceite de chía es el α -linolénico (omega 3), linoleico (omega 6) y oleico con una cantidad de 56,61 g; 17,43 y 15,05 g de ácido graso/100 g de grasa respectivamente, representando el 89,09 % de los ácidos grasos analizados. Sin embargo, para el aceite de cáñamo se invierte el orden, presentó en primer lugar el ácido linoleico (omega 6), seguido de α -linolénico (omega 3) y por último oleico, cuyas cantidades fueron de 54,44; 19,95 y 8,23 g de ácido graso/100 g de grasa, de forma respectiva, conformando un 82,62 % del total de los ácidos grasos analizados. El análisis de su mezcla en proporción 1:1 reveló que, aunque muy cercanos el ácido graso mayoritario es el α -linolénico con 38,04 g/100 g de grasa, el linoleico presentó un valor de 36,11 g/100 g de grasa y la cantidad de oleico fue de 11,60 g/100 g de grasa. En vista de ello, era de esperar que el uso de estos aceites para la elaboración de las emulsiones gelificadas y su posterior incorporación en diversos productos cárnicos mejoraran su perfil lipídico y los hicieran más saludables.

5.2. EVALUACIÓN DE LAS PROPIEDADES QUÍMICAS, FÍSICO-QUÍMICAS Y ESTABILIDAD LIPÍDICA DE LAS EMULSIONES GELIFICADAS ELABORADAS CON ACEITE DE CHÍA O CÁÑAMO Y HARINAS DE PSEUDOCEREALES

Los resultados de este trabajo han sido publicados en la revista Foods (2021), 10, 1463 (Open Access).

Como ya se comentó en el apartado 4.1.2.1., se elaboraron 40 emulsiones gelificadas con la combinación de los aceites de chía (CH), cáñamo (CA), lino (L) y sésamo (S) con las cuatro harinas de pseudocereales: harina de amaranto (A), teff (TE), trigo sarraceno (TS) y quinoa blanca (QB). Así como las mezclas de todos los aceites entre sí en proporción 1:1 (M), con cada una de las harinas anteriormente mencionadas. Se analizaron las 40 formulaciones y de entre todas ellas, se seleccionaron las 12 que mejores características tecnológicas presentaron (capítulo 8.2). Estas 12 fueron la combinación de harina de amaranto con: aceite de chía (ACH), aceite de cáñamo (ACA), aceite de chía-cáñamo en proporción 1:1 (AM4). Harina de trigo sarraceno con: aceite de chía (TSCH), aceite de cáñamo (TSCA), mezcla 1:1 de aceites de chía-cáñamo (TSM4). Harina de teff con: aceite de chía (TECH), aceite de cáñamo (TECA) y mezcla de aceites de chía-cáñamo 1:1 (TEM4). Las cuatro últimas fueron las emulsiones gelificadas obtenidas al mezclar harina de quinoa blanca con: aceite de chía (QBCH), aceite de cáñamo (QBCA), mezcla de aceites 1:1 de chía-cáñamo (QBM4) (formulaciones presentadas anteriormente en la Tabla 6 del apartado 4.1.2.1.).

Las muestras preparadas con harina de teff mostraron pérdidas de aceite, llegando una de ellas (TEM4), a romper totalmente su estructura imposibilitando su análisis.

5.2.1. CARACTERIZACIÓN DE LAS EMULSIONES GELIFICADAS

Desde un punto de vista químico, la humedad de las emulsiones gelificadas analizadas dio valores entre el 44,73 y 49,91 g de agua/100 g de emulsión (Tabla 15). Siendo las muestras elaboradas con las mezclas de aceites las que contenían una humedad superior a las elaboradas con los aceites puros (aceite de chía y aceite de cáñamo). El contenido en grasa fue más variable presentó valores entre 35,55 y 43,86 g de grasa/100 g de emulsión (Tabla 15).

Tabla 15. Composición química de las emulsiones gelificadas.

	Humedad	Grasa	Proteínas	Cenizas
ACH	45,76±0,30 ^{cd}	42,82±0,30 ^{ab}	2,50±0,03 ^c	0,43±0,01 ^c
ACA	46,07±0,14 ^c	42,56±0,10 ^b	2,52±0,01 ^c	0,44±0,01 ^{bc}
AM4	46,10±0,30 ^c	42,24±0,13 ^b	2,52±0,02 ^c	0,48±0,09 ^{bc}
TSCH	45,24±0,36 ^{cd}	42,69±0,32 ^{ab}	2,61±0,01 ^b	0,49±0,08 ^{bc}
TSCA	46,18±0,28 ^c	41,69±0,54 ^c	2,63±0,02 ^{ab}	0,41±0,01 ^c
TSM4	47,59±0,30 ^b	40,41±0,37 ^d	2,61±0,01 ^b	0,45±0,04 ^{bc}
TECH	48,28±1,94 ^{ab}	37,89±1,23 ^e	2,47±0,01 ^c	0,68±0,06 ^a
TECA	49,91±2,57 ^a	35,55±1,52 ^f	2,50±0,02 ^c	0,73±0,05 ^a
TEM4	ND	ND	ND	ND
QBCH	44,73±0,06 ^d	43,86±2,31 ^a	2,62±0,06 ^{ab}	0,46±0,03 ^{bc}
QBCA	45,33±0,21 ^{cd}	43,16±1,97 ^a	2,68±0,01 ^a	0,40±0,03 ^c
QBM4	47,15±0,15 ^b	41,53±0,30 ^c	2,69±0,02 ^a	0,26±0,02 ^d

Resultados expresados en g/100 g. ND: no determinado. ACH: harina de amaranto con aceite de chía; ACA: harina de amaranto con aceite de cáñamo; AM4: harina de amaranto con mezcla de aceites de chía y cáñamo; TSCH: harina de trigo sarraceno con aceite de chía; TSCA: harina de trigo sarraceno con aceite de cáñamo; TSM4: harina de trigo sarraceno con mezcla de aceites de chía y cáñamo; TECH: harina de teff con aceite de chía; TECA: harina de teff con aceite de cáñamo; TEM4: harina de teff con mezcla de aceites de chía y cáñamo; QBCH: harina de quinoa blanca con aceite de chía; QBCA: harina de quinoa blanca con aceite de cáñamo; QBM4: harina de quinoa blanca con mezcla de aceites de chía y cáñamo. Los resultados seguidos de distinta letra minúscula (a-f) representan diferencias significativas ($p < 0,05$) para cada parámetro según el test post-hoc de Tukey. Los datos se presentan como media y DS.

Las muestras que mayor contenido de humedad y menor contenido de grasa ($p < 0,05$) presentaron fueron las elaboradas con harina de teff (Tabla 15), que a su vez fueron las muestras que a simple vista presentaban aceite en la superficie, denotando que la emulsión no se formó de una manera correcta. La cantidad de proteína de las emulsiones gelificadas viene directamente relacionada con la cantidad de proteína de la harina utilizada,

la muestras con mayor contenido en proteínas fueron las elaboradas con harina de quinoa blanca seguidas de las elaboradas con amaranto ($p < 0,05$).

En las propiedades físico-químicas las muestras presentaron un rango de valores de pH entre $5,53 \pm 0,02$ hasta $6,41 \pm 0,02$, pH cercano al de las grasas animales empleadas en la elaboración de productos cárnicos (Tabla 16).

Tabla 16. Propiedades físico-químicas de las emulsiones gelificadas.

	pH	Trabajo de cizalla (N.s)	Firmeza (N)
ACH	$6,38 \pm 0,01^a$	$5,78 \pm 0,63^b$	$6,64 \pm 0,64^b$
ACA	$6,41 \pm 0,02^a$	$4,51 \pm 0,08^c$	$5,26 \pm 0,60^c$
AM4	$6,35 \pm 0,01^a$	$5,22 \pm 0,20^b$	$11,69 \pm 0,52^c$
TSCH	$6,03 \pm 0,02^c$	$0,82 \pm 0,02^f$	$0,83 \pm 0,02^f$
TSCA	$6,06 \pm 0,01^c$	$0,89 \pm 0,12^f$	$0,94 \pm 0,08^f$
TSM4	$6,21 \pm 0,01^b$	$11,49 \pm 1,18^a$	$14,70 \pm 2,25^a$
TECH	$6,14 \pm 0,01^b$	$5,34 \pm 0,20^b$	$6,76 \pm 1,94^b$
TECA	$6,16 \pm 0,01^b$	$3,56 \pm 0,18^d$	$4,08 \pm 0,16^d$
TEM4	ND	ND	ND
QBCH	$5,94 \pm 0,01^d$	$4,15 \pm 0,12^{cd}$	$3,82 \pm 0,14^d$
QBCA	$5,98 \pm 0,01^d$	$2,77 \pm 0,05^e$	$2,71 \pm 0,92^e$
QBM4	$5,53 \pm 0,02^e$	$3,82 \pm 0,03^d$	$7,22 \pm 0,26^b$

ND: no determinado. ACH: harina de amaranto con aceite de chía; ACA: harina de amaranto con aceite de cáñamo; AM4: harina de amaranto con mezcla de aceites de chía y cáñamo, TSCH: harina de trigo sarraceno con aceite de chía; TSCA: harina de trigo sarraceno con aceite de cáñamo; TSM4: harina de trigo sarraceno con mezcla de aceites de chía y cáñamo; TECH: harina de teff con aceite de chía; TECA: harina de teff con aceite de cáñamo; TEM4: harina de teff con mezcla de aceites de chía y cáñamo; QBCH: harina de quinoa blanca con aceite de chía; QBCA: harina de quinoa blanca con aceite de cáñamo; QBM4: harina de quinoa blanca con mezcla de aceites de chía y cáñamo. Los resultados seguidos de distinta letra minúscula (a-f) representan diferencias significativas ($p < 0,05$) para cada parámetro según el test post-hoc de Tukey. Los datos se presentan como media y DS.

5.2.2. ESTABILIDAD DE LAS EMULSIONES GELIFICADAS A LA CONGELACIÓN

5.2.2.1. ESTABILIDAD DE LAS EMULSIONES GELIFICADAS

Un parámetro importante de cara a la incorporación de estas emulsiones gelificadas a productos cárnicos es la estabilidad de la emulsión. Una emulsión estable debe retener la máxima cantidad de fluido en su interior, por ello a medida que el fluido expelido total (FET) aumenta de valor, esto denota que la estabilidad de la emulsión es menor. En este parámetro están implicados diversos factores como las interacciones proteína-proteína,

la estructura formada del gel, la capacidad de retención de agua y/o de aceite, entre otros (Paradiso et al., 2015). La Figura 29, muestra el % de FET de cada emulsión gelificada a tiempo 0 y 15 días de almacenamiento a -23 °C. Se puede observar que no hay un comportamiento claro en la estabilidad de la emulsión respecto al tipo de harina de pseudocereal o al aceite empleado, parece que la relación entre ambos ingredientes definiría el comportamiento de la estabilidad frente a la congelación. A tiempo 0, las muestras de mayor estabilidad fueron harina de amaranto con aceite de chía (ACH), harina de trigo sarraceno con aceite de chía (TSCH), harina de trigo sarraceno con mezcla de aceites de chía y cáñamo (TSM4), harina de quinoa blanca con aceite de chía (QBM4) ($p < 0,05$) y las muestras que menor estabilidad mostraron fueron harina de quinoa blanca con mezcla de aceites de chía y cáñamo y las elaboradas con harina de teff. Se conocen varios factores relacionados con los pseudocereales que afectan en la estabilidad de la emulsión, como por ejemplo un contenido alto de polisacáridos (>70%) puede contribuir a la formación de enlaces cruzados con proteínas y la absorción de estas por parte de la interfase (Fidantsi & Doxastakis, 2001). La presencia de lípidos en los pseudocereales puede afectar negativamente a las propiedades emulsionantes de las proteínas. También una cantidad elevada de proteína puede ser contraproducente para la estabilidad de la emulsión ya que, aunque beneficia a la capacidad emulsionante esto va en detrimento a la estabilidad de esta (Janssen et al., 2017).

La estabilidad de la emulsión se vio afectada por el tratamiento de congelación para todas las muestras, pero las que mantuvieron unos valores menores de %FET tras los 15 días de congelación fueron las muestras harina de quinoa blanca con aceite de chía (QBCH), harina de quinoa blanca con aceite de cáñamo (QBCA) y harina de trigo sarraceno con aceite de chía (TSCH) ($p < 0,05$) y la de menor estabilidad harina de quinoa blanca con

mezcla de aceites de chía y cáñamo (QBM4). La estabilidad de la emulsión a la congelación y descongelación depende de la composición y la estructura principalmente, así como de los puntos de fusión de los aceites relacionado con la temperatura de cristalización de este. Si se emplean aceites que cristalizan antes que la fase acuosa provocará la coalescencia de las gotas de aceite formadas dentro de la emulsión durante los procesos de descongelación, dando lugar a una separación de las fases (Chizawa et al., 2019). A parte de ello, existen muchos factores que afectan a esto como el tipo de emulsionante, la composición de solutos, la estructura, la congelación y las condiciones en sí del proceso (Degner et al., 2014).

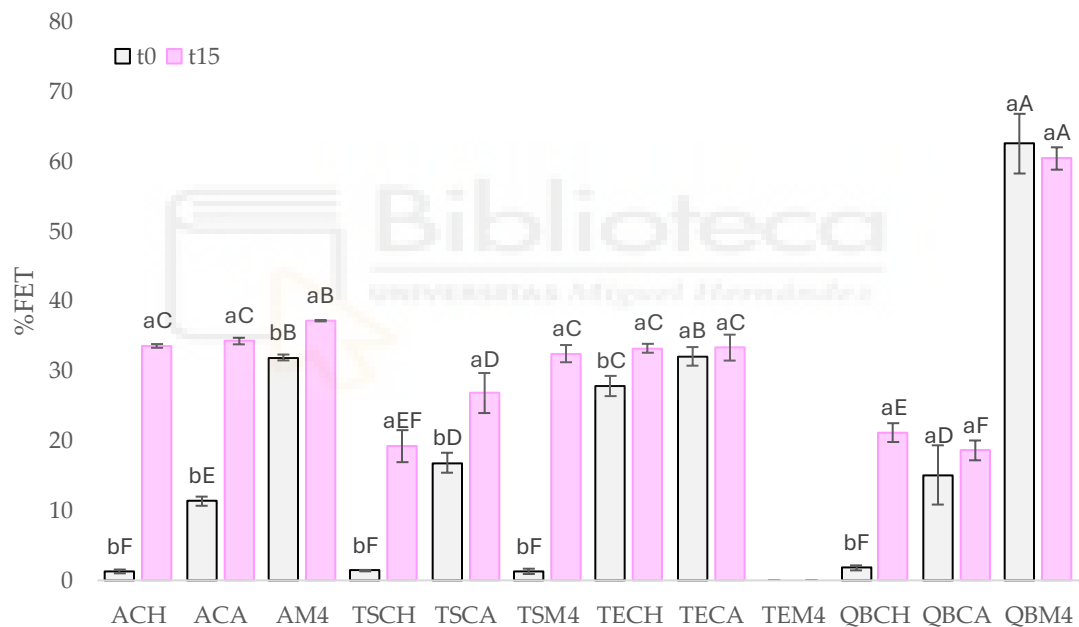


Figura 29. Estabilidad de la emulsión (%fluido expelido total) de las emulsiones gelificadas a día 0 (t_0) y a día 15 (t_{15}) de congelación.

ACH: harina de amaranto con aceite de chía; ACA: harina de amaranto con aceite de cáñamo; AM4: harina de amaranto con mezcla de aceites de chía y cáñamo, TSCH: harina de trigo sarraceno con aceite de chía; TSCA: harina de trigo sarraceno con aceite de cáñamo; TSM4: harina de trigo sarraceno con mezcla de aceites de chía y cáñamo; TECH: harina de teff con aceite de chía; TECA: harina de teff con aceite de cáñamo; TEM4: harina de teff con mezcla de aceites de chía y cáñamo; QBCH: harina de quinoa blanca con aceite de chía; QBCA: harina de quinoa blanca con aceite de cáñamo; QBM4: harina de quinoa blanca con mezcla de aceites de chía y cáñamo. Letras mayúsculas distintas (A-F) sobre cada barra se refiere a la comparación de los valores de estabilidad de la emulsión al mismo tiempo de almacenamiento entre las diferentes muestras de emulsiones gelificadas. Las letras minúsculas (a-b) sobre cada barra se refieren a la comparación de los valores de estabilidad de la emulsión para la misma muestra de emulsiones gelificadas en diferentes tiempos. Los resultados seguidos de distinta letra representan diferencias significativas ($p < 0,05$) para cada parámetro según el test post-hoc de Tukey.

5.2.2.2. OXIDACIÓN LIPÍDICA DE LAS EMULSIONES GELIFICADAS

Debido a que las emulsiones gelificadas desarrolladas tienen una cantidad de grasa por encima del 35% con una proporción de ácidos grasos poliinsaturados superior al 70%, se consideró de mucho interés saber el nivel de oxidación lipídico de las emulsiones gelificadas y como afectaba a este parámetro la congelación de estas. Por ello, se midió el nivel de mg malonaldehído (MDA) por kg de muestra analizada a tiempo 0 y 15 días tras el almacenamiento en condiciones de congelación (Figura 30).

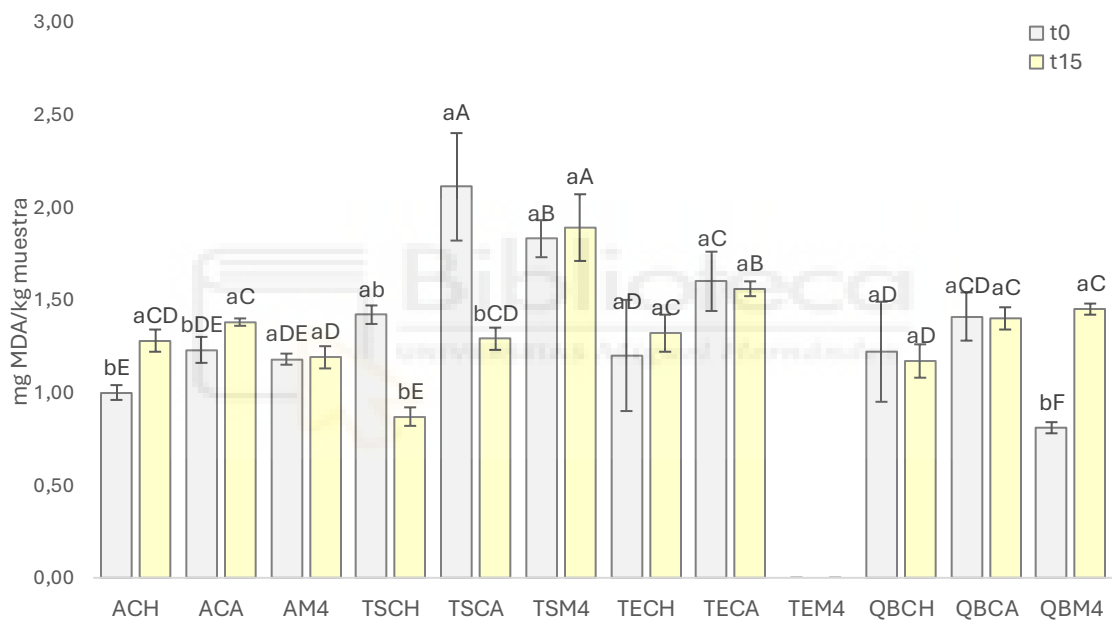


Figura 30. Oxidación lipídica (TBARs; mg malonaldehído/kg de muestra) de emulsiones gelificadas a día 0 (t₀) y después de 15 días de almacenamiento en congelación (t₁₅).

ACH: harina de amaranto con aceite de chía; ACA: harina de amaranto con aceite de cáñamo; AM4: harina de amaranto con mezcla de aceites de chía y cáñamo, TSCH: harina de trigo sarraceno con aceite de chía; TSCA: harina de trigo sarraceno con aceite de cáñamo; TSM4: harina de trigo sarraceno con mezcla de aceites de chía y cáñamo; TECH: harina de teff con aceite de chía; TECA: harina de teff con aceite de cáñamo; TEM4: harina de teff con mezcla de aceites de chía y cáñamo; QBCH: harina de quinoa blanca con aceite de chía; QBCA: harina de quinoa blanca con aceite de cáñamo; QBM4: harina de quinoa blanca con mezcla de aceites de chía y cáñamo. Letras mayúsculas distintas (A-F) sobre cada barra se refiere a la comparación de los valores de oxidación lipídica al mismo tiempo de almacenamiento entre las diferentes muestras de emulsiones gelificadas. Las letras minúsculas (a-b) sobre cada barra se refieren a la comparación de los valores de oxidación lipídica para la misma muestra de emulsiones gelificadas en diferentes tiempos. Los resultados seguidos de distinta letra representan diferencias significativas (p < 0,05) para cada parámetro según el test post-hoc de Tukey.

Todas las muestras de emulsiones gelificadas analizadas presentaron valores por debajo de los 2,5 mg MDA/kg de muestra para ambos días de almacenamiento (Figura 30), valores no muy elevados teniendo en cuenta la cantidad de ácidos grasos poliinsaturados de los aceites usados. Este hecho, podría deberse a que la cantidad de proteína y polisacáridos de las harinas de pseudocereales podrían influir en un incremento de la viscosidad de la fase continua disminuyendo la difusión del oxígeno a la fase lipídica (McClements & Gumus, 2016). Otra causa de estos resultados podría también residir en la cantidad de compuestos bioactivos de diversa naturaleza que presentan las harinas de pseudocereales, como polifenoles, tocotrienoles y tocoferoles, sustancias con capacidad antioxidante (Chizawa et al., 2019; McClements & Gumus, 2016; Pellegrini, Lucas-González, et al., 2018; Zhu, 2018), cuyo efecto en la reducción de la oxidación lipídica se ha observado en la inclusión de diferentes productos por diversos autores (Antoniewska et al., 2018; Jiménez et al., 2020; Rocchetti et al., 2019). No existe un patrón claro sobre la influencia de la congelación en la oxidación lipídica de las emulsiones gelificadas, existieron muestras que aumentaron su oxidación, en otras muestras que disminuyó y otras que mantuvieron durante la congelación los valores de TBARs.

5.3. INCORPORACIÓN DE EMULSIONES GELIFICADAS EN PRODUCTOS CÁRNICOS FRÉSCOS: HAMBURGUESAS DE TERNERA

Los resultados de este trabajo han sido publicados en la revista LWT-Food Science and Technology, (2022), 161,113416 (Open Access)

En este estudio, el objetivo principal fue evaluar la viabilidad tecnológica de usar emulsiones gelificadas, elaboradas con harina de amaranto y aceite de chía (Chía-EG) y harina de amaranto y aceite de cáñamo

(Cáñamo-EG), como sustituyente de grasa animal de forma parcial, en concreto una sustitución del 25% y del 50% para la producción de hamburguesas de ternera. Se analizó la influencia de esta sustitución sobre las propiedades de cocinado, físico químicas, composición química, perfil lipídico, oxidación lipídica y sensoriales de las hamburguesas sustituidas comparando con una formulación control.

La Tabla 17 muestra los resultados de la composición química de las cinco formulaciones elaboradas de hamburguesas sustituidas tanto en crudo como en cocinado.

Tabla 17. Composición química de hamburguesas crudas y cocinadas con un 25% y 50% de sustitución de grasa animal.

	Muestra	Humedad	Cenizas	Grasa	Proteína
CRUDA	Control	62,39±2,52 ^b	2,33±0,20 ^a	14,46±0,65 ^a	17,47±1,78 ^a
	Burger chía25	65,47±2,48 ^a	2,24±0,20 ^a	12,71±5,96 ^b	18,63±0,36 ^a
	Burger chía50	65,90±0,34 ^a	2,38±0,03 ^a	9,18±0,73 ^c	18,06±0,04 ^a
	Burger cáñamo25	65,08±0,89 ^a	2,22±0,02 ^a	12,64±1,01 ^b	18,43±0,28 ^a
	Burger cáñamo50	65,72±0,64 ^a	2,27±0,04 ^a	9,91±0,49 ^c	18,50±1,41 ^a
COCINADA	Control	55,90±0,23 ^v	2,79±0,07 ^v	16,13±0,16 ^v	23,98±0,06 ^v
	Burger chía25	57,70±0,20 ^w	2,77±0,01 ^v	13,51±0,44 ^w	24,42±0,07 ^v
	Burger chía50	57,68±0,00 ^w	2,82±0,02 ^v	9,32±0,32 ^x	24,45±0,51 ^v
	Burger cáñamo25	57,17±0,29 ^w	2,84±0,05 ^v	13,81±0,68 ^w	24,43±0,12 ^v
	Burger cáñamo50	57,41±0,15 ^w	2,73±0,03 ^v	10,05±0,38 ^x	25,05±0,52 ^v

Los resultados vienen expresados como g/100g. Control: muestra control de las hamburguesas con una formulación tradicional; Burger chía25: muestra con un 25% de emulsiones gelificadas con aceite de chía y harina de amaranto como sustituto de grasa; Burger chía50: muestra con un 50% de emulsión gelificada con aceite de chía y harina de amaranto como sustituto de grasa. Burger cáñamo25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa; Burger cáñamo50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa. Los resultados seguidos de distinta letra minúscula (a-c) representan diferencias significativas ($p < 0,05$) para cada parámetro entre las muestras crudas y para cocinadas (v-x) según el test post-hoc de Tukey. Los datos se presentan como media y DS.

Todas las muestras sustituidas mostraron una disminución del contenido de grasa total ($p < 0,05$) (tanto crudas como cocinadas) y un aumento de la humedad ($p < 0,05$) de forma proporcional a la sustitución, en comparación con la respectiva muestra control (Tabla17). Este aumento del contenido en humedad podría deberse al agua añadida durante la preparación de las emulsiones gelificadas, efecto visto con anterioridad por

otros autores que llevaron a cabo una sustitución de grasa en productos cárnicos (Botella-Martínez et al., 2021; Lucas-González et al., 2020).

La reducción de grasa observada para las distintas muestras sustituidas, tanto crudas como cocinadas, siguió una tendencia similar al aumento de humedad mencionado anteriormente. La disminución del contenido en grasa sucedió de forma dependiente con el aumento de sustitución, pero no mostró dependencia con el tipo de emulsión gelificada utilizada (Tabla 17). Se obtuvo una reducción del contenido de grasa de un 12% cuando se empleó la sustitución del 25% y de un 33% cuando la sustitución fue del 50%, comportamiento similar al observado por otros autores (Alejandre et al., 2017; Barros et al., 2021; Lucas-González et al., 2020). Pudiendo considerarse las hamburguesas con un nivel de sustitución del 50% (independiente de la emulsión empleada) “reducidas en grasa” (Parlamento Europeo, 2006).

Las hamburguesas de ternera mostraron diferencias significativas ($p < 0,05$) en el perfil lipídico según el tipo de grasa utilizada y el nivel de reemplazo de grasa animal (Tabla 18). En general disminuyó la cantidad de ácidos grasos saturados (ácido palmítico y esteárico) y monoinsaturados (ácido oleico) y aumentó la cantidad de ácidos grasos poliinsaturados, especialmente el ácido linoleico (C18:2) y α -linolénico (C18:3) ($p < 0,05$).

Tabla 18. Perfil lipídico de de hamburguesas crudas y cocinadas con un 25% y 50% de sustitución de grasa animal.

% Ácidos grasos	CRUDA					COCINADA				
	Control	Burger chía25	Burger chía50	Burger cáñamo25	Burger cáñamo50	Control	Burger chía25	Burger chía50	Burger cáñamo25	Burger cáñamo50
C 14:0	1,16±0,03 ^{aE}	1,09±0,02 ^{bG}	0,93±0,04 ^{eF}	1,02±0,02 ^{cD}	0,96±0,03 ^{dF}	1,17±0,07 ^{aD}	1,09±0,06 ^{bE}	1,03±0,02 ^{cD}	1,09±0,03 ^{bF}	1,03±0,09 ^{cG}
C 16:0	21,86±0,08 ^{aB}	20,68±0,04 ^{bB}	18,83±0,02 ^{dB}	19,95±0,10 ^{eB}	17,47±0,11 ^{eB}	21,86±0,05 ^{aB}	20,50±0,07 ^{bB}	19,04±0,03 ^{cB}	20,39±0,11 ^{bB}	18,46±0,08 ^{dC}
C16:1 trans	0,48±0,03 ^{aH}	0,43±0,07 ^{bj}	0,38±0,02 ^{dH}	0,41±0,05 ^{cE}	0,31±0,02 ^{eH}	0,46±0,00 ^{aG}	0,41±0,00 ^{bGH}	0,35±0,00 ^{dG}	0,40±0,00 ^{cl}	0,32±0,00 ^{ej}
C16:1 cis	2,07±0,02 ^{aD}	1,95±0,04 ^{bF}	1,66±0,09 ^{dE}	1,78±0,12 ^{cD}	1,51±0,07 ^{eE}	2,04±0,02 ^{aD}	1,80±0,02 ^{cE}	1,67±0,02 ^{dD}	1,89±0,02 ^{bF}	1,63±0,02 ^{eF}
C 17:1	0,35±0,01 ^{al}	0,34±0,01 ^{bj}	0,28±0,01 ^{dH}	0,31±0,01 ^{cF}	0,28±0,01 ^{dH}	0,35±0,01 ^{aH}	0,32±0,01 ^{cH}	0,32±0,01 ^{cG}	0,33±0,01 ^{bj}	0,30±0,01 ^{dJ}
C 18:0	12,44±0,02 ^{aC}	11,36±0,06 ^{bD}	10,22±0,01 ^{dD}	10,55±0,02 ^{cC}	10,25±0,06 ^{dC}	12,12±0,00 ^{aC}	12,04±0,00 ^{abC}	11,30±0,00 ^{cC}	11,49±0,00 ^{bcD}	10,92±0,00 ^{bD}
C 18:1cis	43,15±0,09 ^{aA}	42,89±0,08 ^{bA}	38,40±0,07 ^{dA}	40,07±0,10 ^{cA}	32,55±0,11 ^{eA}	45,22±0,02 ^{aA}	40,07±0,02 ^{cA}	37,15±0,01 ^{dA}	41,97±0,01 ^{bA}	35,82±0,01 ^{eA}
C 18:2 (n-6)	12,59±0,02 ^{dC}	12,63±0,04 ^{dC}	13,60±0,02 ^{cC}	17,39±0,06 ^{bB}	23,72±0,08 ^{aB}	12,15±0,01 ^{eC}	12,51±0,02 ^{adC}	12,94±0,09 ^{cC}	15,68±0,12 ^{bC}	21,32±0,02 ^{aB}
C 18:2 (n-3)	0,07±0,00 ^{cM}	0,07±0,00 ^{cM}	0,06±0,00 ^{cJ}	0,55±0,01 ^{bE}	1,26±0,02 ^{aE}	0,07±0,00 ^{cJ}	0,07±0,00 ^{cK}	0,08±0,00 ^c	0,41±0,02 ^{bl}	1,06±0,02 ^{aG}
C 18:3 (n-3)	0,67±0,02 ^{eG}	3,89±0,02 ^{cE}	8,62±0,02 ^{aE}	2,83±0,02 ^{dD}	5,92±0,02 ^{bD}	0,70±0,02 ^{eE}	5,67±0,03 ^{bD}	12,79±0,04 ^{aC}	2,36±0,02 ^{dE}	5,08±0,03 ^{cE}
C18:3 (n-6)	0,11±0,00 ^{dK}	0,13±0,01 ^{cL}	0,09±0,00 ^{eI}	0,17±0,01 ^{bF}	0,43±0,01 ^{aG}	0,13±0,00 ^{bl}	0,14±0,00 ^{al}	0,14±0,00 ^{al}	0,13±0,00 ^b	0,14±0,00 ^{aK}
C 20:0	0,21±0,00 ^{eJ}	0,22±0,00 ^{dK}	0,23±0,00 ^{cH}	0,31±0,00 ^{bF}	0,45±0,01 ^{aG}	0,23±0,02 ^{dH}	0,24±0,02 ^{cH}	0,24±0,02 ^{cH}	0,29±0,02 ^{bj}	0,40±0,02 ^{al}
C 20:1	0,96±0,01 ^{bF}	0,96±0,01 ^{bH}	0,89±0,01 ^{cE}	0,99±0,01 ^{aD}	0,72±0,01 ^{dF}	1,05±0,01 ^{aD}	0,85±0,01 ^{bF}	0,67±0,01 ^{dE}	0,87±0,01 ^{bG}	0,77±0,01 ^{cH}
C20:2 (n-11)	0,60±0,01 ^{aG}	0,59±0,01 ^{abl}	0,53±0,01 ^{cG}	0,58±0,01 ^{bE}	0,43±0,01 ^{dG}	0,58±0,01 ^{aF}	0,53±0,01 ^{bG}	0,41±0,01 ^{cF}	0,54±0,01 ^{bH}	0,41±0,01 ^{cl}
C20:3 (n-11)	0,30±0,01 ^{bl}	0,40±0,01 ^{aj}	0,29±0,01 ^{cH}	0,29±0,01 ^{cF}	0,40±0,01 ^{aG}	0,39±0,02 ^{dG}	0,48±0,02 ^{aG}	0,41±0,02 ^{cF}	0,48±0,02 ^{aH}	0,46±0,02 ^{bl}
ΣAGS	35,89±0,13^a	33,19±0,07^b	30,85±0,03^d	32,50±0,02^c	29,90±0,02^e	36,20±0,03^a	34,71±0,05^b	32,46±0,03^d	34,09±0,01^c	31,65±0,01^e
ΣAGMI	49,51±0,17^a	46,84±0,05^b	41,73±0,07^d	45,44±0,04^c	37,59±0,06^e	49,31±0,10^a	45,38±0,04^c	40,44±0,02^d	45,80±0,08^b	39,23±0,07^e
ΣAGPI	14,56±0,17^e	17,96±0,06^d	27,41±0,08^b	22,07±0,03^c	32,52±0,06^a	14,28±0,01^d	19,67±0,16^c	27,03±0,08^b	19,89±0,13^c	28,80±0,06^a
Σn3	0,74±0,04^e	3,96±0,03^c	12,68±0,06^a	3,39±0,02^d	7,18±0,03^b	0,77±0,04^e	5,75±0,05^c	12,87±0,08^a	2,78±0,02^d	6,14±0,02^b
Σn6	12,69±0,02^d	12,76±0,05^d	13,69±0,03^c	17,56±0,02^b	24,16±0,05^a	12,28±0,02^d	12,65±0,03^d	13,08±0,04^c	15,81±0,02^b	21,47±0,06^a

Resultados expresados en g/100 g de grasa. Control: muestra control de las hamburguesas con una formulación tradicional; Burger chía25: muestra con un 25% de emulsión gelificada con aceite de chía y harina de amaranto como sustituto de grasa; Burger chía50: muestra con un 50% de emulsión gelificada con aceite de chía y harina de amaranto como sustituto de grasa. Burger cáñamo25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa; Burger cáñamo50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa. AGS: ácidos grasos saturados; AGMI: ácidos grasos monoinsaturados; AGPI: ácidos grasos poliinsaturados. Los resultados seguidos de distinta letra mayúscula (A-N) hace referencia a la comparación de los diferentes ácidos grasos sobre la misma muestra y las letras minúsculas (a-e) hace referencia a la comparación del mismo ácido graso sobre las distintas muestras según el test post-hoc de Tukey. Los datos se presentan como media y DS.

La Tabla 18, muestra una dependencia de los resultados del perfil lipídico con el perfil que presentaron los aceites usados para elaborar las emulsiones gelificadas, de modo que las hamburguesas con sustitución de la Chía-EG destacaron por su contenido en ácidos grasos omega 3 (ácido α -linolénico) mientras que las hamburguesas con Cáñamo-EG mostraron mayor cantidad de ácidos grasos omega 6 (ácido linoleico) ($p < 0.05$). Siguiendo los reclamos nutricionales de del Parlamento Europeo, las hamburguesas crudas y cocinadas con un 50% de reemplazo con Chía-EG y las cocinadas con un reemplazo del 25% con Chía-EG podrían etiquetarse con la declaración nutricional de "altas en ácidos grasos omega 3", ya que presentaron más de 0,6 g de ácido α -linolénico por 100 g de producto (Parlamento Europeo, 2006).

La sustitución de grasa animal por emulsiones gelificadas en las hamburguesas de ternera mejoró varios índices de salud. La proporción ácidos grasos poliinsaturados/ácidos grasos saturados (AGPI/AGS) aumentó, cumpliendo con las recomendaciones ($> 0,4$) (Wu et al., 2020), y el índice omega 6/omega 3 disminuyó, siendo aceptable en todas las hamburguesas reformuladas excepto en aquella con una sustitución del 25% con Cáñamo-EG. Los índices trombogénico y aterogénico disminuyeron, y la proporción h/H aumentó, especialmente con un 50% de sustitución y el uso de Cáñamo-EG. Estos cambios indican una mejora en las propiedades saludables de las hamburguesas reformuladas, resultados que están en concordancia con estudios previos sobre el uso de aceites vegetales en productos cárnicos (Barros et al., 2021; Botella-Martinez et al., 2021; Pires et al., 2019).

La oxidación lipídica es un parámetro responsable de la calidad y el deterioro de la carne y los productos cárnicos (Lima et al., 2013), por ello se decidió medir la oxidación lipídica en los productos con sustitución de grasa para ver el efecto que provocaba la sustitución en este parámetro y comparar sus cambios con la muestra control (Figura 31). Las muestras de hamburguesas sustituidas, tanto crudas como cocinadas, presentaron valores mayores de oxidación (TBARs) que la muestra control ($p < 0,05$). Además, se vio un aumento en los valores de oxidación lipídica a medida que la sustitución aumentaba ($p < 0,05$) cuando se incorporó la emulsión gelificada Chía-EG. Las muestras a las cuales se les incorporó Cáñamo-EG como sustituto de grasa animal, presentaron una menor susceptibilidad a la oxidación lipídica frente a las que se les sustituyó por Chía-EG. Las hamburguesas crudas con un 50% de sustitución con Chía-EG presentaron valores de TBARs 3,5 veces superiores a la muestra control cruda (Figura 31). Todas las muestras sustituidas fueron susceptibles al tratamiento térmico (cocinado) a excepción de la muestra control que mantuvo los valores de TBARs ($p > 0,05$). Las muestras de mayor susceptibilidad fueron las que contenían Chía-EG, presentando un aumento del 52% y del 58% para las muestras con un 25% y un 50% de sustitución con Chía-EG respectivamente. Llegando la muestra con un 50% de sustitución con Chía-EG a niveles de oxidación detectables por el consumidor (> 2 mg MDA/kg muestra) (Trindade et al., 2009).

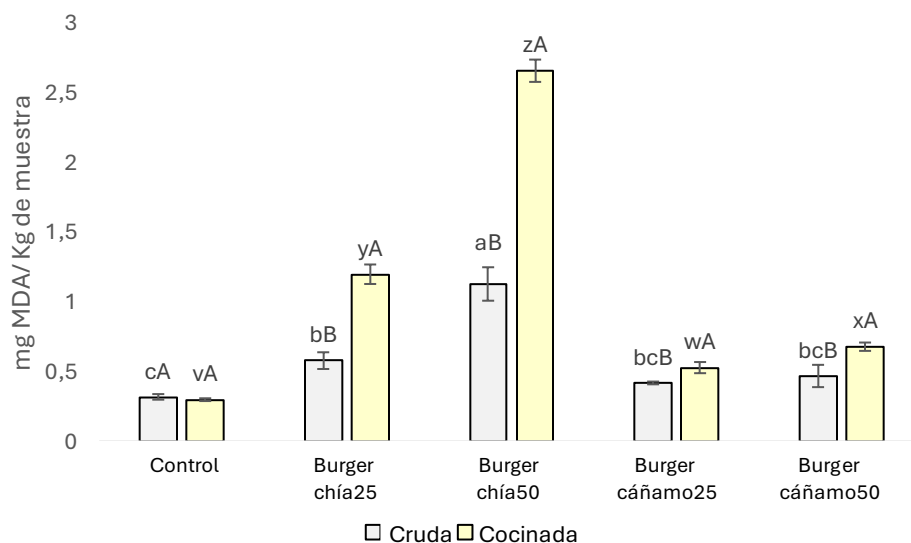


Figura 31. Oxidación lipídica (TBARs; mg malonaldehído/kg de muestra) de hamburguesas crudas y cocinadas con un 25% y 50% de sustitución de grasa animal. Control: muestra control de las hamburguesas con una formulación tradicional; Burger chía25: muestra con un 25% de emulsión gelificada con aceite de chía y harina de amaranto como sustituto de grasa; Burger chía50: muestra con un 50% de emulsión gelificada con aceite de chía y harina de amaranto como sustituto de grasa. Burger cáñamo25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa; Burger cáñamo50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa. Letras en mayúscula (A-B) sobre cada barra del histograma se refiere a la comparación de los valores de TBARs en la misma muestra con distinto tratamiento térmico (cocinadas o crudas) y la letras minúsculas (a-c) a la comparación del mismo tratamiento sobre las distintas muestra cruda; (v-z) para las muestras cocinadas según el test post-hoc de Tukey.

La evaluación sensorial es un parámetro importante que afecta a la decisión de compra del consumidor (Grasso et al., 2022), en concreto en hamburguesas crudas se decidió que esos parámetros a evaluar fueran la apariencia visual del producto, el color y el aroma a rancio (Tabla 19). Todos esos parámetros no presentaron diferencias significativas ($p > 0,05$) entre los 5 lotes de hamburguesas de ternera. Estos resultados estuvieron en concordancia con los resultados obtenidos de los parámetros de color, los cuales mostraron que no existían diferencias ($p > 0,05$) en los parámetros h^* , L^* y b^* , derivando en la no existencia de diferencias significativas ($p > 0,05$) en el parámetro diferencias de color (< 3 unidades).

Tabla 19. Evaluación sensorial de hamburguesas crudas con un 25% y 50% de sustitución de grasa animal.

	Color	Aroma a rancio	Apariencia visual
Control	5,62±1,32 ^a	4,94±2,30 ^a	3,66±2,31 ^a
Burger chía25	6,44±1,61 ^a	4,95±2,40 ^a	4,27±1,72 ^a
Burger chía50	5,83±0,73 ^a	5,36±2,01 ^a	3,74±2,41 ^a
Burger cáñamo25	5,11±1,61 ^a	3,93±1,62 ^a	3,75±2,32 ^a
Burger cáñamo50	6,55±1,02 ^a	4,41±2,30 ^a	4,57±2,61 ^a

Control: muestra control de las hamburguesas con una formulación tradicional; Burger chía25: muestra con un 25% de emulsión gelificada con aceite de chía y harina de amaranto como sustituto de grasa; Burger chía50: muestra con un 50% de emulsión gelificada con aceite de chía y harina de amaranto como sustituto de grasa. Burger cáñamo25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa; Burger cáñamo50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa. Para cada parámetro, los resultados seguidos de la misma letra no presentaron diferencias significativas según el test de Tukey ($p > 0,05$). Los datos se presentan como media y DS.

En el caso de las muestras cocinadas, se evaluaron la masticabilidad, la jugosidad, la sensación de grasa, la granulosidad y se realizó una pregunta final de aceptabilidad general (Figura 32). Solo se presentaron diferencias ($p < 0,05$) entre las 5 muestras evaluadas para la granulosidad, los panelistas señalaron que las muestras con un 25% de sustitución (Burger chía25 y Burger cáñamo25) fueron las que presentaban una granulosidad mayor, sin diferencias entre ellas ($p > 0,05$) y la que menor valoración para este atributo presentó fue la muestra control (Figura 32).

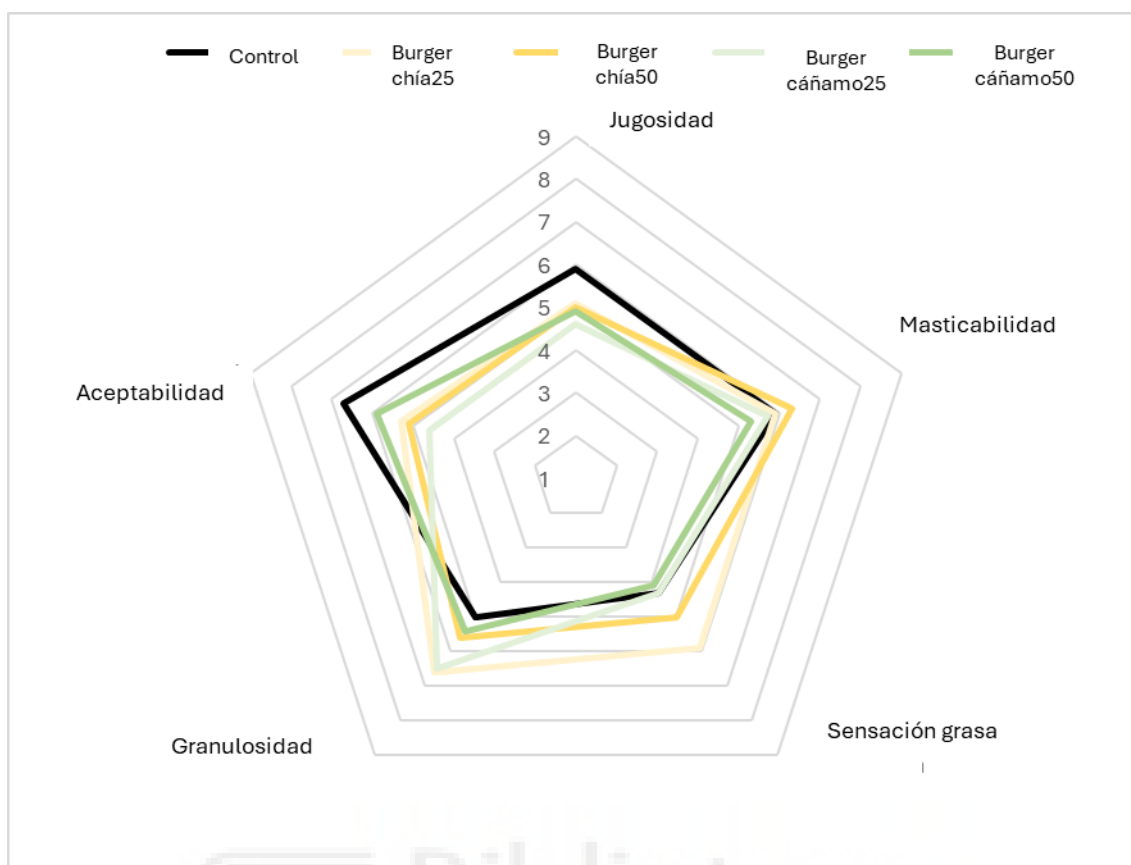


Figura 32. Evaluación sensorial de hamburguesas cocinadas con un 25% y 50% de sustitución de grasa animal.

Control: muestra control de las hamburguesas con una formulación tradicional; Burger chía25: muestra con un 25% de emulsión gelificada con aceite de chía y harina de amaranto como sustituto de grasa; Burger chía50: muestra con un 50% de emulsión gelificada con aceite de chía y harina de amaranto como sustituto de grasa. Burger cáñamo25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa; Burger cáñamo50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de amaranto como sustituto de grasa.

Estos resultados están en concordancia con los valores de textura obtenidos por las muestras en las cuales únicamente hubo diferencias en la cohesividad. En cuanto a la aceptabilidad general, la muestra control seguida de la muestra con un 50% de sustitución de Cáñamo-EG fueron las que obtuvieron mayores puntuaciones. Estos resultados podrían verse influenciados por el conocimiento del perfil nutricional de las hamburguesas reformuladas, lo cual pudo influir en la percepción de su atractivo sensorial por parte de los consumidores (Siegrist, 2008).

5.4. ESTABILIDAD A LA CONGELACIÓN DE HAMBURGUESAS DE TERNERA CON INCORPORACIÓN DE EMULSIONES GELIFICADAS

Los resultados de este trabajo han sido publicados en la revista International Journal of Food Science and Technology, (2022), 58(6), 3234-3243 (Open Access).

Debido a que las hamburguesas son uno de los productos cárnicos más populares y consumidos (GVR, 2020). El objetivo innovador de la industria alimentaria en cuanto a este tipo de productos es hacerlas más saludables, principalmente reduciendo o modificando el contenido en ácidos grasos saturados (FAO, 2010). Una de las formas más comunes de comercialización de las hamburguesas de ternera es de forma congelada, representando el 68% del mercado global de hamburguesas envasadas (Orehov, 2019). Por ello, las hamburguesas congeladas deben ser formuladas teniendo en cuenta su gran susceptibilidad y modificaciones físicas durante la congelación evitando al máximo que la sustitución de grasa animal por emulsiones gelificadas afecte en su vida útil (Degner et al., 2014). En vista de todo lo anterior, el objetivo de este estudio fue evaluar la sustitución parcial de grasa de cerdo con emulsiones gelificadas a base de harina de amaranto con aceite de chía y cáñamo en hamburguesas de ternera y ver si esta sustitución podría afectar a la estabilidad de las hamburguesas en un almacenamiento a $-23\text{ }^{\circ}\text{C}$ durante 60 días.

La evolución de los principales ácidos grasos para las hamburguesas crudas y cocinadas durante el tiempo de congelación se muestra en la Figura 33.

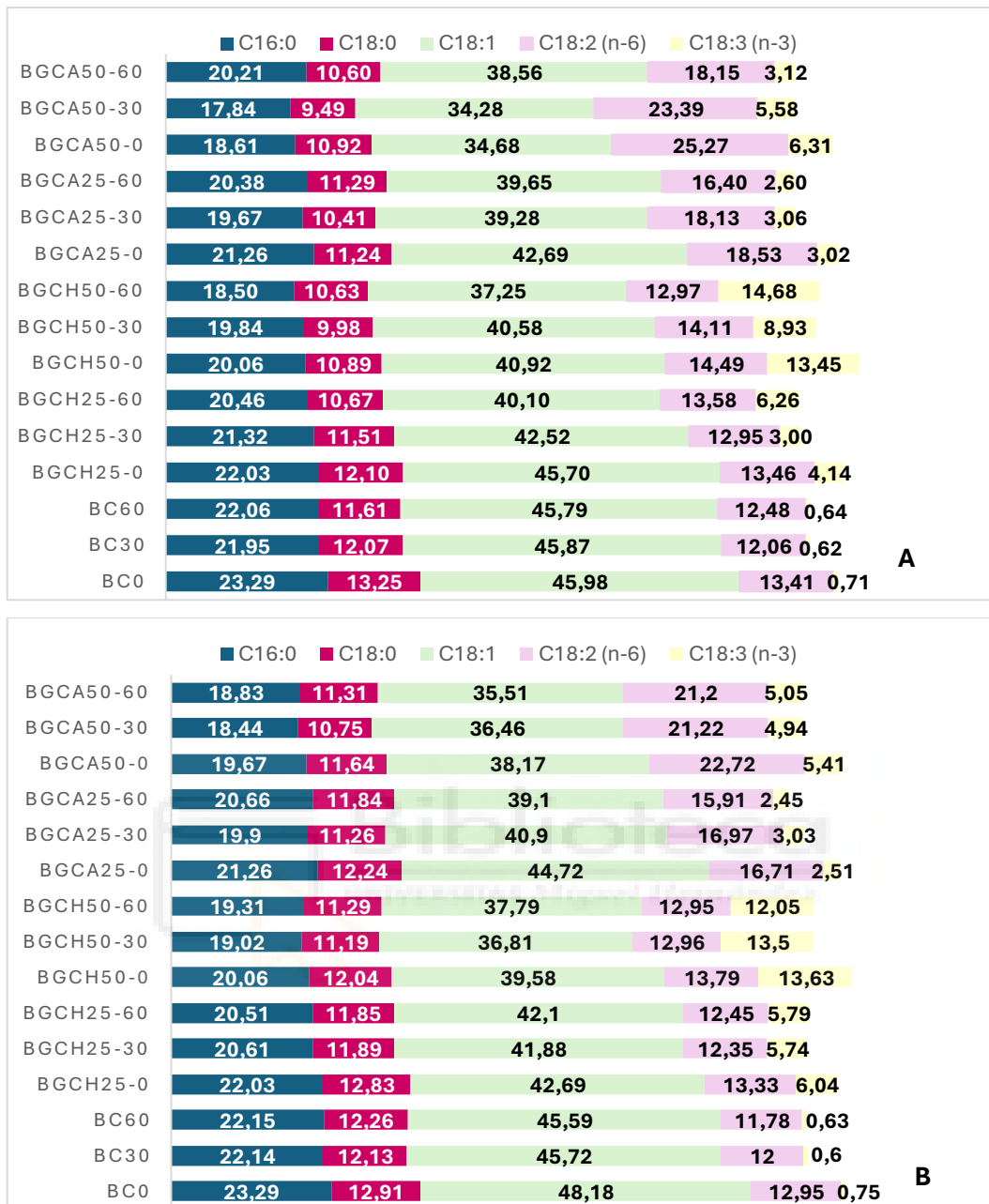


Figura 33. (A) Ácidos grasos mayoritarios de las hamburguesas crudas sustituidas y su evolución a lo largo del tiempo de congelación. **(B)** Ácidos grasos mayoritarios de las hamburguesas cocinadas sustituidas y su evolución a lo largo del tiempo de congelación.

BGCA50: hamburguesa con un 50% de sustitución con la emulsión gelificada de amaranto y aceite de cáñamo; BGCA25: hamburguesa con un 25% de sustitución con la emulsión gelificada de amaranto y aceite de cáñamo. BGCH50: hamburguesa con un 50% de sustitución con la emulsión gelificada de amaranto y aceite de chía; BGCH25: hamburguesa con un 25% de sustitución con la emulsión gelificada de amaranto y aceite de chía. BC: hamburguesa control sin reemplazo de grasa animal.

Las propiedades de cocinado son parámetros importantes para percibir la aceptabilidad de los consumidores teniendo efectos indeseados. Durante el cocinado las pérdidas por cocinado se dan debido a una transferencia de masa (agua y grasa), también se dan debido a la disminución de la capacidad de retención de agua por parte de los productos cárnicos, por la desnaturalización de las proteínas de la carne influyendo esto en la contracción del producto cárnico (Domínguez et al., 2014; Vaskoska et al., 2020). La adición de las emulsiones gelificadas puede influir en las propiedades de cocinado, esto se presenta en la Tabla 20 ya que en los tiempos inicial y final de almacenamiento en congelación (0 y 60 días) no se observó un aumento en las pérdidas por cocinado ($p > 0,05$), siendo mayores las pérdidas a día 30 que a 60. Esto podría explicarse debido a la formación de cristales de hielo durante el almacenamiento en congelación, lo cual puede causar daño físico en la estructura celular de la carne, facilitando esto las pérdidas por cocinado. También, podría estar aumentando la oxidación de las proteínas la inclusión de las emulsiones gelificadas en el producto cárnico, lo cual favorece un aumento de las pérdidas por cocinado respecto a la hamburguesa control.

Tabla 20. Propiedades de cocinado de las hamburguesas a lo largo del periodo de congelación.

Parámetro	Muestra	0	30	60
Pérdidas por cocinado (%)	BC	19,34±0,30 ^{cC}	22,89±0,09 ^{aD}	20,92±0,13 ^{bD}
	BGCH25	21,63±0,47 ^{cBC}	24,93±0,12 ^{aC}	22,50±0,22 ^{bB}
	BGCH50	24,26±0,56 ^{bAB}	28,03±0,24 ^{aA}	22,52±0,13 ^{bB}
	BGCA25	27,13±0,32 ^{aA}	25,33±0,11 ^{bB}	21,96±0,23 ^{cC}
	BGCA50	25,09±0,95 ^{aA}	25,31±0,02 ^{aB}	26,19±0,05 ^{aA}
Encogimiento (%)	BC	19,55±0,96 ^{bC}	20,83±0,12 ^{aD}	16,67±0,23 ^{cB}
	BGCH25	21,64±1,78 ^{aB}	26,27±0,25 ^{aB}	24,99±0,18 ^{aA}
	BGCH50	21,41±0,45 ^{aB}	31,14±1,02 ^{aA}	24,86±0,35 ^{aA}
	BGCA25	25,75±1,81 ^{aA}	24,77±0,08 ^{aC}	24,93±0,14 ^{aA}
	BGCA50	24,19±1,67 ^{aA}	20,77±0,15 ^{aD}	24,89±0,20 ^{aA}
Aumento de espesor (%)	BC	8,13±0,53 ^{aC}	9,50±0,43 ^{aD}	11,18±1,51 ^{aD}
	BGCH25	12,92±0,42 ^{cB}	23,33±0,14 ^{bA}	26,15±0,16 ^{aB}
	BGCH50	11,07±0,90 ^{cB}	21,50±0,33 ^{bB}	31,67±0,42 ^{aA}
	BGCA25	13,02±0,20 ^{bA}	14,57±0,12 ^{aC}	15,57±1,03 ^{aC}
	BGCA50	13,81±0,69 ^{cA}	15,56±0,44 ^{bC}	25,71±0,10 ^{aB}

Tiempo de almacenamiento expresado en días. BGCA50: hamburguesa con un 50% de sustitución con la emulsión gelificada de amaranto y aceite de cáñamo; BGCA25: hamburguesa con un 25% de sustitución con la emulsión gelificada de amaranto y aceite de cáñamo. BGCH50: hamburguesa con un 50% de sustitución con la emulsión gelificada de amaranto y aceite de chía; BGCH25: hamburguesa con un 25% de sustitución con la emulsión gelificada de amaranto y aceite de chía. BC: hamburguesa control sin reemplazo de grasa animal. Letras en minúscula (a-c) se refiere a la comparación de los valores de cada parámetro para la misma muestra en diferente tiempo de almacenamiento en congelación y las letras mayúsculas (A-D) se refiere a la comparación de los valores de cada parámetro para diferentes muestras en el mismo tiempo de almacenamiento según el test post-hoc de Tukey ($p < 0,05$). Los datos se presentan como media y DS.

La Figura 34 muestra la evolución de la oxidación lipídica a lo largo de la conservación en congelación, se observaron cambios significativos ($p < 0,05$) en los niveles de TBARs durante el almacenamiento congelado en las hamburguesas reformuladas con emulsiones gelificadas a base de aceite de chía y cáñamo tanto en las muestras crudas como en las cocinadas. Se encontró que las hamburguesas reformuladas con los niveles de sustitución de grasa más altos (50% de sustitución por Cáñamo-EG y 50 % de sustitución por Chía-EG) presentaron niveles significativamente más altos de TBARs al final del almacenamiento congelado (día 60) que las hamburguesas con niveles más bajos de sustitución independientemente del tratamiento térmico (Figura 34).

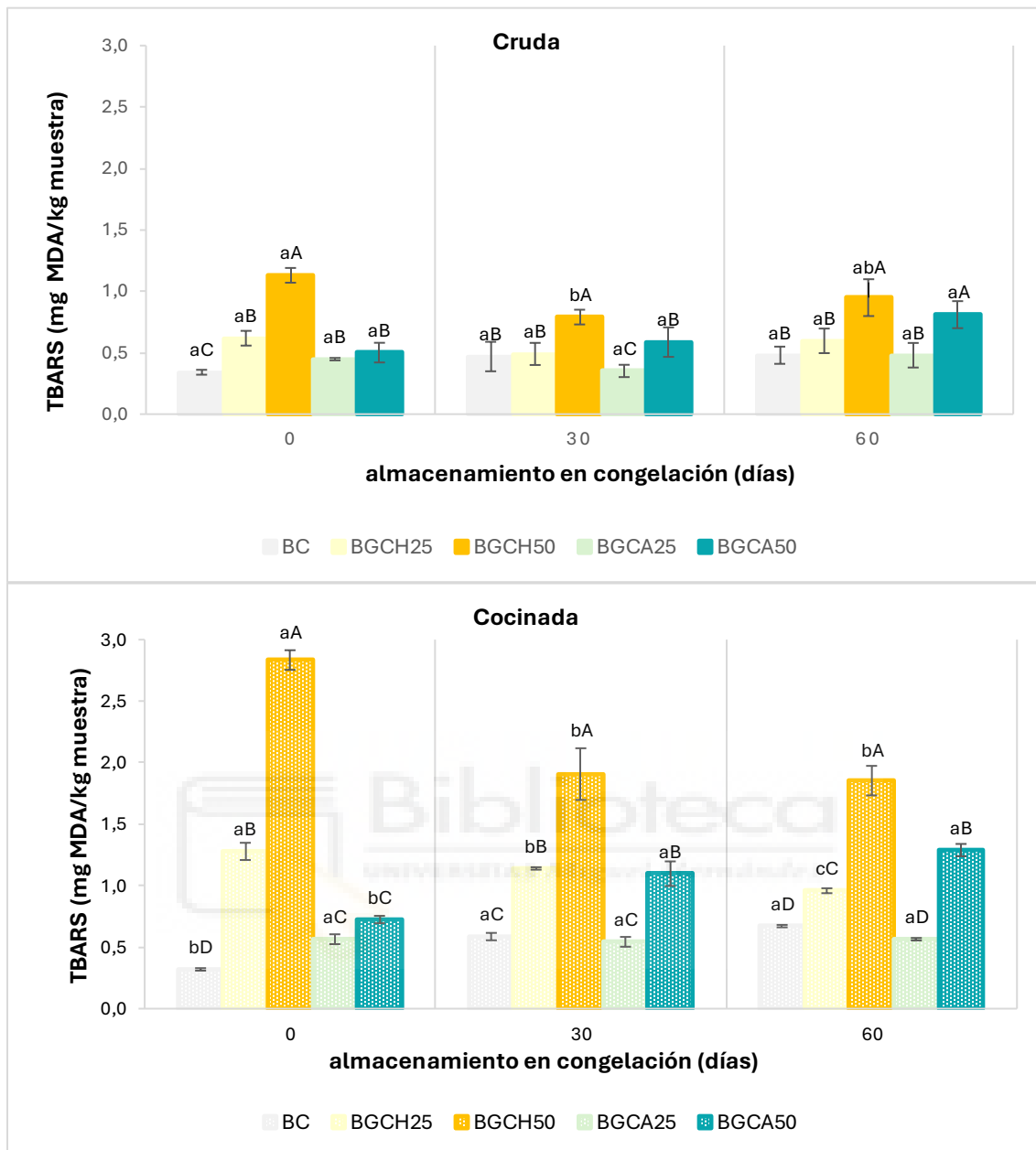


Figura 34. (A) Evolución de los valores de oxidación lipídica en hamburguesas crudas sustituidas a lo largo del tiempo de congelación. **(B)** Evolución de los valores de oxidación lipídica en hamburguesas cocinadas sustituidas a lo largo del tiempo de congelación.

BGCA50: hamburguesa con un 50% de sustitución con la emulsión gelificada de amaranto y aceite de cáñamo; BGCA25: hamburguesa con un 25% de sustitución con la emulsión gelificada de amaranto y aceite de cáñamo. BGCH50: hamburguesa con un 50% de sustitución con la emulsión gelificada de amaranto y aceite de chía; BGCH25: hamburguesa con un 25% de sustitución con la emulsión gelificada de amaranto y aceite de chía. BC: hamburguesa control sin reemplazo de grasa animal. Letras en mayúscula (A-D) se refiere a la comparación de los valores de TBARS para las distintas muestras en el mismo tiempo de almacenamiento en congelación y las letras minúsculas (a-c) a la comparación de los valores de TBARS para la misma muestra a lo largo de los distintos tiempos de almacenamiento según el test post-hoc de Tukey ($p < 0,05$). Los datos se presentan como media y DS.

En las figuras se ve claramente que la hamburguesa con sustitución del 50% de Chía-EG superó tras cocinarse únicamente a tiempo 0 el límite de aceptabilidad de TBARs de 2 mg MDA/kg de muestra publicado por (Campo et al., 2006). Lo que significa que probablemente los consumidores detectarían sabores y olores rancios en esta muestra debido a esos niveles de mg de MDA/kg de muestra alcanzados. Esto nos indica la susceptibilidad de este aceite y estos valores se encuentran en concordancia con los obtenidos en la oxidación lipídica de las emulsiones gelificadas del apartado 5.2.2.2.

5.5. INCORPORACIÓN DE EMULSIONES GELIFICADAS EN EMBUTIDOS COCIDOS: SALCHICHAS TIPO FRANKFURT

Los resultados de este trabajo han sido publicados en la revista Foods (2021), 10, 1681 (Open Access).

Las salchichas tipo Frankfurt son un producto cárnico ampliamente consumido en multitud de regiones, debido a su precio, su sabor, y su conveniencia (Fernández-López et al., 2019). Por otro lado, este tipo de productos suele presentar una cantidad de grasa elevada, algunas veces cercana al 40%. Por ello para mejorar el perfil lipídico de estos productos cárnicos y reducir la cantidad de grasa se propuso el presente estudio donde se reemplazó en un 25%, 50%, 75% y 100% (S25, S50, S75, S100) la cantidad de grasa animal por una emulsión gelificada a base de trigo sarraceno y aceite de cáñamo para estudiar la viabilidad del producto así como la influencia en la composición química, el perfil lipídico, las propiedades físico-químicas, estabilidad de la emulsión, estabilidad lipídica y las propiedades sensoriales de las salchichas tipo Frankfurt reformuladas (S25, S50, S75 y

S100) en comparación con una muestra de salchicha tipo Frankfurt elaborada con una formulación tradicional (SC).

Entre los resultados más destacables se vio que la incorporación de la emulsión gelificada no afectó a la estabilidad de la emulsión (%FET) ni siquiera cuando el remplazo fue total (S100) ($p > 0,05$) (Figura 35).

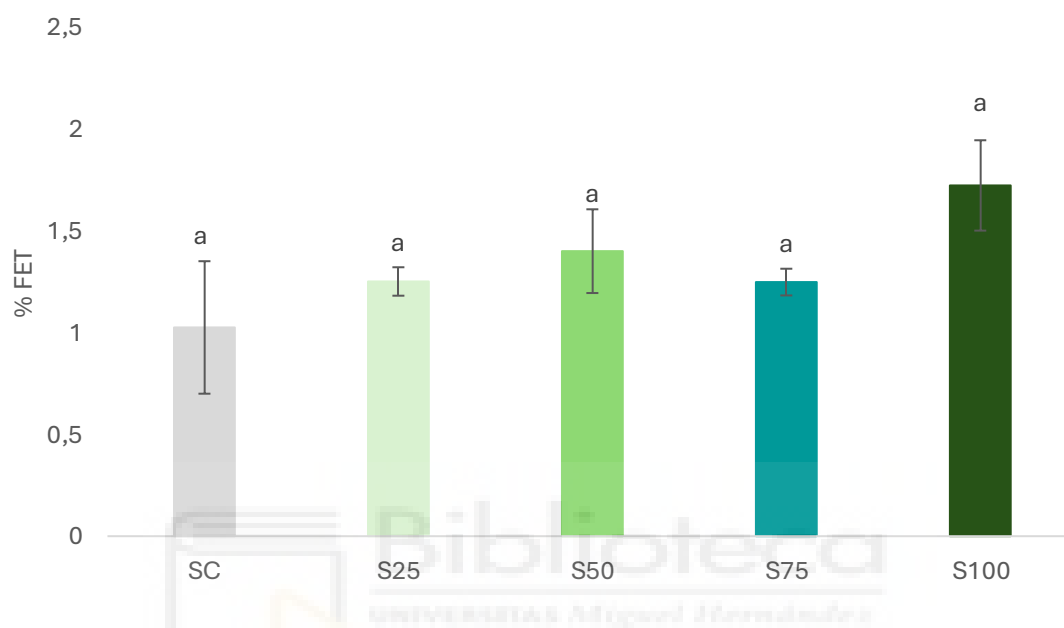


Figura 35. Estabilidad de la emulsión (%fluido expelido total) de las salchichas tipo Frankfurt con sustitución de grasa animal por una emulsión gelificada con aceite de cáñamo y trigo sarraceno.

SC: muestra de salchicha tipo Frankfurt control con una formula tradicional; S25: muestra de salchicha tipo Frankfurt con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S50: muestra de salchicha tipo Frankfurt con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S75: muestra de salchicha tipo Frankfurt con un 75% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S100: muestra de salchicha tipo Frankfurt con un 100% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno. Misma letra minúscula encima de cada barra indica que no existen diferencias significativas entre las diferentes muestras para la misma propiedad (estabilidad de la emulsión), según el test de Tukey ($p > 0,05$).

En la composición química de las salchichas tipo Frankfurt, viene recogida en la Tabla 21, en ella se observa la misma tendencia que se vio en las hamburguesas de ternera sustituidas, un aumento en la cantidad de humedad ($p < 0,05$) a medida que aumentaba la sustitución y una disminución en el contenido de grasa total ($p < 0,05$) relacionado con el aumento del remplazo de grasa animal por la emulsión gelificada. Esta

disminución del contenido de grasa va desde el 17% para la muestra con una sustitución del 25% de grasa animal (S25) hasta el 39% para aquella con un reemplazo total de grasa animal (S100), siendo mayor a medida que lo hace el nivel de reemplazo.

Tabla 21. Composición química de las salchichas tipo Frankfurt con sustitución de grasa animal por una emulsión gelificada con aceite de cáñamo y trigo sarraceno.

	Humedad	Cenizas	Grasa	Proteína
SC	59,66±0,03 ^d	2,34±0,15 ^a	20,75±0,28 ^a	14,59±0,25 ^a
S25	61,65±0,56 ^c	2,15±0,07 ^a	17,20±0,32 ^b	14,17±0,19 ^{ab}
S50	64,94±0,03 ^{ab}	2,14±0,14 ^a	17,02±0,63 ^b	12,61±0,10 ^d
S75	64,87±0,10 ^b	3,11±1,47 ^a	14,78±0,09 ^c	13,48±0,22 ^{bc}
S100	65,81±0,02 ^a	2,39±0,04 ^a	12,69±0,10 ^d	13,41±0,15 ^c

Resultados expresados en g/100 g. SC: muestra de salchicha tipo Frankfurt control con una formula tradicional; S25: muestra de salchicha tipo Frankfurt con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S50: muestra de salchicha tipo Frankfurt con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S75: muestra de salchicha tipo Frankfurt con un 75% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S100: muestra de salchicha tipo Frankfurt con un 100% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno. Los resultados seguidos de distinta letra minúscula (a-d) representan diferencias significativas ($p < 0,05$) para cada parámetro entre las diferentes muestras según el test post-hoc de Tukey. Los datos se presentan como media y DS.

En las propiedades físico-químicas, los parámetros de color presentan mucha variabilidad para todas las muestras en los valores de L*, pero existe una tendencia clara en los parámetros de a* y b*, por ejemplo, los valores de a* disminuyen ($p < 0,05$) a medida que la sustitución aumenta y sin embargo con los valores de b* sucede al contrario. Todo este conjunto de coordenadas CIEL*a*b* dan lugar a que las diferencias de color calculadas para cada una de las muestras con diferente nivel de sustitución de la grasa fueran mayores ($p < 0,05$) a media que la sustitución aumentaba referidas a la muestra control (SC). Las propiedades de textura no se vieron prácticamente afectadas por la sustitución de la grasa animal por la emulsión gelificada, únicamente la elasticidad de la muestra con un 50% de sustitución

(S50) mostró diferencias significativas ($p < 0,05$) respecto a elasticidad presentada por la muestra control (SC).

Tabla 22. Parámetros de color de las salchichas tipo Frankfurt con sustitución de grasa animal por una emulsión gelificada con aceite de cáñamo y trigo sarraceno.

	L*	a*	b*	C*	h	ΔE^*
SC	72,70±0,63 ^a	3,42±0,35 ^a	9,15±0,24 ^e	9,77±0,30 ^e	69,53±1,71 ^e	-
S25	70,42±0,87 ^{bc}	2,88±0,19 ^b	11,32±0,42 ^d	11,68±0,44 ^d	75,70±0,58 ^d	3,30±0,47 ^d
S50	69,53±1,29 ^c	2,80±0,25 ^b	13,06±1,72 ^c	13,36±1,71 ^c	77,72±1,46 ^c	5,36±1,27 ^c
S75	70,99±0,90 ^b	1,21±0,47 ^c	15,23±0,31 ^b	15,29±0,30 ^b	85,45±1,79 ^b	6,77±0,24 ^b
S100	72,18±0,27 ^a	0,38±0,16 ^d	17,01±0,33 ^a	17,01±0,33 ^a	88,73±0,54 ^a	8,45±0,33 ^a

SC: muestra de salchicha tipo Frankfurt control con una formula tradicional; S25: muestra de salchicha tipo Frankfurt con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S50: muestra de salchicha tipo Frankfurt con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S75: muestra de salchicha tipo Frankfurt con un 75% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S100: muestra de salchicha tipo Frankfurt con un 100% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno. Los resultados seguidos de distinta letra minúscula (a-e) representan diferencias significativas ($p < 0,05$) para cada parámetro entre las distintas muestras según el test post-hoc de Tukey. Los datos se presentan como media y DS.

En el análisis del perfil de los ácidos grasos (Tabla 23), la muestra control presentó como ácidos grasos principales el palmítico, oleico y esteárico, lo que representa el 80% de los ácidos grasos totales de esta formulación. En las salchichas tipo Frankfurt con sustitución de grasa animal por parte de la emulsión gelificada, se vio una disminución de la cantidad del oleico, palmítico y esteárico junto a un aumento del ácido graso linoleico. Ya que es el ácido graso predominante del aceite de cáñamo. Esta disminución de los ácidos grasos saturados, ácidos grasos monoinsaturados y el aumento del ácido graso linoleico y del α -linolénico siguió la misma tendencia que la sustitución de grasa en las salchichas tipo Frankfurt.

Tabla 23. Efecto sobre la sustitución de grasa animal por una emulsión gelificada de aceite de cáñamo y harina de trigo sarraceno sobre el perfil lipídico de las salchichas tipo Frankfurt.

Ácido Graso	SC	S25	S50	S75	S100
C 14:0	1,27±0,02 ^{aE}	1,13±0,02 ^{bG}	0,95±0,02 ^{cG}	0,67±0,01 ^{dJ}	0,37±0,00 ^{eJ}
C 16:0	22,92±0,03 ^{aB}	21,10±0,05 ^{bB}	18,26±0,21 ^{cC}	14,39±0,09 ^{dC}	9,96±0,13 ^{eC}
C 16:1cis	2,21±0,01 ^{aD}	1,96±0,05 ^{bF}	1,53±0,01 ^{cF}	1,04±0,00 ^{dH}	0,54±0,01 ^{eI}
C 17:0	0,38±0,01 ^{aG}	0,36±0,01 ^{bJ}	0,31±0,00 ^{cJ}	0,25±0,00 ^{dL}	0,17±0,00 ^{eL}
C 17:1	0,34±0,00 ^{aH}	0,31±0,00 ^{aJ}	0,25±0,02 ^{abJ}	0,19±0,00 ^{bM}	0,07±0,04 ^{cM}
C 18:0	10,94±0,04 ^{aC}	10,36±0,03 ^{bD}	9,47±0,02 ^{cD}	7,07±0,02 ^{dE}	4,44±0,07 ^{eD}
C 18:1	46,71±0,10 ^{aA}	40,45±0,55 ^{bA}	32,38±0,08 ^{cA}	23,53±0,10 ^{dB}	15,56±0,44 ^{eB}
C 18:2 (n6)	11,56±0,01 ^{eC}	17,51±0,38 ^{dC}	25,54±0,13 ^{cB}	35,09±0,01 ^{bA}	45,62±0,06 ^{aA}
C 18:2(n3)	0,09±0,01 ^{eJ}	0,65±0,04 ^{dH}	1,45±0,02 ^{cF}	2,31±0,00 ^{bF}	3,30±0,00 ^{bE}
C 18:3 (n3)	0,68±0,01 ^{eF}	3,29±0,18 ^{dE}	6,79±0,17 ^{cE}	10,81±0,00 ^{bD}	15,38±0,00 ^{aB}
C18:3 (n6)	ND	0,22±0,02 ^{dK}	0,50±0,03 ^{cH}	0,84±0,00 ^{bI}	1,22±0,01 ^{aF}
C 20:0	0,20±0,00 ^{eI}	0,31±0,00 ^{dJ}	0,46±0,01 ^{cH}	0,61±0,00 ^{bJ}	0,75±0,02 ^{aH}
C 20:1	1,10±0,03 ^{aE}	0,95±0,01 ^{bG}	0,84±0,01 ^{cG}	0,67±0,00 ^{dJ}	0,55±0,00 ^{eI}
C20:2	0,54±0,01 ^{aF}	0,47±0,00 ^{bI}	0,40±0,01 ^{cI}	0,32±0,00 ^{dK}	0,24±0,01 ^{eK}
C20:3	0,31±0,01 ^{aH}	0,30±0,00 ^{abJ}	0,27±0,00 ^{abJ}	0,25±0,01 ^{bCL}	0,20±0,01 ^{cK}
n3	0,98±0,02 ^{eG}	3,59±0,03 ^{dG}	7,21±0,07 ^{cD}	11,30±0,17 ^{bE}	15,90±0,07 ^{aD}
n6	12,19±0,05 ^{dE}	18,85±0,08 ^{dE}	27,89±0,11 ^{cC}	38,56±0,01 ^{bC}	50,39±0,05 ^{aC}
n-6/n-3 ratio	12,39±0,01 ^{aE}	5,25±0,02 ^{bF}	3,87±0,01 ^{cE}	3,41±0,03 ^{dG}	3,17±0,05 ^{eF}
ΣAGS	35,96±0,04 ^{aC}	33,49±0,06 ^{bC}	29,66±0,01 ^{cC}	23,17±0,00 ^{dD}	16,03±0,01 ^{eD}
ΣAGI	64,05±0,03 ^{aA}	66,55±0,21 ^{dA}	70,42±0,24 ^{cA}	76,85±0,07 ^{bA}	84,06±0,22 ^{aA}
ΣAGMI	50,88±0,02 ^{aB}	44,10±0,04 ^{bB}	35,33±0,09 ^{cB}	26,98±0,04 ^{dD}	17,78±0,18 ^{eD}
ΣAGPI	13,17±0,01 ^{eD}	22,44±0,02 ^{dD}	35,10±0,06 ^{cB}	49,87±0,02 ^{bB}	66,28±0,13 ^{aB}
ΣAGPI/ΣAGS	0,37±0,01 ^{eH}	0,67±0,03 ^{dI}	1,18±0,02 ^{cF}	2,15±0,07 ^{bG}	4,13±0,02 ^{aF}
IA	0,44±0,01 ^{aH}	0,39±0,00 ^{bJ}	0,31±0,01 ^{cG}	0,22±0,00 ^{dH}	0,14±0,00 ^{eG}
IT	1,02±0,04 ^{aG}	0,77±0,01 ^{bI}	0,54±0,01 ^{cG}	0,33±0,00 ^{dH}	0,18±0,00 ^{eG}
h/H	2,48±0,03 ^{eF}	2,83±0,04 ^{dH}	3,52±0,04 ^{cE}	4,96±0,02 ^{bF}	8,01±0,07 ^{aE}

Resultados expresados en g/100 g de grasa. SC: muestra de salchicha tipo Frankfurt control con una formula tradicional; S25: muestra de salchicha tipo Frankfurt con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S50: muestra de salchicha tipo Frankfurt con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S75: muestra de salchicha tipo Frankfurt con un 75% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S100: muestra de salchicha tipo Frankfurt con un 100% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno. Los resultados seguidos de distinta letra mayúscula (A-N) hace referencia a la comparación de los diferentes ácidos grasos sobre la misma muestra y las letras minúsculas (a-e) hace referencia a la comparación del mismo ácido graso sobre las distintas muestras según el test post-hoc de Tukey. Los datos se presentan como media y DS.

La cantidad de ácidos grasos omega 3, aumenta a medida que lo hace la sustitución, pasando de valores de 0,98 g/100 g de grasa para la muestra control a valores de 15,90 g/100 g de grasa para la muestra con un 100% de reemplazo, con diferencias estadísticas entre todas las muestras ($p < 0,05$).

En cuanto a los ácidos grasos omega 6, estos aumentaron en mayor cantidad pasaron de valores de 12,19 g/ 100 g de grasa a 50,39 g/100 g de grasa, con diferencias estadísticas entre todas las muestras ($p < 0,05$). Los parámetros nutricionales de la relación ácidos grasos poliinsaturados/ácidos grasos saturados (AGPI/AGS) y la relación de ácidos grasos omega 6/omega 3 son importantes para prevenir enfermedades cardiovasculares (Wu et al., 2020). Se recomienda que la relación AGPI/AGS sea superior a 0,4 y que la relación omega 6/omega 3 sea inferior a 4 (Li et al., 2022). Todas las salchichas tipo Frankfurt reformuladas cumplieron con la relación AGPI/AGS, pero solo las muestras con sustitución del 50% en adelante (75% y 100%) cumplieron con la relación mencionada de omega 6/omega 3, mientras que la muestra control no cumplió con ninguna las recomendaciones.

A pesar de realizarse una sustitución de grasa animal, rica principalmente en ácidos grasos monoinsaturados y saturados, por una grasa vegetal, rica en ácidos grasos poliinsaturados, y de ser por ello más susceptible en principio a la oxidación lipídica, todas las muestras evaluadas presentaron valores de oxidación lipídica estadísticamente similares ($p > 0,05$) (Figura 36). Existen diversos estudios que asocian este efecto a la protección por parte de la matriz de gel hacia las gotas de aceite, generando como un efecto de encapsulación y sirviendo de barrera de protección contra la oxidación (Öztürk-Kerimoğlu et al., 2021a).

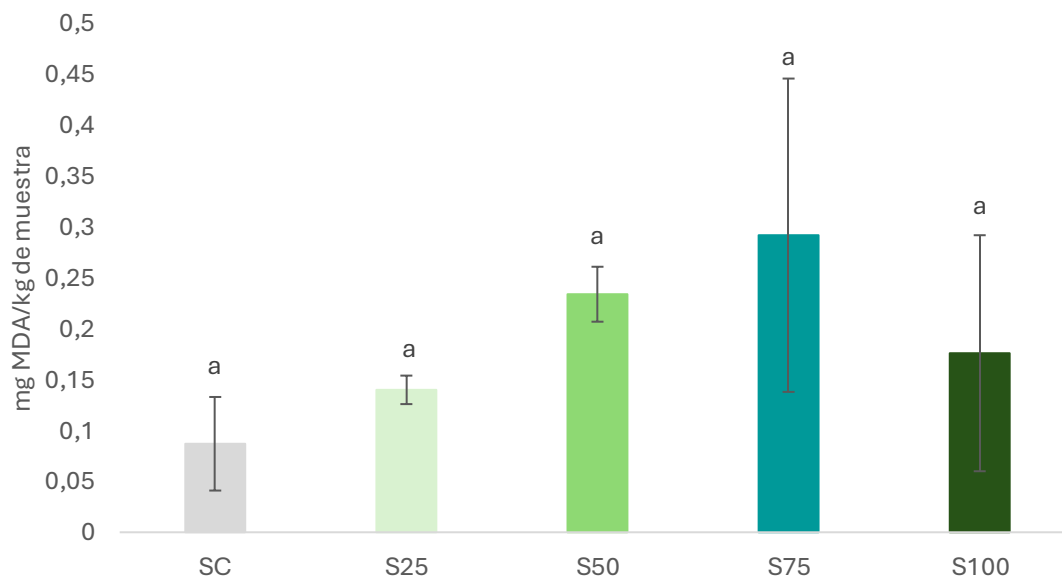


Figura 36. Oxidación lipídica (TBARs; mg malonaldehído/kg de muestra) salchichas tipo Frankfurt con sustitución de grasa animal por una emulsión gelificada con aceite de cáñamo y trigo sarraceno.

SC: muestra de salchicha tipo Frankfurt control con una formula tradicional; S25: muestra de salchicha tipo Frankfurt con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S50: muestra de salchicha tipo Frankfurt con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S75: muestra de salchicha tipo Frankfurt con un 75% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S100: muestra de salchicha tipo Frankfurt con un 100% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno. Misma letra minúscula encima de cada barra indica que no existen diferencias significativas entre las diferentes muestras para la misma propiedad (oxidación lipídica), según el test de Tukey ($p > 0,05$).

En la evaluación sensorial de las salchichas tipo Frankfurt reformuladas, se evaluaron propiedades de color, olor a cáñamo, sabor a cáñamo, sabor salado, jugosidad, dureza y aceptabilidad general (Figura 37). En estos atributos se vio que en el atributo que más diferencias ($p < 0,05$) presentó de entre las 5 muestras evaluadas fue en el parámetro de color. Presentando las muestras con 75% y un 100% de sustitución (S75 y S100) puntuaciones menores y siendo las muestras control y la que tenía 25% (S25) de sustitución de grasa animal las de mayor puntuación para el parámetro de color. En cuanto a los resultados de aceptabilidad general de los productos evaluados (Figura 37), la muestra control (SC) fue la que presentó una mayor aceptabilidad seguida de la muestra con 25% de sustitución (S25)

y por otro lado, la muestra con 100% de reemplazo de grasa animal (S100) fue la que menores puntuaciones de aceptabilidad presentó, el motivo de estas puntuaciones menores podría ser la detección por parte del consumidor del olor y color del aceite de cáñamo con el cual no están caracterizados.

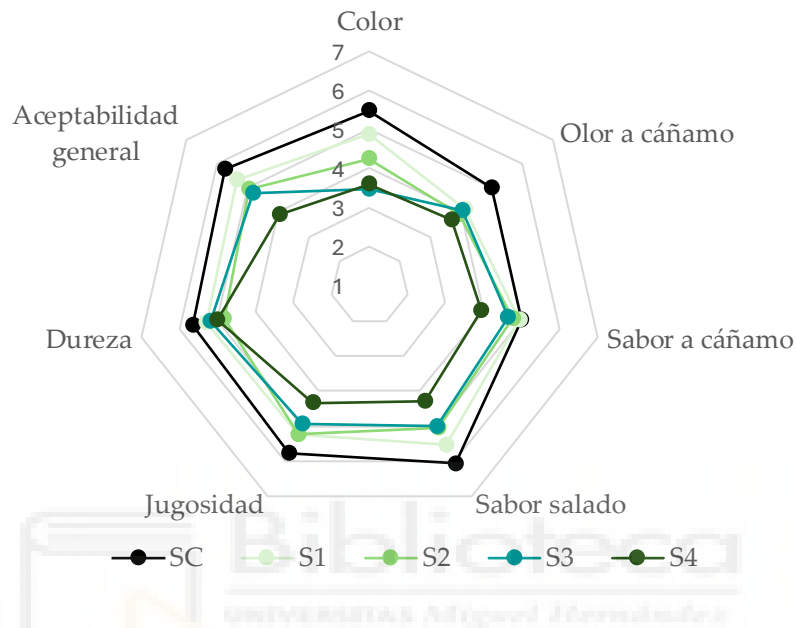


Figura 37. Evaluación sensorial de las salchichas tipo Frankfurt con sustitución de grasa animal por una emulsión gelificada con aceite de cáñamo y trigo sarraceno. SC: muestra de salchicha tipo Frankfurt control con una formula tradicional; S25: muestra de salchicha tipo Frankfurt con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S50: muestra de salchicha tipo Frankfurt con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S75: muestra de salchicha tipo Frankfurt con un 75% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno; S100: muestra de salchicha tipo Frankfurt con un 100% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno.

5.6. INCORPORACIÓN DE EMULSIONES GELIFICADAS EN PRODUCTOS CÁRNICOS COCIDOS UNTABLES: PATÉ

Los resultados de este trabajo han sido publicados en la revista LWT-Food Science and Technology, (2024), 206,116630 (Open Access).

El paté es un plato muy apreciado en la gastronomía europea, éste tiene un elevado contenido de grasa debido a que está compuesto por

hígado (Skalecki et al., 2021). El perfil nutricional del paté es bastante atractivo debido a su contenido en proteínas de alto valor biológico, en vitaminas del grupo B y por ser rico en hierro hemo (Sánchez-Zapata et al., 2013; Lucas-González et al., 2019). Aun con todas estas propiedades beneficiosas, no se debe obviar que es un producto de un elevado aporte calórico ya que contiene entre el 25% y 40% de grasa de origen animal y sobre todo por su composición rica en ácidos grasos saturados. Por todo ello, para mejorar el perfil lipídico de este tipo de productos, en la presente Tesis se propuso el reemplazo de un 10% y un 20% de grasa animal (en concreto la panceta y la papada usada en la elaboración) por una emulsión gelificada a base de harina de trigo sarraceno y de aceite de cáñamo (Cáñamo-EG) para así ver como afectaba esta sustitución de grasa a diversas propiedades.

De entre los resultados obtenidos destaca, a diferencia de otros productos con sustitución de grasa por esta emulsión vistos con anterioridad, que la sustitución de grasa animal por la emulsión gelificada en este producto si afectó a la estabilidad de la emulsión (previa a la cocción) (Figura 38). De hecho, se encontró que la sustitución de grasa produjo un aumento en la cantidad de líquido expulsado ($p < 0,05$), indicando una menor estabilidad de la emulsión, siendo mayor la inestabilidad a niveles más altos de sustitución (20% de reemplazo por Cáñamo-EG (Paté20)). Los valores publicados sobre estabilidad de la emulsión para la formulación control (Control) fueron similares a otros estudios publicados como el llevado a cabo por Gómez-Estaca et al. (2019). Aunque los valores de estabilidad presentados por las muestras fueron mucho menores que los obtenidos por Goemaere et al. (2021) en estudios de salchichas tipo Frankfurt, esto podría justificarse debido a que una mayor cantidad de proteína en la matriz cárnica tiene un efecto de estabilización de la emulsión debido al efecto matriz-gel-

emulsión, así como una influencia en la capacidad de retención de agua y de aceite, por lo que al ser los ingredientes cárnicos empleados en la elaboración del paté, hígado y papada, estos contienen una menor cantidad de proteína y esto podría explicar estas diferencias (Santos et al., 2022).

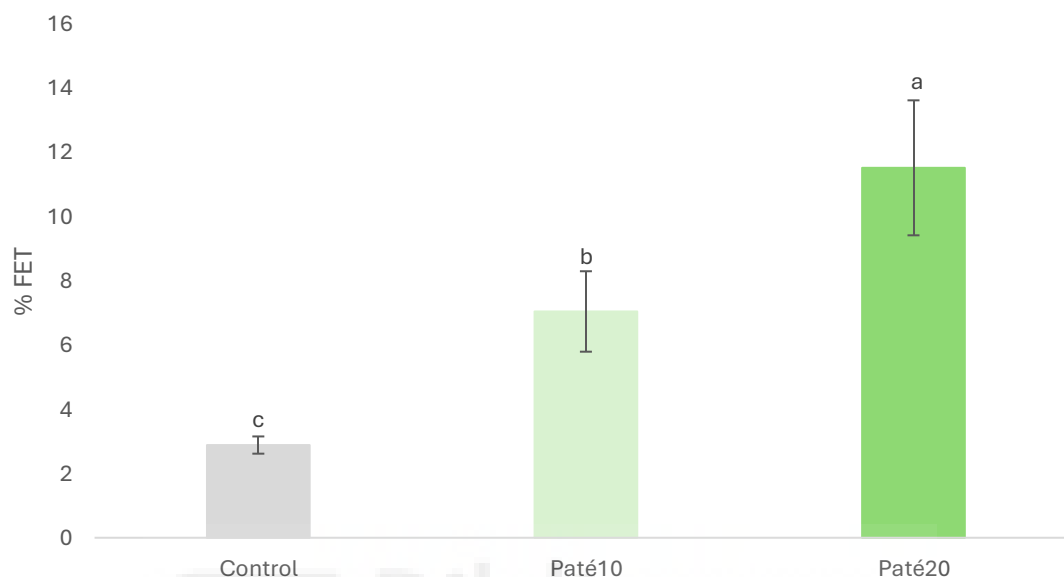


Figura 38. Estabilidad de la emulsión (%fluido expelido total) de las muestras de paté con emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal. Control: paté control; Paté10: Paté con un 10% de emulsión a base de aceite de cáñamo como sustituto de grasa animal; Paté20: paté con un 20% de emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal. Misma letra minúscula encima de cada barra indica que no existen diferencias significativas entre las diferentes muestras para la misma propiedad (estabilidad de la emulsión), según el test de Tukey ($p > 0,05$).

Por otro lado, en la Tabla 24 puede observarse como la sustitución de grasa animal por Cáñamo-EG resultó en unos valores de colesterol menores, a pesar de tener un mayor contenido en grasa por parte de los patés reformulados en comparación con la muestra control ($P < 0,05$). Esta disminución del contenido de colesterol hace que estos productos reformulados estén en concordancia con las recomendaciones nutricionales para desarrollar productos cárnicos más saludables (de Souza Paglarini et al., 2019; Nieto & Lorenzo, 2021). Sin verse influenciado ni en la cantidad de

minerales ni en la proporción de hierro hemo en los patés reformulados (Paté10 y Paté20).

Tabla 24. Composición química de las muestras de paté con emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal.

	Control	Paté10	Paté20
Composición proximal (%)			
Humedad	55,14±0,34 ^a	52,73±0,62 ^b	52,28±0,22 ^b
Cenizas	2,41±0,02 ^a	2,22±0,02 ^b	2,17±0,04 ^b
Grasa	23,83±0,09 ^b	29,10±0,52 ^a	28,32±0,78 ^a
Proteína	13,35±0,78 ^a	10,89±0,03 ^b	10,28±0,09 ^b
Perfil mineral (mg/100 g)			
Calcio	17,88±0,10 ^a	17,48±0,55 ^a	15,38±0,06 ^b
Cobre	0,16±0,00 ^a	0,16±0,00 ^a	0,17±0,00 ^a
Hierro	2,92±0,01 ^b	3,05±0,08 ^b	3,97±0,06 ^a
Potasio	142,78±4,78 ^a	121,81±7,79 ^a	132,98±1,75 ^a
Magnesio	12,75±1,13 ^a	11,66±0,66 ^a	13,92±0,26 ^a
Manganeso	0,09±0,00 ^b	0,10±0,00 ^b	0,16±0,01 ^a
Sodio	797,18±17,16 ^a	824,25±12,26 ^a	834,09±3,18 ^a
Zinc	2,06±0,05 ^a	1,86±0,02 ^a	1,86±0,03 ^a
Colesterol	69,29±3,30 ^a	58,01±0,31 ^b	60,61±0,85 ^b
Hierro hemo	5,79±0,04 ^b	6,17±0,06 ^a	5,87±0,11 ^b

Control: paté control; Paté10: Paté con un 10% de emulsión a base de aceite de cáñamo como sustituto de grasa animal; Paté20: paté con un 20% de emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal. Los datos se presentan como la media ± la desviación estándar. Diferentes letras (a-b) en la misma fila indican diferencias significativas ($p < 0,05$) determinadas mediante el test de Tukey.

Referente al perfil de ácidos grasos (Tabla25), se volvió a ver la tendencia observada en la incorporación de este tipo de emulsión gelificada como en los anteriores productos cárnicos, se ve una disminución de los ácidos grasos monoinsaturados principalmente el ácido graso oleico (disminución del 6% y 21% para las muestras reformuladas Paté10 y Paté20, respectivamente) junto a una disminución de los ácidos grasos saturados (esteárico y palmítico principalmente) presentando la formulación de mayor sustitución los valores menores de estos ácidos grasos. Por otro lado, se observa un aumento de los ácidos grasos poliinsaturados α -linolénico y linoleico a medida que la sustitución incrementa, llegando a un aumento del

45% y del 70% para los patés sustituidos un 10% y un 20% (Paté10 y Paté20 respectivamente), respectivamente, comparado con el paté Control. Estos resultados se encuentran en concordancia con el perfil de ácidos grasos del aceite de cáñamo visto con anterioridad y con los resultados obtenidos en otros estudios en los cuales se realizó una sustitución de grasa animal por aceites vegetales (Barbut et al., 2019; Cittadini et al., 2022).

Tabla 25. Perfil lipídico (g/100 g de ácidos grasos) de las muestras de paté con emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal.

	Control	Paté10	Paté20
C14:0	1,38±0,06 ^a	1,32±0,00 ^a	1,07±0,01 ^b
C16:0	23,95±0,23 ^a	22,06±0,06 ^b	18,48±0,11 ^c
C16:1n-7	2,55±0,11 ^a	2,44±0,00 ^a	1,95±0,02 ^b
C17:0	0,67±0,01 ^a	0,59±0,00 ^b	0,50±0,00 ^c
C17:1n-7	0,57±0,01 ^a	0,51±0,00 ^b	0,42±0,00 ^c
C18:0	12,20±0,31 ^a	10,49±0,02 ^b	8,91±0,03 ^c
C18:1n-9	40,02±0,31 ^a	37,63±0,03 ^b	31,78±0,12 ^c
C18:1n-7	3,18±0,03 ^a	2,99±0,00 ^b	2,47±0,02 ^c
C18:2n-6	10,91±0,26 ^c	15,82±0,01 ^b	18,62±0,02 ^a
C20:0	0,15±0,01 ^c	0,18±0,00 ^b	0,22±0,00 ^a
C20:1n-9	0,90±0,03 ^a	0,81±0,00 ^b	0,70±0,01 ^c
C20:2n-6	0,57±0,01 ^a	0,52±0,00 ^a	0,45±0,00 ^b
C20:4n-6	0,82±0,04 ^a	0,72±0,01 ^b	0,61±0,01 ^c
C18:3n-6	0,04±0,00 ^b	0,10±0,00 ^a	0,15±0,01 ^a
C18:3n-3	0,51±0,02 ^c	2,23±0,00 ^b	3,56±0,01 ^a
C20:2n-6	0,57±0,01 ^a	0,52±0,00 ^a	0,45±0,00 ^b
C20:3n-6	0,14±0,00 ^a	0,12±0,00 ^a	0,10±0,00 ^a

Control: paté control; 10% CAEG: Paté con un 10% de emulsión a base de aceite de cáñamo como sustituto de grasa animal; 20% CAEG: paté con un 20% de emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal. Los resultados seguidos de distinta letra minúscula (a-c) representan diferencias significativas ($p < 0,05$) para cada ácido graso o parámetro entre las diferentes muestras según el test post-hoc de Tukey. Los datos se presentan como media y DS.

La sustitución de grasa animal por cáñamo-EG en los patés mejoró significativamente los índices de nutricionales (Tabla 26), como el índice de aterogenicidad (IA), el índice de trombogenicidad (IT) y la relación hipocolesterolémico/hipercolesterolémico (h/H). En particular, los patés reformulados (Paté10 y Paté20) mostraron una mejor relación AGPI/AGS, superando el valor mínimo recomendado de 0,4, a diferencia del paté de

control. Además, la relación omega 6/omega 3 disminuyó en estos patés sustituidos, lo que es beneficioso para la salud, ya que una menor proporción de esta relación está asociada con la reducción de riesgos de enfermedades metabólicas y cardiovasculares (Wu et al., 2020).

Tabla 26. Parámetros nutricionales e índices de salud de las muestras de paté con emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal.

	Control	Paté10	Paté20
Σ AGS	38,82±0,18 ^a	35,07±0,05 ^b	29,54±0,11 ^c
Σ AGMI	47,60±0,25 ^a	44,74±0,02 ^c	46,36±0,11 ^b
Σ AGPI	13,59±0,32 ^c	20,19±0,04 ^b	24,09±0,02 ^a
Σ n3	0,78±0,02 ^c	2,53±0,01 ^b	3,83±0,01 ^a
Σ n6	12,81±0,30 ^c	17,66±0,02 ^b	20,27±0,00 ^a
Σ AGPI/ Σ AGS	0,35±0,01 ^c	0,58±0,00 ^b	0,82±0,00 ^a
n6/n3	16,33±0,11 ^a	6,97±0,02 ^b	5,30±0,02 ^c
IA	0,48±0,01 ^a	0,42±0,00 ^b	0,32±0,00 ^c
IT	1,15±0,01 ^a	0,87±0,00 ^b	0,63±0,00 ^c
h/H	2,24±0,03 ^a	2,60±0,01 ^b	2,98±0,02 ^c

Control: paté control; 10% CAEG: Paté con un 10% de emulsión a base de aceite de cáñamo como sustituto de grasa animal; 20% CAEG: paté con un 20% de emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal. Los resultados seguidos de distinta letra minúscula (a-c) representan diferencias significativas ($p < 0,05$) para cada ácido graso o parámetro entre las diferentes muestras según el test post-hoc de Tukey. Los datos se presentan como media y DS.

Las propiedades físico-químicas se muestran en la Tabla 27, en concreto los valores de pH y las diferencias de color no se vieron afectadas por la sustitución de la grasa animal por la Cáñamo-EG. Sin embargo, si se vio una disminución en los parámetros de textura de las muestras sustituidas (Paté10 y Paté20) sin diferencias significativas ($p > 0,05$) entre ellas. Lo que se traduce en un aumento de la untabilidad al disminuir los parámetros de firmeza y de trabajo de cizalla respecto a la formulación Control.

Tabla 27. Propiedades físico-químicas y texturales de las muestras de paté con emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal.

	Control	Paté10	Paté20
pH	6,21±0,01 ^a	6,23±0,01 ^a	6,18±0,01 ^b
Aw	0,891±0,00 ^a	0,893±0,00 ^a	0,891±0,00 ^a
L*	58,56±1,67 ^a	57,64±0,29 ^{ab}	57,19±0,84 ^b
a*	6,98±0,26 ^a	6,46±0,18 ^b	6,35±0,51 ^b
b*	12,95±0,59 ^a	13,06±0,22 ^a	13,00±0,53 ^a
C*	14,71±0,60 ^a	14,57±0,19 ^a	14,48±0,55 ^a
h	61,65±0,92 ^b	63,66±0,83 ^a	63,98±1,91 ^a
ΔE	-	2,07±0,59 ^a	1,16±0,51 ^b
Firmeza (N)	0,38±0,05 ^a	0,21±0,03 ^b	0,18±0,02 ^b
Trabajo de cizalla (N.s)	0,40±0,07 ^a	0,22±0,03 ^b	0,19±0,02 ^b

Control: paté control; Paté10: Paté con un 10% de emulsión a base de aceite de cáñamo como sustituto de grasa animal; Paté20: paté con un 20% de emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal. Los resultados seguidos de distinta letra minúscula (a-b) representan diferencias significativas ($p < 0,05$) para cada parámetro entre las diferentes muestras según el test post-hoc de Tukey. Los datos se presentan como media y DS.

La sustitución de grasa animal por la Cáñamo-EG en los patés aumentó los valores de oxidación lipídica sin diferencias significativas entre ellos ($p > 0,05$) (Figura 39). El paté es un producto de una susceptibilidad elevada a la oxidación debido a que es un producto altamente procesado y con elevado contenido en grasa, estos procesos de oxidación afectan a múltiples propiedades sensoriales, de seguridad y de calidad, como el color, la textura y el valor nutricional (Cittadini et al., 2022; Skafęcki et al., 2020). Pero en el caso de la sustitución aún aumentó más los valores de TBARs de los patés, el aumento fue de un 63% para el Paté10 y un 83% para el Paté20.

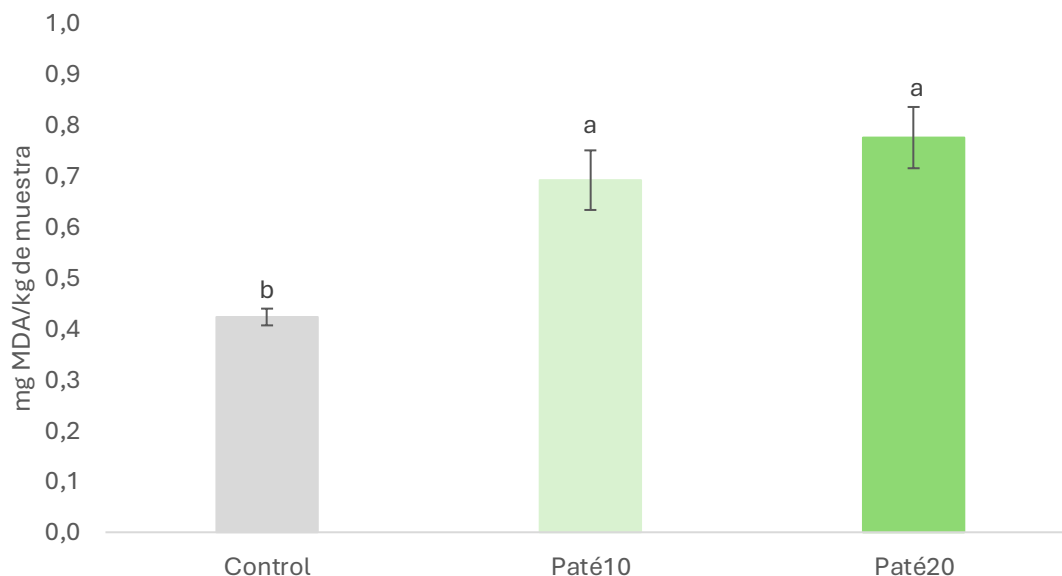


Figura 39. Oxidación lipídica (TBARS; mg malonaldehído/kg de muestra) de las muestras de paté con emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal.

Control: paté control; Paté10: Paté con un 10% de emulsión a base de aceite de cáñamo como sustituto de grasa animal; Paté20: paté con un 20% de emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal. Diferente letra minúscula encima de cada barra (a-b) indica que existen diferencias significativas entre las diferentes muestras para la misma propiedad (oxidación lipídica), según el test de Tukey ($p < 0,05$).

En la evaluación sensorial de los productos reformulados es un parámetro importante, sobre todo lo referente a la aceptación de dicho producto reformulado. Ya que los atributos de sabor, aroma y la apariencia del producto son los factores más influyentes a la hora de tomar la decisión de compra (Skatecki et al., 2020; Smarzyński et al., 2019). En el análisis sensorial de los patés control y reformulados medidos (Figura 40) se obtuvieron solo diferencias estadísticamente significativas ($p < 0,05$) en los atributos de jugosidad, rancidez, olor general y untabilidad entre la muestra control y la muestra Paté20. Entre el control y la muestra sustituida un 10% por Cáñamo-EG no se obtuvieron diferencias en ninguno de los 10 parámetros evaluados. Referente a la aceptabilidad general evaluada por los

panelistas, todas las muestras presentaron un nivel de aceptabilidad similar sin diferencias ($p > 0,05$) entre ellas.



Figura 40. Evaluación sensorial del paté con sustitución parcial de grasa animal por una emulsión gelificada de aceite de cáñamo.

Control: paté control; 10% Paté10: Paté con un 10% de emulsión a base de aceite de cáñamo como sustituto de grasa animal; Paté20: paté con un 20% de emulsión gelificada a base de aceite de cáñamo como sustituto de grasa animal.

5.7. INCORPORACIÓN DE EMULSIONES GELIFICADAS EN UN PRODUCTO CÁRNICO TÍPICO PORTUGUES: ALHEIRAS

Los resultados de este trabajo han sido publicados en la revista [European Food Research and Technology](#) (2023), 249:2273-2285 (Open Access).

La alheira es una salchicha fermentada tradicional típica de las zonas rurales del *Tras-os-Montes* portugués, elaborada con una combinación de carnes de ave, cerdo, pan regional, aceite de oliva, sal y especias. A pesar de que la alheira es un producto cárnico bien aceptado, los consumidores la

perciben como poco saludable debido a su alto contenido de grasa y es en este punto donde la industria alimentaria ha de incidir, intentando reducir o eliminando las grasas saturadas en las formulaciones de los productos cárnicos para así obtener productos cárnicos de alta calidad, nutritivos y más saludables (de Carvalho et al., 2020; Ferreira et al., 2022). Para ello, en la presente Tesis Doctoral, se realizó un estudio donde se sustituyó en una formulación tradicional de alheira un 25% (Alheira25) y un 50% (Alheira50) de grasa animal, por una emulsión gelificada a base de trigo sarraceno y aceite de cáñamo y así ver su influencia sobre la composición química y el perfil de ácidos grasos, las propiedades físico-químicas, el perfil lipídico y los valores de oxidación lipídica.

Entre los resultados más destacables del análisis de las alheiras tanto crudas como cocinadas, se obtuvo que el contenido en grasa disminuía ($p < 0,05$) a medida que la sustitución aumentaba y esta tendencia se observó tanto en las muestras crudas como cocinadas (Tabla 28). Suponiendo una reducción del 7,5% y del 16,65% en el contenido de grasa total para las muestras Alheira25 y Alheira50 respectivamente para las muestras crudas y del 11,01% y del 37,82% para las muestras cocinadas Alheira25 y Alheira50 respectivamente (Tabla 28). La humedad también experimentó un aumento tanto en las muestras de alheiras crudas como cocinadas relacionado con la sustitución, a mayor sustitución mayor cantidad de humedad, tendencia similar a la que se ha visto con anterioridad para otros productos cárnicos en los cuales se incluyó este tipo de emulsiones gelificadas (hamburguesas de ternera y salchichas tipo Frankfurt) y estos resultados están en concordancia con los resultados obtenidos por otros autores en estudios similares (Carvalho et al., 2019; Patarata et al., 2008; Teixeira et al., 2020).

Tabla 28. Composición química de las alheiras reformuladas (crudas y cocinadas)

		Humedad	Cenizas	Grasa	Proteína	Cloruro
CRUDA	Control	57,58±1,06 ^b	1,17±0,06 ^b	15,07±0,74 ^a	9,85±0,39 ^c	0,78±0,05 ^b
	Alheira25	58,03±0,59 ^b	1,35±0,03 ^a	13,82±0,97 ^a	11,63±1,37 ^b	0,89±0,04 ^a
	Alheira50	59,56±0,31 ^a	1,38±0,01 ^a	15,38±3,99 ^a	12,18±0,36 ^a	0,91±0,01 ^a
COCINADA	Control	57,30±0,91 ^x	1,26±0,02 ^y	17,71±1,43 ^x	10,36±0,27 ^z	0,89±0,04 ^y
	Alheira25	57,46±0,76 ^x	1,45±0,04 ^x	15,76±0,81 ^x	11,98±0,58 ^y	1,01±0,02 ^x
	Alheira50	58,39±1,05 ^x	1,45±0,04 ^x	12,85±0,72 ^x	12,61±0,71 ^x	1,07±0,03 ^x

Los resultados vienen expresados como g/100g. Control: muestra de alheira control con una formulación tradicional; Alheira25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal; Alheira50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal. Los resultados seguidos de distinta letra minúscula (a-c) representan diferencias significativas ($p < 0,05$) para cada parámetro entre las distintas muestras crudas y para las muestras cocinadas (x-z) según el test post-hoc de Tukey. Los datos se presentan como media y DS.

En la Tabla 29 se presentan las propiedades físico-químicas de las alheiras crudas y cocinadas, destacó la existencia de diferencias ($p < 0,05$) en los parámetros de color, sobre todo en las coordenadas L^* y a^* , lo cual se traduce en unas ligeras diferencias de color más evidentes en las muestras crudas que en las muestras cocinadas. Como ya se comentó con anterioridad, para que existan diferencias de color es necesario que haya una diferencia superior a 3 unidades, por ello para las muestras de alheiras cocinadas a simple vista no serían perceptibles dichas diferencias entre ninguna de las tres muestras. Por otro lado, las muestras crudas presentaron una diferencia ($p < 0,05$) de 4 unidades para la muestra Alheira25 y casi 7 unidades para las Alheira50, siendo pues más evidente a simple vista el cambio de coloración entre muestras.

Tabla 29. Parámetros físico-químicos de las alheiras control y reformuladas (crudas y cocinadas).

	CRUDA			COCINADA		
	Control	Alheira25	Alheira50	Control	Alheira25	Alheira50
pH	5,95±0,09 ^a	5,79±0,05 ^b	5,92±0,05 ^a	5,83±0,03 ^x	5,74±0,05 ^y	5,85±0,08 ^x
Aw	0,963±0,00 ^a	0,958±0,01 ^a	0,957±0,01 ^a	0,959±0,00 ^x	0,958±0,01 ^x	0,961±0,01 ^x
L*	68,24±3,51 ^a	66,54±3,75 ^{ab}	60,56±1,97 ^b	65,06±1,86 ^x	65,43±2,15 ^x	63,63±2,54 ^x
a*	11,61±0,62 ^a	11,48±0,60 ^a	10,20±0,35 ^b	11,29±0,50 ^x	11,66±0,69 ^x	11,44±0,75 ^x
b*	26,54±1,20 ^a	26,89±1,46 ^a	24,96±0,77 ^a	25,53±0,97 ^x	26,70±1,14 ^x	26,37±1,20 ^x
C*	28,90±1,31 ^a	29,24±1,50 ^a	26,96±0,85 ^b	27,92±1,07 ^x	29,14±1,28 ^x	28,75±1,38 ^x
h	1,16±0,01 ^a	1,17±0,02 ^a	1,18±0,00 ^a	1,15±0,01 ^x	1,16±0,01 ^x	1,16±0,01 ^x
ΔE*	-	4,00±1,89 ^b	6,87±0,84 ^a	-	2,57±1,20 ^x	3,15±1,15 ^x

Control: muestra de alheira control con una formulación tradicional; Alheira25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal; Alheira50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal. Los resultados seguidos de distinta letra minúscula (a-b) representan diferencias significativas ($p < 0,05$) para cada parámetro entre las muestras crudas y para cocinadas (x-y) según el test post-hoc de Tukey. Los datos se presentan como media y DS.

Se evaluaron las pérdidas por cocinado de las alheiras y como resultado no se obtuvieron diferencias significativas ($p > 0,05$) en las pérdidas al cocinar con valores entre 6,36% y 8,54% de pérdidas (Figura 41). Este parámetro medido para controlar la calidad de los productos cárnicos con sustitución es muy variable dependiendo del material con el cual se haga la sustitución, el porcentaje de sustitución y el producto en el cual se realice, por ello existe mucha variabilidad para este parámetro vista en la literatura científica (Araujo et al., 2021; Choe & Kim, 2019; Salejda et al., 2022).

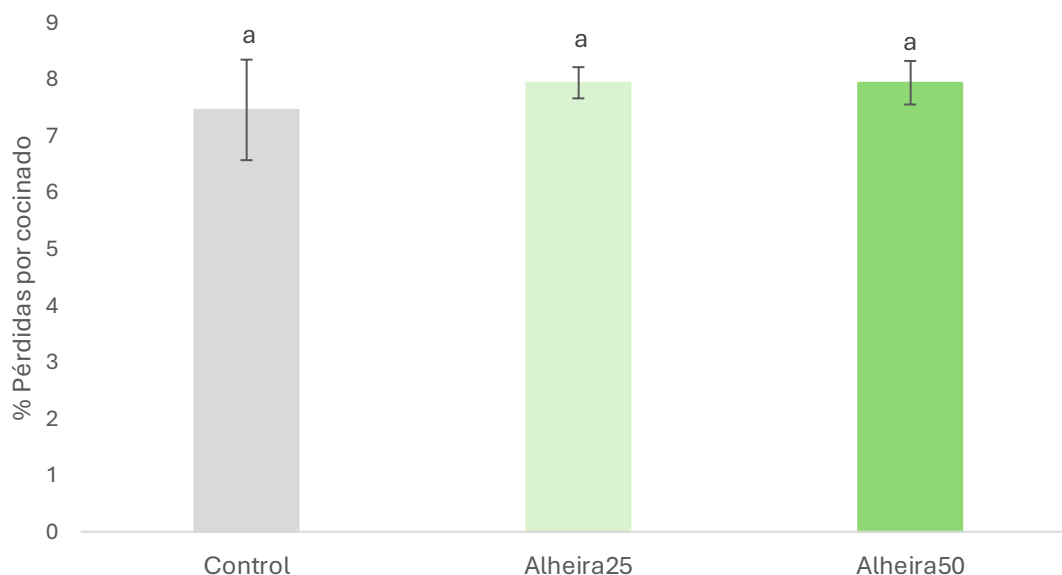


Figura 41. Pérdidas por cocinado de las alheiras control y reformuladas.

Control: muestra de alheira control con una formulación tradicional; Alheira25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal; Alheira50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal. Misma letra minúscula encima de cada barra indica que no existen diferencias significativas entre las diferentes muestras para la misma propiedad (pérdidas por cocinado), según el test de Tukey ($p > 0,05$).

En la Tabla 30 se muestra el análisis del perfil de los ácidos grasos, la muestra control presentó como ácidos grasos principales el palmítico, oleico y esteárico, lo que representa el 81,22% de los ácidos grasos totales de esta formulación cruda y de 79,54% para la muestra control cocinada. En las alheiras con sustitución de grasa animal por parte de la emulsión gelificada, se vio una disminución de la cantidad de los ácidos grasos oleico, palmítico y esteárico junto a un aumento del ácido graso linoleico (debido a que es el ácido graso predominante del aceite de cáñamo). Esta disminución de los ácidos grasos saturados y monoinsaturados y el aumento del ácido graso linoleico y del α -linolénico siguió la misma tendencia que la sustitución de grasa en las alheiras tanto crudas como cocinadas. La cantidad de ácidos grasos omega 3, aumenta a medida que lo hace la sustitución, pasando de valores de 0,54 g/100 g de grasa para la muestra control a valores de 3,68

g/100 g de grasa para la muestra cruda Alheira50, con diferencias estadísticas entre ambas muestras sustituidas ($p < 0,05$) (Alheira25 y Alheira50).

Tabla 30. Perfil lipídico de las alheiras control y reformuladas (crudas y cocinadas).

	Cruda			Cocinada		
	Control	Alheira25	Alheira50	Control	Alheira25	Alheira50
C 14:0	0.97±0.04 ^{aF}	0.86±0.01 ^{bG}	0.71±0.04 ^{cG}	0.92±0.02 ^{xF}	0.84±0.01 ^{yF}	0.73±0.02 ^{zF}
C16:0	24.49±0.11 ^{aB}	22.79±0.09 ^{bB}	20.43±0.02 ^{cC}	24.38±0.13 ^{xB}	22.90±0.14 ^{yB}	21.12±0.70 ^{zB}
C16:1n-7	2.24±0.06 ^{aE}	1.96±0.02 ^{bE}	1.79±0.00 ^{cF}	2.12±0.05 ^{xE}	1.88±0.01 ^{yE}	1.87±0.16 ^{zE}
C 17:0	0.19±0.00 ^{aK}	0.18±0.01 ^{aj}	0.16±0.00 ^{bl}	0.19±0.00 ^{xl}	0.18±0.01 ^{xH}	0.12±0.06 ^{yH}
C 17:1n-7	0.17±0.01 ^{aK}	0.15±0.00 ^{bl}	0.13±0.00 ^{cl}	0.16±0.00 ^{xl}	0.16±0.00 ^{xH}	0.12±0.04 ^{yH}
C 18:0	10.79±0.26 ^{aD}	10.25±0.08 ^{bD}	8.80±0.01 ^{cD}	11.30±0.20 ^{xD}	10.68±0.04 ^{yD}	7.56±3.37 ^{zC}
C 18:1	45.44±0.18 ^{aA}	43.01±0.18 ^{bA}	39.59±0.01 ^{cA}	45.37±0.12 ^{xA}	43.02±0.13 ^{yA}	40.66±1.35 ^{zA}
C 18:2n-6	13.08±0.25 ^{cC}	16.71±0.17 ^{bC}	22.56±0.02 ^{aB}	12.71±0.10 ^{zC}	16.29±0.30 ^{yC}	22.14±1.05 ^{zB}
C 20:0	0.18±0.01 ^{cK}	0.23±0.01 ^{bl}	0.28±0.00 ^{aH}	0.20±0.01 ^{zl}	0.24±0.00 ^{yG}	0.28±0.02 ^{zG}
C 18:3n-6	0.82±0.03 ^{aG}	0.76±0.04 ^{bH}	0.67±0.02 ^{cG}	0.88±0.03 ^{xF}	0.82±0.05 ^{yF}	0.70±0.04 ^{zF}
C18:3n-3	0.53±0.02 ^{cH}	1.84±0.04 ^{bF}	3.65±0.00 ^{aE}	0.51±0.02 ^{zG}	1.75±0.04 ^{yE}	3.45±0.17 ^{zD}
C20:2n-6	0.34±0.01 ^{al}	0.32±0.01 ^{al}	0.27±0.01 ^{bH}	0.34±0.00 ^{xH}	0.33±0.00 ^{xG}	0.27±0.02 ^{yG}
C20:3n-6	0.06±0.03 ^{aL}	0.06±0.00 ^{aK}	0.06±0.00 ^{aj}	0.07±0.01 ^{xJ}	0.05±0.02 ^{xl}	0.06±0.00 ^{xJ}
C22:1n-9	0.07±0.03 ^{aL}	0.08±0.01 ^{aK}	0.07±0.00 ^{aj}	0.08±0.01 ^{xJ}	0.07±0.02 ^{xl}	0.07±0.01 ^{xl}
C23:0	0.24±0.02 ^{bl}	0.27±0.04 ^{abl}	0.30±0.01 ^{aH}	0.25±0.01 ^{yH}	0.27±0.01 ^{yG}	0.34±0.02 ^{xG}
ΣAGS	36.95±0.11 ^a	34.75±0.09 ^b	30.90±0.02 ^c	37.37±0.13 ^{xA}	35.28±0.14 ^{yB}	30.37±0.17 ^{zC}
ΣAGMI	48.18±0.18 ^a	45.50±0.18 ^b	41.83±0.01 ^c	48.05±0.12 ^{xA}	45.42±0.13 ^{yB}	42.95±0.25 ^{zC}
ΣAGPI	14.86±0.25 ^c	19.76±0.17 ^b	27.27±0.01 ^a	14.57±0.10 ^{zA}	19.30±0.30 ^{yB}	26.66±0.32 ^{zC}
Σn3	0.54±0.04 ^c	1.87±0.03 ^b	3.68±0.06 ^a	0.53±0.04 ^{zC}	1.78±0.05 ^{yB}	3.46±0.08 ^{xA}
Σn6	14.30±0.02 ^c	17.85±0.05 ^b	23.56±0.03 ^a	14.00±0.02 ^{zA}	17.49±0.03 ^{yA}	23.16±0.04 ^{xA}

Los resultados vienen expresados como g/100g. Control: muestra de alheira control con una formulación tradicional; Alheira25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal; Alheira50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal. Los resultados seguidos de distinta letra mayúscula (A-L) hace referencia a la existencia de diferencias significativas ($p < 0,05$) entre los diferentes ácidos grasos sobre la misma muestra y las letras minúsculas (a-c) hace referencia a la comparación del mismo ácido graso sobre las distintas muestras crudas y las letras minúsculas (x-z) hace referencia a la comparación del mismo ácido graso sobre las distintas muestras cocinadas según el test post-hoc de Tukey. Los datos se presentan como media y DS.

A pesar de que el aceite de cáñamo es rico en ácidos grasos poliinsaturados, los resultados mostraron que no existieron diferencias significativas ($p > 0,05$) en la oxidación lipídica de las alheiras crudas (Figura 42). Si se vio un efecto sobre la oxidación lipídica debido al cocinado en las

muestras sustituidas, la muestra control no presentó variación en los valores de TBARs ($p > 0,05$) entre la misma muestra con diferente tratamiento térmico (cruda y cocinada). Sin embargo, en la muestra Alheira25 tras el cocinado se dio un incremento de los valores de TBARs del 30% y un 65% para la muestra Alheira50. Así pues, aunque con el tratamiento térmico si se vio un cambio en los valores de oxidación lipídica de las muestras, este no superó los niveles de detección por parte de los consumidores.

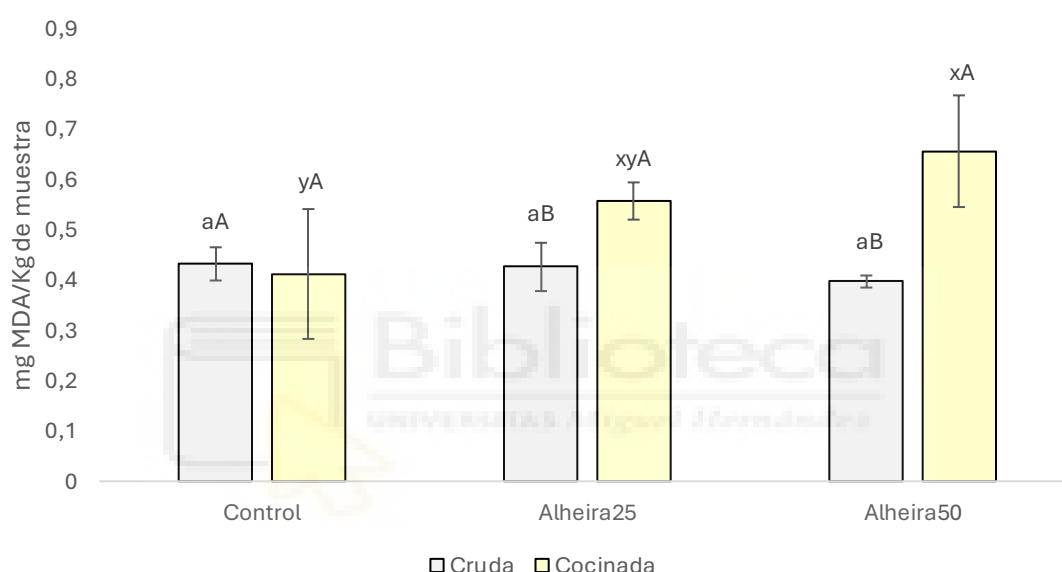


Figura 42. Oxidación lipídica (TBARs; mg malonaldehído/kg de muestra) de las alheiras control y reformuladas (crudas y cocinadas).

Control: muestra de alheira control con una formulación tradicional; Alheira25: muestra con un 25% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal; Alheira50: muestra con un 50% de emulsión gelificada con aceite de cáñamo y harina de trigo sarraceno como sustituto de grasa animal. Letras en mayúscula (A-B) sobre cada barra del histograma se refiere a la comparación de los valores de TBARs en la misma muestra con distinto tratamiento térmico (cocinadas o crudas) y la letras minúsculas (a) a la comparación del mismo tratamiento sobre las distintas muestra cruda; (x-y) para las muestras cocinadas según el test post-hoc de Tukey.

5.8. INCORPORACIÓN DE EMULSIONES GELIFICADAS EN ANÁLOGOS DE CARNE: HAMBURGUESAS VEGANAS

Los resultados de este trabajo han sido publicados en la revista LWT-Food Science and Technology, (2022), 161,113416 (Open Access).

Debido al rápido crecimiento en el consumo de análogos de carne, las hamburguesas veganas están ganando popularidad tanto en los hogares como en los restaurantes. Esto se debe a la creciente preocupación por el impacto del consumo de alimentos de origen animal y la conciencia por el medio ambiente de ellos consumidores (van Vliet et al., 2020; Willett et al., 2019). Por ello, existe un nicho de mercado en expansión para estos productos, que no se limita solo a personas veganas o vegetarianas (Fernández-López et al., 2021). Para ser una alternativa atractiva, las hamburguesas veganas deben mantener compuesto nutritivos valiosos presentes en la carne, como proteínas con un perfil de aminoácidos equilibrado, y evitar el uso de compuestos poco saludables como las grasas saturadas (Badar et al., 2021; Kyriakopoulou et al., 2021). Por todo ello, el objetivo de este estudio fue la evaluación del efecto del uso de dos emulsiones gelificadas, una elaborada con aceite de chía y harina de trigo sarraceno y otra con aceite de cáñamo y harina de trigo sarraceno como fuente de grasa y de la combinación de zumo de remolacha fresco y comercial como agente colorante para conseguir una apariencia similar a la carne, evaluando las propiedades químicas, nutricionales, físico químicas, de cocinado y sensoriales de las hamburguesas veganas.

Los valores de la composición química se muestran a continuación en la Tabla 31, se observó una diferencia notable ($p < 0,05$) en los valores de humedad de las hamburguesas veganas dependiendo de si en su

formulación se había utilizado el zumo comercial o el zumo fresco de remolacha e independientemente de la emulsión gelificada empleada.

Tabla 31. Composición química de las hamburguesas veganas con adición de EG como fuente de grasa.

		Humedad	Cenizas	Grasa	Proteína	FDT
CRUDA	PBFCH	57,47±0,13 ^{aX}	3,39±0,00 ^{aY}	2,90±0,26 ^{aY}	19,52±0,27 ^{aY}	14,10±0,35 ^{bY}
	PBCCH	54,22±0,30 ^{bX}	3,44±0,01 ^{aY}	2,87±0,21 ^{aY}	18,68±0,28 ^{abY}	16,15±0,50 ^{aY}
	PBFCA	57,11±0,13 ^{aX}	3,37±0,06 ^{aY}	2,09±0,21 ^{bY}	18,59±0,09 ^{bY}	14,54±0,43 ^{bY}
	PBCCA	53,94±0,12 ^{bX}	3,41±0,05 ^{aY}	2,91±0,41 ^{aY}	18,68±0,35 ^{abY}	16,19±0,40 ^{aY}
COCINADA	PBFCH	48,74±0,27 ^{aY}	4,17±0,02 ^{aX}	5,58±0,30 ^{bX}	21,94±0,03 ^{bX}	16,40±0,45 ^{bX}
	PBCCH	43,74±0,62 ^{bY}	4,24±0,15 ^{aX}	5,57±0,12 ^{bX}	22,44±0,27 ^{aX}	18,21±0,60 ^{aX}
	PBFCA	47,71±0,52 ^{aY}	4,11±0,17 ^{aX}	4,87±0,21 ^{cX}	22,21±0,13 ^{abX}	16,74±0,66 ^{bX}
	PBCCA	44,97±0,27 ^{bY}	4,07±0,37 ^{aX}	5,98±0,35 ^{abX}	21,94±0,01 ^{bX}	18,18±0,52 ^{aX}

Todos los resultados vienen expresados como g/100 g de muestra. PBFCH: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBCCH: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBFCA: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo. PBCCA: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo. Los resultados seguidos de distinta letra minúscula (a-b) representan diferencias significativas ($p < 0,05$) para cada parámetro entre las muestras crudas como cocinadas, las letras mayúsculas (X-Y) compara la influencia del tratamiento térmico para la misma muestra y el mismo parámetro, según el test post-hoc de Tukey. Los datos se presentan como media y DS.

Estas diferencias podrían deberse a la dilución del zumo fresco para su uso durante la hidratación de la soja texturizada, lo que hizo que existieran diferencias en el resto de la composición química (grasa, proteína, fibra dietética total) debido a este aporte diferente de humedad por parte de los zumos. Por otro lado, el tipo de emulsión usada no influyó en dicho contenido de humedad ni en el de grasa total de las formulaciones de las hamburguesas veganas, únicamente según los resultados obtenidos (Tabla 31), la influencia se debe al tipo de zumo empleado. Las hamburguesas elaboradas con zumo fresco (el cual iba diluido en una proporción 1:3) mostraron un mayor contenido de proteína y un menor contenido de grasa y fibra dietética total ($p < 0,05$) que las otras hamburguesas elaboradas con

zumado comercial independientemente de la emulsión gelificada incorporada (Tabla 31).

Se analizaron un total de 17 ácidos grasos para las hamburguesas veganas, aunque en la Figura 43 se muestran los ácidos grasos mayoritarios, los cuales fueron el palmítico, esteárico, oleico, α -linolénico y linoleico, representando estos 5 mencionados el 95% del total de los ácidos grasos presentes en las muestras analizadas. En general, se puede observar que la fracción predominante en todas las muestras es la de ácidos grasos poliinsaturados con una cantidad superior al 57%, en concreto se observa una dependencia con el aceite con el cual se realizó la emulsión gelificada incorporada. Por ello, para las hamburguesas veganas con incorporación de Cáñamo-EG, el ácido graso predominante fue el linoleico (C18:2) lo que coincide con el mayoritario del aceite de cáñamo. Por el contrario, para las hamburguesas elaboradas con Chía-EG, su ácido graso mayoritario fue el α -linolénico (C18:3) tal y como se obtuvo en el análisis del perfil lipídico para el aceite de chía. Esto difiere con otros estudios donde la fracción mayoritaria fue la de ácidos grasos monoinsaturados, pero esto se debió a que los aceites usados fueron el de colza o el de oliva con un perfil lipídico distinto (He et al., 2021). Los ácidos grasos trans se detectaron únicamente en las muestras cocinadas y en cantidades muy bajas (0,6%) en comparación con las publicadas para las hamburguesas comerciales veganas (2,5%) o incluso para las hamburguesas tradicionales con ingredientes cárnicos (5-6%) (He et al., 2021). Aunque la formación de AGT se ha asociado con condiciones de cocinado severas, también se han observado pequeños cambios en el contenido de AGT durante el de cocinado en unas condiciones más suaves (Tsuzuki et al., 2010).

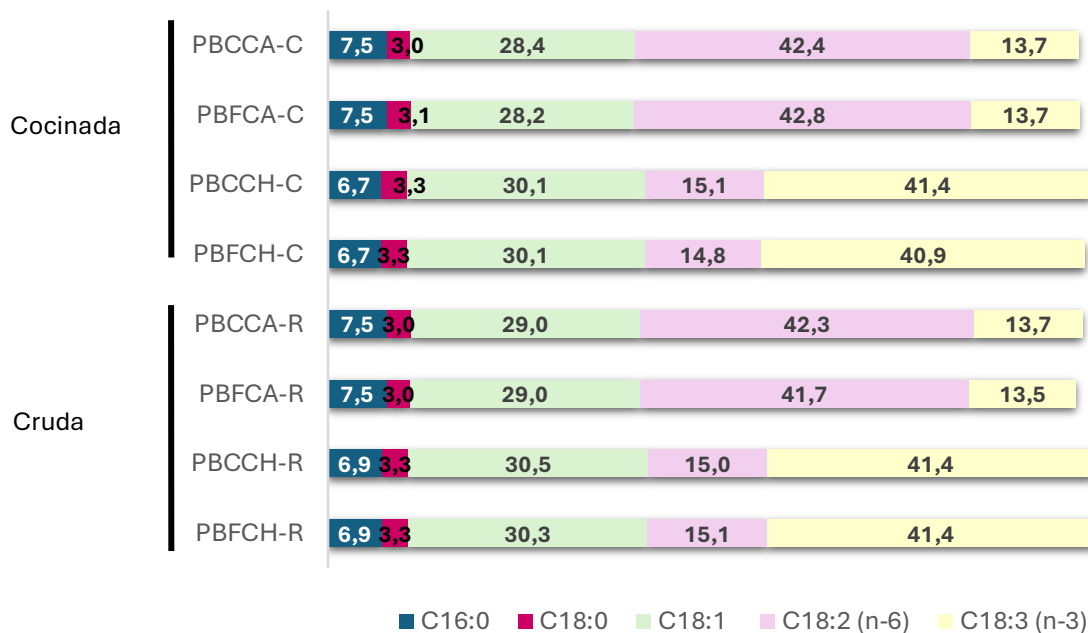


Figura 43. Ácidos grasos mayoritarios en las hamburguesas veganas con adición de emulsión gelificada como fuente de grasa.

PBFCH: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBCCH: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBFCA: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo. PBCCA: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo.

El análisis por HPLC de los zumos de remolacha fresco y comercial mostró la presencia de betalaínas (pigmento bioactivo). Se vio un contenido en betacianinas (isobetanina, betanina y betanidina) para el zumo de remolacha fresco y de betaxantinas en forma de vulgaxantina I. En el zumo comercial de remolacha únicamente se detectó neobetanina como betacianina. El contenido de pigmentos total también fue diferente en un zumo u otro, en el zumo fresco se obtuvo una cantidad de 27 a 35 mg/100 g de peso seco y en el zumo comercial fue inferior a los 5 mg/100 g de peso seco. La presencia de estos pigmentos se ven afectados tanto por el pH como por el tratamiento térmico, por ello el zumo comercial que presentó un pH ácido (3,71) y llevaba un tratamiento térmico obtuvo menor cantidad de

pigmentos y betalaínas. Esto sugiere, en vista de los compuestos bioactivos, que lo más recomendable es usar el zumo fresco para simular el color de la carne en las hamburguesas veganas.

Las hamburguesas veganas mostraron una pérdida durante el cocinado entre un 14% y un 17%, así como una reducción del diámetro del 3% al 5%, sin un aumento en el grosor de las muestras, para todas las propiedades no se obtuvieron diferencias significativas entre las distintas muestras de hamburguesas veganas sustituidas ($p > 0,05$) (Tabla 32). Estos valores son menores que los encontrados en las hamburguesas de carne tradicionales publicados por otros autores, lo que sugiere un efecto positivo de los ingredientes vegetales en la reducción de las pérdidas por cocinado. Ni el tipo de zumo ni la emulsión gelificada utilizada tuvieron un efecto significativo sobre las proteínas de las hamburguesas veganas. Esto se podría deber a que la soja texturizada, utilizada como principal fuente de proteína en las mismas, ya está desnaturalizada antes del cocinado, lo que limita los cambios en la microestructura y las propiedades de retención de fluidos de la matriz de la hamburguesa.

Tabla 32. Propiedades de cocinado en las hamburguesas veganas con adición de emulsión gelificada como fuente de grasa.

	Pérdidas por cocinado (%)	Acortamiento (%)
PBFCH	15,58±0,76 ^a	3,85±1,28 ^a
PBCCH	16,81±1,14 ^a	3,79±0,76 ^a
PBFCA	17,36±0,34 ^a	4,75±0,42 ^a
PBCCA	14,59±0,61 ^a	5,11±0,26 ^a

PBFCH: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBCCH: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBFCA: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo. PBCCA: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo. Para cada parámetro, los resultados seguidos por la misma letra minúscula no presentan diferencias significativas ($p > 0,05$) según el test de Tukey entre las diferentes muestras cocinadas. Los datos se presentan como media y DS.

En los parámetros de textura únicamente en la dureza existieron diferencias significativas ($p < 0,05$) entre las distintas formulaciones, habiendo una diferencia entre las hamburguesas con adición de zumo comercial o fresco, las elaboradas con zumo comercial presentaron mayores valores de dureza respecto a las elaboradas con zumo fresco ($p < 0,05$) (Tabla 33). Esto podría deberse a lo anteriormente mencionado, el hecho que, durante la elaboración de las hamburguesas con zumo fresco, este se tuviera que diluir para obtener la coloración deseada, provocando una diferencia en el valor de humedad de partida de las hamburguesas.

Tabla 33. Parámetros de textura de las hamburguesas veganas con adición de emulsión gelificada como fuente de grasa.

	Dureza (N)	Elasticidad	Cohesividad	Masticabilidad (N.mm)
PBFCH	23,33±1,66 ^b	0,11±0,01 ^a	0,53±0,05 ^a	1,36±0,19 ^a
PBCCH	32,88±1,99 ^a	0,11±0,01 ^a	0,44±0,06 ^a	1,59±0,26 ^a
PBFCA	22,38±2,03 ^b	0,12±0,01 ^a	0,51±0,09 ^a	1,33±0,26 ^a
PBCCA	33,30±2,08 ^a	0,10±0,01 ^a	0,50±0,04 ^a	1,60±0,36 ^a

PBFCH: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBCCH: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBFCA: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo. PBCCA: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo. Los resultados seguidos de distinta letra minúscula (a-b) representan diferencias significativas ($p < 0,05$) para cada parámetro según el test post-hoc de Tukey. Los datos se presentan como media y DS.

En la Figura 44 se pueden observar los resultados de evaluación sensorial de las hamburguesas veganas, entre los distintos parámetros evaluados no se presentaron diferencias significativas ($p > 0,05$) para la textura y la aceptación general de las cuatro muestras analizadas. Las hamburguesas veganas elaboradas con zumo comercial obtuvieron puntuaciones significativamente más altas ($p < 0,05$) en color y apariencia, mientras que las elaboradas con zumo fresco destacaron en sabor ($p < 0,05$).

No se encontraron diferencias sensoriales atribuibles al tipo de emulsión gelificada utilizada ($p > 0,05$). Aunque en estudios previos se han observado variaciones sensoriales en hamburguesas de carne donde la grasa animal fue sustituida por emulsiones gelificadas, en este caso, la combinación de ingredientes (proteínas de soja, fibra de guisante, zumo de remolacha, especias, etc.) podrían haber enmascarado las diferencias de sabor atribuibles al tipo de emulsión gelificada. Las diferencias observadas en color y sabor, dependiendo del tipo de zumo usado, parecen ser las responsables de la ausencia de variaciones en la aceptación general de las hamburguesas veganas.

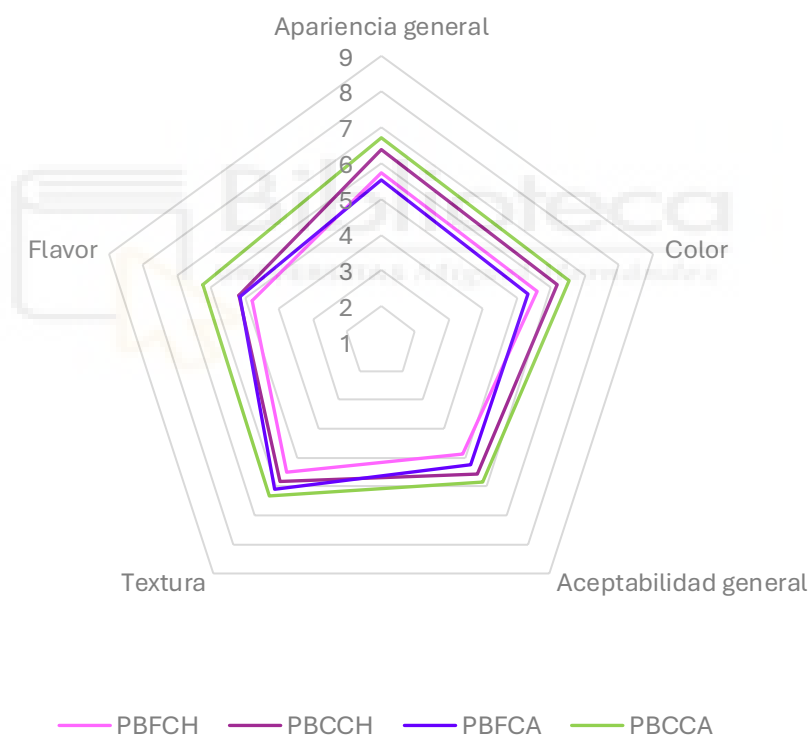


Figura 44. Evaluación sensorial de las hamburguesas veganas con adición de emulsión gelificada como fuente de grasa.

PBFCH: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBCCH: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de chía. PBFA: hamburguesa vegana con zumo fresco de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo. PBCCA: hamburguesa vegana con zumo comercial de remolacha y adición de emulsión gelificada de harina de trigo sarraceno y aceite de cáñamo.

CAPÍTULO 6

CONCLUSIONES/
CONCLUSIONS



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6. CONCLUSIONES/CONCLUSIONS

6.1. CONCLUSIONES

1. Las emulsiones gelificadas elaboradas con harinas de pseudocereales (amaranto, trigo sarraceno, quinoa y teff) y aceites vegetales (chía, cáñamo y la mezcla en proporción 1:1 de ambos) se presentan como una alternativa adecuada para sustituir la grasa animal, desde un punto de vista tecnológico y con un perfil lipídico más saludable (caracterizado por un alto contenido en ácidos grasos poliinsaturados) que las grasas de origen animal.

2. Las emulsiones gelificadas formuladas con harina de amaranto, harina de trigo sarraceno y aceites de chia y cáñamo presentan mejores resultados en términos de estabilidad oxidativa y de estabilidad de emulsión (a lo largo de 15 días de almacenamiento en congelación), en comparación con las emulsiones gelificadas desarrolladas con harina de teff y quinoa blanca.

3. El uso de emulsiones gelificadas de harina de amaranto con aceite de chia o cáñamo como sustituto (hasta el 50%) de la grasa animal permite alcanzar una reducción de hasta el 33% de grasa en hamburguesas de ternera, sin afectar a las propiedades texturales, color y pH.

4. En hamburguesas de ternera, la sustitución parcial de grasa animal por la emulsión gelificada a base de amaranto con aceite de cáñamo consigue incrementar el contenido de ácidos grasos omega 6, mientras que cuando se usó aceite de chia se incrementaron los ácidos grasos omega 3. Alcanzándose en este último caso, valores de los ácidos grasos omega 3

superiores a 0,6 g/100 g, pudiendo ser etiquetadas como “hamburguesas altas en ácidos grasos omega 3”.

5. Sensorialmente, la utilización de las emulsiones de harina de amaranto y aceite de cáñamo como sustituto parcial de grasa animal (hasta el 50%) en hamburguesas de ternera, no provoca diferencias respecto a las hamburguesas control.

6. La conservación en congelación (durante 60 días) de las hamburguesas elaboradas con las emulsiones gelificadas de amaranto con aceite de chía o cáñamo no provoca incrementos en los niveles de oxidación, aunque las hamburguesas en las que se utilizó el aceite de chía muestran mayor susceptibilidad.

7. El empleo de emulsiones gelificadas con aceite de cáñamo y trigo sarraceno en salchichas tipo Frankfurt permite una reducción de entre el 17% y el 39% de la grasa total con una mejora del perfil lipídico, sin comprometer la estabilidad oxidativa. También se consigue una reducción de los ácidos grasos saturados y un incremento de los poliinsaturados, al igual que en el resto de los productos cárnicos desarrollados.

8. En las salchichas tipo Frankfurt, la sustitución de hasta un 75% de grasa animal por emulsión gelificada (de trigo sarraceno y aceite de cáñamo) no provoca diferencias sensoriales con las muestras control. La peor evaluación sensorial la presentan las salchichas en las que se alcanzó el 100% de sustitución de grasa animal por la emulsión gelificada.

9. La incorporación de la emulsión gelificada a base de aceite de cáñamo y harina de trigo sarraceno es tecnológicamente viable para la sustitución de grasa animal en el desarrollo de un paté de hígado de cerdo con un perfil nutricional mejorado.

10. La utilización de emulsiones gelificadas (de harina de trigo sarraceno y aceite de cáñamo) como sustitutos de grasa animal en paté, reduce el aporte de colesterol (hasta un 16%), permitiendo alcanzar las directrices nutricionales establecidas para el desarrollo de productos cárnicos más saludables. También se consigue incrementar el contenido total de ácidos grasos poliinsaturados y de los ácidos grasos omega 3 en particular.

11. La reformulación de patés utilizando las emulsiones gelificadas mejora su untabilidad y jugosidad. Sensorialmente, presentan una aceptación general similar a la de los patés tradicionales.

12. Las emulsiones gelificadas se presenta también como una alternativa viable para la reformulación de productos tradicionales portugueses, sin efectos negativos en las pérdidas por cocinado ni en las propiedades físico-químicas.

13. Aunque en fresco las alheiras reformuladas con emulsiones gelificadas presentan propiedades de color diferentes a las alheiras tradicionales, tras el cocinado previo a su consumo, dichas diferencias se vuelven no detectables por los consumidores.

14. A pesar de que las alheiras cocinadas resultan ser más susceptibles a la oxidación lipídica, presentan valores de oxidación (mg MDA/kg de muestra) por debajo del límite de detección de rancidez por parte de los consumidores.

15. La combinación de las emulsiones gelificadas con el zumo de remolacha (fresco o comercial) permite desarrollar hamburguesas veganas con potencial para cubrir las demandas de la población que sigue una dieta vegana, vegetariana o flexitariana.

16. Las hamburguesas veganas desarrolladas presentan un alto contenido en ácidos grasos poliinsaturados, destacando los ácidos grasos α -linolénico y linoleico (dependiendo del aceite usado para la elaboración de la emulsión gelificada).

17. Los índices nutricionales utilizados para evaluar la calidad de las grasas como el índice aterogénico, trombogénico y la relación hipocolesterolémica/hipercolesterolémica, relacionados con la prevención y tratamiento de enfermedades, mejoran cuando se usa la emulsión gelificada de chía en comparación con las hamburguesas veganas que contienen la emulsión con aceite de cáñamo.

6.2. CONCLUSIONS

1. Gelled emulsions made with pseudocereal flours (amaranth, buckwheat, quinoa and teff) and vegetable oils (chia, hemp and a 1:1 mixture of both) are presented as a suitable alternative as a fat replacement from a technological

point of view and with a healthier lipid profile (characterized by a high content of polyunsaturated fatty acids) compared animal fats.

2. Gelled emulsions formulated with amaranth flour, buckwheat flour, chia and hemp oils show better results in terms of oxidative stability and emulsion stability (over 15 days of frozen storage) compared to gelled emulsions developed with teff and white quinoa flours.

3. The use of gelled emulsions of amaranth flour with chia or hemp oil as a replacer (up to 50%) for animal fat allows a reduction of up to 33% fat in beef burgers, without affecting textural properties, colour and pH.

4. In beef burgers, the partial replacement of animal fat with amaranth-based gelled emulsions with hemp oil increased the omega 6 fatty acid content, while when chia oil was used, omega 3 fatty acids were increased. In the latter case, omega 3 fatty acid values above 0.6 g/100 g were achieved and could be labelled as 'burgers high in omega 3 fatty acids'.

5. Sensorially, the use of amaranth flour and hemp oil emulsions as a partial fat replacer for animal fat (up to 50%) in beef burgers, does not cause differences compared to control burgers.

6. Frozen storage (for 60 days) of burgers made with the gelled amaranth emulsions with chia or hemp oil does not cause increases in oxidation levels, although the burgers in which chia oil was used show higher susceptibility.

7. The use of gelled emulsions with hemp and buckwheat oil in Frankfurters sausages allows a reduction between 17% and 39% of total fat with an improved lipid profile, without compromising oxidative stability. A reduction in saturated fatty acids and an increase in polyunsaturated fatty acids is also achieved, as in the other meat products developed.

8. In Frankfurter sausages, the substitution up to 75% of animal fat by gelled emulsion (buckwheat and hemp oil) does not cause sensory differences with the control samples. The worst sensory evaluation is in the case of sausages where 100% replacement of animal fat by gelled emulsion was achieved.

9. The incorporation of the gelled emulsion based on hemp oil and buckwheat flour is technologically feasible for the replacement of animal fat in the development of a pork liver pâté with an improved nutritional profile.

10. The use of gelled emulsions (buckwheat flour and hemp oil) as animal fat substitutes in pâté decrease of the cholesterol (up to 16%), making it possible to achieve the nutritional guidelines established for the development of healthier meat products. It also increases the total content of polyunsaturated fatty acids and omega 3 fatty acids.

11. Reformulation of pâté using the gelled emulsions improves spreadability and juiciness. Sensorially, they have a similar general acceptance as traditional pâté.

12. Gelled emulsions are also presented as a viable alternative for the reformulation of traditional Portuguese products, without negative effects on cooking losses and physico-chemical properties.

13. Although the reformulated alheiras with gelled emulsions have different color properties compared to traditional alheiras when fresh, after cooking prior to consumption, these differences become undetectable to consumers.

14. Although cooked alheiras are more susceptible to lipid oxidation, they show oxidation values (mg MDA/kg of sample) below the limit of detection of rancidity by consumers.

15. The combination of the gelled emulsions with beetroot juice (fresh or commercial) allows the development of a new plant-based burger with the potential to meet the demands of the population following a vegan, vegetarian or flexitarian diet.

16. The developed plant-based burgers have a high content of polyunsaturated fatty acids, highlighting α -linolenic and linoleic fatty acids (depending on the oil used to make the gelled emulsion).

17. The nutritional indices used to evaluate fat quality, such as the atherogenic index, thrombogenic index and hypocholesterolemic/hypercholesterolemic ratio, related to the prevention and treatment of diseases, improve when using the chia gelled emulsion compared to the plant-based burgers containing the emulsion with hemp oil.



CAPÍTULO 7

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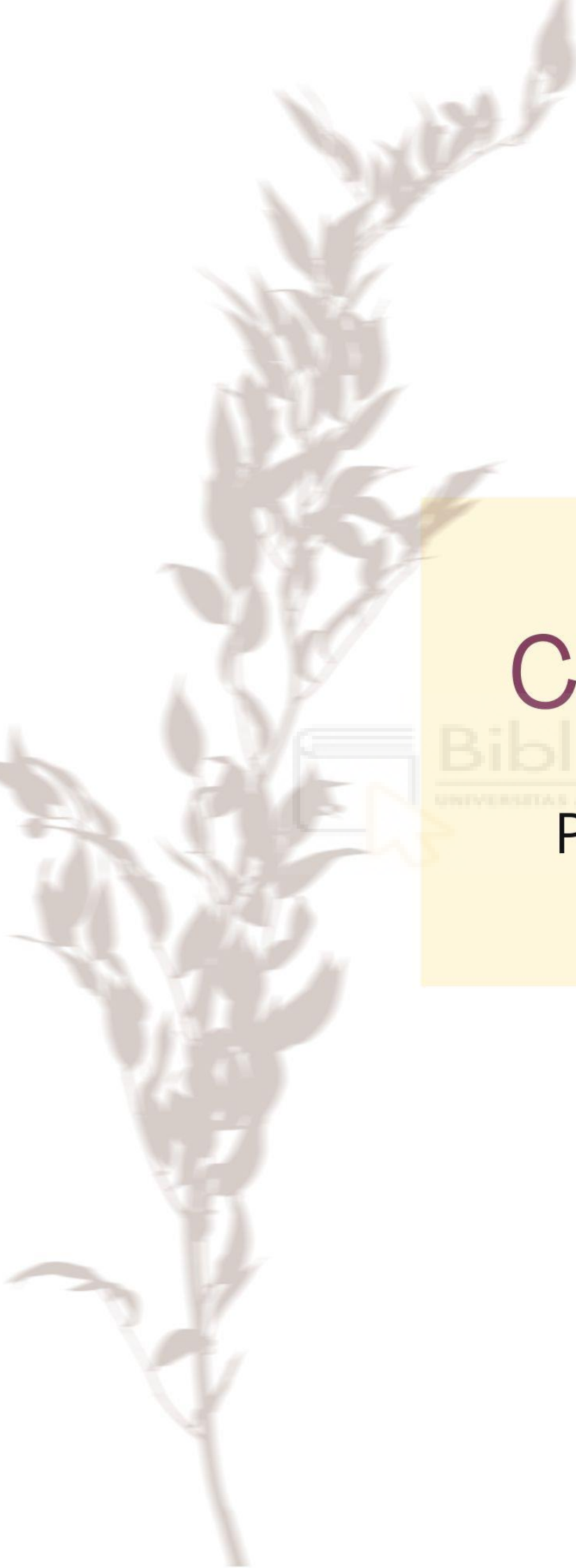
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CAPÍTULO 8

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PUBLICACIONES



8.1. PUBLICACIÓN 1

Healthier oils: a new scope in the development of functional meat and dairy products: a review

Autores: Carmen Botella-Martínez, José Ángel Pérez-Álvarez, Estrella Sayas-Barberá, Casilda Navarro Rodríguez de Vera, Juana Fernández-López y Manuel Viuda-Martos.

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Review

Healthier Oils: A New Scope in the Development of Functional Meat and Dairy Products: A Review

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Abstract: In the present day, it has been widely established that a high intake of animal fat that contains a high content of saturated fatty acids may cause several life-threatening diseases, including obesity, diabetes-type 2, cardiovascular diseases, as well as several types of cancer. In this context, a great number of health organizations and government agencies have launched campaigns to reduce the saturated fat content in foods, which has prompted the food industry, which is no stranger to this problem, to start working to develop foods with a lower fat content or with a different fatty acid profile. Nevertheless, this is not an easy task due to the fact that saturated fat plays a very important role in food processing and in the sensorial perception of foods. Actually, the best way to replace saturated fat is with the use of structured vegetable or marine oils. The main strategies for structuring oils include pre-emulsification, microencapsulation, the development of gelled emulsions, and the development of oleogels. This review will examine the current literature on the different (i) healthier oils and (ii) strategies that will be potentially used by the food industry to reduce or replace the fat content in several food products.

Keywords: reformulation; vegetable oils; marine oils; pre-emulsification; microencapsulation; gelled emulsion; oleogels; meat products; dairy products



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1. Introduction

Non-communicable diseases are the principal cause of death around the world. Globally in 2019, non-communicable diseases were responsible for 73.6% of deaths, which is higher than all other causes combined [1]. Among non-communicable diseases, cardiovascular diseases account for the highest number of deaths [2]. In this sense, out of the 17 million premature deaths (under the age of 70) due to non-communicable diseases in 2019, 38% were caused by cardiovascular diseases. Cardiovascular disease comprises coronary heart disease (heart attacks), cerebrovascular disease (strokes), heart rhythm problems (arrhythmias), and pulmonary embolism, among others [3].

Atherosclerosis, which is linked to dyslipidaemias and obesity, is the principal risk factor in the development of heart attacks and ischemic strokes [4]. Atherosclerosis can be considered a multifactorial disease in which modifiable factors, including tobacco smoking, alcohol consumption, no physical activity, bad dietary habits including high intake of salt and saturated fats, and a deficiency in the consumption of vegetables and fruit [5,6], and non-modifiable factors, such as age, gender, ethnicity, and family history of atherosclerosis, interfere in its development [7].

Among the modifiable factors, saturated fat intake reduction is the principal issue that can be addressed. Meat, meat products, and dairy products are the main sources of saturated fat. In this sense, in the last decade a huge number of countries have applied diverse initiatives, including food labelling schemes, healthy eating promotion campaigns, risk assessment measures, and trade consultations, to reduce the intake of foods with a

high saturated fat content [8]. Thus, the World Health Organization recommends that the total fat consumption must be lower than 30% of total energy intake (TEI), the saturated fat consumption must be lower than 10% of TEI, and the total *trans*-fats consumption must be lower than 1% of TEI, with the ultimate objective of replacing saturated fats and *trans*-fats with mono- and polyunsaturated fats [9].

However, it is important to highlight that in the organism, fat not only plays a role as an energy source but also plays a very important role as (i) a structural component of the body (ii) in the transport of fat-soluble vitamins as well as (iii) through intervening in physiological processes of the organism. Additionally, it is essential for the correct functioning of a series of biological functions during growth and development [10,11]. In food products, fat has both technological and sensory functions. Regarding the technological functions, fat content is related to an increase in emulsion stability, a reduction of cooking loss, and the regulation of the drying process in dry-cured products, besides exerting a great influence on the rheological and structural properties [12,13]. In reference to sensorial properties, fat improves the palatability of products, improves the overall texture of products, increases juiciness, decreases hardness, enhances the color properties of products, and increases the flavor of products [13–15]. For these reasons, reducing or eliminating fat from food, especially meat and dairy products, is time-consuming and labor-intensive. The agri-food industry, together with the scientific community, has already started to look for alternatives to replace saturated fat with other types of fat that have better fatty acid profiles that are rich in monounsaturated and polyunsaturated fatty acids. Thus, low-fat reformulated products with better fatty acid profiles are increasingly available on the market.

The principal sources of fat rich in monounsaturated and polyunsaturated fatty acids are vegetable (seeds and fruits) and marine (fish and seaweed) oils [16]. Due to their composition, these fats are more susceptible to rancidity, which leads to a loss of nutritional and sensory value of the product, as well as a reduction in shelf life, making their use in the preparation of meat or dairy foods more difficult [17]. These side effects may be reduced using diverse strategies that could stabilize the system to avoid oil separation in the food matrix [18]. These strategies include the transformation of liquid oils obtained from vegetables, seaweeds, or fishes into solid-like fats without the formation of artificial *trans*-fat [15]. The main techniques for structuring oils and improving their technological and functional properties include pre-emulsification, microencapsulation, the development of gelled emulsions, and the development of oleogels [13,19–21].

In the scientific literature, it is possible to find a high number of works that have informed of the use of pre-emulsification, microencapsulation, oleogels, and gelled emulsions prepared with vegetable or marine oils (with a high content of polyunsaturated fatty acids) as saturated fat replacers in several foods, including meat and dairy products [22–26] (Figure 1).

The aim of this work is to examine the current literature on different (i) healthier oils and (ii) strategies that will be potentially used by the food industry to reduce or replace the fat content in several types of food products.

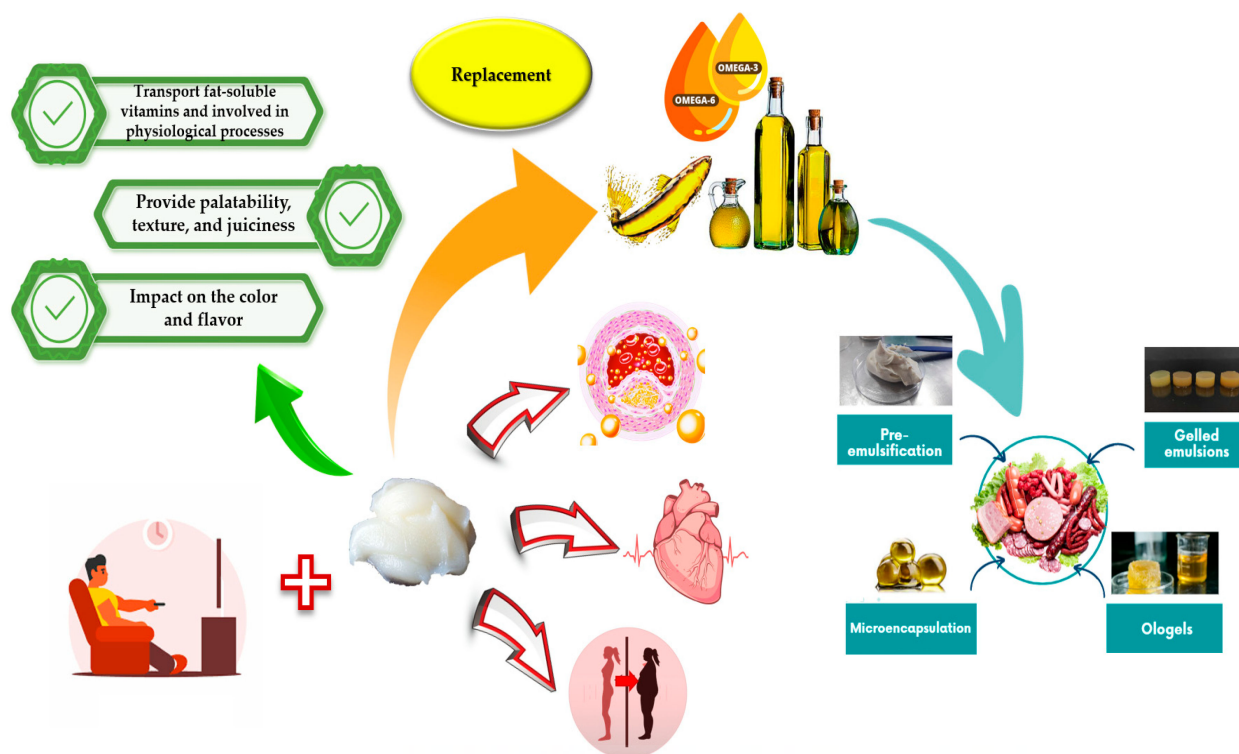


Figure 1. Effects of animal fat in the body and the main strategies to replace it in food products.

2. Healthier Oils

2.1. Vegetable Oils

Global vegetable oil production has increased in the last few years to reach 217.7 million metric tons in 2022 [27]. An important feature common to most vegetable oils is the high percentage of unsaturated fatty acids in triacylglycerols. Although this fact is beneficial for health, deep frying vegetable oils for a longer period, as occurs with saturated fats from animal origin, converts them into compounds responsible for off-type flavors such as short-chain hydroperoxides, aldehydes, and keto derivatives [28]. Table 1 shows the lipid profile of some edible vegetable oils.

Table 1. Lipid profile of several vegetable and animal oils.

	Oils from Oilseeds						Ref.
	Saturated Fatty Acids	Monounsaturated Fatty Acids		Polyunsaturated Fatty Acids			
		Oleic Acid	Other	Linoleic Acid (<i>w-6</i>)	Linolenic Acid (<i>w-3</i>)	Others	
Soybean	15.6–16.2	21.4–22.6	0.2–1.0	52.0–54.5	10.0–12.8	0.8–1.2	[29,30]
Canola	7.4–8.2	61.8–63.5	1.5–2.4	18.6–20.2	9.1–10.4	0.4–0.7	[31,32]
Sunflower	15.5–17.0	22.4–23.3	0.2–0.8	55.0–57.8	3.6–4.2	0.9–1.4	[33,34]
Cottonseed	25.9–26.8	17.8–18.6	1.2–1.8	52.1–54.0	0.6–1.0	0.1–0.5	[35,36]
Corn	15.6–17.0	25.3–26.7	0.3–0.5	52.0–53.4	1.0–1.2	4.4–4.9	[37,38]
Peanut	13.5–15.4	53.0–54.2	0.3–0.6	29.8–32.0	0.2–0.4	0.8–1.0	[39–41]
Chia	8.0–8.5	4.0–4.8	—	20.0–22.7	68.0–69.5	—	[42,43]
Hemp	7.0–7.8	8.3–9.0	—	52.6–54.0	21.7–22.0	8.0–8.6	[44,45]
Date	44.5–47.0	42.6–45.0	—	8.0–8.6	n.d.	n.d.	[46]

Table 1. Cont.

Oils from Oilseeds							
	Saturated Fatty Acids	Monounsaturated Fatty Acids			Polyunsaturated Fatty Acids		Ref.
Oils from fruits							
Olive	11.1–12.7	72.3–78.6	1.0–1.5	8.8–9.1	0.7–0.9	5.1–5.5	[31,47,48]
Coconut	80.0–82.5	6.0–7.8	5.0–6.3	—	—	1.7–2.1	[49,50]
Avocado	9.60–12.5	58.6–61.5	8.7–10.5	9.0–12.5	1.5–2.4	1.5–1.9	[31,51]
Oils from marine origin							
Seaweed	50	11.2	11.2–12.0	5.0–5.8	1.5–1.8	26.5–28.0	[52–54]
Fish (<i>Sardina pilchardus</i>)	32	25	7.2–7.8	3.5–4.2	1.0–1.2	29.5–31.3	[55,56]
Oils from Insects							
<i>Tenebrio mollitor</i>	33.4	35.8	2.1	22.8	0.1	5.8	[57]
<i>Acheta domesticus</i>	31.2	20.2	0.8	41.4	1.1	5.3	[58]

2.1.1. Oils from Oilseeds

Oilseed plants are one of the largest crop groups in world production because their seeds have a high percentage of high-quality fatty acids and proteins. Among the most commonly used seeds for the extraction of edible oils are soybeans, rapeseed (canola), sunflower, cottonseed, corn, and peanut, among others. Oils from rapeseed, soybeans, and sunflower seeds account for 87% of global vegetable oil production [27]. They are rich in ω -3 and ω -6 fatty acids, vitamins A, D, E, and K, and minerals such as zinc, calcium, magnesium, potassium, copper, and iron. Most of them are principally obtained by solvent (particularly *n*-hexane) extraction or mechanical expellers. Once the oils are extracted from the seeds, they need a refining process (compulsory if they are chemically extracted) prior to consumption to improve the conservation and nutritional conditions, since some seeds contain a series of substances called antinutrients that can be toxic.

Soybean Oil

Soybean oil is extracted from the seeds of the soybean (*Glycine max*). The composition of soybean oil is 14–16% saturated fatty acids (10% of palmitic acid (16:0) and 4% of stearic acid (18:0)), 20–24% monounsaturated fatty acids (mainly oleic acid (18:1) approx. 18–20%), and 62–66% polyunsaturated fatty acids (54% of linoleic acid (18:2) and 12% linolenic acid (18:3)) [29,30]. Like other vegetable oils, soybean oil also contains phytosterols (β -sitosterol, campesterol, stigmaterols, etc.), tocopherols (α -tocopherol and β -tocopherol), β -carotene, lutein, and chlorophylls [30].

Canola Oil

Canola oil is produced from rapeseed (mainly *Brassica napus* L). This oil exhibits the best fatty acid composition among all common oils. It has the ideal combination of the lowest level of saturated fatty acids (7–8%), a high content of monounsaturated fatty acids (63–66%), and an excellent ratio of ω -6 (linoleic acid) to ω -3 (α -linolenic acid) polyunsaturated fatty acids (19.0/9.1 g/100 g) [31]. This composition is responsible for the health properties associated with canola oil consumption. Among the non-glyceridic content, canola oil also contains phytosterols (mainly β -sitosterol) and vitamins E (as α and β -tocopherol) and K [32].

Sunflower Seed Oil

Sunflower oil is extracted from the seeds of sunflowers (*Helianthus annuus*). The main fatty acid fraction is polyunsaturated fatty acids (44–75% linoleic acid), followed by monounsaturated fatty acids (22–24% oleic acid) and saturated fatty acids (15%: 7% palmitic acid and 8% stearic acid). It is also a rich source of vitamin E [33]. Depending on plant breeding and industrial processing, four types of sunflower oil have been obtained: high-linoleic (69% linoleic acid), high-oleic (82% oleic acid), mid-oleic (65% oleic acid), and high-stearic combined with high-oleic (18% stearic acid and 72% oleic acid) [34]. However, from these four types of sunflower oil, the most used are the high-oleic and the mid-oleic sunflower oils.

Cottonseed Oil

Cottonseed oil is extracted from the seeds of cotton plants from various species, mainly *Gossypium hirsutum* and *Gossypium herbaceum*. It has been reported that yield, nutritional value, and chemical composition seed oils are affected by several factors, mainly including genetic constitution [59]. The main fatty acid fraction in cottonseed oil is polyunsaturated fatty acids (52–55%; mainly linoleic acid 52% and a small amount of (<1%) linolenic acid), followed by saturated fatty acids (25–30%; palmitic acid 26% and stearic acid 2%) and monounsaturated fatty acids (18%, oleic acid) [35].

Corn Oil

Corn oil is extracted from the germ of the seeds of the corn plant (*Zea mays* L.), and for this reason it is commonly known as “corn germ oil.” The germ represents between 9–11% of the seed weight and contains about 80% of the lipids found in the whole seed [60].

Corn oil belongs to the group of vegetable oils with high levels of linoleic and oleic acids (similar to sunflower oil). Specifically, it has been reported that about 60% of its fatty acids are polyunsaturated fatty acids (52% linoleic acid and only 1% of linolenic acid), 25% are monounsaturated fatty acids (oleic acid), and 15–17% are saturated fatty acids (palmitic acid as the predominant one) [37,38]. Corn oil also represents an important source of minor bioactive lipids, such as phytosterols (β -sitosterol 55–67%, campesterol 19–24%, stigmasterol 4–8%, and Δ -5-avenasterol 4–8%), tocopherols, tocotrienols, and carotenoids (especially xanthophylls, lutein, and zeaxanthin) [61].

Peanut Oil

Peanut oil is the oil from the seed (peanut) of the peanut plant (*Arachis hypogaea*). It is predominantly perceived as a valuable source in relation to edible-oil along with the protein source that mainly remains in the peanut cake after oil extraction [62]. Peanut oil has oleic acid as the predominant fatty acid, accounting for 48 to 57%, with a mean value of 53%. In addition, it contains linoleic acid, accounting for 27 to 38%, with a mean value of 32%. Peanut oil also contains appreciable amounts of saturated fatty acids (10–15%), especially palmitic (8–11%) and stearic (2–4%) acids [39]. Like other oilseeds, medium and high oleic peanut varieties comprising 66–69% and 78–80% of monounsaturated fatty acids, respectively, have been developed [40,41]. Peanut oil contains a considerable amount of phytosterols (207 mg/100 g), which is even greater than olive oil’s phytosterol level [63]. In addition, peanut oil provides a valuable source of lipid-soluble vitamins, such as tocopherols (Vitamin E) and pantothenate [64].

Walnut Oil

Walnut oil is generally extracted by cold pressing from the fruit of the walnut tree (*Juglans regia*). Walnut seeds generally contain between 52% and 70% of oil, which depends on the cultivar [65]. In particular, walnut oil contains between 2.4–5.3% palmitic acid, between 1.4–4.1% stearic, between 17.66–20.7% oleic acid, between 48.50–53.204% linoleic acid, and between 13.7–15.90% linolenic acid [66,67]. Walnut oil also contains a great

number of phytosterols (106.5 mg/100 g) than others seed oils [68]. Additionally, walnut oil has a high content of α -, β -, γ -, and δ -tocopherols (Vitamin E) and pantothenate [69].

Chia Oil

Chia (*Salvia hispanica* L.) is native to Central America. The oil content in chia seeds is about 34%, which presents the highest percentage of α -linolenic acid known so far (62–64%) as well as the highest content (82.3%) of essential fatty acids (α -linolenic acid and linoleic acid) [70]. The α -linolenic acid constitutes more than 60% of all total fatty acids in chia seeds, making this product one of the most important sources of α -linolenic acid in our diet [43]. Moreover, chia oil has the additional advantage of having a low content of saturated fatty acids; in particular, the oil contains 6.9% palmitic acid and 2.8% stearic acid [70].

Oil from Non-Traditional Oilseeds

Recently, it is possible to find oils obtained from seeds that are not usually cultivated for this purpose, but which are attracting increasing interest due to the special fatty acid compositions of the oils obtained. Regarding this, hemp oil (extracted from the seeds of *Cannabis sativa* L.) contains more than 80% of polyunsaturated fatty acids, including essential fatty acids usually not contained in traditional oils used for the human diet, consisting mainly of *w*-6 linoleic acid (50–55%) and *w*-3 α -linolenic acid (13–15%) [44]. In addition, the *w*-3/*w*-6 ratio is 3:1, which agrees with European Food Safety Agency recommendations [71]. Its unsaponifiable fraction is also a source of interesting minor bioactive compounds such as tocopherols, vitamins D and E, and phytosterols [45]. Linseed oil (*Linum usitatissimum* L.) is an important component in the development of functional foods, as it is rich in polyunsaturated fatty acids and phenolic compounds [72]. Linseed oil contains a low amount of saturated fatty acids (around 9% of total fatty acids), a moderate content of monounsaturated fatty acids (around 18%), and a high amount of polyunsaturated fatty acids (73%) [73]. Date seed oil, extracted from the seeds of the fruit of date palm, is liquid at room temperature, yellowish in color, and has a pleasant odor. This oil is considered a source of oleic acid (41–48%), with an important amount of saturated fatty acids (45–50%, mainly lauric acid 19%) and small amounts of polyunsaturated fatty acids (8%, mainly linoleic acid) [46]. It is also a good source of tocopherols and tocotrienols (74 mg/100 g), phytosterols (mainly β -sitosterol, campesterol, and Δ 5-avenasterol), and polyphenols, containing an even higher amount of polyphenols than olive oil [74].

2.1.2. Oils from Fruits

Olive Oil

Olive oil is extracted from the fruit of the olive tree (*Olea europaea* L.), which was one of the first plants to be cultivated for oil production. The main fatty acid in olive oil is oleic acid (65–85%), accounting for 55–83% of total fatty acids. It also contains variable amounts of linoleic acid (3–21%) and linolenic acid (<1%), with small saturated fatty acids contents (8–13%) [31,47]. Concerning bioactive compounds, their main representatives are the same of oil in general, namely tocopherols and phenolic compounds such as hydroxytyrosol and oleuropein, but also other compounds as pigments, such as provitamin A compounds and chlorophylls [48].

Coconut Oil

Coconut oil is an edible oil derived from the wick, meat, and milk of the fruit of the coconut palm (*Cocos nucifera*). The oil is a rich source of saturated fatty acids (85–90%), with short- and medium-chain fatty acids accounting for 70% of these fatty acids. The predominant fatty acid is lauric acid, representing 44–50% of the total fatty acids. It has a low content of unsaturated fatty acids (6–11%), oleic acid being the majority (5–8%), with a negligible content of both *w*-6 and *w*-3 polyunsaturated fatty acids and a low *w*-6/*w*-3 ratio (<4) [49].

Avocado Oil

Avocado oil is one of few edible oils not derived from seeds; it is extracted from the pulp of avocados, the fruit of *Persea Americana*, with an oil content of about 60%. Avocado oil has a similar monounsaturated fat profile (>60%) to olive oil. It can contain up to 71% of monounsaturated fatty acids (61% oleic acid and 10% palmitoleic acid), 13% of polyunsaturated fatty acids (12% linoleic acid and 1% linolenic acid), and 16% of saturated fatty acids (15% palmitic acid and less than 1% of stearic acid) [31,51]. Avocado oil has a high concentration of phytosterols (3.3 g to 4.5 mg/g of oil, higher than in olive oil), of which the most abundant is β -sitosterol, followed by sitostanol, cycloartenol, cycloeucalenol, and D7-avenasterol [75].

2.2. Oils from Marine Origin

2.2.1. Seaweed Oils

Seaweed oil is extracted from seaweed (also known as macroalgae or marine algae) and can be considered an alternative source of edible oils. Although its lipid content is low (0.1–10%), its use has raised considerable interest in recent years due to its high polyunsaturated fatty acids content (15–30%), specifically α -linolenic (w -3), octadecatetraenoic (w -3), arachidonic (w -6), and eicosapentaenoic acids (w -3) [76]. However, the saturated fatty acid fraction is the main fraction (45–55%), of which more than half consists of palmitic acid (C16). The monounsaturated fatty acids content (19–25%) is a little less than that of the polyunsaturated fatty acids (25–40%) [52]. It must be noted that the lipid fatty acid composition depends on the type of algae [53]. Red seaweed species (*Rhodophyta*) contain significant amounts of polyunsaturated fatty acids. Their two main polyunsaturated fatty acids are eicosapentaenoic (C20:5 w -3) and arachidonic acids (C20:4, w -6), and they also have a high oleic acid content. Brown seaweeds (*Phaeophyta*) show the highest relative concentration of monounsaturated fatty acids. Green seaweeds (*Chlorophyta*) are characterized by C16 and C18 polyunsaturated fatty acids, with a high C18/C20 polyunsaturated fatty acids ratio and a high degree of unsaturation. Unlike red and brown algae, green algae contain large amounts of hexadecatrienoic (16:3) and hexadecatetraenoic (16:4) polyunsaturated fatty acids and high contents of C18 polyunsaturated fatty acids (C18:2 and C18:3). Linoleic acid is the main polyunsaturated fatty acid found in most chlorophytes and α -linolenic acid is the main polyunsaturated fatty acid found in Ulvales algae [54]. Algae oil is also rich in some bioactive compounds found in its unsaponifiable fraction, mainly phytosterols, tocopherols, and carotenoids [52].

2.2.2. Fish Oils

Fish oil is derived from the tissues of fish (liver in lean fish and flesh in fatty fish). The main sources of fish oil are pelagic species caught in large quantities, particularly those with oily flesh (salmon, tuna, mackerel, and herring) or small fish (anchovies and capelin). Like seaweed oil, fish oil is characterized by a high level of the very long-chain, highly unsaturated ω -3 fatty acids eicosapentaenoic acid (C20:5, ω -3), docosapentaenoic acid (C22:5, ω -3), and docosahexaenoic acid (C22:6, ω -3). The growing awareness about the importance of ω -3 fatty acids in nutrition and health has led to a significant increase in fish oil consumption. The global fish oil market size is estimated to reach 284,412 million dollars by 2027, with a compound annual growth rate of 5.79% from 2021 to 2027 [55].

The lipid content in seafood species varies between 0.3–20%, depending on factors such as species, nutrition, geographical region, season, biological condition, age, gender, maturity, reproduction, and temperature [56]. Total saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids percentages of the total lipids range from 28% to 37%, from 18% to 38%, and from 11% to 35%, respectively. Palmitic acid is a dominant saturated fatty acid, followed by stearic acid. Palmitoleic and oleic acids are the main monounsaturated fatty acids (Table 1). The ω -3 polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic fatty acids are predominant [77], and therefore fish oil is an important dietary source of these essential fatty acids.

2.3. Insect Oils

Edible insects have been revealed as a sustainable and alternative source of nutrients, mainly proteins (trying to address human food demand), but also lipids, which are the second largest fraction and are sometimes regarded as a co-product. Lipid content in insects ranges between 10–35% in dry matter. Both lipid content and fatty acid composition can vary depending on species, state of growth, and extraction technologies, among other factors. The lipid content of *T. molitor* in the larvae state is 33%, clearly much higher than the content reported for the adult states of other insects (15% for *A. domesticus*) [57]. Most insect lipids are liquid at room temperature, which indicates that they are rich in UFA. The oil from *T. molitor* has a light-yellow color; oleic acid is the predominant fatty acid (32–38%), followed by linoleic acid (*w-6*, 20–25%) and palmitic acid (18–23%). The fatty acid profile of *A. domesticus* lipids is similar to that of *T. molitor* [58], but in this case the proportion of linoleic acid is higher than that of oleic acid [57] (Table 1).

3. Strategies for Structuring Oils

As mentioned above, beyond esterification or hydrogenation, there are many ways of structuring oils for their subsequent incorporation into food matrices. Additionally, the strategies attempt to mimic the appearance, plasticity, and rheological properties of animal fat in addition to improving nutritional quality and the lipid profile [13,78]. In this regard, as shown in Figure 2, pre-emulsification, oil encapsulation, oleogels, and gelled emulsions are several varieties of solid oil structured systems that offer promising results as fat substitutes in the development of healthier foods [79–84].

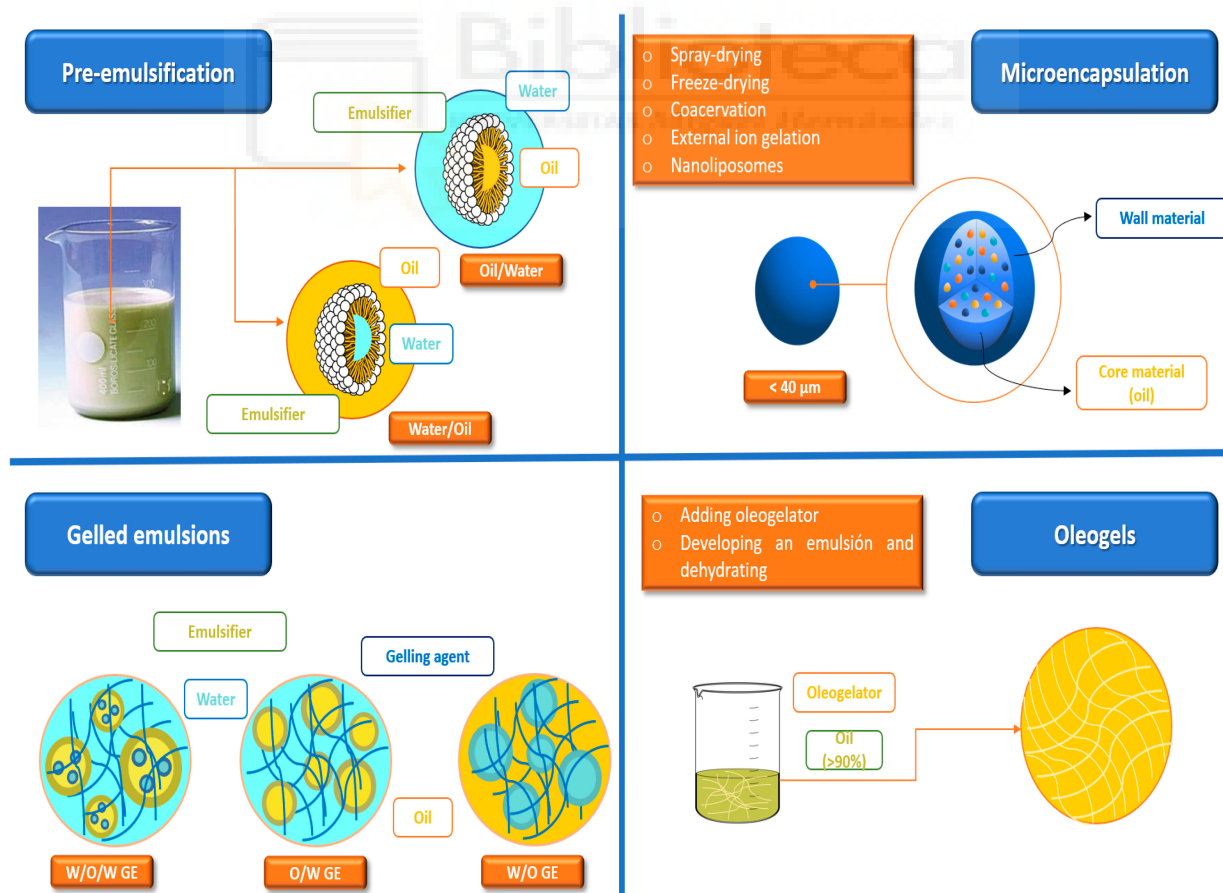


Figure 2. The main strategies to structure vegetable or marine oils.

Table 2 presents several studies where vegetable and marine oils were structured using different strategies to be applied in foods.

Table 2. Technological strategies to structure vegetable and marine oils.

Structure Components	Structuring Strategy	Procedure	Application	Ref.
<ul style="list-style-type: none"> - Sunflower oil (47 g/100 g) - Water (51 g/100 g) - Cellulose ethers (2 g/100 g) 	Pre-emulsification	First, the cellulose ether was dispersed in the oil and then the water was gradually added. Finally, the mixture was homogenized until the emulsion was obtained.	Cocoa creams	[85]
<ul style="list-style-type: none"> - Soy protein - Water - Rapeseed oil - Ratio (1:4:4) 	Pre-emulsification	Initially, soy protein was added into the water and mixed. Then, the oil was added gradually in a bowl chopper fitted with three blades and operating at two speeds.	UK-style sausages	[86]
<ul style="list-style-type: none"> - Whey protein powder - Hazelnut oil - Ratio (1:2) 	Pre-emulsification	The whey protein was mixed with hazelnut oil with a hand blender until the emulsion was obtained.	Sucuk Turkish fermented sausages	[87]
<ul style="list-style-type: none"> - Fish protein isolate (4%) - Soybean oil 	Pre-emulsification	Fish protein isolate was dissolved in phosphate buffer (10 mM, pH 7). The solution was mixed with soybean oil, in equal amounts (oil volume fraction 0.5), using the homogenizer at 19,000 rpm for 5 min.	Pork sausages	[88]
<ul style="list-style-type: none"> - Poppy oil or chia oil 100 mL - Maltodextrin 22 g 	Pre-emulsification	Oil and maltodextrin were mixed using a spoon and kept at 4 °C for 24 h.	Fresh chorizo	[23]
<ul style="list-style-type: none"> - Water (650 mL) - Tiger nut oil (50 g) - Lactose (50 g) - sodium caseinate (50 g) 	Encapsulation	Dry sprayer conditions were: feed rate 30%, inlet temperature 145 °C, and the aspirator at 80%.	Deer pâté	[89]
<ul style="list-style-type: none"> - Fish oils (1 kg) - Water (12 kg) - Maltodextrin (1.95 kg) - Gum arabic (0.9 kg) - Caseinate (0.15 kg) 	Encapsulation	Dry sprayer conditions were: feed rate 75 L/h, inlet temperature 180 °C, and outlet temperature 80 °C. Drying process lasted 3 h.	Frankfurters	[90]
<ul style="list-style-type: none"> - Chia oil - Sodium alginate solution (2%) 	Encapsulation	External ionic gelation technique.	Pork burgers	[91]
<ul style="list-style-type: none"> - Fish oil 10% - Lecithin 3% - Chitosan 2% 	Encapsulation	Dry sprayer conditions were: feed rate 1 L/h, inlet temperature 180 °C, and outlet temperature ranged 85–90 °C.	Pork burgers	[92]

Table 2. Cont.

Structure Components	Structuring Strategy	Procedure	Application	Ref.
<ul style="list-style-type: none"> - Linseed oil (40%) - Carrageenan (1.5) - Water (58.5%) - Polysorbate 80 (0.12 g/emulsion) 	Gelled emulsion	The oil phase with the surfactant was added to the aqueous phase (water + carrageenan) and homogenized.	Dry fermented sausages	[93]
<ul style="list-style-type: none"> - Water (56%) - Alginate-based hydrogels (6.7%) - Olive oil (37.3%) 	Gelled emulsion	Water and olive oil were mixed for 1 min. Then, gelling agent was added and homogenized during 3 min and then left to rest for 2 h. After that the mixture was cooled at 4 °C.	Deer fermented sausages	[94]
<ul style="list-style-type: none"> - Açai oil 200 mL - Water 100 mL - Konjac flour (0.86 g) - Sodium alginate (2 g) 	Gelled emulsion	Sodium alginate and konjac flour were dissolved in water at 60 °C with constant stirring. The emulsion (2:1 oil:water) was homogenized in the biocomposite obtained.	Beef burgers	[95]
<ul style="list-style-type: none"> - Water (45%) - Chia or hemp oils (45%) - Buckwheat flour (9%) - Carrageenans and locust bean gum (1%) 	Gelled emulsion	Carrageenans and locust bean gum were dissolved in water at 80 °C with constant stirring. Chia or hemp were added to aqueous solution and homogenized.	Plant-based burgers	[82]
<ul style="list-style-type: none"> - Sesame oil - Beeswax (10%) 	Direct oleogel	Oil and beeswax mixture were heated at 70 °C with continuous stirring until complete dissolution of beeswax. After that the blend was cooled at room T ^a .	Beef burgers	[96]
<ul style="list-style-type: none"> - High oleic sunflower oil (90%) - Rice bran wax (10%) 	Direct oleogel	Sunflower oil was combined with rice brand wax and the mixture was heated at 80 °C and stirred (5 min). After that the blend was cooled at room T ^a .	Ice cream	[97]
<ul style="list-style-type: none"> - Water - High oleic sunflower oil - Pork skin - Ratio 1:1.5:1.5 	Direct oleogel	Water, high oleic sunflower oil, and pork skin (cooked at 80 °C) were mixed in a blender.	Bologna sausages	[98]
<ul style="list-style-type: none"> - Linseed oil - Mixture of oryzanol and β-sitosterol (8%) 	Direct oleogel	Oryzanol and β -sitosterol were dispersed under stirring until solubilization in linseed oil at 80 °C for 30 min.	Pork burgers	[99]
<ul style="list-style-type: none"> - Carnauba wax - Canola oil - Ratio 1:9 	Direct oleogel	Carnauba wax and canola oil were heated at 90 °C with continuous agitation. Then the sample was cooled at room T ^a .	Cakes	[100]

3.1. Pre-Emulsification

Pre-emulsification of oils with a high content of monounsaturated and polyunsaturated fatty acids can be considered a good method to carry them into several food matrices such as meat, bakery, and dairy products without reducing their technological and physico-chemical properties [79,80]. To obtain this structure, the oils must be blended with different emulsifiers, mainly proteins, including whey protein, lecithin, soy proteins, and caseinates, among others [81], or with carbohydrates such as cellulose ethers [85]. The methodologies utilized to elaborate the pre-emulsified oils, the emulsifiers selected, as well as the concentrations of oil, water, and protein used are very different among the studies present in the scientific literature, making it very difficult to standardize a process. Thus, Bolger et al. [79] carried out a study on pre-emulsified flaxseed oil and vitamin E to be used as a fat replacer in low-fat chicken sausages. For the study, one part of soy protein concentrate was mixed with four parts of ice water with a hand-blender for 1 min. Then four parts of flaxseed oil were added and mixed for 5 min. To elaborate a low-fat pork patty, Lee et al. [12] prepared an oil-in-water emulsion by mixing water (70%), tween 80 (3.5%), canola oil (30%), and lecithin (1%) in a homogenizer at 4000 rpm for 3 min. In a similar study, Urgu-Öztürk et al. [81] designed a healthier beef sausage elaborated with a pre-emulsion made by emulsifying hazelnut oil (51.6%) with water (42.1%), using sodium caseinate (5.3%) and sodium chloride (1%) as emulsifier agents, and using a food processor at 5800 rpm for 2 min. In the same sense, to elaborate low-fat cooked lamb sausages de Carvalho et al. [101] made three pre-emulsions with chia, olive, and linseed oil, water, and sodium caseinate as an emulsifying agent in a proportion of 5:5:1 in a homogenizer at 5000 rpm for 3 min. Li et al. [102] made a pre-emulsion for reduced-fat filling in steamed buns. For that, they used soybean oil (30%) and aqueous solution (70%), adding whey protein isolate (0.2%), modified starch (0.5%), and composite gum (0.2%). More recently, to replace the animal fat in sheep meat sausages, Santos-Lima et al. [103] elaborated a pre-emulsion of linseed oil with isolated soy protein in a proportion of one part oil and two parts isolated soy protein.

3.2. Encapsulation

An alternative to pre-emulsification consists in encapsulating the oils. The encapsulation provides several advantages when they are added to food matrices. For instance, the encapsulating technique allows the masking of off-flavors, reduces lipid oxidation, and eases handling and precision when they are incorporated [78,104]. Oil encapsulation can be classified in several ways based on the chemical and physical transformations of the walls into capsules [105]. Some of the techniques used for the encapsulation of oils are spray-drying, freeze-drying, coacervation, and, to a lesser extent, the use of external ionic gelation and nanoliposomes [106]. The spray-drying process of oils has been the most used technique to obtain particles sized lower than 40 μm . This technique involves the oils' dispersion into a polymeric solution and a posterior atomization and dehydration, forming microparticles [104,107,108]. The freeze-drying encapsulation consists of vacuum dehydration before freezing the samples for subsequent incorporation in food matrices [109,110]. Coacervation is one of the most common methods to encapsulate oils. In this method, 1 to 10% of the polymer (coacervate) is dissolved in water and the oil is dispersed into a solution at 40–50 °C. The coating (liquid polymer) is deposited and stabilized in the oil phase [111]. Ionic gelation is the most popular extrusion process, with alginate and calcium interaction used for gellification. Nanoliposomes are made by phospholipids as a wall material and have a diameter lower than 100 nm. Thus, Venturini et al. [109] elaborated cookies with 30% fat replacement using chia oil with sodium caseinate and carnauba wax as wall materials by applying freeze-drying as the encapsulation methodology. Ullah et al. [104] carried out a study where they elaborated microcapsules of chia oil utilizing chitosan as the encapsulating material by means of spray-drying with its subsequent application in butter. In the same sense, Ojagh and Hasani [107] investigated the effect of fish oil encapsulated in nanoliposomes using wall materials of lecithin and sunflower oil. Heck et al. [91] studied

the microencapsulation of chia oil enriched with rosemary in sodium alginate and CaCl_2 using ionic gelation as the encapsulating technique (sodium alginate).

3.3. Gelled Emulsions

A gelled emulsion is an emulsion with a gel-like network structure and solid-like textural properties [112]. In this type of structure, the emulsions and gel co-exist and have stable rheological properties due to their similarity to animal fat in properties and characteristics [113]. Another important property is that these structures allow the inclusion of both hydrophobic and hydrophilic functional ingredients.

For the elaboration of gelled emulsions, an emulsifier is necessary, which could be of a polysaccharide nature (xanthan gum, konjac matrix, arabic gum, carrageenan, dietary fibre, etc.), a protein nature (sodium caseinate, soybean protein isolate, whey protein isolate, etc.) or a polysaccharide–protein combination [114]. The purpose of the emulsifier is to bind together the water-soluble matrix with the hydrophobic substances, creating water in oil (W/O) or oil in water (O/W) emulsions. Another main component is a gelling agent (pectin, gelatin, gellan gum, alginate methylcellulose, inulin, etc.), which creates the necessary network structure to enable gelation to take place [112].

In the scientific literature, there are several different combinations of emulsifiers, gelling agents, and oils used in the development of gelled emulsion [13,82,84]. In this sense, de Souza Paglarini et al. [115] elaborated a gelled emulsion made with soybean oil (50%), soy protein isolate (4%) as an emulsifier, inulin (16.5%), and water (29.5%) to be utilized as a fat replacer in bologna sausage. Öztürk-Kerimoğlu et al. [116] obtained a gelled emulsion with a 46.8% mixture of peanut oil and linseed oil (10:1) and 3.2% of polyglycerol polyricinoleate as an emulsifier and 50% of the aqueous phase with 40.3% water, 5% lyophilized powder of albumin egg and gelatin, 4% inulin, and 0.7% microbial transglutaminase. Botella-Martínez et al. [21] prepared gelled emulsions to be used as fat replacers in beef burgers based on chia oil or hemp oil (40%) with amaranth flour (10%) as an emulsifier and gellan gum and gelatine (3%) as gelling agents. Lee et al. [117] elaborated a gelled emulsion of canola oil (40%) using k-carrageenan (0, 0.5% *w/w*) and methylcellulose (0, 3% *w/w*) as gelling agents; the emulsifiers selected were tween 80 (1% *w/w*) and polyglycerol polyricinoleate (5% *w/w*). A more recent study carried out by Khan et al. [84] generated double water in oil-in-water (W/O/W) emulsions gelled with sunflower oil, whey protein isolate (WPI, as an emulsifier), pectin, and L-ascorbic acid (as gelling agents).

3.4. Oleogels

Oleogels are liquid oils turned to be semi-solid structures with viscoelastic properties and hydrophobic natures due to the establishment of a three-dimensional oleogelator network [118]. Oleogel formation allows a high concentration of liquid oil (>90%) to be structured into a “gel-like” system [119]. These new oil structures can be obtained directly by adding the oleogelator to the oil to be structured or indirectly by developing an emulsion beforehand and then dehydrating this structure to form the oleogel [83,120]. Several compounds can be used for oleogelators; the most widely utilized in food products include lipid-based gelators (bee wax, rice bran wax, candelilla wax, fatty alcohols, etc.) [121] and polymers (chitosan, ethylcellulose, hydroxypropylmethylcellulose, methylcellulose, etc.) [122]. Other compounds used as oleogelators include sphingolipids, tocopherols, phytosterols, and lecithin [123].

As regards direct gelation methods, the lipid-based gelators are widely used to elaborate the oleogels [83,120,124,125]. Therefore, in a study carried out by Malvano et al. [120] to replace the butter in the preparation of sponge cake, an oleogel was made by dissolving beeswax at 3 g/100 g in olive oil (97 g/100 g) at 85 °C and continuously agitating it in a vortex at 100 rpm. After that, the blend was cooled at 25 °C until the oleogel was formed. Similarly, Zbikowska et al. [125] made oleogels by dissolving different concentrations of beeswax (2, 4, 6 and 8 g/100 g) in peanut oil at 80 °C. This mixture was then cooled until

the oleogel was obtained. In the study conducted by Dent et al. [83], rice bran wax was added as an oleogelator in different proportions (2, 6, and 10 g/100 g *w/w*) to corn oil at 90 °C with or without added curcumin. Then, after complete dissolution, the mixture was cooled to obtain the oleogel. Pintado and Cofrades [126] showed how to obtain an oleogel based on a mixture of olive oil and chia oil for application in a fermented dry sausage with 10% beeswax as an oleogelator under constant stirring at 65 °C. More recently, in a study carried out by Oliveira et al. [127] an oleogel system was prepared using phytosterols and lecithin in various ratios (5:5; 6:4; 7:3; 8:2) dispersed in sunflower oil at 85 °C for 30 min under magnetic stirring.

In reference to indirect methodologies used to elaborate oleogels, Wang et al. [128] investigated through two indirect methodologies the preparation of oleogels with hydroxypropylmethylcellulose and methylcellulose as oleogelators and sunflower oil. In the emulsion, the oil content was 18, 33, and 47%, combined with 1.5% cellulose, and then the emulsions were dehydrated at 60 °C for 48 h, resulting in final concentrations of 92, 96, and 97%. For the foam template, 6 g of cellulose was dissolved in 94 g of water at 85 °C and the samples were freeze-dried. A final concentration of 92, 96, and 97% of oil was achieved by adding sunflower oil.

4. Food Application

4.1. Incorporation of Structured Healthier Oils in Meat Products

Improvements in the lipid profiles and quantity of meat products should be seen by the meat industry as an excellent opportunity for the development of functional products promoting health, differentiation, and competitive advantages. The animal fat of processed meat products is, as mentioned above, one of the ingredients that consumers are most concerned about due to its link with negative health implications (including obesity, cardiovascular diseases, and other diseases in modern society) [129–131]. The reformulation of processed meat products to develop healthier meat foods is focused mainly on modifying the lipids of meat products (reduction of total fat, reduction of total cholesterol intake, and improved fatty acid profile) [132,133]. Vegetable oils have been used to achieve reformulation strategies and to develop functional meat products based on lipid profile modification (lower content of saturated fatty acids, higher amounts of mono- and polyunsaturated fatty acids) of processed meat products [134].

Recent studies have shown that the partial and total replacement of animal fat with structuring vegetable oils is an efficient strategy to improve high-fat foods, especially with regard to meat product reformulation with healthier lipids [17,129,135].

It is known that animal fat plays a significant role in the functional characteristics of meat products, such as stability, heat transfer, and texture [13,136], and in the sensory properties (color, taste, elasticity, viscosity, and hardness) [137]. Pork backfat (animal fat widely used in the processing of meat products) is a semi-solid ingredient, but when it is directly replaced by oils, some undesired changes take place, including increased lipid oxidation, changed texture properties, and reduced water holding capacity [14,134]. As mentioned above, meat products' reformulation, such as the use of healthier oils using several strategies to simulate animal fat properties [130], is in focus. The encapsulation of oils in different matrices [79,89] and the use of oleogels [25,138] and structured emulsions (gelled emulsions or emulsions gel) [139,140] have been reported as viable strategies which are able to stabilize and structure liquid oils in a semi-solid system comparable to animal fat characteristics and thus avoid negative impacts on final products [130,134].

Several researchers have reported the effect of the reformulation of meat products using structured vegetable oils in fresh, cooked, and dry-cured meat products as a good strategy to replace and improve fat content (quantity and type) [14,139–141]. In these studies, the vegetable oils used to replace animal fats were sunflower, soybean, olive, perilla, and chia oils, among others, which improved fatty acid profiles, reducing the level of saturated fatty acids (SFA) and increasing the level of polyunsaturated fatty acids, but

also accelerated lipid oxidation reactions and reduced shelf life, in addition to reducing the loss of sensory properties [141].

Table 3 shows different studies that apply vegetable oils through different strategies as animal fat replacers to improve the fat content (quantity and type) of several reformulated fresh meat products.

Table 3. Summary of studies assessing effects on quality parameters of meat products with total or partial substitution of animal fat by vegetable oils with different incorporation strategies.

Meat Products	Vegetable Oil	Incorporation Strategy	Replacement (%)	Response on Meat Product	Ref.
Burger	Chia and linseed oils	Microencapsulation (external ionic gelation)	Replacement at 50%	Improved important technological properties (cooking loss and fat retention). Low fat, higher content of healthier polyunsaturated fatty acids/saturated fatty acids, and ω -6/ ω -3 ratio ratios. Acceptable sensory properties.	[142]
Burger	Chia and linseed oils	Hydrogelled emulsion	Replacement at 20, 40, 60, 80, and 100%	Increased protein:lipid ratio. Improved the fatty acid profile of raw burgers. Increased TBARs. Non-affected technological properties.	[80]
Burger	Chia oil	Microencapsulation (enriched with rosemary)	Replacement at 50%	Non-impact on the volatiles profile. Decreased in volatiles from lipid and protein oxidation. Decreased sensory descriptors related to lipid oxidation.	[143]
Burger	Chia and hemp oil	Gelled emulsions (amaranth flour, gellan gum, and gelatin)	Replacement at 25 and 50%	Improved nutritional characteristics of burgers. Non-affected technological or sensory properties. More susceptible to lipid oxidation.	[21]
Fresh chorizo	Melon and pumpkin seed oils	Oil emulsions	Replacement at 50%, 75%, and 100%	Softer texture. Better fatty acid profile, decreased in saturated fatty acids, and increased linoleic and linolenic fatty acids.	[23]
Fresh sausages	Olive oil	Gelled emulsions prepared with chia and oats	Replacement at 90%	Improved fat, minerals, and amino acid contents. Cooking loss was lower. Higher Kramer shear force values. Affected sensory properties, but were judged acceptable.	[144]

Table 3. Cont.

Meat Products	Vegetable Oil	Incorporation Strategy	Replacement (%)	Response on Meat Product	Ref.
Emulsified Meat Products					
Frankfurter	Soybean oil	Emulsion gels (EG) prepared, sonicated and non-sonicated soy protein isolate dispersions, carrageenan, and inulin	Replacement at 100% The pork backfat (10 and 20%) was replaced by the gelled emulsion	Good source of fiber. A reduction of 19–29% in energy value. A reduction of 35, 72, and 63% in ω -6/ ω -3 ratio, atherogenic index (AI), and thrombogenic index (TI), respectively.	[145]
Frankfurter	Soybean oil	Oleogels structured with rice bran wax	Replacement at 100%	Less dark and less red. Higher in the essential polyunsaturated fatty acids linoleic (18:2n6) and α -linolenic (18:3n3). Non-negatively influence the technological quality.	[24]
Frankfurter	Linseed oil	Oleogel gelled with beeswax	Replacement at 0% 25% and 50%	The fatty acid profile was substantially improved and saturated fatty acid content, as ω -6/ ω -3 ratio and cholesterol were reduced. Increased the yellowness with linseed oleogel. Increased cohesiveness, gumminess, and chewiness.	[25]
Frankfurter	Canola/soy/flaxseed oil	Oleogels	Replacement of 100%	Higher hardness values. Springiness was lower. Flaxseed oil provided the highest b*. Reduced cooking loss.	[26]
Emulsified sausages	Peanut and linseed oil	Gelled emulsion	Replacement of up to 40%	Healthier lipid composition and improved nutritional ratios: Decreased saturated fatty acids and cholesterol and increased mono and polyunsaturated fatty acids. Improved emulsion stability and cooking behaviors. Alterations in color and texture: higher yellowness and increased the hardness. Decreased oxidative stability.	[146]
Bologna sausage	Soybean oil	Emulsion gels prepared with chia flour and/or soy protein isolate, inulin, carrageenan, sodium caseinate, and sodium tripolyphosphate	Replacement at 50 and 100%	Improved lipid profile of the sausage. Lower fat content. Affected the color of sausages: increased L* and reduced a*. More homogeneous batter and a compact structure. Greater hardness, chewiness, and shear force.	[145]

Table 3. Cont.

Meat Products	Vegetable Oil	Incorporation Strategy	Replacement (%)	Response on Meat Product	Ref.
Deer pâté	Tigernut, chia, or linseed oils	Microencapsulated	Replacement at 50%	Decreased fat and cholesterol contents. Decreased the total amount of saturated fatty acids and increased polyunsaturated fatty acids (chia and linseed pâtés) or monosaturated fatty acids contents (tigernut pâtés). Modification of color parameters. Softer textures.	[89]
Bologna	High oleic sunflower oil	Oleogel prepared with pork skin, water, and high oleic sunflower oil	Replacement at 25, 50, 75, and 100%	Healthier lipid profile: reduction of approximately 10% cholesterol levels. Increased the proportion of oleic acid and decreased the proportion of linoleic acid. Non-changes in the oxidative stability. The acceptance and the sensory profile of the samples were not affected by the substitution of up to 50%. Decrease in cooking loss.	[98]
Pâtés	Mixture of olive, linseed, and fish oil	Oleogels produced with ethyl cellulose and beeswax oleogel	Replacement at 15%	Optimal fatty acid profile from a health standpoint (high polyunsaturated fatty acids/saturated fatty acids ratio and low as ω -6/ ω -3 ratio). Emulsion stability, texture, and color of pâtés not affected. Increased lipid oxidation. Sensory attributes similar.	[147]
Traditional Fermented Meat Products					
Dry fermented sausages	Linseed oil	Gelled emulsion	Replacement at 26.3%, 32.8%, and 39.5%	Increased polyunsaturated fatty acids supply (up to 10.3%) and reductions in ω -6/ ω -3 ratio (75, 82, and 84%, respectively). Non-affected peroxides and Thiobarbituric reactive substances (TBARs) values.	[93]

Table 3. Cont.

Meat Products	Vegetable Oil	Incorporation Strategy	Replacement (%)	Response on Meat Product	Ref.
Dry fermented sausage (<i>Salchichón</i>)	Linseed oil	Oleogels produced with 8% γ -oryzanol, β -sitosterol, and beeswax	Replacement at 20 and 40%	Improvement of the fatty acid profile. Color and sensory parameters were strongly affected. Quality parameters such as pH and color also changed with the inclusion of oleogels. Changed in the sensory quality.	[148]
Dry fermented sausage	Linseed oil	Gelled emulsion	Replacement at 65%	Lower saturated fatty acids and monounsaturated fatty acids and higher polyunsaturated fatty acids content with an improved ω -6/ ω -3 ratio α - and linolenic acid increment. Decreased in springiness, chewiness, and hardness and increase in adhesiveness. Lower L* and higher a*. Higher susceptibility to oxidation and lipolysis.	[149]
Dry fermented sausage	Grapeseed oil	Liquid, encapsulated, and pre-emulsified	Replacement at 20%	Higher weight loss. Lower hardness, chewiness, cohesiveness.	[150]
Dry fermented meat product (<i>Fuet</i>)	Olive and chia oil	Oleogels and gelled emulsion	Replacement at 80%	Improved fatty acid profile. Decrease of ω -6/ ω -3 ratio. Emulsion gel as animal fat replacer had similar hardness to the control whereas those with oleogel were softer.	[126]

4.1.1. Fresh Meat Products

Among the fresh meat products, burgers are the most studied meat products due to the importance of their intake among young people. Most of the studies are focused on reducing burgers' usual content of animal fat (up 20–30%) or replacing the animal fat content with healthier oil [140]. However, the use of vegetable oils in these meat products just affects the technological and sensory properties and shelf life (increased lipid oxidation); therefore, it would be advisable to use different strategies, such as microencapsulation, oleogels, and gelled emulsions. In fresh meat products (patties, fresh sausages, etc.), the appearance and structure of fat replacers are more important and they can influence visual sensory properties [151].

Microencapsulation allows the incorporation of vegetable oils with high ω -3 polyunsaturated fatty acids. Heck et al. [142] replaced 50% of the animal fat by encapsulated chia and linseed oils by using external ionic gelation and concluded that the microencapsulation was an effective strategy to produce healthier burgers (low fat, higher content of healthier polyunsaturated fatty acids/saturated fatty acids and as ω -6/ ω -3 ratios) [80]. Natural antioxidants are recommended when chia oil is used to avoid further lipid oxidation [143].

Recent studies have shown microencapsulation as a novel strategy for the protection of ω -3 polyunsaturated fatty acids from liquid oil against lipid oxidation [78].

Other strategies for the reformulation of burgers with healthy properties are gel emulsions, which show higher stability against oxidation and acceptable sensory properties [152]. Lucas-González et al. [139] evaluated the partial replacement of animal fat by an emulsion gel prepared with chestnut flour and chia oil in the reformulation pork burger, concluding that there was no negative impact on pork burger quality (except for the increase in lipid oxidation, as would be expected), and that the health properties of the reformulated healthy burgers were significantly improved (atherogenicity and thrombogenicity indexes). In two different works, Botella-Martínez et al. [21,153] used gelled emulsions based on chia and hemp oils as partial (25% and 50%) fat replacers in beef burgers during frozen storage and reported better nutritional quality than the control, mainly due to the increase in polyunsaturated fatty acids (specifically α -linolenic (C18:3) and linoleic (C18:2) fatty acids) and the decrease in saturated fatty acids. The burger showed no negative impact during frozen storage. Pintado et al. [144] used emulsion gels with olive oil prepared with chia flour or oat bran as animal fat replacers in reduced-fat fresh sausages, obtaining final meat products suitable for “reduced fat content” and “energy-reduced” claims without strongly affecting the sensory and technological properties. Martínez et al. [23] concluded that chorizos reformulated by replacing pork fat with emulsified seed oils from seeds (50%, 75%, and 100%) presented a better fatty acid profile and were positively evaluated in all the parameters studied.

4.1.2. Emulsified Meat Products

Current studies have concluded that pork backfat substitution by different vegetable oil inclusion strategies in emulsified meat products is a viable option for nutritional enhancement, decreased cholesterol, and a lowered atherogenicity index, thrombogenicity index, and ω -6/ ω -3 ratio of cooked meat products [146]. Several authors reported the difficulty of incorporating vegetable or marine oils rich in ω -3 polyunsaturated fatty acids in the heat treatment of meat products due to the higher degree of oxidation and technical and sensorial complications in the final product [91,136,137,154]. Likewise, the composition of the oil and the fat: protein ratio could affect the quality of the final products because they affect the emulsion stability [137].

Different effects have been reported in reformulated cooked meat products, such as improved lipid profile, softer texture through the increase of unsaturated fatty acid content [89], a better dispersion and distribution of the vegetable oils [89,155], and increased yellowness values due to the effect of vegetable oils [141,156]. Gelled emulsions with olive oil, chia oil, hemp oil, soybean oil, or linseed oil have been used as animal fat replacers in cooked products with an optimization in the lipid profile either through an increase in the monounsaturated fatty acids content, e.g., when olive oil is used [126], or through the increase in levels of polyunsaturated fatty acids, e.g., hemp oil [136] or chia oil [89]. Botella-Martínez et al. [136] studied the incorporation of gelled emulsion prepared with hemp oil and buckwheat flour in sausages at different percentages of substitution of pork fat (25%, 50%, 75%, and 100%). The reformulated frankfurters exhibited an improved lipid profile, without affecting the technological properties and lipid oxidation, despite the high PUFA content in hemp oil. Several authors have attributed this to the capsulated oil droplets in the gel matrix, which would act as a protective barrier against oxidation [156]. In other studies, an increase in lipid oxidation was observed in vegetable oils with a high content of ω -3 polyunsaturated fatty acids (e.g., chia oil), but vegetable oils rich in monounsaturated fatty acids did not show this increase [89,156]. De Souza Paglarini et al. [145] replaced the pork backfat of reduced-fat frankfurters with emulsion gels formulated with soybean oil, sonicated soy protein isolate dispersion, inulin, and carrageenan. They concluded that reformulated frankfurters could be considered a good source of fiber and as high in unsaturated lipids; however, some of the sensorial properties were affected (juiciness, soft texture).

Several works have studied the substitution of animal fat with different structured vegetable oils in the development of Frankfurt sausages as a viable strategy for developing healthier meat products [24–26,98]. Barbut and Marangoni [26] observed a reduction in hardness, which may be due to different interactions between the vegetable oil organogels within the meat matrix system. They concluded that the type of fat/oil and its degree of saturation play a major role in determining the hardness/texture of the final meat product. Da Silva et al. [98] developed an oleogel with pork skin, water, and high oleic sunflower oil. They showed a significant reduction in cooking loss and an increase in emulsion stability when the backfat was replaced by this oleogel in Bologna, in contrast to other works where oleogels were used as fat replacers. They reported that this optimum impact of technological properties may be due to the pork skin collagen, which interacts with proteins, developing a more rigid gel matrix and preventing the exudation of water and fat from the meat system during cooking [24,98].

4.1.3. Traditional Fermented Meat Products

The improvement in the lipid profile has promoted the reformulation of traditional meat products. Most studies have focused on the replacement of animal fat by non-fat ingredients (inulin, cellulose gel, konjac gel, or boiled quinoa) or vegetable oils (olive, soybean, linseed, grapeseed) as liquid, encapsulated, or pre-emulsified fat [25,150,157]. It should be emphasized that fat is a critical ingredient in the development of the flavor, texture, and juiciness of dry-cured sausages [158,159].

The direct replacement by healthier oil or integration by vegetable oil in water emulsion systems to replace animal fat is not technologically viable because of technological difficulties and its negative impact on texture and sensorial attributes [160,161]. New strategies are required to preserve the textural properties of the final product, especially considering the complex ripening process and the strong role of fat in moisture loss during the drying process [93]. At present, studies are directed towards the incorporation of vegetable oils into fermented sausage in edible structured gels (oleogels) or encapsulated forms.

Stajić et al. [150] replaced 20% of the backfat of dry fermented sausages with grapeseed oil prepared as encapsulated and reported changes in the texture characteristics (lower hardness, chewiness, and cohesiveness) which were perceived as negative characteristics in a sensory evaluation. Alejandre et al. [93] substituted different levels of animal fat by a gelled emulsion of linseed oil in dry fermented sausages during the chopping step. The reformulated dry sausages showed a small defect in the appearance of the slices and did not show oxidation problems. In addition, from a sensorial point of view, no perceptible differences for taste and juiciness were obtained.

Recently studies have shown the potential of the edible oleogels elaborated with vegetable oils to be used as substitutes for pork backfat in fermented sausages [126,148]. This substitution provides an improvement of the polyunsaturated fatty acids/saturated fatty acids ratio and ω -6/ ω -3 ratio, although the impact was negative for sensory properties. Franco et al. [148] studied the oleogels of linseed oil based on mixtures of γ -oryzanol, β -sitosterol, and beeswax for the replacement of pork backfat in fermented sausages. They concluded that the drying process was dependent on the oleogelator and the level of replacement, affecting textural and sensorial attributes. The fermented sausages with beeswax oleogel presented a higher moisture content due to lower drying speed, which indicates that oleogel based on beeswax would behave like a barrier, avoiding water loss during the drying process. Pintado and Cofrades [126] concluded that oleogels or emulsion gels, elaborated with a chia and olive oil mixture, were optimal strategies for the development of dry healthier fermented sausages with an improved lipid profile. They recommended that more studies should be carried out to improve the negative impacts on the sensory characteristics of meat products due to the fact that fat is a basic component since it contributes to technological and sensory properties (softness and juiciness), water holding capacity, emulsion stability, and shear strength [17].

4.2. Incorporation of Structured Healthier Oils in Dairy Products

Milk is a complete and balanced food as a source of nutrients [162] but it also contains a considerable amount of saturated fat. This high fat content has been the subject of various reduction strategies to diminish potential health risks. Moreover, there is a trend towards the consumption of plant-based foods, which are analogues of many dairy products, by the total or partial substitution of dairy components, mainly fats [163–165].

In addition to the reduction of milk fat content, products have also been developed where the lipid fraction of dairy products has been replaced or enriched. One of the most commonly used methods has been microencapsulation, which shows great potential for the protection of bioactives or vegetable oils incorporated during processing, storage, and transportation [163]. Substituting or enriching the lipid fraction of dairy products by integrating functional chia and sunflower oils in spray-dried emulsions is a suitable way to incorporate these ingredients to increase their added value [166]. Both the high nutritional value and the expensive price of dairy products have encouraged one of the most common frauds: replacing milk fat with cheaper vegetable oils due to the economic advantage of such substitution. Dairy fat may be replaced or mixed with cheap oils such as palm, sunflower, and corn oils [167–169]. Several research works assess the effects on quality parameters of dairy products with total or partial substitution of milk fat by vegetable oils with different incorporation strategies, as shown in Table 4. The most frequently modified dairy products are cheese, yoghurt, and ice cream, which coincide with those with the highest consumption.

Table 4. Summary of studies assessing effects on quality parameters of dairy products with total or partial substitution of milk fat by vegetable oils with different incorporation strategies.

Dairy Product	Vegetable Oil	Incorporation Strategy	Effect on Dairy Product	Ref.
Processed cheese	Chia oil	Microcapsules versus free oil	Microencapsulation process masked the flavor of the oil.	[170]
Cheddar cheese	Chia oil	Incorporated in mixture of ingredients	No effect on sensory attributes; antioxidant properties improved.	[171]
Queso blanco cheese	Flaxseed oil	Incorporated during homogenization, coagulation, or salting	The best scores are obtained when oil is incorporated during homogenization.	[172]
Yoghurt	Soybean oil	Emulsion O/W for dissolving phytosterols as functional ingredient	No significant difference in texture, appearance, flavor, and overall acceptance; lower syneresis, higher firmness, and lower apparent viscosity.	[173]
Yoghurt	Extra virgin olive oil (EVOO)	Microcapsules of EVOO and synbiotic bacteria	Increased antioxidant activity.	[174]
Yoghurt	Pequi oil	Microcapsules of pequi oil	Increase in the percentage of oleic acid; delayed oxidation.	[175]
Yoghurt	Corn oil	Emulsion gel microparticles from corn oil	Improvement in textural and rheological properties, water holding capacity, and storage stability; sensory defects were reduced.	[176]

Table 4. Cont.

Dairy Product	Vegetable Oil	Incorporation Strategy	Effect on Dairy Product	Ref.
Yoghurt	Flaxseed	Nanoemulsion	Increase solubility, bioavailability, and protection of ω -3.	[177]
Ice cream	Vegetal oil	Oleogels	Greater overrun, which implies an improvement in texture and appearance.	[178]
Ice cream	Grape seed oil	Incorporated in mixture of ingredients	High nutritional antioxidant activity.	[179]
Ice cream	Basil oil	Encapsulated by Spray-drying	High antioxidant and phenolic content; sensorial attributes were not affected.	[180]
Ice cream	Extra virgin olive	Incorporated in mixture of ingredients	Matrix masked EVOO bitterness; higher content of biophenols.	[181]
Ice cream	Hazelnut and olive oil	Incorporated in mixture of ingredients	Similar or even better quality characteristics.	[182]

4.2.1. Cheese

Various studies have been carried out modifying different types of cheese by including vegetable oils as dairy fat replacers in order to alter their sensory and nutritional attributes and modify their lipid profiles [171,183,184]. The resulting cheese has less cholesterol and different a composition of saturated and unsaturated fatty acids. The inclusion of vegetable oils in cheese has become a good opportunity to increase unsaturated fatty acids [185] and to improve antioxidant properties with effects on sensory attributes [171]. In most cheeses, milk fat is one of the major components, which results in many consumers having to limit their consumption for health reasons [184]. The incorporation of vegetable oils, such as high oleic sunflower oil, could help to solve this problem [186]. In a study carried out by Bermúdez-Aguirre and Barbosa-Cánovas [187], cheese was fortified with ω -3 fatty acids obtained from vegetable sources (canola, flaxseed, and soybean). These authors found that the reformulated cheese had some differences in texture but had good scores in sensory evaluation. Achachlouei et al. [183] replaced dairy fat in white brine cheese by half or total substitution with emulsified canola and olive oils. The cheese containing these vegetable oils had lower amounts of saturated fatty acids and higher amounts of unsaturated fatty acids compared with the original cheese and also received better sensory scores. The substitution of milk fat with palm oil decreased dry matter, increased texture parameters, and decreased sensory quality in a white brine cheese, as reported by Sulejmani et al. [188].

Vegetable oils, such as soybean oil, palm oil, peanut oil, canola oil, coconut oil, and corn oil, are also used in the elaboration of most processed cheeses. Processed cheeses are products elaborated from cheeses unmarketable due to defects in shape or ripeness; they are produced to reduce costs and food waste and can meet special dietary needs through changes in their formulation [184,189].

Processed cheeses (which cannot be called cheese as they are not produced solely from milk, milk protein, milk fat, or other milk solids) can be classified as:

- Analogue or imitation cheese, made from dairy and/or nondairy ingredients, formulated with specific nutritional and/or functional properties, according to consumer needs [184,189]. In imitation cheese, the fat source can come from soybean oil, rapeseed oil, palm oil, canola oil, sunflower oil, and corn oil [165,186].
- Functional processed cheese, made from dairy and/or nondairy ingredients and fortified with some bioactive compounds with functional properties.

- Plant-based processed cheese, made from nondairy ingredients [189].

As important as the type of vegetable oil incorporated is the mode of incorporation. Garbin Cardoso et al. [170] reported that microencapsulation by ionic gelation, without the need for heating, is a technology used to stabilize vegetable oils. The results of their study, which incorporated microencapsulated chia oil in a processed cheese from Grana Padano and mozzarella cheese, showed that microencapsulation was able to mask the oil taste.

4.2.2. Yoghurt

The incorporation of vegetable oils into yoghurt can be due to various reasons, such as improving the lipid profile of the dairy product or using the oil as a transporter for another ingredient through the formation of emulsions or microencapsulation. Izadi et al. [173] used soybean oil to create an oil-in-water emulsion to produce an enriched yoghurt with phytosterol as a functional ingredient due to its cholesterol-lowering properties. The formation of this emulsion avoids a chalky taste due to phytosterol enrichment. The results showed no significant differences between the enriched yoghurt and the control with regard to texture, appearance, flavor, and overall acceptance; also, the enriched yoghurt had lower syneresis, higher firmness, and lower apparent viscosity. In a study carried out by El Sayed et al. [174], stirred yoghurt was fortified with two models of microcapsules based on synbiotic extra virgin olive oil (EVOO). The results showed that the addition of nanoemulsion microcapsules to yoghurts increased antioxidant activity more than in plain yoghurt. Pequi (*Caryocar coriaceum* Wittm.) oil was also incorporated due to its high levels of unsaturated fatty acids. To avoid its strong taste, the microencapsulation method was employed, which also protects against oxidation [175]. Li et al. [176] applied whey protein emulsion gel microparticles from corn oil to yoghurt as a fat substitute, finding that there was an improvement in textural and rheological properties, water holding capacity, and storage stability, while the sensory defects associated with fat reduction were reduced. When palm oil was incorporated in the mixture of ingredients to make yoghurt, the yoghurt obtained showed equal sensory attributes and retained probiotic properties [190].

4.2.3. Ice Cream

The fact that ice cream is one of the most popular types of dairy products worldwide and the concern about the effect of the food on health have both led to an increase in demand for lower calorie or better lipid profile ice creams, alongside the consideration that these modifications must not spoil the sensory attributes and storage stability of ice cream [178,179,181]. Among the strategies carried out to achieve the substitution of milk fat is the incorporation of vegetable oils, which in some cases happen to be by-products of other agri-food processes. This is accompanied by nutritional benefits and, sometimes, as in the case of the use of oleogels, the improvement of some technological characteristics, such as greater overrun, with enhancements in texture and appearance [178,179]. Paul et al. [180] investigated the incorporation of basil oil microcapsules in ice cream. They found that sensorial attributes were not affected and the antioxidant properties were improved due to the polyphenolic content; meanwhile, the direct use of basil oil has been found to cause adverse effects in sensorial and antioxidant properties. However, when extra virgin olive oil (EVOO) is incorporated in the mixture of ice cream ingredients, the ice cream matrix masks EVOO bitterness and there is a higher content of biophenols. Güven et al. [182] demonstrated that similar or even better quality characteristics were obtained when the dairy fat of ice cream was replaced with hazelnut oil and olive oil.

4.2.4. Butter

More than 82% of butter is fat, comprising mostly saturated fat with good technological properties. However, the consumption of butter in excess is associated with health problems [191]. In addition to this association, the environmental impact butter generates as a food of animal origin has led to the development of alternatives of vegetable origin, such as echium oil or extra virgin olive oil. Gutiérrez-Luna et al. [164] have developed gel

emulsions from both oils that could be used as potential butter analogues, both for their technological properties and their nutritional value, although more in-depth studies are needed for their full development and commercial use.

5. Conclusions

This review highlights the interest of the scientific community in reducing the saturated fat content of meat and dairy products and replacing it with vegetable and marine oils with healthier fatty acid profiles. It shows how the different strategies used to structure these oils affect their mode of application and how the chemical composition, techno-functional, physicochemical, and sensory properties of the reformulated products are modified.

As a future perspective, more research should be carried out in two directions. On the one hand, the protection of these polyunsaturated oils from oxidative processes should be investigated, as they can lead to changes in the organoleptic properties that occur during the production process of meat and dairy products. On the other hand, more research has to be performed to know the bioaccessibility and bioavailability of these fatty acids when they are ingested and to know the possible interactions with other components of the food matrix that could affect or promote their release and/or absorption.

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8.2. PUBLICACIÓN 2

Assessment of chemical, physicochemical, and lipid stability properties of gelled emulsions elaborated with different oils chia (*Salvia hispanica* L.) or hemp (*Cannabis sativa* L.) and pseudocereals

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Assessment of Chemical, Physicochemical, and Lipid Stability Properties of Gelled Emulsions Elaborated with Different Oils Chia (*Salvia hispanica* L.) or Hemp (*Cannabis sativa* L.) and Pseudocereals

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Abstract: Gelled emulsion (GE) systems are one of the novel proposals for the reformulation of meat products with healthier profiles. The aims of this research were (i) to develop gelled emulsions using pseudocereal flours (amaranth, buckwheat, teff, and quinoa) and vegetable oils (chia oil, hemp oil, and their combination), (ii) to determine their chemical composition, physicochemical properties, and lipid stability, and (iii) to evaluate their stability during frozen storage. The results showed that GEs are technologically viable except for the sample elaborated with teff flour and a mix of oils. The lipid oxidation was not greater than 2.5 mg malonaldehyde/kg of sample for any of the samples analyzed. The physicochemical properties analyzed showed both the pH and color values of the GEs within the range of values obtained for the fat of animal origin. The texture properties were affected by the type of oil added; in general, the firmness and the work of shear increased with the addition of the mixture of both oils. The samples elaborated with buckwheat and chia oil and quinoa and chia oil had the highest emulsion stability values, which remained among the highest after freezing. The results showed that gelled emulsions, based on chia oil, hemp, and their mixture with pseudocereal flours, are a viable alternative as a possible substitute of saturated fat in the development of novel foods.

Keywords: gelled emulsion; hemp oil; chia oil; pseudocereals; fat replacer

1. Introduction

In developed countries, and even in developing countries, there is a rising concern on the part of health authorities on how diet can cause numerous diseases [1]. Therefore, several epidemiological studies have exposed that the consumption of diets with high quantities of fat (>40% of energy from fat) and with a high content in saturated fatty acids induces many health-related disorders [2]. Thus, one of the most effective behaviors to reduce the risk to develop several diseases is restraining the consumption of saturated fats. In this sense, meat and meat products are one of the principal dietary sources of saturated fats. These fats, which comprised between 30 and 50% of the product, are rich in saturated fatty acids and cholesterol, and they are considered a promoting factor in the development of several diseases including coronary heart disease, metabolic syndrome, obesity and overweight, inflammation, oxidative stress, etc. [2,3].

As a result of these undesirable health effects of excessive fat consumption, the meat industry has had to adapt to these consumers' requirements developing low-fat meat products with healthier lipid profiles. To achieve this objective, besides the reduction of fat content, numerous strategies have been tried including (i) the direct addition of vegetable oils with healthier lipid profiles [4,5], (ii) the incorporation of vegetable oils with healthier

profile encapsulated in several matrices [6,7], (iii) the use of oleogels [8,9], and (iv) the use of gelled emulsions [10,11]. A gelled emulsion is a colloidal material in which oil-in-water emulsion (O/W) coexists within a gel network. Its formation consists of two stages; in the first, the O/W emulsion is elaborated, and in the second stage, the gelled emulsion is properly obtained with the formation in the aqueous phase of a drop structure of the emulsion inside of the cross-linked structure of biopolymers [12]. To elaborate these O/W emulsions, several vegetable or marine oils, as well as mixes of them with a healthier fatty acid profile have been utilized, including chia oil, linseed oil, tiger nut oil, and algal oil, among others [10–14]. Nevertheless, it should be borne in mind that reducing or replacing the fat content in a meat product is not an easy task. Animal fat is a basic ingredient in the processing of meat products due to its technological (to improve emulsion stability, the impact on rheological and structural capacities, and the adjustment of the drying process in dry-cured meat products, among others) and sensory properties (positive effects on hardness, juiciness, color, tenderness, palatability, and so on) [15,16]. Additionally, the addition of vegetables or marine oils with a healthier fatty acid profile may cause an acceleration of lipid oxidation reactions, which can lead to a decrease in the product shelf life as well as a deterioration of their sensorial and nutritional properties [17].

As mentioned above, several vegetable oils can be used to elaborate gelled emulsion. Chia (*Salvia hispanica* L.) oil is a significant oilseed due to its nutritional composition, consisting of up to 65% α -linolenic acid and 20% linoleic acid in the unsaturated fatty acid fraction [18]. Since 2014, it can be marketed in the European Union. On the contrary, hemp (*Cannabis sativa* L.) oil is not widespread on the market, although it is also characterized by an interesting fatty acid composition with a high content of polyunsaturated fatty acids. Thus, in this composition, it is possible to find a high content, up to 75%, of polyunsaturated fatty acids and the unique ratio of 3:1 between omega-6 and omega-3. Hemp oil highly contains linoleic acid and α -linolenic acid in the range of 50–60% and 20–25%, respectively [19]. In addition, there are high amounts of chlorophyll in the oil due to the harvesting of high amounts of immature seeds [20].

With the objective to stabilize the O/W emulsion formed, several ingredients (mainly starchy ingredients) have been used. Pseudocereal flours (from quinoa, amaranth, buckwheat, teff, etc.) seem to be excellent candidates for this application [21,22]. They contain high-quality proteins, abundant amounts of starch with unique characteristics, large quantities of micronutrients such as minerals, vitamins, and bioactive compounds, and they are gluten-free, which makes them suitable for people suffering from various gluten intolerances. Their main component, starch, has many interesting features such as very small granules ready to form cross-link structures, which made them useful for stabilizing emulsions [23]. For these reasons, interest in pseudocereals has increased immensely since the turn of the century, and research efforts have been intensified to include them in our diet. Therefore, the objective of this work was (i) to develop gelled emulsions using pseudocereal flours (amaranth, buckwheat, teff, and quinoa) and vegetable oils (chia oil, hemp oil, and their combination at 50%), (ii) to determine their chemical composition, physico-chemical properties, and lipid stability, and (iii) to evaluate their stability during frozen storage.

2. Materials and Methods

2.1. Plant Material

Chia oil (CH) was obtained from Herbolarios Navarro, (Alicante, Spain), while hemp oil (H) was purchased from Laboratorios Almond, S.L. (Librilla, Spain). Amaranth flour (A) was obtained from Tentorium Energy S.L. (Tarragona, Spain); buckwheat flour (BW) and white quinoa flour (WQ) were purchased from Biogran S.L. (Madrid, Spain), and whole teff flour (T) was obtained from El granero integral, S.L. (Madrid, Spain). The gelling agent was gellan gum (an extracellular polysaccharide excreted by microorganism *Pseudomonas elodea*). It is a water-soluble linear structure with a repeating unit of tetrasaccharide and instant gel (gelatin of animal origin (pork) with 180 bloom), which was obtained from Sosa Ingredients S.L. (Barcelona, Spain).

2.2. Lipid Profile of Vegetable Oils

The identification of fatty acids was carried out according to the method 969.33 [24]. For that, fatty acids of all samples were transmethylated producing fatty acid methyl esters (FAME). The FAMEs were analyzed on HP 6890 chromatography equipment with a flame ionizer detector and a Suprewax-280 capillary column (30 m, 0.25 μm of film, 0.25 mm internal diameter; Tecknokroma Barcelona, Spain). The injector and detector temperatures were 250 and 270 $^{\circ}\text{C}$ respectively. The temperature program was as follows: the initial temperature was 60 $^{\circ}\text{C}$, and this was maintained for 1 min after the injection; subsequently, it was raised at a rate of 10 $^{\circ}\text{C}/\text{min}$ until reaching 170 $^{\circ}\text{C}$ and was kept at this temperature for 2 min. After these 2 min, it was raised at a speed of 3 $^{\circ}\text{C}/\text{min}$ until reaching 230 $^{\circ}\text{C}$, and it was kept at this temperature for 10 min, and finally, it was raised at a speed of 2 $^{\circ}\text{C}/\text{min}$ until reaching 260 $^{\circ}\text{C}$ and maintained for 1 min at this temperature. The carrier gas was helium with an internal column pressure of 11 psi. The injector volume was 0.2 μL in splitless. The response factors were calculated using fatty acid standards, and their identification was made by comparison with the retention times of these FAME standards (Supelco 37 component FAME Mix, Bellefonte, PA, USA). With the data obtained from the chromatograms, the following parameters were calculated: total saturated fatty acids (SFAs), total unsaturated fatty acids (UFAs), total monounsaturated fatty acids (MUFAs), total polyunsaturated fatty acids (PUFAs), the ratio between saturated and unsaturated fatty acids (SFAs/UFAs), and the ratio between omega-3 and omega-6 fatty acids (ω -3/ ω -6). All analyses were carried out in triplicate (three independent batches), and the results were expressed as g fatty acid/100 g oil.

2.3. Gelled Emulsions Preparation

Gelled emulsion (GE) preparation essentially involves producing a protein-stabilized emulsion using emulsifying agents and incorporating a gelling agent such as a hydrocolloid or other ingredients with the gelling capacity to convert the emulsion into a GE. Twelve different types of oil-in-water (O/W) GE samples were formulated, as shown in Table 1. Eight GE samples were made combining each of the flours with each of the oils: amaranth flour with chia oil or hemp oil (ACH and AH, respectively); buckwheat flour with chia oil or hemp oil (BWCH and BWH, respectively); whole teff flour with chia oil or hemp oil (TCH and TH, respectively) and finally, white quinoa flour with chia oil or hemp oil (WQCH and WQH, respectively). For the other four GE, a blend of chia oil and hemp oil (50:50 v/v) was made, and each flour was combined with this oil blend. The other four GEs elaborated were amaranth flour with chia and hemp oils blend (AM); buckwheat flour with chia and hemp oils blend (BWM); whole teff flour and with chia and hemp oils blend (TM), and white quinoa flour with chia and hemp oil blends (WQM).

The O/W GE samples were prepared as follows. For each type of GE, first the gelling agent “instant gel” was mixed in a homogenizer (Thermomix 31, Vorwerk-España M.S.L., S.C., Spain) with water for 2 min at 60 $^{\circ}\text{C}$ at high speed. Then, the flour was added and mixed for 1 min at medium speed. In the next step, the temperature was turned down to 37 $^{\circ}\text{C}$ and gellan gum was added and mixed for 2.5 min at 250 rpm. In the last step, the mixture was mixed with the gradual addition of the appropriate amount of oils or their blends for 5 min, at 37 $^{\circ}\text{C}$ and 1100 rpm. The elaborated GEs were placed in metal containers and stored at 4 $^{\circ}\text{C}$ for 20 h until use. The whole process was replicated three times (three independent batches).

2.4. Gelled Emulsion Analysis

2.4.1. Proximate Composition

Protein, fat, ash, and moisture content were determined on GE samples using the appropriate methodology from the Association of Official Analytical Chemist [24]. Protein content was determined by the Kjeldahl method with a factor of nitrogen of 6.25. The Soxhlet method was used for fat content determination, with petroleum ether as the

extractant. Ash content was determined by incinerating the samples at 525 °C, while moisture was determined by heating the samples in an oven until constant weight.

Table 1. Formulation of oil-in-water gelled emulsion (GE) samples.

Samples	Water	Instant Gel	Gellan Gum	Amaranth Flour	Buckwheat Flour	Teff Flour	Quinoa Flour	Chia Oil	Hemp Oil
ACH	47	1.5	1.5	10	-	-	-	40	-
AH	47	1.5	1.5	10	-	-	-	-	40
AM	47	1.5	1.5	10	-	-	-	20	20
BWCH	47	1.5	1.5	-	10	-	-	40	-
BWH	47	1.5	1.5	-	10	-	-	-	40
BWM	47	1.5	1.5	-	10	-	-	20	20
TCH	47	1.5	1.5	-	-	10	-	40	-
TH	47	1.5	1.5	-	-	10	-	-	40
TM	47	1.5	1.5	-	-	10	-	20	20
WQCH	47	1.5	1.5	-	-	-	10	40	-
WQH	47	1.5	1.5	-	-	-	10	-	40
WQM	47	1.5	1.5	-	-	-	10	20	20

Values expressed as g/100 g. ACH: amaranth flour with chia oil; AH: amaranth flour with hemp oil; AM: amaranth flour with a mix of both chia and hemp oils; BWCH: buckwheat flour with chia oil; BWH: buckwheat flour with hemp oil; BWM: buckwheat flour with a mix of both oils; TCH: teff flour with chia oil; TH: teff flour with hemp oil; TM: teff flour with a mix of both oils; WQCH: white quinoa flour with chia oil; WQH: white quinoa flour with hemp oil; WQM: white quinoa with a mix of both oils.

2.4.2. Physicochemical Properties

The pH of GE samples was measured using a Crison combination electrode connected to a pH-meter Crison model 510, (Barcelona, Spain). These measures were made directly into the emulsion.

The texture of each sample was evaluated using a TA-XT2i texturometer (Stable Micro Systems, Surrey, England). The “Measure Force in Compression” Test was selected, and the accessory TTC spreadability rig (HDP/SR, Stable Micro Systems) was used. It is composed of a 90° male cone probe and five cone-shaped product holders that were precisely matched females. Both cones were 25 mm apart, and the sample was placed into the female cone and pressed down to eliminate air pockets. Any excess sample was scraped off with a knife to leave a flat test area. GE samples were stabilized at 5 °C for 30 min before testing and were forced to flow out at 45° with a test speed of 3 mm/s. During compression, the force increases up until the point of maximum penetration depth. This force value was taken as the “firmness (N)” at this specified depth. The “work of shear (N.s)” represents the total amount of force required to perform the shearing process [25,26].

2.5. Stability of Gelled Emulsion during Frozen Storage

Since GE samples should be kept frozen until their application to avoid quick oxidation (high unsaturated fat content), it has been decided to assess the influence of freezing time on several properties related to their stability (resistance of emulsion characteristics to changes over time) such as emulsion stability (retention of fluids in the system at maximum levels), color, and lipid oxidation. For that, each emulsion was placed into Petri dishes that were covered, sealed with parafilm, and frozen at −23 °C in an air freezer W7 8210 0X (Whirlpool, MI, USA) for 15 days. After that, samples were thawed in refrigeration conditions (1 h), and color parameters, emulsion stability, and lipid oxidation were assessed as described below.

2.5.1. Emulsion Stability

The emulsion stability was determined following the procedure from [27] with slight modifications. Samples were introduced into centrifuge tubes of 15 mL and centrifuged at 3000 rpm for 1 min. Then, they were heated in a water bath for 30 min at 70 °C and cooled at room temperature; after that, they were centrifuged again at 3000 rpm for 3 min. The samples were left standing upside down to release the separated fat and water onto filter paper. The results are expressed in g of total fluid expelled/100 g of sample and were calculated using the following expression:

$$\%TEF = \frac{\text{Weight of tube with sample} - \text{Weight of tube with pellet}}{\text{Weight of sample}} \times 100, \quad (1)$$

2.5.2. Instrumental Color Analysis

The instrumental color parameters of GE samples were measured in the CIEL*a*b* color space using a Minolta CM-700 (Minolta Camera Co., Osaka, Japan), with illuminant D65, SCI mode, and an observer angle of 10°. Low reflectance glass (Minolta CR-A51/1829-752) was placed between the samples and the equipment. The CIEL*a*b* coordinates determined were L^* (lightness), a^* (red/green), and b^* (yellow/blue). The magnitudes h° (hue) and C^* (chrome) were calculated with Equations (2) and (3), respectively.

$$C^* = \sqrt{a^{*2} + b^{*2}}, \quad (2)$$

$$h^{\circ} = \arctg\left(\frac{b^*}{a^*}\right), \quad (3)$$

2.5.3. Oxidative Stability

The oxidative stability of emulsions was evaluated by measuring changes in thio-barbituric acid reactive substances (TBARs). TBARs determination for each sample was performed in triplicate by the method described by Rosmini et al. [28]. TBARs values were calculated from a malonaldehyde (MA) standard curve and were expressed as mg MA/kg sample.

2.6. Statistical Assay

The whole process was replicated three times (three independent batches). Each replication was performed on a different production day, and each batch was analyzed in triplicate. Means and standard deviations of data obtained from the analysis of GE samples are shown in corresponding tables. A one-way ANOVA test and the Tukey-b post hoc test were used to determine significant differences in both the different types of GE samples and the different times of frozen storage. SPSS version 24.0 was used (SPSS Inc., Chicago, IL, USA) for the evaluations at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Fatty Acid Profile of Oils Used for Gelled Emulsions Preparation

The fatty acid profile of chia oil, hemp oil, and their blend is shown in Table 2. Linoleic acid was the most abundant ($p < 0.05$) fatty acid in hemp oil (54.44%) followed by α -linolenic acid (19.95%) and oleic acid (8.23%). On the other hand, chia oil showed mainly ($p < 0.05$) α -linolenic acid (56.61%) followed by linoleic acid (17.43%) and oleic acid (15.05%). However, in the blend of these oils, the predominant fatty acids were α -linolenic acid (38.04%) and linoleic acid (36.11%), followed by oleic acid (11.60%).

Table 2. Fatty acid profile of hemp oil, chia oil, and their blend, which were used as ingredients for the development of gelled emulsions.

Fatty Acid	Hemp Oil	Chia Oil	Chia/Hemp Oils Mix
C14:0	0.04 ± 0.01 ^a	0.04 ± 0.01 ^a	0.04 ± 0.01 ^a
C16:0	6.17 ± 0.08 ^a	5.84 ± 0.04 ^b	6.03 ± 0.01 ^a
C17:0	0.06 ± 0.01 ^b	0.05 ± 0.01 ^b	0.10 ± 0.03 ^a
C18:0	2.3 ± 0.01 ^c	3.63 ± 0.09 ^a	2.98 ± 0.01 ^b
C20:0	0.94 ± 0.03 ^a	0.19 ± 0.02 ^c	0.57 ± 0.02 ^b
C22:0	0.41 ± 0.01 ^a	0.1 ± 0.01 ^c	0.27 ± 0.03 ^b
C24:0	0.32 ± 0.01 ^a	0.15 ± 0.01 ^b	0.14 ± 0.01 ^b
C16:1 cis	0.1 ± 0.01 ^c	0.05 ± 0.01 ^b	0.08 ± 0.01 ^a
C16:1 trans	0.02 ± 0.01 ^a	0.01 ± 0.01 ^a	0.02 ± 0.01 ^a
C17:1	0.03 ± 0.01 ^a	0.03 ± 0.01 ^a	0.04 ± 0.01 ^a
C18:1 cis	8.23 ± 0.01 ^c	15.05 ± 0.07 ^a	11.60 ± 0.04 ^b
C18:1 trans	0.56 ± 0.01 ^b	0.62 ± 0.02 ^{ab}	0.68 ± 0.03 ^a
C18:2 (ω-6)	54.44 ± 0.01 ^a	17.43 ± 0.09 ^c	36.11 ± 0.14 ^b
C18:2 (ω-3)	4.26 ± 0.01 ^a	0.01 ± 0.00 ^c	2.16 ± 0.01 ^b
C18:3 (ω-3)	19.95 ± 0.01 ^c	56.61 ± 0.12 ^a	38.04 ± 0.06 ^b
C18:3 (ω-6)	1.62 ± 0.01 ^a	0.02 ± 0.01 ^c	0.81 ± 0.03 ^b
C20:1	0.45 ± 0.01 ^a	0.13 ± 0.02 ^c	0.29 ± 0.02 ^b
C20:2	0.09 ± 0.01 ^a	0.05 ± 0.01 ^{ab}	0.08 ± 0.02 ^a
C20:3 (ω-11)	0.02 ± 0.01	ND	ND
SFA	10.24 ± 0.08 ^a	10.00 ± 0.03 ^a	10.12 ± 0.04 ^a
UFA	89.77 ± 0.06 ^a	90.01 ± 0.02 ^a	89.83 ± 0.09 ^a
MUFA	9.39 ± 0.02 ^c	15.89 ± 0.09 ^a	12.70 ± 0.03 ^b
PUFA	80.38 ± 0.07 ^a	74.12 ± 0.08 ^c	77.20 ± 0.05 ^b
SFA/UFA	0.11 ± 0.01 ^a	0.11 ± 0.01 ^a	0.11 ± 0.01 ^a
ω-3/ω-6 ratio	0.43 ± 0.03 ^c	3.24 ± 0.01 ^a	1.09 ± 0.02 ^b

Results are expressed as g/100 g. ND: not detected. SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Results followed by the same lowercase letter are not significantly different according to Tukey's HSD post hoc test ($p > 0.05$).

Polyunsaturated fatty acids (PUFAs) were the most abundant fatty acids in all samples. Hemp oil showed the highest PUFAs content—hardly 6% more than chia oil, which showed the lowest content. In contrast, the ratio between saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) was the same, without significant differences between samples ($p > 0.05$). Due to the particular composition of these oils, the ω-3/ω-6 was higher for chia oil (3.24) than for hemp oil (0.43). Thus, the blend of both oils showed an intermediate ratio of 1.09. There is an agreement regarding the need to lower the ω-6/ω-3

ratio, and according to some authors, the ideal ratio may be 1:1 or 2:1. However, it can be stated that an adequate intake of both fatty acids, ω -6 and ω -3, is essential for good health and for reducing the percentage of cardiovascular diseases—although it is not clear whether the ratio between them is of any use [29]. The American Heart Association (AHA) published a review recommending the amount of ω -6 to represent between 5 and 10% of total energy consumed. The AHA indicates that the consumption of ω -6 from vegetable oils, nuts, and seeds is beneficial when forming part of a healthy diet plan in which saturated and trans-fats are replaced by PUFAs [30].

Regarding the fatty acid composition in chia oil, higher amounts of most of the saturated fatty acids compared with those obtained in this work have been reported in studies carried out with chia oil directly extracted from chia seeds [31,32]. These authors also reported lower amounts of stearic acid and similar amounts of behenic acid than the values obtained in this work. In general, regarding unsaturated fatty acids, a greater amount of oleic acid, linoleic acid, and α -linolenic acid was obtained in the present study compared to those reported by these authors [31–33]. Regarding hemp oil, a similar fatty acid profile has been reported by Abdollahi et al. [19] for oils obtained from four hemp cultivars in the north of Iran. However, the study of Montserrat de la Paz et al. [34] on refined hemp oil showed a higher amount of saturated fatty acids and monounsaturated fatty acids. The fatty acid profile of oils is highly influenced by the raw material (variety, growth conditions, harvest conditions, etc.) and extraction procedure [35]. Despite this, the relationship between omega-3 and omega-6 for both oils was similar to that reported in the scientific literature.

Comparing the lipid profile of these oils with those of the main animal fats used as a fat source in meat products (Table 3), it is easy to verify their healthier composition. Animal fats showed SFA content higher than 25% compared to percentages not higher than 10.3 in these oils, and their PUFA content was lower than 22% compared to more than 74% found in these oils. Given that, it is expected that their use (as GE) for fat replacement in meat products would improve their lipid profile toward healthier one.

Table 3. Fatty acid profile, color parameters, and pH of the main animal fats used in meat products.

Parameters	Beef Tallow ⁽¹⁾	Pork Back Fat ⁽²⁾	Poultry Skin ⁽³⁾
Lipid profile (% of total lipids)			
C14:0	1–1.5	1–1.5	-
C16:0	24–28	24–28	20–24
C16:1	2–3	2–3	5–9
C18:0	20–24	13–14	4–6
C18:1 (ω -9)	40–43	43–47	33–44
C18:2 (ω -6)	2–4	8–11	18–20
C18:3 (ω -3)	<1	<1	1–2
SFA	46–55	38–43.5	25–31.5
MUFA	42–46	45–50	38–53
PUFA	2–4	8–11	19–22
SFA/UFA	1.0	0.7	0.4
Color parameters			
<i>L</i> *	71.4	71.9	64.6
<i>a</i> *	1.2	3.3	2.6
<i>b</i> *	24.5	7.8	9.9
pH	5.3	6.3	6.0

⁽¹⁾ Motram et al. [36]; Alm [37]; Daly et al. [38]; ⁽²⁾ Motram et al. [36]; Ospina-E et al. [39]; Jiménez-Colmenero et al. [40]; Méndez-Cid [41]. ⁽³⁾ Sheu and Chen [42]; Feddern et al. [43]; Alm [37]; Peña-Saldarriaga et al. [44].

3.2. Gelled Emulsions

Figure 1 shows the twelve GE samples obtained. As can be seen in the figure, the only formula that did not achieve a correct emulsion of its ingredients was TM. It can be clearly seen that the oil was not integrated into the structure of GE. For this reason, TM was no longer subjected to the following analyses and was not considered for further studies.

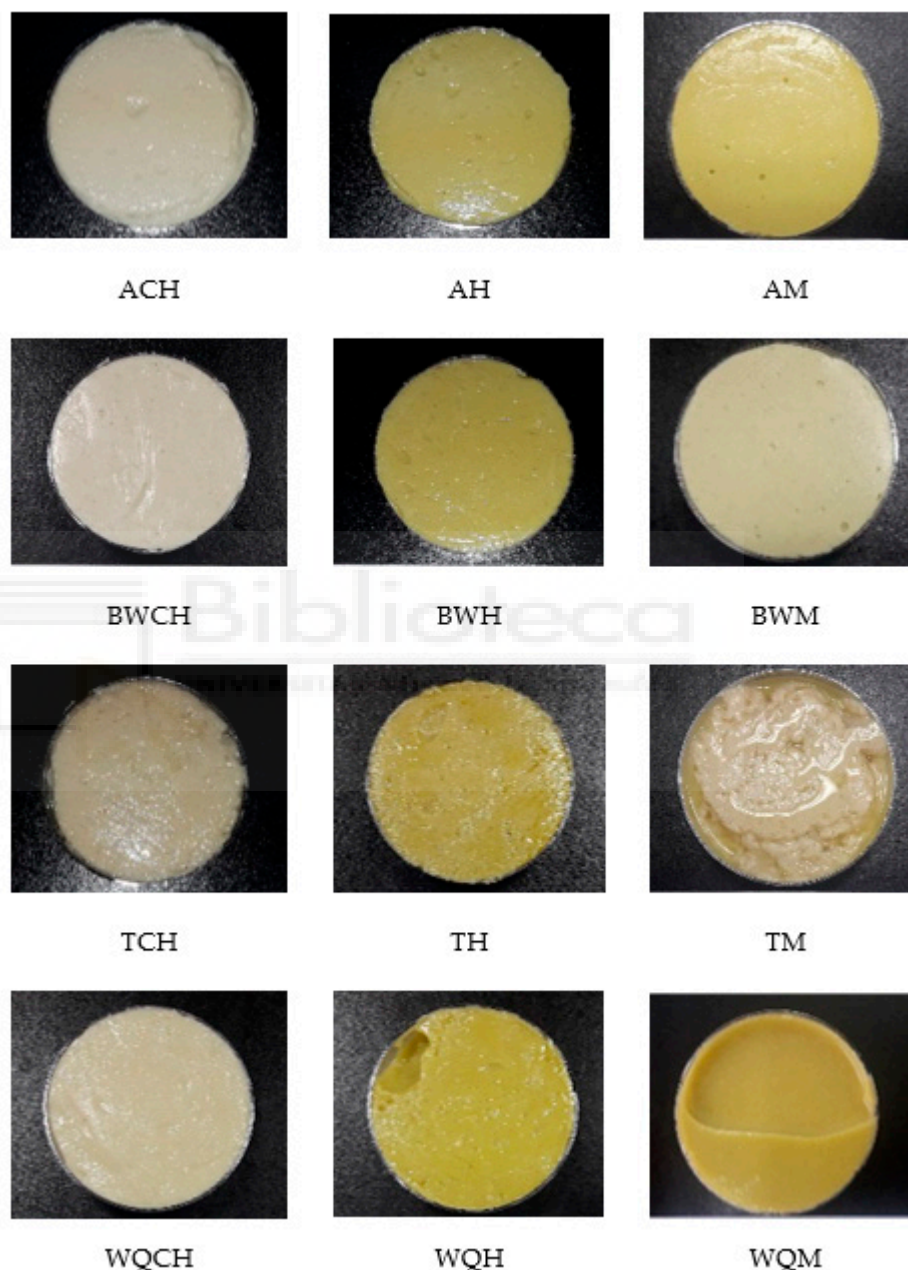


Figure 1. Appearance of the twelve oil-in-water GE samples developed. ACH: amaranth flour with chia oil; AH: amaranth flour with hemp oil; AM: amaranth flour with a blend of both chia and hemp oils; BWCH: buckwheat flour with chia oil; BWH: buckwheat flour with hemp oil; BWM: buckwheat flour with a mix of both oils; TCH: teff flour with chia oil; TH: teff flour with hemp oil; TM: teff flour with a mix of both oils; WQCH: white quinoa flour with chia oil; WQH: white quinoa flour with hemp oil; WQM: white quinoa with a mix of both oils.

In the other samples made with teff flour (TCH and TH), a slight oil release can be seen, although both GEs maintained their structure. The samples made with amaranth (AM, ACH, and AH) and buckwheat (BWM, BWCH, and BWH) showed a firmer consistency

and showed no noticeable syneresis or oil release. In the same way, very similar GEs were obtained with samples elaborated with white quinoa (WQM, WQH, and WQCH). The structure of each GE and the interactions between its different components are very complicated, and this fact determines their physical properties; any disequilibrium between them seems to be enough to destabilize the systems, among other characteristics [12,27,45]. In this sense, the high molecular weight, as well as branching degree of polysaccharides, plays the role of emulsifying capacity through steric hindrance and charge repulsion [46]; it should be noted that the stability of emulsifiers agents is also affected by many factors such as the heat variability of free proteins and sensitivity to pH [47].

3.2.1. Proximal Composition of Gelled Emulsions

The proximal composition of GE samples is shown in Table 4. The moisture content of the GEs ranged from 44.73% to 49.91%. For the same flour, moisture content increased ($p < 0.05$) with the addition of the oils blend, except for the amaranth flour that did not present statistically significant differences ($p > 0.05$).

Table 4. Chemical composition of GE.

Sample	Moisture	Fats	Proteins	Ash
ACH	45.76 ± 0.30 ^{cd}	42.82 ± 0.30 ^{ab}	2.50 ± 0.03 ^c	0.43 ± 0.01 ^c
AH	46.07 ± 0.14 ^c	42.56 ± 0.10 ^b	2.52 ± 0.01 ^c	0.44 ± 0.01 ^{bc}
AM	46.10 ± 0.30 ^c	42.24 ± 0.13 ^b	2.52 ± 0.02 ^c	0.48 ± 0.09 ^{bc}
BWCH	45.24 ± 0.36 ^{cd}	42.69 ± 0.32 ^{ab}	2.61 ± 0.01 ^b	0.49 ± 0.08 ^{bc}
BWH	46.18 ± 0.28 ^c	41.69 ± 0.54 ^c	2.63 ± 0.02 ^{ab}	0.41 ± 0.01 ^c
BWM	47.59 ± 0.30 ^b	40.41 ± 0.37 ^d	2.61 ± 0.01 ^b	0.45 ± 0.04 ^{bc}
TCH	48.28 ± 1.94 ^{ab}	37.89 ± 1.23 ^e	2.47 ± 0.01 ^c	0.68 ± 0.06 ^a
TH	49.91 ± 2.57 ^a	35.55 ± 1.52 ^f	2.50 ± 0.02 ^c	0.73 ± 0.05 ^a
TM	ND	ND	ND	ND
WQCH	44.73 ± 0.06 ^d	43.86 ± 2.31 ^a	2.62 ± 0.06 ^{ab}	0.46 ± 0.03 ^{bc}
WQH	45.33 ± 0.21 ^{cd}	43.16 ± 1.97 ^a	2.68 ± 0.01 ^a	0.40 ± 0.03 ^c
WQM	47.15 ± 0.15 ^b	41.53 ± 0.30 ^c	2.69 ± 0.02 ^a	0.26 ± 0.02 ^d

Results are expressed as g/100 g. ND: not determined. ACH: amaranth flour with chia oil; AH: amaranth flour with hemp oil; AM: amaranth flour with a mix of both chia and hemp oils; BWCH: buckwheat flour with chia oil; BWH: buckwheat flour with hemp oil; BWM: buckwheat flour with a mix of both oils; TCH: teff flour with chia oil; TH: teff flour with hemp oil; TM: teff flour with a mix of both oils; WQCH: white quinoa flour with chia oil; WQH: white quinoa flour with hemp oil; WQM: white quinoa with a mix of both oils. For each assessment, results followed by the same lowercase letter (^{a–f}) are not significantly different according to Tukey's HSD post hoc test ($p > 0.05$).

The highest fat content was 43.86% for the WQCH sample (at the same significance level as WQH, ACH, and BWCH) and the lowest was 35.55% for the TH sample. In general, the highest moisture content and the lowest fat content ($p < 0.05$) were for both oils with teff flour (TCH and TH). Considering that all the emulsions have the same amount of water and oil (47 and 40%, respectively), the differences in water and fat content found seem to be related to the process of the emulsion's formation. It is possible that the oil or water added to elaborate the emulsion was not perfectly trapped in the gel structure and at the time of sampling, it was not homogeneous. As regards the protein content, GEs elaborated with buckwheat or white quinoa flours as emulsifier agents showed higher values ($p < 0.05$) than EG samples made with amaranth or teff flours. The protein content

of GE is determined by the protein content of flour used to make the GE. Regarding the ash content, the GE made with teff flours had the highest ($p < 0.05$) values. No statistical differences ($p > 0.05$) were found between GE samples elaborated with amaranth flour, buckwheat flour, and quinoa flour except for the GE sample elaborated with quinoa flour and blend oils that had the lowest ($p < 0.05$) ash content.

3.2.2. Physicochemical Properties of Gelled Emulsions

Taking into account that the purpose of these oil-in-water GEs is for them to be used as fat replacers in meat products, is crucial to know their pH value and texture because of the effect on the meat batter formation on the final quality of the meat product. The pH and texture parameters of the GEs are shown in Table 5.

Table 5. Physicochemical properties of GEs.

Sample	pH	Work of Shear (N·s)	Firmness (N)
ACH	6.38 ± 0.01 ^a	5.78 ± 0.63 ^b	6.64 ± 0.64 ^b
AH	6.41 ± 0.02 ^a	4.51 ± 0.08 ^c	5.26 ± 0.60 ^c
AM	6.35 ± 0.01 ^a	5.22 ± 0.20 ^b	11.69 ± 0.52 ^a
BWCH	6.03 ± 0.02 ^c	0.82 ± 0.02 ^f	0.83 ± 0.02 ^f
BWH	6.06 ± 0.01 ^c	0.89 ± 0.12 ^f	0.94 ± 0.08 ^f
BWM	6.21 ± 0.01 ^b	11.49 ± 1.18 ^a	14.70 ± 2.25 ^a
TCH	6.14 ± 0.01 ^b	5.34 ± 0.20 ^b	6.76 ± 1.94 ^b
TH	6.16 ± 0.01 ^b	3.56 ± 0.18 ^d	4.08 ± 0.16 ^d
TM	ND	ND	ND
WQCH	5.94 ± 0.01 ^d	4.15 ± 0.12 ^{cd}	3.82 ± 0.14 ^d
WQH	5.98 ± 0.01 ^d	2.77 ± 0.05 ^e	2.71 ± 0.92 ^e
WQM	5.53 ± 0.02 ^e	3.82 ± 0.03 ^d	7.22 ± 0.26 ^b

ND: not determined. ACH: amaranth flour with chia oil; AH: amaranth flour with hemp oil; AM: amaranth flour with a mix of both chia and hemp oils; BWCH: buckwheat flour with chia oil; BWH: buckwheat flour with hemp oil; BWM: buckwheat flour with a mix of both oils; TCH: teff flour with chia oil; TH: teff flour with hemp oil; TM: teff flour with a mix of both oils; WQCH: white quinoa flour with chia oil; WQH: white quinoa flour with hemp oil; WQM: white quinoa with a mix of both oils. For each parameter, results followed by same lowercase letter (a–f) are not significantly different according to Tukey's HSD post hoc test ($p > 0.05$).

The pH values of all GE samples are in the range of 5.53 to 6.41, which is included within the pH range of the main animal fats (Table 3). The pH of GE samples seems to be related to the type of pseudocereal flour ($p < 0.05$) more than to the type of oil. The lowest pH value ($p < 0.05$) was observed in samples with white quinoa flour (WQM, WQCH, and WQH) and the highest value was observed in samples with amaranth flour (ACH, AH, and AM). The values obtained were lower than those reported by Öztürk-Kerimoğlu et al. [48], who reported that pH values of GEs elaborated with peanut oil and linseed oil as healthier oils and animal protein and inulin as gelling agents were 6.58. Similarly, a study carried out by Verheyen et al. [49] found that the pH value of GEs containing sunflower oil, calcium carbonate, and glucono delta-lactone was 6.34.

Regarding texture parameters, they seem to be mainly affected by the type of oil ($p < 0.05$). For all GE samples, the use of the oils mix (M) significantly increased their firmness ($p < 0.05$) in comparison with the values obtained when only one oil was added. In addition, for the same flour, the GE firmness was higher when chia oil was used than

when the oil used was the hemp oil ($p < 0.05$), except for buckwheat flour that did not show differences ($p > 0.05$).

Both texture parameters seem to have similar behavior and have been affected in a similar way for the type of flour and oil. In this way, BWM showed the highest firmness and “work of shear” and WQH showed the lowest ($p < 0.05$). It has been reported that a firmer sample also shows a correspondingly larger area that represents the total amount of force required to perform the shearing process. Both of these values have been shown to rank samples in the same order, but for some samples, many prove to be more suitable than the others [26]. In this case, there are only two samples (AM and WQM) that were not showing the same behavior for firmness and “work of shear”. This could represent that these GE samples need a high peak of force for shearing (high firmness), but once it has been reached, they shear easily and quickly (low “work of shear”).

The rheological behavior of GEs differs widely depending on their composition, structure, droplet interactions, droplet size, etc. [12,50]. Ingredients used for GEs differed in terms of protein content and type, starch content and type, lipid profile, and the presence of other compounds. For example, it has been reported that the proteins in the different pseudocereals used possess suitable emulsifying and gel-forming capabilities [51–53]. In this way, protein–protein, protein–oil, and oil–oil interactions driven by hydrogen and covalent bonds, electrostatic, hydrophobic, and electrostatic interactions affect gel strength (protein–protein, protein–oil, and oil–oil) [54]. Finally, oil droplet size also has a considerable effect on the texture properties of emulsion gels [45,55]. In view of that, the formation of a stronger network structure in BWM, as evidenced by the highest firmness and “work of shear”, could be due to the synergic effect of both factors, the specific compounds present in buckwheat flour, and the droplet size of the oils mix, contributing to a stronger gel network.

3.3. Stability of Gelled Emulsion during Frozen Storage

3.3.1. Emulsion Stability of Gelled Emulsions

A stable emulsion should retain fluids in the system and also show stable structure at maximum levels: the higher the emulsion stability, the lower the total expressible fluid value. This value is related to several factors such as the water and oil retention capacity, protein–protein interrelations, the amphiphilic properties of proteins, gel structure, cross-linked structure of starch granules, and unsaturated acid fats contents (melting point), among others [56,57]. Some of these factors depend on the flour composition, while others depend on the oil composition and others depend on their interrelation. Other components in pseudocereal matrices can also affect the emulsion properties and stability. Thus, polysaccharides, which are present in a concentration higher than 70 g/100 g in the pseudocereal flours analyzed in this work, can contribute to emulsion stability by crosslinking proteins and adsorbing them at the interface [58]. The presence of lipids in pseudocereals negatively affects the protein emulsifying properties, especially at pH values higher than 6 [59], as occurs in this work. In addition, it is important to notice that at higher protein concentrations in pseudocereal flours (around 12 g/100 g for all flours analyzed in this work), the emulsification properties increases; however, the obtained emulsions are less stable [60]. Figure 2 shows the %TEF of each GE at time 0 (freshly made) and after 15 days of frozen storage. There is not a clear behavior of emulsion stability concerning the type of pseudocereal flour or oil used; it seems that the interrelation between both ingredients would define their effect on the emulsion stability. At time 0, ACH, BWCH, BWM, and WQCH showed the highest ($p < 0.05$) emulsion stability (%TEF < 2.5%), and WQM showed the lowest (TEF > 50%).

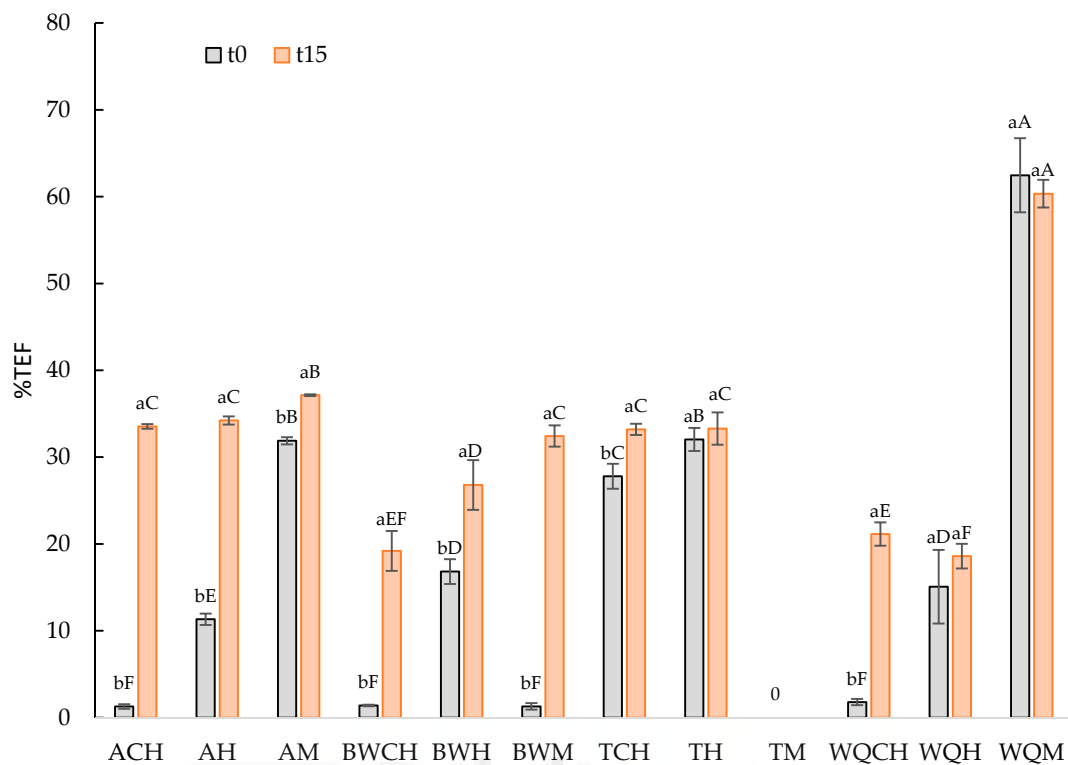


Figure 2. Emulsion stability (%Total Expressible Fluid) of gelled emulsions at day 0 (t_0) and after 15 days of frozen storage (t_{15}). Uppercase letters (A–F) refer to the comparison of the same emulsion stability values and storage time between the different GE samples; lowercase letters (a,b) refer to the comparison of the same emulsion stability values and GE samples between times. ACH: amaranth flour with chia oil; AH: amaranth flour with hemp oil; AM: amaranth flour with a mix of both chia and hemp oils; BWCH: buckwheat flour with chia oil; BWH: buckwheat flour with hemp oil; BWM: buckwheat flour with a mix of both oils; TCH: teff flour with chia oil; TH: teff flour with hemp oil; TM: teff flour with a mix of both oils; WQCH: white quinoa flour with chia oil; WQH: white quinoa flour with hemp oil; WQM: white quinoa with a mix of both oils.

Frozen storage decreased ($p < 0.05$) the emulsion stability in all the GEs except in TH, WQH, and WQM, which kept the same values ($p > 0.05$). ACH and BWM samples showed the highest decrease in emulsion stability due to frozen storage (TEF > 30%). After 15 days of frozen storage, the highest emulsion stability ($p < 0.05$) was found in WQCH, WQH, and BWCH (TEF < 20%) and the lowest was found in WQM (TEF > 50%). The stability of emulsion to freezing and thawing depends on their composition and structure, as well as on the freezing, storage, and defrosting conditions used. The freezing of GEs may crystallize both the oil and water phases, and these phase transitions play an important role in determining the properties of the final products. Depending on the melting point of the fat phase, the fat droplets may crystallize before the water, or vice versa, which can have a major impact on the freeze–thaw stability of a product [61]. In this case, both hemp and chia oils had different melting points, which as mentioned above can affect the stability of the emulsion. Thus, the destabilization of an O/W emulsion using an oil with a high melting point, in which the oil phase crystallizes before the aqueous phase, could be explained due to the coalescence of oil droplets mediated by crosslinking progress during the thawing process, and repeated coalescence eventually leads to the separation of oil and water [62]. The chemical and physical stability of emulsions are influenced by the polymorphism and degree of crystallinity of the lipids, and the phase behavior of water [63]. In turn, these are determined by several factors including emulsifier types, solutes' composition, and structural, freezing, and processing conditions [64].

3.3.2. Lipid Oxidation of GE

In order to monitor the potential oxidation of the new GE developed, which is rich in PUFA, TBARs values at time 0 and after 15 days of frozen storage were measured (Figure 3). It is very important to notice that GE samples had a fat content exceeding 35% with a proportion of PUFA higher than 70%, so a high level of lipid oxidation would be expected. The GE samples showed TBARs values lower than 2.5 mg MA/kg sample both at time 0 and after 15 days of frozen storage. This fact could be explained due to the protein and/or polysaccharide emulsifiers present in pseudocereal flours, which may increase the viscosity of the continuous phase reducing oxygen diffusion and therefore preventing lipid oxidation [65].

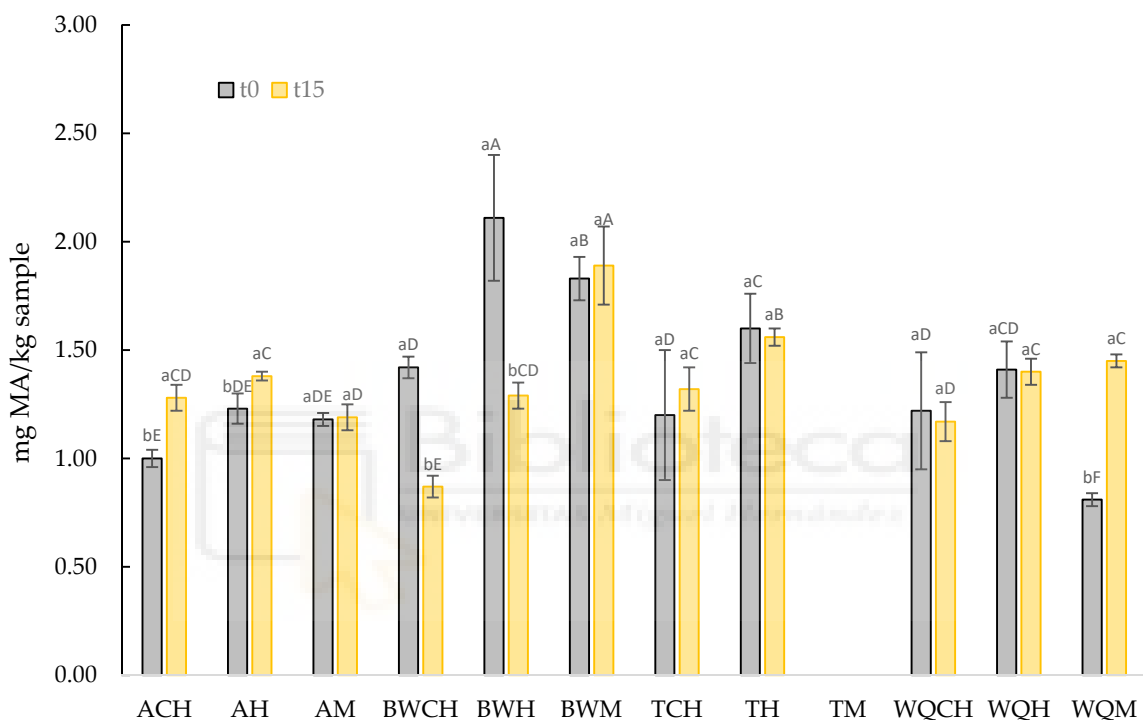


Figure 3. Lipid oxidation (TBARs; mg malonaldehyde/kg sample) of gelled emulsions at day 0 (t_0) and after 15 days of frozen storage (t_{15}). Uppercase letters (A–F) refer to the comparison of the same lipid oxidation values and storage time between the different gelled emulsion samples; lowercase letters (a,b) refer to the comparison of the same lipid oxidation values and GE samples between times; results followed by the same lower/uppercase letter are not significantly different according to Tukey’s HSD post hoc test ($p > 0.05$). ACH: amaranth flour with chia oil; AH: amaranth flour with hemp oil; AM: amaranth flour with a mix of both chia and hemp oils; BWCH: buckwheat flour with chia oil; BWH: buckwheat flour with hemp oil; BWM: buckwheat flour with a mix of both oils; TCH: teff flour with chia oil; TH: teff flour with hemp oil; TM: teff flour with a mix of both oils; WQCH: white quinoa flour with chia oil; WQH: white quinoa flour with hemp oil; WQM: white quinoa with a mix of both oils.

In general, GE samples containing amaranth flour (ACH, AH, and AM) or quinoa flour (WQCH, WQH, and WQM) showed lower ($p < 0.05$) TBARs values than GE samples containing buckwheat flour (BWCH, BWT, and BWM) or teff flour (TCH and TH). Considering that the predominant fatty acids are unsaturated fatty acids, which are easily oxidized and that any antioxidant compound has been added in GE formulation, the TBARs values do not seem too high. It must be considered that pseudocereal flours have bioactive compounds, mainly polyphenols as well as tocopherols and tocotrienols, with antioxidants properties, which could be protecting against lipid oxidation [52,53,66–68].

In this sense, Antoniewska et al. [69] reported that the addition of a buckwheat/amaranth flours blend into muffins reduces the lipid oxidation degree due to the phenolic compounds as well as phytosterols and tocopherols presents in these flours. Similarly,

Jimenez et al. [70] informed that baby dehydrated purees formulated with quinoa and amaranth flours had more fat oxidative stability than control samples due to the bioactive content, mainly tocopherols and tocotrienols, of pseudocereal flours. Previously, Rocchetti et al. [71] reported that pseudocereal flours including quinoa, amaranth, teff, and buckwheat had a high antioxidant capacity measured with FRAP and ORAC assays, and this antioxidant capacity was directly correlated with the content of polyphenolic compounds such as flavonoids (i.e., flavonols) and phenolic acids (hydroxycinnamic). There is not a clear pattern to describe the effect of frozen storage on TBARS; in some cases, their value was increased (ACH, AH, and WQM) or not modified (AM, BWM, TCH, TH, WQCH, and WQH) or even reduced (BWCH, and BWH).

3.3.3. Color Properties of GE

As the purpose of these GE applications is to be used as the replacement of animal fat, their colors must be as close as possible to the color of pork backfat or lard or even poultry fat (traditional fat sources in meat products). The visual appearance of these GEs (Figure 1) could indicate that GE samples have colors into this range but with clear differences between them.

Knowing that the color is highly influenced by the development of lipid oxidation, it has been considered interesting to assess their color changes during frozen storage. The color parameters of GEs at day 0 and after 15 days of frozen storage are shown in Table 6.

The lightness values of GE samples ranged between 58.78 and 78.07. All these L^* values are in the range of L^* reported for animal fats (Table 3). It could be said that L^* depends on both main ingredients (flour and oil) because there is not a clear pattern of any of them. L^* depends on the water and oil free on the ultrastructure of the product surface: the higher the amount of this ingredient on the surface, the higher the L^* values [72]. The water and oil-holding capacity attributed to each flour and the special oil composition could be responsible for these L^* variations. All GE samples showed lower L^* values after frozen storage. The frozen process modifies the ultrastructure; there are water migrations inside the samples, and also, the water and oil-holding capacity could be modified [64]. All these factors could be affecting L^* changes.

Redness (a^*) values ranged between 0.19 and 1.48 with differences between them ($p < 0.05$), although it must be considered that differences lower than 1 unit have no practical effect upon visual color. GE samples containing amaranth or quinoa flour seem to have the same behavior in relation to the type of oil added: the addition of hemp oil increased a^* values ($p < 0.05$) in relation to chia oil. On the contrary, a^* values of GE samples containing buckwheat or teff flour decreased ($p < 0.05$) when hemp oil was added. Hemp oil has a high content of chlorophyll, which could be affecting a^* values [20]. These changes could be attributed to the subtraction or addition effect upon red color components determined by the type of oil. All GE samples showed redness values into the range reported for animal fats (Table 3). Although frozen storage caused some variations (increase, reduce, or not variation) in a^* values compared to the same values at time 0, any of these differences were higher than 1 unit, so this has no practical importance.

Yellowness (b^*) values ranged between 8.78 and 29.73 with significant differences between them ($p < 0.05$). In this case, a clear effect (increasing) of hemp oil on b^* values can be observed in reference to chia oil. The yellow components present in hemp oil would seem to be responsible for this b^* increase. Several authors have reported a high content in total chlorophylls (up to 57.66 mg/kg) and carotenes (up to 146.80 mg/kg) in hemp oil [73]. This high carotene content could be contributing to yellowness increase. All GEs showed yellowness values into the range of that reported for animal fats (Table 3).

Frozen storage caused a slight modification in the b^* values of GE samples. The behavior of C^* in GE samples seems to be related to the b^* coordinate (b^* -dependent) in both cases: before and after frozen storage.

Table 6. Color parameters of GE samples (freshly, t_0) and after 15 days of frozen storage (t_{15}).

Samples	t_0					t_{15}				
	L^*	a^*	b^*	C^*	h	L^*	a^*	b^*	C^*	h
ACH	74.58 ± 1.52 ^{Ba}	0.33 ± 0.06 ^{Ba}	10.77 ± 0.28 ^{Fb}	10.77 ± 0.28 ^{Fb}	88.22 ± 0.35 ^{Da}	70.77 ± 0.60 ^{Ab}	0.34 ± 0.08 ^{Ca}	11.82 ± 0.30 ^{Da}	11.83 ± 0.30 ^{Da}	88.35 ± 0.34 ^{Ca}
AH	69.45 ± 1.91 ^{Ca}	−1.03 ± 0.22 ^{Fb}	23.52 ± 0.54 ^{BCa}	23.54 ± 0.54 ^{BCa}	92.52 ± 0.54 ^{Aa}	61.83 ± 2.29 ^{CDb}	−0.53 ± 0.28 ^{Ga}	23.22 ± 1.62 ^{Ba}	23.23 ± 1.62 ^{Ba}	91.27 ± 0.65 ^{Aa}
AM	64.68 ± 2.86 ^{Da}	−1.04 ± 0.08 ^{Fb}	25.15 ± 2.69 ^{Ba}	25.17 ± 2.69 ^{Ba}	92.41 ± 0.46 ^{Aa}	61.74 ± 0.79 ^{Da}	−0.12 ± 0.27 ^{Fa}	25.54 ± 1.31 ^{Aa}	25.55 ± 1.31 ^{Aa}	90.25 ± 0.59 ^{Bb}
BWCH	74.27 ± 1.98 ^{Ba}	1.23 ± 0.20 ^{Ab}	8.78 ± 0.47 ^{Ga}	8.87 ± 0.49 ^{Ga}	82.57 ± 0.86 ^{Fa}	65.28 ± 2.35 ^{Cb}	1.51 ± 0.4 ^{Aa}	8.85 ± 0.27 ^{Fa}	8.98 ± 0.26 ^{Fa}	80.28 ± 1.02 ^{Eb}
BWH	64.35 ± 0.52 ^{Da}	0.56 ± 0.18 ^{Eb}	23.20 ± 0.57 ^{BCb}	23.21 ± 0.57 ^{BCb}	91.37 ± 0.42 ^{Ba}	60.09 ± 0.87 ^{DEb}	−0.15 ± 0.24 ^{Fa}	24.98 ± 2.31 ^{ABa}	24.98 ± 2.31 ^{ABa}	90.32 ± 0.50 ^{Bb}
BWM	78.07 ± 1.73 ^{Aa}	0.45 ± 0.14 ^{Db}	17.56 ± 0.53 ^{Da}	17.57 ± 0.53 ^{Da}	91.48 ± 0.47 ^{Ba}	65.65 ± 1.30 ^{Cb}	0.05 ± 0.18 ^{Ea}	16.95 ± 0.94 ^{Cb}	16.95 ± 0.94 ^{Cb}	89.81 ± 0.62 ^{Cb}
TCH	64.50 ± 1.77 ^{Da}	1.23 ± 0.12 ^{Aa}	11.75 ± 0.17 ^{Ea}	11.81 ± 0.16 ^{Ea}	84.03 ± 0.64 ^{Ea}	59.56 ± 1.39 ^{Eb}	0.81 ± 0.10 ^{Bb}	11.67 ± 0.36 ^{Da}	11.69 ± 0.37 ^{Da}	84.50 ± 0.36 ^{Da}
TH	58.78 ± 0.80 ^{Fa}	0.19 ± 0.09 ^{Ca}	22.25 ± 1.01 ^{Cb}	22.25 ± 1.01 ^{Ca}	89.50 ± 0.22 ^{Ca}	54.63 ± 1.76 ^{Fb}	0.10 ± 0.13 ^{EDb}	22.98 ± 1.35 ^{Ba}	22.98 ± 1.35 ^{Ba}	90.25 ± 0.33 ^{Ba}
TM	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
WQCH	72.38 ± 2.09 ^{BCa}	0.34 ± 0.08 ^{Da}	11.70 ± 0.56 ^{Ea}	11.71 ± 0.56 ^{Ea}	91.69 ± 0.41 ^{Bb}	68.98 ± 0.50 ^{Bb}	−0.51 ± 0.07 ^{Gb}	10.80 ± 0.29 ^{Eb}	10.82 ± 0.29 ^{Eb}	92.74 ± 0.43 ^{Aa}
WQH	62.00 ± 1.02 ^{DEa}	−1.16 ± 0.20 ^{Fb}	24.67 ± 0.58 ^{Bb}	24.70 ± 0.58 ^{Bb}	92.70 ± 0.44 ^{Aa}	54.12 ± 2.82 ^{Fb}	−0.89 ± 0.28 ^{Ha}	26.17 ± 1.40 ^{Aa}	26.19 ± 1.39 ^{Aa}	91.95 ± 0.62 ^{Ab}
WQM	60.68 ± 0.50 ^{Ea}	1.48 ± 0.14 ^{Hb}	29.73 ± 0.77 ^{Aa}	29.77 ± 0.77 ^{Aa}	92.85 ± 0.23 ^{Aa}	58.89 ± 2.25 ^{Eb}	−1.09 ± 0.15 ^{Ia}	23.41 ± 1.15 ^{Bb}	23.44 ± 1.15 ^{Bb}	92.66 ± 0.30 ^{Aa}

Uppercase letters (A–I) refer to the comparison of the same color parameter and storage time between the different GE samples; lowercase letters (a,b) refer to the comparison of the same color parameter and GE samples between times; results followed by the same lower/uppercase letter are not significantly different according to Tukey's HSD post hoc test ($p > 0.05$). ND: not determined. ACH: amaranth flour with chia oil; AH: amaranth flour with hemp oil; AM: amaranth flour with a mix of both chia and hemp oils; BWCH: buckwheat flour with chia oil; BWH: buckwheat flour with hemp oil; BWM: buckwheat flour with a mix of both oils; TCH: teff flour with chia oil; TH: teff flour with hemp oil; TM: teff flour with a mix of both oils; WQCH: white quinoa flour with chia oil; WQH: white quinoa flour with hemp oil; WQM: white quinoa with a mix of both oils.

GE samples containing quinoa flour (WQCH, WQH, and WQM) showed hue values in the range of yellow hue (90.00° – 97.50°). GEs containing teff flour (TCH and TH) showed hue values in the range of yellow-orangish (82.50° – 89.99°). GE samples containing amaranth or buckwheat flour showed a hue values range dependent on the type of oil: with chia oil, the hue values were in the range of yellow-orangish, whereas with hemp oil (alone or in the mix with chia oil), their hue values were in the range of yellow hue [74]. In all GE samples, frozen storage caused slight modifications of hue values, or they were not modified.

4. Conclusions

The use of pseudocereal flours (amaranth, buckwheat, teff, and white quinoa) and vegetable oils (hemp oil, chia oil, and a blend of both) results in a technologically viable option to elaborate gelled emulsions with a healthier lipid profile (>70% PUFA). The only combination that is not suitable for further application is the use of teff flour with the blend of both oils. Several combinations of all these ingredients allow the elaboration of GE with different firmness that will be useful for their application in different types of foods. If the oxidative stability of the GE is taken as a quality criterion, AM (amaranth flour + blend oils) and WQCH (white quinoa flour + chia oil) samples are the most suitable for the substitution of fat in the development of new foods low in fat or with a healthier lipid profile. On the other hand, TH (teff flour + hemp oil), and WQH (white quinoa flour + hemp oil) show better behavior (emulsion stability) under the frozen and thawing process, which made them suitable for frozen foods. In any case, more studies are needed to improve the stability of the emulsion. Possible alternatives to improve this stability could be to (i) increase the concentration of the emulsifying agent (pseudocereal flours) and reduce the water content, (ii) increase the concentration of the gelling agents and reduce the water content, or (iii) increase the concentration of the emulsifying agent and the gelling agents and reduce the water content. To sum up, the use of these gelled emulsions in foods development brings a new strategy to produce healthy foods.

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8.3. PUBLICACIÓN 3

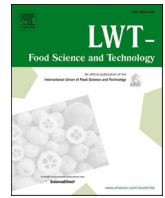
Improving the lipid profile of beef burgers added with chia oil (*Salvia hispanica* L.) or hemp oil (*Cannabis sativa* L.) gelled emulsions as partial animal fat replacers

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Improving the lipid profile of beef burgers added with chia oil (*Salvia hispanica* L.) or hemp oil (*Cannabis sativa* L.) gelled emulsions as partial animal fat replacers

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ABSTRACT

New gelled emulsions (GE) based on amaranth flour mixed with chia or hemp oil were developed and used as partial pork back-fat replacer (25 and 50%) in beef burgers. The addition of GE decreased the fat content in the burgers between 12% and 33%. The use of GE decreased the amount of palmitic, stearic, and oleic fatty acids and increased the amount of linolenic (higher in amaranth-hemp GE) and α -linolenic (higher in amaranth-chia GE) fatty acids. Both GE improved the n-6/n-3 and PUFA/SFA nutritional ratios in burgers and the AI, TI, h/H indices related to healthy properties of lipid fractions. Color, water activity, pH, and texture were not affected by the addition of GE but cooking loss, shrinkage, and thickness changes were increased (higher in amaranth-hemp GE). Burgers containing amaranth-chia GE (both raw and cooked) resulted in more susceptibility to lipid oxidation than the others and also resulted in lower sensorial acceptability. As a general conclusion, the use of amaranth-hemp GE as pork backfat substitute improve nutritional characteristics of the burgers without affecting technological or sensory properties.

1. Introduction

Animal fat is an important ingredient in meat products with a high impact on their technological and sensorial properties. However, its high percentage in saturated fatty acids (SFA) which has been associated with a series of diseases like obesity, cardiovascular and chronic diseases (Food and Agriculture Organization of the United Nations FAO, 2016), is being a real problem for nowadays consumers who are really worried about their health, requesting healthier foods. A way to please this demand is by reformulating meat products with healthier lipid sources (rich in unsaturated fatty acids), especially from vegetable oils (Gómez-Estaca, Herrero, et al., 2019; Vargas-Ramella et al., 2020). The type of fat and lipid composition are not only interesting from a nutritional point of view but also have a significant role in the structure, texture, sensorial and technological properties of the final product (Barros et al., 2021; Öztürk-Kerimoğlu, Urgu-Öztürk, & Serdaroglu, 2021). For this reason, several strategies have been applied to replace animal fat with vegetable oils minimizing both, their effect on the physicochemical and sensorial properties of the final product, to ensure

their acceptability by consumers, but also on their technological characteristics, to ensure their technological viability in the meat industry (de Souza-de Souza Paglarini et al., 2019; de Carvalho et al., 2020; Tarté, Paulus, Acevedo, Prusa, & Lee, 2020). One way of doing this substitution, with minimal technological effects, could be the use of gelled emulsions (GEs) (Alejandre, Passarini, Astiasarán, & Ansorena, 2017; Pintado, Ruiz-Capillas, Jiménez-Colmenero, Carmona, & Herrero, 2015). Several vegetable oils with healthy lipid profiles (wheat germ, tiger nut, chia, flaxseed, linseed, olive, canola, and soybean oils, among others) and emulsifier or gelling agents (gelatin, alginate, chia mucilage flour, protein soy, carrageenan, chestnut flour, gums, and inulin, among others) have been successfully used in the development of these GEs (Lucas-González et al., 2020; de Souza-de Souza Paglarini et al., 2019; Vargas-Ramella et al., 2020; Barros et al., 2021; Öztürk et al., 2021). Moreover, previous studies have shown the potential of these GEs as animal fat replacers in several meat products, mainly cooked meat products (Barros et al., 2020; de Carvalho et al., 2020). Among the variety of meat products, burgers and patties seem to be a compelling choice for both fat reduction and lipid profile improvement since they

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are popular products sold as ready-to-eat and fast food consumption, easy to prepare at home, and so, with a high impact in our diet.

Although GEs are been highly applied as animal fat replacers in meat products, to our knowledge, there are no published data using the combination of hemp (*Cannabis sativa* L.) oil or chia (*Salvia hispanica* L.) oil with amaranth flour to elaborate GEs for fat replacer in burgers production. Chia oil is composed of a well-balanced fatty acids profile, consisting of up in the unsaturated fatty acid fraction with 65% linolenic acid and 20% linoleic acid (Villanueva-Bermejo, Calvo, Castro-Gómez, Fornari, & Fontecha, 2019). Hemp oil is rich in polyunsaturated fatty acids, mainly linoleic acid and also contains gamma and alpha linolenic acids (Tura et al., 2022). On the other hand, amaranth is a highly nutritious pseudocereal known to be a dietary source of proteins, vitamins, minerals and dietary fiber (Tafadzwa et al., 2021). Additionally, amaranth proteins provide emulsifying and gelling properties, both highly useful for the development of gelled emulsions (Alejandre, Ansorena, Calvo, Caverro, & Astiasaran, 2019).

Therefore, the aims of this work were to evaluate the technological viability of using GEs, elaborated with hemp or chia oil with amaranth flour, as partial animal fat replacer (25% and 50%) for beef burgers production and study the effect of these two partial fat substitutions on proximate composition, lipid profile, lipid oxidation, and physicochemical, cooking, and sensory properties of reformulated beef burgers.

2. Materials and methods

2.1. Materials

For gelled emulsions (GEs) preparation the following ingredients were used: chia oil (56.61% α -linolenic acid, 17.43% linoleic acid, and 15.05% oleic acid) and hemp oil (54.44% linoleic acid, 19.95% α -linolenic acid, 8.23% oleic acid) purchase from Laboratorios Almond, S.L. (Murcia, Spain); amaranth flour was obtained from Tentorium Energy S. L. (Tarragona, Spain); gellan gum (a polysaccharide excreted by microorganism *Pseudomonas elodea*) and gelatin of animal origin (pork) named "instant gel" were obtained from Sosa Ingredients S.L. (Barcelona, Spain). Meat ingredients [beef meat (74.02% moisture, 24.51% protein, 2.88% lipids, and 1.01% ash) and pork backfat (84.05% lipids, 3.18% proteins, 12.51% moisture, and 0.26% ash)] were purchased from a local supermarket.

2.2. Preparation of oil in water gelled emulsions

The gelled emulsions were elaborated as described by Botella-Martínez Pérez-Álvarez, Sayas-Barberá, Fernández-López, and Viuda-Martos (2021). Thus, for each type of GE, first the gelling agent was mixed in a homogenizer (Thermomix 31, Vorwerk-España, Spain) with water for 2 min at 60 °C at high speed. Then, the flour was added and mixed for 1 min at medium speed. In the next step, the temperature was turned down to 37 °C and gellan gum was added and mixed for 2.5 min at 250 rpm. In the last step, the mixture was mixed with the gradual addition of the appropriate amount of oil for 5 min, at 37 °C and 1100 rpm. The elaborated GE were placed in metal containers and stored at 4 °C until use. The chemical composition of GE elaborated with amaranth flour and chia oil was 45.76% moisture, 42.82% fats, 2.50% proteins, 8.49% carbohydrates, and 0.43% ashes while the chemical composition of GE elaborated with amaranth flour and hemp oil was 46.07% moisture, 42.56% fats, 2.52% protein, 8.41% carbohydrates, and 0.44% ashes.

2.3. Processing of burgers containing gelled emulsions

Burgers (twelve for each formulation) were made according to the traditional formula. This original formula was used as a formula control whereas the other four formulations, where different proportions of pork backfat (25 or 50%) fat (50 or 100%) were replaced by gelled emulsion

made with amaranth flour and chia oil (BCh) or amaranth flour and hemp oil (BH), were elaborated as shown in Table 1.

Beef burgers were elaborated following the procedure described by Botella-Martínez et al., (2021). Beef meat and pork backfat were ground through 8 mm plate in a mincer. Then the mixer, water, salt and pepper were added into a bowl and mixed with the spiral dough hook at 80 rpm for 5 min. For each formulation, the corresponding proportions of fat (25% or 50%) were replaced by gelled emulsion elaborated with amaranth/chia oil or gelled emulsion elaborated with amaranth/hemp oil and mixed again for 5 min. The samples were shaped using a commercial burger maker to obtain burgers of approximately 1 cm thickness and 80 g. Burgers were packed into bags and storage at 4 °C until analysis (Raw burgers). Six burgers from each formulation were cooked in a griddle until reaching an internal temperature of 72 °C, approximately 4 min for each side (Cooked burgers).

2.4. Evaluation of beef burgers

2.4.1. Proximate analysis

Proximate analysis (moisture, protein, fat and ash content) were evaluated by AOAC (2010) in raw and cooked burgers.

2.4.2. Lipid profile and nutritional parameters

Lipid extraction from the samples was conducted according to Folch, Less and Sloane (1957). The lipid phase was methylated according to method 969.33 of AOAC (2010). The fatty acids methylated (FAMES) were determined according to the chromatographic conditions described by Pellegrini et al. (2018). Results were expressed as g fatty acid/100 g of fat.

Using equations developed by Ulbricht and Southgate (1991), the atherogenic index (AI) and thrombogenic index (TI) were calculated (Eq. (1) and Eq. (2)), respectively.

$$AI = \frac{C12 : 0 + (4x C14 : 0) + C16 : 0}{\sum MUFA + \sum n - 6 + \sum n - 3} \quad (1)$$

$$TI = \frac{C14 : 0 + C16 : 0 + C18 : 0}{(0,5x \sum MUFA) + (0,5x \sum n - 6) + (3x \sum n - 3) + \left(\frac{\sum n - 3}{\sum n - 6} \right)} \quad (2)$$

The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated using equation (3), as described by Fernández et al. (2007).

$$h/H = \frac{C18 : 1n9 + C18 : 1n7 + \sum PUFA}{C14 : 0 + C16 : 0} \quad (3)$$

2.4.3. Physicochemical properties

The pH values of raw and cooked burgers were measured using a penetration probe, at different sites of the sample, connected to a pH-meter Crison model 510 (Barcelona, Spain).

The water activity (a_w) was measured in raw burgers using an

Table 1

Formulation of beef burgers burgers reformulated with both amaranth-chia oil (BCh) or amaranth-hemp oil (BH) gelled emulsion used as partial animal fat replacers.

	Treatments (%)				
	CS	BCh25	BCh50	BH25	BH50
Beef	80	80	80	80	80
Pork backfat	20	15	10	5	10
Water	5	5	5	5	5
Salt	1.5	1.5	1.5	1.5	1.5
White pepper	0.05	0.05	0.05	0.05	0.05
GECW	0	5	10	5	10

Percentages of non-meat ingredients are related to 100% meat. CS: control sample. GECW: gelled emulsion elaborated with cocoa bean shell flour and walnut oil.

electrolytic hygrometer (Novasina TH-500, Novasina, Pfaeffikon, Switzerland) at 25 °C.

Texture profile analysis- TPA was performed in cooked beef burgers samples using a TA-XT2i Texture Analyzer (Stable Micro Systems, Surrey, England). Samples of 1 cm³ were submitted to two-cycle compression to 75% at a constant velocity of 1 mm/s at room temperature. The parameters calculated were: Hardness (N) springiness (mm), cohesiveness, and chewiness (N x mm) (Claus, 1995).

Color measurements were performed using a CM-700 spectrophotometer (Minolta Camera Co., Osaka, Japan) with illuminant D65, observer angle 10°, SCI mode and a low reflectance glass placed between surface of samples and the equipment. For Color assessment, the CIE-L*a*b* color coordinates determined were: lightness (L*), red/green coordinate (a*) and yellow/blue coordinate (b*). The psychophysical magnitudes hue (h*) and chrome (C*) were calculated with equations (4) and (5), respectively, in both raw and cooked burgers.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (4)$$

$$h^* = \arctg(b^* / a^*) \quad (5)$$

Total color differences (ΔE) of each sample reformulate with respect to control burger (BC) were also calculated with equation (6).

$$\Delta E = \sqrt{(L_S^* - L_{CON}^*)^2 + (a_S^* - a_{CON}^*)^2 + (b_S^* - b_{CON}^*)^2} \quad (6)$$

2.4.4. Cooking characteristics

The weight, thickness, and diameter of the beef burgers from each batch were measured at room temperature before and after cooking. To estimate the dimensional changes, the shrinkage and the thickness increases were calculated with equations (7) and (8).

$$\% \text{ Thickness increase} = \frac{\text{cooked thickness} - \text{raw thickness}}{\text{cooked thickness}} \times 100 \quad (7)$$

$$\% \text{ Shrinkage} = \frac{\text{raw diameter} - \text{cooked diameter}}{\text{raw diameter}} \times 100 \quad (8)$$

The cooking loss was calculated according to equation (9):

$$\% \text{ Cooking loss} = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100 \quad (9)$$

2.4.5. Lipid oxidation

The Thiobarbituric Acid Reactive substances (TBARS) values were determined according to Rosmini et al. (1996) in both, raw and cooked samples. Results, expressed as mg malondialdehyde (MDA)/kg sample.

2.4.6. Sensory analysis

The sensory evaluation was carried out in a sensory analysis laboratory of Miguel Hernández University (Orihuela, Spain). The sensory panel was formed by 37 members, from the staff and students. Five samples from each formulation were shown to panellist to evaluate the raw burger attributes and later the samples were cooked in a griddle and submitted on pieces of 2 cm³ approximately. The sensory analysis scheme was developed for raw samples with a hedonic scale of 9 levels: color intensity (1: extremely light and 9: extremely dark), for rancid aroma (1: imperceptible and 9: extremely rancid), and visual aspect (1: dislike extremely and 9: like extremely). In the case of cooked samples, the following attributes: general acceptability, juiciness, chewiness, fat sensation and graininess, were evaluated with a hedonic scale of 9 levels being 1: dislike extremely and 9: like extremely:

2.5. Statistical analysis

The full process (gelled emulsion elaboration and burger manufacture) was replicated three times (three independent batches). Each replication was done on a different production day and each batch was

analyzed in triplicate. Data were evaluated by one-way analysis of variance (ANOVA) and Tukey-b post-hoc test was performed at 5% significance level ($p < 0.05$) using SPSS software (version 24.0, SPSS Inc., Chicago, USA). Means and standard deviations of data are shown in corresponding tables and figures.

3. Results and discussion

3.1. Proximate composition of beef burgers

Table 2 showed the proximal composition of raw and cooked beef burgers reformulated with either amaranth-chia oil or amaranth-hemp oil gelled emulsion used as partial animal fat replacers.

In raw burger, the effect of replacing animal fat with GEs did not cause any effect ($p > 0.05$) on ash and protein content. However, the moisture values increased while the fat values decreased ($p < 0.05$) in beef burgers containing GEs, compared to control ones. The increase in moisture content was due to the water added to prepare the GEs. The same finding has been reported by several authors when used GEs as animal fat replacers in meat products (Lucas-González et al., 2020; Botella-Martínez, Viuda-Martos, Pérez-Álvarez, & Fernández-López, 2021). The reduction in fat content when the animal fat was replaced by the GEs was not influenced by the type of GE used ($p > 0.05$) but occur in a concentration-dependent manner ($p < 0.05$). When both GEs were used at 25% of fat substitution in burgers, the level of fat reduction achieved was 12%, while GEs were used at 50% of fat substitution, the level of reduction increased until 33% compared to control burgers, without differences between gelled emulsion elaborated with chia oil (GCh) and gelled emulsion elaborated with hemp oil (GH). This behavior was also observed by several authors (Alejandro et al., 2017; Barros et al., 2020; Lucas-González et al., 2020). Thus, Lucas-González et al. (2020) who replaced animal fat with chia-chestnut gelled emulsion (5 and 10%) in pork burgers reported a reduction of the fat content when

Table 2

Proximate composition of raw and cooked beef burgers reformulated with both amaranth-chia oil or amaranth-hemp oil gelled emulsion used as partial animal fat replacers.

	Sample	Protein	Fat	Ash	Moisture
Raw	BC	17.47 ± 1.78 ^a	14.46 ± 0.65 ^a	2.33 ± 0.20 ^a	62.39 ± 2.52 ^b
	BCh25	18.63 ± 0.36 ^a	12.71 ± 5.96 ^b	2.24 ± 0.20 ^a	65.47 ± 2.48 ^a
	BCh50	18.06 ± 0.04 ^a	9.18 ± 0.73 ^c	2.38 ± 0.03 ^a	65.90 ± 0.34 ^a
	BH25	18.43 ± 0.28 ^a	12.64 ± 1.01 ^b	2.22 ± 0.02 ^a	65.08 ± 0.89 ^a
	BH50	18.50 ± 1.41 ^a	9.91 ± 0.49 ^c	2.27 ± 0.04 ^a	65.72 ± 0.64 ^a
Cooked	BC	23.98 ± 0.06 ^v	16.13 ± 0.16 ^v	2.79 ± 0.07 ^v	55.90 ± 0.23 ^v
	BCh25	24.42 ± 0.07 ^v	13.51 ± 0.44 ^w	2.77 ± 0.01 ^v	57.70 ± 0.20 ^w
	BCh50	24.45 ± 0.51 ^v	9.32 ± 0.32 ^x	2.82 ± 0.02 ^v	57.68 ± 0.00 ^w
	BH25	24.43 ± 0.12 ^v	13.81 ± 0.68 ^w	2.84 ± 0.05 ^v	57.17 ± 0.29 ^w
	BH50	25.05 ± 0.52 ^v	10.05 ± 0.38 ^x	2.73 ± 0.03 ^v	57.41 ± 0.15 ^w

Values expressed in g/100 g of sample. For each parameter, results followed by same letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as mean ± standard deviation.

BC: control burgers with a traditional formula; BCh25: sample with 25% animal fat replaced by GE with chia oil and amaranth flour; BCh50: sample with 50% animal fat replaced by GE with chia oil and amaranth flour. BH25: sample with 25% animal fat replaced by GE with hemp oil and amaranth flour as fat replacer. BH50: sample with 50% animal fat replaced by GE with hemp oil and amaranth flour. A lower-case letter refers to the comparison of the same parameter between the different raw samples (a-e) and for cooked samples (v-z).

substitution level increased. Similarly, Regarding nutritional claims, only burgers with the highest GEs substitution level (BCh50 and Bh50) can be considered as “reduced fat content” (at least 30% reduction compared to the original product (European Parliament, 2006).

In cooked burger, again no statistical differences ($p > 0.05$) were found among the control sample and reformulated samples for protein and ash content while in the case of fat and moisture content the same behavior than raw burger was observed.

3.2. Fatty acids profile and health parameters of beef burgers

3.2.1. Fatty acids profile

Table 3 shows the fatty acids profile of beef burgers (raw and cooked). Regarding raw burgers, as expected, significant differences ($p < 0.05$) were detected in the fatty acid profile of burgers depending on both, the type of fat used (animal fat, GCh or GH) and the level of pork fat replacement (25 or 50%). From the total of fatty acids identified in control burgers, oleic (C18:1), palmitic (C16:0), linoleic (C18:2), stearic (C18:0), and palmitoleic (C16:1) fatty acids make up 91% of total fatty acids. To reach this level, in the case of reformulated burgers, the contribution of the α -linolenic fatty acid (C18:3) must be considered. In general, it could say that the use of GE as partial animal fat replacer in burgers decreased the amount of palmitic (C16:0), stearic (C18:0) and oleic (C18:1) fatty acids and increased the amount of linolenic (C18:2) and α -linolenic (C18:3) fatty acids ($p < 0.05$). The most evident difference between burgers due to the type of GE used was the amount of linolenic (C18:2) and α -linolenic (C18:3) fatty acids. Burgers with amaranth-chia GE showed the highest amount of α -linolenic (C18:3) fatty acid while burgers with amaranth-hemp GE showed the highest ($p < 0.05$) amount of linolenic (C18:2) fatty acid. This is in accordance with the fatty acid composition of the corresponding vegetable oils. According to European Association, raw and cooked BCh50 and cooked BCh25 could be labeled with the nutritional claim as “high n-3 fatty acids”, since they contained more than 0.6 g α -linolenic acid per 100 g of the product (European Parliament, 2006).

For the cooked samples, the trend is very similar regarding the influence of the percentage of substitution and the gelled emulsion used. Some small variations in the values and so in the statistical significance in cooked sample respect to obtained in raw samples could be attributed to the loss of fat and water during cooking. Among the saturated fatty acids (SFA), in all burgers, the largest proportions ($p < 0.05$) were palmitic (C16:0), stearic (C18:0), and myristic (C14:0) fatty acids. The replacement of animal fat by GE in burgers decreased the SFA content ($p < 0.05$) depending on both, the substitution level (higher decrease at 50% substitution level) and type of GE used (higher decrease when amaranth-hemp oil GE was used). This fact has also been reported by other authors in the case of replacement of animal fat by vegetable or marine oils in several meat products (Domínguez et al., 2017; Heck et al., 2019; Pires, dos Santos, Barros, & Trindade, 2019; Tarté et al., 2020; Vargas-Ramella et al., 2020). Control burgers (raw and cooked) showed the highest amount of SFA, (35.89% and 36.20% respectively), therefore BH50 (raw and cooked) showed the lowest, with a decrease of 17% and 12.5% respectively, with respect to control ones.

In the case of monounsaturated fatty acid (MUFA) content, a reduction was also reported due to the use of GE, showing control samples (raw and cooked) the highest content ($p < 0.05$). It is important to notice that MUFA was the predominant fraction in all burgers (raw and cooked) being oleic acid (C18:1) the predominant. On the contrary, polyunsaturated fatty acid (PUFA) fraction increased in reformulated burgers, compared to control ones, being this increase higher at higher GE replacement level and also when amaranth-hemp GE was used ($p < 0.05$). Linoleic (C18:2) and α -linolenic (C18:3) fatty acids are responsible (in a high way) for this increase.

3.2.2. Health indices of burgers

Table 4 shows the health indices of cooked beef burgers (control and

reformulated burgers). In relation to the PUFA/SFA ratio, it is observed an increase when animal fat is replaced by GE, due to both, the decrease in SFA and the increase in PUFA contents. This increase depends on both, substitution level (higher at 50% than at 25% replacement level) and type of GE (higher when amaranth-hemp GE was used) ($p < 0.05$). All reformulated burgers are in accordance with the recommendations of the PUFA/SFA ratio that should be above than 0.4 (Wood et al., 2008). Regarding the n-6/n-3 index, all reformulated burgers, except BH25 are in accordance with the recommended value which must be less than 4 (Simopoulos, 2004). As can be seen in Table 4, this index was widely improved (decreased) ($p < 0.05$) by the use of GE with respect to control burgers.

The indices TI, AI and h/H have been proposed as good indicators of healthy food products and have been widely calculated and discussed to address the healthy characteristics of fats in meat products (Botella-Martínez, Lucas-González, et al., 2021; de Souza-de Souza Paglarini et al., 2019; Pintado et al., 2015). Regarding that, TI and AI should be as low as possible and h/H ratio the other way around, as higher as possible. In view of that, the influence of pork back fat replacement by GE in burgers was positive considering that TI and AI indices decreased ($p < 0.05$) and h/H ratio increased ($p < 0.05$). All these changes observed are directly related to the percentage of pork backfat replace: the most positive values in the three indices were shown in burgers with 50% substitution (BCh50 and BH50). Several authors have used these indices to highlight the healthy properties of using vegetable oils (added in different ways) in substitution of animal fats in meat products (Barros et al., 2021; Botella-Martínez, Lucas-González, et al., 2021; Pires et al., 2019).

3.3. Physicochemical characteristics of beef burgers

The physicochemical properties of raw and cooked beef burgers formulated with amaranth flour and chia or hemp oil gelled emulsions as partial pork backfat replacers were shown in Table 5. Regarding raw burgers, the main values of pH and Aw were not affected ($p > 0.05$) by addition of GEs in burgers as partial substitute of pork backfat. Similarly, Lucas-González et al. (2020) found no differences on pH and Aw values in burgers when emulsion gels formulated with chestnut flour and chia oil were used as a substitute of pork backfat. Lightness (L^*), yellowness (b^*) and hue (h^*) of burgers were not influenced ($p > 0.05$) by the used of gelled emulsions. Quite the opposite, redness (a^*) and chroma (C^*) were significantly affected by this replacement although their variation was not quantitatively relevant. In fact, redness values ranged from 4.06 (control burger) to 5.72 (BCh25). Similarly, Barros et al. (2021) found no differences in the color parameters of the beef burgers added with oil emulsions. On the contrary, several authors (Lucas-González et al., 2020; de Souza-de Souza Paglarini et al., 2019; Barros et al., 2020) reported that the addition of gelled emulsions, in different meat products, were able to affect all color parameters. All these differences could be due to the different oil characteristics and composition, as well as the emulsion properties and the rest of ingredients used in the meat product formulation. In addition, taking into account that color differences (ΔE^*) lower than 3 units cannot be detected by human eye (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001), only burgers formulated with gelled emulsion elaborated with chia oil as partial fat replacement (BCh25 and BCh50) could be detected as different from control burgers.

In reference to cooked burgers, the cooking process resulted in a slight pH increase (ranging from 6.27 to 6.38) respect to the corresponding raw samples, but without differences ($p > 0.05$) between samples.

As regards to color properties, during heating of meat products several reactions occur, including the Maillard reaction, protein denaturation, and fat and water loss and these reactions are responsible for color and taste development of cooked products (Fennema, Damodaran, & Parkin, 2017). In this case, some of the color changes detected in raw burgers due to the addition of gelled emulsions have not been noted after

Table 3

Lipid profile of raw and cooked beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion used as partial animal fat replacers.

% Fatty acids	Raw					Cooked				
	BC	BCh25	BCh50	BH25	BH50	BC	BCh25	BCh50	BH25	BH50
C10:0	0.05 ± 0.00 ^{aM}	0.05 ± 0.00 ^{aM}	0.04 ± 0.00 ^{bJ}	0.05 ± 0.00 ^{aH}	0.04 ± 0.00 ^{bK}	0.05 ± 0.00 ^{vJ}	0.05 ± 0.00 ^{vL}	0.04 ± 0.00 ^{wK}	0.05 ± 0.00 ^{vM}	0.04 ± 0.00 ^{wN}
C12:0	0.05 ± 0.00 ^{bM}	0.06 ± 0.00 ^{bM}	0.05 ± 0.00 ^{bJ}	0.05 ± 0.00 ^{bH}	0.05 ± 0.00 ^{bK}	0.06 ± 0.00 ^{vJ}	0.05 ± 0.00 ^{wL}	0.05 ± 0.00 ^{wK}	0.05 ± 0.00 ^{wM}	0.05 ± 0.00 ^{wN}
C14:0	1.16 ± 0.03 ^{aE}	1.09 ± 0.02 ^{bG}	0.93 ± 0.04 ^{aF}	1.02 ± 0.02 ^{cD}	0.96 ± 0.03 ^{dF}	1.17 ± 0.07 ^{vD}	1.09 ± 0.06 ^{wE}	1.03 ± 0.02 ^{xD}	1.09 ± 0.03 ^{wF}	1.03 ± 0.09 ^{xG}
C4:1 cis	0.05 ± 0.00 ^{bM}	0.05 ± 0.00 ^{bM}	0.03 ± 0.00 ^{cJ}	0.03 ± 0.00 ^{cH}	0.07 ± 0.00 ^{aJ}	0.05 ± 0.00 ^{wJ}	0.02 ± 0.00 ^{yH}	0.04 ± 0.00 ^{xK}	0.04 ± 0.00 ^{xM}	0.08 ± 0.00 ^{wM}
C15:0	0.09 ± 0.00 ^{cL}	0.10 ± 0.02 ^{bL}	0.08 ± 0.00 ^{dI}	0.08 ± 0.00 ^{dG}	0.13 ± 0.02 ^{aI}	0.10 ± 0.02 ^{xI}	0.11 ± 0.02 ^{wJ}	0.14 ± 0.02 ^{yI}	0.11 ± 0.02 ^{wL}	0.14 ± 0.02 ^{wK}
C15:1	0.08 ± 0.00 ^{cL}	0.10 ± 0.03 ^{bL}	0.02 ± 0.00 ^{dJ}	0.02 ± 0.00 ^{dH}	0.16 ± 0.07 ^{aI}	0.03 ± 0.00 ^{zK}	0.17 ± 0.02 ^{xI}	0.12 ± 0.05 ^{yI}	0.18 ± 0.02 ^{wK}	0.19 ± 0.01 ^{vK}
C16:0	21.86 ± 0.08 ^{aB}	20.68 ± 0.04 ^{bB}	18.83 ± 0.02 ^{dB}	19.95 ± 0.10 ^{cB}	17.47 ± 0.11 ^{eB}	21.86 ± 0.05 ^{wB}	20.50 ± 0.07 ^{wB}	19.04 ± 0.03 ^{xB}	20.39 ± 0.11 ^{wB}	18.46 ± 0.08 ^{yC}
C16:1 trans	0.48 ± 0.03 ^{aH}	0.43 ± 0.07 ^{bJ}	0.38 ± 0.02 ^{dH}	0.41 ± 0.05 ^{cE}	0.31 ± 0.02 ^{eH}	0.46 ± 0.00 ^{vG}	0.41 ± 0.00 ^{wGH}	0.35 ± 0.00 ^{yG}	0.40 ± 0.00 ^{xI}	0.32 ± 0.00 ^{zJ}
C16:1 cis	2.07 ± 0.02 ^{aD}	1.95 ± 0.04 ^{bF}	1.66 ± 0.09 ^{dE}	1.78 ± 0.12 ^{cD}	1.51 ± 0.07 ^{eE}	2.04 ± 0.02 ^{vD}	1.80 ± 0.02 ^{xE}	1.67 ± 0.02 ^{yD}	1.89 ± 0.02 ^{wF}	1.63 ± 0.02 ^{zF}
C17:0	0.39 ± 0.02 ^{abI}	0.37 ± 0.01 ^{abJ}	0.32 ± 0.01 ^{bH}	0.34 ± 0.02 ^{abF}	0.40 ± 0.01 ^{aG}	0.41 ± 0.00 ^x	0.40 ± 0.00 ^{yG}	0.43 ± 0.00 ^{yF}	0.40 ± 0.00 ^{yI}	0.42 ± 0.00 ^{wI}
C17:1	0.35 ± 0.01 ^{aI}	0.34 ± 0.01 ^{bJ}	0.28 ± 0.01 ^{dH}	0.31 ± 0.01 ^{cF}	0.28 ± 0.01 ^{dH}	0.35 ± 0.01 ^{vH}	0.32 ± 0.01 ^{xH}	0.32 ± 0.01 ^{xG}	0.33 ± 0.01 ^{wJ}	0.30 ± 0.01 ^{yJ}
C18:0	12.44 ± 0.02 ^{aC}	11.36 ± 0.06 ^{bD}	10.22 ± 0.01 ^{dD}	10.55 ± 0.02 ^{cC}	10.25 ± 0.06 ^{dC}	12.12 ± 0.00 ^{vC}	12.04 ± 0.00 ^{wC}	11.30 ± 0.00 ^{xC}	11.49 ± 0.00 ^{wD}	10.92 ± 0.00 ^{wD}
C18:1cis	43.15 ± 0.09 ^{aA}	42.89 ± 0.08 ^{bA}	38.40 ± 0.07 ^{dA}	42.89 ± 0.10 ^{cA}	32.55 ± 0.11 ^{eA}	45.22 ± 0.02 ^{vA}	40.07 ± 0.02 ^{xA}	37.15 ± 0.01 ^{yA}	41.97 ± 0.01 ^{WA}	35.82 ± 0.01 ^{ZA}
C18:2 (n-6)	12.59 ± 0.02 ^{dC}	12.63 ± 0.04 ^{dC}	13.60 ± 0.02 ^{cC}	17.39 ± 0.06 ^{bB}	23.72 ± 0.08 ^{aB}	12.15 ± 0.01 ^{zC}	12.51 ± 0.02 ^{vyC}	12.94 ± 0.09 ^{xC}	15.68 ± 0.12 ^{wC}	21.32 ± 0.02 ^{zB}
C18:2 (n-3)	0.07 ± 0.00 ^{cM}	0.07 ± 0.00 ^{cM}	0.06 ± 0.00 ^{cJ}	0.55 ± 0.01 ^{bE}	1.26 ± 0.02 ^{aE}	0.07 ± 0.00 ^{xJ}	0.07 ± 0.00 ^{xK}	0.08 ± 0.00 ^{xK}	0.41 ± 0.02 ^{wI}	1.06 ± 0.02 ^{vG}
C18:3 (n-3)	0.67 ± 0.02 ^{eG}	3.89 ± 0.02 ^{eE}	8.62 ± 0.02 ^{aE}	2.83 ± 0.02 ^{dD}	5.92 ± 0.02 ^{bD}	0.70 ± 0.02 ^{zE}	5.67 ± 0.03 ^{wD}	12.79 ± 0.04 ^{vC}	2.36 ± 0.02 ^{yE}	5.08 ± 0.03 ^{zE}
C18:3 (n-6)	0.11 ± 0.00 ^{dK}	0.13 ± 0.01 ^{cL}	0.09 ± 0.00 ^{eI}	0.17 ± 0.01 ^{bF}	0.43 ± 0.01 ^{aG}	0.13 ± 0.00 ^{wI}	0.14 ± 0.00 ^{vi}	0.14 ± 0.00 ^{vi}	0.13 ± 0.00 ^w	0.14 ± 0.00 ^{wK}
C20:0	0.21 ± 0.00 ^{eJ}	0.22 ± 0.00 ^{dK}	0.23 ± 0.00 ^{eH}	0.31 ± 0.00 ^{bF}	0.45 ± 0.01 ^{aG}	0.23 ± 0.02 ^{yH}	0.24 ± 0.02 ^{yH}	0.24 ± 0.02 ^{xH}	0.29 ± 0.02 ^{wJ}	0.40 ± 0.02 ^{vi}
C20:1	0.96 ± 0.01 ^{bF}	0.96 ± 0.01 ^{bH}	0.89 ± 0.01 ^{eE}	0.99 ± 0.01 ^{aD}	0.72 ± 0.01 ^{dF}	1.05 ± 0.01 ^{vD}	0.85 ± 0.01 ^{wF}	0.67 ± 0.01 ^{yE}	0.87 ± 0.01 ^{wG}	0.77 ± 0.01 ^{hH}
C20:2 (n-11)	0.60 ± 0.01 ^{aG}	0.59 ± 0.01 ^{abI}	0.53 ± 0.01 ^{cG}	0.58 ± 0.01 ^{bE}	0.43 ± 0.01 ^{dG}	0.58 ± 0.01 ^{vF}	0.53 ± 0.01 ^{wG}	0.41 ± 0.01 ^{xF}	0.54 ± 0.01 ^{WH}	0.41 ± 0.01 ^{xi}
C20:3 (n-8)	0.12 ± 0.01 ^{aK}	0.14 ± 0.01 ^{bI}	0.10 ± 0.01 ^{aL}	0.11 ± 0.01 ^{bG}	0.13 ± 0.01 ^{aI}	0.13 ± 0.00 ^{xI}	0.15 ± 0.00 ^{vi}	0.13 ± 0.00 ^{xI}	0.15 ± 0.00 ^{vK}	0.14 ± 0.00 ^{wK}
C20:3 (n-11)	0.30 ± 0.01 ^{bI}	0.40 ± 0.01 ^{aJ}	0.29 ± 0.01 ^{cH}	0.29 ± 0.01 ^{cF}	0.40 ± 0.01 ^{aG}	0.39 ± 0.02 ^{vG}	0.48 ± 0.02 ^{vG}	0.41 ± 0.02 ^{xF}	0.48 ± 0.02 ^{vH}	0.46 ± 0.02 ^{vi}
C20:4	0.09 ± 0.01 ^{abL}	0.10 ± 0.01 ^{aL}	0.09 ± 0.01 ^{abI}	0.09 ± 0.00 ^{bG}	0.08 ± 0.00 ^{cJ}	0.09 ± 0.00 ^{vi}	0.09 ± 0.00 ^{vi}	0.06 ± 0.00 ^{yJ}	0.08 ± 0.00 ^{wL}	0.07 ± 0.00 ^{zM}
C20:5	0.01 ± 0.00 ^{eN}	0.02 ± 0.00 ^{dN}	0.03 ± 0.00 ^{cJ}	0.06 ± 0.00 ^{bG}	0.14 ± 0.02 ^{aI}	0.02 ± 0.00 ^{yK}	0.03 ± 0.00 ^{xL}	0.06 ± 0.00 ^{wJ}	0.06 ± 0.00 ^{wM}	0.11 ± 0.00 ^{wL}
C23:0	0.15 ± 0.02 ^{aK}	0.10 ± 0.02 ^{bL}	0.08 ± 0.00 ^{cI}	0.08 ± 0.00 ^{cG}	0.07 ± 0.00 ^{dJ}	0.10 ± 0.00 ^{wI}	0.11 ± 0.00 ^{vJ}	0.08 ± 0.00 ^{xJ}	0.10 ± 0.00 ^{wL}	0.08 ± 0.00 ^{zM}
C 24:0	0.10 ± 0.02 ^{aKL}	0.09 ± 0.02 ^{bL}	0.07 ± 0.00 ^{dI}	0.07 ± 0.00 ^{dG}	0.08 ± 0.00 ^{cJ}	0.09 ± 0.01 ^{xI}	0.11 ± 0.00 ^{wJ}	0.12 ± 0.00 ^{vi}	0.11 ± 0.00 ^{wL}	0.11 ± 0.00 ^{wL}
∑SFA	35.89 ± 0.13 ^a	33.19 ± 0.07 ^b	30.85 ± 0.03 ^d	32.50 ± 0.02 ^c	29.90 ± 0.02 ^e	36.20 ± 0.03 ^v	34.71 ± 0.05 ^w	32.46 ± 0.03 ^y	34.09 ± 0.01 ^x	31.65 ± 0.01 ^z
∑MUFA	49.51 ± 0.17 ^a	46.84 ± 0.05 ^b	41.73 ± 0.07 ^d	45.44 ± 0.04 ^c	37.59 ± 0.06 ^e	49.31 ± 0.10 ^x	45.38 ± 0.04 ^x	40.44 ± 0.02 ^y	45.80 ± 0.08 ^w	39.23 ± 0.07 ^z
∑PUFA	14.56 ± 0.17 ^e	17.96 ± 0.06 ^d	27.41 ± 0.08 ^b	22.07 ± 0.03 ^c	32.52 ± 0.06 ^a	14.28 ± 0.01 ^y	19.67 ± 0.16 ^x	27.03 ± 0.08 ^w	19.89 ± 0.13 ^x	28.80 ± 0.06 ^v
∑n3	0.74 ± 0.04 ^e	3.96 ± 0.03 ^c	12.68 ± 0.06 ^a	3.39 ± 0.02 ^d	7.18 ± 0.03 ^b	0.77 ± 0.04 ^z	5.75 ± 0.05 ^x	12.87 ± 0.08 ^y	2.78 ± 0.02 ^y	6.14 ± 0.02 ^w
∑n6	12.69 ± 0.02 ^d	12.76 ± 0.05 ^d	13.69 ± 0.03 ^c	17.56 ± 0.02 ^b	24.16 ± 0.05 ^a	12.28 ± 0.02 ^y	12.65 ± 0.03 ^y	13.08 ± 0.04 ^x	15.81 ± 0.02 ^w	21.47 ± 0.06 ^v

Results are expressed as g/100g. Data are presented as mean ± standard deviation. BC: control burgers with a traditional formula; BCh25: sample with 25% animal fat replaced by GE with chia oil and amaranth flour; BCh50: sample with 50% animal fat replaced by GE with chia oil and amaranth flour. BH25: sample with 25% animal fat replaced by GE with hemp oil and amaranth flour as fat replacer. BH50: sample with 50% animal fat replaced by GE with hemp oil and amaranth flour. SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. For each parameter, results followed by same letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). A lower-case letters refers to the comparison of the same fatty acid or parameters between the different raw samples (a-e) and for cooked samples (v-z), while an upper-case letter (A-N) refers to the comparison of the different fatty acids in the same sample.

Table 4

Health indices cooked beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion used as partial animal fat replacers.

Sample	\sum PUFA/ \sum SFA	n6/n3	AI	TI	h/H
BC	0.39 ± 0.02 ^x	15.89 ± 0.04 ^y	0.43 ± 0.01 ^v	1.06 ± 0.03 ^v	2.59 ± 0.02 ^y
BCh25	0.57 ± 0.02 ^w	2.20 ± 0.02 ^x	0.39 ± 0.01 ^w	0.72 ± 0.03 ^x	2.85 ± 0.02 ^x
BCh50	0.83 ± 0.04 ^v	1.02 ± 0.02 ^v	0.35 ± 0.01 ^x	0.47 ± 0.02 ^z	3.20 ± 0.01 ^w
BH25	0.58 ± 0.02 ^w	5.69 ± 0.02 ^w	0.38 ± 0.01 ^w	0.84 ± 0.03 ^w	2.89 ± 0.02 ^x
BH50	0.91 ± 0.05 ^v	3.50 ± 0.04 ^v	0.34 ± 0.01 ^y	0.62 ± 0.02 ^y	3.32 ± 0.01 ^v

For each parameter, results followed by same letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data were presented as mean ± standard deviation.

BC: control burgers with a traditional formula; BCh25: sample with 25% animal fat replaced by GE with chia oil and amaranth flour; BCh50: sample with 50% animal fat replaced by GE with chia oil and amaranth flour. BH25: sample with 25% animal fat replaced by GE with hemp oil and amaranth flour as fat replacer. BH50: sample with 50% animal fat replaced by GE with hemp oil and amaranth flour. AI: atherogenic index; TI: thrombogenic index; h/H: hypocholesterolemic/hypercholesterolemic index. A lower-case letter refers to the comparison of the same parameter between the different cooked samples (v-z).

cooking. It could be said that cooking has masked these changes, resulting in similar values for all burgers in all color parameters. These results were in agreement than those reported by Lucas-González et al. (2020) and Summo, De Angelis, Difonzo, Caponio, and Pasqualone (2020) who observed that the color differences were higher in raw burger than in cooked burgers where the fat was partially replaced by gelled emulsions. It is important to notice that, after cooking, all values for color differences (ΔE^*) were lower than 3 units and so they could not be detected by the human eye (Martínez et al., 2001).

The texture properties of cooked burgers were shown in Table 6. There were no significant differences ($p > 0.05$) for hardness, springiness, and chewiness between all samples analyzed. Cohesiveness was the only parameter that significantly varied between samples ($p < 0.05$). Cohesiveness differences were mainly influenced by the fat replacement level (25% or 50%) and not by the type of GE used; the higher the fat replacement level, the lower cohesiveness values. However, it must be noted that burgers with the highest fat substitution levels (BCh50 and BH50) showed cohesiveness values similar to control burgers ($p > 0.05$). This trend could indicate that if these GE were used at higher fat substitution levels, burgers cohesiveness will be expected to be significantly reduced. In the scientific literature, contradictory results have been

Table 5

Physico-chemical parameters of raw and cooked beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion used as partial animal fat replacers.

	Sample	pH	Aw	L*	a*	b*	C*	h	ΔE
Raw	BC	6.20 ± 0.03 ^a	0.887 ± 0.01 ^a	47.38 ± 4.29 ^a	4.06 ± 1.40 ^b	7.76 ± 1.87 ^a	8.90 ± 1.75 ^b	61.85 ± 9.27 ^a	–
	BCh25	6.15 ± 0.03 ^a	0.889 ± 0.01 ^a	47.53 ± 2.73 ^a	5.72 ± 1.23 ^a	9.08 ± 1.87 ^a	10.81 ± 1.81 ^a	57.45 ± 6.72 ^a	3.60 ± 2.02
	BCh50	6.18 ± 0.03 ^a	0.889 ± 0.01 ^a	47.06 ± 3.86 ^a	5.10 ± 1.27 ^{ab}	8.69 ± 1.41 ^a	10.17 ± 1.32 ^{ab}	59.43 ± 7.41 ^a	4.01 ± 2.11
	BH25	6.16 ± 0.03 ^a	0.888 ± 0.00 ^a	46.55 ± 1.91 ^a	5.17 ± 1.14 ^{ab}	8.95 ± 1.21 ^a	10.36 ± 1.53 ^{ab}	60.30 ± 3.84 ^a	2.95 ± 1.02
	BH50	6.17 ± 0.03 ^a	0.889 ± 0.00 ^a	47.83 ± 2.36 ^a	4.36 ± 0.73 ^{ab}	9.41 ± 1.40 ^a	10.41 ± 1.31 ^{ab}	64.87 ± 4.64 ^a	2.79 ± 1.82
Cooked	BC	6.38 ± 0.02 ^v	–	44.17 ± 1.78 ^v	3.96 ± 0.52 ^v	9.06 ± 0.98 ^v	10.01 ± 0.98 ^v	65.82 ± 2.9 ^v	–
	BCh25	6.33 ± 0.02 ^v	–	43.02 ± 3.13 ^v	4.19 ± 1.06 ^v	8.64 ± 1.26 ^v	9.90 ± 1.53 ^v	65.11 ± 3.42 ^{vw}	2.98 ± 1.84 ^v
	BCh50	6.34 ± 0.01 ^v	–	44.67 ± 2.20 ^v	4.11 ± 0.59 ^v	8.22 ± 0.77 ^v	9.20 ± 0.85 ^v	66.47 ± 2.83 ^{vwx}	2.44 ± 1.13 ^v
	BH25	6.28 ± 0.02 ^w	–	43.44 ± 2.40 ^v	4.34 ± 0.66 ^v	8.59 ± 0.70 ^v	9.33 ± 0.76 ^v	64.57 ± 3.97 ^x	2.92 ± 1.73 ^v
	BH50	6.27 ± 0.01 ^w	–	43.49 ± 3.07 ^v	4.33 ± 0.88 ^v	8.66 ± 2.25 ^v	9.38 ± 2.20 ^v	61.21 ± 6.28 ^{wx}	2.83 ± 2.23 ^v

For each parameter, results followed by same letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as mean ± standard deviation.

BC: control burgers with a traditional formula; BCh25: sample with 25% animal fat replaced by GE with chia oil and amaranth flour; BCh50: sample with 50% animal fat replaced by GE with chia oil and amaranth flour. BH25: sample with 25% animal fat replaced by GE with hemp oil and amaranth flour as fat replacer. BH50: sample with 50% animal fat replaced by GE with hemp oil and amaranth flour. A lower-case letter refers to the comparison of the same parameter between the different raw samples (a-e) and for cooked samples (v-z).

reported on textural properties, depending on the concentration and types of emulsions used as fat replacers in burgers (Afshari, Hosseini, Khaneghah & Khaksar, 2017; Barros et al., 2021; Cittadini et al., 2021; Heck et al., 2019; Lucas-González et al., 2020). For example, in the study carried out by Cittadini et al. (2021) where 100% of pork fat was replaced by two hydrogels (avocado-algal oil mixed and pumpkin seed-algal oil mixed) in foal burgers, no differences ($p > 0.05$) in hardness or springiness were found compared to control burgers, but cohesiveness and chewiness were significantly reduced ($p < 0.05$). On the contrary, Afshari et al. (2017) reported that the use of an emulsion (canola/olive oil, soy protein, inulin and β -glucan) to replace the animal fat in burgers significantly reduced hardness of samples in comparison with control ones. On the other hand, Alejandre, et al. (2019), de Souza-de Souza Paglarini et al. (2019), Barros et al. (2020) and Vargas-Ramella et al. (2020) informed that there were no differences in textural properties of reformulated meat products with oil emulsions used as fat replacers. These differences could be attributed to the different physicochemical characteristics between animal fat and gelled emulsions and their interaction with meat.

3.4. Cooking characteristics

Cooking loss, shrinkage and increase in thickness of beef burgers

Table 6

Texture profile (TPA) of cooked beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion used as partial animal fat replacers.

Sample	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N x mm)
BC	13.00 ± 4.55 ^a	0.23 ± 0.08 ^a	0.59 ± 0.15 ^{ab}	1.69 ± 0.77 ^a
BCh25	11.45 ± 4.23 ^a	0.22 ± 0.02 ^a	0.65 ± 0.11 ^a	1.39 ± 0.91 ^a
BCh50	9.40 ± 4.34 ^a	0.18 ± 0.04 ^a	0.40 ± 0.03 ^b	1.15 ± 0.79 ^a
BH25	8.71 ± 2.33 ^a	0.21 ± 0.03 ^a	0.65 ± 0.10 ^a	1.16 ± 0.37 ^a
BH50	8.36 ± 3.25 ^a	0.26 ± 0.04 ^a	0.47 ± 0.06 ^{ab}	1.02 ± 0.65 ^a

For each parameter, results followed by same letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$).

Data are presented as mean ± standard deviation.

BC: control burgers with a traditional formula; BCh25: sample with 25% animal fat replaced by GE with chia oil and amaranth flour; BCh50: sample with 50% animal fat replaced by GE with chia oil and amaranth flour. BH25: sample with 25% animal fat replaced by GE with hemp oil and amaranth flour as fat replacer. BH50: sample with 50% animal fat replaced by GE with hemp oil and amaranth flour.

(control and reformulated) were shown in Table 7. The use of gelled emulsions affected all cooking properties ($p < 0.05$) in different ways. In general, the burgers reformulation with GEs increased cooking loss, shrinkage and thickness increase ($p < 0.05$), being this effect higher when GE elaborated with hemp oil were used. Burgers with hemp oil (BH25 and BH50) showed the highest cooking loss, shrinkage and thickness increase, without differences ($p > 0.05$), between the levels of replacement applied. The cooking process leads to water evaporation and lipid migration in samples, and the intensity of these changes affects product acceptance (Fernández-López et al., 2019; Lucas-González et al., 2020). In the scientific literature, there is not a clear trend in the behavior of this parameter in reformulated burgers with vegetable oils added with gelled emulsions: increase (Dias et al., 2021), decrease (Heck et al., 2017; Lucas-González et al., 2020) and not modifications (Barros et al., 2021; Heck et al., 2019). In most of the cases, these modifications although significant compared to control burgers does not seem to be quantitatively very important (2–10%). These variations could be attributed to the specific behavior of the ingredients used for the GE preparation (type and percentage of oil, flour, emulsion agent, and gelling agent), their stability, and their interrelation with the meat matrix. Regarding that, the higher cooking loss found in burgers with GE with hemp oil (BH25 and BH50) compared to burgers with GE with chia oil (BCh25 and BCh50) could be related to the lower emulsion stability and firmness reported for these GE with hemp oil (Botella-Martínez, Pérez-Álvarez, Sayas-Barberá, Fernández-López, & Viuda-Martos, 2021) which would allow lower water and oil retention capacity into the emulsion structure.

Reformulated burgers with the highest cooking loss (BH25 and BH50) showed also the highest shrinkage ($p < 0.05$) and the reasons would seem to be the same as reported for cooking loss. Cooking shrinkage has been mainly attributed to meat protein denaturation, giving off water and fat from meat batter (Pathare & Roskilly, 2016). It has been reported that the most important physical change occurs during meat product grilling (Tabarestani & Tehrani, 2014).

3.5. Lipid oxidation of beef burgers (TBARS)

Lipid oxidation is the main process responsible for the quality deterioration of meat and meat products. This process affects color, texture, nutritional value, taste, and aroma leading to rancidity, which are important reasons for consumer rejection (Lima, Rangel, Urbano, Mitzi, & Moreno, 2013).

In order to monitor the effects of reformulation and heating treatment on the lipid oxidation of beef burgers, lipid oxidation was measured in all samples, before and after cooking (Fig. 1). Significant differences ($p < 0.05$) were obtained with the addition of GEs, in both

Table 7

Cooking properties of cooked beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion used as partial animal fat replacers.

Sample	Cooking loss (%)	Shrinkage (%)	Thickness increase (%)
BC	19.34 ± 0.30 ^c	19.55 ± 0.96 ^c	8.13 ± 0.53 ^c
BCh25	21.63 ± 0.47 ^{bc}	21.64 ± 1.78 ^b	12.92 ± 0.42 ^b
BCh50	24.26 ± 0.56 ^{ab}	21.41 ± 0.45 ^b	11.07 ± 0.90 ^b
BH25	27.13 ± 0.32 ^a	25.75 ± 1.81 ^a	13.02 ± 0.20 ^a
BH50	25.09 ± 0.95 ^a	24.19 ± 1.67 ^a	13.81 ± 0.69 ^a

For each parameter, results followed by same letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as mean ± standard deviation.

BC: control burgers with a traditional formula; BCh25: sample with 25% animal fat replaced by GE with chia oil and amaranth flour; BCh50: sample with 50% animal fat replaced by GE with chia oil and amaranth flour. BH25: sample with 25% animal fat replaced by GE with hemp oil and amaranth flour as fat replacer. BH50: sample with 50% animal fat replaced by GE with hemp oil and amaranth flour.

raw and cooked samples. In raw samples, burgers reformulated with chia oil (BCh25 and BCh50) registered higher TBARS values ($p < 0.05$) than the control sample, being burgers with the highest replacement level (BCh50) which showed the highest ($p < 0.05$) TBARS values (1.13 mg MDA/kg of sample). In fact, BCh50 samples showed 3.5 times more oxidation than the control sample. Burgers reformulated with GE with hemp oil (BH25 and BH50) showed similar TBARS values (0.42 and 0.47 mg MDA/kg of sample, respectively) ($p > 0.05$) than control (0.32 mg MDA/kg of sample).

The TBARS values of cooked samples was higher ($p < 0.05$) in burgers with GCh (52% and 58% for BCh25 and BCh50, respectively) than GH (21% and 31% for BH25 and BH50, respectively). This fact was in concordance with several authors who reported that the use of GE elaborated with vegetable oils as animal fat replacement in meat products might be complex due to the high oxidation susceptibility of these unsaturated oils (Lucas-González et al., 2020; Moghtadaei, Soltanizadeh, Goli, & Sharifimehr, 2021). The differences in the lipid profile of the oils, the content of polyunsaturated fatty acids, and the temperature used to generate oleogels or gelled emulsions could affect the MDA levels (Gómez-Estaca et al., 2019).

It must be noticed that TBARS values in burgers reformulated with GE elaborated with amaranth flour and hemp oil (both raw and cooked) as well as the burgers reformulated (both raw and cooked) with GE elaborated chia oil (25%) were below the malonaldehyde limit for acceptability reported by Trindade, Mancini-Filho, and Villavicencio (2009) (2 mg MDA/kg) for loss of sensory attributes and perception of oxidation by consumers. However, it is important to highlight that cooked burgers reformulated with GE elaborated with amaranth flour and chia oil showed values above the threshold limit for consumer acceptability.

3.6. Sensorial analysis

The influence of the addition of GEs on sensory attributes of raw beef burgers is shown in Table 8. Relevant parameters affecting consumer purchase were measured, such as "color", "rancid aroma" and "product appearance" (mainly influenced by the product's optical properties, its physical form and its mode of presentation). Panelists did not detect differences between control and reformulated burgers ($p > 0.05$) for any of the three evaluated parameters. This result agreed with the instrumental color parameters, where L^* , b^* and h^* values had no differences ($p > 0.05$) between samples and the rest of color parameters (a^* and C^*) showed small differences which were statistically significant but without practical significance (< 3 units).

In the case of cooked samples, juiciness, chewiness, fat sensation, graininess and general acceptability were evaluated (Fig. 2). The only attribute that showed differences ($p < 0.05$) between samples was graininess: BCh25 and BH25 showed the highest ($p < 0.05$) score (6.60 and 6.50, respectively) without statistical differences between them ($p > 0.05$), while control sample had the lowest (5.00). These results agreed with the instrumental analysis since textural analysis revealed only differences in cohesiveness between some samples. For the preference test, control sample (6.70) and BH50 (5.90) were the most chosen. It has to be mentioned that the information about the nutritional improvement (healthier lipid profile) achieved in reformulated burgers was not communicated to panellists and that could be relevant and affect their sensory attractiveness (Siegrist, 2008).

4. Conclusions

This study suggests that the reformulation of beef burgers using gelled emulsion (based on amaranth-chia oil or amaranth-hemp oil) as a partial (up to 50%) pork back-fat substitute is feasible and can be seen as a viable alternative for improving nutritional composition without adversely affecting either the physicochemical properties (color, pH and texture) or the typical appearance of the resulting burgers. A reduction

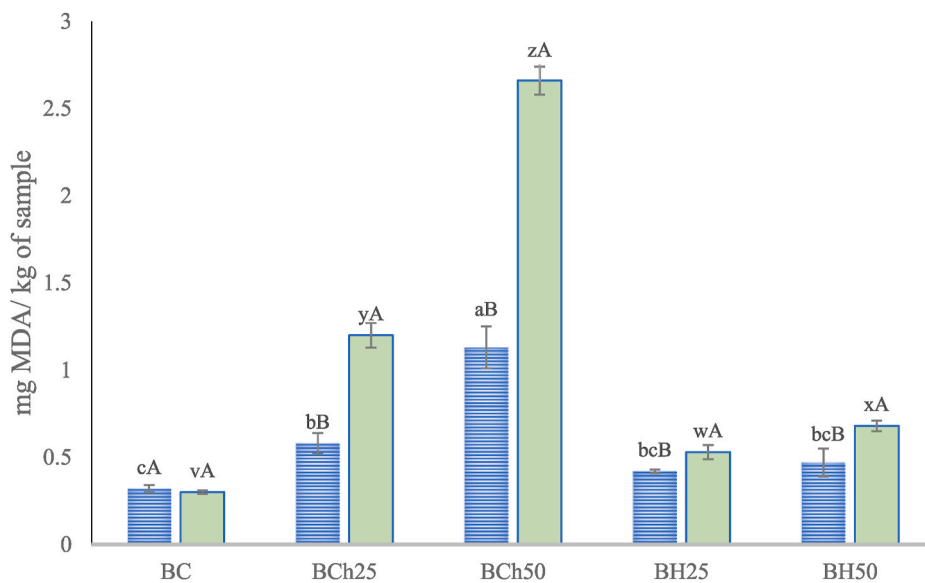


Fig. 1. Lipid oxidation (TBARS values) of raw and cooked beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion used as partial animal fat replacers.

For each parameter, results followed by same letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as mean \pm standard deviation. A lower-case letter refers to the comparison of the same treatment between the different samples (a–e) for raw samples and (v–z) for cooked samples, while an upper-case letter (A–B) refers to the comparison of the different TBARS values in the same sample depending on treatment (raw or cooked). BC: control burgers with a traditional formula; BCh25: sample with 25% animal fat replaced by GE with chia oil and amaranth flour; BCh50: sample with 50% animal fat replaced by GE with chia oil and amaranth flour. BH25: sample with 25% animal fat replaced by GE with hemp oil and amaranth flour as fat replacer. BH50: sample with 50% animal fat replaced by GE with hemp oil and amaranth flour. Blue histogram is for raw beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion. Green histogram is for cooked beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion. . (For

interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 8

Sensory analysis of raw cooked beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion used as partial animal fat replacers.

Sample	Color	Rancid aroma	Product appearance
BC	5.62 \pm 1.32 ^a	4.94 \pm 2.30 ^a	3.66 \pm 2.31 ^a
BCh25	6.44 \pm 1.61 ^a	4.95 \pm 2.40 ^a	4.27 \pm 1.72 ^a
BCh50	5.83 \pm 0.73 ^a	5.36 \pm 2.01 ^a	3.74 \pm 2.41 ^a
BH25	5.11 \pm 1.61 ^a	3.93 \pm 1.62 ^a	3.75 \pm 2.32 ^a
BH50	6.55 \pm 1.02 ^a	4.41 \pm 2.30 ^a	4.57 \pm 2.61 ^a

For each parameter, results followed by same letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as mean \pm standard deviation.

BC: control burgers with a traditional formula; BCh25: sample with 25% animal fat replaced by GE with chia oil and amaranth flour; BCh50: sample with 50% animal fat replaced by GE with chia oil and amaranth flour. BH25: sample with 25% animal fat replaced by GE with hemp oil and amaranth flour as fat replacer. BH50: sample with 50% animal fat replaced by GE with hemp oil and amaranth flour.

of 12–33% of total fat was achieved with an improved lipid profile (lower saturated fatty acids and higher polyunsaturated fatty acids than control). Burgers with amaranth-hemp gelled emulsion were especially rich in linolenic fatty acid while burgers with amaranth-chia gelled emulsion was in α -linolenic fatty acid. These last burgers (with amaranth-chia gelled emulsion) were more susceptible to lipid oxidation than control and amaranth-hemp gelled emulsions (despite the use of hemp oil which was expected more susceptible to oxidation). Panelists did not detect differences in color, rancid aroma, or appearance in raw burgers but when they were cooked, control and burgers with amaranth-hemp gelled emulsions received the highest score.

CRedit authorship contribution statement

Carmen Botella-Martínez: Investigation, Writing – original draft. **Aarón Gea-Quesada:** Investigation. **Estrella Sayas-Barberá:** Formal analysis, Data curation. **José Ángel Pérez-Álvarez:** Data curation, Supervision. **Juana Fernández-López:** Formal analysis, Writing – review & editing. **Manuel Viuda-Martos:** Conceptualization, Writing – review

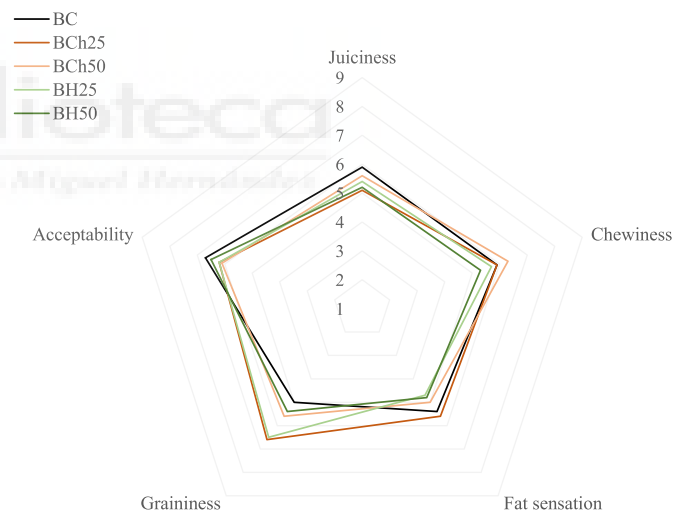


Fig. 2. Sensory analysis of cooked beef burgers reformulated with both amaranth/chia oil or amaranth/hemp oil gelled emulsion used as partial animal fat replacers.

BC: control burgers with a traditional formula; BCh25: sample with 25% animal fat replaced by GE with chia oil and amaranth flour; BCh50: sample with 50% animal fat replaced by GE with chia oil and amaranth flour. BH25: sample with 25% animal fat replaced by GE with hemp oil and amaranth flour as fat replacer. BH50: sample with 50% animal fat replaced by GE with hemp oil and amaranth flour.

& editing.

Declaration of competing interest

The authors declared that they have no conflicts of interest to this work.

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8.4. PUBLICACIÓN 4

Chia and hemp oils-based gelled emulsions as replacers of pork backfat in burgers: effect on lipid profile, technological attributes and oxidation stability during frozen storage

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Summary Gelled emulsions based on chia and hemp oils were used as partial (25% and 50%) fat replacer in beef burgers. The effect of formulation, frozen storage during 60 days and cooking process was assessed on lipid profile, oxidation susceptibility and technological attributes (cooking properties). Reformulated burgers showed better nutritional quality (in reference to dietary fats) than control, mainly due to the increase in PUFAs (specifically α -linolenic (C18:3) and linoleic (C18:2) fatty acids) and decrease in SFAs which was higher in burgers with hemp-GE than in burgers with chia-GE and also dependent on the substitution level (the highest at 50%). This pattern was not modified by frozen storage for 60 days or by cooking process. In addition, cooking increased the susceptibility of reformulated burgers to oxidation in a more intense way than 60 days of frozen storage, being burgers with 50% chia-GEs the most susceptible.

Keywords burgers, chia oil, fat replacer, gelled emulsion, hemp oil, lipid oxidation.

Introduction

Burgers are one of the most popular and commonly consumed meat products (GVR, 2020). Currently, the innovation in burger production is related to making them healthier, mainly due to concerns regarding their fat content and specifically to the high amount of saturated fatty acids. Both parameters have been associated with a high risk to develop some noncommunicable diseases such as obesity, hypertension, coronary heart and cardiovascular diseases (Chen & Liu, 2020; Badar *et al.*, 2021), and so international food safety agencies have made recommendations in view of decreasing or limit their consumption (FAO, 2010).

The reformulation of burgers to make them healthier, therefore, represents an important technological strategy. Solid fat (animal fat) is going to be substituted by vegetable oils (liquids, with low saturated fatty acids and high unsaturated ones) which usually have a great technological impact. In addition, these vegetable oils are more susceptible to lipid oxidation, and so prevention actions must be implemented to control it. To avoid this, new structuring methods have been developed to provide vegetable oils with a

similar solid structure to animal fats, but keeping stable their healthy lipid profile (Ospina-E *et al.*, 2010; Ospina-E *et al.*, 2015; da Silva *et al.*, 2019; Guo *et al.*, 2020; Badar *et al.*, 2021; Botella-Martínez *et al.*, 2021a). Among these strategies, gelled emulsions (GE) show a great potential as animal fat substitution in meat products in order to make them healthier (Herrero *et al.*, 2017; de Souza-Paglarini *et al.*, 2019; Lucas-González *et al.*, 2020; Nacak *et al.*, 2021; Botella-Martínez *et al.*, 2021b, 2021c, 2022).

Hemp (*Cannabis sativa* L.) and chia (*Salvia hispanica* L.) oils are interesting for this substitution in view not only of their lipid profile (high PUFA/SFA ratio, with a high amount of essential fatty acids such as α -linolenic and linoleic acids) (Ixtaina *et al.*, 2011; Zajac *et al.*, 2019) but also their content in antioxidant compounds (mainly phenolic compounds but also tocopherols and phytosterol in hemp) which could protect them to prone oxidation (Bodoira *et al.*, 2017; Leonard *et al.*, 2020). This protection against lipid oxidation have been reported in several meat products added with vegetable oils-GE in which rancidity was not sensorial detected although showed lipid oxidation values slightly higher than control products (Poyato *et al.*, 2015; Lucas-González *et al.*, 2020; Botella-

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Martínez *et al.*, 2021c). Although chia oil has been studied for its application in the development of GE for meat products, mainly emulsified meat products, like Frankfurt-type sausages and burgers, with interesting nutritional improvement (fat reduction and healthier lipid profile) and sensorial acceptance (Heck *et al.*, 2019; Pintado *et al.*, 2019; Lucas-González *et al.*, 2020), scarce studies about the use of hemp oil for GE preparation have been found and all of them are from our research group (Botella-Martínez *et al.*, 2021a, 2021b, 2022), demonstrating its feasibility, technological suitability and nutritional quality to be used as a fat replacement in the development of healthier foods.

One of the most common ways to commercialise beef burgers is as frozen burgers, which accounted for more than 68% of the global packaged burgers market (Orehov, 2019). Frozen hamburgers must be formulated taking care that their great susceptibility to physical destabilisation during freezing, especially referring to GE, does not affect their long shelf life (Degner *et al.*, 2014), in addition to the highest risk to develop lipid oxidation reactions.

In view of these findings, the objective of this article is to evaluate if the partial replacement of pork backfat by chia and hemp oil-based GE in beef burgers could affect burgers' stability (lipid profile, cooking properties and oxidation stability) during frozen storage (60 days).

Materials and methods

Preparation of gelled emulsions

Chia and hemp gelled emulsions were elaborated with 47 g of water/100 g of GE, 40 g of oil (chia oil or hemp oil in each case)/100 g of GE, 10 g of amaranth flour/100 g of GE and 3 g of gelling agent/100 g of GE, following the elaboration process described by Botella-Martínez *et al.* (2021a). The gelling agent was a mix of gellan gum and instant gel (pork gelatin with 180 bloom).

Beef burgers manufacture

Five batches of beef burgers were processed by triplicate (at different days) at the Food Pilot Plant of EPSO-Miguel Hernández University. Batch 1 (control) was a control model system with beef lean meat (80 g/100 g) and pork backfat (20 g/100 g); the rest of ingredients are referred to 100% meat batter: 5% of cold water, 1.5% salt and 0.05% white pepper. Batches 2 and 3 were made replacing 25% and 50% of pork backfat with the GE made with chia oil (batches Chia25 and Chia50, respectively). Batches 4 and 5 were

obtained replacing the same levels of pork backfat (25% and 50%) with the hemp oil gelled emulsion (batches Hemp25 and Hemp50, respectively). Burgers (80 g approx.) were aerobically packaged in plastic bags (sterile) and stored in freezing at -18 ± 1 °C. Sampling was made at 0, 30 and 60 days of storage. Half of the burgers for each batch were randomly selected for cooking. For cooking, burgers were grid-dled (at 180 °C until reaching 72 °C in the inner). After that, samples were cooled to room temperature (20–25 °C) before analysis.

Beef burgers analysis

Lipid profile

For fatty acid analysis, total fat was previously extracted and methylated (AOAC, 2010) obtaining the corresponding fatty acids methylated (FAMES), which were separated and quantified using an HP 6890 gas chromatography (GC) (Agilent Technologies, Inc. Santa Clara, California, USA). Detailed working conditions have been reported by Botella-Martínez *et al.* (2021b). Standard fatty acids (Supelco 37 component FAME Mix, Bellefonte, USA) were used to identify individual fatty acids (comparing their retention times). Peak areas were calculated (GC ChemStation Software; Agilent Technologies), and results are expressed as g fatty acid/100 g of total fatty acids. Final values per sample were obtained as the average of 3 reads.

Nutritional indices (from lipid profile)

To assess the nutritional quality of lipids in burgers, the following indices were calculated only in cooked burgers (because is the form in which they are consumed): the atherogenic index (AI) and the thrombogenic index (TI) following the formula described by Ulbricht & Southgate (1991); the hypocholesterolaemic/hypercholesterolaemic ratio (h/H) following the procedure reported by Fernández *et al.* (2007); and the nutritional value (NV) applying the formula reported by Estévez *et al.* (2004).

Cooking properties

Weight (g), diameter and thickness (mm) of burgers before and after cooking were measured. From these measures, cooking loss (%), thickness increased (%) and shrinkage (%) were calculated (Botella-Martínez *et al.*, 2022).

Lipid oxidation

The 2-thiobarbituric acid reactive substances index (TBARs) was calculated to evaluate lipid oxidation in burgers (Rosmini *et al.*, 1996). Triplicate samples were analysed from each batch. Results were shown as mg malondialdehyde (MDA)/kg of sample.

Statistical analysis

All analyses were made in triplicate in raw and cooked burgers. Data were submitted for a two-way (batches and storage time) analysis of variance (ANOVA) and Tukey-*b post hoc* test when ANOVA showed a significant effect ($P < 0.05$). These analyses were performed using SPSS software (version 24.0, SPSS Inc., Chicago, Illinois, USA).

Results and discussion

General aspects

The feasibility of burgers development in which pork backfat has been replaced with hemp and chia oil-based GE (at 25% and 50%) has been previously assessed by our research group. These reformulated burgers showed lower fat content (12% fat reduction when these GE were used at 25% and 33% fat reduction when they were used at 50%) and higher moisture than control burgers. Although all reformulated raw burgers showed good sensorial acceptance, it was reduced in burgers with chia oil-GE when they were evaluated after cooking (Botella-Martínez *et al.*, 2022).

Fatty acid profile

The effect of fat replacement and frozen storage time on fatty acid profile of beef burgers (raw and cooked) is shown in Table 1. From the 26 fatty acids identified in the burgers, only those that represented a proportion greater than 0.5% of total fat content (in any treatment or storage time) are shown. However, for the calculation of the corresponding sums (Σ SFA, Σ MUFA, Σ PUFA, $\Sigma n3$ and $\Sigma n6$) and indices (AI, TI, h/h and NV), all the fatty acids identified were used. The use of GE significantly improved the lipid profile of burgers, which can be observed at all frozen storage times and in both, raw and cooked samples. In control burgers (raw and cooked and at all storage times), the main fatty acids fraction were MUFA (ranging between 49.31% and 49.90%), followed by SFA (35.85%–36.66%) and PUFA (13.93%–14.56%). Reformulation with GEs resulted in a fatty acid profile modified compared to control burgers, that is, a reduction in SFA and MUFA fractions as well as an increase in PUFA ($P < 0.05$). This trend can be observed in raw and cooked burgers at all storage times.

Regarding SFA fraction, it was reduced ($P < 0.05$) in reformulated burgers depending on the type of GE used (higher reduction in hemp-GE based burgers; 9%–17%) but also on the substitution level (higher reduction at the highest substitution level; 14%–17%). However, in all burgers (control and reformulated,

raw and cooked and at all storage times), the predominant saturated fatty acids were palmitic (C16:0), stearic (C18:0) and myristic (C14:0) fatty acids (from highest to lowest proportion). The MUFA fraction was the majority in all the burgers (control and reformulated, raw and cooked and at all storage times) being one of its fatty acids, the oleic acid (C18:1), the main MUFA in all of them ($P < 0.05$). The highest ($P < 0.05$) MUFA content was shown in control burgers (raw and cooked and at all storage times) and it was reduced in reformulated ones, although in this case the reduction depended on the level of substitution (25% or 50%) and not so much on the type of GE used (hemp-GE or chia-GE). The increase in PUFA content in reformulated burgers respect to control ones follows the same trend as SFA: It was higher in burgers with hemp-GE (Hemp25 and Hemp50) than in burgers with chia-GE (Chia25 and Chia50) and also dependent on the replacement level (higher at 50% than at 25%) ($P < 0.05$). In addition, it can be observed that this increase in PUFA content in reformulated burgers was mainly due to α -linolenic (C18:3) and linoleic (C18:2) fatty acids. The linoleic fatty acid (C18:2) was the most abundant PUFA in all burgers (control and reformulated, raw and cooked, at all storage times) except in Chia50 treatment in which the high increase in α -linolenic acid (C18:3) resulted in changes in its predominance. However, it should be highlighted that the amount of these 2 essential fatty acids (C18:2n-6 and C18:3n-3) in reformulated burgers ranged from 17% to 30% (depending on treatment) in comparison with those observed in control burgers (aprox. 14%).

These variations in the fatty acid profile of burgers are due to the specific fatty acid composition of the main fat source used (pork backfat or GE based on hemp or chia oils), considering that lean meat used was the same in all of them (lean beef meat). MUFA (47%) and SFA (35%) are the main fractions in pork backfat, being PUFA fraction (18%) the minority (Ospina-E *et al.*, 2010). On the contrary the main fraction in chia and hemp oil is PUFA (82%) being the α -linolenic acid (C18:3) the predominant in chia oil, while in hemp oil is the linoleic acid (C18:2) (Ixtaina *et al.*, 2011; Bodoira *et al.*, 2017; Zajac *et al.*, 2019; Leonard *et al.*, 2020; Botella-Martínez *et al.*, 2021a). The fact that the lipid profile in burgers is directly related to the FA composition of the fat source used has been widely reported by other authors in several reformulated meat products (Ospina-E *et al.*, 2015; Heck *et al.*, 2017; Da Silva *et al.*, 2019; De Carvalho *et al.*, 2020; Botella-Martínez *et al.*, 2021b).

In quantitative terms and in reference to individual fatty acids, it has been noted that from the 26 fatty acids identified in burgers, the content of 5 of them (oleic, palmitic, linoleic, stearic and palmitoleic fatty

Table 1 Lipid profile of beef burgers (5 formulations, raw and cooked) during frozen storage

% Fatty acids (n = 3)	Raw					Cooked				
	Control	Chia25	Chia50	Hemp25	Hemp50	Control	Chia25	Chia50	Hemp25	Hemp50
Storage day 0										
C 14:0	1.16 ± 0.03 ^{aE}	1.09 ± 0.02 ^{bG}	0.93 ± 0.04 ^{eG}	1.02 ± 0.02 ^{eG}	0.96 ± 0.03 ^{dG}	1.17 ± 0.07 ^{aE}	1.09 ± 0.06 ^{bF}	1.03 ± 0.02 ^{dE}	1.09 ± 0.03 ^{bG}	1.03 ± 0.09 ^{cG}
C16:0	21.86 ± 0.08 ^{aB}	20.68 ± 0.04 ^{bB}	18.83 ± 0.02 ^{bB}	19.95 ± 0.10 ^{eB}	17.47 ± 0.11 ^{eC}	21.86 ± 0.05 ^{aB}	20.50 ± 0.07 ^{bB}	19.04 ± 0.03 ^{eB}	20.39 ± 0.11 ^{bB}	18.46 ± 0.08 ^{cC}
C16:1	2.07 ± 0.02 ^{aD}	1.95 ± 0.04 ^{bF}	1.66 ± 0.09 ^{dF}	1.78 ± 0.12 ^{eF}	1.51 ± 0.07 ^{eF}	2.04 ± 0.02 ^{aD}	1.80 ± 0.02 ^{eE}	1.67 ± 0.02 ^{dD}	1.89 ± 0.02 ^{bF}	1.63 ± 0.02 ^{eF}
C 18:0	12.44 ± 0.09 ^{aA}	11.36 ± 0.06 ^{bD}	10.22 ± 0.01 ^{eE}	10.55 ± 0.10 ^{eA}	10.25 ± 0.06 ^{dD}	12.12 ± 0.00 ^{eC}	12.04 ± 0.00 ^{bC}	11.30 ± 0.00 ^{cC}	11.49 ± 0.01 ^{bA}	10.92 ± 0.00 ^{bD}
C 18:1	43.15 ± 0.09 ^{aA}	42.89 ± 0.08 ^{bA}	38.40 ± 0.07 ^{bA}	40.07 ± 0.10 ^{eA}	32.55 ± 0.11 ^{eA}	45.22 ± 0.02 ^{aA}	40.07 ± 0.02 ^{aA}	37.15 ± 0.01 ^{dA}	41.97 ± 0.01 ^{bA}	35.82 ± 0.01 ^{eA}
C 18:2 (n-6)	12.59 ± 0.02 ^{cC}	12.63 ± 0.04 ^{dC}	13.60 ± 0.02 ^{cC}	17.39 ± 0.06 ^{bC}	23.72 ± 0.08 ^{aB}	12.15 ± 0.01 ^{eC}	12.51 ± 0.02 ^{aC}	12.94 ± 0.09 ^{cC}	15.68 ± 0.12 ^{bC}	21.32 ± 0.02 ^{aB}
C 18:2 (n-3)	0.07 ± 0.00 ^{dI}	0.07 ± 0.00 ^{cJ}	0.06 ± 0.00 ^{cJ}	0.55 ± 0.01 ^{bH}	1.26 ± 0.02 ^{aF}	0.07 ± 0.00 ^{dH}	0.07 ± 0.00 ^{dH}	0.08 ± 0.00 ^{dH}	0.41 ± 0.02 ^{bI}	1.06 ± 0.02 ^{aG}
C 18:3 (n-3)	0.67 ± 0.02 ^{eG}	3.89 ± 0.02 ^{eE}	8.62 ± 0.02 ^{aD}	2.83 ± 0.02 ^{dE}	5.92 ± 0.02 ^{bE}	0.70 ± 0.02 ^{eF}	5.67 ± 0.03 ^{bD}	12.79 ± 0.04 ^{eC}	2.36 ± 0.02 ^{dE}	5.08 ± 0.03 ^{eE}
C 20:1	0.96 ± 0.01 ^{bF}	0.96 ± 0.01 ^{bG}	0.89 ± 0.01 ^{eG}	0.99 ± 0.01 ^{aG}	0.72 ± 0.01 ^{dH}	1.05 ± 0.01 ^{aE}	0.85 ± 0.01 ^{bF}	0.67 ± 0.01 ^{dF}	0.87 ± 0.01 ^{bG}	0.77 ± 0.01 ^{cH}
C20:2 (n-11)	0.60 ± 0.01 ^{aG}	0.59 ± 0.01 ^{aBH}	0.53 ± 0.01 ^{cH}	0.58 ± 0.01 ^{bH}	0.43 ± 0.01 ^{dI}	0.58 ± 0.01 ^{aF}	0.53 ± 0.01 ^{bG}	0.41 ± 0.01 ^{eG}	0.54 ± 0.01 ^{bH}	0.41 ± 0.01 ^{cI}
C20:3 (n-11)	0.30 ± 0.01 ^{bH}	0.40 ± 0.01 ^{aI}	0.29 ± 0.01 ^{dI}	0.29 ± 0.01 ^{dI}	0.40 ± 0.01 ^{aI}	0.39 ± 0.02 ^{dG}	0.48 ± 0.02 ^{aG}	0.41 ± 0.02 ^{cG}	0.48 ± 0.02 ^{aH}	0.46 ± 0.02 ^{bI}
ΣSFA	35.89 ± 0.13 ^a	33.19 ± 0.07 ^b	30.85 ± 0.03 ^d	32.50 ± 0.02 ^c	29.90 ± 0.02 ^e	36.20 ± 0.03 ^a	34.71 ± 0.05 ^b	32.46 ± 0.03 ^d	34.09 ± 0.01 ^c	31.65 ± 0.01 ^e
ΣMUFA	49.51 ± 0.17 ^a	46.84 ± 0.05 ^b	41.73 ± 0.07 ^d	45.44 ± 0.04 ^c	37.59 ± 0.06 ^e	49.31 ± 0.10 ^a	45.38 ± 0.04 ^b	40.44 ± 0.02 ^c	45.80 ± 0.08 ^b	39.23 ± 0.07 ^c
ΣPUFA	14.56 ± 0.17 ^e	17.96 ± 0.06 ^d	27.41 ± 0.08 ^b	22.07 ± 0.03 ^c	32.52 ± 0.06 ^a	14.28 ± 0.01 ^d	19.67 ± 0.16 ^d	27.03 ± 0.08 ^b	19.89 ± 0.13 ^c	28.80 ± 0.06 ^a
Σn3	0.74 ± 0.04 ^e	3.96 ± 0.03 ^c	12.68 ± 0.06 ^a	3.39 ± 0.02 ^d	7.18 ± 0.03 ^b	0.77 ± 0.04 ^e	5.75 ± 0.05 ^c	12.87 ± 0.08 ^a	2.78 ± 0.02 ^d	6.14 ± 0.02 ^b
Σn6	12.69 ± 0.02 ^d	12.76 ± 0.05 ^d	13.69 ± 0.03 ^c	17.56 ± 0.02 ^b	24.16 ± 0.05 ^a	12.28 ± 0.02 ^d	12.65 ± 0.03 ^d	13.08 ± 0.04 ^c	15.81 ± 0.02 ^b	21.47 ± 0.06 ^a
Storage day 30										
C 14:0	1.17 ± 0.02 ^{aE}	1.06 ± 0.03 ^{bG}	1.00 ± 0.03 ^{bF}	0.99 ± 0.02 ^{bF}	0.87 ± 0.04 ^{eG}	1.18 ± 0.07 ^{aE}	1.11 ± 0.06 ^{aE}	0.99 ± 0.02 ^{bE}	1.05 ± 0.03 ^{abG}	1.00 ± 0.09 ^{bG}
C16:0	21.95 ± 0.07 ^{aB}	21.32 ± 0.05 ^{aB}	19.84 ± 0.03 ^{bB}	19.67 ± 0.08 ^{bB}	17.84 ± 0.09 ^{cC}	22.14 ± 0.05 ^{aB}	20.61 ± 0.07 ^{bB}	19.02 ± 0.03 ^{eB}	19.90 ± 0.11 ^{eB}	18.44 ± 0.08 ^{cC}
C16:1	2.07 ± 0.02 ^{aD}	1.85 ± 0.04 ^{bF}	1.81 ± 0.06 ^{bE}	1.77 ± 0.10 ^{eE}	1.50 ± 0.04 ^{dF}	2.07 ± 0.02 ^{aD}	1.86 ± 0.02 ^{bE}	1.62 ± 0.02 ^{eE}	1.80 ± 0.02 ^{bF}	1.62 ± 0.02 ^{eF}
C 18:0	12.07 ± 0.04 ^{aC}	11.51 ± 0.05 ^{bD}	9.98 ± 0.02 ^{cD}	10.41 ± 0.02 ^{bC}	9.49 ± 0.04 ^{dD}	12.13 ± 0.00 ^{aC}	11.89 ± 0.00 ^{bC}	11.19 ± 0.00 ^{bD}	11.26 ± 0.00 ^{bD}	10.75 ± 0.00 ^{cD}
C 18:1	45.87 ± 0.05 ^{aA}	42.52 ± 0.06 ^{bA}	40.58 ± 0.06 ^{aA}	39.28 ± 0.09 ^{bA}	34.28 ± 0.10 ^{dA}	45.72 ± 0.02 ^{aA}	41.88 ± 0.02 ^{bA}	36.81 ± 0.01 ^{cA}	40.90 ± 0.01 ^{bA}	36.46 ± 0.01 ^{cA}
C 18:2 (n-6)	12.06 ± 0.03 ^{dC}	12.95 ± 0.05 ^{dC}	14.11 ± 0.03 ^{cC}	18.13 ± 0.05 ^{bB}	23.39 ± 0.07 ^{aB}	12.00 ± 0.01 ^{cC}	12.35 ± 0.02 ^{cC}	12.96 ± 0.09 ^{cC}	16.97 ± 0.12 ^{bC}	21.22 ± 0.02 ^{aB}
C 18:2 (n-3)	0.08 ± 0.00 ^{cH}	0.07 ± 0.00 ^{cJ}	0.06 ± 0.00 ^{cI}	0.60 ± 0.01 ^{bG}	1.16 ± 0.03 ^{aF}	0.07 ± 0.00 ^{cH}	0.07 ± 0.00 ^{cH}	0.01 ± 0.00 ^{cH}	0.56 ± 0.02 ^{bH}	1.03 ± 0.02 ^{aG}
C 18:3 (n-3)	0.62 ± 0.02 ^{dF}	3.00 ± 0.02 ^{eE}	8.93 ± 0.02 ^{aD}	3.06 ± 0.02 ^{dD}	5.58 ± 0.02 ^{bE}	0.60 ± 0.02 ^{dF}	5.74 ± 0.03 ^{bD}	13.50 ± 0.04 ^{eC}	3.03 ± 0.02 ^{eE}	4.94 ± 0.03 ^{bE}
C 20:1	0.94 ± 0.02 ^{eE}	1.00 ± 0.02 ^{aG}	0.87 ± 0.02 ^{bF}	0.88 ± 0.02 ^{bF}	0.81 ± 0.02 ^{bG}	0.93 ± 0.01 ^{eE}	0.82 ± 0.01 ^{bF}	0.65 ± 0.01 ^{dF}	0.86 ± 0.01 ^{bG}	0.74 ± 0.01 ^{cH}
C20:2 (n-11)	0.59 ± 0.01 ^{aF}	0.62 ± 0.01 ^{aH}	0.55 ± 0.01 ^{aG}	0.56 ± 0.01 ^{aG}	0.47 ± 0.01 ^{bH}	0.58 ± 0.01 ^{aF}	0.51 ± 0.01 ^{bG}	0.40 ± 0.01 ^{cG}	0.50 ± 0.01 ^{bH}	0.42 ± 0.01 ^{cI}
C20:3 (n-11)	0.31 ± 0.01 ^{bG}	0.30 ± 0.01 ^{bI}	0.27 ± 0.01 ^{bH}	0.35 ± 0.01 ^{aH}	0.28 ± 0.01 ^{bI}	0.31 ± 0.02 ^{bG}	0.45 ± 0.02 ^{aG}	0.36 ± 0.02 ^{bG}	0.43 ± 0.02 ^{aH}	0.42 ± 0.02 ^{aI}
ΣSFA	36.20 ± 0.13 ^a	34.80 ± 0.07 ^b	31.70 ± 0.03 ^d	32.06 ± 0.02 ^c	29.24 ± 0.02 ^e	36.41 ± 0.03 ^a	34.70 ± 0.05 ^b	32.25 ± 0.03 ^d	33.35 ± 0.01 ^c	31.42 ± 0.01 ^e
ΣMUFA	49.00 ± 0.17 ^a	47.92 ± 0.05 ^b	44.13 ± 0.07 ^c	44.51 ± 0.04 ^c	39.00 ± 0.06 ^e	49.82 ± 0.10 ^a	45.46 ± 0.04 ^b	40.04 ± 0.02 ^d	44.54 ± 0.08 ^c	39.78 ± 0.07 ^d
ΣPUFA	14.90 ± 0.17 ^e	17.25 ± 0.06 ^d	24.22 ± 0.08 ^b	23.19 ± 0.03 ^c	31.57 ± 0.06 ^a	13.93 ± 0.01 ^e	19.55 ± 0.16 ^d	27.62 ± 0.08 ^b	21.93 ± 0.13 ^c	28.67 ± 0.06 ^a
Σn3	0.70 ± 0.04 ^e	3.07 ± 0.03 ^c	8.99 ± 0.06 ^a	3.66 ± 0.02 ^d	6.73 ± 0.03 ^b	0.67 ± 0.04 ^e	5.81 ± 0.05 ^c	13.52 ± 0.08 ^a	3.58 ± 0.02 ^d	5.96 ± 0.02 ^b
Σn6	12.19 ± 0.02 ^d	13.04 ± 0.05 ^d	14.19 ± 0.03 ^c	18.33 ± 0.02 ^b	23.79 ± 0.05 ^a	12.12 ± 0.02 ^d	12.49 ± 0.03 ^d	13.10 ± 0.04 ^c	17.16 ± 0.02 ^b	21.56 ± 0.06 ^a
Storage day 60										
C 14:0	1.19 ± 0.03 ^{aF}	1.02 ± 0.02 ^{bG}	0.95 ± 0.04 ^{eF}	1.07 ± 0.04 ^{eG}	1.03 ± 0.02 ^{cG}	1.19 ± 0.03 ^{dE}	1.11 ± 0.06 ^{bF}	1.00 ± 0.02 ^{dF}	1.08 ± 0.03 ^{bG}	0.97 ± 0.09 ^{cG}
C16:0	22.06 ± 0.08 ^{aB}	20.46 ± 0.04 ^{bB}	18.50 ± 0.02 ^{bB}	20.38 ± 0.02 ^{bB}	20.21 ± 0.10 ^{cB}	22.15 ± 0.11 ^{bB}	20.51 ± 0.07 ^{bB}	19.31 ± 0.03 ^{eB}	20.66 ± 0.11 ^{bB}	18.83 ± 0.08 ^{cC}
C16:1	2.08 ± 0.02 ^{aE}	1.78 ± 0.04 ^{bF}	1.55 ± 0.09 ^{dF}	1.78 ± 0.09 ^{bF}	1.81 ± 0.12 ^{eF}	2.05 ± 0.07 ^{eD}	1.86 ± 0.02 ^{eE}	1.60 ± 0.02 ^{dE}	1.81 ± 0.02 ^{bF}	1.57 ± 0.02 ^{eF}
C 18:0	11.61 ± 0.02 ^{aD}	10.67 ± 0.06 ^{bD}	10.63 ± 0.01 ^{dD}	11.29 ± 0.01 ^{dD}	10.60 ± 0.02 ^{dD}	12.26 ± 0.06 ^{cD}	11.85 ± 0.00 ^{bC}	11.29 ± 0.00 ^{dD}	11.84 ± 0.00 ^{bD}	11.31 ± 0.00 ^{bD}
C 18:1	45.79 ± 0.09 ^{aA}	40.10 ± 0.08 ^{bA}	37.25 ± 0.07 ^{dA}	39.65 ± 0.07 ^{dA}	38.56 ± 0.10 ^{eA}	45.59 ± 0.11 ^{aA}	42.10 ± 0.02 ^{cA}	37.79 ± 0.01 ^{dA}	39.10 ± 0.01 ^{bA}	35.51 ± 0.01 ^{eA}
C 18:2 (n-6)	12.48 ± 0.02 ^{dC}	13.58 ± 0.04 ^{dC}	12.97 ± 0.02 ^{cC}	16.40 ± 0.02 ^{cC}	18.15 ± 0.06 ^{bC}	11.78 ± 0.08 ^{eC}	12.45 ± 0.02 ^{aC}	12.95 ± 0.09 ^{cC}	15.91 ± 0.12 ^{bC}	21.20 ± 0.02 ^{aB}

Table 1 (Continued)

	Raw					Cooked				
	0.06 ± 0.00 ^{cj}	0.06 ± 0.02 ^{ei}	0.07 ± 0.00 ^{cl}	0.06 ± 0.00 ^{cj}	0.48 ± 0.00 ^{dh}	0.64 ± 0.01 ^{bh}	0.07 ± 0.02 ^{ah}	0.07 ± 0.00 ^{ch}	0.43 ± 0.02 ^{bh}	1.02 ± 0.02 ^{ag}
C 18:2 (n-3)	6.26 ± 0.02 ^{ei}	6.26 ± 0.02 ^{ei}	0.64 ± 0.02 ^{eg}	14.68 ± 0.02 ^{ei}	2.60 ± 0.02 ^{ae}	3.12 ± 0.02 ^{de}	0.63 ± 0.02 ^{bf}	12.05 ± 0.04 ^{cd}	2.45 ± 0.02 ^{de}	5.05 ± 0.03 ^{ce}
C 18:3 (n-3)	0.91 ± 0.01 ^{bg}	0.91 ± 0.01 ^{bg}	0.94 ± 0.01 ^{bf}	0.77 ± 0.01 ^{cg}	0.90 ± 0.01 ^{sg}	0.84 ± 0.01 ^{de}	0.92 ± 0.01 ^{de}	0.72 ± 0.01 ^{df}	0.87 ± 0.01 ^{sg}	0.71 ± 0.01 ^{ch}
C 20:1	0.58 ± 0.01 ^{abh}	0.58 ± 0.01 ^{abh}	0.59 ± 0.01 ^{ag}	0.45 ± 0.01 ^{ch}	0.55 ± 0.01 ^{dh}	0.50 ± 0.01 ^{bh}	0.55 ± 0.01 ^{df}	0.43 ± 0.01 ^{cg}	0.52 ± 0.01 ^{bg}	0.40 ± 0.01 ^{cl}
C20:2 (n-11)	0.29 ± 0.01 ^{ai}	0.29 ± 0.01 ^{ai}	0.27 ± 0.01 ^{bh}	0.28 ± 0.01 ^{ci}	0.30 ± 0.01 ^{di}	0.26 ± 0.01 ^{ci}	0.37 ± 0.01 ^{ag}	0.42 ± 0.02 ^{cg}	0.51 ± 0.02 ^{ah}	0.46 ± 0.02 ^{bl}
C20:3 (n-11)	33.02 ± 0.07 ^b	33.02 ± 0.07 ^b	35.85 ± 0.13 ^a	31.00 ± 0.03 ^d	33.86 ± 0.02 ^c	32.85 ± 0.02 ^e	36.66 ± 0.03 ^a	34.53 ± 0.05 ^b	35.54 ± 0.01 ^c	32.36 ± 0.01 ^e
ΣSFA	45.86 ± 0.05 ^b	45.86 ± 0.05 ^b	49.79 ± 0.17 ^a	40.34 ± 0.07 ^d	45.23 ± 0.04 ^b	43.83 ± 0.06 ^c	49.58 ± 0.10 ^a	45.89 ± 0.04 ^b	44.81 ± 0.08 ^c	38.63 ± 0.07 ^e
ΣMUFA	21.08 ± 0.06 ^d	21.08 ± 0.06 ^d	14.38 ± 0.17 ^e	28.77 ± 0.08 ^b	20.75 ± 0.03 ^c	23.13 ± 0.06 ^a	13.77 ± 0.01 ^e	19.73 ± 0.16 ^d	20.24 ± 0.13 ^c	28.79 ± 0.06 ^a
ΣPUFA	6.32 ± 0.03 ^c	6.32 ± 0.03 ^c	0.71 ± 0.04 ^e	14.74 ± 0.06 ^a	3.08 ± 0.02 ^d	3.76 ± 0.03 ^b	0.70 ± 0.04 ^e	5.87 ± 0.05 ^c	2.88 ± 0.02 ^d	6.07 ± 0.02 ^b
Σn3	13.67 ± 0.05 ^d	13.67 ± 0.05 ^d	12.59 ± 0.02 ^d	13.08 ± 0.03 ^c	16.55 ± 0.02 ^b	18.36 ± 0.05 ^a	11.90 ± 0.02 ^d	12.59 ± 0.03 ^d	16.03 ± 0.02 ^b	21.54 ± 0.06 ^a
Σn6										

Results are expressed as g/100 g of fat. Data are presented as mean ± standard deviation. Control: control burgers with a traditional formula; Chia25: sample with 25% animal fat replaced by gelled emulsion with chia oil; Chia50: sample with 50% animal fat replaced by gelled emulsion with chia oil. Hemp25: sample with 25% animal fat replaced by gelled emulsion with hemp oil. Hemp50: sample with 50% animal fat replaced by gelled emulsion with hemp oil. For each parameter, results followed by same letter are not significantly different according to Tukey's HSD *post hoc* test ($P > 0.05$). Lower-case letters refer to the comparison of the same fatty acid or parameters between the different samples (a–e), while an upper-case letter (A–J) refers to the comparison of the different fatty acids in the same sample.

acids) add up to 90% of total fatty acids in control burgers, in comparison to reformulated burgers in which one more fatty acid (α -linolenic fatty acid) must be incorporated to reach similar identification levels. These results hold throughout frozen storage in both, raw and cooked burgers. In general, it could be said that pork back fat substitution by GE in burgers decreased the content in palmitic, stearic, palmitoleic and oleic fatty acids and increased the content in linoleic and α -linolenic fatty acids. On the one hand, the greater ($P < 0.05$) content of palmitic and stearic acids in control burgers than in reformulated ones could be associated with the high content of both fatty acids in animal tissues (adipose and muscle tissue) (Ospina-E *et al.*, 2010). On the other hand, the higher ($P < 0.05$) content of linoleic and α -linolenic fatty acids in reformulated burgers than in control could be linked to their greater content in chia and hemp oils used for GE formation (Wood *et al.*, 2008). The lipid profile of control burgers is in accordance with those reported in beef burgers elaborated with pork backfat (Heck *et al.*, 2017; Sayas-Barberá *et al.*, 2021).

Some nutritional and health indices of cooked burgers along with frozen storage are shown in Table 2. In this case, it has been decided to calculate these indices only for cooked burgers with the intention of being able to carry out a more precise nutritional evaluation to the real way in which they are consumed. The PUFA/SFA ratio was greater ($P < 0.05$) in reformulated burgers than in control ones which is due to both facts, SFA reduction and PUFA increase, induced by the pork backfat substitution by GEs. Also in this case the PUFA/SFA increase was dependent on the type of GE (higher in the case of hemp-GE than chia-GE) and on the level of fat substitution (higher at higher substitution levels). Taking into account the recommendation established by Wood *et al.* (2008) that PUFA/SFA ratio should be higher than 0.4, all reformulated burgers at all frozen storage times meet this requirement. However, a more recent recommendation increase this index at 0.85 (FAO, 2010), and so in this case, only Hemp50 and Chia50 burgers would meet this level. In any case, it could be said that the higher this ratio, the more positive the effect. Although in general, the increase in the proportion of PUFA in the diet has been recommended as healthy, recently, several studies have concluded that for the development of healthier meat products, not only the increase in PUFA fraction is important but also the reduction in n-6 fatty acids and so in the n-6/n-3 ratio. This is because some lipid mediators derived from n-6 PUFA have been related to several pathogenic processes such as inflammation, platelet aggregation and vasoconstriction, while those derived from n-3 PUFA seem to be implied with opposite effects. In addition, the pathogenesis of many modern diet-related chronic diseases

Table 2 Health indices of beef burgers (5 formulations, cooked) during frozen storage

Parameter	Sample	Storage time (days)		
		0	30	60
ΣPUFA/ ΣSFA	Control	0.39 ± 0.02 ^{aC}	0.38 ± 0.01 ^{aD}	0.38 ± 0.03 ^{aD}
	Chia25	0.57 ± 0.02 ^{aB}	0.56 ± 0.02 ^{aC}	0.57 ± 0.02 ^{aC}
n6/n3	Chia50	0.83 ± 0.04 ^{abA}	0.86 ± 0.04 ^{aA}	0.81 ± 0.01 ^{bB}
	Hemp25	0.58 ± 0.02 ^{bB}	0.66 ± 0.01 ^{aB}	0.57 ± 0.02 ^{bC}
	Hemp50	0.91 ± 0.05 ^{aA}	0.91 ± 0.02 ^{aA}	0.89 ± 0.01 ^{aA}
	Control	15.89 ± 0.04 ^{cA}	18.00 ± 0.02 ^{aA}	16.89 ± 0.02 ^{bA}
	Chia25	2.20 ± 0.02 ^{aD}	2.15 ± 0.03 ^{bD}	2.15 ± 0.01 ^{bD}
AI	Chia50	1.02 ± 0.05 ^{c^{abE}}	0.97 ± 0.02 ^{bE}	1.08 ± 0.03 ^{aE}
	Hemp25	5.69 ± 0.02 ^{aB}	4.79 ± 0.01 ^{cB}	5.57 ± 0.01 ^{bB}
	Hemp50	3.50 ± 0.04 ^{bC}	3.62 ± 0.03 ^{aC}	3.55 ± 0.02 ^{bC}
	Control	0.43 ± 0.01 ^{aA}	0.43 ± 0.02 ^{aA}	0.43 ± 0.02 ^{aA}
	Chia25	0.39 ± 0.01 ^{a^{AB}}	0.39 ± 0.01 ^{a^B}	0.39 ± 0.01 ^{a^B}
TI	Chia50	0.35 ± 0.01 ^{a^B}	0.35 ± 0.03 ^{a^C}	0.35 ± 0.02 ^{a^C}
	Hemp25	0.38 ± 0.01 ^{a^{AB}}	0.37 ± 0.01 ^{a^{BC}}	0.39 ± 0.03 ^{a^{BD}}
	Hemp50	0.34 ± 0.01 ^{a^B}	0.33 ± 0.02 ^{a^C}	0.34 ± 0.01 ^{a^C}
	Control	1.06 ± 0.03 ^{aA}	1.07 ± 0.01 ^{aA}	1.08 ± 0.03 ^{aA}
	Chia25	0.72 ± 0.03 ^{a^C}	0.72 ± 0.02 ^{a^C}	0.71 ± 0.02 ^{a^C}
h/H	Chia50	0.47 ± 0.02 ^{a^E}	0.46 ± 0.04 ^{a^E}	0.49 ± 0.01 ^{a^E}
	Hemp25	0.84 ± 0.03 ^{a^B}	0.77 ± 0.01 ^{b^B}	0.86 ± 0.02 ^{a^B}
	Hemp50	0.62 ± 0.02 ^{a^D}	0.62 ± 0.02 ^{a^D}	0.64 ± 0.01 ^{a^D}
	Control	2.59 ± 0.02 ^{a^E}	2.56 ± 0.02 ^{a^{bE}}	2.54 ± 0.02 ^{b^E}
	Chia25	2.85 ± 0.02 ^{c^D}	2.83 ± 0.05 ^{b^D}	2.86 ± 0.01 ^{a^C}
NV	Chia50	3.20 ± 0.01 ^{a^{bB}}	3.22 ± 0.02 ^{a^B}	3.16 ± 0.03 ^{a^B}
	Hemp25	2.89 ± 0.02 ^{a^C}	3.00 ± 0.01 ^{a^C}	2.73 ± 0.01 ^{a^{AD}}
	Hemp50	3.32 ± 0.01 ^{b^A}	3.35 ± 0.03 ^{a^A}	3.25 ± 0.02 ^{a^{AA}}
	Control	0.51 ± 0.01 ^{a^B}	0.51 ± 0.01 ^{a^B}	0.51 ± 0.01 ^{a^B}
	Chia25	0.54 ± 0.01 ^{a^A}	0.52 ± 0.01 ^{a^B}	0.51 ± 0.01 ^{a^B}
NV	Chia50	0.54 ± 0.01 ^{a^A}	0.54 ± 0.01 ^{a^A}	0.54 ± 0.01 ^{a^A}
	Hemp25	0.51 ± 0.01 ^{a^B}	0.51 ± 0.01 ^{a^B}	0.53 ± 0.01 ^{a^B}
	Hemp50	0.55 ± 0.01 ^{a^A}	0.53 ± 0.01 ^{a^A}	0.56 ± 0.01 ^{a^A}

For each parameter, results followed by same letter are not significantly different according to Tukey's HSD *post hoc* test ($P > 0.05$). Data were presented as mean ± standard deviation. Control: control burgers with a traditional formula; Chia25: sample with 25% animal fat replaced by gelled emulsion with chia oil; Chia50: sample with 50% animal fat replaced by gelled emulsion with chia oil. Hemp25: sample with 25% animal fat replaced by gelled emulsion with hemp oil. Hemp50: sample with 50% animal fat replaced by gelled emulsion with hemp oil. A lower-case letter refers to the comparison of the same sample between the different days of storage (a-c), while an upper-case letter (A-D) refers to the comparison of the different samples in the same day of storage.

AI, atherogenic index; TI, thrombogenic index; h/H, hypocholesterolaemic/hypercholesterolaemic index; NV, nutritional value.

has been strongly associated with low intake of n-3 PUFAs and overconsumption of n-6 PUFAs (Mariamenatu & Abdu, 2021). In view of all these findings, FAO has recommended that this ratio should be less than 4.0 (FAO, 2010). The substitution of pork back-fat by GEs significantly improved the n-6/n-3 ratio in burgers, changing from values between 16 and 18 in control burgers to values below 4.0 in reformulated

ones, except in Hemp25 treatment (ranging from 4.8 to 5.7). On the other hand, although this requirement was not achieved in Hemp25 samples, also in this case the reduction of n-6/n-3 ratio with respect to control burgers was noticeable (65%–70%).

Several authors have proposed the calculation of other indices (based on lipid profile of foods) as indicators of healthy foods which have ended up widely used to address the healthy characteristics of fats in meat products (Pintado *et al.*, 2015; de Souza-Paglarini *et al.*, 2019; Botella-Martínez *et al.*, 2021c). These indices are atherogenic index (AI) and thrombogenic index (TI), as good indicators of the relationship between diet and coronary heart disease (the lower these ratios, the more positive the effect) (Bohrer, 2019) and the hypocholesterolaemic/hypercholesterolaemic ratio that is specifically related to the functional effects of diet fats on cholesterol metabolism (the higher this ratio, the more positive the effect). In addition, another index has been proposed as indicator of the nutritional value (NV) of diet fats.

Analysing these four ratios, it could be said that reformulated burgers (at all storage times) are healthier than control ones considering that reformulated burgers showed h/H and NV index values higher than control and TI and AI ratios lower than control ($P < 0.05$). In this case, the behaviour of these indices seems to be dependent on the replacement level (higher effect at 50% than at 25% or even only significant effect at 50%) ($P < 0.05$) and not on the type of GE used ($P > 0.05$). In addition, the storage time did not change this trend.

The positive behaviour (healthier) of these four indices in reformulated burgers could be indicating a decrease in vascular risk factors together with a healthy trend in cholesterol metabolism due to their consumption (higher amount of fatty acids considered as hypocholesterolaemic and lower content of those hypercholesterolaemic) compared to control.

Cooking properties

Table 3 shows cooking loss and dimensional changes (shrinkage and thickness increase) in reformulated burgers during frozen storage. Cooking loss and dimensional changes in meat products due to cooking are used to be perceived by consumers as undesirable effects, decreasing their acceptance. Domínguez *et al.* (2014) reported that cooking loss is related to mass transfer (water and fat) during thermal treatment. Denaturation of meat proteins (myofibrillar and connective) during cooking is responsible for meat shrinkage and loss of water holding capacity (Vas-koska *et al.*, 2020). Taking into account that all these parameters are influenced by the ingredients used, the impact of new ingredients (as GE) in dimensional changes should be evaluated.

Table 3 Cooking properties of beef burgers (5 formulations, raw and cooked) during frozen storage

Parameter	Sample	Storage time (days)		
		0	30	60
Cooking loss (%)	Control	19.34 ± 0.30 ^{cC}	22.89 ± 0.09 ^{aD}	20.92 ± 0.13 ^{bD}
	Chia25	21.63 ± 0.47 ^{cBC}	24.93 ± 0.12 ^{aC}	22.50 ± 0.22 ^{bB}
	Chia50	24.26 ± 0.56 ^{bAB}	28.03 ± 0.24 ^{aA}	22.52 ± 0.13 ^{bB}
	Hemp25	27.13 ± 0.32 ^{aA}	25.33 ± 0.11 ^{bB}	21.96 ± 0.23 ^{cC}
	Hemp50	25.09 ± 0.95 ^{aA}	25.31 ± 0.02 ^{aB}	26.19 ± 0.05 ^{aA}
Shrinkage (%)	Control	19.55 ± 0.96 ^{bC}	20.83 ± 0.12 ^{aD}	16.67 ± 0.23 ^{cB}
	Chia25	21.64 ± 1.78 ^{aB}	26.27 ± 0.25 ^{aB}	24.99 ± 0.18 ^{aA}
	Chia50	21.41 ± 0.45 ^{cAB}	31.14 ± 1.02 ^{aA}	24.86 ± 0.35 ^{aA}
	Hemp25	25.75 ± 1.81 ^{aA}	24.77 ± 0.08 ^{aC}	24.93 ± 0.14 ^{aA}
	Hemp50	24.19 ± 1.67 ^{aA}	20.77 ± 0.15 ^{aD}	24.89 ± 0.20 ^{aA}
Thickness increase (%)	Control	8.13 ± 0.53 ^{aC}	9.50 ± 0.43 ^{aD}	11.18 ± 1.51 ^{aD}
	Chia25	12.92 ± 0.42 ^{cB}	23.33 ± 0.14 ^{bA}	26.15 ± 0.16 ^{aB}
	Chia50	11.07 ± 0.90 ^{cB}	21.50 ± 0.33 ^{bB}	31.67 ± 0.42 ^{aA}
	Hemp25	13.02 ± 0.20 ^{bA}	14.57 ± 0.12 ^{aC}	15.57 ± 1.03 ^{aC}
	Hemp50	13.81 ± 0.69 ^{cA}	15.56 ± 0.44 ^{bC}	25.71 ± 0.10 ^{aB}

For each parameter, results followed by same letter are not significantly different according to Tukey's HSD *post hoc* test ($P > 0.05$). Data were presented as mean ± standard deviation. Control: control burgers with a traditional formula; Chia25: sample with 25% animal fat replaced by gelled emulsion with chia oil; Chia50: sample with 50% animal fat replaced by gelled emulsion with chia oil. Hemp25: sample with 25% animal fat replaced by gelled emulsion with hemp oil. Hemp50: sample with 50% animal fat replaced by gelled emulsion with hemp oil. A lower-case letter refers to the comparison of the same sample between the different days of storage (a–c), while an upper-case letter (A–D) refers to the comparison of the different samples in the same day of storage.

In general, it could be said that fat substitution by GE in burgers resulted in higher ($P < 0.05$) cooking loss and dimensional changes than control burgers, at the beginning (time 0) and at the end of storage (time 60), although not all these differences were keeping at the middle of frozen storage (time 30). In addition, there is no clear trend in the behaviour of these parameters either as a function of frozen storage time or as a function of the formulation (type of GE used or substitution level). In the literature review carried out about the effect of using GE (with several vegetable oils) as fat substitution in burgers on dimensional changes during their cooking, contradictory results have been found: increasing (Dias *et al.*, 2021), decreasing (Heck *et al.*, 2017; Lucas-González *et al.*, 2020) and not variations (Barros *et al.*, 2021). All these modifications have been attributed to the cooking effect on water evaporation and lipid migration (Pathare & Roskilly, 2016), and it seems clear that both actions can be affected by the replacement of backfat by GEs. Regarding frozen storage effect on cooking loss and dimensional changes, it could be said that the intensity of these changes (with respect to the values at time 0) was higher ($P < 0.05$) at day 30 than at day 60. In this case, the formation and evolution of ice crystals during freezing and frozen storage and the corresponding physical damage on the muscle cell structure together with the protein oxidation (affecting protein structure and functional properties) are reasons contributing to cooking loss (Leygonie

et al., 2012; Utrera *et al.*, 2014). As reformulated burgers have shown higher lipid oxidation values (Fig. 1) and so a higher protein oxidation would be expected, it could also favour their higher cooking loss and dimensional changes with respect to control burgers.

Lipid oxidation

Reformulated meat products should be evaluated about the effect of new ingredients and process on lipid oxidation reaction, mainly if the composition of these new ingredients (high PUFA content) might enhance their oxidation susceptibility.

The variations in the TBARS content in reformulated burgers (raw and cooked) throughout frozen storage are shown in Fig. 1. These results indicated that TBARS values were affected ($P < 0.05$) by both, treatment (animal fat substitution by GEs) and storage time.

Regarding raw burgers and at time 0, the fat substitution by chia-GE (Chia25 and Chia50) increased ($P < 0.05$) TBARS values, being this increase higher at the highest substitution level (Chia50). On the contrary, there were no differences ($P > 0.05$) in TBARS values between control and hemp-GE burgers (at any substitution level). In this case, it could be said that TBARS values depend on the type of GE used and not on the substitution level. These differences could be attributed to variations in the lipid profile of the

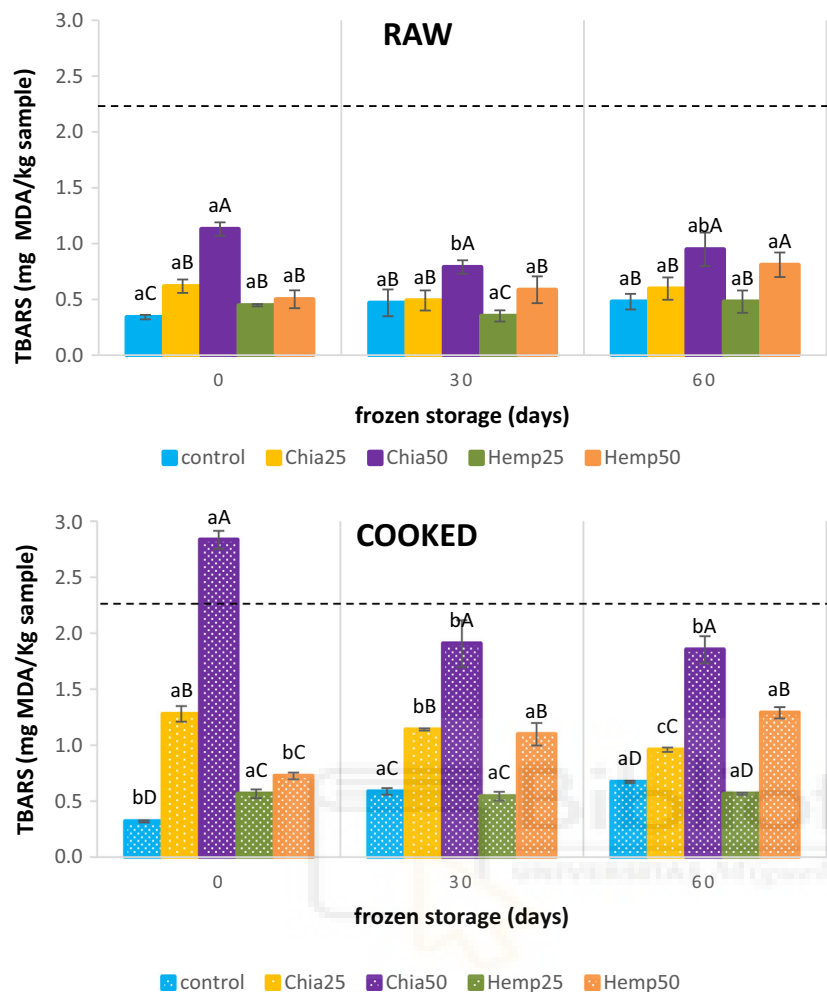


Figure 1 Lipid oxidation (TBARS values) of beef burgers (5 formulations, raw and cooked) during frozen storage. For each parameter, results followed by same letter are not significantly different according to Tukey's HSD *post hoc* test ($P > 0.05$). Data are presented as mean \pm standard deviation. Control: control burgers with a traditional formula; Chia25: sample with 25% animal fat replaced by gelled emulsion with chia oil; Chia50: sample with 50% animal fat replaced by gelled emulsion with chia oil; Hemp25: sample with 25% animal fat replaced by gelled emulsion with hemp oil; Hemp50: sample with 50% animal fat replaced by gelled emulsion with hemp oil. A lower-case letter refers to the comparison of the same parameter between the different days of storage (a–c), while an upper-case letter (A–D) refers to the comparison of the different samples in the same day of storage.

oils, in fact in the level of PUFA (Ixtaina *et al.*, 2011; Zajac *et al.*, 2019) as well as in their content in antioxidant compounds (Bodoira *et al.*, 2017; Leonard *et al.*, 2020). However, during frozen storage, significant changes ($P < 0.05$) in TBARS were observed only in burgers with the highest GEs substitution levels (Hemp50 and Chia50) being in this case not significant ($P > 0.05$) the type of GE used. TBARS values in control, Chia25 and Hemp25 burgers did not change ($P > 0.05$) during 60 days of frozen storage. Chia50 and Hemp50 burgers showed at the end of frozen storage higher ($P < 0.05$) TBARS values than the others. Despite these changes in TBARS values of raw burgers along frozen storage, none of them exceeded the limit of acceptability (2.28 mg MDA/kg) reported by Campo *et al.* (2006). Similar findings have been reported in burgers reformulated with GE based on vegetable oils (Poyato *et al.*, 2015; Lucas-González *et al.*, 2020; Botella-Martínez *et al.*, 2021c) and also in burgers during storage (Fernández-López *et al.*, 2016; Sayas-Barberá *et al.*, 2021). During frozen storage,

lipid oxidation reactions continue but at slower rate which could indicate that 60 days of frozen storage in burgers are not enough to increase TBARS values above the limit indicated as detectable by consumers (Campo *et al.*, 2006).

As could be expected, cooked burgers showed higher TBARS values ($P < 0.05$) than corresponding raw ones, except control burgers. Heat treatment acts as a promoting effect in the propagation phase of oxidation reaction, causing the decomposition of hydroperoxides and generating radical peroxides (Domínguez *et al.*, 2014). It is true that, depending on the treatment, cooking effect on lipid oxidation was more or less intense. Burgers with chia-GE (Chia25 and Chia50) showed higher ($P < 0.05$) increase in TBARS values (more than 100% increase) due to cooking than the others (control, Chia25 and Chia50). Also in this case, the effect of cooking on lipid oxidation was stronger than the frozen storage. All cooked burgers showed TBARS values lower than 2.28 mg MAD/kg sample during the whole frozen storage, except Chia50 burgers that exceeded this limit

already from the beginning of frozen storage. Maybe the antioxidant compounds in chia oil are not enough to control the oxidation process at the highest chia-GE substitution level (50%) when strong oxidative processes (cooked) are applied.

Conclusions

Partial replacement of pork backfat by optimised GEs (based on chia and hemp oils) is a suitable strategy to obtain healthier burgers in relation to the quality of dietary fats (increase in PUFAs and decrease in SFAs). These variations are dependent on the type of GE (higher with GE with hemp oil) and on the replacement level (higher at 50 than 25%). This trend was not modified by frozen storage for 60 days or by the cooking process. In addition, cooking increased the susceptibility of reformulated burgers to oxidation in a more intense way than frozen storage, and this effect was stronger when chia-GE was used. However, only burgers with 50% fat substitution by chia-GE exceeded the TBARs values that could be indicative of consumer-detectable rancidity in meat products. These findings will contribute to increasing the meat industry competitiveness by being able to offer products that meet the requirements of world food safety agencies as well as the increasing consumer demand for healthier products.

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AUTHOR CONTRIBUTIONS

Carmen Botella-Martínez: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Estrella Sayas:** Conceptualization (equal); formal analysis (equal); supervision (equal). **Jose Angel Pérez-Alvarez:** Funding acquisition (equal); resources (equal); supervision (equal); visualization (equal). **Manuel Viuda-Martos:** Conceptualization (equal); investigation (equal); supervision (equal); writing – review and editing (equal). **Juana Fernández-López:** Conceptualization (equal); investigation (equal); supervision (equal); writing – review and editing (equal).

Conflict of interest

The authors declare that they have no conflicts of interest to this work.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors

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8.5. PUBLICACIÓN 5





Total and partial fat replacement by gelled emulsion (hemp oil and buckwheat flour) and its impact on the chemical, technological and sensory properties of frankfurters

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Total and Partial Fat Replacement by Gelled Emulsion (Hemp Oil and Buckwheat Flour) and Its Impact on the Chemical, Technological and Sensory Properties of Frankfurters

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Abstract: A gelled emulsion (GE) prepared with hemp oil and buckwheat flour was used to replace pork back fat in frankfurters. Five different formulations were prepared: control (with 35% pork back fat—SC), and the following four to achieve 25%, 50%, 75%, and 100% pork back fat substitution by GE (S1, S2, S3, and S4, respectively). Nutritional, technological, and sensorial characteristics of frankfurters were evaluated. Sausages containing GE presented a lower total fat content with a higher amount of polyunsaturated fatty acids, increased omega 3 content, and reduced saturated fat by up to 55%. The incorporation of GE did not significantly modify technological properties such as emulsion stability or lipid oxidation in spite of using vegetable oils highly susceptible to oxidation. The reformulation of the frankfurters presented a greater effect on the texture and sensory properties when GE was used as total substitution for the pork back fat (S4). When GE was used only as partial substitution for the pork back fat, sausages similar to control frankfurter were obtained. So this study demonstrated that the use of GE could be a promising strategy in the reformulation of healthier meat products.

Keywords: hemp oil; fat replacer; buckwheat flour; frankfurter; healthy meat products



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1. Introduction

Frankfurter is a meat product widely consumed in different regions of the world, mainly attributed to its convenience, low price, nutritional value, and flavor [1,2]. On the other hand, it is well known that a relevant amount of animal fat (pork back fat) is used during its elaboration, reaching in some cases levels close to 40% of fat in the final product [3]. Pork backfat is the most appreciated and valued source of fat in meat products processing mainly due to its chemical composition and saturated fatty acid (SFA) content (approx. 40%) which results not only in technological advantages during processing, but also texture and taste improvement in the final product [4–6]. The relationship between saturated fat consumption and certain diseases (mainly obesity and cardiovascular diseases) is moving the meat industry to develop healthier meat products, not only by reducing the amount of fats but also by replacing saturated fats by others with a healthier lipid profile [7–9]. Therefore, studies are being carried out in which unsaturated or polyunsaturated oils are used as animal fat replacement in these types of meat product to make them healthier. In any case, this replacement is not an easy task, mainly due to both, technical and sensorial problems in the product, making them difficult to cut and more prone to oxidation [2,4,7]. Within this scene, gelled emulsion (GE) systems have been revealed as a suitable alternative to create animal fat substitutes that provide and improve stability, textural and oxidative properties or without detriment [6,7,10–23]. A GE is a colloidal material in which oil in water emulsions (O/W) coexists within a gel network with mechanical properties similar to a viscoelastic solid [12,13]. For the elaboration of GE, different vegetable oils (chia, hemp,

linseed, among others) have been assayed, together with other protein/starchy ingredients such as pseudo-cereal flours (quinoa, amaranth, buckwheat, teff, etc.), with the aim of stabilizing these O/W emulsions [9,12–14]. In one of our previous studies, 12 combinations of these vegetable oils and pseudo-cereal flours were used to obtain GE, selecting the combination of hemp oil and buckwheat flour because it was technologically feasible to obtain and showed the best (healthy) fatty acid composition. It presented an interesting contribution of ω -3 and ω -6 fatty acids that could be attractive for reformulating meat products [15,24].

Hemp oil (*Cannabis sativa* L.) is composed of a well-balanced fatty acids and antioxidant profile, is rich in polyunsaturated fatty acids, principally the omega-6 linoleic acid (55.41–56.94%) and contains gamma and alpha linoleic acids (0.64–1.10%, 16.51–20.40%) [25,26]. Buckwheat is a pseudo-cereal with a high nutritional quality known for being a dietary source of dietary fiber, vitamins, minerals, and antioxidants. Its flour contains high levels of essential nutrients and bioactive flavonoids. In reference to that, quercetin and quercetin glycoside, such as rutin and iso-quercetin, have been identified as the major flavonoids in buckwheat, which provide several pharmacological advantages. Many polyphenolics exhibit antioxidative properties, especially oxygen species scavenging [27–30]. Buckwheat proteins have a high biological value, with a high lysine content, approximately 6 mg/100 g of proteins [31]. The average of protein distribution for buckwheat is 18%–22% of albumin, 15%–70% of globulins, 0%–5% prolamins, and 4%–23% glutens. The protein composition of buckwheat provides emulsifying and gelling properties to buckwheat flour used in the elaboration of the GE [30,32].

No studies have been conducted on the use of this kind of GE in meat products. The objectives of this work were (i) to evaluate the technological viability of producing frankfurters with different pork back fat substitution levels by a GE elaborated with hemp oil and buckwheat flour; (ii) to investigate the effects of different percentages of fat substitution (25%, 50%, 75%, and 100%) on their chemical composition, lipid profile, physico-chemical properties, emulsion and lipid stability, and sensorial properties of frankfurters.

2. Materials and Methods

2.1. Materials

The ingredients used to make gelled emulsions were: hemp oil (54.44% linoleic acid, 19.95% α -linolenic acid, 8.23% oleic acid, 6.17% palmitic acid, 4.26% linoleic acid, 2.3% stearic acid, 1.62% γ -linolenic acid, and 3.03% others, according to the information given by the supplier) from Laboratorios Almond, S.L. (Murcia, Spain); buckwheat flour (BW) distributed by Biogran S.L. (Madrid, Spain); gellan gum (a polysaccharide excreted by microorganism *Pseudomonas elodea*: this is a water-soluble linear structure with a repeating unit of tetra-saccharide), and instant gel (gelatin from animal origin (pork) with 180 bloom), supplied from Sosa Ingredients S.L. (Barcelona, Spain).

2.2. Preparation of Oil-in-Water Gelled Emulsions

Oil-in-water gelled emulsions (GE) were prepared as follows: first the gelling agent “instant gel” was mixed in a homogenizer (Thermomix 31, Vorwerk-España M.S.L., S.C, Spain) with water for 2 min at 60 °C at high speed. Then, the buckwheat flour was added and mixed for 1 min at medium speed. In the next step, the temperature was turned down to 37 °C and “gellan gum” was added and mixed for 2.5 min at 250 rpm. In the last step, the mixture was mixed with the gradual addition of the appropriate amount of hemp oil for 5 min, at 37 °C and 1100 rpm. The GE was then transferred to a refrigerator and stored at 4 °C prior to sausage production. The GE was made combining 47% water, 40% hemp oil, 10% buckwheat flour, 1.5% gellan gum and 1.5% instant gel.

2.3. Preparation of Frankfurters

Frankfurter-like sausages were made according to a traditional formula (meat percentages add up to 100% and percentages of others ingredients are related to that of meat): pork lean meat (65%) and pork backfat (35%), 15% water (ice form), 3% potato starch, 1.5% sodium chloride, 300 mg/Kg sodium tripolyphosphate, 150 mg/Kg sodium nitrite, 1.5% casein, 0.2 mL/kg liquid smoke, and spices (a mixture of white pepper and nutmeg).

Five different frankfurter sausage batches (4 kg) were designed to modify lipid concentration, as follows: batch 1 was used as control (SC) with the traditional formula described above. The other four batches were formulated replacing different amounts of animal fat by the GE previously prepared: in the second batch (S1), 25% of pork backfat was replaced by GE; the following batches (S2, S3, and S4) were obtained replacing 50%, 75%, and 100%, respectively, of pork backfat by GE. Three replications of this elaboration process were performed on different days.

The products were prepared in the IPOA Research Group Pilot Plant at the Miguel Hernández University, following an industrial processing protocol. Briefly, meat ingredients were ground in a cutter (1094-Homogeneizer, Tekator, Höganäs, Sweden) and mixed with the sodium chloride and the rest of the ingredients for 2 min (temperature below 12 °C). After homogenization, the resulting meat batter was stuffed using a piston stuffer EM-12 (Mainca, Granollers, Barcelona, Spain) into 20 mm diameter cellulose casings (Fibran, Girona, Spain). Samples were hand linked and cooked in a water bath (80 °C) until the core temperature reached 72 °C. When the endpoint temperature was achieved, the sausages were immediately chilled in ice for 5 min, packed, and stored at 4 °C under darkness conditions.

2.4. Emulsion Stability

Emulsion stability of the meat batters (before cooking) was evaluated by means of total expressible fluid (TEF) according to Pintado et al. [16] with slight modifications. Samples were centrifugated into centrifuge tubes of 15 mL at 3000 rpm for 1 min. Then they were heated in a water bath at 70 °C for 30 min and cooled at room temperature. Next, they were recentrifuged for 3 min at 3000 rpm. The samples were left standing upside down to release the expressible fluid (fat and water) onto filter paper. The determinations were made in triplicate for each sample. The results are expressed in g of total fluid expelled/100g of sample and were calculated using the following expression (Equation (1)):

$$\%TEF = \frac{\text{Weight of tube with sample} - \text{Weight of tube with pellet}}{\text{Weight of sample}} \times 100. \quad (1)$$

2.5. Frankfurters' Characterization

2.5.1. Proximate Composition

Total moisture, ash, protein, and fat content of the sausages were determined according to the Association of Official Analytical Chemist analysis [33]. The determinations were made in triplicate for each sample.

2.5.2. Fatty Acid Profile

Lipid extraction from the samples was conducted according to Folch et al. [34], and the lipid phase was methylated according to the AOAC [33] method 969.33. The fatty acid methyl esters (FAMES) were injected into an HP 6890 gas chromatography equipment with a flame ionizer detector and a Suprewax-280 capillary column (30 m, 0.25 µm of film, 0.25 mm internal diameter; Tecknokroma Barcelona, Spain). The temperature program was as follows: the initial temperature was 60 °C for 1 min, then raised at a rate of 10 °C/min until reaching 170 °C and was kept at this temperature for 2 min. Then it was raised at a speed of 3 °C/min until 230 °C for 10 min, and finally, it was raised at a speed of 2 °C/min until 260 °C and maintained for 1 min at this temperature. Helium was the carrier gas with an internal column pressure of 11 psi. The injector volume was 0.2 µL in splitless.

The response factors were calculated using fatty acid standards and their identification was made by comparison with the retention times of these FAME standards (Supelco 37 component FAME Mix, Bellefonte, PA, USA). Acetonitrile and formic acid (1%) were used as mobile phase and the flow rate was set at 1 mL/min. All analyzes were carried out in triplicate and the results were expressed as g fatty acid/100 g oil.

Atherogenic index (*AI*) and thrombogenic index (*TI*) were calculated by Equations (2) and (3), respectively, developed by Ulbricht & Southgate [35].

$$AI = \frac{C12 : 0 + (4 \times C14 : 0) + C16 : 0}{\sum AGMI + \sum n6 + \sum n3} \quad (2)$$

$$TI = \frac{C14 : 0 + C16 : 0 + C18 : 0}{(0,5 \times \sum MUFA) + (0,5 \times \sum n6) + (3 \times \sum n3) + \left(\frac{\sum n3}{\sum n6} \right)} \quad (3)$$

The hypo-cholesterolemic/hypercholesterolemic ratio (*h/H*) was calculated using Equation (4), (Equation (4)) as described by Fernández et al. [36].

$$\frac{h}{H} = \frac{C18 : 1n9 + C18 : 1n7 + \sum PUFA}{C14 : 0 + C16 : 0} \quad (4)$$

2.5.3. Physico-Chemical Properties

pH

The pH of the final products was measured in triplicate with a pH-meter Crison model 510, (Barcelona, Spain) using a penetration probe at different sites of the sample (6 measures).

Water Activity

Water activity (*aw*) was measured at 25 °C using an electrolytic hygrometer (Novasina TH-500, Novasina, Axair Ltd., Pfaeffikon, Switzerland). Three replications of each sample were made.

Texture

Texture measurements (texture profile analysis, TPA) were performed using a TA-XT2i Texture Analyzer (Stable Micro Systems, Surrey, England). Sausage sections of 2 cm height in horizontal were submitted to two compression cycles up to 75% at a constant velocity of 1 mm/s at 15–20 °C. From the curves obtained (force-time deformation), the following parameters were calculated: hardness (N), as the maximum peak force during the first compression; adhesiveness (N s), as the negative work between the two compression cycles; springiness (mm), as the height that the food recovers during the time between the end of the first compression and the beginning of the second compression; cohesiveness (dimensionless), as the ratio of the positive force area during the second compression to that during the first compression; and chewiness (N mm), as the product of hardness times, cohesiveness times and springiness [37]. Five measurements per sample were made.

Color

The instrumental color parameters were performed directly on the cross-sections of frankfurters using a CM-700 spectrophotometer (Minolta Camera Co., Osaka, Japan), operating with D65 illuminant, 10° observer angle, SCI mode, and with a low reflectance glass (Minolta CR-A51/1829-752) placed between the samples and the equipment. The CIELAB coordinates determined were: *L** (lightness), *a** (+/− red/green), *b** (+/− yellow/blue). Nine readings of each sample were performed at room temperature (25 °C).

The magnitudes *h** (hue) and *C** (chrome) were calculated with Equations (5) and (6) (Equations (5) and (6)), respectively.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (5)$$

$$h^* = \arctg(b^*/a^*) \quad (6)$$

Total color differences (ΔE) of each sample (S) with respect to control sausage (C) were also calculated (Equation (7)).

$$\Delta E = \sqrt{(L_S^* - L_{CON}^*)^2 + (a_S^* - a_{CON}^*)^2 + (b_S^* - b_{CON}^*)^2} \quad (7)$$

2.5.4. Lipid Oxidation

The TBARs (thio-barbituric acid reactive substances) values were determined in triplicate according to Rosmini et al. [38]. Results, expressed as mg malondialdehyde (MDA)/kg sample, were calculated from a malonaldehyde (MDA) standard curve.

2.6. Sensory Assessment

The sensory evaluation was carried out in a sensory analysis laboratory with partitioned cabinets under white lights in individual booths. A 17-member sensory panel aged 18–55 years and with no specific training in the sensory analysis of frankfurters, were recruited from the staff and students at the Miguel Hernández University. The sensory analysis scheme was developed with a hedonic scale consisted of 7 levels (1: dislike extremely and 7: like extremely) on pieces of 2.0 cm approx. thickness (cutting from the frankfurter), evaluating the following attributes: color, hardness, juiciness, hemp smell, hemp flavor, salty flavor, and general acceptability.

2.7. Statistical Analysis

From each elaboration process (3), three samples were obtained from each of the five batches (formulation). Means and standard deviations of data obtained from the analysis of frankfurters are shown in corresponding tables and figures. Data were evaluated by one-way analysis of variance (ANOVA); if statistically significant differences were found, a Tukey-b post-hoc test was performed at 5% significance level ($p < 0.05$) using SPSS software (version 24.0, SPSS Inc., Chicago, IL, USA).

3. Results & Discussion

3.1. Properties of Gelled Emulsion

The total characterization of the GE prepared with hemp oil and buckwheat flour has been previously published [15]. Some of the main properties with special relevance in this study are pH 6.06 ± 0.01 , protein content 2.63 ± 0.02 g/100 g of sample, appropriate stability against centrifugation forces ($16.83 \pm 1.43\%$ TEF) with very little separation between phases after centrifugation. The amount of saturated and unsaturated fatty acids for this GE are $10.24 \pm 0.08\%$ and $89.77 \pm 0.06\%$, respectively. The main fatty acids are linoleic acid (54.44%) followed by α -linolenic acid (19.95%), oleic acid (8.23%), and palmitic acid (6.17%). This GE contains approximately 20% of fatty acids n-3.

3.2. Emulsion Stability of Meat Batters

The stability of the emulsion was determined on the raw dough before the products were stuffed and cooked and is shown in Figure 1. The higher the TEF percentage, the lower the emulsion stability. The replacement of animal fat by GE (at any percentage) did not cause significant differences ($p > 0.05$) in emulsion stability, values ranging from 1.03 to 1.72 % (Figure 1) in all samples, which is according to normal values for these type of sausages [17]. Some authors have reported that the simple reduction of back fat in cooked sausages decreased their stability [13]. In this case, the water holding capacity of the hydrocolloids present in the GE (gellan gum and instant gel) could be counteracting this negative effect. In addition, it has been reported that the use of a pre-emulsion fat as fat replacement in frankfurters increased their stability reducing the amount of liquid exudate [18,19,39]. In this case, it could be said that all the formulations were stable, as the TEF was low ($p < 4\%$) [13].

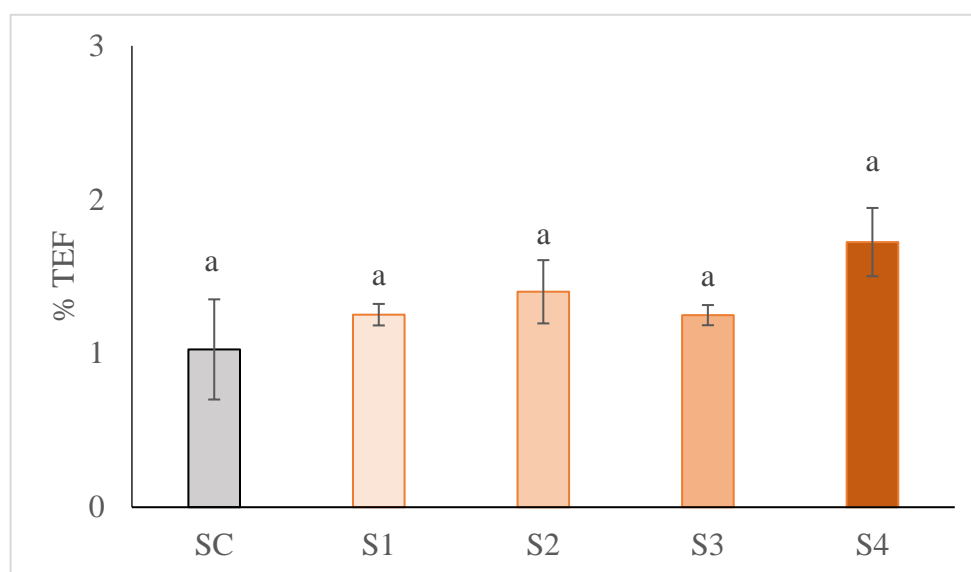


Figure 1. Effect of partial and total replacement of pork back fat by GE, from hemp oil and buckwheat flour, on emulsion stability of frankfurters. Results are expressed as % total expressible fluid (%TEF). SC: frankfurter without GE; S1: frankfurter formulated with GE as 25% fat replacer; S2: frankfurter formulated with GE as 50% fat replacer; S3: frankfurter formulated with GE as 75% fat replacer, and S4: frankfurter formulated with GE as 100% fat replacer. For each parameter, results followed by the same case letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as the mean values of replications \pm SD.

3.3. Properties of Frankfurters Prepared with GE

3.3.1. Proximate Composition

Total moisture, protein, fat, and ash contents of frankfurters are shown in Table 1. The moisture content of sausages ranged from 65.81% to 59.66%, control samples showing the lowest value ($p < 0.05$). The replacement of animal fat by GE increased moisture content without significant differences ($p > 0.05$) between samples with higher substitution percentages (S2 (50% substitution), S3 (75% substitution), and S4 (100% of fat replacement)). A significant reduction in total fat content was achieved in frankfurters as the level of fat replacement (by GE) increased. The decrease in fat contents ranged from 17% to 39%, at different levels of fat replacement. Protein content varied between samples although there was not a clear pattern related to fat replacement: SC and S1 showed the highest protein content and S2 the lowest ($p < 0.05$). No differences were observed in the ash contents regardless of the fat substitution level by GE.

Table 1. Effect of partial and total replacement of pork back fat by the gelled emulsion from hemp oil and buckwheat flour, on proximate composition of frankfurters.

Sample	Moisture	Ash	Fat	Protein
SC	59.66 \pm 0.03 ^d	2.34 \pm 0.15 ^a	20.75 \pm 0.28 ^a	14.59 \pm 0.25 ^a
S1	61.65 \pm 0.56 ^c	2.15 \pm 0.07 ^a	17.20 \pm 0.32 ^b	14.17 \pm 0.19 ^{a,b}
S2	64.94 \pm 0.03 ^{a,b}	2.14 \pm 0.14 ^a	17.02 \pm 0.63 ^b	12.61 \pm 0.10 ^d
S3	64.87 \pm 0.10 ^b	3.11 \pm 1.47 ^a	14.78 \pm 0.09 ^c	13.48 \pm 0.22 ^{bc}
S4	65.81 \pm 0.02 ^a	2.39 \pm 0.04 ^a	12.69 \pm 0.10 ^d	13.41 \pm 0.15 ^c

Results are expressed as g/100 g. SC: frankfurter without GE; S1: frankfurter formulated with GE as 25% fat replacer; S2: frankfurter formulated with GE as 50% fat replacer; S3: frankfurter formulated with GE as 75% fat replacer, and S4: frankfurter formulated with GE as 100% fat replacer. For each parameter, results followed by the same case letter (a–d) are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as the mean values of replications \pm SD.

Regarding proximate composition, it is important to highlight that interesting fat reduction levels can be achieved using this GE as fat replacement in frankfurters, which

could contribute to decreasing the energy value of this type of meat product, according to new consumer trends. Similar results of fat content reduction have been reported by other authors in different types of meat product (beef patties, hot-dog style sausages or chicken sausages) applying GEs as partial animal fat replacement [21–23].

3.3.2. Lipid Profile and Nutritional Parameters

The impact of pork back fat replacement by GE on the lipid profile and nutritional parameters of frankfurters is shown in Table 2. It is known that vegetable oils are rich in unsaturated fatty acids, both monounsaturated (MUFAs) and polyunsaturated (PUFAs). As expected, significant differences ($p < 0.05$) were detected in the fatty acid profile of frankfurters depending on the pork back fat substitution level. In control samples (SC) 21 fatty acids were identified, among which palmitic (C16:0), oleic (C18:1), and linoleic fatty acids (C18:2) make up approximately 80% of the total fat. Together with stearic acid (C18:0), this figure rises to 92% of the total fatty acids, which is in accord with the fatty acid composition of pork back fat reported by other authors [4]. Regarding these four fatty acids, the substitution of pork back fat by GE in frankfurters resulted in a decrease in oleic, palmitic and stearic fatty acids together with an increase in linoleic fatty acid. In addition, to rise to more than 90% of the total fat, it is necessary to add the amount of other fatty acid, linolenic acid (C18:3). All these changes in fatty acid composition are higher as fat substitution levels increase. At the highest fat substitution level (S4), linoleic acid showed the highest percentage, followed by oleic, and linolenic acid (without differences between them, $p > 0.05$), palmitic acid and stearic acid.

Table 2. Effect of partial and total replacement of pork back fat by the gelled emulsion from hemp oil and buckwheat flour, on lipid profile and nutritional parameters of frankfurters.

Fatty Acids (%)	SC	S1	S2	S3	S4
C6:0	0.01 ± 0.00 aL	0.01 ± 0.00 aM	0.02 ± 0.00 aM	0.02 ± 0.00 aN	0.02 ± 0.00 aN
C8:0	0.02 ± 0.00 aL	0.03 ± 0.00 aM	0.03 ± 0.00 aM	0.04 ± 0.00 aN	0.03 ± 0.00 aN
C10:0	0.06 ± 0.01 aK	0.06 ± 0.01 aL	0.05 ± 0.00 bL	0.04 ± 0.00 cN	0.02 ± 0.00 dN
C12:0	0.08 ± 0.00 aK	0.07 ± 0.00 aL	0.06 ± 0.00 bL	0.05 ± 0.00 cN	0.02 ± 0.00 dN
C13:0	ND	ND	ND	ND	0.02 ± 0.00 N
C14:0	1.27 ± 0.02 aE	1.13 ± 0.02 bG	0.95 ± 0.02 cG	0.67 ± 0.01 dJ	0.37 ± 0.00 eJ
C15:0	0.07 ± 0.00 aK	0.06 ± 0.01 aL	0.05 ± 0.01 bL	0.05 ± 0.00 bN	0.03 ± 0.00 cN
C16:0	22.92 ± 0.03 aB	21.10 ± 0.05 bB	18.26 ± 0.21 cC	14.39 ± 0.09 dC	9.96 ± 0.13 eC
C16:1trans	0.42 ± 0.00 aG	0.35 ± 0.01 bJ	0.26 ± 0.01 cJ	0.22 ± 0.00 dL	0.14 ± 0.00 eL
C16:1cis	2.21 ± 0.01 aD	1.96 ± 0.05 bF	1.53 ± 0.01 cF	1.04 ± 0.00 dH	0.54 ± 0.01 eI
C17:0	0.38 ± 0.01 aG	0.36 ± 0.01 bJ	0.31 ± 0.00 cJ	0.25 ± 0.00 dL	0.17 ± 0.00 eL
C17:1	0.34 ± 0.00 aH	0.31 ± 0.00 aJ	0.25 ± 0.02 abJ	0.19 ± 0.00 bM	0.07 ± 0.04 cM
C18:0	10.94 ± 0.04 aC	10.36 ± 0.03 bD	9.47 ± 0.02 cD	7.07 ± 0.02 dE	4.44 ± 0.07 eD
C18:1 cis	46.71 ± 0.10 aA	40.45 ± 0.55 bA	32.38 ± 0.08 cA	23.53 ± 0.10 dB	15.56 ± 0.44 eB
C18:1 trans	0.10 ± 0.00 bJ	0.09 ± 0.00 bL	0.07 ± 0.00 bL	1.34 ± 0.00 aG	0.92 ± 0.16 aG
C18:2 (n-6)	11.56 ± 0.01 eC	17.51 ± 0.38 dC	25.54 ± 0.13 cB	35.09 ± 0.01 bA	45.62 ± 0.06 aA
C18:2(n-3)	0.09 ± 0.01 eJ	0.65 ± 0.04 dH	1.45 ± 0.02 cF	2.31 ± 0.00 bF	3.30 ± 0.00 aE
C18:3 (n-3)	0.68 ± 0.01 eF	3.29 ± 0.18 dE	6.79 ± 0.17 cE	10.81 ± 0.00 bD	15.38 ± 0.00 aB
C18:3 (n-6)	ND	0.22 ± 0.02 dK	0.50 ± 0.03 cH	0.84 ± 0.00 bI	1.22 ± 0.01 aF
C20:0	0.20 ± 0.00 eI	0.31 ± 0.00 dJ	0.46 ± 0.01 cH	0.61 ± 0.00 bJ	0.75 ± 0.02 aH
C20:1	1.10 ± 0.03 aE	0.95 ± 0.01 bG	0.84 ± 0.01 cG	0.67 ± 0.00 dJ	0.55 ± 0.00 eI
C20:2	0.54 ± 0.01 aF	0.47 ± 0.00 bI	0.40 ± 0.01 cI	0.32 ± 0.00 dK	0.24 ± 0.01 eK
C20:3	0.31 ± 0.01 aH	0.30 ± 0.00 abJ	0.27 ± 0.00 abJ	0.25 ± 0.01 bcL	0.20 ± 0.01 cK
C20:5	ND	ND	0.15 ± 0.00 cK	0.24 ± 0.00 bL	0.32 ± 0.01 aJ
C24:0	ND	ND	ND	ND	0.20 ± 0.03 K
Σ n-3	0.98 ± 0.02 eG	3.59 ± 0.03 dG	7.21 ± 0.07 cD	11.30 ± 0.17 bE	15.90 ± 0.07 aD
Σ n-6	12.19 ± 0.05 dE	18.85 ± 0.08 dE	27.89 ± 0.11 cC	38.56 ± 0.01 bC	50.39 ± 0.05 aC
n-6/n-3 ratio	12.39 ± 0.01 aE	5.25 ± 0.02 bF	3.87 ± 0.01 cE	3.41 ± 0.03 dG	3.17 ± 0.05 eF

Table 2. Cont.

Fatty Acids (%)	SC	S1	S2	S3	S4
Σ SFA	35.96 ± 0.04 ^{aC}	33.49 ± 0.06 ^{bC}	29.66 ± 0.01 ^{cC}	23.17 ± 0.00 ^{dD}	16.03 ± 0.01 ^{eD}
Σ UFA	64.05 ± 0.03 ^{eA}	66.55 ± 0.21 ^{dA}	70.42 ± 0.24 ^{cA}	76.85 ± 0.07 ^{bA}	84.06 ± 0.22 ^{aA}
Σ MUFA	50.88 ± 0.02 ^{aB}	44.10 ± 0.04 ^{bB}	35.33 ± 0.09 ^{cB}	26.98 ± 0.04 ^{dD}	17.78 ± 0.18 ^{eD}
Σ PUFA	13.17 ± 0.01 ^{eD}	22.44 ± 0.02 ^{dD}	35.10 ± 0.06 ^{cB}	49.87 ± 0.02 ^{bB}	66.28 ± 0.13 ^{aB}
Σ PUFA/ Σ SFA	0.37 ± 0.01 ^{eH}	0.67 ± 0.03 ^{dI}	1.18 ± 0.02 ^{cF}	2.15 ± 0.07 ^{bG}	4.13 ± 0.02 ^{aF}
AI *	0.44 ± 0.01 ^{aH}	0.39 ± 0.00 ^{bJ}	0.31 ± 0.01 ^{cG}	0.22 ± 0.00 ^{dH}	0.14 ± 0.00 ^{eG}
TI *	1.02 ± 0.04 ^{aG}	0.77 ± 0.01 ^{bI}	0.54 ± 0.01 ^{cG}	0.33 ± 0.00 ^{dH}	0.18 ± 0.00 ^{eG}
h/H *	2.48 ± 0.03 ^{eF}	2.83 ± 0.04 ^{dH}	3.52 ± 0.04 ^{cE}	4.96 ± 0.02 ^{bF}	8.01 ± 0.07 ^{aE}

Results are expressed as g/100g. ND: not detected. SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. * AI (atherogenic index), TI (thrombogenic index), h/H (hypocholesterolemic/hypercholesterolemic index). A lower-case letter (a–e) refers to the comparison of the same fatty acid between the different oil samples, while an upper-case letter (A–N) refers to the comparison of the different fatty acids in the same sample; results followed by the same lower/upper-case letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as the mean values of replications ± SD.

The replacement of animal fat by GE reduced ($p < 0.05$) the amount of saturated fatty acids (SFAs) in frankfurters depending on the substitution level. SC formulated with pork back fat contained the highest amount of SFAs (35.96%), mainly palmitic acid (C16:0) and stearic acid (C18:0). On the other hand, S4 showed the lowest amount of SFAs (16.03%) (palmitic acid and stearic acid) which means a 60% reduction in SFAs. This is due to the acid profile of GE that showed oleic acid (C18:1) as the major MUFA, and linoleic and linolenic acids as the major PUFAs [15]. MUFA content in frankfurters decreased from 50.88% to 17.78% ($p < 0.05$), depending on the fat substitution level. As a result, PUFA content of frankfurters with GE (S1, S2, S3, and S4) increased ($p < 0.05$) as fat substitution level increased. This increase was mainly due to the linoleic acid content that increased from 11.56% (SC) to 45.62% (S4), which means an increase of 75%. In addition, the increase in α -linolenic acid must be noticed (from 0.68% in SC to 15.38% in frankfurters with the highest fat substitution level, S4), which means an increase of 96%. Moreover, according to European regulations [40], S3 and S4 samples could be assigned as sausages with “reduced in saturated fat” as they reached a reduction of more than 25%, while all GE added samples could be labeled with the nutritional claim as “high n-3 fatty acids”, since they contained more than 0.6 g α -linolenic acid per 100 g of the product (1–1.95 g α -linolenic acid per 100 g of frankfurter).

Both the PUFA/SFA and the n-6/n-3 fatty acid ratios are widely studied nutritional parameters because of their association with cardiovascular diseases. It is recommended that the PUFA/SFA ratio be above 0.4 and the n-6/n-3 ratio below 4 [41]. While all reformulated frankfurters (S1, S2, S3, and S4) fulfilled the PUFA/SFA recommendation, only S2, S3 and S4 samples (50, 75, and 100% pork back fat substitution level, respectively) also fulfilled the n-6/n-3 ratio. By contrast, control sample does not fulfill any requirement regarding these ratios.

The atherogenic index (AI) and thrombogenic index (TI) are significant parameters to describe possible healthier appeal in meat products [16]. The appearance of various diseases is associated with high values of these indexes [13]. The influence of pork back fat replacement in these parameters was positive considering that both parameters decreased ($p < 0.05$) as pork back fat substitution level increased, indicating a reduction in these cardiovascular risk factors. On the other hand, the hypocholesterolemic/hypercholesterolemic index (h/H) also showed a healthy trend depending on the pork back fat substitution level, because it has been described that the better is the highest. As can be seen in Table 2, this index increased as pork back fat substitution level increased, S4 samples (100% substitution) showing the highest value ($p < 0.05$).

3.3.3. pH and Water Activity

pH and water activity values of frankfurters are shown in Table 3. All frankfurters showed pH values in the range considered normal for this type of cooked sausages [2,12,19] with slight differences between some. The substitution of animal fat by GE increased pH values ($p < 0.05$) although this increase was only significant at low substitution level ($p < 0.05$). Several authors have associated higher pH values in cooked sausages with vegetable oil addition, although in other cases this relation was not significant [4]. In spite of the statistical differences between some formulations, these variations do not affect the final quality of the product.

Table 3. Effect of partial and total replacement of pork back fat by the gelled emulsion, from hemp oil and buckwheat flour, on pH and water capacity of frankfurters.

Sample	pH	Aw
SC	5.88 ± 0.01 ^c	0.947 ± 0.003 ^c
S1	5.94 ± 0.01 ^b	0.953 ± 0.001 ^{bc}
S2	6.09 ± 0.01 ^a	0.956 ± 0.002 ^{bc}
S3	5.91 ± 0.01 ^{bc}	0.960 ± 0.003 ^b
S4	5.90 ± 0.01 ^c	0.969 ± 0.001 ^a

SC: frankfurter without GE; S1: frankfurter formulated with GE as 25% fat replacer; S2: frankfurter formulated with GE as 50% fat replacer; S3: frankfurter formulated with GE as 75% fat replacer, and S4: frankfurter formulated with GE as 100% fat replacer. For each parameter, results followed by the same case letter (a–c) are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as the mean values of replications ± SD.

For Aw values, the highest value was found for samples with 100% of fat replacement (S4) and the lowest for control samples (SC) ($p < 0.05$), increasing Aw values depending on the fat substitution level. In any case, the highest Aw value, which was obtained for S4 samples, is in the range of normal Aw values reported for cooked sausages [2,42]. So the pork back fat substitution by GE in frankfurters would not result in a problem related to frankfurter shelf-life.

3.3.4. Color

Color is one of the most studied physical properties in meat products and its objective measurement allows the evaluation of the immediate impact of pork back fat substitution on frankfurters. The color parameters of frankfurters are shown in Table 4. All color parameters were affected ($p < 0.05$) depending on the percentage of fat substitution, all showing a clear behavior (increase or decrease) except for lightness: control frankfurters (SC) and samples with the highest fat substitution level (S4) showed similar ($p < 0.05$) L* values, decreasing toward intermediate pork back fat substitution levels ($p < 0.05$). However, it is important to highlight that the differences showed for L* values between samples were really small (1–2 units) and so without technical importance.

Table 4. Effect of partial and total replacement of pork back fat by the gelled emulsion from hemp oil and buckwheat flour on color parameters of frankfurters.

Sample	L*	a*	b*	C*	h	ΔE*
SC	72.7 ± 0.63 ^a	3.42 ± 0.35 ^a	9.15 ± 0.24 ^e	9.77 ± 0.30 ^e	69.53 ± 1.71 ^e	-
S1	70.42 ± 0.87 ^{bc}	2.88 ± 0.19 ^b	11.32 ± 0.42 ^d	11.68 ± 0.44 ^d	75.70 ± 0.58 ^d	3.30 ± 0.47 ^d
S2	69.53 ± 1.29 ^c	2.80 ± 0.25 ^b	13.06 ± 1.72 ^c	13.36 ± 1.71 ^c	77.72 ± 1.46 ^c	5.36 ± 1.27 ^c
S3	70.99 ± 0.90 ^b	1.21 ± 0.47 ^c	15.23 ± 0.31 ^b	15.29 ± 0.30 ^b	85.45 ± 1.79 ^b	6.77 ± 0.24 ^b
S4	72.18 ± 0.27 ^a	0.38 ± 0.16 ^d	17.01 ± 0.33 ^a	17.01 ± 0.33 ^a	88.73 ± 0.54 ^a	8.45 ± 0.33 ^a

SC: frankfurter without GE; S1: frankfurter formulated with GE as 25% fat replacer; S2: frankfurter formulated with GE as 50% fat replacer; S3: frankfurter formulated with GE as 75% fat replacer, and S4: frankfurter formulated with GE as 100% fat replacer. For each parameter, results followed by the same case letter (a–e) are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as the mean values of replications ± SD.

On the other hand, redness values decreased ($p < 0.05$), and yellowness, chroma, and hue values increased ($p < 0.05$) as fat substitution levels increase. This behavior has been previously reported in cooked sausages due to the replacement of pork back fat by several vegetable oils [4,16,19]. The pigment content in these vegetable oils has been attributed as the main reason for this. As the substitution percentage increases, the contribution of red pigments (myoglobin and other derivatives) to the final color decreases, resulting in lower a^* and higher b^* values in frankfurters. In addition, it has been reported that hemp oil has a high content in total chlorophylls (up to 57.66 mg/kg) and carotenes (up to 146.80 mg/kg) [43], and both pigments would be responsible for this increase in b^* values. In this case, it is also clear that the behavior of color saturation (C^*) is dependent only on the b^* evolution.

Nevertheless, taking into consideration the color differences (ΔE^*) with respect to control frankfurter (SC), it could be said that all show differences easily detected by the human eye (>3 units; Martínez et al. [44]). These color differences would be expected because of the GE color, which could be defined as greenish and yellowish, mainly because of the color of the hemp oil and buckwheat flour. In any case, the color of the frankfurters with GE, although different from the control, is still a typical color of frankfurter-like sausages, for example of the Bratwurst type, and so it should not be a reason to reject them.

3.3.5. Texture

Texture is a considerable factor for consumers of meat products and one of the quality parameters generally most affected due to the replacement of pork back fat (solid) by vegetal oils (liquid). To avoid this problem, GE has been developed in order to retain solid-like properties allowing oil stabilization and structuring [14,15,20,24]. The results of texture profile analysis are given in Table 5. Adhesiveness, cohesiveness, and chewiness did not differ between formulations ($p > 0.05$). Hardness and springiness were affected ($p < 0.05$) by the substitution of pork back fat by GE. In the case of hardness, this effect was only significant at the highest substitution level (S4; 100%) which showed the lowest value ($p < 0.05$). This could be related to the chemical composition because increased moisture together with decreased fat makes the final product less dense [45]. Only springiness in S2 sample (0.34 ± 0.02) differed ($p < 0.05$) from the control (0.26 ± 0.02) and, although the result was significant, quantitatively this effect was very small. Controversial results in textural properties have been reported in cooked sausages due to the partial/total pork back fat replacement by GEs. In most of the cases hardness was the property most affected, but sometimes this was greater and at other times reduced [10,11,13,14]. So it could be concluded that differences in texture properties may be related to the specific characteristics of each GE and its impact in the meat matrix.

Table 5. Effect of partial and total replacement of pork back fat by the gelled emulsion from hemp oil and buckwheat flour, on texture profile of frankfurters.

Sample	Hardness (N)	Adhesiveness (N s)	Springiness (mm)	Cohesiveness	Chewiness (N mm)
SC	87.11 ± 8.86^{ab}	-0.67 ± 0.34^a	0.26 ± 0.02^b	0.78 ± 0.04^a	17.36 ± 2.12^a
S1	93.51 ± 9.73^a	-0.72 ± 0.36^a	0.29 ± 0.04^{ab}	0.75 ± 0.03^a	20.83 ± 4.45^a
S2	83.91 ± 11.22^{ab}	-0.52 ± 0.32^a	0.34 ± 0.02^a	0.75 ± 0.02^a	21.37 ± 3.71^a
S3	89.86 ± 20.73^{ab}	-0.49 ± 0.32^a	0.33 ± 0.05^{ab}	0.76 ± 0.03^a	22.82 ± 7.66^a
S4	63.22 ± 9.72^b	-0.91 ± 0.34^a	0.25 ± 0.04^b	0.81 ± 0.02^a	12.92 ± 3.27^a

SC: frankfurter without GE; S1: frankfurter formulated with GE as 25% fat replacer; S2: frankfurter formulated with GE as 50% fat replacer; S3: frankfurter formulated with GE as 75% fat replacer, and S4: frankfurter formulated with GE as 100% fat replacer. For each parameter, results followed by the same case letter (a,b) are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as the mean values of replications \pm SD.

3.3.6. Lipid Oxidation

As can be seen in Figure 2, all samples showed a low lipid oxidation level, without differences between them ($p > 0.05$). All samples showed TBARS values ranging from 0.09 to 0.29 mg MA/ Kg of sample, lower than the rancidity detection limit (>1.0 mg MA/Kg of sample, Verna & Sahoo [46]). This is a very positive result taking into account that vegetable oils are more susceptible to lipid oxidation, due to their higher PUFA content. Therefore, in the case of these reformulated frankfurters (S1, S2, S3, and S4), although the amount of PUFA has increased (Table 2), this has not increased their lipid oxidation. Several authors have attributed this to the capsulated oil droplets in the gel matrix, which would act as a protective barrier against oxidation [39]. According to this positive result, even a total replacement of pork back fat by this GE would not cause a significant increase in oxidation. However, a shelf-life study would be necessary to confirm this behavior in reformulated frankfurters. Alejandre et al. [22] reported a similar trend for lipid oxidation in beef patties in which fat was totally replaced with a gel emulsion containing microalgal oil and blackthorn branch extract. They also suggested that the lower fat content of the reformulated patties could explain this lipid oxidation control.

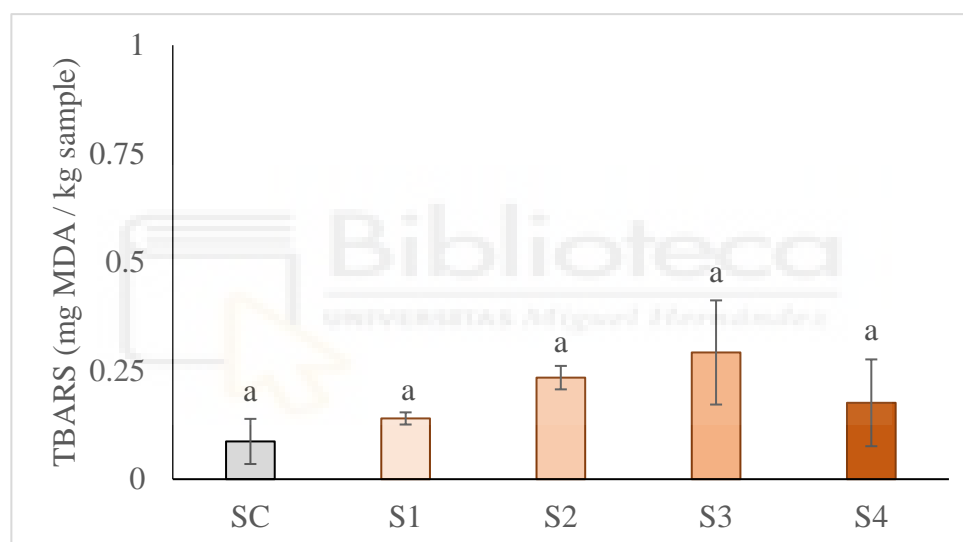


Figure 2. Effect of partial and total replacement of pork back fat by the gelled emulsion, from hemp oil and buckwheat flour, on lipid oxidation of frankfurters. SC: frankfurter without GE; S1: frankfurter formulated with GE as 25% fat replacer; S2: frankfurter formulated with GE as 50% fat replacer; S3: frankfurter formulated with GE as 75% fat replacer, and S4: frankfurter formulated with GE as 100% fat replacer. For each parameter, results followed by the same case letter are not significantly different according to Tukey's HSD post-hoc test ($p > 0.05$). Data are presented as the mean values of replications \pm SD.

3.4. Sensory Evaluation

Sensory quality should be evaluated because the modification of fatty acids in meat products is a challenging procedure. Figure 3 shows the results of the sensory quality of lipid-modified samples for color, hemp smell, hemp flavor, salty flavor, juiciness, hardness, and acceptability. Hemp flavor, juiciness, and hardness scores were similar ($p > 0.05$) for all samples. Hemp smell and salty flavor scores followed the same pattern: only S4 samples scored significantly lower ($p < 0.05$) than control. Color was the attribute influenced mostly by frankfurter reformulation; in this case, S3 and S4 samples presented lower scores ($p < 0.05$) than control and S1. The color score of S3 and S4 were significantly lower ($p < 0.05$) than control and S1 samples. So# it seems clear that the inclusion of GE (at high percentages) altered visual sensory features, which could be due to the color of the hemp oil as has been discussed in Section 3.3.4. The color and hemp smell (scores <4) are the main reasons for the lower sensory acceptance of the frankfurters with high

GE substitution percentages (S4, 100%). Other researchers using GE as partial and total substitutes for pork back fat in cooked sausages also verified that color and flavor were the attributes most rejected by consumers [47]. Most authors reported unpleasant sensory characteristics in different types of meat products in which the animal fat replacement by GE was made at high percentages [13,19,21].

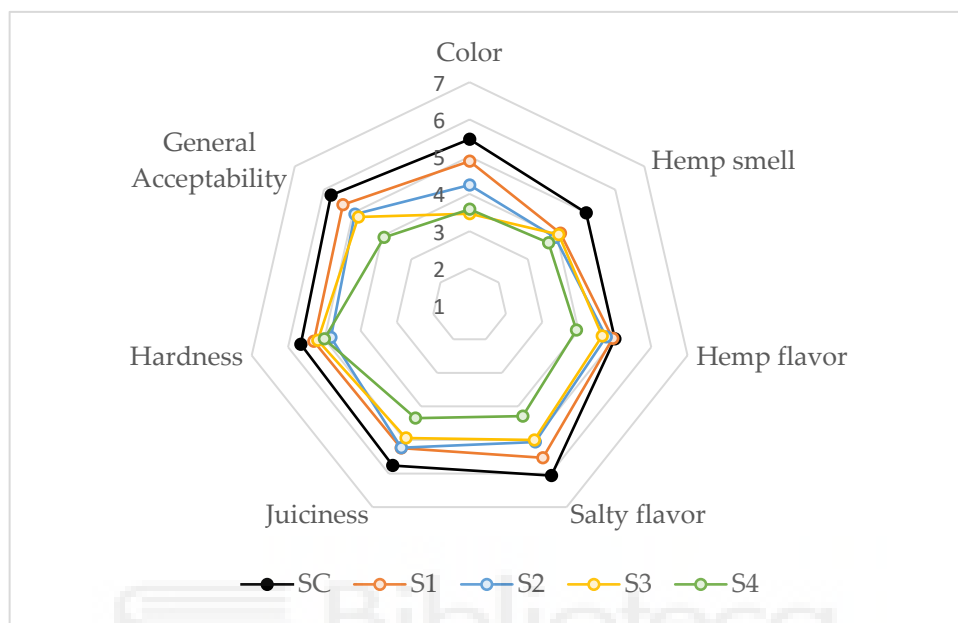


Figure 3. Effect of partial and total replacement of pork back fat by the gelled emulsion, from hemp oil and buckwheat flour, on sensory parameters. SC: frankfurter without GE; S1: frankfurter formulated with GE as 25% fat replacer; S2: frankfurter formulated with GE as 50% fat replacer; S3: frankfurter formulated with GE as 75% fat replacer, and S4: frankfurter formulated with GE as 100% fat replacer.

4. Conclusions

This research suggests that the reformulation of cooked sausages (frankfurters) using hemp oil and buckwheat flour as a gelled emulsion as pork back fat substitute is feasible and represents a viable alternative for improving nutritional composition, without adversely affecting either the technological properties or the typical appearance of the resulting product, mainly when this gelled emulsion was used as partial replacement of pork back fat. A reduction of 17%–39% of total fat was obtained with an improved lipid profile (reduction in saturated and increase in polyunsaturated fatty acids). In general, although sensorial differences were detected for frankfurters in which pork back fat was totally replaced by this GE (S4), all the others were evaluated as acceptable by panelists. However, even in the case of a total replacement, the sensory limitations in terms of flavor negative attributes could be easily overcome by reformulating the product with other spice mixtures or increasing its smoked flavor. Other than the quality aspects, these reformulation strategies did not result in higher lipid oxidation, despite the substitution by more susceptible oils. In any case, shelf-life studies would be necessary to confirm these results in reformulated frankfurters, and to assess their stability and microbiological quality during storage.

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8.6. PUBLICACIÓN 6

Innovative formulation in pâté using a gelled emulsion of hemp oil
(*Cannabis Sativa* L.) as fat replacer

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Innovative formulation in pâté using a gelled emulsion of hemp oil (*Cannabis sativa L.*) as fat replacer

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ABSTRACT

The effect of the partial replacement (10 and 20%) of animal fat by a gelled emulsion based on hemp oil and buckwheat flour (hemp-GE) in a traditional pâté was assessed. For that, the nutritional composition, physicochemical properties, lipid profile, lipid oxidation and sensory properties were evaluated. Hemp-GE had a positive effect in the reduction of cholesterol and improved the n-6/n-3 and PUFA/SFA nutritional ratios which is in line with the nutritional recommendations for the development of healthier meat products. Although reformulated pâtés resulted more susceptible to lipid oxidation and some of their physicochemical properties were modified, these changes were not sufficiently intense to diminish the sensory acceptance of the new product. Structuring healthy vegetal oils employing gelled emulsions in view of using them as partial animal fat replacers in traditional meat products seems to be a useful strategy to make them healthier.

1. Introduction

Liver pâté is a highly appreciated dish that is mostly made of liver and fat and has its roots in European gastronomy heritage (Skalecki et al., 2021). Not only does it give a rich and flavourful sensation, but it also acts as a useful supply of biologically relevant proteins, along with critical vitamins such as B1, B12, folic acid, and heme iron (Brito et al., 2006; Lucas-González et al., 2019). This nutritional profile renders liver pâtés particularly attractive, especially for individuals like children and women susceptible to iron deficiency-induced anemia, a prevalent global health concern (Lucas-González et al., 2019; Sánchez-Zapata et al., 2012). The selection and quality of the primary ingredients in the formulation determine the majority of the nutritional value of pâtés. Although the nutritional value of pâté is indisputable, the innovation of the meat industry towards the incorporation of healthier raw materials of natural origin is opening up a new field of attractive research opportunities that might lead to improved product quality as well as the encouragement of healthy eating choices (Barbut et al., 2021). The high-calorie value, high-fat content (25–40%), and unfavorable fatty acid composition (mainly saturated fatty acids typical of animal fats) mean that regular use of these products may damage the health (Lucas-González et al., 2019; Skalecki et al., 2021). Therefore, the meat

industry and the scientific community are concentrating on creative ways to produce healthier meat products with additional value, without compromising on textural and sensory aspects in order to increase the nutritional worth of this type of food (Cittadini et al., 2022). In this regard, has been reported that certain comminuted meat products (such as sausages and frankfurters) replace saturated animal fats with liquid vegetable oils (such as canola, olive, sunflower, peanut, fish) with or without the inclusion of hydrocolloid gums to alter the fatty acid content of the final product (Domínguez et al., 2017; Öztürk-Kerimoğlu et al., 2021).

The elaboration of a gelled emulsion using hemp seed oil (*Cannabis sativa L.*) and buckwheat flour as an emulsifying agent is the main topic of this study in view to enhance the nutritional profile of a traditional pâté and encourage the use of sustainable and alternative ingredients. The high protein, vitamin, mineral, and dietary fiber content of buckwheat flour (Zhou et al., 2015) is enhanced by the added hemp seed oil, which is known for its nutritional value and content in polyunsaturated fatty acids (PUFA), particularly long-chain n-3 PUFA, which are known to improve health in the neurological, immunological, and cardiovascular systems (Heck et al., 2022). The use of gelled emulsions, made with different vegetable oils and flours, as animal fat replacers in different meat products such as burgers and cooked sausages has been

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previously studied (Botella-Martínez, Viuda-Martos, et al., 2021, 2022; Hanula et al., 2022; Nacak et al., 2021). However, the combination of hemp seed oil and buckwheat flour to obtain gelled emulsions and their application in a meat product like a pâté, as fat replacement, has not been previously reported. This combination has been carefully chosen based on factors such as technical viability, physicochemical characteristics, and fatty acid content. This study aims (i) to assess the viability of using a gelled emulsion made of hemp seed oil and buckwheat flour as partial fat replacement of pâtés, and (ii) to evaluate the impact of this substitution on the chemical composition, fatty acid profiles, physicochemical properties, lipid oxidation, and sensory attributes.

2. Materials and methods

2.1. Materials

The following ingredients were used for the elaboration of the hemp seed oil gelled emulsions (Hemp-GE): water (40%), hemp seed oil (40%), buckwheat flour (15%), and gelatine “instant gel” (5%) from pork origin. The preparation of the gelled emulsion and materials employed for this elaboration was carried out according to the procedure described by Botella-Martínez, Pérez-Álvarez, et al., (2021). Briefly, water (60 °C) was mixed with instant gel and then the flour was added and mixed again. When the temperature was turned down to 37 °C, the hemp oil was gradually added. Once the GE was obtained, it was refrigerated and stored at 4 °C until its use. The meat ingredients (pork liver, dewlap, and pork fat) were purchased from a local butchery and the additives and spices were provided by an authorized supplier of food ingredients Suministros River S.L.U. (Alicante, Spain) (Table 1).

2.2. Pâté manufacturing and sampling

For pâté elaboration, three batches (1 Kg of each) were prepared: control pâté (CP), pâté with 10 % of hemp-GE as replacer of pork backfat (PGEH10), and pâté with 20% of hemp-GE as replacer of pork backfat plus 10 % of dewlap (PGEH20) (Table 1). The rest of the ingredients (liver, water, additives, and spices) used following a traditional pâté formulation were the same in the three batches and are specified in Table 1. The pâtés were elaborated in the food pilot plant of the Miguel Hernández University in the Polytechnic School of Orihuela (Orihuela, Alicante, Spain). The pâté processing as well as packaging and preservation methods employed were identical to those utilized in the study conducted by Lucas-González et al. (2019). Once the desired temperature was reached, the samples were chilled in ice/water for subsequent analysis. The entire process was conducted in triplicate.

Table 1

Formulation of pâtés with hemp oil gelled emulsion as partial animal fat replacer.

Ingredients	CP	PGEH10	PGEH20
Dewlap (%)	65	65	55
Liver (%)	25	25	25
Backfat (%)	10	0	0
Hemp-GE (%)	0	10	20
Water (%)	15	15	15
Salt (%)	2	2	2
Caseinate (%)	1	1	1
Polyphosphates (mg/Kg)	300	300	300
Nitrite (mg/Kg)	125	125	125
White pepper (%)	0.05	0.05	0.05
Nutmeg (%)	0.03	0.03	0.03
Thyme (%)	0.03	0.03	0.03

Percentages of non-meat ingredients are related to 100% meat block (dewlap, liver and backfat). CP: control pâté; PGEH10: pâté with 10% hemp oil gelled emulsion as animal fat substitute; PGEH20: pâté with 20% hemp oil gelled emulsion as animal fat substitute.

2.3. Total expelled fluid of raw pâté

The emulsion stability of the pâtés (pre-cooking) was assessed using the total expressible fluid (TEF) method as outlined by Pintado et al. (2015), with minor adjustments. Samples were centrifuged in 15 mL centrifuge tubes at 3000 rpm for 1 min. Subsequently, they underwent heating in a water bath at 70 °C for 30 min, followed by cooling to room temperature. Afterward, the samples were centrifuged again for 3 min at 3000 rpm. The expressible fluid (comprising fat and water) was allowed to drain onto filter paper by placing the tubes upside down. Each sample was analyzed in triplicate, and the results were presented in grams of total fluid expelled per 100 g of the sample. The calculation was based on the following expression (Equation (1)):

$$\%TEF = \frac{\text{Weight of tube with sample} - \text{Weight of tube with pellet}}{\text{Weight of sample}} \times 100 \quad (\text{Eq. 1})$$

2.4. Chemical composition

Fat, protein, ash, and moisture content were determined according to AOAC methods (AOAC, 2000). Heme iron was determined by a spectrometric method with acidified acetone and water as reagents (Sánchez-Zapata et al., 2013). All assays were taken on three samples of each formulation. Total cholesterol was determined by HPLC using 2-propanol and hexane (2:98) in mode isocratic (1 mL/min) with a photodiode array detector (DAD) at 208 nm, following the procedures described by Domínguez et al. (2016). The analysis of mineral content was made using a MS-2030 (Shimadzu, Tokyo, Japan) inductively coupled plasma mass spectrometry (ICP-MS).

2.5. Fatty acids composition, nutritional parameters, and health indices analysis

The method described by Folch et al. (1957) was applied for the lipid extraction from the pâté samples. The fatty acids were transesterified following the method reported by Domínguez et al. (2015). As an internal standard, the nonadonic acid (C19:0) at 0.3 mg/mL was added to the samples before methylation. Results regarding FAME composition were expressed in g/100 g of fat.

The nutritional parameters refer to the sum of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), the sum of omega 3 (n-3) and omega 6 (n-6) fatty acids. Health indices including n-6 and n-3 ratio, PUFA, and SFA ratio were calculated. The index of atherogenicity (AI) and the thrombogenicity index (TI) were calculated following the formula described by Ulbricht and Southgate (1991). The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated as described by Fernández et al. (2007).

2.6. Physico-chemical analysis

The pH of pâté samples was determined using a Crison micro pH-meter model GLP 21 equipped with a penetrating electrode (Crison Instrument S.A., Barcelona, Spain). The water activity was assessed using a Novasina TH-500 analyzer (Novasina, Axair LTD., Pfaeffikon, Switzerland). Color analysis of pâté samples was determined on their surfaces utilizing a Minolta spectrophotometer Model CM-700 with 10° observer angle and D65 illumination conditions (Konica Minolta Sensing, Inc., Tokyo, Japan). CIEL*a*b* color space was used to obtain the color coordinates (L*-lightness, a*- red/green coordinate and b*-yellow/blue coordinate) from which hue (H*) and Chroma (C*) were calculated. The total color differences (ΔE) for each reformulated pâté relative to the control sample were determined with the following Equation (2).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (\text{Eq. 2})$$

2.7. Texture analysis

For the texture analysis of the pâtés a TA.XT2i universal texture analyzer (Stable Microsystems, United Kingdom) equipped with a measuring head having a maximum pressure force of 5 kg and a conical plexiglass sensor designed for spreadability testing (HDP/SR, Stable Microsystems, United Kingdom) was used. The analysis involved a 45° angle formed by the cone base and slant height, with the measuring head moving at a speed of 3.0 mm/s. The spreadability test had two phases, recording force over time at a rate of 200 measurement points per second. In the first phase, the upper cone was immersed in the test sample within a fixed inverted cone-shaped container to a depth of 23 mm. The maximum force recorded, termed firmness, and the area under the curve, representing the total force for the initial phase, termed spreadability, were measured. In the subsequent phase, the head moved in the opposite direction, with the maximum force recorded as firmness and the area under the curve representing the work of shear. Measurements were conducted at a temperature of 18 ± 2 °C, and texture parameters were determined based on five sample replicates.

2.8. Lipid oxidation

The assessment of lipid oxidation in pâté samples through the thiobarbituric acid reactive substances (TBARS) assay involved extracting malondialdehyde (MDA) following the method described by Rosmini et al. (1996). The obtained results were quantified and expressed as milligrams of MDA per kilogram of sample.

2.9. Sensory assessment

Seventy panellists aged between 20 and 65 years old selected from staff and students of the Polytechnic School of Orihuela (Miguel Hernández University) carried out the sensory assessment of the pâté samples. Before starting the analyses, each subject was informed about the specific characteristics of the product to be tasted and about what the analysis would consist of, and a written informed consent was signed by the participants. This project was approved by the Responsible Research Office of the Miguel Hernández University (OIR- Reg. 211019105733, Ref. PRL. DTA.MVM.02.21, UMH, Elche, Alicante, Spain). The test took place in the tasting room of Miguel Hernández University (Orihuela, Alicante, Spain). Three slices of pâté (1 cm thickness approx.), once from each batch, were provided to each panelist at room temperature. They were asked to rate their preference for general appearance, color, brightness, overall odor, rancidity, spreadability, juiciness, cohesiveness, hardness, and overall flavor. At the end, the panellists were asked about the overall acceptance of the product they were testing. Preference was expressed on a 7-point hedonic scale ranging from “dislike extremely” (1) to “like extremely” (7).

2.10. Statistics analysis

The entire process, encompassing the development of hemp seed oil emulsion (Hemp-GE) and the preparation of liver pork pâté samples, was replicated three times (3 independent batches). Repetitions were conducted on different production days, and each batch underwent triplicate analyses. Data analysis employed SPSS software (version 24.0, SPSS Inc., Chicago, USA) selecting one-way analysis of variance (ANOVA) and Tukey-b post-hoc tests at a 5% significance level ($p < 0.05$).

3. Results and discussion

3.1. Emulsion stability of raw pâté dough

The stability of the emulsion was assessed on raw dough before the samples were stuffed and cooked, and the results are shown in Fig. 1. The substitution of animal fat by the Hemp-GE resulted in an increasing amount of total liquid expelled (lower emulsion stability), higher at higher substitution levels ($p < 0.05$). Control pâtés showed TEF values similar to those reported by other authors (Gómez-Estaca et al., 2019). It should be noted that these values were higher than those reported as normal for cooked sausages (0.9–1.7%) (Botella-Martínez, Viuda-Martos, et al., 2021; Goemaere et al., 2021), which could be due to the behavior (lower emulsion capacity) of two specific ingredients used in pâté elaboration (liver and dewlap). In addition, the substitution of animal fat by the Hemp-GE resulted in a 150% increase in TEF for PGEH10 and 310% for PGEH20. Depending on the ingredients used as fat substitutes, the stability of meat emulsion could be increased, decreased or not modified (Botella-Martínez, Viuda-Martos, et al., 2021; Momchilova et al., 2023). In general, the higher the amount of protein in the meat matrix, the higher the emulsion stability, mainly due to its effect on the gel/emulsion matrix. In addition, when the ingredients used for the fat replacement have high WHC or/and OHC, the emulsion stability was improved (Fernández-Martín et al., 2009; Sánchez-Zapata et al., 2013). The relation SFA/UFA has been also proposed as a significant factor in the emulsion stability; it has been pointed out that a higher UFA content (with a lower melting point than saturated fats) may reduce emulsion stability (Martin et al., 2008). In the case of pâté, Delgado-Pando et al., 2011 reported significant increases in TEF in both, low-fat pâtés and pâtés in which the animal fat was replaced by O/W made with olive, linseed, and fish oils. In both cases, the TEF values reported (10.5% and 11.9%) are in line with our values. These authors also reported significant reductions in these TEF values with the addition of konjac gel. Gómez-Estaca et al. (2019) also reported that the replacement of animal fat by ethyl cellulose and beeswax oleogels increased the total fluid loss (%) of reformulated pâtés respect to control. In our case, the high emulsion stability found in control pâté could be associated with its highest protein control (Table 2) and saturated fatty acids content (Table 3).

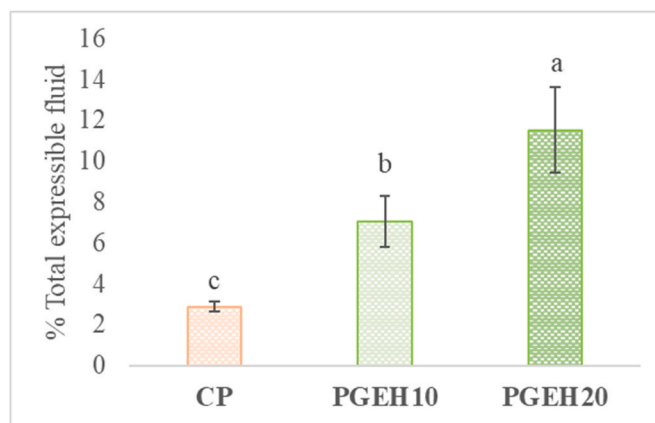


Fig. 1. Total expressible fluid of raw pâté with hemp oil gelled emulsion as partial animal fat replacer

CP: control pâté; PGEH10: pâté with 10% hemp oil gelled emulsion as animal fat substitute; PGEH20: pâté with 20% hemp oil gelled emulsion as animal fat substitute.

Data are presented as mean \pm SD. Different letters (a–c) indicate statistically significant differences as determined by Tukey's HSD post-hoc test ($p < 0.05$).

Table 2

Chemical composition of pâté with hemp oil gelled emulsion as partial animal fat replacer.

	CP	PGEH10	PGEH20
Proximate composition (%)			
Moisture	55.14 ± 0.34 ^a	52.73 ± 0.62 ^b	52.28 ± 0.22 ^b
Ash	2.41 ± 0.02 ^a	2.22 ± 0.02 ^b	2.17 ± 0.04 ^b
Fat	23.83 ± 0.09 ^b	29.10 ± 0.52 ^a	28.32 ± 0.78 ^a
Protein	13.35 ± 0.78 ^a	10.89 ± 0.03 ^b	10.28 ± 0.09 ^b
Mineral profile (mg/100g)			
Sodium	797.18 ± 17.16 ^a	824.25 ± 12.26 ^a	834.09 ± 3.18 ^a
Potassium	142.78 ± 4.78 ^a	121.81 ± 7.79 ^a	132.98 ± 1.75 ^a
Calcium	17.88 ± 0.10 ^a	17.48 ± 0.55 ^a	15.38 ± 0.06 ^b
Magnesium	12.75 ± 1.13 ^a	11.66 ± 0.66 ^a	13.92 ± 0.26 ^a
Iron	2.92 ± 0.01 ^a	3.05 ± 0.08 ^a	3.08 ± 0.06 ^a
Zinc	2.06 ± 0.05 ^a	1.86 ± 0.02 ^a	1.86 ± 0.03 ^a
Copper	0.16 ± 0.00 ^a	0.16 ± 0.00 ^a	0.17 ± 0.00 ^a
Manganese	0.09 ± 0.00 ^b	0.10 ± 0.00 ^b	0.16 ± 0.01 ^a
Heme iron (mg/100g)	2.36 ± 0.14 ^a	2.43 ± 0.16 ^a	2.49 ± 0.11 ^a
Cholesterol (mg/100g)	69.29 ± 3.30 ^a	58.01 ± 0.31 ^b	60.61 ± 0.85 ^b

CP: control pâté; PGEH10: pâté with 10% hemp oil gelled emulsion as animal fat substitute; PGEH20: pâté with 20% hemp oil gelled emulsion as animal fat substitute.

Data are presented as mean ± SD. Different letters (a-b) in the same row indicate statistically significant differences as determined by Tukey's HSD post-hoc test ($p < 0.05$).

Table 3

Lipid profile (g/100 g fatty acids), nutritional parameters and health indices of control and reformulated pâtés.

	CP	PGEH10	PGEH20
C14:0	1.38 ± 0.06 ^a	1.32 ± 0.00 ^a	1.07 ± 0.01 ^b
C16:0	23.95 ± 0.23 ^a	22.06 ± 0.06 ^b	18.48 ± 0.11 ^c
C16:1n-7	2.55 ± 0.11 ^a	2.44 ± 0.00 ^a	1.95 ± 0.02 ^b
C17:0	0.67 ± 0.01 ^a	0.59 ± 0.00 ^b	0.50 ± 0.00 ^c
C17:1n-7	0.57 ± 0.01 ^a	0.51 ± 0.00 ^b	0.42 ± 0.00 ^c
C18:0	12.20 ± 0.31 ^a	10.49 ± 0.02 ^b	8.91 ± 0.03 ^c
C18:1n-9	40.02 ± 0.31 ^a	37.63 ± 0.03 ^b	31.78 ± 0.12 ^c
C18:1n-7	3.18 ± 0.03 ^a	2.99 ± 0.00 ^b	2.47 ± 0.02 ^c
C18:2n-6	10.91 ± 0.26 ^c	15.82 ± 0.01 ^b	18.62 ± 0.02 ^a
C20:0	0.15 ± 0.01 ^c	0.18 ± 0.00 ^b	0.22 ± 0.00 ^a
C20:1n-9	0.90 ± 0.03 ^a	0.81 ± 0.00 ^b	0.70 ± 0.01 ^c
C20:2n-6	0.57 ± 0.01 ^a	0.52 ± 0.00 ^a	0.45 ± 0.00 ^b
C20:4n-6	0.82 ± 0.04 ^a	0.72 ± 0.01 ^b	0.61 ± 0.01 ^c
C18:3n-6	0.04 ± 0.00 ^b	0.10 ± 0.00 ^a	0.15 ± 0.01 ^a
C18:3n-3	0.51 ± 0.02 ^c	2.23 ± 0.00 ^b	3.56 ± 0.01 ^a
C20:2n-6	0.57 ± 0.01 ^a	0.52 ± 0.00 ^a	0.45 ± 0.00 ^b
C20:3n-6	0.14 ± 0.00 ^a	0.12 ± 0.00 ^a	0.10 ± 0.00 ^a
Σ SFA	38.82 ± 0.18 ^a	35.07 ± 0.05 ^b	29.54 ± 0.11 ^c
Σ MUFA	47.60 ± 0.25 ^a	44.74 ± 0.02 ^c	46.36 ± 0.11 ^b
Σ PUFA	13.59 ± 0.32 ^c	20.19 ± 0.04 ^b	24.09 ± 0.02 ^a
Σ n3	0.78 ± 0.02 ^c	2.53 ± 0.01 ^b	3.83 ± 0.01 ^a
Σ n6	12.81 ± 0.30 ^c	17.66 ± 0.02 ^b	20.27 ± 0.00 ^a
Σ PUFA/ Σ SFA	0.35 ± 0.01 ^c	0.58 ± 0.00 ^b	0.82 ± 0.00 ^a
n6/n3	16.33 ± 0.11 ^a	6.97 ± 0.02 ^b	5.30 ± 0.02 ^c
AI	0.48 ± 0.01 ^a	0.42 ± 0.00 ^b	0.32 ± 0.00 ^c
TI	1.15 ± 0.01 ^a	0.87 ± 0.00 ^b	0.63 ± 0.00 ^c
h/H	2.24 ± 0.03 ^a	2.60 ± 0.01 ^b	2.98 ± 0.02 ^c

CP: control pâté; PGEH10: pâté with 10% hemp oil gelled emulsion as animal fat substitute; PGEH20: pâté with 20% hemp oil gelled emulsion as animal fat substitute. AI: atherogenic index; TI: thrombogenic index; h/H: hypocholesterolemic/hypercholesterolemic index.

Data are presented as mean ± SD. Different letters (a-b) in the same row indicate statistically significant differences as determined by Tukey's HSD post-hoc test ($p < 0.05$).

3.2. Chemical composition

The results of the chemical composition of the control and reformulated pâtés are shown in Table 2. The control sample (CP) showed the highest values ($p < 0.05$) for moisture, and protein content and the

lowest for fat content. It should be expected that reformulated pâtés showed higher moisture content and lower fat content than the control because the animal fat was replaced by the Hemp-GE containing 40% water. In this case, the low water content of reformulated pâtés could be related to their low emulsion stability and so, high water losses. In addition, these differences in moisture content among samples would be responsible for the differences in fat and protein content. It is important to notice that although reformulated pâtés (PGEH10 and PGEH20) showed the highest fat content, their cholesterol content was the lowest ($p < 0.05$) (Table 2). In this case, the substitution of animal fat by the Hemp-GE had a positive effect in the reduction of cholesterol in pâtés which is in line with the nutritional recommendations for the development of healthier meat products. Undoubtedly, cholesterol reduction is related to the decrease in the animal fat content in the reformulated pâtés (Domínguez et al., 2016; Martins et al., 2020; Vargas-Ramella et al., 2020). Pâtés are considered good sources of minerals mainly Fe, Mg, and K (Brito et al., 2006; Lucas-González et al., 2019). The reformulation of pâtés had no significant effect on their mineral profile ($p > 0.05$). Only slight differences in the content of Mn resulted statistically significant ($p < 0.05$). Heme iron is an essential micronutrient that promotes growth, supports neurocognitive development, and ensures the proper functioning of the immune system (Assandri et al., 2018). Additionally, iron is actively engaged in the synthesis of essential proteins such as hemoglobin, myoglobin, and specific enzymes. It should be noticed that this reformulation didn't affect ($p < 0.05$) the proportion of heme iron in pâté samples. This is important because the possible increase in non-heme iron could have negative consequences in both, the nutritional and technological properties of pâtés. Regarding that, heme iron is more bioavailable than non-heme iron (Hunt & Roughead, 2000) and, furthermore, non-heme iron (released from the heme molecule) could have a greater effect on promoting oxidation processes in pâtés (Estévez & Cava, 2004).

3.3. Main fatty acids composition and nutritional analysis

Forty-one fatty acids were measured (g/100 g), of which the 17 major ones are showed in Table 3, accounting for 94.91 % of the total fatty acids in control sample (CP), 95.22 % in PGEH10 and 95.76 % in PGEH20. Pâté samples showed statistical differences ($p < 0.05$) in fatty acids profile depending on the replacement level (0, 10 % or 20 %). The use of the Hemp-GE decreased the amount of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 n-9), vaccenic acid (C18:1 n-7) and increased linoleic acid (C18:2 n-6) and alpha-linolenic acid (C18:3 n-3) ($p < 0.05$). However, palmitoleic acid (C16:1 n-7) and myristic acid (C14:0) content did not show differences ($p > 0.05$) between PGEH10 and control sample but did show respect to PGEH20 ($p < 0.05$). The 4 fatty acids predominant in all samples were oleic, miristic, palmitic, and linoleic acid but in different order depending on the % of fat replacement: in CP the order was oleic > palmitic > stearic > linoleic acid; in PGEH10 the order was oleic > palmitic > linoleic > stearic; in PGEH20 the order was oleic > linoleic = palmitic > stearic. The higher the fat replacement level by Hemp-GE, the higher the predominance of linoleic acid.

MUFA represented the most abundant fraction in all samples, with levels between 44.74 % (PGEH10) to 47.60 % (CP). Oleic acid decreased by about 6% in PGEH10 and 21% in PGEH20, compared to the control. The increase in PUFA content due to the use of Hemp-GE ranged from 49% (PGEH10) to 77% (PGEH20). Quantitatively, linoleic acid was the predominant fatty acid in this fraction (PUFA), representing in all samples more than 50% and reaching increase level of 45 % for PGEH10 and 70 % for PGEH20, respect to CP. In the case of alpha-linolenic acid (the second one more important in PUFA fraction), the increase was from values of 0.51 g/100 g fat for the CP sample to values of 2.23 g/100 g fat in PGEH10 and to 3.56 g/100 g fat in PGEH20, representing fourfold and sevenfold increase, respectively, compared to the CP. This fact agreed with the fatty acid composition of the hemp oil

used to obtain the gelled emulsion (Botella-Martínez, Pérez-Álvarez, et al., 2021; Cittadini et al., 2022; Domínguez et al., 2017) and it is also responsible for the increase in the omega 3 (n3) and omega 6 (n6). Similar values were obtained in the study carried out by Zajac and Świątek (2018), in which the addition of hemp seed products to poultry liver pâtés resulted in similar values to those obtained for the PGEH20 sample of the present study in terms of the fatty acid profile, especially in the values of linoleic and alpha-linolenic acid.

A similar trend was also reported, with a decrease in SFA and an increase in MUFA and PUFA depending on the fat source used in several studies in which pâtés were reformulated using fat substitutes with different oils and structuring technologies (microencapsulation, oleogels and hydrogels) (Barbut et al., 2019; Cittadini et al., 2022).

3.4. Health indices of reformulated pâté

Table 3 shows the health indices of pâtés. The PUFA/SFA ratio is considered a good indicator of nutritional quality and in this case, only PGEH10 and PGEH20 showed values higher than 0.4 (PGEH20 >PGEH10), the limit recommended for WHO and FAO in meat products (Oliveira et al., 2023). Control pâté, with a value of 0.35, is under this recommended PUFA/SFA limit ($p < 0.05$).

For the omega-6 to omega-3 (n6/n3) ratio a decrease was observed in reformulated pâtés, indicating a positive result. Because the n-6/n-3 ratio has been linked to a number of pathogenic processes, recent research has emphasized the significance of both lowering it and increasing the amount of polyunsaturated fatty acids (PUFA) in the diet (Mariamenatu & Abdu, 2021). Research demonstrates that PUFA, especially n-3, can relieve symptoms of metabolic syndrome and lower the risk of heart disease by oxidizing fat instead of storing it (Clarke, 2001). It is commonly known that n-3 PUFA intake has health advantages for both treating and avoiding metabolic syndrome and inflammation (Wei et al., 2021).

The directional impact of lipids eaten may be shown by the fatty acid indices (AI, TI, and h/H). Several unhealthy effects have been attributed to different SFA, for example, C14:0, C16:0, and C18:0 have been associated with thrombogenic effects, and C12:0, C14:0, and C16:0 have demonstrated atherogenic effects (increasing total cholesterol and LDL fraction (FAO, 2010)). The atherogenic and thrombogenic indices and the hypocholesterolemic-hypercholesterolemic ratio are recommended to be as low as possible for the first two (AI and TI) and as high as possible for the last one (h/H) (FAO, 2008; Abid et al., 2021). Table 3 shows that the use of Hemp-GE as an animal fat replacer, significantly improved the AI, TI, and h/H indices respect to control pâté, being this improvement greater at a higher substitution level ($p < 0.05$).

3.5. Physico-chemical analysis

Slight pH differences between samples were found resulting in statistically different ($p < 0.05$) only for PGEH20 samples (the lowest pH value). In addition, all pâtés showed pH values ranging from 6.18 to 6.23 (Table 4) which is according with normal pH values for pâtés (Lucas-González et al., 2019; Sánchez-Zapata et al., 2013). In this case, it could be said that the substitution of animal fat for Hemp-GE didn't cause important pH changes. In line with that, this fat replacement also did not lead to changes in water activity ($p > 0.05$) for the pâtés. The water activity values (Aw) did not show statistically significant differences ($p > 0.05$) for any of the three samples tested.

Regarding the color of liver pâtés, it is principally derived from the color displayed by the fats, livers, and muscles used for their manufacture (Estévez & Cava, 2004; Vargas-Ramella et al., 2022). Therefore, as the source of fat seems to be relevant in the color of pâtés and trying to ensure that color changes were as few as possible or that such changes did not result in such color modifications that they could not be associated with the original meat product, to assess the color modifications in this reformulation is of great importance. In this case, the replacement

Table 4

Physicochemical parameters and textural properties of pâté with hemp oil gelled emulsion as partial animal fat replacer.

Sample	CP	PGEH10	PGEH20
pH	6.21 ± 0.01 ^a	6.23 ± 0.01 ^a	6.18 ± 0.01 ^b
Aw	0.891 ± 0.00 ^a	0.893 ± 0.00 ^a	0.891 ± 0.00 ^a
L*	58.56 ± 1.67 ^a	57.64 ± 0.29 ^{ab}	57.19 ± 0.84 ^b
a*	6.98 ± 0.26 ^a	6.46 ± 0.18 ^b	6.35 ± 0.51 ^b
b*	12.95 ± 0.59 ^a	13.06 ± 0.22 ^a	13.00 ± 0.53 ^a
C*	14.71 ± 0.60 ^a	14.57 ± 0.19 ^a	14.48 ± 0.55 ^a
h	61.65 ± 0.92 ^b	63.66 ± 0.83 ^a	63.98 ± 1.91 ^a
ΔE	–	2.07 ± 0.59 ^a	1.16 ± 0.51 ^b
Firmness (N)	0.38 ± 0.05 ^a	0.21 ± 0.03 ^b	0.18 ± 0.02 ^b
Shear work (N*s)	0.40 ± 0.07 ^a	0.22 ± 0.03 ^b	0.19 ± 0.02 ^b

CP: control pâté; PGEH10: pâté with 10% hemp oil gelled emulsion as animal fat substitute; PGEH20: pâté with 20% hemp oil gelled emulsion as animal fat substitute.

Data are presented as mean ± SD. Different letters (a-b) in the same row indicate statistically significant differences as determined by Tukey's HSD post-hoc test ($p < 0.05$).

of animal fat by Hemp-GE in pâtés didn't result in strong color changes. Yellowness and saturation of pâtés were not affected ($p > 0.05$) by fat replacement. Although PEGH10 and PEGH20 showed L* and a* values lower but higher h* values than control samples ($p < 0.05$), it should be highlighted that in any case, these differences were no higher than 2 units, and so have not practical meaning. Moreover, based on h* values, all pâtés samples showed a hue color defined as orange-yellowish (IRANOR, 1981). Similar results have been observed in other studies such as the one carried out by Skalecki et al. (2021); Zajac & Świątek (2018); Županjac et al. (2023a) using other sources of fat and other levels of substitution in pâtés, where statistically significant differences did not exceed the 2 units mentioned above. The differences in color between the three samples evaluated were determined with ΔE. This parameter showed that the change in the color between the CP sample and PGEH10 was noticeable with 2.07 units of difference and less difference there were between the CP sample and PGEH20 with 1.16 units of difference. Showing statistical differences ($p > 0.05$) among PGEH10 and PGEH20. It is crucial to emphasize that the human eye is incapable of perceiving color variations (ΔE*) smaller than three units (Goswami et al., 2015; Martínez et al., 2001).

3.6. Texture profile

Regarding texture, pâté should show a fairly homogeneous mass because it is a finely comminuted meat product composed of a mixture of protein, fat globules, water, salt, and spices. This mixture has a paste-like texture in the raw state that gradually should change into a more "rigid" structure by gelation of proteins (denaturation) throughout the cooking process allowing their participation in protein-protein interactions. Microstructurally, the presence of pores and the degree of packing of this protein structure have been related to the spreadability of the pâté (Terrasa et al., 2016). Table 4 displays the impact of the replacement of animal fat by Hemp-GE in pâtés. The reformulated pâtés (PGEH10 and PGEH20) exhibited a noteworthy decrease (without differences between them) in both texture parameters (firmness and shear work) ($p < 0.05$) respect to those of the control sample. From a physical perspective, the decrease in firmness and shear work in meat batters have been related to an improvement in their spreadability (Terrasa et al., 2016) because it refers to the capacity of elasto-viscoplastic materials to deform (Rezler et al., 2021). Consequently, it should be said that the use of Hemp-GE as animal fat replacers in pâtés enhanced their spreadability. Although there are a lot of factors that can influence the textural parameters of a meat product like pâté, such as the raw materials used (type and quantity), the fat source used and how it is incorporated (animal fat, gelled emulsion, oleogel, oil liquid, oil encapsulated, etc.), how the meat proteins interact with fat, and the

procedures followed during the pâté processing (Martins et al., 2020; Morales-Irigoyen et al., 2012; Rezler et al., 2021) it seems clear that the substitution of animal fat by vegetable oils reduces the firmness, hardness, penetration test, and shear work of pâtés and so enhancing spreadability. For example, Županjac et al. (2023) reported that the replacement of animal fat (20 and 40%) by oleogel with sunflower oil reduced the firmness and work of shear in 16 and 40% respectively each substitution.

3.7. Lipid oxidation

The assessment of liver pâtés' oxidative stability was conducted using the TBARS index, which results are shown in Fig. 2. This index is commonly employed as an indicator of lipid oxidation (Estévez et al., 2005). Lipid oxidation carries negative implications in meat products due to the development of undesirable rancid flavors and a decline in nutritional value (Estévez et al., 2005; Martin et al., 2008). Regarding lipid stability, traditional pâté is typically regarded as a type of meat product susceptible to oxidation since it is a highly processed product with high fat content and low natural antioxidant levels, affecting their safety, sensory and quality, including color, texture and nutritional value (Cittadini et al., 2022; Skalecki et al., 2021). Several factors, including the complex matrix, the manufacturing process (grinding and heating), and the high fat content, can influence the lipid oxidation of pâtés (Lima et al., 2013). The substitution of animal fat by the Hemp-GE in pâtés resulted in increased TBARS values (both PGEH10 and PGEH20, without differences between them) in comparison with CP ($p < 0.05$). Oils with a high content of PUFAs are more susceptible to oxidation than animal fat, despite being incorporated in a gelled structure and a meat matrix, as previously observed in other products where some gelled emulsions were used as fat replacement (Botella-Martínez, Viuda-Martos, et al., 2021, 2022; Martins et al., 2020; Nacak et al., 2021). However, it's important to emphasize that all pâté samples showed TBARS below the sensory range (2.0 mg MDA/kg sample) at which consumers detect rancidity taste, or odor. This same tendency was observed by other researchers in several meat products like burgers, sausages, frankfurter and dry fermented sausages, among others, when an oleogel or gelled emulsion was used as replacement of animal fat (Glisic et al., 2019; Heck et al., 2019; Öztürk-Kerimoğlu et al., 2021). In the case of pâtés, when the oil (fish oil) was incorporated microencapsulated, no differences were found in the lipid oxidation between reformulated pâtés and control (Vargas-Ramella et al., 2022). In other studies, such as that of Vargas-Ramella et al. (2020), great variability of

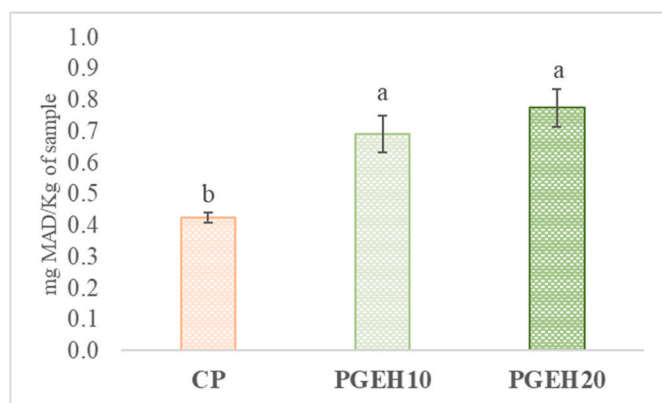


Fig. 2. Lipid oxidation of pâté with hemp oil gelled emulsion as partial fat replacer

CP: control pâté; PGEH10: pâté with 10% hemp oil gelled emulsion as animal fat substitute; PGEH20: pâté with 20% hemp gelled emulsion as animal fat substitute.

Data are presented as mean \pm SD. Different letters indicate statistically significant differences as determined by Tukey's HSD post-hoc test ($p < 0.05$).

TBARS values of pâté samples, between 0.26 and 0.64 mg MDA/Kg of product was observed, depending on the oil used for the microencapsulation.

3.8. Sensory evaluation

Consumer acceptance is a fundamental requirement for the economic viability of innovative food components and, consequently, food products. The appearance, flavor, and aroma of the product stand out as the main factors influencing the consumer's purchasing decisions (Skalecki et al., 2021; Smarzyński et al., 2019). The outcomes of the sensory assessment are depicted in Fig. 3a. Among the sensory attributes evaluated (color, brightness, overall odor, rancidity, spreadability, juiciness, cohesiveness, hardness, overall flavor, and general appearance), only the overall odor, rancidity, spreadability, and juiciness showed significant differences ($p < 0.05$) between PGEH20 and control samples. CP and PGEH10 samples did not show significant differences ($p > 0.05$) for any of the 10 evaluated attributes. The decrease in the scores for spreadability and juiciness in PGEH20 may be due to the higher presence of unbound lipids in 100% pork fat pâtés (CP) compared to the PGEH20 pâtés. It has been reported that vegetable oil tends to form bonds within the gelled emulsion (GE) network, reducing the likelihood of rapid expulsion from the structure (Barbut et al., 2021). This fact is in accordance with the decrease in emulsion stability of the reformulated pâtés (Fig. 1). Panellist detected rancidity in PGEH20, although, as has been previously discussed, this pâté showed TBARS values below the reported detection limit of consumers (section 3.7). In this case, maybe the low scores for overall odor obtained in PGEH20, mainly associated to the particular odor of hemp oil, could be identified as rancidity odors. Furthermore, there were no significant differences in overall acceptance between pâtés ($p > 0.05$) with scores in all cases higher than 5 (7 points hedonic scale) ($p > 0.05$). These results should indicate no changes in the acceptability of pâtés with animal fat replacers (by Hemp-GE emulsion) compared to traditional pâtés. Different results in overall acceptance in reformulated pâtés changing the fat source have been obtained. For example Vargas-Ramella et al. (2022), did not find differences in overall acceptance in pâtés in which the 25 and 50% of the animal fat were replaced with microencapsulated fish oil. But Cittadini et al. (2022) found differences in overall acceptance in pâtés in which 50% of foal fat was replaced by two mixture of microencapsulates oils; walnut oil-seaweed oil and pistachio-seaweed oil.

4. Conclusion

The reformulation of pâté by the partial replacement (10 and 20%) of animal fat by a gelled emulsion made with hemp oil and buckwheat flour is a technologically feasible option and an effective way to enhance their nutritional quality, mainly focus on a healthier lipid profile (increasing PUFA and an omega-3-fatty acid contents and decreasing cholesterol content), without significantly affecting the sensorial acceptability. Although the increase in PUFA resulted in a faster lipid oxidation, in any case the resulting compounds were higher than the sensorial detection limit by consumers. The reformulation of traditional meat products with the aim of making them healthier but without modifying their typical sensory characteristics can contribute to maintaining the gastronomic heritage of the different regions adapted to new scientific knowledge and consumer demands.

CRedit authorship contribution statement

Carmen Botella-Martínez: Writing – original draft, Methodology, Investigation. **José Ángel Pérez-Álvarez:** Validation, Resources, Funding acquisition, Conceptualization. **Juana Fernández-López:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Manuel Viuda-Martos:** Writing – review & editing, Supervision, Investigation, Conceptualization.

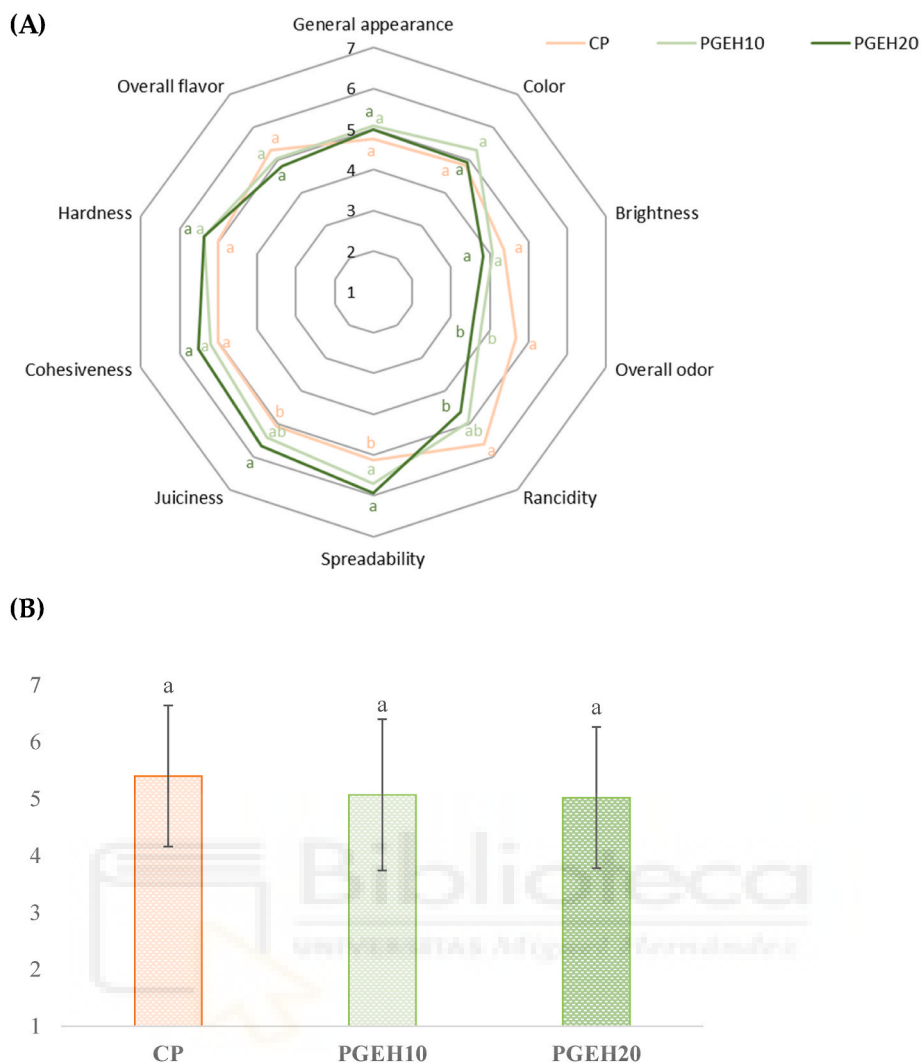


Fig. 3. Sensory evaluation (A) and overall acceptance (B) of pâtés with hemp oil gelled emulsion as partial animal fat replacer.

CP: control pâté; PGEH10: pâté with 10% hemp gelled emulsion as animal fat substitute; PGEH20: pâté with 20% hemp gelled emulsion as animal fat substitute. Data are presented as mean \pm SD. Different letters indicate statistically significant differences as determined by Tukey's HSD post-hoc test ($p < 0.05$).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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8.7. PUBLICACIÓN 7

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Alheiras with animal fat replacement: application of a gelled emulsion based on hemp oil (*Cannabis sativa* L.) and buckwheat

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Abstract

A gelled emulsion elaborated using hemp seed oil and buckwheat flour was utilized as pork backfat substitute in a typical Portuguese meat product named Alheira. Three different formulations were prepared: Alheiras control (AC) with a fat content of 13%, alheiras where the 25% pork backfat was substituted with a hemp seed oil-GE (AH25), and alheiras where the 50% pork backfat was substituted with a hemp seed oil-GE (AH50). The chemical composition as well as the physicochemical properties and lipid stability of different samples were assessed. Reformulated samples showed similar protein, moisture, and fat content ($p < 0.05$) that AC. However, AH25 and AH50 had a higher amount of linoleic acid (16.29 and 22.14 g/100 g, respectively) and linolenic acid (1.75 and 3.45 g/100 g, respectively) than AC (12.71 and 0.51 g/100 g). Similarly, AH25 and AH50 showed lower saturated fatty acids (35.28 and 30.37 g/100 g, respectively) than AC (37.37 g/100 g). The substitution of pork backfat for hemp seed oil-GE did not modify significantly the physicochemical properties of samples. On the other hand, the lipid oxidation values increased by 30 and 65% in AH25 and AH50 respectively, in comparison to AC due to the use of polyunsaturated oils, which are highly susceptible to oxidation. This work established that the use of gelled emulsions elaborated using hemp oil and buckwheat flour may be a promising strategy to obtain meat products with a better healthier profile.

Keywords Reformulation · Fat substitution · Healthier oil · Polyunsaturated fatty acids · Health indices · Healthy meat product

Introduction

Alheira is a traditional Portuguese fermented sausage produced with a combination of poultry meats (from different types including duck, turkey, quail, partridge, and hen), pork (pork backfat), regional bread, olive oil, salt and spices including garlic, and paprika. This product is a highly appreciated and long-established practise in Northeast Portugal (Trás-os-Montes region). Although alheiras are commonly consumed in the rural areas of northern Portugal, today these products are becoming trendy in urban cities whose commercialization is growing significantly. Several studies have been conducted to examine the processing conditions [1–3], physicochemical and sensory properties [4], microbiological characteristics and safety [5–8], composition and meat species identification of alheiras [9], besides even the characteristics of a non-meat-based alheira has also been studied [10].

Despite the fact that alheira is a well-accepted meat product, it is perceived by consumers as unhealthy due

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to the high-fat content. This fact has become increasingly important for the meat industry, which is trying to develop several strategies to reduce or even eliminate saturated fat in product formulations and thereby to obtain high quality, nutritious, and healthier meat products while maintaining taste traditions and consumer expectations. Numerous approaches have been used to substitute saturated fat of animal origin with vegetable oils to minimize their effects on the physicochemical and sensorial characteristics of the final product, ensuring consumer acceptance as well as the technological characteristics, and enabling them to be economically viable in the meat industry [11–17]. Within these approaches, the use of gelled emulsion (GE) has been shown as a feasible strategy to structure liquid oils from different origins (marine, vegetable, or seeds) that have healthier fatty acid profiles trying to mimic the technological properties of pork backfat [18, 19]. In addition, several emulsifiers (chestnut flour, chia mucilage flour, soy protein, etc.) or gelling agents (gelatin, alginate, carrageenan, gums, etc.) have been utilized to develop these gelled emulsions [11, 12, 14, 20, 21]. In this manner, gelled emulsions prepared with diverse vegetable oils and gelling agents have been successfully utilized as fat substitutes in the development of cooked and fresh meat products with better effects on health [13, 16, 21–23]. Although the use of gelled emulsions as a saturated animal fat substitute in meat products is widespread, there are no studies in the scientific literature that indicate the use of these gelled emulsions to replace the animal fat content in alheiras, a traditional Portuguese meat product.

In this work, the gelled emulsion was elaborated with hemp seed oil (*Cannabis sativa* L.) using buckwheat flour as an emulsifier agent. Hemp seed oil shows an elevated content [24]. On the other hand, buckwheat flour has a high content of proteins, vitamins, minerals as well as dietary fibre [25]. In addition, the buckwheat proteins as well as the carbohydrate contents offer good emulsifying and gelling properties, which are necessary to develop the gelled emulsion [26]. In previous studies, gelled emulsion elaborated with several vegetable oils and flours obtained from pseudocereal were analysed [27, 28]. From all of them, due to the technological feasibility, physicochemical properties, and fatty acids composition the use of hemp seed oil with buckwheat flour had been selected to elaborate the gelled emulsion.

This study aims to (i) assess the technological viability of gelled emulsions made with hemp seed oil and buckwheat flour, as substitutes (25% and 50%) of animal fat for alheira production and (ii) study the effect of partial substitution of animal fat of alheiras on chemical composition and fatty acid profile, physicochemical properties, lipid profile, and lipid oxidation values.

Materials and methods

Materials

The following ingredients were used for hemp seed oil-gelled emulsions (Hemp-GE) elaboration: hemp seed oil (544.4 mg/g linoleic acid, 199.5 mg/g α -linolenic acid, and 82.3 mg/g oleic acid) was supplied by Laboratorios Almond, S.L. (Murcia, Spain); buckwheat flour was obtained from Biogran S.L. (Madrid, Spain); and gelatine “instant gel” from pork origin was purchased from Sosa Ingredients S.L. (Barcelona, Spain). The meat ingredients (hen (breast and thighs), duck (breast and thighs), and pork backfat after *rigor mortis*) were purchased from a local Portuguese butchery. A Tras-os-Montes Protected Designation of Origin olive oil (752.0 mg/g oleic acid, 33.0 mg/g stearic acid, and 77.0 mg/g linoleic acid) was used [29].

Gelled emulsion preparation

The gelled emulsion was elaborated with a proportion of 40% water, 40% hemp seed oil, 15% buckwheat flour, and 5% gelatine (Hemp-GE). Firstly, at high speed (5600 rpm), the gelling agent (instant gel) was mixed with the water at room temperature using a hand blender (Moulinex quickchef, France) for 1 min. In the next step, the buckwheat flour was incorporated and mixed for 1 min at high speed (5600 rpm) again. Finally, the hemp seed oil was gradually added at 15 ml/min to the mixture and mixed for 3 min at high speed (5600 rpm). The elaborated Hemp-GEs were deposited in flasks and stored at $-18\text{ }^{\circ}\text{C}$ until their subsequent incorporation into the meat product.

Alheiras manufacturing and sampling

For alheiras elaboration, all meats (big pieces) were cooked (at $100\text{ }^{\circ}\text{C}$) in water (2% salt) with proportion water: meat of 2.2:1 during 45 min. Afterward, the bread was finely sliced and immersed in the broth generated during the meat boiling. Then, when the bread was adequately soft, the chopped meat, salt, garlic, paprika, and olive oil were added to the batter. This batter was mixed for 10 min. Finally, the fat was added (at $4\text{ }^{\circ}\text{C}$, Hemp-GE previously thawed) and the mixture was mixed until all the ingredients were integrated. At the end of this process, the paste was stuffed into cattle intestinal casings and submitted to a dry process at $15\text{ }^{\circ}\text{C}$ and 75% relative humidity for 10 days without smoking process. Figure 1 shows the flowchart of the alheira production process.

Three types of alheiras were prepared (Table 1): in the control treatment, pork backfat was used (AC), and in

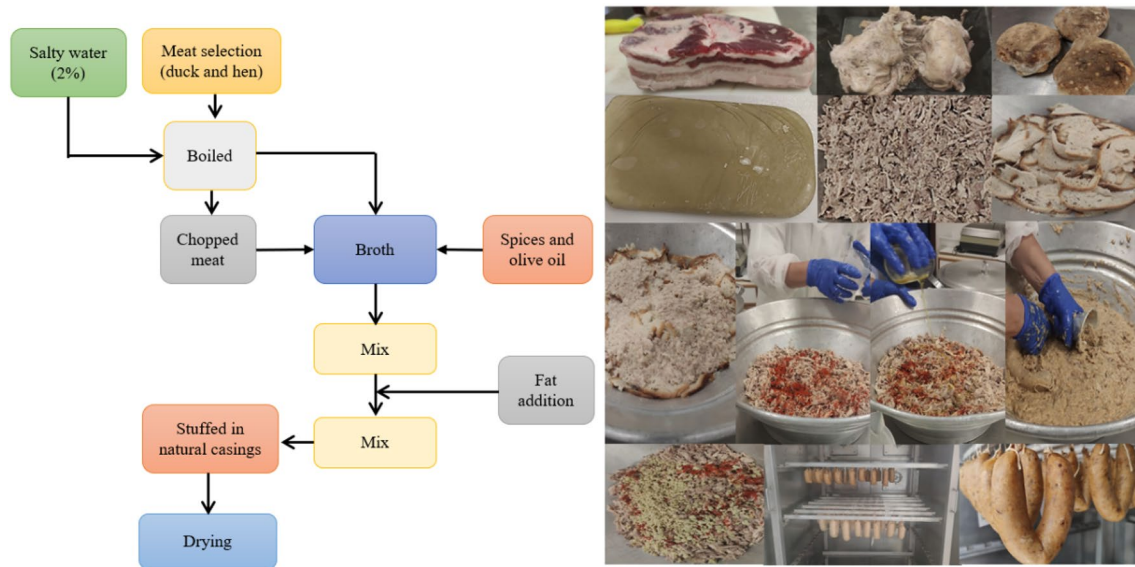


Fig. 1 Flowchart of the alheira production process

Table 1 Composition of control and reformulated alheiras

	AC	AH25	AH50
Bread	17.08	17.08	17.08
Hen, duck	21.36	21.36	21.36
Pork backfat	12.81	9.61	6.41
Hemp-GE	0.00	3.20	6.41
Salt	0.64	0.64	0.64
Garlic	0.17	0.17	0.17
Olive oil	0.78	0.78	0.78
Paprika	0.17	0.17	0.17
Broth	46.98	46.98	46.98

*Values are expressed as g/100 g. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour

the other two treatments, the pork backfat was partially replaced with Hemp-GE (AH25: 25% pork backfat was substituted with Hemp-GE; AH50: Alheira where the 50% pork backfat was substituted with Hemp-GE). The samples were developed according to the diagram of the production process (Fig. 1) and the formulation as shown in Table 1. Three alheiras from each formulation were cooked at 180 °C in an electric grill until the internal

temperature, measured at the geometrical centre of each alheira, reached 72 °C.

Chemical composition

For ash content, the alheiras were incinerated in muffle at 550 °C, according to Portuguese standards 1615 [30] while the moisture content was assessed following the methodology described by the Portuguese standards NP1614 [31]. Briefly, approximately 3 g of sample were added with 5 mL of ethanol and heated at 70 °C until its complete evaporation; after, the samples were oven-dried at 103 °C ± 2 °C until constant weight. The fat content was determined according to the recommendations of Folch et al. [32]. Protein content was analysed using the Kjeldahl method (%N × 6.25) in agreement with NP 1612 [33] using a Kjeldahl System equipment a gas neutralizer Buchi K-415 coupled to the Buchi K-446 Mineralizer and a Buchi auto Kjeldahl Unit K-370 distiller. The total chloride content was assessed using the methodology detailed in Portuguese standard NP 1845 [34]. For the titration the Titrino Plus brand was used with 0.1 N silver nitrate solution. The chloride content (NaCl) of the sample is expressed as a percentage, by mass by the equipment itself. All assays were taken on three raw and cooked samples of each formulation.

Physicochemical analyses

The pH analysis was measured directly in the different alheira samples following the Portuguese standard NP 344 [35] recommendations, using a Crison 507 pH-metre equipped with a 52-32 puncture electrode at different sites

of the sample. Water activity was determined according to AOAC [36]. Colour parameters were obtained as CIEL*a*b* coordinates using a colorimeter CM-2600D (Minolta Camera Co., Osaka, Japan) with illuminant D₆₅, 10° observer, SCI mode, 11 mm aperture of the instrument for illumination and 8 mm for measurement. Spectrally pure glass (CR-A51: Minolta Co.) was put between the sample and the equipment. Each alheira was evaluated six times on several points of surfaces for lightness (L*), redness (a* coordinate), and yellowness (b* coordinate), and the psychophysical parameters hue (h*) and chroma (C*) which were calculated according to the Eqs. (1) and (2), respectively.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

The total colour differences (ΔE^*) of each sample where the pork backfat was replaced with Hemp-GE (AH) with respect to the control sample (AC) were also calculated following the Eq. (3):

$$\Delta E^* = \sqrt{(L_s^* - L_c^*)^2 + (a_s^* - a_c^*)^2 + (b_s^* - b_c^*)^2} \quad (3)$$

Cooking loss

The weight of three samples, control and two partial fat replacement alheiras were measured at room temperature before and after cooking. The cooking loss was calculated with Eq. 4. Measurements were taken on three samples of each formulation.

$$\% \text{ Cooking loss} = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100 \quad (4)$$

Fatty acids composition analysis

To analyse the fatty acid profile, of both raw and cooked alheiras, the method described by Folch et al. [32] was applied for the lipid extraction from the samples. The fatty acids were transesterified following the method reported by Domínguez et al. [37] while Teixeira et al. [38] described the chromatographic conditions. As an internal standard, nonadecanoic acid (C19:0) at 0.3 mg/mL was added to the samples.

Individual Fatty acid methyl esters were identified by comparing their retention times with those of authenticated standards. The results obtained were expressed as g/100 g of total fatty acids. To analyse the nutritional value of alheiras where the pork backfat was replaced by Hemp-GE, several

health indices including omega-6 and omega-3 fatty acids ratio, and polyunsaturated fatty acids and saturated fatty acids ratio were calculated. In addition, the index of atherogenicity (AI) and the index of thrombogenicity (IT) were calculated according to the equations proposed by Ulbricht and Southgate [39]:

$$AI = \frac{C12 : 0 + (4 * C14 : 0) + C16 : 0}{\sum MUFA + \sum n6 + \sum n3} \quad (5)$$

$$TI = \frac{C14 : 0 + C16 : 0 + C18 : 0}{(0.5 * \sum MUFA) + (0.5 * \sum n6) + (3 * \sum n3) + \frac{(\sum n3)}{(\sum n6)}} \quad (6)$$

The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated using Eq. (7), as described by Fernández et al. [40].

$$\frac{h}{H} = \frac{C18 : 1n - 9 + C18 : 1n - 7 + \sum PUFA}{C14 : 0 + C16 : 0} \quad (7)$$

Lipid oxidation

The lipid oxidation values of both raw and cooked alheiras, analysed by the thiobarbituric acid reactive substances (TBARs) assay, were assessed by extraction of malondialdehyde (MDA) according to NP-ISO-3356 [41]. The results obtained were expressed as mg of MDA/kg of the sample.

Statistics analysis

All the process (elaboration of Hemp-GE and elaboration of alheiras) was replicated three times (three independent batches). The repetitions were carried out on different production days, and each batch was analysed in triplicate. SPSS software (version 24.0, SPSS Inc., Chicago, USA) was used to analyse the data using one-way or two-way analysis of variance (ANOVA) and Tukey-b post-hoc tests at 5% significance level ($p < 0.05$).

Results and discussion

Chemical composition

The results of the chemical composition of the control and reformulated alheiras are shown in Table 2. In raw samples, AC had the lowest values ($p < 0.05$) for moisture, ash, proteins, and chlorides. The moisture values varied between 56.52 and 59.87 g/100 g, being AH50, the sample that showed the highest ($p < 0.05$) value. In this product, high moisture values are characteristic since it is made of boiled

Table 2 Chemical composition of control and reformulated alheiras (raw and cooked)

	Sample	Moisture	Ash	Fat	Protein	Chlorides
Raw	AC	56.52 ± 1.06 ^c	1.17 ± 0.06 ^b	15.07 ± 0.74 ^a	9.85 ± 0.39 ^c	0.78 ± 0.05 ^b
	AH25	58.03 ± 0.39 ^b	1.35 ± 0.03 ^a	13.94 ± 0.97 ^b	11.63 ± 1.37 ^b	0.89 ± 0.04 ^a
	AH50	59.87 ± 0.31 ^a	1.38 ± 0.01 ^a	12.56 ± 0.83 ^c	12.18 ± 0.36 ^a	0.91 ± 0.01 ^a
Cooked	AC	54.23 ± 0.56 ^x	1.26 ± 0.02 ^y	17.71 ± 1.43 ^x	10.36 ± 0.27 ^z	0.89 ± 0.04 ^y
	AH25	54.62 ± 0.85 ^x	1.45 ± 0.04 ^x	15.76 ± 0.81 ^y	11.98 ± 0.58 ^y	1.01 ± 0.02 ^x
	AH50	55.24 ± 0.92 ^x	1.45 ± 0.04 ^x	12.85 ± 0.72 ^z	12.61 ± 0.71 ^x	1.07 ± 0.03 ^x

*Results were expressed as g/100 g. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour. For raw samples (a–c) and cooked samples (x–z); values followed by a different letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$)

poultry meats blended with bread which is soaked in the broth of the poultry meat cooking. Additionally, the increase in moisture content might be explained as a result of the water added to prepare the gelled emulsions. However, in cooked samples, the moisture content showed no statistical differences ($p > 0.05$) between AC, AH25, and AH50 with values ranging from 54.23 to 55.24 g/100 g of cooked sample. In this instance, the total moisture content of samples was affected by heat treatment. The moisture values found were in line with those reported in similar works by Teixeira et al. [38] and Patarata et al. [4] but higher than those reported by Carvalho et al. [7].

In raw alheiras, the rise in moisture content was accompanied by a reduction in the fat content ($p < 0.05$) when the Hemp-GE was utilized as a fat substitute. These changes represented a fat reduction, concerning the control sample, about 7.5% and 16.65% for AH25 and AH50, respectively. In the case of cooked alheiras, the same behaviour was observed. Thus, as the degree of substitution increased, the fat content decreased ($p < 0.05$). The fat reduction obtained with respect to AC was 11.01% and 27.44% for AH25 and AH50, respectively. These results were in concordance with those reported de Souza Paglarini et al. [42], and Botella-Martínez et al. [23] who reported a reduction in fat content in meat products where the pork backfat content was substituted by different gelled emulsions made with vegetable oils. In raw and cooked alheiras, the protein content increased with the substitution of pork backfat by Hemp-GE with statistical differences ($p < 0.05$) between samples. This fact might be explained as a result of the protein content (12.23 g/100 g) of buckwheat flour as well as the protein content of the gelling agent used to elaborate the Hemp-GE. The values obtained are close to those stated by Patarata et al. [4], Ferreira et al. [5] and Teixeira et al. [38] due to the substantial variation in sausage formulations available on the market.

In this study, the results reflected the changes in the initial formulation. The substitution of pork backfat (with high-fat content) for gelled emulsion elaborated with 40/40% (v/v)

of water/oil is expected (as occurs) that reduce the fat and increase moisture contents. Additionally, the use of gelatine in the formulation also produces a significant rise in protein content in the reformulated samples. Thus, the changes in the composition reflect the changes and proportions of the raw materials used in the alheira formulation.

Regarding ash content (Table 2), in raw samples, there were statistical differences ($p < 0.05$) between AC and the reformulated alheiras (AH25 and AH50), with the control alheira containing 15% fewer ashes than the partial fat substitution samples. The same tendency was obtained in the cooked samples, but with an overall 7% increase when cooked treatment was applied. This increase, in the ash content of reformulate samples with respect to control, could be due to the gelled emulsions containing buckwheat flour that may contribute to the increase of these parameters in both raw and cooked AH25 and AH50 samples [25, 43–45]. In any case, the values obtained for ash content were lower than those reported by Campos et al. [2] and Teixeira et al. [38] who reported values around 2.0 g/100 g but within the range of values presented by Patarata et al. [4]. In reference to sodium chloride content expressed as % chlorides (Table 2), no statistical differences ($p > 0.05$) were found between AH25 and AH50 in raw samples with values around 0.9 g /100 g of sample. In cooked samples, AC showed the lowest ($p < 0.05$) values whilst for AH25 and AH50, as occurs in raw samples, no statistical differences ($p < 0.05$) were observed. The values obtained, how it happens with those obtained for the ashes were lower than those reported in the literature [2, 4, 46].

Fatty acids composition

The fatty acids profile of raw and cooked alheiras (control, AH25 and AH50) are shown in Table 3. Regarding raw alheiras, statistical differences ($p < 0.05$) were found in the fatty acids profile of samples depending on the replacement level (25 or 50%) and the kind of fat used (pork backfat or Hemp-GE). In both raw and cooked control, AH25 and

Table 3 Lipid profile and nutritional parameters of control and reformulated alheiras (raw and cooked)

Fatty acid profile	Raw			Cooked		
	AC	AH25	AH50	AC	AH25	AH50
C14:0	0.97 ± 0.04 ^{aF}	0.86 ± 0.01 ^{bG}	0.71 ± 0.04 ^{cG}	0.92 ± 0.02 ^{xF}	0.84 ± 0.01 ^{yF}	0.73 ± 0.02 ^{zF}
C16:0	24.49 ± 0.11 ^{aB}	22.79 ± 0.09 ^{bB}	20.43 ± 0.02 ^{cC}	24.38 ± 0.13 ^{xB}	22.90 ± 0.14 ^{yB}	21.12 ± 0.70 ^{zB}
C16:1n-7	2.24 ± 0.06 ^{aE}	1.96 ± 0.02 ^{bE}	1.79 ± 0.00 ^{cF}	2.12 ± 0.05 ^{xE}	1.88 ± 0.01 ^{yE}	1.87 ± 0.16 ^{yE}
C17:0	0.19 ± 0.00 ^{aK}	0.18 ± 0.01 ^{aJ}	0.16 ± 0.00 ^{bI}	0.19 ± 0.00 ^{xI}	0.18 ± 0.01 ^{xH}	0.12 ± 0.06 ^{yH}
C17:1n-7	0.17 ± 0.01 ^{aK}	0.15 ± 0.00 ^{bJ}	0.13 ± 0.00 ^{cI}	0.16 ± 0.00 ^{xI}	0.16 ± 0.00 ^{xH}	0.12 ± 0.04 ^{yH}
C18:0	10.79 ± 0.26 ^{aD}	10.25 ± 0.08 ^{bD}	8.80 ± 0.01 ^{cD}	11.30 ± 0.20 ^{xD}	10.68 ± 0.04 ^{yD}	7.56 ± 3.37 ^{zC}
C18:1n-9	45.44 ± 0.18 ^{aA}	43.01 ± 0.18 ^{bA}	39.59 ± 0.01 ^{cA}	45.37 ± 0.12 ^{xA}	43.02 ± 0.13 ^{yA}	40.66 ± 1.35 ^{zA}
C18:2n-6	13.08 ± 0.25 ^{cC}	16.71 ± 0.17 ^{bC}	22.56 ± 0.02 ^{aB}	12.71 ± 0.10 ^{zC}	16.29 ± 0.30 ^{yC}	22.14 ± 1.05 ^{xB}
C20:0	0.18 ± 0.01 ^{cK}	0.23 ± 0.01 ^{bI}	0.28 ± 0.00 ^{aH}	0.20 ± 0.01 ^{zI}	0.24 ± 0.00 ^{yG}	0.28 ± 0.02 ^{xG}
C18:3n-6	0.82 ± 0.03 ^{aG}	0.76 ± 0.04 ^{bH}	0.67 ± 0.02 ^{cG}	0.88 ± 0.03 ^{xF}	0.82 ± 0.05 ^{yF}	0.70 ± 0.04 ^{zF}
C18:3n-3	0.53 ± 0.02 ^{cH}	1.84 ± 0.04 ^{bF}	3.65 ± 0.00 ^{aE}	0.51 ± 0.02 ^{zG}	1.75 ± 0.04 ^{yE}	3.45 ± 0.17 ^{xD}
C20:2n-6	0.34 ± 0.01 ^{aI}	0.32 ± 0.01 ^{aI}	0.27 ± 0.01 ^{bH}	0.34 ± 0.00 ^{xH}	0.33 ± 0.00 ^{xG}	0.27 ± 0.02 ^{yG}
C20:3n-6	0.06 ± 0.03 ^{aL}	0.06 ± 0.00 ^{aK}	0.06 ± 0.00 ^{aJ}	0.07 ± 0.01 ^{xJ}	0.05 ± 0.02 ^{xI}	0.06 ± 0.00 ^{xJ}
C22:1n-9	0.07 ± 0.03 ^{aL}	0.08 ± 0.01 ^{aK}	0.07 ± 0.00 ^{aJ}	0.08 ± 0.01 ^{xJ}	0.07 ± 0.02 ^{xI}	0.07 ± 0.01 ^{xI}
C23:0	0.24 ± 0.02 ^{bJ}	0.27 ± 0.04 ^{abI}	0.30 ± 0.01 ^{aH}	0.25 ± 0.01 ^{yH}	0.27 ± 0.01 ^{yG}	0.34 ± 0.02 ^{xG}
ΣSFA	36.95 ± 0.11 ^{aA}	34.75 ± 0.09 ^{bB}	30.90 ± 0.02 ^{cC}	37.37 ± 0.13 ^{xA}	35.28 ± 0.14 ^{yB}	30.37 ± 0.17 ^{zC}
ΣMUFA	48.18 ± 0.18 ^{aA}	45.50 ± 0.18 ^{bB}	41.83 ± 0.01 ^{cC}	48.05 ± 0.12 ^{xA}	45.42 ± 0.13 ^{yB}	42.95 ± 0.25 ^{zC}
ΣPUFA	14.86 ± 0.25 ^{cC}	19.76 ± 0.17 ^{bB}	27.27 ± 0.01 ^{aA}	14.57 ± 0.10 ^{zC}	19.30 ± 0.30 ^{yB}	26.66 ± 0.32 ^{xA}
Σn3	0.54 ± 0.04 ^{cC}	1.87 ± 0.03 ^{bB}	3.68 ± 0.06 ^{aA}	0.53 ± 0.04 ^{zC}	1.78 ± 0.05 ^{yB}	3.46 ± 0.08 ^{xA}
Σn6	14.30 ± 0.02 ^{cC}	17.85 ± 0.05 ^{bB}	23.56 ± 0.03 ^{aA}	14.00 ± 0.02 ^{zA}	17.49 ± 0.03 ^{yA}	23.16 ± 0.04 ^{xA}

*Values were expressed as g/100 g fatty acids. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour. For raw (a–c) or cooked (x–z) alheiras, values followed by the same small letter in the same row were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$). Values followed by the same capital letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

AH50 alheiras, a total of 24 fatty acids were detected. From the total of fatty acids identified in control alheira, oleic acid (C18:1n-9), palmitic acid (C16:0), linoleic acid (C18:2n-6), stearic acid (C18:0), and palmitoleic acid (C16:1n-7) represent more than 96% of total acids. In the case of reformulated alheiras, there was evidence to suggest that Hemp-GE as a partial replacer of animal fat decreased palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1n-9) and increase significantly ($p < 0.05$) linoleic acid (C18:2n-6) and α -linolenic acid (C18:3n-3). The major variance between all alheiras samples was the linoleic acid (C18:2n-6) and α -linolenic acid (C18:3n-6) contents. Thus, the alheiras with the highest animal fat replacement (AH50) had the highest ($p < 0.05$) content of α -linolenic acid and linoleic acid with values of 3.65 and 22.56 g/100 g of fatty acids respectively. This fact agreed with the fatty acid composition of hemp seed oil used to obtain the gelled emulsion. The cooked alheiras showed the same fatty acid profile as the raw samples, with no statistical differences ($p > 0.05$). The results of control sample agreed with the fatty acids profile of raw alheiras, recorded by Campos et al. [2] who reported that the main fatty acids found in traditional alheiras were oleic acid (34.76%) and palmitic acid (29.37%) and Teixeira et al. [38]

who informed that the main fatty acids present in alheiras were oleic acid (46.10%) and palmitic acid (23.90%). The change, in the lipid profile of samples where the animal fat was substituted with Hemp-GE (AH25 and AH50 samples), was consistent with previous studies where, in other kinds of meat products including beef burgers [16, 23] and Frankfurt sausages [47], the pork backfat was replaced by gelled emulsions.

All samples (cooked and raw) were mainly composed of monounsaturated fatty acids (MUFA) with levels between 41.83 and 48.18% for AH50 and AC, respectively with statistical differences between samples ($p < 0.05$). The use of gelled emulsions also resulted in a MUFA reduction, with the control samples (raw and cooked) containing the highest levels ($p < 0.05$). Oleic acid (C18:1) was the predominant fatty acid (39.59–45.44%) followed by palmitoleic acid (C16:1) with values between 1.79 and 2.24%. Regarding saturated fatty acids (SFA), it was reduced ($p < 0.05$) in AH25 and AH50 alheiras depending on the level of replacement (6 and 16%, higher reduction at the highest substitution level). The principal saturated fatty acids were palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0). Polyunsaturated fatty acids (PUFA) increased their content

in AH25 and AH50 alheiras with respect to control in 32% and 83% for AH25 and AH50 raw and cooked respectively. It can be seen that this increase in polyunsaturated fatty acid content in AH25 and AH50 alheiras was mostly as a result of α -linolenic acid and linoleic acid. Consequently, substituted alheiras had higher levels of omega-3 and omega-6 than control alheiras. The amount of omega-3 fatty acids was three times greater with the 25% substitution while for the 50% substitution the amount of omega-3 fatty acids was 6 times greater. For AH25 and AH50 samples, the omega-6 fatty acids increased a 25% and 65%, respectively. In both raw and cooked alheiras, this behaviour was observed with no differences between them ($p > 0.05$). The values of MUFA, SFA, and PUFA of control alheiras obtained in this study were higher than those reported by Marcos et al. [46] on different Portuguese alheiras. It should be remembered that alheira is a very variable product in terms of meat composition, quantity, and type of fat. Several authors have also reported this fact when replacing animal fat with vegetable or marine oils in several meat products [15, 20, 48, 49]. Thus, as a general conclusion, the fatty acids profile, and the proportions of individual and the SFA, MUFA and PUFA depends only on the fatty acids profile and the proportions of the animal fat and GE used in the alheira formulation.

Health indices of reformulated alheiras

In scientific literature, there are several works that reported that the substitution of animal fats, in the development of healthier meat products, using vegetable oils (added in different ways including oleogels and/or gelled emulsions) have important health benefits [16, 23, 49, 50]. Table 4 shows the health indices of raw and cooked alheiras. Human diets may be evaluated using the PUFA/SFA ratio as an indicator of nutritional quality. The mean ratio of PUFA/SFA

recommended by World health organization (WHO) and Food and Agriculture Organization (FAO) experts is above 0.4 [51].

All reformulated alheiras (AH25 and AH50) were in accordance with the mentioned recommendations of PUFA/SFA ratio with values of 0.57 and 0.55 for AH25 raw and cooked respectively, and values of 0.88 for raw and cooked AH50. However, the AC sample presented values within the recommended limit. Regarding the n6/n3 index, all the samples analysed (AC, AH25, and AH50) had a higher value than the proposed recommendations by Simopoulos, [52] who indicates that to have a healthy effect the ratio n6/n3 must be 4/1. Despite this, the decrease in the raw and cooked AH50 samples was 24% and 25% respectively. Although, based on scientific evidence and conceptual limitations, WHO [53] reported that no convincing scientific rational recommendation exists for n6/n3 ratios.

There has been a lot of discussion about the healthy properties of fats in meat products, using the atherogenic index, thrombogenic index, and/or hypocholesterolemic/hypercholesterolemic ratio as indicators [12, 16, 18]. Table 4 showed that the use of Hemp-GE as pork backfat replacer, significantly improved ($p < 0.05$) all indices except the atherogenic index. Thus, the thrombogenic index decreased while hypocholesterolemic/hypercholesterolemic ratio increased with the replacement compared with control alheira. All changes obtained in the health indices were directly linked to the amount of pork backfat substituted, the most positive values in the three indices were shown in alheiras with 50% substitution (AH50). On the other hand, the atherogenic index showed no statistically significant differences ($p > 0.05$) between the different samples of raw alheiras and between different samples of cooked alheiras. The heat treatment used also did not cause the atherogenic index to change. Compared to control alheira, reformulated samples showed an increased hypocholesterolemic/hypercholesterolemic

Table 4 Health indices of control and reformulated alheiras (raw and cooked)

	Sample	\sum PUFA/ \sum SFA	n6/n3	Atherogenic index	Thrombogenic index	Hypocholesterolemic/hypercholesterolemic index
Raw	AC	0.40 ± 0.02 ^{cA}	26.73 ± 0.04 ^{aA}	0.45 ± 0.03 ^{aA}	1.10 ± 0.06 ^{cA}	2.37 ± 0.02 ^{cA}
	AH25	0.57 ± 0.03 ^{bA}	9.57 ± 0.02 ^{bB}	0.40 ± 0.07 ^{aA}	0.91 ± 0.04 ^{bA}	2.65 ± 0.02 ^{bA}
	AH50	0.88 ± 0.04 ^{aA}	6.41 ± 0.02 ^{cB}	0.34 ± 0.03 ^{aA}	0.68 ± 0.02 ^{aA}	3.16 ± 0.01 ^{aA}
Cooked	AC	0.39 ± 0.02 ^{zA}	26.32 ± 0.14 ^{xA}	0.45 ± 0.03 ^{xA}	1.12 ± 0.07 ^{zA}	2.37 ± 0.02 ^{zA}
	AH25	0.55 ± 0.03 ^{yA}	9.85 ± 0.02 ^{yA}	0.41 ± 0.07 ^{xA}	0.93 ± 0.10 ^{yA}	2.62 ± 0.02 ^{yA}
	AH50	0.88 ± 0.04 ^{xA}	6.69 ± 0.02 ^{zA}	0.35 ± 0.03 ^{xA}	0.68 ± 0.11 ^{xA}	3.08 ± 0.01 ^{xB}

AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substitute with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substitute with a GE elaborated with hemp seed oil and buckwheat flour. Values followed by different small and capital letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$). A lower-case letter refers to the comparison of the same parameter between raw samples (a–c) and cooked samples (x–z). Capital letters refers to the comparison of the same sample depending on treatment (raw or cooked)

ratio. According to Barros et al. [50], high hypocholesterolemic/hypercholesterolemic ratios indicate healthier meat products than those with low ratios. In raw samples, the hypocholesterolemic/hypercholesterolemic ratio of AH50 was 3.16, while for AC was 2.37, with significant differences ($p < 0.05$). The same trend was observed in cooked samples. The values obtained for the different nutritional indices of alheiras, in the case of alheira control were similar to parameters described by Campos et al. [2] and Teixeira et al. [38].

Physicochemical analysis

The results of the physicochemical analyses of raw and cooked alheiras formulated with a gelled emulsion made with hemp seed oil and buckwheat flour used as a partial substitute for pork backfat were shown in Table 5. Regarding pH values, in both raw and cooked alheiras no statistical differences were found ($p > 0.05$) between AC and AH50 whilst AH25 had the lowest ($p < 0.05$).

Water activity values for both raw and cooked alheiras were not significantly altered ($p > 0.05$) by the partial substitution of pork backfat by the hemp seed oil-GE, with values around 0.960. The results of the physicochemical analyses made by Azevedo et al. [10] of several traditional and reformulated alheiras showed no differences in pH values ranging between 4.2 and 6.0 and 4.0 and 6.0 for traditional and reformulated alheiras respectively. This range includes the samples analysed in this study. Also, water activity values for traditional (0.959–0.988) and reformulated alheiras (0.961–0.991) were included in the water activity of this study. Teixeira et al. [38] found similar values of water activity ranging between 0.939 and 0.961 and similar values of pH. Otherwise, Patarata et al. [4] mentioned lower values of water activity (0.920–0.940) and pH (4.32–5.71). This variability in the physicochemical values could be due to the different manufacturing processes carried out, and/or the different raw materials used to elaborate this traditional meat product. In reference to colour parameters, in raw alheiras, the yellowness (b^*) coordinate, which ranged from 24.96 to

26.89, and hue (h^*) which ranged from 1.15 to 1.18, were not influenced ($p > 0.05$) by the use of Hemp-GE as pork backfat replacer. On the other hand, lightness (L^*), redness (a^*), and chroma (C^*) of raw alheiras were significantly ($p < 0.05$) affected by this replacement although their variation was not quantitatively relevant. There can be several reasons for these differences, including different oil characteristics and compositions, different emulsion properties, and different meat ingredients. As a general trend, only the raw sample with higher substitution (AH50) showed differences ($p < 0.05$) in the parameters lightness, redness, and chroma in reference with the control sample (AC) and AH25 sample. Redness values, ranged from 10.20 (AH50) to 11.61 (AC), and chroma values between 26.96 (AH50) and 29.24 (AH25), with very small differences. Therefore, lightness presented 8 points of differences between raw sample AH50 and AC. All these colour changes could be attributed to the characteristic colour of Hemp-GE, which is different in comparison with animal fat. In terms of colour properties, when meat products are heated, several reactions occur, such as the Maillard reaction, protein denaturation, and fat and water loss, which result in colour and taste changes [54]. Raw alheiras had some colour changes due to gelled emulsions, but cooking does not reveal these changes ($p > 0.05$) for all colour parameters. Similarly, Barros et al. [50] reported that in beef burgers added with oil emulsions no differences were found in the colour parameters. In contrast, several studies reported that colour parameters of different meat products were affected by the use of gelled emulsions used as fat replacer [11, 12, 50]. A study carried out by Summo et al. [55] revealed that raw meat products showed greater colour differences than cooked meat products where the fat was partially replaced by gelled emulsions.

It is very important to highlight that colour differences (ΔE^*) lower than 3 units can not be perceived by the human eye [56]; thus, all raw alheiras formulated with gelled emulsion as a partial pork backfat replacement (AH25 and AH50) might be perceived as different from control alheira. Since the minimum difference ($p < 0.05$) between AH25 and AC

Table 5 Physicochemical parameters of control and reformulated alheiras (raw and cooked)

	Sample	pH	Aw	L^*	a^*	b^*	C^*	h	ΔE^*
Raw	AC	5.95 ± 0.09 ^a	0.963 ± 0.00 ^a	68.24 ± 3.51 ^a	11.61 ± 0.62 ^a	26.54 ± 1.20 ^a	28.90 ± 1.31 ^a	1.16 ± 0.01 ^a	–
	AH25	5.79 ± 0.05 ^b	0.958 ± 0.01 ^a	66.54 ± 3.75 ^{ab}	11.48 ± 0.60 ^a	26.89 ± 1.46 ^a	29.24 ± 1.50 ^a	1.17 ± 0.02 ^a	4.00 ± 1.89 ^b
	AH50	5.92 ± 0.05 ^a	0.957 ± 0.01 ^a	60.56 ± 1.97 ^b	10.20 ± 0.35 ^b	24.96 ± 0.77 ^a	26.96 ± 0.85 ^b	1.18 ± 0.00 ^a	6.87 ± 0.84 ^a
Cooked	AC	5.83 ± 0.03 ^x	0.959 ± 0.00 ^x	65.06 ± 1.86 ^x	11.29 ± 0.50 ^x	25.53 ± 0.97 ^x	27.92 ± 1.07 ^x	1.15 ± 0.01 ^x	–
	AH25	5.74 ± 0.05 ^y	0.958 ± 0.01 ^x	65.43 ± 2.15 ^x	11.66 ± 0.69 ^x	26.70 ± 1.14 ^x	29.14 ± 1.28 ^x	1.16 ± 0.01 ^x	2.57 ± 1.20 ^x
	AH50	5.85 ± 0.08 ^x	0.961 ± 0.01 ^x	63.63 ± 2.54 ^x	11.44 ± 0.75 ^x	26.37 ± 1.20 ^x	28.75 ± 1.38 ^x	1.16 ± 0.01 ^x	3.15 ± 1.15 ^x

AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour. For raw samples (a–c) and cooked samples (x–z), values followed by a different letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$)

samples is 4 units. However, the cooking process would have masked the changes, resulting in very similar colour parameters in all the samples. This fact meant that the ΔE^* values obtained for the cooked samples showed no differences ($p > 0.05$) between them, with values close to the limit of 3 units. Consequently, once cooked, the consumer could not distinguish between control and reformulated samples.

Cooking loss

Alheiras were cooked at 180 °C (grilled) and the cooking loss was evaluated. The cooking process produces water evaporation as well as lipid migration in samples. Thus, the magnitude of these changes may affect the product acceptance [11, 57]. As can be seen in Fig. 2, all alheiras (control, AH25 and AH50) showed similar cooking loss, without statistical differences between them ($p > 0.05$). The cooking loss values ranged between 6.36 and 8.54%. So, incorporation of the gelled emulsion to substitute pork backfat in alheira samples does not affect negatively this parameter. Based on their stability and interrelationship with the meat matrix, these variations could be attributed to the nature and amount of oil, flour, and gelling agent used in the gelled emulsion elaboration. The cooking loss values had not been measured in alheira before. However, the analysis of this parameter is very common in meat products in which animal fat, totally or partially, had been replaced by other non-meat ingredients, generally dietary fibre. Therefore, in the scientific literature, the weight loss in cooked meat products where several dietary fibres were used as fat replacers had a contradictory behaviour. In this sense, Salejda et al. [58] informed that the weight loss of Frankfurt sausage added with 3% buckwheat co-products used as fat replacers was 15%. However, De Araujo et al. [59] carried out a work to analyse the weight loss of chicken sausages where the

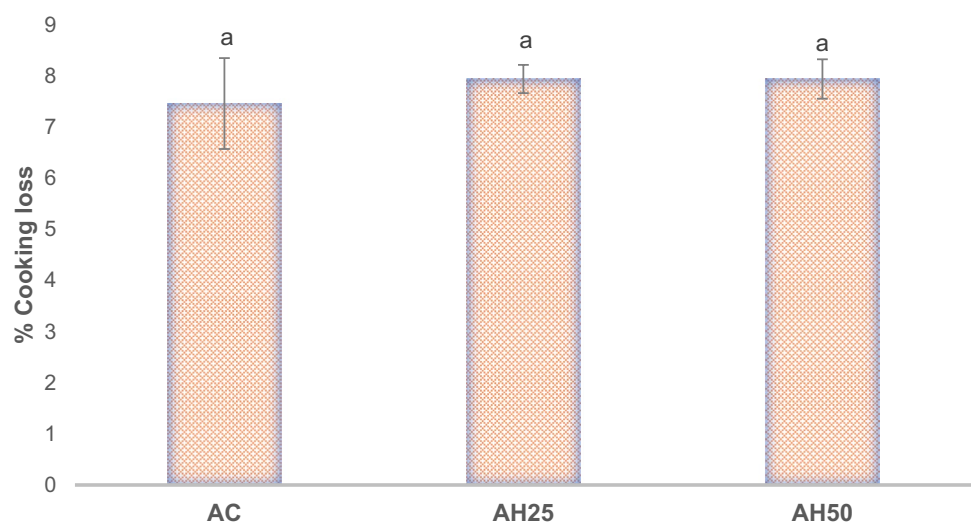
animal fat was substituted by inulin. These authors informed the weight loss values around 2.32% for the total substitution of animal fat. Similar values to those obtained in the present study were found by Choe and Kim [44] on chicken sausages where a chicken skin and wheat fibre mixture was used as a fat substitute. They found that for animal fat replacement levels of 20% there was no difference in cooking losses with the control sample.

Lipid oxidation of alheiras (TBARS)

Lipid oxidation is one of the principal causes of deterioration and quality loss of meat and meat products. In reformulated meat products, the rancidity process negatively affects different parameters such as colour, texture, nutritional value, flavour, and aroma, all of which are important factors that cause consumers to reject the products [60]. Figure 3 showed the lipid oxidation values in all samples analysed, before and after cooking. In raw samples, the substitution of pork backfat for Hemp-GE had no significant impact on the evolution of TBARS values. Thus, there were no significant differences ($p > 0.05$) in TBARS values between the control alheira (0.43 mg malondialdehyde (MDA)/kg of sample) and the AH25 and AH50 alheiras with values of 0.43 and 0.40 mg MDA/kg sample, respectively. The findings were encouraging since hemp seed oil is a rich source of polyunsaturated fatty acids which are much more susceptible to oxidation than pork backfat.

Cooking treatment is the principal factor that will trigger the oxidative processes. Thus, if the effect of the heat treatment is analysed for each of the samples, it is observed that the control sample (AC) was not affected by thermal treatment. On the other hand, after cooking, the AH25 oxidation values increased by 30% with respect to control sample whilst for AH50 the oxidation values increased by

Fig. 2 Cooking weight loss of control and reformulated alheiras. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a gelled emulsion elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a gelled emulsion elaborated with hemp seed oil and buckwheat flour. Bars with different letters were significantly different in accordance with Tukey's HSD post-hoc test ($p < 0.05$)



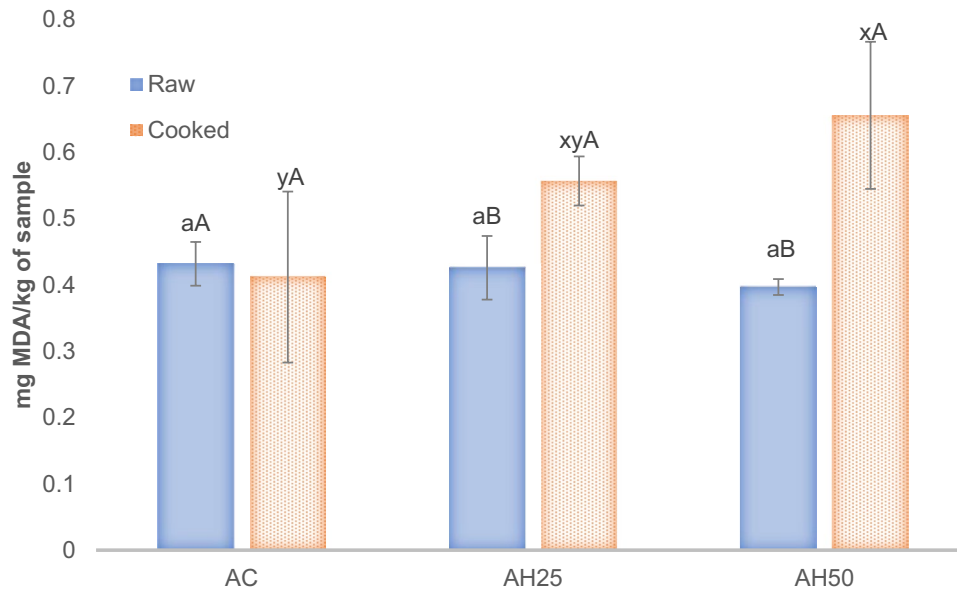


Fig. 3 Lipid oxidation values (TBARS) of control and reformulated alheiras (raw and cooked). Results were expressed as mg of malondialdehyde/kg of sample. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a gelled emulsion elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a gelled emulsion elaborated with hemp seed

oil and buckwheat flour. Bars with different letters were significantly different in accordance with Tukey's HSD post-hoc test ($p < 0.05$). A lower-case letter refers to the comparison of the same parameter between raw samples (a) and cooked samples (x–y). Capital letters (A, B) refers to the comparison of the same sample depending on cooking (raw or cooked)

65% with statistical differences ($p < 0.05$) between samples. Thus, the greater the degree of substitution, the greater the degree of oxidation. The fatty acid profile of the hemp seed oil which had a high content of polyunsaturated fatty acids and the thermal treatment used to cook the alheiras affected the malondialdehyde content.

In the scientific literature, there are no studies where the lipid oxidation of alheiras had been measured. However, it can be seen that the trend obtained in this study is similar to that of other meat products in which pork back fat was replaced by a gelled emulsion based on vegetable oils [16, 23, 47]. Nevertheless, it is important to note that in all alheiras analysed the lipid oxidation values obtained were lower than the malondialdehyde threshold for acceptability. Thus, Domínguez et al. [61] reported that the limit which indicates a loss of sensorial attributes as well as the perception of rancidity by consumers is 2 mg malonaldehyde/kg sample.

Conclusion

The results obtained in this work suggests that the reformulation of alheiras by means of gelled emulsion elaborated with buckwheat flour and hemp seed oil as a partial (25% and 50%) pork backfat replacer is a feasible strategy to obtain healthier meat products in relation to the quality of dietary fats (increase in polyunsaturated fatty acids and

reduce in saturated fatty acids). Alheiras where animal fat was replaced at 50% with gelled emulsions a high content of α -linolenic fatty acid was obtained. It is important to highlight, that colour differences between control sample and reformulated alheiras were obtained only in raw samples. The cooked alheiras, where 50% of pork backfat was replaced by gelled emulsions were more disposed to the lipid oxidation than the control but it did not exceed the limit indicative of rancidity in meat products detectable by consumer. In addition to offering meat products that satisfy the requirements of food safety agencies, these findings will also increase the competitiveness of the meat industry.

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Data availability Data available on request from the authors.

Declarations

Conflict of interest Authors declare no conflict of interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Compliance with ethics requirements This study does not involve research on human participants or animals.

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8.8. PUBLICACIÓN 8

Development of plant-based burgers using gelled emulsions as fat source and beetroot juice as colorant: Effects on chemical, physicochemical, appearance and sensory characteristics

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Development of plant-based burgers using gelled emulsions as fat source and beetroot juice as colorant: Effects on chemical, physicochemical, appearance and sensory characteristics

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ABSTRACT

The aim of this study was to develop plant-based burgers using gelled emulsions (GE, with chia and hemp oil) as fat source and, beetroot juice (fresh and commercial) as colorant ingredient and to assess their quality properties. Burgers with low fat content (<3%) and remarkable protein (18.6–19.5%) and dietary fiber content (14.5–16.2%) were obtained. The use of GE allows improving their lipid profile being PUFAs the main fraction (>57%, PUFA/SFA >4.5, $n-6/n-3 < 4$) with differences in the main fatty acid (>40%) depending on the GE used: α -linolenic in the case of chia-GE and linoleic when hemp-GE was used. The use of beetroot fresh juice allows to obtain burgers with a redness similar to that of traditional meat burgers (16–21), with higher betalains content (27–38 mg/100 g dw) but more susceptible to color changes during cooking than when commercial juice was used. Plant-based burgers suffer less cooking loss (14–17%) and dimensional changes (shrinkage 3–5% and not thickness increase) than reported for traditional meat burgers. According to the results of sensory evaluation, although all plant-based burgers were scored with a good overall acceptability, it could be enhanced by the ingredient optimization because each of the ingredients studied either improved or worsened the different attributes assessed.

1. Introduction

Plant-based burgers are getting rapidly popular worldwide which is due on the one hand, to the fact that its consumption has become widespread in the population (not only as fast food but also in gourmet restaurants and shops) and on the other hand, to the increasing concerns about the impact of animal food consumption on human health, climate change and animal welfare (Willett et al., 2019; van Vliet, Kronberg, & Provenza, 2020). More and more people in the world choose plant-based products over animal-based nutrition, occasionally or permanently. The plant-based burgers market is predicted to rise exponentially, exhibiting a Compound Annual Growth Rate of over 22% between 2020 and 2030 (FMI, 2020). This prediction seems easily achievable just by looking at the breadth of the current plant-based burgers offer and the number of new and innovative options launched on the market by food companies

(Fernández-López, Paya, et al., 2021).

Although global plant-based burgers market started as a niche industry catering only to vegan, vegetarian and flexitarian community, now it is growing into a mainstream food industry trying to increase the acceptance also by omnivores. Plant-based burgers must be designed to have properties (physicochemical, functional, and sensory) close to that of original meat burgers. It means that these products should mimic the appearance, texture, mouthfeel, flavor, cookability, and nutritional profile of original ones (He, Evans, Liu, & Shao, 2020; Lee, Yong, Kim, Choi, & Jo, 2020). Nutritionally, these plant-based burgers should also be designed keeping the most valuable nutritious compounds found in meat (high protein content with a well-balanced amino acid profile) and avoiding the unhealthy ones (saturated fats and cholesterol) to reach advantage for human health purposes (Badar, Liu, Chen, Xia, & Kong, 2021; Kyriakopoulou, Dekkers, & van der Goot, 2019; Kyriakopoulou,

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Kepler, & van der Goot, 2021). In addition, their enrichment in dietary fiber and bioactive compounds (typical compounds from vegetable sources) (Fernández-López, Botella-Martínez, et al., 2020; Viuda-Martos et al., 2010) can provide these plant-based burgers with desirable functional and nutritive attributes which are not found in original meat burgers (Zhou, Vu, Gong, & McClements, 2022).

In order to provide an alternative source of protein (vegetable), different available options have been studied, being in the case of burgers, more attractive their use in the form of textured protein substances (from pea, soy, quinoa, etc.) which allow to take on the texture of whatever ground meat it is substituting (Delizar, Saldivar, Germani, Benassi, & Cabral, 2002; Maningat, Jeradechachai, & Buttshaw, 2022). In addition to this, a long list of ingredients (water, seasonings, salt, binders and coloring agents) has been used to maintain the taste and color of the desired product (He, Liu, Balamurugan, & Shao, 2021). For more natural and clean label products, the selection of ingredients that naturally contain compounds with these activities is being promoted. In these sense, protein-rich flours and vegetable fibers can be used as binders (Pietrasik, Sigvaldson, Soladoye, & Gaudette, 2020) and beetroot or red fruits juices as colorants (Kyriakopoulou et al., 2021), trying to mimic the meat red color. Although in smaller quantities, salt changes the structure of proteins and toughens the burgers (Rios-Mera et al., 2020), while binders provide water and fat retention, and improve the texture and appearance of the product (Pietrasik et al., 2020).

The fat source used has also a great importance not only technologically, but also from the sensorial, nutritional and healthy point of view (Badar et al., 2021). As animal fat substitute, several vegetable oils with healthier lipid profile have also been studied (sunflower, canola, palm, coconut, etc) (Dominguez et al., 2022). Trying to minimize the negative effects on burger batter formation due to the use of these vegetable oils (liquids and easily oxidizable), several structuring methods have been developed to provide vegetable oils a similar solid structure to animal fats, but keeping stable their healthy lipid profile (Ospina-E, Cruz-S, Pérez-Álvarez, & Fernández-López, 2010; Guo et al., 2020; Badar et al., 2021; Botella-Martínez, Pérez-Álvarez, Sayas-Barberá, Fernández-López, & Viuda-Martos, 2021; Herrero & Ruiz-Capillas, 2021; Oztürk-Kerimoglu, Uргу-Oztürk, & Serdaroglu, 2021). Among these strategies, gelled emulsions (GE) show a great potential as animal fat substitution in meat products in order to make them healthier (Botella-Martínez et al., 2022; Botella-Martínez, Viuda-Martos, Pérez-Álvarez, & Fernández-López, 2021; De Souza Paglarini et al., 2019; Nacak, Oztürk-Kerimoglu, Yildiz, Çagindi, & Serdaglou, 2021). A GE is a colloidal material in which oil in water emulsions (O/W) coexists within a gel network providing them mechanical and visual properties similar to solid fat (Herrero, Ruiz-Capillas, Pintado, Carmona, & Jiménez-Colmenero, 2017). For the elaboration of GE different vegetable oils (chia, hemp, linseed, among others) have been assayed, together with other protein/starchy ingredients as pseudocereal flours (quinoa, amaranth, buckwheat, teff, etc) with the aim to stabilize these GE (Botella-Martínez, Pérez-Álvarez, et al., 2021; De Souza Paglarini et al., 2019; Fernández-López, Viuda-Martos, & Pérez-Álvarez, 2021; Pintado, Herrero, Jiménez-Colmenero, Pasqualin-Calvalheiro, & Ruiz-Capillas, 2018). From all these GE, the ones that have shown most interesting both for their technological feasibility and for the lipid profile (high polyunsaturated fatty acids (PUFA)) have been those made with hemp and chia oil, and buckwheat flour (Botella-Martínez, Pérez-Álvarez, et al., 2021). Hemp (*Cannabis sativa* L.) and chia (*Salvia hispanica* L.) oils show high PUFA/SFA ratio (high amount of essential fatty acids, α -linolenic acid (ALA) and linoleic acid (LA)) thus demonstrating their potential as a good alternative for animal fat substitution. Chia oil contains around 60% ALA, while in hemp oil the most abundant fatty acid is LA (55–60%) (Ayerza & Coates, 2004; Leonard, Zhang, Ying, & Fang, 2019; Vodolazska & Lauridsen, 2020). Antioxidant phytochemicals, such as tocopherols, phenols, polyphenols, and lignanamides have been found in hemp oil (Leonard et al., 2019) and also in chia oil (tocopherols, phenolic compounds and carotenoids) (Itxaina et al.,

2011; Bodoira, Penci, Ribotta, & Martínez, 2017) which could contribute to control lipid instability associated with its high PUFA content.

Although several scientific references regarding plant-based burgers development, formula, properties and characterization have been found (De Marchi, Costa, Pozza, Goi, & Manuelian, 2021; He et al., 2021; Keerthana-Priya, Rawson, Vidhyalakshmi, & Jagan-Mohan, 2022; Saget et al., 2021; Smetana, Profeta, Voigt, Kircher, & Heinz, 2021; Tremlova et al., 2022), in none of them GE was used as fat source. For this reason, the purpose of this study was to evaluate the effect of using GE (with chia and hemp oil) as fat source and, beetroot juice (fresh or commercial) as coloring agent in plant-based burgers assessing their chemical, nutritional physicochemical, cooking, appearance and sensory properties.

2. Materials and methods

2.1. Materials

For GEs preparation the following ingredients were used: chia oil (56.61 g/100g α -linolenic acid, 17.43 g/100g linoleic acid, and 15.05 g/100g oleic acid) and hemp oil (54.44 g/100g linoleic acid, 19.95 g/100g α -linolenic acid, 8.23 g/100g oleic acid) from Laboratorios Almond, S.L. (Murcia, Spain); buckwheat flour from HLT S.A. (Madrid, Spain); carrageenans (a polysaccharide extracted from seaweeds such as *Eucheima* species, *Chondrus crispus* and *Gigartina* species (Tarté, 2009) and locust bean gum (a galactomannan vegetable gum used as gelling agent extracted from carob tree) from Innovative Cooking S.L. (Madrid, Spain).

Plant-based ingredients: textured soya (>90 g/100g proteins) and pea fiber (>55 g/100g dietary fiber) from Suministros River S.L.U (Alicante, Spain); peanut flour (12.50 g/100g lipids, 49.59 g/100g protein, 14.10 g/100g fiber and 31.94 g/100g carbohydrates of which sugar 7.2 g/100g) from ViperCo Group Ltd (Batley, UK); commercial beetroot juice (13.27 °Brix, 3.71 pH, CIELAB color coordinates: 28.08 L*, 9.67 a*, 3.26 b*) from Juver Alimentación S.L.U. (Murcia, Spain); beetroots from Naturally Organic S.L. (Murcia, Spain) were used to obtain fresh beetroot juice that was subsequently diluted with tap water in a 1:3 ratio (4.27 °Brix, 6.64 of pH, CIELAB color coordinates: 24.56 L*, 0.66 a*, -0.51 b*).

2.2. Preparation of oil-in-water gelled emulsions (GE)

Chia-GE and hemp-GE were elaborated using water (45%), chia or hemp oils (45%), buckwheat flour (9%), and carrageenans and locust bean gum as gelling agents (1%), following the procedure described by Botella-Martínez, Pérez-Álvarez, et al. (2021).

2.3. Preparation of plant-based burgers

Plant-based burgers were elaborated without pre-processing treatments following formula showed in Table 1. Firstly, textured soya was hydrated during 30 min by adding fresh or commercial beetroot juice. Freshly prepared beetroot juice was previously diluted with tap water in a ratio 1:3, whereas commercial juice was directly used. Then, peanut flour and pea fiber were added and mixed with the hydrated soya. After that, GEs were minced until rice grain size and until a homogenous distribution in the batter. As a last step, salt and spices (parsley powder, onion powder, garlic powder and black pepper) were added. So, four different batches were obtained: two batches with chia-GE [one with fresh beetroot juice (PBFCh) and other with commercial juice (PBCCh)], and two batches with hemp-GE (PBFH with fresh juice and PBCH with commercial juice). There is not a “control” sample in view of the difficult to identify the appropriate formulation to achieve this role. The samples were shaped using a commercial burger maker to obtain plant-based burgers of approximately 1 cm thickness and 80 g. Samples were packed into bags and storage at 4 °C until analysis (raw burgers). Six

Table 1
Plant-based burgers formulation (g/1000g).

INGREDIENTS (g)	PBFCh	PBCCh	PBFH	PBCH
Beetroot juice				
<i>Freshly prepared</i>	525.8	–	525.8	–
<i>Commercial</i>	–	525.8	–	525.8
Texturized soya	214.2	214.2	214.2	214.2
Peanut flour	113.9	113.9	113.9	113.9
Gelled emulsion				
<i>Chia-GE</i>	107.1	107.1	–	–
<i>Hemp-GE</i>	–	–	107.1	107.1
Pea fiber	12.7	12.7	12.7	12.7
Salt	14.6	14.6	14.6	14.6
Spices				
Parsley powder	3.9	3.9	3.9	3.9
Onion powder	2.9	2.9	2.9	2.9
Garlic powder	2.9	2.9	2.9	2.9
Black pepper	1.9	1.9	1.9	1.9
TOTAL	1000.0	1000.0	1000.0	1000.0

burgers from each formulation were cooked in a griddle until reaching an internal temperature of 72 °C, approximately 4.5 min for each side (cooked burgers).

2.4. Characterization of plant-based burgers

2.4.1. Proximate composition

Total ash (AOAC 923.03), protein (AOAC 981.10), fat (AOAC 991.36), dietary fiber (AOAC 985.29) and moisture content (AOAC 925.45) of plant-based burgers were determined using AOAC methods (AOAC, 2010). All determinations were made in triplicate for both raw and cooked plant-based burgers.

2.4.2. Fatty acids analysis

Total fat was extracted and methylated (AOAC, 2010) to obtain the corresponding fatty acid methyl esters (FAMES). The FAMES were analyzed using a Hewlett-Packard 6890 with an ionization detector and a Suprewax 280 capillary column (30 m, 0.25 µm film thickness 0.25 mm i.d.; (Teknokroma Barcelona, Spain)). Working conditions reported by Pellegrini et al. (2018) were applied. Standard fatty acids (Supelco 37 component FAME Mix, Bellefonte, USA) were used to identify individual fatty acids (comparing their retention times). Next, the percentage of each FAME in the samples (g fatty acid/100 g fat) was reported based on their peak area in the chromatogram. All analysis were made in triplicate for both raw and cooked plant-based burgers.

2.4.3. Nutritional indices (from fatty acids analysis)

To evaluate the nutritional value of fatty acids (FA) in plant-based burgers and to explore their potential usage in disease prevention and treatment, several indices can be applied. All these indices have been performed only in cooked hamburgers because that is how they are consumed. The indices of atherogenicity (AI) and the thrombogenic index (TI) to characterize the atherogenic and thrombogenic potential (respectively) of FAs have been calculated following equations proposed by Ulbricht and Southgate (1991). The hypocholesterolaemic/hypercholesterolaemic (h/H) ratio was also calculated using the equation described by Fernández et al. (2007)

$$AI = \frac{[C12 : 0 + (4 \times C14 : 0) + C16 : 0]}{[\sum MUFA + \sum n6 + \sum n3]}$$

$$TI = \frac{[C14 : 0 + C16 : 0 + C18 : 0]}{\left[\left(\frac{\sum MUFA}{2} \right) + \left(\frac{\sum n6}{2} \right) + (3 \times \sum n3) + \left(\frac{\sum n3}{\sum n6} \right) \right]}$$

$$h/H = \frac{[C18 : 1n9 + C18 : 1n7 + \sum PUFA]}{[C14 : 0 + C16 : 0]}$$

2.4.4. Mineral composition

Minerals were quantified after mineralization of the lyophilised raw samples (0.5 g) with 67% nitric acid and 33% hydrogen peroxide by a microwave system using Inductively Coupled Plasma Mass Spectrometry (ICPMS-2030-Shimadzu). The final value per sample was the average of 3 reads; two burgers per batch (n = 6) were analyzed. Minerals were expressed in mg/100g of raw product.

2.4.5. Betalains

Betalain pigments were extracted from the plant burgers with ethanol-water (20:80). Extracts were then clarified by centrifugation at 15,000 × g for 10 min in a Z383K Hermle centrifuge (Wehingen, Germany), and the supernatant passed through a 0.45 µm nylon filter. Red beet juices were pre-diluted and also filtered prior to betalain analysis.

Betalain content was quantitated for each sample as previously described by Fernández-López, Castellar, Obón, and Almela (2002). Total betalain concentration was estimated as the sum of the concentrations of betacyanins and betaxanthins. Betacyanin content was determined as betanin using an extinction molar coefficient (ε) of 60,000 L mol⁻¹·cm⁻¹ at 535 nm. Betaxanthin content was determined as vulgaxanthin I using an extinction molar coefficient (ε) of 48,000 L mol⁻¹·cm⁻¹ at 485 nm (Wruss et al., 2015). Individual betalain pigments were analyzed by HPLC with a Waters modular liquid chromatographic system (Waters, Milford, MA, USA) and a Spherisorb ODS2 5 µm, 250 × 4,6 mm column (Teknokroma, Barcelona, Spain). Program elution followed the method previously proposed by Fernández-López et al. (2002), using a gradient between 175 mmol/L acetic acid in H₂O and 175 mmol/L acetic acid in acetonitrile as mobile phase, with a flow rate of 1 mL min⁻¹.

2.4.6. pH and water activity

pH was determined on both raw and cooked burgers by means of a penetration test carried out with a Crison model 510 pH-meter (Barcelona, Spain) on different areas of each sample. Water activity (aw) was measured in triplicate on burgers (at 25 °C) before and after cooking, using an electrolytic hygrometer (Novasina TH-500, Novasina, Axair Ltd. Pfaeffikon, Switzerland). In both cases three burgers from each batch were used.

2.4.7. Texture

Texture profile analysis (TPA) was carried out on cooked burgers using a TA-XT2i Texture Analyser (Stable Micro Systems, Surrey, England). Samples were uniformly cut into 2 × 2 × 2 cm and compressed (crosshead speed of 1 mm/s) to 75% of their initial height, through a two-cycle sequence with a cylindrical probe of 10 cm diameter. The following TPA parameters were calculated from the recorded force × distance curves: Hardness (N), springiness (mm), cohesiveness and chewiness (N*mm) (Claus, 1995). Nine burgers from each batch were analyzed.

2.4.8. Color parameters

The color of raw and cooked burgers were determined using a Minolta CM-700 spectrophotometer (Minolta Camera Co., Osaka, Japan) with the following settings (illuminant D₆₅, SCI mode and, observation angle 10°). The following CIELAB color coordinates were obtained: Lightness (L*), redness (a*) and yellowness (b*). A low reflectance glass (Minolta CR-A51/1829-752) was placed between the sample and the equipment. From color coordinates, psychophysical magnitudes, hue (h*) and chroma (C*) were calculated. Determinations were performed in triplicate.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad h^* = \arctg \frac{b^*}{a^*}$$

2.4.9. Cooking properties

Cooking loss (%), thickness increase (%) and shrinkage (%) of plant-

based burgers were calculated with the following equations. For that, the weight, thickness and diameter of 3 burgers from each batch were measured before (raw) and after cooking.

$$\text{Cooking loss (\%)} = \frac{(\text{raw weight} - \text{cooked weight})}{\text{raw weight}} \times 100$$

$$\text{Thickness increase (\%)} = \frac{(\text{raw diameter} - \text{cooked diameter})}{\text{raw diameter}} \times 100$$

$$\text{Shrinkage (\%)} = \frac{(\text{raw diameter} - \text{cooked diameter})}{\text{raw diameter}} \times 100$$

2.4.10. Sensory evaluation

A 64-members panel (without specific training) from the CIAGRO-UMH, includes students and researchers, assessed sensory evaluation of plant-based burgers. Protocols for sensory analysis were approved (ref. PRL.DTA.MVM.02.21) by the Project Evaluation Office of the Miguel Hernández University (OEP,UMH, Elche, Alicante, Spain). Sensory analysis was performed under white fluorescent lights in individual booths. Burgers were cooked in a griddle and served in 3 cm³ pieces, approximately. Unsalted crackers and mineral water were provided to clean the palate between samples. A hedonic scale of 9 levels (1:dislike extremely and 9:like extremely) was used in the tasting sheet to evaluate the following attributes: general appearance, color, hardness, flavor and overall acceptance.

2.5. Statistical analysis

Two-way analysis of variance (ANOVA) was performed to evaluate the effect of sample formulation and cooking (statistical significance $p < 0.05$) on burgers properties using the SPSS software v. 27.0 (SPSS Inc., Chicago, USA). For sensory evaluation, panellists were considered random factors. Post-hoc Tukey-b test was applied for means comparison and differences were considered significant at $p < 0.05$. Data are reported as means \pm standard deviation.

3. Results and discussion

3.1. Chemical characterization of plant-based burgers

Some differences in the proximate composition of plant-based

Table 2
Proximate composition of raw and cooked plant-based burgers.

Sample	Ash (g/ 100g)	Protein (g/ 100g)	Fat (g/ 100g)	Moisture (g/ 100g)	TDF (g/ 100g)
RAW BURGERS					
PBFCh	3.39 \pm 0.00 ^{aY}	19.52 \pm 0.27 ^{aY}	2.90 \pm 0.26 ^{aY}	57.47 \pm 0.13 ^{aX}	14.10 \pm 0.35 ^{bY}
PBCCh	3.44 \pm 0.01 ^{aY}	18.68 \pm 0.28 ^{abY}	2.87 \pm 0.21 ^{aY}	54.22 \pm 0.30 ^{bX}	16.15 \pm 0.50 ^{aY}
PBFH	3.37 \pm 0.06 ^{aY}	18.59 \pm 0.09 ^{bY}	2.09 \pm 0.21 ^{bY}	57.11 \pm 0.13 ^{aX}	14.54 \pm 0.43 ^{bY}
PBCH	3.41 \pm 0.05 ^{aY}	18.68 \pm 0.35 ^{abY}	2.91 \pm 0.41 ^{aY}	53.94 \pm 0.12 ^{bX}	16.19 \pm 0.40 ^{aY}
COOKED BURGERS					
PBFCh	4.17 \pm 0.02 ^{aX}	21.94 \pm 0.03 ^{bX}	5.58 \pm 0.30 ^{bX}	48.74 \pm 0.27 ^{aY}	16.40 \pm 0.45 ^{bX}
PBCCh	4.24 \pm 0.15 ^{aX}	22.44 \pm 0.27 ^{aX}	5.57 \pm 0.12 ^{bX}	43.74 \pm 0.62 ^{bY}	18.21 \pm 0.60 ^{aX}
PBFH	4.11 \pm 0.17 ^{aX}	22.21 \pm 0.13 ^{abX}	4.87 \pm 0.21 ^{cX}	47.71 \pm 0.52 ^{aY}	16.74 \pm 0.66 ^{bX}
PBCH	4.07 \pm 0.37 ^{aX}	21.94 \pm 0.01 ^{bX}	5.98 \pm 0.35 ^{abX}	44.97 \pm 0.27 ^{bY}	18.18 \pm 0.52 ^{aX}

^{a-b}Different superscript letter in each column indicate a significant difference ($p < 0.05$) for raw or cooked burgers. ^{X-Y}Different superscript letter in each column indicate a significant difference ($p < 0.05$) for the same sample raw and cooked. Data are presented as mean \pm standard deviation.

burgers due to both formulation and cooking process were observed (Table 2). Burgers made with fresh beetroot juice (PBFCh and PBFH) showed higher moisture content ($p < 0.05$) than those made with commercial juice (PBCCh and PBCH), regardless of the GE used, in both raw and cooked samples. This difference could be due to the juice used in the hydration of the textured soybean, with fresh juice providing a greater amount of moisture than commercial juice. Slight differences in fat, protein and total dietary fiber content between formulations can be attributed to this moisture differences. In fact, if these values were showed in dry basis (data not shown), differences in proximate composition were only due to the type of juice used and not to the type of gelled emulsion (burgers made with fresh juice showed higher protein and lower fat and TDF content ($p < 0.05$) than the others).

All samples showed fat contents lower than 3% which is below the fat content reported for commercial plant-based burgers (4–15%; De Marchi et al., 2021; Fernández-López, Paya, et al., 2021; He et al., 2021). In addition, remarkable protein (18.6–19.5%) and dietary fiber contents (14.5–16.2%) were achieved. In this case, protein content was into the range reported for commercial plant-based burgers but dietary fiber content was higher (0.3–11.3%; Curtain & Grafenauer, 2019; Fernández-López, Paya, et al., 2021; He et al., 2021). As could be expected cooked burgers showed lower ($p < 0.05$) moisture content than corresponding raw ones, which is due to water losses during cooking. This moisture reduction in cooked burgers would be responsible for the observed increase ($p < 0.05$) in the rest of nutrients in comparison to raw ones.

Ash content did not show statically significant differences ($p > 0.05$) between formulation for either raw or cooked samples (Table 2). However, significant differences in the mineral profile in raw samples (Table 3) have been detected. In all formulation, the most abundant minerals were K and Na, followed by Mg and Ca, being Fe, Mn, Zn and Cu, which showed the lowest content ($p < 0.05$). Similar trend have been reported by other authors in several plant-based burgers (De Marchi et al., 2021). Regarding formulation, the main differences seem to be due to the type of juice rather than to the GE used. In this sense, plant-based burgers elaborated with fresh juice (PBFCh and PBFH) showed higher amounts ($p < 0.05$) of Mn, Zn, Na and Cu than those made with commercial juice (PBCCh and PBCH). The only mineral in which significant differences regarding the GE used has been detected was iron, showing burgers made with chia-GE higher iron content ($p < 0.05$) than those made with hemp-GE. Although both seeds (chia and hemp) are considered good sources of iron, higher iron content has been reported for chia seeds (Alonso-Esteban, Torija-Isasa, & Sánchez-Mata, 2022; Pereira da Silva, Kolba, Stampini, Hart, & Tako, 2019).

FA profiles of plant-based burgers (raw and cooked) are shown in Table 4. A total of 17 FA were detected in plant-based burgers ranging from C14 to C24, although only the sum of 5 of them (C16:0, C18:0, C18:1, C18:2 and C18:3) represents more than 95% of the total fat content (Fig. 1). In general, it could be say that cooking process has not a significant effect ($p > 0.05$) on lipid profile of plant-based burgers and so, the main differences are due to formulation. Lipid profile in burgers follows a similar trend in both raw and cooked burgers, which is in agreement with other authors (Botella-Martínez et al., 2022; He et al., 2021).

The predominant fraction of lipids in all formulations were the polyunsaturated fatty acids (PUFA) achieving percentages higher than 57% (in raw and cooked samples), with the saturated lipid fraction (SFA) being the minority in all of them (percentages lower than 12.5%). The monounsaturated fatty acids fraction (MUFA) represents 30% approx. of the total fat content.

These results are very interesting because most of the studies reporting the lipid profile of different types of commercial plant-based burgers founded MUFA as the main fraction which is due to the use of canola or olive oil as lipid ingredient (He et al., 2021). Undoubtedly, the use of these GEs (with chia and hemp oil) as fat source in plant-based burgers is responsible for these findings. In addition, as can be seen in

Table 3
Mineral composition of raw plant-based burgers (mg/100g dw).

Sample	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
PBFCh	255.0 ± 13.8 ^a	1.38 ± 0.02 ^a	8.37 ± 1.77 ^a	1517 ± 25 ^b	256.0 ± 5.0 ^b	3.30 ± 0.09 ^a	1277 ± 21 ^b	3.27 ± 0.07 ^a
PBCCh	235.3 ± 2.3 ^{ab}	1.21 ± 0.01 ^c	8.54 ± 2.17 ^a	1440 ± 36 ^c	235.7 ± 5.0 ^c	2.95 ± 0.05 ^b	1167 ± 30 ^c	2.99 ± 0.02 ^b
PBFH	235.3 ± 8.7 ^{ab}	1.27 ± 0.05 ^b	6.69 ± 0.66 ^b	1613 ± 29 ^a	275.7 ± 3.2 ^a	3.14 ± 0.08 ^a	1340 ± 17 ^a	3.17 ± 0.10 ^a
PBCH	227.0 ± 8.7 ^b	1.20 ± 0.03 ^c	5.46 ± 0.11 ^c	1537 ± 12 ^b	258.3 ± 2.3 ^b	2.85 ± 0.07 ^b	1127±6 ^c	2.76 ± 0.09 ^c

Different superscript letter in each column indicate a significant difference ($p < 0.05$). Data are presented as mean ± standard deviation.

Table 4
Lipid profile of raw and cooked plant-based burgers.

	RAW plant-based burgers				COOKED plant-based burgers			
	PBFCh	PBCCh	PBFH	PBCH	PBFCh	PBCCh	PBFH	PBCH
C14:0	0.04 ± 0.00 ^{aX}	0.04 ± 0.00 ^{aX}	0.04 ± 0.00 ^{aX}	0.04 ± 0.00 ^{aX}	0.04 ± 0.00 ^{aX}	0.04 ± 0.00 ^{aX}	0.04 ± 0.00 ^{aX}	0.04 ± 0.00 ^{aX}
C15:0	0.02 ± 0.00 ^{aY}	0.03 ± 0.00 ^{aX}	0.03 ± 0.01 ^{aX}	0.02 ± 0.00 ^{aX}	0.04 ± 0.01 ^{aX}	0.02 ± 0.00 ^{aY}	0.02 ± 0.01 ^{aY}	0.02 ± 0.00 ^{aX}
C16:0	6.90 ± 0.02 ^{bX}	6.87 ± 0.01 ^{bX}	7.49 ± 0.17 ^{aX}	7.52 ± 0.04 ^{aX}	6.73 ± 0.02 ^{bY}	6.74 ± 0.05 ^{bY}	7.51 ± 0.00 ^{aX}	7.45 ± 0.00 ^{aX}
C16:1	0.07 ± 0.00 ^{bX}	0.07 ± 0.00 ^{bX}	0.10 ± 0.01 ^{aX}	0.10 ± 0.00 ^{aX}	0.07 ± 0.00 ^{bX}	0.07 ± 0.01 ^{bX}	0.10 ± 0.00 ^{aX}	0.10 ± 0.00 ^{aX}
C17:0	0.08 ± 0.00 ^{aX}	0.10 ± 0.02 ^{aX}	0.08 ± 0.01 ^{aX}	0.08 ± 0.00 ^{aX}	0.08 ± 0.01 ^{aX}	0.09 ± 0.00 ^{aY}	0.08 ± 0.01 ^{aX}	0.08 ± 0.01 ^{aX}
C17:1	0.06 ± 0.00 ^{aX}	0.05 ± 0.00 ^{aX}	0.04 ± 0.01 ^{aX}	0.05 ± 0.01 ^{aX}	0.06 ± 0.00 ^{aX}	0.05 ± 0.00 ^{aX}	0.04 ± 0.01 ^{aX}	0.05 ± 0.00 ^{aX}
C18:0	3.30 ± 0.00 ^{aX}	3.30 ± 0.00 ^{aX}	2.99 ± 0.05 ^{bY}	3.01 ± 0.00 ^{bY}	3.28 ± 0.00 ^{bY}	3.31 ± 0.01 ^{aX}	3.05 ± 0.00 ^{cX}	3.04 ± 0.01 ^{dX}
C18:1 (cis)	30.28 ± 0.08 ^{aX}	30.46 ± 0.15 ^{aX}	29.02 ± 0.39 ^{bX}	28.99 ± 0.05 ^{bX}	30.11 ± 0.03 ^{aY}	30.10 ± 0.08 ^{aY}	28.16 ± 0.03 ^{bY}	28.40 ± 0.02 ^{bY}
C18:1 (TRANS)	0.00	0.00	0.00	0.00	0.63 ± 0.00 ^{aX}	0.61 ± 0.00 ^{aX}	0.63 ± 0.00 ^{aX}	0.60 ± 0.02 ^{aX}
C18:2 (n 6,9)	15.13 ± 0.03 ^{bX}	14.97 ± 0.06 ^{bY}	41.72 ± 0.78 ^{aY}	42.30 ± 0.04 ^{aX}	14.80 ± 0.04 ^{dY}	15.12 ± 0.02 ^{cX}	42.78 ± 0.00 ^{aX}	42.44 ± 0.00 ^{bX}
C18:2 (n 3,6)	0.41 ± 0.00 ^{bX}	0.42 ± 0.00 ^{bX}	0.42 ± 0.01 ^{aX}	0.43 ± 0.00 ^{aX}	0.41 ± 0.00 ^{aX}	0.41 ± 0.00 ^{aX}	0.43 ± 0.00 ^{aX}	0.43 ± 0.00 ^{aX}
C18:3 (n 3,6,9)	41.43 ± 0.05 ^{aX}	41.42 ± 0.11 ^{aX}	13.46 ± 0.26 ^{bY}	13.75 ± 0.04 ^{bX}	40.93 ± 0.05 ^{bY}	41.37 ± 0.01 ^{aX}	13.69 ± 0.01 ^{cX}	13.73 ± 0.03 ^{cX}
C18:3 (n 6,9,12)	0.48 ± 0.01 ^{bX}	0.49 ± 0.01 ^{bX}	0.87 ± 0.02 ^{aX}	0.88 ± 0.00 ^{aX}	0.49 ± 0.01 ^{bX}	0.50 ± 0.00 ^{bX}	0.88 ± 0.00 ^{aX}	0.89 ± 0.01 ^{aX}
C20:0	0.52 ± 0.00 ^{bX}	0.52 ± 0.00 ^{bX}	0.72 ± 0.03 ^{aX}	0.71 ± 0.01 ^{aX}	0.53 ± 0.00 ^{bX}	0.53 ± 0.00 ^{bX}	0.71 ± 0.00 ^{aX}	0.70 ± 0.01 ^{aX}
C20:5 (n 5,8,11,14,17)	0.41 ± 0.01 ^{aY}	0.32 ± 0.01 ^{bX}	0.21 ± 0.02 ^{cX}	0.24 ± 0.02 ^{cX}	0.95 ± 0.02 ^{aX}	0.17 ± 0.00 ^{bY}	0.12 ± 0.04 ^{bY}	0.09 ± 0.02 ^{bY}
C22:0	0.76 ± 0.01 ^{aY}	0.77 ± 0.01 ^{aY}	0.88 ± 0.02 ^{aX}	0.88 ± 0.01 ^{aX}	0.75 ± 0.01 ^{aX}	0.80 ± 0.01 ^{aX}	0.87 ± 0.01 ^{aX}	0.89 ± 0.01 ^{aX}
C22:2	0.06 ± 0.01 ^{aX}	0.08 ± 0.01 ^{aX}	0.06 ± 0.01 ^{aX}	0.07 ± 0.01 ^{aX}	0.05 ± 0.01 ^{aX}	0.06 ± 0.01 ^{aX}	0.07 ± 0.01 ^{aX}	0.07 ± 0.01 ^{aX}
C24:0	0.47 ± 0.01 ^{aX}	0.47 ± 0.00 ^{aX}	0.49 ± 0.01 ^{aX}	0.48 ± 0.01 ^{aX}	0.46 ± 0.01 ^{aX}	0.47 ± 0.01 ^{aX}	0.46 ± 0.01 ^{aX}	0.49 ± 0.01 ^{aX}
∑SFA	12.09 ± 0.04 ^{aX}	11.63 ± 0.04 ^{bY}	12.23 ± 0.24 ^{aX}	11.36 ± 0.07 ^{cX}	11.91 ± 0.06 ^{aX}	12.00 ± 0.08 ^{aX}	11.41 ± 0.04 ^{bY}	11.32 ± 0.05 ^{bX}
∑MUFA	30.41 ± 0.08 ^{aX}	30.57 ± 0.15 ^{aX}	29.16 ± 0.41 ^{bX}	29.14 ± 0.06 ^{bX}	30.87 ± 0.03 ^{aX}	30.82 ± 0.09 ^{aX}	28.93 ± 0.04 ^{bY}	29.15 ± 0.14 ^{bX}
∑PUFA	57.93 ± 0.11 ^{aX}	57.70 ± 0.20 ^{aX}	57.54 ± 1.10 ^{aX}	57.60 ± 0.11 ^{aX}	57.62 ± 0.06 ^{bX}	57.64 ± 0.04 ^{bX}	57.89 ± 0.06 ^{aX}	57.49 ± 0.03 ^{cX}
∑N6	15.62 ± 0.04 ^{bX}	15.46 ± 0.07 ^{bX}	42.59 ± 0.80 ^{aY}	43.18 ± 0.04 ^{aX}	15.29 ± 0.05 ^{bY}	15.62 ± 0.02 ^{bX}	43.66 ± 0.00 ^{aX}	43.33 ± 0.00 ^{aX}
∑N3	41.84 ± 0.05 ^{aX}	41.84 ± 0.11 ^{aX}	13.88 ± 0.27 ^{bY}	14.18 ± 0.04 ^{bX}	41.34 ± 0.05 ^{bY}	41.78 ± 0.01 ^{aX}	14.11 ± 0.03 ^{cX}	14.16 ± 0.03 ^{cX}
∑PUFA/∑SFA	4.79 ± 0.10 ^{bX}	4.96 ± 0.08 ^{bX}	4.70 ± 0.30 ^{bY}	5.07 ± 0.05 ^{aX}	4.84 ± 0.06 ^{bX}	4.80 ± 0.08 ^{bX}	5.07 ± 0.06 ^{aX}	5.08 ± 0.05 ^{aX}
∑N6/∑N3	0.37 ± 0.05 ^{aX}	0.37 ± 0.08 ^{aX}	3.07 ± 0.42 ^{bX}	3.05 ± 0.01 ^{bX}	0.37 ± 0.01 ^{aX}	0.37 ± 0.02 ^{aX}	3.09 ± 0.00 ^{aX}	3.06 ± 0.02 ^{aX}

^{a-b}Different superscript letter in each row indicate a significant difference ($p < 0.05$) for raw or cooked burgers. ^{X-Y}Different superscript letter in each row indicate a significant difference ($p < 0.05$) for the same sample raw and cooked. Data are presented as mean ± standard deviation.

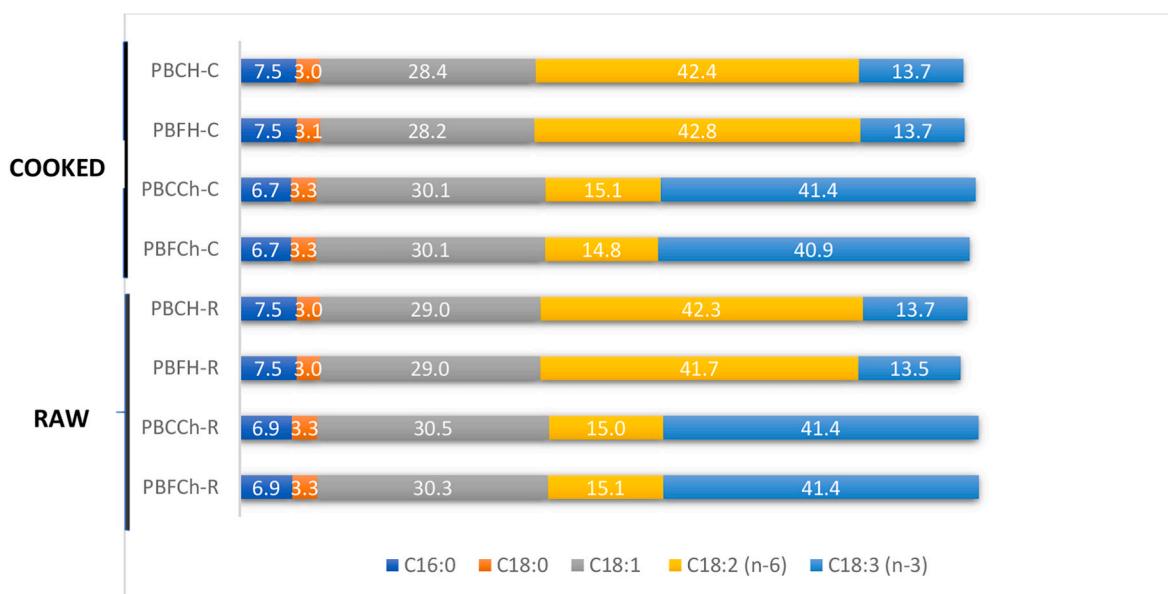


Fig. 1. Percentages of the five main fatty acids detected in plant-based burgers (raw and cooked).

Fig. 1, also the predominant PUFA was totally dependent on the type of GE used: in burgers made with chia-GE, the predominant was the α -linolenic acid (C18:3) while in burgers with hemp-GE the predominant was the linoleic fatty acid (C18:2), which is in accordance with the fatty acid composition of the corresponding vegetable oils (Ayerza & Coates, 2004; Leonard et al., 2019; Vodolazska & Lauridsen, 2020). In all cases, the corresponding predominant fatty acid achieved percentages higher than 40% in both raw and cooked burgers.

The main MUFA detected in plant-based burgers was the oleic acid, which was found in higher amount in burgers with chia-GE than with hemp-GE (in both raw and cooked burgers). On the contrary, the main SFA detected was the palmitic acid and it was found in higher amount ($p < 0.05$) in burgers with hem-GE than chia-GE.

Trans-Fatty acids (*t*-FA) were only detected in cooked samples and at very low amounts (0.6%) compared to those reported for commercial plant-based burgers (2.5%) or even for traditional ones (5–6%) (He et al., 2021). Although the formation of *t*-FA has been linked to severe cooking conditions, small changes in the *t*-FA content has also been observed during normal cooking process (Tsuzuki, Matsuoka, & Ushida, 2010).

The balance of dietary fatty acids, mainly in terms of PUFA vs SFA or even n-6 vs n-3 FA has been highly related to human health (Chen & Liu, 2020) and so recommendations for a healthy diet has been given. Regarding PUFA/SFA ratio, all samples showed values higher than 4.5 (for all formulations without differences between them, in both raw and cooked samples) which is well above the minimum recommended (>0.85) by international agencies (FAO, 2010). In relation to the n-6/n-3 index, the recommendation is that it should be lower than 4, and also in this case all burgers meet it but with significant differences between samples ($p < 0.05$). Plant-based burgers with hemp-GE (PBFH and PBCH; raw and cooked) showed values around 3, while in chia-GE burgers (PBFCh and PBCCh; raw and cooked) this index is approximately 0.4. This behaviour is due to the main PUFA in each burger depending on the GE used: chia-GE is especially rich in α -linolenic acid (n-3) while hemp-GE has linoleic acid (n-6) as the main one. Also in this case all plant-based burgers made with GE (raw and cooked) showed better PUFA/SFA and n-6/n-3 ratios than reported for some commercial plant-based burgers and traditional burgers (De Marchi et al., 2021; He et al., 2021). Several reasons have been given to explain this behaviour: in the case of traditional burgers the high percentage of SFA in animal fats and in the case of plant-based burgers may be the presence of other lipid ingredients, such as coconut oil (which higher SFA content) and also a higher susceptibility to oxidation of PUFAs when vegetables oils are directly added than were they are added as GE (Botella-Martínez et al., 2022; De Marchi et al., 2021).

Several nutritional indices have been proposed as indicators of healthy characteristics of fats in foods, all of them based on their fatty acids profile. The AI and TI indices characterizes the atherogenic and thrombogenic potential (respectively) of FAs and should be as low as

possible. All cooked plant-based burgers showed AI values lower than 0.10 (without differences between formulations; $p > 0.05$) and TI values lower than 0.12. For TI index, differences between formulations were detected ($p < 0.05$) showing burgers with chia-GE the lowest TI values (0.07) (Fig. 2). The h/H index as a relation between some hypo- and hypercholesterolemic FAs should be as high as possible. Also in this case significant differences ($p < 0.05$) between samples were detected showing burgers with chia-GE the highest values (12.9) (Fig. 2). In any case, the comparison of the values of any of these three nutritional indices with those reported for several meats and meat products included traditional burgers (Barros et al., 2021; Chen & Liu, 2020; Lucas-González et al., 2020; Pires et al., 2020) is always favorable (in a high way) to our plant-based burgers. The consumption of foods or products with low AI and TI and high h/H may reduce the risk of coronary heart disease (Chen & Liu, 2020).

Betalains are regarded as bioactive pigments and their inclusion in the dietary intake may be an alternative to prevent certain diseases (Fernández-López, Roca, Angosto, & Obón, 2018). The HPLC chromatographic pigment pattern corresponding to the fresh red beet juice revealed the presence of betanin, isobetanin and betanidin as main betacyanins, while vulgaxanthin I was detected between betaxanthins (Fig. 3). In commercial red beet juices only neobetanin was detected as betacyanin. The pigment content in plant-based burgers with fresh red beet juice was much more higher (27–35 mg/100 g dw) than in those obtained with commercial juice (<5 mg/100 g dw) (Table 5). According to these results, it could be recommended to use fresh red beet juice as colorant ingredient in plant-based burgers, in order to increase the content of betacyanins in these plant-based alternatives, which are considered health-promoting substances.

3.2. Physicochemical properties of plant-based burgers

Physicochemical properties (pH, Aw and color parameters) of raw and cooked plant-based burgers are shown in Table 6 pH and Aw values are highly related to food safety but in the case of plant-based burgers pH has also a relevant effect on the final color because most of the vegetable pigments can change their color depending on the pH. Aw values in raw plant-based burgers depended on the type of juice used which is related to their moisture content: burgers with fresh juice added showed the highest moisture content (Table 2) and also the highest Aw values ($p < 0.05$). Aw values in raw burgers ranging between 0.883 and 0.893, which are included into the range of intermediate moisture foods. As it could be expected, cooking decreased Aw values, following the same trend that reported for raw burgers. Regarding pH, it also depended on the type of juice used: commercial juice showed the lowest pH (3.71) and so burgers with this type of juice (PBCCh and PBCH) showed lower pH values than burgers with fresh juice (PBFCh and PBFH). pH of plant-based burgers ranging between 5.43 and 6.06, in both raw and cooked burgers, without significant differences due to cooking process.

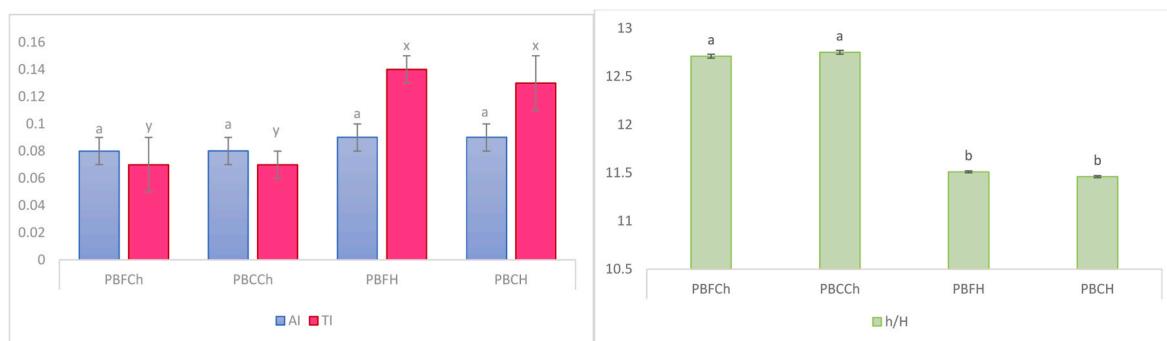


Fig. 2. Nutritional indices [Atherogenic Index (AI), Thrombogenic index (TI) and hypocholesterolemic/hypercholesterolemic ratio (h/H) of cooked plant-based burgers. ^{a-b, X-Y} For the same index, different letter indicate a significant difference ($p < 0.05$) between samples.

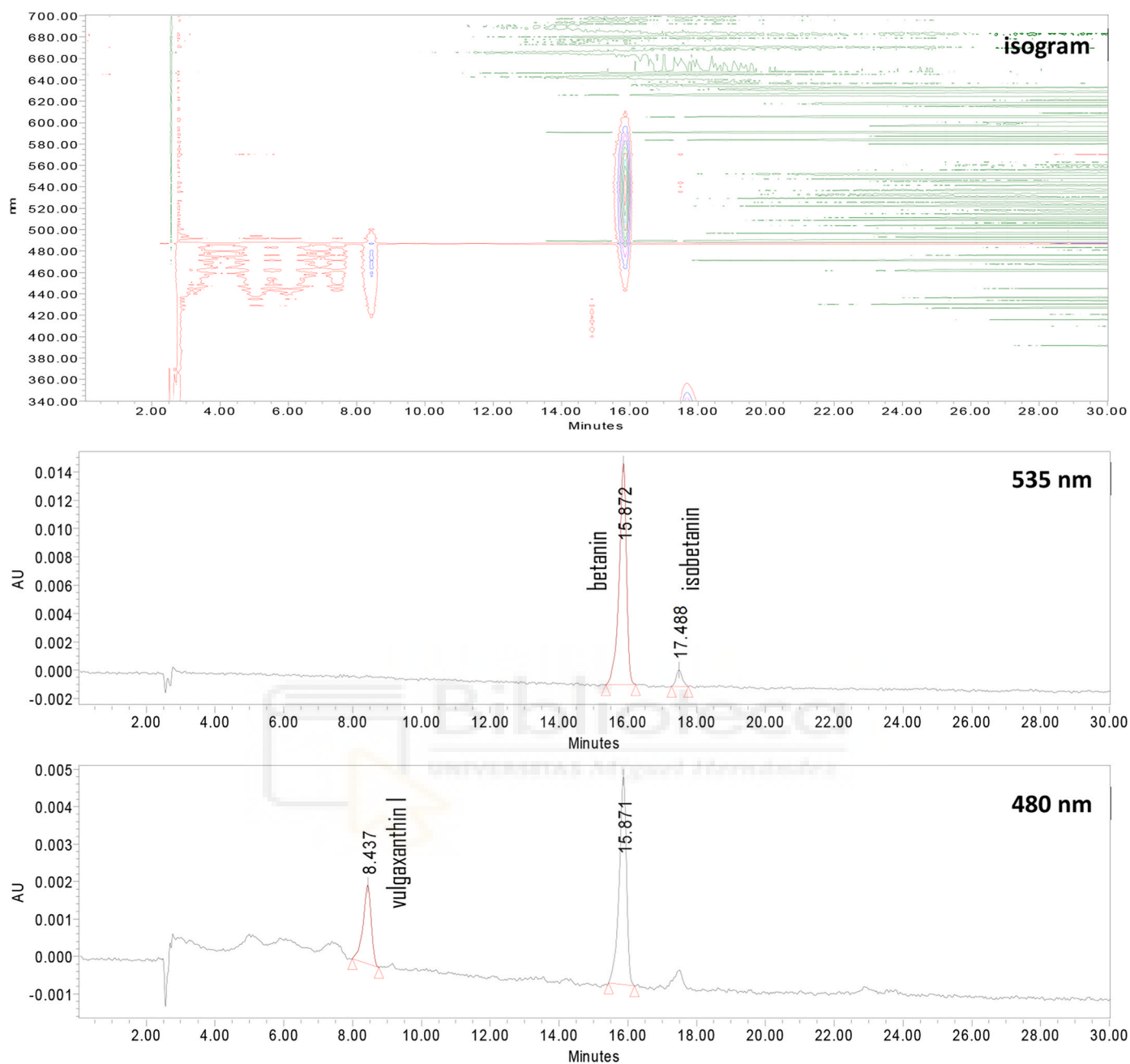


Fig. 3. Isochrom (340–700 nm) and HPLC patterns (535 and 480 nm) of betalains from plant-based burger (PBFH).

Similar pH values have been reported for plant-based burgers and its variability have been attribute to the alkalinity/acidity and diversity of the ingredients used (De Marchi et al., 2021). For both parameters (pH and A_w) no differences due to the type of GE used have been found.

During the development of new meat analogues (plant-based burgers) it must be taken into account that their acceptance is largely determined by their visual appearance. So, after providing the right texture and shape, the next focus should be on color or color changes during preparation and cooking. As the main ingredients used in the formulation of these plant-based burgers (soy protein, pea fiber and peanut flour) are beige or yellow colour, the use of beetroot juice was necessary trying to resemble the typical reddish colour of the traditional burgers. In addition, the proper red colour achieved should be stable at the pH value of the burgers and also be degrade or brown upon heating. In this sense, beetroot extracts (due to betalains content) have been proposed as interesting ingredients attributing a “raw meat” colour and

undergo colour changes due to thermal degradation (Herbach, Stintzing, & Carle, 2006; Kyriakopoulou et al., 2021). The main differences in color parameters between raw samples were due to the type of juice used what supports the fact that beetroot juice is the key factor in the color of plant-based burgers. Burgers with commercial juice (PBCCh and PBCH) showed higher lightness, yellowness and hue values but lower redness ($p < 0.05$) and chroma values than samples with fresh juice (PBFCh and PBFH). The use of fresh juice (even diluted) was useful to obtain plant-based burgers with a^* values similar to that reported for traditional burgers (16–21; De Marchi et al., 2021). The pronounced thermal stability of betanin extracts is well known (Fernández-López, Fernández-Lledó, & Angosto, 2020), which results in color changes that become more evident as the intensity of the heat treatment increases. It is advisable that products with these extracts are subjected to mild heat treatments in order not to alter either their chromatic characteristics or their bioactive properties. On the contrary, redness values obtained for

Table 5
Betain content in red beetroot juices and plant-based burgers.

	Betacyanins	Betaxanthins
Beetroot juice	<i>betanin</i> (mg/L)	<i>vulgaxanthin I</i> (mg/l)
Fresh	570.1 ± 17.8 BETANIN-93,2% ISOBETANIN-4,7% BETANIDIN-2,1%	208.2 ± 13.2
Commercial	318,3 ± 18,2 NEOBETANIN	134.2 ± 10.2
Burgers	<i>betanin</i> (mg/100 g dw)	<i>vulgaxanthin I</i> (mg/100 g dw)
PBFCh	19.08 ± 1.20 BETANIN-94,2% ISOBETANIN-5,8%	16.47 ± 1.05
PBCCCh	2.32 ± 0.35 NEOBETANIN	2.01 ± 0.31
PBFH	14.83 ± 1.50 BETANIN-94,1% ISOBETANIN-5,9%	12.11 ± 1.63
PBCH	0.14 ± 0.02 NEOBETANIN	–

Data are presented as mean ± standard deviation.

plant-based burgers with commercial juice were very low even in comparison with those reported for commercial plant-based burgers (15–17; De Marchi et al., 2021). The heat treatment applied during juice processing would be contributing to thermal degradation of betanins with the corresponding decrease in a^* values. The use of fresh juice increased ($p < 0.05$) the color saturation (C^*) of burger which means a higher purity of color, irrespective of GE used. Regarding hue values, burgers made with fresh juice showed redish hue (14–16°) in contrast with the orange-yellowish hue (61–63°) observed in burgers with commercial juice.

Regarding cooked burgers, all color parameters showed the same trend than reported for raw burgers except L^* and b^* values that did not show differences ($p > 0.05$) between burgers. It is interesting to note that the effect of cooking process on color parameters of burgers were more intense (higher color parameter variations between raw and cooked burgers) when fresh juice was used (PBFCh and PBFH) which could be due to a higher lability of the colorants components (betanins) in fresh beetroot juice comparing to commercial juice. In addition, other reactions that take place in foods during heat treatment (mainly protein denaturation and aggregation, water evaporation, fat crystal melting and Maillard reaction) could be contributing to these color changes during cooking (Fennema, Damodaran, & Parkin, 2017; Zhou et al., 2022). In meat products, all these reactions also affect the system ability to bind water and fat and so, are responsible for cooking loss and dimensional changes (shrinkage and thickness) of the cooked product. Regarding that, it is very interesting to evaluate how the substitution of meat proteins and animal fat by vegetable proteins and oils (as GE), with chemical and physical properties completely different, could affect cooking properties in plant-based burgers. Plant-based burgers showed

Table 6
Physicochemical properties (A_w , pH and CIELAB color parameters) of raw and cooked plant-based burgers.

Sample	A_w	pH	L^*	a^*	b^*	C^*	h^*
RAW BURGERS							
PBFCh	0.892 ± 0.001 ^{aX}	6.06 ± 0.02 ^{aX}	35.44 ± 0.67 ^{bX}	20.04 ± 0.79 ^{aX}	5.71 ± 0.70 ^{bY}	20.85 ± 0.89 ^{aX}	15.88 ± 1.59 ^{bY}
PBCCCh	0.883 ± 0.000 ^{cX}	5.70 ± 0.09 ^{bX}	43.74 ± 0.96 ^{aX}	6.37 ± 0.33 ^{bY}	11.92 ± 0.92 ^{aX}	13.52 ± 0.94 ^{bX}	61.81 ± 1.23 ^{aX}
PBFH	0.893 ± 0.000 ^{aX}	6.05 ± 0.03 ^{aX}	34.96 ± 1.25 ^{bX}	21.39 ± 1.03 ^{aX}	5.32 ± 0.61 ^{bY}	22.05 ± 1.04 ^{aX}	13.96 ± 1.54 ^{bY}
PBCH	0.888 ± 0.001 ^{bX}	5.67 ± 0.05 ^{bX}	44.47 ± 0.90 ^{aX}	6.23 ± 0.40 ^{bY}	12.33 ± 1.17 ^{aX}	13.82 ± 1.18 ^{bX}	63.11 ± 1.55 ^{aX}
COOKED BURGERS							
PBFCh	0.885 ± 0.000 ^{aY}	6.00 ± 0.04 ^{bX}	33.22 ± 1.22 ^{aY}	13.18 ± 0.81 ^{aY}	9.40 ± 1.53 ^{aX}	16.22 ± 1.46 ^{aY}	35.27 ± 3.32 ^{bX}
PBCCCh	0.875 ± 0.003 ^{bCY}	5.44 ± 0.02 ^{cY}	34.87 ± 2.02 ^{aY}	7.92 ± 1.04 ^{bX}	9.50 ± 2.29 ^{aY}	12.40 ± 2.36 ^{bX}	49.51 ± 4.22 ^{aY}
PBFH	0.881 ± 0.001 ^{aBY}	6.06 ± 0.01 ^{aX}	33.03 ± 0.84 ^{aX}	12.21 ± 1.13 ^{aY}	8.14 ± 1.00 ^{aX}	14.69 ± 1.40 ^{aY}	33.65 ± 2.19 ^{bX}
PBCH	0.871 ± 0.001 ^{cY}	5.43 ± 0.03 ^{cY}	34.72 ± 1.58 ^{aY}	7.88 ± 0.65 ^{bX}	9.72 ± 1.76 ^{aY}	12.54 ± 1.71 ^{bX}	50.52 ± 3.84 ^{aY}

a-bDifferent superscript letter in each column indicate a significant difference ($p < 0.05$) for raw or cooked burgers. X-YDifferent superscript letter in each column indicate a significant difference ($p < 0.05$) for the same sample raw and cooked. Data are presented as mean ± standard deviation.

mean cooking loss ranging from 14 to 17%, shrinkage values ranging from 3 to 5% and no thickness increase, in all cases without significant differences ($p > 0.05$) between samples (data not shown). These values are lower than reported for traditional meat burgers (Botella-Martínez et al., 2022; Kamani, Meera, Bhaskar, & Modi, 2019; Zhou et al., 2022) which is in consistent with previous studies reporting positive effects of plant ingredients on reduction of cooking loss in meat batters (Kamani et al., 2019; Zhou et al., 2022) mainly attributed to differences in the structural organization and molecular interactions of the ingredients in the plant-based burger matrix compared to those in the meat matrix. In this case, neither the type of juice nor the GE used appears to have any effect ($p > 0.05$) on vegetable proteins. Taking into account that texturized soya has been used as the main source proteins in plant-based burgers, and that it may already be denatured prior to heating, less changes in the overall microstructure and fluid holding properties of the burger matrix should be expected, which is in accordance with our results.

The texture properties (TPA) of cooked burgers are shown in Table 7. There were no significant differences ($p > 0.05$) for springiness, cohesiveness and chewiness between all samples analyzed. Hardness was the only parameter affected by plant-based burger formulation: the use of commercial beetroot juice resulted in harder ($p < 0.05$) burgers than those made with fresh juice. No differences due to the type of GE used were detected ($p < 0.05$). It has been reported that food hardness tend to decrease when the moisture content of food increases (Wi, Bae, Kim, Cho, & Choi, 2020) which is in agreement with our results since plant-based burgers made with fresh juice retained more water after cooking (Table 2). The higher fiber content found in cooked burgers made with commercial juice (Table 2) could be also contributing to their high hardness. But not only moisture or fiber content would be responsible for the mechanical properties of plant-based burgers, crosslinks (number, strength and type) between vegetable proteins, fibers and starch should be expected as relevant factors.

Table 7
Textural properties (TPA) of cooked plant-based burgers.

Sample	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N. mm)
PBFCh	23.33 ± 1.66 ^b	0.11 ± 0.01	0.53 ± 0.05	1.36 ± 0.19
PBCCCh	32.88 ± 1.99 ^a	0.11 ± 0.01	0.44 ± 0.06	1.59 ± 0.26
PBFH	22.38 ± 2.03 ^b	0.12 ± 0.01	0.51 ± 0.09	1.33 ± 0.26
PBCH	33.30 ± 2.08 ^a	0.10 ± 0.01	0.50 ± 0.04	1.60 ± 0.36

Different superscript letter in each column indicate a significant difference ($p < 0.05$) Data are presented as mean ± standard deviation.

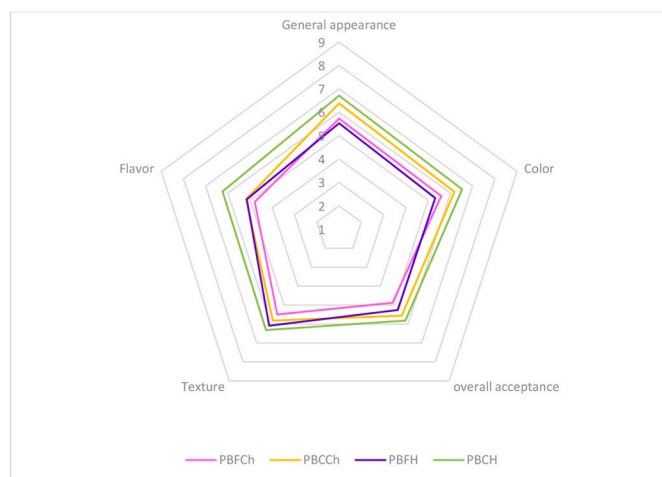


Fig. 4. Sensorial analysis of cooked plant-based burgers.

3.3. Sensorial analysis of plant-based burgers

The results of sensory assessment are shown in Fig. 4. No significant differences were observed in the scores of texture and overall acceptance between samples. Plant-based burgers made with commercial juice showed higher ($p < 0.05$) scores for color and appearance than those obtained for burgers made with fresh juice. On the contrary, flavour scores were higher ($p < 0.05$) in plant-based burgers made with fresh juice. No sensory differences ($p > 0.05$) were detected between burgers due to the type of GE used. Several authors have reported sensorial differences (mainly in flavour) in traditional meat burgers, in which fat animal was substituted by GE, depending on the type of GE used (Lucas-González et al., 2020; Botella-Martínez et al., 2022). On the contrary, in this case, the typical flavour of some ingredients (soy proteins, pea fiber, beetroot juice, ...) together with the spice mix used, would be masking flavour differences due to the GE remaining only those can be attributed to fresh beetroot juice. In addition, it seems clear that color and flavour attributes (with opposite scores depending on the type of juice used) are responsible for the lack of differences in the overall acceptance of plant-based burgers.

4. Conclusions

This study suggests that the reformulation of plant-based burgers using gelled emulsion (with chia or hemp oil) as fat source and, beetroot juice (fresh or commercial) as colorant ingredient is feasible and represents a useful alternative to develop healthier and sensory accepted plant-based burgers. The use of both ingredients enhance the nutritional composition, without adversely affecting the technological properties of these plant-based meat alternatives. In particular, the use of GE allows to reduce the fat content (<3%) and to improve their lipid profile in comparison with commercial plant-based burgers [PUFAs was the main fraction (>57%) with differences in the main fatty acid depending on the oil used: α -linolenic fatty acid in the case of burgers with chia-GE and linoleic when hemp-GE was used]. The most favorable nutritional indexes in cooked plant-based burgers are obtained when chia-GE was used (the lowest TI and the highest h/H). The use of beetroot fresh juice allow to obtain a final product with a redness similar to that of a traditional meat burgers and with an interesting content in health-promoting substances (betalains) but causing more intense color changes during cooking than when commercial juice is used. Plant-based burgers suffer less cooking loss and dimensional changes than traditional meat burgers. According to the results of sensory evaluation, although plant-based burgers were scored with a good overall acceptability, it could be enhanced by the ingredient optimization.

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CRediT authorship contribution statement

Carmen Botella-Martínez: Methodology, Formal analysis, Writing – original draft, Visualization. **Manuel Viuda-Martos:** Conceptualization, Investigation, Visualization, Supervision. **Jose A. Fernández-López:** Methodology, Visualization. **Jose A. Pérez-Álvarez:** Validation, Visualization. **Juana Fernández-López:** Conceptualization, Investigation, Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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