



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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Keppler, & 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.

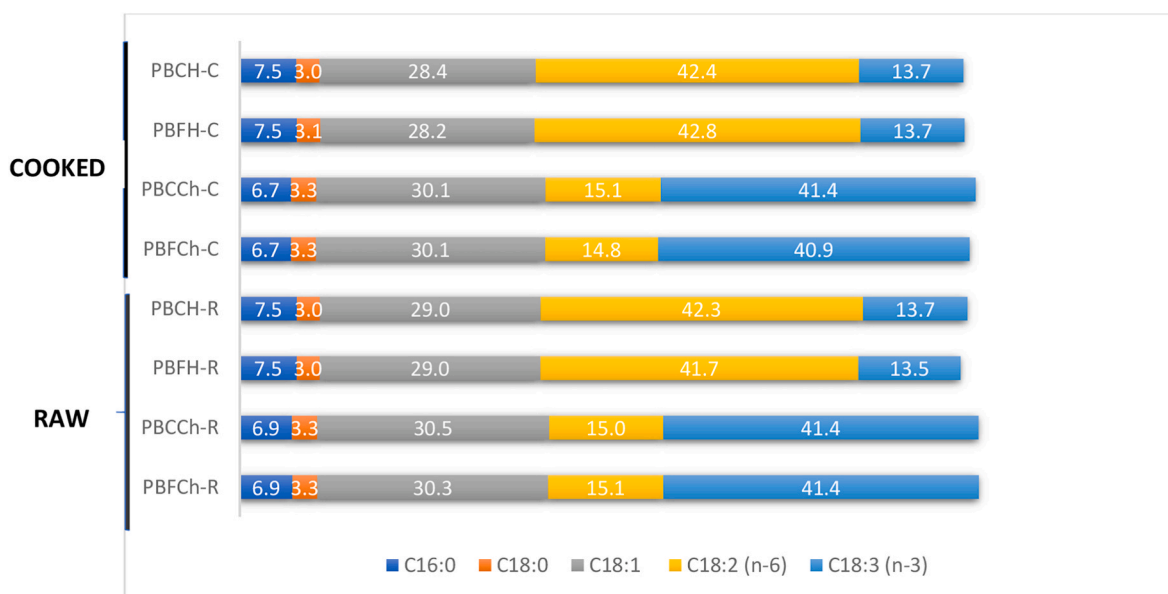


Fig. 1. Percentages of the five main fatty acids detected in plant-based burgers (raw and cooked).

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.

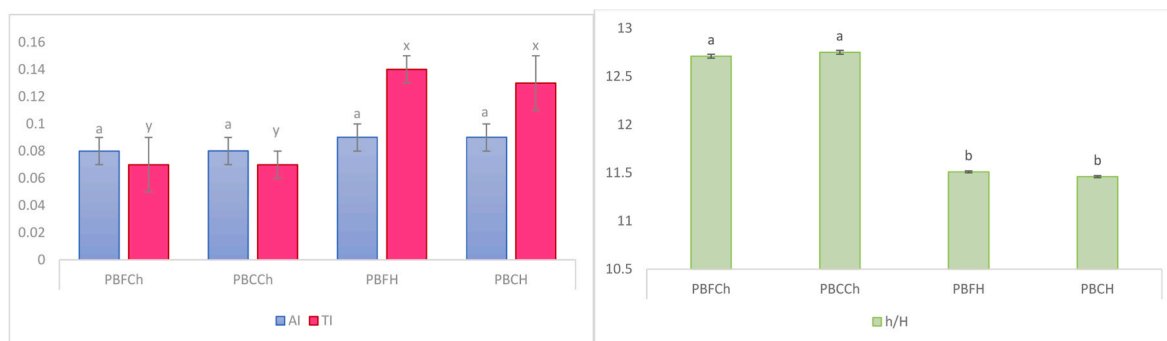


Fig. 2. Nutritional indices [Atherogenic Index (AI), Thrombogenic index (TI) and hypocholesterolaemic/hypercholesterolaemic ratio (h/H) of cooked plant-based burgers. ^{a-b, X-Y} For the same index, different letter indicate a significant difference ($p < 0.05$) between samples.

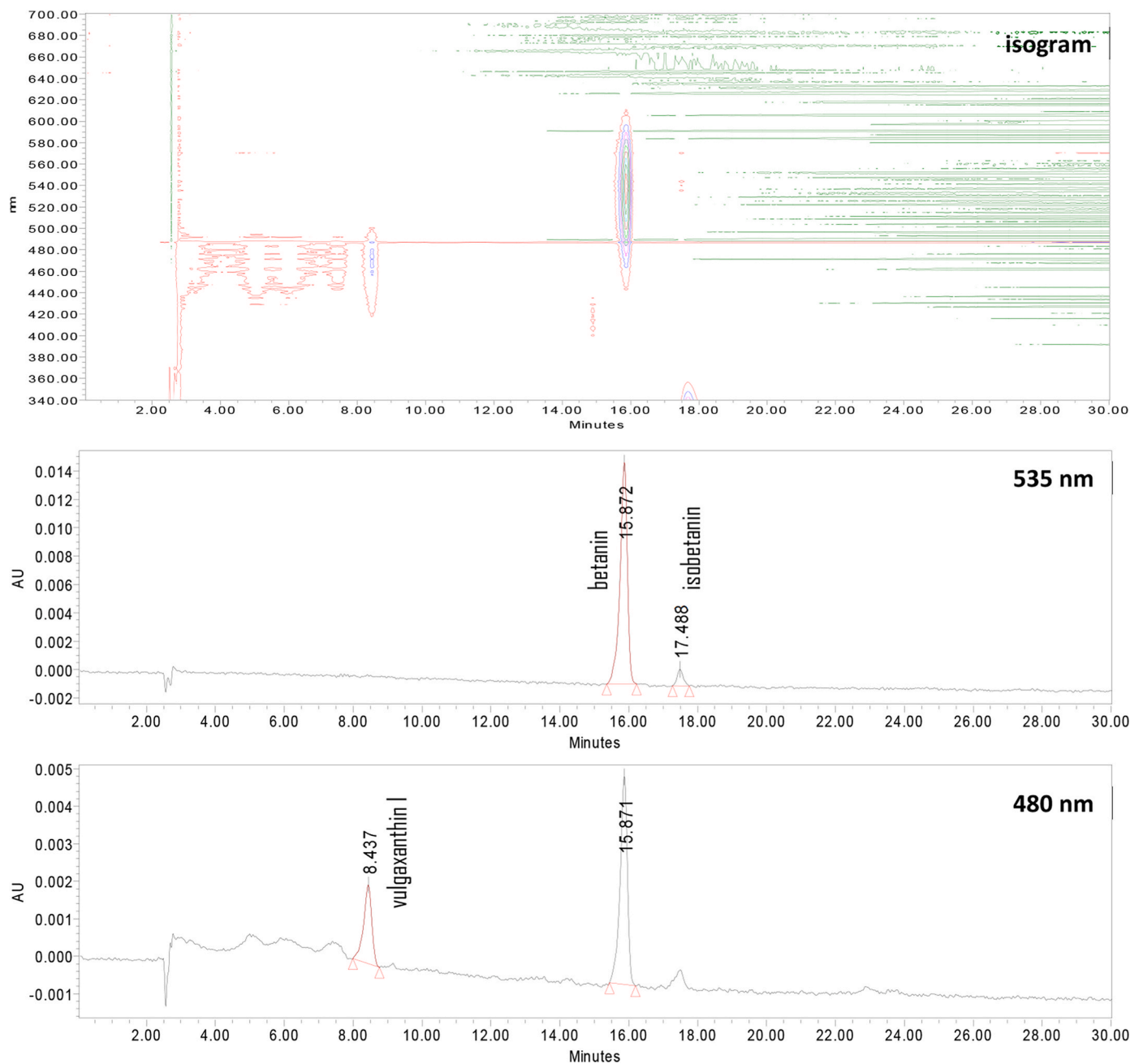


Fig. 3. Isochrom (340–700 nm) and HPLC patterns (535 and 480 nm) of betalains from plant-based burger (PBFH).

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 (mg/L)</i>	<i>vulgaxanthin I (mg/l)</i>
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 (mg/100 g dw)</i>	<i>vulgaxanthin I (mg/100 g dw)</i>
PBFCh	19.08 ± 1.20 BETANIN-94,2% ISOBETANIN-5,8%	16.47 ± 1.05
PBCCh	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}
PBCCh	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}
PBCCh	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
PBCCh	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.

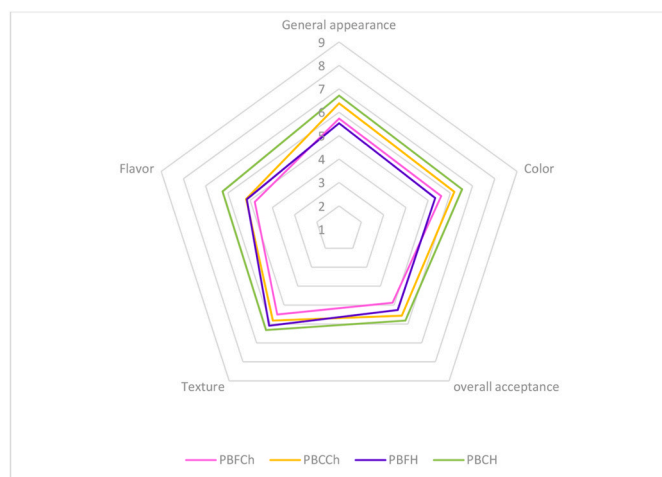


Fig. 4. Sensorial analysis of cooked plant-based burgers.

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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References

- Alonso-Esteban, J. I., Torija-Isasa, M. E., & Sánchez-Mata, M. C. (2022). Mineral elements and related antinutrients, in whole and hulled hemp (*Cannabis sativa* L.) seeds. *Journal of Food Composition and Analysis*, 109. <https://doi.org/10.1016/j.jfca.2022.104516>. Article 104516.
- AOAC. (2010). *Official methods of analysis of AOAC international* (18th ed.). Rockville, MD, USA: Association of Official Analytical Chemistry.
- Ayerza, R., & Coates, W. (2004). Composition of chia (*Salvia hispánica*) grown in six tropical and subtropical ecosystems of South America. *Tropical Science*, 44, 131–135. <https://doi.org/10.1002/ts.154>
- Badar, I. H., Liu, H., Chen, Q., Xia, X., & Kong, B. (2021). Future trends of processed meat products concerning perceived healthiness: A review. *Comprehensive Reviews in Food Science and Food Safety*, 20, 4739–4778. <https://doi.org/10.1111/1541-4337.12813>
- Barros, J. C., Munekeata, P. E. S., de Carvalho, F. A. L., Domínguez, R., Trindade, M. A., Pateiro, M., et al. (2021). Healthy beef burgers: Effect of animal fat replacement by algal and wheat germ oil emulsions. *Meat Science*, 10, 2706. <https://doi.org/10.1016/j.meatsci.2020.108396>
- Bodoira, R. M., Penci, M., Ribotta, P. D., & Martínez, M. L. (2017). Chia (*Salvia hispánica* L.) oil stability: Study of the effect of natural antioxidants. *LWT - Food Science and Technology*, 75, 107–113. <https://doi.org/10.1016/j.lwt.2016.08.031>
- Botella-Martínez, C., Gea-Quesada, A., Sayas-Barberá, E., Pérez-Álvarez, J. A., Fernández-López, J., & Viuda-Martos, M. (2022). Improving the lipid profile of beef burgers added with chia oil (*Salvia hispánica* L.) or hemp oil (*Cannabis sativa* L.) gelled emulsions as partial animal fat replacers. *LWT—Food Science and Technology*, 161. <https://doi.org/10.1016/j.lwt.2022.113416>. Article 113416.
- Botella-Martínez, C., Pérez-Álvarez, J. A., Sayas-Barberá, E., Fernández-López, J., & Viuda-Martos, M. (2021a). Assessment of chemical, physicochemical, and lipid stability properties of gelled emulsions elaborated with different oils chia (*Salvia hispánica* L.) or hemp (*Cannabis sativa* L.) and pseudocereals. *Foods*, 10. <https://doi.org/10.3390/foods10071463>. Article 1463.
- Botella-Martínez, C., Viuda-Martos, M., Pérez-Álvarez, J. A., & Fernández-López, J. (2021b). 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. <https://doi.org/10.3390/foods10081681>. Article 1681.
- Chen, J., & Liu, H. (2020). Nutritional indices for assessing fatty acids: A mini-review. *Molecular Science*, 21. <https://doi.org/10.3390/jms21165695>. Article 5695.
- Claus, J. R. (1995). Methods for the objective measurement of meat product texture. *Reciprocal Meat Conference Proceedings*, 48, 96–101.
- Curtain, F., & Grafenauer, S. (2019). Plant-based meat substitutes in the flexitarian age: An audit of products on supermarket shelves. *Nutrients*, 11. <https://doi.org/10.3390/nu11112603>. Article 2603.

- De Marchi, M., Costa, A., Pozza, M., Goi, A., & Manuelian, C. L. (2021). Detailed characterization of plant-based burgers. *Scientific Reports*, *11*. <https://doi.org/10.1038/s41598-021-81684-9>. Article 2049.
- De Souza Paglarini, C., de Figueiredo Frutado, G., Raissa Honório, A., Mokarzel, L., da Silva Vidal, V. A., Badan Ribeiro, A. P., et al. (2019). Functional emulsion gels as pork back fat replacers in Bologna sausage. *Food Structure*, *20*. <https://doi.org/10.1016/j.foosr.2019.100105>. Article 100105.
- Delizar, R., Saldívar, S. S., Germani, R., Benassi, V., & Cabral, L. (2002). The effects of colored textured soybean protein (TSP) on sensory and physical attributes of ground beef patties. *Journal of Sensory Studies*, *17*, 121–132. <https://doi.org/10.1111/j.1745-459X.2002.tb00337.x>
- Domínguez, R., Lorenzo, J. M., Pateiro, M., Munekate, P. E. S., Alves dos Santos, B., Basso Pintor, M., et al. (2022). Main animal fat replacers for the manufacture of healthy processed meat products. *Critical Reviews in Food Science and Nutrition*. <https://doi.org/10.1080/10408398.2022.2124397>
- FAO. (2010). Food and agriculture organization of the united nations. Fat and fatty acid requirements for adults. Fats and fatty acids in human nutrition. Report of an expert consultation. *FAO Food and Nutrition*, *91*, 55–62 (Chapter 5).
- Fennema, O. R., Damodaran, S., & Parkin, K. L. (2017). Introduction to food chemistry. In *Fennema's food chemistry* (pp. 1–16). CRC Press. <https://doi.org/10.1201/9781315372914>.
- Fernández-López, J., Botella-Martínez, C., Navarro-Rodríguez de Vera, C., Sayas-Barberá, M. E., Viuda-Martos, M., Sánchez-Zapata, E., et al. (2020a). Vegetable soups and creams: Raw materials, processing, health benefits, and innovation trends. *Plants*, *9*. <https://doi.org/10.3390/plants9121769>. Article 1769.
- Fernández-López, J. A., Castellar, R., Obón, J. M., & Almela, L. (2002). Screening and mass-spectral confirmation of betalains in cactus pears. *Chromatographia*, *56*, 591–595.
- Fernández-López, J. A., Fernández-Lledó, V., & Angosto, J. M. (2020b). New insights into red plant pigments: More than just natural colorants. *RSC Advances*, *10*, 24669–24682. <https://doi.org/10.1039/D0RA03514A>
- Fernández-López, J., Paya, L., Botella-Martínez, C., Lucas-González, R., Viuda-Martos, M., Sayas-Barberá, E., et al. (2021a). Plant-based meat substitutes in the Spanish market: Type of products, main plant protein substitutes and health and nutrition claims. In *Conference book from the 35th Effost International Conference: Healthy individuals, resilient communities and global food security*. November, (Lausanne, Sw).
- Fernández-López, J. A., Roca, M. J., Angosto, J. M., & Obón, J. M. (2018). Betaxanthin-rich extract from cactus pear fruits as yellow water-soluble colorant with potential application in foods. *Plant Foods for Human Nutrition*, *73*, 146–153. <https://doi.org/10.1007/s11130-018-0664-3>
- Fernández-López, J., Viuda-Martos, M., & Pérez-Álvarez, J. A. (2021b). Quinoa and chia products as ingredients for healthier processed meat products: Technological strategies for their application and effects on the final product. *Current Opinion in Food Science*, *40*, 26–32. <https://doi.org/10.1016/j.cofs.2020.05.004>
- Fernández, M., Ordoñez, J. A., Cambero, I., Santos, C., Pin, C., & Hoz, L. (2007). Fatty acids compositions of selected varieties of Spanish dry ham related to their nutritional implications. *Food Chemistry*, *101*, 107–112. <https://doi.org/10.1016/j.foodchem.2006.01.006>
- FMI. (2020). *Plant-based burger market* Accessed March 22, 2022 <https://www.futuremarketinsights.com/reports/plant-based-burger-market>.
- Guo, Y., Cai, Z., Xie, Y., Ma, A., Zhang, H., Rao, P., et al. (2020). Synthesis, physicochemical properties, and health aspects of structured lipids: A review. *Comprehensive Reviews in Food Science and Food Safety*, *19*, 759–800. <https://doi.org/10.1111/1541-4337.12537>
- He, J., Evans, N. M., Liu, H., & Shao, S. (2020). A review of research on plant-based meat alternatives: Driving forces, history, manufacturing, and consumer attitudes. *Comprehensive Reviews in Food Science and Food Safety*, *19*(5), 2639–2656. <https://doi.org/10.1111/1541-4337.12610>
- He, J., Liu, H., Balamurugan, S., & Shao, S. (2021). Fatty acids and volatile flavor compounds in commercial plant-based burgers. *Journal of Food Science*, *86*, 293–305. <https://doi.org/10.1111/1750-3841.15594>
- Herbach, K. M., Stintzing, F. C., & Carle, R. (2006). Impact of thermal treatment on color and pigment pattern of red beet (*Beta vulgaris* L.) preparations. *Journal of Food Science*, *69*, 491–498. <https://doi.org/10.1111/j.1365-2621.2004.tb10994.x>
- Herrero, A. M., & Ruiz-Capillas, C. (2021). Novel lipid materials based on gelling procedures as fat analogues in the development of healthier meat products. *Current Opinion in Food Science*, *39*, 1–6. <https://doi.org/10.1016/j.cofs.2020.12.010>
- Herrero, A. M., Ruiz-Capillas, C., Pintado, T., Carmona, P., & Jiménez-Colmenero, F. (2017). Infrared spectroscopy used to determine effects of chia and olive oil incorporation strategies on lipid structure of reduced-fat frankfurters. *Food Chemistry*, *221*, 1333–1339. <https://doi.org/10.1016/j.foodchem.2016.11.022>
- Itxaina, V. Y., Marín, M. L., Spotorno, V., Mateo, C., Maestri, D., Diehl, B., et al. (2011). Characterization of chia seed oils obtained by pressing and solvent extraction. *Journal of Food Composition and Analysis*, *24*, 166–174. <https://doi.org/10.1016/j.jfca.2010.08.006>
- Kamani, M. H., Meera, M. S., Bhaskar, N., & Modi, V. K. (2019). Partial and total replacement of meat by plant-based proteins in chicken sausage: Evaluation of mechanical, physico-chemical and sensory characteristics. *Journal of Food Science & Technology*, *56*, 2660–2669. <https://doi.org/10.1007/s13197-019-03754-1>
- Keerthana-Priya, R., Rawson, A., Vidhyalakshmi, R., & Jagan-Mohan, R. (2022). Development of vegan sausage using banana floret (*Musa paradisiaca*) and jackfruit (*Artocarpus heterophyllus* Lam.) as a meat substitute: Evaluation of textural, physico-chemical and sensory characteristics. *Journal of Food Processing and Preservation*, *46*. <https://doi.org/10.1111/jfpp.16118>. Article e16118.
- Kyriakopoulou, K., Dekkers, B., & van der Goot, A. J. (2019). Plant-based meat analogues. In C. M. Galanakis (Ed.), *Sustainable meat production and processing* (pp. 103–126). Academic Press.
- Kyriakopoulou, K., Keppler, J. K., & van der Goot, A. J. (2021). Functionality of ingredients and additives in plant-based meat analogues. *Foods*, *10*(3). <https://doi.org/10.3390/foods10030600>. Article 600.
- Lee, H. J., Yong, H. I., Kim, M., Choi, Y.-S., & Jo, C. (2020). Status of meat alternatives and their potential role in the future meat market- A review. *Asian-Australasian Journal of Animal Sciences*, *33*(10), 1533–1543. <https://doi.org/10.5713/ajas.20.0419>
- Leonard, W., Zhang, P., Ying, D., & Fang, Z. (2019). Hempseed in food industry: Nutritional value, health benefits, and industrial applications. *Comprehensive Reviews in Food Science and Food Safety*, *19*, 282–308. <https://doi.org/10.1111/1541-4337.12517>
- Lucas-González, R., Roldán-Verdú, A., Sayas-Barberá, E., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2020). Assessment of emulsion gels formulated with chestnut (*Castanea sativa* M.) flour and chia (*Salvia hispanica* L) oil as partial fat replacers in pork burger formulation. *Journal of the Science of Food and Agriculture*, *100*, 1265–1273. <https://doi.org/10.1002/jsfa.10138>
- Maningat, C. C., Jeradechachai, T., & Buttshaw, M. R. (2022). Textured wheat and pea proteins for meat alternative applications. *Cereal Chemistry*, *99*, 37–66. <https://doi.org/10.1002/ccche.10503>
- Nacac, B., Öztürk-Kerimoglu, B., Yildiz, D., Çagindi, Ö., & Serdaglou, M. (2021). Peanut and linseed oil emulsion gels as potential fat replacer in emulsified sausages. *Meat Science*, *176*. <https://doi.org/10.1016/j.meatsci.2021.108464>. Article 108464.
- Ospina-E, J. C., Cruz-S, A., Pérez-Álvarez, J. A., & Fernández-López, J. (2010). Development of combinations of chemically modified vegetable oils as pork backfat substitutes in sausages formulation. *Meat Science*, *84*, 491–497. <https://doi.org/10.1016/j.meatsci.2009.10.003>
- Öztürk-Kerimoglu, B., Urgan-Oztürk, M., & Serdaroglu, M. (2021). A new inverse olive oil emulsion plus carrot powder to replace animal fat in model meat batters. *LWT-Food Science and Technology*, *135*. <https://doi.org/10.1016/j.lwt.2020.110044>. Article 110044.
- Pellegrini, M., Lucas-Gonzales, R., Ricci, A., Fontecha, J., Fernandez-López, J., Pérez-Álvarez, J. A., et al. (2018). Chemical, fatty acid, polyphenolic profile, technofunctional and antioxidant properties of flours obtained from quinoa (*Chenopodium quinoa* Willd) seeds. *Industrial Crops and Products*, *111*, 38–46. <https://doi.org/10.1080/19476337.2018.1525433>
- Pereira da Silva, B., Kolba, N., Stampini, D. M. H., Hart, J., & Tako, E. (2019). Soluble extracts from chia seed (*Salvia hispanica* L.) affect brush border membrane functionality, morphology and intestinal bacterial populations in vivo (*Gallus gallus*). *Nutrients*, *11*. <https://doi.org/10.3390/nu11102457>. Article 2457.
- Pietrasik, Z., Sigvaldson, M., Soladoye, O. P., & Gaudette, N. J. (2020). Utilization of pea starch and fibre fractions for replacement of wheat crumb in beef burgers. *Meat Science*, *161*, Article 107974. <https://doi.org/10.1016/j.meatsci.2019.107974>
- Pintado, T., Herrero, A. M., Jiménez-Colmenero, F., Pasqualin-Calvalheiro, C., & Ruiz-Capillas, C. (2018). Chia and oat emulsion gels as new animal fat replacers and healthy bioactive sources in fresh sausages formulation. *Meat Science*, *135*, 6–13. <https://doi.org/10.1016/j.meatsci.2017.08.004>
- Pires, M. A., Rodrigues, I., Barros, J. C., Carnauba, G., de Carvalho, F. A., & Trindade, M. A. (2020). Partial replacement of pork fat by Echium oil in reduced sodium bologna sausages: Technological, nutritional and stability implications. *Journal of the Science of Food and Agriculture*, *100*, 410–420. <https://doi.org/10.1002/jsfa.10070>
- Rios-Mera, J. D., Saldaña, E., Cruzado-Bravo, M. L., Martins, M. M., Patinho, I., Selani, M. M., et al. (2020). Impact of the content and size of NaCl on dynamic sensory profile and instrumental texture of beef burgers. *Meat Science*, *161*. <https://doi.org/10.1016/j.meatsci.2019.107992>. Article 107992.
- Saget, S., Porto-Costa, M., Sancho-Santos, C., Vasconcelos, M., Styles, D., & Williams, M. (2021). Comparative life cycle assessment of plant and beef-based patties, including carbon opportunity costs. *Sustainable Production and Consumption*, *28*, 936–952. <https://doi.org/10.1016/j.spc.2021.07.017>
- Smetana, S., Profeta, A., Voigt, R., Kircher, C., & Heinz, V. (2021). Meat substitution in burgers: Nutritional scoring, sensorial testing, and life cycle assessment. *Future Foods*, *4*. <https://doi.org/10.1016/j.fufo.2021.100042>. Article 100042.
- Tarté, R. (2009). *Ingredients in meat products: Properties, functionality and applications*. Springer Science & Business Media.
- Tremlova, B., Havlova, L., Benes, P., Zemancova, J., Buchtova, H., Tesikova, K., et al. (2022). Vegetarian “Sausages” with the addition of grape flour. *Applied Sciences*, *12*. <https://doi.org/10.3390/app12042189>. Article 2189.
- Tsuzuki, W., Matsuoka, A., & Ushida, K. (2010). Formation of trans fatty acids in edible oils during the frying and heating process. *Food Chemistry*, *123*, 976–982. <https://doi.org/10.1016/j.foodchem.2010.05.048>
- Ulbricht, T., & Southgate, D. (1991). Coronary heart disease: Seven dietary factors. *Lancet*, *338*, 985–992. [https://doi.org/10.1016/0140-6736\(91\)91846-m](https://doi.org/10.1016/0140-6736(91)91846-m)
- Viuda-Martos, M., López-Marcos, M. C., Fernández-López, J., Sendra, E., López-Vargas, J. H., & Pérez-Álvarez, J. A. (2010). Role of fiber in cardiovascular diseases: A review. *Comprehensive Reviews in Food Science and Food Safety*, *9*, 240–258. <https://doi.org/10.1111/j.1541-4337.2009.00102.x>
- van Vliet, S., Kronberg, S. L., & Provenza, F. D. (2020). Plant-based meats, human health, and climate change. *Frontiers in Sustainable Food Systems*, *4*. <https://doi.org/10.3389/fsufs.2020.00128>. Article 128.
- Vodolazska, D., & Lauridsen, C. (2020). Effects of dietary hemp seed oil to sows on fatty acid profiles, nutritional and immune status of piglets. *Journal of Animal Science and Biotechnology*, *11*. <https://doi.org/10.1186/s40104-020-0429-3>. Article 28.

- Wi, G., Bae, J., Kim, H., Cho, Y., & Choi, M.-J. (2020). Evaluation of the physicochemical and structural properties and the sensory characteristics of meat analogues prepared with various non-animal based liquid additives. *Foods*, *9*. <https://doi.org/10.3390/foods9040461>. Article 461.
- Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the anthropocene: The EAT–lancet commission on healthy diets from sustainable food systems. *Lancet*, *393*, 447–492. [https://doi.org/10.1016/S0140-6736\(18\)31788-4](https://doi.org/10.1016/S0140-6736(18)31788-4)
- Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P., Müller, U., et al. (2015). Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *Journal of Food Composition and Analysis*, *42*, 46–55. <https://doi.org/10.1016/j.jfca.2015.03.005>
- Zhou, H., Vu, G., Gong, X., & McClements, D. J. (2022). Comparison of the cooking behaviors of meat and plant-based meat analogues: Appearance, texture, and fluid holding properties. *ACS Food Science & Technology*, *2*, 844–851. <https://doi.org/10.1021/acscfoodscitech.2c00016>