



Original article

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; Zając *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 griddled (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, Σ n3 and Σ 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 (approx. 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

	Raw						Cooked					
	Control	Chia25	Chia50	Hemp25	Hemp50	Control	Chia25	Chia50	Hemp25	Hemp50		
% Fatty acids (n = 3)												
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 ^{cE}	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 ^{cB}	20.39 ± 0.11 ^{bB}	18.46 ± 0.08 ^{dC}		
C16:1	2.07 ± 0.02 ^{dD}	1.95 ± 0.04 ^{bF}	1.66 ± 0.09 ^{dF}	1.78 ± 0.12 ^{eF}	1.51 ± 0.07 ^{aF}	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 18:0	12.44 ± 0.02 ^{aC}	11.36 ± 0.06 ^{bD}	10.22 ± 0.01 ^{dE}	10.55 ± 0.02 ^{cD}	10.25 ± 0.06 ^{cD}	12.12 ± 0.00 ^{aC}	12.04 ± 0.00 ^{aB}	11.30 ± 0.00 ^{cC}	11.49 ± 0.00 ^{bC}	10.92 ± 0.00 ^{bD}		
C 18:1	43.15 ± 0.09 ^{aA}	42.89 ± 0.08 ^{bA}	38.40 ± 0.07 ^{aA}	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 ^{bC}	23.72 ± 0.08 ^{aB}	12.15 ± 0.01 ^{eC}	12.51 ± 0.02 ^{aD}	12.94 ± 0.09 ^{cC}	15.68 ± 0.12 ^{bC}	21.32 ± 0.02 ^{aB}		
C 18:2 (n-3)	0.07 ± 0.00 ^{cI}	0.07 ± 0.00 ^{cJ}	0.06 ± 0.00 ^{cJ}	0.55 ± 0.01 ^{bH}	1.26 ± 0.02 ^{bF}	0.07 ± 0.00 ^{cH}	0.07 ± 0.00 ^{bH}	0.08 ± 0.00 ^{cH}	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 ^{aF}	5.67 ± 0.03 ^{bD}	12.79 ± 0.04 ^{eC}	2.36 ± 0.02 ^{dE}	5.08 ± 0.03 ^{cE}		
C20:1	0.96 ± 0.01 ^{bF}	0.96 ± 0.01 ^{bG}	0.89 ± 0.01 ^{cG}	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 ^{aB}	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 ^{cG}	0.54 ± 0.01 ^{bH}	0.41 ± 0.01 ^{cI}		
C20:3 (n-11)	0.30 ± 0.01 ^{bH}	0.40 ± 0.01 ^{eI}	0.29 ± 0.01 ^{cI}	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 ^e	29.90 ± 0.02 ^e	36.20 ± 0.03 ^a	34.71 ± 0.05 ^b	32.46 ± 0.03 ^d	34.09 ± 0.01 ^e	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 ^d	3.96 ± 0.03 ^c	12.68 ± 0.06 ^a	3.39 ± 0.02 ^d	7.18 ± 0.03 ^b	0.77 ± 0.04 ^d	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 ^{eE}	1.06 ± 0.03 ^{bG}	1.00 ± 0.03 ^{bF}	0.99 ± 0.02 ^{bF}	0.87 ± 0.04 ^{cG}	1.18 ± 0.07 ^{eE}	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 ^{cB}	19.90 ± 0.11 ^{bB}	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 ^{cE}	1.80 ± 0.02 ^{bF}	1.62 ± 0.02 ^{cF}		
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 ^{cD}	12.13 ± 0.00 ^{aC}	11.89 ± 0.00 ^{bC}	11.79 ± 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 ^{cA}	39.28 ± 0.09 ^{cA}	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 ^{cC}	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 ^{bH}	0.07 ± 0.00 ^{cJ}	0.06 ± 0.00 ^{cI}	0.60 ± 0.01 ^{bG}	1.16 ± 0.03 ^{aF}	0.07 ± 0.00 ^{bH}	0.07 ± 0.00 ^{bH}	0.01 ± 0.00 ^{bH}	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 ^{cE}	4.94 ± 0.03 ^{bE}		
C 20:1	0.94 ± 0.02 ^{aE}	1.00 ± 0.02 ^{aG}	0.87 ± 0.02 ^{bF}	0.88 ± 0.02 ^{bF}	0.81 ± 0.02 ^{bG}	0.93 ± 0.01 ^{aE}	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}	0.45 ± 0.01 ^{bG}	0.40 ± 0.01 ^{cG}	0.50 ± 0.01 ^{bH}	0.42 ± 0.01 ^{dI}		
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 ^e	29.24 ± 0.02 ^e	36.41 ± 0.03 ^a	34.70 ± 0.05 ^b	32.25 ± 0.03 ^d	33.35 ± 0.01 ^e	31.42 ± 0.01 ^e		
ΣMUFA	49.90 ± 0.17 ^a	47.92 ± 0.05 ^b	44.13 ± 0.07 ^c	44.51 ± 0.04 ^c	39.00 ± 0.06 ^d	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	14.00 ± 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 ^{cF}	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 ^{dB}	20.21 ± 0.10 ^{bB}	22.15 ± 0.11 ^{eB}	20.51 ± 0.07 ^{bB}	19.31 ± 0.03 ^{eB}	20.66 ± 0.11 ^{bB}	18.83 ± 0.08 ^{dC}		
C16:1	2.08 ± 0.02 ^{aE}	1.78 ± 0.04 ^{bF}	1.55 ± 0.09 ^{dF}	1.78 ± 0.09 ^{dF}	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 ^{cD}	12.26 ± 0.06 ^{cD}	11.85 ± 0.00 ^{aB}	11.29 ± 0.00 ^{cD}	11.84 ± 0.00 ^{bC}	11.31 ± 0.00 ^{bD}		
C 18:1	45.79 ± 0.09 ^{aA}	40.10 ± 0.08 ^{bA}	37.25 ± 0.07 ^{aA}	39.65 ± 0.07 ^{dA}	38.56 ± 0.10 ^{eA}	45.59 ± 0.11 ^{eA}	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 ^{cC}	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																																																																																													
	C 18:2 (n-3)	0.07 ± 0.00 ^{cl}	0.06 ± 0.00 ^{cl}	0.06 ± 0.00 ^{cl}	0.48 ± 0.00 ^{ch}	0.64 ± 0.01 ^{bh}	0.07 ± 0.02 ^{ah}	0.07 ± 0.00 ^{gh}	0.07 ± 0.00 ^{gh}	0.43 ± 0.02 ^{bh}	1.02 ± 0.02 ^{ag}	C 18:3 (n-3)	0.64 ± 0.02 ^{og}	14.68 ± 0.02 ^{oe}	14.68 ± 0.02 ^{oe}	2.60 ± 0.02 ^{oe}	3.12 ± 0.02 ^{de}	0.63 ± 0.02 ^{bf}	5.79 ± 0.03 ^{bd}	12.05 ± 0.04 ^{cd}	2.45 ± 0.02 ^{de}	C 20:1	0.94 ± 0.01 ^{bf}	0.77 ± 0.01 ^{cg}	0.77 ± 0.01 ^{cg}	0.90 ± 0.01 ^{cg}	0.84 ± 0.01 ^{ag}	0.82 ± 0.01 ^{bf}	0.72 ± 0.01 ^{df}	0.87 ± 0.01 ^{bg}	C20:2 (n-11)	0.59 ± 0.01 ^{ag}	0.58 ± 0.01 ^{abh}	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}	C20:3 (n-11)	0.27 ± 0.01 ^{bh}	0.29 ± 0.01 ^{al}	0.28 ± 0.01 ^{cl}	0.30 ± 0.01 ^d	0.26 ± 0.01 ^{cl}	0.37 ± 0.01 ^{ag}	0.49 ± 0.02 ^{cg}	0.42 ± 0.02 ^{gh}	0.51 ± 0.02 ^{ah}	0.46 ± 0.02 ^{bl}	ΣSFA	35.85 ± 0.13 ^a	33.02 ± 0.07 ^b	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	32.62 ± 0.03 ^d	35.54 ± 0.01 ^c	32.36 ± 0.01 ^e	ΣMUFA	49.79 ± 0.17 ^a	45.86 ± 0.05 ^b	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	41.07 ± 0.02 ^d	44.81 ± 0.08 ^c	38.63 ± 0.07 ^e	ΣPUFA	14.38 ± 0.17 ^e	21.08 ± 0.06 ^f	28.77 ± 0.08 ^b	20.75 ± 0.03 ^c	23.13 ± 0.06 ^a	13.77 ± 0.01 ^e	19.73 ± 0.16 ^f	20.24 ± 0.13 ^c	28.79 ± 0.06 ^a	Σn3	0.71 ± 0.04 ^e	6.32 ± 0.03 ^c	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	12.13 ± 0.08 ^a	2.88 ± 0.02 ^d	6.07 ± 0.02 ^b	Σn6	12.59 ± 0.02 ^d	13.67 ± 0.05 ^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	13.06 ± 0.04 ^c	16.03 ± 0.02 ^b

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 ^{aB}	0.39 ± 0.01 ^{aB}
TI	Chia50	0.35 ± 0.01 ^{aB}	0.35 ± 0.03 ^{aC}	0.35 ± 0.02 ^{aC}
	Hemp25	0.38 ± 0.01 ^{a^{AB}}	0.37 ± 0.01 ^{a^{BC}}	0.39 ± 0.03 ^{a^{AB}}
	Hemp50	0.34 ± 0.01 ^{aB}	0.33 ± 0.02 ^{aC}	0.34 ± 0.01 ^{aC}
	Control	1.06 ± 0.03 ^{aA}	1.07 ± 0.01 ^{aA}	1.08 ± 0.03 ^{aA}
	Chia25	0.72 ± 0.03 ^{aC}	0.72 ± 0.02 ^{aC}	0.71 ± 0.02 ^{aC}
h/H	Chia50	0.47 ± 0.02 ^{aE}	0.46 ± 0.04 ^{aE}	0.49 ± 0.01 ^{aE}
	Hemp25	0.84 ± 0.03 ^{aB}	0.77 ± 0.01 ^{bB}	0.86 ± 0.02 ^{aB}
	Hemp50	0.62 ± 0.02 ^{aD}	0.62 ± 0.02 ^{aD}	0.64 ± 0.01 ^{aD}
	Control	2.59 ± 0.02 ^{aE}	2.56 ± 0.02 ^{abE}	2.54 ± 0.02 ^{bE}
	Chia25	2.85 ± 0.02 ^{cD}	2.83 ± 0.05 ^{bD}	2.86 ± 0.01 ^{aC}
NV	Chia50	3.20 ± 0.01 ^{abB}	3.22 ± 0.02 ^{aB}	3.16 ± 0.03 ^{aB}
	Hemp25	2.89 ± 0.02 ^{aC}	3.00 ± 0.01 ^{aC}	2.73 ± 0.01 ^{b^{AD}}
	Hemp50	3.32 ± 0.01 ^{bA}	3.35 ± 0.03 ^{aA}	3.25 ± 0.02 ^{c^{AA}}
	Control	0.51 ± 0.01 ^{aB}	0.51 ± 0.01 ^{aB}	0.51 ± 0.01 ^{aB}
	Chia25	0.54 ± 0.01 ^{aA}	0.52 ± 0.01 ^{aB}	0.51 ± 0.01 ^{aB}
NV	Chia50	0.54 ± 0.01 ^{aA}	0.54 ± 0.01 ^{aA}	0.54 ± 0.01 ^{aA}
	Hemp25	0.51 ± 0.01 ^{aB}	0.51 ± 0.01 ^{aB}	0.53 ± 0.01 ^{aB}
	Hemp50	0.55 ± 0.01 ^{aA}	0.53 ± 0.01 ^{aA}	0.56 ± 0.01 ^{aA}

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 (Mariamanu & 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 (Vaszkoska *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 ^{cC}	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

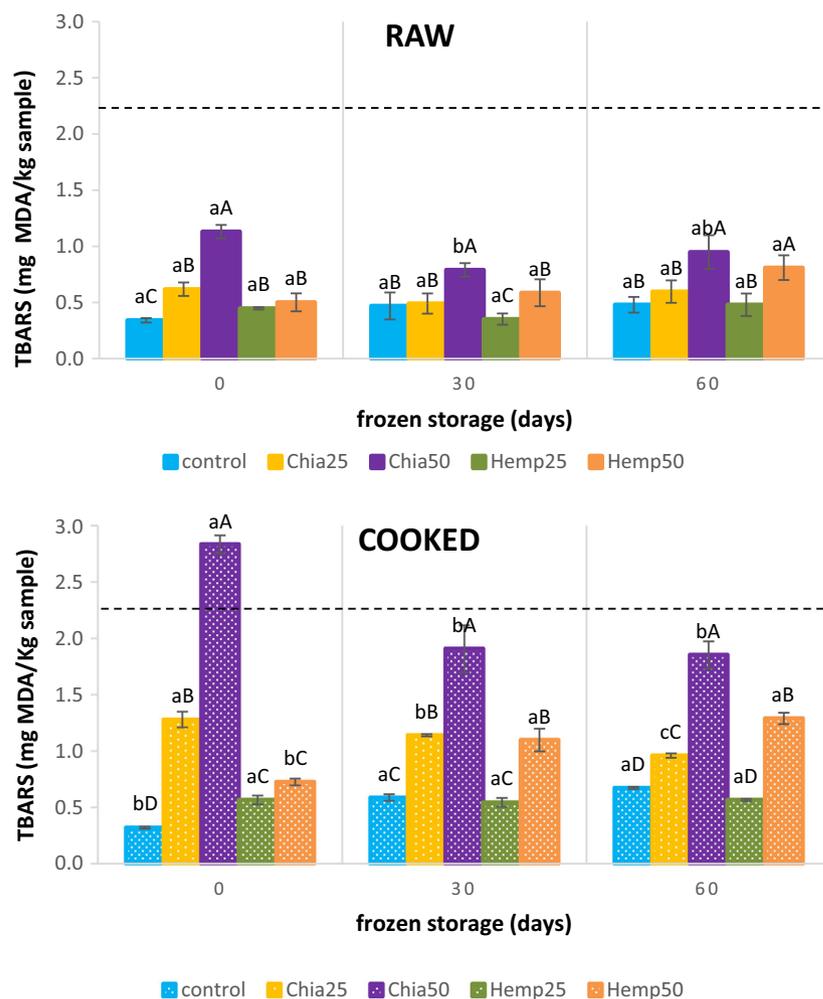


Figure 1 Lipid oxidation (TBARS values) of beef burgers (5 formulations, raw and cooked) during frozen storage. For each parameter, results followed by same letter are not significantly different according to Tukey's HSD *post hoc* test ($P > 0.05$). Data are presented as mean \pm standard deviation. 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 (Bodaira *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.

Peer review

The peer review history for this article is available at <https://publons.com/publon/10.1111/ijfs.15907>.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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