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Effects of flour, starch and pea (*Pisum sativum* L.) protein as fat substitutes during storage of pork sausages

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Abstract: Efforts are being made to replace the fat in meat products such as sausages with vegetable compounds to generate healthier foods. In this work, the effects of including flour, starch, and proteins isolated from pea seeds as partial fat substitutes in pork sausages was evaluated by studying the proximate composition, energy content, total cholesterol, lipid oxidation, and physicochemical, textural, and structural properties during refrigerated storage. The results showed significant differences in the composition of the sausages. Low-fat flour (LFF), starch (LFS), and pea protein (LFP) sausages had approximately 18% lower energy content than high-fat (HF) sausages. Cholesterol content was not significantly different in the treatments. Cooking yield, pH, and water activity were not affected by the inclusion of the replacements. LFF sausages had the highest purge losses and LFP sausages the lowest. The addition of pea starch improved the luminosity of the sausages, but the addition of pea protein resulted in darker sausages. After 12 days of storage, no differences were found between the hardness of LFP and HF sausages. The replacements did not affect lipid oxidation. The results suggest that replacing fat with pea seed components may be an alternative to producing low-fat sausages with health benefits.

Keywords: fat replacer; low-fat sausage; pea seeds; plant-based ingredients; hybrid foods

Sausages are the most consumed meat product worldwide. This food is a source of protein, vitamins, and minerals, however, due to its high content of saturated fat and cholesterol (Hu et al. 2021), they have been associated with chronic-degenerative diseases such as obesity, type 2 diabetes, cancer, and cardiovascular diseases (Li et al. 2023). The reformulation of meat products is one of the strategies most used in the industry to satisfy consumer demand for healthier foods. Therefore, current research evaluates the possibility of totally or partially replacing saturated fats in these foods with plant-based compounds (oils, proteins, and carbohydrates) that provide health benefits and allow for the generation of functional foods (Colomer et al. 2021). On the other hand, plant compounds added to meat products can reduce cooking losses, increase emulsion formation, and improve the texture and oxidative stability of these foods (Ferreira et al. 2023; Theóphilo et al. 2024). Plant compounds can prevent lipid oxidation due to their antioxidant activity (Estévez 2021). According to some authors, the antioxidant capacity of these compounds can be present even after cooking (Gallego et al. 2021). On the other hand, it has also been described that unsaturated fatty acids from vegetable oils added to meat emulsions can be susceptible to oxidation (Hadidi et al. 2022).

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The addition of chickpea flour (Thushan et al. 2010) and polysaccharides such as κ-carrageenan (Fontes-Candia et al. 2023) have been reported as substitutes for animal fat in sausages. Pea (*Pisum sativum* L.) is an herbaceous plant that from the legume family. Its seeds contain protein (20–25 g·100 g^{-1}), carbohydrates (24–49 g·100 g^{-1}), and fibre (60–65 g·100 g^{-1}) including 10–15% insoluble fibre and 2–9% soluble fibre (Shanthakumar et al. 2022). Starch is the most abundant carbohydrate in pea seeds. This starch is high quality due to its amylose content (30–60 g·100 g^{-1}) (Gao et al. 2023). Pea protein is also valued for its amino acid profile, and for being hypoallergenic, non-transgenic, and inexpensive compared to soy and milk protein (Zhang et al. 2023). Due to their nutritional characteristics and functional properties, the flour, starch, and proteins of pea seeds can be used as fat replacements in meat products. Pietrasik and Janz (2010) evaluated the addition of pea flour, starch and fibre fractions to produce low-fat mortadella and reported that this addition reduced cooking losses and improved product texture. Pietrasik and Soladoye (2021) incorporated pea starch into low-fat bologna and noted that the starch provided greater chewiness and hardness. On the other hand, Marti-Quijal et al. (2019) added pea proteins to pork sausages as an additional ingredient to improve the quality of the product rather than as a fat replacement. Although there are some studies on the addition of flour and pea starch to sausages, there are no reports evaluating their properties after refrigerated storage and the incorporation of pea protein as a fat substitute in fresh sausages has also not been evaluated. The aim of this work was to evaluate the effect of incorporating flour, starch, and proteins isolated from dried yellow pea seeds as partial fat substitutes in pork sausages on proximate composition, energy content, cholesterol content, lipid oxidation, and physicochemical, textural, and structural properties during refrigerated storage.

MATERIAL AND METHODS

Raw material

Fresh pork meat (20.73 ± 0.05% of protein; 2.16 ± 0.03% of fat) and backfat (71.76 ± 1.5% of fat) were purchased at a local market (Cordoba, Veracruz, Mexico). Both were ground in a meat grinder (Torrey, Mexico) with a 3.0 mm sieve and stored for 2 h in the freezer (REF2117A15, Thermo Fisher Scientific, USA). Frozen meat was used to avoid temperature increases during emulsion generation in the food processor. Dried yellow pea seeds were collected in San Miguel Tulancingo, Oaxaca, Mexico. The seeds were washed and dried in a food dehydrator (FD-32, Migsa, Mexico) for 2 h at 60 °C. Nitrites, phosphates, and seasonings were food grade and were purchased from the company Bekarem (Mexico). Sodium hydroxide, hydrochloric acid, isopropanol 99.5% purity, methanol 99.8% purity, thiobarbituric acid 98% purity, trichloroacetic acid 99.0% purity, and 1,1,3,1-tetraethoxypropane 96% purity were purchased from Sigma-Aldrich (USA).

Obtaining flour, starch, and isolated protein

Pea seeds were ground in a seed mill (700, Wangyyds, China) and sieved through a 60-mesh sieve to produce flour. The flour was kept at 25 °C until use. Pea flour had 68.3 ± 0.9% of carbohydrates, 23.4 ± 0.8% of proteins, 1.2 ± 0.0% of fat, and 2.9 ± 0.0% of ash. Starch was extracted using the method described by Beta et al. (2001). Briefly, pea flour was ground with distilled water. The suspension was filtered through an 80-mesh screen. The remaining material was rinsed with distilled water. The filtrate was washed with 0.05 M·NaOH. Then the starch was washed with distilled water and dried in a food dehydrator (FD-32, Migsa, Mexico) for 24 h at 45 °C. The starch presented 79.6 ± 0.9% of carbohydrates, 2.8 ± 0.0% of proteins, 4.7 ± 0.4% of fat, and 0.7 ± 0.1% of ash. Pea protein isolate was obtained using the method described by Xu et al. (2020). Pea flour was dispersed in water, adjusted to a pH 9.5 with 2.0 M·NaOH using a pH-meter equipped with a penetration probe (HI5521-02, Hanna Instruments, Mexico). The solution was kept under magnetic stirring for 1 h at 25 °C and centrifuged (5810 R, Eppendorf, Germany) at 6 000 rpm for 20 min. The supernatant was adjusted to pH 4.5 with 1.0 M HCl and centrifuged at 6 000 rpm for 10 min. The precipitate was resuspended in water and the pH was adjusted to 7 with 2.0 M·NaOH. The protein isolate was dried for 24 h at 45°C. The protein isolate presented 85.4 ± 0.0% of protein, 0.7 ± 0.0% of fat, 2.6 ± 0.5% of carbohydrates and 3.8 ± 0.2% of ash.

Preparation of the sausages

The formulation of the five treatments prepared is shown in Table 1. These included high-fat (HF) and low-fat (LF) sausages, and low-fat sausages with added flour (LFF), starch (LFS), or pea protein isolate (LFP). To prepare the emulsion, the meat was blended in a food processor (BL770AMZ, Ninja Blender, USA) with nitrites, phosphates, and 33% of the water/ice for 0.5 min. The fat, seasonings, and 33% of the water/ice were then added, and the mixture was blended

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Table 1. Formulation of the sausages

Ingredients (%)	Formulations treatments				
	HF	LF	LFF	LFS	LFP
Pork meat	45.00	45.00	45.00	45.00	45.00
Pork back-fat	20.00	10.00	10.00	10.00	10.00
Pea flour	–	–	3.00	–	–
Pea starch	–	–	–	3.00	–
Pea protein isolate	–	–	–	–	3.00
Water/ice	32.00	42.00	39.00	39.00	39.00
Sodium nitrite	0.32	0.32	0.32	0.32	0.32
Sodium phosphate	0.32	0.32	0.32	0.32	0.32
Seasoning	2.36	2.36	2.36	2.36	2.36

HF – high-fat sausages; LF – low-fat sausages; LFF – low-fat sausages added with pea flour; LFS – low-fat sausages added with pea starch; LFP–low-fat sausages added with pea protein isolate

for 2 min. The fat replacer and the remaining water/ice were then added, and the mixture was emulsified for 2 min. The prepared emulsions were filled into a 22 mm diameter collagen casing using a manual vertical stuffer (Migsa, Mexico). Sausages were produced with a length of 10 cm and were cooked at 68 °C in a water bath (VWR, USA) for 15 min. They were then placed in a water/ice bath to stop the cooking. The sausages were placed in stainless steel containers, covered with plastic film and placed in the refrigerator (Torrey, Mexico) at 3.0 ± 1.0 °C. Proximate analysis and cholesterol determination were performed 24 h (day 0) after preparation of the sausages. The physicochemical, textural properties and lipid oxidation were determined at day 0 and during the 12 days of refrigerated storage.

Proximate analysis and energy content

The moisture, ash, protein and fat contents of the raw material, sausages, and compounds isolated from pea seeds were obtained using AOAC methods (AOAC 1998). Carbohydrate content was calculated by difference of the other components. The energy content (kcal·100 g^{-1}) was calculated using the factors 9 kcal·g^{-1} for fat; 4.02 kcal·g^{-1} for protein; and 3.87 kcal·g^{-1} for carbohydrate.

Total cholesterol content

Total cholesterol was quantified using an enzymatic method with the Bioanalysis kit (Cat. No. 10139050035; Boehringer Mannheim/Biopharm, Germany). In brief, samples (2.5 g) were mixed with 0.1 M·KOH in methanol. They were heated under a reflux condenser for 25 min. The supernatant was transferred to a 25 mL flask and made up to volume with isopropanol. The mixture was filtered, and the clear solution was used for analysis. A total of 2.5 mL of the filtered solution was taken and mixed with 0.020 mL of the cholesterol oxidase reagent included in the kit. The sample, along with the blank, was incubated in a water bath at 37 °C for 60 min. The sample was allowed to cool to 25 °C, and the absorbance at 405 nm was determined with a UV-Visible spectrophotometer (Thermo Fisher Scientific, USA). The cholesterol concentration was obtained using the Equations (1) and (2):

$$\text{Cholesterol}\left(\text{g}\cdot\text{L}^{-1}\right)=\frac{V\times MW}{\varepsilon\times d\times v\times 1000}\times\Delta A \quad (1)$$

$$\text{Cholesterol}\left(\text{mg}\cdot 100\ \text{g}^{-1}\right)=\frac{\text{Cholesterol}\left(\text{mg}\cdot\text{L}^{-1}\right)}{\text{weight sample}\left(\text{mg}\cdot\text{L}^{-1}\right)}\times 100 \quad (2)$$

where: V – final volume; v – sample volume; MW – molecular weight of the substance to be assayed; d – light path; ε – extinction coefficient of the lutidine-dye at 405 nm.

Cooking yield

The raw sausages were weighed after the stuffing process and re-weighed after cooking. Cooking yield (%) was calculated as the percentage weight difference.

Purge loss during refrigeration storage

Purge loss was calculated by reweighing the sausage samples on days 3, 6, 9, and 12 of storage. The

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results were expressed as a percentage of the initial weight of the sample on day 0. The measurement was performed on sausages stored under refrigeration at 3.0 ± 1.0 °C without vacuum packaging.

Physicochemical properties

pH. The pH was measured with a pH-meter (Hanna Instruments, Mexico). The samples were homogenised with distilled water (pH 6.0 ± 0.1) in a ratio of 1 : 20 for 2 min using a blender (OBL245X, Oster, Mexico).

Water activity (a_w). An Aqualab Pawkit (Meter Group Inc., USA) was used. Samples measuring 2.0 × 1.0 × 1.0 cm and were obtained from the center of the sausages. Measurements were taken in triplicate at 25 °C.

Colour. The colour of the samples was measured using a portable spectrophotometer (CS 520 Sphere, Colorspec, China). The colour was quantified using the CIE LAB system (1976). The parameters L^* – lightness; a^* – yellowness and b^* – redness were evaluated. Chroma, Hue angle, and Euclidean distance (ΔE^*ab) were calculated using Equations (3), (4), and (5):

$$\text{Hue angle} = \tan^{-1}\left(b^*/a^*\right) \quad (3)$$

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}} \quad (4)$$

$$\Delta E^* ab = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \quad (5)$$

Texture properties

Texture profile analyses were performed using a texture analyser (EZ test, Shimadzu, Japan). The samples measured 2.0 cm in height by 2.2 cm in diameter. The analysis conditions were set as follows: pre-test speed 2.0 mm·s^{-1}; post-test speed 4.0 mm·s^{-1}; maximum load 2 kg; head speed 2.0 mm·s^{-1}; distance 8.0 mm; and force 5 g. Hardness, elasticity, gumminess, and chewiness were evaluated.

Structural analysis

Sausage samples were dehydrated in a food dehydrator (FD-32, Migsa, Mexico) at 40 °C for 3 h and were characterised using FTIR spectroscopy (Vertex 70v, Bruker, Germany) with an ATR accessory. Measurements were made from 4 000–500 cm^{-1}.

Lipid oxidation

Determination of the substances reactive to thiobarbituric acid (TBARS) was carried out with the methodology proposed by Du and Ahn (2002) with some modifications. The sample (20 g) was homogenised with 30 mL of distilled water. A volume of 1 mL of the homogenate was removed and mixed with 50 µL of 0.3 M of butylhydroxytoluene (BHT) in ethanol and 2 mL of thiobarbituric acid (15 mM) in trichloroacetic acid (0.9 M). The capped tubes were incubated in a water bath (VWR, USA) at 100 °C for 15 min, cooled in a cold-water bath, and centrifuged at 8 000 rpm for 15 min. The absorbance of the supernatant was measured at 531 nm with a UV-visible spectrophotometer (Thermo Fisher Scientific, USA). The TBARS value was expressed as mg malonaldehyde·kg^{-1} sample using a calibration curve with 1,1,3,3-tetraethoxypropane.

Statistical analysis

Two independent batches of each treatment were prepared in different months. All parameters were measured in triplicate for each batch. Textural properties were analysed on ten samples from each treatment. Outliers were removed using visual inspection techniques. The results were expressed as mean ± standard error of the mean (SEM) and compared using ANOVA and Tukey's test ($P < 0.05$) with the GraphPad Prim program version 10.1.1 (Dotmatics, USA). Statistical comparisons were made between each treatment per day of storage.

RESULTS AND DISCUSSION

Proximate analysis, energy content, and total cholesterol

Table 2 shows the proximate analysis, energy content, and total cholesterol of the sausages. No significant differences were found in the moisture of the HF, LFS, and LFP sausages. However, LF samples had higher moisture levels. Similar results were reported by Pietrasik and Janz (2010). Adding water to the sausages to replace the fat increased their moisture. The protein content was greater in the LFP samples compared to the other treatments, demonstrating the incorporation of the pea protein isolate into the product. There were no statistically significant differences in the protein content of the HF, LF, LFF, and LFS samples. Pietrasik and Janz (2010) reported an increase in the protein content of sausages with added fractions rich in starch and fibre obtained from pea seeds. The differences may be due to the purity of the incorporated isolates and the percentages of their inclusion.

LF, LFF, LFS, and LFP sausages were made with 50% less fat compared to HF sausages, so they had a lower fat content (Table 1). Significant differences were found in the ash content of LFP samples. Proximate analysis

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Table 2. Proximate analysis, energy content, and total cholesterol of the sausages

Samples	Moisture	Protein	Fat	Ash	Carbohydrates	Energy content (kcal·100 g^{-1})	Total cholesterol (mg·100 g^{-1})
	(%)						
HF	68.9 ± 0.06^{a}	9.8 ± 0.07^{a}	12.8 ± 0.17^{a}	1.6 ± 0.00^{a}	6.71 ± 0.20^{a}	182.9 ± 1.15^{a}	12.8 ± 0.35^{a}
LF	72.8 ± 0.13^{b}	10.7 ± 0.06^{a}	6.8 ± 0.06^{b}	1.7 ± 0.02^{a}	7.86 ± 0.14^{a}	137.3 ± 0.84^{b}	15.6 ± 1.11^{a}
LFF	71.5 ± 0.15^{b,c}	10.6 ± 0.07^{a}	7.8 ± 0.03^{b}	1.7 ± 0.03^{a}	8.27 ± 0.04^{b}	147.0 ± 0.77^{b,c}	13.1 ± 0.39^{a}
LFS	70.0 ± 0.37^{a,c}	10.8 ± 0.14^{a}	7.2 ± 0.11^{b}	1.7 ± 0.01^{a}	10.13 ± 0.26^{c}	149.5 ± 1.54^{c}	10.9 ± 0.14^{a}
LFP	70.5 ± 0.30^{a,c}	12.4 ± 0.19^{b}	7.4 ± 0.10^{b}	1.9 ± 0.02^{b}	7.56 ± 0.01^{a}	148.4 ± 1.60^{c}	14.5 ± 0.42^{a}

Values are the mean ± standard error mean (SEM); means followed by different letters in the column are significantly different ($P < 0.05$); HF — high-fat sausages; LF — low-fat sausages; LFF — low-fat sausages added with pea flour; LFS — low-fat sausages added with pea starch; LFP — low-fat sausages added with pea protein isolate

of the protein isolate indicated a higher percentage of ash than starch and pea flour, suggesting that the difference may be due to the purity of the isolate. Regarding carbohydrate content, the highest values were found in sausages with pea starch added, followed by sausages formulated with pea flour. In the proximate analysis, both fat substitutes had the highest carbohydrate content. No significant differences were found in carbohydrate content between HF, LF, and LFP sausages. On the other hand, no significant differences were found in the cholesterol content of the sausages (Table 2). Sampaio et al. (2004) reported similar results for sausages with added whey protein and oat bran as fat replacements. Some authors suggest that the cholesterol content in meat products may not be significant relationship with the amount of fat, suggesting that reducing fat may not be an effective strategy for lowering cholesterol in such products (Jiménez-Colmenero and Cofrades 2001). Cengiz and Gokoglu (2005) reported decreases in cholesterol of 38.6% and 45.7% in sausages with added citrus fibre and soy protein concentrate, respectively. It has been observed that incorporating a higher proportion of plant-based compounds in meat products could have a more substantial impact on the cholesterol content; however, this may negatively influence the sensory properties of the product. Reducing fat content and the addition of flour, starch, and protein isolated from pea seeds in sausages resulted in a reduction of around 18% of the caloric content of the sausage, and in the LF sausages the reduction was approximately 25% compared to HF sausages (Table 2). In LFF and LFS sausages, the increase in energy content can be explained by the increase in carbohydrates provided by the flour and starch. In contrast, in LFP sausages, the calorie content increases due to the addition of protein. Although the caloric content of LFF, LFS, and LFP sausages were higher than that of the LF sausage, the inclusion of vegetable compounds can bring health benefits to the consumer. Pea flour is a source of vitamins, minerals, antioxidants, and fibre. Pea starch is slowly digested and has a low glycemic index compared to other starches. Furthermore, the peptides in raw and cooked pea protein have antioxidant, antimicrobial, antihypertensive, and antidiabetic properties (Gallego et al. 2021; Wang et al. 2022).

Cooking yield and purge loss

The reduction of the fat content and the addition of flour, starch, and pea protein isolate did not significantly affect the yield of the product (Figure 1A). Similar results were reported by Ozturk-Kerimoglu et al. (2022) for sausages added with whey protein as a substitute for fat. Sausages are meat products with a high-water content, and their cooking yield is related to their components' ability to retain water and fat during cooking. The results showed that compounds obtained from pea seeds prevented water loss in sausages, maintaining the cooking yield. The plant-isolated compounds present techno-functional properties such as water/oil retention, emulsifying, and gelling properties (Badia-Olmos et al. 2023). These properties improve the production process and the yields of meat products.

Purge loss on the third day of storage of the sausages were less than 4%, with no significant differences observed between the samples (Figure 1B). During the following days of storage, the purge loss increased. The LFF samples had the highest loss of 15% after 12 days. Cooking yields showed that pea flour interacts with water molecules, allowing water retention during cooking, but water loss occurs during storage. This may indicate that the interactions between flour components and water molecules are weak, as reported by some authors in sausages with vegetable flours (Leonard 2019). In contrast, LFP sausages had the low-

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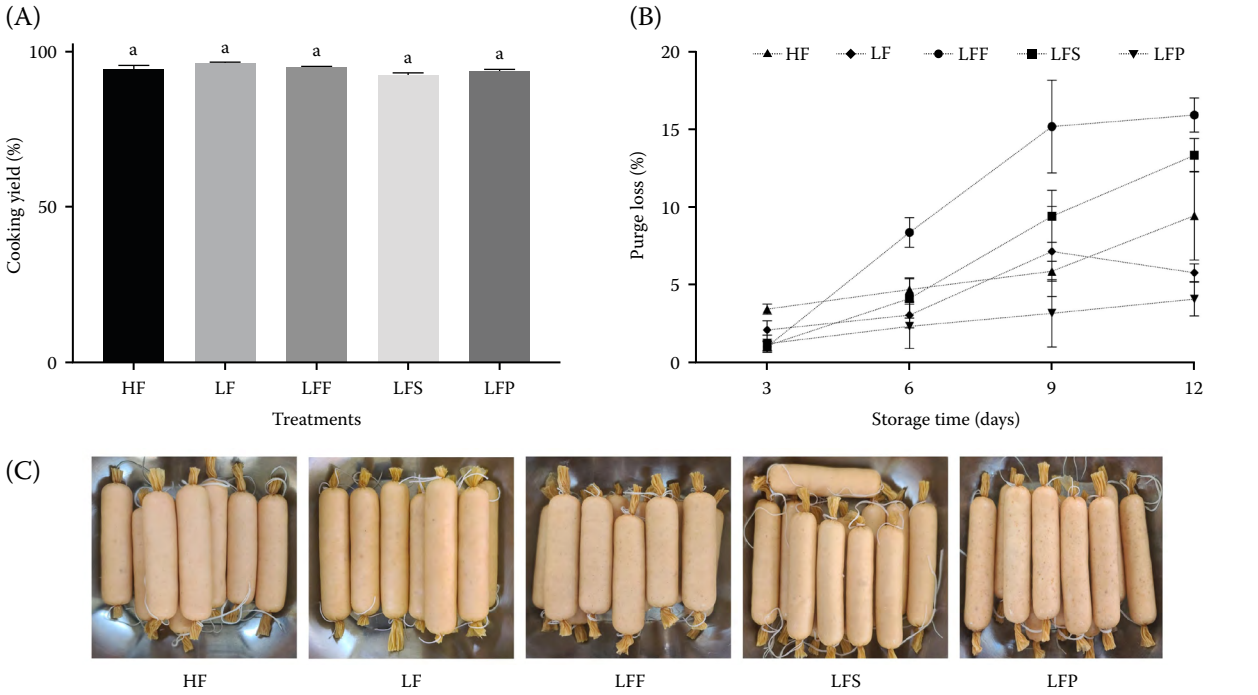

Figure 1. Cooking yield (A) and purge loss (B) during storage of the processed sausages, fresh processed sausages (C)

Values are mean ± standard error mean; means followed by different letters are significantly different ($P < 0.05$); HF – high-fat sausage; LF – low-fat sausages; LFF – low-fat sausages added with pea flour; LFS – low-fat sausages added with pea starch; LFP – low-fat sausages added with pea protein isolate

est losses. Pea proteins are known for their high-water absorption capacity (Lam et al. 2018). The addition of pea protein isolate contributed to better water retention during storage of the sausages compared to pea flour. The values obtained in the loss of purge were higher than those reported in previous studies (Pietrasik and Janz 2010; Pietrasik and Soladoye 2021). This study simulated the storage conditions used by consumers when purchasing sausages in bulk, where the products were not vacuum-packed. Consequently, purge loss may be higher compared to those stored in vacuum-sealed packaging.

Physicochemical properties

pH and a_w. The pH registered during the days of storage of the sausages are shown in Table 3. On day 0, the pH was within the range of 6.03–6.30. No significant differences were found between the treatments. Marti-Quijal et al. (2019) reported similar pH values in sausages with added pea protein isolate. Pietrasik and Janz (2010) reported higher pH values; however, they found no differences in pH between the control sausages and those with added fractions rich in flour, starch, and pea fibre. Some authors have reported that the bioactive compounds present in plant isolates can lower the pH of meat products during storage because they favour the release of fatty acids (Fontes-Candia et al. 2023), however, in this study no variations in pH were found. At 12 days of storage, the pH of the LFF and LFP sausage was slightly lower, but this was not statistically significant. The a_w of the processed sausages was between 0.85 and 0.89 on day 0. No statistically significant differences were found between the aw of the applied treatments. Furthermore, there were no significant changes during storage.

Colour. Figure 1C shows the appearance of freshly prepared sausages and the parameters of colour are shown in Figure 2. The L^* values of samples ranged between 70 and 80% (Figure 2A). The highest luminosities were observed in HF sausages. The results are consistent with the literature, as higher fat content generally increases lightness (Li et al. 2023). In contrast, LF and LFP samples exhibited significantly lower L^* values compared to the HF treatment. The lower fat concentration in LF sausages contributed to their darker colour. Additionally, the pea protein isolate, which has a naturally dark color, further reduced the L^* value when incorporated into the sau-

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Table 3. pH, water activity, and textural properties of the sausages during refrigerated storage

Parameter	Samples	Storage time (days)				
		0	3	6	9	12
pH	HF	6.30 ± 0.03^{a}	6.14 ± 0.01^{a}	6.11 ± 0.01^{a}	6.08 ± 0.01^{a}	6.08 ± 0.01^{a}
	LF	6.01 ± 0.01^{a}	6.12 ± 0.00^{a}	6.10 ± 0.01^{a}	6.07 ± 0.00^{a}	6.01 ± 0.00^{a}
	LFF	6.04 ± 0.00^{a}	6.13 ± 0.00^{a}	6.10 ± 0.01^{a}	6.05 ± 0.01^{a}	5.97 ± 0.03^{a}
	LFS	6.17 ± 0.03^{a}	6.14 ± 0.02^{a}	6.11 ± 0.01^{a}	6.10 ± 0.01^{a}	6.09 ± 0.01^{a}
	LFP	6.03 ± 0.01^{a}	6.05 ± 0.00^{a}	6.00 ± 0.00^{a}	5.99 ± 0.00^{a}	5.98 ± 0.00^{a}
a_w	HF	0.86 ± 0.00^{a}	0.88 ± 0.00a	0.84 ± 0.00^{a}	0.85 ± 0.00^{a}	0.85 ± 0.00^{a}
	LF	0.87 ± 0.00^{a}	0.89 ± 0.00^{a}	0.86 ± 0.00^{a}	0.86 ± 0.00^{a}	0.85 ± 0.00^{a}
	LFF	0.89 ± 0.00^{a}	0.87 ± 0.00^{a}	0.87 ± 0.00^{a}	0.86 ± 0.00^{a}	0.86 ± 0.00^{a}
	LFS	0.85 ± 0.00^{a}	0.85 ± 0.00^{a}	0.84 ± 0.00^{a}	0.84 ± 0.00^{a}	0.84 ± 0.00^{a}
	LFP	0.86 ± 0.00^{a}	0.86 ± 0.00^{a}	0.85 ± 0.00^{a}	0.86 ± 0.00^{a}	0.86 ± 0.00^{a}
Hardness (*N*)	HF	71.66 ± 2.94^{a}	53.58 ± 3.47^{a}	64.71 ± 4.49^{a}	69.75 ± 4.94^{a}	72.61 ± 6.64^{a}
	LF	65.46 ± 1.85^{b}	55.00 ± 3.15^{a}	59.77 ± 3.95^{a}	77.69 ± 5.32^{a}	130.64 ± 3.21^{b}
	LFF	90.38 ± 2.05^{a}	87.14 ± 5.86^{a}	98.64 ± 4.41^{b}	92.01 ± 2.09^{a}	116.96 ± 4.80^{b}
	LFS	69.19 ± 3.48^{a}	81.15 ± 4.48^{a}	119.20 ± 6.49^{a}	91.02 ± 7.25^{a}	110.65 ± 4.47^{b}
	LFP	88.75 ± 4.08^{a}	94.92 ± 2.43^{b}	66.01 ± 2.47^{a}	59.20 ± 2.75^{a}	93.23 ± 2.46^{a}
Gumminess (*N*)	HF	17.75 ± 1.29^{a}	14.79 ± 1.32^{a}	16.60 ± 1.44^{a}	17.76 ± 0.79^{a}	20.50 ± 2.27^{a}
	LF	17.47 ± 0.65^{a}	14.55 ± 0.95^{a}	16.84 ± 1.09^{a}	21.95 ± 1.92^{a}	36.23 ± 1.11^{b}
	LFF	26.40 ± 1.13^{b}	25.41 ± 1.72^{a}	30.98 ± 1.68^{b}	27.57 ± 0.99^{b}	38.83 ± 2.72^{b}
	LFS	19.18 ± 1.40^{a}	24.10 ± 1.25^{a}	35.56 ± 1.75^{b}	23.54 ± 1.93^{a}	32.00 ± 1.49^{b}
	LFP	24.42 ± 1.04^{a}	27.32 ± 1.06^{b}	17.65 ± 0.79^{a}	16.50 ± 0.65^{a}	27.55 ± 1.01^{a}
Elasticity (%)	HF	0.99 ± 0.00^{a}	1.05 ± 0.05^{a}	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}
	LF	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}	1.00 ± 0.00^{a}
	LFF	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}	1.00 ± 0.00^{a}	0.99 ± 0.00^{a}
	LFS	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}	1.00 ± 0.00^{a}	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}
	LFP	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}	0.99 ± 0.00^{a}	0.98 ± 0.01^{a}	0.99 ± 0.00^{a}
Chewiness (*N*)	HF	17.70 ± 1.09^{a}	27.20 ± 0.01^{a}	16.57 ± 1.45^{a}	17.74 ± 0.78^{a}	20.44 ± 2.24^{a}
	LF	17.45 ± 0.60^{a}	14.53 ± 0.96^{b}	16.80 ± 1.09^{a}	21.93 ± 1.91^{a}	36.23 ± 1.11^{b}
	LFF	26.39 ± 1.14^{b}	25.41 ± 1.72^{a}	30.96 ± 1.67^{b}	27.57 ± 1.00^{b}	38.73 ± 2.73^{b}
	LFS	19.18 ± 1.41^{a}	24.07 ± 1.25^{a}	35.57 ± 1.75^{b}	24.43 ± 1.63^{a}	31.91 ± 1.50^{b}
	LFP	24.38 ± 1.04^{a}	26.23 ± 1.17^{a}	17.63 ± 0.79^{a}	16.48 ± 0.65^{a}	27.55 ± 1.03^{a}

Values are mean ± standard error means; means followed by different letters in columns are significantly different ($P < 0.05$); a_w – water activity; HF – high-fat sausages; LF – low-fat sausages; LFF – low-fat sausages added with pea flour; LFS – low-fat sausages added with pea starch; LFP – low-fat sausages added with pea protein isolate

sages. However, in LFS samples, the inclusion of pea starch improved the L^* value, resulting in luminosity similar to that of HF sausages by day 3 of storage. These results agree with those reported by Pietrasik and Soladoye (2021). Throughout the storage period (days 0, 3, 6, and 9), the a^* values (redness) were higher in the LF and LFP samples (Figure 2B). However, the a^* value of the LF samples decreased on day 12 and there was no significant difference ($P < 0.05$) compared to the HF sausages. The a^* value of the LFP samples remained above the values of the high-fat sausages. These results are different from those reported by Marti-Quijal et al. (2019), who found that the addition of pea proteins did not affect a^* values. The differences may be due to the level of inclusion of the protein in the formulation and the extraction methodology of the proteins, which can influence the colour of the protein isolates. Among the HF, LFS, and LFF samples, no significant differences were found in the a^* values. In terms of b^* (yellowness), HF sam-

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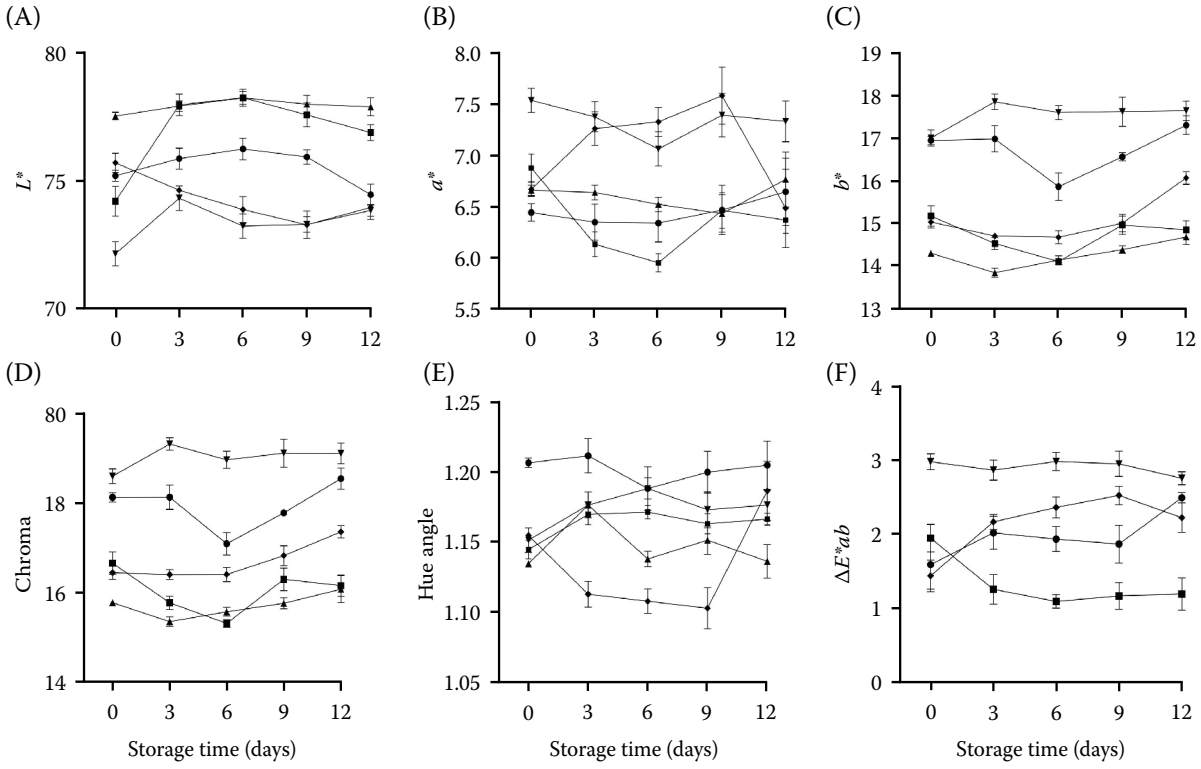

Figure 2. Colour parameters L^* (A), a^* (B), b^* (C); chroma (D); Hue angle (E) and ΔE^*ab (F) during the storage of processed sausages

Values are mean ± standard error mean; HF – high-fat sausage; LF – low-fat sausages; LFF – low-fat sausages added with pea flour; LFS – low-fat sausages added with pea starch; LFP – low-fat sausages added with pea protein isolate

ples showed the lowest values (Figure 2C). Statistically significant differences ($P < 0.05$) were observed between the samples containing pea flour, starch, and pea protein isolate. The sausages with pea protein and flour presented the highest b^* values due to the coloration of these substitutes. These results agree with those reported by Pietrasik and Soladoye (2021). The inclusion of pea protein isolates considerably affected this parameter. Similarly, the Chroma values (Figure 2D) were higher in the LFP sausages. The inclusion of some proteins isolated from legumes such as lentils and broad beans has been reported to modify the colour of sausages due to the legume coloration or the presence of pigments (Marti-Quijal et al. 2019).

The ΔE^*ab values are a useful tool to evaluate the total difference in the colour of the samples, and they were calculated considering the values of the HF treatment. The lowest values of ΔE^*ab were found in the LFS treatment (Figure 2F). Pea starch helped keep L^* and b^* values close to those of HF sausages. As explained above, fat generates greater luminosity, and pea starch, being white in colour, adds luminosity to the product and keeps yellowness values low. On the contrary, the addition of the pea proteins generated more differences in the colour parameter.

Textural properties

At the beginning of storage (day 0), LF samples had a lower hardness (Table 3). No fat replacement was added to these low-fat sausages, resulting in a product with less hardness. dos Santos et al. (2020) and Pietrasik and Janz (2010) reported similar results in the hardness of low-fat sausages. No statistically significant differences were found between the hardness of the HF, LFF, LFS, and LFP treatments on day 0. The inclusion of flour, starch, or isolated pea protein improved the hardness of the sausages on day 0. After 12 days of storage, the hardness of the sausages was greater in the LF, LFF, and LFS treatments. On each day the texture was measured, the hardness of the sausages produced increased, a fact that can also be explained by the water losses that occurred during storage. It was observed that the samples with the highest purge losses had greater hardness. Estévez et al. (2005) described that the increase in hard-

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ness in meat products during refrigerated storage is the result of emulsion instability caused by the separation of water and fat from the protein matrix. The inclusion of flour and pea starch resulted in tougher sausages following storage. Pietrasik and Soladoye (2021) reported that the incorporation of modified corn and pea starch increased the hardness of meat products due to the gelatinisation processes of starch granules. No significant differences were found between the hardness of LFP and HF sausages. Isolated pea proteins have a greater capacity to maintain interactions with water and fat, which improves the texture of the product (Broucke et al. 2022). Kang et al. (2022) reported that the emulsifying capacity of proteins in meat products is mainly due to water-soluble proteins that promote interaction with the fat molecule, allowing the generation of a more stable structure. Ozturk-Kerimoglu et al. (2022) explained that a lower hardness in food demonstrates a better stability of the emulsion. Initially (day 0), greater gumminess was observed in the LFF sausages, although no statistically significant differences were found in the gumminess of the HF, LFS, and LFP sausages (Table 3). After 12 days of storage, higher gumminess values were obtained, and no significant differences were found between the HF and LFP samples. No significant differences were found in the elasticity of the samples at the beginning and during storage. Similar results on elasticity were reported by Marti-Quijal et al. (2019) of sausages that incorporated different protein sources such as soybeans, peas, and lentils. On day 0, the samples prepared with pea flour had the highest chewiness values. No significant differences were found between the HF, LF, LFS, and LFP samples. Pietrasik and Janz (2010) reported that the inclusion of pea flour in bologna sausages did not modify chewiness compared to controls, although the level of flour inclusion was lower than that of this study. After 12 days of storage, the chewiness of the sausages increased. No statistically significant differences were found between the chewiness of HF and LFP sausages, and the inclusion of protein isolated from pea seeds did not affect this parameter.

Structural analysis

The Fourier transform infrared (FTIR) spectra of the sausages are presented in Figure 3A. Similar absorption bands were observed, but with different intensities, highlighting the bands of lipids, proteins, and carbohydrates. This indicates that the composition was similar, but the concentration of each component was different. Lipids had bands at 2 925 cm^{-1} and 2 854 cm^{-1} associated with the CH_2 group, and at 1 746 cm^{-1}, which is related to the C=O bond of the carbonyl group of the triacylglycerol ester bonds (Guntarti et al. 2019). Proteins exhibited absorption bands at 1 657 cm^{-1} (amide I), 1 542 cm^{-1} (amide II) cm^{-1} and 3 300 cm^{-1} (amide A). These bands were observed with greater intensity in the LFP samples. The absorption

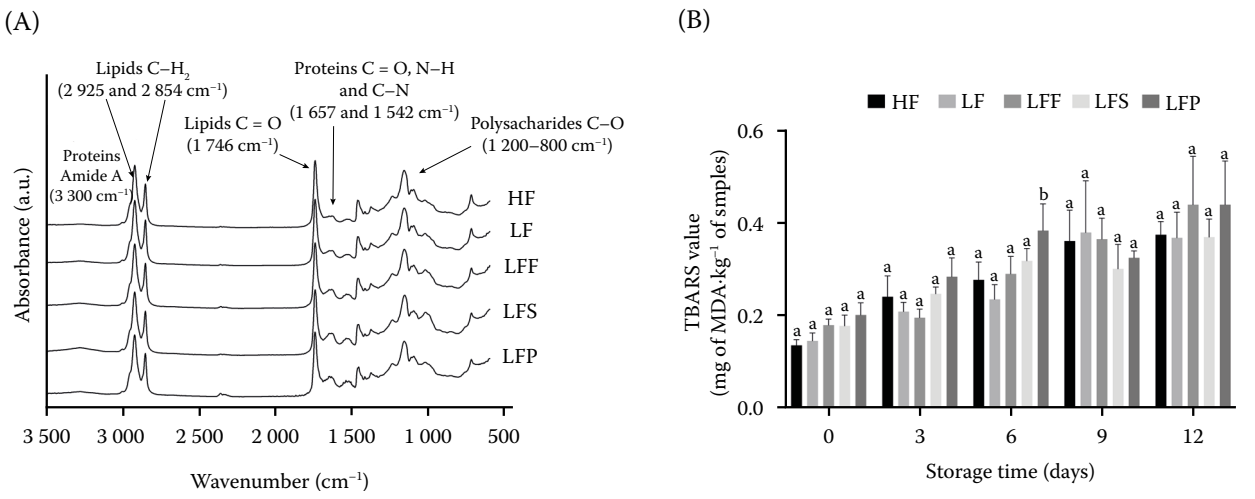

Figure 3. (A) Fourier transform infrared (FTIR) spectra of high-fat (HF); low-fat (LF); low-fat added with pea flour (LFF); low-fat added with pea starch (LFS) and low-fat added with pea proteins isolate sausages (LFP); (B) thiobarbituric acid (TBARS) values of sausages during refrigerated storage

Values are mean ± standard error mean; means followed by different letters in the day columns are significantly different ($P < 0.05$); MDA – malondialdehyde

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bands associated with carbohydrates were identified in the region between 1 200 and 800 cm^{-1}. The band at 1 000 cm^{-1} was more intense in the LFS spectra due to the presence of pea starch.

Lipid oxidation

TBARS values during refrigerated storage of sausages are presented in Figure 3B. On day 0, no statistically significant differences were found between the TBARS values of the treatments. The reduction of fat in some treatments did not prevent lipid oxidation from occurring. This behaviour has been reported by other authors (dos Santos et al. 2020; Ozturk-Kerimoglu et al. 2022). On day 12 of storage, the samples had TBARS values of 0.36 to 0.44 mg of malondialdehyde (MDA)·kg^{-1} of sample, and no statistically significant differences were found between the treatments. Some authors have reported that certain compounds such as sugars and other components of meat (ketones, acid, imides, amide, amino acids, and pyridine) interfere with the TBARS reaction, which may contribute to generating high TBARS values (dos Santos et al. 2020). Despite this, the TBARS levels found in the treatments were low and do not indicate an advanced state of product oxidation, as reported by Souza et al. (2021).

CONCLUSION

The results demonstrate that partial replacement of animal fat with pea seed components can produce low-fat meat products. The addition of the substitutes resulted in changes in the proximate composition and energy content of the sausages, resulting in a calorie reduction of up to 18%. Cholesterol content, pH, a_w, and lipid oxidation were not affected by the inclusion of flour, starch, and pea protein. The starch in the sausages improved product brightness compared to low-fat sausages, but the addition of pea protein resulted in darker sausages. The textural properties of sausages with pea protein were similar to those of high-fat sausages. In contrast, sausages with pea flour and starch had greater hardness during storage. Using pea proteins as a fat substitute may be the most suitable alternative, as it maintains the texture and physicochemical properties, generates fewer purging losses during storage, and increases the protein content of the sausages. However, it affects the product's colour. Future research recommends evaluating microbial quality, antioxidant properties, and performing sensory evaluation to generate more information on reformulated sausages with pea seed compounds as partial fat substitutes.

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