



Alheiras with animal fat replacement: application of a gelled emulsion based on hemp oil (*Cannabis sativa* L.) and buckwheat

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Abstract

A gelled emulsion elaborated using hemp seed oil and buckwheat flour was utilized as pork backfat substitute in a typical Portuguese meat product named Alheira. Three different formulations were prepared: Alheiras control (AC) with a fat content of 13%, alheiras where the 25% pork backfat was substituted with a hemp seed oil-GE (AH25), and alheiras where the 50% pork backfat was substituted with a hemp seed oil-GE (AH50). The chemical composition as well as the physicochemical properties and lipid stability of different samples were assessed. Reformulated samples showed similar protein, moisture, and fat content ($p < 0.05$) that AC. However, AH25 and AH50 had a higher amount of linoleic acid (16.29 and 22.14 g/100 g, respectively) and linolenic acid (1.75 and 3.45 g/100 g, respectively) than AC (12.71 and 0.51 g/100 g). Similarly, AH25 and AH50 showed lower saturated fatty acids (35.28 and 30.37 g/100 g, respectively) than AC (37.37 g/100 g). The substitution of pork backfat for hemp seed oil-GE did not modify significantly the physicochemical properties of samples. On the other hand, the lipid oxidation values increased by 30 and 65% in AH25 and AH50 respectively, in comparison to AC due to the use of polyunsaturated oils, which are highly susceptible to oxidation. This work established that the use of gelled emulsions elaborated using hemp oil and buckwheat flour may be a promising strategy to obtain meat products with a better healthier profile.

Keywords Reformulation · Fat substitution · Healthier oil · Polyunsaturated fatty acids · Health indices · Healthy meat product

Introduction

Alheira is a traditional Portuguese fermented sausage produced with a combination of poultry meats (from different types including duck, turkey, quail, partridge, and hen), pork (pork backfat), regional bread, olive oil, salt and spices including garlic, and paprika. This product is a highly appreciated and long-established practise in Northeast Portugal (Trás-os-Montes region). Although alheiras are commonly consumed in the rural areas of northern Portugal, today these products are becoming trendy in urban cities whose commercialization is growing significantly. Several studies have been conducted to examine the processing conditions [1–3], physicochemical and sensory properties [4], microbiological characteristics and safety [5–8], composition and meat species identification of alheiras [9], besides even the characteristics of a non-meat-based alheira has also been studied [10].

Despite the fact that alheira is a well-accepted meat product, it is perceived by consumers as unhealthy due

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to the high-fat content. This fact has become increasingly important for the meat industry, which is trying to develop several strategies to reduce or even eliminate saturated fat in product formulations and thereby to obtain high quality, nutritious, and healthier meat products while maintaining taste traditions and consumer expectations. Numerous approaches have been used to substitute saturated fat of animal origin with vegetable oils to minimize their effects on the physicochemical and sensorial characteristics of the final product, ensuring consumer acceptance as well as the technological characteristics, and enabling them to be economically viable in the meat industry [11–17]. Within these approaches, the use of gelled emulsion (GE) has been shown as a feasible strategy to structure liquid oils from different origins (marine, vegetable, or seeds) that have healthier fatty acid profiles trying to mimic the technological properties of pork backfat [18, 19]. In addition, several emulsifiers (chestnut flour, chia mucilage flour, soy protein, etc.) or gelling agents (gelatin, alginate, carrageenan, gums, etc.) have been utilized to develop these gelled emulsions [11, 12, 14, 20, 21]. In this manner, gelled emulsions prepared with diverse vegetable oils and gelling agents have been successfully utilized as fat substitutes in the development of cooked and fresh meat products with better effects on health [13, 16, 21–23]. Although the use of gelled emulsions as a saturated animal fat substitute in meat products is widespread, there are no studies in the scientific literature that indicate the use of these gelled emulsions to replace the animal fat content in alheiras, a traditional Portuguese meat product.

In this work, the gelled emulsion was elaborated with hemp seed oil (*Cannabis sativa* L.) using buckwheat flour as an emulsifier agent. Hemp seed oil shows an elevated content [24]. On the other hand, buckwheat flour has a high content of proteins, vitamins, minerals as well as dietary fibre [25]. In addition, the buckwheat proteins as well as the carbohydrate contents offer good emulsifying and gelling properties, which are necessary to develop the gelled emulsion [26]. In previous studies, gelled emulsion elaborated with several vegetable oils and flours obtained from pseudocereal were analysed [27, 28]. From all of them, due to the technological feasibility, physicochemical properties, and fatty acids composition the use of hemp seed oil with buckwheat flour had been selected to elaborate the gelled emulsion.

This study aims to (i) assess the technological viability of gelled emulsions made with hemp seed oil and buckwheat flour, as substitutes (25% and 50%) of animal fat for alheira production and (ii) study the effect of partial substitution of animal fat of alheiras on chemical composition and fatty acid profile, physicochemical properties, lipid profile, and lipid oxidation values.

Materials and methods

Materials

The following ingredients were used for hemp seed oil-gelled emulsions (Hemp-GE) elaboration: hemp seed oil (544.4 mg/g linoleic acid, 199.5 mg/g α -linolenic acid, and 82.3 mg/g oleic acid) was supplied by Laboratorios Almond, S.L. (Murcia, Spain); buckwheat flour was obtained from Biogran S.L. (Madrid, Spain); and gelatine “instant gel” from pork origin was purchased from Sosa Ingredients S.L. (Barcelona, Spain). The meat ingredients (hen (breast and thighs), duck (breast and thighs), and pork backfat after *rigor mortis*) were purchased from a local Portuguese butchery. A Tras-os-Montes Protected Designation of Origin olive oil (752.0 mg/g oleic acid, 33.0 mg/g stearic acid, and 77.0 mg/g linoleic acid) was used [29].

Gelled emulsion preparation

The gelled emulsion was elaborated with a proportion of 40% water, 40% hemp seed oil, 15% buckwheat flour, and 5% gelatine (Hemp-GE). Firstly, at high speed (5600 rpm), the gelling agent (instant gel) was mixed with the water at room temperature using a hand blender (Moulinex quickchef, France) for 1 min. In the next step, the buckwheat flour was incorporated and mixed for 1 min at high speed (5600 rpm) again. Finally, the hemp seed oil was gradually added at 15 ml/min to the mixture and mixed for 3 min at high speed (5600 rpm). The elaborated Hemp-GEs were deposited in flasks and stored at $-18\text{ }^{\circ}\text{C}$ until their subsequent incorporation into the meat product.

Alheiras manufacturing and sampling

For alheiras elaboration, all meats (big pieces) were cooked (at $100\text{ }^{\circ}\text{C}$) in water (2% salt) with proportion water: meat of 2.2:1 during 45 min. Afterward, the bread was finely sliced and immersed in the broth generated during the meat boiling. Then, when the bread was adequately soft, the chopped meat, salt, garlic, paprika, and olive oil were added to the batter. This batter was mixed for 10 min. Finally, the fat was added (at $4\text{ }^{\circ}\text{C}$, Hemp-GE previously thawed) and the mixture was mixed until all the ingredients were integrated. At the end of this process, the paste was stuffed into cattle intestinal casings and submitted to a dry process at $15\text{ }^{\circ}\text{C}$ and 75% relative humidity for 10 days without smoking process. Figure 1 shows the flowchart of the alheira production process.

Three types of alheiras were prepared (Table 1): in the control treatment, pork backfat was used (AC), and in

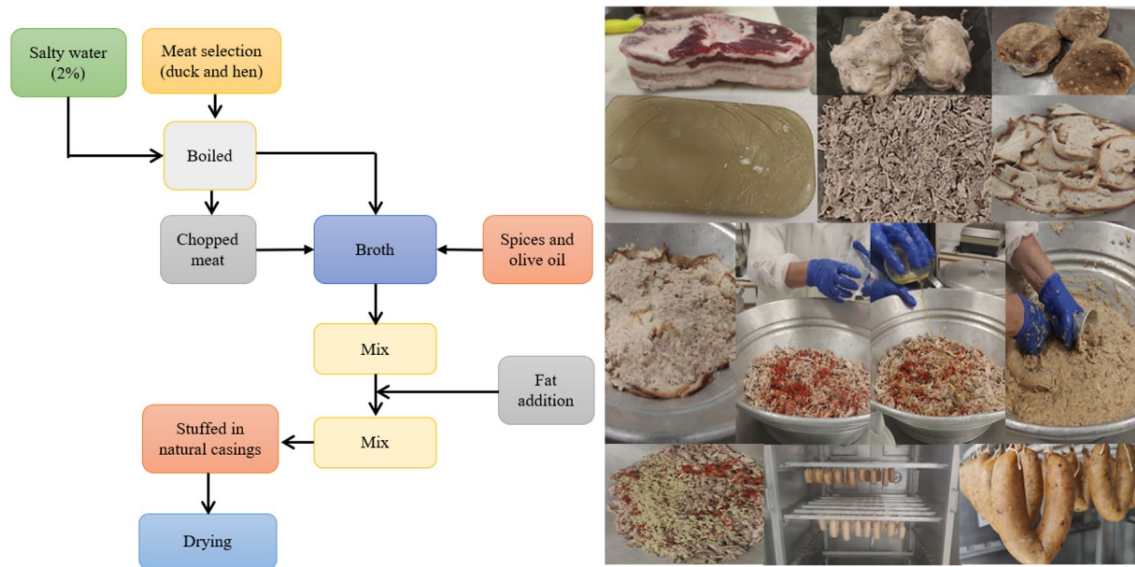


Fig. 1 Flowchart of the alheira production process

Table 1 Composition of control and reformulated alheiras

	AC	AH25	AH50
Bread	17.08	17.08	17.08
Hen, duck	21.36	21.36	21.36
Pork backfat	12.81	9.61	6.41
Hemp-GE	0.00	3.20	6.41
Salt	0.64	0.64	0.64
Garlic	0.17	0.17	0.17
Olive oil	0.78	0.78	0.78
Paprika	0.17	0.17	0.17
Broth	46.98	46.98	46.98

*Values are expressed as g/100 g. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour

the other two treatments, the pork backfat was partially replaced with Hemp-GE (AH25: 25% pork backfat was substituted with Hemp-GE; AH50: Alheira where the 50% pork backfat was substituted with Hemp-GE). The samples were developed according to the diagram of the production process (Fig. 1) and the formulation as shown in Table 1. Three alheiras from each formulation were cooked at 180 °C in an electric grill until the internal

temperature, measured at the geometrical centre of each alheira, reached 72 °C.

Chemical composition

For ash content, the alheiras were incinerated in muffle at 550 °C, according to Portuguese standards 1615 [30] while the moisture content was assessed following the methodology described by the Portuguese standards NP1614 [31]. Briefly, approximately 3 g of sample were added with 5 mL of ethanol and heated at 70 °C until its complete evaporation; after, the samples were oven-dried at 103 °C ± 2 °C until constant weight. The fat content was determined according to the recommendations of Folch et al. [32]. Protein content was analysed using the Kjeldahl method (%N × 6.25) in agreement with NP 1612 [33] using a Kjeldahl System equipment a gas neutralizer Buchi K-415 coupled to the Buchi K-446 Mineralizer and a Buchi auto Kjeldahl Unit K-370 distiller. The total chloride content was assessed using the methodology detailed in Portuguese standard NP 1845 [34]. For the titration the Titrino Plus brand was used with 0.1 N silver nitrate solution. The chloride content (NaCl) of the sample is expressed as a percentage, by mass by the equipment itself. All assays were taken on three raw and cooked samples of each formulation.

Physicochemical analyses

The pH analysis was measured directly in the different alheira samples following the Portuguese standard NP 344 [35] recommendations, using a Crison 507 pH-metre equipped with a 52-32 puncture electrode at different sites

of the sample. Water activity was determined according to AOAC [36]. Colour parameters were obtained as CIEL*a*b* coordinates using a colorimeter CM-2600D (Minolta Camera Co., Osaka, Japan) with illuminant D₆₅, 10° observer, SCI mode, 11 mm aperture of the instrument for illumination and 8 mm for measurement. Spectrally pure glass (CR-A51: Minolta Co.) was put between the sample and the equipment. Each alheira was evaluated six times on several points of surfaces for lightness (L*), redness (a* coordinate), and yellowness (b* coordinate), and the psychophysical parameters hue (h*) and chroma (C*) which were calculated according to the Eqs. (1) and (2), respectively.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

The total colour differences (ΔE^*) of each sample where the pork backfat was replaced with Hemp-GE (AH) with respect to the control sample (AC) were also calculated following the Eq. (3):

$$\Delta E^* = \sqrt{(L_s^* - L_c^*)^2 + (a_s^* - a_c^*)^2 + (b_s^* - b_c^*)^2} \quad (3)$$

Cooking loss

The weight of three samples, control and two partial fat replacement alheiras were measured at room temperature before and after cooking. The cooking loss was calculated with Eq. 4. Measurements were taken on three samples of each formulation.

$$\% \text{ Cooking loss} = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100 \quad (4)$$

Fatty acids composition analysis

To analyse the fatty acid profile, of both raw and cooked alheiras, the method described by Folch et al. [32] was applied for the lipid extraction from the samples. The fatty acids were transesterified following the method reported by Domínguez et al. [37] while Teixeira et al. [38] described the chromatographic conditions. As an internal standard, nonadecanoic acid (C19:0) at 0.3 mg/mL was added to the samples.

Individual Fatty acid methyl esters were identified by comparing their retention times with those of authenticated standards. The results obtained were expressed as g/100 g of total fatty acids. To analyse the nutritional value of alheiras where the pork backfat was replaced by Hemp-GE, several

health indices including omega-6 and omega-3 fatty acids ratio, and polyunsaturated fatty acids and saturated fatty acids ratio were calculated. In addition, the index of atherogenicity (AI) and the index of thrombogenicity (IT) were calculated according to the equations proposed by Ulbricht and Southgate [39]:

$$AI = \frac{C12 : 0 + (4 * C14 : 0) + C16 : 0}{\sum MUFA + \sum n6 + \sum n3} \quad (5)$$

$$TI = \frac{C14 : 0 + C16 : 0 + C18 : 0}{(0.5 * \sum MUFA) + (0.5 * \sum n6) + (3 * \sum n3) + \frac{(\sum n3)}{(\sum n6)}} \quad (6)$$

The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated using Eq. (7), as described by Fernández et al. [40].

$$\frac{h}{H} = \frac{C18 : 1n - 9 + C18 : 1n - 7 + \sum PUFA}{C14 : 0 + C16 : 0} \quad (7)$$

Lipid oxidation

The lipid oxidation values of both raw and cooked alheiras, analysed by the thiobarbituric acid reactive substances (TBARs) assay, were assessed by extraction of malondialdehyde (MDA) according to NP-ISO-3356 [41]. The results obtained were expressed as mg of MDA/kg of the sample.

Statistics analysis

All the process (elaboration of Hemp-GE and elaboration of alheiras) was replicated three times (three independent batches). The repetitions were carried out on different production days, and each batch was analysed in triplicate. SPSS software (version 24.0, SPSS Inc., Chicago, USA) was used to analyse the data using one-way or two-way analysis of variance (ANOVA) and Tukey-b post-hoc tests at 5% significance level ($p < 0.05$).

Results and discussion

Chemical composition

The results of the chemical composition of the control and reformulated alheiras are shown in Table 2. In raw samples, AC had the lowest values ($p < 0.05$) for moisture, ash, proteins, and chlorides. The moisture values varied between 56.52 and 59.87 g/100 g, being AH50, the sample that showed the highest ($p < 0.05$) value. In this product, high moisture values are characteristic since it is made of boiled

Table 2 Chemical composition of control and reformulated alheiras (raw and cooked)

	Sample	Moisture	Ash	Fat	Protein	Chlorides
Raw	AC	56.52 ± 1.06 ^c	1.17 ± 0.06 ^b	15.07 ± 0.74 ^a	9.85 ± 0.39 ^c	0.78 ± 0.05 ^b
	AH25	58.03 ± 0.39 ^b	1.35 ± 0.03 ^a	13.94 ± 0.97 ^b	11.63 ± 1.37 ^b	0.89 ± 0.04 ^a
	AH50	59.87 ± 0.31 ^a	1.38 ± 0.01 ^a	12.56 ± 0.83 ^c	12.18 ± 0.36 ^a	0.91 ± 0.01 ^a
Cooked	AC	54.23 ± 0.56 ^x	1.26 ± 0.02 ^y	17.71 ± 1.43 ^x	10.36 ± 0.27 ^z	0.89 ± 0.04 ^y
	AH25	54.62 ± 0.85 ^x	1.45 ± 0.04 ^x	15.76 ± 0.81 ^y	11.98 ± 0.58 ^y	1.01 ± 0.02 ^x
	AH50	55.24 ± 0.92 ^x	1.45 ± 0.04 ^x	12.85 ± 0.72 ^z	12.61 ± 0.71 ^x	1.07 ± 0.03 ^x

*Results were expressed as g/100 g. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour. For raw samples (a–c) and cooked samples (x–z); values followed by a different letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$)

poultry meats blended with bread which is soaked in the broth of the poultry meat cooking. Additionally, the increase in moisture content might be explained as a result of the water added to prepare the gelled emulsions. However, in cooked samples, the moisture content showed no statistical differences ($p > 0.05$) between AC, AH25, and AH50 with values ranging from 54.23 to 55.24 g/100 g of cooked sample. In this instance, the total moisture content of samples was affected by heat treatment. The moisture values found were in line with those reported in similar works by Teixeira et al. [38] and Patarata et al. [4] but higher than those reported by Carvalho et al. [7].

In raw alheiras, the rise in moisture content was accompanied by a reduction in the fat content ($p < 0.05$) when the Hemp-GE was utilized as a fat substitute. These changes represented a fat reduction, concerning the control sample, about 7.5% and 16.65% for AH25 and AH50, respectively. In the case of cooked alheiras, the same behaviour was observed. Thus, as the degree of substitution increased, the fat content decreased ($p < 0.05$). The fat reduction obtained with respect to AC was 11.01% and 27.44% for AH25 and AH50, respectively. These results were in concordance with those reported de Souza Paglarini et al. [42], and Botella-Martínez et al. [23] who reported a reduction in fat content in meat products where the pork backfat content was substituted by different gelled emulsions made with vegetable oils. In raw and cooked alheiras, the protein content increased with the substitution of pork backfat by Hemp-GE with statistical differences ($p < 0.05$) between samples. This fact might be explained as a result of the protein content (12.23 g/100 g) of buckwheat flour as well as the protein content of the gelling agent used to elaborate the Hemp-GE. The values obtained are close to those stated by Patarata et al. [4], Ferreira et al. [5] and Teixeira et al. [38] due to the substantial variation in sausage formulations available on the market.

In this study, the results reflected the changes in the initial formulation. The substitution of pork backfat (with high-fat content) for gelled emulsion elaborated with 40/40% (v/v)

of water/oil is expected (as occurs) that reduce the fat and increase moisture contents. Additionally, the use of gelatine in the formulation also produces a significant rise in protein content in the reformulated samples. Thus, the changes in the composition reflect the changes and proportions of the raw materials used in the alheira formulation.

Regarding ash content (Table 2), in raw samples, there were statistical differences ($p < 0.05$) between AC and the reformulated alheiras (AH25 and AH50), with the control alheira containing 15% fewer ashes than the partial fat substitution samples. The same tendency was obtained in the cooked samples, but with an overall 7% increase when cooked treatment was applied. This increase, in the ash content of reformulate samples with respect to control, could be due to the gelled emulsions containing buckwheat flour that may contribute to the increase of these parameters in both raw and cooked AH25 and AH50 samples [25, 43–45]. In any case, the values obtained for ash content were lower than those reported by Campos et al. [2] and Teixeira et al. [38] who reported values around 2.0 g/100 g but within the range of values presented by Patarata et al. [4]. In reference to sodium chloride content expressed as % chlorides (Table 2), no statistical differences ($p > 0.05$) were found between AH25 and AH50 in raw samples with values around 0.9 g /100 g of sample. In cooked samples, AC showed the lowest ($p < 0.05$) values whilst for AH25 and AH50, as occurs in raw samples, no statistical differences ($p < 0.05$) were observed. The values obtained, how it happens with those obtained for the ashes were lower than those reported in the literature [2, 4, 46].

Fatty acids composition

The fatty acids profile of raw and cooked alheiras (control, AH25 and AH50) are shown in Table 3. Regarding raw alheiras, statistical differences ($p < 0.05$) were found in the fatty acids profile of samples depending on the replacement level (25 or 50%) and the kind of fat used (pork backfat or Hemp-GE). In both raw and cooked control, AH25 and

Table 3 Lipid profile and nutritional parameters of control and reformulated alheiras (raw and cooked)

Fatty acid profile	Raw			Cooked		
	AC	AH25	AH50	AC	AH25	AH50
C14:0	0.97 ± 0.04 ^{aF}	0.86 ± 0.01 ^{bG}	0.71 ± 0.04 ^{cG}	0.92 ± 0.02 ^{xF}	0.84 ± 0.01 ^{yF}	0.73 ± 0.02 ^{zF}
C16:0	24.49 ± 0.11 ^{aB}	22.79 ± 0.09 ^{bB}	20.43 ± 0.02 ^{cC}	24.38 ± 0.13 ^{xB}	22.90 ± 0.14 ^{yB}	21.12 ± 0.70 ^{zB}
C16:1n-7	2.24 ± 0.06 ^{aE}	1.96 ± 0.02 ^{bE}	1.79 ± 0.00 ^{cF}	2.12 ± 0.05 ^{xE}	1.88 ± 0.01 ^{yE}	1.87 ± 0.16 ^{yE}
C17:0	0.19 ± 0.00 ^{aK}	0.18 ± 0.01 ^{aJ}	0.16 ± 0.00 ^{bI}	0.19 ± 0.00 ^{xI}	0.18 ± 0.01 ^{xH}	0.12 ± 0.06 ^{yH}
C17:1n-7	0.17 ± 0.01 ^{aK}	0.15 ± 0.00 ^{bJ}	0.13 ± 0.00 ^{cI}	0.16 ± 0.00 ^{xI}	0.16 ± 0.00 ^{xH}	0.12 ± 0.04 ^{yH}
C18:0	10.79 ± 0.26 ^{aD}	10.25 ± 0.08 ^{bD}	8.80 ± 0.01 ^{cD}	11.30 ± 0.20 ^{xD}	10.68 ± 0.04 ^{yD}	7.56 ± 3.37 ^{zC}
C18:1n-9	45.44 ± 0.18 ^{aA}	43.01 ± 0.18 ^{bA}	39.59 ± 0.01 ^{cA}	45.37 ± 0.12 ^{xA}	43.02 ± 0.13 ^{yA}	40.66 ± 1.35 ^{zA}
C18:2n-6	13.08 ± 0.25 ^{cC}	16.71 ± 0.17 ^{bC}	22.56 ± 0.02 ^{aB}	12.71 ± 0.10 ^{zC}	16.29 ± 0.30 ^{yC}	22.14 ± 1.05 ^{xB}
C20:0	0.18 ± 0.01 ^{cK}	0.23 ± 0.01 ^{bI}	0.28 ± 0.00 ^{aH}	0.20 ± 0.01 ^{zI}	0.24 ± 0.00 ^{yG}	0.28 ± 0.02 ^{xG}
C18:3n-6	0.82 ± 0.03 ^{aG}	0.76 ± 0.04 ^{bH}	0.67 ± 0.02 ^{cG}	0.88 ± 0.03 ^{xF}	0.82 ± 0.05 ^{yF}	0.70 ± 0.04 ^{zF}
C18:3n-3	0.53 ± 0.02 ^{cH}	1.84 ± 0.04 ^{bF}	3.65 ± 0.00 ^{aE}	0.51 ± 0.02 ^{zG}	1.75 ± 0.04 ^{yE}	3.45 ± 0.17 ^{xD}
C20:2n-6	0.34 ± 0.01 ^{aI}	0.32 ± 0.01 ^{aI}	0.27 ± 0.01 ^{bH}	0.34 ± 0.00 ^{xH}	0.33 ± 0.00 ^{xG}	0.27 ± 0.02 ^{yG}
C20:3n-6	0.06 ± 0.03 ^{aL}	0.06 ± 0.00 ^{aK}	0.06 ± 0.00 ^{aJ}	0.07 ± 0.01 ^{xJ}	0.05 ± 0.02 ^{xI}	0.06 ± 0.00 ^{xJ}
C22:1n-9	0.07 ± 0.03 ^{aL}	0.08 ± 0.01 ^{aK}	0.07 ± 0.00 ^{aJ}	0.08 ± 0.01 ^{xJ}	0.07 ± 0.02 ^{xI}	0.07 ± 0.01 ^{xI}
C23:0	0.24 ± 0.02 ^{bJ}	0.27 ± 0.04 ^{abI}	0.30 ± 0.01 ^{aH}	0.25 ± 0.01 ^{yH}	0.27 ± 0.01 ^{yG}	0.34 ± 0.02 ^{xG}
ΣSFA	36.95 ± 0.11 ^{aA}	34.75 ± 0.09 ^{bB}	30.90 ± 0.02 ^{cC}	37.37 ± 0.13 ^{xA}	35.28 ± 0.14 ^{yB}	30.37 ± 0.17 ^{zC}
ΣMUFA	48.18 ± 0.18 ^{aA}	45.50 ± 0.18 ^{bB}	41.83 ± 0.01 ^{cC}	48.05 ± 0.12 ^{xA}	45.42 ± 0.13 ^{yB}	42.95 ± 0.25 ^{zC}
ΣPUFA	14.86 ± 0.25 ^{cC}	19.76 ± 0.17 ^{bB}	27.27 ± 0.01 ^{aA}	14.57 ± 0.10 ^{zC}	19.30 ± 0.30 ^{yB}	26.66 ± 0.32 ^{xA}
Σn3	0.54 ± 0.04 ^{cC}	1.87 ± 0.03 ^{bB}	3.68 ± 0.06 ^{aA}	0.53 ± 0.04 ^{zC}	1.78 ± 0.05 ^{yB}	3.46 ± 0.08 ^{xA}
Σn6	14.30 ± 0.02 ^{cC}	17.85 ± 0.05 ^{bB}	23.56 ± 0.03 ^{aA}	14.00 ± 0.02 ^{zA}	17.49 ± 0.03 ^{yA}	23.16 ± 0.04 ^{xA}

*Values were expressed as g/100 g fatty acids. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour. For raw (a–c) or cooked (x–z) alheiras, values followed by the same small letter in the same row were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$). Values followed by the same capital letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

AH50 alheiras, a total of 24 fatty acids were detected. From the total of fatty acids identified in control alheira, oleic acid (C18:1n-9), palmitic acid (C16:0), linoleic acid (C18:2n-6), stearic acid (C18:0), and palmitoleic acid (C16:1n-7) represent more than 96% of total acids. In the case of reformulated alheiras, there was evidence to suggest that Hemp-GE as a partial replacer of animal fat decreased palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1n-9) and increase significantly ($p < 0.05$) linoleic acid (C18:2n-6) and α -linolenic acid (C18:3n-3). The major variance between all alheiras samples was the linoleic acid (C18:2n-6) and α -linolenic acid (C18:3n-6) contents. Thus, the alheiras with the highest animal fat replacement (AH50) had the highest ($p < 0.05$) content of α -linolenic acid and linoleic acid with values of 3.65 and 22.56 g/100 g of fatty acids respectively. This fact agreed with the fatty acid composition of hemp seed oil used to obtain the gelled emulsion. The cooked alheiras showed the same fatty acid profile as the raw samples, with no statistical differences ($p > 0.05$). The results of control sample agreed with the fatty acids profile of raw alheiras, recorded by Campos et al. [2] who reported that the main fatty acids found in traditional alheiras were oleic acid (34.76%) and palmitic acid (29.37%) and Teixeira et al. [38]

who informed that the main fatty acids present in alheiras were oleic acid (46.10%) and palmitic acid (23.90%). The change, in the lipid profile of samples where the animal fat was substituted with Hemp-GE (AH25 and AH50 samples), was consistent with previous studies where, in other kinds of meat products including beef burgers [16, 23] and Frankfurt sausages [47], the pork backfat was replaced by gelled emulsions.

All samples (cooked and raw) were mainly composed of monounsaturated fatty acids (MUFA) with levels between 41.83 and 48.18% for AH50 and AC, respectively with statistical differences between samples ($p < 0.05$). The use of gelled emulsions also resulted in a MUFA reduction, with the control samples (raw and cooked) containing the highest levels ($p < 0.05$). Oleic acid (C18:1) was the predominant fatty acid (39.59–45.44%) followed by palmitoleic acid (C16:1) with values between 1.79 and 2.24%. Regarding saturated fatty acids (SFA), it was reduced ($p < 0.05$) in AH25 and AH50 alheiras depending on the level of replacement (6 and 16%, higher reduction at the highest substitution level). The principal saturated fatty acids were palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0). Polyunsaturated fatty acids (PUFA) increased their content

in AH25 and AH50 alheiras with respect to control in 32% and 83% for AH25 and AH50 raw and cooked respectively. It can be seen that this increase in polyunsaturated fatty acid content in AH25 and AH50 alheiras was mostly as a result of α -linolenic acid and linoleic acid. Consequently, substituted alheiras had higher levels of omega-3 and omega-6 than control alheiras. The amount of omega-3 fatty acids was three times greater with the 25% substitution while for the 50% substitution the amount of omega-3 fatty acids was 6 times greater. For AH25 and AH50 samples, the omega-6 fatty acids increased a 25% and 65%, respectively. In both raw and cooked alheiras, this behaviour was observed with no differences between them ($p > 0.05$). The values of MUFA, SFA, and PUFA of control alheiras obtained in this study were higher than those reported by Marcos et al. [46] on different Portuguese alheiras. It should be remembered that alheira is a very variable product in terms of meat composition, quantity, and type of fat. Several authors have also reported this fact when replacing animal fat with vegetable or marine oils in several meat products [15, 20, 48, 49]. Thus, as a general conclusion, the fatty acids profile, and the proportions of individual and the SFA, MUFA and PUFA depends only on the fatty acids profile and the proportions of the animal fat and GE used in the alheira formulation.

Health indices of reformulated alheiras

In scientific literature, there are several works that reported that the substitution of animal fats, in the development of healthier meat products, using vegetable oils (added in different ways including oleogels and/or gelled emulsions) have important health benefits [16, 23, 49, 50]. Table 4 shows the health indices of raw and cooked alheiras. Human diets may be evaluated using the PUFA/SFA ratio as an indicator of nutritional quality. The mean ratio of PUFA/SFA

recommended by World health organization (WHO) and Food and Agriculture Organization (FAO) experts is above 0.4 [51].

All reformulated alheiras (AH25 and AH50) were in accordance with the mentioned recommendations of PUFA/SFA ratio with values of 0.57 and 0.55 for AH25 raw and cooked respectively, and values of 0.88 for raw and cooked AH50. However, the AC sample presented values within the recommended limit. Regarding the n6/n3 index, all the samples analysed (AC, AH25, and AH50) had a higher value than the proposed recommendations by Simopoulos, [52] who indicates that to have a healthy effect the ratio n6/n3 must be 4/1. Despite this, the decrease in the raw and cooked AH50 samples was 24% and 25% respectively. Although, based on scientific evidence and conceptual limitations, WHO [53] reported that no convincing scientific rational recommendation exists for n6/n3 ratios.

There has been a lot of discussion about the healthy properties of fats in meat products, using the atherogenic index, thrombogenic index, and/or hypocholesterolemic/hypercholesterolemic ratio as indicators [12, 16, 18]. Table 4 showed that the use of Hemp-GE as pork backfat replacer, significantly improved ($p < 0.05$) all indices except the atherogenic index. Thus, the thrombogenic index decreased while hypocholesterolemic/hypercholesterolemic ratio increased with the replacement compared with control alheira. All changes obtained in the health indices were directly linked to the amount of pork backfat substituted, the most positive values in the three indices were shown in alheiras with 50% substitution (AH50). On the other hand, the atherogenic index showed no statistically significant differences ($p > 0.05$) between the different samples of raw alheiras and between different samples of cooked alheiras. The heat treatment used also did not cause the atherogenic index to change. Compared to control alheira, reformulated samples showed an increased hypocholesterolemic/hypercholesterolemic

Table 4 Health indices of control and reformulated alheiras (raw and cooked)

	Sample	\sum PUFA/ \sum SFA	n6/n3	Atherogenic index	Thrombogenic index	Hypocholesterolemic/hypercholesterolemic index
Raw	AC	0.40 ± 0.02 ^{cA}	26.73 ± 0.04 ^{aA}	0.45 ± 0.03 ^{aA}	1.10 ± 0.06 ^{cA}	2.37 ± 0.02 ^{cA}
	AH25	0.57 ± 0.03 ^{bA}	9.57 ± 0.02 ^{bB}	0.40 ± 0.07 ^{aA}	0.91 ± 0.04 ^{bA}	2.65 ± 0.02 ^{bA}
	AH50	0.88 ± 0.04 ^{aA}	6.41 ± 0.02 ^{cB}	0.34 ± 0.03 ^{aA}	0.68 ± 0.02 ^{aA}	3.16 ± 0.01 ^{aA}
Cooked	AC	0.39 ± 0.02 ^{zA}	26.32 ± 0.14 ^{xA}	0.45 ± 0.03 ^{xA}	1.12 ± 0.07 ^{zA}	2.37 ± 0.02 ^{zA}
	AH25	0.55 ± 0.03 ^{yA}	9.85 ± 0.02 ^{yA}	0.41 ± 0.07 ^{xA}	0.93 ± 0.10 ^{yA}	2.62 ± 0.02 ^{yA}
	AH50	0.88 ± 0.04 ^{xA}	6.69 ± 0.02 ^{zA}	0.35 ± 0.03 ^{xA}	0.68 ± 0.11 ^{xA}	3.08 ± 0.01 ^{xB}

AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substitute with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substitute with a GE elaborated with hemp seed oil and buckwheat flour. Values followed by different small or capital letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$). A lower-case letter refers to the comparison of the same parameter between raw samples (a–c) and cooked samples (x–z). Capital letters refers to the comparison of the same sample depending on treatment (raw or cooked)

ratio. According to Barros et al. [50], high hypocholesterolemic/hypercholesterolemic ratios indicate healthier meat products than those with low ratios. In raw samples, the hypocholesterolemic/hypercholesterolemic ratio of AH50 was 3.16, while for AC was 2.37, with significant differences ($p < 0.05$). The same trend was observed in cooked samples. The values obtained for the different nutritional indices of alheiras, in the case of alheira control were similar to parameters described by Campos et al. [2] and Teixeira et al. [38].

Physicochemical analysis

The results of the physicochemical analyses of raw and cooked alheiras formulated with a gelled emulsion made with hemp seed oil and buckwheat flour used as a partial substitute for pork backfat were shown in Table 5. Regarding pH values, in both raw and cooked alheiras no statistical differences were found ($p > 0.05$) between AC and AH50 whilst AH25 had the lowest ($p < 0.05$).

Water activity values for both raw and cooked alheiras were not significantly altered ($p > 0.05$) by the partial substitution of pork backfat by the hemp seed oil-GE, with values around 0.960. The results of the physicochemical analyses made by Azevedo et al. [10] of several traditional and reformulated alheiras showed no differences in pH values ranging between 4.2 and 6.0 and 4.0 and 6.0 for traditional and reformulated alheiras respectively. This range includes the samples analysed in this study. Also, water activity values for traditional (0.959–0.988) and reformulated alheiras (0.961–0.991) were included in the water activity of this study. Teixeira et al. [38] found similar values of water activity ranging between 0.939 and 0.961 and similar values of pH. Otherwise, Patarata et al. [4] mentioned lower values of water activity (0.920–0.940) and pH (4.32–5.71). This variability in the physicochemical values could be due to the different manufacturing processes carried out, and/or the different raw materials used to elaborate this traditional meat product. In reference to colour parameters, in raw alheiras, the yellowness (b^*) coordinate, which ranged from 24.96 to

26.89, and hue (h^*) which ranged from 1.15 to 1.18, were not influenced ($p > 0.05$) by the use of Hemp-GE as pork backfat replacer. On the other hand, lightness (L^*), redness (a^*), and chroma (C^*) of raw alheiras were significantly ($p < 0.05$) affected by this replacement although their variation was not quantitatively relevant. There can be several reasons for these differences, including different oil characteristics and compositions, different emulsion properties, and different meat ingredients. As a general trend, only the raw sample with higher substitution (AH50) showed differences ($p < 0.05$) in the parameters lightness, redness, and chroma in reference with the control sample (AC) and AH25 sample. Redness values, ranged from 10.20 (AH50) to 11.61 (AC), and chroma values between 26.96 (AH50) and 29.24 (AH25), with very small differences. Therefore, lightness presented 8 points of differences between raw sample AH50 and AC. All these colour changes could be attributed to the characteristic colour of Hemp-GE, which is different in comparison with animal fat. In terms of colour properties, when meat products are heated, several reactions occur, such as the Maillard reaction, protein denaturation, and fat and water loss, which result in colour and taste changes [54]. Raw alheiras had some colour changes due to gelled emulsions, but cooking does not reveal these changes ($p > 0.05$) for all colour parameters. Similarly, Barros et al. [50] reported that in beef burgers added with oil emulsions no differences were found in the colour parameters. In contrast, several studies reported that colour parameters of different meat products were affected by the use of gelled emulsions used as fat replacer [11, 12, 50]. A study carried out by Summo et al. [55] revealed that raw meat products showed greater colour differences than cooked meat products where the fat was partially replaced by gelled emulsions.

It is very important to highlight that colour differences (ΔE^*) lower than 3 units can not be perceived by the human eye [56]; thus, all raw alheiras formulated with gelled emulsion as a partial pork backfat replacement (AH25 and AH50) might be perceived as different from control alheira. Since the minimum difference ($p < 0.05$) between AH25 and AC

Table 5 Physicochemical parameters of control and reformulated alheiras (raw and cooked)

	Sample	pH	Aw	L^*	a^*	b^*	C^*	h	ΔE^*
Raw	AC	5.95 ± 0.09 ^a	0.963 ± 0.00 ^a	68.24 ± 3.51 ^a	11.61 ± 0.62 ^a	26.54 ± 1.20 ^a	28.90 ± 1.31 ^a	1.16 ± 0.01 ^a	–
	AH25	5.79 ± 0.05 ^b	0.958 ± 0.01 ^a	66.54 ± 3.75 ^{ab}	11.48 ± 0.60 ^a	26.89 ± 1.46 ^a	29.24 ± 1.50 ^a	1.17 ± 0.02 ^a	4.00 ± 1.89 ^b
	AH50	5.92 ± 0.05 ^a	0.957 ± 0.01 ^a	60.56 ± 1.97 ^b	10.20 ± 0.35 ^b	24.96 ± 0.77 ^a	26.96 ± 0.85 ^b	1.18 ± 0.00 ^a	6.87 ± 0.84 ^a
Cooked	AC	5.83 ± 0.03 ^x	0.959 ± 0.00 ^x	65.06 ± 1.86 ^x	11.29 ± 0.50 ^x	25.53 ± 0.97 ^x	27.92 ± 1.07 ^x	1.15 ± 0.01 ^x	–
	AH25	5.74 ± 0.05 ^y	0.958 ± 0.01 ^x	65.43 ± 2.15 ^x	11.66 ± 0.69 ^x	26.70 ± 1.14 ^x	29.14 ± 1.28 ^x	1.16 ± 0.01 ^x	2.57 ± 1.20 ^x
	AH50	5.85 ± 0.08 ^x	0.961 ± 0.01 ^x	63.63 ± 2.54 ^x	11.44 ± 0.75 ^x	26.37 ± 1.20 ^x	28.75 ± 1.38 ^x	1.16 ± 0.01 ^x	3.15 ± 1.15 ^x

AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a GE elaborated with hemp seed oil and buckwheat flour. For raw samples (a–c) and cooked samples (x–z), values followed by a different letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$)

samples is 4 units. However, the cooking process would have masked the changes, resulting in very similar colour parameters in all the samples. This fact meant that the ΔE^* values obtained for the cooked samples showed no differences ($p > 0.05$) between them, with values close to the limit of 3 units. Consequently, once cooked, the consumer could not distinguish between control and reformulated samples.

Cooking loss

Alheiras were cooked at 180 °C (grilled) and the cooking loss was evaluated. The cooking process produces water evaporation as well as lipid migration in samples. Thus, the magnitude of these changes may affect the product acceptance [11, 57]. As can be seen in Fig. 2, all alheiras (control, AH25 and AH50) showed similar cooking loss, without statistical differences between them ($p > 0.05$). The cooking loss values ranged between 6.36 and 8.54%. So, incorporation of the gelled emulsion to substitute pork backfat in alheira samples does not affect negatively this parameter. Based on their stability and interrelationship with the meat matrix, these variations could be attributed to the nature and amount of oil, flour, and gelling agent used in the gelled emulsion elaboration. The cooking loss values had not been measured in alheira before. However, the analysis of this parameter is very common in meat products in which animal fat, totally or partially, had been replaced by other non-meat ingredients, generally dietary fibre. Therefore, in the scientific literature, the weight loss in cooked meat products where several dietary fibres were used as fat replacers had a contradictory behaviour. In this sense, Salejda et al. [58] informed that the weight loss of Frankfurt sausage added with 3% buckwheat co-products used as fat replacers was 15%. However, De Araujo et al. [59] carried out a work to analyse the weight loss of chicken sausages where the

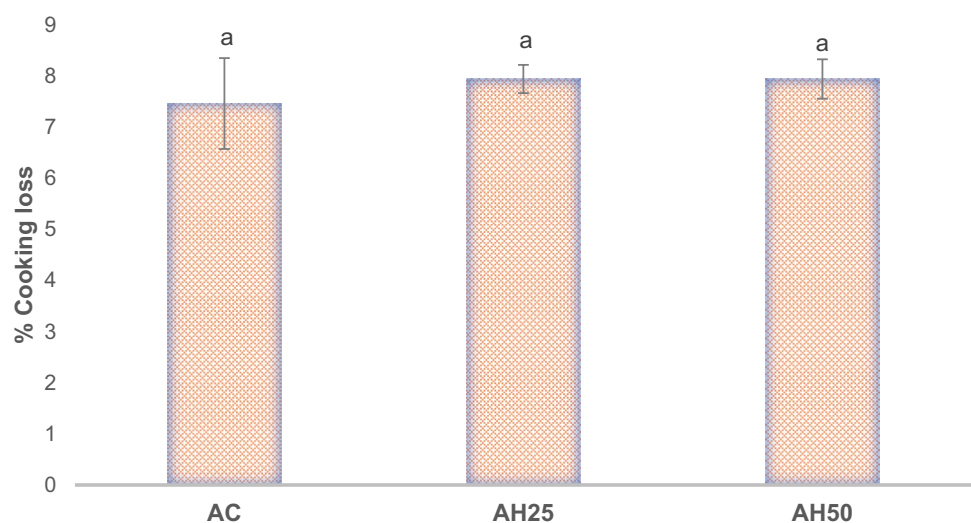
animal fat was substituted by inulin. These authors informed the weight loss values around 2.32% for the total substitution of animal fat. Similar values to those obtained in the present study were found by Choe and Kim [44] on chicken sausages where a chicken skin and wheat fibre mixture was used as a fat substitute. They found that for animal fat replacement levels of 20% there was no difference in cooking losses with the control sample.

Lipid oxidation of alheiras (TBARS)

Lipid oxidation is one of the principal causes of deterioration and quality loss of meat and meat products. In reformulated meat products, the rancidity process negatively affects different parameters such as colour, texture, nutritional value, flavour, and aroma, all of which are important factors that cause consumers to reject the products [60]. Figure 3 showed the lipid oxidation values in all samples analysed, before and after cooking. In raw samples, the substitution of pork backfat for Hemp-GE had no significant impact on the evolution of TBARS values. Thus, there were no significant differences ($p > 0.05$) in TBARS values between the control alheira (0.43 mg malondialdehyde (MDA)/kg of sample) and the AH25 and AH50 alheiras with values of 0.43 and 0.40 mg MDA/kg sample, respectively. The findings were encouraging since hemp seed oil is a rich source of polyunsaturated fatty acids which are much more susceptible to oxidation than pork backfat.

Cooking treatment is the principal factor that will trigger the oxidative processes. Thus, if the effect of the heat treatment is analysed for each of the samples, it is observed that the control sample (AC) was not affected by thermal treatment. On the other hand, after cooking, the AH25 oxidation values increased by 30% with respect to control sample whilst for AH50 the oxidation values increased by

Fig. 2 Cooking weight loss of control and reformulated alheiras. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a gelled emulsion elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a gelled emulsion elaborated with hemp seed oil and buckwheat flour. Bars with different letters were significantly different in accordance with Tukey's HSD post-hoc test ($p < 0.05$)



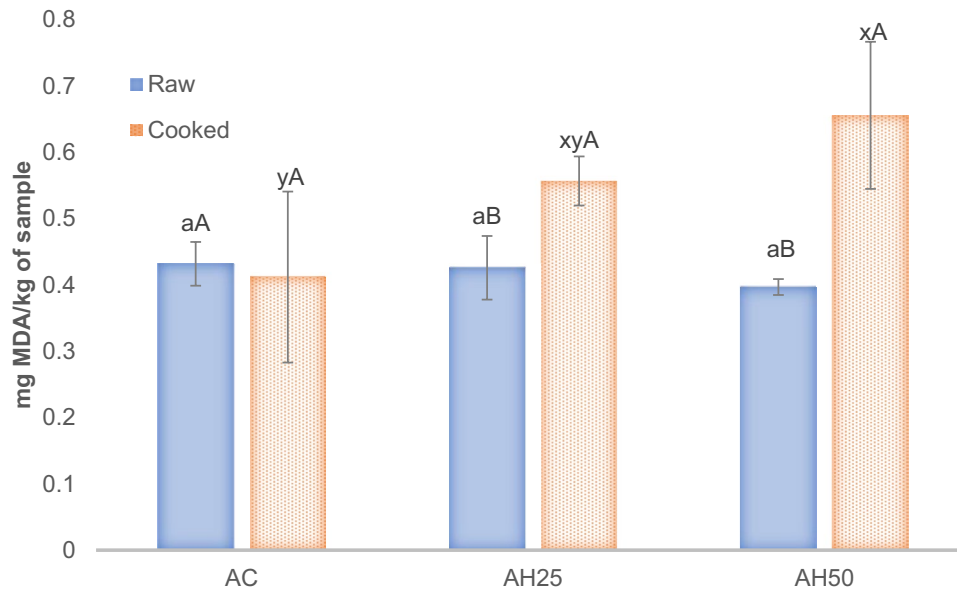


Fig. 3 Lipid oxidation values (TBARs) of control and reformulated alheiras (raw and cooked). Results were expressed as mg of malondialdehyde/kg of sample. AC: Alheira control elaborated with the traditional formula; AH25: Alheira where the 25% pork backfat was substituted with a gelled emulsion elaborated with hemp seed oil and buckwheat flour; AH50: Alheira where the 50% pork backfat was substituted with a gelled emulsion elaborated with hemp seed

oil and buckwheat flour. Bars with different letters were significantly different in accordance with Tukey's HSD post-hoc test ($p < 0.05$). A lower-case letter refers to the comparison of the same parameter between raw samples (a) and cooked samples (x–y). Capital letters (A, B) refers to the comparison of the same sample depending on cooking (raw or cooked)

65% with statistical differences ($p < 0.05$) between samples. Thus, the greater the degree of substitution, the greater the degree of oxidation. The fatty acid profile of the hemp seed oil which had a high content of polyunsaturated fatty acids and the thermal treatment used to cook the alheiras affected the malondialdehyde content.

In the scientific literature, there are no studies where the lipid oxidation of alheiras had been measured. However, it can be seen that the trend obtained in this study is similar to that of other meat products in which pork back fat was replaced by a gelled emulsion based on vegetable oils [16, 23, 47]. Nevertheless, it is important to note that in all alheiras analysed the lipid oxidation values obtained were lower than the malondialdehyde threshold for acceptability. Thus, Domínguez et al. [61] reported that the limit which indicates a loss of sensorial attributes as well as the perception of rancidity by consumers is 2 mg malonaldehyde/kg sample.

Conclusion

The results obtained in this work suggests that the reformulation of alheiras by means of gelled emulsion elaborated with buckwheat flour and hemp seed oil as a partial (25% and 50%) pork backfat replacer is a feasible strategy to obtain healthier meat products in relation to the quality of dietary fats (increase in polyunsaturated fatty acids and

reduce in saturated fatty acids). Alheiras where animal fat was replaced at 50% with gelled emulsions a high content of α -linolenic fatty acid was obtained. It is important to highlight, that colour differences between control sample and reformulated alheiras were obtained only in raw samples. The cooked alheiras, where 50% of pork backfat was replaced by gelled emulsions were more disposed to the lipid oxidation than the control but it did not exceed the limit indicative of rancidity in meat products detectable by consumer. In addition to offering meat products that satisfy the requirements of food safety agencies, these findings will also increase the competitiveness of the meat industry.

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Data availability Data available on request from the authors.

Declarations

Conflict of interest Authors declare no conflict of interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Compliance with ethics requirements This study does not involve research on human participants or animals.

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