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Is it possible to produce a low-fat burger with a healthy n − 6/n − 3 PUFA ratio without affecting the technological and sensory properties?

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ABSTRACT

Burgers subjected to lipid reformulation were made by replacing 50% of the fat component by microparticles containing chia (CO) and linseed (LO) oils obtained by external ionic gelation. The microparticles presented high n − 3 PUFAs levels and were resistant to the pH and temperature conditions commonly used in burger processing. The lipid reformulation did not affect hardness and improved important technological properties, such as cooking loss and fat retention. In addition to reducing the fat content of burgers by up to 50%, the lipid reformulation led to healthier PUFA/SFA and n − 6/n − 3 ratios, and lower atherogenicity and thrombogenicity indices. The burgers with CO microparticles showed a higher lipid oxidation and a lower sensory quality compared to the other treatments. However, the substitution of pork back fat by LO microparticles did not impair the sensory quality of burgers. Therefore, the microencapsulation of n − 3 PUFA-rich oils by external ionic gelation can be considered an effective strategy to produce healthier burgers.

1. Introduction

Burger is a meat product widely consumed in several countries. However, its nutritional quality is questioned by health experts, since it contains a high amount of animal fat (up to 30%). Besides increasing the energy value, animal fat also increases the saturated fatty acid (SFA) concentration of the processed products. Thus, frequent consumption of burgers may increase the incidence of obesity, cardiovascular disease and some cancers (Kaeferstein & Clugston, 1995). In addition, the n − 6/n − 3 ratio is higher in animal fat due to the higher content of n − 6 PUFAs rather than n − 3 PUFAs (Valencak, Gamsjäger, Ohrnberger, Culbert, & Ruf, 2015). This imbalance of PUFA levels may lead to the onset of several chronic diseases (Beecher, 1999).

Lipid reformulation by replacing a portion of the animal fat by fat substitutes containing n − 3 PUFA-rich oils may provide healthier characteristics to the food product, thus meeting the demands of health-conscious consumers. Due to their low SFA content and healthy n − 6/n − 3 ratio (Ayerza & Coates, 2005; Rubilar et al., 2012), chia and linseed oils may be an interesting alternative to improve the nutritional quality of burgers. However, the use of liquid oils rich in n − 3 PUFA in meat products may impair important technological and sensory attributes (Valencia, O'Grady, Ansorena, Astiasaran, & Kerry, 2008), as well as reducing the shelf life of the product due to increased lipid oxidation (Juárez et al., 2012; Triki, Herrero, Rodríguez-Salas, Jimenez-Colmenero, & Ruiz-Capillas, 2013).

Microencapsulation is an effective technique to increase oxidative stability and to prevent thermal degradation of fatty acids in n − 3 PUFA-rich oils (Bakry et al., 2016). This technique consists basically in the production of microparticles by coating the core material with a microencapsulating agent (Champagne & Fustier, 2007). Studies have shown that microencapsulation by external ionic gelation using alginate as microencapsulating agent allowed the production of microparticles resistant to high temperatures (Onwulata, 2013) and with controlled release of the active compounds in the human intestine (Soliman, El-Moghazy, El-Din, & Massoud, 2013).

In spite of the features of oil microencapsulation, only a few studies have been proposed using microencapsulation as a way to incorporate n − 3 PUFA-rich oils into meat products. Pelser, Linssen, Legger, and Houben (2007) used microencapsulated fish and linseed oils in dry fermented sausages. The use of fish oil microparticles was also studied by Josquin, Linssen, and Houben (2012) and Lorenzo, Munekata, Pateiro, Campagnol, and Domínguez (2016) in fermented meat products and by Keenan et al. (2015) in burgers. Those authors have shown that the use of microencapsulated oils may be a viable alternative of

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enriching food products with n − 3 PUFAs. However, those studies have not evaluated the effect of the heat treatment on the fatty acid composition and lipid oxidation. Therefore, n − 3 PUFA-rich oil (chia and linseed) microparticles were produced by external ionic gelation for use as a fat substitute in burgers. The encapsulation efficiency, proximate composition, fatty acid profile, thermal resistance, and stability of the microparticles under different pHs were assessed. The oxidative stability and the technological, nutritional, and sensory quality of the raw and cooked burgers were also evaluated.

2. Material and methods

2.1. Production of microparticles

The microparticles were produced by the external ionic gelation technique, according to Liserre, Ré, and Franco (2007) and Etchepare et al. (2016) with adaptations. In this way, 25% chia oil (T1) and linseed oil (T2) were mixed with 2.0% sodium alginate solution. Then, the mixture was atomized in 0.1 M $CaCl_2$ solution using a dual fluid atomizer nozzle (0.1 mm) at a distance of 12 cm from the solution, under air pressure of 0.125 kg/cm. After atomization, the microparticles were kept under constant stirring for 30 min, and then sieved in a wire mesh sieve (150 μm in diameter) and washed with sterile distilled water.

2.2. Analysis of oil microparticles

2.2.1. Encapsulation efficiency

The encapsulation efficiency (EE%) was calculated in triplicate according to Eq. (1). The total oil content (TO) in the microparticles was quantified according to the methodology described by Bligh and Dyer (1959), and the extractable oil (SO), commonly referred to as surface oil, was determined according to the methodology of Davidov-Pardo, Roccia, Salgado, Leon, and Pedroza-Islas (2008).

$$EE = \frac{(TO - SO)}{TO} \times 100 \quad (1)$$

2.2.2. Proximate composition, pH, and a_w

The proximate composition, pH and a_w of the microparticles were determined in triplicate. Moisture, ash and protein contents were determined according to AOAC (2005). The lipid content was determined by the method of Bligh and Dyer (1959). The pH values were measured using a pH meter (130 MA; Mettler Toledo, SP, Brasil), and a_w was measured using an Aqualab apparatus (Decagon Devices Inc., Pullman, USA).

2.2.3. Fatty acids profile

The fatty acid profile of both the microencapsulated oils and liquid chia and linseed oils was determined in triplicate. The lipids were extracted according to Bligh and Dyer (1959) method, and then 50 mg of sample was subjected to methylation as described by Hartman and Lago (1973), based on the saponification with a 0.4 M of NaOH methanolic solution (100 °C for 10 min) and acid-catalyzed esterification using 1 M H_2SO_4 methanolic solution (100 °C for 10 min). The fatty acid methyl esters (FAME) were quantified using a gas chromatograph equipped with a flame ionization detector (GC-FID, Varian Star 3400CX, Walnut Creek, USA). Aliquots of 1 μL were injected in split mode at a 50:1 ratio in 250 °C. The carrier gas was hydrogen at a constant pressure of 15 psi. The FAMEs were separated on CP-Wax 52 CB capillary column (Agilent, Middelburg, The Netherlands, 50 m × 0.32 mm × 0.20 μm). The initial column temperature was 50 °C, remaining for 1 min, increasing to 180 °C at 10 °C/min, with an increase rate of 2 °C/min after 200 °C, and then 10 °C/min until reaching 230 °C, which temperature was maintained for 5 min. The detector was maintained at 240 °C.

The FAME identification was performed by comparing the retention times of the analytes with FAME Mix-37 standards (P/N 47885-U; Sigma-Aldrich, St. Louis, USA). The results were expressed in grams/100 g of fatty acids. The atherogenicity (AI) and the thrombogenicity (TI) indices were calculated according to Ulbricht and Southgate (1991), as shown in Eqs. (2) and (3), respectively.

$$AI = \frac{C12{:}\,0 + (4{*}C14{:}\,0) + C16{:}\,0}{(\Sigma PUFA) + (\Sigma MUFA)} \quad (2)$$

$$TI = \frac{C14{:}\,0 + C16{:}\,0) + C18{:}\,0}{(0,5{*}\Sigma MUFA) + (0,5{*}\Sigma n - 6) + (3{*}\Sigma n - 3) + (\frac{n-3}{n-6})} \quad (3)$$

2.2.4. Thermal stability

To verify whether the microparticles would remain intact in the burgers when subjected to conditions similar to those used during preparation, test tubes containing approximately 10 g microparticles were heated in a water bath set to 80 °C until reaching the internal temperature of 72 °C, which was maintained for 20 min. Subsequently, the integrity of the microparticles were analyzed under an optical microscope. This analysis was performed in triplicate.

2.2.5. Resistance of microparticles at different pH values

The resistance of the microparticles at different pH values was determined in triplicate according to the methodology proposed by Holkem et al. (2016). The microparticles were mixed with phosphate (pH 7.5 and 6.0) and acetate (pH 4.5) buffers. The solutions with the microparticles were stirred at 150 rpm at 37 °C in a shaking incubator (TE-421, Tecnal, Piracicaba, SP, Brazil). Aliquots were removed after 60, 120, and 180 min to determine the release of the microencapsulated oil. The integrity of the particles was monitored by light microscopy before and after buffer addition.

2.3. Burgers formulation and processing

Beef (*rectus femoris*) (moisture: 72.9%; protein: 21.7%; and fat: 4.5%), pork back fat (moisture: 11.6%; protein: 8.5%; and fat 80.3%), and spices were purchased from local market. Chia and linseed oils were obtained from Giroil S.A. (Santo Ângelo, Brazil). Three burger formulations were processed in a pilot plant, as follows: a control treatment was produced with beef (78.4%), pork back fat (20.0%), salt (1.5%), and garlic (0.1%), and the modified treatments (T1 and T2) were prepared by replacing 50% of pork back fat with microparticles containing chia (T1) and linseed (T2) oils.

To produce the burgers (5 kg per batch), beef and pork back fat were ground separately (Model PJ22, Jamar Ltda, São Paulo, Brazil) using a 3 mm disc. Beef was then mixed with salt to extract myofibrillar proteins. Subsequently, the remaining ingredients were added and the mixture was mixed until complete homogenization. Burgers (100 g), 11 cm in diameter and 2.5 cm thick were produced using a burger machine (HP 112, Picelli, São Paulo, Brazil). The burgers were immediately frozen and stored at − 18 °C until analysis. Some measurements were performed in both raw and cooked burgers. The samples were cooked in an electric grill (Multi Grill, Britânia, São Paulo, Brazil), preheated to 150 °C, until reach an internal temperature of 72 °C in the geometric center of each burger, which was measured by a spit thermometer (HM-600, Highmed, São Paulo, Brazil) inserted in the center of each burger.

2.4. Physicochemical evaluation of burgers

The proximate composition (moisture, protein, lipids, and ash), pH, and a_w of raw and cooked burgers were determined in triplicate using three samples for each treatment according to procedures described above.

The color of the raw and cooked burgers was measured just after

manufacturing (day 1) and after 120 days of storage. A Minolta CR-400 colorimeter (Minolta Sensing Inc. Konica, Japan) was used with spectral reflectance included as calibration mode, illuminant D65 and observation angle of 10°, operating in the CIE (L* a* b*) system. Color variables were measured at six points on each side of the samples. The L* (lightness), a* (intensity of the red color), and b* values (intensity of the yellow color) were determined.

2.5. Technological properties

The technological properties were determined in triplicate using three samples for each treatment. The raw samples were weighed and their diameter was measured. These procedures were repeated after cooking and cooling the samples to room temperature (25 °C). The cooking loss and diameter reduction were calculated using Eqs. (4) and (5), respectively.

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

$$\text{Diameter reduction } (\%) = \frac{\text{diameter}_{raw} - \text{diameter}_{cooked}}{\text{diameter}_{raw}} \quad (5)$$

The moisture retention and fat retention were determined according to Eqs. (6) and (7), respectively.

$$\text{Moisture retention } (\%) = \frac{(100 - \text{cooking loss } (\%)) \times (\text{moisture}_{cooked})}{100} \quad (6)$$

$$\text{Fat retention } (\%) = \frac{(\text{weight}_{cooked} \times \text{fat}_{cooked})}{(\text{weight}_{raw} \times \text{fat}_{raw})} \times 100 \quad (7)$$

2.6. Texture profile

The texture profile analysis (TPA) was performed as described by Bourne (1978). Three burgers from each treatment were cooked, and then cooled to room temperature (25 °C). With the help of a cylindrical knife, four cylinders (2 cm thick and 2 cm in diameter) of each burger (12 cylinders per treatment) were sampled. TPA was performed with a TA-TX2 texture analyzer (Stable Micro Systems Ltd., Surrey, England) equipped with a 25 kg load cell. A 36 mm probe was used, at a constant speed of 1 mm/s, and the cylinders were subjected to two consecutive cycles of 50% compression. The parameters hardness (N), springiness (mm), cohesiveness, and chewiness (N) were calculated.

2.7. Fatty acids profile

The fatty acid profile was determined in both raw and cooked burgers, using three samples for each treatment, according to the procedures previously described in Section 2.2.3.

2.8. Lipid oxidation (TBARS)

Lipid oxidation was evaluated in triplicate in raw and cooked burgers, by determination of TBARS values. The results were expressed in milligrams of malonaldehyde per kg of sample (Bruna, Ordonez, Fernández, Herranz, & Hoz, 2001). TBARS analyses were performed just after manufacture (day 1) and at 30, 60, 90, and 120 days of storage at − 18 °C.

2.9. Sensory evaluation

The sensory evaluation was performed for the control, T1, and T2, and the treatments containing chia (T3) and linseed (T4) liquid oils. The treatments T3 and T4 were prepared using similar manufacturing procedure and formulation as the treatments T1 and T2, except for the addition of microparticles, which was replaced by liquid oils (2.5%) and water (7.5%).

This study was approved by the Research Ethics Committee of the Federal University of Santa Maria (RS, Brazil) (CAAE: 57433316.8.0000.5346). A three-digit code was assigned to the samples, which were evaluated by each consumer in a monadic order, following a balanced design (Ares, Barreiro, Deliza, Giménez, & Gámbaro, 2010). The tests were performed in individual booths with fluorescent lighting. The cooked burgers were cut into 4 × 4 × 2.5 cm, individually wrapped in foil, and kept at 60 °C in an oven. Water at room temperature and cracker biscuits were provided to consumers for palate cleansing. One hundred habitual consumers of burgers (56% female, 44% male, aged 18–55) participated in the sensory tests.

2.9.1. Check-all-that-apply (CATA) evaluation

Consumers were asked to complete a check all that apply (CATA) questionnaire with 21 descriptors related to the sensory characteristics of the burgers. The descriptors used in the CATA questionnaire were based on studies about sensory descriptors of low-fat meat products (Alves et al., 2016). The sensory attributes appearance (pale color, ideal color, strange, and oily), aroma (soft, rancid, pleasant, and greasy), flavor (soft, rancid, pleasant, bitter, unpleasant, seasoning in the right amount, and greasy) and texture (ideal, rubbery, difficult to chew, juicy, and hard) were used to characterize the sensory profile of the burgers.

2.9.2. Acceptance tests

A sensory acceptance test was performed using a 9-point structured hedonic scale, varying from “disliked very much” to “liked it very much” (Stone, Bleibaum, & Thomas, 2012). The attributes color, aroma, flavor, texture, and overall acceptability were evaluated (Meilgaard, Carr, & Civille, 2006).

2.10. Statistical analysis

A randomized block design was used, and the whole experiment was repeated three times. Data (except sensory evaluation) were analyzed by analysis of variance (ANOVA) using a general linear model considering the treatments as fixed effect and the replicates as random effect (n = 3). Tukey's test was used at the 5% level of significance.

Analysis of correspondence was used to analyze data from CATA questionnaire, considering the chi-square distance (Vidal, Tárrega, Antúnez, Ares, & Jaeger, 2015), calculated on the matrix containing the frequency of use of each term for each sample. For the consumer test, a two-way ANOVA (consumers x samples) followed by Tukey's test at 5% significance level ($P < 0.05$) was carried out.

3. Results and discussion

3.1. Analysis of the microparticles

An efficiency of about 86% was observed in the microencapsulation of chia and linseed oils (86.2% and 86.5% for the T1 and T2, respectively, $P > 0.05$, SEM: 0.01), thus proving that the process was effective to retain most of the oil inside the microparticles. Similar encapsulation efficiency was reported by Chang, Yarankovich, and Nickerson (2016) in canola oil microencapsulated with sodium alginate. Chia and linseed oil microparticles had a similar chemical composition ($P > 0.05$). Moisture, protein, lipids, and ash contents ranged from 73.3 to 74.3%, 0.35 to 0.36%, 21.7 to 22%, and 0.81 to 1.14%, respectively. In addition, no significant differences ($P > 0.05$) were observed in pH (from 5.84 to 5.86) and a_w (0.99) values between the microparticles.

Thermal resistance analysis indicated that the temperature of 72 °C for 20 min was not able to disrupt the microparticles (Fig. 1). This result

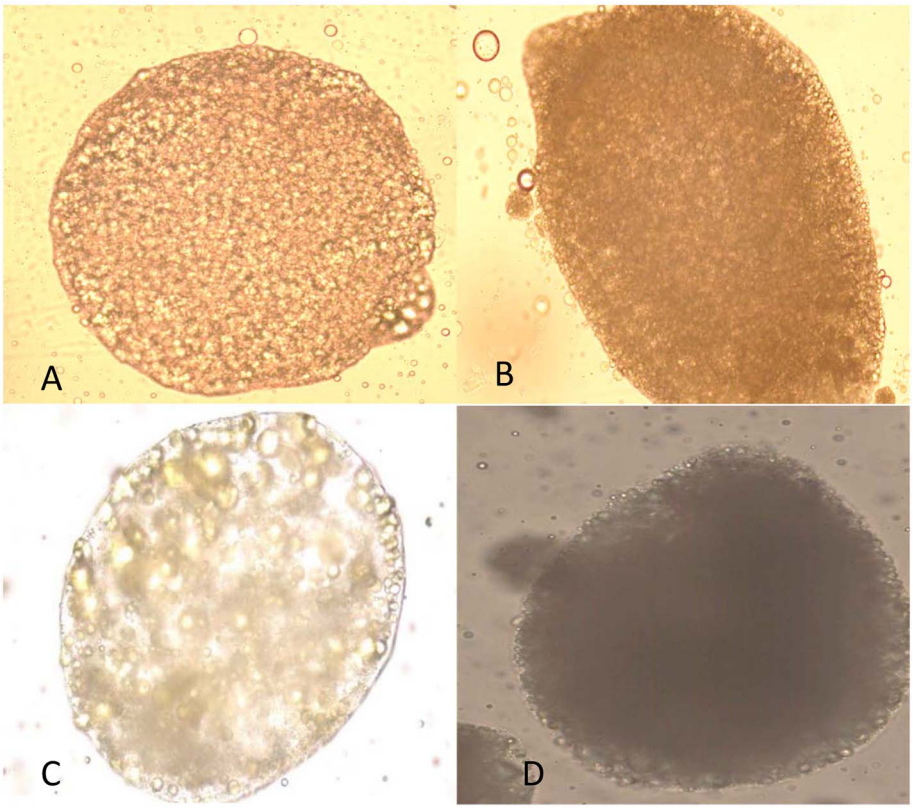

Fig. 1. Optical microscopy of microparticles of chia and linseed oils (150 ×). (A) Chia oil microparticles before thermal treatment; (B) chia oil microparticles after reaching the internal temperature of 72 °C; (C) linseed oil microparticles before thermal treatment; (D) linseed oil microparticles after reaching the internal temperature of 72 °C.

suggests that microparticles are able to withstand the heat treatment traditionally used in burgers. The high thermal stability observed in this study may be due to the combination of sodium alginate and Ca^{+2} ions that form a strongly thermostable gel (Vos et al., 2009). Cheow and Hadinoto (2013) have also reported a high thermal stability of alginate microparticles.

Although no release or dissolution of the microparticles was observed at pH 4.5 and 6.0 (Fig. 2), the microparticles disintegrated after remaining for 180 min at pH 7.5. Hydrated alginate could be converted into an insoluble layer of alginic acid during the encapsulation process, which dissolves only at alkaline pH values (George & Abraham, 2006). Therefore, these results suggested that the

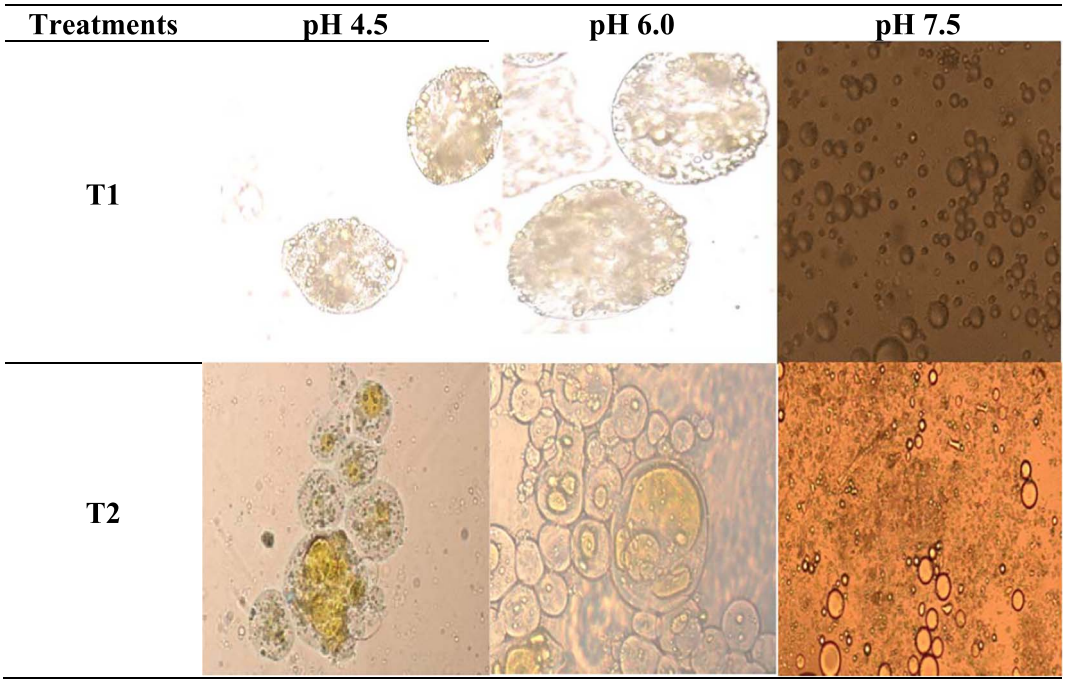

Fig. 2. Images of microparticles obtained by optical photomicroscopy for different pH values during in vitro assay (100 ×).Batches: T1: Chia oil microparticles; T2: Linseed oil microparticles.

Table 1
Fatty acids profile (expressed as g/100 g of fatty acids) of chia (CO) and linseed oil (LO), and chia and linseed oil microparticles (COM and LOM, respectively). Results are expressed as mean of 9 replicates.

	Batch				SEM	Sig.
	CO	LO	COM	LOM		
C14:0	0.05^{a}	0.05^{a}	0.05^{a}	0.04^{a}	0.007	n.s.
C15:0	0.03^{a}	0.03^{a}	0.02^{a}	0.02^{a}	0.002	n.s.
C16:0	9.35^{a}	6.71^{b}	9.17^{a}	6.26^{b}	0.03	***
C16:1	0.08^{a}	0.07^{a}	0.12^{a}	0.06^{a}	0.02	n.s.
C17:0	0.06^{b}	0.09^{a}	0.06^{b}	0.08^{ab}	0.005	**
C18:0	3.73^{c}	4.83^{a}	3.73^{c}	4.52^{b}	0.007	***
C18:1n − 9c	6.89^{c}	22.63^{a}	7.18^{c}	21.76^{b}	0.06	***
C18:2n − 6c	20.16^{a}	15.15^{b}	20.0^{a}	15.3^{b}	0.013	***
C18:3n − 6	0.09	ND	0.09	ND	–	–
C18:3n − 3	58.65^{a}	51.29^{b}	58.65^{a}	46.66^{c}	0.27	***
C20:0	0.38^{a}	0.23^{b}	0.36^{a}	0.19^{b}	0.001	***
C20:1	0.17^{ab}	0.14^{b}	0.18^{a}	0.15^{ab}	0.001	*
C20:2	0.06^{a}	0.04^{b}	0.05^{a}	0.03^{c}	0.001	***
C20:3n − 3	0.05^{b}	0.06^{a}	0.05^{b}	0.06^{a}	0.001	**
C22:0	0.11^{b}	0.18^{a}	0.12^{b}	0.16^{a}	0.01	**
C22:1	0.01	ND	0.01	ND	–	–
C24:0	0.12^{a}	0.13^{a}	0.12^{a}	0.12^{a}	0.004	n.s.
ΣSFA	13.8^{a}	12.3^{b}	13.6^{a}	11.4^{c}	0.08	***
ΣMUFA	7.1^{c}	22.8^{a}	7.4^{c}	21.9^{b}	0.06	***
ΣPUFA	79.0^{a}	64.9^{c}	78.9^{a}	66.6^{b}	0.3	***
PUFA/SFA	5.7^{ab}	5.3^{b}	5.8^{a}	5.9^{a}	0.03	*
n − 6/n − 3	0.35^{a}	0.30^{b}	0.34^{a}	0.30^{b}	0.002	***
AI	0.11^{a}	0.08^{b}	0.11^{a}	0.07^{b}	0.002	***
TI	0.14^{a}	0.14^{a}	0.12^{ab}	0.12^{b}	0.003	*

$^{a–c}$ Mean values in the same row not followed by a common letter differ significantly ($P < 0.05$).
SEM: standard error of the mean.
Sig.: significance; n.s. (not significant).
SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n − 6 = omega-6; n − 3 = omega-3. AI: atherogenic index; TI: thrombogenic index.
ND: not detected.
*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

microparticles produced could not be degraded in the pH ranges commonly found in meat products, but they could release chia and linseed oils in the human intestine. In agreement with these results, Villamizar and Martínez (2008) also observed stability of alginate microparticles subjected to similar pH values of our study.

The microparticles presented high ($P < 0.001$) PUFAs (Table 1) (66.6–78.9 g/100 g fatty acids) and low SFAs levels ($P < 0.001$) (11.4–13.6 g/100 g fatty acids). The n − 6/n − 3 ratio ranged from 0.30 to 0.34 ($P < 0.001$), and low atherogenicity (0.07–0.11) ($P < 0.001$) and thrombogenicity (0.12) ($P < 0.05$) indices were observed in chia and linseed oil microparticles. The results demonstrated that the microencapsulation process by external ionic gelation did not modify the fatty acid profile of chia and linseed oils.

3.2. Physicochemical characterization of burgers

The lipid reformulation significantly affected the proximate composition of burgers (Table 2). The replacement of 50% pork back fat by microencapsulated chia and linseed oils increased moisture ($P < 0.001$) and reduced the lipid content ($P < 0.001$) of raw burgers. A reduction of about 48% (T1) and 50% (T2) in the fat content was also observed for the modified raw burgers when compared to the control. However, the protein and ash contents were not affected ($P > 0.05$) by lipid reformulation in the raw burgers. After cooking, a reduction in moisture content between 11.5 and 14% was observed in the burgers, leading to an increase in the other constituents, except for the fat content of the control sample, which had a decrease of about 23%. This decrease in lipids during cooking is expected, as the distance between the fat globules decreases with increasing fat content, which leads to coalescence of the fat globules and subsequent release of the product, as reported by Tabarestani and Tehrani (2014). Probably, this phenomenon did not occur in the modified treatments (T1 and T2) due to the high thermal stability of the microparticles (Fig.1). However, the cooked burgers still showed a fat reduction between 15.7 and 25.4% ($P < 0.01$) when compared to the control. The pH and a_w values of raw and cooked burgers were not affected ($P > 0.05$) by the lipid reformulation (Table 2).

No significant differences ($P > 0.05$) were observed between the control and the modified burgers (T1 and T2) for the color parameters (a* and b* values) after the manufacturing (day 1) (Table 2). However, a greater ($P < 0.001$) lightness (L*) was found in T1 and T2 batches in relation to the control group, probably due to the greater water loss during cooking ($P < 0.001$) (7.1; 9.7; and 8.0 for the control, T1 and T2, respectively). Youssef and Barbut (2011) have also reported an increase in L* values of burgers with moisture reduction after cooking. Similar behavior was verified in the L* and a* values of the raw and cooked burgers after 120 days of storage (Table 2). However, the modified treatments presented an increase ($P < 0.001$) in b* values when compared to the control, suggesting, therefore, a higher degree of lipid oxidation, as also reported by Ozvural, Huang, and Chikindas (2016).

3.3. Technological properties and texture profile

The technological parameters and texture profile of burgers with lipid reformulation are presented in Table 3. The substitution of pork back fat by lean meat or water is one of the most common alternatives to reduce the fat content of burgers. However, this approach may decrease moisture retention and increase diameter reduction after cooking (Soncu et al., 2015; Carpena, Morcuende, & Estévez, 2011). In this study, no significant differences in moisture retention and diameter reduction were observed between the control and the modified treatments, thus showing that the lipid reformulation was effective to maintain these important technological parameters. The control presented a higher cooking loss ($P < 0.05$) and a lower fat retention ($P < 0.001$) when compared to the modified burgers. In agreement with these results, Bilek and Turhan (2009) have also reported a similar behavior in high-fat burgers. Besides the lower fat levels (Table 2), the lower cooking loss and higher fat retention in the modified burgers may be due to the high thermal stability of the microparticles (Fig. 1), which led to a fat retention during cooking. These outcomes are very important because the fat retention in the meat matrix after thermal processing is indispensable to guarantee a high sensory quality of the products (Serdaroglu, 2006).

Changes in texture are one of the main challenges for the development of meat products with reduced fat and a healthier lipid profile. As reported by Selani et al. (2016), the substitution of animal fat for vegetable oils has increased the hardness of burgers. According to Youssef and Barbut (2009), this may be due to the lower fat globule of vegetable oil when compared to animal fat and the resulting higher protein-protein and protein-lipid interaction, which results in an increased compressive strength. An effective lipid reformulation was observed in the present study, once similar hardness ($P > 0.05$) was observed in the modified burgers when compared to the control (74.9; 73.8; 75.0 for the control, T1 and T2, respectively) (Table 3). This result is extremely important considering the impact of this attribute on the sensory quality (Bastos et al., 2014), and demonstrates the effectiveness of the microencapsulation as a strategy to use vegetable oils in cooked meat products. Although the lipid reformulation did not affect springiness and chewiness parameters ($P > 0.05$), more cohesive burgers were obtained (Table 3). The lower fat to protein ratio of the modified burgers (0.47) as compared to the control (0.91) may explain this result, once the higher amount of proteins available can form a protein

Table 2
Effect of the partial replacement of pork back fat by linseed or chia oil microparticles on proximate composition, pH, Aw, and color parameters of beef burger (mean of nine replicates).

	Raw			SEM	Sig.	Cooked			SEM	Sig.
	Control	T1	T2			Control	T1	T2		
Proximate composition (%)										
Moisture	62.4^{b}	68.1^{a}	68.2^{a}	0.8	***	55.2^{c}	58.5^{b}	60.3^{a}	0.5	***
Fat	17.4^{a}	9.1^{b}	8.7^{b}	0.4	***	13.4^{a}	10.0^{b}	11.3^{b}	0.7	**
Protein	19.0^{a}	19.0^{a}	18.4^{a}	0.3	n.s.	25.1^{a}	25.1^{a}	23.5^{b}	0.7	**
Ash	2.5^{a}	2.6^{a}	2.4^{a}	0.1	n.s.	3.4^{a}	3.3^{a}	3.2^{a}	0.02	n.s.
Physico-chemical parameters										
pH	6.2^{a}	6.4^{a}	6.3^{a}	0.004	n.s.	6.5^{a}	6.48^{a}	6.6^{a}	0.03	n.s.
a_w	0.99^{a}	0.99^{a}	0.99^{a}	0.001	n.s.	0.97^{a}	0.97^{a}	0.97^{a}	0.001	n.s.
Color parameters										
Day 1										
L*	41.1^{ab}	43.1^{a}	40.7^{b}	1.9	*	39.2^{b}	43.2^{a}	44.4^{a}	5.1	***
a*	16.6^{a}	17.6^{a}	17.0^{a}	1.7	n.s.	6.6^{a}	6.5^{ab}	6.0^{b}	0.3	*
b*	15.5^{a}	15.9^{a}	15.4^{a}	1.6	n.s.	15.7^{b}	16.5^{ab}	17.0^{a}	1.3	*
Day 120										
L*	44.6^{a}	45.3^{a}	43.9^{a}	1.2	n.s.	41.8^{b}	46.1^{a}	47.2^{a}	2.9	***
a*	16.0^{a}	14.5^{a}	15.6^{a}	0.7	n.s.	10.7^{a}	11.2^{a}	11.1^{a}	0.3	n.s.
b*	13.5^{b}	14.5^{a}	14.5^{a}	0.7	***	15.4^{b}	17.3^{a}	17.4^{a}	1.5	***

[a–c] Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly ($P < 0.05$).
Batches: control: 20% pork back fat; T1: 50% substitution of pork back fat by chia oil microparticles; T2: 50% substitution of pork back fat by linseed oil microparticles.
SEM: standard error of the mean.
Sig.: significance; n.s. (not significant).
*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

Table 3
Effect of the partial replacement of pork back fat by chia or linseed oil microparticles on technological and textural parameters of beef burger (mean of nine replicates).

	Batch			SEM	Sig.
	Control	T1	T2		
Technological parameters (%)					
Moisture retention	60.1^{a}	61.5^{a}	62.1^{a}	3.9	n.s.
Diameter reduction	21.4^{ab}	20.2^{b}	23.1^{a}	2.8	*
Cooking loss	32.1^{a}	28.4^{b}	28.9^{b}	3.4	*
Fat retention	53.8^{b}	85.8^{a}	88.4^{a}	2.1	***
Textural parameters					**
Hardness (N)	74.9^{a}	73.8^{a}	75.0^{a}	4.1	n.s.
Springiness (mm)	0.7^{a}	0.74^{a}	0.73^{a}	0.01	n.s.
Cohesiveness	0.47^{b}	0.53^{a}	0.52^{a}	0.002	*
Chewiness (N × mm)	24.4^{a}	27.2^{a}	28.3^{a}	1.9	n.s.

[a–b] Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly ($P < 0.05$).
Batches: control: 20% pork back fat; T1: 50% substitution of pork back fat by chia oil microparticles; T2: 50% substitution of pork back fat by linseed oil microparticles.
SEM: standard error of the mean.
Sig.: significance; n.s. (not significant).
*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

network, increasing the cohesiveness, as previously reported by Youssef, Barbut, and Smith (2011).

3.4. Fatty acids profile

The fatty acid profile of raw and cooked burgers is shown in Table 4. In quantitative terms, palmitic acid (16:0) and stearic acid (18:0) were the major SFAs found in raw and cooked burgers, while oleic acid (18:1n − 9c) was the most abundant MUFA. The lipid reformulation decreased ($P < 0.001$) the SFA and MUFA levels and increased ($P < 0.001$) the PUFA content. This finding is in agreement with data reported by Pintado, Capillas, Colmenero, Carmona, and Herrero (2015) and Ciriano et al. (2010) who noticed an increased in PUFA levels in meat products with pork back fat replaced by liquid chia and linseed oils, respectively. Linoleic acid (C18:2n6c) was the main PUFA found in raw and cooked burgers. As the chia and linseed oil microparticles had high levels of linolenic acid (C18:3n3) (Table 1), an increase of > 1000% of this fatty acid was observed in the raw and cooked treatments ($P < 0.001$). Thus, the consumption of a 100 g portion of cooked burgers can provide about 49.1 (T1) and 47.7% (T2) of the daily amount of linolenic acid recommended by Simopoulos, Leaf, and Salem (1999). This high linolenic acid levels led to an increase in PUFA levels ($P < 0.001$) and a higher PUFA/SFA ratio ($P < 0.001$) in the raw and cooked treatments when compared to the control. Thus, the lipid reformulation improved the nutritional quality of burgers, since frequent consumption of foods with a PUFA/SFA ratio lower than 0.45 may increase the incidence of cardiovascular diseases (Wood et al., 2004).

Burgers, as well as most meat products, have a n − 6/n − 3 PUFA ratio much higher than the values considered healthy (1:1 to 2:1) (Simopoulos, 2011). This fact is extremely worrying for human's health, since high n − 6 PUFA and low n − 3 PUFA intakes are related to the increased incidence of inflammatory and autoimmune diseases, various cancers, and cardiovascular diseases (Lee et al., 2012). Thus, the reduction of this index is one of the main challenges for the development of healthier meat products. In this study, as expected, the control sample had a high n − 6/n − 3 PUFA ratio (raw: 12.78; cooked: 12.46), which was similar to that obtained by other researchers (Baggio & Bragagnolo, 2006; Kamei, Ki, Kawagoshi, & Kawai, 2002). However, due to the high n − 3 PUFA levels of the microparticles (Table 1), the lipid reformulation was effective for obtaining burgers with a healthier n − 6/n − 3 PUFA ratio (Table 4). Finally, in addition to improving the PUFA/SFA and n − 6/n − 3 ratio, the lipid reformulation also reduced the atherogenicity (AI) and thrombogenicity (TI) indices in raw and cooked burgers (Table 4).

3.5. Lipid oxidation (TBARS)

The evolution of TBARS values of raw and cooked burgers during 120 days of frozen storage is shown in Fig. 3. It is well known that the replacement of animal fat by liquid oils with high n − 3 PUFA levels increases the lipid oxidation of meat products (Josquin et al., 2012;

Table 4
Effect of the partial replacement of pork back fat by chia or linseed oil microparticles on fatty acids profile (expressed as g/100 g of fatty acids) of beef burger (mean of nine replicates).

	Raw			SEM	Sig.	Cooked			SEM	Sig.
	Control	T1	T2			Control	T1	T2		
C12:0	0.06^{a}	0.05^{b}	0.05^{b}	0.001	***	0.06^{a}	0.05^{b}	0.05^{b}	< 0.001	***
C14:0	1.35^{a}	1.22^{c}	1.3^{b}	< 0.001	***	1.38^{a}	1.25^{b}	1.16^{c}	0.002	***
C14:1	0.07^{b}	0.07^{b}	0.08^{a}	< 0.001	*	0.08^{a}	0.08^{a}	0.07^{a}	< 0.001	n.s.
C15:0	0.2^{a}	0.2^{a}	0.2^{a}	0.001	n.s.	0.2^{b}	0.24^{a}	0.22^{a}	< 0.001	***
C16:0	24.3^{a}	22.0^{b}	21.9^{b}	0.107	***	24.5^{a}	22.3^{b}	21.6^{c}	0.014	***
C16:1	1.5^{a}	1.3^{b}	1.3^{b}	0.003	***	1.53^{a}	1.37^{b}	1.35^{b}	0.002	***
C17:0	0.69^{a}	0.64^{b}	0.67^{ab}	0.001	*	0.7^{a}	0.65^{a}	0.65^{a}	< 0.001	***
C17:1	0.45^{a}	0.40^{b}	0.41^{b}	< 0.001	***	0.5^{a}	0.45^{b}	0.45^{b}	< 0.001	***
C18:0	15.01^{a}	13.32^{c}	14.03^{b}	0.17	***	15.1^{a}	13.4^{b}	13.7^{b}	0.05	***
C18:1n9c	39.2^{a}	32.9^{c}	36.1^{b}	0.1	***	39.5^{a}	33.2^{c}	36.2^{b}	0.05	***
C18:2n6c	12.2^{a}	12.5^{a}	11.2^{b}	0.14	***	11.8^{b}	12.3^{a}	11.5^{b}	0.08	***
C18:3n3	0.8^{c}	11.3^{a}	8.4^{b}	1.4	***	0.8^{c}	10.8^{a}	9.3^{b}	0.2	***
C20:0	0.26^{a}	0.28^{a}	0.29^{a}	0.006	n.s.	0.28^{a}	0.27^{a}	0.25^{b}	< 0.001	*
C20:1	0.91^{a}	0.65^{b}	0.63^{b}	0.04	*	0.83^{a}	0.64^{b}	0.66^{b}	0.001	***
C20:2	0.66^{a}	0.49^{b}	0.49^{b}	0.001	***	0.65^{a}	0.48^{b}	0.49^{b}	< 0.001	***
C20:3n6	0.12^{a}	0.11^{b}	0.11^{b}	< 0.001	**	0.13^{a}	0.12^{b}	0.12^{b}	< 0.001	***
C20:4n6	0.34^{a}	0.33^{a}	0.32	0.001	n.s.	0.39^{ab}	0.37^{b}	0.40^{a}	< 0.001	**
C20:3n3	0.09^{a}	0.07^{b}	0.07^{b}	< 0.001	***	0.09^{a}	0.07^{b}	0.07^{b}	< 0.001	***
C20:5n3	0.08^{a}	0.09^{a}	0.09^{a}	< 0.001	n.s.	0.1^{a}	0.09^{b}	0.11^{a}	< 0.001	***
C23:0	1.61^{b}	2.07^{a}	2.2^{a}	0.09	**	1.52^{b}	1.65^{a}	1.58^{ab}	0.009	*
C24:0	0.04^{c}	0.06^{a}	0.05^{b}	< 0.001	***	0.04^{c}	0.05^{b}	0.06^{a}	< 0.001	***
ΣSFA	43.62^{a}	39.93^{c}	40.82^{b}	0.33	***	43.8^{a}	40.01^{b}	39.36^{c}	0.09	***
ΣMUFA	42.13^{a}	35.35^{c}	38.51^{b}	1.1	***	42.39^{a}	35.78^{c}	38.69^{b}	0.035	***
ΣPUFA	14.32^{c}	24.8^{a}	20.8^{b}	1.7	***	13.93^{c}	24.29^{a}	22.02^{b}	0.15	***
PUFA/SFA	0.33^{c}	0.62^{a}	0.51^{b}	0.002	***	0.32^{c}	0.61^{a}	0.56^{b}	< 0.001	***
n − 6/n − 3	12.78^{a}	1.16^{b}	1.36^{b}	0.3	***	12.46^{a}	1.18^{b}	1.26^{b}	0.016	***
AI	0.53^{a}	0.45^{b}	0.46^{b}	< 0.001	***	0.54^{a}	0.46^{b}	0.44^{c}	< 0.001	***
TI	0.68^{a}	0.58^{c}	0.63^{b}	0.001	***	0.69^{a}	0.59^{b}	0.59^{b}	< 0.001	***

[a–c] Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly ($P < 0.05$).
Batches: control: 20% pork back fat; T1: 50% substitution of pork back fat by chia oil microparticles; T2: 50% substitution of pork back fat by linseed oil microparticles.
SEM: standard error of the mean; Sig.: significance; n.s. (not significant).
SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n − 6 = omega-6; n − 3 = omega-3. AI: atherogenic index; TI: thrombogenic index.
*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

Delgado-Pando, Cofrades, Ruiz-Capillas, Triki, & Jiménez-Colmenero, 2012; Domínguez, Pateiro, Munekata, Campagnol, & Lorenzo, 2016; Lorenzo et al., 2016). This fact was also observed in this study, since the lipid reformulation significantly increased the lipid oxidation of the burgers, especially after cooking, demonstrating the difficulty of the addition of n − 3 PUFA-rich oils in cooked meat products even in the microencapsulated form. The treatment containing microencapsulated chia oil (T1) had the highest TBARS values throughout storage in both raw and cooked burgers, probably due to the higher PUFAs of this treatment as compared to control and T2 (Table 4). This result agrees with Guillevic, Kouba, and Mourot (2009), who reported a positive correlation between high PUFA levels and increased lipid oxidation in meat products.

3.6. Check-all-that-apply (CATA)

Correspondence analysis (CA) used to evaluate the descriptors generated by the CATA questionnaire is presented in Fig. 4. The CA explained 91.56% of the total variance, with 81.73% and 9.84% in the first and second dimensions, respectively. The burgers were separated on the sensory map into four distinct groups. The first group consisted of the treatment containing the microencapsulated chia oil (T1); the second group was characterized by the treatment containing liquid chia oil (T3); the third group contained linseed oil (T4), and the fourth group

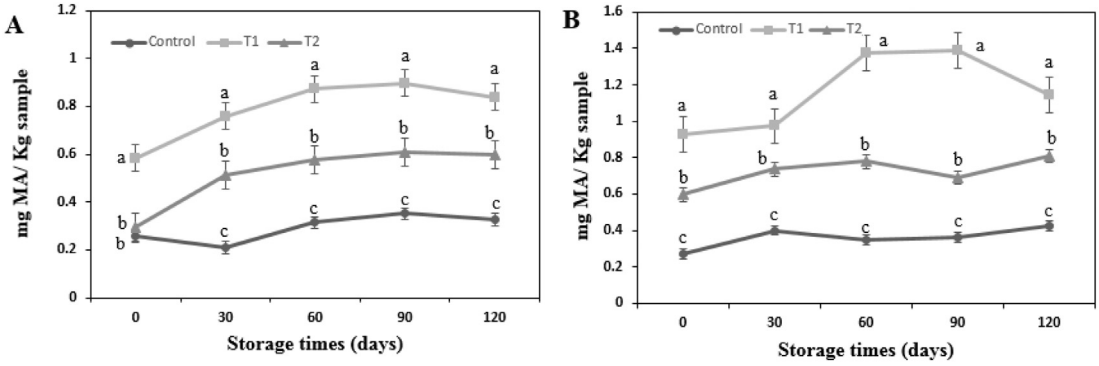

Fig. 3. Malonaldehyde (MA) content (mg/kg) of raw (A) and cooked (B) low-fat burgers. Different letters among treatments, in the same storage period, differ significantly ($P < 0.05$) by the Tukey's test. Batches: control: 20% pork back fat; T1: 50% substitution of pork back fat by chia oil microparticles; T2: 50% substitution of pork back fat by linseed oil microparticles.

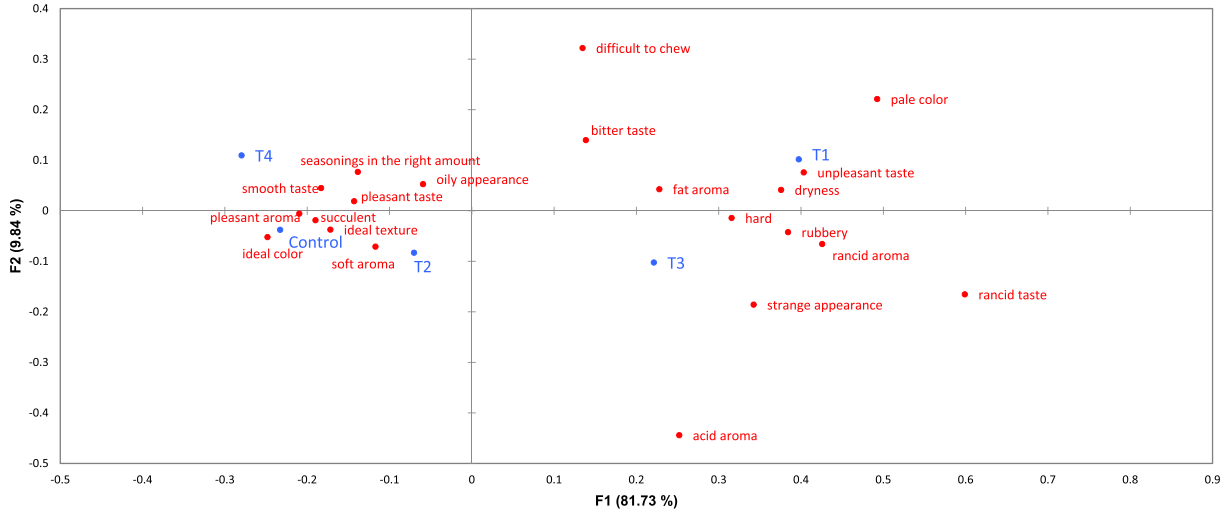

Fig. 4. Representation of the samples and the terms in the first and second dimensions of correspondence analysis performed on data questions check-all-that-apply (CATA). Lots: batches: control: 20% pork back fat; T1: 50% substitution of pork back fat by microencapsulated chia oil; T2: 50% substitution of pork back fat by microencapsulated linseed oil; T3: 50% substitution of pork backfat by water and unencapsulated chia oil; T4: 50% substitution of pork backfat by water and unencapsulated linseed oil.

consisted of both the control and the treatment with microencapsulated linseed oil (T2).

The treatment with microencapsulated chia oil (T1) was characterized by the descriptors bitter taste, unpleasant taste, dryness, fat aroma, pale color, and difficult to chew. The treatment with liquid chia oil (T3) was characterized by the attributes rancid taste, hard, rubbery, rancid aroma, strange appearance, and acid aroma. Thus, CATA results showed that the use of 2.5% chia oil in both liquid and microencapsulated forms impaired the sensory quality of the burgers. This fact was also reported by Borneo, Aguirre, and León (2010), who studied the addition of chia oil to bakery products, probably because the higher susceptibility of this oil to lipid oxidation due to its high PUFA level (Table 1). The treatment with linseed oil (T4) was characterized by the attributes seasonings in the right amount, smooth taste, and pleasant taste. However, T4 has also been described as having oily appearance, probably due to the oil migration to the product's surface during cooking (Braeckman, Ronsse, Cueva Hidalgo, & Pieters, 2009).

Finally, the CATA results indicated the effectiveness of microencapsulation to reduce the sensory defects caused by the use of linseed oil, since the control and the treatment containing the microencapsulated linseed oil (T2) were characterized by the descriptors pleasant aroma, succulent, ideal texture, ideal color, and soft aroma.

3.7. Acceptance test

The results of the acceptance test are presented in Table 5. As reported by Tudose, Iordachescu, Stan, Cercel, and Alexe (2014), the addition of liquid oils with high n − 3 PUFA levels may decrease the sensory quality of meat products, which was also observed in this study, since the burgers with liquid chia (T3) and linseed oils (T4) presented significantly lower scores when compared to the control for all sensory attributes. Microencapsulation was effective to provide similar sensory acceptance ($P > 0.05$) to the control for the attribute texture of the modified burgers. This result corroborates the hardness values found in the texture profile analysis (Table 3). However, the microencapsulation was not effective to suppress all the sensory defects caused by the addition of liquid chia oil, since T1 obtained significantly lower scores than the control in the attributes color, aroma, flavor, and overall acceptability. These results are well correlated with the negative descriptors (bitter taste, unpleasant taste, dryness, fat aroma, pale color, and difficult to chew) generated in the CATA test (Fig. 4), probably due to the higher lipid oxidation (Fig. 3) observed in this treatment. On the other hand, all the sensory defects caused by the addition of liquid linseed oil were minimized by the microencapsulation process, since no significant differences ($P > 0.05$) were observed between the control and T2 for the other attributes (color, aroma, flavor, and overall acceptance). These results are also consistent with the descriptors generated in the CATA test (Fig. 4).

Table 5
Results of consumer study of low-fat burger formulations with microencapsulated and unencapsulated chia and linseed oils.

	Batches					SEM	Sig.*
	Control	T1	T2	T3	T4		
Color	7.39^{a}	6.89^{b}	7.17^{ab}	6.3^{c}	7.03^{b}	0.22	**
Aroma	7.39^{a}	6.66^{b}	7.03^{ab}	6.0^{c}	6.84^{b}	0.22	***
Flavor	7.67^{a}	6.98^{b}	7.25^{ab}	6.08^{c}	7.03^{b}	0.24	***
Texture	7.58^{a}	7.21^{ab}	7.37^{a}	6.29^{c}	6.98^{b}	0.21	***
Overall acceptability	7.69^{a}	7.12^{b}	7.29^{ab}	6.24^{c}	7.07^{b}	0.21	***

[a–c] Mean values in the same row not followed by a common letter differ significantly ($P < 0.05$).
Batches: control: 20% pork back fat; T1: 50% substitution of pork back fat by microencapsulated chia oil; T2: 50% substitution of pork back fat by microencapsulated linseed oil; T3: 50% substitution of pork backfat by water and unencapsulated chia oil; T4: 50% substitution of pork backfat by water and unencapsulated chia oil.
SEM: standard error of the mean.
Sig.: significance; n.s. (not significant).
*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

4. Conclusion

Chia and linseed oil microparticles presented a lipid profile that was nutritionally favorable to human health. In addition, the microparticles were resistant to the temperatures commonly used during burger cooking, and were only degraded at pH values found in the human gut.

Besides reducing the fat content of the burgers by up to 50%, the lipid reformulation proposed in this study provided an increase of up to 90% in the PUFA/SFA ratio, and a decrease of up to 18.5 and 14.7% in the atherogenicity and thrombogenicity indices, respectively. In addition, the modified burgers presented a healthy n − 6/n − 3 PUFA ratio. The lipid reformulation reduced cooking loss and increased fat retention, with no changes in hardness of the burgers. The results also indicated that the replacement of 50% pork back fat by linseed oil microparticles can be done without loss of the sensory quality of the

burgers. However, the fat replacement with chia oil microparticles should be performed with caution, as it may adversely affect important sensory attributes. Thus, this study demonstrated that the microencapsulation by external ionic gelation can be a promising alternative to incorporate vegetable oils with high n − 3 PUFA levels in burgers, without affecting their technological and sensory quality. However, further studies are needed to improve the oxidative stability of modified burgers.

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