Innovations in the development of healthier chicken sausages formulated with different lipid sources

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ABSTRACT Long-chain polyunsaturated n-3 fatty acids are critical nutrients for human health and the fortification of foods with these fatty acids is an important emerging area from the commercial and academic point of view. Development, characterization, and changes during refrigerated vacuum storage of low-fat chicken sausages formulated with preemulsified squid oil were examined and compared with those formulated with beef tallow. Physicochemical analysis and process yield after heat treatment were determined; the heat-treated sausages were evaluated by purge loss, color, texture, microstructure by SEM, microbial counts, fatty acid profile, lipid oxidation, and sensory analysis during refrigerated vacuum storage. Process yield of both formulations was higher than 97% and purge losses during storage were lower than 7%. Purge losses of oil-formulated sausages were lower than those with beef tallow. Sausages with squid oil resulted in higher lightness, lower redness and yellowness, and lower texture profile analysis parameters than the formulation prepared with beef tallow. Microstructure of both formulations was similar, except for the fat droplets that microscopic observations showed in the sausages made with beef tallow. Low lipid oxidation was detected in formulation with squid oil due to the the combination of ingredients and storage conditions. Microbial counts of both products were less than 5 log cfu/g at the end of 90 d of storage. The sausage formulated with squid oil presented more than 30 and 40 g/100 g of monounsaturated and polyunsaturated fatty acids, respectively. Docosahexaenoic acid was the predominant polyunsaturated fatty acid, followed by eicosapentaenoic acid and linoleic acid. Both products showed safe sanitary conditions, good sensory acceptability, and presented very good stability and quality attributes, but sausages formulated with squid oil showed a better fatty acid profile according to nutritional criteria.

Key words: low-fat sausage, squid oil, texture, storage stability

2009 Poultry Science 88:1755–1764
doi:10.3382/ps.2008-00495

INTRODUCTION

Meat and meat products can be modified by adding ingredients considered beneficial for health or by eliminating or reducing components that are considered harmful. The use of these ingredients in meat products offers processors the opportunity to improve the nutritional and health qualities of their products (Fernandéz-Ginés et al., 2005). Because fat is the main contribution to the overall energy level of processed meat products, the reduction of fat content contributes to a healthier diet (Vandendriessche, 2008).

Healthier meat product formulations need to contain less saturated fat or promote the presence of specific healthy compounds (nonmeat ingredients), or both; however, this affects the quality attributes of cooked meat emulsions (Ayo et al., 2008). Partially replacing fat with water in these products has been reported to increase cooking and purge losses (Claus et al., 1989, 1990; Gregg et al., 1993); moreover, increasing water may affect the texture and juiciness of the product (Matulis et al., 1995). Gums and nonmeat proteins are commonly used, with salt, to increase binding and improve the yield of these products (Barbut and Somboonpanyakul, 2007). Andrés et al. (2006a,b, 2008) had characterized and studied the storage stability of low-fat chicken sausages formulated with whey protein and guan and xanthan gums as functional ingredients, obtaining products with good thermal stability, process yield, and quality attributes.

Received November 13, 2008.
Accepted April 15, 2009.
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Long-chain polyunsaturated n-3 fatty acids (**PUFA**) have been implicated as critical nutrients for human health. These fatty acids (**FA**) constitute a minority of all the FA classes present in the human diet. Oils rich in n-3 PUFA are present mainly in cold-water fish and seafood. Fortification of foods with these FA is an emerging area of commercial and academic interest (Connor, 2000; Lee et al., 2005; Jiménez-Colmenero, 2007).

Reformulation of meat derivatives is one of the strategies that has been studied to develop meat-based functional foods. Where the lipid fraction is concerned, reformulation is generally based on the replacement of the animal fat normally present in the product with other lipids whose characteristics are more in line with health recommendations. There are various plant and marine lipid sources that can help supply such nutritional and functional benefits to varying degrees (Jiménez-Colmenero, 2007).

There is a need to develop foods that can deliver the n-3 PUFA of fish in convenience foods consumed on a regular basis (Garg et al., 2006). Yilmaz et al. (2002) studied different low-fat meat or chicken sausages formulated with sunflower oil with no negative sensory characteristics. Hoz et al. (2004) manufactured dry-fermented sausages enriched in n-3 PUFA without adverse effects on their composition, lipid stability, and textural and sensory properties. Park et al. (1989) studied the properties of low-fat meat frankfurters containing high-oleic acid sunflower oil and fish oil, finding that those formulated with fish oil resulted in objectionable off-flavor and those formulated with sunflower oil had objectionable texture when compared with the regular product containing 30 g/100 g of product of animal fat. Park et al. (1990) formulated low-fat meat frankfurters with simultaneous incorporation of water and high-oleic acid sunflower oil with properties similar to the regular product. Incorporation of n-3 PUFA into food systems is potentially problematic due to their propensity to readily oxidize. Furthermore, muscle foods are susceptible to oxidation, and meat processing operations facilitate particle size reduction and exposure of increased surface area, addition of potential prooxidant ingredients, and heat-induced changes that decrease oxidative stability (Lee et al., 2005).

Poultry products are universally popular and they have a high potential to reduce their fat content and to include PUFA. The development of low-fat products rich in PUFA to replace traditional ones may contribute to a more healthful diet.

Chicken breast meat is appropriate to formulate low-fat sausages because of its low lipid content. In these types of products, it is important to consider the FA profile of the added fat. Beef tallow (**BT**) or pork fat contains a high ratio of saturated FA (**SFA**) to unsaturated FA; thus, replacing them with vegetable or seafood oils would produce a healthier product provided that quality attributes such as texture, color, and flavor are maintained.

The objectives of the present work were as follows: 1) to develop a healthier low-fat chicken sausage enriched in PUFA by replacing animal fat with squid oil (**SO**); 2) to characterize their physicochemical, textural, and sensory properties; 3) to compare the quality attributes of low-fat chicken sausages containing BT as a lipid source with those containing SO, analyzing process yield and storage stability of the vacuum-packaged product by measuring color, texture, microstructure, purge loss, and microbial counts of products over refrigerated storage (4°C); and 4) to evaluate FA profile and rancidity in the case of SO-formulated products.

MATERIALS AND METHODS

Materials

Low-fat sausages were developed using fresh chicken breast meat obtained from local processors (pH 5.7 to 5.9). Deodorized refined SO (Omega Sur S.A., Mar del Plata, Argentina) with 1 g/kg of synthetic vitamin E or commercial BT was used as a lipid source. Fatty acid composition of the SO was as follows: monounsaturated FA (**MUFA**), 31.81%; SFA, 24.97%; PUFA, 40.88%; and n-3 PUFA, 37.87% [eicosapentaenoic acid (**EPA**), 9.96% and docosahexaenoic acid (**DHA**), 24.86%], and for BT was as follows: SFA, 56.10%; MUFA, 35.8%; and PUFA, 5.7%. Whey protein concentrate (**WPC**) containing 80 g of protein/100 g of product (Arla Foods Ingredients S.A., Martínez, Argentina), food-grade commercial preparation of xanthan and guar gums (Sigma Chemical Co., St. Louis, MO), and analytical-grade NaCl, $NaNO_2$, sodium erythorbate, and tripolyphosphate were used. Cold distilled water was employed (4°C).

Sausage Manufacture

Chicken breast meat without skin (<1.1 g of fat/100 g) was ground with a commercial food processor (Universo, Rowenta, Germany) equipped with a 14-cm blade for 10 min at the highest speed. Salt was slowly added to the ground meat while processing.

For manufacturing sausages with BT (5 g/100 g of raw batter) as a fat source, dry ingredients (powders) were slowly added to the ground chicken meat while processing. Afterwards, cold water (25 g/100 g of raw batter) was incorporated and finally ground fat at room temperature was added.

For producing sausages with SO as the lipid source, dry ingredients were solubilized in cold water and then homogenized with the oil using a hand-held food processor (Braun, Buenos Aires, Argentina) for 1 min to form a coarse emulsion. The obtained emulsion was added to the ground meat, processing all ingredients for 5 min afterwards.

In both cases, final temperature of batter varied between 12 and 15°C. The batter was manually stuffed in collagen-reconstituted casing (27-mm diameter)

and hand-linked to form links approximately 8 cm in length. The sausages were then placed in cook-in bags at 23°C (Cryovac CN510, Sealed Air Co., Buenos Aires, Argentina), containing 2 sausages per bag. The bags were heat-processed in a temperature-controlled water-bath (Haake L, Haake Buchler Instruments, Karlsruhe, Germany) maintained at 80°C until a final internal temperature of 74°C was reached, according to the recommendations of the US Department of Health and Human Services (2001). Temperature was monitored by a type-T (copper-constantan) thermocouple inserted in the center of a link and connected to an acquisition system (Testo175, Testo AG, Lenzkirch, Germany). Then, samples were cooled immediately in an ice-water bath, vacuum-packaged in Cryovac BB4L bags (permeability to oxygen of the film: 35 $cm^3/(m^2/day$ per bar) at 23°C; Sealed Air Co.) and stored at 4°C during 45 d.

The components of both formulations are listed in Table 1, where percentages of contents are expressed as grams per 100 g of raw batter. The concentration of $NaNO_2$ was selected according to the level permitted by the Argentinean Regulations (0.015 g/100 g of poultry product; Código Alimentario Argentino, 1996), being this value lower than the maximum permitted by the US Code of Federal Regulations (USDA-FSIS, 1999), 0.0645 g/100 g of poultry product. The process was replicated twice.

Physicochemical Analysis

Moisture, ash, and protein contents of the sausages were determined according to AOAC (1980) methods 24.003, 24.009, and 24.027, respectively, in triplicates. Fat content was determined on samples previously dried with SO_4Na_2 by Soxhlet method, using ethyl ether and petroleum ether (boiling point: 35 to 60°C) in a 1:1 relationship as extraction solvent (García et al., 2002). Carbohydrates were calculated by difference; pH was measured using a spear-tip glass electrode (U-05998-20, Cole-Palmer, Vernon Hills, IL) on a pH meter (EC30, Hacht, Loveland, CO). Total calories (kcal) were calculated using the Atwater values corresponding to lipids (9 kcal/g), proteins (4.02 kcal/g), and carbohydrates (3.87 kcal/g) (Cáceres et al., 2006).

Table 1. Formulation to prepare 100 g of raw batter of low-fat sausages with beef tallow or squid oil as a lipid source

Ingredients (g)	Raw batter (g/100 g)
Breast chicken meat	66.7
Beef tallow or squid oil	5
Water	25
NaCl	1.5
Whey protein concentrate	0.97
Guar gum	0.096
Xanthan gum	0.224
Sodium tripolyphosphate	0.2
White pepper	0.2
Ground nutmeg	0.05
$NaNO_2$	0.015
Erythorbic acid	0.045

Process Yield

Process yield was determined in duplicate by weighing the product before (w_{before}) and after thermal treatment (w_{after}) and corresponds to weight loss due to heating. The percentage of loss in weight during the precooking treatment (process yield %; Candogan and Kolsarici, 2003) was calculated as:

$$\text{process yield } \% = \frac{\left(w_{before} - w_{after}\right)}{w_{before}} \times 100. \quad [1]$$

Purge Loss

For each formulation, purge loss was measured by removing 2 packages from refrigerated storage at different times. The sausages were removed from the package, placed in funnels to drain (1 min), carefully bloated with filter paper to eliminate any liquid on the surface of the links, and weighed (w_t). The initial weight of the links was recorded at the beginning of refrigerated storage (w_i). Purge loss was reported as a percentage of w_i:

$$\text{purge loss } \% = \frac{\left(w_i - w_t\right)}{w_i} \times 100. \quad [2]$$

Color

Color was measured at room temperature on the surface of transversal slices, recently cut, at different times during the storage at 4°C using a Chroma Meter CR-300 colorimeter (Minolta Co., Ramsey, NJ) and the CIE-LAB parameters (lightness, L*, redness, a*, and yellowness, b*) were determined. Five measures were taken for each data point.

Texture Measurements

Texture profile analysis (Bourne, 1978; Brennan and Bourne, 1994) was performed on chilled (4°C) sausages at 7-d intervals during storage. Two repeated measurements were taken for each replicate and mean values were reported. Samples 1.5 cm thick and 1.7 cm in diameter were cut from the center of the links and compressed twice to 30% of their original height between flat plates using a TAXT2i Texture Analyzer (Stable Micro Systems, Surrey, UK) interfaced with a computer, using the software supplied by Texture Technologies Corp. (Scarsdale, NY). In these experiments, the head was operated at 0.5 mm/s. Hardness (peak force of first compression cycle, N), springiness (distance of the detected height of the product on the second compression divided by the original compression distance, mm/mm), cohesiveness (ratio of positive areas of second cycle to area of first cycle, J/J), adhesiveness (negative force area of the first byte represented the work necessary to

pull the compressing plunger away from the sample, J), chewiness (hardness × cohesiveness × springiness, N), and resilience (area during the withdrawal of the first compression divided by the area of the first compression, J/J) were determined.

Microstructure Determination

Small pieces of sausages of 0.5 cm in diameter and 0.2 to 0.3 cm thick were used for electron microscopy analysis. The samples were fixed during 24 h and dehydrated using a series of gradually increasing concentrations of ethyl alcohol according to Andrés et al. (2006b). Samples were mounted on aluminum stubs using double-sided tape and were then coated with a layer of gold (40 to 50 nm), allowing surface and cross-section visualization. Micrographs of the samples were obtained with scanning electron microscope (SEM 505, Philips, Eindhoven, the Netherlands).

Microbial Analysis

Bacterial counts were determined using the pour-plate method at different times during refrigerated storage. The initial dilution was made by aseptically blending in a Stomacher blender (West Sussex, UK) 20 g of sample with 180 mL of 1 g/L of peptone solution for 60 s. Appropriate serial dilutions were plated in duplicate with plate count agar (**PCA**, Oxoid, Hampshire, UK) for total mesophilic aerobic count (incubated at 30°C for 2 d) and total psychrotrophic aerobic count (incubated at 4°C for 7 d), with violet red bile agar (Merck KGaA, Darmstadt, Germany) for Enterobacteriaceae (incubated at 37°C for 24 h), and with de Man, Rogosa, and Sharpe agar (Oxoid) for lactic acid bacteria (incubated at 30°C for 2 d). Yeast glucose cloranfenicol agar (Merck KGaA) was used for mold and yeast counts (incubated for 5 d at 30°C). Data were expressed as log colony-forming units per gram of sample.

The products were tested at 2-wk intervals for total coliform counts using the most probable number method according to AOAC (1984) method 46016, and sulfite-reducing *Clostridium* counts were enumerated in tryptone-sulfite-neomycin agar (Biokar Diagnostics, Pantin Cedex, France) incubated at 37°C for 48 h in anaerobic condition.

Lipid Oxidation and FA Profile Determination

Squid oil is highly susceptible to lipid oxidation due to its high PUFA content; therefore, it was necessary to control whether the incorporated synthetic vitamin E was enough to prevent product deterioration.

Lipid oxidation depends on the instrumental method utilized for its measurement. The meat industry prefers results based on sensory tests; however, this technique is expensive and time-consuming. Selection of the TBA reactive substances (**TBARS**) test was based on results from a work done by Yang et al. (2000) that reported a highly significant correlation between TBARS and sensory determinations.

The TBARS values of the SO sausages were determined according to Tironi et al. (2007) to evaluate the extent of oxidative rancidity development in sausages formulated with SO during vacuum storage at 4°C for up to 90 d. Results were expressed as milligrams of malonaldehyde (**MDA**) per kilogram of product.

For FA analysis of the same formulation, total lipids were extracted using chloroform:methanol (2:1, vol/vol) according to the procedure of Folch et al. (1957) and were methylated with 10 g/100 g of boron trifluoride methanol complex in methanolic solution (Morrison and Smith, 1964). The lipid composition was determined at the Programa de Prevención del Infarto en Argentina laboratory of the Universidad Nacional de La Plata by gas chromatography in a Hewlett-Packard 6890 gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with fused silica capillary column (Chrompack CP SIL 88, length 50 m, i.d. 0.25 mm, 0.1-μm film; Varian Inc., Palo Alto, CA) and a flame-ionization detector.

Sensory Analysis

Sensory analyses were conducted by 25 panelists that included graduate students and faculty members in our institute who were experienced in sensory evaluation of foods but who received no specific training relevant to these products. Panelists were asked to indicate how much they liked or disliked both formulations on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) according to flavor, texture, and overall acceptability characteristics. Samples that had been stored for 30 d at 4°C were prepared by steeping the sausages in boiling water for 3 min, draining the liquid, and holding on a warming tray in covered plates for no longer than 30 min. Warm 2-cm-long pieces were distributed in white polystyrene plates and presented to the panelist with 3-digit codes and in random order for evaluation. Tap water was supplied to the panelist for rinsing between samples. Experiments were conducted in an appropriately designed and lighted room.

Statistical Analysis

Analyses of variance were conducted separately on the dependent variables of instrumental texture analysis (hardness, cohesiveness, adhesiveness, springiness, chewiness, resilience), color parameters (L*, a*, and b*), pH, purge loss, microbial counts, and TBARS values. The type of lipid and storage time were the factors considered. For simultaneous pairwise comparisons, least significanct differences test was chosen. Differences in means and F-tests were considered significant when $P < 0.05$. All statistical procedures were computed using

Table 2. Characterization of low-fat chicken sausages formulated with 5 g/100 g of different lipid sources

Analysis[1]	Lipid source	
	Beef tallow	Squid oil
Moisture (g/100 g of product)	72.3 ± 1.7	74.1 ± 0.13
Protein (g/100 g of product)	14.4 ± 0.3	16.5 ± 0.32
Lipids (g/100 g of product)	5.8 ± 0.1	5.70 ± 0.06
Ash (g/100 g of product)	2.9 ± 0.2	2.26 ± 0.02
Carbohydrates (g/100 g of product)	4.7	1.5
Energy value (kcal/100 g)	128.6	123.1
pH	6.17 ± 0.03	6.15 ± 0.03
Process yield (%)	97.0 ± 1.5	97.39 ± 0.03

[1]Mean value ± SEM.

the Systat software (Systat Inc., Evanston, IL). Standard error of the mean was reported in each case.

RESULTS AND DISCUSSION

Physicochemical Characterization

In previous works, Andrés et al. (2006b) found that low-fat chicken sausages prepared with 5 g/100 g of added BT obtained higher overall acceptability scores than 2 and 0 g/100 g of added fat. Thus, in the present work, a 5 g/100 g of fat content was chosen; however, to make a healthier product, BT was replaced by deodorized SO.

Proximate composition of precooked sausages is shown in Table 2. Fat contents of both formulations were less than 6 g/100 g. Lipid content reflected the amount of fat or oil added plus the lipids contained in the meat, indicating that fat losses that occurred during processing were very low. Protein contents were higher than 14 g/100 g of product, and due to their origin (muscle and dairy products), they were of high biological value. The ash contents were low because the salt added in the formulations was less than the levels used in this type of product.

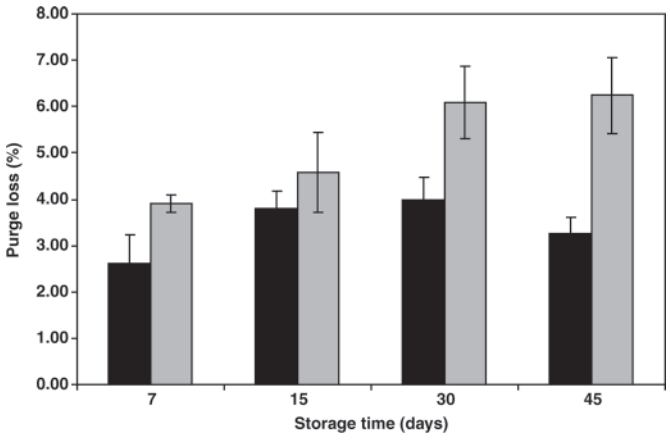

Figure 1. Changes in purge losses (%) during refrigerated vacuum storage for squid oil (black bar) or beef tallow (gray bar) chicken sausages. Error bars indicate SEM.

Energy value ranged between 123 and 128 kcal/100 g, less than half the 315 kcal/100 g of the traditional products (Andrés et al., 2006b). The pH values of the formulation were 6.15 and 6.17 for sausages with oil and tallow, respectively, and did not change during the vacuum-packaged refrigerated storage.

Process yields for both types of sausages were high (>97%). Initial purge losses were small (<4%) and increased slightly during storage (Figure 1). The SO sausages showed significantly less purge losses than those containing BT, indicating that water and lipids were better retained when WPC, tripolyphosphate, and gums were previously dissolved in water than when they were added to the meat as dry ingredients.

The small amount of total fluids released during heating and storage indicates a high water- and fat-holding

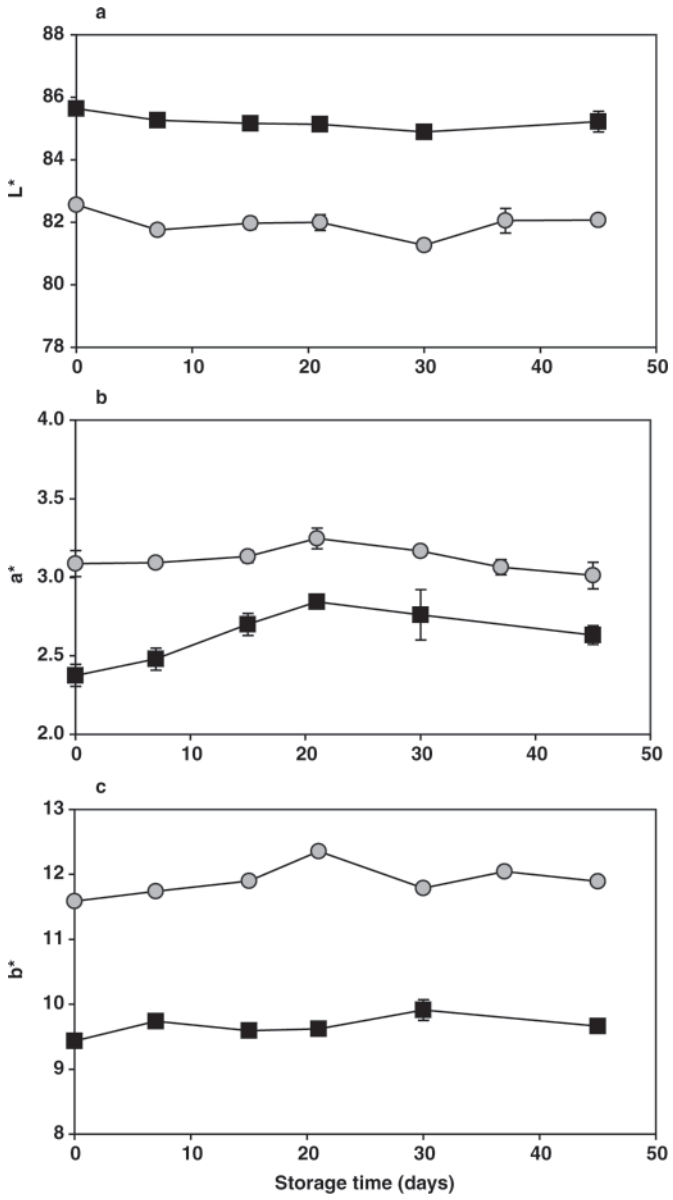

Figure 2. Color parameters along refrigerated vacuum storage for squid oil (black square) or beef tallow (gray circle) chicken sausages: a) lightness (L*), b) redness (a*), c) yellowness (b*). Error bars indicate SEM.

capacity of both systems, in spite of the inclusion of substantial amounts of low-melting fat (SO; Park et al., 1989). The addition of WPC and hydrocolloids had beneficial effects in the chicken sausages. Other authors had described the lowest cook loss-highest yield obtained by addition of certain dairy proteins in meat systems (Barbut, 2008).

Color Properties

The instrumental color of both types of sausages determined at different storage times is given in Figure 2 a, b, and c for lightness (L*), redness (a*), and yellowness (b*), respectively. The SO sausages showed larger L* and smaller a* and b* values than BT sausages ($P < 0.05$). The inclusion of preemulsified oil in the formulation probably caused a lighter product. Similar observations were made by Cáceres et al. (2008) that pointed out that the incorporation of preemulsified oil causes color changes increasing lightness and decreasing redness values.

Lightness of both formulation slightly decreased during storage ($P < 0.01$), whereas redness and yellowness increased ($P < 0.01$).

Textural Properties and Microstructure

The SO chicken sausages showed lower values of hardness, cohesiveness, adhesiveness, chewiness, and resilience (Figure 3) than BT sausages ($P < 0.05$). Only springiness of both formulations was equivalent ($P > 0.05$; Figure 3 e).

Storage time significantly ($P < 0.05$) changed all textural parameters of both SO and BT sausages (Figure 3). Hardness slightly increased in both products during vacuum-refrigerated storage at 4°C because as purge

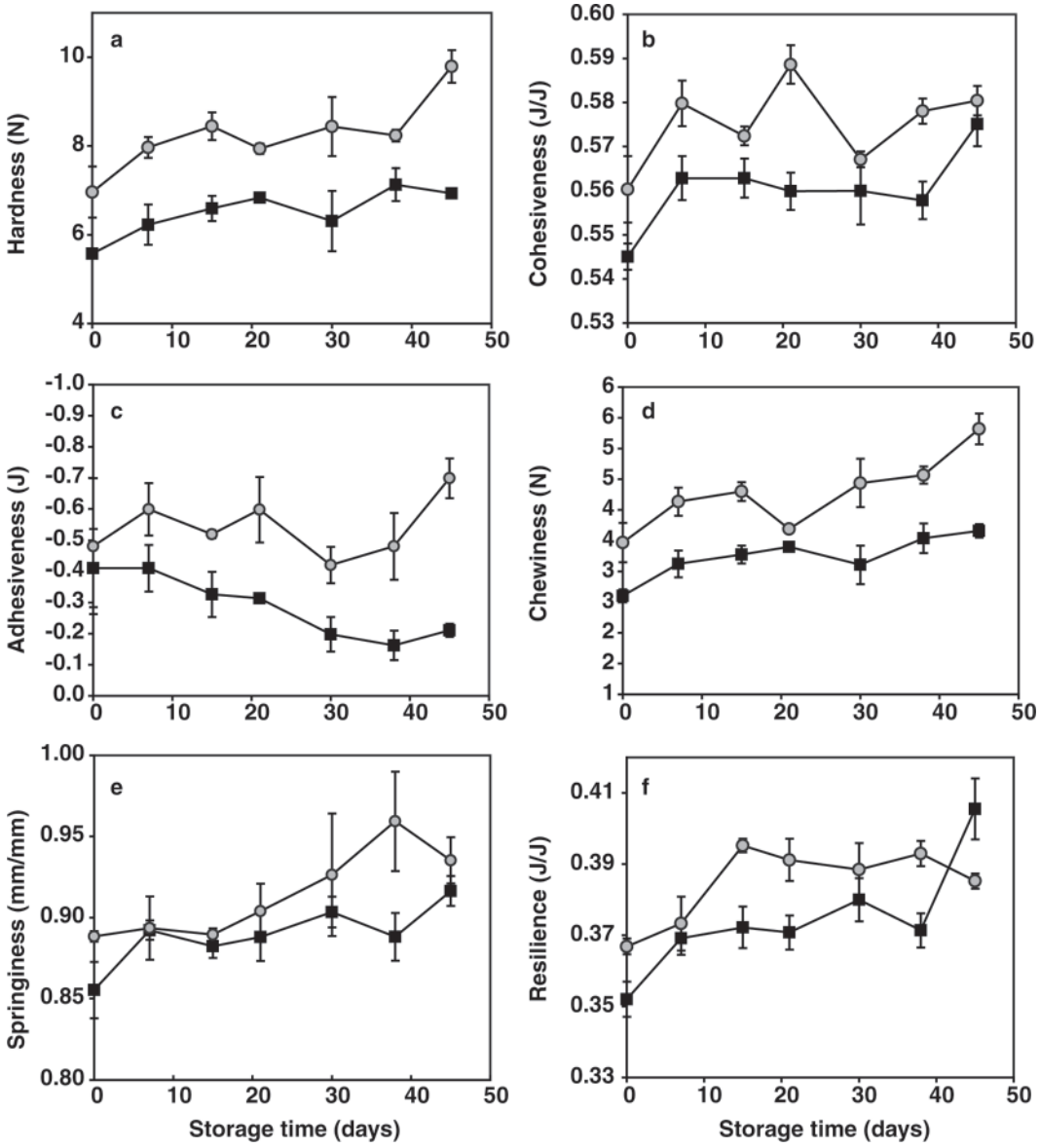

Figure 3. Effect of refrigerated vacuum storage time on texture profile analysis parameters of squid oil (black square) or beef tallow (gray circle) chicken sausages: a) hardness, b) cohesiveness, c) adhesiveness, d) chewiness, e) springiness, f) resilience. Error bars indicate SEM.

losses increased, there was less water available to act as plasticizer of the matrix; hardness of SO sausages was less affected because changes in purge losses were smaller than those of BT sausages. These results agree with Candogan and Kolsarici (2003), who also reported a small increase in hardness during refrigerated storage of low-fat beef frankfurters.

Micrographs of BT and SO sausages are shown in Figure 4. Cohesive and somewhat granular protein matrixes were observed. Fine strands and sheets with gel-like appearance were observed, probably caused by the addition of whey protein and hydrocolloids to the formulation. Fat globules were visible in BT sausages; however, there were not visible in SO ones. Barbut (2008) found that the addition of dairy proteins as dry powders to lean meat systems produced dairy protein islands within the meat protein gel, probably due to the lower initial water solubility. These protein structures were not observed neither in the sausages with preemulsified oil (when WPC was previously dissolved in water) nor in the others that contained BT, and WPC was added as powder to the chicken meat.

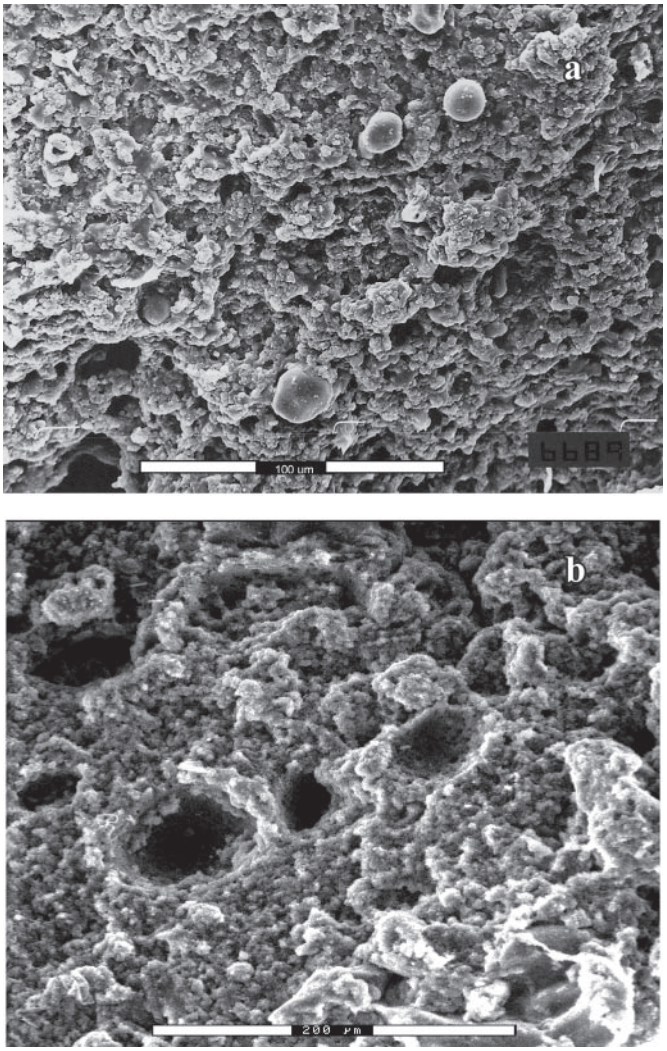

Figure 4. Scanning electron micrographs of beef tallow (a) or squid oil (b) chicken sausages. a) Bar = 100 µm; b) bar = 200 µm.

Microbiological Analyses

Figure 5 shows lactic acid bacteria, total mesophilic, and total psychrotrophic aerobic counts as a function of refrigerated storage time for low-fat sausages formulated with different lipid sources. Low initial microbial counts were found for the different tested culture conditions in both products; they were less than 5 log cfu/g at the end of the storage, indicating a successful thermal treatment that inactivated most of the microorganisms.

The differences between PCA (4°C), PCA (30°C), and MRS average counts, which corresponded to a given

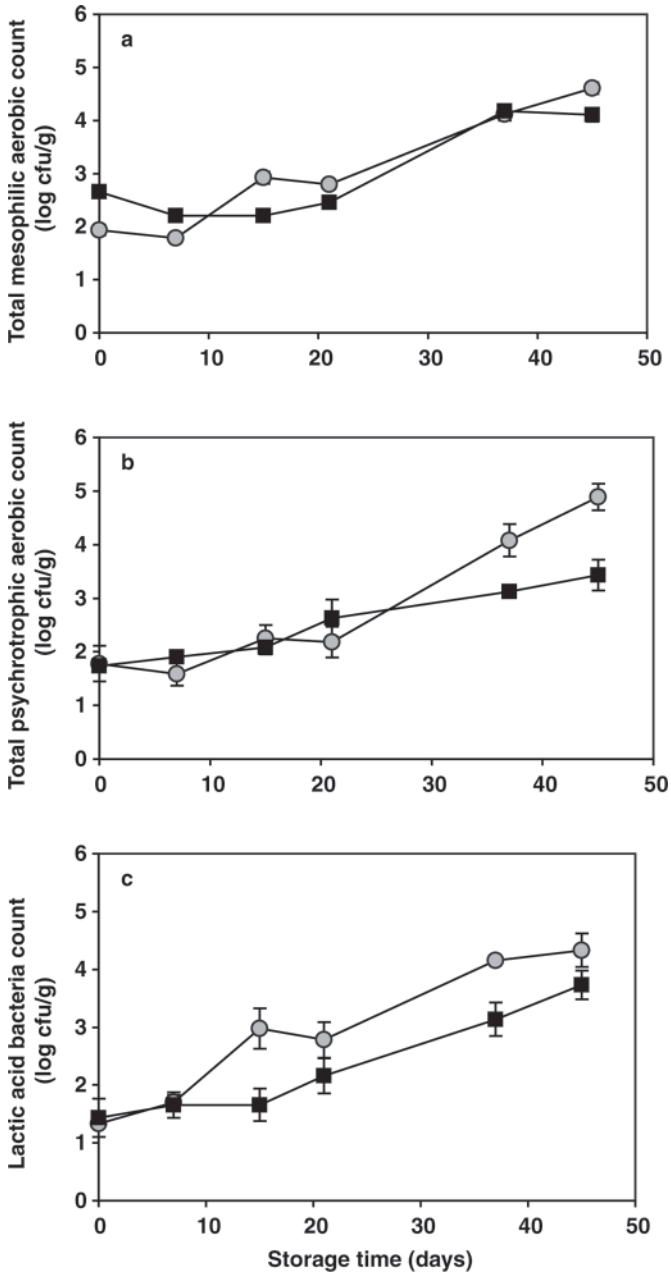

Figure 5. Effect of refrigerated storage time on microbial growth of vacuum-packaged squid oil (black square) or beef tallow (gray circle) chicken sausages: a) total mesophilic aerobic count, b) total psychrotrophic aerobic count, c) lactic acid bacteria counts. Error bars indicate SEM.

Table 3. Fatty acid composition of sausages formulated with squid oil at different storage times

	Storage times (d)			
Fatty acids (g/100 g)	0	15	45	90
Myristic C14:0	2.5[c]	3.5[ab]	3.1[b]	3.7[a]
Pentadecanoic C15:0	0.7[a]	0.7[a]	1.0[a]	0.8[a]
Palmitic C16:0	17.1[b]	15.8[c]	17.2[b]	18.7[a]
Palmitoleic C16:1n-7	7.8[c]	7.9[c]	9.2[a]	8.5[b]
Margaric C17:0	0.7[a]	0.7[a]	0.7[a]	0.6[a]
Stearic C18:0	3.4[ab]	3.0[b]	3.1[b]	3.7[a]
Oleic C18:1n-9 *cis*	27.9[a]	27.1[b]	22.8[c]	23.1[c]
Linoleic C18:2n-6	5.8[b]	6.1[b]	7.3[a]	7.3[a]
Linolenic C18:3n-3	4.8[a]	5.0[a]	3.6[b]	3.9[b]
C20:3n-6	0.4[a]	0.2[a]	0.3[a]	0.2[a]
C20:4n-6	2.5[a]	2.5[a]	3.0[a]	2.7[a]
Eicosapentaenoic C20:5n-3	7.3[c]	7.5[c]	9.9[a]	8.8[b]
C22:5n-3	2.5[a]	2.5[a]	2.3[a]	2.3[a]
Docosahexaenoic C22:6n-3	16.5[c]	16.8[c]	18.1[a]	17.4[b]
SFA[1]	24.4[a]	23.7[a]	25.1[a]	27.6[a]
MUFA[2]	35.7	35.0	32.0	31.6
PUFA[3]	39.8	40.5	44.5	42.8
n-6 PUFA	8.7[b]	8.7[b]	10.5[a]	10.3[a]
n-3 PUFA	31.1[a]	31.8[a]	33.9[a]	32.5[a]
n-6:n-3	0.3	0.3	0.3	0.3

[a–c]Different superscripts within the same row for the products indicate that average values differ significantly ($P < 0.05$).

[1]SFA = saturated fatty acid.

[2]MUFA = monounsaturated fatty acid.

[3]PUFA = polyunsaturated fatty acid.

time, did not differ significantly ($P > 0.05$); this result could indicate that the dominant flora in the product was psychrotrophic lactic acid bacteria. Enteriobacteriaceae and mold and yeast counts were always lower than 2 log cfu/g during the refrigerated storage.

No coliforms were found in the product throughout 45 d of storage at 4°C due to the adequate heat treatment during production, which destroyed heat-sensitive coliforms (Hung and Zayas, 1991). No sulfite-reducing *Clostridium* was noted in the sausages during the storage period, indicating safe sanitary conditions.

FA Composition and Lipid Oxidation

Our main concern regarding the SO sausages was related to their susceptibility to lipid oxidation, which could diminish the quality of the product; thus, changes in FA composition and TBARS values were measured during a longer storage period.

The FA profiles of SO sausages evaluated at 0, 15, 45, and 90 d of refrigerated vacuum storage are presented in Table 3. The FA profiles of the products strongly resembled the FA profile of the added SO because fat content of the chicken breast meat used was very low (<1.1 g/100 g of meat). Thus, the SFA content of the sausages was lower than 30 g/100 g of the total, and the unsaturated FA content was higher than 70 g/100 g. The predominant SFA was palmitic acid (C16:0), followed by stearic acid (C18:0). The MUFA content was always less than the PUFA content, with the main MUFA compound being *cis* oleic acid (>22 g/100 g of the total). The n-3 PUFA content remained fairly constant and higher than 30 g/100 g, with the predominant PUFA (16 to 18 g/100 g) being DHA (C22:6n-3), followed by EPA (C20:5n-3, 7 to 9 g/100 g) and linoleic acid (C18:2n-6, 5 to 7 g/100 g). These high levels of long-chain FA detected in the SO sausages are a consequence of the SO incorporation.

The low n-6:n-3 ratio (0.3) achieved in SO sausages was in accordance with the recommended values for healthy products (Simopoulos, 1999, 2000; WHO, 2004; Jiménez-Colmenero, 2007). Consumption of 100 g of these products would provide a reasonable intake for EPA + DHA of approximately 1.4 g per day (Jiménez-Colmenero, 2007).

The TBARS values of the SO products are shown in Figure 6. The TBARS values ranged from an initial minimum of 0.20 to a maximum of 0.50 mg of MDA/kg after 90 d, indicating a low lipid oxidation mainly due to the combined effect of the antioxidant (synthetic vitamin E) included in the SO to stabilize the n-3 PUFA, vacuum-packaging, and low storage temperature. Whey protein concentrate might also have a protective effect against lipid oxidation because proteins have been found to inhibit lipid oxidation in oil-in-water emulsions when they are at the emulsion droplet interface or in the continuous phase (Tong et al., 2000; Faraji et al., 2004; Djordjevic et al., 2008). The TBARS values remained constant up to 30 d of storage, rising thereafter at approximately 0.0064 mg of MDA/kg per day. These values are similar to those reported for mortadella formulated with fish oil by Cáceres et al. (2008). Park et al. (1989) also found low TBA values after 12 wk of vacuum storage of low-fat frankfurters containing high-oleic acid sunflower oil. Observed TBARS values were much lower than those described as the minimum de-

Table 4. Sensory flavor, texture, and acceptability scores of low-fat chicken sausages containing squid oil or beef tallow at 30 d of refrigerated storage[1]

Source of lipids	Flavor	Texture	Acceptability
Beef tallow	6.9[a] (78.9)	7.6[a] (89.4)	7.4[a] (89.4)
Squid oil	6.5[a] (73.7)	6.9[b] (78.9)	6.8[a] (84.2)

[a,b]Different superscripts within the same column for the products indicate that average values differ significantly ($P < 0.05$).

[1]Nine-point hedonic scale (9 = like extremely; 1 = dislike extremely). Percentage of panelists who scored each tested property between 6 and 9 is given in parentheses.

tected of rancid taste in meat burgers (2 mg of MDA/kg; Georgantelis et al., 2007) and in fermented sausages enriched with n-3 FA (3 mg of MDA/kg; Cáceres et al., 2008).

Sensory Analysis

Mean scores given by the panelists are shown in Table 4. Both SO and BT sausages had acceptable sensory scores (greater than 5) for flavor, overall acceptability, and texture. The 2 sets of formulations only differ in average texture scores ($P < 0.05$). Observations were also classified into 3 perception sensorial groups: the first one corresponded to those that disliked the product (scores 1 to 4, dislike extremely to slightly), the second one was indifferent (scores 5), and the third group expressed that they liked the samples (scores 6 to 9, like slightly to extremely). More than 73.7% of the panelists liked the flavor of the products, 78.9% liked the texture, and over 84.2% of the panelists liked the products, considering the overall acceptability of the SO sausages. These results showed that the presence of the SO did not adversely affect the product.

In conclusion, low-fat chicken sausages containing 5% of refined SO with antioxidants was compared with those containing 5% of BT. Both products showed very good stability and quality attributes. The incorporation of gums and WPC contributed to obtain high process yields and low purge losses during storage. Sausages with SO presented higher lightness and lower redness and yellowness than the formulation prepared with solid fat, and they showed lower values of hardness, cohesiveness, adhesiveness, chewiness, and resilience. Both products showed microbial counts lower than 5 log cfu/g at the end of storage and safe sanitary conditions. Low lipid oxidation was detected in formulation with SO due to the combination of ingredients and storage conditions. Sausages formulated with SO will be healthier due to the high MUFA and PUFA, low SFA contents, and low total fat content. The predominat n-3 PUFA were DHA (16 to 18 g/100 g), followed by EPA (7 to 9 g/100 g) and linoleic acid (C18:2n-6, 5 to 7 g/100 g). Oxidation of PUFA was extremely reduced by the combination of vitamin E as antioxidant in the oil and subsequent storing of the product under vacuum at 4°C. The good sensory results obtained for flavor, texture, and overall acceptability of SO-formulated sausages showed that the presence of this unsaturated FA-rich oil did not adversely affect the product, leading to an innovative and healthier product.

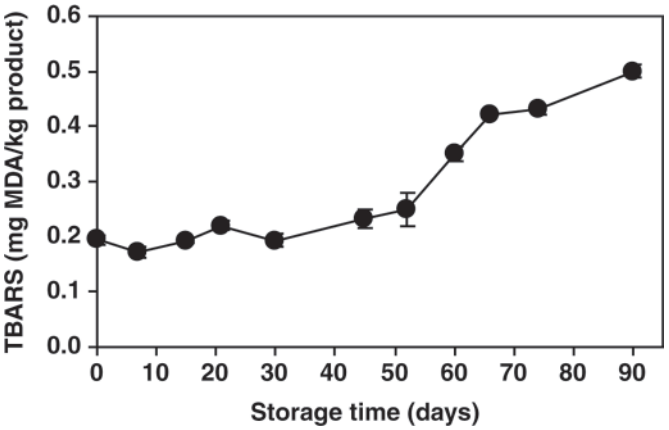

Figure 6. Changes in TBA reactive substance (TBARS) values of chicken sausages with 5% squid oil during storage expressed as milligrams of malonaldehyde (MDA) per kilogram of product. Error bars indicate SEM.

ACKNOWLEDGMENTS

We acknowledge the financial support of the Consejo Nacional de Investigaciones Científicas y Tecnológicas, Agencia Nacional de Promoción Científica y Tecnológica (Buenos Aires, Argentina), and Universidad Nacional de La Plata. Arla Foods Ingredients S.A. provided WPC and Omega Sur S.A. the SO. We thank Graciela Peterson from Programa de Prevención del Infarto en Argentina for the determination of FA profiles.

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