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PHYSICOCHEMICAL, MICROBIOLOGICAL AND OXIDATIVE CHANGES DURING REFRIGERATED STORAGE OF N-3 PUFA ENRICHED COOKED MEAT SAUSAGES WITH PARTIAL NACL SUBSTITUTION

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ABSTRACT

Storage stability of cooked meat sausages with 50 g marine oil/kg and two salt combinations: (1) 14.00 g NaCl/kg and 2.0 g sodium tripolyphosphate (TPP)/kg, (2) sodium reduced formulation with 6.08 g NaCl/kg, 4.92 g KCl/kg and 5.00 g TPP/kg were studied. In addition, effect of BHA or tocopherols as antioxidants was tested. Changes in process yield, purge loss, texture, color, microbial growth and pH during vacuum refrigerated storage were monitored. Partial substitution of sodium did not affect matrix stability, maintaining high process yields and low purge losses ($\leq$5.5%). The products with marine oil used as fat source resulted in: high PUFA levels and lower risks indicators associated with cardiovascular events. Tocopherols prevented the oxidation process; n-6/n-3 ratio remained unchanged throughout the storage, establishing a natural alternative to BHA. Moreover, the consumption of 15–18 g of this product would cover the recommended daily intake of EPA + DHA.

PRACTICAL APPLICATIONS

In previous works, we developed formulations replacing the beef fat with pre-emulsified and deodorized marine oil. We also study an alternative formulation with low sodium content. These characteristics are a necessity for the consumers who are demanding better nutritional quality products, and the producers must attend that demand. Other authors have studied different low fat and/or low sodium meat systems or meat emulsions with different fat sources to enhance the nutritional quality. Nevertheless there is not much knowledge of the stability of these new meat systems, containing more water, and more PUFA. Thus, the aim of this research was to study the storage stability of different cooked meat sausages with fish oil from different approaches (microbial, physicochemical and oxidative). Assuring the stability of these products is essential to the producers to maximize the shelf-life.

INTRODUCTION

Meat products reformulation is one of the strategies that have been studied in order to develop meat-based functional foods, generally based on animal fat replacement with other lipids such as plant and/or marine oils (Berasategi *et al.* 2014). The high polyunsaturated fatty acids (PUFA) present in marine oils have numerous beneficial health effects associated with its consumption (Funahashi *et al.* 2006; Coates *et al.* 2009), particularly of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). WHO and USDA (WHO 2008; USDA 2010) recommend a dairy intake of 250 mg of long-chain n-3 PUFA in persons with and without cardiovascular diseases.

Muscle foods are susceptible to oxidation. Meat processing operations that increase surface area, addition of potential

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PUFA, and heat treatments decrease oxidative stability (Lee *et al.* 2005). The use of antioxidants could prevent the oxidative spoilage of n-3 PUFA enriched foods, however, similar antioxidants show different effects on same food matrix (Jacobsen *et al.* 2008). Previous works have shown that it is possible to develop new PUFA enriched meat products with preemulsified oils and antioxidants to improve its nutritional properties (Asuming-Bediako *et al.* 2014; Berasategi *et al.* 2014; Marchetti *et al.* 2015).

Synthetic antioxidants (BHT, BHA, PG, TBHQ) are widely used in food industry. However, in several studies they have been related with tumors development and other negative effects (Amadasi *et al.* 2008; Gharavi and El-Kadi 2005). Nowadays consumers encourage food manufacture from natural sources and with the so-called green technologies (Valenzuela *et al.* 2011). Natural antioxidants are polyphenolic compounds that can be found in herbs, spices and other vegetables. In 2013, the World Health Assembly (WHO 2013) agreed nine global voluntary targets for the prevention and control of Noncommunicable Diseases, which include a 30% relative reduction in the intake of salt by 2025. According to WHO (2013) reducing salt intake has been identified as one of the most cost-effective measures that countries can take to improve population health outcomes. However, in meat products, NaCl promotes the solubilization of myofibrillar proteins, increasing the hydration and water retention capacity, thus reducing cooking and exudate losses. If the NaCl content of the formulation is reduced, it might adversely affect such properties. Potassium chloride (KCl) is the most commonly used substitute in low/reduced sodium foods. Feltrin *et al.* (2015) reported that KCl was the only salt replacer that showed temporal sensory profile similar to NaCl. However, at blends over 50:50 potassium chloride/sodium chloride in solution, a significant increase in bitterness and loss of saltiness was observed (Desmond 2006; Soglia *et al.* 2014). Both, fat and salt play an important role in this product so alternatives must be carefully chosen to reduce both components.

Cooling, vacuum packaging and edible coating are common techniques to maintain the quality of agri-food products. For cooked meats, cooling is also a very important process to ensure product safety before consumption (Feng and Sun 2013). During vacuum refrigerated storage of cooked meat emulsions changes in their quality parameters (weight loss, texture, color, microbial growth and fatty acid profile) that may limit shelf-life may occur. Andrés *et al.* (2009) found that low-fat chicken sausages containing squid oil with synthetic vitamin E had good stability and quality attributes during the storage. In a previous work we studied low-fat meat emulsions with preemulsified fish oil with different hydrocolloids added, optimized the carrageenan and milk proteins levels (Marchetti *et al.* 2014), and then optimized the formulation in order to reduce its sodium content (Marchetti *et al.* 2014, 2015). Although they contained 46 and 71% less sodium than a commercial sausage, both formulations presented good sensory scores, but it is still necessary to study their storage stability, particularly the inhibition of lipid oxidation that keep n-3 PUFA unaltered, the possibility of larger exudates values when less Na is present in the system.

In the present paper, the objective was to study changes in physicochemical characteristics (purge loss, color, textural), microbial counts and pH during 45 days of vacuum refrigerated storage (4C) of two low-fat sausage formulations, where deodorized marine oil has been used for replacing saturated animal fat, and containing milk protein concentrate and κ/ι carrageenans. In one of the formulations a partial NaCl replacement with KCl and sodium tripolyphosphate (TPP) was carried out. The experimental design included different levels of natural tocopherols or BHA to prevent lipid oxidation and assure an adequate shelf-life. Changes in their fatty acids (FA) profile and lipid oxidation were also studied, and its effect on different health related indexes.

MATERIALS AND METHODS

Materials

Low-fat sausages were prepared using fresh lean beef meat (*adductor femoris* and *semimembranosus muscles*) obtained from local market (pH: 5.48 ± 0.01, fat content: 13 ± 1.7 g/kg). Meat (18 kg, from eight different carcasses for each batch of experiments) without visible fat and connective tissue was passed through a grinder with a 0.95 cm plate (Meifa 32, Buenos Aires, Argentina). Thirty-six lots of 500 g was vacuum packed in Cryovac BB4L bags (PO_2: 0.35 cm^3/m^2/d/kPa at 23C, Sealed Air Co., Buenos Aires, Argentina), frozen and stored at −20C until used (no more than 3 weeks).

Fat source was commercial deodorized marine oil (Omega Sur S.A., Mar del Plata, Argentina). As stabilizer or emulsifier agents food-grade commercial preparations of milk proteins concentrate (802 g/kg proteins (caseins + whey proteins, solubility 97.3 ± 0.4%; Milkaut, Santa Fe, Argentina) and synergistic 2:1 κ/ι carrageenans mixture (ADAMA S.A., Buenos Aires, Argentina) were used (Marchetti *et al.* 2014). Cold distilled water was used in all formulations (4C). Mixed phytosterols (Advasterol 90, AOM S.A., Buenos Aires, Argentina) were included. Analytical grade sodium chloride (NaCl), nitrite ($NaNO_2$), erythorbate and tripolyphosphate (TPP) salts were employed. Sodium nitrite concentration was selected according to the level permitted by Argentinean food law (0.15 g/kg, Código Alimentario Argentino (1999)).

The following components were included to prepare 1 kg of uncooked meat batter: meat (666.5 g), water (250 g), deodorized

TABLE 1. SALT AND ANTIOXIDANTS LEVELS OF THE SAUSAGE MEAT BATTERS*

Code	Sodium chloride (g/kg)	Potassium chloride (g/kg)	Sodium tripolyphosphate (g/kg)	Tocopherols (mg/kg)	BHA (mg/kg)
Na-C	14.00	–	2.00	–	–
Na-BHA	14.00	–	2.00	–	5.0
Na-T1	14.00	–	2.00	37.5	–
Na-T2	14.00	–	2.00	50.0	–
Na/K-C	6.08	4.92	5.00	–	–
Na/K-T1	6.08	4.92	5.00	37.5	–
Na/K-T2	6.08	4.92	5.00	50.0	–

* Units are expressed per kg of raw meat batter.
Codes: Na = sodium formulations, Na/K = partial Na replaced formulations, C = control without antioxidant, BHA = butylatedhydroxyanisole, T1-2 = tocopherols levels.

marine oil (50 g), sodium erythorbate (0.45 g), $NaNO_2$ (0.15 g), 2:1 κ/ι-carrageenans (5.93 g), milk proteins concentrate (3.20 g), phytosterols (5.00 g), monosodium glutamate (0.20 g); ground pepper (2.00 g), nutmeg (0.50 g) and carminic acid (0.032 g, Naturis S.A., Buenos Aires, Argentina).

The experiment included two different salts combinations levels, which corresponded to the optimized systems studied by Marchetti *et al.* (2014, 2015). Formulations were codified as Na (14.00 g NaCl + 2.00 g TPP/kg), and partially NaCl replaced (Na/K: 6.08 g NaCl/kg + 4.92 KCl g/kg + 5.00 g TPP/kg). In any case, the total amount of these salts was 16.00 g/kg, a content lower than traditional products (Desmond 2006).

Two levels of natural tocopherols (T, Tocomix 70, AOM SA, Buenos Aires, Argentina, with d-γ-/d-β-tocopherol 43.81%, d-δ-tocopherol 19.31% and d-α-tocopherol 7.40%,) were evaluated. Formulations without antioxidants were included as controls for both salt combinations (Table 1). One formulation of Na sausages with butylated hydroxyanisole (BHA, Fagron S.A., Madrid, Spain) at maximum permitted level (0.5 mg/100 g product, Código Alimentario Argentino (1999) was also included in the design. The sample size of each formulation was 80–100 links (28–33 g per sausage) and the study was run in duplicate.

Product Manufacture

Elaboration of the sausages was according to Marchetti *et al.* (2014, 2015). Briefly, 500 g grounded meat was homogenized in a commercial food processor (Universo, Rowenta, Germany, 14 cm blade) with Na or Na/K mixture according to the design (Table 1). Carrageenans, milk proteins, sodium nitrite and erythorbate were dissolved in cold water and then homogenized with the deodorized marine oil using a hand-held food processor (Braun, Buenos Aires, Argentina) during 2 min to form a coarse emulsion. The obtained emulsion was added to ground meat, processing all ingredients during 5 min afterward. Final temperature of batter varied between 12 and 15C. Samples were stuffed (vertical piston stuffer, Santini s.n.c., Marostica, Italy; into cellulose casing 22 mm diameter, Farmesa, Buenos Aires, Argentina), thermally treated in a hot water bath (80C) until the center reached 74C, cooled, vacuum packaged in Cryovac BB4L bags and stored at 4C during 45 days (typical shelf-life of commercial products).

Physicochemical Determinations

Process yield and purge loss were performed by triplicate according to Andrés *et al.* (2009). The methodology of Brennan and Bourne (1994) was followed to determined Texture Profile Analysis (TPA), analyzing 10 replicates per point. Color was determined at room temperature on the surface of transversally slices, recently cut, according to Marchetti *et al.* (2015). Five measures were taken for each data point. Finally, pH of the samples was measuring in triplicate using a spear tip glass electrode with Ag/AgCl reference (Phoenix 557-3512, USA) on a pHmeter (EC30, Hacht, Loveland, CO, USA).

Microbial Analysis

Bacterial counts were determined using the pour plate method at different times during refrigerated storage according to Andrés *et al.* (2009). 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 1 min. Appropriate serial dilutions were plated with Plate Count Agar (PCA, Oxoid, Hampshire, UK) for total mesophilic aerobic count (incubated at 30C for 2 d) and total psychrotrophic aerobic count (incubated at 4C for 7 d), with Violet Red Bile Glucose Agar (Merck KGaA, Darmstadt, Germany) for *Enterobacteriaceae* (incubated at 37C for 24 h), and with de Man, Rogosa, Sharpe agar (MRS agar, Oxoid) for lactic acid bacteria (incubated at 30C for 2 d). Yeast Extract Glucose Chloramphenicol Agar (YGC agar, Merck KGaA) was used for mold and yeast counts (incubated for 5 d at 30C). At the end of the storage, the products were also tested for total coliform counts using the most probable number method (MPN) according to AOAC (AOAC 1984) method 46016, and sulfite-reducing *Clostridium* were enumerated in Tryptone Sulfite Neomycin Agar (TNS agar, Oxoid) (incubated at 30C for 2 d). Data were expressed as log colony-forming units per gram of sample.

Lipid Oxidation and Fatty Acids Profile Determination

TBARS values were determined by quadruplicate according to Pennisi Forell *et al.* (2010) to evaluate the lipid oxidation in the sausages. Results were expressed as mg malonaldehyde (MDA)/kg product.

For fatty acid (FA) analysis of Na-T2, Na/K-T2, Na-BHA and Na/K-C formulations at initial and final storage time, total lipids were extracted using chloroform-methanol mix (2:1, v/v) according to Folch *et al.* (1957) procedure, and were methylated with 100 g/kg boron trifluoride methanol complex in methanolic solution. FA composition was determined at the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP, Mar del Plata), following (Pennisi Forell *et al.* 2010), in a Shimadsu 2010 gas chromatograph (Hewlett-Packard, USA) equipped with capillary column Omegawax 320 (30 m/0.32 mm id/0.25 µm) and mass detector. FA profiles were obtained by comparison of the retention times with a standard of 37 fatty acids (Supelco 37 Component FAME Mix, Cat. No. 18919-1 AMP, Sigma-Aldrich) previously analyzed in same conditions. Fatty acids were identified by comparison of the retention times.

Changes in Health Lipid Indexes During Storage

Based on the FA results the atherogenic index (AI, Eq. (1)) and the thrombogenic index (TI, Eq. (2)) were calculated according to Ulbricht and Southgate (1991) to assess the nutritional quality of the products, as a measure of the propensity of the product consumption influence the incidence of coronary heart disease:

$$AI=\frac{C_{12:0}+4\times C_{14:0}+C_{16:0}}{[PUFA_{n-6}+PUFA_{n-3}+MUFA]} \tag{1}$$

$$TI=\frac{[C_{12:0}+C_{14:0}+C_{16:0}]}{\left[\frac{1}{2}PUFA_{n-6}+3\times PUFA_{n-3}+\frac{1}{2}MUFA+\frac{PUFA_{n-3}}{PUFA_{n-6}}\right]} \tag{2}$$

where $C_{n:i}$ corresponds to each fatty acid content expressed as % FA.

Also the nutritional fat index (NFI = PUFA + MUFA)/SFA) was calculated (Amine *et al.* 2002).

Statistical Analysis

Analysis of variance (ANOVA, SYSTAT, Inc., Evanston, IL, USA) was carried out to test the significance of independent variables. Experimental data were reported as mean values ± the corresponding standard error of the mean (SEM) when appropriate. For simultaneous pairwise comparisons, least significance differences (LSD) test was chosen. Differences in means and *F* tests were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Physicochemical Properties

Process yield was not affected by salt contents or antioxidants. Formulations exhibited an average value of 985 ± 3 g/kg ($P > 0.05$), indicating high liquid retention of the matrix during the thermal treatment, even in Na/K formulations. These results were in agreement with Triki *et al.* (2013), who found no differences in process yields between merguez sausage formulation with 50% of NaCl replacement. Purge losses could be a serious problem, besides the fact of an unpleasant aspect of the product, by stimulating the microbial growth resulting in a lower shelf-life (López-López *et al.* 2009). Purge loss varied between 12 ± 1 g/kg at the beginning of storage for both Na content, and 43 ± 2 or 53 ± 2 g/kg for Na or Na/K formulations, respectively, for the final storage time (Fig. 1). These values were similar to those reported by other authors for lean sausages (Candogan and Kolsarici 2003; Andrés *et al.* 2009). Sodium reduced and nonreduced formulations showed different behavior (Fig. 1). Up to 14 days, purge loss exhibited a sharp increase and no significant differences among formulations. After 20 days, the effect of sodium replacement becomes significant. Those formulations with KCl added, released more liquid than the formulations without Na replacement that remained fairly constant. Low NaCl level could decrease the concentration of extracted/solubilized proteins involved in the formation of the emulsified gel. Low purge losses could be related with high liquid retention by the matrix throughout the storage, which was not modified by the antioxidant added to the product. Similar results have been reported by Colmenero *et al.* (2005), who studied the effect of NaCl, KCl, and transglutaminase in low-fat sausages formulations and found that the partial NaCl replacement decreased water binding properties.

Texture profile could reflect the possible changes that if noticed by the consumers may impact in their acceptance of the product. In Fig. 2, the obtained results of textural parameters, hardness, chewiness and resilience of formulated sausages during refrigerated storage are showed. Hardness was significantly affected by sausage formulation and

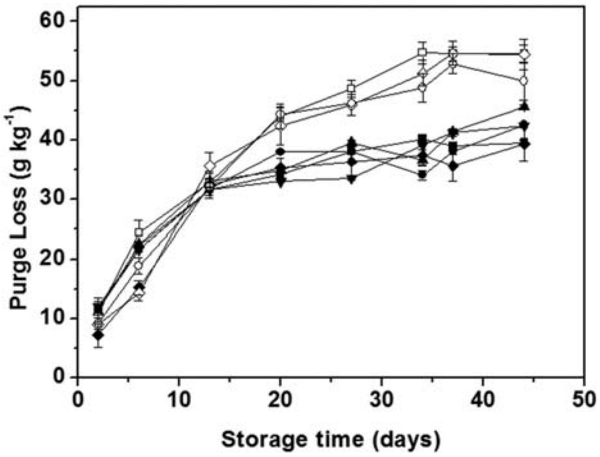

FIG. 1. PURGE LOSS (g/kg) OF MEAT SAUSAGES FORMULATED WITH MARINE OIL DURING REFRIGERATED VACUUM STORAGE
Codes: (1) Na formulations (14.00 g NaCl + 2.00 g TPP/kg product): (◆, 37.5 g tocopherols/kg (Na-T1); (•, 50 g tocopherols/kg (Na-T2); ▼, 5 g BHA/kg (Na-BHA); ■, control without antioxidant (Na-C); (2) Na/K formulations (6.08 g NaCl + 4.92 g KCl + 5.00 g TPP/kg product): (◇, 37.5 g tocopherols/kg (Na-T2); (○, 50 g tocopherols/kg (Na-T2); □, control without antioxidant (Na/K-C). Error bars indicate SEM.

storage time (Fig. 2a). Initial hardness of reduced sodium sausages (Na/K) was lower than nonreplaced ones. Literature shows diverse texture results depending on meat system, type and salt level used as NaCl partial replacer. Horita *et al.* (2011) found similar variations in emulsified meat products texture when NaCl was 50% reduced, with a hardness decrease when NaCl was reduced up to 75%. Besides, Marchetti *et al.* (2015) working with sodium-reduced lean sausages with fish oil found that for a given KCl level, hardness increased with TPP fraction, probably because changes in ionic strength and protein solubility affected meat texture.

Both sets of formulations increased its hardness with storage time, and after 30 days, no significant differences between formulations were observed; thus, there was a marked hardness increase when potassium chloride and TPP were added (28.3%) with respect to formulations without KCl (11.1%). This could be explained by the differences observed in purge loss, partially replaced sodium sausages (Na/K) lost more liquid and increased their hardness more rapidly that the nonreplaced formulations (Na), resulting in less water available to act as matrix plasticizer. Therefore, a possible relationship between hardness and purge loss was investigated (Fig. 3), finding a significant correlation ($P < 0.05$) between both parameters for each salt mixture (sodium-replaced and nonreplaced). Nevertheless, hardness values (9–12 N) were similar to those measured for Argentinean commercial products containing 20% fat. These results agree with other authors who had informed increases in hardness during refrigerated storage of cooked meat emulsions (Estévez *et al.* 2005; Hassaballa *et al.* 2009).

Chewiness showed a similar tendency to hardness (Fig. 2b). On the other hand, cohesiveness and springiness were not significantly altered by storage time or formulation. The obtained mean values were 0.873 ± 0.007 (mm/mm) for springiness and 0.573 ± 0.004 for cohesiveness (J/J).

Color is one of the main factors that affect the acceptability of a meat product by consumers. Chromaticity parameters (a^* and b^*) showed neither changes during storage nor between formulations ($P > 0.05$); the obtained mean values

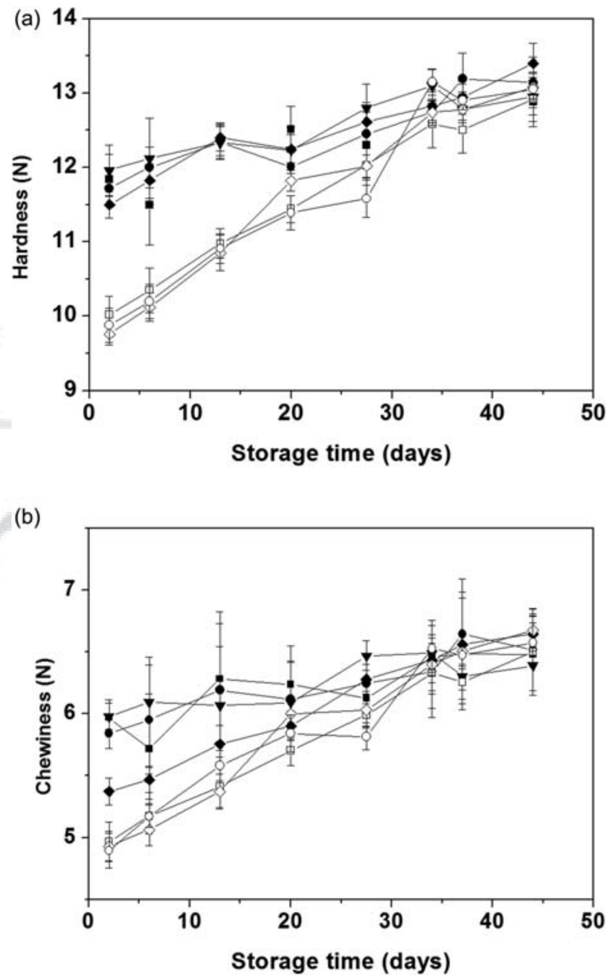

FIG. 2. EFFECT OF REFRIGERATED VACUUM STORAGE TIME ON TEXTURE PROFILE ANALYSIS PARAMETERS OF MEAT SAUSAGES FORMULATED WITH MARINE OIL
(a) Hardness, (b) chewiness. Codes: (1) Na formulations (14.00 g NaCl + 2.00 g TPP/kg product): (◆, 37.5 g tocopherols/kg (Na-T1); (•, 50 g tocopherol/kg (Na-T2); ▼, 5 g BHA/kg (Na-BHA); ■, control without antioxidant (Na-C); (2) Na/K formulations (6.08 g NaCl + 4.92 g KCl + 5.00 g TPP/kg product): (◇, 37.5 g tocopherols/kg (Na-T1); (○, 50 g tocopherols/kg (Na-T2); □, control without antioxidant (Na/K-C). Error bars indicate SEM.

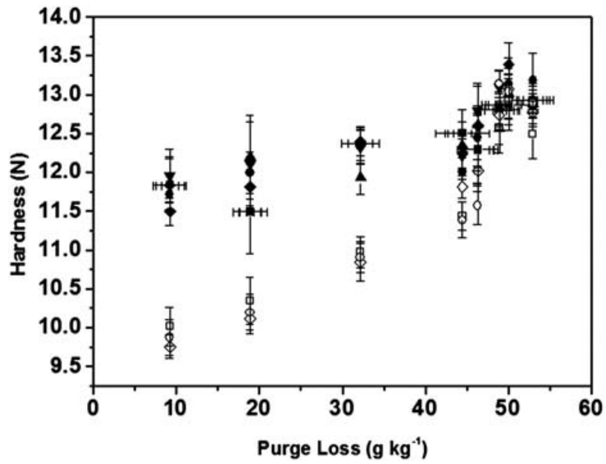

FIG. 3. CORRELATION BETWEEN HARDNESS AND PURGE LOSS
Codes: (1) Na formulations (14.00 g NaCl + 2.00 g TPP/kg product): (◆, 37.5 g tocopherols/kg (Na-T1); (•, 50 g tocopherols/kg (Na-T2); ▼, 5 g BHA/kg (Na-BHA); ■, control without antioxidant (Na-C); (2) Na/K formulations (6.08 g NaCl + 4.92 g KCl + 5.00 g TPP/kg product): (◇, 37.5 g tocopherols/kg (Na-T1); (○, 50 g tocopherols/kg (Na-T2); □, control without antioxidant (Na/K-C). Error bars indicate SEM.

were 10.3 ± 0.9 and 13.3 ± 0.8 for a^* and b^*, respectively. These color parameters result in agreement with those reported by García-García and Totosaus (2008). However, luminosity of all formulations significantly decreased after 20 days of storage ($P < 0.05$), as shown in Table 2. These changes could be related to the higher solid content of the product as a result of liquid lost as purge.

Microbial Quality

Sodium reduction did not significantly affect the microbial growth, because KCl has shown similar antimicrobial effect than NaCl (Bidlas and Lambert 2008). Soglia *et al.* (2014) informed that replacing up to 30% of NaCl by KCl did not change microbiological traits (total aerobic mesophilic and lactic LAB counts) in vacuum-packaged rabbit meat. Dominant flora in these products was psychrotrophic lactic acid bacteria, in concordance with other authors (Nychas and Drosinos 1999; Andrés *et al.* 2009). This spoilage might significantly affect product quality due to the acidification in anaerobic conditions. Table 2 shows average microbial counts of the formulations analyzed at different storage time. All formulations presented low initial microbial counts for total mesophilic and psychrotrophic microorganisms, and lactic acid bacteria (LAB), in consequence of the adequate thermal treatment done in their production. At the end of storage, total mesophilic levels were lower than 5 log cfu/g, maximum level permitted by Argentinean regulations (Código Alimentario Argentino 1999). No lag phase was observed for the microbial growths for mesophilic, psychrotrophic and LAB, Feng *et al.* (2014) reported similar trends in refrigerated Irish sausages. Regarding the pH evolution of the samples during storage pH decreased from 5.82 to 5.34 between initial and final time, related to LAB development (Table 2). Cayré *et al.* (2005) proposed that the vacuum storage of meat products limited the growth of *Pseudomonas* spp., resulting in lactic acid bacteria as the main component of the flora. In these products, its development and metabolism depend of different factor (pH, temperature, atmospheric composition within package, substrate availability) (Yan *et al.* 2008).

Enteriobacteriaceae and yeast and molds counts were below the detection limit of the technique (2 log cfu/g) during the refrigerated storage of all the analyzed formulations. Total coliforms counts were <2 MPN/g in all formulations at the end of storage. These results were in accordance to Argentinean regulations (Código Alimentario Argentino 1999). In addition, no sulfite-reducing *Clostridium* was noted in the sausages during the storage period, indicating safe sanitary conditions, and related to the inclusion of $NaNO_2$, which is a key component to avoid *Clostridium* spp. growth (Christiansen *et al.* 1975).

TABLE 2. CHANGES IN AVERAGE LUMINOSITY (L^*), PH AND MICROBIAL COUNTS DURING REFRIGERATED STORAGE OF LOW-FAT MEAT EMULSIONS PREPARED WITH MARINE OIL

Time (days)	Luminosity (L^*)	pH	Total mesophilic counts (log cfu/g)	Total psychrotrophic counts (log cfu/g)	Lactic acid bacteria (log cfu/g)
1	61.8 ± 0.2a	5.82 ± 0.01a	2.98 ± 0.08e	1.87 ± 0.05g	2.03 ± 0.1f
7	61.5 ± 0.3ab	5.79 ± 0.02ab	3.33 ± 0.07e	2.22 ± 0.3f	2.44 ± 0.2e
14	60.9 ± 0.2b	5.74 ± 0.02bc	3.70 ± 0.1d	2.61 ± 0.07e	2.81 ± 0.09e
22	60.5 ± 0.2bc	5.69 ± 0.01d	3.98 ± 0.06cd	2.99 ± 0.08d	3.27 ± 0.3d
28	60.1 ± 0.2cd	5.61 ± 0.01d	4.26 ± 0.1bc	3.10 ± 0.1cd	3.4 ± 0.07cd
34	60.0 ± 0.3cde	5.51 ± 0.03e	4.51 ± 0.1ab	3.45 ± 0.1bc	3.71 ± 0.2bc
41	59.9 ± 0.1de	5.42 ± 0.02f	4.69 ± 0.2a	3.58 ± 0.08ab	3.89 ± 0.1b
45	59.6 ± 0.2e	5.34 ± 0.01g	4.78 ± 0.08a	3.88 ± 0.03a	4.29 ± 0.9a

Average values ± standard error of the mean (SEM), different superscripts within the same column indicate that average values differ significantly ($P < 0.05$).

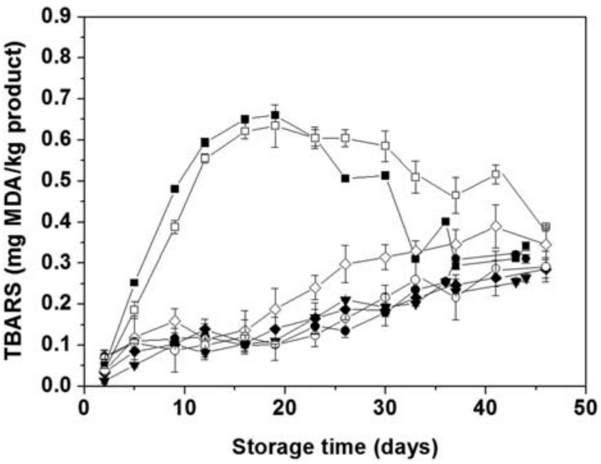

FIG. 4. TBARS OF MEAT SAUSAGES WITH 5% DEODORIZED MARINE OIL DURING VACUUM REFRIGERATED STORAGE EXPRESSED AS MILLIGRAMS OF MALONALDEHYDE (MDA) PER KILOGRAM OF PRODUCT

Codes: (1) Na formulations (14.00 g NaCl + 2.00 g TPP/kg product): (◆, 37.5 g tocopherols/kg (Na-T1); (•, 50 g tocopherols/kg (Na-T2); ▼, 5 g BHA/kg (Na-BHA); ■, control without antioxidant (Na-C); (2) Na/K formulations (6.08 g NaCl + 4.92 g KCl + 5.00 g TPP/kg product): (◇, 37.5 g tocopherols/kg (Na-T1); (○, 50 g tocopherols/kg (Na-T2); □, control without antioxidant (Na/K-C). Error bars indicate SEM.

Lipid Oxidation

The TBARS evolution is related with malonaldehyde (MDA) formation as an intermediary product in oxidation. In a first step, the MDA formation rate is higher than its extinction rate, and after a certain point the contrary happens. Jamora and Rhee (2002) reported that the formed MDA during storage of meat products may undergo intermolecular reactions (polymerization) or react with other components, especially amino acids/proteins and consequently the MDA loss rate during storage could exceed the production rate through lipid oxidation. de Ciriano *et al.* (2010) and Rhee and Myers (2004) reported this trend in TBARS for meat systems with fat sources composed of an O/W emulsion with algae oil (*Crypthecodinium cohnii*). Also, according to Shahidi (1992), TBARS in meat products tend to increase during the storage period, reaching a maximum value and then decreasing due to an additional reaction of MDA with amino groups.

In the present work, this behavior was observed in control formulations without antioxidant (Na-C and Na/K-C, Fig. 4). TBARS increased significantly until day 19, reaching a maximum value (0.66 mg MDA/kg product), thereafter, TBARS decreased. The addition of 37.5 mg of tocopherols/kg[1] to the products (Na-T1 and Na/K-T1) delayed lipid oxidation, but Na/K-T1 showed an increase in TBARS number at the end of the storage period. Lipid oxidation was adequately inhibited in formulations with BHA (Na-BHA) or 50 mg tocopherols/kg (Na-T2 and Na/K-T2), with a slight increase in TBARS at the end of the storage (<0.4 mg MDA/kg product), without significant differences between both antioxidants ($P > 0.05$). This implies an adequate inhibition of lipid oxidation in the studied meat systems, showing that the synthetic antioxidant could be replaced with a natural one with similar results.

Several physicochemical or sensory TBARS limits in meat products or systems have been reported. Campo *et al.* (2006) informed that levels > 2 mg MDA/kg are not accepted in bovine meat. Otherwise, Georgantelis *et al.* (2007) established a maximum limit of 0.6 mg MDA/kg over which it is detectable a rancid flavor in meat products. Lanari *et al.* (1995) proposed a limit of 0.50 mg MDA/kg for the start of unpleasant flavor due to rancidity in pork. Therefore, according to the obtained results formulations with natural tocopherols or BHA presented TBARS values lower than even the strictest limits suggested in the literature during the 45 days of storage. However, it was necessary to add at least 37.5 and 50 mg tocopherols/kg to Na and Na/K formulations, respectively, to achieve the inhibition obtained with BHA in sausages containing 14 Na/kg.

These results agree with those reported by Kim (2012) who obtained a reduction of TBARS and improved color stability of a meat emulsion system by using 67 and 134 mg tocopherols/kg product. Also it has been reported that the addition of 50 and 100 mg tocopherols/kg to stuffed cooked meat product reduced the peroxide value, free fatty acids and TBARS number (Aksu 2007). Cáceres *et al.* (2008) reported low lipid oxidation (TBARS 0.37–0.52 mg MDA/kg) during cooling of bologna made with commercial fish oil with α-tocopherol, resulting in similar values to those obtained in this work.

Fatty Acid Profile

The results of fatty acid composition are consistent with the type of ingredients used in the formulation. Table 3 shows the obtained fatty acids profiles from the lipid phases of several formulations (sodium reduced or not) made with marine oil with different antioxidants (BHA or tocopherols) at the initial and end (45 days) of the storage period. In addition, it was included a FA profile of a reduced sodium formulation without antioxidants (control) and a traditional product with animal fat (USDA 2015).

The obtained FA profiles are within the current diet recommendations, due to marine oil incorporation. In addition to considerations of individual fatty acids, scientific evidence suggests that ratios such as PUFA/SFA (recommended > 0.4) and n-6/n-3 PUFAs (recommended < 4) are the main parameters currently used to assess the nutritional quality of the lipid fraction of foods. In 45 g (1 commercial sausage link) of the products studied in this work, saturated

TABLE 3. FATTY ACID (FA) PROFILES OF DIFFERENT SAUSAGES FORMULATED WITH MARINE OIL AT INITIAL OR END OF STORAGE. TP DENOTES A TRADITIONAL PRODUCT ACCORDING TO USDA (2015)

	(14 g NaCl + 2 g TPP)/kg				(6.08 g NaCl + 4.92 g KCl + 5 g TPP)/kg				
	Na-BHA (5 mg BHA/kg)		Na-T2 (50 mg T/kg)		Na/K-C (no antioxidant)		Na/K-T2 (50 mg T/kg)		
Fatty acid (% of total FA)	0 days	45 days	0 days	45 days	0 days	45 days	0 days	45 days	TP
Lauric C12:0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.7
Myristic C14:0	3.8b	4.2ab	4.0b	4.0b	0.9c	1.1c	1.0c	1.1c	4.4
Palmitic C16:0	16.9d	18.1b	17.0c	17.2cd	17.5c	18.6b	15.9e	16.3e	20.6
Palmitoleic C16:1 n-7	5.2c	5.5c	5.2c	5.2c	7a	6.4b	7.3a	6.8ab	4.8
Stearic C18:0	4.8c	4.8c	4.8c	4.9c	6.1b	5.9b	6.0b	6.2b	22.1
Oleic C18:1 n-9 *cis*	27.1c	27.2c	26.6cd	27.1c	25.8e	24.3f	28.6b	26.1de	41.1
Linoleic C18:2 n-6	2.7b	2.8b	2.8b	2.8b	2.5b	1.6c	2.5b	2.5b	3.3
Linolenic C18:3 n-3	2.1a	2.1a	2.2a	2.1a	2.4a	0.7b	2.4a	2.2a	0.4
C20:1 (undefined)	5.2a	5.2a	5.3a	5.2a	3.5b	3.4b	3.4b	3.6b	0.6
Arachidonic C20:4 n-6	1.6a	1.4a	1.6a	1.4a	1.4a	0.7b	1.5a	1.4a	N.D.
Eicosapentaenoic C20:5 n-3	10.9a	9.9b	10.8a	9.8b	8.9c	7.0e	8.8cd	8.4d	N.D.
Docosahexaenoic C22:6 n-3	17b	16.2c	16.9b	16.2c	17.7a	13.0a	17.6a	16.7b	N.D.
SFA	25.5bc	27.1b	25.8bc	26.1bc	24.5bc	25.6bc	22.9c	23.6c	49.8
MUFA	37.5bc	37.9bc	37.1c	37.5bc	36.3cd	34.1d	39.3b	36.5c	46.5
PUFA	34.3a	32.4ab	34.3a	32.1bc	32.9ab	23.8d	32.8ab	31.2c	3.7
n-6/n-3	0.14b	0.09b	0.10b	0.09b	0.15b	0.12b	0.15b	0.16b	8.33
NFI	2.82a	2.59ab	2.77a	2.67ab	2.82a	2.26b	3.15a	2.87a	1.01
PUFA/SFA	1.35ab	1.20c	1.33a	1.23c	1.34b	0.93d	1.43a	1.32b	0.07
Aterogenicity Index	0.45b	0.50b	0.46b	0.48b	0.30c	0.40b	0.28c	0.31c	0.81
Trombogenicity Index	0.18b	0.19b	0.17b	0.18b	0.16b	0.22b	0.15b	0.16b	1.06

N.D. = Not detected. Different superscripts within the same row f indicate that average values differ significantly ($P < 0.05$).

(SFA) and monounsaturated (MUFA) fatty acids were lower than those corresponding to a traditional formulation (659 mg versus 4219 mg, and 959 mg versus 3939 mg, respectively). In addition, one serving (45 g) of low-fat sausages with marine oil contained 820 mg PUFA, providing 241 mg of EPA and 419 mg of DHA, contrasting with the traditional product with pork fat, which presents 313 mg of PUFA per 45 g sausage (USDA 2015), with no EPA or DHA.

The FA profile of the reformulated products results in a significantly lower n-6/n-3 ratio. Furthermore, the PUFA/SFA ratio was always >1.2, thus replacement of pork or beef fat by marine oil with antioxidants, significantly increased this ratio from the commonly found for these products (Delgado-Pando *et al.* 2011) (about 0.34, Table 3).

EFSA dietary recommendations (EFSA 2012) for EPA and DHA based on cardiovascular diseases risk considerations for adults are between 250 and 500 mg/d. This product could easily sum up for the daily intake of EPA and DHA; an intake of one serving of this product would greatly exceed the minimum 250 mg required.

The formulation without antioxidant (Na/K-C) showed a noteworthy decrease ($P < 0.05$) of EPA, DHA, and total PUFA (21.3, 26.6 and 27.7% reduction, respectively), also, in oleic, linoleic and linolenic acid contents at 45 days of storage. With the antioxidants addition, the oxidation of the last fatty acids was inhibited, while EPA and DHA oxidation was reduced. The n-6/n-3 ratio of the products remained unchanged throughout the storage period (range: 0.09–0.16).

FA profiles and their changes at the end of vacuum-packaged refrigerated storage are in agreement with the results obtained in the TBARS assay, where inclusion of tocopherols in the formulation were able to delay lipid oxidation, establishing a natural alternative to BHA.

Average values of AI and TI for sausages manufactured with marine oil were 0.40 and 0.17, respectively, significantly lower than the traditional product indexes, in agreement with the literature reports (Ulbricht and Southgate 1991; Higgs 2000; Senso *et al.* 2007; Afonso *et al.* 2013), indicating less risk of cardiovascular event. Moreover, all cooked sausages achieved the World Health Organization's recommendation (Amine *et al.* 2002) on the nutritional fat index ((NFI = PUFA + MUFA)/SFA $\geq$ 2) which is very relevant to the development of healthier formulations since the calculated values ranged between 2.26 and 3.15. Besides three indexes remained unchanged during storage when antioxidants were added (formulations Na-BHA, Na-T2 and Na/K-T2).

In previous works sensory assays showed that neither the deodorized fish oil inclusion nor the partial substitution of

NaCl had a negative impact over the flavor, color, texture and overall acceptability (Marchetti *et al.* 2014, 2015). It may be concluded that these products would present good storage stability if natural tocopherols were added in at least 50 mg/kg.

CONCLUSIONS

A significant reduction of sodium content did not alter process high yields (985 g/kg) and low purge losses ($\leq$5.5%). Reducing Na content initially produced harder sausages, but hardness increased during storage at a different rate that depended on Na content, reaching similar values at the end of the 45 days period, within the commercial products hardness range. Sodium replacement significantly affected the oxidative stability of the products, although 50 mg natural tocopherols/kg successfully prevented rancidity in products with and without NaCl partial replacement. The resulting fatty acid profile was associated with a reduction in risks of different cardiovascular diseases (lower TI and AI).

Thus, it is possible to obtain cooked meat emulsions (sausages) with low sodium, low saturated fat, and high amounts of n-3 PUFA by applying a combination of carrageenans, milk proteins concentrate and preemulsified marine oil, without significant adverse effects over the quality of the products for at least 45 days of refrigerated storage.

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