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Sodium-reduced lean sausages with fish oil optimized by a mixture design approach

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ABSTRACT

A partial NaCl replacement by KCl and sodium tripolyphosphate on low-fat meat sausages formulated with fish oil was studied using a mixture design. Thermal behavior by modulated differential scanning calorimetry, physicochemical, and textural properties were determined; afterwards they were mathematically modeled as a function of salts content. The thermo-rheological behavior of the different formulations was also studied in a control-stress rheometer. The optimal sodium reduction was found employing a desirability function approach. This formulation was experimentally validated and employed for microstructure analysis by environmental scanning microscopy. The results obtained in this work revealed that partial sodium replacement affected the matrix microstructure, but this change had no impact on sensory acceptability. In comparison with US and Argentinean commercial sausages, our product has 58% and 70% less Na^+ respectively.

1. Introduction

It is well established that excess dietary salt consumed throughout life causes blood pressure to rise with age and greatly increases the risk of cardiovascular diseases, becoming the leading causes of death and disability in many Western countries. In Argentina they are responsible for 30% of diseases (Ministerio de Salud de la Nación, 2013). There is a consensus that a reduction in salt consumption will lower blood pressure, with great potential to produce significant individual and population health benefits (Angell, 2010).

Processed meat products contain in general relatively high amounts of saturated fats and sodium, and production of healthier animal protein foods requires that these two elements would be reduced. Direct reduction of fats and sodium can lead to technological difficulties, making these reductions a serious technological issue in the meat industry (García-García & Totosaus, 2008). Since sodium intake generally exceeds nutritional recommendations in industrialized countries and approximately 20–30% of common salt intake comes from meat products, there is increasing interest among consumers and processors in reducing the use of salt (minimizing sodium) in meat processing (Cofrades, López-López, Solas, Bravo, & Jiménez-Colmenero, 2008).

Salt is necessary during meat processing to induce structural changes through electrostatic interactions between muscle proteins and sodium and chloride ions; these cause swelling of myofibrils, depolymerization of myofilaments, and dissociation of the actomyosin complex. Reduced salt concentrations also lead to decreases in extracted and solubilized myofibrillar proteins, affecting the functionality of the entire meat system. The addition of calcium, magnesium, or potassium chloride salts to meat batters in the presence of NaCl enhances protein extraction and solubility, emulsion stability, and favors the orderly gelation of proteins. Moreover emulsified meat products such as mortadella require specific concentrations of NaCl in the original formulations to promote the extraction of myofibrillar proteins, especially actomyosin complex, which are soluble only in solutions of high ionic strength (Totosaus & Pérez-Chabela, 2009). Myofibrillar proteins extracted in the comminuting process in the presence of NaCl are responsible for the water-holding capacity, emulsification and fat binding properties in the batter and formation of stable gels in the cooking stage (Desmond, 2006).

Potassium chloride has been the most investigated substitute for NaCl in low- or reduced salt/sodium foods and its intake in various

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studies has reduced blood pressure in humans, although in some vulnerable group of population a diet rich in potassium could have damaging effects. From a technological point of view, this salt has been used to ensure the necessary ionic strength to develop stable emulsions; however, its use alone results in a bitter, astringent and metallic taste. At blends over 50:50 sodium chloride/potassium chloride in solution, show a significant increase in bitterness and loss of saltiness (Desmond, 2006). Thus, it is necessary to optimize product formulations with the use of other ingredients and additives besides KCl capable of stabilizing the emulsion.

Ingredient functionality in processed foods can be studied by mixture design methodology, in which the systematic experimental design validates the importance of ingredient interactions. Applying this methodology, García-García and Totosaus (2008) studied the effect of potato starch, locust bean gum and κ-carrageenan mixture interaction on the properties of low-fat sodium reduced sausages; they found that a low proportion of starch can be used as extender if κ-carrageenan and locust bean gum are included in similar proportions.

In a previous work, (Marchetti, Andrés, & Califano, 2014) a response surface methodology was applied in order to optimize carrageenans and milk proteins concentrate levels in low-fat meat sausages with pre-emulsified fish oil (5 g/100 g). Salts levels employed (NaCl = 1.4 g/100 g and sodium tripolyphosphate = 0.2 g/100 g) were below average contents of commercial products. The obtained product had similar physicochemical properties to a commercial product with 20 g/100 g of beef tallow. Moreover the optimized formulation had much better nutritional quality; nevertheless the salt level employed could be reduced to enhance nutritional benefits.

Thus, considering this optimized formulation as the non-sodium replaced one, in the present work the objectives were: i) to study the effect of the partial sodium replacement by potassium on the thermal and thermo-rheological properties of the batters and on the quality parameters of low-fat sausages prepared with pre-emulsified fish oil, ii) to find the optimum combination of NaCl, KCl, and sodium tripolyphosphate that results in a product with similar quality parameters to the non-replaced one, iii) to validate the sodium-reduced optimized formulation by manufacturing it and measuring its quality parameters, comparing the obtained results with the values predicted by mathematical models.

2. Materials and methods

2.1. 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: 1.3 ± 0.17 g/100 g). Meat (9 kg, muscles from four different carcasses) without visible fat and connective tissue was passed through a grinder with a 0.95 cm plate (Meifa 32, Buenos Aires, Argentina). Lots of 500 g were vacuum packed in Cryovac BB4L bags (PO_2: 0.35 $cm^3 m^{-2} day^{-1} kPa^{-1}$ at 23 °C, Sealed Air Co., Buenos Aires, Argentina), frozen, and stored at −20 °C until used, no more than three weeks (Ayo, Carballo, Solas, & Jiménez-Colmenero, 2005).

As fat source, deodorized refined fish oil with 1000 ppm tocopherols added (Omega Sur S.A., Mar del Plata, Argentina) was used; its fatty acid (FA) composition was: monounsaturated FA (MUFA), 36.89%; saturated FA (SFA), 26.78%; total polyunsaturated FA (PUFA), 36.27%; and n-3 PUFA, 24.47% (EPA, 9.69% and DHA, 15.63%). As stabilizer or emulsifier agents food-grade commercial preparations of milk proteins concentrate (MPr, Milkaut, Santa Fe, Argentina) and 2:1 κ/ι carrageenans mixture (Carr, ADAMA S.A., Buenos Aires, Agentina) a synergistic combination (Candogan & Kolsarici, 2003) were used in previous optimized levels (Marchetti et al., 2014). Cold distilled water was used in all formulations (4 °C). Mixed phytosterols (Advasterol 90% with 16–24% campesterol, 19–32% stigmasterol and 32–50% β-sitosterol, AOM SA, Buenos Aires, Argentina) were included.

Analytical grade sodium chloride (NaCl), potassium chloride (KCl), sodium nitrite ($NaNO_2$) sodium erythorbate, monosodium glutamate and sodium tripolyphosphate (TPP) were employed. The concentration of sodium nitrite was selected according to the level permitted by the Argentinean Regulations (0.015 g/100 g, Código Alimentario Argentino (1999)).

The following components were included to prepare 100 g of uncooked meat batter: meat (66.65 g), water (25 g); fish oil (5 g); salts mixture (NaCl + KCl + TPP = 1.6 g), sodium erythorbate (0.045 g); $NaNO_2$ (0.015 g); κ/ι carrageenans (0.593 g), milk protein concentrate (0.32 g), phytosterols (0.5 g), monosodium glutamate (0.02 g), ground pepper (0.2 g); nutmeg (0.05 g), carminic acid (0.0032 g, Naturis S.A., Buenos Aires, Argentina).

2.2. Experimental design

In a mixture experiment, the measured response is assumed to depend only on the relative proportion of ingredients or components present in the mixture, which usually sum 100%.

To diminish the sodium content of the product NaCl and TPP were partially replaced by KCl, using a three-component constrained simplex lattice mixture design. The mixture of components consisted of NaCl, KCl, and TPP, and the sum of the three salts was 1.6 g/100 g. Consequently a mixture design with constrains was chosen. This type of design is suggested when the proportions of some or all of the components are restricted by upper and lower bounds; the experimental region is just a sub-region of the entire mixture simplex (Cornell, 2011). Combinations of NaCl (0.5–1.4 g/100 g), KCl (0–0.7 g/100 g), and TPP (0–0.5 g/100 g) were used in a simplex-centroid augmented design. The maximum KCl was established according to Desmond (2006) to reach a replacement up to 50% of NaCl. Maximum level of TPP was fixed taken into account that Liu, Booren, Gray, and Crackel (1992) employed 0.5 g/100 g sodium tripolyphosphate in pork meat systems without adverse results.

The design consisted of eleven runs. The centroid point formulation (0) was prepared three times. Table 1 shows all the tested formulations; formulation 2 corresponded to a non-replaced sodium formulation (1.4 g/100 g NaCl and 0.2 g/100 g TPP, without KCl, (Marchetti et al., 2014).

2.3. Product manufacture

All formulations were manufactured following the methodology of Andrés, Zaritzky, and Califano (2009) and Marchetti et al. (2014). Briefly, after meat packages were thawed (approximately 18 h at 4 °C), each batch was homogenized and grounded in a commercial food processor (Universo, Rowenta, Germany, 14 cm blade) with the mixed salt according to the design. Carrageenans and milk proteins concentrate, sodium

Table 1
Actual and codified NaCl, KCl, and tripolyphosphate values according with the mixture design used.

Formulation	Actual values (g/100 g)			Codified values		
	NaCl	KCl	TPP	NaCl	KCl	TPP
0	0.966	0.366	0.266	0.604	0.229	0.167
1	1.1	0	0.5	0.688	0.000	0.313
2	1.4	0	0.2	0.875	0.000	0.125
3	0.5	0.6	0.5	0.313	0.375	0.313
4	0.9	0.7	0	0.563	0.438	0.000
5	1.4	0.2	0	0.875	0.125	0.000
6	1.15	0.45	0	0.719	0.281	0.000
7	0.8	0.3	0.5	0.500	0.188	0.313
8	0.733	0.533	0.333	0.458	0.333	0.208
9	1.183	0.183	0.233	0.740	0.115	0.146
10	0.7	0.7	0.2	0.438	0.438	0.125

nitrite, and erythorbate were dissolved in cold water and then homogenized with fish 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 afterwards. Final batter temperature varied between 12–15 °C. Batters were immediately stuffed (vertical piston stuffer, Santini s.n.c., Marostica, Italy) into cellulose casing (22 mm diameter, Farmesa, Buenos Aires, Argentina), hand-linked and placed in "cook-in" bags (3 or 4 sausages/bag) (Cryovac CN510, Sealed Air Co., Buenos Aires, Argentina) to thermal treatment in a temperature-controlled water-bath (Haake L, Haake Buchler Instruments, Karlsruhe, Germany) at 80 °C until a final internal temperature of 74 °C according to the recommendations of the US Food Code (U.S. Food and Drug Administration (2013). Then, samples in the bags were cooled immediately in an ice-water-bath and stored at 4 °C until further analysis.

2.4. Proximal analyses

Moisture, ash and protein contents of sausages were determined according to the AOAC methods 24.003, 24.009, and 24.027, respectively, in triplicates. Fat content was determined on triplicate samples previously dried with sodium sulphate anhydrous (SO4Na2) by Soxhlet method, using petroleum ether (Bp: 35–60 °C) as extraction solvent (Andrés et al., 2009).

2.5. Modulated Scanning differential calorimetry (MDSC)

Thermal behavior of raw meat systems was monitored by MDSC using a previously calibrated differential scanning calorimeter (DSC Q100, TA Instruments, New Castle, USA) according to Marchetti, Andrés, and Califano (2013). Sample weight was around 12 mg (±0.002) determined by an electronic balance. Samples were encapsulated in hermetically sealed aluminum pans. An empty sample pan was used as reference. The gas employed was N2 with a 50 ml/min flow rate. Duplicate samples were annealed in order to allow ice crystallization according to the following protocol: samples were cooled to −50 °C, heated to −25 °C at 5 °C/min, equilibrated at −25 °C for 3 min, cooled to −50 °C at 10 °C/min, and then scanned from −50 °C to 120 °C at a heating rate of 5 °C/min, with a modulation ±1 °C every 60 s. After each MDSC run a pinhole was made on the cover of every sample and dried at 105 °C for water content determination (Fernández-Martín, Cofrades, Carballo, & Jiménez-Colmenero, 2002).

Enthalpies (ΔH), expressed as J/g protein, associated with the water melting, and protein denaturation were determined by integrating the area under the MDSC curve, using software for thermal analysis (TA Instruments Universal Analysis 2000; TA Instruments, USA), and dividing this area by the weight of the sample. From the different baseline options offered by the software, a sigmoid baseline was selected to calculate peak areas and compensate changes in specific heat that occurs when water changes from solid to liquid state (Bourne, 1978). Peak transition temperatures were also determined from MDSC thermograms. Appropriate enthalpy changes integrated from −40 °C to 20 °C were evaluated for meat batters (ΔH_{mb}) and pure water (ΔH_w), and then the frozen and non-frozen water fractions were calculated (Marchetti et al., 2013).

2.6. Thermo-rheological assays

All rheological measurements were performed using a controlled stress rheometer (Haake RS600, Thermoelectron, Germany) provided with a temperature control unit (K-15 Haake, Thermoelectron, Germany). After positioning the sample on the sensor system, it was allowed to rest for 3 min before starting the corresponding measurement. Samples perimeters were covered with a thin film of silicone oil and the measuring system was covered with a special device to prevent evaporation during the temperature sweeps. Raw batter was placed between serrated parallel plates (35 mm diameter, 1 mm gap). After equilibration at 20 °C, the samples were sheared at a fixed frequency of 6.28 rad/s with a stress of 5.0 Pa during all the thermal ramps and isothermal process. The thermal scanning started with a heating to 75 °C with a heating rate of 4.8 °C/min. After this heating stage the sample was held isothermally at 75 °C for 5 min. Then, a cooling stage was done from 75 °C to 20 °C at 10 °C/min. Lastly an isothermal step at 20 °C was performed for 5 min.

Changes in the dynamic storage modulus, G' (Pa), loss modulus, G'', and loss tangent (δ) were monitored continuously throughout the simulated gelling process at 6.28 rad/s (1 Hz) frequency. All measurements were performed within the linear viscoelastic range which has been previously determined at 25 °C, 50 °C, and 75 °C. The thermo-rheograms presented correspond to mean values of two replicates per formulation.

2.7. Process yield

Process yield was determined in duplicate by weighing the product before and after thermal treatment, and corresponds to weight loss due to heating. The percent loss in weight during the precooking treatment is reported as "process yield, g/100 g" (Andrés et al., 2009; Candogan & Kolsarici, 2003).

2.8. Texture Profile Analysis

Texture Profile Analysis (TPA) (Bourne, 1978; Brennan & Bourne, 1994) was performed on sausages in a controlled temperature room (20 °C). Ten repeated measurements were taken and mean values were reported. Samples (1.5 cm thick and 1.7 cm diameter) were cut from the centre of the links and compressed twice to 30% of their original height between flat plates using a TAXT2i Texture Analyzer (Stable Micro Systems, UK) with a 75 mm diameter probe at 0.5 mm/sec (SMSP/75), interfaced with a computer, using the software supplied by Texture Technologies Corp.. Hardness (peak force of first compression cycle, N), cohesiveness (ratio of positive areas of second cycle to area of first cycle, J/J, dimensionless), adhesiveness (negative force area of the first byte represented the work necessary to pull the compressing plunger away from the sample, J), chewiness (hardness x cohesiveness x springiness, N), springiness (distance of the detected height of the product on the second compression divided by the original compression distance, mm/mm, dimensionless), and resilience (area during the withdrawal of the first compression divided by the area of the first compression, J/J, dimensionless) were determined.

2.9. Color

Color was measured at room temperature on the surface of transversally slices recently cut, using a Chroma Meter CR-400 colorimeter (Minolta Co., Ramsey, New Jersey, USA) and the CIE-LAB parameters (lightness, L*, redness, a*, and yellowness, b*) were determined. Five measures were taken for each date point.

2.10. Microstructure

Recently cut small pieces of sausages (0.5 cm diam., 0.2–0.3 cm thick) were used for electron microscopy analysis. Samples were placed on aluminum stubs using double-sided tape. Micrographs of the samples were obtained with a microscope FEI-Quanta 200 (Hillsboro, Oregon, USA) using an environmental mode.

2.11. Surface response analysis

Models for analyzing data from mixture-process variable experiments are usually obtained by combining traditional Scheffé type models for the mixture variables with response surface models for the process variables. In the present work the following second order polynomial model was fitted to the data:

$$\hat{Y} = \sum_{i=1}^{k} \beta_i x_i + \sum_{\substack{i,j \\ i \neq j}}^{k} \beta_{ij} x_i x_j + \beta_{ijk} x_i x_j x_k + \sum_{\substack{i,j \\ i \neq j}}^{k} \beta_{i-j} x_i \left(x_i - x_j\right) \quad (1)$$

where $\hat{Y}$ was the response variable, X_i were the proportion of components expressed in percentage (X_1 = NaCl, X_2 = KCl, X_3 = TPP) and β_i, β_{ij}, β_{ijk} and β_{i-j} were the regression coefficients obtained for each variable (first order, double, triple, and complex interaction respectively). A stepwise methodology was followed to determine the significant terms in Eq. (1). Differences in the computed parameters were considered significant when the computed probabilities were less than 0.05 ($P < 0.05$).

After model fitting was performed, residual analysis was conducted to validate the assumptions used in the analysis of variance. This analysis included calculating case statistics to identify outliers and examining diagnostic plots such as normal and residual plots. The proportion of variance explained by the polynomial models obtained was given by the multiple coefficients of determination, and the quality of the developed models was verified using a "lack of fit" test (Walpole, Myers, Myers, & Ye, 1993) and the "adequate precision" coefficient which measures the signal to noise ratio (Vohra & Satyanarayana, 2002).

2.12. Optimization

Optimization main objective was to determine the independent variables levels (formulation components) that would give the best low-fat sausage characteristics, considering the quality parameters of the target product. Based on the ingredients effects on each product characteristic the overall desirability criterion was used (Derringer, 1980). The general approach was to first convert each response ($\hat{Y}_i$) into an individual desirability function (d_i) that varied from 0 to 1, where, if the response was at its goal or target, then $d_i = 1$ representing a completely desirable or ideal response value, and if the response was outside an acceptable region, then $d_i = 0$ representing a completely undesirable value of $\hat{Y}_i$. Eq. (2) expresses the global desirability function, D, defined as the geometric mean of the individual desirability functions that correspond to each parameter. With that function, a multiple response problem could be transformed into a one response, throughout mathematical transformations (Derringer, 1980).

$$D = \sqrt[n]{d_1 x\ d_2 x \ldots x\ d_i x \ldots x\ d_n} \quad (2)$$

The optimum values of factors are determined from the value of individual desired functions that maximizes D. The optimum formulation was used for calculating the predicted values of response variables using the predictive equations derived by RSM. Also, a batch of sausages was prepared using the optimal ingredient levels; determining process yield, color, and texture as described above and results were statistically compared to the predicted values.

2.13. Sodium and potassium quantification

Ashes were obtained from formulation 2 and low-Na optimized formulation according to 2.13 (AOAC, 1984, 24.003). They were dissolved in HNO_3 and deionized water, making the necessary dilutions. Sodium and potassium were determined using a flame photometer (Corning FP-210, UK). Measurements were carried out according to the manufacturer's recommendations for maximum sensitivity, with methane and compressed air as the combustion gases, employing polypropylene vases to avoid Na^+ contamination. The aspiration rate was adjusted to 1.00 mL/min of working standard solutions or samples.

Each measurement was performed in duplicate. High purity deionized water (resistivity 26 MΩcm, Milli-Q system, Millipore, Bedford, MA, USA) and analytical grade nitric acid (Merck, USA) were used for the aqueous solutions.

2.14. Sensory analysis

Sensory analysis 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. Two samples were given to the panelists: non-sodium replaced (formulation 2; 1.4 g/100 g NaCl, 0 g/100 g KCl, 0.2 g/100 g TPP), and the optimized sodium-reduced formulation. The panelist were asked to indicate how much they liked or disliked the products on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) according to appearance, color, flavor, texture, and overall acceptability characteristics. Samples 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.

2.15. Statistical analysis

Analyses of variance were conducted separately on the dependent variables studied considering each formulation as a level in a one-way factorial design. For simultaneous pairwise comparisons, least significance differences (LSD) test was chosen. Differences in means and F-tests were considered significant when $P < 0.05$. These statistical procedures were computed using the SYSTAT software (SYSTAT, Inc., Evanston, IL). Experimental data were reported as mean values ± standard errors of the mean (SEM).

Mixture design, generation of response surfaces, desirability functional analysis, optimization, and contour plots were accomplished using the Expert Design (trial version 7.1.6, Stat-Ease Inc., Minneapolis, USA) statistical software.

3. Results and discussion

3.1. Proximal composition

No significant differences were found in the chemical composition of the studied formulations ($P > 0.05$). Average protein content was 12.9 ± 0.3 g/100 g, and due to the source (beef muscle and dairy products), they were proteins of high biological value. Low salts addition to the batters produced low ash levels (2.8 ± 0.1 g/100 g). Mean lipid content was 6.3 ± 0.3 g/100 g and reflected the amount of fat or oil added plus the lipids contained in the meat, indicating that fat losses occurred during processing were low, thus the obtained emulsions were stable.

3.2. Effect of salt content on protein denaturation and non-frozen water fraction

Although endothermic events of meat proteins are typically grouped in three regions, with peak temperatures around 54–58 °C related to myosin (region I), 65–67 °C collagen and sarcoplasmic proteins (region II), and 80–83 °C related to actin (region III) (Graiver, Pinotti, Califano, & Zaritzky, 2006; Martens, Stabursvik, & Martens, 1982; Tornberg, 2005; Wright, Leach, & Wilding, 1977), Fernández-Martín, López-López, Cofrades, and Colmenero (2009) informed that salts (NaCl, TPP, KCl) addition to meat comminuting can decrease the myofibrillar-proteins thermal stability, making the myosin and actin converge to the same denaturation temperature. This type of biphasic thermogram produced by

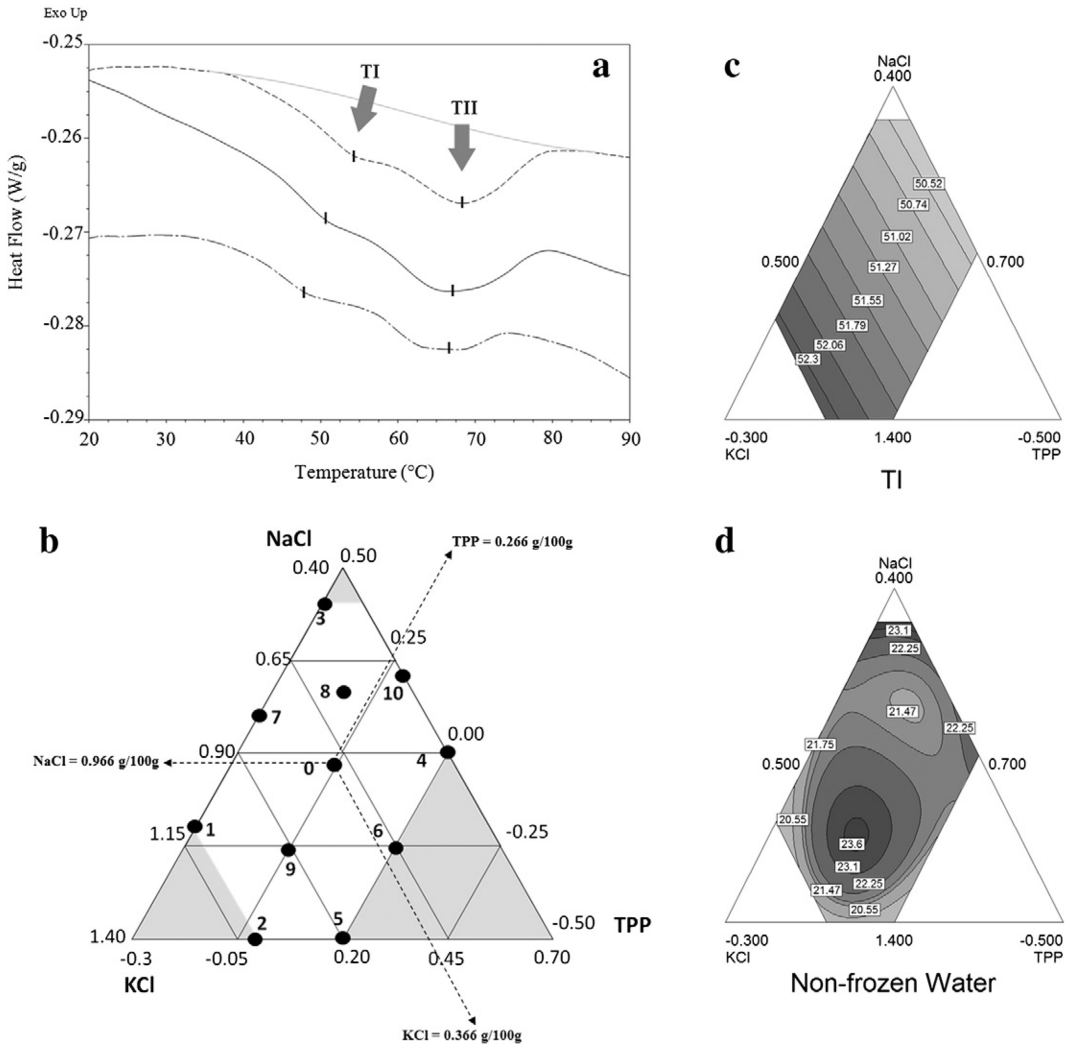

Fig. 1. Effect of salts concentration on a) MDSC thermograms, Codes: (— — —) Formulation 2; (— - —); Formulation 7; (——) Formulation 10. b) Example of reading for the triangular contour plot. c) protein denaturation temperature (TI, °C) and d) non-frozen water (g/100 g batter). Darker colors indicate higher values of the modeled parameters.

the salt added during comminution or by diffusion process was also informed by several authors. (Graiver et al., 2006) and Giménez, Graiver, Califano, and Zaritzky (2015) studying the diffusion of sodium chloride in pork tissue found that when NaCl concentration in the brine was increased to 20 g/l only two peaks were observed in the thermograms; peaks II and III were contracted to one peak which corresponds mainly to actin thermal transition. Thorarinsdottir, Arason, Geirsdottir, Bogason, and Kristbergsson (2002) found that the salt-curing of cod muscle decreased, made broader, and less separable the transitions peaks compare to the fresh tissue. They reported that after brine salting the peaks shifted to lower temperatures and it was not possible to distinguish between the respective transitions of sarcoplasmic proteins and myosin. This was likely due to an expected decrease in sarcoplasmic proteins and partial breakdown of the myosin during brining, indicating disassociation of the myosin heavy chain. The destabilizing effect of NaCl on whole beef muscles and isolated myofibrils was also informed in the research article of Pighin, Sancho, and Gonzalez (2008)

Fig. 1a shows DSC traces obtained for several sausages formulations with different amount of added KCl. It is easily seen that a complex biphasic endotherm appeared in the protein denaturation zone; TI and TII peaks are indicated. Marchetti et al. (2013) working on thermal properties of low-lipid meat emulsions formulated with different binders also informed this type of behavior. These changes in the appearance of the denaturation endotherm could be attributed to a partial breakdown of the myosin, indicating disassociation of the myosin heavy chain (Thorarinsdottir et al., 2002). As both peaks were very close, only the total enthalpy of transitions for the protein zone was calculated.

The partial substitution of NaCl by KCl and/or TPP significantly ($P < 0.05$) affected protein denaturation temperature TI, attributed to myosin gelation, which involves partial denaturation of myosin head portions followed by aggregation through disulphide bonds and a three-dimensional network formations as a result of thermal unfolding of the helical tail portion (Damodaran, 1997; Sun & Holley, 2010); Fig. 1b shows, as an example, the interpretations of the salts levels of

Table 2
Denaturation temperature TI, and non-frozen water content obtained by MDSC.

Formulation	TI (°C)	Non-frozen water content (g non-frozen H2O/100 g H2O)
0	51.6 ± 0.3	21.8 ± 0.3
1	52.1 ± 0.4	18.9 ± 0.4
2	53.4 ± 0.5	18.9 ± 0.4
3	50.9 ± 0.4	23.7 ± 0.8
4	49.9 ± 0.3	23.6 ± 0.9
5	52.4 ± 0.3	21.8 ± 0.5
6	50.5 ± 0.3	20.9 ± 0.3
7	51.8 ± 0.3	20.7 ± 0.2
8	51.3 ± 0.1	21.2 ± 0.6
9	51.2 ± 0.5	21.5 ± 0.7
10	49.8 ± 0.4	24.6 ± 0.7

± SEM values.

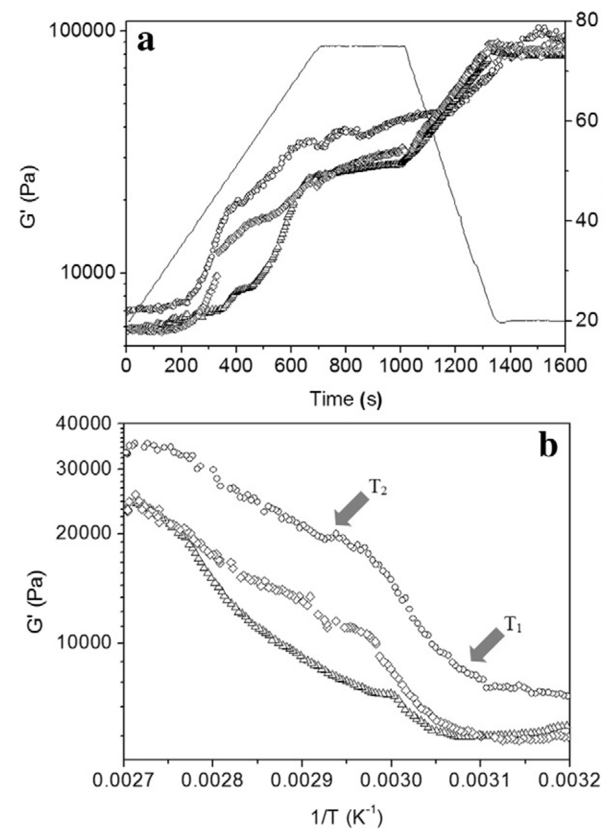

Fig. 2. a) Elastic modulus (G') evolution for different assayed formulations as a function of time during the thermo-rheological tests, b) Elastic modulus (G') evolution as a function of 1/T. Codes: 8 Formulation 2; M Formulation 8; – Formulation 10; (——) Thermal history.

the formulation 0 in the triangular contour plot. Fig. 1c shows the obtained contour plot; the corresponding values are shown in Table 2.

The highest denaturation temperature TI (53.4 ± 0.5 °C) corresponded to formulation 2, which contained the highest NaCl level (1.4 g/100 g), while lower temperatures were obtained for high KCl content (formulations 4 and 10, TI = 49.9 ± 0.3 °C and 49.8 ± 0.4 °C respectively). It can be seen that higher potassium levels, associated with a higher chaotropic effect, affected the stability of these myofibrillar proteins, resulting in lower values of TI (Fig. 1c). However proteins associated with the region II (mainly actin) were not significantly affected, reflecting its greater stability and resistance to environmental modification. Total denaturation enthalpy (ΔH_d) was not significantly affected ($P > 0.05$) by the partial sodium replacement either. Average values were: TII = 66.08 ± 0.19 °C, $\Delta H_d = 8.3 \pm 0.1$ J/$g_{protein}$ These results agree with previous studies on low-fat meat sausages without KCl (Marchetti et al., 2013). Lack of fit and precision adequacy values of TI and non-frozen water content revealed that the models were convenient for the predictions.

A complex effect of salts on non-frozen water content was observed (Fig. 1d), reaching a maximum at 1.14 g/100 g NaCl, 0.22 g/100 g KCl, and 0.24 g/100 g TPP, while an abrupt decrease occurs at higher values of NaCl or TPP, and also at higher potassium content (NaCl = 0.75 g/100 g, KCl = 0.57 g/100 g and TPP = 0.28 g/100 g); finally, a significant increase was observed above 0.57 g of KCl g/100 g. Higher non-frozen water content implies that water is more tightly associated with proteins through charged groups and polar sites and it is correlated with higher water holding capacity of the product (Marchetti et al., 2013) since it is influenced by the structure of myofibrils.

These results agree with Colmenero, Ayo, and Carballo (2005) who reported that the KCl addition improved water interaction properties of sodium reduced sausages. Differences could be related to diverse cation (K^+ and Na^+) levels and their location in the Hofmeister series (Hofmeister, 1888). The Hofmeister series mechanism is not entirely clear but may not be the result of changes in the overall structure of the water, but the result of more specific interactions between ions and proteins and ions and water molecules in direct contact with proteins instead (Zhang & Cremer, 2006).

3.3. Effect of Na^+ partial substitution on thermo-rheological experiments

The major changes observed in the thermo-rheograms corresponded to the elastic modulus, evidencing the sol–gel transition which is mainly reflected by changes in this modulus. As an example Fig. 2 shows the evolution of the elastic modulus obtained for three raw formulations containing different combinations of salts.

Fig. 2a shows that during the heating stage, there was a noticeable increase in G′ between 45 °C and 60 °C related to an elastic gel like matrix formation as a result of proteins denaturation. This behavior is typical of myofibrillar proteins gelation in presence of salts (Tahergorabi & Jaczynski, 2012). Given the denaturation temperature range of myosin (50–60 °C) which is a multidomain protein where the domains unfold independently of each other (Privalov & Gill, 1987), it is likely that the increase in the elastic characteristics of the samples could be attributed to this transition. However, the constant rise of G' indicates that the new structure formation was not completed before reaching 75 °C. These observations agree with the description of Fernández-Martín et al. (2009) who evaluated the gelation of several meat systems.

Among the factors affecting gelation properties of myofibrillar proteins, the ionic strength is considered important because it affects their solubility. However, Feng and Hultin (2001) informed that elastic gels with high water-holding capacity can be formed directly from water-washed minced muscle at physiological ionic strength (0.15) without first solubilizing myofibrillar proteins at high salt

Table 3
Regression coefficients of the proposed model for the variables: process yield, and, color parameters (L* and b*), texture variables (hardness, springiness, adhesiveness, and chewiness), expressed in terms of the coded concentrations of the salts.

Regression coefficients	Process yield	L*	b*	Hardness	Springiness	Adhesiveness	Chewiness
NaCl	61.38 ± 0.12	37.53 ± 0.41	7.88 ± 0.31	6.27 ± 0.29	0.518 ± 0.01	0.06 ± 0.008	2.32 ± 0.2
KCl	58.79 ± 0.78	35.39 ± 2.91	13.85 ± 1.88	3.94 ± 0.64	0.387 ± 0.073	0.0041 ± 0.001	3.33 ± 0.53
TPP	60.11 ± 0.46	67.54 ± 5.8	16.46 ± 3.54	7.61 ± 0.97	−0.0983 ± 0.013	0.03 ± 0.003	4.03 ± 0.78
NaCl x KCl	2.67 ± 0.91	4.33 ± 0.5	−8.27 ± 2.4	-	0.213 ± 0.092	-	-
NaCl x TPP	4.37 ± 1.23	−36.92 ± 6.4	11.19 ± 3.75	-	0.704 ± 0.014	-	−2.29 ± 0.70
KCl x TPP	61.38 ± 0.12	−38.67 ± 6.3	−22.51 ± 8.0	-	1.09 ± 0.32	-	0.15 ± 0.082
NaCl x KCl x TPP	-	35.78 ± 3.8	20.07 ± 7.62	-	−0.842 ± 0.32	-	-
NaCl x KCl x (NaCl - KCl)	-	-	-	-	-	-	-
NaCl x TPP x (NaCl - TPP)	-	15.62 ± 1.9	-	-	-	-	6.24 ± 3.12
KCl x TPP x (KCl - TPP)	-	−0.87 ± 2.9	-	-	-	-	−12.94 ± 4.14
Lack of Fit (P)	0.144	0.58	0.96	0.38	0.35	0.77	0.1
Adequate Precision	7.33	11.71	6.71	7.25	8.78	4.6	7.89

± Standard deviation.

Table 4
Thermo-rheological parameters of raw and cooked meat product with different salt contents.*

Formulation	T_1 (°C)	T_2 (°C)	G' (Pa)		
			uncooked (20 °C)	cooked (75 °C)	cooked (20 °C)
0	$55.9^{f} \pm 0.3$	$68.9^{d} \pm 0.5$	$6175^{d} \pm 5$	$27086^{d} \pm 13$	$73078^{d} \pm 230$
1	$62.8^{ab} \pm 0.5$	$71.7^{a} \pm 0.2$	$6936^{b} \pm 2$	$28667^{c} \pm 9$	$88498^{a} \pm 100$
2	$60.4^{e} \pm 0.2$	$68.2^{e} \pm 0.3$	$5779^{f} \pm 2$	$28847^{c} \pm 8$	$79136^{c} \pm 81$
3	$63.0^{a} \pm 0.3$	$71.1^{b} \pm 0.2$	$5721^{f} \pm 3$	$31082^{b} \pm 14$	$84447^{b} \pm 80$
4	$51.1^{h} \pm 0.2$	$69.4^{c} \pm 0.5$	$6528^{c} \pm 3$	$23129^{f} \pm 5$	$82508^{bc} \pm 153$
5	$62.3^{b} \pm 0.4$	$71.0^{b} \pm 0.4$	$5865^{ef} \pm 3$	$25347^{e} \pm 32$	$82160^{bc} \pm 163$
6	$61.2^{c} \pm 0.4$	$69.3^{cd} \pm 0.6$	$5914^{de} \pm 3$	$23488^{f} \pm 5$	$78599^{c} \pm 87$
7	$59.4^{e} \pm 0.3$	$69.7^{c} \pm 0.3$	$7396^{a} \pm 4$	$29074^{bc} \pm 6$	$74693^{d} \pm 75$
8	$63.3^{a} \pm 0.1$	$72.2^{a} \pm 0.2$	$5801^{ef} \pm 4$	$27996^{c} \pm 6$	$87399^{a} \pm 208$
9	$54.1^{g} \pm 0.3$	$67.6^{f} \pm 0.2$	$7230^{ab} \pm 3$	$26420^{d} \pm 9$	$58070^{e} \pm 130$
10	$51.2^{h} \pm 0.6$	$69.8^{c} \pm 0.3$	$6952^{b} \pm 4$	$38100^{a} \pm 16$	$83121^{b} \pm 170$

± SEM values.
* Means with same superscript within same column do not differ significantly (P > 0.05).

concentrations. This could explain the variably of the obtained results with the different salts combination employed.

Transition temperatures ranges during the heating stage were determined from the log (G') vs. 1/T data; Fig. 2b shows that two characteristic temperatures (T_1 and T_2) can be determined at the intersection of the tangent lines, which are shown in Table 4. T_1 ranged between 51.1 °C and 63.3 °C which indicates that the transition was strongly affected by formulation, in agreement with the analysis of protein denaturation temperatures (TI) by MDSC. Temperature range for T_2 was smaller (67.6–72.2 °C). Although temperature ranges of T_1 and T_2 were consistently higher than those determined by MDSC, a significant correlation was found between TI and T_1 ($R^2 = 0.81$, $P < 0.05$).

Besides to analyze the different events that occur during the test, average values of the elastic modulus G' at several characteristic temperatures were determined and compared a: i) sample at 20 °C; ii) isothermal stage at 75 °C; iii) cooked sample at 20 °C, after thermal treatment (Table 4).

During the 75 °C isothermal stage those formulations containing TPP and a high content of KCl exhibited the highest solid-like behavior (highest G', Table 4). Gel strength of salt-soluble beef protein was the strongest after addition of potassium chloride, followed by sodium chloride, and then calcium chloride (Sun & Holley, 2010). Also Totosaus, Alfaro-Rodriguez, and Pérez-Chabela (2004) informed that κ-carrageenan addition improved quality and sensorial parameters of low-fat meat products due to the incorporation of K^+ or Ca^{2+} which stabilize the carrageenan molecules. On the other hand, partially sodium-replaced formulations without TPP (4, 5, and 6) were less elastic than samples containing TPP (0, 1, 7, 8 and 9) indicating the TPP importance in gel structure development.

Samples cooling from 75 to 20 °C increased their solid characteristics, which could be attributed to the formation of H-bonds at lower temperatures. Finally, at 20 °C, thermally treated samples presented G' values between 73078 and 88498 Pa, except formulation 9 with a markedly smaller elastic modulus (58070 Pa).

3.4. Effect of NaCl partial replacement on quality parameters

All the formulations showed high process yield values (≥98 g/100 g) that significantly varied with salts content (Fig. 3a and Table 3). Other authors have reported similar results consistent with this work. Totosaus et al. (2004) informed that the NaCl partial replacement by other salts (KCl or $CaCl_2$) did not negatively affect process yield or water retention capacity of low fat sausages. Moreover Trius, Sebranek, Rust, and Carr (1994) reported a process yield increase in meat system when KCl was used as substitute of NaCl. These authors suggested that the KCl presence induces formation of a structure capable of retaining water. The κ-carrageenan incorporation to meat systems with low ionic strength containing KCl produced a strong gel where the interaction between K^+ and carrageenan make up for the lower solubility of myofibrillar proteins without adverse effects in the product (Totosaus et al., 2004).

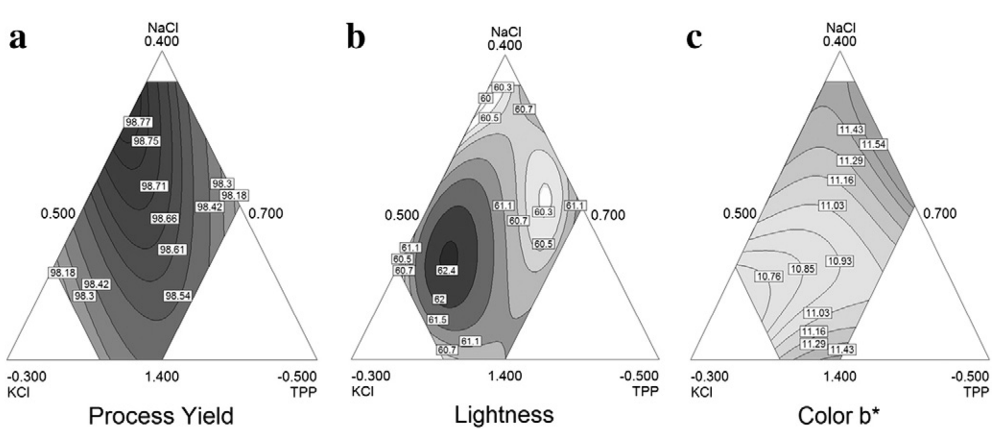

Fig. 3. Contour plots of a) process yield (g/100 g); color parameters: b) lightness (%), and c) b* (%), as a function of NaCl, KCl, and TPP content (g/100 g batter). Darker colors indicate higher values of the modeled parameters.

Table 5
Resilience, cohesiveness and color parameter a* of different studied formulations.*

Formulation	a*	Resilience (J/J)	Cohesiveness (J/J)
0	$16.85^{bcd} \pm 0.09$	$0.385^{bcd} \pm 0.004$	$0.546^{c} \pm 0.004$
1	$16.76^{bcd} \pm 0.04$	$0.435^{ab} \pm 0.004$	$0.585^{a} \pm 0.004$
2	$16.90^{bc} \pm 0.08$	$0.401^{bcd} \pm 0.004$	$0.559^{bc} \pm 0.004$
3	$18.30^{a} \pm 0.03$	$0.386^{bcd} \pm 0.004$	$0.559^{bc} \pm 0.004$
4	$17.74^{a} \pm 0.05$	$0.346^{ef} \pm 0.005$	$0.521^{d} \pm 0.005$
5	$17.84^{a} \pm 0.04$	$0.338^{f} \pm 0.004$	$0.521^{d} \pm 0.004$
6	$16.51^{bcd} \pm 0.1$	$0.374^{e} \pm 0.005$	$0.551^{bc} \pm 0.005$
7	$16.20^{d} \pm 0.08$	$0.404^{bc} \pm 0.004$	$0.563^{b} \pm 0.004$
8	$16.31^{cd} \pm 0.05$	$0.386^{cd} \pm 0.004$	$0.557^{bc} \pm 0.004$
9	$16.92^{b} \pm 0.05$	$0.412^{ab} \pm 0.005$	$0.564^{ab} \pm 0.005$
10	$18.08^{a} \pm 0.04$	$0.378^{d} \pm 0.005$	$0.547^{bc} \pm 0.005$

± SEM values.
* Means with same superscript within same column do not differ significantly ($P > 0.05$).

Significant differences in the three color parameters, lightness (L*), redness (a*), and yellowness (b*), were found. However, only L* and b* data could be mathematically fitted by the model proposed (Fig. 3b, c, and Table 3). A marked effect of KCl was observed. Lower b* values were obtained with medium and low KCl levels. Average a* values (Table 5) ranged from 16.2 to 18.3, resulting in a red color typical of these products. Carraro, Machado, Espindola, Campagnol, and Pollonio (2012) reported that NaCl reduction in mortadella caused a significant variation of the color parameters with no alteration of a*. Similar results were reported by Gimeno, Astiasarán, and Bello (1999) who studied the effect of the NaCl replacement by KCl and $CaCl_2$ in the formulation of fermented sausages obtaining a significant variation of b* and L*.

In the present work hardness, elasticity, adhesiveness, and chewiness were significantly affected by formulation ($P < 0.05$) and could be mathematically fitted with the proposed model (Fig. 4 and Table 3). Hardness ranged from 8.22 - 10.62 N and presented a linear dependence with the three salts content. The lowest regression coefficient corresponded to KCl, reflecting its smaller contribution to product hardness than NaCl or TPP, as shown in Fig. 4a. For a given KCl level, hardness increased with TPP fraction.

Both springiness and chewiness presented a complex dependence with salts combination (Table 3). The first order coefficient for TPP was negative, which could be explained by the interaction with the meat matrix increasing the protein-water interactions and decreasing the protein-protein ones (Ruusunen & Puolanne, 2005) resulted in a more difficult structure recovery after deformation. However, third order interaction coefficient (NaCl x KCl x TPP) had stronger impact than the linear coefficient, so that salts addition together with TPP results in a product with more springiness, confirming the synergistic effect of the three salts.

Adhesiveness was low for all formulations, typical of these products (Andrés et al., 2009). It showed a linear dependence with salts addition, being KCl the component with more impact on this property (Fig. 4c). The resulting model for chewiness included first order terms, double,

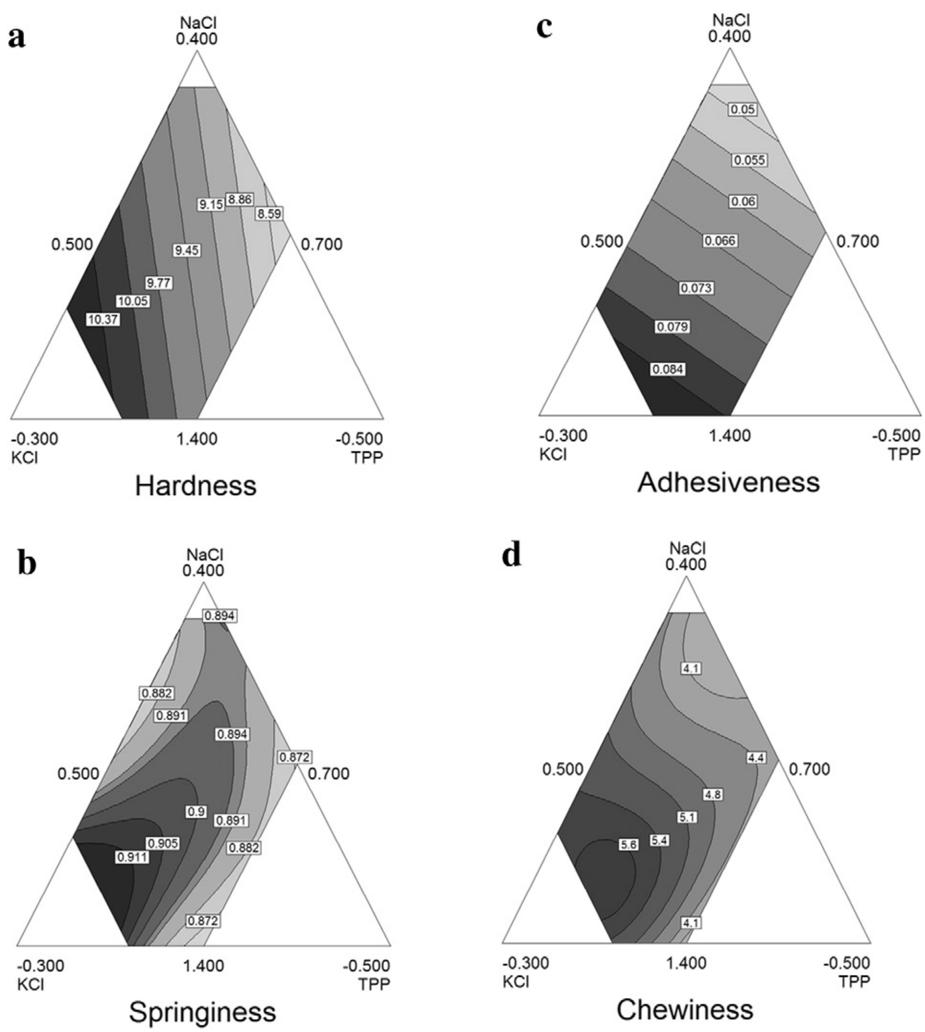

Fig. 4. Contour plots corresponding to the variation of: a) hardness (N), b) springiness (J/J), c) adhesiveness ($Jx10^{-3}$), and d) chewiness (N) with the NaCl, KCl and TPP content (g/100 g batter). Darker colors indicate higher values of the modeled parameters.

triple, and complex interaction. Fig. 4d shows that KCl had an important effect on this parameter. Chewiness decreased when KCl increased and this can be related with the negative interactions terms KCl x TPP and KCl x TPP x (KCl - TPP).

Resilience and cohesiveness also showed significant differences between treatments, however they could not be mathematically adjusted by the proposed model. The parameters values are given in Table 5.

Literature shows diverse texture results depending on meat system, type, and salt level used as NaCl partial replacer. Horita, Morgano, Celeghini, and Pollonio (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%. The amount of extracted or solubilized protein can also decrease when NaCl is reduced or replaced (Gordon & Barbut, 1991), thus affecting water retention capacity and gel strength (Whiting, 1984).

Sofos (1983) reported significant changes in sausages texture when NaCl replacement was over 20%, the hardness and elasticity decreased when NaCl levels became lower 1 g/100 g. Gou, Guerrero, Gelabert, and Arnau (1996) reported a 14 % reduction in hardness when NaCl (2.6 g/100 g) was replaced by NaCl (1 g/100 g), KCl (0.552 g/100 g), and $CaCl_2$ (0.74 g/100 g) mixture in fermented meat products.

Besides salt reduction (2.5 to 1.25 g/100 g) resulted into a soft texture in mortadella (Seman, Olson, & Mandigo, 1980) and sausage (Hand, Hollingsworth, Calkins, & Mandigo, 1987; Matulis, McKeith, Sutherland, & Brewer, 1995). On the other hand Jiménez-Colmenero, Fernández, and Carballo (1998) found no clear effect on texture parameters with different salt levels in meat products. Walstra (2002) reported that a 25% of NaCl replacement by KCl did not significantly affect sausages textural properties. However, these authors informed that with a 50% NaCl replacement by KCl a lower hardness and chewiness and unchanged elasticity and cohesiveness were obtained.

Regarding cohesiveness, bibliographic data reflect an ambiguous trend. Colmenero et al. (2005) reported that NaCl replacement by KCl in lean sausages produced an increase in cohesiveness. However Horita et al. (2011) reported a decreased in this parameter when NaCl was partially substituted for KCl (50%) in low fat mortadella.

3.5. Optimization and verification

Individual predictive equations were calculated (Eq. (1), Table 3); computed lack of fit tests were always non significant, and "adequate precision" values were in all cases above 4 indicating a high significance and adequacy of the models. Five objective functions were defined for the variables that were significantly affected by the salt content: process yield, hardness, adhesiveness, springiness, and lightness. Several studies reported that lightness (L^*) was the color parameter that better reflects the color changes in meat products (Carraro et al., 2012; Mielnik & Slinde, 1983; Oellingrath & Slinde, 1985). Therefore, this parameter was considered in order to optimize the formulations, in detriment of the color parameter b^*, which was not used. Besides chewiness was not included in this procedure because it is a linear function of hardness, springiness, and cohesiveness; therefore to incorporate this parameter would be redundant (Marchetti et al., 2014).

Individual desirability functions for each response variable and the overall desirability function (D) were calculated. Then D was maximized with respect to the NaCl, KCl, and TPP levels. Table 6 shows the optimization criteria used for each response. Process yield was maximized; hardness, adhesiveness, springiness, and lightness were set in a range similar to an Argentinean commercial product containing 20 g/100 g of beef fat. According to the obtained results the optimum combination of salts was NaCl = 0.608 g/100 g; KCl = 0.492 g/100 g; and TPP = 0.500 g/100 g. The overall desirability function value was D = 0.892; this value is quite satisfactory as it is close to 1.

Table 6 shows experimental and predicted values for process yield, texture, and color parameters for the optimum Na reduced formulation, which were not statistically different ($P > 0.05$) for all the analyzed variables. Therefore, it may be concluded that the chosen mathematical procedure adequately predicted the quality attributes for the sodium-reduced meat sausage.

Table 6
Optimization criteria and predicted and experimental values of process yield, hardness, springiness, adhesiveness, chewiness, and lightness obtained for the optimized sodium-reduced formulation.

Response variables	Optimization criteria	Predicted values	Experimental	
			Mean	Confidence Interval ($\alpha = 0.05$)
Process yield (g/100 g)	Maximum	98.78	98.6	98.1–99.1
Hardness (N)	9.00–10.00	9.55	9.97	9.50–10.44
Adhesiveness (J)	0.02–0.6	0.054	0.3	0.02–0.579
Springiness (J/J)	0.845–0.973	0.877	0.902	0.869–0.925
Lightness	58–61	59.99	59.75	59.35–60.15
Chewiness	-	4.61	5.02	4.60–5.44

3.6. Microstructure

Microstructure of optimized (NaCl = 0.608 g/100 g; KCl = 0.492 g/100 g; and TPP = 0.500 g/100 g.) and control formulation (2; NaCl = 1.4 g/100 g; KCl = 0 g/100 g; TPP = 0.4 g/100 g) were observed by environmental scanning electronic microscopy. The resulting structures were characteristic of a cooked meat-emulsion systems, as describe by several authors (Andrés, García, Zaritzky, & Califano, 2006; Andrés et al., 2009; Ayadi, Kechaou, Makni, & Attia, 2009; Carballo,

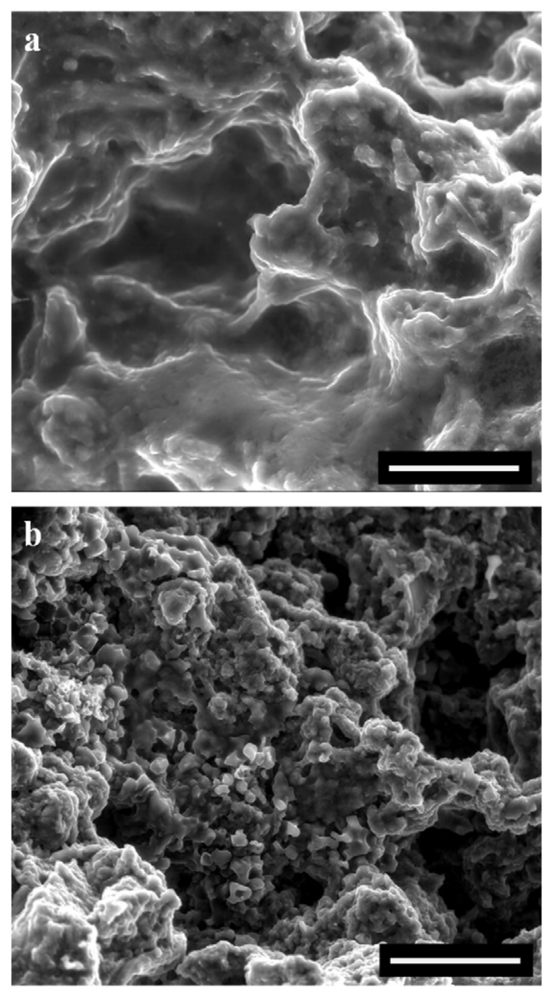

Fig. 5. Environmental scanning microscopy of cooked matrices of a) control (formulation 2) and b) reduced sodium formulation. White bars indicate 50 µm.

Table 7
Sensory appearance, color, flavor, texture, and overall acceptability scores of low-fat meat sausages containing fish oil with (Optimized) or without (Control, non-replaced) partial NaCl replacement.*

Formulation	Appearance	Color	Flavor	Texture	Overall acceptability
Control	$7.3^a \pm 0.2$ (100)	$7.7^a \pm 0.2$ (96.3)	$5.3^a \pm 0.3$ (59.3)	$5.7^a \pm 0.3$ (74.1)	$5.8^a \pm 0.3$ (70.4)
Optimized	$6.7^a \pm 0.2$ (88.9)	$6.3^b \pm 0.3$ (85.2)	$5.2^a \pm 0.3$ (55.6)	$5.7^a \pm 0.3$ (77.8)	$5.4^a \pm 0.3$ (63)

± SEM values.
* 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.

Fernandez, Barreto, Solas, & Colmenero, 1996; Chen, Xu, & Wang, 2007). A gel-like compact structure was observed, with a three-dimensional network with numerous cavities, tending to a sponge-like structure. The formation of these cavities may be due to the expansion of a number of components during the heat treatment of the product, mainly water, oil, or air (Cavestany, Jiménez Colmenero, Solas, & Carballo, 1994). This structure resulted coincident with that reported by Ayadi et al. (2009) for turkey sausage with carrageenan and by Verbeken, Neirinck, Van Der Meeren, and Dewettinck (2005) who studied the influence of κ-carrageenan on sarcoplasmic proteins gels.

The morphological observations in these products indicate that the characteristics of the continuous protein matrix would be slightly affected by the type and levels of salts used (Fig. 5 a, and b). The formulation without NaCl replacement (formulation 2, Fig. 5a) exhibited a continuous microstructure where protein aggregates were embedded into a homogeneous matrix. On the other hand, the partial sodium substitution presented a less embedded matrix with a coarse appearance (Fig. 5b). This would indicate that the KCl presence may alter the meat system structure, decreasing the amount of extracted proteins (solubility), thus generating a particulate aspect. This effect could explain the above-mentioned differences in textural parameters. However, in all cases, obtained gels had adequate quality parameters for sausages. Totosaus et al. (2004) postulated that a complete solubilization of myofibrillar proteins is not necessary for proper gel formation.

3.7. Sensory analysis

NaCl is a multifunctional component in meat products acting as a preservative, ensuring favorable functional characteristics, and improving the flavor (Gillette, 1985). Besides suppress off-flavors by the Na ability to inhibit the bitter taste, increasing positive flavor attributes without only contributing to salty perception. Therefore, reducing salt must deal not only with the decreased perceived saltiness, but also how salt reduction impacts over flavor attributes. Ruusunen and Puolanne (2005) stated that NaCl level in the traditional diet of a population, with its associated salty taste, determines the maximum NaCl amount that can be reduced. Consequently, reducing the sodium content of the products may not be equally acceptable for all the consumers.

Mean attribute scores given by the panelists are shown in Table 7. Both control and optimized sausages had acceptable sensory scores (greater than 5) for appearance, flavor, texture, and overall acceptability. Only color scores showed significant differences ($P < 0.05$) between formulations. 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 94% of the panelists liked product appearance, 90% liked the color, 60% liked the flavor, 76% liked the texture and 66.7% liked the products, considering the overall acceptability of both sausages formulations. These results showed that the NaCl partial replacement did not adversely affect the product. In conclusion, low-fat, low-sodium meat sausages containing 5% of refined fish oil with tocopherols and phytosterols showed very good sensory attributes.

4. Conclusions

Sodium chloride partial replacement in meat sausages containing 5 g/100 g fish oil and 100 mg/100 g phytosterols was achieved with the addition of KCl and TPP to obtain good physicochemical and quality characteristics. The effect of salts on several quality and thermal parameters was studied and accurately modeled employing a mixture design. An optimal formulation was proposed with 58% and 70% sodium reduction when comparing to US (USDA, 2014) and Argentinean commercial sausages (Vienissima) and no negative effects due to Na^+ replacement. Rheological and microstructural studies determined that the gel structure was altered with the salts composition, but sensory studies showed that salts modification did not have a negative impact on the tested attributes leading to an innovative and healthier product.

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