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Low-fat meat sausages with fish oil: Optimization of milk proteins and carrageenan contents using response surface methodology

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ABSTRACT

Response surface methodology was used to analyze the effect of milk proteins and 2:1 κ:ι-carrageenans on cooking loss (CL), weight lost by centrifugation (WLC) and texture attributes of low-fat meat sausages with pre-emulsified fish oil. A central-composite design was used to develop models for the objective responses. Changes in carrageenans affected more the responses than milk proteins levels. Convenience functions were calculated for CL, WLC, hardness, and springiness of the product. Responses were optimized simultaneously minimizing CL and WLC; ranges for hardness and springiness corresponded to commercial products (20 g of pork fat/100 g). The optimum corresponded to 0.593 g of carrageenans/100 g and 0.320 g of milk proteins and its total lipid content was 6.3 g/100 g. This formulation was prepared and evaluated showing a good agreement between predicted and experimental responses. These additives could produce low-fat meat sausages with pre-emulsified fish oil with good nutritional quality and similar characteristics than traditional ones.

1. Introduction

Recommendations about lipid consumption (WHO, 2010) encourage to diminish the intake of saturated fat and to increase the unsaturated lipids. Meat products are one of the main sources of dietary saturated fat, so, changes in its fat content and fatty acid (FA) profile could help to increase its nutritional quality (Fernandéz-Ginés, Fernández-Lopez, Sayas-Barbera, & Perez-Alvarez, 2005). The increase in the consumption of n-3 polyunsaturated FA (n-3 PUFA: α-linolenic acid, ALA, eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) had been related in several studies with potential healthy effects as keep normal cholesterol blood level (European Commission, 2012). Recently, there has been a growing interest in developing products with a health concept such as a high omega-3 fatty acid product (Lee, Joaquin, & Lee, 2007). A way to increase the intake of n-3 PUFA is by incorporating them in traditional products.

Manufacture of low-fat sausages is usually achieved by two basic principles: the use of lean meats (which increases cost) and/or reduction of fat and caloric content by adding water ingredients that introduce less or no calories.

In emulsified meat products, diminishing fat without increasing water content results in a harder product (Hand, Hollingsworth, Calkins, & Mandigo, 1987), while fat replacement with water increases exudative and cooking losses (Su, Bowers, & Zayas, 2008) and also affects texture and juiciness of the product (Cierach, Modzelewska-Kapitula, & Szaciło, 2009). However Ordoñez, Rovira, and Jaime (2001) indicated that low-fat (10%) frankfurters with a texture profile similar to standard ones could be manufactured.

In order to reduce these problems different strategies had been applied such as: add proteins (soy proteins, whey protein concentrate, gluten, sodium caseinate), gums (xanthan, locust bean gum, carrageenans, microcellulose, pectins, konjac), and/or sodium polyphosphate in different combinations (Ayadi, Kechaou, Makni, & Attia, 2009; Brewer, 2012; Candogan & Kolsarici, 2003; Hsu & Sun, 2006; Jiménez-Colmenero et al., 2012; Lurueña-Martínez, Vivar-Quintana, & Revilla, 2004; Muchenje et al., 2009; Pietrasik, 2003; Youssef & Barbut, 2010, 2011).

In a previous work, we studied low-fat meat emulsions with pre-emulsified deodorized fish oil as a source of unsaturated fatty acids. The addition of 1 g/100 g of several binders (milk proteins, whey protein concentrate, thermally treated whey protein concentrate, ovoalbumin, hydroxypropyl methylcellulose (HPMC), methylcellulose, mixtures of κ:ι carrageenans or xanthan-locust bean gums (XLBG)) to meat emulsions containing 5 g of fish oil/100 g, 25 g of water/100 g and 1.4 g NaCl/100 g, were compared to control formulations with 5 g/100 g of beef tallow or fish oil without additives. Cooking losses were lower than 4 g/100 g of meat emulsion, except for the addition of HPMC; formulations with hydrocolloids presented less weight lost by centrifugation than both controls. Addition of milk proteins, XLBG, or mix of carrageenans gave the highest hardness, similar to fat control (Marchetti, Andrés, & Califano, 2013).

Carrageenans addition in lean sausages had also been described by several authors (Ayadi et al., 2009; Candogan & Kolsarici, 2003) who

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obtained products with good physic-chemical characteristics. Besides, Atughonu, Zayas, Herald, and Harbers (1998) decreased cooking losses of sausages with milk proteins due to its high emulsifying capacity.

Regarding the replacement of animal fat by vegetable or marine oils in emulsified meat products, Park, Rhee, Keeton, and Rhee (1989) found that frankfurters with fish oil had undesirable flavor while those prepared with high oleic sunflower oil showed texture problems. Later, Park, Rhee, and Zipri (1990) developed a product adding high oleic sunflower oil and water simultaneously, with similar properties than the traditional. Andrés, García, Zaritzky, and Califano (2006) developed low-fat chicken sausages with pre-emulsified squid oil using whey protein concentrate as binder and obtained good functional properties and acceptable sensory scores. Cáceres et al. (2008) partially replaced beef tallow with pre-emulsified fish oil, caseinates, and water in cooked meat emulsions. More recently Ospina, Cruz, Pérez-Álvarez, and Fernández-López (2010) replaced pork fat with a mix of modified vegetable oils, while Álvarez et al. (2012) studied the viscoelastic and textural properties of pork sausages manufactured with a mix of canola and olive oils.

Response surface methodology (RSM) is a powerful mathematical and statistical technique for testing multiple process variables and their interactive and quadratic effects. It can help in investigating the interactive effect of several variables and in building a mathematical model that accurately describes the overall process (Myers & Montgomery, 2002; Velioğlu, Velioğlu, Boyaci, Yilmaz, & Kurultay, 2010). It has been effectively used to optimize formulations in a variety of food products such as low-fat frankfurters, bologna, and pork sausages or batters (Carballo, Barreto, & Jiménez-Colmenero, 1995; Murphy, Gilroy, Kerry, Buckley, & Kerry, 2004; Pietrasik & Li-Chan, 2002).

There is no universal replacement of fat; systems are usually formulated with more than one component, and their interactions must be considered with the purpose of making a product similar to the traditional one. The aims of this research were: i) to study the effect of the different levels of a combination of κ:ι carrageenans and milk proteins as stability agents in low-fat sausages containing pre-emulsified fish oil on quality parameters such as cooking loss, weight lost by centrifugation, and texture, ii) to model the effect of those additives on the mentioned parameters by Response Surface Methodology, iii) to use the desirability function as multicriteria tool to optimize the content of both milk proteins and carrageenans.

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 processors (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, & Jiménez-Colmenero, 2005).

As fat source, deodorized refined fish oil (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, Fluka BioChemika, Denmark) a synergistic combination (Candogan & Kolsarici, 2003) were used.

Cold distilled water was used in all formulations (4 °C). Analytical grade sodium chloride (NaCl), sodium nitrite ($NaNO_2$), sodium erythorbate 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, CAA, 1996).

The following components were included to prepare 100 g of uncooked meat batter: water (25 g); fish oil (5 g); NaCl (1.4 g); TPP (0.2 g); sodium erythorbate (0.045 g); $NaNO_2$ (0.015 g); ground pepper (0.2 g); and nutmeg (0.05 g). The content of Carr + MPr varied from 0.41 g to 2.40 g, thus the meat content varied from 65.69 g to 67.69 g accordingly to complete 100 g of batter.

2.2. Sausage formulation and processing

Elaboration of the sausages was according to Andrés, Zaritzky, and Califano (2008). 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 NaCl and TPP. Carrageenans, milk proteins, sodium 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 temperature of batter 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 Department of Health and Human Services (2009). Then, samples in the bags were cooled immediately in an ice-water-bath and stored at 4 °C until further analysis.

2.3. Lipid content

Lipid content was determined by triplicate on samples previously dried with anhydrous sodium sulfate (SO_4Na_2) by Soxhlet method (AOAC 24.003), using petroleum ether (Bp: 35–60 °C) as extraction solvent (Andrés, Zaritzky, & Califano, 2009).

2.4. Cooking loss (CL)

Cooking loss was determined (five replicates) by weighing the product before and after thermal treatment, and expressed as g/100 g of initial sample weight (Andrés et al., 2009; Candogan & Kolsarici, 2003).

2.5. Weight lost by centrifugation (WLC)

A modified method of Ayadi et al. (2009) was performed (eight replicates) to evaluate the stability of the matrices. Cylindrical sausage samples, previously weighted (approximately 0.4 g), were placed in Falcon tubes over glass beads and were centrifuged at 12,000 x*g* for 5 min at 4 °C. Liquids removed during centrifugations drained through the glass beads and were collected in the bottom of the centrifuge tube (Eide, Børresen, & Strøm, 1982). Weight lost by centrifugation (WLC) was calculated as a percentage of weight of liquid extracted from 100 g of sausage (Verbeken, Neirinck, Van Der Meeren, & Dewettinck, 2005).

2.6. Texture measurements

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 center 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 (SMSP/75), interfaced with a computer, using the software supplied by Texture Technologies Corp. In these experiments the probe was operated at 0.5 mm/s. 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.

2.7. Experimental design and statistical analysis

Nine different formulations of low-fat sausages were manufactured following a two-factors central composite design (Box & Draper, 1987) for the κ/ι carrageenans (Carr) and milk proteins (MPr) contents, with the center point replicated three times. Table 1 shows the actual and codified compositions, being the factors Carr and MPr. κ/ι carrageenans varied from 0 to 0.8 g/100 g and MPr from 0 to 2 g/100 g.

The runs were randomized in order to exclude block effects. Once the responses were obtained, the analysis of variance (ANOVA) was carried out to test the significance of the effects, (SYSTAT, Inc., Evanston, IL, USA). Experimental data were reported as mean values with the corresponding standard errors of the mean (SEM) given between parenthesis when appropriate. For simultaneous pairwise comparisons, least significance differences (LSD) test was chosen. Differences in means and F-tests were considered significant when $P < 0.05$.

A response surface methodology (RSM) was used to analyze the effect of the two independent variables (Carr and MPr) on the responses (Y) using the second-order polynomial response surface with the corresponding interactions:

$$Y = \beta_o + \beta_{Carr} x_{Carr} + \beta_{MPr} x_{MPr} + \beta_{CarrMPr} x_{Carr} x_{MPr} + \beta_{CarrCarr} x^2_{Carr} + \beta_{MPrMPr} x^2_{MPr} \quad (1)$$

where Y is the corresponding response variable; x_{Carr} and x_{MPr} are the codified independent variables (Carr and MPr, respectively) and βs are the coefficients of the model. 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 coefficient of determination, R^2, and the adequacy of the model was verified using a “lack of fit” test.

All statistical analysis, generation of response surfaces, desirability functional analysis, optimization, 3D and contour plots were accomplished using the Expert Design (trial version 7.1.6, Stat-Ease Inc., Minneapolis, USA) statistical software.

Table 1
κ/ι carrageenans and milk proteins levels added to low-fat sausages formulated with pre-emulsified fish oil.

Formulation	κ/ι Carrageenans		Milk proteins	
	g/100 g	Codified	g/100 g	Codified
1	0.117	−1	0.293	−1
2	0.117	−1	1.707	1
3	0.683	1	0.293	−1
4	0.683	1	1.707	1
5	0	−1.414	1.000	0
6	0.800	1.414	1.000	0
7	0.400	0	0.000	−1.414
8	0.400	0	2.000	1.414
9*	0.400	0	1.000	0

* The center point (0,0) was replicated three times (n = 3).

2.8. Optimization and verification

A multiresponse optimization was used to found the optimum formulation, using a desirability function approach which is based on the idea that the “quality” of a product or process has multiple quality characteristics, with none of them outside some “desired” limits. The method finds operating conditions that provide the “most desirable” response values (Myers & Montgomery, 2002).

For each response $Y_j(x)$, a desirability function $d_j(Y_j)$ assigns numbers between 0 and 1 to the possible values of Y_j, with $d_j(Y_j) = 0$ representing a completely undesirable value of Y_j and $d_j(Y_j) = 1$ representing a completely desirable or ideal response value. The individual desirabilities are then combined using the geometric mean, which gives the overall desirability D. With that function, a multiple response problem could be transformed into a one response, throughout mathematical transformations (Derringer & Suich, 1980). Desirability functions for each response variable that was significantly affected by milk proteins and/or carrageenan contents and the overall desirability function were calculated. Then D was maximized with respect to the carrageenans and milk proteins concentration.

The optimum formulation was used for calculating the predicted values of response variables using the predictive equations derived by RSM. A batch of sausages was prepared using the optimal ingredient levels and cooking loss, weight lost by centrifugation, and texture parameters were determined as described in Materials and Methods section. Finally results were statistically compared to the values predicted by the mathematical model.

3. Results and discussion

3.1. Water binding properties of the low-fat sausages

Cooking loss (CL) depends on the ability of the protein matrix to immobilize both fat and water. However, in meat batters with extremely low fat content, gelling ability and water retention capacity of non-meat ingredients may play a greater role rather than emulsion formation in determining thermal and storage stability of the products (Marchetti, Andrés, & Califano, 2011; Su et al., 2008).

Table 2
Cooking loss (CL), weight lost by centrifugation (WLC), hardness, springiness, and chewiness for low-fat sausages with fish oil and different κ/ι carrageenans and milk proteins levels.

Formulation	CL (g/100 g) (n = 5)	WLC (g/100 g) (n = 8)	Hardness (N) (n = 10)	Springiness (J/J) (n = 10)	Chewiness (N) (n = 10)
1	$2.2(0.03)^b$	$9.50(0.16)^a$	$8.09(0.06)^f$	$0.85(0.002)^h$	$3.73(0.04)^g$
2	$3.4(0.16)^a$	$8.34(0.08)^{abc}$	$8.74(0.06)^g$	$0.86(0.004)^g$	$4.09(0.04)^f$
3	$1.0(0.06)^d$	$4.23(0.2)^{de}$	$11.02(0.1)^c$	$0.94(0.003)^c$	$5.81(0.07)^b$
4	$1.5(0.08)^{c\ d}$	$3.22(0.06)^e$	$11.65(0.05)^b$	$0.97(0.001)^a$	$6.48(0.04)^a$
5	$1.2(0.11)^a$	$8.87(0.14)^{ab}$	$8.13(0.05)^f$	$0.86(0.002)^g$	$3.79(0.03)^g$
6	$1.1(0.07)^{cd}$	$2.98(0.2)^e$	$12.02(0.06)^a$	$0.96(0.001)^b$	$6.53(0.04)^a$
7	$2.1(0.11)^b$	$7.21(0.1)^{bcd}$	$9.35(0.07)^e$	$0.87(0.001)^f$	$4.47(0.03)^e$
8	$1.5(0.16)^{cd}$	$6.20(0.07)^{cd}$	$9.56(0.06)^e$	$0.89(0.0005)^e$	$4.69(0.03)^d$
9	$1.7(0.04)^{bc}$	$5.92(0.04)^d$	$10.05(0.06)^d$	$0.91(0.001)^d$	$5.14(0.03)^c$

Different superscripts in the same column indicate significant differences ($P < 0.05$). Standard error of the mean is given between parentheses. “n” indicates the number of replicates performed.

The obtained results for cooking loss and weight lost by centrifugation (WLC) of the studied formulations are showed in Table 2. Cooking losses were lower than 4 g/100 g, showing an adequate thermal stability of all the formulations. They also presented good water retention of the network producing low weight losses by centrifugation (<10 g/100 g). Hughes, Cofrades, and Troy (1997) found that the addition of 1 g/100 g of a combination of κ- and λ-carrageenan reduced cooking loss and enhanced water holding capacity and emulsion stability of frankfurters with 5, 12, and 30 g/100 g fat content. García-García and Totosaus (2008) found a positive correlation between water holding capacity and κ-carrageenan (0.245 g/100 g–1.5 g/100 g) added to low-fat sodium-reduced sausages. Lin and Mei (2000) found that the addition of 0.41 g/100 g of iota carrageenan to meat batters improved the emulsion stability and water-holding capacity and could possibly be due to the formation of a more stable complex with denatured meat proteins during heating. The carrageenans interact with polarity groups of protein and integrate them in its gel systems, then form stronger dimensional structure-gel. Also, the structure of carrageenan molecule is negatively charged leading to hydrogen bonds formation with free water (Li & Jiang, 2004). However, in some cases, the addition of carrageenan seems to have no or a very limited effect on the water holding capacity of meat gels (Barbut & Mittal, 1992; Bernal, Smajda, Smith, & Stanley, 1987; Foegeding & Ramsey, 1986).

The proposed models for both response variables were highly adequate because they had satisfactory levels of R^2 and no significant lack of fit was found (Gan et al., 2007). Table 3 shows the significant linear and quadratic regression coefficients obtained for CL and WLC. Quadratic approximations to the real function response were used because it was found that the interaction terms were not significant ($P > 0.05$), regardless of the response variable analyzed.

Water binding properties were strongly affected by carrageenan as shown in Fig. 1a and b, reflecting the higher values of the corresponding regression coefficients (Table 3). At high Carr content water binding properties of the system tend to increase resulting in lower CL and WLC (Fig. 1). At high and low MPr contents weight losses by centrifugation were higher and cooking loss diminished. Dexter, Sofos, and Schmidt (1993), Pietrasik and Duda (2000), and Pietrasik (2003) reported similar results in cooking loss and water holding capacity of meat batters with the addition of carrageenan. Jones and Mandigo (1982) proposed that a thick and rigid interfacial protein film (related with higher amounts of MPr) would not allow fat to expand during heating and would result in rupture holes and therefore fat separation/emulsion breakdown. Another possibility is that a high protein level forms a denser highly aggregated protein network (during cooking) which could put pressure on the fat globules to coalesce and squeeze out some of the protein matrix (Youssef & Barbut, 2010).

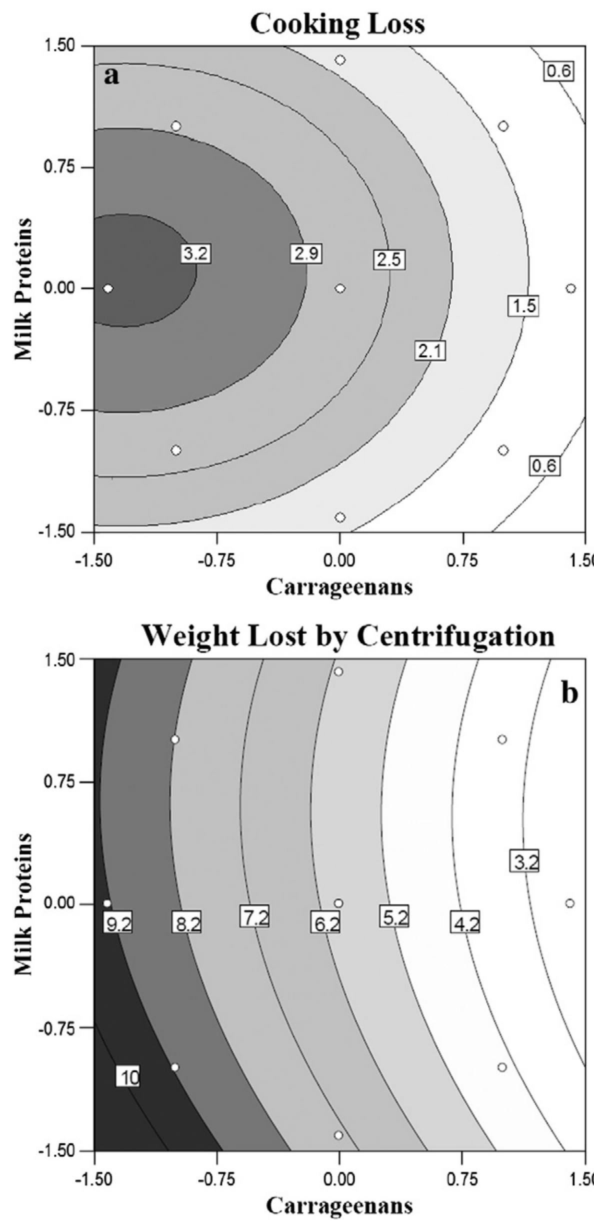

Fig. 1. Contour plots of: a) cooking loss and b) weight lost by centrifugation as a function of carrageenans and milk proteins contents expressed as codified values. The darkest shade indicates highest value and lines correspond to isoparametric values; ○ indicates the design points.

3.2. Texture of low-fat sausages with fish oil

The most important texture parameters to define and differentiate the texture of frankfurters are hardness, juiciness and those relating to them. A sausage of medium hardness and high juiciness was considered by the consumers to have the best texture (Ordóñez et al., 2001).

Regarding TPA parameters, cohesiveness and resilience were not significantly affected by changes in Carr and MPr levels ($P > 0.05$), being their mean values 0.554 (0.001) and 0.422 (0.002), respectively. Hardness, springiness, and chewiness were significantly affected by formulation ($P < 0.05$); mean values of these parameters are shown in Table 2.

Table 3
Regression coefficients of the proposed model for the variables: cooking loss (CL), weight lost by centrifugation (WLC), hardness, springiness, and chewiness.

β Coefficients	CL	WLC	Hardness	Springiness	Chewiness
Constant	2.75(0.3)	5.91(0.25)	10.04(0.13)	0.91(0.009)	5.19(0.08)
Carr	−0.76(0.1)	−3.31(0.17)	2.00(0.09)	0.06(0.006)	1.05(0.09)
MPr	0.11(0.1)	−0.64(0.17)	0.28(0.09)	0.01(0.006)	0.18(0.09)
Carr × Carr	−0.29(0.02)	0.02(0.33)	0.09(0.17)	–	–
MPr × MPr	−0.46(0.02)	0.80(0.33)	−0.53(0.17)	−0.03(0.012)	−0.27(0.1)
Lack of fit (P)	0.21	0.15	0.38	0.37	0.28
R^2	0.838	0.987	0.990	0.962	0.981

Standard error of the mean is given between parentheses.

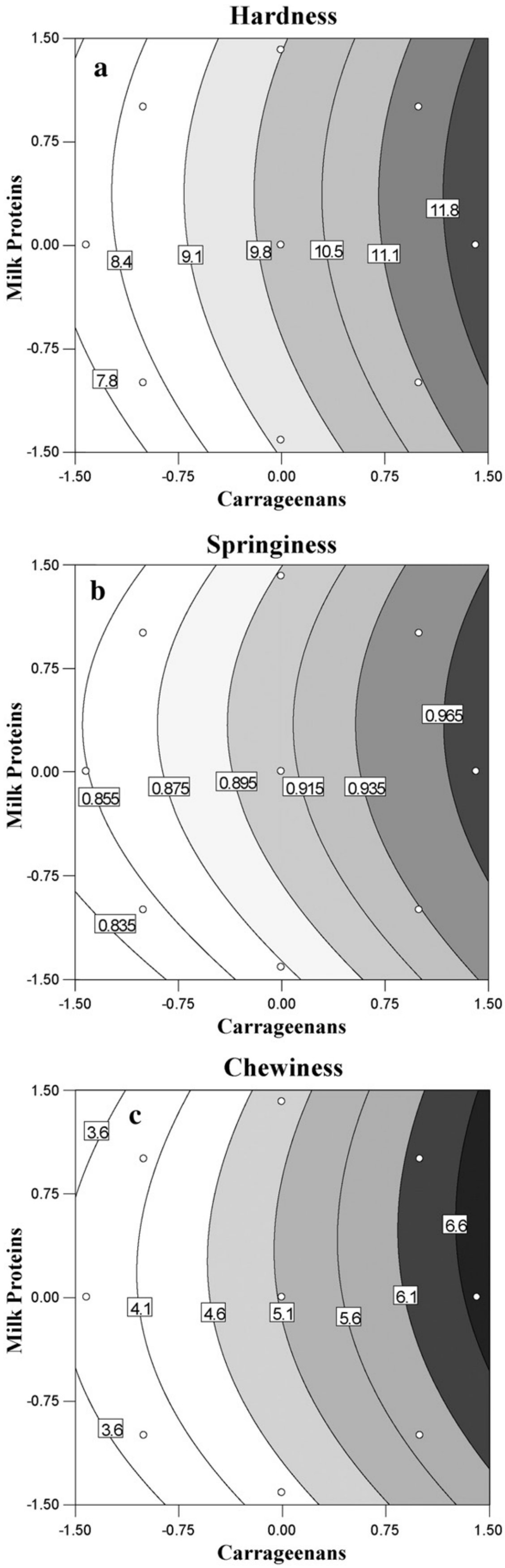

Fig. 2. Contour plots of: a) hardness, b) springiness, and c) chewiness as a function of carrageenans and milk proteins contents expressed as codified values. The darkest shade indicates highest value and lines correspond to isoparametric values; ○ indicates the design points.

It can be observed in Fig. 2 that the amount of κ/ι carrageenan mix added had a higher influence on the three parameters than the milk proteins level (larger β coefficients). Concerning the effect of MPr, the highest values corresponded to intermediate levels (MPr > 1) due to the negative quadratic β coefficient (Table 3). Youssef and Barbut (2009) informed that milk proteins affected elasticity of meat batters in a parabolic way. Hongsprabhas and Barbut (1997) reported a similar tendency of the penetration force with the addition of whey protein concentrate to poultry meat batters.

Our results also agree with Ayadi et al. (2009) who reported that κ/ι-carrageenan addition to turkey sausages increased hardness and water binding capacity. Pietrasik (2003) working with meat batters containing κ-carrageenan obtained an increase in hardness and fracturability of beef gels, but springiness and cohesiveness were not modified.

3.3. Optimization of basic formulation

The lack of fit test is a measure of the failure of a model to represent data in the experimental domain (Varnalis, Brennan, Macdougall, & Gilmour, 2004). Models for all the response variables were highly adequate because they had satisfactory levels of R^2 (>85%) and there were no significant lack of fit in all the response variables (Gan et al., 2007).

Once the individual predictive equations were known (Table 3), four desirability functions were defined for the variables that were significantly affected by formulation. Chewiness, as it is a lineal function of hardness, springiness, and cohesiveness, was not included in this procedure.

Table 4 shows the optimization criteria for each response. Cooking loss and weight lost by centrifugation were minimized, and textural parameters of the desirable ranges correspond to a commercial product with 20 g/100 g pork fat content (hardness: 9.9 ± 0.1 N, springiness: 0.922 ± 0.002 J/J, cohesiveness: 0.440 ± 0.001 J/J, chewiness 4.05 ± 0.29 N, resilience: 0.484 ± 0.001 J/J, adhesiveness: 0.49 ±0.08 J$\times 10^{-4}$, as reported by Marchetti et al., 2011).

The mathematical model used to found a global desirability value, D, is the geometric media of individual desirability values (Derringer & Suich, 1980; Harrington, 1965). The optimum combination of ingredients to add in low-fat sausages with pre-emulsified fish oil with a final fat content of 6.2 ± 0.2 g/100 g product corresponded to 0.593 g/100 g of κ/ι carrageenan mix and 0.320 g/100 g of milk proteins, with a global or overall desirability D = 0.794. Table 4 also shows the predicted responses for this composition and the corresponding d_j function values.

3.4. Verification of the model

Once the optimum formulation was determined, it was used to manufacture low-fat sausages and all the response variables of this product were analyzed. Experimental values of each response were compared with those predicted by the models and both set of values are presented in Table 5. Experimental and predicted values were not statistically different (P > 0.05). Thus, the chosen mathematical procedure adequately predicted the studied attributes. The meat sausages containing 5 g/100 g fish oil, 0.593 g/100 g of κ/ι carrageenan mix,

Table 4
Criteria used to obtain the desirability functions, predicted responses, and individual desirability functions (d_i).

Response variables	Optimization criteria	Predicted Response	d_j
Cooking loss (g/100 g)	Minimum	1.24	0.872
Weight lost by centrifugation (g/100 g)	Minimum	5.12	0.646
Springiness (J/J)	Range: 0.845–0.973	0.922	1.000
Hardness (N)	Range: 8.0–12.0	10.59	0.703

Table 5
Predicted and experimental cooking loss, weight lost by centrifugation, hardness, springiness, and chewiness obtained for the formulation containing 0.593 g of κ:ι carrageenans/100 g and 0.320 g of milk proteins/100 g.

Parameters	Predicted	Experimental validation	
		Mean	Confidence interval ($\alpha = 0.05$)
Cooking loss (g/100 g) (n = 5)	1.24	0.90	0.5–1.3
Weight lost by centrifugation (g/100 g) (n = 8)	5.12	7.54	4.91–10.17
Hardness (N) (n = 10)	10.59	10.31	10.03–10.59
Springiness (J/J) (n = 10)	0.922	0.925	0.900–0.950
Chewiness(N) (n = 10)	5.84	5.36	4.86–5.86

"n" indicates the number of replicates performed.

and 0.320 g/100 g of milk proteins showed characteristics similar to a commercial product with 20 g/100 g pork fat content.

4. Conclusions

Response surface methodology enabled definition of quadratic models as a function of milk proteins and carrageenans contents of meat sausages containing fish oil. Responses of interest were cooking loss, weight lost by centrifugation, hardness, springiness, and chewiness. These attributes were more affected by changes in 2:1 κ:ι-carrageenans than in milk proteins added. Desirability methodology was applied to find the optimum formulation and the predicted responses were experimentally validated. The optimized formulation produced a low-fat meat product with similar physicochemical characteristics to a traditional one but having better nutritional quality as a consequence of fat substitution with fish oil.

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