"Optimization of a gelled emulsion intended to supply ω-3 fatty acids into meat products by means of Response Surface Methodology"

Candelaria Poyato[1], Diana Ansorena[1]*, Izaskun Berasategi[1], Íñigo Navarro-Blasco[2], Iciar Astiasarán[1]

[1] Department of Nutrition, Food Science and Physiology, Faculty of Pharmacy, University of Navarra, Irunlarrea s/n, 31008-Pamplona, Spain.

[2] Department of Chemistry and Soil Science, Faculty of Sciences, University of Navarra. Irunlarrea s/n, 31008-Pamplona, Spain.

*Corresponding author: Tel.: +34 948 42 56 00 (ext. 6263); Fax: +34 948 42 56 49.

E-mail address: dansorena@unav.es

ABSTRACT

The optimization of a gelled oil-in-water emulsion was performed for use as fat replacer in the formulation of ω-3 PUFA-enriched cooked meat products. The linseed oil content, carrageenan concentration and surfactant-oil ratio were properly combined in a surface response design for maximizing the hardness and minimizing the syneresis of the PUFA delivery system. The optimal formulation resulted in a gelled emulsion containing 40 % of oil and 1.5 % of carrageenan, keeping a surfactant-oil ratio of 0.003. The gel was applied as a partial fat replacer in a Bologna-type sausage and compared to the use of an O/W emulsion also enriched in ω-3. Both experimental sausages contributed with higher ω-3 PUFA content than the control. No sensory differences were found among formulations. The selected optimized gelled oil-in-water emulsion was demonstrated to be a suitable lipophilic delivery system for ω-3 PUFA compounds and applicable in food formulations as fat replacer.

Key words: fat replacer, hydrocolloids, delivery system, gelled emulsion.

1. INTRODUCTION

The characteristics of fat analogues intended to replace animal fats are needed in order to achieve the appearance and the technological, rheological and sensory properties required for use in the food industry (Tye, 1991). In fact, the use of fat replacers can cause, in some cases, technological problems due to the fact that fat has a great impact on flavour, palatability and texture of foods (Hort & Cook, 2007; Delgado-Pando, Cofrades, Ruiz-Capillas & Jiménez-Colmenero, 2010; Horita, Morgano, Celeghini & Pollonio, 2011).

The use of emulsion based delivery systems is a suitable technology for protection and release of lipids in food (McClements, Decker & Weiss, 2007; Salminen, Herrmann & Weiss, 2013). There has been an increasing interest in improving the functional performance of foods using a wide variety of novel types of emulsion delivery systems, including solid lipid particles, filled hydrogel particles and conventional, multiple and multilayer emulsions (McClements, 2010). These systems are able to incorporate lipophilic functional agents with beneficial health effects into food products (Valencia, O'Grady, Ansorena, Astiasarán & Kerry, 2008; Taneja & Singh, 2012; Chung, Degner, & McClements, 2013; Nielsen & Jacobsen, 2013; Poyato, Navarro-Blasco, Calvo, Cavero, Astiasarán & Ansorena, 2013). Some of these emulsion delivery systems have been used as fat replacers to produce high ω-3 products for improving the nutritional quality of new products. In this sense, the potential development of functional meat products using reformulation strategies has been attempted with the aid of emulsion based systems. The substitution of pork back fat with pre-emulsified oils ω-3 type PUFA oils has been demonstrated to be a good strategy to achieve healthier lipid profiles in these products (García-Iñiguez de Ciriano et al., 2010; Berasategi et al., 2011).

40 Recently, some papers (Triki, Herrero, Jiménez-colmenero & Ruiz-Capillas, 2013a;
41 Triki, Herrero, Rodríguez-Salas, Jiménez-Colmenero & Ruiz-Capillas, 2013b; Triki,
42 Herrero, Jiménez-Colmenero & Ruiz-Capillas, 2013c; Jiménez-Colmenero, Triki,
43 Herrero, Rodríguez-Salas & Ruiz-Capillas, 2013; Salcedo-Sandoval, Cofrades, Ruiz-
44 Capillas, Solas & Jiménez-Colmenero, 2013) have used konjac gel and oil stabilized in
45 a complex konjac matrix as potential fat analogues to reduce or improve the lipid
46 fraction of different meat products, obtaining good results.
47 In comparison to oil-in-water emulsions, gelled emulsions could be a better option to
48 mimic hardness and water holding capacity of pork back fat used in most of the
49 currently consumed meat products.
50 The objective of our research was to optimize the formulation of a gelled oil-in-water
51 emulsion prepared with oil rich in ω-3 fatty acids (linseed oil), carrageenan, a surfactant
52 and water, in order to obtain a successful functional ingredient by means of a factorial
53 design of response surface. This optimized gelled oil-in-water emulsion was used as
54 partial fat replacer in a meat product (Bologna type sausages), and its nutritional,
55 sensory and technological properties were assessed.

56 **2. MATERIAL AND METHODS**

57 **2.1. Materials**

58 Fresh pork meat (shoulder and front leg) and back fat were obtained from a local meat
59 market. The meat was trimmed of visible fat and connective tisue. Linseed oil (Biolasi
60 Productos Naturales, Guipúzcoa, Spain) was obtained in a local market. BDRom Carne
61 (a mixture of typical aromatic compounds) and the red colorant Carmin de Cochenille
62 50% (E-120) were obtained from BDF Natural Ingredients S.L. (Girona, Spain).
63 Carrageenan (kappa-carrageenan) was kindly donated by Cargill (San Sebastián, Spain)
64 and Curavi (a mixture of curing agents: NaCl, E-250, E-252 and antioxidant E-331)

65 BHA, polyphosphates, monosodium glutamate, sodium ascorbate and garlic were
66 kindly donated by ANVISA (Arganda del Rey, Madrid, Spain). All the chemical
67 reagents and Polysorbate 80 were obtained from Sigma-Aldrich Chemical Co. (MO,
68 USA).

69 **2.2. Gelled emulsion design**

70 Response Surface Methodology (RSM) was applied to optimize the formulation of an
71 oil-in-water gelled emulsion. The effect of three independent variables including oil
72 concentration, carrageenan concentration and surfactant-oil ratio (SOR) were studied in
73 order to maximize hardness and minimize syneresis of the obtained gels. The first
74 approach for the optimization was the delimitation of the ranges for the three
75 ingredients used in the preparation of the gels. The maximum oil concentration
76 technologically able to produce a gelled oil-in-water emulsion was selected as the upper
77 limit for this ingredient (70%), whereas the lowest limit (40%) was the minimum oil
78 content needed for achieving a significant amount of fatty acids based on nutritional
79 value. The lowest (0.5%) and upper (1.5%) limit for carrageenan concentration were the
80 minimum and maximum carrageenan concentration able to form a gelled oil-in-water
81 emulsion with the lowest and highest amount of oil, respectively. In the case of
82 polysorbate 80, the limits were expressed as the ratio between surfactant and oil amount
83 (SOR). The lowest limit for SOR was that needed for obtaining a stable gelled oil-in-
84 water emulsion (0.003), whereas the upper limit was the maximum concentration whose
85 bitterness was not detected in the gelled emulsion formed (0.005).

86 Taking to account these limits, the application of the central composition design (2^3 +
87 star, including 2 central points, Statgraphics Centurion XV software), resulted in a
88 design of 16 experimental settings, which were carried out in triplicate, and in random
89 order (Table S1, supplementary material).

90 **2.3. Gelled emulsion preparation and analysis**

91 50 ml of every 16 types of gelled emulsions were prepared as follows: the oil phase
92 containing the hydrophobic surfactant (Polysorbate 80) was added to the aqueous phase
93 that included the corresponding percentage of carrageenan and homogeneized. Both
94 phases were previously heated separately to 70°C. After the homogenization process
95 (16.000 rpm, Ultra-Turrax® T25basic), the emulsions were cooled to room temperature
96 in a sealed flask, allowing the k-carrageenan to polymerize. The gels were kept
97 overnight under refrigeration (4 ºC) before analysis.

98 For the determination of hardness and syneresis, gel samples were cut into cylinders
99 (D= 2.8 cm, h= 1 cm). Hardness was measured using a universal texture analyzer (TA-
100 XT2i, Stable Micro Systems, Surrey, United Kingdom) with a P 0.5R probe to
101 determine the textural characteristics of gels. Cylindrical samples were placed under the
102 probe and underwent compression under a 5 Kg load cell at a deformation rate of 30%.
103 Force-time curves were recorded at a crosshead speed of 0.5 mm/s. Ten measurements
104 were performed in each type of sample.

105 For the determination of syneresis, each sample was weighed (W_0) inside Petri dishes,
106 and placed in a cabinet at 25 ºC for 3 days. The water that condensed on the container
107 walls was removed before weighing the gels (W_t). The syneresis of the gels was
108 calculated as follows: Syneresis (%) = $[(W_0 - W_t)/C_0] \times 100$, were C_0 is the initial water
109 content in the sample, expressed in percentage. The experiment was performed in
110 triplicate.

111 The application of the Multiple Response Optimization to hardness and syneresis results
112 let us to conclude that the optimum combination of the gel ingredients was: 40% oil,
113 1.5% carrageenan and 0.003 SOR. This was the gel used as partial fat replacer in
114 Bologna type sausages elaborated in the second part of the work.

115 **2.4. Sausage formulation and processing**

116 Three different formulations (Table S2, supplementary material) of Bologna-type
117 sausages were manufactured in a pilot plant according to the procedure described by
118 Berasategi et al. (2011). Control products (Control) contained 16% pork back fat,
119 whereas in the two experimental batches, half of the pork back-fat was substituted by a
120 conventional oil-in-water emulsion (Emulsion) or by the previously optimized gelled
121 oil-in-water emulsion (Gel) rich in ω-3 fatty acids. The conventional oil-in-water (O/W)
122 emulsion was prepared according to the procedure described by García-Íniguez de
123 Ciriano et al. (2010) and the gelled oil-in-water emulsion was prepared as previously
124 described (Section 2.3). The conventional and gelled emulsions were kept under
125 refrigeration until their use.

126 Previous experiments (Berasategi et al., 2011) demonstrated the need for the addition of
127 extra antioxidants when cooked meat products contained high PUFA fat sources. Thus,
128 in both experimental batches, 200 mg of BHA/Kg meat batter were added in the mixture
129 of all additives. The control type was manufactured free of extra antioxidants. The
130 formulations were carried out in triplicate. Additionally, samples from every type of
131 formulation were stored under refrigeration (4ºC) for 35 days.

132 **2.5. Analysis of sausages**

133 Colour of sausages was measured using a digital colorimeter (Chromameter-2 CR-200,
134 Minolta, Osaka, Japan) to obtain the colour coordinates L*, a* and b*. These values are
135 used to calculate the euclidean distance value
136 ($\Delta E = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2}$) of products along the storage. The texture
137 (TPA) was measured using a Universal TA-XT2i texture analyzer. Conditions applied
138 for colour and texture were those described by Berasategi, Navarro-Blasco, Calvo,
139 Cavero, Astiasarán & Ansorena (2014).

140 The method of Folch, Lees & Stanley (1957) was used for the extraction of fat.

141 In order to assess the oxidation status of the Bologna-type sausages, TBARS
142 (Thiobarbituric acid value) value was determined in all three types of sausages over
143 storage time, using 0.25 g fat, according to the method described by Masqsood &
144 Benjakul (2010) with slight modifications (Poyato, Ansorena, Navarro-Blasco &
145 Astiasarán, 2014). Results were expressed in mg of malondialdehyde (MDA)
146 equivalents/ Kg sausage.

147 The fatty acids were determined in the lipid extracts by gas chromatography FID
148 detection according to the procedure described by Valencia et al. (2008). Moisture,
149 protein and fat content were analyzed using official methods (AOAC 2002a, 2002b,
150 2002c).

151 Fat extraction, TBARS, colour and texture were measured every 7 days of storage.

152 **2.6. Sensory analysis of meat products**

153 A triangle test was performed to determine the existence of perceptible sensory
154 differences in hardness, taste and appearance between control and the gel containing
155 products (Gel) and between the two experimental products (Emulsion and Gel). A total
156 of 21 semi-trained panellists participated in the sessions. Three samples, of which two
157 were identical, were presented to each panellist, and they were asked to indicate which
158 sample differed from the others. The number of correct answers was collected.
159 According to the Spanish norm UNE 87-006-92 (1992), for a 21-member panel, the
160 difference among samples was significant if the number of correct answers were 12 (*
161 $p<0.05$), 13 (** $p<0.01$) and 15 (*** $p<0.001$).

2.7. Statistical analysis

Gelled emulsion design and optimization

The experimental data (corresponding to the measures of hardness and syneresis) were analyzed by multiple regressions to the independent variables to a polynomial model with Expert Design 8 software. The goodness of fit of the model was evaluated by the determination coefficient (R^2), the adjusted determination coefficient (adjusted R^2), the coefficient of variation (CV) and the lack-of-fit. The value R^2 (0.9302 for hardness and 0.9810 for syneresis) indicated a good correlation between the experimental and the predicted values of the responses. In addition, the value of adjusted R^2 (0.9049 and 0.9741 for hardness and syneresis, respectively) suggested that a high percentage of the total variation (90.5% and 97.4% respectively) would be explained by the independent variables. The non-significant value for lack of fit ($p > 0.05$) revealed that the quadratic model was statistically significant for the response and therefore, its use was allowed in the study.

Also, analysis of variance (ANOVA) was performed to determine the statistically significant factors and their interactions in the regression model at the confidence level of 95% ($\alpha = 0.05$) (Table 1). Stepwise regression was used to eliminate the insignificant model terms and final equations were proposed, which are discussed in results and discussion section. Finally, a Multiple Response Optimization was performed in order to determine the combination of experimental factors which simultaneously optimized both responses: maximizing the hardness and minimizing the syneresis.

Bologna type sausages

Means and standard deviations of data obtained from the analysis of sausages are shown in corresponding tables. A one-way ANOVA test and the Tukey-*b* post-hoc test were used to determine significant differences in both the different types of Bologna-type

187 sausages and the different times of storage. SPSS version 15.0 was used (SPSS inc.
188 Chicago, Illinois, USA) for the evaluations at a significance level of $p \leq 0.05$.

189 **3. RESULTS AND DISCUSSION**

190 **3.1. Optimization of the gelled emulsion**

191 The 16 types of gel formulations characterized for hardness and syneresis revealed a
192 wide dispersion of experimental data, with a clear interaction between the amount of oil
193 and carrageenan (Table S1, supplementary material). For hardness, the maximum
194 experimental value (response) obtained was 1606 g (corresponded to the 50% of oil and
195 1.64% of carrageenan formulation) whereas the minimum for syneresis was 19.90% (for
196 the 40% of oil and 1.5% of carrageenan formulation).

197 Among the different regression models tested to explain the behaviour of the parameters
198 studied, hardness and syneresis, the quadratic polynomial model was found to be the
199 best fit for the experimental data both for hardness ($R^2 = 0.834$) and syneresis ($R^2 =$
200 0.978).

201 The analysis of variance of the empirical model for each variable is listed in Table 1.
202 ANOVA showed that there were 4 terms of the model that had p-values lower than
203 0.05, indicating a significant impact (95 % of confidence level) on the final responses.
204 The four terms were: oil concentration (A), carrageenan concentration (B), interaction
205 between oil and carrageenan concentration (AB) and quadratic term of oil concentration
206 (A^2). Even though a minimum ratio of surfactant-oil (C term) is required to produce the
207 gelled emulsion, this factor seems to have no influence on the responses at the studied
208 range.

209 The polynomial equations in terms of coded factors that resulted from these models
210 were:
211 (1) Hardness (g) = $838.7 – 245.8A + 307.5B – 426.9AB – 362.6A^2$
212 (2) Syneresis (%) = $37.34 + 24.22A + 8.88B + 14.77AB + 16.22A^2$
213 These models showed that hardness and syneresis were influenced in different ways by
214 the same factors (A, B, AB and A^2), so significant interactions could be expected among
215 them, as also shown by the high F-values obtained in the ANOVA test. According to the
216 interaction plots among studied variables (Figure 1A and 1B) hardness decreased when
217 the oil content increased at high carrageenan concentration (1.5%). This fact could be
218 because in k-carrageenan and in mixed k/i-carrageenan gels, the emulsion droplets are
219 not connected to the matrix and weaken the gel network when the amount of oil is too
220 high (Sala et al., 2008). In contrast, at lower carrageenan concentration (0.5%) the
221 hardness increased to a maximum point after which a decrease was again observed. The
222 syneresis increased when the oil content increased at high carrageenan concentrations as
223 the oil was not retained by the system. On the other hand, the syneresis decreased,
224 reaching a minimum at the lower carrageenan concentration before increasing again.
225 As the gel was intended to be used as partial pork fat replacer, the objective was to get a
226 gel with a maximum hardness (simulating the texture of pork back fat) and minimum
227 syneresis (to avoid technological problems during the meat product elaboration). For
228 this, a RSM was performed, estimating the desirability as the combination of maximum
229 hardness and minimum syneresis. According to Jung and Joo (2013), the desirability
230 function approach is one of the most widely used methods for the optimization of the
231 multiple response process. It is based on the idea that the quality of a product or process
232 that has multiple quality characteristics is unacceptable when one of them stays out-side
233 of some desired range. As it can be seen in Figure 1C, the combination of factor levels

234 which maximized the desirability on the studied led to values close to 40% of oil, 1.5%
235 of carrageenan and 0.003 for the surfactant-oil ratio, resulting in a desirability value of
236 0.931. This combination was used for the gelled oil-in-water emulsion tested as partial
237 fat replacer in the second part of the work.

238 **3.2. Comparative study among Bologna type sausages**

239 Once the optimum gelled emulsion formulation was achieved, a practical application
240 was designed in order to confirm the usefulness of the new ingredient. It consisted on
241 comparing a traditional meat product (Control) with other two formulations enriched in
242 ω-3 fatty acids by means of the incorporation of the developed gelled emulsion (Gel)
243 and also of conventional emulsions (Emulsion) previously used in different works.
244 These two ingredients were added for the replacement of ~~replacing a~~ 50% ~~of~~ the pork
245 back fat.

246 Regarding the sensory results for texture, no significant differences were found in the
247 triangle test between Control and the Gel-type products, or between the Gel-type and
248 Emulsion-type products, as panellist were not able to differentiate between samples ($p >$
249 0.05) (Table S3, supplementary material). TPA results (Figure 2) revealed that during
250 the first 10 days, hardness was similar between Control and Gel-type products, whereas
251 emulsion-type showed lower values ($p < 0.05$). These results led to conclude that the
252 gelled emulsion ingredient could be more efficient to maintain the hardness of the
253 original product. The three products showed an increase of hardness during storage,
254 probably as a consequence of a slight water loss, and giving rise to similar values
255 among the three products from the 15th day of storage. Similarly, other authors (Rubio
256 et al., 2007; Ayadi, Kechaou, Makni & Attia, 2009; Cierach, Modzelewska-Kapitula &
257 Szacilo, 2009; Triki et al., 2013a) reported increments in hardness during the storage of
258 meat products in which partial fat replacements were done.

The triangle test led us to conclude that the use of the gelled emulsion did not show sensory problems related to odour, taste and juiciness, showing no significant differences when compared to Control products or the traditional Emulsion-type products ($p > 0.05$).

Previous works have shown that colour differences are noticed in meat products when substituting pork back fat by a conventional oil-in-water emulsion (Jiménez-Colmenero, Herrero, Pintado, Solas & Ruiz-Capillas., 2010; Youssef & Barbut, 2011; Berasategi et al, 2013). As this finding was expected, sensory evaluation in this work was done under red light conditions, in order to avoid biased evaluation of the rest of parameters. Colour was not consequently included among the parameters assessed by panellists. In any case, the instrumental colour data of the three formulations (Figure 3) confirmed that lightness (L*), yellowness (b*) and redness (a*) were significantly higher in the emulsion containing products compared to control ones, as expected. In comparing the gel containing products with the control, significant differences were also found for L*, a* and b* values pointing out that the use of the gel instead of the emulsion does not perfectly reproduce the colour contribution of lard to the new formulation. These colour modifications can be probably related to the much smaller oil globule diameter in emulsions, which reflect more light than the larger animal fat globules. Nevertheless, these differences do not affect the evaluation of the general acceptability of the new products. Additionally, the three products maintained constant colour during storage, as ΔE in the three cases was lower than 2 (Francis & Clydesdale, 1975).

From the nutritional point of view, the use of the gelled emulsion gave the same advantages as the traditional emulsion when both modified formulations were compared to ~~traditional~~ Control products. A significant decrease in total fat content was found as well as for every lipid fraction analyzed (Table 2). Despite the fact that the gel or the

284 emulsion were added at a 8% of the total formulation, a significant supply of α-linolenic
285 acid, which is abundant in linseed oil, was noted for both modified products. This
286 represents approximately 17-18-fold more fatty acid in the Emulsion- and Gel-type
287 products ~~as~~ compared to the control. This modification reduced significantly the ω-6/ω-
288 3 ratio from 14 for control products to 0.75, on average, in the modified products. These
289 α-linolenic amounts allowed claiming "high ω-3"for the products developed, which is
290 set at 0.6 g α-linolenic per 100 g and 100 Kcal by EU Regulation (EFSA, 2009).
291 In order to monitor the potential oxidation of the new formulation, rich in PUFA,
292 TBARS during storage were measured (Figure 4). Both experimental products showed
293 no lipid oxidation events during storage, having consistent values lower than 0.2 mg
294 MDA/kg product, thus, confirming the effectiveness of BHA to control lipid oxidation
295 in the gel containing product. On the contrary, control products showed incremental
296 increases in TBARS from day 15, reaching values of approximately 0.27 mg MDA/kg
297 product by day 20 and continuing to the end of the storage period. These results
298 demonstrated the viability of modified products regarding oxidative stability despite
299 their high content of unsaturated fatty acids.
300 In conclusion, the optimized gelled emulsion seems to be an effective ingredient as
301 partial pork back fat replacer in cooked meat products, showing good technological
302 properties, nutritional advantages and without negative influence on the sensory
303 properties of the final product. More studies related to the stability of this ingredient and
304 its efficiency when used at different concentrations as a fat replacer are needed.
305 Evaluation of the bioavailability of the lipid compounds delivered by this product
306 should be carried out to determine the efficacy of the nutrient delivery system.

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FIGURE CAPTIONS

Figure 1. (a) Interaction plots of the hardness and (b) the syneresis. (c) Response surface plot of the multiple optimization of hardness and syneresis.

Figure 2. Hardness values (g) obtained for the three formulations of Bologna-type sausages during the storage.

Figure 3. Color coordinates of the three types of Bologna-type formulations along the storage.

Figure 4. TBARS (mg MDA/kg product) obtained for the three formulations of Bologna-type sausages during the storage.

TABLE CAPTIONS

Table 1. ANOVA items of regression equations.

Table 2. Chemical composition (g/100 g product) of the different formulated Bologna-type sausages.

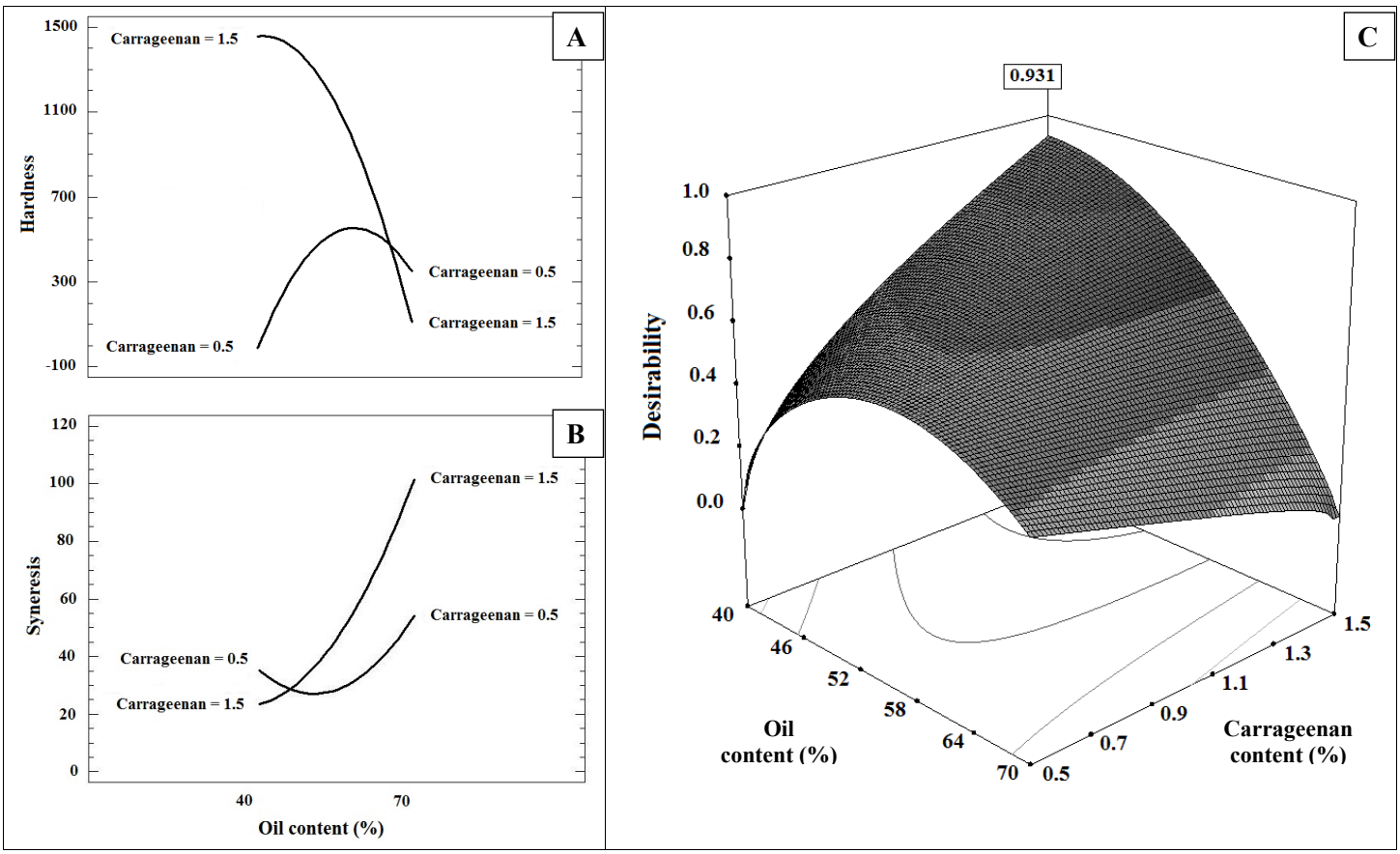
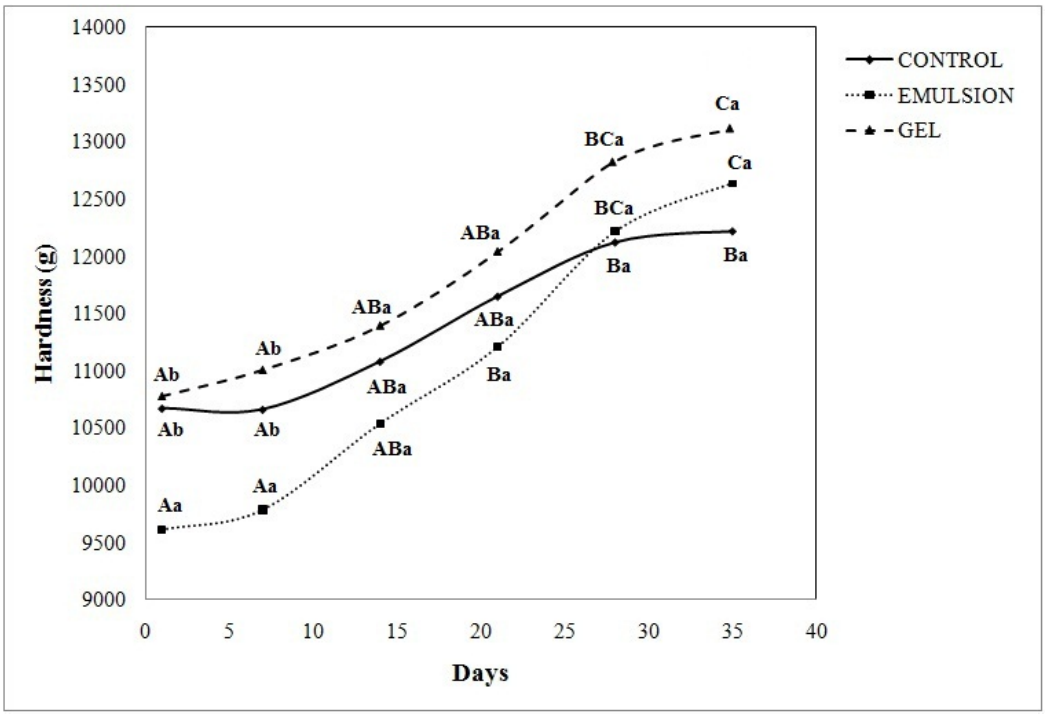


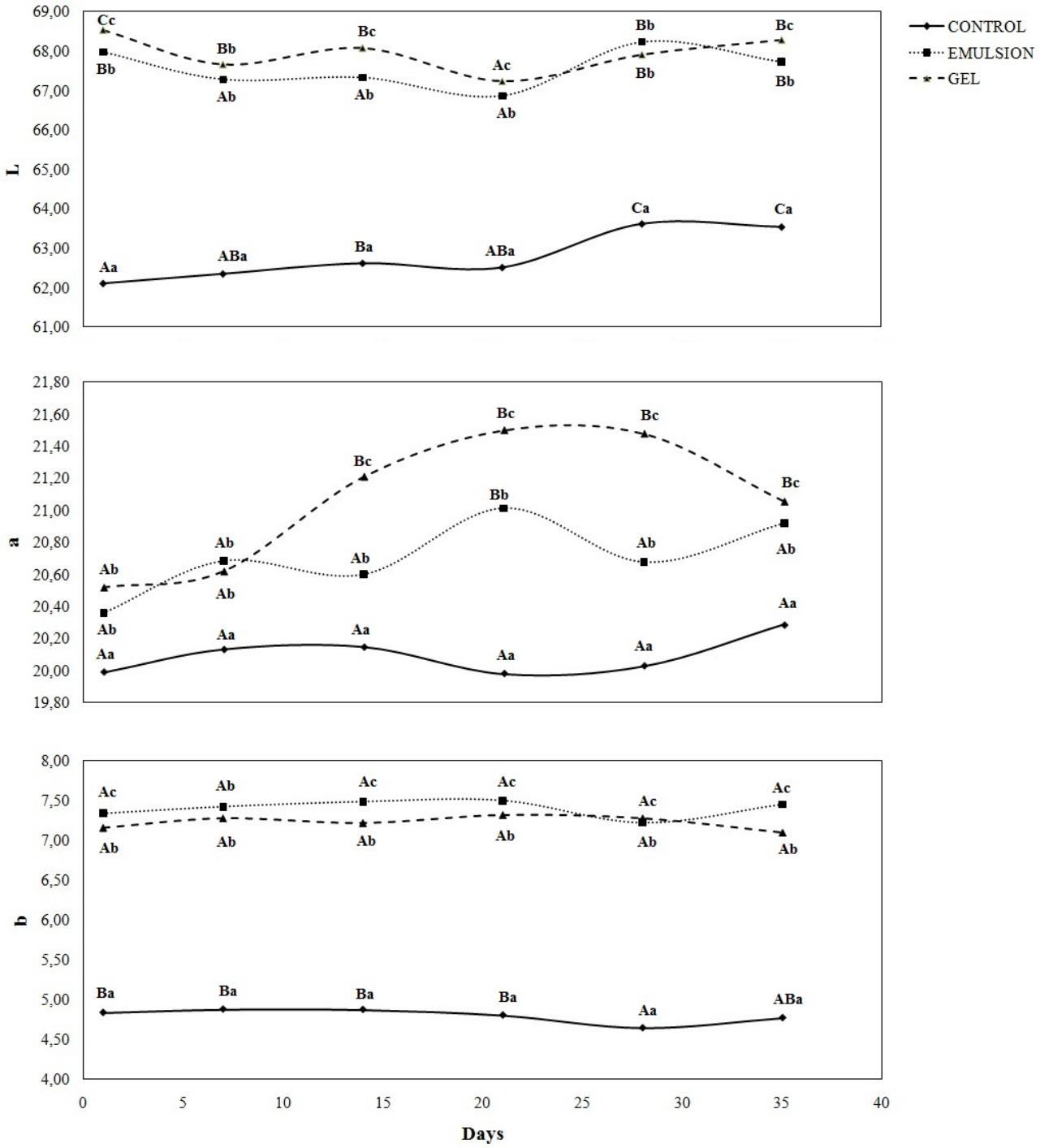
Figure 1. (A) Interaction plots of the hardness (g) and (B) the syneresis (%). (C) Response surface plot of the multiple optimization of hardness and syneresis.

Figure 2. Hardness values (g) obtained for the three formulations of Bologna-type sausages during the storage.



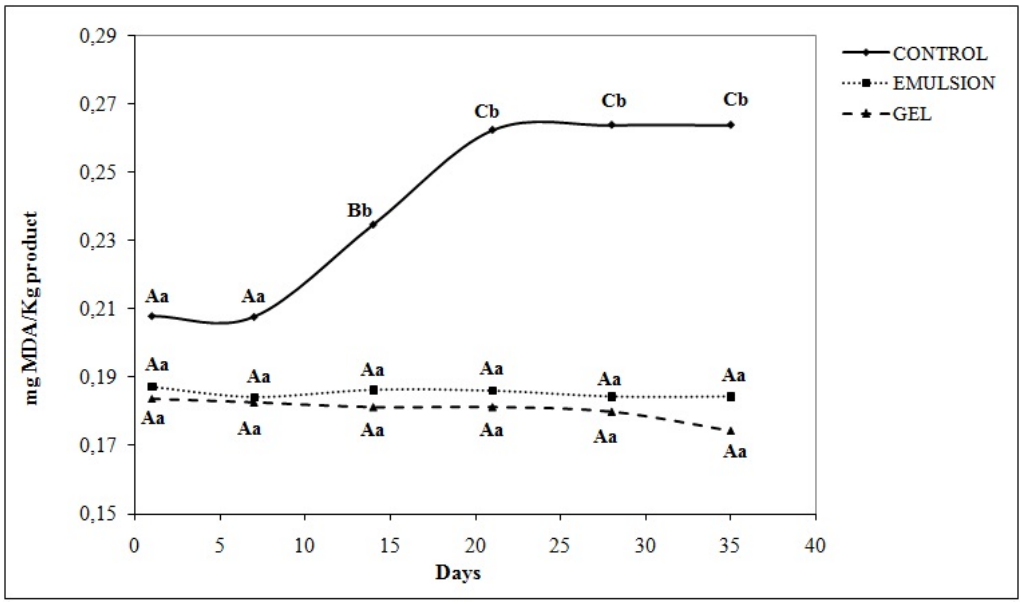
Within each type of formulation different capital letters denote significant differences along the storage, and within each sampling time, different small letters denote significant differences among types of formulation ($p < 0.05$).

Figure 3. Color coordinates of the three types of Bologna-type formulations along the storage.



Within each type of formulation different capital letters denote significant differences along the storage, and within each sampling time, different small letters denote significant differences among types of formulation ($p < 0.05$).

Figure 4. TBARS (mg MDA/kg product) obtained for the three formulations of Bologna-type sausages during the storage.



Different capital letters denote significant differences for each type during storage and different small letters denote significant differences among types at each day ($p < 0.05$).

Table 1. ANOVA items of regression equations.

Source of variations	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
HARDNESS						
Model	3948911	9	438768	9.39	0.007**	significant
A-Oil	683754	1	683754	14.63	0.009**	
B-Carrageenan	1069946	1	1069946	22.89	0.003**	
C-Polysorbate 80	759	1	759	0.016	0.903	
AB	1458364	1	1458364	31.21	0.001***	
AC	148	1	148	0.003	0.957	
BC	91	1	91	0.002	0.966	
A^2	722156	1	722156	15.45	0.008**	
B^2	13566	1	13566	0.290	0.609	
C^2	131	1	131	0.003	0.959	
Residual	280367	6	46728			
Lack of Fit	249631	5	49926	1.62	0.5318	not significant
Pure Error	30735	1	30735			
Cor Total	4229278	15				
SYNERESIS						
Model	10830	9	1203	77.89	< 0.001***	significant
A-Oil	6635	1	6635	429	< 0.001***	
B-Carrageenan	892	1	892	57.79	< 0.001***	
C-Polysorbate 80	7.62	1	7.62	0.49	0.509	
AB	1744	1	1744	112	< 0.001***	
AC	41.78	1	41.78	2.70	0.151	
BC	0.12	1	0.12	0.01	0.934	
A^2	1443	1	1443	93.44	< 0.001***	
B^2	24.23	1	24.23	1.57	0.257	
C^2	40.71	1	40.71	2.63	0.156	
Residual	92.70	6	15.45			
Lack of Fit	74.14	5	14.83	0.80	0.686	not significant
Pure Error	18.57	1	18.57			
Cor Total	10923	15				

Table 2. Chemical composition (g/100 g product) of the different formulated Bologna-type sausages.

	Control	Emulsion	Gel
Moisture	69.09 ± 0.07[a]	70.87 ± 0.06[b]	71.48 ± 0.07[c]
Protein	15.06 ± 0.65[a]	13.91 ± 0.78[a]	14.88 ± 0.57[a]
Fat content	13.24 ± 0.15[b]	12.09 ± 0.15[a]	11.89 ± 0.04[a]
Caprilic C8:0	nd	nd	nd
Capric C10:0	0.03 ± 0.01[b]	0.02 ± 0.01[a]	0.02 ±0.01[a]
Lauric C12:0	0.02 ± 0.01[b]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Myristic C14:0	0.17 ± 0.01[b]	0.09 ± 0.01[a]	0.10 ± 0.01[a]
Palmitic C16:0	3.08 ± 0.01[c]	2.11 ± 0.01[b]	2.06 ± 0.01[a]
t-Palmitoleic C16:1	0.01 ± 0.00[b]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Palmitoleic C16:1	0.22 ± 0.01[c]	0.16 ± 0.01[b]	0.15 ± 0.01[a]
Stearic C18:0	1.67 ± 0.01[c]	1.16 ± 0.01[b]	1.12 ± 0.01[a]
Elaidic C18:1	0.07 ± 0.01[c]	0.04 ± 0.01[b]	0.03 ± 0.01[a]
Oleic C18:1 (ω-9)	5.10 ± 0.02[c]	4.29 ± 0.01[b]	4.00 ± 0.01[a]
c-Vaccenic C18:1 (ω-7)	0.34 ± 0.01[c]	0.27 ± 0.01[b]	0.25 ± 0.01[a]
t-Linoleic C18:2	nd	nd	nd
c-t linoleic C18:1	0.01 ± 0.00[b]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
t-c linoleic C18:1	0.01 ± 0.00[b]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Linoleic C18:2 (ω-6)	2.23 ± 0.01[c]	1.90 ± 0.01[a]	1.93 ± 0.01[b]
Arachidic C20:0	nd	0.01 ± 0.00[a]	0.01 ± 0.00[a]
γ-linolenic C18:3 (ω-6)	0.01 ± 0.00[c]	0.01 ± 0.00[a]	0.01 ± 0.00[b]
Eicosenoic C20:1 (ω-9)	0.10 ± 0.01[c]	0.07 ± 0.01[b]	0.07 ± 0.01[a]
α-linolenic C18:3 (ω-3)	0.11 ± 0.01[a]	1.84 ± 0.01[b]	1.99 ± 0.01[c]
Behenic C22:0	nd	nd	nd
Brassidic C20:1	nd	nd	nd
Erucic C22:1	nd	nd	nd
Eicosatrienoic C20:3 (ω-3)	0.02 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Arachidonic C20:4 (ω-6)	0.04 ± 0.01[a]	0.04 ± 0.01[a]	0.05 ± 0.01[b]
Eicosapentaenoic C22:5 (ω-3)	nd	0.01 ± 0.00[a]	0.01 ± 0.00[a]
Nervonic C24:1 (ω-9)	nd	nd	nd
Docosatrienoic C22:3 (ω-3)	nd	nd	nd
Docosapentaenoic C22:5 (ω-6)	nd	0.02 ± 0.01[a]	0.02 ± 0.01[a]
Lignoceric C24:0	nd	nd	nd
SFA	4.97 ± 0.02[c]	3.41 ± 0.01[b]	3.33 ± 0.01[a]
MUFA	5.76 ± 0.02[c]	4.79 ± 0.01[b]	4.48 ± 0.01[a]
PUFA	2.41 ± 0.01[a]	3.82 ± 0.02[b]	4.01 ± 0.01[c]
ω-3	0.12 ± 0.01[a]	1.87 ± 0.02[b]	2.03 ± 0.01[b]
ω-6	2.28 ± 0.01[c]	1.95 ± 0.01[a]	1.98 ± 0.01[b]
ω-6/ω-3	14.08[b]	0.78[a]	0.73[a]
Trans	0.11 ± 0.01[b]	0.07 ± 0.01[a]	0.05 ± 0.01[a]

[1]*Different letters in the same row denote significant differences among samples ($p<0.05$). nd: not detected*

SUPPLEMENTARY MATERIAL

Optimization of a gelled emulsion intended to supply ω-3 fatty acids into meat products by means of RSM

Candelaria Poyato, Diana Ansorena, Izaskun Berasategi, Íñigo Navarro-Blasco, Iciar Astiasarán

Table S1. Central composition design (2^3+ star; including 2 central points) for the three variables and observed responses.

Run	Linseed oil (%)	Carrageenan (%)	SOR	Hardness[a] (g)	Syneresis[a] (%)
1	35.69	1.00	0.0040	548	32.06
2	55.00	1.64	0.0040	1606	53.36
3	40.00	1.50	0.0050	1383	27.77
4	55.00	1.00	0.0040	822	32.88
5	55.00	1.00	0.0053	894	32.52
6	55.00	1.00	0.0027	873	36.78
7	74.31	1.00	0.0040	2.3	99.01
8	55.00	0.36	0.0040	310	31.93
9	40.00	1.50	0.0030	1364	19.90
10	40.00	0.50	0.0030	69.0	30.81
11	70.00	1.50	0.0050	0.01	98.65
12	40.00	0.50	0.0050	65.3	39.47
13	55.00	1.00	0.0040	874	38.97
14	70.00	1.50	0.0030	0.01	99.20
15	70.00	0.50	0.0030	376	53.11
16	70.00	0.50	0.0050	426	51.36

[a] Average value of triplicate experiments
SOR: surfactant oil ratio

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Table S2. Formulation of the three types of Bologna-type sausages.

Ingredients	Control	Modified products	
		Emulsion	Gel
Pork meat (%)	55	55	55
Pork fat (%)	16	8	8
Ice (%)	29	29	29
Emulsion (%)	0	8	0
Gel (%)	0	0	8
BHA (mg/Kg)	0	200	200
Iodized NaCl (g/kg of meat-fat batter)	26	26	26
Powdered milk (g/kg)	12	12	12
Garlic (g/kg)	3	3	3
Curavi[1] (g/kg)	3	3	3
Polyphosphates[2] (g/kg)	2	2	2
Sodium ascorbate (g/kg)	0.5	0.5	0.5
BDRom Carne (g/kg)	1	1	1
Monosodium glutamate (g/kg)	1	1	1
Carmin de Cochenille 50% (E-120) (g/kg)	0.1	0.1	0.1

[1]*Curavi: a mixture of curing agents: NaCl, E-250, E-252 and antioxidant E-331.*
[2] *Mixture of E-430i, E-454i and E-451i.*

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Table S3. Scores obtained in the triangular sensory analysis.

	Odour	Taste	Juiciness	Texture
Control Vs. Gel				
Correct replies	10 ^{ns}	8 ^{ns}	7 ^{ns}	9 ^{ns}
Incorrect replies	11	13	14	12
Emulsion Vs. Gel				
Correct replies	2 ^{ns}	3 ^{ns}	3 ^{ns}	4 ^{ns}
Incorrect replies	19	18	18	17

For n=21, the difference between samples was significant if the number of correct answers was 12 (= $p<0.05$), 13 (** = $p<0.01$) and 15 (*** = $p<0.001$). ns: not significant.*