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

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1 **ABSTRACT**

2 The optimization of a gelled oil-in-water emulsion was performed for use as fat replacer

3 in the formulation of ω-3 PUFA-enriched cooked meat products. The linseed oil

4 content, carrageenan concentration and surfactant-oil ratio were properly combined in a

5 surface response design for maximizing the hardness and minimizing the syneresis of

6 the PUFA delivery system. The optimal formulation resulted in a gelled emulsion

7 containing 40 % of oil and 1.5 % of carrageenan, keeping a surfactant-oil ratio of 0.003.

8 The gel was applied as a partial fat replacer in a Bologna-type sausage and compared to

9 the use of an O/W emulsion also enriched in ω-3. Both experimental sausages

10 contributed with higher ω-3 PUFA content than the control. No sensory differences

11 were found among formulations. The selected optimized gelled oil-in-water emulsion

12 was demonstrated to be a suitable lipophilic delivery system for ω-3 PUFA compounds

13 and applicable in food formulations as fat replacer.

14 **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 (%) = $[(W_0 - W_t)/C_0]$ x 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.

2.4. Sausage formulation and processing

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

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

2.5. Analysis of sausages

Colour of sausages was measured using a digital colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan) to obtain the colour coordinates L*, a* and b*. These values are used to calculate the euclidean distance value

$(\Delta E = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2}$) of products along the storage. The texture (TPA) was measured using a Universal TA-XT2i texture analyzer. Conditions applied for colour and texture were those described by Berasategi, Navarro-Blasco, Calvo, 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.

259 The triangle test led us to conclude that the use of the gelled emulsion did not show

260 sensory problems related to odour, taste and juiciness, showing no significant

261 differences when compared to Control products or the traditional Emulsion-type

262 products ($p > 0.05$).

263 Previous works have shown that colour differences are noticed in meat products when

264 substituting pork back fat by a conventional oil-in-water emulsion (Jiménez-Colmenero,

265 Herrero, Pintado, Solas & Ruiz-Capillas., 2010; Youssef & Barbut, 2011; Berasategi et

266 al, 2013). As this finding was expected, sensory evaluation in this work was done under

267 red light conditions, in order to avoid biased evaluation of the rest of parameters. Colour

268 was not consequently included among the parameters assessed by panellists. In any

269 case, the instrumental colour data of the three formulations (Figure 3) confirmed that

270 lightness (L*), yellowness (b*) and redness (a*) were significantly higher in the

271 emulsion containing products compared to control ones, as expected. In comparing the

272 gel containing products with the control, significant differences were also found for L*,

273 a* and b* values pointing out that the use of the gel instead of the emulsion does not

274 perfectly reproduce the colour contribution of lard to the new formulation. These colour

275 modifications can be probably related to the much smaller oil globule diameter in

276 emulsions, which reflect more light than the larger animal fat globules. Nevertheless,

277 these differences do not affect the evaluation of the general acceptability of the new

278 products. Additionally, the three products maintained constant colour during storage, as

279 ΔE in the three cases was lower than 2 (Francis & Clydesdale, 1975).

280 From the nutritional point of view, the use of the gelled emulsion gave the same

281 advantages as the traditional emulsion when both modified formulations were compared

282 to ~~traditional~~ Control products. A significant decrease in total fat content was found as

283 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.

5. REFERENCES

AOAC. (2002a). Fat (crude) or ether extract in meat. 960.39. In W. Horwitz (Ed.), *Official method of analysis.* (17th ed., pp. 12-13). Gaithersburg, Maryland: Association of Official Analytical Chemists.

AOAC. (2002b). Determination of moisture content. 950.46. In W. Horwitz (Ed.), *Official method of analysis.* (17th ed., pp. 12-13). Gaithersburg, Maryland: Association of Official Analytical Chemists.

AOAC. (2002c). Crude protein in meat. 981.10. In W. Horwitz (Ed.), *Official method of analysis.* (17th ed., pp. 7-8). Gaithersburg, Maryland: Association of Official Analytical Chemists.

Ayadi, M., Kechaou, A., Makni, I. & Attia, H. (2009) Influence of carrageenan addition on turkey meat sausages properties. *Journal of Food Engineering,* 93, 278-283.

Berasategi, I., Legarra, S., de Ciriano, M., Rehecho, S., Calvo, M., & Cavero, R. (2011). High in omega-3 fatty acids Bologna-type sausages stabilized with an aqueous-ethanol extract of *Melissa officinalis. Meat Science, 88*(4), 705-711.

Berasategi, I., García-Íñiguez de Ciriano, M., Navarro-Blasco, Í., Calvo, M. I., Cavero, R. Y., Astiasarán, I., et al. (2014). Reduced-fat bologna sausages with improved lipid fraction. *Journal of the Science of Food and Agriculture, 94*(4), 744-751.

Berasategi, I., Navarro-Blasco, Í., Calvo, M. I., Cavero, R. Y., Astiasarán, I., & Ansorena, D. (2014). Healthy reduced-fat bologna sausages enriched in ALA and DHA and stabilized with *Melissa officinalis* extract. *Meat Science, 96*(3), 1185-1190.

Chung, C., Degner, B., & McClements, D J. (2013). Designing reduced-fat food emulsions: Locust bean gum–fat droplet interactions. *Food Hydrocolloids*, 32(2), 263-270.

Cierach, M., Modzelewska-Kapitula, M. & Szacilo, K. (2009) The influence of carrageenan on the properties of low-fat frankfurters. *Meat Science,* 82, 295-299.

Delgado-Pando, G., Cofrades, S., Ruiz-Capillas, C., Jimenez-Colmenero, F. (2010). Healthier lipid combination as functional ingredient influencing sensory and technological properties of low-fat frankfurters. *European Journal of Lipid Science and Technology, 112*(8), 859-870.

EFSA. (2009). Scientific opinion of the panel on dietetic products, nutrition and allergies on a request from the commission related to labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids. *The EFSA Journal*, 1176, 1-11.

Folch, J., Lees, M., & Stanley, G. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry, 226*(1), 497-509.

Francis, F.J., & Clydesdale, F.M. (1975). Food colorimetry: theory and application. New York, USA: Chapman & Hall.

Garcia-Iniguez de Ciriano, M., Rehecho, S., Calvo, M., Cavero, R., Navarro, I., Astiasarán, I., Ansorena, D. (2010). Effect of lyophilized water extracts of *Melissa officinalis* on the stability of algae and linseed oil-in-water emulsion to be used as a functional ingredient in meat products. *Meat Science, 85*(2), 373-377.

Horita, C., Morgano, M., Celeghini, R., Pollonio, M. (2011). Physico-chemical and sensory properties of reduced-fat mortadella prepared with blends of calcium, magnesium and potassium chloride as partial substitutes for sodium chloride. *Meat Science, 89*(4), 426-33.

Hort, J., & Cook, D. (2007). Formulating low-fat food: The challenge of retaining flavour quality. In Taylor, A. & Hort, J., *Modifying flavour in food* (pp. 131-143). Cambridge, England: Woodhead Publishing in Food Science, Technology and Nutrition.

Jiménez-Colmenero, F., Herrero, A., Pintado, T., Solas, M. T., Ruiz-Capillas, C. (2010). Influence of emulsified olive oil stabilizing system used for pork backfat replacement in frankfurters. *Food Research International, 43*(8), 2068-2076.

Jiménez-Colmenero, F., Triki, M.; Herrero, AM., Rodríguez-Salas, L., Ruiz-Capillas, C. (2013). Healthy oil combination stabilized in a konjac matrix as pork fat replacement in low-fat, PUFA-enriched, dry fermented sausages. *Food Science and Technology, 51*(1), 158-163.

Jung, E. & Joo, N. (2013). Roselle (*Hibiscus sabdariffa* L.) and soybean oil effects on quality characteristics of pork patties studied by response surface methodology. *Meat Science, 94*(3), 391-401.

Maqsood, S. & Benjakul, S. (2010). Comparative studies of four different phenolic compounds on in vitro antioxidative activity and the preventive effect on lipid oxidation of fish oil emulsion and fish mince. *Food Chemistry, 119*(1), 123-132.

McClements, D. (2010). Emulsion design to improve the delivery of functional lipophilic components. *Annual Review of Food Science and Technology, 1*(1), 241-269.

McClements, D., Decker, E., & Weiss, J. (2007). Emulsion-based delivery systems for lipophilioc bioactive components. *Journal of Food Science, 72*(8), R109-R124.

Nielsen, N. S., & Jacobsen, C. (2013). Retardation of lipid oxidation in fish oil-enriched fish pâté-combination effects. *Journal of Food Biochemistry*, 37(1), 88-97.

Poyato, C., Ansorena, D., Navarro-Blasco, I., & Astiasarán, I. (2014). A novel approach to monitor the oxidation process of different types of heated oils by using chemometric tools. *Food Research International, 57*(0), 152-161.

Poyato, C., Navarro-Blasco, I., Calvo, M. I., Cavero, R. Y., Astiasarán, I., & Ansorena, D. (2013). Oxidative stability of O/W and W/O/W emulsions: Effect of lipid composition and antioxidant polarity. *Food Research International, 51*(1), 132-140.

Rubio, B., Martínez, B., Sánchez, M., García-Cachan, M., Rovira, J., Martínez, B., Sánchez, M. & Jaime, I. (2007). Study of the shelf life of a dry fermented sausage "salchichón" made from raw material enriched in monounsaturated and polyunsaturated fatty acids and stored under modified atmospheres. *Meat Science, 76*(1), 128-37.

Sala, G., de Wijk, R. A., van de Velde, F. & van Aken, G. A. (2008). Matrix properties affect the sensory perception of emulsion-filled gels. *Food Hydrocolloids, 22*(3), 353-363.

Salcedo Sandoval, L., Cofrades, S., Ruiz-Capillas Pérez, C., Solas, M. T. & Jiménez-Colmenero, F. (2013). Healthier oils stabilized in konjac matrix as fat replacers in n-3 PUFA enriched frankfurters. *Meat Science, 93*(3), 757-66.

Salminen, H., Herrmann, K. & Weiss, J. (2013). Oil-in-water emulsions as a delivery system for n-3 fatty acids in meat products. *Meat Science, 93*(3), 659-67.

Taneja, A. S. & Harjinder. (2012). Challenges for the delivery of long-chain n-3 fatty
acids in functional foods. *Annual Review of Food Science and Technology, 3*, 105-
23.

Triki, M., Herrero, A. M., Jiménez-Colmenero, F., Ruiz-Capillas, C. (2013a). Effect of
preformed konjac gels, with and without olive oil, on the technological attributes
and storage stability of merguez sausage. *Meat Science, 93*(3), 351-60.

Triki, M., Herrero, A. M., Rodríguez-Salas, L., Jiménez-Colmenero, F., Ruiz-Capillas,
C. (2013b). Chilled storage characteristics of low-fat, n-3 PUFA-enriched dry
fermented sausage reformulated with a healthy oil combination stabilized in a
konjac matrix. *Food Control, 31*(1), 158-165.

Triki, M., Herrero, A. M., Jiménez-Colmenero, F., Ruiz-Capillas, C. (2013c). Storage
stability of low-fat sodium reduced fresh merguez sausage prepared with olive oil
in konjac gel matrix. *Meat Science, 94*(4), 438-46.

Tye, R.J. 1991. Konjac flour: Properties and applications. *Food Technology*. 45: 86-92

UNE 87-006-92. Norma Española. Metodología Prueba Triangular. (1992).

Valencia, I., O'Grady, M.N., Ansorena, D., Astiasarán, I. & Kerry, J. (2008).
Enhancement of the nutritional status and quality of fresh pork sausages following
the addition of linseed oil, fish oil and natural antioxidants. *Meat Science, 80*(4),
1046-54.

Youssef, M. K. & Barbut., S. (2011). Fat reduction in comminuted meat products-
effects of beef fat, regular and pre-emulsified canola oil. *Meat Science, 87*(4), 356-
60.

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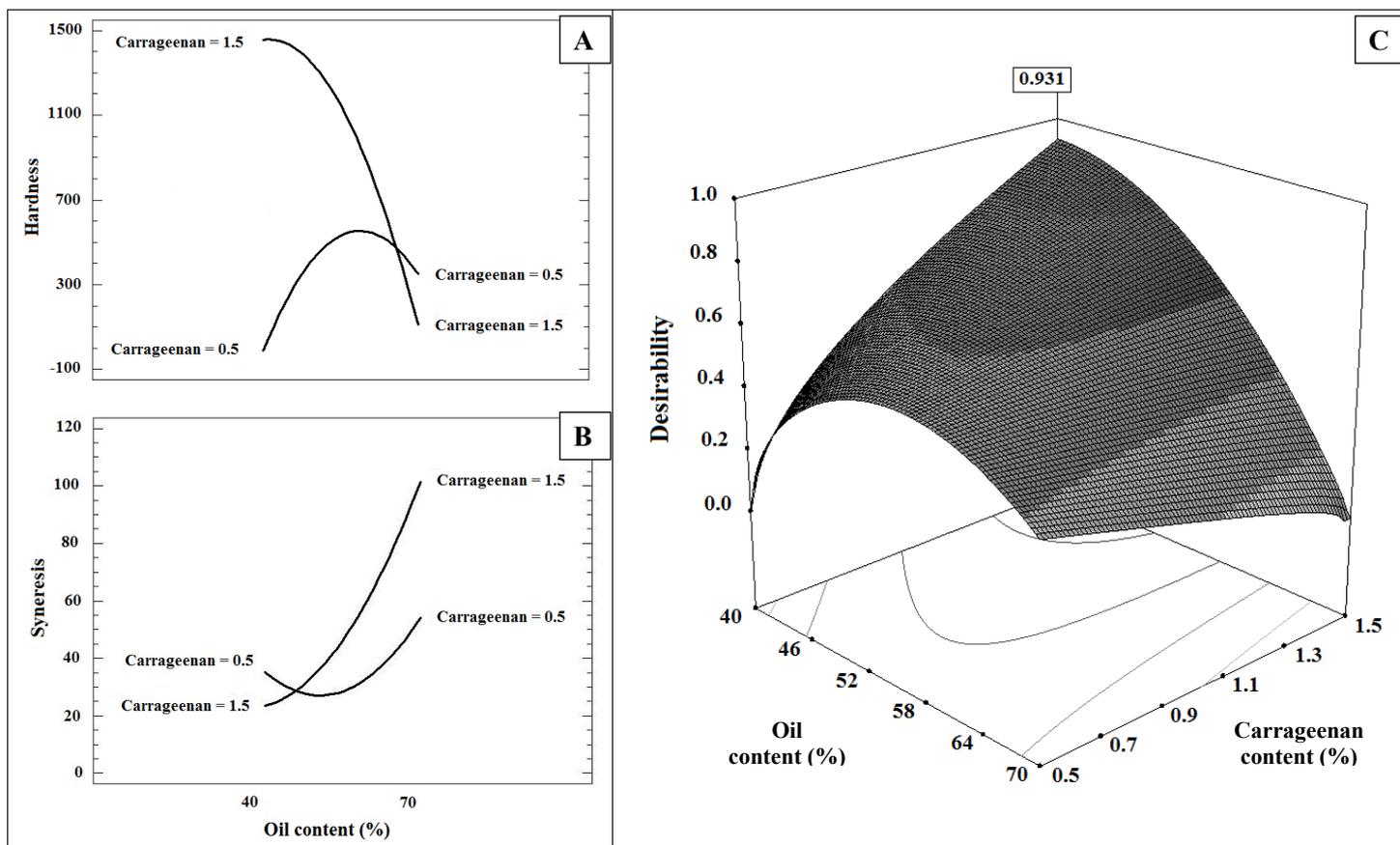
Figure 1. (A) Interaction plots of the hardness (g) and (B) the syneresis (%). (C) Response surface plot of the multiple optimization of hardness and syneresis.

Figure 2. Hardness values (g) obtained for the three formulations of Bologna-type sausages during the storage.
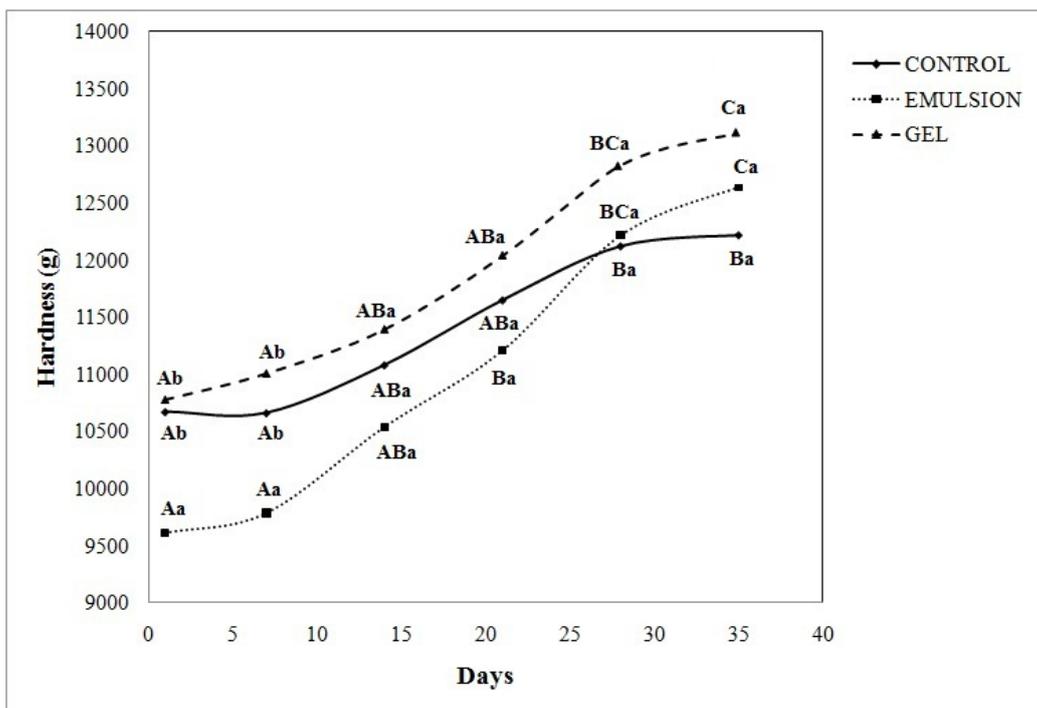
Within each type of formulation different capital letters denote significant differences along the storage, and within each sampling time, different small letters denote significant differences among types of formulation (p< 0.05).

Figure 3. Color coordinates of the three types of Bologna-type formulations along the storage.
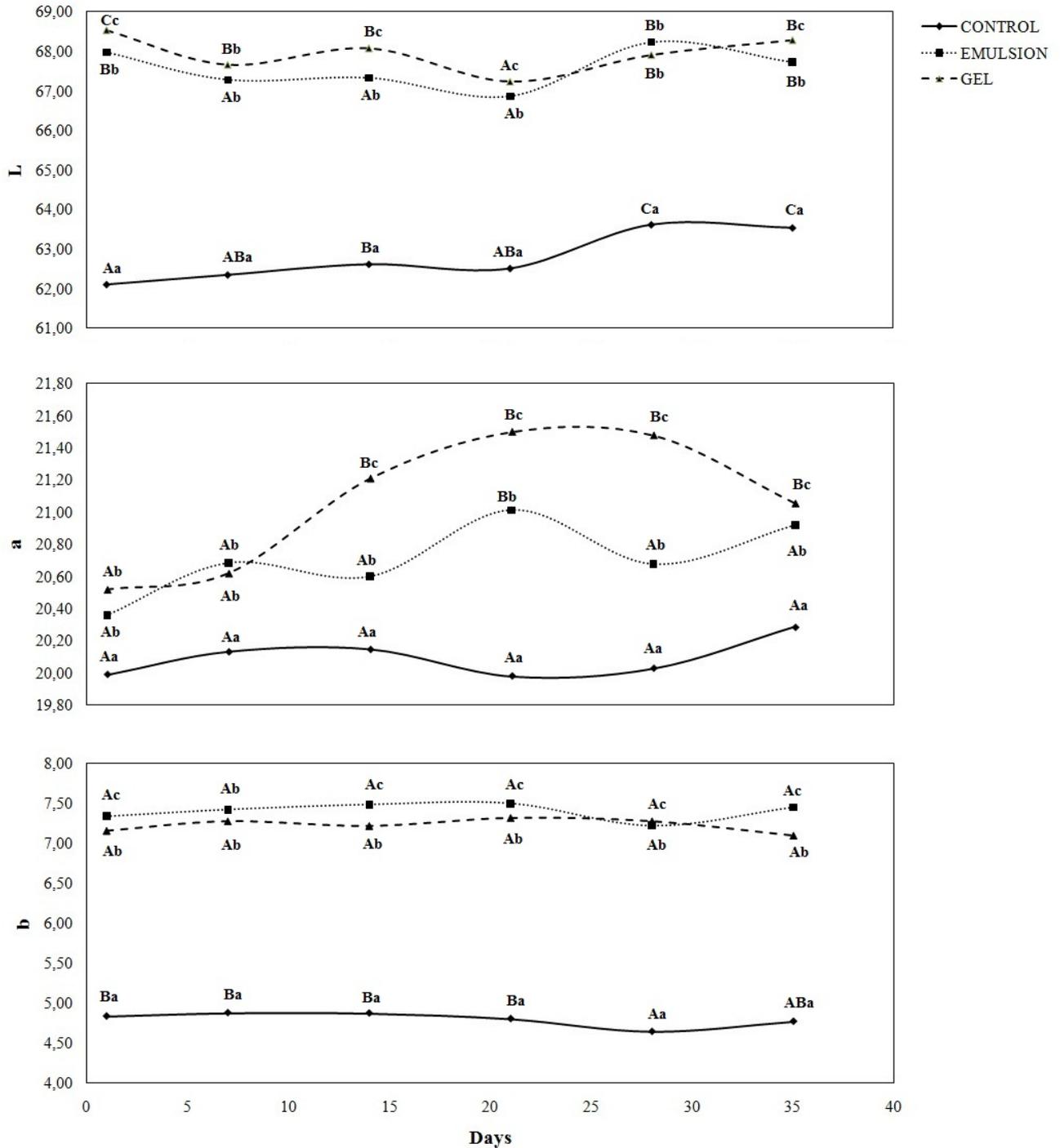
Within each type of formulation different capital letters denote significant differences along the storage, and within each sampling time, different small letters denote significant differences among types of formulation (p< 0.05).

Figure 4. TBARS (mg MDA/kg product) obtained for the three formulations of Bologna-type sausages during the storage.
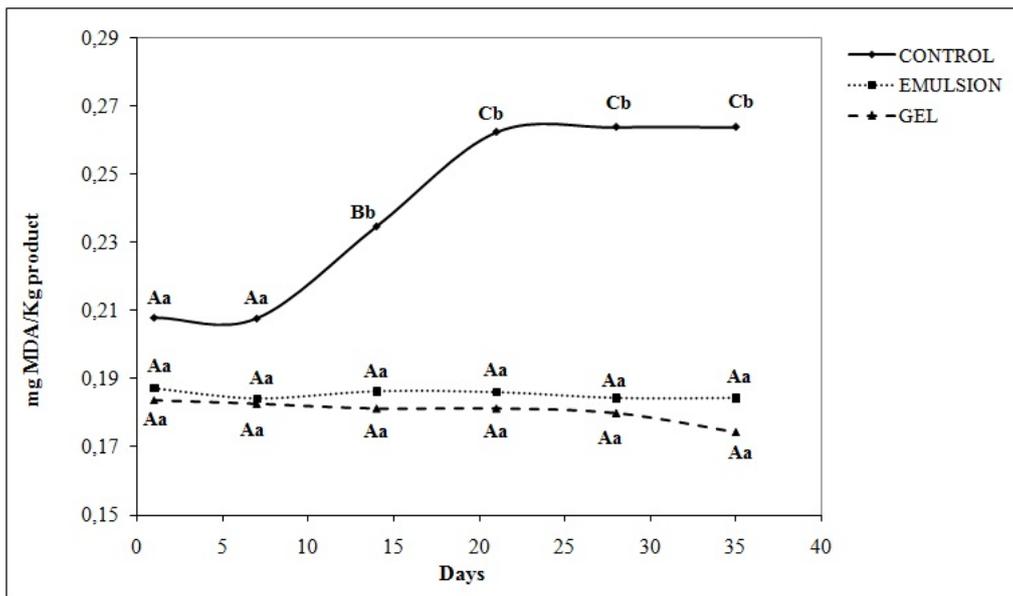
Different capital letters denote significant differences for each type during storage and different small letters denote significant differences among types at each day (p< 0.05).

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

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.