“The effect of low-fat beef patties formulated with a low-energy fat analogue enriched in long-chain polyunsaturated fatty acids on lipid oxidation and sensory attributes”

Marta Alejandre[1], Denis Passarini[2], Iciar Astiasarán[1], Diana Ansorena[1*]

[1] Department of Nutrition, Food Science and Physiology, Faculty of Pharmacy and Nutrition, University of Navarra, Irunlarrea s/n, 31008, IDISNA – Instituto de Investigación Sanitaria de Navarra, Pamplona, Spain

[2]Department of Agricultural and Food Sciences, Alma Mater Studiorum–Università di Bologna, Viale Fanin 40, 40127 Bologna (BO), Italy

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

E-mail address: dansorena@unav.es

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ABSTRACT

A new low-energy gelled emulsion containing algae oil was developed as animal fat replacer. Its stability was evaluated under different storage conditions: 4V (4°C/vacuum), 4NV (4°C/no vacuum), 25V (25°C/vacuum) and 25NV (25°C/no vacuum). According to moisture, hardness, color and lipid oxidation data, 4°C under vacuum (4V) was selected as the best condition. Once the gelled emulsion was characterized, its effectiveness as fat analogue was demonstrated in beef patties. Reformulated patties were produced with 100% of animal fat replacement and compared to conventional patties (9%fat). A 70%fat reduction was achieved in the new patties, mainly due to a reduction in the saturated fatty acids. Also, decreased n-6 (76%lower content) and increased eicosapentaenoic and docosahexaenoic acids (55%higher content) were noticed in the new formulation. The incorporation of the gelled emulsion containing reduced amount of n-6 fatty acids and increased amounts of long chain n-3 fatty acids (EPA+DHA) reduced the oxidation status of the patties and their sensory evaluation resulted in acceptable scores.

Key words: beef patties, gelled emulsion, algae oil, fat replacer, EPA, DHA.

1. INTRODUCTION

A wide literature is available about the effect of high-energy-diet on health, especially coming from fat intake that should not exceed 30% of total energy intake to avoid unhealthy weight gain. Also, the risk of developing noncommunicable diseases (NCDs) is lowered by reducing saturated fats to less than 10% of total energy intake, goal that can be achieved by replacing them with unsaturated fats (WHO, 2003; FAO, 2010).

Burgers or patties are one of the most popular processed meat products with significant animal fat content. Strategies that involve fat reduction and fat replacement in these products are interesting choices for reformulation to make them healthier (Keenan et al., 2015; Selani et al., 2016).

The 2008 Expert Consultation highlighted the beneficial role of long-chain polyunsaturated fatty acids (LC-PUFA) in the maintenance of long-term health and prevention of specific chronic diseases. In particular, the omega-3 LC-PUFA docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) may contribute to the prevention of coronary heart diseases (CHD) and possibly other degenerative diseases associated to aging (FAO, 2010).

Marine (algae and fish) oils are excellent sources of omega-3 fatty acids, mainly due to the high levels of DHA and EPA. Some of the strategies to increase these fatty acids in meat and meat products have been through animal feeding (Ponnampalam et al., 2016) or as ingredients in animal fat replacers. However, the direct incorporation of these oils has sometimes had negative implications due to the fishy taste reported, leading to a reduction of the sensory acceptance of the new products (Valencia, Ansorena, & Astiasaran, 2006).

The creation of emulsions is an interesting option for stabilizing liquid oils. In particular, oil in water (O/W) emulsion hydrogel is a complex solid structure generated by gelling from a stable liquid emulsion (Jimenez-Colmenero et al., 2015). The technological properties of gelled emulsions are more similar to animal fat than conventional O/W emulsions, thus their use as fat analogue is gaining interest in the development of healthier meat products. On the other hand, to our knowledge, an oil bulking system based on konjac gel (20% of olive, linseed and fish oils mixed) has been the only stabilizing lipid model previously used as animal fat replacer in patties (Salcedo-Sandoval, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2014; Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Carballo, & Jimenez-Colmenero, 2015).

In previous works, our research group has demonstrated the viability of a gelled emulsion containing 40% of linseed oil, 1.5 % of carrageenan and surfactant, as partial fat replacer in bologna sausages, burger patties and dry fermented sausages (Poyato, Ansorena, Berasategi, Navarro-Blasco, & Astiasaran, 2014b; Poyato, Astiasaran, Barriuso, & Ansorena, 2015; Alejandre, Poyato, Ansorena, & Astiasarán, 2016). These products showed healthier lipid profiles without negative influence on the sensory properties. Due to the success of this gelled emulsion in meat products, this research aimed to formulate a new low-energy gelled emulsion with algae oil as lipid source, as total fat replacer in beef patties to achieve a significant fat reduction, but also improved lipid profile of the reformulated patties. The stability of the selected gelled emulsion under different conditions of storage was assessed for a clear understanding of the behavior of this fat analogue. Finally, nutritional, technological and sensory properties were assessed in the new reformulated patties.

2.MATERIALS AND METHODS

2.1 Formulation and preparation of the gelled emulsion

In a first phase of the work, different proportions of carrageenan and algae oil were tested for the formulation of a low energy gelled emulsion aiming to obtain a combination of ingredients that could allow obtaining an optimized product with the maximum hardness and the minimum syneresis. 1%-3% was the range selected for kappa-carrageenan concentrations in which a firm gelled emulsion without gelation problems could be obtained. Range selected for the algae oil was 1%-4%. The maximum oil concentration (4%) was selected as the upper limit to produce a gelled emulsion that could be declared 'low in energy'. The lowest limit (1%) was the minimum oil content needed to obtain a gel that can be declared ´source of omega-3 fatty acids´ (Regulation (EC) No 1924/2006) reducing to the minimum its energy value. Based on these considerations, different formulations of gelled emulsions within the established ranges of carrageenan and oil were prepared as follows.

The different gelled emulsions were prepared as described by Poyato et al. (2014b). Algae oil was provided by DHASCO® oil, commercially available oil obtained from *Crypthecodinium cohnii (*Martek Biosciences Corporation, Columbia, USA). Kappa-carrageenan was provided by Cargill (San Sebastian, Spain) and polysorbate 80 was obtained from Sigma-Aldrich Chemical Co. (MO, USA). Two solutions (pre-heated at 70°C), the first one containing the algae oil and the surfactant (polysorbate 80, used at the following proportion: 0.12 g/100 g oil) and the second one containing the aqueous phase and carrageenan, were mixed. Subsequently to an homogenization treatment

87 (16000 rpm, Ultra-Turrax T25basic), the emulsions were cooled to room temperature in
88 sealed flasks, and stored at 4°C to allow the polymerization of kappa-carrageenan.

89 The gelled emulsions were cut into cylinders (D=2.8 cm, h=1 cm) and the variables
90 hardness and syneresis were measured. Hardness analysis was performed the day after
91 the preparation of the gelled emulsions. Cylindrical samples were placed under the
92 probe and underwent compression under a 5 kg load cell at a deformation rate of 30%.
93 Force-time curves were recorded at a crosshead speed of 0.5 mm/s. The equipment
94 used was a Texture Analyzer (TA-XT2i, Stable Micro Systems, Surrey, United Kingdom).
95 For the determination of syneresis, each sample (5 g) was weighed (W_0) inside Petri
96 dishes, and placed in a cabinet at 25 ºC for 7 days. The water that condensed on the
97 container walls was removed before weighing the gelled emulsion (W_t). The syneresis
98 was calculated as follows: syneresis (%) = $[(W_0–W_t)/C_0] \times 100$, where C_0 is the initial
99 water content in the sample, expressed in percentage.
100 Taking into account all the results of hardness and syneresis on the different gelled
101 emulsions elaborated with the different combinations of algae oil and kappa-
102 carrageenan, an optimized gel was selected for further analysis and application (see
103 Discussion section).

104 **2.2 Assessment of physical and chemical stability of the optimized gelled emulsion**
105 **during storage**

106 The stability of the optimized gelled emulsion was studied on four batches stored at
107 different conditions for 31 days. For each batch, several portions (50 g each) were
108 prepared for subsequent analyses. Portions of batches 1 and 2 were aerobically packed
109 (no vacuum, NV) in plastic bags and portions of batches 3 and 4 were placed in plastic
110 bags and sealed under vacuum (V). Also, the batches were stored at two different

111 temperatures: portions of batches 1 and 3 were stored at 4°C (4NV and 4V), whereas
112 portions of batches 2 and 4 were stored at 25°C (25NV and 25V).
113 Determination of moisture, hardness, color and TBARS was carried out at the beginning
114 (day 0) and after different days of storage (at day 3, 7, 15, 31) in samples of the four
115 batches (4V, 4NV, 25V and 25NV).
116 Moisture was determined according to the harmonised international protocol AOAC
117 Official Method (AOAC, 2002). Hardness was evaluated as explained above (section 2.1).
118 Color changes were analyzed in samples (cylinders D=2.8 cm and h=1cm) using a
119 colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan). Calibration was done
120 using a standard white porcelain with Y = 93.7, x = 0.3160 and y = 0.3323. The following
121 parameters were determined: lightness (L*), redness (a* ± red-green), and yellowness
122 (b* ± yellow-blue). Color coordinates were obtained using the CIE L*a*b* system, angle
123 10°, illuminant D65. Hue (H*) and Chroma (C*) were calculated according to equations
124 1 and 2:
125 1)$\text{Hue} = \tan^{-1} b^*/a^*$
126 2)$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$
127 TBARS were determined on gelled emulsion samples (0.5 g) according to the method
128 described by Maqsood & Benjakul (2010) with slight modifications reported in Poyato,
129 Ansorena, Navarro-Blasco, & Astiasaran (2014c).
130 Microbiological analysis was also performed in the four batches after 60 days of storage
131 by an external laboratory. *Salmonella* spp., *Listeria monocytogenes,* mesophilic aerobic
132 bacteria, yeasts and molds were determined. ISO 6579 (ISO, 2002) and ISO 11290-
133 1:1997/A1 (ISO, 2005) were used respectively to detect *Salmonella* spp. and *Listeria*

134 *monocytogenes* and the results were expressed as absence in 25 g of sample. ISO 4833
135 (ISO, 2003) and ISO 7954 (ISO, 1988) were used respectively to enumerate mesophilic
136 aerobic bacteria and yeasts and molds, and the results were expressed as logarithm of
137 colony-forming-units (CFU) per gram.

138 **2.3 Beef patties formulation and processing**

139 Fresh minced beef loin and fresh minced pork back fat were used as raw materials
140 obtained from a local meat market. Two different formulations of beef patties were
141 manufactured in a pilot plant. Each formulation was replicated twice, on different days.
142 The first formulation corresponded to the control (C), in which the fat content was
143 adjusted to 9% by the addition of pork back fat. In the second formulation (M), the pork
144 backfat was totally replaced by the gelled emulsion, freshly prepared. Both formulations
145 also included the following common ingredients per kilogram of minced beef meat: 8 g
146 salt, 5 g red pepper, 4 g dehydrated onion, 2 g garlic powder and 1.5 g black pepper. The
147 minced meat (2.5 kg) and the spices were thoroughly hand mixed. The meat mixture
148 was divided into two halves: pork back-fat was added to the first half, corresponding to
149 the control formulation (C), and the gelled emulsion, cut in small cubes (5x5mm), was
150 added to the other half meat mixture to obtain the modified formulation (M).

151 From each formulation, minced meat patties (80 g portions) were formed compressing
152 with the appropriate tool until a compacted and homogenized form was obtained (9 cm
153 diameter and 1.5 cm thickness each patty). Half of the patties from each formulation
154 were randomly selected for being cooked in a hot air oven, (8 min at 180 °C). After
155 cooling to room temperature, the patties (raw and cooked) were aerobically packaged
156 and stored at −20 °C for a period of maximum 4 days until all analyses were carried out.

157 The sensory evaluation of cooked products was performed just after manufacturing and
158 cooking the patties (day 0).

159 **2.4 Analysis of beef patties**

160 **2.4.1 Technological and nutritional analysis**

161 The analyses were performed on raw and cooked patties of each replicate and
162 formulation, with three measurements per sample.
163 Quantification of moisture, protein, ash and fat was done using official methods (AOAC,
164 2002). Extraction of lipids was carried out using fresh sample (120 g) and a chloroform:
165 methanol mixture (2:1), according to Folch, Lees, & Stanley, (1957). Fatty acid profile
166 was determined in the lipid extracts by gas chromatography (Ansorena, Echarte, Olle, &
167 Astiasaran, 2013). Briefly, 500 mg fat were weighed and mixed with boron
168 trifluoride/methanol (AOAC, 2002). After methylation, FAME were solved in hexane (5
169 mL). 1 mL of this solution was added to 1 mL of internal standard solution (7 mg/mL),
170 just prior to injection. This sample (0.5 µL) was injected in the gas chromatograph
171 (Perkin-Elmer Clarus 500 equipped with a capillary column SP^{TM} – 2560 (100 m x 0.25
172 mm x 0.2 µm) and flame ionization detection. The injector was set at 250 °C and the
173 detector temperature was set at 260 °C. The temperature of the column oven was
174 established at 175 °C for 10 minutes increasing up to 200 °C at a pace of 10 °C/min,
175 followed by an increase up to 220 °C at a pace of 4 °C/min and finally maintained at that
176 temperature for 15 minutes. The gas for the flame ionization detector was compressed
177 synthetic gas (O_2-N_2) mixed with hydrogen at a pressure of 20.5 psi. Hydrogen was used
178 as a carrier gas (mobile phase).
179 The identification of the fatty acid methyl esters was done by comparison of the
180 retention times of the peaks in the sample with those of individual standard pure

compounds from Sigma-Aldrich Chemical Co. (MO, USA) and by spiking the sample with each standard individually. The quantification of individual fatty acids was based on the internal standard method, using heptadecanoic acid methyl ester (Sigma, St. Louis, MO, USA). After the quantification of the individual fatty acids, the ω-6/ ω-3 ratio was calculated, as well as the following sums: EPA+DHA; polyunsaturated (PUFA: ω-3: α-linolenic, eicosapentaenoic, docosahexaenoic acid; ω-6: linoleic, γ-linoleic, arachidonic acid); saturated (SFA: caprilic, capric, lauric, myristic, palmitic, stearic and arachidic acid); monounsaturated (MUFA: palmitoleic, oleic, c-vaccenic, erucic and eicosenoic acid) and trans, (t-palmitioleic, elaidic and brassidic acid).

2.4.2TBARS

TBARS were determined on the extracted fat according to the method described by Maqsood & Benjakul (2010) with slight modifications reported in Poyato et al. (2014c). The analysis was performed on raw and cooked patties of each replicate and formulation, with tree measurements per sample.

2.4.3Sensory evaluation

A hedonic test (Anzaldua-Morales, 1994) was performed to evaluate the acceptability of the cooked patties. 38 non-trained panellists scored control and modified patties (C and M) with a 9-point scale. The scores ranged from 1 to 9 (9. like extremely; 8. like too much; 7. like considerably; 6. like slightly; 5. not like no dislike; 4. dislike slightly; 3. dislike considerably; 2. dislike too much; 1. dislike extremely). Each point marked was converted to a numerical value (from 0 to 10) assigned to the descriptive terms of the questionnaire so that further statistical analyses of data could be performed. The sessions were carried out in normalized testing booths and under controlled red light to

neutralize possible differences in color or appearance of the samples, although they were quite similar (see Figure S1). Patties were given to the panelists with a three-digit number chosen randomly. Water and neutral crackers were served to the panelists to rinse the mouth between the samples. The tests included a section in which panelists could describe any particular note detected during the sensory evaluation.

2.5 Statistical analysis

The statistical analysis of data was done using the STATA/IC 12.1 program (StataCorp LP, Texas, USA). All the experimental design was done in duplicate. Differences between the two replicates were not significant ($P < 0.05$) so this term was removed from the model. The values in the tables were given in terms of mean values and standard error of the mean (SEM). Differences among mean values for the burger patties were determined using one-way analysis of variance (ANOVA). TBARS, hardness and color of the gelled emulsions were analyzed using 2x2 factorial ANOVA with “days of storage” as repeated measurements. Multiple comparisons of means were done in all cases using Bonferroni Post Hoc procedure to evaluate the statistical significance ($P < 0.05$) for assessing the storage conditions of the gelled emulsion (4V, 4NV, 25V and 25NV) and the burger treatments (C raw, C cooked, M raw and M cooked). The numerical values obtained in the sensory test were evaluated by ANOVA. Significant differences ($P < 0.05$) among samples and panelists were also identified by the Bonferroni Post Hoc procedure.

3. RESULTS AND DISCUSSION

The formulation of a gelled emulsion was selected in order to obtain a low energy ingredient with increased supply of long chain omega-3 fatty acids (EPA+DHA) and technological properties (hardness and syneresis) similar to those of animal fat. The oil content and the carrageenan content were the two variables that needed to be considered.

Poyato et al. (2014b) reported that, depending on the carrageenan and oil concentrations added during their preparation, this type of gelled emulsions can have different values of hardness and syneresis. In fact, in our study, the carrageenan concentration had greater influence on the responses of hardness and syneresis as compared to the effect of oil concentration (data not shown). After the preparation and assessment of different gelled emulsions, the selected gelled emulsion contained 3% carrageenan and 1% algae oil, giving rise to values of 41.22 N for hardness and 1.14% for syneresis.

3.1 Stability of the optimized gelled emulsion during storage

Once the formulation of the gelled emulsion was selected (3% carrageenan and 1% algae oil), its stability was studied by means of physicochemical and lipid oxidation parameters in samples subjected to different storage conditions (4V, 4NV, 25V, 25NV).

Initial moisture content of the gelled emulsion was 95.86% (Figure 1). Sample stored under 4V (4°C/Vacuum), 4NV (4°C/No Vacuum) and 25V (25°C/Vacuum) conditions, remained in similar values ($P > 0.05$) during the storage. However, 25NV sample (25°C/No Vacuum) significantly decreased the water content at day 31 in 7.25% ($P<0.05$). This finding was attributed to the water evaporation of the sample.

246 Regarding the hardness of the gels, all the samples showed reductions in hardness
247 during storage. In the case of 4V, 25V and 4NV, the reductions (from 0 to 31 days) were
248 4, 8 and 11%, whereas in the case of 25 NV, the reduction was much more intense (30%).
249 This last sample (25 NV) was the one with the highest moisture loss, pointing out to a
250 possible effect of destabilization of the network of the gel due to the break of some of
251 the links between water molecules and the polymer chains.

252 Color is considered another important parameter to be controlled in the gels during the
253 storage, because modifications in this parameter could cause color differences in the
254 food matrix where the gel is incorporated. The initial values of the gel were: L*: 59.8, a*:
255 -3.8 and b*: 17.3. Similar values of L* and a* were found for pork backfat and a konjac
256 gel reported by Jimenez-Colmenero et al. (2012). However, b* parameter, indicative of
257 yellowness, showed a higher value, also when was compared to a gelled emulsion with
258 40% of linseed oil and 1.5% of carrageenan developed previously by our group (Poyato,
259 Ansorena, & Astiasarán, 2014a). The high b* value in this gel can be attributed to the
260 carotenoids present in the algae oil. Hue angle, indicative of the tone, and Chroma value,
261 measure of color intensity, were calculated from a* and b* parameters (figure 3). Higher
262 values ($P< 0.05$) of Hue angle were observed in 25NV as compared to the other
263 conditions during all the storage. On the contrary, Chroma value in 25NV was
264 significantly reduced (from 17.7 to 11.8) during the storage ($P< 0.05$), showing lower
265 values than in the other conditions. As a result, the gel showed a perceptible loss of color
266 intensity. These changes in Hue and Chroma values in 25NV sample were mainly caused
267 by the decrease of b* parameter, indicative of yellowness. Loss of yellow intensity might
268 be related to isomerization and potential degradation of carotenoids (Khoo, Prasad,
269 Kong, Jiang, & Ismail, 2011) caused by processes such as drying and oxygen reaction

(Boon, McClements, Weiss, & Decker, 2010). Thus, these color changes could be explained by a combined effect of loss of moisture and a possible lipid oxidation of 25NV sample.

In fact, lipid oxidation is a crucial parameter to guarantee the stability of LC-PUFA enriched products. TBARS were determined in samples stored under the four conditions at day 0, 7, 15 and 31. As shown in table 1, conditions of storage and storage times had significant effect on TBARS. The interaction between them was also significant ($P <$ 0.000). Initial TBARS value of the gel was 0.33 mg MDA/100 g gel. Temperature and vacuum packaging affected lipid oxidation susceptibility. At the end of the storage, significant differences ($P< 0.05$) were found comparing samples at the same packaging condition (4V vs. 25V) and (4NV vs. 25 NV), finding higher TBARS values at 25°C. In particular, when vacuum was not applied (25NV), highest TBARS value was observed (0.81). The significant increase of lipid oxidation in 25NV observed during the entire storage period ($P< 0.05$), could lead to the color changes previously described. In any case, all TBARS values could be considered indicative of low lipid oxidation. The low percentage of lipid fraction (1%), the antioxidants present in the oil, and the fact that the immobilized networked structure acts as a barrier against the lipid oxidation were possibly the reasons for this certain stability. Nevertheless, it could be interesting to add some extra antioxidants in the gel in order to control the slight increase of TBARS observed during the storage, even under vacuum conditions.

Regarding microbiological analyses, higher levels of total mesophilic microorganisms were detected in samples stored at 25°C (5 log CFU/g) than in samples stored under refrigeration (2 log CFU/g), which means that cooling storage was an important factor

293 to avoid the growth of mesophilic microorganisms (Table S1, Supplementary Material).
294 Regardless the application of vacuum, the growth of yeast and molds was almost halved
295 in 4V (2.66 log CFU/g) in comparison with the rest of samples (4NV, 25V, 25NV) noticing
296 here the effect of condition of storage.

297 To conclude about the stability of the gel, gels stored at 4ºC under vacuum packaging
298 showed the best results in microbiological stability and lipid oxidation, without giving
299 rise to significant changes in color and gel consistency. Nevertheless, good physical and
300 chemical stability was also noticed in 4NV and 25V gels.

301 **3.2 Application in beef patties**

302 In the second part of the work, the optimized gel (3% carrageenan and 1% algae oil) was
303 incorporated into beef patties in order to assess the viability of the new ingredient as an
304 animal fat replacer. Analyses of control and modified patties were done before and after
305 a cooking process (C Raw, C Cooked, M Raw and M Cooked).

306 Incorporation of the gel affected the general composition of raw and cooked patties
307 (Table 2). As it was expected, the moisture content of modified patties (M) increased
308 significantly (11%) as compared to the control ones (C) because of the high content of
309 water in the gel (96%). This fact was also observed in previous studies where gel systems
310 were incorporated in patties (Salcedo-Sandoval et al., 2015; Poyato et al., 2015) or when
311 vegetable oils were used as animal fat replacers (Dzudie, Kouebou, Essia-Ngang, &
312 Mbofung, 2004; Youssef & Barbut, 2011). Nevertheless, cooking process led to similar
313 reduction in moisture content (11%) in both patties (C and M).

314 Fat content of the control patties (C) was around 9%, within the range reported in
315 previous works for conventional patties (Martinez et al., 2012; Rodriguez-Carpena,

316 Morcuende, & Estevez, 2011; Realini, Guardia, Diaz, Garcia-Regueiro, & Arnau, 2015).
317 Modified patties (M) showed 2.62% fat, giving rise to a considerable level of fat
318 reduction (70%) as compared to the control ones (C). In addition, cooking process did
319 not affect the fat content because no significant differences (P<0.05) between raw and
320 cooked patties were found. As a consequence, the energy value of the modified raw
321 patties (M Raw) was 343 kJ/100g, meaning a 45% of energy reduction in these products
322 (712 kJ/100g in C Raw). Consequently, under the current Regulation (EC) No 1924/2006
323 on nutrition claims, the following statements could be made for modified products:
324 'energy reduced' (because the fat content in these patties was reduced more than 30%
325 compared to a similar product) and 'low-fat' (they contained no more than 3 g of fat per
326 100 g of solids).

327 Evaluation of the lipid composition of the modified products is crucial to confirm their
328 potential nutrition and health benefits (Table 3). Cooking process did not significantly
329 affect the lipid profile of the patties. In agreement with the proposal of the EFSA related
330 to the decrease of saturated fat in the diet (EFSA 2010), the SFA content was reduced
331 about a 69% in modified patties (M) as compared to the control (C) (3665 and 1146
332 mg/100g, respectively). Therefore, modified patties could also be claimed as 'reduced
333 saturated fat' (Regulation (EC) No 1924/2006). A low-level of total PUFA content was
334 also noticed in modified patties due to lower values of linoleic and alfa-linolenic acids.
335 Omega-3 content was also reduced in modified patties, mainly, due to lower values of
336 alfa-linolenic acid (Table S2, Supplementary material). However, the significant decrease
337 in omega-6 content of modified patties (P< 0.05) contributed to reduce the omega-
338 6/omega-3 ratio of the product. Control patties (C) showed values close to 16, far from
339 current nutritional recommendations, while modified patties (M) halved this ratio (7.1)

340 (Table 3). There is scientific evidence suggesting that a high omega-6/omega-3 PUFA
341 ratio is associated with the pathogenesis of numerous disorders, among them
342 cardiovascular diseases (CVD) or cancer. A lower ratio of omega-6/ omega-3 fatty acids
343 is more desirable in reducing the risk of many of the chronic diseases (Simopoulos, 2004).

344 Although total omega-3 content was reduced with the incorporation of the gelled
345 emulsion, the main omega-3 LC-PUFA in the algae oil, docosahexaenoic (DHA) and
346 eicosapentaenoic (EPA) fatty acids, were increased in the patties containing the gelled
347 emulsion. Consequently, EPA+DHA content increased in modified patties in 55%, almost
348 two fold the value detected in the Control (C) (24.4 and 13.5 mg, respectively). As far as
349 the authors are aware, the fat reduction and the improved lipid profile in these modified
350 beef patties as compared to a control with 9% of fat, has not been achieved in previous
351 works.

352 TBARS were determined in the patties before and after the cooking process, in order to
353 monitor the oxidation status (Table 4). Overall, all the samples showed oxidation values
354 within acceptable limit. However, a considerable decrease of lipid oxidation was
355 achieved in modified products (M) as compared to the control ones (C). This effect was
356 noticed both when results were expressed per kg of product, but also when expressing
357 data per amount of fat. The efficiency of the gelled emulsion to protect the lipid fraction
358 and the low oil amount in the system (1% algae oil) could be the reasons for the low lipid
359 oxidation status. Antioxidants present in the algae oil could be sufficient to avoid a
360 significant degree of oxidation in modified patties. Moreover, it has been reported that
361 increasing dietary omega-3 fatty acid in meat did not adversely affect lipid oxidation and
362 sensory attributes when enough amount of vitamin E concentration was present in the

363 muscle (Ponnampalam et al., 2014). Other authors have shown the same trend in their
364 studies when a solid non-meat fat system was used as fat replacer in different meat
365 products. Triki, Herrero, Jimenez-Colmenero, & Ruiz-Capillas, (2013) and Salcedo-
366 Sandoval et al. (2015) reported decreased lipid oxidation when pork backfat was
367 replaced by a konjac gel matrix. Poyato et al. (2015) showed the same behavior in
368 patties where the pork backfat was replaced with a linseed oil gelled emulsion, reporting
369 no differences before and after cooking among patties.

370 Consumers' opinion about functional meat products is highly appreciated. It has been
371 stated that consumers are prone to purchase this type of products if the price and taste
372 remain uncompromised (Shan et al., 2016). The mean score received by control patties
373 (C) with 8.80% fat was 6.37, whereas the modified patty (M), with 2.67% fat, scored 5.47.
374 These scores were not particularly high probably due to the low fat content in these
375 products (10 %), when typically these commercial products ranged between 10-20 %.
376 Moreover, none of the panelists detected a negative note on the patties, and when they
377 were asked if they would consume the products, a positive answer was reported both
378 for the control (68% of panelists would consume them) and for the modified patties (55%
379 of positive answers). It has to be mentioned that the panelists were not aware of the
380 nutritional benefits or the food technology used in the new formulation and this fact
381 might be relevant because it has been reported that providing this information to
382 consumers may affects its sensory appeal (Siegrist, 2008). All these results allowed us to
383 conclude about the positive evaluation of the reformulated product.

384 **4. CONCLUSIONS**

385 The optimized algae oil gelled emulsion (3% carrageenan and 1% algae oil) was an
386 efficient functional ingredient, technologically stable over time under vacuum packaging
387 and refrigeration storage. It has proved to be a successful total animal fat replacer in
388 beef patties, in which 70% fat reduction (low-fat energy patties) was achieved without
389 negative impact on its acceptability. Moreover, the modified patty increased EPA+DHA
390 content, while SFA and omega-6/omega-3 ratio were reduced, particularly interesting
391 from a health point of view.

392 **5. ACKNOWLEDGMENTS**

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401

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524

525 **FIGURE CAPTIONS**

526 Figure 1. Moisture (%) of the gelled emulsions at different conditions (4V, 4NV, 25V and
527 25NV) and days of storage (0, 3, 7, 15, 31).

528 Figure 2. Hardness (N) of the gelled emulsions at different conditions (4V, 4NV, 25V and
529 25NV) and days of storage (0, 15 and 31).

530 Figure 3. Hue and Chroma values of gelled emulsions at different conditions (4V, 4NV,
531 25V and 25NV) and days of storage (0, 3, 7, 15, 31).

532 **TABLE CAPTIONS**

533 Table 1. TBARS (mg MDA/100 g gel) of the gelled emulsions at different conditions (4V,
534 4NV, 25V and 25NV) and days of storage (0, 7, 15 and 31).

535 Table 2. General composition and energy values of Control (C) and Modified (M) beef
536 patties.

537 Table 3. Fatty acid profile of Control (C) and Modified (M) burger patties expressed in mg
538 per 100 g of product.

539 Table 4. Lipid oxidation of Control (C) and Modified (M) burger patties.

540

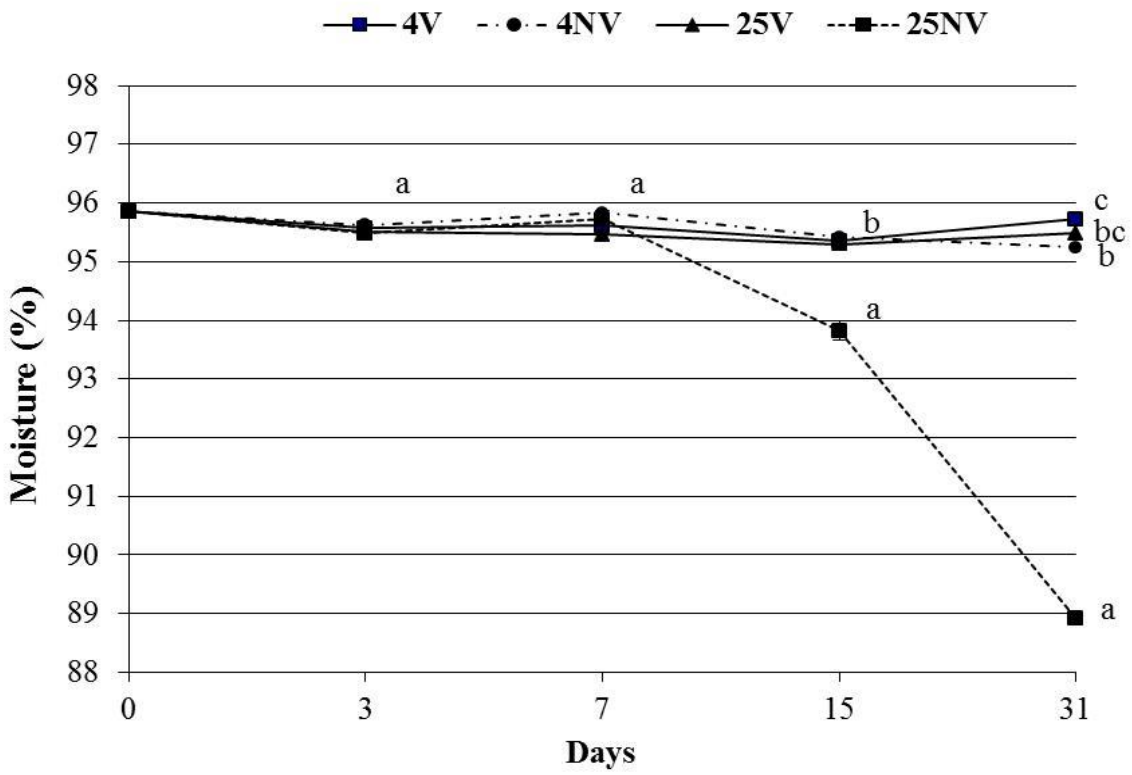
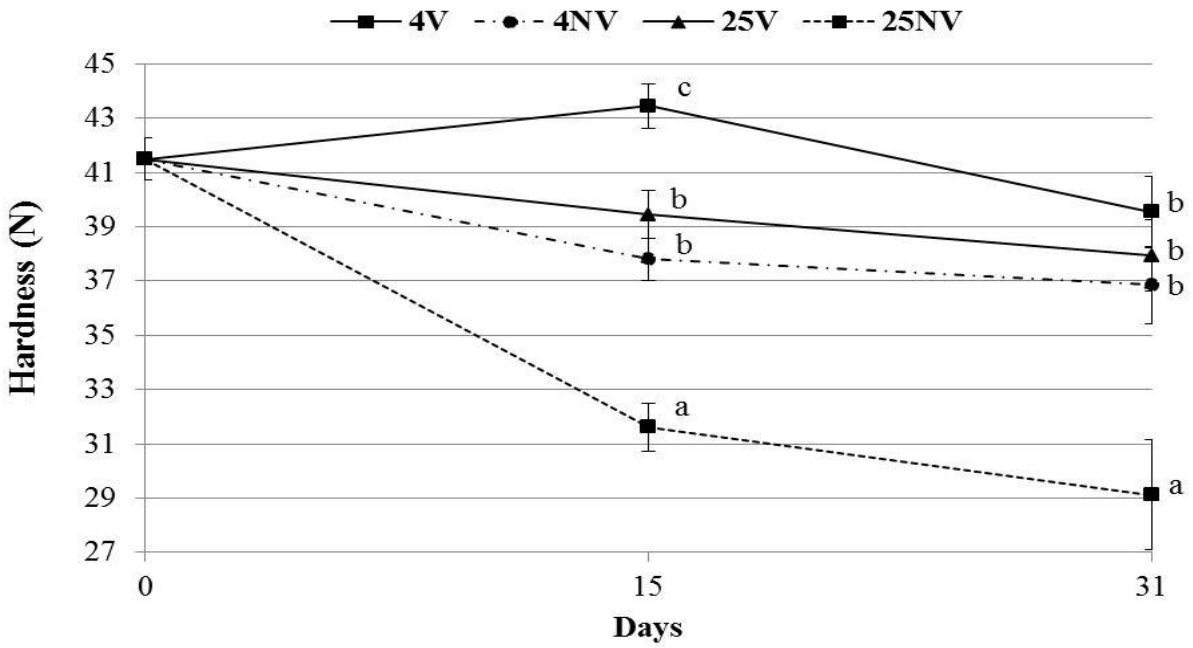


Figure 1. Moisture (%) of the gelled emulsions at different conditions (4V, 4NV, 25V and 25NV) and days of storage (0, 3, 7, 15, 31). Error bars denote standard error of the mean (SEM). Different letters (a,b,c) indicate significant differences ($P < 0.05$) among conditions by post hoc Bonferroni test. Common SEM for all values was 0.23.



548

549 **Figure 2.** Hardness (N) of the gelled emulsions at different conditions (4V, 4NV, 25V and 25NV) and days of
550 storage (0, 15 and 31). Error bars denote standard error of the mean (SEM). Different letters (a,b,c) indicate
551 significant differences (P< 0.05) among conditions by post hoc Bonferroni test. Common SEM for all values was
552 0.38.

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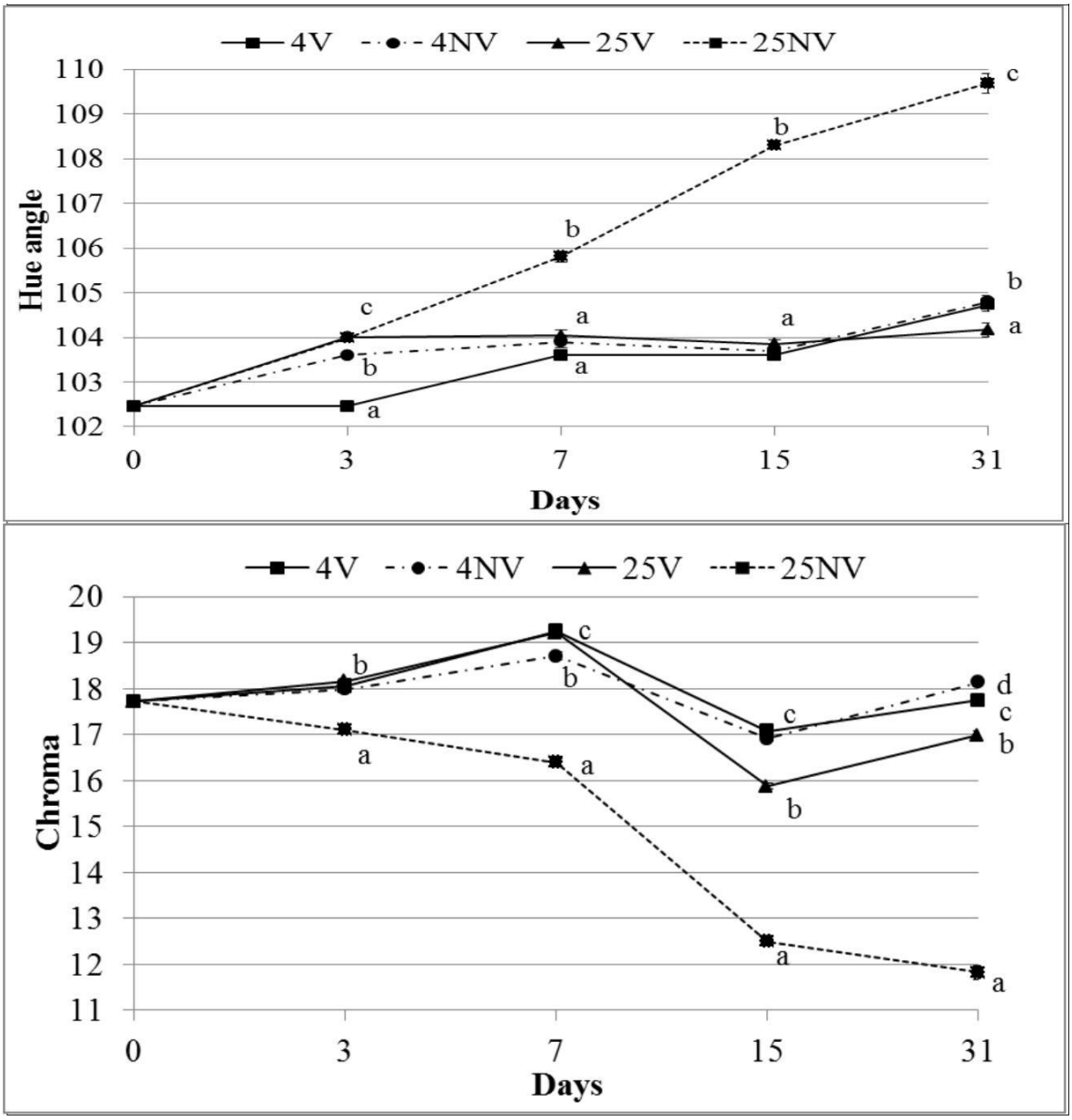

Figure 3. Hue and Chroma values of gelled emulsions at different conditions (4V, 4NV, 25V and 25NV) and days of storage (0, 3, 7, 15, 31). Error bars denote standard error of the mean (SEM). Different letters (a,b,c,d) indicate significant differences ($P< 0.05$) among conditions by post hoc Bonferroni test. Common SEM for Hue and Chroma were 0.09 and 0.08, respectively.

564 **Table 1.** TBARS (mg MDA/100 g gel) of the gelled emulsions at different conditions (4V, 4NV, 25V and 25NV)
565 and days of storage (0, 7, 15 and 31).

Conditions	Days of storage (Days)				SEM	*P*-value*		
	Day 0	Day 7	Day 15	Day 31		Condition	Day	C x Day
4V	0.33aA	0.54bC	0.53aC	0.49aB	0.01	0.000	0.000	0.000
4NV	0.33aA	0.57cC	0.60bC	0.52abB	0.02			
25V	0.33aA	0.52aB	0.58bB	0.54bBC	0.02			
25NV	0.33aA	0.53aB	0.70cC	0.81cD	0.03			

*SEM: standard error of the mean. Small letters within the same time of analysis (same column) indicate significant differences (P< 0.05) among storage conditions. Capital letters within the same condition (same row) indicate significant differences (P< 0.05) among days of storage by post hoc Bonferroni test. *Results from 2x2 factorial ANOVA.*

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569 **Table 2.** General composition and energy values of Control (C) and Modified (M) beef patties.

	C Raw	C Cooked	M Raw	M Cooked	SEM	*P* -value
Moisture (%)	68.57 b	63.57 a	77.09 d	71.65 c	1.04	0.001
Fat (%)	8.99 b	8.80 b	2.62 a	2.67 a	0.67	0.001
Protein (%)	22.39 b	26.36 c	18.58 a	22.99 b	0.63	0.001
Ash (%)	1.55 a	1.71 b	1.57 a	1.80 b	0.03	0.001
Energy value (kcal/100g)	170 c	186 c	98 a	116 b	8	0.001
Energy value (kJ/100g)	712 d	647 c	343 a	404 b	51	0.001
Energy from fat (kcal/100g)	81 b	79 b	24 a	24 a	6	0.001
Energy from fat (%)	47 c	43 b	24 a	21 a	3	0.001
Fat reduction (%)	-	-	70 a	70 a	2	1
Energy value reduction (%)	-	-	43 a	37 a	3	1

SEM: standard error of the mean. Per each parameter, different letters in the same row (a,b,c) indicate significant differences (P< 0.05) based on post hoc Bonferroni test.

570

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572

573 **Table 3**. Fatty acid profile of Control (C) and Modified (M) burger patties expressed in mg per 100 g of
574 product.

	C Raw	C Cooked	M Raw	M Cooked	SEM	*P*-value
EPA	3.4 b	4.1 b	2.7 a	2.7 a	0.17	0.04
DHA	10.5 a	11.6 a	21.7 b	24.3 b	2.55	0.001
EPA+DHA	13.5 a	14.9 a	24.4 b	26.9 b	2.38	0.04
Omega-3	68.9 b	72.6 b	37.5 a	34.6 a	7.74	0.003
Omega-6	1108 b	1091 b	268 a	264 a	144	0.001
Omega-6/Omega-3	16.1 b	15.0 b	7.1 a	7.6 a	1.06	0.001
PUFA	1116 b	1239 b	357 a	303 a	147	0.001
SFA	3665 b	3530 b	1146 a	1102 a	477	0.001

575 *SEM: standard error of the mean. Per each parameter, different letters in the same row (a,b) indicate*
576 *significant differences (P< 0.05) based on post hoc Bonferroni test.*

577

578 **Table 4.** Lipid oxidation of Control (C) and Modified (M) burger patties.

	C Raw	C Cooked	M Raw	M Cooked	SEM	*P*-value
TBARS (mg MDA/100 g fat)	0.87 b	0.83 b	0.52 a	0.59 a	0.03	0.001
TBARS (mg MDA/kg product)	0.70 b	0.88 b	0.14 a	0.14 a	0.09	0.001

SEM: standard error of the mean. Per each parameter, different letters in the same row (a,b) indicate significant differences (P< 0.05) based on post hoc Bonferroni test.

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