**“A new polyunsaturated gelled emulsion as replacer of pork back-fat in burger patties: Effect on lipid composition, oxidative stability and sensory acceptability”**

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**ABSTRACT**

A new gelled carrageenan containing emulsion developed as ingredient was used as fat replacer in burger patties. Increasing amounts (25, 50, 75 and 100 %) of this gelled emulsion were added into the product in order to reduce the fat content while improving its fatty acid profile. A 41% reduction of the total fat content with an increment of the 74.5% of the unsaturated fatty acids, and a significant decrease in cholesterol (47%) and saturated fat (62%) were achieved in the product with the highest level of substitution. These products showed significantly lower thiobarbituric acid reactive substances (TBARS) and cholesterol oxidation products (COPs) compared to control. Additionally, when samples were subjected to thermal treatment (180 ºC, 15 min, oven conditions) higher lipid oxidation rates were found when increasing amounts of the gelled emulsion were incorporated into the new formulations, without impairment of their final sensory properties.

**Key words:** fat replacer, gelled emulsion, lipid oxidation, cholesterol oxidation, meat patties

## 1. INTRODUCTION

The development of nutritionally improved meat products using reformulation strategies has been accomplished with the aid of emulsion based systems, being the improvement of the fat quality one of the most important goals. The substitution of pork back-fat with PUFA (polyunsaturated fatty acids) emulsified oils has been demonstrated to be a good strategy to achieve healthier lipid profiles in these products (Garcia-Iñiguez de Ciriano, Rehecho, Calvo, Cavero, Navarro, Astiasarán & Ansorena, 2010; Berasategi, Legarra, García-Íñiguez de Ciriano, Rehecho, Calvo, Cavero, Navarro-Blasco, Ansorena & Astiasarán, 2011; Rodríguez-Carpena, J. G., Morcuende, D., & Estévez, M., 2012). When using fat replacers a careful approach is needed in order to achieve the appearance and the technological, rheological and sensory properties required in the food industry (Tye, 1991). In this sense, polysaccharides can be used to create a variety of gel structures suitable for immobilizing oil droplets and forming gelled emulsions able to incorporate lipophilic agents with beneficial health effects into foodstuffs. (Herrero, Carmona, Jiménez-Colmenero & Ruíz-Capillas, 2014; Poyato, Ansorena, Berasategi, Navarro-Blasco & Astiasarán, 2014a). Polysaccharidic biopolymers such as carrageenan, konjac, inulin, dextrin and alginate have been used as potential fat analogues to reduce or improve the lipid fraction of different meat products, obtaining good results (Triki, Herrero, Jiménez-Colmenero & Ruíz-Capillas, 2013a; Salcedo-Sandoval, Cofrades, Ruíz-Capillas, Solas & Jiménez-Colmenero, 2013; Ruíz-Capillas, Carmona, Jiménez-Colmenero & Herrero, 2013; Utrilla, García-Ruiz & Soriano, 2014; Herrero, Carmona, Jiménez-Colmenero & Ruíz-Capillas, 2014). In comparison to conventional (oil-in-water) emulsions, gelled emulsions could be a better option to mimic hardness and water holding capacity of pork back fat used in most of the currently consumed meat products. Additionally, the use of gelled emulsion as fat

replacer may be a suitable technology not only for delivering, but also for protecting lipids in food products. Recently, our group has optimized the processing conditions for obtaining a carrageenan containing gel rich in polyunsaturated fatty acids, which successfully served as fat replacer ingredient in a Bologna-type sausage (Poyato et al., 2014a). However, a clear understanding of the gelled emulsion behaviour under different processing conditions is needed in order to highlight its potential use as animal fat replacer in a variety of products. This work was focused on adapting the use of this novel ingredient in a fresh meat product and on the evaluation of the behaviour of the new product (burger patty) after usual cooking.

The behaviour of the fat replacer on the samples was studied in raw and cooked conditions, by assessing nutritional and sensory properties of the resulting products, and by evaluating their stability against oxidation.

## 2. MATERIAL AND METHODS

### 2.1. Materials

Fresh beef and pork meat (loin) and pork back fat were obtained from a local meat market. Sunflower oil (Urzante S.L, Navarra, Spain) was obtained in a local market. Carrageenan (kappa-carrageenan) was kindly donated by Cargill (San Sebastián, Spain). Cholesterol, 5α-cholestane, thiobarbituric acid, fatty acid methyl esters and Polysorbate 80 were purchased from Sigma-Aldrich Chemical (Steinheim, Germany). 19-hydroxycholesterol was obtained from Steraloids (Wilton, NH, USA). Tri-sil® reagent was obtained from Pierce (Rockford, IL, USA). Acetone, chloroform, ethyl acetate, methanol, hexane, 2-propanol, hydrochloric acid, cyclohexanone, trichloroacetic acid, potassium chloride, potassium hydroxide, anhydrous sodium sulphate and sodium phosphate were obtained from Panreac (Barcelona, Spain). Hexane for gas chromatography, dichloromethane for gas chromatography and boron

trifluoride/methanol were from Merck (Whitehouse Station, NJ, USA). Strata $NH_2$ (55 μm, 70 A) 500 mg/3 mL Solid Phase Extraction cartridges were obtained from Phenomenex (Torrance, CA, USA).

**2.2. Gelled emulsion preparation**

The gelled emulsion was prepared according to the method described by Poyato et al. (2014a) using sunflower oil as the oil phase. The oil phase (40 g/100 g emulsion) containing the Polysorbate 80 as surfactant (0.12 g/100 g oil), was added to the aqueous phase (that included 1.5 g carrageenan/100 g emulsion) and homogenized. Both phases were previously heated separately to 70°C. After the homogenization process (16.000 rpm, Ultra-Turrax® T25basic), the emulsion was cooled to room temperature in a sealed flask, allowing the κ-carrageenan to polymerize. The gel was kept overnight under refrigeration (4 ºC) until being used (see supplementary material figure SM1, showing the gel aspect). The physical appearance was maintained and no syneresis was noticed during the burger meat patties processing.

**2.3. Meat patties formulation and processing**

Five different formulations of meat patties were manufactured in a pilot plant. About 5 kg of loin (2.5 kg beef and 2.5 kg pork) was minced (10 mm plate) using a meat mincer. After mincing, the samples were assigned to one of the following five treatments (G0, G25, G50, G75 and G100). In the Control (G0) 20 g/100 g of pork back fat was added, whereas in the four experimental batches different percentages, 25% (G25), 50% (G50), 75% (G75) and 100% (G100), of the pork back-fat were substituted by the gelled emulsion. Salt (0.2 g/100 g) was also added in all batches, samples were thoroughly hand mixed. Minced meat patties (80 g portions) were formed compressing with the appropriate tool until a compacted and homogenized form was obtained (8.9 cm diameter and 1.5 cm thickness each patty). Half of the patties from each batch were

randomly selected for being cooked in a hot air oven, (15 min at 180ºC). After cooling to room temperature, the patties were aerobically packaged and stored at -20ºC. The meat patties were analyzed in raw and cooked, in triplicate. The sensory evaluation was performed just after manufacturing the products on the first day. See supplementary material figure SM2, showing the G0 and G100, raw and cooked samples.

**2.4. Analysis of samples**

Moisture, protein and fat content were analyzed using official methods (AOAC 2002a, 2002b, 2002c). The method of Folch, Lees & Stanley (1957) was used for the extraction of fat. The fatty acids were determined in the lipid extracts by gas chromatography FID detection according to the procedure described by Valencia et al. (2008). The identification of the fatty acid methyl esters was done by comparison of the retention times of the peaks in the sample with those of standard pure compounds 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. After the quantification of the individual fatty acids, the sums of saturated, SFA, (capric, lauric, myristic, palmitic, stearic, arachidic, and behenic acid), monounsaturated, MUFA, (palmitoleic, oleic, vaccenic, erucic, nervonic and eicosenoic acid), polyunsaturated, PUFA, (ω-3: α-linolenic, eicosadienoic, eicosatrienoic, docosapentaenoic, docosahexahenoic acid; ω-6: linoleic, γ-linoleic, arachidonic, docosapentaenoic) were calculated.

*2.4.1. TBARS value*

TBARS values were determined on fat according to the method described by Maqsood and Benjakul (2010) with slight modifications. Briefly, the TBARS reagent was prepared by mixing trichloroacetic acid (15 g/100 mL), 2-thiobarbituric acid (0.375 g/100 mL) and hydrochloric acid (0.25 g/100 g). The fat (0.25 g), distillate water (250

µL), BHT (20 µL, 1 g /100 mL) and the TBARS reagent (1 mL) were vortexed in a centrifuge tube (20 s), placed in a boiling water bath for exactly 15 min and then cooled in an ice bath to room temperature. Cyclohexanone (2 mL) and 4 M ammonium sulphate (500 µL) were added to the mixture and were vortexed for 30 s. The mixture was centrifuged at room temperature at 4000 rpm for 10 min to allow separation of phases. After centrifugation, the supernatant was collected and the absorbance was measured between 300 and 600 nm (FLUOStar Omega spectrofluorometric analyzer, BMG Labtechnologies, Offenburg, Germany). The spectra were collected with a resolution of 2 nm. The quantification was performed using TEP (tetraethoxypropane) (calibration range: $2.6\times10^{-6}$ – $8.30\times1^{-5}$ mmol/g; LOD= $6.0\times10^{-7}$ mmol/g; LOQ=$1.8\times10^{-6}$ mmol/g) and 2,4-Decadienal (calibration range: $6.9\times10^{-3}$ – $2.6\times10^{-2}$ mmol/g, LOD= $8.3\times10^{-4}$; LOQ= $1.8\times10^{-3}$) as external standards, measuring the absorbance at 532 nm and 390, nm respectively. Results were expressed in mg MDA/kg product and in mmol 2,4-Decadienal/kg product, in each case.

*2.4.2. Cholesterol determination*

Meat patties (3 g) were added to 20 mL ethanol (95%), 5 mL KOH (50 g/100 mL) and 1 mL 5α-cholestane as internal standard (2 mg/mL in chloroform), and heated at 50ºC for 1 h until complete saponification. Then, water (13 mL) was added and the unsaponifiable material was extracted with hexane (6x20 mL). After filtering through anhydrous sodium sulphate, organic solvent was evaporated using a rotatory vacuum evaporator at 30 ºC. For derivatization purposes, Tri-Sil® reagent (400 µL) was added to each aliquot and they were kept at 60 ºC for 45 min in a water bath. The solvent was evaporated under a stream of nitrogen and the TMS-ether derivates were solved in hexane (400 µL) for gas chromatography. This solution was filtrated with a syringe and a filter (0.45 µm) and poured to a glass vial prior to GC-FID analysis. A Perkin-Elmer

Autosystem gas chromatograph equipped with an HP1 column (30 m x 0.25 mm x 0.1 μm) was used. The oven temperature was maintained isothermally at 265ºC. The temperature of both the injection port and detector was 300 ºC. The sample size was 1 μl.

Peak identification was based on comparison of the retention time of analytical standard. Quantification was made with calibration curve, with 5α-cholestane as the internal standard. Perkin-Elmer Turbochrom programme was used for the integration.

*2.4.4. Cholesterol Oxidation Products (COP) determination*

Approximately 0.5 g of the previously extracted fat was weighted in a flask containing 1M KOH in methanol and 1 mL 19-hydroxycholesterol (20 μg /mL in hexane:isopropanol 3:2) and kept at room temperature during 20 h to complete the cold saponification. The unsaponifiable material was extracted with diethyl ether (3x10 mL). The whole organic extract was washed with water (3×5 mL) and filtered through anhydrous sodium sulphate. Then it was recovered in a round-bottom flask, and the solvent was evaporated under a stream of nitrogen. Purification with amino-propyl cartridges and derivatization to trimethyl silyl ethers and analysis by GC-MS were performed as described in Ansorena et al. (2013). Quantification was done as described in Barriuso, Otaegui-Arrazola, Menendez-Carreño, Astiasarán & Ansorena (2012). The results were expressed as μg/100 g product and also μg/100 g dry matter of the sample. The oxidation rate was also calculated as follows, using data on μg/100 g dry matter:

$$OxidationRate = \frac{(COP_{cooked} - COP_{raw})}{COP_{cooked}} \times 100$$

**2.6. Sensory analysis of meat products**

A hedonic test (Anzaldúa-Morales, 1994) was performed on raw samples. The degree of appearance acceptability was evaluated for 33 non-trained panellists. A 7-point scale

was used for scoring the samples (3. I really like; 2. I like; 1. I slightly like; 0. I rather like or dislike; -1. I slightly dislike; -2. I dislike; -3. I really dislike).

In addition, a multiple comparison test (Anzaldúa-Morales, 1994) was performed on cooked products to determine the existence of perceptible sensory differences in colour, odour, taste, hardness, juiciness and fatness between the control and the gel containing products. This test was used to determine the effect of the possible changes caused by the substitution of an ingredient for another. A total of 11 trained panellists participated in the sessions to consider differences in the studied parameters and to assess the magnitude of the difference. Five samples were presented to each panellist, who were asked to indicate which sample differed from the control (G0). The scores (1. very much less; 2. much less; 3. considerably less; 4. slightly less; 5. not differences; 6. slightly more; 7. considerably more; 8. much more; 9. very much more) were collected and the statistical analysis of the results was carried out by an analysis of variance, in which numerical values were assigned to the descriptive terms of the questionnaire.

**2.7. Statistical analysis**

Mean and standard deviation of results obtained from the three replicates made per type of product were calculated. For each parameter, one factor ANOVA with Bonferroni post hoc multiple comparisons was used in order to evaluate the significant differences among samples. Within each type of sample, the differences between raw and cooked were evaluated by Student t-test.

Regarding the sensory tests the scores obtained in the multiple comparison tests and the hedonic test were evaluated by ANOVA. The effect of each variable and the variability among judges were evaluated. Pearson correlation test was used to determine correlations among variables.

The statistical analysis of data was done using the STATA/IC 12.1 program (StataCorp LP, Texas, USA) Significance level of $p \leq 0.05$ was used for all evaluations.

## 3. RESULTS AND DISCUSSION

### *3.1. Sensory evaluation*

The gelled emulsion developed in a previous work (Poyato et al., 2014a) showed good technological properties when added to Bologna-type sausages, where it did not cause sensory problems. Taking into account that sensory acceptability is a crucial factor when testing the incorporation of new ingredients in foodstuffs, another challenge was faced with this gel, being used in fresh meat product. A hedonic test was performed in raw samples in order to evaluate consumer acceptability of the new formulations. The scores reported by panellists for the gel containing products were higher than for the control one, meaning that the gel tend to improve the appearance of the new meat patties compared to the control, although no significant differences were found. After cooking, samples were subjected to multiple comparison tests between G0 and the gel-type products (G25, G50, G75 and G100) (Table 1). Results showed no significant differences ($p > 0.05$) for odour, colour, taste, hardness, juiciness and fatness between the experimental batches and the control in these cooked products and neither among the gel-containing samples, pointing out that the white colour and the consistence of the gel perfectly mimicked the appearance of lard in the new products. These results led to conclude that all replacements were satisfactory for the panellist, so that the gelled emulsion ingredient could be a good fat replacer for obtaining burger patties similar to the original product. Similarly, other authors (Gao, Zhang & Zhou, 2014) reported improvements or no significant differences in meat products in which partial fat replacements were done.

### *3.2. Overall nutritional value*

Once the sensory acceptability was ensured, we evaluated the improvement of the nutritional quality of the developed products by the added gelled emulsion.

The use of the gelled emulsion significantly reduced the total fat content of reformulated products (up to 41% in G100 raw samples compared to control ones) increasing at the same time the PUFA supply (up to 63%) in a dose-dependant manner (Table 2). In fact, the higher the gel used, i.e., the lower the fat content, the higher the PUFA fraction (r = 0.959**), and the lower the SFA one (r = 0.957**). These modifications resulted in improved PUFA/SFA and PUFA+MUFA/SFA ratios. These achievements were highly relevant from the nutritional point of view, because it has been demonstrated that an increased PUFA consumption as a replacement for SFA reduced the occurrence of coronary heart disease events (Mozaffarian, Micha & Wallace, 2010).

Moreover, a quantitative noticeable reduction in cholesterol content (up to 16.9% decrease) was noticed when increasing the gel content in the product from G0 to G100. The combined action of the reduction of the fat content, SFA and cholesterol and the PUFA increment resulted in healthier products as long as more gel was incorporated.

As it was expected, the fat decrease was accompanied by a moisture increase, although these differences were reduced after the heat treatment due to the water loss. In fact, moisture loss during the heat treatment was slightly higher in the samples elaborated whit the gelled emulsion. No significant differences ($p > 0.05$) for protein content were noticed among the different formulations in raw products, and only significant differences ($p < 0.05$) between G0 and G100 were detected in cooked samples. Additionally, net protein content increased with heat treatments as consequence of the water loss.

Table 3 gathered the potential nutrition claims that could be applied to the developed burger patties referred to raw products according to the EU Regulation (Regulation 1924/2006). A claim that a food is “high in protein” may be made in all products due to the fact that at least 20 % of the energy value of the product is provided by protein. Depending on the amount of gelled emulsion incorporated in the formulations several nutrition and health claims could be made. The reduction of 30% in the energy supply in the G100 product compared to a conventional formulation could allow it to declare “energy reduced”. G75 and G100 products, in which the reduction in fat content was at least 30 % compared to the control (G0), can be declared as “reduced fat”. On the other hand, the claim "reduced saturated fat" can be made in the G50, G75 and G100 products because the sum of SFA and trans were, in all cases, at least 30 % less than in the G0; and the content in trans in the products was significantly lower ($p<0.05$) than in a conventional product. The PUFA+MUFA content allowed claiming “high unsaturated fat content” for the G75 and G100 products, in which unsaturated fatty acids were at least 70% of the fatty acids present in the product and provided more than 20% of energy of the product. As it can be seen, the G100 product could be labelled with five nutrition claims: “energy reduced”, “high protein”, “reduced fat”, “reduced saturated fat”, and “high unsaturated fat”. Additionally, according to the Commission Regulation (EU) 432/2012, these nutritional modifications allowed to claim in G75 and G100 products that “Replacing saturated fats in the diet with unsaturated fats contributes to the maintenance of normal blood cholesterol levels”, “Linoleic acid contributes to the maintenance of normal blood cholesterol levels; the beneficial effect is obtained with a daily intake of 10 g of LA” and “Reducing consumption of saturated fat contributes to the maintenance of normal blood cholesterol levels”. This last claim could be also made for G50.

### *3.3. Oxidative stability*

#### *3.3.1. TBARS assessment*

Increasing the PUFA content of products might enhance the oxidation susceptibility of the gelled emulsion containing formulations during the heating treatment (Poyato et al., 2014a). The most important cause of deterioration of oils and fats is oxidation, which does not only reduce shelf life, sensory acceptance and the nutritional value of food, but also produces toxic compounds. In order to monitor the oxidation status, TBARS at 390 nm (mmol 2,4-Decadienal/kg product) and at 532 nm (mg MDA/kg product) were measured, before and after the heating treatment (Table 4). A progressive reduction of the oxidation products in raw patties were observed at 390 and 532 nm, which was statistically significant in the products with the highest substitution level compared to the control one. This was caused probably as a merged action of the reduction of fat content and the lower oxidative status of the fat in these products. In fact, if the results were expressed over kg fat, significant differences were noted among formulations at 390 nm, and a trend in the reduction of the oxidation compounds at 532 nm was observed. Poyato et al. (2014b) reported that the sensitivity of the measure of the oxidation compounds, depending on the lipid profile, was higher at 390 than at 532, nm allowing us to detect these differences. As expected, cooking increased lipid oxidation in all products, having all burger patties higher values of mg MDA/kg product and mmol 2,4-Decadienal/kg product in cooked samples compared to raw ones. Moreover, in cooked samples a dose dependent effect was noticed with the substitution level. Thus, the higher the amount of gel added (it means, the lower the fat present in the product), the higher the oxidation rate found. A negative correlation was found between fat content and mg MDA/g fat (-0.784*) or mmol 2,4-Decadienal/kg fat (-0.843**) and a positive correlation between PUFA content and mg MDA/g fat (0.773**) or mmol 2,4-

Decadienal/kg fat (0.834**) was noticed. Thus, compared to raw products, oxidation values increased with cooking a 19% (G0) and a 95% (G100), when analysing values at 390 nm and 71% (G0) to 197% (G100) at 532 nm. This is in accordance with other authors (Jacobsen, Timm, & Meyer, 2001; Taherian, Britten, Sabik, & Fustier, 2011) who reported that the use of vegetable oils as functional ingredients in food lipid emulsions might be complex due to the high oxidation susceptibility of these unsaturated oils.

*3.3.2. COPs assessment*

As the selected food was from animal origin the study of the oxidative stability included also the cholesterol oxidation products (COPs) determination. These compounds have been used as a measure of markers of the oxidation process (Rodríguez-Estrada, Penazzi, Caboni, Bertacco & Lecker, 1997), besides their known role in some harmful effects for human health (Otaegui-Arrazola, Menendez-Carreño, Ansorena & Astiasarán, 2010). In agreement to the lipid oxidation trend detected by the classical TBARS method, significantly lower total cholesterol oxidation compounds were observed in those products with the highest content of gelled emulsion (Pearson correlation between fat content and COP µg/100 g was 0.888** and correlation between cholesterol content and COPs was 0.707**). COPs were 2-fold lower in G100 samples than in the control patties (G0), in both raw and cooked samples when expressed per 100 g of product (Table 5). In order to avoid bias caused by water loss by cooking, results of COPs were also calculated on dry matter of the samples. These results pointed out that during heating, the cholesterol oxidation percentage gradually increased with the gelled emulsion incorporation from 15.3 % to 36.5% in G0 and G100, respectively. The most abundant COPs, in all samples, were 5,6-β-cholesterol epoxide and 7-ketocholesterol. Rodríguez-Estrada et al. (1997) reported that 7-ketocholesterol was

used as a marker of the degree of cholesterol oxidation, due to its fast and continuous formation and its relatively high amounts with respect to the other oxidation products. However, the higher oxidation rate (referred to the amount in raw samples) was for 7α-hydroxycholesterol and 7β-hydroxycholesterol that gradually increased with the incorporation of gelled emulsion from 35.4% (G0) to 88.9 % (G100) and from 49.2% (G0) to 96.2% (G100), respectively. These results were in agreement with the initial states of the thermal cholesterol oxidation in which 7α-hydroxycholesterol and 7β-hydroxycholesterol are two of the major oxidation products (Smith, 1987). Moreover epoxides significantly increased during the heating being the 5,6-β-cholesterol epoxide the predominating compound as expected (Lampi, Juntunen, Toivo, & Piironen, 2002; Yen, Lu, Inbaraj, & Chen, 2011; Ansorena et al., 2013). Finally, both epoxy compounds (5,6-β-cholesterol and 5,6-α-cholesterol) can give 3,5,6-cholestanetriol by hydration when the epoxy compounds had already started their decline (Ansorena, et al., 2013). Because of the short heat treatment applied to these samples (15 min) the 3,5,6-cholestanetriol had the lowest oxidation rate (from 8.1 (G0) to 18.6 (G100)), as this COP is formed at advanced stages of oxidation.

The results showed that higher PUFA content of the gel contributes to create a pro-oxidant environment for cholesterol causing the formation of COPs (Pearson correlation between COPs oxidation rate and PUFA content was 0.942**), despite the presence of antioxidant compounds in the sunflower oil such as tocopherols (72 mg/100 g, data obtained from the label). Other studies also showed that the higher unsaturation degree of the lipid matrix promotes cholesterol oxidation (Pignoli, Rodriguez-Estrada, Mandrioli, Barbantui, Rizzi & Lecker, 2009; Boselli, Rodriguez-Estrada, Ferioli, Caboni & Lecker, 2010) with the presence of free radicals and hydroperoxides (Ohshima, 2002).

## 4. CONCLUSIONS

In conclusion, the optimized gelled emulsion was an effective ingredient as partial or total pork back fat replacer in meat patties, showing nutritional advantages and without negative influence on the sensory properties of the final products. Additionally, results also showed that cooking increased the susceptibility of the highly unsaturated ingredient to oxidation, being advisable to control this process in future applications.

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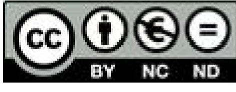

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