The effect of household storage and cooking practices on quality attributes of pork burgers formulated with PUFA- and curcumin-loaded oleogels as healthy fat substitutes

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ABSTRACT

Structuring healthy oils into oleogels is a feasible way to develop functional ingredients with solid-like properties, similar to those of animal fat; being therefore adequate in the development of meat products. Due to the highly unsaturated nature of the oils employed, lipid oxidation is a major concern regarding product quality. In the present work, the effect of household practices (chilled storage and cooking) on quality attributes of low fat PUFA-enriched pork burgers has been studied as function of the oleogelation system (ethyl cellulose or beeswax) and the presence of curcumin as an antioxidant. Curcumin effectively reduced the lipid oxidation process derived from chilled storage or cooking regardless of the oleogelation method; the resulting products showed a lipid composition which meets recommendations for fatty acid intake. The samples formulated with beeswax oleogel presented adequate technological properties and good overall sensory acceptability. Further studies are needed to adapt ethyl cellulose oleogels prepared with highly unsaturated lipids for the successful development of fresh meat products, due to

24 the extent of lipid oxidation during refrigeration and cooking, and to the lower sensory
25 acceptability.

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27 **Keywords:** functional food; meat product; beeswax; ethyl cellulose; reformulation

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1. INTRODUCTION

Improving the lipid content of meat products (reducing fat level, enriching with PUFAs) has been object of intensive study by researchers (Alejandre, Astiasarán, Ansorena, & Barbut, 2019; Ansorena & Astiasarán, 2004b; Gómez-Estaca, Pintado, Jiménez-Colmenero, & Cofrades, 2019b; Jimenez-Colmenero et al., 2015). Replacing animal solid fat with vegetal or fish oils with a healthier composition is a technical challenge, as they induce changes in product texture, flavor and oxidative stability. A suitable solution is structuring oils into solid-like structures (Jimenez-Colmenero et al., 2015). Also, these new lipid materials could be used as carriers of several compounds, such as antioxidants, fiber, minerals, etc. with technological and/or nutritional advantages (Alejandre, Ansorena, Calvo, Cavero, & Astiasarán, 2019; Flaiz et al., 2016). One of the possible strategies to structure liquid oils into solid-like systems is organogelation. Several oils structured in ethyl cellulose or beeswax have been successfully employed in the development of raw or cooked meat products (sausages, burgers, pâtés, etc.) presenting a healthier lipid profile and adequate technological properties (Barbut, Wood, & Marangoni, 2016; Gómez-Estaca et al., 2019a; Gómez-Estaca et al., 2019b; Moghtadaei, Soltanizadeh, & Goli, 2018). However, some aspects should be improved, for instance the stability of the lipid fraction: manufacturing processes involve temperatures up to 160 °C, which have been proved to induce lipid oxidation and could therefore affect meat product quality (Gómez-Estaca et al., 2019a; Gómez-Estaca et al., 2019b). Furthermore, common household practices such as chilled storage and cooking also induce lipid oxidation, resulting in loss of quality (Jiménez-Colmenero, Cofrades, Herrero, & Ruiz-Capillas, 2018)

The most feasible way to inhibit lipid oxidation during oleogel manufacturing process may be the addition of antioxidants; however, the body of literature is scarce in this

aspect. In a recent work by Martinović, Poklar Ulrih, and Abramovič (2019), sinapic acid and its derivatives were evaluated as antioxidants in sunflower oil oleogels prepared with beeswax, and it was found that the most apolar compounds exerted the most active antioxidant effect. In the present work, curcumin has been included in the formulation of ethyl cellulose and beeswax oleogels, due to its good solubility in apolar solvents and its antioxidant properties (Jayaprakasha, Rao, & Sakariah, 2006). Furthermore, this antioxidant may play a role not only during organogel formation, but also during product development, in addition to household storage and cooking practices, improving product quality. Therefore, the objective of the present work is to evaluate the effect of household practices (chilled storage and cooking) on quality attributes of low fat PUFA-enriched pork burgers, as function of the oleogelation system (ethyl cellulose or beeswax) and the presence of curcumin as an antioxidant.

2. Materials and Methods

2.1. Materials and reagents

The lipid sources for oleogel development were: olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain), linseed oil (Natursoy SL, Alimentos Ecológicos, Castellterçol, Spain) and fish oil (Omevital 18/12 TG Gold, Cognis GmbH, Illertissen, Germany). Ethyl cellulose (Aqualon N10 EC) was kindly donated by Ashland (The Netherlands). Beeswax was acquired from Manuel Riesgo SA (Madrid, Spain). Sorbitan monostearate and curcumin were from Sigma-Aldrich (Madrid, Spain). Pork meat, back-fat, salt and spices were acquired at a local market and prepared as described by (Salcedo-Sandoval, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2014).

2.2. Oleogel development

Oleogels made using ethyl cellulose or beeswax with or without added curcumin were prepared according to our previous work (Gómez-Estaca et al., 2019a) with slight modifications. These included the incorporation of curcumin (0.2 g/100 g oleogel) to the oil/organogelator mixture during oleogel preparation, as well as the application of a sonication step prior to gel cooling. For that, 2 consecutive cycles of 1 min each at 30 s intervals in a Q700 sonicator (Qsonica, Newton, CT, USA) set at 100 Hz were applied. Also, in line with previous works, the lipid phase of oleogels consisted of a mixture of olive, linseed and fish oils (44.39%, 37.87%, and 17.74%, respectively), which was prepared to attain an optimal lipid profile from a health standpoint (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2010).

2.3. Burger formulation

The formulation of the five batches of burgers is shown in Table 1. Five different batches were prepared: pork back-fat (C batch), ethyl cellulose oleogel (EC batch), ethyl cellulose oleogel with curcumin added (EC-Cu batch), beeswax oleogel (W batch), and beeswax oleogel with curcumin added (W-Cu batch). Meat and pork back-fat packages were thawed (approx. 18 h in refrigeration, reaching ≈ -5 °C), and the oleogels were placed in a freezer (-18 ± 1 °C) for approximately 2 h before burger preparation. Burgers were prepared according to Salcedo-Sandoval, 2014). Briefly, meat and fatty ingredients were minced (4.5 mm) (Van Dall Srl, model FTSIII, Treviglio, Italy) and mixed for 2 min (MAINCA, Spain) with the other ingredients. Burgers weighting ≈ 85 g were prepared using a manual burger press, packaged in trays in air atmosphere, and stored at 4 ± 2 °C.

The effect of formulation and common household practices on this kind of products, such as chilled storage and grill cooking, were evaluated regarding composition, technological properties, oxidative status, microbiological stability and sensory acceptability.

Determinations to evaluate the effect of formulation and cooking were performed on day 1 after preparation, whereas the effect of chilled storage (4 ± 2 °C) on stability was determined at different time intervals (days 1, 3 and 6).

2.4. Composition

Proximate analysis (moisture, protein, fat and ash) was carried out in triplicate in both raw and cooked burgers according to methodologies described elsewhere (Gómez-Estaca et al., 2019b; Salcedo-Sandoval et al., 2014). Fatty acid contents were determined (in triplicate) in raw and cooked burgers previously freeze-dried by saponification and bimethylation as described by Lee, Tweed, Kim, and Scollan (2012), using C13:0 as internal patron. Fatty acid methyl ester (FAME) was analyzed by gas chromatography on an Agilent gas chromatograph (Model 7820A, CA-USA) fitted with a GC-7 Agilent HP-88 capillary column ($60\text{ m} \times 250\text{ }\mu\text{m} \times 0.2\text{ }\mu\text{m}$), and a flame ionization detector was used. Injector and detector temperatures were 250 °C and 260 °C respectively. The temperature profile of the oven was 125 °C, increasing by 8 °C/min to 145 °C (held for 26 min) and 2 °C/min to 220 °C (held for 5 min). Fatty acids were identified by comparison of retention times with a fatty acid standard (Supelco 37 FAME Mix 47885-U, USA) and expressed as g of FAME/100 g burger.

2.5. Technological properties

For texture analysis in raw and cooked burgers, Kramer shear force (KSF) was determined (six replications) in a TA-XTplus Texture Analyzer (Stable Micro Systems Ltd., UK) equipped with a 50 N load cell, using a miniature Kramer/Ottawa cell (HDP/MK05). Samples measuring 2×2 cm were cut from two burgers, accurately weighed, and placed into the cell, at room temperature. Samples were penetrated 20 mm,

at a speed of 0.8 mm/s. KSF values were calculated as the maximum force per g of sample (N/g).

Color parameters were measured (ten times) in raw and cooked samples using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan) set to D65 illuminant/10° observer. Results are expressed as chromaticity (C^*) and hue angle (h°) chromatic parameters using simple transformations (Gómez-Estaca et al., 2019b).

Cooking loss was calculated as weight loss after cooking and expressed in %. Burgers were cooked in an electric griddle set (Princess classic multigrill type 2321, The Netherlands) at 180 °C, for 2 min per side, the time being previously optimized to reach 70 °C in the thermal center.

2.6. Curcumin content and antioxidant activity

These determinations (triplicate) were performed in both raw and cooked burgers. For curcumin extraction, ≈ 2 g of sample were accurately weighted and homogenized in 40 mL HPLC grade methanol using an Ultraturrax blender (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 14,000 rpm, for 1 min. After 30 min of agitation (200 rpm), the supernatant was collected by filtration through Whatman no. 1 filter paper. Curcumin was determined at 428 nm using a Shimadzu UV/VIS 1203 spectrophotometer and quantification performed by interpolation in a standard calibration curve for curcumin dissolved in methanol prepared in advance. Results are expressed as mg curcumin/100 g burger.

Antioxidant activity was determined by the photochemiluminescence assay, using the PHOTOCHEM system (Analytik Jena AG). This assay involves the photochemical generation of superoxide O_2^- free radical combined with chemiluminescence detection of

luminol, which acts as a photosensitizer and also as an oxygen radical detection reagent. Results are expressed as mg eq. Trolox/100 g burger.

2.7. Lipid oxidation

Thiobarbituric acid reactive substances (TBARs) were determined in triplicate in both raw and cooked burgers using the method described by Delgado-Pando, Cofrades, Ruiz-Capillas, Triki, and Jiménez-Colmenero (2012). Results were expressed as mg malonaldehyde (MDA)/kg burger, based on a standard curve prepared from 1,1,3,3-tetraethoxypropane in advance.

2.8. Microbial counts

Microbiological analyses were carried out in a vertical laminar-flow cabinet (model AV 30/70 Telsar, Spain). 10 g of each raw burger were aseptically taken and mixed with 90 mL of buffered peptone water (Panreac, Germany) to give 1/10 dilution. After 2 min in a stomacher blender (Stomacher Colworth 400, Seward, London, UK), appropriate decimal dilutions were plated in duplicate on Violet Red Bile Glucose Agar (VRBG) (Panreac, Germany) with a double layer for *Enterobacteriaceae* (37 °C for 24 h) and on Plate Count Agar (PCA) (Panreac, Germany) for the total viable counts (TVC) (30 °C for 72 h). All microbial counts were converted to logarithms of colony-forming units per gram (log cfu/g).

2.9. Sensory analysis

A hedonic sensory analysis was performed by an untrained panel of 40 people selected from the staff of ICTAN-CSIC. Each burger was cut into 4 pieces immediately after being cooked on an electric griddle for 4 min (2 min per side) and the five samples (C, W, W-Cu, EC, EC-Cu) were presented to the panelists, who were instructed to rinse their mouths

with bread and water between samples. Odor, color, texture and overall acceptability were evaluated on a 10-point scale, being 0 considered as “strongly dislike” and 10 as “strongly like.” The panelists were also asked to make any comments they considered appropriate about their sensory perception of the samples.

2.10. Statistical analysis

Statistical tests were performed using the SPSS computer program (SPSS Statistical Software, Inc., Chicago, IL, USA). One-way and/or two-way analyses of variance (ANOVA) were conducted. Differences between pairs of means were assessed on the basis of confidence intervals using the Tukey-b test. The level of significance was $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of formulation

In accordance to target formulation (Table 1), all raw samples showed the same proximate composition ($p > 0.05$) (Table 2), being the low fat content (7.5% in average) especially remarkable. An improvement in the fatty acid profile in reformulated samples (W/W-Cu, EC/EC-Cu batches) was observed, showing an increase ($p \leq 0.05$) of MUFA and PUFA and a concurrent SFA reduction ($p \leq 0.05$) compared with the control batch (Figure 1). The most abundant PUFA in the oleogel-added burgers was ALA, followed by EPA and DHA, respectively (Table 3). According to this, reformulated burgers could be labelled with several nutritional and health claims according to European Regulations. In this regard, reformulation strategies resulted in an increase in the Σ PUFA/ Σ SFA ratio from 0.4 (control batch) to ≈ 1.3 (W/W-Cu and EC/EC-Cu) and a marked decrease of the Σ PUFA-6/ Σ PUFA-3 ratio from 15 to ≈ 0.65 (Table 3). These ratios are one of the main parameters

used to assess the nutritional quality of the lipid fraction in foods and they are recommended to be greater than 0.4 for Σ PUFA/ Σ SFA and lower than 4 for Σ PUFAn-6/ Σ PUFAn-3 ratio, respectively (Simopoulos, 2002; Wood et al., 2004). Furthermore, the amount of calories provided by PUFAS was approximately 2-fold in the reformulated samples compared with the control ones (Table 3). These results are similar to those of previous works in which animal fat was substituted with the same lipid mixture, structured by different methods such as oleogels, oil-in-water emulsions or konjac-based bulking system in different meat products (burgers, frankfurters, pâtés) (Delgado-Pando et al., 2010; Gómez-Estaca et al., 2019a; Gómez-Estaca et al., 2019b; Salcedo-Sandoval et al., 2014).

Table 4 shows some technological properties of the raw burgers. Formulation had a significant effect ($p \leq 0.05$) on optical properties, being curcumin the factor with the greatest influence. Thus, W-Cu and EC-Cu batches showed a higher hue and chroma; hence, they were significantly yellower than the others. Reformulation also had an effect on hardness, regardless of the lipid analogue used, the resulting burgers were softer than the control batch ($p \leq 0.05$). The results obtained for optical properties and texture are in accordance to the literature (Gómez-Estaca et al., 2019b; Mancini et al., 2015; Moghtadaei et al., 2018).

The effect of reformulation on the oxidative status of the burgers is shown in Figure 2A (black bars). As shown in a previous work by the authors, animal fat substitution by the developed oleogels (without curcumin) resulted in a product with a higher TBARS content, which was especially remarkable in the case of ethyl cellulose oleogels (Gómez-Estaca et al., 2019b). This is due to the polyunsaturated nature of the lipid mixture, as compared to pork back-fat, as well as to the high processing temperatures employed

during oleogel manufacturing. However, when curcumin was added during oleogel development, a lower lipid oxidation was observed in the burgers (W-Cu and EC-Cu), as compared with the corresponding counterparts. Thus, the TBARS content in EC-Cu was similar to that of the C batch ($p>0.05$) and in the W-Cu batch it was even lower ($p\leq 0.05$). This is largely attributed to the antioxidant properties of curcumin, which has been attributed both, radical scavenging and iron chelating capacities (Hatcher, Planalp, Cho, Torti, & Torti, 2008). The curcumin content and antioxidant activity of the burgers are shown in Table 5. As expected, there were no differences in the curcumin content between batches W-Cu and EC-Cu ($p>0.05$), as the amount of curcumin added to the oleogels and the amount of oleogel added to the formulations were identical (Table 1). The presence of curcumin induced an increase of antioxidant activity in these batches, compared with those without curcumin ($p\leq 0.05$); there was no difference between batches W-Cu and EC-Cu ($p\leq 0.05$). Thus, these results show that curcumin is available in the burgers to exert its antioxidant activity during burger preparation and eventually to prevent lipid oxidation that may derive from storage or cooking.

3.2. Effect of chilled storage

All raw batches showed the accumulation of lipid oxidation products during storage, although differences in the kinetics and magnitude were observed as function of the lipid ingredients used (Figure 2B). The C batch was stable during the first 3 days of storage, but after 6 days lipid oxidation had increased significantly ($p\leq 0.05$). A similar trend was observed for batches W and W-Cu, although the former reached higher absolute values at the end of storage ($p\leq 0.05$) owing to the presence of polyunsaturated fatty acids and the absence of curcumin, as compared with C and W-Cu. The EC batch, which was the one that showed the highest lipid oxidation initially, was also the one that showed the lowest stability, with significant increases in TBARS both on day 3 and 6 of storage

($p \leq 0.05$). Also, curcumin was effective at reducing lipid oxidation in burgers containing EC oleogel (EC-Cu batch), however, lipid oxidation values were higher than those of the other batches during storage ($p \leq 0.05$). According to the literature, the value obtained (≤ 1.1 mg/kg) would not be associated to rancid flavors (Ansorena & Astiasarán, 2004a; Delgado-Pando et al., 2012). Although some fluctuations were found in curcumin content and antioxidant activity during chilled storage, changes were generally not significant (Table 5).

Initial counts for TVC and *Enterobacteriaceae* in C batch were 6.9 and 3.9 log cfu/g, respectively (Figure 3). These values are relatively high, but in accordance with a product prepared under artisanal conditions (Mizi et al., 2019) without preservatives. Reformulated samples showed a lower microbial load ($p \leq 0.05$) for both microorganism groups when compared with the control sample, without relevant differences among them. As all batches were processed in the same way and the only difference among them was the fatty ingredient, it can be deduced that the microbial load provided by the pork back-fat used is the main cause of the differences found. As expected, microbial counts raised during chilled storage in all batches, although it was the control batch the one that showed the highest TVC, probably due to the higher initial load. After 6 days of storage, all batches showed high counts that were between 8-9 log cfu/g for TVC and 5.5-6 log cfu/g for *Enterobacteriaceae*. Fresh meat products, such as burgers, are highly perishable products and a favorable medium for microorganism growth; furthermore, no preservatives were added and the samples were stored in air atmosphere, emulating household conditions, which prompted microbial growth. Under these conditions, the shelf life of the burgers developed is approximately 3 days; however, this may be improved by an adequate control of raw materials, by adding preservatives and/or by packing under modified atmosphere, among others. The antimicrobial properties of

curcumin have been reported elsewhere (Jayaprakasha, Jagan Mohan Rao, & Sakariah, 2005), however, they largely depend on its water solubility and dispersion in the food matrix (Bhawana, Basniwal, Buttar, Jain, & Jain, 2011; Gómez-Estaca, Balaguer, López-Carballo, Gavara, & Hernández-Muñoz, 2017). Curcumin was completely solubilized in the developed oleogels and efficiently dispersed in the food matrix; nevertheless, its low water solubility seemed to limit its antimicrobial properties.

3.3. Effect of cooking

Common household cooking practices may modify the content of the active compounds added to a functional food, or promote the formation of undesirable compounds, such as lipid oxidation products (Jiménez-Colmenero et al., 2018). This is a very relevant aspect, as raw functional foods need to be cooked before consumption. The absolute content in all fatty acid groups (Σ SFA, Σ MUFA, Σ PUFA) increased after cooking (Figure 1), due to their concentration in the meat matrix derived from water loss ($p \leq 0.05$). In spite of this, reformulated cooked samples kept showing higher ($p \leq 0.05$) MUFA and PUFA levels than the cooked control batch. The presence of healthy fatty acids (ALA, EPA, DHA) in W/W-Cu and EC/EC-Cu was higher in the cooked burgers, as compared with their raw counterparts, so the consumption of these burgers could make an important contribution to the recommended intake of dietary n-3 PUFA, which is 1.4-3 g/day (Tetens, 2007).

Only minor differences in cooking loss were observed, being $\approx 20\%$ in all samples other than EC-Cu ($p > 0.05$), which showed a lower cooking loss ($p \leq 0.05$) (Table 4). It may be due to the higher thermal stability of EC oleogel, which is not expected to melt at cooking temperatures (Gómez-Estaca et al., 2019b), together with the effect of curcumin, which has been shown to reduce cooking loss in rabbit burgers presumably due to an improvement in its water holding capacity (Mancini et al., 2015). As expected, cooking

losses gave rise to a reduction of moisture content in burgers, with the concomitant concentration of ash, protein and fat (Table 2). However, no significant differences ($p>0.05$) in proximate composition were found among the cooked burgers, indicating that the lipid analogues designed showed a similar behavior to that of animal fat. Objective measurement of color revealed that cooking induced changes that tended to reduce the differences found in the raw burgers as function of formulation. Thus, hue generally shifted towards lower values (moved away from the yellow region) in reformulated samples ($p\leq 0.05$) (Table 2). In the case of chromaticity, burgers remained stable or became slightly darker. In consequence, after cooking, only the W-Cu batch was significantly different from the control batch with regard to optical properties. Regarding texture, as expected, cooking increased hardness, as an effect of protein denaturation and water loss (Salcedo-Sandoval et al., 2014).

The effect of cooking on lipid oxidation is depicted in Figure 2A. It can be shown that thermal treatment induced the accumulation of lipid oxidation products in all batches, independently from the lipid composition, oil structuration method or curcumin presence. However, in all cases the values attained were low and below the limit to develop rancid flavors (≤ 1.1 mg MDA/kg.). The formulation showing the highest lipid oxidation was the one containing ethyl cellulose oleogel (EC batch) ($p\leq 0.05$), as it was the one that was initially more oxidized before cooking. On the contrary, formulations containing curcumin were the ones with the lowest lipid oxidation (EC-Cu and especially W-Cu), showing values that were even lower than that of the control after cooking (C batch) ($p\leq 0.05$). So, curcumin was effective at reducing the lipid oxidation derived from cooking, in spite of the high unsaturation level of the lipids of the burgers. Regarding curcumin content, it was reduced in EC-Cu and W-Cu after cooking, and the same was also true for the remaining antioxidant activity, although these effects were significant

only in the latter ($p \leq 0.05$) (Table 5). This observation could be partially attributable to its depletion when acting as an antioxidant, but some leaking of fat and curcumin in W-Cu batch during cooking is probable, as the beeswax oleogel melts at ≈ 54 °C, whereas the burgers were cooked until the thermal center reached 70 °C. On the contrary, EC-Cu oleogel does not melt at cooking temperatures up to 200 °C (Gómez-Estaca et al., 2019b). In any case, antioxidant activity of the remaining curcumin is available to exert its *in vivo* beneficial effects when consumed.

The scores obtained from sensory evaluation of the different batches developed are shown in Table 6. The highest overall acceptability was for the burger prepared with animal fat (C batch), although no significant differences were found with batches elaborated with beeswax oleogel (W and W-Cu). On the other hand, batches containing ethyl cellulose oleogel (EC and EC-Cu) showed overall acceptability values below the “neutral scale”, being flavor the sensory parameter that was mostly affected in a negative way. Regarding the presence of curcumin, both batches (W-Cu and EC-Cu) attained lower ratings for color than the C batch and their corresponding counterparts (W and EC), pointing to a negative effect of this compound, in spite of the little differences observed by the objective measurement of color. Indeed, turmeric/curcumin is traditionally used as a colorant that provides an intense yellow color (Hatcher et al., 2008). Regarding texture, no differences ($p > 0.05$) in acceptability were observed among batches. From these results it can be deduced that ethyl cellulose and curcumin impair burger flavor and color respectively, which are negatively perceived by panelists (Table 6). However, in the case of curcumin-added samples, overall acceptability was similar ($p > 0.05$) to that of the control sample when beeswax was used as structuration agent. Reformulating the seasoning may be a way to overcome the objections to product flavor.

4. CONCLUSION

Beeswax oleogels containing a healthier lipid mixture (olive, linseed, fish oils) and curcumin are a realistic alternative to animal fat for the development of functional fresh meat products, such as low-fat burgers. Curcumin reduced the lipid oxidation derived from chilled storage or cooking and the resulting product showed a lipid composition that meets recommendations for fatty acid intake. The product presented adequate technological properties and good overall sensory acceptability. Further studies are needed to adapt ethyl cellulose oleogels prepared with this lipid mixture for the successful development of fresh meat products, due to the extent of lipid oxidation during refrigeration and cooking, and to the lower sensory acceptability observed under the present experimental conditions.

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Figure captions

Figure 1. SFA, MUFA and PUFA content (g/100g) of raw and cooked burgers. For simplification purposes, results are presented as means of W/W-Cu and EC/EC-Cu, as no significant differences were found as function of curcumin addition. Different letters (a, b) indicate significant differences as function of formulation for each fatty acid group. Different numbers (1, 2) indicate significant differences among raw and cooked samples for each fatty acid group.

Figure 2. Thiobarbituric acid reactive substances (TBARS) (mg MDA/kg sample) as function of cooking (A) or refrigerated storage (raw samples) (B). Sample denomination as per Table 1. Different letters indicate significant differences among raw (x, y, z) and cooked samples (a, b, c, d, e). An asterisk indicates significant differences as function of cooking.

Figure 3. Total viable counts (A) and Enterobacteriaceae counts (B) (log cfu/g) of raw patties during refrigerated storage ($4\text{ }^{\circ}\text{C} \pm 2$). Sample denomination as per Table 1.

467

468

469 Figure 1

470

Figure 2

Figure 3

482 Table 1. Formulation (g/100 g) of the different burgers developed.

Samples	Meat	Pork backfat	Oleogel	NaCl	Water/ice mixture	Spice mixture*
C	84.0	6.0	0.0	1.0	8.0	1.0
W	84.0	0.0	6.0	1.0	8.0	1.0
W-Cu	84.0	0.0	6.0	1.0	8.0	1.0
EC	84.0	0.0	6.0	1.0	8.0	1.0
EC-Cu	84.0	0.0	6.0	1.0	8.0	1.0

483 B/C: control sample formulated with pork backfat; B/W: sample formulated with total pork backfat
 484 replacement by oleogel elaborated with beeswax; B/W-Cu; sample formulated with total pork backfat
 485 replacement by oleogel elaborated with beeswax and with curcumin; B/EC: sample formulated with total
 486 pork backfat replacement by oleogel elaborated with ethylcellulose; B/EC-Cu: sample formulated with total
 487 pork backfat replacement by oleogel elaborated with ethylcellulose and with curcumin.

488 *The spice mixture consisted in black pepper, white pepper, dried garlic and dried onion in equal parts.

Table 2. Proximate analysis (%) of raw and cooked pork patties. Batches denomination as per Table 1.

	C	W	W-Cu	EC	EC-Cu
Moisture (%)					
<i>Raw</i>	72.29 ± 0.37 ^{a2}	71.92 ± 0.19 ^{a2}	71.62 ± 0.36 ^{a2}	71.835 ± 0.21 ^{a2}	71.97 ± 0.21 ^{a2}
<i>Cooked</i>	65.25 ± 0.64 ^{a1}	65.48 ± 0.15 ^{a1}	65.48 ± 0.08 ^{a1}	64.808 ± 0.17 ^{a1}	66.65 ± 0.05 ^{b1}
Ash (%)					
<i>Raw</i>	1.87 ± 0.08 ^{a1}	1.88 ± 0.057 ^{a1}	2.03 ± 0.08 ^{a1}	2.03 ± 0.03 ^{a1}	2.13 ± 0.19 ^{a1}
<i>Cooked</i>	2.26 ± 0.01 ^{a2}	2.37 ± 0.38 ^{a2}	2.23 ± 0.08 ^{a1}	2.30 ± 0.02 ^{a1}	2.18 ± 0.08 ^{a1}
Protein (%)					
<i>Raw</i>	18.32 ± 0.17 ^{a1}	17.87 ± 0.08 ^{a1}	18.81 ± 0.30 ^{a1}	18.04 ± 0.40 ^{a1}	18.23 ± 0.28 ^{a1}
<i>Cooked</i>	23.26 ± 0.37 ^{a2}	22.71 ± 0.16 ^{a2}	22.62 ± 0.25 ^{a2}	22.51 ± 0.05 ^{a2}	21.41 ± 0.09 ^{a2}
Fat (%)					
<i>Raw</i>	7.21 ± 0.28 ^{a1}	7.72 ± 0.15 ^{a1}	7.20 ± 0.56 ^{a1}	7.88 ± 0.06 ^{a1}	7.92 ± 0.05 ^{a1}
<i>Cooked</i>	9.23 ± 0.26 ^{a2}	9.44 ± 0.53 ^{a2}	9.67 ± 0.67 ^{a2}	10.39 ± 1.56 ^{a2}	9.66 ± 0.19 ^{a2}

Different letters in the same row indicate significant differences ($P \leq 0.05$) among samples. Different numbers in the same column indicate significant differences ($P \leq 0.05$) as function of cooking. Results are expressed as mean ± standard deviation.

Table 3. PUFAs profile (g/100g), and nutritional ratios of raw and cooked burgers. Batches denomination as per Table 1.

		C	W/W-Cu	EC/EC-Cu
<i>Linoleic acid</i>	<i>raw</i>	0.75±0.01 ^a	0.76±0.02 ^a	0.74±0.01 ^a
<i>C18:2n6</i>	<i>cooked</i>	0.95±0.01 ^{c*}	0.88±0.01 ^{a*}	0.89±0.01 ^{a*}
<i>ALA</i>	<i>raw</i>	0.03±0.00 ^a	1.04±0.03 ^b	0.99±0.03 ^b
<i>C18:3n3</i>	<i>cooked</i>	0.03±0.00 ^a	1.17±0.02 ^{b*}	1.19±0.03 ^{b*}
<i>EPA</i>	<i>raw</i>	0.00±0.00 ^a	0.17±0.01 ^b	0.15±0.01 ^b
<i>C20:5n3</i>	<i>cooked</i>	0.00±0.00 ^a	0.19±0.00 ^{b*}	0.19±0.01 ^{b*}
<i>DHA</i>	<i>raw</i>	0.00±0.00 ^a	0.11±0.01 ^b	0.10±0.00 ^b
<i>C22:6n3</i>	<i>cooked</i>	0.00±0.00 ^a	0.12±0.00 ^{b*}	0.12±0.00 ^{b*}
<i>ΣPUFA/ΣSFA</i>	<i>raw</i>	0.36±0.01 ^a	1.32±0.04 ^b	1.31±0.03 ^b
	<i>cooked</i>	0.36±0.00 ^a	1.30±0.02 ^b	1.26±0.04 ^b
<i>Σn-6/Σn-3</i>	<i>raw</i>	15.01±0.07 ^b	0.64±0.02 ^a	0.64±0.02 ^a
	<i>cooked</i>	14.97±0.02 ^b	0.65±0.01 ^a	0.65±0.02 ^a
% calories	<i>raw</i>	6.2	14.6	13.7
from PUFAs	<i>cooked</i>	6.1	13.2	13.3

Data of W/W-Cu and EC/EC-Cu are presented as means, as no significant differences ($p>0.05$) were observed as function of adding curcumin. Results are expressed as means±standard deviation. Different letters in the same row indicate significant differences ($p\leq0.05$) among samples. An asterisk indicates significant differences ($p\leq0.05$) as function of cooking. ALA: α -linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Table 4. Some technological properties (color parameters, Kramer share force and cooking loss) of raw and/or cooked patties. Samples denomination as per Table 1.

	Samples	Raw	Cooked
Hue (°)	C	76.9 ± 1.9b	77.0 ± 2.0a
	W	76.9 ± 1.9b	74.0 ± 2.5a*
	W-Cu	88.9 ± 0.9d	84.1 ± 4.5b*
	EC	73.7 ± 1.7a	72.9 ± 2.8a
	EC-Cu	87.1 ± 1.7c	76.3 ± 6.3a*
Chroma	C	9.5 ± 0.8a	15.2 ± 0.9a*
	W	11.3 ± 1.0b	16.5 ± 1.2a*
	W-Cu	24.4 ± 1.3d	24.4 ± 1.3c
	EC	12.0 ± 0.9b	15.2 ± 0.8a*
	EC-Cu	21.8 ± 1.6c	21.2 ± 1.5b
KSF (N)	C	3.480± 0.457c	22.465± 1.804c*
	W	2.232±0.229a	15.282± 0.739a*
	W-Cu	2.482±0.186ab	19.064± 0.711b*
	EC	2.607±0.213ab	26.490± 0.973d*
	EC-Cu	2.969±0.0.486b	21.493± 1.761c*
Cooking loss (%)	C	-	19.96±2.72b
	W	-	20.03±0.22b
	W-Cu	-	18.86±0.82ab
	EC	-	21.09±0.8b
	EC-Cu	-	17.03±1.04a

Means±standard deviation. Different letters in the same column (a, b, c, d) indicate significant differences (p≤0.05) as function of formulation for raw or cooked burgers. An asterisk indicates significant differences (p≤0.05) as function of cooking.

Table 5. Curcumin content and antioxidant activity by photochem of raw burgers during refrigerated storage and cooked burgers after 1 day of preparation (data in parenthesis).

	Curcumin content (mg/100 g)			Antioxidant activity (mg eq. Trolox/100 g)		
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6
C	–	–	–	2.7±0.2 ^{a/x} (3.4±0.3)	4.2±1.4 ^{a/x}	3.3±0.2 ^{a/x}
W	–	–	–	5.9±0.3 ^{a/x} (5.4±0.3)	4.8±1.4 ^{a/x}	4.8±0.0 ^{a/y}
W-Cu	9.4±0.3 ^{ab/x} (8.2±0.2*)	10.1±0.5 ^{b/x}	8.4±0.2 ^{a/x}	18.3±0.9 ^{a/y} (15.8±0.6*)	20.0±3.2 ^{a/y}	17.0±0.7 ^{a/z}
EC	–	–	–	5.1±0.1 ^{a/x} (5.7±0.1)	4.7±0.2 ^{a/x}	5.0±0.3 ^{a/y}
EC-Cu	8.3±0.2 ^{a/x} (8.0±0.3)	9.7±0.8 ^{a/x}	8.2±0.4 ^{a/x}	15.9±2.0 ^{a/y} (15.6±0.3)	17.3±0.6 ^{a/y}	17.3±0.1 ^{a/z}

Different letters (a, b) in the same row indicate significant differences as function of storage time. Different letters (x, y, z) in the same column indicate significant differences ($p \leq 0.05$) as function of formulation at each storage day. An asterisk indicates significant differences as function of cooking ($p \leq 0.05$).

Table 6. Sensory evaluation of the different burgers.

Samples	Flavor	Texture	Color	Overall acceptability
C	6.99±1.54 ^c	6.84±1.51 ^a	7.32±1.44 ^b	6.89±1.60 ^c
W	5.73±2.42 ^{bc}	6.79±1.60 ^a	7.37±1.30 ^b	5.74±2.33 ^{bc}
W-Cu	5.63±2.03 ^{bc}	6.35±1.49 ^a	5.22±2.26 ^a	5.50±1.93 ^{bc}
EC	3.89±2.67 ^a	5.88±2.15 ^a	7.22±3.1 ^b	3.95±2.57 ^a
EC-Cu	4.57±2.19 ^{ab}	5.94±1.90 ^a	5.11±2.16 ^a	4.50±2.16 ^{ab}

Means±sd. Different letters (a, b, c) in the same column indicate significant differences ($p \leq 0.05$). Sample denomination as per Table 1.