Assessment of a healthy oil combination structured in ethyl cellulose and beeswax oleogels as animal fat replacers in low-fat, PUFA-enriched pork burgers

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ABSTRACT

The present work evaluates the suitability of ethyl cellulose and beeswax oleogels prepared with a healthy lipid mixture (olive, linseed, and fish oils) as fat replacers for fresh meat product development. The texture, color, thermal properties, and fatty acid composition of the oleogels indicated their suitability for the intended use, and they were stable for at least one month of chilled storage (3 ± 1 °C). However, the oleogels suffered some lipid oxidation during refrigerated storage, especially in the case of ethyl cellulose. Low-fat pork burgers formulated with total substitution of pork backfat by the oleogels developed were softer and without important changes in optical properties, as compared to the control. Although some lipid oxidation was observed, especially when ethyl cellulose oleogel was used, the fatty acid profile of the reformulated burgers was significantly improved, with a 3.6-fold increase of the PUFA/SFA ratio and a 23-fold decrease of the n-6/n-3 ratio, as compared to the control. A sensory acceptability test showed high ratings for the burgers made with beeswax oleogel, in contrast to the ones made with ethyl cellulose, which scored values below the neutral point. Results from this work indicate the potential of the ingredients developed for the formulation of

healthier fresh meat products with an improved fatty acid profile, and the need for research on strategies to improve oxidative stability and sensory properties.

Keywords: functional food; meat product; PUFA; n-3; reformulation

Introduction

In response to recommendations for optimal intake of total and unsaturated fatty acids proposed by a number of scientific authorities and nutritional organizations (McNeill 2014; Anonymous 2003), various approaches have been considered to optimize the lipid contents and fatty acid profiles of various foods, including meat products, in order to achieve a composition more in line with nutrient intake goals. In this regard, various oils (of plant and marine origin) have been widely used as animal fat replacers in meat product reformulation strategies and to produce healthier lipid content in meat-based functional foods (Domínguez et al. 2017; Barbut et al. 2016a; Delgado-Pando et al. 2014; Salcedo-Sandoval et al. 2015; Muguerza et al. 2004; Jiménez-Colmenero 2007). However, as the functionality and texture of the solid animal fat present in meat products are quite different from the characteristics of oil (liquid at room temperature), and these properties have a major effect on several product characteristics (mouthfeel, juiciness, texture, bite, heat transfer, etc.), the replacement of animal fat with liquid oils presents a considerable technical challenge, often resulting in a negative effect on the desired quality attributes of the reformulated products (Grasso et al. 2014; Muguerza et al. 2004).

One of the first developments in this topic was the addition of oils in pre-emulsified form, as in the work by Martínez et al. (2012), who developed beef burgers with a mixture of olive, corn and fish oils. Other structuration method that has been employed for meat products development is encapsulation. Thus, Jiménez-Martín et al. (2016) developed chicken nuggets containing microencapsulated omega-3 fish oil, achieving good results for oxidative stability and sensory quality. Enzymatic intersterification and structuration using mono- and diglycerides have been also employed as strategies to improve the physical performance of liquid fats (Chen et al. 2012; Lupi et al. 2012).

However, newer proposals for oil structuration have recently been considered in order to create a plastic fat that retains solid-like properties (very similar to those of animal fat, while possessing a healthier fatty acid profile), and that can be used as an approach to improving the fat content of meat products (Jimenez-Colmenero et al. 2015). Organogelation is one of the most promising techniques to give liquid oils solid-fat functionality. Various organogelators have been used to obtain organogels (oleogels), including small molecules that crystallize to form colloidal or fibrillar networks, and hydrophobic polymers that self-assemble under specific processing conditions (Davidovich-Pinhas et al. 2016). Waxes and ethyl cellulose (EC) are examples of these two organogelation-lipid structuring systems, respectively. Wax esters previously solubilized into liquid oil may crystallize upon cooling, trapping the oil molecules in a structured network. The properties of the resulting oleogels depend on the type of wax used (candelilla, carnauba, rice bran, beeswax, etc.) and concentration levels (Martins et al. 2016; Yilmaz and Öğütcü 2014; Öğütcü and Yilmaz 2014; Mert and Demirkesen 2016; Zetzl and Marangoni 2011; Öğütcü et al. 2015). EC oil structuring is based on the ability of polymers to dissolve in the oil phase while the temperature is increased above its glass transition, approximately ~140°C, followed by cooling below the gel point temperature, approximately ~120°C.

EC oleogels have been used to replace animal fat in the reformulation of fresh and cooked meat products such as frankfurters, breakfast sausages, and pâtés (Zetzl et al. 2012; Barbut et al. 2016a, b, c; Gómez-Estaca et al. 2019). The characteristics of oleogels, and therefore their suitability to be used as animal fat replacers, depends on factors associated basically with the nature of the compound (polymer molecular weight), concentration, presence of surfactants, and type of oil (Zetzl et al. 2012; Barbut et al. 2016a; Gravelle et al. 2014). The use of wax oleogels as fat substitutes in meat

79 products is not common and is very recent. Moghtadaei et al. (2018) formulated beef
80 burgers in which animal fat was partially replaced by sesame oil-beeswax oleogels,
81 finding a reduction in cooking loss and fat absorption, although cooking shrinkage and
82 lipid oxidation increased significantly. Wolfer et al. (2018) substituted pork backfat
83 with soybean oil oleogels structured with rice bran wax in the formulation of
84 frankfurter-type sausages, with good results for textural and sensory properties.

85 The characteristics and stability of new lipid materials are fundamental for
86 understanding and improving their suitability as animal fat replacers in meat products.
87 The characteristics need to be considered in order to modulate their composition
88 (healthier lipid presence), understand their role in the meat matrix, and achieve the
89 appearance and the technological, rheological, and sensory properties required for use
90 as raw materials to replace animal fats. Further studies are needed because, whether
91 they are used as non-meat ingredients or as part of meat products, these novel materials
92 can be affected by various common processing treatments such as chilling storage,
93 which can alter both their technological suitability and the quality attributes of
94 reformulated meat products. Therefore, the objective of the present work is to evaluate
95 the suitability of ethyl cellulose and beeswax oleogels prepared with an olive-linseed-
96 fish oil mixture as fat replacers in low-fat, PUFA-enriched pork burgers, considering
97 aspects related to the lipid ingredients (fatty acid profile, texture, thermal properties, and
98 chilling stability) and the products (composition, technological and sensory properties).

99 **Materials and Methods**

100 **Materials and reagents**

101 The lipid sources for oleogel development were: olive oil (Carbonell Virgen Extra, SOS
102 Cuétara SA, Madrid, Spain), linseed oil (Natursoy SL, Alimentos Ecológicos,

Castellterçol, Spain), and fish oil (Omevital 18/12 TG Gold) from Cognis GmbH, Illertissen, Germany. Ethyl cellulose with a viscosity of 10 cP (Aqualon N10 EC) was kindly donated by Ashland (The Netherlands). Beeswax was acquired from Manuel Riesgo SA (Madrid, Spain). Sorbitan monostearate was from Sigma-Aldrich (Madrid, Spain). Raw materials, pork meat, and backfat were acquired and prepared as described by Salcedo-Sandoval et al. (2015). Finally, the spice mixture and salt were obtained at a local market.

Oleogel development

Two different oleogels were prepared, using ethyl cellulose (EC-OG) and beeswax (W-OG) as organogelators. In both, the lipid phase was prepared to attain an optimal lipid profile from a health standpoint (Delgado-Pando et al. 2010), and consisted of a mixture of olive, linseed, and fish oils in a proportion of 44.39%, 37.87%, and 17.74%, respectively. Ethyl cellulose oleogel (EC-OG) and beeswax oleogel (W-OG) were prepared on the basis of the method described by Gómez-Estaca et al. (2019).

Burger formulation

Three batches of burgers, each batch weighing 3.5 kg, were prepared as described by Salcedo-Sandoval et al. (2015), with slight modifications. All of them contained 84% meat, 8% water/ice mixture, 1% salt, 1% spice mixture (black pepper, white pepper, dried garlic, dried onion), and 6% fatty ingredient: pork backfat (C-B batch), ethyl cellulose oleogel (EC-B), or beeswax oleogel (W-B). For the preparation of the burgers, meat and pork backfat 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. Meat and fatty ingredients (pork backfat or oleogel) were minced through a grinder with a 4.5 mm plate (Van Dall Srl, model FTSIII, Treviglio,

Italy). For each formulation, the chopped meat and the fatty ingredient were placed in a mixer (MAINCA, Spain) and homogenized for 1 min. The other ingredients were added and mixed for 1 min more. The mince temperature was controlled (Daqpro-5300 thermocouple from Omega, Spain) during the process and was always below 3 °C. Burgers weighting ≈85 g were prepared using a manual burger former, packaged in trays under air atmosphere, and stored at 2 ± 2 °C until analysis (Figure 1).

Oleogel characterization

Fatty acid determination

Ten mg of the oil mixture or oleogel was derivatized into fatty acid methyl esters (FAMEs) in triplicate using 0.5 M sodium methoxide in anhydrous methanol and acetyl chloride in anhydrous methanol. FAMEs were extracted with 4 mL hexane and used for GC analysis (1 µL). The fatty acid profile was determined in an Agilent 7820A gas chromatograph with FID detector. Separation was performed in an Agilent HP-88 column (60 m, 0.32 mm i.d., 0.25 µm film thickness, ref. 112-8867) with split injection (40:1) and helium at a constant flow of 1.2 mL/min. Detector temperature was set at 260 °C and injector temperature at 250 °C. The temperature profile of the oven was 125 °C for 1 min, then increased by 8 °C/min to 145 °C for 26 min, then increased to 220 °C for 5 min. Identification was done by comparing retention times with a standard of 37 fatty acids (Supelco 37 FAME Mix 47885-U, USA). The internal standard used for quantification was C13:0, which was added to the sample in the non-methylated state before methylation. Results were expressed as mg fatty acid/g oil.

Instrumental color and texture analysis

Color parameters were measured (ten times) using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan) set to D65 illuminant/10° observer. The CIELAB color space was used to obtain the color coordinates L* [black (0) to white (100)], a* [green (–) to red (+)], and b* [blue (–) to yellow (+)]. Determinations were performed at different time intervals during 28 days of chilled storage (3 ± 1 °C).

Texture analysis was performed in a TA-XTplus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 50 N load cell. A penetration test was carried out at room temperature on each sample placed in a cylindrical-shaped container (35 mm in diameter and 30 mm high), immediately after refrigeration at 3 °C (six replications). The analysis was performed with a 12 mm diameter flat probe that penetrated 10 mm into the sample at a velocity of 0.8 mm/s. The penetration force (PF, N) was calculated as the force exerted at 10 mm (for the EC-OG sample) or the force at the point of gel fracture (for the W-OG sample), according to Herrero et al. (2011). Determinations were performed at different time intervals during 28 days of refrigerated storage (3 ± 1 °C).

Lipid oxidation

Thiobarbituric acid reactive substances (TBARS) of the oleogels were determined in triplicate, based on the method described by Maqsood and Benjakul (2010), with modifications. One gram of finely comminuted oleogel was dispersed with 3 mL of 0.26% butylhydroxytoluene (BHT) by sonication (Q-Sonica sonicator equipped with a microtip) at 75 Hz for 5 s; the test tube containing the sample was immersed in an ice bath to avoid heating. Afterwards, 8 mL of TBARS reagent (15% trichloroacetic acid, 0.375% thiobarbituric acid, 0.25% hydrochloric acid) was added, and the mixture was

vortexed and sonicated again (75 Hz, 5 s). The test tubes were placed in a water bath set at 100 °C for 15 min and then immediately transferred to an ice-water bath to cool down. Afterwards, 4 mL of 4 M ammonium sulfate and 4 mL of hexane were sequentially added and the mixture was vigorously vortexed for 30 s. The lower phase was collected after centrifugation in a Heraeus Multifuge 3L centrifuge (DJB Labcare Ltd., Buckinghamshire, England) set at 5000 rpm/10 °C/30 min, and the absorbance at 532 nm was measured in a Shimadzu UV-1800 spectrophotometer (Shimadzu Inc., Kyoto, Japan). Results were expressed as mg malonaldehyde (MDA)/kg oil, based on a standard curve prepared from 1,1,3,3-tetraethoxypropane in advance. Determinations were performed at different time intervals during 28 days of refrigerated storage (3 ± 1 °C).

Microscopy

Oleogel morphology was studied by optical microscopy with a Leica AF6000 LX microscope. After organogelator dissolution and prior to setting, samples were poured directly into the holders and then allowed to gel at room temperature in the same conditions as described above. Micrographs were taken at 40× magnification after two days of refrigerated storage (3 ± 1 °C).

Differential Scanning Calorimetry (DSC)

DSC analysis of the oleogels was performed with a previously calibrated model TA-Q1000 Differential Scanning Calorimeter (TA Instruments, New Castle, DE, USA). Pure ethyl cellulose, pure beeswax, and oleogel samples of approximately 5–8 mg (± 0.002 mg) were weighed out using a model ME235S electronic balance (Sartorius, Goettingen, Germany), tightly encapsulated in hermetic aluminum pans, and scanned under dry nitrogen (50 mL/min) purge. An empty capsule was used as a reference. A

heating ramp and a subsequent cooling ramp were performed from 5 to 100 or 200 °C (W-OG and EC-OG samples, respectively) at 5 °C/min. A second run was also performed in order to check the reversibility of thermal events. Melting temperatures (Tm, °C), crystallization temperatures (Tc, °C), and transition enthalpies (ΔH, J/g, by linear baseline integration) were calculated. Samples were analyzed in triplicate. Determinations were performed at different time intervals during 28 days of refrigerated storage (3 ± 1 °C).

Burger characterization

Composition

Proximate analysis was carried out in triplicate. Moisture and ash content of the burgers were determined according to AOAC (2005). Protein content was measured with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, USA), and fat content was evaluated following the method described by Bligh and Dyer (1959).

Fatty acid composition was determined in freeze-dried burgers as described previously for the oleogels. Results were expressed as g fatty acid/100 g burger.

Technological properties

Color parameters were measured (ten times) after one day of preparation and in refrigerated conditions in raw burgers as described for the oleogels. For the burgers, simple transformations were used to convert a* and b* coordinates to C* and h° chromatic parameters (Gómez-Estaca et al. 2015).

For texture analysis, Kramer shear force (KSF) was determined (six replications) in a TA-XTplus Texture Analyzer (Stable Micro Systems Ltd., UK) equipped with a 50 N

219 load cell using a miniature Kramer/Ottawa cell (HDP/MK05). Samples measuring 2×2
220 cm were cut from two burgers, accurately weighed, and placed into the cell, at room
221 temperature. Samples were penetrated 20 mm, at a speed of 0.8 mm/s. KSF values were
222 calculated as the maximum force per g of sample (N/g).

223 For lipid oxidation analysis, TBARs were determined in triplicate using the method
224 described by Delgado-Pando et al. (2012). Briefly, 4 g of each sample was placed in
225 centrifuge tubes, 1 mL of distilled water and 10 mL of 10% trichloroacetic acid were
226 added, and the mixture was homogenized for 30 s with a vortex stirrer. Then 5 mL of 20
227 mM 2-thiobarbituric acid was added and the mixture was stirred again for 30 s. Each
228 tube was centrifuged for 5 min at 2600 *g* (Heraeus Multifuge 3 L-R, DJB Labcare Ltd.,
229 Buckinghamshire, UK), and the supernatant was collected and kept in darkness for 20 h
230 at 20 ± 2 °C. The absorbance at 532 nm was measured in a Shimadzu UV/VIS 1203
231 spectrophotometer, and the results were expressed as mg malonaldehyde (MDA)/kg
232 burger, based on a standard curve prepared from 1,1,3,3-tetraethoxypropane in advance.

233 *Sensory analysis*

234 A hedonic sensory analysis was performed by an untrained panel of 40 people selected
235 from staff of ICTAN-CSIC, in two independent sessions. Immediately after being
236 cooked on a grill plate for 4 min (2 min per side), each burger was cut into 4 pieces and
237 the three samples (C-B, EC-B, W-B) were presented to the panelists, who were
238 instructed to rinse their mouth with bread and water between samples. Odor, color,
239 texture, and overall acceptability were evaluated on a 10-point scale, 0 being considered
240 as “dislike strongly” and 10 as “like strongly.” The panelists were also asked to make
241 any comments they considered appropriate about their sensory perception of the
242 samples.

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 $p \leq 0.05$.

Results and Discussion

Fatty acid composition, characterization, and chilling stability of oleogels

A study of the fatty acid composition is not generally conducted in the literature, because it seems evident that it corresponds to the composition of the lipid material of which it consists, but in this case it is useful to make this analysis because the preparation conditions required (high processing temperatures) may induce changes. The fatty acid profiles of the oleogels developed and the oil mixture are shown in Table 1. The fatty acid composition of the oil mixture was similar to that previously reported by Delgado-Pando et al. (2010), showing 15.5% saturated fatty acid (SFA), 48.5% monounsaturated fatty acid (MUFA), and 36% polyunsaturated fatty acid (PUFA) according to the composition of the oils in the mixture (olive, linseed, and fish). The fatty acid profile was scarcely affected by organogelation, the main change being an increase in ΣSFA ($p \leq 0.05$). A possible explanation is that these fatty acids were formed from the degradation of other fatty acids, not only by oxidative reactions, but also by decarboxylation or degradation by carbon-carbon cleavage (Nawar 1969), as the content of ΣMUFA and ΣPUFA decreased concomitantly, although the decrease was not significant ($p > 0.05$). In consonance with these findings, Tenyang et al. (2017) found that roasting two varieties of sesame (120 °C/30 min) caused a decrease in ΣPUFA and/or ΣMUFA and a related increase in ΣSFA. Along the same lines, Blasi et

267 al. (2018) reported an increase in ΣSFA of olive oil during frying. As a result of these
268 fatty acid modifications, the ΣPUFA/ΣSFA ratio decreased ($p \leq 0.05$) for both oleogels,
269 especially for EC-OG, but the ΣPUFAn-6/ΣPUFAn-3 ratio remained unchanged. As the
270 changes in the fatty acid profile were small, the lipid composition of the oleogels
271 developed was optimal from a nutritional standpoint, with a view to their intended use
272 as ingredients for the development of healthier meat products.

273 A fuller knowledge of oleogel characteristics (appearance, morphology, mechanical
274 properties, thermal behavior, oxidation, etc.) will therefore facilitate their use, help to
275 elucidate their role in the protein matrix structure, and help to improve the quality of
276 healthy meat-based food systems in which they are used. Both oil-structuring methods
277 produced solid-like structures that showed a yellowish color to the naked eye (Figure 2).
278 The objective measure of color showed that W-OG was lighter (Table 2) and more
279 yellowish ($p \leq 0.05$), whereas EC-OG showed higher redness ($p \leq 0.05$). With regard to
280 the effect of storage time, no changes ($p > 0.05$) were observed, irrespective of the type
281 of organogelator used, indicating the stability of the oleogels developed in terms of the
282 optical properties considered. For this reason and for further simplification, Table 2
283 shows the mean value of all the determinations performed during storage.

284 The microscopic analysis of the oleogels is shown in Figure 3. EC-OG had a compact,
285 granular structure in which no crystalline structures were observed (2A). This was
286 especially evident in the photograph taken under polarized light (2C). Zetzl et al. (2012)
287 reported that the ethyl cellulose gel consists of an extensive polymer network with small
288 pockets or holes where oil would be entrapped; the image observed here may be
289 consistent with the microstructure reported there. The beeswax oleogel had needle-like
290 structures (2B and 2D), which are characteristic of oleogels prepared with this type of
291 wax (Yilmaz and Öğütcü 2014; Martins et al. 2016). The gelation process of waxes is

292 the result of the association of microcrystalline structures that build up a three-
293 dimensional network that, if strong enough, is capable of restraining the oil phase,
294 resulting in an oleogel (Toro-Vazquez et al. 2007). Needle-like microcrystals enable
295 large volumes of oil to become entrapped between the crystalline strands, unlike other
296 waxes such as candelilla or carnauba, which form smaller spherulitic crystals (Zetzl and
297 Marangoni 2011).

298 Initially, EC-OG showed higher ($p < 0.05$) oxidation levels than W-OG (Figure 4),
299 probably owing to the higher temperatures (up to 170 °C) needed to unfold ethyl
300 cellulose for gel formation (Gravelle et al. 2012), in contrast to W-OG, which is
301 produced at 65 °C, a temperature that is sufficient to ensure effective wax melting and
302 mixing with the oil. The two samples had similar oxidation rates during storage, with a
303 progressive accumulation of TBARS that was significantly different from the initial
304 point after 28 days of storage and with higher values for EC-OG, although the oxidation
305 values were below the reported minimum needed to detect objectionable flavors
306 (Ockerman 1985). Lipid oxidation is an important aspect to be taken into consideration
307 when producing plastic fats from unsaturated oils owing to the susceptibility of these
308 kinds of lipids to oxidation and to the high processing temperatures used, especially in
309 the case of ethyl cellulose oleogels. Surprisingly, there is very little literature about the
310 chilling stability of these materials, which can be processed in a similar way to other
311 raw materials used in food (meat) processing. Gravelle et al. (2012) reported a
312 progressive accumulation of lipid oxidation products (hydroperoxides and TBARS)
313 when canola oil was heated to 140 °C during a 120 min experiment. They found that
314 holding heating times beyond 20 min resulted in an accumulation of ≈10 meq
315 hydroperoxides/kg oil, which, according to the authors, is the upper limit for oil to be
316 considered “fresh.” On the basis of these results, the authors stated that a processing

317 time of 15–20 min would be optimum for ethyl cellulose unfolding and mixing with the
318 oil while limiting lipid oxidation. However, to date no studies have been done on the
319 stability of ethyl cellulose oleogels during storage. Yilmaz and Öğütcü (2014) found
320 that beeswax-structured hazelnut oil stored at 4 °C did not oxidize after 3 months
321 (according to the peroxide value), whereas lipid oxidation was observed when it was
322 stored at 20 °C.

323 For a better understanding of the mechanical properties of ethyl cellulose and beeswax
324 oleogels, examples of the typical penetration curves obtained are plotted in Figure 5.
325 EC-OG showed a typical viscoelastic pattern, with an elastic region followed by a
326 plateau, whereas W-OG behaved quite differently, with plunger penetration producing a
327 breaking point characteristic of gel fracture. W-OG attained a significantly ($p \leq 0.05$)
328 higher penetration force (measured at the breaking point, 11.7 ± 2.3 N) than EC-OG
329 (measured at 10 mm of penetration, 6.5 ± 0.8 N). These results are in accordance with a
330 previous work in which a rheological characterization of these oleogels was performed,
331 finding higher values of complex modulus (G^*) and lower values of γ_{max} for beeswax
332 oleogels, which indicated that beeswax produced denser but less deformable gels than
333 those produced with ethyl cellulose (Gómez-Estaca et al. 2019).

334 Other researchers working with ethyl cellulose oleogels observed similar mechanical
335 behavior to that observed in this work: using back extrusion, they found a plateau zone
336 in which the force was constant from a certain penetration depth onwards; however,
337 texture profile analysis revealed elastic behavior (Zetzl et al. 2012). In other works, the
338 plateau zone was not observed when the mechanical properties of ethyl cellulose
339 oleogels were determined, the gel being clearly fractured as the probe penetrated
340 (Gravelle et al. 2014; Gravelle et al. 2013). These different results may be related to the
341 fact that the mechanical properties of ethyl cellulose oleogels are strongly dependent on

342 processing and compositional parameters such as processing time and temperature,
343 heating/cooling rates, ethyl cellulose molecular weight and concentration, the presence,
344 type, and concentration of plasticizer, and the degree of oil unsaturation (Gravelle et al.
345 2014; Davidovich-Pinhas et al. 2015; Gravelle et al. 2012). As in the case of lipid
346 oxidation, studies of the effect of chilling storage on EC oleogel mechanical properties
347 have not been reported. After 28 days of chilled storage, neither the mechanical profile
348 nor the maximum penetration force or gel strength had changed, irrespective of the type
349 of organogelator used (results not shown), bearing witness to the high stability of the
350 oleogels developed. In consonance with this experiment, Yilmaz and Öğütcü (2014),
351 who studied the effect of storage time (up to 3 months) on the mechanical properties of
352 hazelnut oil-beeswax oleogels, did not find significant changes, although some
353 fluctuations were observed during the storage period.

354 The thermal transitions of the oleogels were studied by DSC, owing to the importance
355 of their physical state on the behavior during production, storage, and cooking of the
356 meat product (Figure 6). The oil mixture, which was analyzed as a control, exhibited an
357 exothermic event at 151.5 ± 10.4 °C (Figure 6A) in the first heating scan, which did not
358 appear either in the cooling or the second heating scans (data not shown) and is
359 attributable to partial thermal degradation. EC-OG showed a similar trend, with an oil
360 thermal degradation peak at 147.5 ± 9.8 °C (Figure 6A). The same behavior was
361 observed by Dey et al. (2011) working with flaxseed oil structured in 22 cP ethyl
362 cellulose oleogel. In that case, the thermal degradation peak was found at 130–135 °C
363 for unstructured oil, whereas it appeared at lower temperatures in the oleogel (125–130
364 °C). The absence of any other thermal transition event in EC-OG during heating or
365 melting indicates that the gelation mechanism and structure do not involve a highly
366 ordered secondary structure formation (Davidovich-Pinhas et al. 2015). Radically

367 different thermal behavior was observed in the beeswax oleogel, showing melting and
368 crystallization peaks at 54.2 ± 0.1 °C and 49.0 ± 0.2 °C, respectively. The thermal
369 behavior of the pure beeswax revealed a similar trend, with melting and crystallization
370 peaks ≈10 °C higher (63.9 ± 0.2 °C and 58.5 ± 0.7 °C, respectively) (traces not shown).
371 Melting and crystallization enthalpies were also higher for pure polymer than for W-
372 OG: 155.2 ± 3.2 J/g and 136.9 ± 0.4 J/g, respectively, for pure beeswax versus 8.9 ± 1.0
373 J/g and 8.4 ± 0.3 J/g, respectively, for W-OG owing to a dilution effect of the polymer.
374 It was observed that melting enthalpies are dependent on organogelator concentration,
375 suggesting the possibility of adjusting the organogelator concentration depending on the
376 intended application of the oleogel (Yilmaz and Öğütcü 2014; Yi et al. 2017). These
377 results are in agreement with a previous report by Yilmaz and Öğütcü (2014), who
378 found Tm and Tc peak values and enthalpies for beeswax and 10% hazelnut oil-
379 beeswax oleogels quite similar to those found in this paper. As for the effect of storage
380 time (28 days) on the thermal properties of the oleogels, no differences were observed
381 for thermal profile or melting/crystallization temperatures or enthalpies (data not
382 shown). Therefore, the ingredients developed could be stored for at least one month
383 without significant changes in their thermal behavior. From these results it can be
384 deduced that both organogels retain their solid-like properties at room temperature, but
385 the beeswax oleogel will melt upon cooking or thermal processing. However, upon
386 cooling, the oil will be structured again to form the oleogel.

387 **Suitability of oleogels as fat substitutes for healthier burgers**

388 According to the target formulation, generally the three burgers had a similar proximate
389 composition (Table 3). Although some significant variations were observed in fat and
390 ash contents, they can be considered of minor technological or nutritional importance
391 owing to their small magnitudes.

392 The fatty acid profile of the burgers is shown in Figure 7. Significant differences were
393 observed among the samples for all fatty acid groups. Thus, ΣSFA decreased and
394 ΣMUFA and ΣPUFA increased in the EC-B and W-B batches, as compared to the
395 control batch formulated with animal fat (C-B). MUFAs were the most abundant fatty
396 acid group in all samples, which is consistent with the lipid composition of the pork
397 meat used. There was a notable increase in ΣPUFA in the EC-B and W-B samples,
398 mainly attributed to linolenic acid, and this increase, together with a concomitant
399 decrease in ΣSFA, resulted in an increase in the ΣPUFA/ΣSFA ratio by ≈3.6-fold.
400 Furthermore, the increase in the content of n-3 fatty acids in oleogel-added batches, as
401 compared to the control, gave rise to a considerable decrease in the ΣPUFAn-
402 6/ΣPUFAn-3 ratio (≈23-fold). These changes are consistent with the fat substitution
403 level and the fatty acid composition of the oleogels developed (Table 1). According to
404 European regulations (1924/2006 and 432/2012), nutritional claims may be made
405 concerning burgers made with oleogels, such as *high α-linolenic acid content* and *high*
406 *omega-3 fatty acids content*, and the corresponding health claims, among others. There
407 is abundant evidence associating a higher n-6/n-3 ratio with the promotion of
408 pathogenesis of many diseases, including cardiovascular diseases, cancer, etc., and
409 lower ratios with a suppressive effect (Simopoulos 2002). The results obtained are in
410 line with a previous work in which functional pork patties were developed by
411 substitution of animal fat by the same oil mixture as in the present work, structured in a
412 konjac-based bulking system (Salcedo-Sandoval et al. 2014). However, the ratios
413 obtained here were even better, owing to the higher animal fat substitution level. Similar
414 results were obtained for cooked meat products (frankfurters and pâtés) in which animal
415 fat was totally or partially replaced by an oil-in-water emulsion or ethyl cellulose or
416 beeswax oleogels containing the same vegetable/fish oil combination as in the present

417 work (Delgado-Pando et al. 2010; Delgado-Pando et al. 2011; Gómez-Estaca et al.
418 2019).

419 With regard to the effect of lipid substitution on the optical properties of the burgers, no
420 significant differences in lightness were found between the samples ($p > 0.05$), but
421 higher ($p \leq 0.05$) redness and yellowness were observed in the EC-B and W-B samples
422 compared with the control sample (Table 4). In order to make a deeper analysis and
423 interpretation of the optical properties of the burgers developed, hue angle and
424 chromaticity were calculated, showing that the W-B sample did not differ significantly
425 from the control formulated with animal fat in hue ($p > 0.05$), but the color was
426 significantly more intense. In the case of the EC-B sample, the hue shifted to lower
427 values (nearer to the yellow region) ($p \leq 0.05$) and the color was more intense than that
428 of the control burger ($p \leq 0.05$), indicating a higher impact of the ethyl cellulose oleogel
429 on the optical properties of the burgers than the beeswax one. In any case, the changes
430 in the optical properties were of low magnitude and of little importance from a
431 technological point of view.

432 The Kramer shear force of the burgers developed is also shown in Table 4. The control
433 burger was firmer than the one formulated in previous works (Freire et al. 2017;
434 Salcedo-Sandoval et al. 2015), which is consistent with the lower fat content of the
435 present formulation (≈7% to ≈15%). The incorporation of oleogels produced a decrease
436 in shear force ($p \leq 0.05$), without differences as a function of organogelator system ($p >$
437 0.05). The texture of restructured meat products is affected both by matrix
438 characteristics (protein/water/fat composition) and by the physicochemical properties of
439 the fat (Jiménez-Colmenero et al. 1995). The substitution of animal fat by fish or
440 vegetable oils in burgers also resulted in a decrease in hardness (Keenan et al. 2015;

441 Lurueña-Martínez et al. 2004), whereas when oils were incorporated into a konjac oil
442 bulking system no significant differences were found (Salcedo-Sandoval et al. 2015).
443 There is little literature on the substitution of animal fat by oleogels in fresh
444 comminuted meat products. Recently, Moghtadaei et al. (2018) developed beef burgers
445 in which animal fat was partially substituted by sesame oil-beeswax oleogel, finding a
446 decrease in hardness that was directly related to the substitution level in the raw
447 samples. The authors attributed this to the lower fat globule size in oleogels as
448 compared to animal fat. With regard to the use of ethyl cellulose oleogels as fat
449 substitutes in meat products, the literature is limited to finely comminuted cooked
450 products. Barbut et al. (2016a) formulated frankfurters in which beef fat was substituted
451 by ethyl cellulose oleogels made from canola oil, obtaining similar hardness values in
452 both samples. Similarly, Gómez-Estaca et al. (2019) did not find differences in pork
453 liver pâtés formulated by partial or total substitution of pork backfat by ethyl cellulose
454 or beeswax oleogels. From the few results found in the literature and those of the
455 present work, it seems that the effect of the ethyl cellulose oleogel on texture is
456 dependent on the type of product developed.

457 The oxidative status of the burgers developed was determined. The control sample
458 showed the lowest value among the three samples, followed by the sample with
459 beeswax oleogel and the one with ethyl cellulose (Table 4). This is consistent with the
460 results of lipid oxidation of the oleogels shown in Figure 4, as the ethyl cellulose
461 oleogel was the one that showed the highest lipid oxidation, probably owing to the
462 higher processing temperature and time during the manufacturing process, as compared
463 to the beeswax oleogel. These results indicate the need to make a deeper study of the
464 oxidative stability of meat products containing oleogels with this oil mixture. Despite
465 this, the oxidation values were relatively low and below the reported minimum needed

466 to detect objectionable flavors in processed meat products (Delgado-Pando et al. 2012),
467 although it is expected that they would increase with storage time. A possible way to
468 ameliorate this effect would be the addition of antioxidant compounds to the oleogels,
469 as shown for other animal fat analogues containing unsaturated lipids (Flaiz et al. 2016).

470 The sensory evaluation of the cooked burgers is shown in Figure 8. The control sample
471 showed a relatively good overall acceptability, in spite of its low fat content.
472 Substituting animal fat by the oleogels developed had no effects on color and texture
473 acceptability ($p > 0.05$), and these two samples also obtained good ratings. With regard
474 to flavor, animal fat substitution by the two oleogels developed had a negative effect (p
475 ≤ 0.05); this was especially evident in the EC-B batch, which attained values below 5
476 (considered as "neutral"). The overall acceptability of the burgers showed a similar
477 trend, i.e., a lower acceptability in the reformulated samples than in the control ($p \leq$
478 0.05), but no differences were observed between the two oleogelation systems ($p >$
479 0.05). From these results it can be deduced that the oleogelation systems employed in
480 the present work were successful for the development of reformulated burgers whose
481 color and texture resemble those of a control product, but the lipid source has a major
482 impact on flavor that ultimately impairs overall acceptability. Salcedo-Sandoval et al.
483 (2015), who developed pork burgers by substitution of pork backfat by an oil bulking
484 system based on konjac and including the same oil mixture as in the present work,
485 found similar results and attributed them to the intrinsic sensory properties of the lipid
486 material, especially fish oil. The negative effect on sensory properties could be
487 mitigated by reformulating the seasoning. In a previous work in which pork liver pâtés
488 were formulated by substituting pork backfat by the same oleogels as those used in the
489 present work good acceptability was achieved, as compared to a sample without

substitution, probably owing to the addition of seasoning in the formulation (Gómez-Estaca et al. 2019).

CONCLUSION

These results demonstrate the suitability of ethyl cellulose and beeswax organogelation systems to structure an oil combination with an optimal fatty acid profile from a health standpoint into solid-like structures that behave in a similar way to animal fat when incorporated in a fresh comminuted product such as pork burgers. From this study and under the experimental conditions used, beeswax seems to be a better alternative than ethyl cellulose for the development of substitutes for animal fat to be employed in the reformulation of fresh comminuted meat products with an improved fatty acid profile. Despite this, further research on this topic is being conducted, focusing on improving the oxidative stability and sensory properties of beeswax and ethyl cellulose oleogels.

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References

Anonymous (2003). Diet, nutrition and the prevention of chronic diseases. World Health Organization technical report series, 916, i-viii, 1-149, back cover.

AOAC (2005) Official methods of analysis of AOAC International. AOAC international.

Barbut S., Wood J. & Marangoni A. (2016a). Potential use of organogels to replace animal fat in comminuted meat products. Meat Science, 122, 155-162.

Barbut S., Wood J. & Marangoni A. (2016b). Quality effects of using organogels in breakfast sausage. Meat Science, 122, 84-89.

Barbut S., Wood J. & Marangoni A. (2016c). Effects of Organogel Hardness and Formulation on Acceptance of Frankfurters. Journal of Food Science, 81(9), 2183-2188.

519 Blasi F., Rocchetti G., Montesano D., Lucini L., Chiodelli G., Ghisoni S., Baccolo G.,
520 Simonetti M.S. & Cossignani L. (2018). Changes in extra-virgin olive oil added
521 with Lycium barbarum L. carotenoids during frying: Chemical analyses and
522 metabolomic approach. Food Research International, 105, 507-516.
523 Bligh E.G. & Dyer W.J. (1959). A rapid method of total lipid extraction and
524 purification. Canadian Journal of Biochemistry and Physiology, 37(8), 911-917.
525 Chen B., Zhang H., Cheong L.Z., Tan T. & Xu X. (2012). Enzymatic Production of
526 ABA-Type Structured Lipids Containing Omega-3 and Medium-Chain Fatty
527 Acids: Effects of Different Acyl Donors on the Acyl Migration Rate. Food and
528 Bioprocess Technology, 5(2), 541-547.
529 Commission Regulation (EU) No 1924/2006 of 20 December 2006 on nutrition and
530 health claims made on foods. In: Official Journal of the European Union, vol 12.
531 pp 3-18.
532 Commission Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of
533 permitted health claims made on foods, other than those referring to the
534 reduction of disease risk and to children′s development and health. In: Official
535 Journal of the European Union, vol 50. pp. 1-40.
536 Chen B., Zhang H., Cheong L.Z., Tan T. & Xu X. (2012). Enzymatic Production of
537 ABA-Type Structured Lipids Containing Omega-3 and Medium-Chain Fatty
538 Acids: Effects of Different Acyl Donors on the Acyl Migration Rate. Food and
539 Bioprocess Technology, 5(2), 541-547.
540 Davidovich-Pinhas M., Barbut S. & Marangoni A.G. (2015). The gelation of oil using
541 ethyl cellulose. Carbohydrate Polymers, 117, 869-878.
542 Davidovich-Pinhas M., Barbut S. & Marangoni A.G. (2016). Development,
543 characterization, and utilization of food-grade polymer oleogels. Annual Review
544 of Food Science and Technology, 7, 65-91.
545 Delgado-Pando G., Celada P., Sánchez-Muniz F.J., Jiménez-Colmenero F. &
546 Olmedilla-Alonso B. (2014). Effects of improved fat content of frankfurters and
547 pâtés on lipid and lipoprotein profile of volunteers at increased cardiovascular
548 risk: A placebo-controlled study. European Journal of Nutrition, 53(1), 83-93.
549 Delgado-Pando G., Cofrades S., Rodríguez Salas L. & Jiménez Colmenero F. (2011). A
550 healthier oil combination and konjac gel as functional ingredients in low-fat
551 pork liver pâté. Meat Science, 88(2), 241-248.
552 Delgado-Pando G., Cofrades S., Ruiz-Capillas C., Triki M. & Jiménez-Colmenero F.
553 (2012). Enriched n-3 PUFA/konjac gel low-fat pork liver pâté: Lipid oxidation,
554 microbiological properties and biogenic amine formation during chilling storage.
555 Meat Science, 92(4), 762-767.
556 Delgado-Pando G., Cofrades S., Ruiz Capillas C., Solas M. & Jiménez-Colmenero F.
557 (2010). Healthier lipid combination oil-in-water emulsions prepared with
558 various protein systems: an approach for development of functional meat
559 products. European Journal of Lipid Science and Technology, 112(7), 791-801.
560 Dey T., Kim D.A. & Marangoni A.G. (2011). Ethylcellulose oleogels. Edible oleogels:
561 structure and health implications AOCS Press, Urbana, 295-312.
562 Domínguez R., Pateiro M., Sichetti Munekata P.E., Bastianello Campagnol P.C. &
563 Lorenzo J.M. (2017). Influence of partial pork backfat replacement by fish oil on
564 nutritional and technological properties of liver pâté. European Journal of Lipid
565 Science and Technology, 119(5).
566 Flaiz L., Freire M., Cofrades S., Mateos R., Weiss J., Jiménez-Colmenero F. & Bou R.
567 (2016). Comparison of simple, double and gelled double emulsions as
568 hydroxytyrosol and n-3 fatty acid delivery systems. Food Chemistry, 213, 49-57.

569 Freire M., Cofrades S., Serrano-Casas V., Pintado T., Jimenez M.J. & Jimenez-
570 Colmenero F. (2017). Gelled double emulsions as delivery systems for
571 hydroxytyrosol and n-3 fatty acids in healthy pork patties. Journal of Food
572 Science and Technology, 54(12), 3959-3968.
573 Gómez-Estaca J., Calvo M.M., Sánchez-Faure A., Montero P. & Gómez-Guillén M.C.
574 (2015). Development, properties, and stability of antioxidant shrimp muscle
575 protein films incorporating carotenoid-containing extracts from food by-
576 products. LWT-Food Science and Technology, 64(1), 189-196.
577 Gómez-Estaca J., Herrero A.M., Herranz B., Álvarez M.D., Jiménez-Colmenero F. &
578 Cofrades S. (2019). Characterization of ethyl cellulose and beeswax oleogels
579 and their suitability as fat replacers in healthier lipid pâtés development. Food
580 Hydrocolloids, 87, 960-969.
581 Grasso S., Brunton N.P., Lyng J.G., Lalor F. & Monahan F.J. (2014). Healthy processed
582 meat products - Regulatory, reformulation and consumer challenges. Trends in
583 Food Science & Technology, 39(1), 4-17.
584 Gravelle A.J., Barbut S. & Marangoni A.G. (2012). Ethylcellulose oleogels:
585 Manufacturing considerations and effects of oil oxidation. Food Research
586 International, 48(2), 578-583.
587 Gravelle A.J., Barbut S. & Marangoni A.G. (2013). Fractionation of ethylcellulose
588 oleogels during setting. Food and Function, 4(1), 153-161.
589 Gravelle A.J., Barbut S., Quinton M. & Marangoni A.G. (2014). Towards the
590 development of a predictive model of the formulation-dependent mechanical
591 behaviour of edible oil-based ethylcellulose oleogels. Journal of Food
592 Engineering, 143, 114-122.
593 Herrero A.M., Carmona P., Pintado T., Jiménez-Colmenero F. & Ruíz-Capillas C.
594 (2011). Olive oil-in-water emulsions stabilized with caseinate: Elucidation of
595 protein-lipid interactions by infrared spectroscopy. Food Hydrocolloids, 25(1),
596 12-18.
597 Jiménez-Colmenero F. (2007). Healthier lipid formulation approaches in meat-based
598 functional foods. Technological options for replacement of meat fats by non-
599 meat fats. Trends in Food Science and Technology, 18(11), 567-578.
600 Jiménez-Colmenero F., Barreto G., Mota N. & Carballo J. (1995). Influence of protein
601 and fat content and cooking temperature on texture and sensory evaluation of
602 bologna sausage. LWT-Food Science and Technology, 28(5), 481-487.
603 Jimenez-Colmenero F., Salcedo-Sandoval L., Bou R., Cofrades S., Herrero A.M. &
604 Ruiz-Capillas C. (2015). Novel applications of oil-structuring methods as a
605 strategy to improve the fat content of meat products. Trends in Food Science &
606 Technology, 44(2), 177-188.
607 Jiménez-Martín E., Pérez-Palacios T., Carrascal J.R. & Rojas T.A. (2016). Enrichment
608 of Chicken Nuggets with Microencapsulated Omega-3 Fish Oil: Effect of
609 Frozen Storage Time on Oxidative Stability and Sensory Quality. Food and
610 Bioprocess Technology, 9(2), 285-297.
611 Keenan D.F., Resconi V.C., Smyth T.J., Botinestean C., Lefranc C., Kerry J.P. &
612 Hamill R.M. (2015). The effect of partial-fat substitutions with encapsulated and
613 unencapsulated fish oils on the technological and eating quality of beef burgers
614 over storage. Meat Science, 107, 75-85.
615 Lupi F.R., Gabriele D. & de Cindio B. (2012). Effect of Shear Rate on Crystallisation
616 Phenomena in Olive Oil-Based Organogels. Food and Bioprocess Technology,
617 5(7), 2880-2888.

618 Lurueña-Martínez M.A., Vivar-Quintana A.M. & Revilla I. (2004). Effect of locust
619 bean/xanthan gum addition and replacement of pork fat with olive oil on the
620 quality characteristics of low-fat frankfurters. Meat Science, 68(3), 383-389.
621 Maqsood S. & Benjakul S. (2010). Comparative studies of four different phenolic
622 compounds on in vitro antioxidative activity and the preventive effect on lipid
623 oxidation of fish oil emulsion and fish mince. Food Chemistry, 119(1), 123-132.
624 Martínez B., Miranda J.M., Vázquez B.I., Fente C.A., Franco C.M., Rodríguez J.L. &
625 Cepeda A. (2012). Development of a hamburger patty with healthier lipid
626 formulation and study of its nutritional, sensory, and stability properties. Food
627 and Bioprocess Technology, 5(1), 200-208.
628 Martins A.J., Cerqueira M.A., Fasolin L.H., Cunha R.L. & Vicente A.A. (2016).
629 Beeswax organogels: Influence of gelator concentration and oil type in the
630 gelation process. Food Research International, 84, 170-179.
631 McNeill S.H. (2014). Inclusion of red meat in healthful dietary patterns. Meat Science,
632 98(3), 452-460.
633 Mert B. & Demirkesen I. (2016). Reducing saturated fat with oleogel/shortening blends
634 in a baked product. Food Chemistry, 199, 809-816.
635 Moghtadaei M., Soltanizadeh N. & Goli S.A.H. (2018). Production of sesame oil
636 oleogels based on beeswax and application as partial substitutes of animal fat in
637 beef burger. Food Research International, 108, 368-377.
638 Muguerza E., Gimeno O., Ansorena D. & Astiasarán I. (2004). New formulations for
639 healthier dry fermented sausages: a review. Trends in Food Science &
640 Technology, 15(9), 452-457.
641 Nawar W.W. (1969). Thermal degradation of lipids. Journal of Agricultural and Food
642 Chemistry, 17(1), 18-21.
643 Ockerman H.W. (1985) Quality control of post-mortem muscle tissue. Dept. of Animal
644 Science, Ohio State University.
645 Öğütcü M., Arifoglu N. & Yilmaz E. (2015). Preparation and characterization of virgin
646 olive oil-beeswax oleogel emulsion products. Journal of the American Oil
647 Chemists Society, 92(4), 459-471.
648 Öğütcü M. & Yilmaz E. (2014). Oleogels of virgin olive oil with carnauba wax and
649 monoglyceride as spreadable products. Grasas y Aceites, 65(3).
650 Salcedo-Sandoval L., Cofrades S., Ruiz-Capillas C., Carballo J. & Jiménez-Colmenero
651 F. (2015). Konjac-based oil bulking system for development of improved-lipid
652 pork patties: Technological, microbiological and sensory assessment. Meat
653 Science, 101, 95-102.
654 Salcedo-Sandoval L., Cofrades S., Ruiz-Capillas C. & Jiménez-Colmenero F. (2014).
655 Effect of cooking method on the fatty acid content of reduced-fat and PUFA-
656 enriched pork patties formulated with a konjac-based oil bulking system. Meat
657 Science, 98(4), 795-803.
658 Simopoulos A.P. (2002). The importance of the ratio of omega-6/omega-3 essential
659 fatty acids. Biomedicine and Pharmacotherapy, 56(8), 365-379.
660 Tenyang N., Ponka R., Tiencheu B., Djikeng F.T., Azmeera T., Karuna M.S.L., Prasad
661 R.B.N. & Womeni H.M. (2017). Effects of boiling and roasting on proximate
662 composition, lipid oxidation, fatty acid profile and mineral content of two
663 sesame varieties commercialized and consumed in Far-North Region of
664 Cameroon. Food Chemistry, 221, 1308-1316.
665 Toro-Vazquez J.F., Morales-Rueda J.A., Dibildox-Alvarado E., Charó-Alonso M.,
666 Alonzo-Macias M. & González-Chávez M.M. (2007). Thermal and textural

properties of organogels developed by candelilla wax in safflower oil. JAOCS, Journal of the American Oil Chemists' Society, 84(11), 989-1000.
Wolfer T.L., Acevedo N.C., Prusa K.J., Sebranek J.G. & Tarté R. (2018). Replacement of pork fat in frankfurter-type sausages by soybean oil oleogels structured with rice bran wax. Meat Science, 145, 352-362.
Yi B.R., Kim M.J., Lee S.Y. & Lee J.H. (2017). Physicochemical properties and oxidative stability of oleogels made of carnauba wax with canola oil or beeswax with grapeseed oil. Food Science and Biotechnology, 26(1), 79-87.
Yilmaz E. & Öğütcü M. (2014). Properties and stability of hazelnut oil organogels with beeswax and monoglyceride. JAOCS, Journal of the American Oil Chemists' Society, 91(6), 1007-1017.
Yilmaz E. & Öğütcü M. (2014). Comparative analysis of olive oil organogels containing beeswax and sunflower wax with breakfast margarine. Journal of Food Science, 79(9), E1732-E1738.
Zetzl A. & Marangoni A. (2011). Novel strategies for nanostructuring liquid oils into functional fats. Edible oleogels: Structure and health implications, 19-47.
Zetzl A.K., Marangoni A.G. & Barbut S. (2012). Mechanical properties of ethylcellulose oleogels and their potential for saturated fat reduction in frankfurters. Food and Function, 3(3), 327-337.

687 **Figure captions**

688 Figure 1. Schematic drawing of burger preparation.

689 Figure 2. Pictures of ethyl cellulose (A) and beeswax (B) oleogels.

690 Figure 3. Microscopic images of ethylcellulose (A, C) and beeswax (B, D) oleogels.
691 Pictures C and D were taken under polarized light.

692 Figure 4. TBARS of ethyl cellulose (EC-OG) and beeswax (W-OG) oleogels as a
693 function of storage time at 3 ± 1 °C. Different letters indicate significant differences for
694 each oleogel as a function of storage time.

695 Figure 5. Typical penetration force analysis curves of ethyl cellulose (EC-OG, solid
696 line) and beeswax (W-OG, dotted line) oleogels.

697 Figure 6. DSC traces of ethyl cellulose (A) and beeswax (B) oleogels during heating
698 (solid lines) and cooling (dotted lines) ramps (1 day after preparation). The DSC trace
699 of the oil mixture during the heating ramp is also plotted (A).

700 Figure 7. ΣSFA, ΣMUFA, ΣPUFA, and ALA (g /100 g product) and nutritional ratios of
701 the burgers developed.

702 Figure 8. Sensory evaluation of the burgers developed. C-B, control with pork backfat;
703 EC-B, olive oil/linseed oil/fish oil structured with ethyl cellulose; W-B, olive oil/linseed
704 oil/fish oil structured with beeswax.

705 **Table captions**

706 Table 1. Most abundant fatty acids (mg/g oil) and nutritional ratios of ethyl cellulose
707 (EC-OG) and beeswax (W-OG) oleogel samples compared to those of the oil mixture.

708 Table 2. Optical properties (lightness, L*; redness, a*; yellowness, b*).

709 Table 3. Proximate composition (%) of burgers formulated with different lipid sources
710 or oil structuring methods: C-B, control with pork backfat; EC-B, olive oil/linseed
711 oil/fish oil structured with ethyl cellulose; W-B, olive oil/linseed oil/fish oil structured
712 with beeswax.

713 Table 4. Some physicochemical properties of the burgers developed: luminosity (L*),
714 redness (a*), yellowness (b*), hue angle, chromaticity, Kramer shear force, and
715 TBARS. Batch denominations as per Table 3.

716

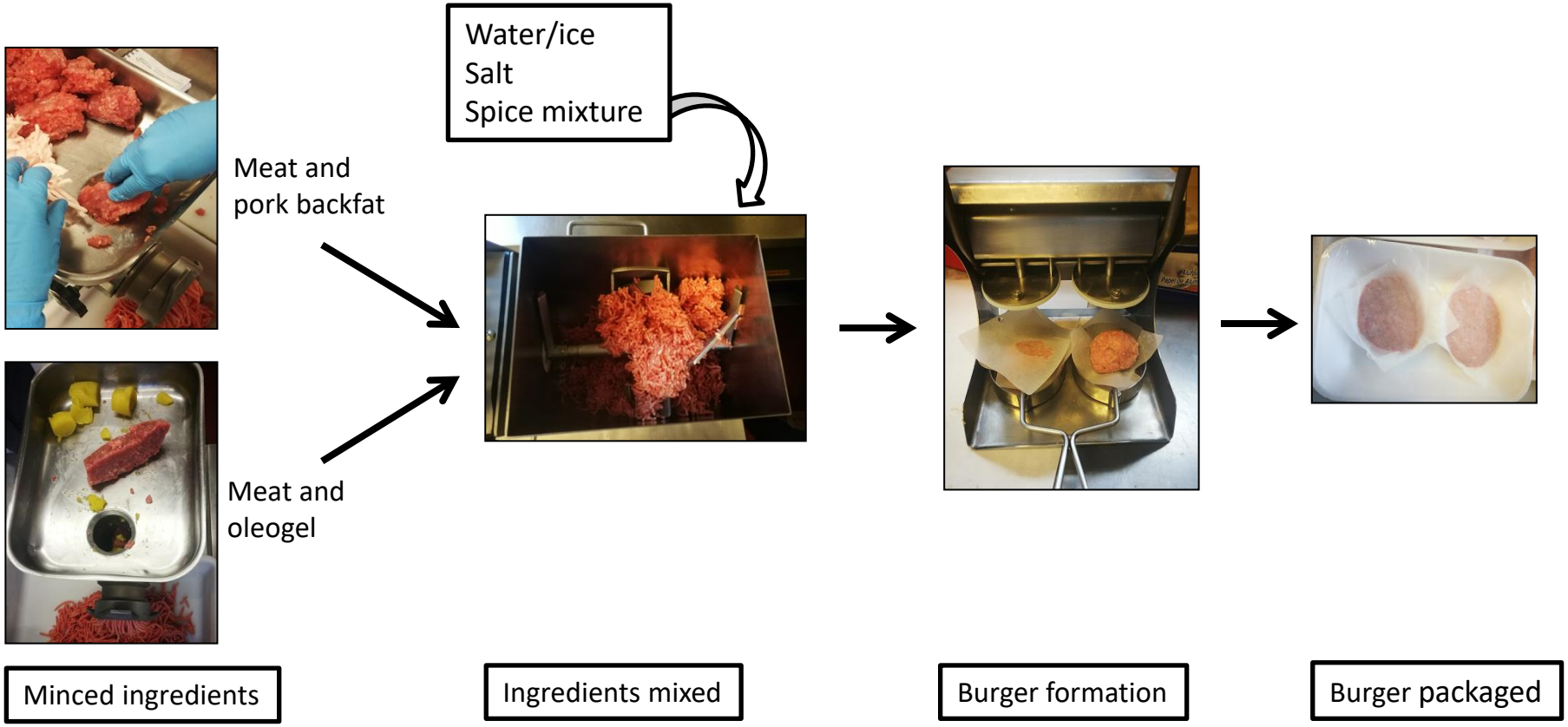


Figure 1. Schematic drawing of burger preparation

Table 1. Most abundant fatty acids (mg/g oil) and nutritional ratios of ethylcellulose (EC-OG) and beeswax (W-OG) oleogel samples compared to those of the oil mixture.

	Oil mixture	**EC-OG**	**W-OG**
Palmitic C16:0	87.8 ± 1.8a	102.9 ± 0.2c	98.1 ± 1.7b
Stearic C18:0	38.3 ± 0.8a	53.0 ± 0.04b	38.4 ± 0.6a
ΣSFA	147.2 ± 2.8a	177.1 ± 0.1c	163.1 ± 2.5b
Oleic C18:1n9c	421.6 ± 8.7a	412.4 ± 0.6a	412.6 ± 7.1a
ΣMUFA	459.3 ± 9.8a	449.8 ± 1.4a	452.2 ± 8.1a
Linoleic C18:2n6c	82.9 ± 1.7a	81.0 ± 0.2a	80.9 ± 1.3a
Linolenic C18:3n3	209.2 ± 4.6a	203.4 ± 0.5a	204.4 ± 3.5a
EPA C20:5n3	28.9 ± 0.6a	28.1 ± 0.1a	28.4 ± 0.5a
DHA C22:6n3	19.7 ± 0.5a	19.5 ± 0.4a	19.7 ± 0.5a
ΣPUFA	341.1 ± 7.6a	332.5 ± 0.8a	334.9 ± 5.8a
ΣPUFA/ΣSFA	2.32 ± 0.01c	1.88 ± 0.01a	2.05 ± 0.01b
ΣPUFAn-3	267.8 ± 6.2a	260.9 ± 0.2a	263.2 ± 5.0a
ΣPUFAn-6	85.6 ± 1.7a	83.7 ± 0.3a	84.2 ± 1.5a
Σn-6/Σn-3	0.32 ± 0.0a	0.32 ± 0.0a	0.32 ± 0.0a

ΣPUFA: total amount of polyunsaturated fatty acids. ΣPUFAn-3: total amount of n-3 polyunsaturated fatty acids. ΣPUFAn-6: total amount of n-6 polyunsaturated fatty acids. ΣPUFA/ΣSFA: polyunsaturated/saturated fatty acid ratio. Σn-6/Σn-3: n-6/n-3 polyunsaturated fatty acid ratio. Means ± standard deviation. Different letters (a, b, c) in the same row indicate significant differences ($p \leq 0.05$).

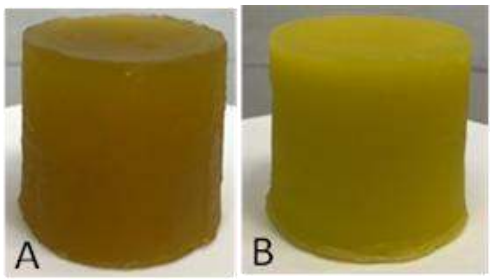
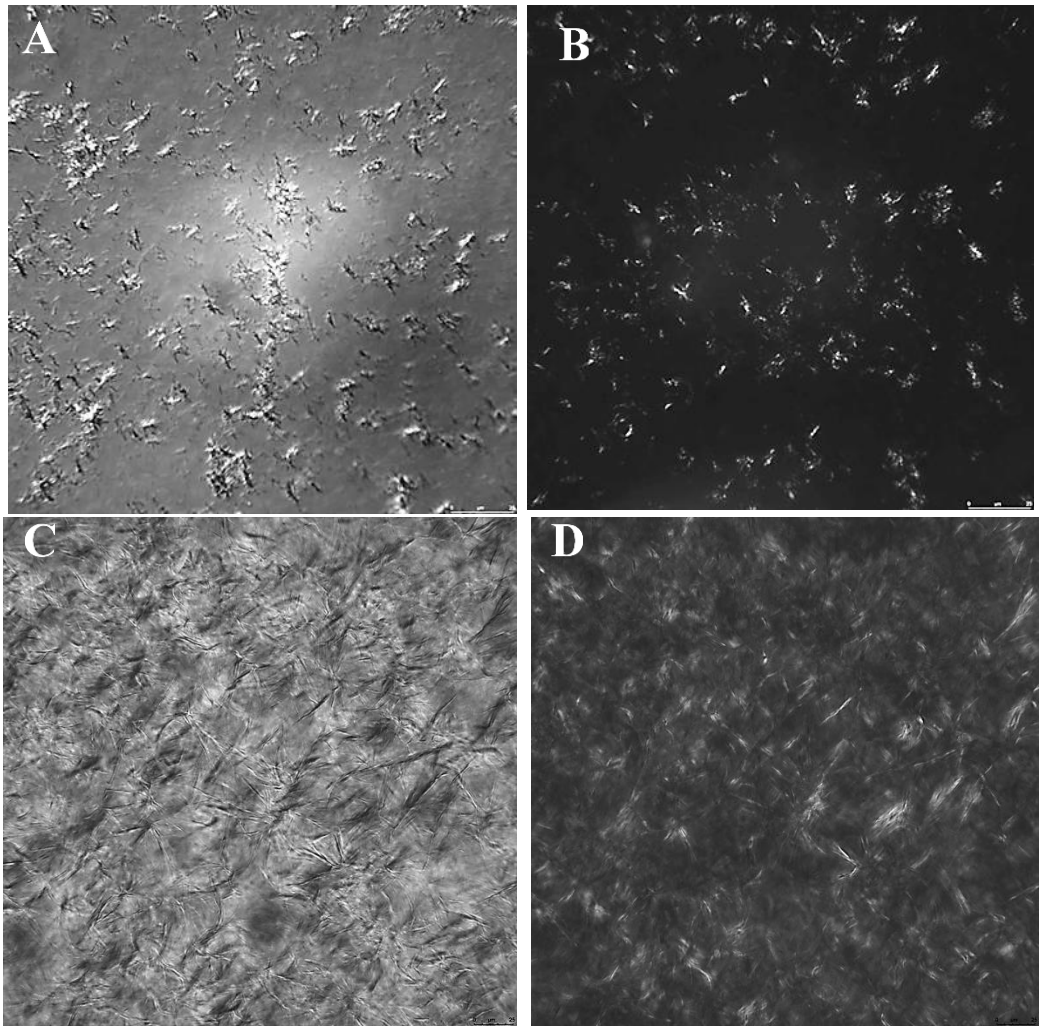


Figure 2. Pictures of ethyl cellulose (A) and beeswax (B) oleogels.

Table 2. Optical (lightness, L*; redness, a*; yellowness, b*).

		EC-OG	W-OG
Optical properties	L*	25.9 ± 0.1a	36.7 ± 0.1b
	a*	-0.1 ± 0.1b	-1.40 ± 0.01a
	b*	2.7 ± 0.1a	20.9 ± 0.1b

As no significant differences were observed as function of storage time (28 days at 3 ºC ± 1), results are expressed means of all sampling dates ± standard deviation. Different letters (a, b) indicate significant differences ($p \leq 0.05$) as a function of the oleogelator system.



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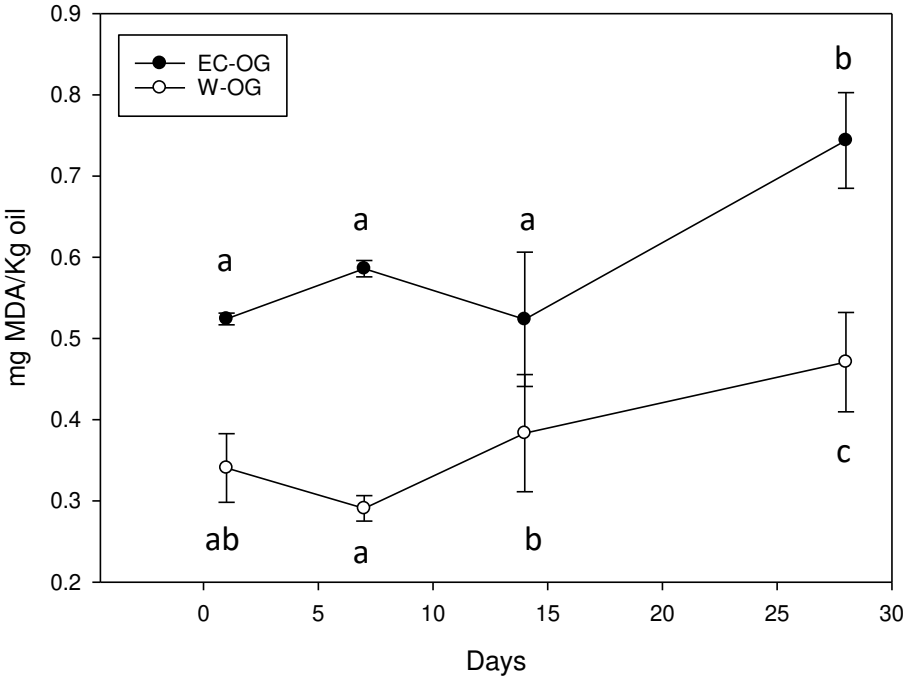

Figure 4. TBARS of ethyl cellulose (EC-OG) and beeswax (W-OG) oleogels as a function of storage time at 3 ± 1 °C. Different letters indicate significant differences for each oleogel as function of storage time.

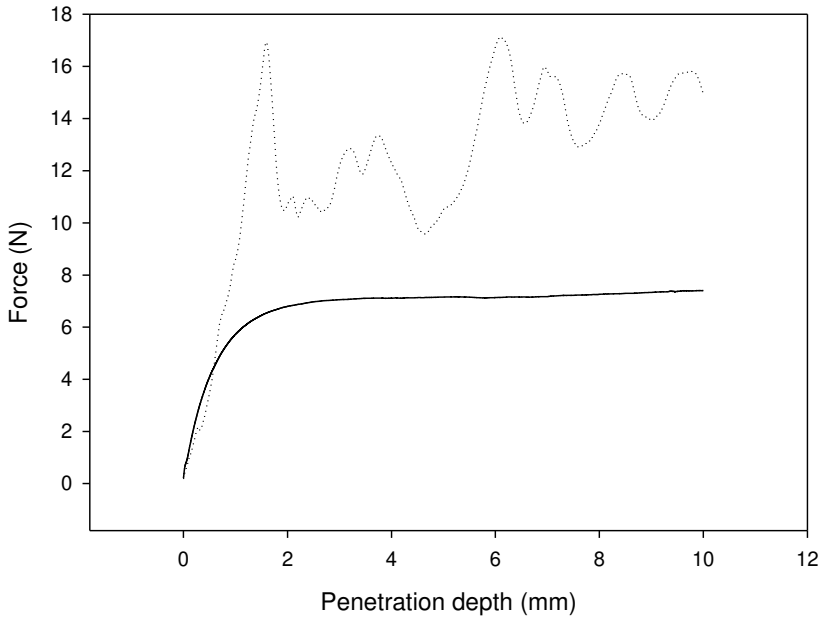

Figure 5. Typical penetration force analysis curves of ethyl cellulose (EC-OG, solid line) and beeswax (W-OG, dotted line) oleogels.

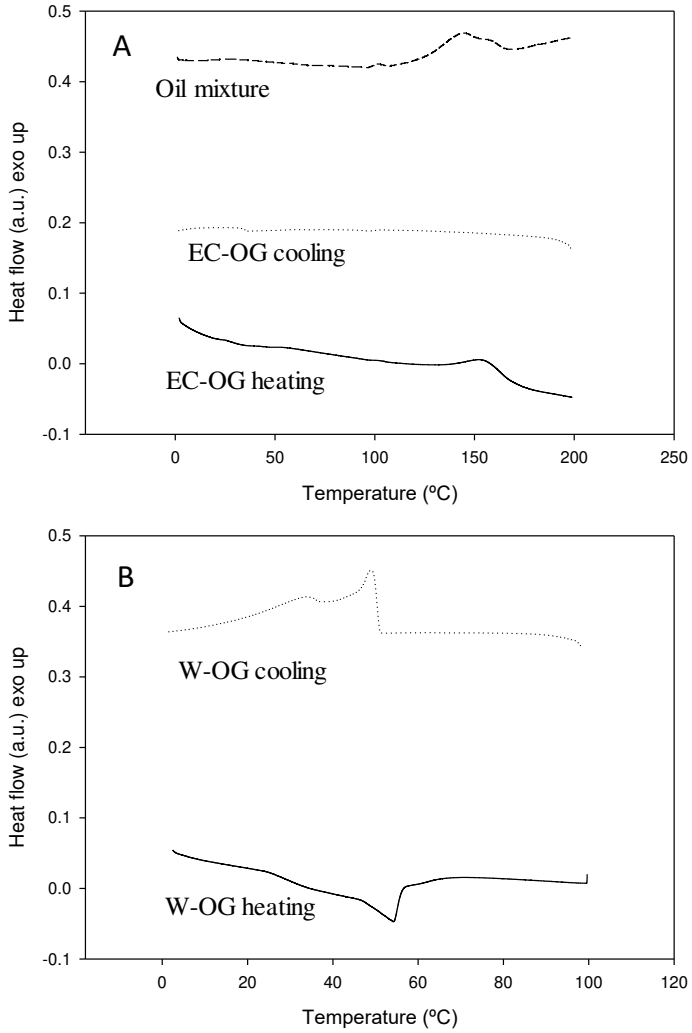

Figure 6. DSC traces of ethyl cellulose (A) and beeswax (B) oleogels during heating (solid lines) and cooling (dotted lines) ramps (1 day after preparation). DSC trace of the oil mixture during heating ramp is also plotted (A).

Table 3. Proximate composition (%) of burgers formulated with different lipid source or oil structuring method: C-B, control with pork backfat; EC-B, olive oil/linseed oil/fish oil structured with ethylcellulose; W-B, olive oil/linseed oil/fish oil structured with beeswax.

	Moisture	**Fat**	**Ash**	**Protein**
C-B	72.30±0.37a	7.21±0.20a	1.87±0.08a	18.31±0.17a
EC-B	71.84±0.21a	7.87±0.04b	2.03±0.03b	18.04±0.40a
W-B	71.92±0.19a	7.72±0.15b	1.88±0.06a	17.87±0.08a

Means ± standard deviation. Different letters in the same column (a, b) indicate significant differences ($p \leq 0.05$).

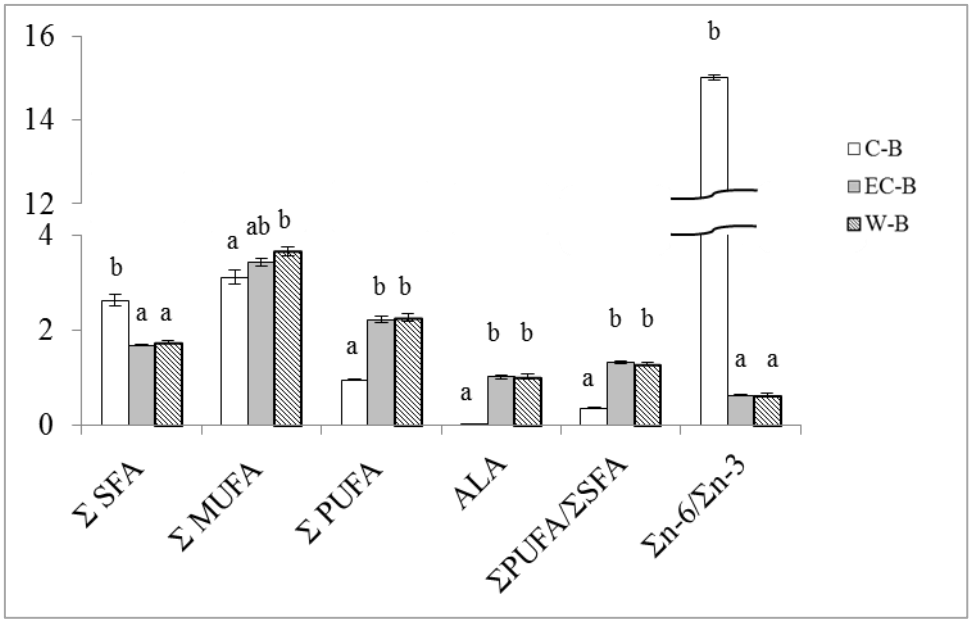

Figure 7. ΣSFA, ΣMUFA, ΣPUFA and ALA (g /100 g product) and nutritional ratios of the burgers developed

Table 4. Some physico-chemical properties of the burgers developed: luminosity (L*), redness (a*), yellowness (b*), hue angle, chromaticity, Kramer shear force, and TBARS. Batches denomination as per Table 3.

	C-B	EC-B	W-B
L*	51.31±1.77a	52.32±1.67a	52.12±2.07a
a*	2.13±0.28a	3.35±0.29c	2.55±0.40b
b*	9.22±0.82a	11.49±0.89b	11.00±0.95b
Hue angle (º)	76.9 ± 1.9b	73.7 ± 1.7a	76.9 ± 1.9b
Chromaticity	9.5 ± 0.8a	12.0 ± 0.9b	11.3 ± 1.0b
Kramer shear force (N/g)	3.48±0.46b	2.61±0.21a	2.23±0.23a
TBARS (mg MDA/Kg sample)	0.11±0.02a	0.30±0.13c	0.14±0.01b

Means ± standard deviation. Different letters (a, b, c) in the same row indicate significant ($p \leq 0.05$) differences among samples.

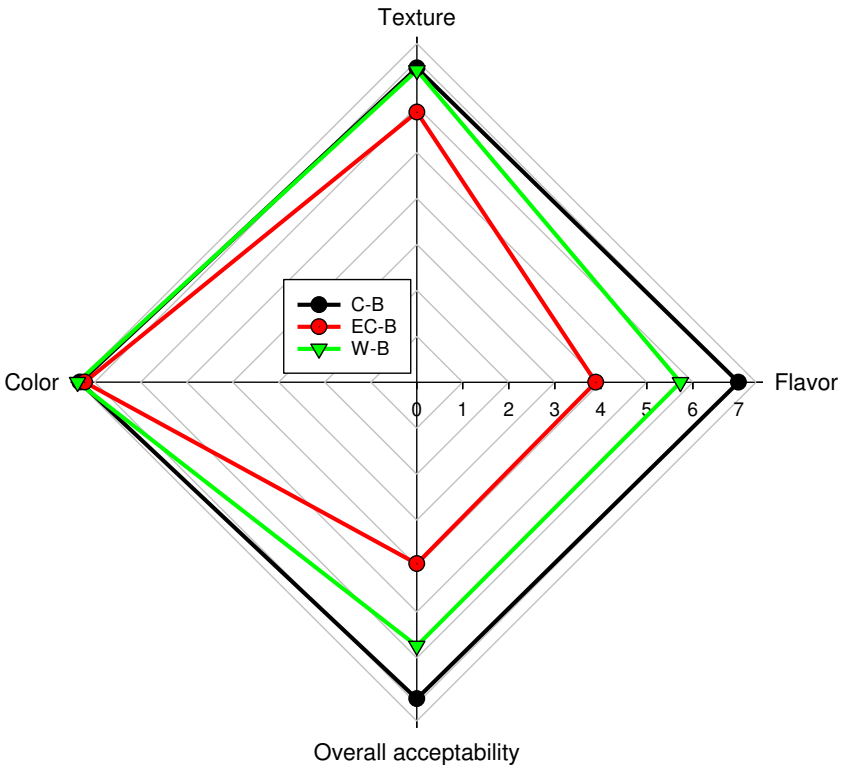

Figure 8. Sensory evaluation of the burgers developed. C-B, control with pork backfat; EC-B, olive oil/linseed oil/fish oil structured with ethylcellulose; W-B, olive oil/linseed oil/fish oil structured with beeswax