# Phenolic compounds in emulsion gel-based delivery systems applied as animal fat replacers in frankfurters: physico-chemical and structural approach

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**Abstract**

This article evaluates the use of emulsion gels (EGs) containing two different solid polyphenol extracts [from grape seed (R-EPG) or grape seed and olive (R-EPGO)] as animal fat replacers in the development of frankfurters. The incorporation of EGs improved their lipid content, particularly R-EPG and R-EPGO also contained high levels of phenolic compounds (hydroxytyrosol and flavanols). These frankfurters were judged acceptable by the panellists and showed good thermal and storage stability. Colour parameters, pH and textural properties were affected ($p<0.05$) by the formulation, being significant the influence of polyphenols extracts. Spectroscopic results showed greater ($p<0.05$) inter- and intramolecular lipid disorder in the frankfurters with EGs, irrespective of the presence of polyphenol extracts. Comparing the reduced-fat samples, R-EPG and R-EPGO showed the lowest ($p<0.05$) total viable counts. Significant changes in pH and texture parameters were observed during chilled storage while lipid structure was not affected.

**Keywords**: phenolic compounds; antioxidants; antimicrobial activity; emulsion gels; physico-chemical properties; lipid structure.

## 1. Introduction

There is growing interest in phenolic compounds due to the benefits they can bring to consumer health, related to their capacity to decrease the risk of several diseases, and also because of their technological advantages, mainly related to their antioxidant activity (Hygreeva, Pandey, & Radhakrishna, 2014; Papuc, Goran, Predescu, Nicorescu, & Stefan, 2017). Such compounds are important secondary plant metabolites that are found in fresh and processed fruit and vegetables (Papuc, et al., 2017). The recommendation to eat five servings of fruit and vegetables daily could result in a total polyphenol intake of over 500 mg per day (Williamson, & Holst, 2008). Grapes, and particularly their seed, are a rich source of phenolic compounds such as gallic acid and flavanol monomers, together with their derivatives: proanthocyanidins. These compounds have been related with strong antioxidant effects and benefits for the cardiovascular system (Rodríguez-Montealegre, Romero-Peces, Chacon-Vozmediano, Martínez-Gascuena, & García-Romero, 2006), and some studies have also suggested that grape seed has beneficial effects on the cardiovascular system. For these reasons, it has been advised that we consume 100–300 mg/day of grape seed extract rich in proanthocyanidins (Feringa, Laskey, Dickson, & Coleman, 2011). Olives, and especially olive oils, contain hydroxytyrosol: a phenolic compound (averaging 200-500 mg/kg) with strong antioxidant properties and with EFSA-approved health benefits that is deemed safe (EFSA, 2017). The incorporation of these sources of phenolic compounds into other foods could therefore represent an opportunity to improve their nutritional contribution to the diet of those how consume them while furthermore extending the shelf life of products (Aziz & Karboune, 2018; Hygreeva, et al., 2014; Nowak, Czyzowska, Efenberger, & Krala, 2016; Papuc, et al., 2017).

In meat and meat products, in order to enhance the content of phenolic compounds due to their beneficial properties, different strategies have been adopted, focusing mainly on their inclusion in animal diets (Nardoia, et al., 2018) or using reformulation processes (Aziz & Karboune, 2018; Hygreeva, et al., 2014). Focus in the reformulation process of meat products are mainly based on

the direct addition of phenol-rich ingredients (berries, grape seed, tea, olive, cloves, sage, essential oils, etc.), with a physico-chemical purpose to transfer to the reformulated product both antimicrobial and antioxidant effects (Papuc, et al., 2017). However, polyphenols that are added directly are prone to undesired inactivation or degradation under conditions that typically occur in the processing of meat products; moreover, some have an unpleasant colour, bitter taste and astringency. It has been indicated that protecting phenols using emulsion procedures could overcome such drawbacks (Fang, & Bhandari, 2010). Among the different emulsion procedures, an interesting and unexplored option to protect phenolic compounds could be the use of emulsion gels (EGs) to delivery bioactive compounds due to their special characteristics (Pintado, Ruiz-Capillas, Jiménez-Colmenero, Carmona, & Herrero, 2015). Moreover, the EGs has been applied as animal fat replacers to development healthier meat products preserving their physico-chemical properties (Paglarini, Martini, & Pollonio, 2019; Pintado, Herrero, Jiménez-Colmenero, & Ruiz-Capillas, 2016a; Pintado, Herrero, Jiménez-Colmenero, Cavalheiro, & Ruiz-Capillas, 2018). Particularly, some authors have explored the possibility of including polyphenols in EGs with the aim of evaluating their potential as antioxidants when they are incorporated into meat products (Alejandre, Ansorena, Calvo, Cavero, & Astiasarán, 2019; Flaiz, et al., 2016; Wang, Xie, Li, Liu, & Yan, 2018).

The potential of EGs make it interesting to study their application on reformulation of meat products enriched with polyphenols. Therefore, the aim of the present work was to evaluate the use of EGs containing solid polyphenol extracts from grape seed and a mixture of grape seed and olives, as animal fat replacers, to develop different frankfurters. This strategy to incorporate phenolic compounds in frankfurters entailed to ensure health benefits when they are consumed in common portions. Physico-chemical properties, lipid structural characteristics (using attenuated total reflectance (ATR)–FTIR spectroscopy), oxidative and microbiological stability were evaluated over storage. Composition, energy content and sensory characteristics were also studied.

## 2. Material and Methods

### 2.1. Preparation of oil-in-water emulsion gels

Three O/W EGs were prepared similarly, in duplicate, with a combination of water, extra virgin olive oil (40%) (Aceites del sur-Coosur S.A., Spain), soy protein isolate (5%) (Wilpro G300; Vicoprot, TRADES S.A., Spain), and a gelling agent based on alginate (4%), which included sodium alginate (1.46%) (Tradissimo, TRADES S.A., Spain), calcium sulphate 2-hydrate (1.46%) and tetra-sodium pyrophosphate 10-hydrate (1.08%) (Panreac Química, S.A., Spain). Based on these components, one type of EG was prepared and used as the reference: EC. Two further EGs also included either 2.32% of grape seed extract (EPG) (ExGrape® seed extract, Inquiaroma S.A., Spain) or 1.95% of extract from grape seed and olive (EPGO) (OleoGrape® seed extract, Inquiaroma S.A., Spain). Both EPG and EPGO were designed to ensure a high content of phenolic compounds in the frankfurter, into which they were to be incorporated as animal fat replacers, considering technological limitations and polyphenol recommendations for consumer health benefits. Previously, total phenolic compounds were determined by means of double aqueous–organic extraction, following the method of Nardoia et al., 2018, in both the solid extracts used and estimated as 1.8% of total extractable polyphenols.

The O/W EGs were prepared following Muñoz-González, Ruiz-Capillas, Salvador, & Herrero (2019) and Pintado, et al., (2015). Previously, the gelling agent was individually dissolved (in ~5% of the total water). Similarly, the solid polyphenol extracts added to EPG and EPGO were dissolved in 30% of the total water. Briefly, the preparation of EGs was based on mixing soy protein isolate with the rest of the water using a homogenizer (Thermomix TM 31, VorwerkEspaña M.S.L., S.C, Spain). The previously dissolved solid polyphenol extracts were incorporated into the EPG and EPGO samples and mixed. In all the samples, the gelling agent was then added and mixed again. Promptly, olive oil was gradually added to the mixture for 3 min at approx. 5600 rpm. Finally, the EGs were placed in a metal container and stored at 2 ºC until use.

100 **2.2. Preparation of frankfurters**

101 Frankfurters were prepared with sufficient (20 kg) fresh post-rigor pork meat (mixture of
102 *biceps femoris*, *semimembranosus*, *semitendinosus*, *gracilis and adductor* M) (22.1% ± 0.4%
103 protein, 5.1% ± 0.6% fat) and pork back fat (2 kg) (7.5% ± 0.7% protein, 86.7% ± 1.9% fat), from
104 different animals, obtained from a local market. Both meat and fat were passed through a 0.6 mm
105 mincer. Lots of approximately 1 kg of meat and 0.5 kg of fat were prepared, vacuum packed, frozen
106 and stored (-20 ºC) until use.

107 Five different frankfurters were prepared (Table 1). Two of these were formulated with all pork
108 back fat (F) as reference sausages: one with normal (N) fat content (~23%) designated N-F; and the
109 other with a reduced (R) fat level (~12%) (R-F). Moreover, three different reduced-fat (~12%)
110 frankfurters were formulated by totally replacing the pork back fat with the same proportion of the
111 corresponding O/W EG (EC, EPG and EPGO), which we labelled: R-EC, R-EPG and R-EPGO,
112 respectively (Table 1).

113 The procedure followed to elaborate these frankfurters was as described by Jiménez-
114 Colmenero, Herrero, Pintado, Solas, & Ruiz-Capillas (2010) (Table 1). Briefly, raw meat and non-
115 meat materials, added at different times, were homogenized under vacuum conditions for a total of
116 5 minutes in a UM5 Stephan Universal Machine (Stephan Söhne GmbH and Co., Germany), and
117 then the resulting meat batter (< 14 ºC) was stuffed into 20 mm diameter Nojax cellulose casings
118 (Viscase S.A., France). The samples were hand linked and heat processed in a smokehouse (model
119 Unimatic 1000, Micro 40 Eller, Italy). The frankfurters were chilled overnight, then the casings
120 were removed, and samples were vacuum packed in plastic bags (Cryovac® BB3050, Spain) and
121 stored (4 ± 1 °C). The composition, energy content and sensory evaluation were carried out after 1
122 day. Physico-chemical properties, structural characteristics, lipid oxidation and microbiology were
123 determined at different times over 60 days of chilled storage (4 ± 1 °C).

## 2.3. Composition and energy content

Moisture and ash contents were determined by AOAC (2005). According to Bligh, & Dyer (1959) was evaluated fat content. Protein level was measured by a Nitrogen Determinator LECO FP-2000, (Leco Co., USA). All determinations were carried out in triplicate.

Fatty acid composition was determined in freeze-dried (Lyophilizer Telstar Cryodos Equipment, Spain) frankfurters, performed (in triplicate) by gas chromatography, as reported by Pintado et al., (2018). Results are expressed as g of fatty acid per 100 g of product.

The energy content was calculated based on 9 kcal/g for fat and 4 kcal/g for protein and carbohydrates.

## 2.4. Sensory evaluation

A hedonic sensory analysis was performed by a panel who regularly consume this kind of product. Samples (2.5 cm long) from each formulation were heated for 15 s in a microwave, and then immediately presented to the panellists. These judges were instructed to evaluate colour, flavour, texture, juiciness and general acceptability in a rating test with fixed extremes (0=intensely dislike, 10= intensely like). Each point was later converted to a numerical scale.

## 2.5. Physico-chemical properties

### 2.5.1. Processing and purge losses

The processing loss in the frankfurters was calculated in ten samples as the weight loss (expressed as a percentage of the initial sample weight) occurring after heat processing and chilling overnight at 2 ºC.

To evaluate purge loss, surface exudate (tiny drops) was wiped from the frankfurters with paper towels and weighed. The purge loss was calculated from the weight difference, and expressed as a percentage of the initial weight. Two vacuum packs per formulation were selected.

### 2.5.2. Colour and pH measurement

The colour of the frankfurters was measured in cross-sections (2 cm) using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing, Inc., Japan). The CIELAB colour space was used to obtain the colour coordinates L* [black (0) to white (100)], a* [green (–) to red (+)], and b* [blue (–) to yellow (+)]. Ten measurements were taken per sample.

The pH was determined in quadruplicate using an 827 Metrohm pH Meter (MetrohmAG, Switzerland) at room temperature on homogenates (1:10 w/v sample/distilled water).

### 2.5.3. Texture profile analysis (TPA)

Textural properties were analysed by texture profile analysis (TPA) performed in a TA-XT.plus Texture Analyzer (Texture Technologies Corp., USA) as described by Bourne (1978). Five cores (length = 20 mm) from each sample were axially compressed to 40% of their original length. Force–time deformation curves were obtained with a 5 kg load cell, applied at a crosshead speed of 1 mm/s. Hardness (N), cohesiveness (without dimensions), springiness (mm) and chewiness (N*mm) were the parameters calculated.

## 2.6. Structural Characteristics

*2.61. Attenuated Total Reflectance (ATR)-FTIR spectroscopy*

Infrared spectra were recorded for each sample using a Perkin-Elmer SpectrumTM 400 spectrometer (Perkin Elmer Inc., Spain) in mid-infrared mode, equipped with an ATR sampling device (Pintado, Herrero, Ruiz-Capillas, et al., 2016). Approximately 25 mg of each sample (with no previous preparation) was analysed and nine measurements were made per sample. A total of three sum spectra (72 accumulations) were analysed for each type of frankfurter. The 3000-2800 $cm^{-1}$ spectral region was analysed to study the lipid structure. To avoid any spectral influence from water and other ingredients, the corresponding aqueous solution spectrum was appropriately

subtracted using the 2125 $cm^{-1}$ association band of water as an internal intensity standard (Vincent, Steer, & Levin, 1984).

### 2.7. Lipid oxidation

The frankfurters were assessed for oxidative stability on the basis of changes in concentrations of lipid hydroperoxides and thiobarbituric acid-reactive substances (TBARS) as measures of primary and secondary oxidation products, respectively.

Lipid hydroperoxides were measured in triplicate as described by Salcedo-Sandoval et al., (2015) and the results expressed as mmol hydroperoxides/kg of sample. TBARS were determined in triplicate as reported by Pintado, Herrero, Ruiz-Capillas, et al. (2016) and the results were expressed as mg MDA/kg of sample.

### 2.8. Microbiological analysis

Microbiological analysis of the frankfurters was carried out in a vertical laminar-flow cabinet (model AV 30/70 Telsar, Spain) following the methodology described by Pintado et al., 2016 using Plate Count Agar (PCA) (Panreac, Germany) for the total viable counts (TVC) (30 ºC for 72 h) and Violet Red Bile Glucose Agar (VRBG) (Panreac, Germany) with a double layer for *Enterobacteriaceae* (37 ºC for 24 h). The results are expressed as logarithms of colony-forming units per gram (log cfu/g).

### 2.9. Statistical analysis

Statistical analysis was performed using the SPSS® computer program (v.22 IBM, SPSS Statistical Software, Inc., USA). One-way analysis of variance (ANOVA) was performed to evaluate the statistical significance ($p<0.05$) of the effect of the frankfurter formulation, and two-way ANOVA as a function of formulation and storage. Formulation and storage time were assigned as fixed effects and the replicate was assigned as a random effect. Least squares differences were

used to compare mean values between formulations, and Tukey's HSD test to identify significant differences ($p<0.05$) between formulations and storage time.

## 3. Results and Discussion

### 3.1. Composition and energy

Proximate frankfurter composition was affected by the reduction in fat content and the incorporation of EGs as animal fat replacers (Table 1). Normal-fat samples showed the lowest ($p<0.05$) moisture (56.32 %) and ash (3.14 %) contents.. Significant differences were observed in the moisture and ash content of reduced-fat samples, although no clear formulation dependence was observed. The moisture content for these samples ranged between 65.34 and 66.51 %) and that of ash amongst 3.23 and 3.75 %. Normal-fat samples (N-F) showed the highest ($p<0.05$) protein content (20.21 %), while no differences ($p>0.05$) were observed between reduced-fat samples whose value was close to 18%. Consistent with the target levels, two fat proportions were observed in frankfurters, ~24% in normal-fat samples and approximately half of that (~12%), which was similar ($p>0.05$) in all the reduced-fat samples (R-F, R-EC, R-EPG and R-EPGO). Based on this, irrespective of the formulation process, reduced-fat samples could indeed be labelled with the claim "*reduced fat*" according to Regulation (EC) No 1924/2006 (European Commission, 2010). Based on the estimated total phenolic content in the EGs elaborated with solid polyphenol extracts (EPG and EPGO), the corresponding frankfurters (R-EPG and R-EPGO) contained about 414 mg/100 g. This was chiefly hydroxytyrosol and gallic acid, flavanol monomers and their derivatives (Muñoz-Gonzalez et al., 2019). Although the polyphenol content of R-EPG and R-EPGO frankfurters is high when compared with other foods, and particularly meat products, it is difficult to establish whether consuming them supplies sufficient quantity to offer positive health effects. Some companies that sell different nutritional supplements that are rich in polyphenols recommend a consumption of 100-300 mg per day of grape seed extract (Mennen, Walker, Bennetau-Pelissero, & Scalbert, 2005). Meanwhile, the recommendation to eat five servings of fruit and vegetables daily

would result in a total polyphenol intake of approximately 500 mg/day, of which 150-300 mg/day would be flavonoids (Williamson & Holst, 2008). The protective role of polyphenols against degenerative diseases is supported by many studies carried out on animals, and different mechanisms of action have been proposed to explain such protective effects. However, the most appropriate levels of intake need to be determined for both the general population and populations at risk of developing particular diseases (Scalbert, Manach, Morand, Remesy, & Jimenez, 2005). The subject of this work, the use of EGs as delivery systems of phenolic compounds in the development of frankfurters, could be an interesting alternative to obtain products with an appropriate polyphenol content to provide health benefits for the consumer.

According to the approximate composition of frankfurters (Table 1), the energy value of the normal-fat samples (N-F) was approximately 254 kcal/100 g. In reduced-fat samples, the energy value was between 196 and 174 kcal/100 g (R-EC and R-EPGO, respectively). Thus, the energy value was reduced by almost 30% as a result of the different reformulation strategies used.

The fatty acid profile of the different samples, which was affected by the fat content and the use of EGs as animal fat replacers, is shown in Table 2. All the frankfurters formulated with EGs (R-EC, R-EPG and R-EPGO) contained similar proportions and types of fat; and so the data reported are the mean values for these samples. In all-animal-fat products (N-F and R-F) the proportions of SFAs were 39.11 and 35.09 % respectively; whereas in the modified samples, this was reduced to almost half (18.84 %) (Table 2). Oleic acid was predominant in all the samples, with values between 5.89 and 9.94 g/100 g of product (Table 2). This is consistent with reports of the fatty acid composition of pork fat (Wood, et al. 2004) and of olive oil (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2010) the lipid source used in the development of the EGs. The MUFA and PUFA proportions were higher ($p<0.05$) in samples with EGs (R-EC, R-EPG and R-EPGO); although the quantity of each was significantly higher (in general) in normal-fat frankfurters (N-F) (Table 2). Moreover, as at least 70% of the fatty acids present in the samples elaborated with EGs were derived from unsaturated fat, then under the condition that unsaturated fat

provide more than 20% of the energy of the product, the R-EC, R-EPGO and R-EPG frankfurters could be labelled with the nutritional claim "*high unsaturated fat*" and the corresponding health claim (European Commission, 2010, 2012). Meanwhile, the PUFA/SFA ratio was higher ($p<0.05$) in samples elaborated with EGs (Table 2). This ratio is one of the main parameters used to assess the nutritional quality of the lipid fraction in foods, and it is recommended that it be greater than 0.4 (Wood, et al., 2004). Similar results in relation to an improvement in the fatty acid profile have been reported for meat products elaborated with different oils stabilized in EGs (Paglarini, et al., 2019; Pintado, Herrero, Jiménez-Colmenero, et al., 2016a; Pintado et al., 2018).

### 3.2. Sensory evaluation

The sensory scores awarded by the panel of panellists are shown in figure 3. For all the sensory parameters evaluated, no differences were observed in frankfurters as a consequence of reducing the animal fat content. In contrast, samples formulated with EGs (R-EC, R-EPG and R-EPGO) obtained lower scores than N-F and R-F samples. Similar results have been obtained in other sausages containing different types of EGs, using different reformulation strategies (Jiménez-Colmenero et al., 2010, Pintado, Herrero, Ruiz-Capillas, et al, 2016). Fig. 3 shows that the frankfurters elaborated with EGs presented similar ($p>0.05$) scores for colour, flavour, juiciness, texture and general acceptability; so no significant differences were observed as a result of the phenolic extracts added to EGs. Thus, these EGs are appropriate as phenol delivery systems, in relation to their sensory attributes. The incorporation of excessive amounts of plant extracts may result in unpleasant sensory characteristics in meat products when they are added directly, as some authors have observed with grape seed extracts in frankfurters (Özvural, & Vural, 2012). In spite of this, for cooked meat products, other authors have reported that direct addition of plant extracts rich in phenols did not have any clear effect on the sensory attributes (Nowak et al., 2016). In general, according to the scores of our sensorial panel (Fig. 3), all the products were acceptable as they obtained above average scores on the sensory scale.

### 3.3. Physico-chemical properties

### 3.3.1. Processing and purge losses

Processing loss values ranged between 11.0% and 13.7% and the frankfurters with reduced animal fat content (R-F) were those with the highest ($p<0.05$) values. Frankfurters elaborated with EGs (R-EC, R-EPGO and R-EPG), irrespectively of the presence of phenolic compounds, showed similar ($p>0.05$) processing losses (~12%) with lower values than their counterparts elaborated with animal fat (R-F). Therefore, comparing reduced-fat samples, the strategy based on the use of EGs as animal fat replacers causes a decrease in processing losses, thus improving product yield during its processing.

In general, purge losses in the frankfurters was below 1.3% and no significant differences were observed during chilled storage. Similar results for processing and purge losses have been reported in other lipid-reformulated healthier frankfurters elaborated with EGs as fat replacers (Pintado, Herrero, Jiménez-Colmenero, et al., 2016a; Pintado, Herrero, Ruiz-Capillas, et al., 2016). This indicates good thermal and storage stability in terms of fat and water binding properties of the meat matrix associated with a strategy based on replacing animal fat by EGs.

### 3.3.2. Colour and pH measurement

The colour parameters lightness (L*), redness (a*) and yellowness (b*) were affected ($p<0.05$) to the formulation (Table 3). As a consequence of reducing animal fat, the samples showed lower ($p<0.05$) lightness and yellowness, but higher ($p<0.05$) redness. The same has previously been reported for lightness and yellowness in frankfurters as consequence of animal fat reduction (Paglarini, et al., 2019; Pintado, Herrero, Ruiz-Capillas, et al., 2016). The use of EGs as animal fat replacers significantly conditioned the colour parameters (Table 3), probably due to differences between animal fat colour and that of EGs (Jiménez-Colmenero, et al., 2012; Pintado et al., 2015). The higher b* values in all samples with EGs, regardless of the presence of phenolic compounds, should be noted, due to the yellowish-green colour of olive oil. Meanwhile, the

294 presence of polyphenolic extracts in EGs gave rise to frankfurters with the lowest ($p<0.05$)
295 lightness, and to redness higher than in samples with control EG (R-EC) (Table 3). Differences in
296 colour parameters in cooked meat products have been reported as a consequence of the
297 incorporation of phenolic compounds; however, the specific modification in each colour parameter
298 (L*, a* or b*) depends on the type and amount of the ingredients incorporated (Özvural, & Vural,
299 2012; 2014; Vivar-Vera, et al., 2018).

300 Regarding chilled storage, no clear effect on colour parameters was observed according to
301 the formulation strategy used in frankfurter reformulation, which included the presence of
302 polyphenols in the EGs (Table 3). In general, no significant changes in colour parameters were
303 found during storage in any frankfurters (Table 3). By contrast, some authors have reported a
304 decrease in redness during chilled storage of frankfurters formulated with different concentrations
305 of polyphenol extracts or oils that were added directly during chilled storage (Özvural, & Vural,
306 2012).

307 Table 3 shows pH values, which may be considered normal for this kind of product
308 (Jiménez-Colmenero et al., 2010). The replacement of pork back fat by EGs caused a decrease
309 ($p<0.05$) in pH, and samples with EGs that contained solid polyphenol extracts (R-EPG and R-
310 EPGO, respectively) showed the lowest pH values. Other authors have reported an increase in
311 (Pintado, Herrero, Jiménez-Colmenero, et al., 2016a; Wang, et al., 2018) or no effect on (Paglarini,
312 et al., 2019; Pintado, Herrero, Ruiz-Capillas, et al., 2016) pH values as a consequence of replacing
313 animal fat by different types of EGs in frankfurters, independently of the degree of substitution.
314 This behaviour could be attributed to the variation in pH values observed in different EGs,
315 according to their formulation (Pintado et al., 2015).

316 During chilled storage, the pH values showed a significant decrease, except in samples
317 elaborated with EGs containing grape seed extract, whose pH was similar ($p>0.05$) over the whole
318 period (Table 3). A decrease in pH values during chilled storage has been reported in cooked

sausages in which animal fat was replaced by camellia oil EGs (Wang et al., 2018). This was also observed in frankfurters with grape seed extracts added directly (Özvural & Vural, 2012).

### 3.3.3. Texture profile analysis (TPA)

The texture profile analysis indicated that both the formulation and chilled storage affected ($p<0.05$) the hardness, cohesiveness, springiness and chewiness of the frankfurters (Table 4). As a consequence of animal fat content reduction, the samples suffered a significant decrease in hardness and chewiness values, similar to observations by other authors (Paglarini, et al., 2019; Pintado, Herrero, Ruiz-Capillas, et al., 2016); although the cohesiveness and springiness values in R-F samples were higher than those in N-F samples. Comparing all the reduced-fat samples, the use of EGs as animal fat replacers caused a significant increase in hardness and chewiness of the frankfurters. These values were even higher ($p<0.05$) in samples with EGs containing polyphenol extracts (R-EPG and R-EPGO) (Table 4). These last frankfurters (containing EGs with polyphenols) also showed the highest ($p<0.05$) values of cohesiveness while no clear trend in springiness was observed in these samples (Table 4). Considering that the protein–moisture ratio and lipid content were similar in all the reduced-fat samples, the differences in texture would appear to be due to the presence of oil-in-water EGs including the solid polyphenol extracts (Table 4). By contrast, the use of camellia oil EGs as animal fat replacers in cooked sausages has been found to produce a decrease in hardness and chewiness (Wang et al., 2018). Meanwhile, regardless of the presence of ingredients with phenol compounds, increases of hardness in cooked sausages formulated with different sources of polyphenols (starfruit dietary fibre concentrate, grape seed extracts, etc.) added directly have been reported, although no differences were observed in cohesiveness or springiness (Özvural & Vural, 2012; Vivar-Vera et al., 2018).

During chilled storage, in general, all the frankfurters underwent an increase ($p<0.05$) of hardness, springiness and chewiness; while cohesiveness remained constant during this period except in R-F samples (Table 4). The incorporation of natural antioxidants from the strawberry tree

(*Arbutus unedo*) and the dog rose (*Rosa canina*) added directly to frankfurters caused a decrease in texture parameters over 30 and 60 days under refrigerated conditions (Armenteros, Morcuende, Ventanas, & Estévez, 2013). These differences could be due to the type and strategy of incorporation of polyphenols in this type of meat products.

### 3.4. Structural Characteristics

Fig. 1 shows the acyl chain region, comprised between 2950-2830 $cm^{-1}$, of the ATR–FTIR spectrum of the different frankfurters analysed. This spectral region is dominated by two strong bands that are the result of the asymmetric ($\nu_{as}CH_2$) and the symmetric ($\nu_sCH_2$) stretching vibrations of the acyl $CH_2$ groups (Guillen & Cabo, 1997). Replacement of animal fat by EGs shifts the frequency of $\nu_{as}CH_2$ and $\nu_sCH_2$ from 2921 to 2923 $cm^{-1}$ and from 2852 to 2853 $cm^{-1}$ respectively (Fig. 1). This frequency increase is generally attributed to the diminution of the conformational order of the lipid acyl chains and their more active dynamics (Herrero, Carmona, Pintado, Jiménez-Colmenero, & Ruiz-Capillas, 2011). These differences in frequency imply greater inter- and intramolecular lipid disorder in frankfurters elaborated with EGs attributed to more lipid interactions with the other compounds mainly proteins in these samples (Carmona, Ruiz-Capillas, Jiménez-Colmenero, Pintado, & Herrero, 2011). The lipid chain disorder or increased lipid interactions observed in all the frankfurters elaborated with EGs could account for their small processing loss and their textural alterations (mainly greater hardness and chewiness). No frequency variations were observed in frankfurters as a result of the presence of phenolic compounds in the EGs (Fig. 1); this could be associated with no variation in the conformational order of the lipid acyl chains or their dynamics (Herrero et al., 2011) due to the incorporation of solid polyphenol extracts into the emulsions although, by contrast, there are changes in some physicochemical properties due to the presence of polyphenols in the EGs. Chilled storage produced no significant changes in frequency of the $\nu_{as}CH_2$ and $\nu_sCH_2$ bands (*data not shown*), indicating no modification in acyl lipid chain order or lipid–protein interactions in any of the frankfurters during storage. Similar findings

have been reported in frankfurters elaborated with other types of EGs, in terms of the observed frequency alterations depending on the formulation and the lack of modifications in the frequencies during cold storage (Pintado, Herrero, Ruiz-Capillas, et al., 2016).

### 3.5. Lipid oxidation

The effectiveness of phenolic compounds in the inhibition of oxidative process in meat products is related to the scavenging activity of their reactive species, which are formed during processing and storage, and are conditioned by the product composition (Papuc, et al., 2017). To evaluate their activity in frankfurters, we used lipid hydroperoxide and TBARs values, which were affected ($p<0.05$) by formulation and storage time (Fig. 2). At the initial time, the frankfurters elaborated with normal and reduced animal fat content (N-F and R-F) had similar ($p>0.05$) hydroperoxide and TBARS values, but these were lower ($p<0.05$) than those of frankfurters reformulated with EGs (R-EC, R-EPG and R-EPGO) (Fig. 2). These results could be related with the fact that EGs have higher levels of unsaturated fatty acids (Pintado, Herrero, Jiménez-Colmenero, et al., 2016a). Lipid oxidation in R-EPG was higher than in R-EPGO, despite both samples being designed to obtain similar levels of total phenolic compounds. These differences could be attributed to the concentration of each type of phenol and their antioxidant activity; for example, the greater amounts of hydroxytyrosol in extracts from grape seed and olives (Muñoz-González et al., 2019).

In general, TBARs contents remained constant over the storage time, while there were changes in lipid hydroperoxide values as a function of the formulation (Fig. 2). Samples reformulated with EGs containing grape seed and olive extracts showed no significant increase in lipid oxidation during storage; while an increase ($p<0.05$) of hydroperoxide values was observed in samples formulated with animal fat and EGs without phenolic compounds (N-F, R-F and R-EC). It has previously been reported that flavonoids are free radical scavengers during food oxidation (Papuc et al., 2017); so, the higher amounts of flavonoids in the solid extracts present in R-EPG and R-EPGO (Muñoz-González et al.,

2019) could explain the maintenance of hydroperoxide values in these samples during their storage, and even their high unsaturated fatty acid content (Table 2). Some authors have studied the antioxidant activities of grape seed extracts, added directly to meat products or after being dissolved in water, during chilled storage and found that the antioxidant treatment significantly inhibited lipid oxidation (Karre, López, & Getty, 2013; Özvural & Vural, 2012). Other authors have also reported that the antioxidant activity in beef patties containing EGs with polyphenols, mainly catechins, was doubled, and that stability against oxidation was improved, by reducing the peroxide content more than two-fold, compared to control patties (Alejandre et al., 2019).

### 3.6. Microbiological analysis

The initial levels of microorganism were very low in all the samples (1.63-2.52 log cfu/g data not shown), even for Enterobacteria (< 1 log cfu/g; data not shown). Similar levels were observed by other author (Pintado et al., 2016) by the effect of used emulsion gel in frankfurter. This was presumably a result of the thermal treatment during the processing of these products, which control the levels of this microorganism. Initial levels of TVC was also in relation with the reformulation, sample with all animal fat (N-F) ($1.63\pm0.21^{a}$ log cfu/g) and with EG containing grape seed and olive extract (R-EPGO) showed lower ($p<0.05$) levels ($1.84 \pm0.09^{ab}$ log cfu/g), compare with the reduce fat (R-F) ($2.52 \pm0.03^{c}$ log cfu/g).

Microbial growth ($p<0.05$) was appreciated during storage in all samples. At 60 days of storage, higher ($p<0.05$) TVC were observed in the reduced fat (R-F) sample ($8.78\pm0.06^{d2}$ log cfu/g). However the other samples with reduce fat and EGs (R-EC, R-EPG and R-EPGO), presented significantly lower TVC levels ($7.39\pm0.02^{c2}$, $5.11\pm0.00^{a2}$ and $6.65\pm0.03^{b2}$ log cfu/g respectively), among them R-EPG with grape seed polyphenol extracts showed the lowest ($p<0.05$) TVC. These results could be explain by the antimicrobial effect of the polyphenols and the specific mechanism of antimicrobial activity for each type of phenol; although due to the structural diversity of classes, the mechanisms that explain it have not yet been fully resolved (Papuc et al., 2017). Other author

419 also noted antimicrobial activity by the polyphenols in meat products (Nowak et al., 2016). Papuc et
420 al., 2017 observed the effect of ingredients rich in polyphenols (such as rosemary and clove
421 ethanolic extract, and their combination) reduce the TVC, LAB, and both Pseudomonas spp. and
422 Enterobacterias counts in refrigerated raw chicken meat. These authors also highlight the capacity
423 of polyphenols as promising antimicrobial agents for meat and meat products.

424 **4. Conclusions**

425 The strategy used in this work to develop healthier frankfurters, based on the incorporation of
426 EGs as animal fat replacers and to delivery high levels of polyphenols compounds, could be a
427 feasible option to enhance their nutritional composition and shelf life. The use of EGs as animal fat
428 replacers allows the resulting frankfurters to be labelled with nutritional and health claims related to
429 their lipid content, in accordance with European regulations. Moreover, frankfurters reformulated
430 with EGs containing solid polyphenol extracts also had high levels of hydroxytyrosol and contents
431 of gallic acid, flavanol monomers and their derivatives. None of these nutritional advantages
432 entailed any detrimental changes in the sensorial characteristics, physico-chemical properties or
433 lipid structure of the frankfurters. The main modifications in frankfurters characteristics are due to
434 use of EGs regardless of the polyphenols incorporated in them. The presence of phenolic
435 compounds in the EGs used as animal fat replacers also seems to improve oxidative stability and
436 safety during chilled storage of the reformulated frankfurters.

437 The use of these EGs containing phenolic compounds as animal fat replacers in meat
438 products could be a promising option to enhance product quality, mainly in terms of healthier
439 composition, oxidative stability and microbial grown, and it could help the meat industry to meet
440 consumer demands for high-quality and healthier meat products.

441

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**Author Contributions**

T. P. and AM. H. contributed equally to this work, and they are co-first authors. AM. H and C. R-C. participated in the design of this study. All the authors (AM. H.; C. R-C.; I. M-G; M. S. and T. P.) performed the experiments and collaborated in the statistical analysis and drafted the main manuscript.



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**Figure captions**

Fig. 1. ATR–FTIR spectra in the 2950-2830 $cm^{-1}$ of frankfurters. For sample denomination see Table 1.

Fig. 2.- Hydroperoxides (mmol/kg sample) and thiobarbituric acid-reactive substance (TBARs) values (mg MDA/kg sample) of frankfurters during chilled storage. For sample denominations see Table 1. For hydroperoxides and TBARS analysis different letters between samples and numbers between days, indicates significant differences ($p<0.05$).

Fig. 3.- Sensory evaluation of frankfurters. For sample denominations see Table 1.

583 Table 1.- Formulation (%) of frankfurters

| | | | Emulsion gels ** | | | |
|---|---|---|---|---|---|---|
| Samples* | Meat | Pork back fat | EC | EPG | EPGO | Water |
| N-F | 61.0 | 23.0 | | | | 13.2 |
| R-F | 61.0 | 11.0 | | | | 25.2 |
| R-EC | 61.0 | | 23.0 | | | 13.2 |
| R-EPG | 61.0 | | | 23.0 | | 13.2 |
| R-EPGO | 61.0 | | | | 23.0 | 13.2 |

584 Additives added to all the samples per 100 g of product: 2 g NaCl; 0.5 g flavouring; 0.3 g sodium
585 tripolyphosphate and 0.012 g sodium nitrite.

586 *Frankfurter formulated with pork back fat (F) with two levels, normal (N-F) and reduced (R-F)
587 content. Reduced fat frankfurter reformulated by totally replacing pork back fat with soy emulsion
588 gel (EC), soy emulsion gels with polyphenol solid extracts based on grape seed (EPG) and grape
589 seed and olive (EPGO).

590 ** Emulsion gel elaborated with water, 5 % isolated soy protein, 40 % olive oil, 4 % gelling agent
591 (based on alginate) and: 2.32 % grape seed solid extract (EPG), 1.95 % grape seed and olive solid
592 extract (EPGO) or without polyphenolic extract used as control (EC).

593

594

595 Table 2.- Fatty acid profile (g/100 g product) and significance ratios of frankfurters.

| Parameters | Samples* | | |
|---|---|---|---|
| | N-F | R-F | R-EC; R-EPG and R-EPGO ** |
| Myristic C14:0 | 0.31±0.01 [c] | 0.16±0.01 [b] | 0.03±0.00 [a] |
| Palmitic C16:0 | 5.32±0.14 [c] | 2.79±0.11 [b] | 1.60±0.12 [a] |
| Stearic C18:0 | 2.79±0.08 [c] | 1.28±0.06 [b] | 0.58±0.05 [a] |
| ΣSFA (%) | 39.11±0.04 [c] | 35.09±0.05 [b] | 18.84±0.35 [a] |
| Palmitoleic C16:1 | 0.47±0.01 [c] | 0.31±0.01 [b] | 0.14±0.01 [a] |
| Vaccenic C18:1n7 | 0.68±0.02 [c] | 0.45±0.02 [b] | 0.27±0.02 [a] |
| Oleic C18:1n9 | 9.94±0.28 [c] | 5.89±0.24 [a] | 8.36±0.49 [b] |
| ΣMUFA (%) | 52.15±0.07 [a] | 55.61±0.06 [b] | 71.42±0.30 [c] |
| Linoleic C18:2n6 | 1.62±0.04 [c] | 0.94±0.04 [a] | 1.04±0.06 [b] |
| Linolenic C18:3n3 | 0.07±0.00 [b] | 0.05±0.00 [a] | 0.09±0.00 [c] |
| ΣPUFA (%) | 8.74±0.03 [a] | 9.30±0.05 [b] | 9.74±0.07 [c] |
| ΣPUFA/ΣSFA | 0.22±0.00 [a] | 0.27±0.00 [b] | 0.52±0.01 [c] |
| Σn-6/Σn-3 | 19.45±0.10 [c] | 16.66±0.02 [b] | 12.72±0.14 [a] |

596 *For frankfurters denominations, see Table 1. ** R-EC; R-EPG and R-EPGO contained similar
597 proportions and types of fat and data reported are the mean values of these samples. Means ±
598 standard deviation. Different letters in the same row indicate significant differences ($p<0.05$).

599 Table 3.- Colour parameters [(L*) lightness, (a*) redness and (b*) yellowness] and pH values of
600 frankfurters during chilled storage.

| Parameters | Samples* | Days | | |
|---|---|---|---|---|
| | | 0 | 30 | 60 |
| L* | N-F | 74.56±0.63 d1 | 74.85±0.36 d1 | 74.73±0.35 cd1 |
| | R-F | 73.64±0.55 c1 | 74.17±0.46 c12 | 74.30±0.42 c2 |
| | R-EC | 75.55±0.41 e1 | 75.27±0.28 d1 | 75.11±0.6 d1 |
| | R-EPG | 70.42±0.78 b1 | 70.21±0.42 b1 | 70.29±0.38 b1 |
| | R-EPGO | 68.77±0.55 a1 | 68.82±0.33 a1 | 68.63±0.24 a1 |
| a* | N-F | 6.94±0.49 b1 | 7.22±0.16 b2 | 7.04±0.13 bc12 |
| | R-F | 7.19±0.24 c1 | 7.28±0.22 b1 | 7.24±0.13 c1 |
| | R-EC | 5.97± 0.08 a1 | 6.20±0.13 a2 | 6.29±0.15 a2 |
| | R-EPG | 7.07± 0.13 bc1 | 7.12±0.05 b1 | 6.99±0.08 b1 |
| | R-EPGO | 9.67± 0.12 d2 | 9.62±0.09 c2 | 9.26±0.10 d1 |
| b* | N-F | 11.25±0.28 b1 | 11.27±0.15 b1 | 11.33±0.14 b1 |
| | R-F | 10.66±0.24 a1 | 10.56±0.08 a1 | 10.57±0.17 a1 |
| | R-EC | 14.28±0.19 c1 | 14.26±0.15 d1 | 14.38±0.11 d1 |
| | R-EPG | 14.00±0.71 c2 | 13.56±0.17 c1 | 13.63±0.17 c1 |
| | R-EPGO | 15.23±0.23 d1 | 15.08±0.12 e1 | 15.11±0.17 e1 |
| pH | N-F | 6.43±0.02c2 | 6.43±0.04d2 | 6.27±0.01c1 |
| | R-F | 6.63±0.03d3 | 6.35±0.01b2 | 6.08±0.01a1 |
| | R-EC | 6.40±0.01b2 | 6.38±0.01c2 | 6.10±0.01ab1 |
| | R-EPG | 6.35±0.00a1 | 6.36±0.00bc1 | 6.36±0.01d1 |
| | R-EPGO | 6.35±0.01a3 | 6.26±0.01a2 | 6.12±0.01b1 |

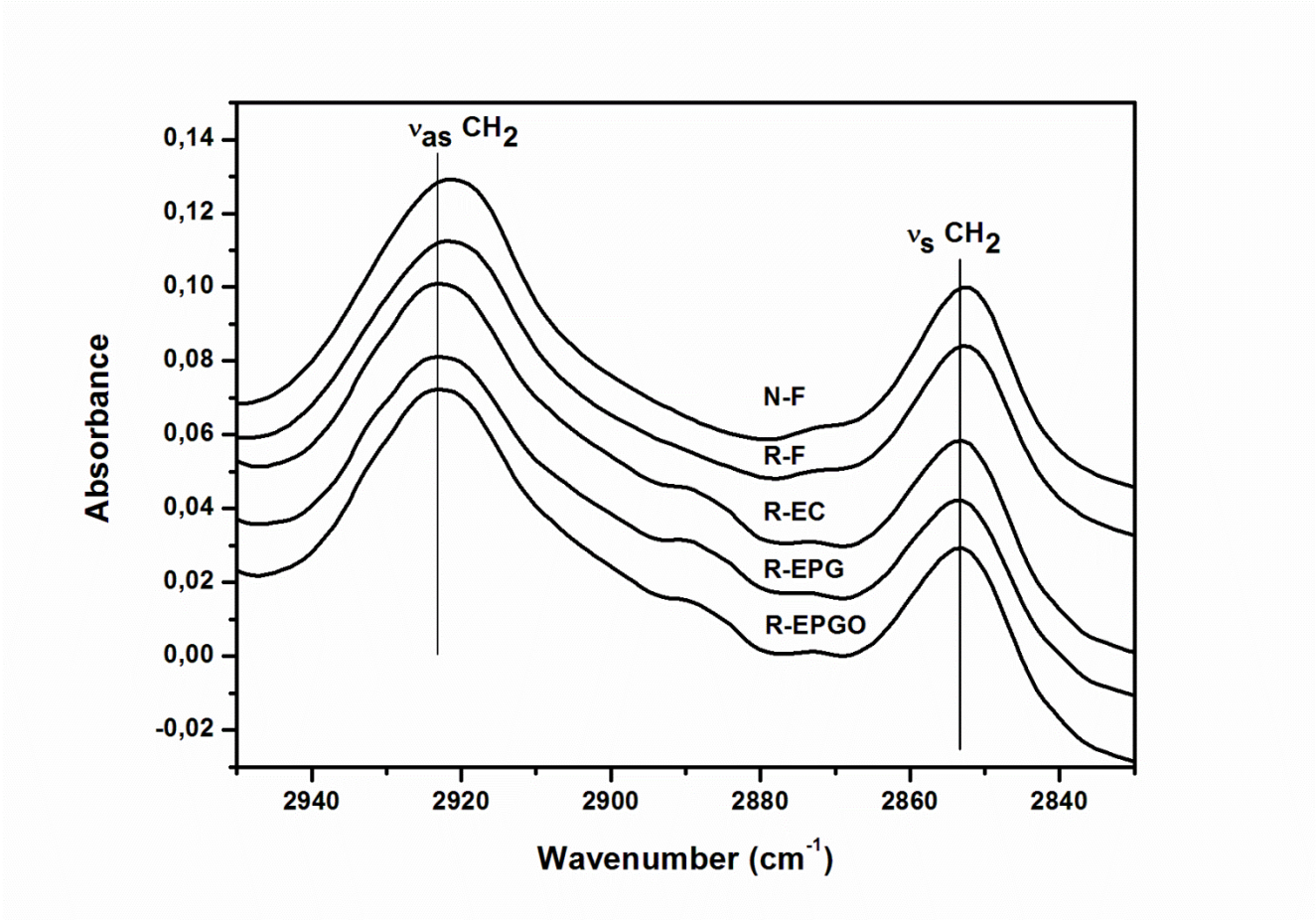
601 *For frankfurters denominations, see Table 1. Means ± standard deviation. Different letters in the
602 same column and different numbers in the same row indicate significant differences ($p<0.05$).

603 Table 4.-Textural profile analysis (TPA) parameters of frankfurters during chilled storage.

| Parameters | Samples* | Days | | |
|---|---|---|---|---|
| | | 0 | 30 | 60 |
| Hardness (N) | N-F | 21.21±0.97 b1 | 23.08±0.59 b2 | 23.74±1.08 a2 |
| | R-F | 17.09±0.31a1 | 20.40±0.62 a2 | 24.26±0.61a3 |
| | R-EC | 22.07±1.18 b1 | 27.44±0.42 c2 | 28.60±0.43 b2 |
| | R-EPG | 29.05±1.32 c1 | 29.17±0.62 c1 | 31.46±1.19 c2 |
| | R-EPGO | 30.06±0.67 c1 | 36.44±0.65 d2 | 40.68±1.48 d3 |
| Cohesiveness | N-F | 0.66±0.01 a1 | 0.67±0.01 b1 | 0.69±0.00 b1 |
| | R-F | 0.70±0.00 b1 | 0.70±0.01 c12 | 0.71±0.01 c2 |
| | R-EC | 0.70±0.01 b1 | 0.71±0.01 c1 | 0.71±0.00 c1 |
| | R-EPG | 0.65±0.01 a1 | 0.65±0.00 a1 | 0.66±0.01 a1 |
| | R-EPGO | 0.66±0.00 a1 | 0.66±0.00 ab1 | 0.67±0.01 a1 |
| Springiness (mm) | N-F | 6.38±0.04 a1 | 6.79±0.08 a2 | 6.84±0.06 a2 |
| | R-F | 6.68±0.15 b1 | 6.98±0.13 ab2 | 7.17±0.09 bc3 |
| | R-EC | 7.00±0.04 c1 | 7.19±0.16 c12 | 7.27±0.09 c2 |
| | R-EPG | 6.77±0.03 b1 | 7.08±0.04 bc2 | 6.98±0.13 ab2 |
| | R-EPGO | 6.85±0.08 bc1 | 7.12±0.10 bc2 | 7.09±0.05 bc2 |
| Chewiness (N*mm) | N-F | 89.70±5.52 b1 | 105.83±1.85 a2 | 111.43±3.88 a2 |
| | R-F | 79.46±3.16 a1 | 99.39±3.58 a2 | 123.68±3.33 b3 |
| | R-EC | 108.94±5.16 c1 | 139.54±4.24 b2 | 147.10±3.10 c2 |
| | R-EPG | 127.60±5.34 d1 | 134.15±2.15 b1 | 144.99±7.19 c2 |
| | R-EPGO | 135.73±4.35 d1 | 171.47±3.63 c2 | 192.30±4.60 d3 |

604 *For frankfurters denominations, see Table 1. Means ± standard deviation. Different letters in the
605 same column and different numbers in the same row indicate significant differences ($p<0.05$).

606 **Fig. 1**


$\nu_{as}$ $CH_2$
$\nu_s$ $CH_2$
N-F
R-F
R-EC
R-EPG
R-EPGO
Absorbance
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Wavenumber ($cm^{-1}$)

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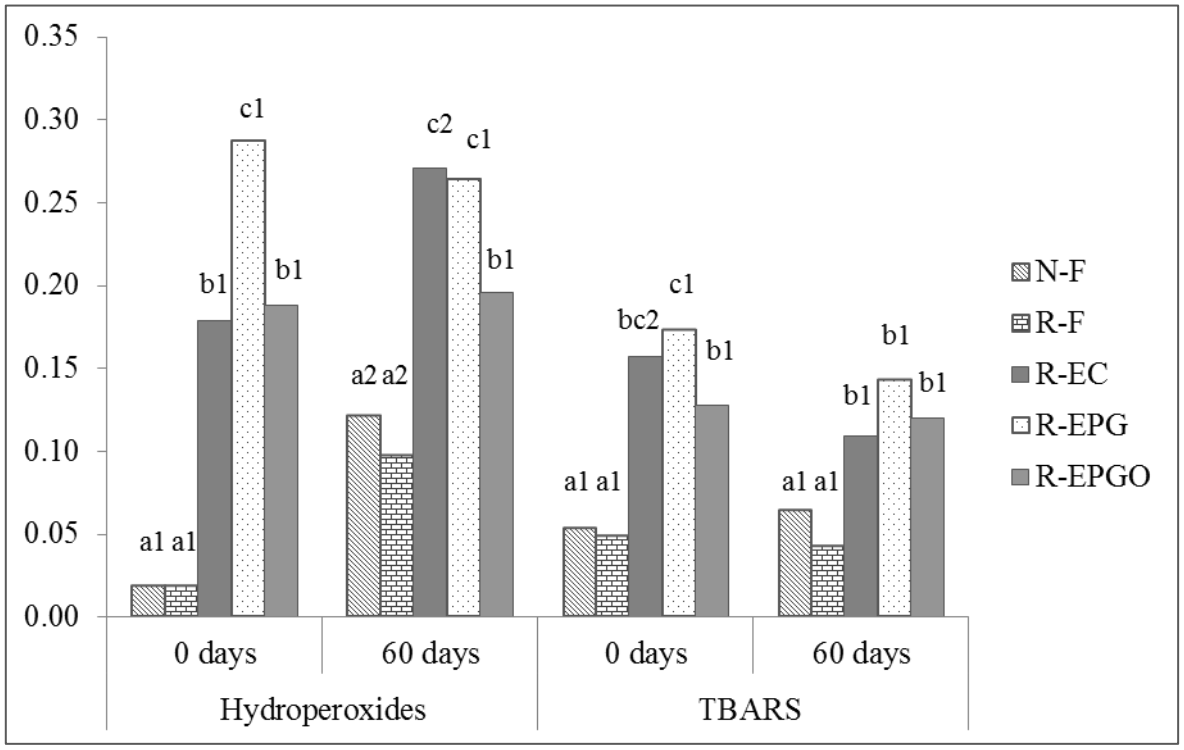
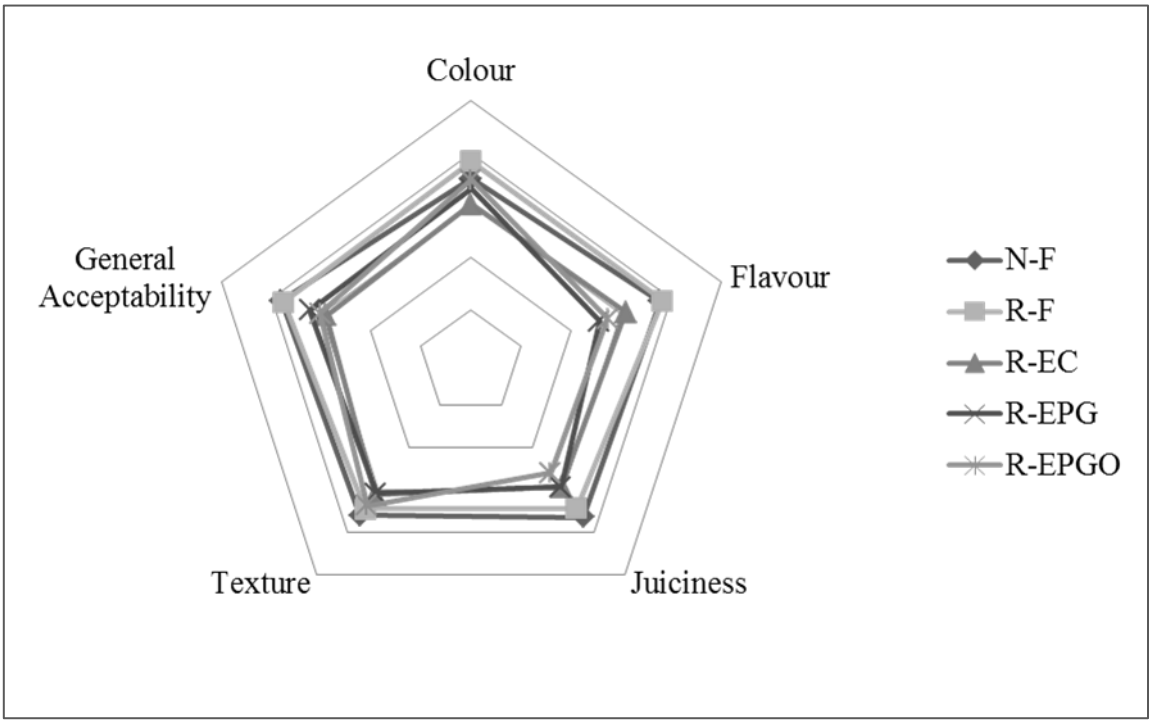
608 **Fig. 2**


0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
a1 a1
b1
c1
b1
a2 a2
c2
c1
b1
a1 a1
bc2
c1
b1
a1 a1
b1
b1
b1
0 days
60 days
0 days
60 days
Hydroperoxides
TBARS
N-F
R-F
R-EC
R-EPG
R-EPGO

609

610 **Fig. 3**


Colour
Flavour
Juiciness
Texture
General Acceptability
N-F
R-F
R-EC
R-EPG
R-EPGO

611