Gelled double emulsions as delivery systems for hydroxytyrosol and n-3 fatty acids in healthy pork patties

M. Freire¹ · S. Cofrades¹ · V. Serrano-Casas¹ · T. Pintado¹ · M. J. Jimenez¹ · F. Jimenez-Colmenero¹

Revised: 28 July 2017 / Accepted: 7 September 2017 / Published online: 14 September 2017
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Abstract Structuring of double emulsion offers the possibility to obtain a system with similar consistency to animal fat which is an interesting approach to improve the fat content of meat products. This article examines the suitability of gelled double emulsions (GDE) for use as a delivery system for n-3 PUFAs and hydroxytyrosol (Hxt) in pork patties. Effect of partial (MF/GDE sample) and total (LF/GDE) replacement of pork backfat with GDE with perilla oil (PO) as lipid phase and Hxt (in W1) on pork patty composition and properties was evaluated. Compared with the control sample (NF/CS), the products with PO contained less SFAs and higher proportions of LNA and ALA, and this difference augmented with the level of GDE. Addition of GDE increased Kramer shear force of cooked patties as the higher percentage of fat replaced was used. Oxidative stability of patties can be interpreted in terms of composition and structural factors, especially in relation with the use of GDE as a fat replacer. Products showed oxidation values greater than 1 after 3 days of refrigeration. NF/GDE and MF/GDE, with similar scores, were the most acceptable for the panellists. This technology is suitable for labelling meat products with nutritional and health claims.

Keywords Double emulsion · Gelled emulsion · Hydroxytyrosol · Perilla oil · n-3 fatty acids · Chilling storage

Introduction

Meat and meat products are some of the most important sources of dietary fat. As such, in view of health recommendations for optimal intake of total and unsaturated fatty acids, changes in fat contents and lipid profiles could help them to contribute more to a healthy and well-balanced diet. Multiple technological strategies to improve the fatty acid profile of meat products have been assayed to that end (Jiménez-Colmenero et al. 2015). Different vegetable oils, marine oils, or combinations of these, have been used to partially replace animal fat in meat products. However, it has been established these lipid materials have different physicochemical characteristics from habitually used meat fats, and these may detract from the quality attributes in the reformulated product.

Novel proposals for liquid phase oil stabilization and structuring have recently been reported as a basis for the development of fat alternatives that can be used to improve the fat content of reformulated systems, including meat products. The modification or restructuring of oils to create a plastic fat that has solid-like properties and also a healthier fatty acid profile is a very important area of research for both academic and industrial purposes (Dickinson 2012). In this context, the development of gelled double (W1/O/W2) emulsion (GDE)-based delivery systems offer interesting possibilities for structuring emulsions that can be used to create novel functional attributes (McClements 2012). Double emulsion (DE) offers other attractive opportunities for the food industry, among them encapsulation of hydrophilic bioactive compounds and improvement of the fat content of foods (Jiménez-Colmenero 2013). The most promising procedure for producing a GDE is to add compounds (gelatin, alginate, or transglutaminase) to the outer aqueous phase of the DE to

✉ S. Cofrades
scofrades@ictan.csic.es

¹ Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), C/José Antonio Novais, 10, 28040 Madrid, Spain

act as gelling agents (Jiménez-Colmenero et al. 2015). Recently our group reported the preparation and some characteristics of gelled double emulsions using a combination of gelatin and MTG (Cofrades et al. 2017).

As an animal fat alternative, perilla oil (PO) is of particular interest. PO, produced from perilla seeds (*Perilla frutescens*), is one of the richest sources of α -linolenic acid (ALA, over 60 g/100 g). It has proven effective in preventing atherosclerosis and chemically-induced cancer and also has beneficial effects on immune and mental functions (Jo et al. 2013). DE has been used with PO as lipid phase to reduce fat and achieve healthier fatty acid profiles in frankfurters (Freire et al. 2016), but unexplored additional possibilities may be achieved through their use in the development of emulsion-based structured delivery systems. However, these n-3 PUFA enriched systems are prone to oxidation and thus need to be protected since their oxidation causes nutritional loss and presents potential negative health implications. Because synthetic antioxidants may exhibit toxic properties, different natural antioxidant sources have been assayed, among them phenolic compounds such as hydroxytyrosol (Hxt). This is a hydrophilic phenolic compound that has received a lot of attention not only because it is a very potent antioxidant, but also because of the anti-inflammatory and cancer-preventive effects attributed to it (Kountouri et al. 2007). Consequently, Hxt has been used as an antioxidant to minimize oxidation in n-3 PUFA-rich foods (DeJong and Lanari 2009; Cofrades et al. 2011), including by incorporation in DEs. Recently, Cofrades et al. (2014) studied the oxidative stability of DEs containing chia oil and Hxt and that of a cooked meat system incorporating these DEs. These authors reported that the DE showed good antioxidant capacity in the early days of storage; and again, the incorporation of DE containing Hxt in cooked meat systems protected from oxidation. Flaiz et al. (2016) compared the oxidative stability of three different emulsion-based systems (simple emulsion, DE and GDE) as delivery systems for n-3 fatty acids (PO) and Hxt. These authors showed that n-3 fatty acids were efficiently protected from oxidation in all systems by the addition of Hxt.

To the best of our knowledge, there have been no studies on the use of a structured emulsion such as a GDE to improve fat content (reduce fat and improve fatty acid profile) in meat products. To verify the suitability of GDE for use as a delivery system for n-3 PUFAs and hydroxytyrosol in meat products, this study assessed the effect of partial (66%) and total (100%) replacement of pork backfat with gelled water-in-oil-in-water ($W_1/O/W_2$) emulsions (with PO as lipid phase and Hxt in the inner aqueous phase, W_1) on the composition (fatty acid profile) and properties (texture, microbiology, oxidative stability and sensory analysis) of pork patties. An all-animal-fat control sample

was used for comparative purpose. The influence of chilling storage (14 days at 2 ± 2 °C) on pork patty characteristics as affected by formulation was also evaluated.

Materials and methods

Meat raw material, ingredients and additives

Meat raw materials (pork meat and pork backfat) were obtained and prepared as described by Salcedo-Sandoval et al. (2015).

Hxt (purity $\geq 99\%$) was purchased from Seprox BIOTECH (Madrid, Spain) and perilla oil (fatty acid composition: 6% 16:0, 2% 18:0, 12% 18:1n9, 14% 18:2n6, 65% 18:3n3, according to supplier) from Grupo Nutracéutico Chiasa, SL (Meliana, Spain). Sodium caseinate (Excellion EM 7) was purchased from FrieslandCampina DMV (Veghel, The Netherlands), polyglycerol polyricinoleate (PGPR) from Bavaro Chemicals S.L. (Sant Cebrià de Vallalta, Spain), gelatin (type B, 200–220 bloom) from Manuel Riesgo, S.A. (Madrid, Spain), and microbial transglutaminase (Activa GS) from Ajinomoto (Tokyo, Japan). According to the supplier the activity of the enzyme was 47–82 units of hydroxamate/g. All other reagents and solvents used were of a suitable grade for spectrophotometric or chromatographic analyses.

Preparation of GDE

The procedure was used to prepare a stable GDE was as reported by Flaiz et al. (2016) with minor modifications in the inner and outer water phases which none of them contained sodium azide.

Design and production of pork patties

Three different formulations with three fat contents were prepared. A pork patty with normal-fat content (all pork fat) as control sample (NF/CS) containing 80% meat and 13.5% pork back fat. Additionally, medium-fat and low-fat improved lipid profile samples (MF/GDE and LF/GDE respectively) were prepared, replacing pork backfat with the same proportion of GDE (66 and 100% respectively). All samples contained NaCl (1%) and added ice-water (5.5%).

Pork patty preparation was as reported by Salcedo-Sandoval et al. (2015) with some modifications. Briefly, pork meat, backfat (previously thawed for 18 h/2 °C) and GDE were passed through a grinder with a 4.5 mm plate (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy). The meat and half of the ingredients were placed in a mixer (Hobart mixer N-506, Hobart MFG. Co., Troy, USA) for 1 min.

After this, the other half of the ingredients was added and the whole mixed again for 1 min. Patties (80 g) were then prepared using a manual burger former. Patties were weighed (initial weight), placed in trays (Evoh PP) covered with a film (PET12 μ PP Evoh PP70 μ NPL; ILPRA Systems España S.L) and stored in modified atmosphere (80% O₂–20% CO₂) at 2 ± 2 °C for 14 days. Analyses were performed at days 1, 3, 8 and 14 of chilling storage. Eight patties (4/raw and 4/cooked) were taken at random for analysis on each of these days: four for raw sample evaluation and four for cooked sample evaluation. Patties were cooked for 2 min per side at 180 ± 2 °C in a contact grill (Princess classic multigrill type 2321, The Netherlands) up to 70 °C (centre of product). The entire patty processing procedure was replicated twice on two different days.

Proximate analysis and fatty acid profile

Moisture and ash contents were determined by the AOAC methods (2005) and fat content according to Bligh and Dyer (1959). Protein content was measured with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). All analyses were done in triplicate.

The fatty acid profiles of raw and cooked patties were determined by gas chromatography as reported by Salcedo-Sandoval et al. (2014). Fatty acids expressed as, % fatty acids methyl esters, FAME, were identified by comparison of the retention times with a fatty acid standard mixture (AccuStandard FAMQ-005 (47015-U. Sigma Chemical Co., St. Louis, MO, USA).

Texture

Kramer shear force (KSF) was performed using a TA-XT.plus Texture Analyzer (Texture Technologies Corp. Scarsdale, NY) with a Kramer shear cell attached to a 30 kg load cell (crosshead speed 80 mm/s). Measurements were made on six portions (2 × 2 cm) per formulation (on raw and cooked samples for each storage period) at room temperature. Results were expressed as maximum load per gram of sample (N/g).

Microbiological analyses

Samples were prepared in a vertical laminar-flow cabinet (model AV 30/70, Telstar, Madrid, Spain). For each patty (raw) 10 g (per replicate) was obtained and located in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 ml of peptone water (0.1%) and 0.85% NaCl (Panreac Química, S.A. Barcelona, Spain). After 2 min in a stomacher blender (Colworth 400, Seward, London, UK), appropriate decimal dilutions were pour-plated on the following media: Plate Count Agar (PCA) (Merck,

Germany) for total viable count (TVC) (30 °C for 72 h) and for Psychrotrophic bacteria (4 °C for 7–10 days); De Man-Rogosa-Sharpe Agar (MRS) (Merck, Germany) for lactic acid bacteria (LAB) (30 °C for 3–5 days); and Violet Red Bile Glucose Agar (Merck, Germany) for Enterobacteriaceae (37 °C for 24 h). The results were expressed as logarithms of colony forming units per gram (log cfu/g).

Lipid oxidation

Pork patties (raw and cooked) 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 of raw and cooked samples were measured in triplicate as described by Salcedo-Sandoval et al. (2015) and the results expressed as mmol hydroperoxides/kg sample. TBARS were determined in triplicate as reported by Serrano et al. (2006) and the results expressed as mg MDA/kg of sample.

Sensory analysis

A trained 19-member sensory panel, recruited among staff of the ICTAN-CSIC with previous experience in descriptive analysis, was specifically trained to evaluate, by scoring, the samples in two sessions. Patties were cooked for 2 min on a grill until the centre of the product reached 70 °C. A portion of each patty was presented to the panelists in random order with randomly assigned three-digit codes. The panel evaluated the perceived intensity of five attributes (characteristic colour, characteristic aroma, characteristic taste, hardness and juiciness) using a 10 cm unstructured line scale (“low intensity” and “high intensity”). The panelists also evaluated the overall acceptability of the pork patties using a 10-point hedonic scale from “dislike extremely” to “like extremely”. Mineral water and bread was provided to panelists to rinse their mouths between each sample. The sensory analyses were performed at day 2 of the storage.

Statistical analysis

One-way analyses of variance (ANOVA) were carried out to evaluate the statistical significance ($P < 0.05$) of the formulation and two-way ANOVA as a function of formulation and storage time using the general linear model (GLM) procedure of SPSS Statistics (v.20, IBM SPSS Inc., Chicago, IL). Formulation and storage time were assigned as fixed effects and replicate as random effect. Least squares differences were used for comparison of mean values between treatments and Tukey's HSD test to

identify significant differences ($P < 0.05$) between formulations and storage time.

Two-way ANOVA (samples and panelists) with interaction was applied to the sensory data obtained for each attribute. Individual differences among panelists were analysed by a fixed model, considering panelists as a fixed factor. One-way analysis of variance was applied to study the effect of the formulation on each of the sensory attributes. Minimum significant differences were calculated using Fisher's least significant difference (LSD) tests with a 95% confidence interval for comparison of sensory attributes. Principal component analysis (PCA) of the sensory data was conducted on the mean values of attribute intensities presenting significant differences using the Pearson's correlation matrix. PCA is a statistical tool that helps to summarize, and hence conveys the results from descriptive panel profiling better (Moussaoui and Varela 2010). Data analyses were carried out with XLSTAT 2009.4.03 (Addinsoft, Barcelona, Spain).

Results and discussion

Of the different strategies that have been reported for stabilizing and structuring edible oils in DEs (Jiménez-Colmenero et al. 2015), in this study a non-thermally-reversible hydrogel was formed on the outer aqueous phase based on a combination of gelatin/MTG. The resulting GDE presented a solid-like structure, containing concentrations of 28.2 g and 28.2 mg/100 g of the PO and Hxt respectively according to preparation conditions. This GDE was physically stable, retaining its physical appearance, and no syneresis was observed during patties processing.

Proximate analysis and fatty acid composition

The proximate analyses of raw samples were generally close to the target levels, with some significant differences from one formulation to another (Table 1). Irrespective of formulation, protein and ash contents were around 18.3 and 2.2% respectively. Moisture content increased and fat level decreased when pork backfat was partially or totally replaced by GDE (Table 1); this is consistent with the fact that the GDE contained a higher proportion of water and a lower proportion of fat than the pork backfat. Thus, moisture content was lowest ($P < 0.05$) in the control sample (NF/CS) and highest ($P < 0.05$) in LF/GDE sample. According to the experimental design, patties presented three different fat contents (Table 1). Fat levels in reformulated samples were considerably lower than in the control, approximately 25% in MF/GDE and over 40% in LF/GDE. Also, while all the lipid in NF/CS came from pork, MF/GDE and LF/GDE samples contained different

Table 1 Proximate analysis and fatty acids profile (%) of pork patties

	NF/CS	MF/GDE	LF/GDE
Moisture	64.59 ^a ± 0.43	70.17 ^b ± 0.15	72.87 ^c ± 0.14
Protein	18.12 ^a ± 0.10	18.53 ^b ± 0.18	18.21 ^{ab} ± 0.04
Fat	15.62 ^c ± 1.13	11.69 ^b ± 0.45	9.20 ^a ± 0.44
Ash	2.23 ^a ± 0.01	2.25 ^a ± 0.04	2.23 ^a ± 0.01
Fatty acid (%)			
C16:0	23.88 ^c ± 0.70	18.54 ^b ± 0.49	13.28 ^a ± 0.14
C18:0	12.26 ^c ± 0.71	9.14 ^b ± 0.65	5.87 ^a ± 0.08
ΣSFA	37.99 ^c ± 0.37	29.00 ^b ± 0.30	20.00 ^a ± 0.04
C18:1n9	44.11 ^c ± 1.06	35.47 ^b ± 0.45	24.92 ^a ± 0.35
Σ MUFA	50.64 ^c ± 0.50	40.82 ^b ± 0.21	28.99 ^a ± 0.17
C18:2n6	9.04 ^c ± 0.34	12.26 ^b ± 0.19	15.84 ^a ± 0.14
C18:3n3	0.83 ^c ± 0.04	16.31 ^b ± 0.53	33.44 ^c ± 0.25
Σ PUFA	11.37 ^c ± 0.13	30.18 ^b ± 0.21	51.01 ^c ± 0.13
PUFA/SFA	0.31 ^c ± 0.04	1.04 ^b ± 0.06	2.53 ^a ± 0.04
Σ n-6 PUFA	10.54 ^c ± 0.15	13.87 ^b ± 0.08	15.56 ^a ± 0.05
Σ n-3 PUFA	0.83 ^c ± 0.04	16.31 ^b ± 0.52	33.44 ^a ± 0.27
n6/n3 ratio	12.66 ^c ± 0.15	0.85 ^b ± 0.21	0.53 ^a ± 0.10

Sample denomination: NF/CS normal-fat content/control sample prepared with all pork fat; MF/GDE medium-fat sample prepared replacing 66% pork backfat with the same proportion of gelled double emulsion (GDE); LF/GDE low-fat sample prepared replacing 100% pork backfat with the same proportion of GDE. Since the % of fatty acid were not influenced ($P > 0.05$) by cooking, this table shows the mean values for raw and cooked products. Mean ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$)

proportions of PO (2.5 and 3.8 g/100 g respectively), and the LF/GDE formulation contained no pork backfat. This means that, whereas NF/CS contained all animal fat, around 21 and 41% of the fat content respectively of MF/GDE and LF/GDE patties was PO.

Various authors have reported the addition of oils in different ways to achieve patties with better lipid profiles (Jiménez-Colmenero 2007). For instance, the amount of pork backfat in pork patties has been halved by direct addition of olive oil (Hur et al. 2008) and olive, avocado and sunflower oils (Rodríguez-Carpena et al. 2011). Healthier beef patties have been made with 10 or 20% fat by adding blended lipids—beef tallow combined with 43% safflower, olive or corn oil (Lowder and Osburn 2010). Lopez-Lopez et al. (2010) made beef patties (10% fat content) in which roughly 25 and 60% of pork backfat was replaced by an olive oil-in-water emulsion (52.6% oil). Patties (9% fat content) have been made with a pre-emulsified mix of olive, corn and fish oils (Martínez et al. 2012). In the line of the present experiment, gelled carrageenan containing simple emulsion have been used as solid-fat replacers in burger patties, reducing up to 41% of the total

fat content (Poyato et al. 2015). And again, Salcedo-Sandoval et al. (2015) reported fat reduction (30–86%) in pork patties by replacement of pork backfat with a healthier oil combination (olive, linseed and fish oils) bulking system based on konjac gel. However, to the best of our knowledge there has been very limited use of PO in meat products, and in no case with application to patty-type products. In one example (Freire et al. 2016), frankfurter properties were analysed to determine how these were affected by replacing pork backfat with two different types of DE in which PO and pork backfat were used as lipid phases.

The fatty acid profile (%) of patties was affected ($P < 0.05$) by formulation but not ($P > 0.05$) by cooking. For this reason Table 1 shows the mean values for raw and cooked products. All-animal-fat sample (NF/CS) presented the highest ($P < 0.05$) levels of SFAs and MUFAs (mainly oleic acid) and the lowest PUFA concentration (Table 1). In this product 62% of total fatty acids consisted of MUFAs and PUFAs, and of the latter linoleic acid (LNA) was the most abundant. This is consistent with fatty acid profiles reported elsewhere in ground pork products (Salcedo-Sandoval et al. 2014). The products formulated with PO contained less ($P < 0.05$) SFAs than the control sample (NF/CS), a difference which increased in line with the proportion of added GDE. In general, the replacement of pork backfat reduced ($P < 0.05$) the concentration of MUFAs (oleic acid) and considerably increased ($P < 0.05$) PUFAs, especially in the case of LF/GDE, with a level over 50% (Table 1). The proportions of LNA and ALA were highest ($P < 0.05$) in low fat sample (LF/GDE) containing no pork backfat. The PUFA/SFA ratio was 0.31 for all pork fat patties, which is within the ranges reported for pork products, including all pork patties (0.7–0.37) (Salcedo-Sandoval et al. 2014). As could be expected from the n-6/n-3 ratios in animal fat, the value found in all-pork-fat sample (12.66) diverged to some extent from the recommended values (<4). The n-6/n-3 ratio of reformulated patties was lower ($P < 0.05$) than in control sample (Table 1), and lowest in LF/GDE. This effect was due mainly to a strong

increase of n-3 PUFA (linolenic acid) content from PO (Table 1). The high n-3 PUFA content (estimated at over 2 g/100 g of patty in LF/GDE) suggests that there may be interesting opportunities for health claims.

Texture

Product texture was studied both raw and after cooking at each specific storage period. Both data sets can be useful in providing technological information that can help to understand the effect of the reformulation process and the cooking consequences, and therefore in the development of a new product.

KSF in raw patties was affected ($P < 0.05$) by formulation and storage time, especially formulation (Table 2). The all-animal-fat sample registered the lowest ($P < 0.05$) values of KSF, which did not change ($P > 0.05$) over storage. In MF/GDE and LF/GDE samples, KSF increased ($P < 0.05$) during storage. After 8 days the highest value ($P < 0.05$) of KSF was registered in LF/GDE. Since the KSF of cooked patties was not influenced ($P > 0.05$) by storage period, Table 2 shows the means of each formulation over the same period. These results indicate that storage did not influence the effect of heating on patty texture, as in previous finding (Salcedo-Sandoval et al. 2015). These authors found that in reformulated patties by replacement of animal fat with a healthier oil combination (olive linseed and fish oils) bulking system based on konjac gel, no differences ($P > 0.05$) were detected in texture as a function of storage time. Addition of GDE as a pork fat replacer increased ($P < 0.01$) KSF in cooked patties in proportion to the percentage of backfat replaced (Table 2), as also reported by Salcedo-Sandoval et al. (2015) using konjac gel for the same purpose.

The effect of formulation on patty texture (Table 2) can be attributed mainly to two factors, the characteristics of the matrix formed (with differences in protein/water/fat ratio), and the physicochemical characteristics of the lipid material (pork backfat versus GDE). A decrease in fat

Table 2 Kramer share force (N/g) of raw and cooked patties over storage

Samples	Raw patties				Cooked patties
	Days at 2 °C				
	1	3	8	14	
NF/CS	1.52 ^{a1} ± 0.15	1.74 ^{a1} ± 0.09	1.72 ^{a1} ± 0.11	1.78 ^{a1} ± 0.11	12.84 ^a ± 2.07
MF/GDE	1.94 ^{b1} ± 0.19	2.20 ^{b1} ± 0.09	2.16 ^{b1} ± 0.14	2.55 ^{b2} ± 0.15	17.82 ^b ± 2.79
LF/GDE	2.03 ^{b1} ± 0.17	2.42 ^{b2} ± 0.29	2.49 ^{c2} ± 0.15	3.33 ^{c3} ± 0.20	21.04 ^c ± 2.20

For sample denomination, see Table 1. Mean ± standard deviation. Since the KSF of cooked patties was not influenced ($P > 0.05$) by storage period, this table shows the means of each formulation over the storage period. Different letters in the same column and numbers in the same row indicate significant differences ($P < 0.05$)

Table 3 Microbiological analysis (log cfu/g) of raw patties over storage

Microorganism	Samples	Days at 2 °C			
		1	3	8	14
TVC	NF/CS	4.62 ^{a1} ± 0.06	4.98 ^{b2} ± 0.09	6.17 ^{a3} ± 0.04	7.37 ^{a4} ± 0.02
	MF/GDE	4.73 ^{a1} ± 0.05	4.81 ^{a1} ± 0.02	6.51 ^{b2} ± 0.03	7.59 ^{b3} ± 0.03
	LF/GDE	5.48 ^{b2} ± 0.02	5.25 ^{c1} ± 0.02	7.08 ^{c3} ± 0.00	7.93 ^{c4} ± 0.02
LAB	NF/CS	3.82 ^{a1} ± 0.02	4.17 ^{a2} ± 0.08	5.10 ^{a3} ± 0.03	6.56 ^{b4} ± 0.03
	MF/GDE	3.80 ^{a1} ± 0.00	4.27 ^{a2} ± 0.05	5.24 ^{b3} ± 0.05	6.59 ^{b4} ± 0.03
	LF/GDE	3.85 ^{a1} ± 0.05	4.28 ^{a2} ± 0.03	5.43 ^{c3} ± 0.00	6.41 ^{a4} ± 0.05
<i>Enterobacteriaceae</i>	NF/CS	3.34 ^{a1} ± 0.01	3.54 ^{a2} ± 0.04	3.98 ^{a3} ± 0.00	5.04 ^{a4} ± 0.03
	MF/GDE	3.40 ^{a1} ± 0.01	3.52 ^{a1} ± 0.03	4.17 ^{b2} ± 0.03	5.11 ^{a3} ± 0.02
	LF/GDE	3.45 ^{a1} ± 0.00	3.83 ^{b2} ± 0.15	4.25 ^{b3} ± 0.03	5.32 ^{b4} ± 0.03

For sample denomination, see Table 1. Mean ± standard deviation. Different letters (a, b, c) in the same column and different numbers (1, 2, 3) in the same row indicate significant differences ($P < 0.05$)

Table 4 Concentrations of hydroperoxides (mmol/kg sample) and thiobarbituric acid-reactive substance (TBARS) concentration (mg MDA/kg sample) of raw and cooked (after each storage period) patties over storage

Days 2 °C	Samples		
	NF/CS	MF/GDE	LF/GDE
Hydroperoxides (mmol/kg sample)			
1	1.57 ^{a1X} ± 0.08 (3.49 ^Y ± 0.14)	0.98 ^{a1X} ± 0.08 (3.81 ^Y ± 0.25)	1.01 ^{a1X} ± 0.04 (5.72 ^Y ± 0.08)
3	1.55 ^{b1X} ± 0.14 (4.14 ^Y ± 0.42)	2.27 ^{c2X} ± 0.14 (4.56 ^Y ± 0.23)	0.87 ^{a1X} ± 0.06 (5.51 ^Y ± 0.34)
8	5.52 ^{b2X} ± 0.41 (6.13 ^X ± 0.27)	4.43 ^{a3X} ± 0.18 (5.00 ^X ± 0.14)	4.34 ^{a2X} ± 0.15 (4.72 ^X ± 0.96)
14	7.36 ^{a3X} ± 0.19 (12.85 ^Y ± 0.36)	9.77 ^{b4X} ± 0.03 (17.48 ^Y ± 0.43)	9.95 ^{b3X} ± 0.16 (17.16 ^Y ± 0.67)
TBARS (mg MDA/kg sample)			
1	2.22 ^{b1X} ± 0.02 (3.01 ^Y ± 0.08)	0.98 ^{a1X} ± 0.03 (3.90 ^Y ± 0.13)	0.59 ^{a1X} ± 0.02 (3.89 ^Y ± 0.11)
3	2.39 ^{ab1X} ± 0.07 (4.48 ^Y ± 0.17)	2.83 ^{b2X} ± 0.03 (4.32 ^Y ± 0.06)	1.50 ^{a2X} ± 0.01 (4.98 ^Y ± 0.05)
8	3.72 ^{a2X} ± 0.43 (5.25 ^Y ± 0.15)	3.01 ^{a2X} ± 0.67 (3.83 ^X ± 0.03)	3.80 ^{a3X} ± 0.93 (4.03 ^X ± 0.30)
14	5.78 ^{a3X} ± 0.06 (9.18 ^Y ± 0.23)	7.17 ^{b3X} ± 0.05 (8.61 ^Y ± 0.13)	9.46 ^{c4X} ± 0.24 (11.08 ^Y ± 0.11)

For sample denomination, see Table 1. Mean ± standard deviation, in parenthesis value for each sample after heating. Raw samples with different letters (a, b, c) within the same row or numbers (1–3) in the same column indicate significant differences ($P < 0.05$). Different letters “X, Y” indicates significant differences ($P < 0.05$) between the raw and cooked sample at each storage period

content and an increase in water, with similar protein content (Table 1) will lower the “effective” concentration of the protein acting to form the meat matrix, and this is generally associated with softer structures (Jiménez Colmenero et al. 1995). The fact that the effect on texture was the opposite (Table 2) would suggest that GDE is instrumental in the formation of firmer structures. These apparent discrepancies can therefore be attributed to differences in the kind of lipid material used. Textures are generally softer when plant oils are added to beef patties and other meat products (Lurueña-Martínez et al. 2004; Keenan et al. 2015). This is presumably because oil is more liquid than the meat fats habitually used, whereas the solid lipid material used in the present experiment mimics the characteristics of these fats better, thus obviating the effect on that quality attribute in the reformulated product. In this regard, a review dealing with the potential use of solid-like

lipid structures to develop alternatives to animal fat replacement to produce healthy meat products, and specially in ground meat type patties (Jiménez-Colmenero et al. 2015), notes that the study of its effects on patty texture has been very limited. Babji et al. (1998), for instance, reported that replacement of animal fat with partially hydrogenated palm oil (as solid fat) produced softer beef burgers. More recently Salcedo-Sandoval et al. (2015) reported that KSF values of pork patties were not affected by replacement (partial or total) of pork backfat with an oil bulking system based on konjac gel.

Microbiological analysis

Microbial counts (Table 3) were affected by formulation and by storage time ($P < 0.05$). Initially all microbial counts were below 6 log cfu/g irrespective of formulation,

as reported elsewhere for this kind of meat product (Lorenzo et al. 2014; Salcedo-Sandoval et al. 2015). Microbial counts increased ($P < 0.05$) during storage, generally less in control sample and more in LF/GDE (Table 3). At the end of storage microorganism levels were lower than 8 log cfu/g and 7 log cfu/g in TVC and LAB respectively. As in this experiment, Salcedo-Sandoval et al. (2015) reported no clear connection between microbial growth and the strategy used to improve fat, either in samples with konjac gel as fat replacer (fat reduction) or in samples containing a healthier oil combination stabilized in a konjac matrix (lower fat plus healthier lipids).

Lipid oxidation

Lipid oxidation was studied both raw and after cooking in each specific storage period. This strategy serves to measure both the effect of chilling storage and the influence of cooking after a specific time in storage. Since final levels of lipid oxidation are affected by both factors, which may be interrelated, this provides information helpful to understanding the effects of reformulation and retail storage and/or the consequences of cooking.

Hydroperoxide concentrations in patties were affected ($P < 0.05$) by formulation and storage time (Table 4). At the outset of the experiment, all samples registered similar hydroperoxide levels ($P > 0.05$) irrespective of fat proportion and source. However, the increases of hydroperoxide concentration during storage were affected by formulation ($P < 0.05$), with the main variations observed at the end of storage, when patties with lower fat content but containing PO (MF/GDE and LF/GDE) registered the higher hydroperoxide level ($P < 0.05$). The difference in rates of formation of hydroperoxides of reformulated samples (as compared with control sample) is due to a combination of different fat proportions, greater susceptibility to oxidation of unsaturated fatty acid, and the antioxidant activity of Hxt. The cooking of patties generally produced an increase ($P < 0.05$) of the hydroperoxide levels in each storage period, which was generally proportionally greater the lower the concentrations of primary oxidation products in the raw sample (Table 4).

TBARS values of the different raw patties were affected ($P < 0.05$) by formulation and storage (Table 4). Initial TBARS concentration in the normal all-pork-fat content patty (NF/CS) was higher ($P < 0.05$) than the in modified sample (with less fat content) containing PO. In these conditions, it seems to have been the quantitative (amount of fat) rather than the qualitative (level of unsaturation) factor that most favoured the formation of secondary oxidation products. This result differs from the report by Salcedo-Sandoval et al. (2015), who reported that when the pork backfat was partially and totally replaced by an oil

bulking system (olive, linseed and fish), lipid oxidation levels rose in proportion with the oil in the formulation. This behaviour may be attributable to the structural characteristics of the fat replacer used in each case and the presence of antioxidant in the GDE. TBARS values increased ($P > 0.05$) during storage in all products (values >1 after 3 days of storage), generally following the same trend, but as in the case of hydroperoxide behaviour, the highest rate of increase of TBARS was observed in samples prepared with medium and reduced fat content, both containing PO. As referenced by initial TBARS values, at the end of storage the formation of secondary oxidation products increased (in %): 260 for NF/CS, 731 for MF/GDE and 1603 for LF/GDE. The higher oxidation rates found are consistent with the greater presence of unsaturated lipids in meat products in which pork backfat was replaced by GDE. As expected in each storage period, cooking generally produced an increase ($P < 0.05$) of TBARS levels, although the change was proportionally smaller in control sample (Table 4).

The oxidative stability of these meat systems can be interpreted in terms of composition and processing factors, especially as they relate to the use of GDE as a fat replacer. The potential contribution of this fat replacer to the oxidative stability of a meat system may be related to various factors. The presence of a PO lipid phase of the GDE increased the level of unsaturation, and hence susceptibility to lipid oxidation, both during storage of fresh patties and as an effect of thermal processing. Hxt has also been reported to inhibit lipid oxidation in $W_1/O/W_2$ emulsions and in different muscle-based matrixes (DeJong and Lanari 2009), including meat matrixes formulated with DE as the animal fat replacer (Cofrades et al. 2014). In this experiment the antioxidant capacity of Hxt, which seems to be related to its location at the oil–water interphase, could help to protect against oxidation of PO outside the inner aqueous phase. This compartmentalization effect means that there is less potential antioxidant capacity for non-DE-lipid materials, so that the antioxidant capacity of Hxt increases when added to the meat matrix (Cofrades et al. 2014). Also, it has been found that Hxt mainly exerts an antioxidant effect at the outset of storage (Cofrades et al. 2014), after which it is spent and hence the effect of unsaturated oils may predominate. Also, it has been found that the caseinate at the interface between the PO and the outer aqueous phase, which is used as a hydrophilic emulsifier, is highly effective in preventing lipid oxidation, due to its ability to scavenge lipid oxidation products in the lipid phase and to chelate transition metals (Matalanis et al. 2012).

The oxidative stability of PUFA-enrichment patties was studied under different retailing conditions, including fresh and pre-cooked products stored under chilling or frozen

conditions. One of the most common consumption conditions necessarily entails first chilling storage of fresh product and then cooking prior to consumption (chilling+cooking), as assayed in this experiment. Compared with chilling+cooking conditions, pre-cooking of the product followed by chilling storage (pre-cooking+chilling) offers some advantages as regards convenience and additionally extends shelf life; however, there are also some limitations in terms of oxidative stability. This is because cooking of PUFA-enriched patties promotes lipid oxidation (Poyato et al. 2015), and also there is an intensive oxidative reaction in cooked meat products during chilling storage (Ganhão et al. 2013), which could be further promoted by reheating. In fact, in chilling+cooking the oxidation level of the product upon ingestion is due to the effects induced by storage and by thermal treatment. However, both these factors are often ignored, and that limits our ability to understand the system and hence to develop more appropriate antioxidant strategies. Note that although cooking promoted lipid oxidation, the extent of that oxidation may depend on the lipid oxidation level in the raw product, which depends the composition and storage conditions (Table 4).

Sensory analysis

The sensory scores for the attributes evaluated, and overall acceptability, were subjected to two-way ANOVA (samples-S and panelists-A) with panelists considered as a fixed effect (data not shown). Panelists receive training, but there is also a significant source of variation outside of characteristic aroma and characteristic taste. It is very difficult to entirely factor out significant variations arising from individual differences (use of scales or differences in sensitivity, motivation and culture) (Carlucci and Monteleone 2001). SxA did not significantly affect the 5 attributes which according to the panel differed perceptibly from sample to sample. This means that the panel members largely agreed in their assessments of these attributes.

The analysis of differences in sensory attributes (Fig. 1) showed that total or partial replacement of pork backfat by GDE did not affect ($P > 0.05$) the juiciness of patties but produced the lowest ($P < 0.05$) score for characteristic taste. The NF/CS samples registered significantly greater colour and hardness than the MF/GDE samples. MF/GDE and NF/CS were most acceptable to the panelists. PCA was only applied to the average scores of significant attributes (aroma, taste, colour, hardness and overall acceptability). Figure 2 shows the relative positioning of the pork patties and attributes on the sensory scale generated by the panel. The biplot explained 90% of the total variability, reflecting a very close agreement among the panelists. With the separate distribution of the samples in a two-dimensional

Fig. 1 Sensory attributes (10-cm unstructured line scale) of the patties. For sample denomination, see Table 1

space, PCA showed that the panelists were able to clearly discriminate among samples on the basis of the target attributes. The first component (65.87%) closely tracked aroma, taste, colour and hardness of the patties, while the second component (24.13%) shows a positive correlation with overall acceptability. The low-fat sample (LF/GDE) diverged from NF/CS to MF/GDE, mainly due to its lower acceptability due to the low scores for aroma and taste in this patty, whilst MF/GDE was situated in upper left part of the biplot, indicating high acceptability. Despite the good discrimination by the judges, a discrepancy was found in reference to the KSF (Table 2). Although hardness is apparently a simple texture property, it is part of a complex set of properties hinging on the rheological and structural (geometric and surface) attributes that are detected through mechanical, tactile and, in some cases, visual and auditory receptors (Foegeding et al. 2011). It might therefore be necessary to provide further training in the use of this descriptor, since a trained panelists can clearly learn to perceive a broader range of textures through experience (Cardello et al. 1985).

Conclusion

The replacement of pork fat by a gelled double emulsion made with PO (rich in PUFA n-3) as lipid phase and Hxt in the inner aqueous phase, as a fat substitute in pork patties proved a suitable strategy for the production of healthier lipid patties. For nutritional purposes, the changes in composition mean that products reformulated in this way (with 30% less fat than the reference product) can qualify for a “reduced fat content” claim pursuant to European Regulation (Regulation (EC) No 1924/2006). Similarly

Fig. 2 Two-dimensional PCA plot of the variations in sensorial analysis among patties. For sample denomination, see Table 1

(pursuant to Commission Regulation No 116/2010), patties with perilla oil may claim “*high omega 3 fatty acid content*” since they contain more than 0.6 g of ALA/100 g. Oxidative stability of the new meat matrix can be interpreted in terms of composition and processing factors, especially in association with the use of GDE as a fat replacer. The presence of PO as the lipid phase in a GDE increases susceptibility to lipid oxidation during storage of fresh patties and as an effect of thermal processing. The antioxidant capacity of Hxt seemed to be related to its location at the oil–water interphase. The sensory quality of patties with partial replacement of pork fat was generally similar to that of normal fat content (all animal fat) samples; however, when pork backfat was entirely replaced by the GDE, sensory acceptability decreased.

Acknowledgements This research was supported by projects AGL2014-53207-C2-1-R and Intramural Project CSIC 201470E056 from the Spanish Ministry of Science and Innovation. M. Freire greatly thanks her FPI fellowship No. BES-2012-057977 from Ministerio de Economía y Competitividad. V. Serrano-Casas thanks Consejo Nacional de Ciencia y Tecnología (CONACyT), Mexico, for the Grant No. 300614 for this research stay in Spain. We are grateful to the Analysis Service Unit facilities of ICTAN for the analysis of Chromatography.

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