Healthier lipid combination as functional ingredient in sensory and technological properties of low-fat frankfurters

Gonzalo Delgado-Pando, Susana Cofrades; Claudia Ruiz-Capillas; Francisco Jiménez-Colmenero*.

Instituto del Frío (CSIC). Ciudad Universitaria, 28040 Madrid, Spain.

* Corresponding author: Tel.: +34 91 549 23 00; fax: +34 91 549 36 27.

E-mail addresses: fjimenez@if.csic.es

Key words: oil-in-water emulsion**,** frankfurter, healthier oil combination, fatty acid profile, technological properties, sensory analysis, chilling storage

Short title: Oil (healthier lipid combination)-in-water emulsions in low-fat frankfurters.

List of Abbreviations: MTG microbial transglutaminase, **SC** sodium caseinate, **SFA** saturated fatty acids, **SPI** soy protein isolate.

Abstract

Oil (healthier lipid combination of olive, linseed and fish oils)-in-water emulsions stabilized with different protein systems (prepared with sodium caseinate [SC], soy protein isolate [SPI], and microbial transglutaminase [MTG]) were used as pork backfat replacers in low-fat frankfurters. Composition (proximate analysis and fatty acid profile), sensory analysis, and technological (processing and purge losses, texture and colour) properties of frankfurters were analysed as affected by the type of oil-in-water emulsion and by chilling storage (2 °C, 41 days). Frankfurters produced with oil combinations had lower levels of saturated fatty acids (SFA) (19.3 %), similar levels of MUFA (46.9 %) and higher levels of PUFA (33.6 %) than control frankfurters (all pork fat) (39.3, 49.5 and 10.6 % respectively). PUFA/SFA and n-6/n-3 PUFA ratios in control sample were 0.27 and 9.27; in reformulated frankfurters the PUFA/SFA ratio was higher (1.7) and the n-6/n-3 PUFA ratio was lower (0.47). In general frankfurters had good fat and water binding properties. Colour parameters were affected by formulation and storage time. Compared to control sample, frankfurters made with oil-in-water emulsions had higher ($P < 0.05$) hardness, springiness and chewiness values. Emulsified oil stabilizing systems did not affect sensory characteristics of frankfurters, and all products were judged as acceptable.

1 Introduction

Comminuted cooked meat products (gel/emulsion systems) are a commercially important group of meat products, of which frankfurters are among the foremost. Frankfurters are popular, frequently-consumed meat products which are of considerable economic importance and enjoy wide consumer acceptance in certain sectors of the population. However, these products present some negative health concerns related to their fat content and fatty acid profiles [1-3]. Reformulation of frankfurters has been used to achieve better lipid compositions by reducing fat content and/or replacing (to a greater or lesser extent) the animal fat normally present in the product with another fat (of plant and/or marine origin) whose characteristics are more in line with health recommendations: i.e. contain smaller proportions of saturated fatty acids (SFA) and larger proportions of MUFA or PUFA fatty acids, especially long chain n-3 PUFA (LC n-3 PUFA), better n-6/n-3 PUFA and PUFA/SFA ratios, and if possible cholesterol-free [3].

A number of studies have been conducted to improve the lipid profile of finely comminuted cooked meat products such as frankfurters. In order to increase the MUFA content, frankfurters have been reformulated with the addition of high-oleic-acid sunflower oil [4, 5] or in most cases olive oil [1, 6-10](among others). Animal fat has been partially replaced with various vegetable oils (cottonseed, corn, soybean, peanut, etc.) to increase PUFA levels, improve fatty acid profiles (PUFA/SFA ratio) and reduce cholesterol contents of frankfurters [3]. Cottonseed and corn oils are very rich in PUFA and contain very high concentrations of linoleic acid (18:2 n-6) (> 56% of total fatty acid); their addition to frankfurters does reduce PUFA/SFA ratios, but it also has the unwanted effect of raising the n-6/n-3 PUFA ratio [2, 8]. Soybean oil contains high levels of both linoleic acid (56.1% of total fatty acid) and ALA (7.3%), and has been used in frankfurter formulation [2]. Vegetable and marine oils have been used to supply substantial amounts of n-3 PUFA in order to produce $n-3$ PUFA-enriched frankfurters [4, 11] and bologna-type sausage [12].

Incorporation of individual lipids (from only one source of plant or marine origin) does improve the fatty acid profile of meat products, but a better approximation to optimal lipid profile it means more in line with health recommendations can be achieved using healthier oil combinations as animal fat replacers. The rationale behind lipid modification to improve the health status of the population is that reducing SFA concentrations and increasing MUFA and PUFA contents, especially n-3 PUFA, will

promote a reduction in the n-6/n-3 PUFA ratio [13-17]. While obviously such a healthier fatty acid composition can only be achieved by combining several types of lipid material (from both plant and marine sources), there have been few studies on reformulation of meat products, including frankfurters. Combinations of vegetable oils (olive, cottonseed and soybean) have been used in frankfurter formulation following dietary guidelines for fatty acids [2]. A healthier lipid formulation (algal and olive oils) produced a low-fat frankfurter enriched with high levels of long n-3 PUFA and MUFA and a good balance of MUFA/SFA, PUFA/SFA and n-6/ n-3 ratios [9, 18].

Oil-in-water emulsion technology has been shown to be feasible as a means of stabilizing the non-meat fats used for incorporation in meat derivates [1, 3, 19, 20]. In a previous paper [21], our group used various protein systems (based on sodium caseinate [SC], soy protein isolate [SPI], meat protein and microbial transglutaminase [MTG]) to stabilize a healthier oil combination formed by vegetable (olive and linseed) and fish oils in suitable amounts and proportions to provide a fatty acid profile better adjusted to healthier intake goals [21]. These plant and marine oils were combined in such a way as to produce an improved fatty acid profile with a low proportion of SFA (15%) and a high proportion of MUFA (47 %, 43 % of oleic acid) and n-3 PUFA (36 %, including a high proportion of long chain n-3 PUFA), with a PUFA/SFA ratio > 2 and a n-6/n-3 PUFA ratio of 0.4, in line with recommendations for optimal intake of total and unsaturated fatty acids [13-15, 17]. These oil-in-water emulsions had different physicochemical characteristics depending on the system used to stabilize the emulsified oil [21]. Because they are added to frankfurters as fat ingredients (for animal fat replacement), their physicochemical characteristics affect their role in the meat system and hence the quality properties of the reformulated product [3]. Differences in physicochemical properties of such systems may determine their suitability for use as fat replacers in meat products such as finely comminuted cooked meat products [21]. Despite their possibilities, as far as the authors know there have been no studies on the use of such healthier lipid combinations/stabilizing systems as fat ingredients in meat product formulation.

The aim of this experiment was to assess the suitability of healthier oil-in-water emulsions stabilized with various protein systems as pork backfat replacers in a comminuted gel/emulsion matrix, in this case frankfurters. The lipid phase of the oil-in-water emulsions was a combination of olive, linseed and fish oils, specially designed with fatty acids in suitable amounts and proportions for purposes of achieving healthier

intake goals [21]. The oil-in-water emulsions were stabilized with various protein systems formulated using SC, SPI, and MTG. Potential nutritional advantages (fatty acid profile) were assessed and sensory analyses conducted. Technological (processing and purge losses, texture, colour) properties of frankfurters were evaluated as affected by the type of oil-in-water emulsion and chilling storage (41 days at 2 °C). Parallel to this study, our group has been assessing the influence of formulation and chilling storage on microstructure, microbiology, biogenic amine formation and lipid oxidation. This will be reported in another paper.

2. Materials and methods

2.1. Materials

Fresh post-rigor pork meat (mixture of *M. biceps femoris*, *M. semimembranosus*, *M. semitendinosus*, *M. gracilis* and *M. adductor*) and pork backfat were obtained from a local meat market. The meat was trimmed of fat and connective tissue and the pork fat was passed through a grinder with a 0.4 mm plate. Lots of approx. 1 kg were vacuum packed, frozen and stored at -20 °C until use, which took place within 2 weeks.

Ingredients used for preparation of oil-in-water emulsions included olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain), linseed oil (Natursoy S.L., Alimentos Ecológicos, Castellterçol, Spain) and fish oil (Omevital 18/12 TG Gold from Cognis GmbH, Illertissen, Germany), according to supplier information containing 160 mg of EPA/g and 115 mg of DHA/g plus a combination of tocopherols as antioxidants. The materials used for oil-in-water emulsion stabilization were SC containing 86.4 % protein (Julio Criado Gómez SA, Alcorcón, Spain), SPI containing 92.1 % protein (Vicoprot, TRADES S.A., Barcelona, Spain) and MTG (ACTIVA WM, Ajinomoto Europe Sales GmbH, Hamburg, Germany). According to supplier information, the enzyme was in a mixture containing 1 % transglutaminase and 99 % maltodextrin, with a standard transglutaminase activity of approximately 100 units/g.

Others additives included sodium chloride (Panreac Química, S.A. Barcelona, Spain), sodium tripolyphosphate (Manuel Riesgo, S.A. Madrid, Spain), sodium nitrite (Fulka Chemie GmbH, Buchs, Germany), and flavouring (Gewürzmüller, GmbH, Münichingen, Germany).

2.2. Preparation of oil-in-water emulsions

Three different types of oil-in-water emulsions were formulated (Table 1) and prepared according to the procedure described by Delgado-Pando *et al*.[21]. The lipid material, which was the same in all cases, consisted of a combination of olive, linseed and fish oils in respective proportions of 44.39, 37.87 and 17.74 %. This oil combination was designed to produce a healthier lipid formulation with a small proportion of SFA, large proportions of MUFA and PUFA (including long chain n-3 PUFA) and balanced n-6/n-3 PUFA and PUFA/SFA ratios as reported by Delgado-Pando *et al*. [21]. The oil-in-water emulsions were stored 24 h at 2 º± 2 ºC before use in frankfurter preparation.

2.3. Design and preparation of healthy frankfurters

Four different frankfurters were formulated (Table 2): a control frankfurter (all pork fat) and three modified frankfurters reformulated by totally replacing pork backfat with one of the oil-in-water emulsions indicated in Table 1. These frankfurters had been designed to produce a healthier fatty acid profile than that of the pork fat: with less SFA, similar MUFA levels and higher n-3 PUFA levels (including long chain n-3 PUFA). The fat level in these frankfurters is lower than normally found in such meat products. Higher levels of pork fat reduction were not considered in this experiment as these would have reduced the amount of healthy oil combination that could be incorporated in place of the fat. One fundamental requirement of design and reformulation of these products with a view to potential health benefits is to assure that the lipid content and profile are such as to make a serious contribution to the recommended intake levels when consumed in normal quantities.

Meat and fat packages were thawed (approx. 18 h at 2 ± 2 ºC) prior to use. Preparation of the frankfurters was as described by Jiménez-Colmenero *et al*. [22]. Briefly, raw meat material was homogenized and ground for 1 min in a chilled cutter (2 ºC) (Stephan Universal Machine UM5, Stephan u. Söhne GmbH and Co., Hameln, Germany). Half of the pork backfat or oil-in-water emulsion (depending on the formulation), NaCl, sodium tripolyphosphate and sodium nitrite (the last two previously dissolved in the added water) were added to the ground meat and mixed again for 1 min. The rest of the additives, the pork backfat and the oil-in-water emulsion were added and the whole homogenized for 1 min. Finally the whole meat batter was homogenized under vacuum for 2 min. Mixing time was standardized at 5 min. The final batter temperature was below 14 ºC in all cases.

The meat batter was stuffed into 20 mm diameter Nojax cellulose casings (Viscase S.A., Bagnold Cedex, France) and hand-linked. Frankfurters were heat processed in an Eller smokehouse (model Unimatic 1000, Micro 40, Eller, Merano, Italy) until the core of the product reached 70 °C. Heat processing conditions were established beforehand, and the internal temperature was monitored throughout heating by means of thermocouples inserted in each frankfurter (thermal centre) and connected to a temperature recorder (Yokogawa Hokuskin Electric YEM, Mod. 3087, Tokyo, Japan). Once heating was complete, the frankfurters were cooled (at room temperature), kept in a cold room (2 °C for 14 h), packed (Cryovac® BB3050) and stored at 2 ºC (± 1 °C) and analysed periodically over 41 days.

2.4. Proximate analysis and fatty acid composition

Moisture and ash contents of the frankfurters were determined [23] in triplicate. Fat content was evaluated (in triplicate) according to Bligh and Dyer [24]. Protein content was measured in quadruplicate by a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI).

Fatty acid composition of frankfurters was determined (in quintuplicate) by gas chromatography as reported by López-López *et al*. [18]. Briefly, boron trifluoride/methanol was used for fatty acid methyl ester (FAME) preparation. A Shimadzu gas chromatograph (Model GC-2014, Kyoto, Japan) fitted with a capillary column SP™-2330 (60 m x 0.25 mm x 0.2μm i.d.) (Supelco, Inc, Bellefonte, USA) was used with a flame ionisation detector. Injector and detector temperatures were 250 and 260 ºC respectively, and the oven temperature was 140 ºC for 5 min, raised to 240 ºC at a rate of 4 ºC/min and held for 20 min. Fatty acids were identified by comparison with a known standard FAME mixture (Supelco, Alltech Associated, Inc. Deerfield, IL, USA).

Based on the FAME results, the atherogenic index (AI) and thrombogenic index (TI) were computed according to Ulbricht and Southgate [25].

AI = (C12:0 + 4xC14:0 + C16:0)/[∑MUFA+ ∑PUFA (n-6) and (n-3)];

TI = (C14:0 + C16:0 + C18:0) / (0.5 x ∑MUFA + 0.5 x ∑PUFA (n-6) + 3 x ∑PUFA (n-3) + (n-3 PUFA)/(n-6 PUFA)].

2.5. Processing loss and purge loss

Processing loss of frankfurters was calculated, in sextuplicate, as the weight loss (expressed as % of initial sample weight) occurring after heat processing and chilling overnight at 2 °C.

Three vacuum packs per formulation were used to determine purge loss during chilling storage. After the frankfurters were removed from the package, the exudate was dried with paper towels and the frankfurters weighed again. The purge loss was calculated by weight difference and expressed as a percentage of the initial weight.

2.6. pH determination

The pH was determined on a Radiometer model PHM 93 pH-meter (Orion 3 Star, Thermo Fisher Scientific Inc., Waltham MA, USA) at room temperature on homogenates of frankfurters in water in a ratio of 1:10 (w/v). Four determinations were performed for each sample.

2.7. *Colour measurement*

Colour, CIE-LAB tristimulus values, lightness, L*; redness, a* and yellowness, b* of frankfurter cross-sections were immediately evaluated on a CR-400 Chroma Meter (Konica Minolta Business Technologies, Inc., Tokyo, Japan). Six determinations were performed from each formulation.

2.8. *Texture Profile Analysis*

Texture Profile Analysis (TPA) was performed in a TA-XT.plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) as described by Bourne [26]. Six frankfurter cores (diam = 22 mm, height = 20 mm) were axially compressed to 40 % of their original height. Force-time deformation curves were obtained with a 5 kN load cell, applied at a crosshead speed of 1 mm/sec. Attributes were calculated as follows: hardness (Hd) = peak force (N) required for first compression; cohesiveness (Ch) = ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp) = distance (mm) the sample recovers after the first compression; chewiness (Cw) = Hd x Ch x Sp (N x mm). Measurement of samples was carried out at room temperature.

2.9. Sensory analysis

Frankfurters were assessed by a 15-member panel using a hedonic test. The panel was selected in preliminary sessions from staff who had received training (two sessions) with the products and terminology. Samples 2.5 cm long from each formulation were heated in a microwave for 15 s then immediately presented to panellists in random order. Judges were instructed to evaluate the juiciness, hardness, flavour and overall acceptability on a non-structured scale without fixed extremes. Each point was converted to a numerical value from: juiciness (0 = very dry, 9 = very juicy); hardness (0 = soft, 9 = hard); off-flavour (0 = no different from the typical flavour, to 9 = not typical flavour); and texture, flavour and overall acceptability (0 = dislike extremely to 9 = like extremely).

2.10. Statistical analysis

Each product was prepared in duplicate. The repeated measures test was used for statistical comparisons between samples. Data were analysed using SPSS Statistics 17.0 (SPSS Inc, Chicago, USA) for one-way and two-way ANOVA. Least squares differences were used for comparison of mean values among treatments and Tukey's HSD test to identify significant differences ($P<0.05$) between formulations and storage times.

3. Results and Discussion

3.1 Proximate analysis.

Proximate analysis of frankfurters showed some significant differences between types of formulation (Table 3). The differences in moisture content were small (range 60.8-62.2 %), with higher ($P<0.05$) levels in control and F/SPI samples. Protein content ranged between 17.8-19.4 % (Table 3). Since all samples were formulated with the same meat content (Table 2), the control sausage contained only muscle protein, mainly from meat, but also a small amount from pork backfat. In the modified frankfurters, on the other hand, because the pork backfat was totally replaced by oil-water emulsion, the sausages contained not only muscle protein (from meat only), but also non-meat proteins (Table 1 and 2). The differences observed in ash content were small, even when significant, the highest ($P<0.05$) being recorded in product F/SC (Table 3).

No differences (P>0.05) were found in fat content of frankfurters; this was around 12 %, which is lower than normally found in this type of meat products. While control frankfurter contained only pork fat, most of the fat in the reformulated sausages (total replacement of pork backfat by oil-water emulsion) were of plant and fish origin (Table 1 and 2). In terms of ingredient composition and formulation, around 80 % of the fat contained by modified frankfurters was supplied by the oil-in-water emulsion, which contained around 4.3 g, 3.6 g and 1.6 g (per 100 g of the products) of olive, linseed and fish oils respectively. A large variety of plant and marine oils (olive, cottonseed, sunflower, soyseed, high-oleic-acid sunflower, palm, fish, etc.) have been used individually to produce frankfurters; they have been added in variable proportions (2-20 g oil/100 g of product) and in different ways: directly during product manufacture (in liquid or solid form at the end of the process), oil-in-water emulsified (pre-emulsified generally with SC) or interesterified. When oil combinations have been incorporated in low-fat frankfurters, pre-emulsified with SC [2], or in liquid form [9, 18], the oil contents in the product have varied by between 4 and 6 g per 100 g of product. These proposals differ from the approach adopted in the present experiment in that the oil combination used is healthier and a higher proportion was added.

Energy content of the samples ranged from 176 to 194 kcal/100 g (Table 3), of which fat accounted for almost 60 % (the remaining 40 % or so from protein). Since in the F/SC, F/SPI and F/SPI+SC+MTG samples all pork backfat was replaced by oil-in-water emulsion, oil combinations accounted for almost 50 % of total frankfurter energy content. The approximate energy supplied by olive, linseed and fish oils is 39, 32 and 14 kcal/100 g, respectively. Generally, differences in proximate analysis between samples are mainly due to differences in ingredients, and formulation and processing conditions.

3.2. Fatty acid profile

The fatty acid composition of frankfurters differed in control and modified samples (Table 4). The most abundant fatty acids in the control sample (all pork fat) were MUFA, followed by SFA and PUFA; MUFA and PUFA together accounted for 60 % of total fatty acids. In control sample, oleic acid was the most abundant fatty acid, followed by palmitic and finally stearic and linoleic acids. These results are consistent with reports for fatty acid composition of frankfurters [11] and for pork fat [27].

Because of the healthy fatty acid composition of the oil-in-water emulsions, characterized by a low proportion of SFA and a high proportion of MUFA (mainly oleic acid) and n-3 PUFA [21], their addition to meat products as pork backfat replacers produced major changes in the fatty acid profiles of the reformulated frankfurters (Table 4). As compared with control sample, the reformulated products contained less ($P<0.05$) SFA, among them palmitic and stearic acids. Although SFA are considered to be the chief risk factor because of their hypercholesterolaemic effect, not all of them act in the same way. While stearic acid is neutral, palmitic and myristic acids produce the greatest atherogenic effect [28]. The concentrations of these fatty acids (palmitic and myristic acids) decreased from 24.5 % to 12.5 % when pork backfat was replaced by the vegetable and marine oil combination.

Although there were significant variations, there was little observable difference in MUFA contents of modified and control frankfurters, while oleic content was similar ($P>0.05$) (around 42 %) in both types of frankfurter (Table 4). These results are consistent with the fact that the pork fat and the oil-in-water emulsions had very similar proportions of oleic acid (43 %) and MUFA [21]. MUFA in the diet have a beneficial effect on the serum lipid profile [16, 29]. The incorporation of oil-in-water emulsions caused considerable changes in PUFA contents (Table 4). As compared with all-pork-fat frankfurters (control), modified samples contained more ($P<0.05$) linoleic, linolenic, docosapentaenoic, EPA, and DHA fatty acids, with total PUFA levels three times higher than in the control samples (Table 4). Total fatty acid in fat [30], and the formulation (Table 2), indicate that total n-3 PUFA were around 2.5 g/100 g (of which approximately 2 g/100 g was ALA and 500 mg/100 g were long chain n-3 PUFA, EPA, docosapentaenoic acid and DHA) in modified frankfurters as opposed to around 0.11 g/100 g in all-pork-fat product. This means that although dietary recommendations vary depending on different factors (population, desired disease prevention, etc.), these products can make a very important contribution to dietary intake as compared to non-fortified frankfurters— considering that the dietary recommendation for total n-3 PUFA is estimated as between 1.4 and 3 g/day or even higher [14, 15, 29], while the estimated daily range for long chain n-3 PUFA is between 180 and 1000 mg [14, 29]. Recommended intakes for fatty acids are often expressed as a proportion of the total daily energy intake. In our reformulated products, the estimated energy contributions (as % of total energy content) of the different types of fatty acids were: SFA 6 %, MUFA 26 %, PUFA 18 %, n-3 PUFA 12 % (long chain n-3 PUFA 2 %) and n-6 PUFA 6 %, as

compared to respective control sample values of 23 %, 28 %, 6 %, 0.5 % and 5 %. Meat products like these reformulated frankfurters (with low energy input from SFA and high energy input from PUFA) thus conform better to dietary recommendations for optimal intake of total, saturated and unsaturated fatty acids according to various national and international organizations [13, 29]. When consumed on a regular basis, such modified frankfurters can supply a significant proportion of recommended long chain n-3 PUFA intakes, higher than most of the reformulated cooked meat products reported in the literature, which generally contain less than 150 mg/100 g of n-3 PUFA [3]. ALA has been associated with a reduced risk of cardiovascular disease (CVD) [31]. Long chain n-3 PUFA are associated with a reduced risk of CVD, certain types of cancer, inflammatory diseases (rheumatoid, arthritis, asthma, lupus and ulcerative colitis), diabetes mellitus, multiple sclerosis and clinical depression [14].

The PUFA/SFA ratio is one of the main parameters currently used to assess the nutritional quality of the lipid fraction of foods. Nutritional guidelines recommend a PUFA/SFA ratio above 0.4 [27]. It has been reported that increasing the dietary PUFA/SFA ratio can lead to a reduction in plasma total cholesterol, and as a result there is a lot of research focusing on ways to improve this ratio in meat [31]. Whereas the PUFA/SFA ratio in control sample was around 0.3 (Table 4), which is consistent with reports by other authors in conventional meat products [11, 12], replacement of pork fat by a combination of plant and marine oils increased this ratio ($P<0.05$) to 1.7 since SFA were reduced and PUFA increased (Table 4). Similar patterns have been reported in meat products incorporating different plant and fish oils [2, 8, 12, 32] or a high-fat ingredient (walnut) [33].

Scientific evidence suggests that a very high n-6/n-3 PUFA ratio promotes the pathogenesis of many diseases, including CVD, cancer, etc., whereas increased n-3 PUFA content (a low n-6/n-3 PUFA ratio) exerts a suppressive effect [17]. The dietary recommendation for prevention of CVD is to reduce this ratio to less than 4. Since not all n-3 PUFA and n-6 PUFA are equal with regard to their effect on health, the usefulness of this ratio has recently been questioned, in favour of more individualized consideration of linolenic acid and long chain n-3 PUFA [31]. Control samples presented a n-6/n-3 PUFA ratio of 9.2, similar to the ratios reported by other authors [12]. The addition of oil considerably reduced this ratio, almost down to 0.5 (Table 4). The reason for this was that while the n-3 PUFA content increased around 22-fold, the

n-6 PUFA contents in control and modified samples were close to 10 (Table 4). These results are consistent with the findings of other authors [12, 34].

Atherogenic index (AI) and thrombogenic index (TI) values were lower ($P<0.05$) in modified samples than in control product (Table 4). Similar AI and TI index values have been reported in all-pork-fat (normal and low fat) frankfurters [11]. As in this experiment, Ayo *et al*. [11] found that replacing animal fat with walnut produced a reduction of both indices.

According to the proposal to regulate nutrition claims concerning n-3 fatty acids, monounsaturated fat, polyunsaturated fat and unsaturated fat made on foods in Europe [29], reformulated frankfurters may be claimed to be *high omega-3 fatty acid* since they contain more than 30 % of the recommended nutritional intake (2 g/day for an adult male) of n-3 fatty acids per 100 g of product (2.5 g/100 g). According to a recent Commission Regulation (European Parliament) nº 116/2010 (amending regulation 1924/2006) regarding the list of nutrition claims, a *high omega-3 fatty acid* claim may only be made where the product contains a minimum of 0.6 g ALA /100 g or a minimum of 80 mg of the sum of EPA and DHA per 100 g [35].

3.3. Processing loss, purge loss and pH

Processing loss of frankfurters ranged from 17.5 to 21 % (Table 5), with highest processing loss values in the F/SC sample and no significant variations among the other samples. Ranges of processing loss for low-fat frankfurters (including those made with vegetable oils) between 10-20 % have been reported [1, 4-6, 8, 10, 18, 36].

Purge accumulation in the packaged product during retail storage is undesirable for aesthetic and microbiological reasons. Purge loss levels over storage (ranging from 1.0 to 1.7 %) were relatively low (Table 5), indicating good storage stability in terms of fat and water binding properties of the meat matrix. This stability was not dependent on the type of lipid used (pork backfat versus oil-in-water emulsion), nor did it vary with the protein system used to stabilize the plant and fish oils. Higher proportions of purge loss in low-fat frankfurters have been reported by other authors [1, 8, 18] . Generally, formulations and chilling storage affected purge loss very little; the differences observed were small, even when significant, and unlikely to be of practical importance. Pappa *et al*. [10] reported that replacing pork backfat with olive oil in low-fat frankfurters had no effect on purge loss. On the contrary, Bishop *et al*. [19] reported that purge loss was higher in bologna sausage containing emulsified oil than in bologna

sausage containing pork backfat. Increasing purge loss with storage time has been reported in low-fat frankfurters [1, 6, 8], although other authors found no changes in purge loss during storage [37], as in this experiment.

Since pH values were not influenced ($P>0.05$) by chilling storage, table 5 shows only the mean values over the storage period. pH values (range 6.2-6.5) may be considered normal in products of this kind [1, 8]. The lowest ($P<0.05$) pH values were recorded in the control frankfurter. The pH was higher ($P<0.05$) in samples with added oil but was not affected ($P>0.05$) by the particular emulsified oil stabilizing system used in the product reformulation (Table 5). This increase, which has been described by other authors [12, 38], is possibly related to the protein component [12] and to the lipid material of the oil-in-water emulsion. The pH values (5.96 ± 0.10) of the pork backfat used in the control formulation were lower than those of the oil-in-water emulsions (7.8-7.9) used in modified sausages [21].

3.4. Colour

Colour parameters were affected by formulation and storage time (Table 6). Generally, lightness, redness and yellowness values were higher ($P<0.05$) in samples where pork backfat had been replaced by oil-in-water emulsions. There were also some differences in colour parameters depending on the protein system used for oil-in-water emulsion stabilization. For instance, the increase in the parameter L* (as compared with control sample) produced by addition of oil-in-water emulsion was smaller when the emulsified oil stabilizing system contained SC (Table 6). Similarly, the presence of SC seemed to influence the effect of the emulsion on frankfurter redness and yellowness, although the effect on lightness was less evident. It has been reported that differences in the colour of pork backfat and oil-in-water emulsions affect the colour parameter of frankfurters (Jiménez-Colmenero et al., submitted for publication), possibly caused by the protein and lipid material in the emulsions. Other authors have reported that incorporation of non-meat proteins such as soy and caseinates as emulsified stabilizing fats in frankfurters had no effect on redness and yellowness, and that although soy protein did not affect lightness, SC reduced it [39]. Caceres *et al*. [12] concluded that the incorporation of pre-emulsified fish oil with SC to bologna-type sausage caused an increase of lightness and a reduction of redness. It is also the case that oil has a significantly larger surface area than finely cut pork fat, and this makes the colour lighter [40]. López-López *et al*. [18] and Bishop *et al*. [19] reported that the replacement

of pork backfat by olive oil increased lightness and reduced redness of sausages, although others authors [8, 41] have observed that oil substitution had no effect on colour. Also, Hong *et al*. [38] reported that redness of sausages increased when pork backfat was replaced with oil. These conflicting results are probably related to the type of sausage used, the product formulation and the characteristics of the oils assayed.

Colour parameters decreased ($P<0.05$) over the storage period in all the different frankfurters (Table 6). The rate of the decrease in lightness was lower at the outset of storage, but both the rate and extent of the decrease were very similar in all samples. In the case of redness the pattern of behaviour over the storage period was similar, although the onset occurred later than in the case of lightness (Table 6). The decrease in b* values over the storage period was greater in control samples than in modified frankfurters. Contrasting with our observations in the present experiment, there have been reports of increasing redness of reduced-fat frankfurters over time [42]. However, there have also been reports that storage time had no effect on the colour characteristics of low-frankfurters with added oils [1, 6, 41].

3.5. *Texture*

Texture profile analyses (TPA) of frankfurters were affected ($P<0.05$) by the presence of oil-in-water emulsions in the meat matrix (type of formulation) and by chilling storage (Table 7). TPA parameters indicated that compared with the control sample (all pork fat), the products with oil-in-water emulsions (F/SC, F/SPI and F/SPI+SC+MTG samples) presented higher ($P<0.05$) hardness, springiness and chewiness values but the same ($P>0.05$) cohesiveness. There are various factors that may help explain this; for instance, given that the same amount of meat was used in all samples (Table 2), in the reformulated products the oil combination was emulsified with non-meat protein and so more meat protein became available to contribute to gel formation [19]. Then again, it has been reported that oils achieve a better distribution than animal fat in meat emulsion matrixes, thus producing firmer sausages due to improved association with the protein [43]. However, conflicting results have been reported for the effect of vegetable oils on the texture of frankfurters. Substitution of pork fat by olive oil (stabilized with SC) in reduced-fat frankfurter (as compared to a normal-fat product) has been reported to produce a harder/firmer product [1, 2, 8] or to have no influence [1]. However, other authors have observed that olive oil addition combined with fat reduction (with similar protein content) caused a decrease in hardness

and chewiness of frankfurters [7]. Marquez *et al*. [41] reported that there were no major changes in the firmness of low-fat frankfurters due to peanut oil treatment. As in the case of fat reduction strategies, the apparent discrepancies regarding the effect of substituting olive oil for animal fat may be related to varying extents to composition factors (moisture and protein content), to which a great deal of importance has been attached [44, 45].

The reformulated frankfurters generally differed little in terms of TPA textural parameters (Table 7). This was despite there being some variations in the protein contents of samples (Table 3) and some differences in the physicochemical characteristics of the oil-in-water emulsions used to replace animal fat [21]. These differences must be related to the protein system used to stabilize the emulsified oil, since the combination of oils was the same in all cases. The emulsion stabilized with SC behaved like a viscous material but lacked a gel-like behaviour; the emulsion with SPI appeared to possess gel-like characteristics, if very weak ones; and addition of MTG conferred a stronger gel-like structure [21]. These characteristics influence the textural properties of frankfurters reformulated with olive oil-in-water emulsions in place of pork backfat in different ways (Jiménez-Colmenero et al., submitted for publication). As was observed in the present experiment, when olive oil-in-water emulsions stabilized with SC or SPI (individually) were used to replace pork backfat in frankfurters, there were no observable effects on the hardness or chewiness of the product. However, conflicting results were observed when an oil-in-water emulsion was stabilized in the presence of MTG; the addition of MTG to the emulsion produced a significant increase in the hardness, cohesiveness and chewiness of the frankfurters (Jimenez-Colmenero et al., submitted for publication). Although the influence of the MTG on the physicochemical properties of the emulsions used in this work was evident [21], there was no such effect on the textural properties of the frankfurters (Table 7). We can therefore state that in the given experimental conditions the physicochemical characteristics of the oil-in-water emulsions used to replace pork backfat had no clear effect on the textural properties of the frankfurters.

Chilling storage had a minor effect on some TPA parameters (Table 7). There was no clear trend associated with the type of sample, but as storage progressed there was some loss ($P<0.05$) of hardness in reformulated products and increasing springiness and cohesiveness in control, F/SC and F/SPI samples. Kao and Lin [46] reported that shear force increased gradually in reduced-fat frankfurters with increasing storage time.

And similarly, hardness has been reported to increase during chilling storage, a development attributed to changes in purge loss [36, 37]. However, texture was not affected by storage time in liver sausage and frankfurters where pork backfat and beef fat respectively were replaced by vegetable oils [38, 41].

3.6. Sensory analysis

Sensory evaluation indicated that pork backfat replacement by oil-in-water emulsions affected ($P<0.05$) some sensory attributes of the frankfurters (Table 8). As compared with control (all pork fat) sample, hardness increased ($P<0.05$) (and texture acceptability decreased significantly) in frankfurters formulated with oil-in-water emulsions. There were no significant differences in relation to the emulsion stabilizing system used. This is consistent with the fact that these samples were harder and chewier (Table 7), although the panellists did not report the textural differences between formulations (with oil-in-water emulsion) that were indicated by TPA.

There were no significant differences among any of the samples in terms of juiciness, although juiciness did tend to decrease as a result of total replacement of pork back fat by a healthy lipid combination. Pork backfat replacement by an oil-in-water emulsion had no effect on flavour appreciation parameters in the frankfurters. While all-pork-fat (control) frankfurters scored higher for flavour acceptability and lower for off-flavour, there were no significant differences between formulations (Table 8).

The panellists considered all products acceptable; the control sample scored highest ($P<0.05$), but F/SC sausage scored almost as high, the lowest scores ($P<0.05$) going to F/SPI and F/SPI+SC+MTG products. It has been reported that low-fat frankfurters produced by replacing pork backfat with olive oil had lower overall palatability or overall acceptability than high-fat frankfurters produced with pork backfat [1, 10].

Conclusion

Low-fat frankfurters (12 %) can be manufactured using a healthier oil (from plant and marine sources) combination stabilized with different non meat protein systems, to give a product with healthy lipid content (amount and fatty acid profile). This type of meat product with a low level of SFA and a high level of PUFA (including LC n-3 PUFA) approximates more to dietary recommendations for optimal intake of

total, saturated and unsaturated fatty acids made by various national and international organizations [13, 29]. The technological properties and sensory characteristics demonstrate that it is possible to produce a healthier frankfurter. Additional studies on microstructure, microbiology, biogenic amine formation and lipid oxidation have been pursued at the same time to gain a clearer understanding of these products and will be reported in another paper.

Acknowledgements

This research was supported by projects AGL2007-61038/ALI and AGL2008-04892-CO3-01 under the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) and the Consolider-Ingenio 2010:CARNISENUSA (CSD2007-00016), Ministerio de Ciencia y Tecnología.

References

[1] J.G. Bloukas, E.D. Paneras: Substituting olive oil for pork backfat affects quality of low-fat frankfurters. *J Food Sci.* 1993, **58**, 705-709.

[2] E.D. Paneras, J.G. Bloukas, D.G. Filis: Production of low-fat frankfurters with vegetable oils following the dietary guidelines for fatty acids. *J Muscle Food*. 1998, **9**, 111-126.

[3] F. Jiménez-Colmenero: Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends Food Sci Techol.* 2007, **18**, 567-578.

[4] J. Park, S.K. Rhee, J.T. Keeton, K.C. Rhee: Properties of low-fat frankfurters containing monounsaturated and omega-3 polyunsaturated oils. *J Food Sci*. 1989, **54**, 500-504.

[5] J. Park, K.S. Rhee, Y.A. Ziprin: Low-fat frankfurters with elevated levels of water and oleic acid. *J Food Sci*. 1990, **55**, 871-872,874.

[6] J.G. Bloukas, E.D. Paneras, G.C. Fournitzis: Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages *Meat Sci.* 1997, **45**, 133-144.

[7] M.A. Lurueña-Martinez, A.M. Vivar-Quintana, I. Revilla: Effect of locust bean/xanthan gum addition and replacement of pork fat with olive oil on the quality characteristics of low-fat frankfurters. *Meat Sci.* 2004, **68**, 383-389.

[8] E.D. Paneras, J.G. Bloukas: Vegetable-oils replace pork backfat for low-fat frankfurters. *J Food Sci.* 1994, **59**, 725-728.

[9] I. Lopez-Lopez, S. Cofrades, F. Jimenez-Colmenero: Low-fat frankfurters enriched with n-3 PUFA and edible seaweed: Effects of olive oil and chilled storage on physicochemical, sensory and microbial characteristics. *Meat Sci.* 2009, **83**, 148-154.

[10] I.C. Pappa, J.G. Bloukas, I.S. Arvanitoyannis: Optimization of salt, olive oil and pectin level for low-fat frankfurters produced by replacing pork backfat with olive oil. *Meat Sci.* 2000, **56**, 81-88.

[11] J. Ayo, J. Carballo, J. Serrano, B. Olmedilla-Alonso, C. Ruiz-Capillas, F. Jimenez-Colmenero: Effect of total replacement of pork backfat with walnut on the nutritional profile of frankfurters. *Meat Sci.* 2007, **77**, 173-181.

[12] E. Caceres, M.L. Garcia, M.D. Selgas: Effect of pre-emulsified fish oil - as source of PUFA n-3 - on microstructure and sensory properties of mortadella, a Spanish bologna-type sausage. *Meat Sci.* 2008, **80**, 183-193.

[13] WHO: Diet, Nutrition and the Prevention of chronic diseases, In: *Technical Report Series*. Ed WHO, Geneve (Switzerland) 2003, No. 916

[14] M.L. Garg, L.G. Wood, H. Singh, P.J. Moughan: Means of delivering recommended levels of long chain n-3 polyunsaturated fatty acids in human diets. *J Food Sci.* 2006, **71**, R66-R71.

[15] W. Kolanowski, F. Swiderski, S. Berger: Posibilities of fish oil application for food products enrichement with ω-3 PUFA. *Int J Food Sci Nutr.* 1999, **50**, 39-49.

[16] F.H. Mattson, S.M. Grundy: Comparison of effects of saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J Lipid Res.* 1985, **26**, 194-202.

[17] A.P. Simopoulos: The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother.* 2002, **56**, 365-379.

[18] I. Lopez-Lopez, S. Cofrades, C. Ruiz-Capillas, F. Jimenez-Colmenero: Design and nutritional properties of potential functional frankfurters based on lipid formulation, added seaweed and low salt content. *Meat Sci.* 2009, **83**, 255-262.

[19] D.J. Bishop, D.G. Olson, C.L. Knipe: Pre-emulsified corn oil, pork fat, or added moisture affect quality of reduced fat bologna quality. *J Food Sci*. 1993, **58**, 484-487.

[20] D. Djordjevic, D.J. McClements, E.A. Decker: Oxidative stability of whey protein-stabilized oil-in-water emulsions at pH 3: Potential ω-3 fatty acid delivery systems (Part B). *J Food Sci.* 2004, **69**, C356-362.

[21] G. Delgado-Pando, S. Cofrades, C. Ruiz-Capillas, M.T. Solas, F. Jiménez Colmenero: Characteristics of oil (healthier lipid combination)-in-water emulsions prepared with various protein systems. An approach for the development of functional meat products. *Eur J Lipid Sci Technol*. in press.

[22] F. Jiménez-Colmenero, G. Barreto, N. Mota, J. Carballo.: Influence of protein and fat content and cooking temperature on texture and sensory evaluation of bologna sausage. *Lebensm. Wiss.-Technol.* 1995, **28**, 481-487.

[23] AOAC: Official methods of analysis of AOAC International. Association of Official Analytical Chemistry, Maryland (USA) 2000.

[24] E.G. Bligh, W.J. Dyer: A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* 1959, **37**, 911-917.

[25] T.L.V. Ulbricht, D.A.T. Southgate: Coronary heart-disease - 7 dietary factors. *Lancet*. 1991, **338**, 985-992.

[26] M.C. Bourne: Texture profile analysis. *Food Technol.* 1978, **32**, 62-65.

[27] J.D. Wood, R.I. Richardson, G.R. Nute, A.V. Fisher, M.M. Campo, E. Kasapidou, P.R. Sheard, M. Enser: Effects of fatty acids on meat quality: a review. *Meat Sci.* 2004, **66**, 21-32.

[28] F.B. Hu, J.E. Manson, W.C. Willett: Types of dietary fat and risk of coronary heart disease: A critical review. *J Am Coll Nutr.* 2001, **20**, 5-19.

[29] EFSA: Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the Commission related to nutrition claims concerning omega-3 fatty acids, monounsaturated fat, polyunsaturated fat and unsaturated fat. *The EFSA Journal*. 2005, **253**, 1-29.

[30] MAFF: Fatty acids. Supplement to McCance & Widdowson´s. The composition of foods. The Royal Society of Chemistry, Cambridge, and Ministry of Agriculture, Fisheries and Food, London 1998.

[31] A.J. McAfee, E.M. McSorley, G.J. Cuskelly, B.W. Moss, J.M.W. Wallace, M.P. Bonham, A.M. Fearon: Red meat consumption: An overview of the risks and benefits. *Meat Sci.* 2010, **84**, 1-13.

[32] H. Vural, I. Javidipour, O.O. Ozbas: Effects of interesterified vegetable oils and sugarbeet fiber on the quality of frankfurters. *Meat Sci.* 2004, **67**, 65-72.

[33] A. Serrano, S. Cofrades, C. Ruiz-Capillas, B. Olmedilla-Alonso, C. Herrero-Barbudo, F. Jimenez-Colmenero: Nutritional profile of restructured beef steak with added walnuts. *Meat Sci.* 2005, **70**, 647-654.

[34] I. Valencia, D. Ansorena, I. Astiasaran: Development of dry fermented sausages rich in docosahexaenoic acid with oil from the microalgae Schizochytrium sp.: Influence on nutritional properties, sensorial quality and oxidation stability. *Food Chem.* 2007, **104**, 1087-1096.

[35] OJEU: *Official Journal of the European Union*. 2010, **L37**, 18.

[36] K. Candogan, N. Kolsarici: The effects of carrageenan and pectin on some quality characteristics of low-fat beef frankfurters. *Meat Sci.* 2003, **64**, 199-206.

[37] S.C. Andres, M.E. Garcia, N.E. Zaritzky, A. Califano: Storage stability of low-fat chicken sausages. *J Food Eng.* 2006, **72**, 311-319.

[38] G. Hong, S. Lee, S. Min: Effects of replacement pork backfat with soybean oil on the quality characteristics of spredeable liver sausage. *Food Sci Biotechnol*. 2004, **13**, 51-56.

[39] Y.K. Su, J.A. Bowers, J.F. Zayas: Physical characteristics and microstructure of reduced-fat frankfurters as affected by salt and emulsified fat stabilized with nonmeat proteins. *J Food Sci.* 2000, **65**, 123-128.

[40] G. Feiner: Meat Products Handbook. Practical science and technology. Woodhead Publishing, Cambridge (England) 2006.

[41] E.J. Marquez, Ahmed, E. M., West, R. L., & Johnson, D. D.: Emulsion stability and sensory quality of beef frankfurters produced at different fat and peanut oil levels. *J Food Sci.* 1989, **54**, 867-870, 873.

[42] C.M. Calhoun, S.J. Eilert, R.W. Mandigo: Connective tissue/acidic phosphate preblend effects on reduced fat frankfurters. *J Food Sci.* 1996, **61**, 459-464.

[43] G. Hammer: Verabeitung pflanzlicher öle zu brüwurst. *Fleischwirtschaft*. 1991, **71**, 1248-1254.

[44] J. Carballo, P. Fernández, G. Barreto, M.T. Solas, F. Jiménez-Colmenero: Morphology and texture of bologna sausage containing different levels of fat, starch and egg white. *J Food Sci*. 1996, **61**, 652-655.

[45] J.R. Claus, M.C. Hunt, C.L. Kastner: Effects of substituting added water for fat on the textural, sensory, and processing characteristics of bologna. *J Muscle Foods*. 1989, **1**, 1-21.

[46] W.T. Kao, K.W. Lin: Quality of reduced-fat frankfurter modified by konjac-starch mixed gels. *J Food Sci.* 2006, **71**, S326-S332.

Table 1. Formulation [g] of different oil-in-water emulsions.

Samples	Oil combination†	Water	SPI†	MTG†	SC†
O/SC	789.47	631.58	-	-	78.95
O/SPI	789.47	631.58	78.95	-	-
O/SPI+SC+MTG	789.47	631.58	78.95	5.37	14.21

†O: oil combination (44.39 % olive oil, 37.87 % linseed oil and 17.74 % fish oil); SC: sodium caseinate; SPI: soy protein isolate; MTG: microbial transglutaminase.

Table 2. Formulation [g] of frankfurters made with pork backfat and the different oil-in-water emulsions described in Table 1.

Sample	Meat	Pork backfat	Oil-in-water emulsion			Water
			O/SC	O/SPI	O/SPI+SC+MTG	
Control	2569.4	477.4	-	-	-	840.6
F/SC	2569.4	-	805.1	-	-	513.0
F/SPI	2569.4	-	-	805.1	-	513.0
F/ SPI+SC+MTG	2569.4	-	-		805.1	513.0

Control: frankfurter formulated with pork backfat. F/SC, F/SPI and F/SPI+SC+MTG: frankfurters formulated with oil-in-water emulsion (O/SC, O/SPI and O/SPI+SC+MTG respectively) as pork backfat replacer. The following were also added to all samples: 2.0 g/100 g NaCl; 0.30 g/100 g sodium tripolyphosphate; 0.012 g/100 g sodium nitrite; 0.50 g/100 g flavouring.

Table 3. Proximate analysis [%] and energy content of frankfurters formulated with pork backfat and different oil-in-water emulsions[†]

Sample	**Control**	**F/SC**	**F/SPI**	**F/SPI + SC+MTG**
Moisture	61.77±0.15^{b}	60.77±0.15^{a}	62.21±0.35^{b}	60.83±0.44^{a}
Protein	17.89±0.16^{a}	19.39±0.28^{c}	17.80±0.16^{a}	18.54±0.40^{b}
Fat	12.30±0.73^{a}	12.05±0.15^{a}	11.39±0.48^{a}	11.33±0.06^{a}
Ash	3.32±0.01^{ab}	3.58±0.01^{c}	3.29±0.03^{a}	3.37±0.00^{b}
Energy content[‡]				
Total [kcal/100 g]	185.2	194.2	176.6	179.1
From fat [kcal/100 g] [§]	111.9 (60.4)	114.7 (59.0)	103.6 (58.6)	103.1 (57.6)
From oils [kcal/100 g][§]	-	91.8 (47.3)	82.9 (46.9)	82.5 (46.0)

[†] Control: frankfurter formulated with pork backfat. F/SC, F/SPI and F/SPI+SC+MTG: frankfurters formulated with oil-in-water emulsion (O/SC, O/SPI and O/SPI+SC+MTG respectively) as pork backfat replacer. Means ± standard deviation. Different letters in the same column indicate significant differences ($P<0.05$).

[‡]Calculation based on 9.1 kcal/g for fat, and 4.1 kcal/g for protein.

[§] Calculated on the basis of formulation (approximately 80 % of fat content in modified samples is healthier oil combination). In brackets, percentage of energy content.

Table 4. Fatty acid profile [as % of total fatty acids] and nutritional significant ratios of frankfurters[†].

Fatty acid	Frankfurters	
	Control	Modified
Myristic C 14:0	1.16±0.05[a]	1.11±0.03[a]
Palmitic C16:0	23.40±0.34[b]	11.42±0.14[a]
Stearic C18:0	13.78±0.79[b]	5.36±0.06[a]
Arachidic C20:0	0.25±0.01[a]	0,36±0.01[b]
Other SFAs	0.77±0.45[a]	1.09±0.53[a]
∑ SFA	39.36±1.19[b]	19.34±0.56[a]
Palmitoleic C16:1	1.84±0.07[b]	1.64±0.03[a]
Oleic C18:1n9	42.49±0.90[a]	42.52±0.39[a]
Vaccenic C18:1n7*c*	3.57±0.17[b]	2.32±0.02[a]
Eicosenoic C20:1n9c	1.17±0.03[b]	0.38±0.01[a]
Other MUFAs	0.46±0.19	-
∑ MUFA	49.53±0.96[b]	46.86±0.42[a]
Linoleic C18:2n6	8.62±0.28[a]	10.78±0.11[b]
Linolenic C18:3n3	0.64±0.05[a]	17,70±0.23[b]
Eicosadienoic C20:2n6	0.53±0.02	-
Arachidonic C20:4n6	0.35±0.07	-
Eicosapentaenoic C20:5n3	-	2.55±0.04
Docosapentaenoic C22:5n3	-	0.35±0.01
Docosahexaenoic C22:6n3	-	1.71±0.03
Other PUFAs	0.47±0.05[a]	0.52±0.02[b]
∑ PUFA	10.61±0.48[a]	33.61±0.31[b]
PUFA/SFA	0.27±0.02[a]	1.74±0.07[b]
∑ n-3	1.04±0.10[a]	22.83±0.28[b]
∑ n-6	9.56±0.39[a]	10.78±0.11[b]
n-6/n-3	9.20±0.58[b]	0.47±0.01[a]
Atherogenic index	0.47±0.02[b]	0.20±0.00[a]
Thrombogenic index	1.17±0.06[b]	0.18±0.00[a]

[†] Samples: Control, frankfurter formulated with all pork fat; Modified, frankfurters reformulated replacing pork backfat by an oil (44.39 % olive oil, 37.87 % linseed oil and 17.74 % fish oil)-in water emulsion. Since the three modified samples (F/SC, F/FSPI and F/SPI+SC+MTG) were formulated with the same lipid material, data reported are the mean values of these frankfurters.

Means ± standard deviation. Different letters in the same row indicate significant differences ($P<0.05$).

Table 5. Processing loss [%], purge loss [%] and pH of frankfurters formulated with pork backfat and different oil-in-water emulsions

Samples	pH	Processing loss	Purge loss (storage days at 2 °C)			
			1	13	27	41
Control	6.25 ± 0.01^{a}	17.45 ± 0.65^{a}	1.59 ± 0.37^{bA}	1.43 ± 0.18^{aA}	1.51 ± 0.17^{aA}	1.75 ± 0.48^{bA}
F/SC	6.44 ± 0.03^{b}	20.97 ± 0.81^{b}	1.04 ± 0.20^{aA}	1.36 ± 0.17^{aA}	1.29 ± 0.10^{aA}	1.33 ± 0.10^{aA}
F/SPI	6.45 ± 0.07^{b}	17.84 ± 0.68^{a}	1.06 ± 0.22^{aA}	1.58 ± 0.25^{aB}	1.47 ± 0.19^{aB}	1.62 ± 0.14^{abB}
F/ SPI+SC+MTG	6.46 ± 0.04^{b}	18.43 ± 0.94^{a}	1.20 ± 0.11^{abA}	1.30 ± 0.18^{aA}	1.41 ± 0.04^{aA}	1.37 ± 0.20^{abA}

Control: frankfurter formulated with pork backfat. F/SC, F/SPI and F/SPI+SC+MTG: frankfurters formulated with oil-in-water emulsion (O/SC, O/SPI and O/SPI+SC+MTG respectively) as pork backfat replacer. Means ± standard deviation. Different letters in the same column (a, b, c,..) and in the same row (A, B, C, ...) indicate significant differences ($P<0.05$).

Table 6. Colour parameters (L* lightness. a * redness and b* yellowness) of frankfurters formulated with pork backfat and different oil-in-water emulsions

	Sample	Storage at 2 °C (days)			
		1	13	27	41
L*	Control	71.24 ± 0.84^{aC}	64.28 ± 0.72^{aB}	62.15 ± 0.26^{aA}	62.94 ± 0.14^{aA}
	F/SC	72.34 ± 0.77^{bC}	66.06 ± 0.87^{bB}	63.13 ± 0.71^{bA}	63.12 ± 0.44^{abA}
	F/SPI	74.32 ± 0.52^{cC}	69.00 ± 0.50^{cB}	64.77 ± 0.55^{cA}	65.42 ± 0.58^{cA}
	F/ SPI+SC+MTG	73.46 ± 0.34^{cC}	66.52 ± 0.89^{bB}	64.14 ± 0.58^{cA}	63.99 ± 0.48^{bA}
a*	Control	8.42 ± 0.48^{aB}	8.32 ± 0.39^{aB}	7.86 ± 0.13^{abA}	7.69 ± 0.11^{aA}
	F/SC	9.81 ± 0.30^{cB}	9.71 ± 0.37^{cB}	8.27 ± 0.25^{bA}	8.42 ± 0.06^{bA}
	F/SPI	8.90 ± 0.27^{bB}	8.99 ± 0.13^{bB}	7.53 ± 0.20^{aA}	7.44 ± 0.12^{aA}
	F/ SPI+SC+MTG	9.12 ± 0.16^{bC}	8.62 ± 0.53^{abB}	7.51 ± 0.11^{aA}	7.62 ± 0.15^{aA}
b*	Control	10.11 ± 0.68^{aB}	8.07 ± 0.08^{aA}	8.38 ± 0.10^{aA}	8.05 ± 0.07^{aA}
	F/SC	11.26 ± 0.25^{bB}	10.05 ± 0.29^{bA}	9.73 ± 0.16^{bA}	9.70 ± 0.11^{bA}
	F/SPI	11.59 ± 0.15^{bcB}	10.56 ± 0.07^{cA}	10.25 ± 0.09^{cA}	10.26 ± 0.30^{cA}
	F/ SPI+SC+MTG	11.96 ± 0.09^{cB}	10.31 ± 0.24^{bcA}	10.19 ± 0.12^{cA}	10.12 ± 0.13^{cA}

Control: frankfurter formulated with pork backfat. F/SC, F/SPI and F/SPI+SC+MTG: frankfurters formulated with oil-in-water emulsion (O/SC, O/SPI and O/SPI+SC+MTG respectively) as pork backfat replacer. Means ± standard deviation. Different letters in the same column (a, b, c,..) and in the same row (A, B, C, ...) indicate significant differences ($P<0.05$).

Table 7. Texture Profile Analysis parameters of frankfurters formulated with pork backfat and different oil-in-water emulsions

	Sample	Storage at 2 °C (days)			
		1	13	27	41
Hardness [N]	Control	15.6 ± 0.6^{aA}	16.3 ± 0.4^{aAB}	17.8 ± 0.5^{aB}	15.1 ± 0.2^{aA}
	F/SC	24.6 ± 0.4^{bC}	23.5 ± 1.3^{bBC}	22.8 ± 0.7^{bAB}	21.9 ± 0.6^{cA}
	F/SPI	24.9 ± 1.9^{bB}	23.1 ± 0.7^{bA}	23.0 ± 0.7^{bA}	23.3 ± 0.7^{cA}
	F/ SPI +SC+MTG	23.8 ± 0.6^{bC}	22.7 ± 1.4^{bBC}	21.4 ± 0.7^{bB}	19.6 ± 0.7^{bA}
Springiness [mm]	Control	6.3 ± 0.1^{aA}	6.5 ± 0.1^{aB}	6.8 ± 0.2^{aC}	6.7 ± 0.0^{aC}
	F/SC	6.6 ± 0.1^{bA}	6.8 ± 0.0^{bB}	6.8 ± 0.0^{aB}	6.9 ± 0.0^{bB}
	F/SPI	6.7 ± 0.0^{bA}	6.8 ± 0.2^{bAB}	6.9 ± 0.0^{aB}	6.9 ± 0.0^{bB}
	F/ SPI+SC+MTG	6.8 ± 0.1^{bA}	6.9 ± 0.1^{bA}	6.9 ± 0.1^{aA}	6.8 ± 0.1^{abA}
Cohesiveness [dimensionless]	Control	0.680 ± 0.005^{aA}	0.691 ± 0.005^{aAB}	0.759 ± 0.073^{bC}	0.727 ± 0.002^{aBC}
	F/SC	0.680 ± 0.007^{aA}	0.696 ± 0.008^{abAB}	0.716 ± 0.003^{aAB}	0.722 ± 0.002^{aB}
	F/SPI	0.697 ± 0.003^{aA}	0.715 ± 0.005^{abAB}	0.762 ± 0.003^{abB}	0.733 ± 0.004^{aAB}
	F/ SPI+SC+MTG	0.715 ± 0.004^{aA}	0.728 ± 0.004^{bA}	0.745 ± 0.009^{abA}	0.747 ± 0.003^{aA}
Chewiness [N*mm]	Control	67.1 ± 3.4^{aA}	73.2 ± 2.0^{aA}	92.8 ± 11.1^{aB}	73.3 ± 0.4^{aA}
	F/SC	110.4 ± 2.6^{bA}	111.6 ± 5.6^{bA}	111.4 ± 2.8^{bA}	108.5 ± 2.6^{bA}
	F/SPI	117.2 ± 9.3^{bA}	112.6 ± 3.6^{bA}	116.7 ± 3.9^{bA}	118.6 ± 3.9^{cA}
	F/ SPI+SC+MTG	115.0 ± 4.0^{bB}	113.3 ± 7.5^{bB}	109.5 ± 5.3^{bAB}	100.1 ± 4.5^{bA}

Control: frankfurter formulated with pork backfat. F/SC, F/SPI and F/SPI+SC+MTG: frankfurters formulated with oil-in-water emulsion (O/SC, O/SPI and O/SPI+SC+MTG respectively) as pork backfat replacer. Means ± standard deviation. Different letters in the same column (a, b, c,..) and in the same row (A, B, C, ...) indicate significant differences ($P<0.05$).

Table 8. Sensory evaluation of frankfurters formulated with pork backfat and different oil-in-water emulsions.

Sample	Juiciness	Hardness	Off flavour	Texture	Flavour	Overall acceptability
Control	5.2±2.5^{a}	3.4±1.7^{a}	3.7±2.7^{a}	6.6±1.9^{b}	6.5±0.9^{a}	7.0±1.2^{b}
F/SC	3.0±2.0^{a}	6.5±1.4^{b}	3.9±2.4^{a}	3.1±1.7^{a}	5.6±1.6^{a}	5.5±1.4^{ab}
F/SPI	4.0±2.4^{a}	6.4±1.3^{b}	5.0±2.3^{a}	3.5±1.9^{a}	5.2±1.9^{a}	4.6±2.1^{a}
F/ SPI+SC+MTG	3.6±1.7^{a}	6.2±2.2^{b}	4.9±2.4^{a}	3.5±2.5^{a}	4.9±1.8^{a}	4.6±2.1^{a}

Control: frankfurter formulated with pork backfat. F/SC, F/SPI and F/SPI+SC+MTG: frankfurters formulated with oil-in-water emulsion (O/SC, O/SPI and O/SPI+SC+MTG respectively) as pork backfat replacer. Means ± standard deviation. Different letters in the same column indicate significant differences ($P<0.05$).