

Low-fat frankfurters formulated with a healthier lipid combination as functional ingredient: Microstructure, lipid oxidation, nitrite content, microbiological changes and biogenic amine formation.

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Key words: frankfurter, healthier oil combination, oil-in-water emulsion, meat-based functional foods, chilling storage, physicochemical properties

1 **Abstract**

2 Oil (healthier lipid combination of olive, linseed and fish oils)-in-water
3 emulsions stabilized with different protein systems (prepared with sodium caseinate
4 (SC), soy protein isolate (SPI), and microbial transglutaminase (MTG) were used as
5 pork backfat replacers in low-fat frankfurters. Microstructure, lipid oxidation, nitrite
6 content, microbiological changes and biogenic amine formation of frankfurters were
7 analysed as affected by the type of oil-in-water emulsion and by chilling storage (2 °C,
8 41 days). Although the lipid oxidation levels attained were low, replacement of animal
9 fat by healthier oil combinations in frankfurter formulation did promote a slight increase
10 in lipid oxidation. Residual nitrite was affected ($P<0.05$) by formulation and storage.
11 Only 51-61% of the added nitrite was detectable in the product after processing and 17-
12 46% at the end of storage. The microbial population was low in all formulations during
13 chilling storage. Spermine was the most abundant amine (19-20 mg/kg), but similar in
14 level in all samples.
15

1. Introduction

Healthier lipid formulation based on processing strategies is one of the most important current approaches to the development of potential meat based functional foods. Reformulation of frankfurters has been used to achieve better lipid compositions by reducing fat content and/or replacing 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 monounsaturated (MUFA) or polyunsaturated (PUFA) fatty acids, especially long chain n-3 PUFA, better n-6/n-3 PUFA and PUFA/SFA ratios, and if possible cholesterol-free (Jiménez-Colmenero, 2007). A number of studies have been conducted to improve the lipid profile of finely comminuted cooked meat products like frankfurters (Bloukas & Paneras, 1993; Jiménez-Colmenero, 2007; Paneras & Bloukas, 1994; Park, Rhee, Keeton, & Rhee, 1989). 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 profiles, meaning one more in line with health recommendations, can be achieved using healthier oil combinations as animal fat replacers (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2010b; García-Iníguez de Ciriano *et al.*, 2010; López-López, Cofrades, & Jiménez-Colmenero, 2009; Paneras, Bloukas, & Filis, 1998). Among the various technological options for replacement of animal fat, oil-in-water emulsion technology (pre-emulsion) has been shown to be viable as a mean of stabilizing the non-meat fats used for incorporation in meat derivatives (Bishop, Olson, & Knipe, 1993; Bloukas & Paneras, 1993; Djordjevic, McClements, & Decker, 2004; Jiménez-Colmenero, 2007). A number of procedures have been reported for producing an oil (plant or marine) pre-emulsion for incorporation in meat derivatives (Jiménez-Colmenero, 2007). Because they are added to frankfurters as fat ingredients, their physicochemical characteristics can affect their role in the meat system and hence the quality properties of the reformulated product (Delgado-Pando *et al.*, 2010b).

In a previous paper our group (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2010a) assessed the suitability of a healthier oil combination stabilized (oil-in-water emulsion) with various protein systems as pork backfat replacers in low-fat frankfurters. The healthier oil combination was formed by vegetable (olive and linseed) and fish oils in suitable amounts and proportions to produce a fatty acid profile more in line with healthier intake goals. The authors reported the nutritional

advantages (fatty acid profile), sensory analyses and technological properties of frankfurters as affected by the type of oil-in-water emulsion and chilling storage. Total n-3 PUFA of the reformulated products were around 2.5 g/100 g, of which approximately 2 g/100 g was α -linolenic acid and 500 mg/100 g were long chain n-3 PUFA, docosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), a composition more in line with dietary recommendations for optimal intake of total, saturated and unsaturated fatty acids. Technological properties and sensory characteristics show that it is possible to produce such healthier frankfurters. However, other aspects such as safety, shelf-life and morphological characteristics need to be considered in order to gain a clearer understanding of these products and a more accurate assessment of the suitability of this strategy for a healthier reformulation of frankfurters. To that end, in parallel to Delgado-Pando *et al.* (2010a), the additional studies described in this paper were carried out to assess the influence of the type of oil-in-water emulsion (as a pork backfat replacer) and chilling storage (41 days at 2 °C) on microstructure, lipid oxidation, nitrite content, microbiological changes and biogenic amine formation in frankfurters. As in the experiment reported in Delgado-Pando *et al.* (2010a), 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.

2. Materials and methods

2.1. Materials, healthy frankfurters preparation and chilled storage

The ingredients used for the manufacture of oil-in-water emulsions and frankfurters, the procedures for preparation of oil-in-water emulsions (Table 1), experimental design, preparation of healthy frankfurters (Table 2) and the chilling storage conditions were as reported by Delgado-Pando *et al.* (2010a). Four different formulations were studied (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 (Delgado-Pando *et al.*, 2010a). The samples were vacuum-packed, stored at 2 °C (\pm 1 °C) and analysed periodically (days 1, 13, 27 and 41).

2.2. Microstructure

Microstructure was analysed by scanning electron microscopy (SEM) as reported by Jiménez-Colmenero, Carballo and Solas (1995). The frankfurters were fixed with a mixture (1:1 v/v) of paraformaldehyde (4 g/100 g) and glutaraldehyde (0.2 g/100g) in 0.1 M phosphate buffer pH 7.2, post-fixed with OsO₄, washed, dehydrated in increasing concentrations of acetone, critical-point-dried, sputter-coated with gold/palladium in a metallizer (Blazer, SCD004) and scanned by SEM (Jeol, JSC 6400, Akishima, Tokyo, Japan) at 20 kV. A large number of micrographs were taken in order to select the most representative ones.

2.3. Lipid oxidation

Oxidative stability was evaluated by changes in thiobarbituric acid-reactive substances (TBARS). The procedure for measurement of TBARS was based on methods used by Serrano, Cofrades, and Jiménez-Colmenero (2006). Briefly, the procedure was as follows: 5 g of each sample was homogenized in 35 ml of 7.5 % trichloroacetic acid for 1 min at high speed in an Ultraturrax blender (Ika-Werke, GmbH & Co, Staufen, Germany). The blender sample was centrifuged (3000 g, 2 min) and 5 ml of the supernatant was mixed with 5 ml of 20 mM thiobarbituric acid; finally the solution was mixed and kept in the dark for 20 h at 20 ±1.5 °C. The pink colour that formed was measured spectrophotometrically (Lambda 15UV/VIS spectrophotometer, Perkin-Elmer, USA) at 532 nm. A calibration curve was plotted with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO, USA) to obtain the malonaldehyde (MDA) concentration and results were expressed as mg MDA/kg of sample. TBARS determinations for each sample were performed in duplicate.

2.4. Determination of residual nitrite

Residual nitrite contents were determined using the flow injection analysis according to Ruiz-Capillas, Aller-Guiote and Jiménez-Colmenero (2007a). Results, expressed as mg/1000 g of sample, were averages of 3 determinations per sample.

2.5. Microbiological analysis

Samples were prepared in a vertical laminar-flow cabinet (model AV 30/70, Telstar, Madrid, Spain). For each sample, 10 g (in replicate) was taken and placed 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 1 minute 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); De Man, Rogosa, Sharp 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 24h). The results were expressed as logarithms of colony forming units per gram (Log cfu/g).

2.6. Analysis of biogenic amines (BA) by ion-exchange chromatography

Tyramine, phenylethylamine, histamine, putrescine, cadaverine, agmatine, spermidine and spermine were determined in an extract prepared by blending 25 g of each sample with 50 mL of 7.5% trichloroacetic acid in an ultraturrax homogenizer (IKA-Werke, Janke, & Kunkel, Staufen, Germany) (20000 rpm, 3 min) and centrifuged at 5000 g for 15 min at 4 °C in a desktop centrifuge (Sorvall RTB6000B, DuPont, USA). The supernatants were filtered through a 0.45 µm Millipore filter, and 10 µL of this filtrate was injected into an HPLC model 1022 (Perkin Elmer) with a Pickering PCX 3100 post-column system (Pickering Laboratories, Mountain View, Ca, USA) following the methodology of Ruiz-Capillas and Moral (2001). The results are averages of at least 2 determinations from two extractions per sample.

2.7. Statistical analysis

The repeated measures test was used for statistical comparisons between samples (Delgado-Pando *et al.*, 2010a). 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

It has been suggested that foods that are strategically or naturally enriched in healthier fatty acids can be used to achieve desired health benefits without the intake of supplements or changes in dietary habit. In a previous paper Delgado-Pando *et al.* (2010a) reported the proximate composition and fatty acid profile, sensory analyses and processing and purge losses, pH, texture profile analysis and colour of frankfurters as affected by the type of oil-in-water emulsion (used as functional ingredients) and

chilling storage (41 days at 2 °C). The following sections deal with different aspects relating to morphological, safety and shelf-life characteristics that are essential to gain a clearer understanding of these products.

3.1. Microstructure

The morphology of the control sausages (Figure 1a) was characteristic of cooked gel/emulsion systems (Carballo, Fernandez, Barreto, Solas, & Jiménez-Colmenero, 1996a; Carballo, Fernández, Barreto, Solas, & Jiménez-Colmenero, 1996b; Katsaras & Peetz, 1989) showing the formation of numerous cavities, producing structures with a spongy (honeycomb-like) appearance. The formation of these cavities may have been due to expansion of a number of constituents, mainly fat, water or air (Cavestany, Jiménez-Colmenero, Solas, & Carballo, 1994; Katsaras & Peetz, 1989).

The morphology of the reformulated frankfurters indicates that the characteristics of the continuous protein matrix and the fat globules are affected by the type of oil-in-water emulsions used in the product formulation. Thus, the matrix generally becomes disorganized and loses some of its spongy appearance (few cavities), showing a more continuous and compact structure. This is particularly apparent in samples F/SC and F/SPI (For sample formulation see Table 2) (Fig. 1b-c). The microstructure of sample (F/SPI+SC+MTG) (For sample formulation see Table 2) formulated with oil-in-water emulsions stabilized using MTG (Fig. 1d) revealed more of a spongy structure (as compared with F/SC and F/SPI), with more cavity formation but was clearly morphologically different (generally smaller) from the control. Similar findings on microstructure have been reported using olive-oil-in-water emulsions as pork backfat replacers (Jiménez-Colmenero, Herrero, Pintado, Solas, & Ruiz-Capillas, 2010), these morphological differences were attributed to variations in the physicochemical characteristics of the oil-in-water emulsions. The morphological characteristics observed in the frankfurters containing the healthier oil combination conferred greater consistency on the product and promoted textural changes, so that these were harder and chewier than the control sample. However the physicochemical characteristics of the oil-in-water had no clear effect on textural properties of the frankfurters (Delgado-Pando *et al.*, 2010b). Since all the frankfurters had very similar compositions (Delgado-Pando *et al.*, 2010a), other factors associated with the nature of the matrix and dependent on meat batter characteristics must be implicated in the morphology-texture relationship (Carballo *et al.*, 1996b), in this case the type of lipid materials. Similar microstructural results have been

reported (Cáceres, García, & Selgas, 2008) as a result of addition of pre-emulsified fish oil (with caseinates) to bologna-type sausage.

3.2. Lipid oxidation

Lipid oxidation is a major cause of deterioration in the quality of stored meat products. One of the main potential problems associated with healthier lipid formulations in meat products is how they may influence the rate and extent of lipid oxidation, which in turn affects quality characteristics and has health implications. There are a number of factors determining the scale of this phenomenon. Susceptibility to lipid oxidation can be augmented by increasing concentrations of unsaturated fatty acids (particularly polyunsaturated), and also by some processing conditions like grinding, cooking, drying, etc. which entail exposure to high temperatures, decompartmentalization of prooxidants and antioxidants or enhanced access of oxygen to the substrate (Lee, Choi, & Moon, 2006).

TBARS values were affected ($P < 0.05$) by the formulation and storage (Table 3), with interaction ($P < 0.05$) between both factors. From the outset of storage TBARS values were higher ($P < 0.05$) in the reformulated samples than in the control (Table 3), indicating a higher rate and a greater extent of lipid oxidation in healthier lipid frankfurters (with higher levels of unsaturates). As reported previously (Delgado-Pando *et al.*, 2010a), in the experimental conditions 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). However, in these products the lipid oxidation varied according to the type of systems used to stabilize the oil (healthier lipid combination)-in-water emulsions used as pork backfat replacers (Table 2). For instance, the use of SPI+SC+MTG produced the highest oxidation levels at all times throughout storage. Except in sample F/SPI+SC+MTG, the TBARS value increased ($P < 0.05$) over storage time (up to day 13 for control and day 27 for F/SC and F/SPI), then decreased as from day 41. Thus, lipid oxidation was greater when MTG was included in the stabilization system of oil-in-water emulsion. The pattern of TBARS behaviour during storage (a peak followed by a decline of the TBARS value) has been reported during frozen storage of ground beef (Bhattacharya, Hanna, &

Mandigo, 1988; Brewer & Wu, 1993). It has been suggested that the decline in TBARS values may have been due to the formation of malonaldehyde as an intermediate product; up to a certain time, the rate of malonaldehyde formation was greater than the rate of disappearance of the product, and after that point the reverse was true. Thus, the rate of disappearance exceeded the rate of formation and hence the TBARS values decreased (Bhattacharya *et al.*, 1988). Similarly, Jamora and Rhee (2002) reported that the malonaldehyde formed during storage of meat products might have undergone intermolecular reactions (polymerization) and reactions with other constituents, especially amino acids/proteins. Therefore the rate of malonaldehyde loss/disappearance during storage may have exceeded the rate of production through lipid oxidation.

Lipid oxidation in healthier fat meat product formulations varies according to the nature of the product, the type, amount and means of addition of non-meat fats, and the antioxidative system used to control rancidity development (Jiménez-Colmenero, 2007). Generally no specific problems have been reported in connection with oxidative stability in gel/emulsion meat based products formulated with healthier lipid profiles. This fact has been put down to a variety of factors: the presence of a curing mixture ingredient containing substances such as nitrite, phosphate or ascorbate which act as antioxidants (Márquez, Ahmed, West, & Johnson, 1989); the natural presence of various antioxidant substances (tocopherols, phenolic compounds) in the plant oils used—for example olive oil (Bloukas, Paneras, & Fournitzis, 1997a) or corn oil (Bishop *et al.*, 1993)—or finally the absence of phospholipids in refined oils (Bishop *et al.*, 1993). Cáceres *et al.* (2008) reported a low level of lipid oxidation (TBARS value of 0.37-0.52 mg MDA/kg) during chilling of bologna-type sausage prepared with fish oil. In the present experiment, although the use of a healthier oil combination to replace animal fat in frankfurter formulation promoted slightly (but significantly over storage) more lipid oxidation than in the control sample (Table 3), the observed TBARS values (< 0.5 mg/kg in all samples except F/SPI+SC+MTG, where it was <0.8) were lower than those described as the minimum needed to detect objectionable flavours in processed meat products (Cáceres *et al.*, 2008; Liu, Lee, & Damodaran, 1999; Mercadante, Capitani, Decker, & Castro, 2010).

Comminuted meat systems contain salt and relatively high concentrations of unsaturated fat. When subjected to thermal treatment, they become prone to lipid oxidation behaviour associated (among other factors) with the pro-oxidative activity of non-heme iron, which catalyzes lipid oxidation in this biological tissue (Bastida *et al.*,

2009). There are several factors that may be implicated in the relatively low lipid oxidation of healthier reformulated frankfurters like the ones studied in this experiment. One may be related to the combined effect of the antioxidants used in sausage manufacture (nitrite) and included in the vegetable and fish (combination of tocopherols) oils used in product manufacture. Another may be related to the protective effect of sodium caseinate and soy protein isolate against lipid oxidation in oil-in-water emulsions through a combination of free radical scavenging and/or metal chelation (Faraji, McClements, & Decker, 2004). It has been reported that SPI has greater oxidative stability than SC, since other antioxidants associated with this protein (such as the isoflavone) could also act as antioxidants (Faraji *et al.*, 2004). This would help to explain the lower rate of lipid oxidation in F/SPI frankfurter as compared to F/SC (Table 3), while the fact that oxidative activity was greatest in F/SPI+SC+MTG sample could be related to interference by transglutaminase in any of the antioxidative mechanisms of those proteins, limiting their ability to inhibit lipid oxidation. This effect may be more pronounced with SC (Table 3) since this protein appears to be a better substrate for MTG (formation of microbial transglutaminase-catalyzed protein crosslinking) than soy protein (Motoki & Seguro, 1998).

3.3 Residual nitrite

Recently, interest in nitrite and its reaction has re-emerged because of its implications for human health (Cassens, 1997). Residual nitrite was affected ($P<0.05$) by the formulation and storage (Table 4), with interaction ($P<0.05$) between both factors. As expected, residual nitrite levels decreased over storage in all samples. Only 51-61% of the added nitrite was detectable in the final product after processing and 17-46% after storage for 41 days at 2 °C (Table 4). Of the healthier lipid formulations, F/SPI+SC+MTG frankfurter had the highest ($P<0.05$) residual nitrite level over storage. Many studies have demonstrated that the added nitrite is rapidly depleted in meat products since nitrite reacts with or binds to constituents (lipids, proteins, etc) of the meat (Carballo, Cavestany, & Jiménez-Colmenero, 1991; Cassens, Greaser, Ito, & Lee, 1979; EFSA, 2003). The rate of loss of nitrite in a product is dependent on a number of factors including the heat process used, the pH of the product, the storage temperature and the addition of ascorbic acid or other reducing agents (Cassens, 1997; EFSA, 2003). In our experimental conditions the lipid material is the main differences in sample formulations (Delgado-Pando *et al.*, 2010a) and the nitrite reacts with lipid components

of meat (Cassens *et al.*, 1979), and therefore the lipid profile can be related to the differences in the residual nitrite behaviour in frankfurters. Several studies have reported the influence of aspects associated with the content and characteristics of the lipid in meat products. Higher residual nitrite levels have been reported in low-fat sausages as compared with high-fat products (Ayo *et al.*, 2007; Jiménez-Colmenero, Carballo, Fernandez, Cofrades, & Cortes, 1997; Jiménez-Colmenero *et al.*, 2010). Unlike the present experiment (Table 4), others have reported that replacement of pork fat by olive oil reduced the residual nitrite as compared with all pork fat sample (López-López, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2009). However, Paneras and Bloukas (1994) found no differences in the nitrite content of frankfurters with vegetable oils.

3.4 Microbiological analysis

Microbiological considerations during chilling storage are known to affect the stability and shelf life of meat products, but there has been hardly any research on reformulated meat products with oils added (Bloukas, Paneras, & Fournitzis, 1997b). Microbial counts were affected by the storage and formulation (Fig. 2). The initial levels of TVC were very low (< 3 Log cfu/g) in all samples (Fig. 2a). This flora consisted mainly of LAB (Fig. 2b), which traditionally predominate in vacuum-packed cooked meat products (Andrés, García, Zaritzky, & Califano, 2006; Holley, 1997; Ruiz-Capillas, Carballo, & Jiménez-Colmenero, 2007b). *Enterobacteriaceae* levels were lower than 1 Log cfu/g over storage. The levels of these microorganisms were within the legal limits and comparable to those detected in other assays on frankfurter and bologna (Andrés *et al.*, 2006; Holley, 1997; Ruiz-Capillas *et al.*, 2007b). This result shows that the mode of product preparation follows good manufacturing practice.

The microbiological changes taking place in stored low-fat frankfurters was most noticeable in F/SC and F/SPI products. The greatest increase in microorganism growth in F/SC and F/SPI products was observed after 27 days, when TVC values exceeded 5 Log cfu/g and LAB levels exceeded 4 Log cfu/g. These were also the products that presented the highest microorganism levels at the end of storage, contrasting with F/SPI+SC+MTG and control samples, where microorganism levels remained low (< 3 Log cfu/g). Similarly, Ruiz-Capillas *et al.* (2007b) reported no changes in the low microorganism levels in vacuum-packed frankfurter until day 48 of chilling storage at 2 °C. Andrés *et al.* (2006) reported microorganism levels of less than 7 log cfc/g in low fat chicken after 50 days of chilling storage at 4 °C. According to

these authors, low fat associated with high moisture provides a better environment for bacterial growth. Bloukas *et al.* (1997b), observed similar microbial behaviour to that reported in the present experiment in low fat frankfurters with olive oil during chilling storage.

Processing (thermal treatment and storage (vacuum-packed, at 2 °C)) conditions account for the fact that the microbial quality of these products was generally adequate.

3.5 Biogenic amines

Biogenic amines (BA) occur in a wide range of foods, among them meat and meat products. These compounds are of interest for two reasons: firstly as possible quality indicators, and secondly because high levels of dietary BA can present a toxic risk to certain consumers (Ruiz-Capillas & Jiménez-Colmenero, 2004). There are numerous studies of BA in meat products (fresh, fermented, cooked, etc) (Ruiz-Capillas *et al.*, 2007b), but the authors know of no studies that analyse the formation of BA in healthier lipid meat products. Factors associated with the reformulation process (ingredient modifications, handling conditions, etc.) decisively influence the factors responsible for the formation of BA such as microorganisms (Lactic acid bacteria, *enterobacteriaceae*, *micrococaceae*), pH, free amino acids, etc., and hence their profile and final concentrations. It is essential to understand how BA formation is affected by the reformulation process in order to assess its potential presence in these healthy meat products.

BA contents in frankfurters (Table 5) were affected ($P < 0.05$) by the storage and formulation, with interaction ($P < 0.05$) between both factors. Putrescine levels were very low, less than 0.5 mg/kg, in all samples. Low putrescine levels have been reported in cooked products such as frankfurters and meat batters (Ruiz-Capillas, Aller-Guiote, Carballo, & Jiménez-Colmenero, 2006; Ruiz-Capillas *et al.*, 2007b). Spermine was the most abundant BA (19-20 mg/kg), and there were generally no differences ($P > 0.05$) in levels between formulations (Table 5). One factor that may explain this is that spermine comes chiefly from the meat that is used. Similar levels of spermine have been found in frankfurters and meat batters (Ruiz-Capillas *et al.*, 2006; Ruiz-Capillas *et al.*, 2007b). In the case of spermidine, initial levels were very low, < 0.80 mg/kg, as compared to control sample, but levels in the reformulated products were lower ($P < 0.05$) (Table 5). There were high levels of agmatine and phenylethylamine, as is usual in meat and frankfurter-type meat products (Ruiz-Capillas *et al.*, 2006), although the agmatine levels

were lower than reported in other studies (Ruiz-Capillas *et al.*, 2007b). The initial levels of tyramine and cadaverine were low (around 2 and 1 mg/kg respectively). Also low was histamine (<1 mg/kg), which is not found in appreciable quantities in meat products (Halász, Barath, Simonsarkadi, & Holzapfel, 1994; Ruiz-Capillas *et al.*, 2006; Ruiz-Capillas *et al.*, 2007b; Ruiz-Capillas & Jiménez-Colmenero, 2004).

The most appreciable changes in the course of storage occurred in tyramine and cadaverine levels, especially at the end of storage (Table 5), mainly as a consequence of microbial activity (Figure 2). For instance, F/SC and F/SPI frankfurters, the ones with the highest TVC levels (Figure 2), presented higher ($P<0.05$) levels of tyramine and cadaverine at the end of storage. On the other hand, the control and F/SPI+SC+MTG samples present similar patterns to one another throughout storage (Table 5), and TVC and LAB levels were also similar (Figure 2). Similar behaviour has been reported in other experiments with frankfurters (Ruiz-Capillas *et al.*, 2006; Ruiz-Capillas *et al.*, 2007b)

Conclusions

Low-fat frankfurters can be manufactured using a healthier oil (from plant and marine sources) combination stabilized with different non meat protein systems as pork backfat replacers, to give a product with healthy lipid content (amount and fatty acid profile). The reformulation process and chilling storage affect product characteristics such as matrix morphology, lipid oxidation, residual nitrite, microbial population and BA formation, but they do not produce safety issues or shelf-life constraints in frankfurters. This in addition to the nutritional advantages (fatty acid profile), sensory attributes and technological properties (Delgado-Pando *et al.*, 2010a) suggests that this can be a suitable strategy for the manufacture healthier frankfurters (potential functional food).

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.

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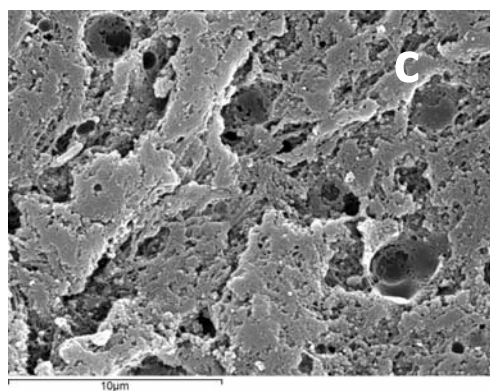
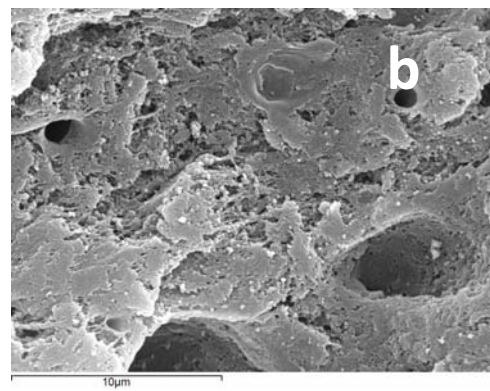
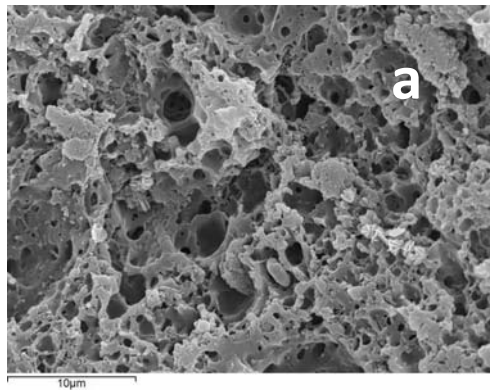
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Figure legends.

Figure 1. Scanning electron micrograph of the different frankfurters: a) Control, b) F/SC, c) F/SPI, d) F/SPI+ SC+MTG. Bar represents 10 μm .

Figure 2. Microorganism counts (a: Total viable count. b: Lactic acid bacteria) in frankfurters during chilling storage.

Figure 1. Delgado-Pando et al.,



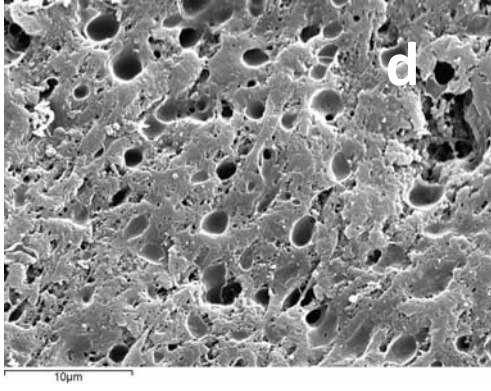


Figure 2. Delgado-Pando et al.,

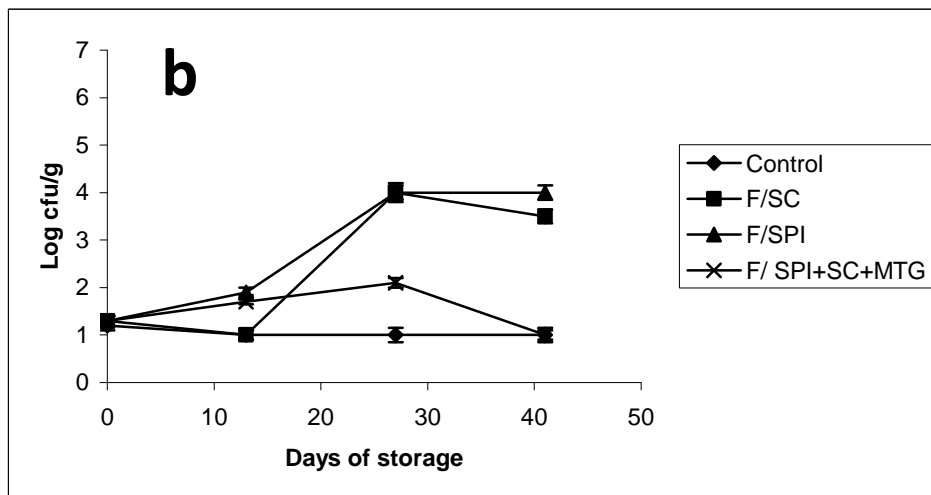
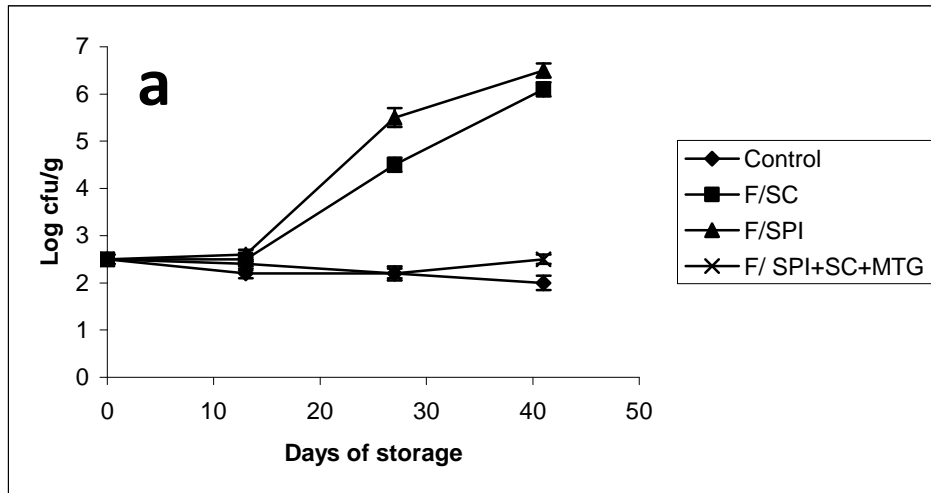


Table 1. Formulation (g) of different oil-in-water emulsions.

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.

Sample	Meat	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. Lipid oxidation (TBARS values, expressed as mg MDA/kg sample) of frankfurters during chilling storage

Sample	Storage (days)			
	1	13	27	41
Control	0.037±0.018 ^{aA}	0.120±0.015 ^{aC}	0.064±0.009 ^{aB}	0.022±0.010 ^{aA}
F/SC	0.164±0.022 ^{cA}	0.424±0.023 ^{cB}	0.498±0.011 ^{cC}	0.419±0.005 ^{cB}
F/SPI	0.102±0.012 ^{bA}	0.352±0.011 ^{bC}	0.400±0.013 ^{bD}	0.313±0.023 ^{bB}
F/SPI+SC+MTG	0.296±0.050 ^{dA}	0.625±0.024 ^{dB}	0.756±0.013 ^{dC}	0.754±0.006 ^{dC}

For sample formulation see Table 2. Mean ± SD. Different letters in the same column (a,b,c...) and in the same row (A,B,C,..) indicate significant differences (P<0.05).

Tabla 4- Concentration of residual nitrite (mg/kg of sample) in frankfurters during chilling storage.

Samples	Storage (days)			
	1	13	27	41
Control	62.80± 3.26 ^{aD}	53.17±0.39 ^{aC}	46.93±0.34 ^{aB}	21.16±0.79 ^{aA}
F/SC	68.57±0.86 ^{bD}	62.69±0.41 ^{bC}	56.09±0.33 ^{bB}	50.18±0.44 ^{bA}
F/SPI	71.01±1.25 ^{cC}	64.49±0.51 ^{bB}	55.23±0.63 ^{bA}	54.41±0.42 ^{cA}
F/SPI+SC+MTG	73.24±1.77 ^{dC}	67.78±0.00 ^{cB}	57.31±1.25 ^{cA}	55.54±1.91 ^{cA}

For sample formulation see Table 2. Mean ± SD. Different letters in the same column (a,b,c...) and in the same row (A,B,C,..) indicate significant differences (P<0.05).

Table 5. Biogenic amines content (mg/kg) in frankfurters during chilled storage.

Biogenic amines	Sample	Storage (days)			
		1	13	27	41
Tyramine	Control	2.48±0.53 ^{aA}	2.57±0.29 ^{bA}	2.08±0.22 ^{bA}	1.86±0.03 ^{aBC}
	F/SC	2.32±0.49 ^{aC}	1.67±0.02 ^{aAB}	1.28±0.10 ^{aA}	3.46±0.159 ^{bB}
	F/SPI	2.41±0.28 ^{aB}	2.07±0.19 ^{abAB}	1.82±0.02 ^{abA}	5.65±0.05 ^{cC}
	F/ SPI+SC+MTG	1.93±0.31 ^{aA}	2.17±0.22 ^{abA}	2.12±0.07 ^{bA}	1.80±0.02 ^{aA}
Phenylethylamine	Control	7.97±0.11 ^{bD}	5.68±0.03 ^{bC}	1.78±0.01 ^{aA}	2.96±0.06 ^{aB}
	F/SC	9.68±0.29 ^{cC}	5.81±0.03 ^{bB}	13.31±0.16 ^{dD}	5.01±0.22 ^{cA}
	F/SPI	7.21±0.02 ^{aC}	5.87±0.07 ^{bB}	8.14±0.16 ^{cD}	3.37±0.02 ^{bA}
	F/ SPI+SC+MTG	9.60±0.07 ^{cD}	4.81±0.01 ^{aB}	2.40±0.21 ^{bA}	7.11±0.05 ^{dC}
Histamine	Control	1.45±0.06 ^{aC}	0.90±0.05 ^{aB}	0.00±0.00 ^{aA}	0.08±0.01 ^{aA}
	F/SC	2.15±0.01 ^{bC}	0.97±0.08 ^{aB}	2.95±0.47 ^{cD}	0.39±0.04 ^{aA}
	F/SPI	1.27±0.04 ^{aBC}	0.98±0.07 ^{aB}	1.56±0.24 ^{bC}	0.12±0.01 ^{aA}
	F/ SPI+SC+MTG	2.22±0.00 ^{bD}	0.69±0.05 ^{aB}	0.08±0.01 ^{aA}	1.05±0.01 ^{bC}
Cadaverine	Control	1.18±0.06 ^{bA}	2.01±0.34 ^{bB}	2.82±0.34 ^{bC}	3.57±0.21 ^{aD}
	F/SC	1.53±0.10 ^{bA}	1.94±0.05 ^{bA}	3.46±0.11 ^{bB}	5.33±0.06 ^{bC}
	F/SPI	1.15±0.07 ^{bA}	2.46±0.30 ^{cB}	3.71±0.31 ^{cC}	5.90±0.10 ^{cD}
	F/ SPI+SC+MTG	0.38±0.02 ^{aA}	0.85±0.01 ^{aB}	1.85±0.06 ^{aC}	3.89±0.06 ^{aD}
Agmatine	Control	7.06±0.01 ^{aBC}	6.66±0.07 ^{aA}	7.04±0.03 ^{aB}	7.22±0.02 ^{aC}
	F/SC	9.37±0.24 ^{dC}	9.17±0.09 ^{dB}	8.90±0.03 ^{cA}	8.96±0.07 ^{cA}
	F/SPI	8.17±0.04 ^{cAB}	8.06±0.01 ^{cA}	8.60±0.03 ^{bC}	8.31±0.05 ^{bB}
	F/ SPI+SC+MTG	7.25±0.00 ^{bB}	7.51±0.07 ^{bC}	7.03±0.05 ^{aA}	7.20±0.05 ^{aAB}
Spermidine	Control	0.78±0.07 ^{bB}	0.69±0.01 ^{bB}	0.59±0.16 ^{bAB}	0.48±0.01 ^{aA}
	F/SC	0.52±0.02 ^{aA}	0.37±0.09 ^{aA}	0.37±0.00 ^{aA}	0.51±0.04 ^{aA}
	F/SPI	0.50±0.03 ^{aA}	0.73±0.01 ^{bB}	0.78±0.01 ^{cBC}	0.92±0.01 ^{bC}
	F/ SPI+SC+MTG	0.55±0.19 ^{aA}	0.62±0.13 ^{bA}	0.53±0.07 ^{abA}	0.85±0.02 ^{bB}
Spermine	Control	20.55±0.07 ^{abAB}	19.32±0.23 ^{aA}	21.04±0.91 ^{bB}	20.48±0.98 ^{aAB}
	F/SC	21.68±0.52 ^{bA}	21.28±0.45 ^{bA}	21.30±1.05 ^{bA}	20.77±0.76 ^{aA}
	F/SPI	20.05±0.17 ^{aA}	19.95±0.13 ^{abA}	22.24±1.05 ^{bB}	21.54±0.19 ^{aB}
	F/ SPI+SC+MTG	19.36±0.04 ^{aA}	19.58±0.37 ^{aAB}	19.40±0.69 ^{aA}	20.94±0.38 ^{aB}

For sample formulation see Table 2. Mean ± SD. Different letters in the same column (a,b,c,...) and in the same row (A,B,C,...) indicate significant differences (P<0.05).