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# 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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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. Microstructure, lipid oxidation, nitrite content, microbiological changes and biogenic amine formation of frankfurters were analyzed and found to be affected by the type of oil-in-water emulsion and by chilling storage (2 °C, 41 days). Although the lipid oxidation levels attained were low, replacement of animal fat by healthier oil combinations in frankfurter formulation did promote a slight increase in lipid oxidation. Residual nitrite was affected ($P<0.05$) by formulation and storage. Only 51–61% of the added nitrite was detectable in the product after processing and 17–46% at the end of storage. The microbial population was low in all formulations during chilling storage. Spermine was the most abundant amine (19–20 mg/kg), but similar in level to all samples.
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## 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 polyunsaturatyed (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, 2010; 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 means of stabilizing the non-meat fats used for incorporation in meat derivates (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, Cofrades, Ruiz-Capillas, Solas, et al., 2010).

In a previous paper our group (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2010) 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

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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, Cofrades, Ruiz-Capillas, and Jimenez-Colmenero (2010), 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, Cofrades, Ruiz-Capillas, and Jimenez-Colmenero (2010), 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, Cofrades, Ruiz-Capillas, and Jimenez-Colmenero (2010). 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, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2010). The samples were vacuum-packed, stored at 2 °C (±1 °C) and analyzed periodically (days 1, 13, 27 and 41).

### 2.2. Microstructure

Microstructure was analyzed 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/100 g) in 0.1 M phosphate buffer pH 7.2, post-fixed with $OsO_4$, 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.

**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 flavoring.

### 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 color that formed was measured spectrophotometrically (Lambda 15 UV/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 min in a stomacher blender (Colworth 400, Seward, London, UK), appropriate decimal dilutions were pour-plated on the following media: Plate Count Agar (PCA) (Merck, Germany) for total viable count (TVC) (30 °C for 72 h); 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 24 h). 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) (20,000 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

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