A healthier oil combination and konjac gel as functional ingredients in low-fat pork liver pâté

Abstract

Healthier lipid pâtés were formulated by reducing the fat content and/or replacing the pork backfat by a combination of healthier oils (olive, linseed and fish oils) and konjac gel (0-15 %). The reformulation results were evaluated by composition (proximate analysis and fatty acid profile), technological properties (emulsion stability, colour, texture), microbiological and sensory parameters of the pâtés. Pâtés with partial or total replacement of pork backfat had lower levels of saturated fatty acids (SFA) (27.4% and 21.3%) and monounsaturated fatty acids (MUFA) (49.8% and 42.5%), and higher levels of polyunsaturated fatty acids (PUFA) (22.4% and 35.6%) compared with control pâtés (32.2%, 58.2% and 9.04% respectively). The n-6/n-3 PUFA ratio was decreased from 6.78 (in control pâtés) to 0.79 and 0.48 when partial and total pork backfat respectively was replaced by a healthier oil combination. Although emulsion stability was affected by the formulation, in general all the pâtés had good fat and water binding properties. Pork fat replacement produced lighter ($P<0.05$) and less red ($P<0.05$) pâté. The fat reduction produced a softer and more spreadable pâté, although no effect on penetration parameters was observed after by pork fat replacement by a healthier oil combination. The addition of 15 % of konjac gel produced stiffer structures (as compared with 0 and 7 %) which are very close to those of the control samples. No microbiological limitations were produced by the reformulation process, obtaining pâtés with acceptable sensory characteristics, similar to the control sample.

INTRODUCTION

Pâté is a very popular and cheap cooked meat product manufactured worldwide. In some countries liver pâtés are traditional products with good sensory properties, which form part of the gastronomic culture, with high levels of consumption as in e.g. Spain, France, Germany and Denmark (Fernández-López, Sayas-Barberá, Sendra, & Pérez-Álvarez, 2004; Martín, Antequera, Muriel, Pérez-Palacios, & Ruiz, 2009). However, these products present some negative health concerns related to their high fat (between 25-40 %) and energetic content (around 300-400 kcal/100 g) and the fatty acid profiles of animal fat. As in other meat products with similar characteristics, (Jiménez-Colmenero, 2007), pâté reformulation processes have been used to achieve better lipid compositions by reducing the fat content and/or replacing the animal fat normally present in the product with another fat with characteristics more in line with health recommendations. The rationale behind lipid modification to improve consumer health is that reducing saturated fatty acid (SFA) concentrations and increasing monounsaturated fatty acid (MUFA) and polyunsaturated fatty acids (PUFA) content, especially n-3 PUFA, will reduce the n-6/n-3 PUFA ratio (Simopoulos, 2002; WHO, 2003) .

A number of studies have been conducted to reduce fat in pâté. Pork backfat replacement by muscle was used to reduce the fat content from normal (31%) to medium (26%) and low (20%) levels (Estévez, Ventanas, & Cava, 2005). Globin and plasma have been used as fat replacements, reducing the fat content in ham pâté from 25 to 16 % (Viana, Silva, Delvivo, Bizzotto, & Silvestre, 2005). Very low fat (3 %) liver pâté and ham pâté were produced with potato flour as fat substitute (Kaack & Pedersen, 2005). Other studies have been also carried out to improve the fatty acid profile of these products. Pork liver pâté (with 39% fat content) with a healthier ω-6/ω-3 PUFA ratio has been manufactured using backfat and meat enriched with ω-3 PUFA, obtained from pigs fed on linseed oil-enriched diets (D'Arrigo et al., 2004). Very low fat (2%) liver pâté from pigs fed on conjugated linoleic acid (CLA) and MUFA was produced without pork lard in the formulation (Martín et al., 2009). Partial replacement of pork fat by CLA and/or olive oil (rich in oleic acid) or soybean oil (with high PUFA) has been used to produce liver pâté (with 24-29% fat content) or spreadable liver sausage (30-32 % fat content), respectively (Hong, Lee, & Min, 2004; Martín, Ruiz, Kivikari, & Puolanne, 2008).

The addition of individual lipids (of plant or marine origin) does improve the fatty acid profile of meat products, but a better approximation to an optimal lipid profile from a health standpoint 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, Ruiz-Capillas, & Jiménez-Colmenero, 2009; Paneras, Bloukas, & Filis, 1998). In a previous paper (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, in press) our group assessed the suitability of oil (healthier lipid combination)-in-water emulsion as pork backfat replacement in frankfurters. The healthier lipid combination was formed by vegetable oils (olive and linseed) and fish oils in suitable amounts and proportions to provide a fatty acid profile better adjusted to healthier intake goals. This oil combination was designed to produce a lipid material 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 (Delgado-Pando et al., 2010). As far as the authors are aware, the use of a healthier oil combination as a functional ingredient in the development of low-fat pâté has not been explored.

Since the processes of reduction and replacement of animal fat with other more unsaturated fats may have implications for the technical and sensorial properties, different strategies have been used to modulate their consequences (Jiménez-Colmenero, 2000). Konjac glucomannan is a neutral polysaccharide produced from the tuber *Amorphophallus konjac.* It is widely used in Asia, where it has been known for centuries. The interest of this ingredient lies in its important technological properties (water retention capacity, as a gelling and thickening agent) and potential health implications (e.g. reducing cholesterol, insulin and glucose levels or its satiating and laxative effect). This is why its use as a fat substitute has been tested in the formulation of low-fat meat products such as frankfurter or bologna sausage (Chin, Keeton, Miller, Longnecker, & Lamkey, 2000; Jiménez-Colmenero et al., 2010; Kao & Lin, 2006; Lin & Huang, 2003, entre otros). We are not aware of its application to pâté-type products.

Although many studies relate to developing healthier lipid formulation in meat products based on processing strategies (Jiménez-Colmenero, 2007), there are very few related to pâtés, particularly with simultaneous tests reducing fat content and replacing the animal fat with another fat which is more compatible with health recommendations. The aim of this paper is to evaluate the nutritional and technological results of a reformulation process for pâtés to produce better lipid compositions by reducing fat content and/or replacing the pork backfat by an oil (healthier lipid combination)-in-water emulsion and konjac gel in different proportions. The healthier lipid combination was 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 (Delgado-Pando et al., in press).

2. Materials and methods

2.1. Materials, oil-in-water emulsion and konjac gel preparation

Fresh post-rigor pork meat (mixture of *M. biceps femoris*, *M. semimembranosus*, *M. semitendinosus*, *M. gracilis* and *M. adductor*) (21.2% and 2.2% protein and fat content, respectively), Iberian pork backfat (3.2% protein, 88.7% fat) and pork liver (21% protein, 5% fat) were obtained from a local market. The visible fat was separated from the meat and this was minced with a 0.4 mm mincer plate. The minced meat, fat and liver were vacuum packed and frozen at -20ºC until used (not more than 14 days later).

An oil-in-water emulsion (O/SPI) was prepared from the following ingredients : 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, Cognis GMBH, Illertissen, Germany), containing according to the producer 160 mg eicosapentaenoic acid (EPA)/g and 115 mg docosahexaenoic acid (DHA)/g; and soy protein isolate (SPI) containing 92.1% protein (Vicoprot, TRADES S.A., Barcelona, Spain). The oil-in-water emulsion was prepared with a 52.63 % oil combination (44.39 % olive oil, 37.87 % linseed oil and 17.74 % fish oil), 42.11 % water and 2.26 % SPI, following the procedure described by Delgado-Pando et al. (2010). The oil-in-water emulsion was produced the day before it was used and stored at 2 ± 2 ºC.

The konjac gel (KG) preparation was based on that of (Osburn & Keeton, 2004) with modifications. Briefly, (for each 900 g of konjac gel preparation)konjac flour (45 g) (glucomannan 83 %, 120 mesh, Trades S.A., Barcelona, Spain) was homogenized (Hobart N- 506, Hobart Corporation, Troy, OH, USA) with water (583.2 ml) for 5 min; i-carrageenan (9 g) (Secolata IP. Hispanagar, Burgos. Spain) was then added and the mixture homogenized again for 3 min. The pre-gelled corn starch powder (27 g)

(Amigel, Julio Criado S. L. Madrid. Spain) was dispersed in 145.8 ml water and homogenized (5 min) with the mixture of konjac flour and i-carrageenan. The mixture was cooled to 10 ºC, then 90 ml $Ca(OH)_2$ solution (1%) (Panreac Química S. A. Barcelona, Spain) was added with gentle stirring at room temperature. It was then cast and left to gel in appropriate containers at 2 ± 2ºC until used (within 24 h of preparation).

Other ingredients and additives used were sodium chloride and sodium ascorbate (Panreac Química, S.A. Barcelona, Spain), sodium tripolyphosphate (STP) (Manuel Riesgo, S.A. Madrid, Spain), sodium nitrite (Fulka Chemie GMBH, Buchs, Germany), sodium caseinate containing 86.4% protein (Julio Criado Gómez SA, Alcorcón, Spain), milk powder (Antonio Villoria S.A., Arganda del Rey, Spain) and flavouring (Gewürzmüller, GMBH, Münichingen, Germany).

2.2. Preparation of pâté

Eight different batches of pâtés were formulated (Table 1) : two control samples - one with normal (30 %) fat content (NFC30) similar to the commercial product and another with low (15 %) fat content (LFC15). Three low fat (15 %) samples were also formulated with partial substitution of pork backfat by the oil-in-water emulsion and with the addition of konjac gel in different proportions : 0 %(LFPO-K0), 7.5 % (LFPO-K7) and 15 % (LFPO-K15). Finally three other low fat (15 %) samples were formulated, with total substitution of pork backfat by oil-in-water emulsion and with the addition of different proportions of konjac gel: 0 % (LFTO-K0), 7.5 % (LFTO-K7) and 15 % (LFTO-K15). At a compositional level, the experimental design for improving the fat content of these products is related to fat reduction and to improving the fatty acid profile, while keeping the meat protein level constant (12%).

The meat, liver and backfat were all thawed before use (18h at 2±2 ºC). The meat, fat and oil-in-water emulsion were heated in a water bath at 85 ºC for 30-35 min and the water to be added was heated to 60 ºC. Meanwhile the chopped liver was placed in a mincer /homogenizer (Stephan Universal Machine UM5, Stephan u. Söhne GMBH and Co., Hameln, Germany), and homogenized for 1min. Then the sodium chloride, sodium nitrite, sodium tripolyphosphate were added (the last two previously dissolved in some of the added water), along with the milk powder, sodium caseinate, sodium ascorbate, flavouring and konjac (depending on the formulation), and homogenized for 1min. The hot meat was then added and the mixture homogenized again for 1min. Then the hot backfat and/or oil-in-water emulsion (depending on the formulation) were mixed for one minute and for 3 minutes more (under vacuum conditions) after finally adding the hot water. The mixture obtained was placed in containers (hermetically closed) and heated in a water bath at 85 °C, until it reached 80 °C at the thermal centre. The heat processing conditions were defined beforehand; in the definition process the internal temperature was monitored throughout heating by means of thermocouples inserted in various containers (product thermal centre) and connected to a temperature recorder (Yokogawa Hokuskin Electric YEM, Mod. 3087, Tokyo, Japan). When the processing was completed, the pâtés were cooled in a water bath (15-18 °C), and stored under refrigeration (2 ± 2 °C) until analysis (within 48 h of preparation).

2.3. Proximate analysis and fatty acid composition

The moisture and ash contents were determined (AOAC, 2000) in triplicate. Fat content was evaluated (in triplicate) according to (Bligh & Dyer, 1959). Protein content was measured in quintuplicate by a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI).

The fatty acid composition of the pâtés was determined (in sextuplicate) by gas chromatography as reported by López-López, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero,(2009). 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^{TM}-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 ºC 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 maintained for 20 min. Fatty acids were identified by comparison with a known standard FAME mixture (Supelco, Alltech Associated, Inc. Deerfield, IL, USA). The quantification of fatty acids was as reported by Delgado-Pando et al. (in press).

Based on the FAME results, the atherogenic index (AI) and thrombogenic index (TI) were computed according to (Ulbricht & Southgate, 1991).

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)].

2.4. Emulsion stability

Emulsion stability (Carballo, Fernandez, Barreto, Solas, & Jiménez-Colmenero, 1996a), as cooking loss (total fluid release, TFR), was expressed as % of initial sample weight. Three determinations for each sample were carried out. The containers (27 mm diameter) containing the sample (45 g) were opened after the heat treatment and left to stand upside down (for 30 min) to release the exudate onto a plate that had been previously weighed. Water loss (WL) was determined as weight loss after heating the total release fluid for 16 h on a stove at 105 ºC and expressed as % of initial sample weight. Fat loss (FL) was calculated as the difference between TFR and WL.

2.5. Colour measurement

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

2.6. pH Determination

The pH was determined on a 827 Metrohm pH-meter (Metrohm AG, Switzerland) at room temperature on homogenates of pâtés in water in a ratio of 1:10 (w/v). Six determinations were performed for each sample.

2.7. Penetration test

The analysis was performed in a TA-XT.plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) as follows. The penetration test was carried out at room temperature (22 ºC) and performed with a 6 mm diameter penetration probe linked to a 5 kg cell, at a velocity of 0.8 mm/s and for a distance of 8 mm. The rheological parameters obtained from the corresponding force-penetration curves were: slope of curve (adimensional), penetration force to rupture (N) and gel strength (mJ), defined as the area below the curve. The analyses were carried out in sextuplicate .

2.8. Microbiological analysis

Samples were prepared in a vertical laminar-flow cabinet (modelAV 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 (30ºC for 72 h) and Violet RedBile Glucose Agar (VRBG) (Merck, Germany) for enterobacteriaceae (37ºC for 24 h). The results were expressed as logarithms of colony forming units per gram (log cfu/g).

2.9. Sensory Analysis

The sensory analysis was performed by 16 panellists previously trained with two training sessions in the products and terminology (Standard UNE 87-001-94). A hedonic scale rating test was carried out where the testers evaluated the following for each sample : flavour (0 = dislike extremely, 10 = like extremely), texture (0 = dislike extremely, 10=like extremely), colour (0=very pale, 10=very dark), fatness (0 = imperceptible, 10 =very intense), hardness (0=soft, 10=firm) and overall acceptability (0=dislike extremely , 10= like extremely). The evaluation was made on a non-structured scale with fixed extremes. Each point was later converted to a numerical scale.

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 PASW Statistics 18.0 (SPSS Inc, Chicago, USA) for one-way ANOVA. Least squares differences were used for comparison of mean values between treatments and Tukey's HSD test to identify significant differences ($P<0.05$) between formulations.

3. Results and Discussion

3.1. Proximate analysis and energy content

The differences in formulation (Table 1) produced significant changes in the proximate composition of the pâtés (Table 2). Significant differences appreciated ($P<0.05$) between the moisture of the normal fat sample (NFC30, 50.1%) and the low-fat samples ranged between 62.8%-66.2%. In the low-fat samples, the highest moisture content ($P<0.05$) corresponded to LFC15, LFPO-K0 and LFPO-K7 samples and the lowest to LFTO-K15. The fat content of the different samples met the target level. It was observed that the normal fat control sample (NFC30) had 30.8 %, while the low-fat samples were near 15%, with no significant differences ($P>0.05$) between them (Table 2). While in both control samples (normal fat-NFC30 and low-fat-LFC15) the fat content is mainly from pork backfat, in the other samples where the pork backfat has been partially replaced (LFPO-K0, LFPO-K7 and LFPO-K15) or totally replaced (LFTO-K0, LFTO-K7, LFTO-K15) by oil-in-water emulsion the fat is mostly vegetable and fish oils (with a lower and constant amount from meat and liver origin). In terms of ingredient composition and formulation (Table 1), around 43 % and 86 % of the fat contained by LFPO and LFTO respectively, were supplied by oil-in-water emulsion. Depending on the pork backfat replacement level (partial or total), the pâtés contained 3-6 g, 2.5-5 g and 1.2-2.4 g (per 100 g of the product) of olive, linseed and fish oils respectively.

All samples had a protein content around 13 % (Table 2), near target level. Since all samples were formulated with the same meat and liver content (Table 1), in the control pâtés (NFC30 and LFC15) the protein was mainly from both meat raw materials, but also a small amount from pork backfat. In the modified low-fat pâté, on the other hand, because the pork backfat was partially or totally replaced by oil-water emulsion, the products contained not only meat proteins, but also SPI. This is why the samples where the pork backfat has been replaced completely by oil-in-water emulsion (LFTO-K0, LFTO-K7 and LFTO-K15), present a higher ($P<0.05$) protein content than the rest of the formulations (Table 2). The differences observed in ash content were small, even when significant (Table 2).

The energy content of the normal fat control sample (NFC30) was 345 kcal/100 g (81 % from fat) while in the low fat samples (around 40% lower than NFC30) it ranged from 201 - 218 kcal/100 g (Table 2), with fat accounting for over 65 %. In samples with partial pork backfat replacement by oil-in-water emulsion (LFPO samples), oil combinations accounted for nearly 30% of the total pâté energy content. In the case of total pork fat replacement (LFTO samples) these values were over 55% of the total pâté energy content. In these samples (LFTO) the approximate energy supplied by olive, linseed and fish oils is 53, 45 and 21 kcal/100 g, respectively.

3.2. Fatty acid profile

Since each group of pâtés, - control samples (NFC30 and LFC15), low-fat samples formulated with partial pork backfat replacement by oil-in-water emulsion (LFPO-K0, LFPO-K7 and LFPO-K15) and low-fat samples formulated with total pork backfat replacement by oil-in-water emulsion (LFTO-K0, LFTO-K7 and LFTO-K15), - were formulated with the same lipid material, in each case the data reported for fatty acid profiles (Table 3) are the mean values of these pâtés. According to these data, the pâté formulation affected the fatty acid profile (Table 3).

The most abundant fatty acids in the control samples (NFC30 and LFC15) (all pork fat) were MUFA (58%), followed by SFA and PUFA; MUFA and PUFA together accounting for 67% of the total fatty acids. In the control samples, oleic acid was the most abundant fatty acid, followed by palmitic and then stearic and linoleic acids (Table 3). These results are consistent with the fact that the backfat from Iberian pigs is rich in these fatty acids, contributing to the fatty acid composition of the liver pâté obtained (Estévez, Ventanas, Cava, & Puolanne, 2005). In the control samples the contribution of specific fatty acids to total diet energy ranged between 20-24% for SFA, 36-43% for MUFA and 5.7-6.7% for PUFA (Table 2).

Replacing the animal fat by the oil-in-water emulsion produces important changes in the fatty acid profile (Table 4) in line with the composition of the oil-in-water emulsion, which had a low proportion of SFA and a large quantity of MUFA and PUFA (Delgado-Pando et al., 2010). Compared with the control samples (32.23%), the products formulated with oil-in-water contained less ($P<0.05$) SFA. This reduction increased with the proportion of pork backfat replaced by the oil-in-water emulsion, so that the LFTO pâtés contain less than 22% SFA (Table 3). 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 (Hu, Manson, & Willett, 2001). Palmitic acid is the main SFA in all the pâtés, but replacing the animal fat by the oil-in-water emulsion produces a decrease ($P<0.05$), so that the 20.48% in the control pâtés is reduced to 17.01 % and 13.58 % in the LFPO and LFTO pâtés, respectively. The concentrations of palmitic and myristic acids decreased from 21.5 % to 14.7 % when the total pork backfat was replaced by the vegetable and marine oil combination (Table 3). This effect is due to changes in the palmitic concentration, since the myristic proportion does not change ($P>0.05$) with the formulation.

Compared with the control samples, partial replacement of pork backfat produced pâté (LFPO) with lower (P<0.05) MUFA and oleic acid contents. The lowest (P<0.05) content of these fatty acids was found with total pork backfat replacement by oil-in-water emulsion (LFTO) (Table 3). Even so, the total amount of oleic acid and MUFA in the reformulated pâtés (LFPO and LFTO), is similar to or even higher than that obtained in meat product reformulation processes by various authors (D'Arrigo et al., 2004; Echarte, Conchillo, Ansorena, & Astiasaran, 2004; Martín et al., 2008). Diets rich in monounsaturated fat have been associated with positive health benefits (López-Miranda, Pérez-Martínez, & Pérez-Jiménez, 2006).

The incorporation of oil-in-water emulsions considerably increased (P<0.05) PUFA proportions, due more to the reduction of SFA than of MUFA (Table 3). The replacement of SFA by PUFA is more effective in reducing serum cholesterol and the risk of coronary heart disease, than simply reducing total fat consumption (Hu et al., 2001). With total or partial pork backfat replacement, the PUFA content of pâtés (LFPO and LFTO) were 2.5 or 4 times higher respectively, as compared with control samples (Table 3). This increase is proportional to the substitution (P<0.05) of the quantity of linoleic acid, linolenic and long-chain n-3 PUFA: EPA, docosapentaenoic (DPA) and DHA. The total n-3 PUFA values are 3.36 g/100 g for the LFTO pâtés, 1.75 g/100 g for the LFPO pâtés and 0.33 and 0.16 g/ 100 g for the two control pâtés (NFC30 and LFC15, respectively). 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 pâtés— considering that the dietary recommendation for total n-3 PUFA is estimated as between 1.4 and 3 g/day or even higher (EFSA, 2005; Garg, Wood, Singh, & Moughan, 2006; Kolanowski, Swiderski, & Berger, 1999). The long chain n-3 PUFA contents of the reformulated low-fat pâtés were 356 and 723 mg/100 g for LFPO and LFTP pâtés respectively, which means higher amounts than those found in other n-3 enriched meat products (Jiménez-Colmenero, 2007). The importance of n-3 PUFA is that its consumption has been linked to reduced risk of cardiovascular disease, which in the case of the long chain n-3 PUFA may also imply a reduced risk of certain types of cancer, diabetes mellitus, inflammatory disease, multiple sclerosis and clinical depression (Garg et al., 2006; McAfee et al., 2010). In terms of the contribution of specific fatty acids to total product energy, and as compared with control samples (NFC30 and LFC15), the pork backfat replacement by oil-in-water emulsions caused a reduction of SFA and MUFA and an increased PUFA contribution (Table 2).

The recommended healthy PUFA/SFA ratio is above 0.4 (Wood et al., 2004). In the control pâtés, the ratio reached is 0.28 (Table 4), a similar value to those found by other authors in this type of product (Echarte et al., 2004; Martín et al., 2008). By replacing the pork fat, the PUFA/SFA ratio increases (P<0.05), clearly exceeding the reference value, in both the LFPO (0.82) and the LFTO (1.67) pâtés (Table 4). There is a direct relationship between the replacement level of pork fat by a healthier lipid combination (of vegetable and marine origin) and an increase in the PUFA/SFA ratio. An increase in this ratio has been related to a reduction of total cholesterol in plasma (McAfee et al., 2010).

Although the n-6 PUFA (mainly linoleic acid) increase (P<0.05) with the partial and total replacement of the animal fat, this occurs to a far lesser extent than with the n-3 PUFA (Table 4). This behaviour means a decrease (P<0.05) in the n-6/n-3 ratio, by a factor of almost 9 in the LFPO products and more than 14 in the LFTO pâtés. These values (< 0.8) are lower than those obtained in liver pâté formulated using materials obtained from pigs fed on linseed oil-enriched diets (near 2) (D'Arrigo et al., 2004) and much lower than those normally found in this type of product (8-18) (D'Arrigo et al., 2004; Echarte et al., 2004; Estévez et al., 2005). The n-6/n-3 PUFA ration reduction is of nutritional importance because it has been linked to a reduced risk of various pathologies including diabetes, cancer, CVD, etc. Accordingly, the recommended ratio is < 4, but the optimal ratio may vary with the disease under consideration (Simopoulos, 2002). At present, the use of this ratio as a nutritional parameter is being reconsidered, as it may mean that the relative amounts of n-3 and n-6 PUFA are ignored, and it does not distinguish between the different fatty acids in each group (McAfee et al., 2010). For this reason, the UK Food Standards Agency has proposed the use of absolute amounts of n-3 and n-6 PUFA, as well as of ALA and long chain n-3 PUFA, as nutritional parameters, instead of the n-6/n-3 ratio (Stanley et al., 2007).

Both the aterogenic index (AI) and the thrombogenic index (TI) are reduced ($P<0.05$) with the partial and total replacement of the pork backfat by the oil-in-water emulsion (Table 4). The most noticeable effects of the reformulation on these indices can be seen in the TI, which decreases ($P<0.05$) by half in the LFPO pâtés and is reduced ($P<0.05$) by a factor of four in the LFTO pâtés.

Similar results have previously been reported in the fatty acid profiles and nutritional significant ratios of frankfurters when the same reformulation strategy was applied (Delgado-Pando et al., in press).

3.3. Emulsion stability

Emulsion stability is affected ($P<0.05$) by formulation (Table 4). Both the normal fat control sample (NFC30), and the low fat pâtés in which all the pork backfat had been replaced by oil-in-water emulsion (LFTO-K0, LFTO-K7 and LFTO-K15) generally had lower ($P<0.05$) cooking losses (TFR). The LFC15 and LFPO-K0 samples present lower water and fat binding properties, with TFR values above 10 %. Independently of the product formulation, the cooking losses were mainly caused by water loss (80-90 % of the total fluid released) (Table 4).

Comparing control samples (NFC30 versus LFC15) it can be seen that the higher the fat content (NFC30), the higher the emulsion stability (Table 4). This behaviour is coherent with the fat being reduced by replacing the pork backfat with added water (Table 1). In contrast to this result, Estévez, Ventanas & Cava (2005) reported that cooking yield improved with lower fat content liver pâté, with fat being the main component loss. However, as is well known, the effect of fat reduction has been attributed to the characteristics of the matrix formed in each case, and to variation in the ionic strength of the medium (Claus, Hunt, & Kastner, 1989; Jiménez-Colmenero, Barreto, Mota, & Carballo, 1995). When the fat reduction is accompanied by an increase in the proportion of moisture and protein levels remain the same (Table 2), there is a lower "effective" concentration of the protein acting to form the gel/emulsion matrix, reducing the water binding properties of samples with lower fat content (Table 4). However, when fat reduction is also accompanied by an increase in the protein content of the matrix, the behaviour is different since the meat systems present better emulsion stability.

In the low fat samples, the amount of added water in each formulation, (decreasing as pork backfat replacement increased) can also affect the characteristics of the matrix formed as previously reported. This may explain why the highest proportion of added water (LFC15 and LFPO-K0) (Table 1) produced the lowest water and fat binding properties (Table 4). Although it has been pointed out that a higher unsaturated fatty acid content (with a lower melting point than saturated fats) may reduce the emulsion stability (Martín et al., 2008), this effect does not seem to influence this experiment. In general, little or no effect has been observed on meat emulsion stability due to the presence of different oils (peanut, high-oleic acid sunflower, sunflower, soy bean) (Ambrosiadis, Theodorakakos, Georgakis, & Lekkas, 1994; Hong et al., 2004; Márquez, Ahmed, West, & Johnson, 1989; Park, Rhee, Keeton, & Rhee, 1989). In the products with added konjac (with lower added water), part of the water present in the product was embedded and bound in the konjac gel and hence was not available for the formation of the protein matrix. In these conditions no clear effect was observed of this component on the water binding properties. Improving emulsion stability of low-fat pork meat batters with added konjac gel has been reported (Fernández-Martin, López-López, Cofrades, & Jiménez-Colmenero, 2009).

3.4. Colour and pH

Colour parameters were affected by the pâté formulation (Table 5). Comparing the two control samples (NFC30 and LFC15), it can be seen that reduction of fat generates decreased ($P<0.05$) lightness and yellowness values, and increased ($P<0.05$) redness. Similar behaviour has been described in other meat products (Carballo et al., 1996a; Estévez et al., 2005; Hughes, Mullen, & Troy, 1998; Jiménez-Colmenero et al., 2010). A reduction of fat levels (and increased added water) generally favours the appearance of darker colouring (higher redness and lower lightness) (Carballo, Mota, Barreto, & Colmenero, 1995). With no difference between the substitution levels, both partial and total backfat replacement by oil-in-water emulsion (LFC15 v. LFPO and LFTO) generally produced an increase in L* and b*. In parallel there was a decrease ($P<0.05$) of a*, which became more acute as the level of substitution increased (Table 5). Hong et al. (2004) found that replacing between 5-20% pork backfat by soybean oil in spreadable liver sausage obtained a product which was redder and less yellow and with no change in luminosity. The replacement of pork backfat by oils (olive or corn) caused a reduction of a* values and an increase of b* values. (Bishop, Olson, & Knipe, 1993; López-López, Cofrades, & Jiménez-Colmenero, 2009), although they did not agree with those reported by other authors (Márquez et al., 1989; Paneras & Bloukas, 1994) who had observed that oil substitution had no effect on colour. These differences may be due to differences in product formulation and composition.

The effect of the presence of konjac varies depending on the formulation (Table 5). Thus, in the formulations with partial replacement of the pork backfat small changes in the colour parameter were observed. In contrast, in LFTO pâtés, the addition of konjac produced less red ($P<0.05$), paler ($P<0.05$) and yellower pâtés. It has generally been reported that the addition of konjac gel to comminuted meat products has little effect on colour parameters (Kao & Lin, 2006; Lin & Huang, 2003).

Although there are significant variations in pH from the effect of the formulation they are of little quantitative relevance (Table 5). The values obtained (6.54-6.57) are similar to those described in this type of product (Estévez et al., 2005; Estévez, Ventanas, & Cava, 2005; Fernández-López et al., 2004; Hong et al., 2004; Silva, Morais, Oliveira, & Silvestre, 2003). As a result, formulation has very little influence on the pH of the pâtés.

3.5 .Penetration test

Texture parameters were affected by the pâté formulation (Table 6). Comparing the two control samples (NFC30 and LFC15), shows that the fat reduction produced a decrease ($P<0.05$) of slope, penetration force and gel strength, which means a softer and more spreadable pâté. This behaviour described in pâté (Viana et al., 2005) and other gel/emulsion systems (Candogan & Kolsarici, 2003; Carballo et al., 1996a; Cofrades, Carballo, & JimenezColmenero, 1997; Crehan, Hughes, Troy, & Buckley, 2000; Gregg, Claus, Hackney, & Marriott, 1993, entre otros), is coherent with fat and water binding properties (Table 4) and with the effect of reducing the fat in the texture of meat based emulsion products as long as the meat protein content is kept constant, as previously reported. However, other authors have observed that fat reduction causes increased hardness of liver pâté (Estévez, Ventanas, & Cava, 2005) and frankfurters (Ayo, Carballo, Solas, & Jimenéz-Colmenero, 2008; Hughes et al., 1998), or even has no effect on the textural parameters (Lurueña-Martínez, Vivar-Quintana, & Revilla, 2004; Ordonez, Rovira, & Jaime, 2001).

As compared with the low fat control samples (LFC15), no effect (P>0.05) was observed on textural parameters by the partial (LFPO-K0) or total (LFTO-K0) replacement of the backfat by oil-in-water emulsion. Although not significant, there seemed to be a tendency for the gel strength to reduce as oils increased and pork fat decreased (Table 6). Liver pâté (29 % fat content) manufactured with olive oil and conjugated linoleic acid as a pork backfat substitute showed a softer consistency than those formulated in the traditional way with pork fat (24 % fat content) as the only fat source (Martín et al., 2008). Conflicting results have been reported for the effect of vegetable oils, and specifically olive oil, on the texture of gel/emulsion meat products. For instance, it has been reported that replacing pork fat by olive or peanut oils in reduced-fat frankfurter (as compared to a normal-fat product) produced a harder/firmer product (Bloukas & Paneras, 1993; Márquez et al., 1989; Paneras & Bloukas, 1994; Paneras et al., 1998) or had no effect (Bloukas & Paneras, 1993). No differences in rheological characteristics have been reported from replacing pork backfat with soybean oil in spreadable liver sausage (with fat content over 30 %) (Hong et al., 2004). However, other authors have reported that the addition of olive oil combined with fat reduction (with similar protein content) caused a decrease in the hardness of frankfurters (Lurueña-Martínez et al., 2004). Various factors may condition the effect on the texture of meat products of substituting vegetable oils for animal fat. On the one hand there are those related with the fat source characteristics, including the intact adipocytic structure of pork backfat, or the higher unsaturated levels of oils (Martín et al., 2008) and their better distribution (as compared with animal fat) in the protein matrix (Hammer, 1991; as cited in Hong et al., 2004). On the other hand, the composition factors of the meat matrix (changes in moisture and protein contents), to which a great deal of importance has been attached (Carballo, Fernández, Barreto, Solas, & Jiménez-Colmenero, 1996b; Claus et al., 1989), may also help to explain the apparent discrepancies regarding the effect of substituting oil for animal fat.

Konjac flour has been used to simulate fat characteristics and produce reduced-fat foods including meat products (Tye, 1991) such as frankfurters (Kao & Lin, 2006; Lin & Huang, 2003; Osburn & Keeton, 2004) and bologna (Chin, Keeton, Longnecker, & Lamkey, 1998a, 1998b; Chin et al., 2000). Compared with low fat pâtés with 0 and 7 % of added konjac gel, the addition of 15 % of konjac gel (LFPO-K15 and LFTO-K-15) produced harder structures (Table 6), which are very close to those of the control samples (NFC30 and LFC15), especially in NFC30 and LFTO-K-15 with similar (P>0.05) textural parameters (Table 6). Although the effect of replacing pork backfat with konjac gels varies according to the nature of the gel and the proportion of fat replaced (López-López et al., 2010), similar effects to those described in this experiment have been reported (Chin, Keeton, Longnecker, & Lamkey, 1999; 2000; Yoo, Kook, Park, Shim, & Chin, 2007).

3.6. Microbiology

The raw meat batters had a microbial population range between 5.8-6.5 log cfu/g for TVC and 3.7-4.9 for enterobacteriaceae (Table 7). The heat treatment resulted in a marked decrease in the microbial population, reaching values lower than 3.3 log/g for TVC and <2 for enterobacteriaceae in the different pâtés. In these products, although there were significant differences, the variation between the formulations was of little importance. The total aerobe values found are similar to, or even lower than those recorded by other authors in this kind of products (Beldarraín, De la Mella, Yánez, García, & Cepero, 2003; Soffer, Margalith, & Mannheim, 1994) and in comminuted meat products (Kao & Lin, 2006; López-López, Cofrades, & Jiménez-Colmenero, 2009).

3.7. Sensory evaluation

Table 8 shows the sensory evaluation of the pâtés, which was affected by formulation. In the control samples (NFC30 and LFC15) fat reduction increased ($P<0.05$) colour, without any significant changes in other sensory parameters. In terms of objective colour parameters (Table 5), fat reduction produced darker products (Table 8). Both the replacement of animal fat by the oil-in-water emulsion and the addition of konjac conditioned the colour evolution of the pâtés. Those with the lowest colour scores ($P<0.05$) were the NFC30, LFPO-K0, LFPO-K7, LFTO-K7 and LFTO-K15 samples. (Table 8). Fat perception was not clearly affected by fat content (type and levels), however in general, as the added konjac increased the fat perception tended to decrease. In terms of texture evaluation, the samples LFPO-K0, LFPO-K15 and LFTO-K15 scored lowest. The rest of the formulations scored above average values on the scale, without significant differences found between them ($P>0.05$). All samples had similar ($P>0.05$) flavour and overall acceptability scores, although LFPO-K15 and LFTO-K15 scored lowest, the only scores below the average scale values.

Conclusion

Based on recommendations for optimal intake of total and unsaturated fatty acids, modifying lipid fractions in meat products has been concerned chiefly with reducing fat content and improving fatty acid profiles. This proposal is more interesting and presents greater technological difficulty in products with high fat and energy contents such as pâté. Reformulation strategies, based on the use of a specifically designed combination of vegetable and fish oils and konjac gel as pork fat replacements, have enabled the production of low fat pâtés with a healthier lipid profile. Compared with normal fat pâté, the modified products have lower fat (50 %) and SFA (33%) content, as well as a higher proportion (4 times more) of n-3 PUFA (ranging between 1.75 and 3.36 g/100 g), including a relevant amount of long chain n-3 PUFA (356 and 723 mg/100 g). This means a very important contribution to dietary intake, generally higher than described in different n-3 enriched meat products, without compromising the technological, microbiological and sensorial properties of the reformulated pâtés. Work is in progress to study the storage stability of these products.

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