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Nutritional improvement and physicochemical evaluation of liver pâté formulations

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Summary

Pâté formulations composed of chicken liver, a by-product of poultry industry, have been produced by replacing pork back fat with sunflower oil and reducing fat content. The characterization of these products was performed, while the oxidative stability, microstructure, texture, colour, and hygienic quality were determined throughout refrigerated storage. The hardness of pâtés with sunflower oil was lower than the other ones. Different microstructures regarding protein matrix, fat globules and pores, were associated with fat type and content. The storage time, fat type and content influenced the colour parameters. In terms of the oxidative stability, no reduction in the product quality was found during the refrigerated storage. Pâtés with 28 % w/w of sunflower oil were the most suitable formulation to increase the nutritional value for this kind of meat products.

Keywords: Chicken liver pâté, physicochemical properties, refrigerated storage

1. **Introduction**

Meat products are essential components of the human diet. However, these products contain high levels of fat, cholesterol, and low (polyunsaturated fatty acids/saturated fatty acids) PUFAs/SFAs ratios, linked with development of obesity, hypercholesterolemia and cardiovascular diseases (Arihara, 2006). Currently, many consumers demand low-fat foods with healthy ingredients. Some vegetable oils are an important source of PUFAs as well as minor components such as phytosterols and tocopherols. They have been employed as saturated fat replacers in meat products (Martin, et al. 2008, Pennisi Forell, et al. 2010). However, the reduction and substitution of lipids can affect the physicochemical characteristics of high fat foods like sausages, burgers and pâtés (Delgado-Pando, et al. 2011). Moreover, in these products development of rancidity could affect quality attributes (odour, taste, colour, texture) reducing nutritional value (Estévez and Cava, 2006). Protein oxidation might produce a loss of essential amino acids (Lund et al., 2007). Besides, the stages of processing and preserving (cooking, refrigerating, freezing, etc.) could release the iron from hem proteins decreasing its bioavailable content and modifying the colour (Estévez and Cava, 2004).

Liver pâté is a traditional food manufactured using liver from pig or calf, porcine back-fat and other characteristic ingredients. It is consumed all over the world, especially in European countries and is generally considered an added value product with high nutritional and sensory qualities (Estévez et al., 2007). In recent years, there has been a very important increase in the production and consumption of poultry meat around the world (USDA, 2014). However, the poultry industry generates by-products which are generally underutilized, for example chicken liver.

The aim of this work was to produce chicken liver pâtés in order to obtain healthy products and to study the influence of fat type and its content on their physicochemical characteristics during refrigerated storage time.

2. Materials and methods

2.1. Manufacturing of liver pâtés

Pork back fat (BF), chicken breasts and livers were obtained from the local market and sunflower oil (SO) was supplied from Aceitera General Deheza (Argentina). Four formulations of pâtés (BF40, BF28, SO40 and SO28) were prepared by replacing pork with chicken liver with different type of fat (BF, SO) and content (40 or 28 % w/w). These fat levels were selected according to the traditional formulations and a 30% reduction of lipid phase to obtain healthy products. Other ingredients were added at the same concentration for all formulations (**Table 1**).

The manufacturing process is shown in **Figure 1.** BF was cut into cubes of about 15 mm side and scalding at 65 °C for 30 min. Liver and muscle free of connective tissue were also cut into cubes and then, washed with chlorinated water and mixed with NaCl, $NaNO_2$ and ascorbic acid to achieve tissue nitrification. The purpose of this step is to preserve, flavour and colour the pâtés. Scalded BF and SO were pre-emulsified with sodium caseinate dissolved in distilled water at 75 °C. The batters were filled in glass flasks of 40 mm diameter and 60 mm height with about 80 g of mixture (or 40 mm height) which were subjected to a heat treatment in a stainless steel autoclave. In the core of the pâtés, temperature remained constant at 80 ± 2 °C for 30 min being monitored with a Cu-Constantan type T thermocouple. Subsequently, the flasks were cooled to room temperature

and stored in the dark at 4 ± 1 °C for 150 days. Samples were taken to perform the assays every 30 d. The procedure was repeated twice for each formulation.

2.2. Chemical composition

2.2.1. Proximate analysis and energy content

Moisture, ash, and protein contents were determined according to AOAC (1984) methods: 24.002, 24.009 and 24.027, respectively. Lipid content was determined by the Soxhlet method (AOAC 1984, 24.005) using ethyl ether as extraction solvent which was evaporated using a Rotavapor R-114 (Büchi, Flawil, Switzerland). Lipid content was expressed as g fat/100 g pâté. All determinations were performed in triplicate with freshly manufactured pâtés. Caloric value (Kcal/100 g pâté) was calculated using the Atwater coefficients corresponding to lipids (9.00 Kcal/g), proteins (4.02 Kcal/g) and carbohydrates (3.87 Kcal/g).

2.2.2. Fatty acid profile

Total lipid extraction from pâtés was performed by method of Folch et al. (1957). Fatty acid methyl esters (FAMEs) were prepared by acid esterification using 10 % BF_3 in methanol (AOAC 1990, official method 969.33). FAMEs were analysed using a Hewlett Packard, mod. HP-5890A, gas chromatograph, equipped with a flame ionization detector (FID) and a capillary column Supelco Omega wax 11090-02A (30 m x 0.25 mm internal diameter and 0.1 mm thick). The temperature program was set from 175 to 220 °C at 3 °C/min. The identification of peaks was performed by comparison with retention times of reference fatty acids (Nu Check Prep, Inc., USA). The fatty acid analysis was carried out in duplicate throughout refrigerated storage. In addition, back fat, sunflower oil, chicken muscle and liver were individually analysed to know the influence of these ingredients on

the pâté fatty-acid profiles. Composition results were expressed as percentage of total fatty acids.

2.2.3. Determination of tocopherols

Tocopherol content in pork back fat and sunflower oil was determined in duplicate by a chromatographic technique based on IUPAC rules 2432 (1992) and AOCS Ce8-89 (1998). Lipids were extracted from adipose tissue using the Soxhlet method with n-hexane. This solvent was removed by a rotary evaporator R-114 (Büchi, Flawil, Switzerland) under vacuum at 40 ˚C. Subsequently, the extracted lipids were dissolved in n-hexane for quantification by HPLC with fluorescence detection (λ excitation: 290 nm, λ emission: 330 nm). A Hewlett Packard HPLC Series 1050 chromatograph equipped with a Lichrosorb normal phase column Si-60 (250 mm x 4 mm and 5 µm particle size) was used. Operating conditions were: mobile phase isopropanol: hexane (0.5:99.5 v/v), a flow rate of 1.5 mL/min and 20 µl of injection volume.

2.3. Assessment of quality attributes throughout refrigerated storage

2.3.1. Texture Measurement

Penetration test was performed on pâté formulations in their flasks with a TA-XT2i texture analyser (Stable Micro Systems, Godalming, UK) at room temperature. Force in compression was measured with a 12.7 mm diameter cylinder probe (P/R 0.5 Delrin) which penetrated the sample to a depth of 15 mm at a constant cross head speed of 1 mm/s. The hardness (maximum force required to penetrate the sample in N) were obtained from the force–time curves recorded in triplicate for each pâté formulation.

2.3.2. Microstructure

Microstructures of pâté formulations were observed by Scanning Electron Microscopy. Samples were fixed with 2.5 % glutaraldehyde in sodium phosphate buffer 0.1 M (pH 7.2). Then, they were dehydrated with acetone and dried by critical point technique with CO2 POLARON equipment. Furthermore, the samples were coated with a gold layer by Pelco equipment 91000 and were observed in a microscope JEOL 35 CF (Tokyo, Japan).

2.3.3. Colour

Colour parameters on the surface of the pâté formulations were measured at room temperature in triplicate. CIE-LAB parameters: Lightness (*L**), redness (*a**) and yellowness (*b**) were determined using a Chroma Meter CR-400 colorimeter (Minolta Co., Osaka, Japan).

2.3.4. Sanitary condition of pâtés

Microbiological analyses were performed in duplicate to evaluate sanitary condition of the pâtés from 0 to 150 d. Every 30 d, 20 g of each pâté formulation was aseptically removed from each package, transferred into sterile stomacher bags, homogenized with 80 mL of 0.1 % of sterile peptone solution and blended in stomacher (West Sussex, UK) for 60 s. Decimal progressive dilutions were prepared. Mesophylic aerobic and Psychrotrophic microorganisms were evaluated on plate count agar (PCA Oxoid, Hampshire, UK), by pour plates aerobic incubation at 30 °C for 48 h and 4 °C for 7 d, respectively. *Enterobacteriaceae* microorganisms were enumerated on violet red bile agar (Merck, KGaA, Darmstadt, Germany) by spread plates aerobic incubation at 37 °C for 24 h. Sulphite-reducing Clostridium microorganisms were enumerated in differential clostridia agar (Britania, Argentina) and incubated at 37 °C for 48 h in anaerobic condition. Results were expressed as the average colony forming units per gram (CFU/g).

144

145 2.4 Oxidative stability

146 2.4.1. Lipid and protein oxidation

147 Lipid oxidation was evaluated by the 2-thiobarbituric acid reactive substances
148 (TBARS) test in duplicate during storage time. TBARS values were determined in
149 duplicate on pâtés according to Rosmini et al. (1996). Results were expressed as mg
150 malonaldehyde (MDA)/kg product. The levels of oxidative modified proteins were
151 determined in duplicate according to Oliver et al. (1987). Carbonyl compounds
152 concentration was expressed as nmol/mg protein.

153 2.4.2. Hem iron content

154 Hem iron content was measured in duplicate by spectrophotometry as described
155 Lombardi-Boccia et al. (2002). Hematin concentration expressed as mg/mL was
156 determined at 640 nm using a calibration curve with pork hematin. The concentration of
157 hem iron was calculated using the conversion factor of 0.082 µg Fe/µg hematin. Hem iron
158 content was expressed in µg Fe Hem/g pâté.

159 2.5. Experimental design and statistical analysis

160 A full factorial randomized experimental design was used and the factors studied
161 were: type of fat (two levels: BF and SO), fat content (two levels: 28 % w/w and 40 %
162 w/w), refrigerated storage time (six levels: 0, 30, 60, 90, 120 and 150 d) and their
163 interactions. Means and SEM (standard error of the mean values) were presented for all
164 assays. Analysis of variance was applied to evaluate the influence of the variables using the
165 SYSTAT software (SYSTAT Inc., USA). For simultaneous pairwise comparisons, Fisher's
166 test was chosen. Differences in means and F-tests were considered significant when $p<0.05$.

3. Results and discussion

3.1. Chemical composition

3.1.1. Proximate analysis and energy content

Total lipid content was constituted essentially by the fat used as well as from the liver and muscle to a lesser extent. In SO formulations lipid content is greater than the amount of oil added and the BF formulations had a lower fat percentage when compared to the amount of BF added (**Table 2**). These facts are related to the composition of SO and BF used in the manufacturing process. SO has 99.90 % of lipids and BF is a tissue composed mainly by fat with proteins and moisture in smaller proportions. Proteins are provided by meat ingredients, essentially muscle and liver, sodium caseinate and by the BF. SO28 and BF28 pâtés presented higher protein contents than SO40 and BF40 since the reduction in fat content was replaced with a higher content of liver. This procedure also influenced the moisture content because SO40 and BF40 formulations had lower moisture values than SO28 and BF28 pâtés. The main contribution to ash content is given by the additives and the different liver/fat ratio used, principally due to the iron supply by liver tissue. Pâtés with a low caloric value for both types of fat utilized were obtained by the reduction of the fat content.

3.1.2 Fatty acid composition

Table 3 shows the fatty acid profiles of the different chicken liver pâtés studied. It was possible to observe that BF40 pâtés had oleic acid as the most abundant fatty acid, which represented 50.49 %, followed by palmitic, linoleic and stearic acids and very low levels of other ones. The reduction of the fat content in BF28 pâtés was accompanied by a

191 decrease in the oleic acid, the main fatty acid provided by the back fat. However, SFAs and
192 PUFAs were more abundant in BF28 pâtés which could be associated with the increased
193 contribution of hepatic tissue rich in those fatty acids (data not shown). SO formulations
194 were constituted by linoleic, oleic, stearic and palmitic acids. Particularly, linoleic acid
195 quadrupled BF pâté contents. However, SFAs and oleic acid contents in SO pâtés were
196 lower than those made with BF. Therefore, replacing back fat with sunflower oil resulted in
197 pâtés with a high proportion (p<0.05) of PUFAs. These types of fatty acids accounted for
198 over 50 % of the total fatty acids in SO pâtés, improving its nutritional value due to the
199 contribution of an essential fatty acid such as linoleic acid. Throughout storage no
200 significant changes in the fatty acids contents were observed. However, only 20:2n-6 fatty
201 acid declined significantly (p=0.021) which could be attributed to lipid oxidation.
202
203 3.2. Quality attributes
204 3.2.1 Textural analysis
205 Pâté is considered a finely comminuted meat product composed of a mixture of
206 proteins (soluble and insoluble proteins with particles of muscle fibres and connective
207 tissue), fat globules, water, salt and spices which are mixed into a fairly homogeneous
208 mass. This mixture has a paste-like texture in the raw state but gradually changes into a
209 more rigid structure by gelation of proteins throughout the cooking process. The structure is
210 formed when the proteins start to denature and participate in protein–protein interactions
211 (Barbut et al., 1996).
212 **Figure 2** shows the hardness of chicken liver pâté formulations as a function of
213 storage time. The type of fat was a significant factor that affected the texture of samples.
214 SO pâtés exhibited significantly lower hardnesses (p=0.001) than those made with back fat

due to the replacement of saturated for unsaturated fats. This effect was also observed by Martin et al. (2008) in pâtés with partial replacement of pork fat by olive oil. The fat content significantly modified the hardness of pâtés. The presence of high amounts of sunflower oil (SO40) resulted in soft pâtés (p=0.014) and the reduction of back fat content produced the opposite effect (**Figure 2**). The storage time did not influence the hardness of pâtés (p>0.05).

3.2.2. Microstructure

Micrographs for pâté formulations show a microstructure constituted by a matrix of proteins with the inclusion of fat globules and pores (**Figure 3**). The matrix is composed of proteins from chicken liver, muscle and sodium caseinate. This protein network consists of both fibrous proteins (collagen, elastin, reticulin, actin and myosin) and globular proteins (cytoplasmic proteins of liver tissue, sarcoplasmic myoglobin and haemoglobin). According to Tornberg (2005), fibrous proteins are denatured by heat, acquire random configurations and are associated with globular proteins forming the matrix. Also, the presence of holes of different size can be observed in these images. The holes were identified as the spaces where fat was placed in the gel matrix; this fat disappears with the preparation of the samples. In addition, a large number of small pores distributed in the gel network were seen in **Figure 3b, c and d**. These small pores could be associated with water or air incorporated throughout the homogenization step in the preparation of the pâté formulations.

The microstructure of pâtés formulations varied with fat type and content. BF pâtés (**Figure 3a and b**) showed larger fat globules with more defined shape than SO pâtés (**Figure 3c and d**). BF40 formulation (**Figure 3a**) exhibited a continuous protein matrix and packed structure that may be associated with increasing instrumental hardness (**Figure**

239 **2**), while BF28 and SO pâtés revealed a more aggregated structure of gels (**Figure 3b**, **c**
240 and **d**). In addition, the reduction of fat content (with a liver-content increase) caused an
241 increase in the number of pores due to the increase of moisture content (**Figure 3b and d,**
242 **Table 2)**. This behaviour is in accordance with findings presented by Totosaus and Pérez-
243 Chabela (2009). Moreover, a growth in connective-tissue liver proteins must be related to
244 water retention contributing also, to raise the number of pores. On the other hand, the
245 storage time did not affect the microstructure of the pâtés.

246 3.2.3. Colour

247 **Table 4** exposes the evolution of surface colour parameters (*L**, *a** and *b**) of pâtés
248 with different fat composition throughout storage. The SO pâtés oil presented higher *L**
249 values than BF pâtés which could attribute to a milky appearance imparted by the oil
250 emulsion. Similar results were obtained by Pennisi Forell et al. (2010) in burgers with high
251 oleic sunflower oil due to the high refractive index of this oil. BF40 and SO40 gave higher
252 *L** values than BF28 and SO28 pâtés ($p<0.01$), that were in agreement with the study in
253 low-fat sausages (Crehan et al., 2000). The storage time produced an increase ($p<0.01$) in
254 lightness of the pâtés, similar to that reported by Estévez and Cava (2004).

255 The redness showed an increase ($p<0.01$) during storage time (**Table 4**). This fact
256 was attributed to the formation of nitrosohaemoglobin and red nitrosomyoglobin as Bozkurt
257 (2006) observed in fermented and cured sausages. Moreover, D'Arrigo et al. (2004) related
258 this behaviour with the exposure to air and surface water loss of samples. The BF pâtés
259 presented lower values than those made with sunflower oil ($p<0.01$). BF28 and SO28
260 exhibited the highest values for this parameter ($p<0.01$). In this sense, a significant negative
261 correlation was found between a* values and fat content for the pâté formulations
262 throughout the storage time ($r \geq -0.87$; $p \leq 0.003$). The increase in *a** with the decrease of

fat percentage can be attributed to the high hem proteins content supplied by the liver, which provide an enhanced reddish tint. Estévez et al. (2005) studied the physicochemical properties of pork liver pâté with different fat contents (45, 40 and 35 % w/w) reporting similar results.

Statistical analysis of the values obtained for the *b** parameter (**Table 4**) revealed that pâtés which include back fat in their formulation were less yellow than those prepared with sunflower oil ($p<0.01$). This behaviour was observed by other researchers when fat was replaced by oil producing yellower meat products (Youssef and Barbut, 2009). BF40 and SO40 pâtés gave higher *b** values than BF28 and SO28 pâtés ($p<0.01$). The storage time produced an increase ($p<0.01$) in yellowness of pâté formulations. Fernández-López et al. (2004) observed that both oxidation and oxygenation of myoglobin could generated increments in the *b** parameter. Considering that the third level interaction was significant ($p<0.05$), the modifications in lightness, redness and yellowness produced by storage time depended on the combination of fat type and content.

3.2.4. Sanitary condition of pâtés

No sulphite- reducing Clostridium was noted in any sample throughout the storage period. The microbial counts did not exceed 60 CFU/g for the other groups of microorganisms analysed: mesophylic aerobic, psychrotrophic and *Enteriobacteriaceae* at final storage time. These results indicated that the heat treatment and the application of low temperatures during the storage time were appropriate operations to maintain safe sanitary conditions for all pâté formulations.

3.3. Oxidative stability

Fat content and refrigerated storage time were significant factors ($P<0.05$) that

influenced lipid oxidation in the pâtés. TBARS values in BF28 and SO28 pâtés presented a significant increase while SO40 pâtés showed a slight increase **(Table 5)**. These behaviours might be explained considering that high moisture content in BF28 and SO28 may promote lipid oxidation. TBARS values in BF40 remained steady during storage time. SO40 pâtés presented the highest TBARS value (0.65 mg MDA/Kg) at initial storage time, possibly because its high content of PUFAs produces an increase in the susceptibility to lipid oxidation in pâté manufacturing steps (disruption of tissues and subsequent heat treatment).

No influence of fat type in TBA values (p>0.05) was observed in statistical analysis. SO pâtés exhibited the TBA values lower than expected, taking into account their fatty acid composition rich in PUFAs **(Table 5)**. This behaviour could be attributed to the high Vitamin E content in sunflower oil. Thus, total tocopherol level in this oil was 502 ± 21 µg/g, with α-tocopherol being the major component (498 ± 20 µg/g) followed by β-tocopherol (4 ± 1 µg/g); γ and δ vitamers were not detected. Muguerza et al. (2003) also observed the influence of the natural antioxidants present in vegetable oils on lipid oxidation when back fat is replaced with soybean oil in Pamplona chorizo. Besides, the total tocopherol content in back fat was also considerable (350 ± 20 µg/g), with α-tocopherol as the only vitamer found.

Protein oxidation is considered to be linked to lipid oxidation. In the presence of oxidized lipids, the protein oxidation is produced by free radical chain reactions similar to those for lipid oxidation (Faustman et al., 2010). The carbonyl compounds content of pâté formulations significantly changed (p<0.05) with refrigerated storage time (**Table 4**). In this case, BF28 and SO28 increased significantly; while BF40 and SO40 fluctuated mildly probably due to the by-products of lipid oxidation could have interacted with proteins. Besides, the fat content was a significant factor since the pâtés with 40 % w/w fat content

had significantly higher levels of carbonyl compounds than pâtés with 28 % w/w fat. On the other hand, the statistical evaluation showed that the type of fat did not significantly affect (p>0.05) the protein oxidation, similar to the case of the TBA test.

Hem iron is another parameter to evaluate oxidative damage in fatty meat products. Greater concentrations of iron and myoglobin are associated with greater rates of lipid oxidation (Faustman et al., 2010). In this work, the hem iron content was affected significantly (p<0.01) by refrigerated storage time. BF40 and SO40 showed a hem iron decrease while BF28 and SO28 pâtés varied around 6.50 and 5.50 µg/g pâté, respectively **(Table 5)**. The fat type and its content significantly affected (p<0.01) the hem iron values obtained. BF pâtés presented higher hem iron contents than those SO pâtés. Furthermore, BF40 and SO40 pâtés showed significantly lower values of hem iron than BF28 and SO28 pâtés, probably due to their higher fat/liver ratios, since liver is the main component that provides iron in BF28 and SO28 formulations. The third level interaction was also significant (p<0.05), indicating that changes produced in TBARS, carbonyl compounds and hem iron levels by the storage time depended on the combination of fat type and content.

Liver pâtés contain high amounts of fat and iron, and therefore, oxidative deterioration of liver pâtés during refrigeration was expected. Georgantelis et al. (2007) reported that the rancid flavour is detected in meat products with TBARS values higher than 0.6 mg MDA/kg, while Campo et al. (2006) considered that the limiting threshold for the acceptability of oxidized beef is around 2.0 mg MDA/kg. In this work, all pâté formulations presented TBARS levels below 1mg MDA/kg after 150 d of storage at 4 ± 1 °C. Moreover, the carbonyl compounds contents were lower than those found in traditional liver pâtés from pork during refrigerated storage by Estévez and Cava (2004). In addition,

334 the decreases in the hem iron contents in BF40 and SO40 pâtés were also lower than those
335 reported by Fernández López et al. (2003).

336 The physicochemical evaluation the shelf life of the healthy chicken liver pates
337 indicated that the lipid and protein oxidation levels were lower than those observed for
338 traditional formulations. No substantial reduction of quality attributes was recorded as a
339 function of refrigerated storage. Hence, the developed chicken liver pâtés would be suitable
340 formulations to diversify the poultry industry.

341

342 **4. Conclusions**

343 This study allowed the global characterization of chicken liver pâtés with a
344 reduction and replacement of the traditional back fat by refined sunflower oil. SO pâtés
345 were obtained with a fatty acid profile healthier than BF pâtés, increasing the
346 PUFAs/MUFAs ratio. Regarding the quality attributes, BF pâtés presented higher hardness
347 values (p<0.05) than those made with sunflower oil. Micrographs of pâtés revealed
348 variations in the protein matrix, distribution pattern of the fat globules and pores with fat
349 type and content. Thus, SO pâtés exhibited a greater number of pores than the others which
350 could be related to a more spreadability for these formulations. Storage time, the type and
351 concentration of the fat phase mildly modified the colour parameters. Microbiological
352 analyses indicated an adequate hygienic quality for all pâté formulations throughout
353 refrigerated storage. In terms of oxidative stability, changes observed in TBARS, carbonyl
354 compounds and hem iron contents were satisfactory considering liver pâtés contain high
355 amounts of fat and iron. Therefore, taking into account chemical composition, quality
356 attributes and oxidative stability, the chicken liver pâté with 28 % w/w of sunflower oil was
357 the most adequate formulation to increase the nutritional value for this kind of meat

358 products.

359

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363

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Table 1

Ingredients (in per cent) used for manufacture the different pâté formulations.

Ingredient (%)	BF40	BF28	SO40	SO28
Liver	28.00	40.00	28.00	40.00
Back fat	40.00	28.00	-	-
Sunflower oil	-	-	40.00	28.00
Chicken breast	5.00	5.00	5.00	5.00
Water	22.62	22.62	22.62	22.62
Sodium caseinate	2.00	2.00	2.00	2.00
Sodium chloride	2.00	2.00	2.00	2.00
Sodium phosphate	0.30	0.30	0.30	0.30
Sodium nitrite	0.03	0.03	0.03	0.03
Ascorbic acid	0.05	0.05	0.05	0.05

Table 2
Proximate analysis (g/100g) and total calories (kcal/100g) of chicken liver pâté formulations

	Formulations				SEM	*p*		
	BF40	**BF28**	**SO40**	**SO28**		**T**	**C**	**TxC**
Lipids	37.40^{c}	27.46^{a}	42.71^{d}	30.80^{b}	2.23	<0.001	0.048	<0.001
Proteins	8.79^{b}	10.33^{c}	7.95^{a}	10.21^{c}	0.38	0.002	0.006	<0.001
Ashes	2.61^{b}	2.84^{c}	2.51^{a}	2.59^{b}	0.05	<0.001	0.005	<0.001
Moisture	50.32^{b}	58.75^{d}	46.44^{a}	56.19^{c}	1.83	<0.001	0.021	<0.001
Total Calories	371.70^{c}	288.49^{a}	416.19^{d}	318.04^{b}	18.60	<0.001	0.045	<0.001

$^{a, b, c, d}$ Means with different letters in the same row indicate significant differences (p<0.05).
Abbreviations: T, type of fat; C, fat content; TxC, second level interaction between T and C.

Table 3
Fatty acid profile (% of total fatty acids) of the pâté formulations (n=2).

	Formulations				SEM	*p*		
	BF40	**BF28**	**SO40**	**SO28**		**T**	**C**	**TxC**
Miristic	1.27[a]	2.02[b]	n.d.	n.d.	0.13	<0.001	0.004	0.004
Palmitic	20.80	24.83	6.53	8.34	1.24	<0.001	0.048	0.183
Palmitoleic	2.14	2.30	n.d.	n.d.	0.19	<0.001	0.142	0.142
Stearic	8.90	9.32	3.10	4.57	0.41	<0.001	0.004	0.240
Oleic	50.49[c]	39.51[b]	28.74[a]	30.28[a]	1.13	<0.001	0.002	<0.001
Linoleic	14.18[a]	16.90[a]	61.63[c]	56.82[b]	3.25	<0.001	0.048	<0.001
Linolenic	0.50[a]	1.09[b]	n.d.	n.d.	0.06	<0.001	0.001	0.001
20:00	1.11	0.88	n.d.	n.d.	0.05	<0.001	0.310	0.310
20:2n-6	0.64	0.73	n.d.	n.d.	0.06	<0.001	0.148	0.148
Arachidonic	0.47	0.32	n.d.	n.d.	0.05	<0.001	0.001	0.001
SFA	32.07	37.05	9.63	12.91	1.82	<0.001	0.011	0.340
MUFA	52.62[c]	44.25[b]	28.74[a]	30.28[a]	1.40	<0.001	0.018	<0.001
PUFA	15.78[a]	19.03[b]	61.63[d]	56.82[c]	3.10	<0.001	0.003	<0.001
Total Unsat	68.40	63.27	90.37	87.10	1.83	<0.001	0.049	0.268
PUFA/SFA	0.49	0.51	6.39	4.40	0.47	<0.001	<0.001	0.004

n.d.: not detected.
[a-d] Different superscripts within the same row indicate that average values differ significantly ($p<0.05$).
Abbreviations:T, type of fat; C, fat content; T x C, second level interaction between T and C; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 4
Evolution of surface color parameters (*L**, *a** and *b**) of pâtés with different compositions during refrigerated (4 ± 1 °C) storage time

	Storage [d]						SEM
	0	**30**	**60**	**90**	**120**	**150**	
L*							
BF40	$60.23^{a,b,\alpha}$	$62.70^{c,\beta,\gamma}$	$60.64^{a,\alpha}$	$63.95^{b,\gamma}$	$61.48^{a,\alpha,\beta}$	$62.46^{a,\beta,\gamma}$	0.27
BF28	$59.19^{a,\alpha}$	$59.77^{a,\alpha,\beta}$	$59.86^{a,\alpha,\beta}$	$61.95^{a,\gamma}$	$61.16^{a,\beta,\gamma}$	$61.33^{a,\beta,\gamma}$	0.26
SO40	$61.58^{b,\alpha}$	$60.81^{a,b,\alpha}$	$64.60^{b,\gamma}$	$63.75^{b,\beta,\gamma}$	$62.32^{a,\alpha,\beta}$	$65.17^{b,\gamma}$	0.31
SO28	$61.45^{b,\alpha}$	$61.45^{b,c,\alpha}$	$63.55^{b,\beta}$	$62.44^{a,b,\alpha,\beta}$	$61.63^{a,\alpha}$	$60.89^{a,\alpha}$	0.20
a*							
BF40	$2.74^{a,\alpha}$	$2.39^{a,\alpha}$	$4.37^{a,\beta}$	$3.81^{a,\alpha,\beta}$	$5.97^{a,\gamma}$	$5.23^{b,\beta,\gamma}$	0.25
BF28	$6.70^{c,\beta}$	$6.07^{c,\alpha}$	$6.35^{b,\alpha,\beta}$	$7.74^{c,\beta}$	$9.16^{b,\gamma}$	$8.57^{c,\beta,\gamma}$	0.21
SO40	$2.18^{a,\alpha}$	$3.98^{b,\beta}$	$6.71^{a,b,\gamma}$	$6.59^{b,\gamma}$	$6.85^{a,\gamma}$	$4.04^{a,\beta}$	0.34
SO28	$5.22^{b,\alpha}$	$6.67^{d,\beta}$	$7.96^{c,\gamma}$	$8.93^{d,\gamma}$	$8.53^{b,\gamma}$	$8.01^{c,\gamma}$	0.19
b*							
BF40	$15.77^{a,\alpha}$	$17.14^{b,\beta}$	$15.34^{a,\alpha}$	$16.53^{a,b,\alpha,\beta}$	$16.38^{a,\alpha,\beta}$	$16.52^{a,\alpha,\beta}$	0.17
BF28	$15.10^{a,\alpha}$	$15.69^{a,\beta}$	$16.17^{a,\gamma}$	$16.04^{a,\gamma}$	$15.79^{a,b,\beta,\gamma}$	$15.94^{a,\beta,\gamma}$	0.21
SO40	$17.81^{b,\alpha}$	$18.61^{c,\alpha,\beta}$	$19.24^{c,\beta}$	$18.87^{c,\beta}$	$19.22^{c,\beta}$	$21.45^{c,\gamma}$	0.26
SO28	$17.48^{b,\alpha}$	$17.54^{b,\alpha}$	$17.94^{b,\alpha}$	$17.27^{b,\alpha}$	$17.86^{b,\alpha,\beta}$	$18.56^{b,\beta}$	0.12

^{a,b,c,d} Different letters within the same column indicate significant differences (p<0.05)
$^{\alpha,\beta,\gamma}$ Different letters within the same row indicate significant differences (p<0.05)

Table 5
Evolution of TBARS values (mg MDA/kg), carbonyl compounds (nmol/mg proteins) and hem iron content (µg/g pâté) in chicken liver pâté formulations during refrigerated storage time.

	Storage [d]						SEM
	0	**30**	**60**	**90**	**120**	**150**	
TBARS							
BF40	$0.47^{b,\alpha}$	$0.51^{a,\alpha}$	$0.57^{a,\alpha}$	$0.57^{a,\alpha}$	$0.50^{a,\alpha}$	$0.50^{a,\alpha}$	0.02
BF28	$0.31^{a,\alpha}$	$0.59^{a,\beta}$	$0.60^{a,\beta}$	$0.74^{b,\beta,\gamma}$	$0.70^{b,\beta}$	$0.95^{c,\beta,\gamma}$	0.03
SO40	$0.65^{c,\alpha,\beta}$	$0.59^{a,\alpha}$	$0.62^{a,\alpha,\beta}$	$0.55^{a,\alpha}$	$0.54^{a,\alpha}$	$0.71^{b,\beta}$	0.03
SO28	$0.40^{a,b,\alpha}$	$0.47^{a,\alpha}$	$0.66^{a,\beta}$	$0.65^{a,b,\beta}$	$0.72^{b,\beta}$	$0.63^{a,\beta}$	0.02
Carbonyl compounds							
BF40	$5.27^{c,\alpha}$	$4.62^{b,c,\alpha}$	$6.73^{b,\beta}$	$4.75^{a,\alpha}$	$5.14^{a,\alpha}$	$6.18^{b,\alpha,\beta}$	0.29
BF28	$2.25^{a,\alpha}$	$3.22^{a,\alpha,\beta}$	$3.99^{a,\beta}$	$5.69^{a,b,\beta,\gamma}$	$4.51^{a,\beta,\gamma}$	$7.13^{c,\delta}$	0.35
SO40	$4.17^{b,c,\alpha}$	$4.69^{c,\alpha}$	$5.45^{b,\alpha,\beta}$	$6.43^{b,\beta}$	$4.95^{a,\alpha}$	$5.37^{a,\alpha,\beta}$	0.26
SO28	$3.22^{a,b,\alpha}$	$3.26^{a,b,\alpha}$	$4.46^{a,\alpha,\beta}$	$4.36^{a,\alpha,\beta}$	$4.60^{a,\alpha,\beta}$	$5.74^{a,b,\beta}$	0.23
Fe Hem							
BF40	$6.30^{a,b,\beta,\gamma}$	$4.63^{a,\beta}$	$4.81^{a,\beta}$	$4.15^{b,\beta}$	$1.81^{a,\alpha}$	$2.23^{a,\alpha}$	0.41
BF28	$7.46^{b,\beta}$	$6.33^{b,\alpha}$	$6.32^{b,\alpha}$	$7.08^{c,\alpha,\beta}$	$7.00^{c,\alpha,\beta}$	$6.02^{b,\alpha}$	0.17
SO40	$5.65^{a,\beta}$	$4.73^{a,\beta}$	$5.14^{a,b,\beta}$	$2.42^{a,\alpha}$	$3.24^{a,\alpha,\beta}$	$2.14^{a,\alpha}$	0.32
SO28	$5.53^{a,\alpha,\beta}$	$4.51^{a,\alpha}$	$6.51^{b,\beta}$	$5.91^{b,c,\alpha,\beta}$	$5.34^{b,\alpha,\beta}$	$4.61^{b,\alpha}$	0.24

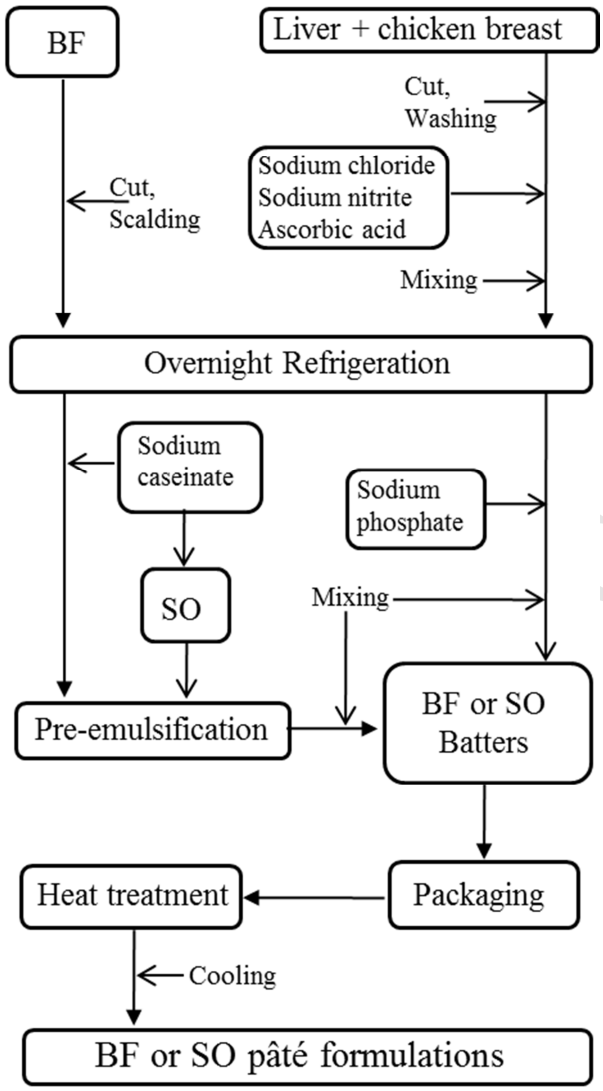
$^{a, b, c}$ Different letters within the same column indicate significant differences ($P<0.05$)
$^{\alpha, \beta, \gamma, \delta}$ Different letters within the same row indicate significant differences ($P<0.05$)

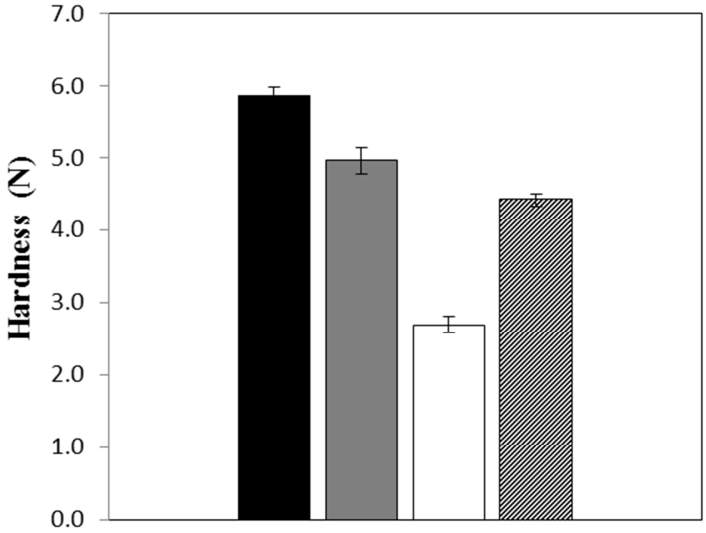
Figure captions

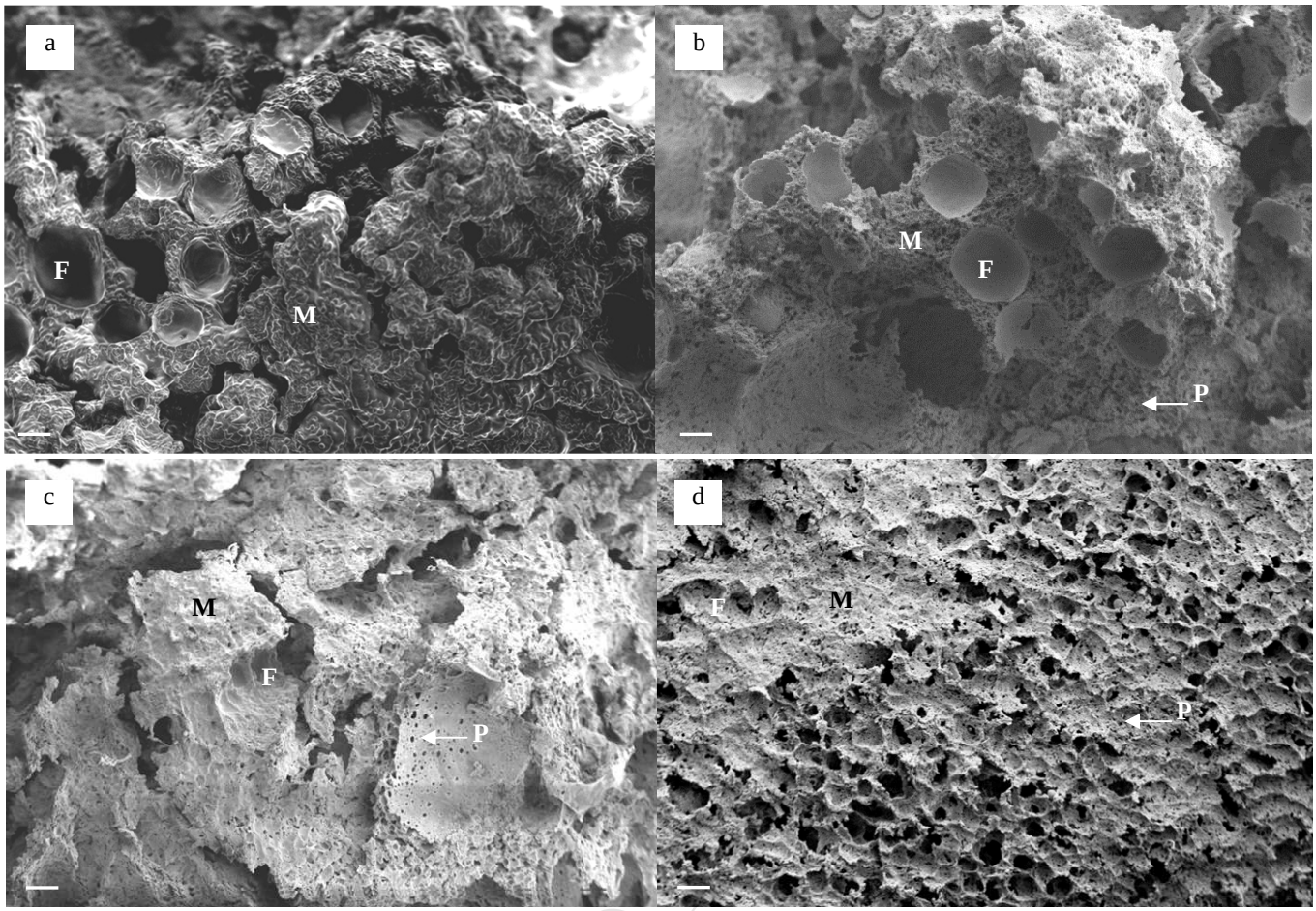
Fig. 1 Flow sheet of chicken liver pâté manufacturing

Fig. 2 Hardness (N) of different chicken liver pâtés. ■ BF40; ▩ BF28; □ SO40; ▨ SO28. The bars correspond to standard deviation of mean values.

Fig. 3 Scanning electron micrographs (x345 magnification) of chicken liver pâtés, a) BF40; b) BF28, c) SO40 and d) SO28. F= fat globules, M= protein matrix, P= pores. The scale bars are 50 μm in length.







Highlights:

Pâtés were developed with chicken liver, a by-product of poultry industry

Pâtés with sunflower oil (28% w/w) raised the nutritional value of this kind of foods

Lipid and protein oxidation were lower than those found in traditional liver pâtés

No substantial reduction in quality attributes were observed during storage