Accepted Manuscript

Nutritional improvement and physicochemical evaluation of liver pâté formulations

A.M. Terrasa, M. Dello Staffolo, M.C. Tomás

Please cite this article as: Terrasa, A.M., Staffolo, M.D., Tomás, M.C., Nutritional improvement and physicochemical evaluation of liver pâté formulations, *LWT - Food Science and Technology* (2015), doi: 10.1016/j.lwt.2015.11.018.

1   **Nutritional improvement and physicochemical evaluation of liver pâté formulations**

2

3   A.M. Terrasa[a], M. Dello Staffolo[b*], M.C. Tomás[b]

4   [a]*Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata (1900), Argentina*

5   [b]*CIDCA, CONICET CCT-La Plata, Facultad de Ingeniería and Facultad de Ciencias Exactas,*
6   *Universidad Nacional de La Plata, La Plata, Argentina*

7

8

9

10

11

[*] Corresponding author: M. Dello Staffolo, CIDCA: 47 esquina 116, La Plata, Argentina. Tel/Fax: +54 221 4254853. E-mail address: marinadellostaffolo@gmail.com

12   **Summary**

13   Pâté formulations composed of chicken liver, a by-product of poultry industry, have been

14   produced by replacing pork back fat with sunflower oil and reducing fat content. The

15   characterization of these products was performed, while the oxidative stability,

16   microstructure, texture, colour, and hygienic quality were determined throughout

17   refrigerated storage. The hardness of pâtés with sunflower oil was lower than the other

18   ones. Different microstructures regarding protein matrix, fat globules and pores, were

19   associated with fat type and content. The storage time, fat type and content influenced the

20   colour parameters. In terms of the oxidative stability, no reduction in the product quality

21   was found during the refrigerated storage. Pâtés with 28 % w/w of sunflower oil were the

22   most suitable formulation to increase the nutritional value for this kind of meat products.

23

24   **Keywords**: Chicken liver pâté, physicochemical properties, refrigerated storage

25

26 1. **Introduction**

27   Meat products are essential components of the human diet. However, these products

28 contain high levels of fat, cholesterol, and low (polyunsaturated fatty acids/saturated fatty

29 acids) PUFAs/SFAs ratios, linked with development of obesity, hypercholesterolemia and

30 cardiovascular diseases (Arihara, 2006). Currently, many consumers demand low-fat foods

31 with healthy ingredients. Some vegetable oils are an important source of PUFAs as well as

32 minor components such as phytosterols and tocopherols. They have been employed as

33 saturated fat replacers in meat products (Martin, et al. 2008, Pennisi Forell, et al. 2010).

34 However, the reduction and substitution of lipids can affect the physicochemical

35 characteristics of high fat foods like sausages, burgers and pâtés (Delgado-Pando, et al.

36 2011). Moreover, in these products development of rancidity could affect quality attributes

37 (odour, taste, colour, texture) reducing nutritional value (Estévez and Cava, 2006). Protein

38 oxidation might produce a loss of essential amino acids (Lund et al., 2007). Besides, the

39 stages of processing and preserving (cooking, refrigerating, freezing, etc.) could release the

40 iron from hem proteins decreasing its bioavailable content and modifying the colour

41 (Estévez and Cava, 2004).

42   Liver pâté is a traditional food manufactured using liver from pig or calf, porcine

43 back-fat and other characteristic ingredients. It is consumed all over the world, especially in

44 European countries and is generally considered an added value product with high

45 nutritional and sensory qualities (Estévez et al., 2007). In recent years, there has been a

46 very important increase in the production and consumption of poultry meat around the

47 world (USDA, 2014). However, the poultry industry generates by-products which are

48 generally underutilized, for example chicken liver.

49   The aim of this work was to produce chicken liver pâtés in order to obtain healthy

50   products and to study the influence of fat type and its content on their physicochemical

51   characteristics during refrigerated storage time.

52

53   **2. Materials and methods**

54

55   2.1. Manufacturing of liver pâtés

56   Pork back fat (BF), chicken breasts and livers were obtained from the local market

57   and sunflower oil (SO) was supplied from Aceitera General Deheza (Argentina). Four

58   formulations of pâtés (BF40, BF28, SO40 and SO28) were prepared by replacing pork with

59   chicken liver with different type of fat (BF, SO) and content (40 or 28 % w/w). These fat

60   levels were selected according to the traditional formulations and a 30% reduction of lipid

61   phase to obtain healthy products. Other ingredients were added at the same concentration

62   for all formulations (**Table 1**).

63   The manufacturing process is shown in **Figure 1.** BF was cut into cubes of about 15

64   mm side and scalding at 65 °C for 30 min. Liver and muscle free of connective tissue were

65   also cut into cubes and then, washed with chlorinated water and mixed with NaCl, $NaNO_2$

66   and ascorbic acid to achieve tissue nitrification. The purpose of this step is to preserve,

67   flavour and colour the pâtés. Scalded BF and SO were pre-emulsified with sodium

68   caseinate dissolved in distilled water at 75 °C. The batters were filled in glass flasks of 40

69   mm diameter and 60 mm height with about 80 g of mixture (or 40 mm height) which were

70   subjected to a heat treatment in a stainless steel autoclave. In the core of the pâtés,

71   temperature remained constant at 80 ± 2 °C for 30 min being monitored with a Cu-

72   Constantan type T thermocouple. Subsequently, the flasks were cooled to room temperature

4

73 and stored in the dark at 4 ± 1 °C for 150 days. Samples were taken to perform the assays

74 every 30 d. The procedure was repeated twice for each formulation.

75

76 2.2. Chemical composition

77 2.2.1. Proximate analysis and energy content

78 Moisture, ash, and protein contents were determined according to AOAC (1984)

79 methods: 24.002, 24.009 and 24.027, respectively. Lipid content was determined by the

80 Soxhlet method (AOAC 1984, 24.005) using ethyl ether as extraction solvent which was

81 evaporated using a Rotavapor R-114 (Büchi, Flawil, Switzerland). Lipid content was

82 expressed as g fat/100 g pâté. All determinations were performed in triplicate with freshly

83 manufactured pâtés. Caloric value (Kcal/100 g pâté) was calculated using the Atwater

84 coefficients corresponding to lipids (9.00 Kcal/g), proteins (4.02 Kcal/g) and carbohydrates

85 (3.87 Kcal/g).

86 2.2.2. Fatty acid profile

87 Total lipid extraction from pâtés was performed by method of Folch et al. (1957).

88 Fatty acid methyl esters (FAMEs) were prepared by acid esterification using 10 % BF_3 in

89 methanol (AOAC 1990, official method 969.33). FAMEs were analysed using a Hewlett

90 Packard, mod. HP-5890A, gas chromatograph, equipped with a flame ionization detector

91 (FID) and a capillary column Supelco Omega wax 11090-02A (30 m x 0.25 mm internal

92 diameter and 0.1 mm thick). The temperature program was set from 175 to 220 °C at 3

93 °C/min. The identification of peaks was performed by comparison with retention times of

94 reference fatty acids (Nu Check Prep, Inc., USA). The fatty acid analysis was carried out in

95 duplicate throughout refrigerated storage. In addition, back fat, sunflower oil, chicken

96 muscle and liver were individually analysed to know the influence of these ingredients on

5

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

98 acids.

99 2.2.3. Determination of tocopherols

100 Tocopherol content in pork back fat and sunflower oil was determined in duplicate

101 by a chromatographic technique based on IUPAC rules 2432 (1992) and AOCS Ce8-89

102 (1998). Lipids were extracted from adipose tissue using the Soxhlet method with n-hexane.

103 This solvent was removed by a rotary evaporator R-114 (Büchi, Flawil, Switzerland) under

104 vacuum at 40 ˚C. Subsequently, the extracted lipids were dissolved in n-hexane for

105 quantification by HPLC with fluorescence detection (λ excitation: 290 nm, λ emission: 330

106 nm). A Hewlett Packard HPLC Series 1050 chromatograph equipped with a Lichrosorb

107 normal phase column Si-60 (250 mm x 4 mm and 5 μm particle size) was used. Operating

108 conditions were: mobile phase isopropanol: hexane (0.5:99.5 v/v), a flow rate of 1.5

109 mL/min and 20 μl of injection volume.

110

111 2.3. Assessment of quality attributes throughout refrigerated storage

112 2.3.1. Texture Measurement

113 Penetration test was performed on pâté formulations in their flasks with a TA-XT2i

114 texture analyser (Stable Micro Systems, Godalming, UK) at room temperature. Force in

115 compression was measured with a 12.7 mm diameter cylinder probe (P/R 0.5 Delrin) which

116 penetrated the sample to a depth of 15 mm at a constant cross head speed of 1 mm/s. The

117 hardness (maximum force required to penetrate the sample in N) were obtained from the

118 force–time curves recorded in triplicate for each pâté formulation.

119 2.3.2. Microstructure

120   Microstructures of pâté formulations were observed by Scanning Electron

121   Microscopy. Samples were fixed with 2.5 % glutaraldehyde in sodium phosphate buffer 0.1

122   M (pH 7.2). Then, they were dehydrated with acetone and dried by critical point technique

123   with CO2 POLARON equipment. Furthermore, the samples were coated with a gold layer

124   by Pelco equipment 91000 and were observed in a microscope JEOL 35 CF (Tokyo,

125   Japan).

126   2.3.3. Colour

127   Colour parameters on the surface of the pâté formulations were measured at room

128   temperature in triplicate. CIE-LAB parameters: Lightness (L^*), redness (a^*) and

129   yellowness (b^*) were determined using a Chroma Meter CR-400 colorimeter (Minolta Co.,

130   Osaka, Japan).

131   2.3.4. Sanitary condition of pâtés

132   Microbiological analyses were performed in duplicate to evaluate sanitary condition

133   of the pâtés from 0 to 150 d. Every 30 d, 20 g of each pâté formulation was aseptically

134   removed from each package, transferred into sterile stomacher bags, homogenized with 80

135   mL of 0.1 % of sterile peptone solution and blended in stomacher (West Sussex, UK) for

136   60 s. Decimal progressive dilutions were prepared. Mesophylic aerobic and Psychrotrophic

137   microorganisms were evaluated on plate count agar (PCA Oxoid, Hampshire, UK), by pour

138   plates aerobic incubation at 30 °C for 48 h and 4 °C for 7 d, respectively.

139   *Enterobacteriaceae* microorganisms were enumerated on violet red bile agar (Merck,

140   KGaA, Darmstadt, Germany) by spread plates aerobic incubation at 37 °C for 24 h.

141   Sulphite-reducing Clostridium microorganisms were enumerated in differential clostridia

142   agar (Britania, Argentina) and incubated at 37 °C for 48 h in anaerobic condition. Results

143   were expressed as the average colony forming units per gram (CFU/g).

7

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 $p<0.05$.

167

3. Results and discussion

169

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

215 due to the replacement of saturated for unsaturated fats. This effect was also observed by

216 Martin et al. (2008) in pâtés with partial replacement of pork fat by olive oil. The fat

217 content significantly modified the hardness of pâtés. The presence of high amounts of

218 sunflower oil (SO40) resulted in soft pâtés (p=0.014) and the reduction of back fat content

219 produced the opposite effect (**Figure 2**). The storage time did not influence the hardness of

220 pâtés (p>0.05).

221 3.2.2. Microstructure

222 Micrographs for pâté formulations show a microstructure constituted by a matrix of

223 proteins with the inclusion of fat globules and pores (**Figure 3**). The matrix is composed of

224 proteins from chicken liver, muscle and sodium caseinate. This protein network consists of

225 both fibrous proteins (collagen, elastin, reticulin, actin and myosin) and globular proteins

226 (cytoplasmic proteins of liver tissue, sarcoplasmic myoglobin and haemoglobin).

227 According to Tornberg (2005), fibrous proteins are denatured by heat, acquire random

228 configurations and are associated with globular proteins forming the matrix. Also, the

229 presence of holes of different size can be observed in these images. The holes were

230 identified as the spaces where fat was placed in the gel matrix; this fat disappears with the

231 preparation of the samples. In addition, a large number of small pores distributed in the gel

232 network were seen in **Figure 3b, c and d**. These small pores could be associated with water

233 or air incorporated throughout the homogenization step in the preparation of the pâté

234 formulations.

235 The microstructure of pâtés formulations varied with fat type and content. BF pâtés

236 (**Figure 3a and b**) showed larger fat globules with more defined shape than SO pâtés

237 (**Figure 3c and d**). BF40 formulation (**Figure 3a**) exhibited a continuous protein matrix

238 and packed structure that may be associated with increasing instrumental hardness (**Figure**

11

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

12

263 fat percentage can be attributed to the high hem proteins content supplied by the liver,

264 which provide an enhanced reddish tint. Estévez et al. (2005) studied the physicochemical

265 properties of pork liver pâté with different fat contents (45, 40 and 35 % w/w) reporting

266 similar results.

267   Statistical analysis of the values obtained for the *b** parameter (**Table 4**) revealed

268 that pâtés which include back fat in their formulation were less yellow than those prepared

269 with sunflower oil ($p<0.01$). This behaviour was observed by other researchers when fat

270 was replaced by oil producing yellower meat products (Youssef and Barbut, 2009). BF40

271 and SO40 pâtés gave higher *b** values than BF28 and SO28 pâtés ($p<0.01$). The storage

272 time produced an increase ($p<0.01$) in yellowness of pâté formulations. Fernández-López et

273 al. (2004) observed that both oxidation and oxygenation of myoglobin could generated

274 increments in the *b** parameter. Considering that the third level interaction was significant

275 ($p<0.05$), the modifications in lightness, redness and yellowness produced by storage time

276 depended on the combination of fat type and content.

277 3.2.4. Sanitary condition of pâtés

278   No sulphite- reducing Clostridium was noted in any sample throughout the storage

279 period. The microbial counts did not exceed 60 CFU/g for the other groups of

280 microorganisms analysed: mesophylic aerobic, psychrotrophic and *Enteriobacteriaceae* at

281 final storage time. These results indicated that the heat treatment and the application of low

282 temperatures during the storage time were appropriate operations to maintain safe sanitary

283 conditions for all pâté formulations.

284

285 3.3. Oxidative stability

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

13

287 influenced lipid oxidation in the pâtés. TBARS values in BF28 and SO28 pâtés presented a

288 significant increase while SO40 pâtés showed a slight increase **(Table 5)**. These behaviours

289 might be explained considering that high moisture content in BF28 and SO28 may promote

290 lipid oxidation. TBARS values in BF40 remained steady during storage time. SO40 pâtés

291 presented the highest TBARS value (0.65 mg MDA/Kg) at initial storage time, possibly

292 because its high content of PUFAs produces an increase in the susceptibility to lipid

293 oxidation in pâté manufacturing steps (disruption of tissues and subsequent heat treatment).

294 No influence of fat type in TBA values ($p>0.05$) was observed in statistical analysis.

295 SO pâtés exhibited the TBA values lower than expected, taking into account their fatty acid

296 composition rich in PUFAs **(Table 5)**. This behaviour could be attributed to the high

297 Vitamin E content in sunflower oil. Thus, total tocopherol level in this oil was 502 ± 21

298 µg/g, with α-tocopherol being the major component (498 ± 20 µg/g) followed by β-

299 tocopherol (4 ± 1 µg/g); γ and δ vitamers were not detected. Muguerza et al. (2003) also

300 observed the influence of the natural antioxidants present in vegetable oils on lipid

301 oxidation when back fat is replaced with soybean oil in Pamplona chorizo. Besides, the

302 total tocopherol content in back fat was also considerable (350 ± 20 µg/g), with α-

303 tocopherol as the only vitamer found.

304 Protein oxidation is considered to be linked to lipid oxidation. In the presence of

305 oxidized lipids, the protein oxidation is produced by free radical chain reactions similar to

306 those for lipid oxidation (Faustman et al., 2010). The carbonyl compounds content of pâté

307 formulations significantly changed ($p<0.05$) with refrigerated storage time (**Table 4**). In

308 this case, BF28 and SO28 increased significantly; while BF40 and SO40 fluctuated mildly

309 probably due to the by-products of lipid oxidation could have interacted with proteins.

310 Besides, the fat content was a significant factor since the pâtés with 40 % w/w fat content

14

311   had significantly higher levels of carbonyl compounds than pâtés with 28 % w/w fat. On

312   the other hand, the statistical evaluation showed that the type of fat did not significantly

313   affect ($p>0.05$) the protein oxidation, similar to the case of the TBA test.

314   Hem iron is another parameter to evaluate oxidative damage in fatty meat products.

315   Greater concentrations of iron and myoglobin are associated with greater rates of lipid

316   oxidation (Faustman et al., 2010). In this work, the hem iron content was affected

317   significantly ($p<0.01$) by refrigerated storage time. BF40 and SO40 showed a hem iron

318   decrease while BF28 and SO28 pâtés varied around 6.50 and 5.50 µg/g pâté, respectively

319   **(Table 5)**. The fat type and its content significantly affected ($p<0.01$) the hem iron values

320   obtained. BF pâtés presented higher hem iron contents than those SO pâtés. Furthermore,

321   BF40 and SO40 pâtés showed significantly lower values of hem iron than BF28 and SO28

322   pâtés, probably due to their higher fat/liver ratios, since liver is the main component that

323   provides iron in BF28 and SO28 formulations. The third level interaction was also

324   significant ($p<0.05$), indicating that changes produced in TBARS, carbonyl compounds and

325   hem iron levels by the storage time depended on the combination of fat type and content.

326   Liver pâtés contain high amounts of fat and iron, and therefore, oxidative

327   deterioration of liver pâtés during refrigeration was expected. Georgantelis et al. (2007)

328   reported that the rancid flavour is detected in meat products with TBARS values higher

329   than 0.6 mg MDA/kg, while Campo et al. (2006) considered that the limiting threshold for

330   the acceptability of oxidized beef is around 2.0 mg MDA/kg. In this work, all pâté

331   formulations presented TBARS levels below 1mg MDA/kg after 150 d of storage at 4 ± 1

332   °C. Moreover, the carbonyl compounds contents were lower than those found in traditional

333   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

16

358   products.

359

363

364   **References**

365   AOAC (1984). *Official methods of analysis* (14th Ed.). Washington, DC: Association of

366   Official Analytical Chemists.

367   AOAC (1990). Methyl esters of fatty acids in oils and fats. In K. Helrich (Ed.), *Official*

368   *methods of analysis* (pp. 963-964). Arlington, USA: Association of Official

369   Analytical Chemists.

370   AOCS (1998). *Official methods and recommended practices of the American Oil Chemists'*

371   *Society*. Champaign, USA: AOCS Press.

372   Arihara, K. (2006). Strategies for designing novel functional meat products. *Meat Science,*

373   74, 219-22.

374   Barbut, S., Gordon, A., & Smith, A. (1996). Effect of Cooking Temperature on the

375   Microstructure of Meat Batters Prepared with Salt and Phosphate. *Lebensm.-Wiss.*

376   *u.-Technol.*, 29, 475–480.

377   Bozkurt, H. (2006). Utilization of natural antioxidants: green tea extract and

378   Thymbraspicata oil in Turkish dry-fermented sausage. *Meat Science*, 73, 442-450.

379   Campo, M.M., Nute, G.R., Hughes, S.I., Enser, M., Wood, J.D., & Richardson, R.I. (2006).

380   Flavour perception of oxidation in beef. *Meat Science*, 72, 303-311.

381   Crehan, C.M., Hughes, E., Troy, D.J., & Buckley, D.J. (2000). Effects of fat level and

382   maltodextrin on the functional properties of frankfurters formulated with 5, 12 and

383   30 % fat. *Meat Science*, 55, 463-469.

384   D'Arrigo, M., Hoz, L., Cambero, I., López-Bote, C.J., Pin, C., & Ordóñez, J.A. (2004).

385   Production of n-3 fatty acid enriched pork liver pâté. *Lebensm.-Wiss. u.-Technol,*

386   37, 585–591.

387   Delgado-Pando, G., Cofrades, S., Rodríguez-Salas, I., & Jiménez-Colmenero, F. (2011). A

388   healthier oil combination and konjac gel as functional ingredients in low-fat pork

389   liver paté. *Meat Science,* 88, 241-248.

390   Estevez, M. & Cava, R. (2004). Lipid and protein oxidation, release of iron from heme

391   molecule and colour deterioration during refrigerated storage of liver pâté. *Meat*

392   *Science,* 68, 551-558.

393   Estévez, M. & Cava, R. (2006). Effectiveness of rosemary essential oil an inhibitor of lipid

394   and protein oxidation: Contradictory effects in different type of frankfurters. *Meat*

395   *Science*, 72, 348-355.

396   Estévez, M., Ramírez, R., Ventanas, S. & Cava, R. (2007). Sage and rosemary essential oils

397   versus BHT for the inhibition of lipid oxidative reactions in liver pâté. *Lebensm.-*

398   *Wiss. u.-Technol*, 40, 58-65.

399   Estévez, M., Ventanas, J. Cava, R. & Puolanne, E. (2005). Characterization of a traditional

400   Finnish liver sausage and different types of Spanish liver pâtés: a comparative

401   study. *Meat Science*, 71, 657-669.

402   Faustman, C., Sun, Q., Mancini, R., Suman, S. P. (2010). Myoglobin and lipid oxidation

403   interactions: Mechanistic bases and control. *Meat Science*, 86, 86–94.

404 Fernández-López, J., Sevilla, I., Sayas-Barberá, E., Navarro, C. Marín, F., & Pérez-
405   Alvarez, J.A. (2003). Evaluation of the antioxidant potential of hyssop (*Hyssopus*
406   *officinalis L.*) and rosemary (*Rosmarinus officinalis L.*) extracts in cooked pork
407   meat. *Journal of Food Science,* 68, 660-664.

408 Fernández-López, J., Sayas-Barberá, E., Sendra, E., & Pérez-Alvarez, J.A. (2004). Quality
409   characteristics of ostrich liver pâté. *Journal of Food Science*, 69, 85-91.

410 Folch, J., Lees, M., & Sloane-Stanley, G.M. (1957). A simple method for the isolation and
411   purification of total lipids from animal tissue. *Journal of Biological Chemistry,* 226,
412   497-508.

413 Georgantelis, D., Blekas, G., Katikou, P., Ambrosiadis, I., & Fletouris, D.J. (2007). Effect
414   of rosemary extract, chitosan and α-tocopherol on lipid oxidation and colour
415   stability during frozen storage of beef burgers. *Meat Science*, 75, 256-264.

416 IUPAC (1992). Standard Methods for the Analysis of Oils, Fats and Derivates. In C.
417   Paquot, & A. Hautffenne (Eds.), International Union of Pure and Applied Chemistry
418   (7th ed.). Oxford, UK: Backwell Scientific Publications Inc.

419 Jiménez Colmenero, F. (2007). Healthier lipid formulation approaches in meat-based
420   functional foods. Technological options for replacement of meat fats by non-meat
421   fats. *Trends in Food Science & Technology*, 18, 567-578.

422 Lombardi-Boccia, G., Martínez-Domínguez, B., Aguzzi, A., & Rincón-León, F. (2002).
423   Optimization of heme iron analysis in raw and cooked red meat. *Food Chemistry*,
424   78, 505-510.

425 Lund, M. N., Hviid, M.S., & Skibsted, L.H. (2007).The combined effect of antioxidants
426   and modified atmosphere packaging on protein and lipid oxidation in beef patties
427   during chill storage. *Meat Science*, 76, 226-233.

428 Martin, D., Ruiz, J., Kivikari, R., & Puolanne, E. (2008). Partial replacement of pork fat by

429   conjugated linoleic acid and/or olive oil in liver pâtés: Effect on physicochemical

430   characteristics and oxidative stability. *Meat Science*, 80, 496-504.

431 Muguerza, E., Ansorena, D., & Astiasarán, I. (2003). Improvement of nutritional properties

432   of Chorizo de Pamplona by replacement of pork back fat with soy oil. Meat

433   Science, 65, 1361-1367.

434 Oliver, C.N., Ahn, B.W., Moerman, E.J., Goldstein, S., & Stadtman E.R. (1987). Age-

435   related changes in oxidized proteins. *The Journal of Biological Chemistry,* 262,

436   5488-5491.

437 Pennisi Forell, S.C., Ranalli, N., Zaritzky, N.E., Andrés, S.C., & Califano, A.N. (2010).

438   Effect of type of emulsifiers and antioxidants on oxidative stability, colour and fatty

439   acid profile of low-fat beef burgers enriched with unsaturated fatty acids and

440   phytosterols. *Meat Science,* 86, 364-370.

441 Rosmini, M.R., Perlo, F., Pérez-Alvarez, J.A., Pagan-Moreno, M.J., Gago-Gago, A.,

442   López-Santovena, F., & Aranda-Catalá, V. (1996). TBA test by an extractive

443   method applied to pâté. *Meat Science,* 42, 103-110.

444 Totosaus, A. & Pérez-Chabela, M.L. (2009). Textural properties and microstructure of low-

445   fat and sodium-reduced meat batters formulated with gellan gum and dicationic

446   salts. *LWT-Food Science and Technology*, 42, 563-569.

447 Tornberg, E. (2005). Effects of heat on meat proteins-Implications on structure and quality

448   of meat products. *Meat Science,* 70, 493-508.

449 USDA (2014). [http://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf]

450   Youssef, M.K. & Barbut, S. (2009). Effects of protein level and fat/oil on emulsion

451   stability, texture, microstructure and color of meat batters. *Meat Science*, 82, 228-

452   233.

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

[a,b,c,d] Different letters within the same column indicate significant differences ($p<0.05$)
[α,β,γ] 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)

[α, β, γ, δ] Different letters within the same row indicate significant differences (P<0.05)

Figure captions

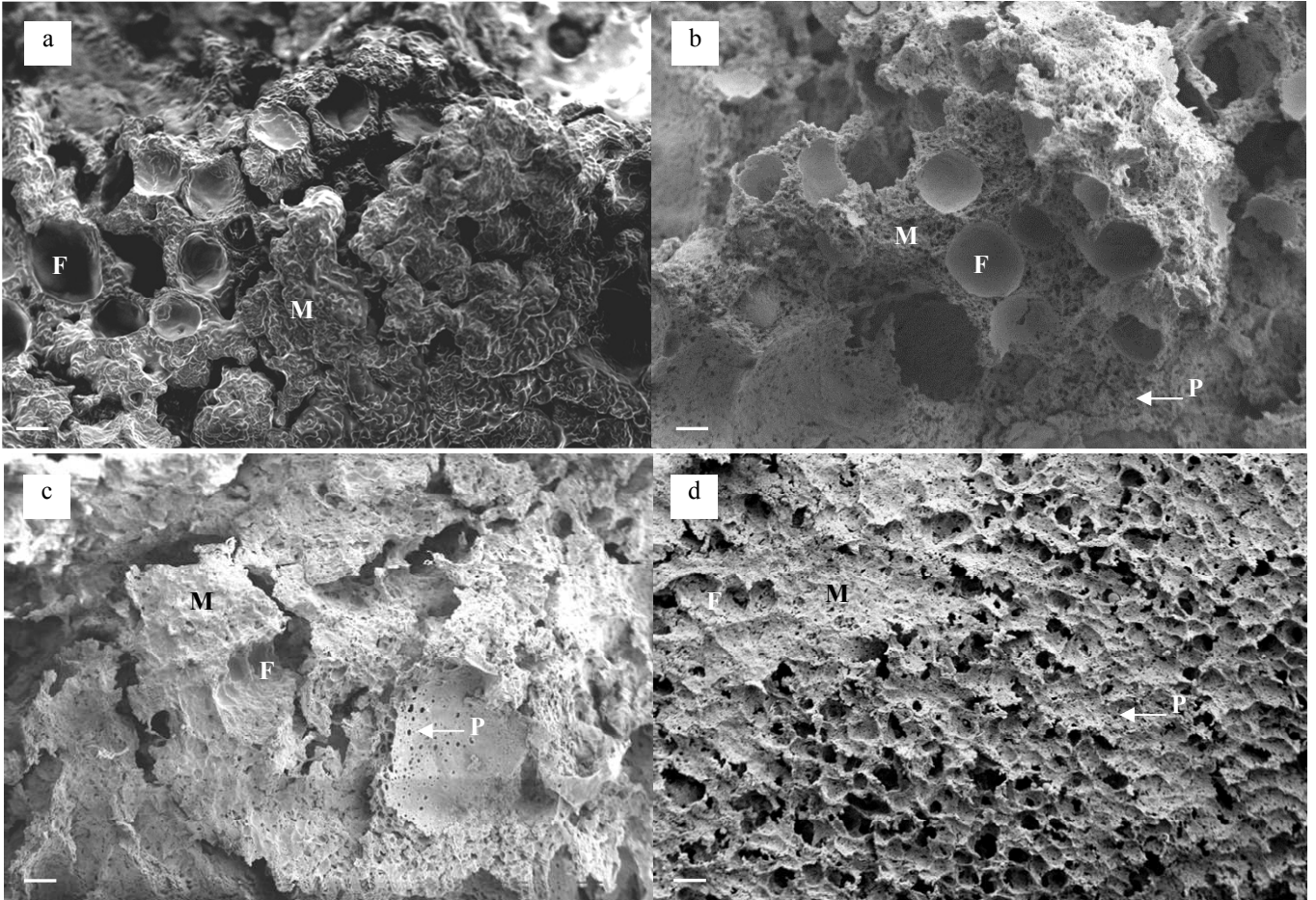**Fig. 1** Flow sheet of chicken liver pâté manufacturing

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.
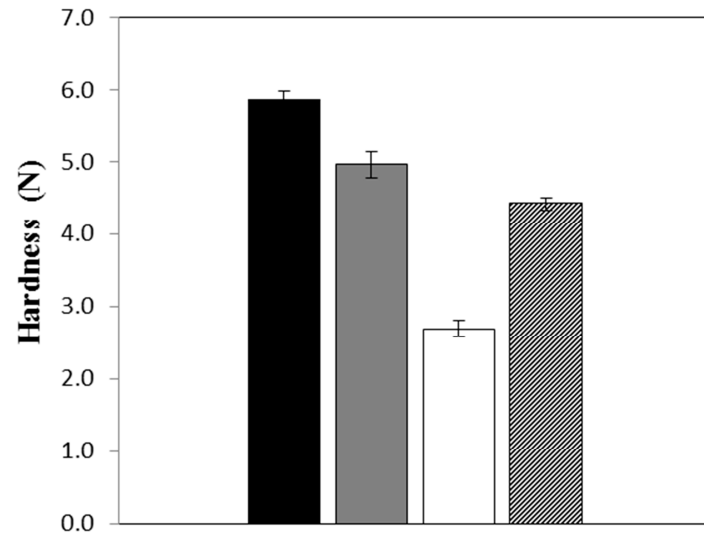
Highlights:

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