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Evaluation of the nutritional aspects and cholesterol oxidation products of pork liver and fish pates

Maider Echarte, Ana Conchillo, Diana Ansorena, Iciar Astiasarán *

Departamento de Bromatología, Tecnología de Alimentos y Toxicología, Facultad de Farmacia, Universidad de Navarra, 31080 Pamplona, Spain

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Abstract

A comparison of traditional pork liver and fish pates (salmon, anchovy and cod) was carried out. Nutritional value and cholesterol oxidation products (COP) were evaluated. Salmon pate showed similar fat content (24–28%) and energy value (300 kcal/100 g) to pork liver pates, whereas pates made with anchovy and cod showed less fat (13–16%) and calories (200–236 kcal/100 g). PUFA/ SFA ratios were much higher in all fish patés (1.55–4.95) than in liver pork patés (0.36–0.44). No great differences were found in ω -6/ ω -3 ratio between salmon and pork liver pates (11.3–18.4), this ratio being even higher in anchovy (32.3) and cod pates (62.8). EPA and DHA supply was around 0.63 for salmon, 0.21 for anchovy and 0.07 for cod pates. Cholesterol concentrations were lower in fish patés (31–37 mg/100 g) than in pork liver patés (77–102 mg/100 g). Total COP ranged from 0.38 to 2.83 ppm, without clear differences between pork liver and fish patés.

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Keywords: COP; Salmon; Anchovy; Cod; Canned foodstuffs

1. Introduction

Pate is a cooked product with important gastronomic traditions and good sensory properties. Traditionally pate has been elaborated with goose liver (''foie-grass'') or pork liver. However, during recent years, many of new products have been launched on the market, especially those including fish, due to the nutritional advantages shown by these products.

Epidemiological studies carried out by Dyerberg and Bang (1979) showed the low incidence of cardiovascular pathologies in the Eskimo population, who are great fish consumers. This fact was related to the abundance of ω -3 long chain fatty acids in the fish fat, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Further reports have demonstrated that the beneficial effects of these fatty acids are due to a complex interaction of a number of mechanisms, such as reduction of the plasma levels of triglycerides, decrease in platelet aggregation, their antiarrithmic effect and their

beneficial effect on endothelial dysfunction (DeCaterina, Liao, & Libby, 2000; Goodfellow, Bellamy, Ramsey, Jones, & Lewis, 2000; Harris, 1989; Kang & Leaf, 2000; Von Shacky, 2000).

Consequently, many studies show positive effect of the ω -3 fatty acids in relation to the prevalence of rheumatic arthritis, cancer, development of metastasis, hypertension and cardiac arrhythmia (Caygill & Hill, 1995; Simopoulos, 1997). Furthermore, an increased intake of ω -3 polyunsaturated fatty acids from fish may have substantial implications for public health and health economy by decreasing the risk of coronary events and sudden cardiac death (Schmidt, Skou, Christensen, & Dyerberg, 2000).

Other epidemiological studies show that increasing fish intake from one to two servings per week to five or six servings per week does not substantially reduce the risk of coronary heart disease among men who are initially free of cardiovascular disease. (Ascherio, Rimm, Stampfer, Giovannucci, & Willlet, 1995). However, this higher intake has been positively correlated with a reduction of the risk of mortality in patients who have already had a myocardial stroke (Hu, Manson, & Willet,

^{*} Corresponding author. Tel.: +34-948-425600; fax: +34-948-425649. E-mail address: [iastiasa@unav.es](mail to: iastiasa@unav.es) (I. Astiasarán).

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2001). Furthermore, there is evidence that the incorporation of a dietary supplement containing EPA and DHA significantly reduces the risk factors for cardiovascular disease (Yam, Bott-Kanner, Genin, Shinitzky, & Klainman, 2002).

Other types of compounds clearly involved in the development of cardiovascular diseases, in this case with a negative effect, are cholesterol oxides (Smith, 1996). Some experiments in humans show their ability to be absorbed from the diet (Emanuel, Hassel, Addis, Bergmann, & Zavoral, 1991; Linseisen & Wolfram, 1998). Many undesirable biological effects, such as cytotoxicity, mutagenicity, carcinogenicity, angiotoxicity, atherogenicity and cell membrane damage have been attributed to them (Guardiola, Codony, Addis, Rafecas, & Boatella, 1996; Tai, Chen, & Chen, 1999).

Yan (1999), reviewing COP formation in foodstuffs, concluded that there is a need to re-examine processing conditions in order, not only to produce organoleptically superior products, but also products with low levels of COP. Tai, Chen, and Chen (2000), in a review about the presence of cholesterol oxides in different food, concluded that these compounds were formed in most processed food containing cholesterol, their formation being difficult to prevent. Heating, which is the main technological step for the adequate elaboration and preservation of cooked products, including pate, is one of the main causes of their synthesis.

The presence of unsaturated fatty acids enhances cholesterol oxidation, through the development of free radicals and peroxides during heating (Korytowski, Bachowski, & Girotti, 1992; Osada, Kodama, Cui, Yamada, & Sugano, 1993). As fish fat is characterised by higher proportions of long chain unsaturated fatty acids than other food products, it is more susceptible to oxidation (Tai et al., 2000). Osada et al. (1993) demonstrated that levels of cholesterol oxides in processed fish products were higher than those found in other processed food products, such as dairy products or egg derivatives. The objective of this work was to study the lipid fraction of different samples of pork liver pate and fish pate, taking into account two points of view: the potential nutritional advantages of fish pates over pork liver pates and the evaluation of the intensity of the cholesterol oxidation processes in these products.

2. Materials and methods

2.1. Commercial samples

Six different types of pates were purchased from local supermarkets. Three of them corresponded to traditional products elaborated with liver pork. The rest were patés elaborated with different fish species: salmon, anchovy and cod.

Ingredients used in the patés (according to the respective labelling) were:

Pork liver patés. Trade 1: pork liver, pork backfat, pork fat and meat, water, milk protein, rice flour, salt, stabilisers (carrageenates, disodium diphosphate), spices, taste enhancer (monosodium glutamate), potassium sorbate, E-217, sodium nitrite, potassium nitrate and flavourings. Trade 2: pork backfat, liver and meat, water, wheat flour, salt, spices, stabilizer (E-450), sugar, taste enhancer (E-621), antioxidant (E-330) and conservant (E-250). Trade 3: pork liver, pork backfat, pork meat, water, wheat flour, salt, milk protein, antioxidant (E-330) and conservant (E-250).

Fish patés. Salmon paté: salmon (40%), skimmed milk, proteins and vegetable oils, dewlap, salt, spices, stabilizers (E-407). Anchovy pate: anchovy (25%), milk, mashed potatoes, water, vegetable oil, protein, flour, flavourings, gelifiers (E-407, E-412, E-410 y E-415) and colorant (E-127). Cod pate: cod (37%), milk, water, vegetable oil, potato starch, salt, garlic, milk proteins, vegetable fibre, flavourings, stabiliser (carrageenan) and spices.

2.2. Chemical analysis

2.2.1. Proximal analysis

Moisture was determined by desiccation (AOAC, 2002a). Protein was analysed using the Kjeldahl method for the determination of nitrogen (AOAC, 2002b), using 6.25 as the factor to transform nitrogen into protein. Fat was determined by the Soxhlet method with petroleum ether (AOAC, 2002c). Ashes were determined by incineration using the method of the AOAC (AOAC, 2002d).

2.2.2. Analysis of fatty acids

Quantitative fat extraction was carried out for subsequent analysis of fatty acids with a chloroform/ methanol mixture, using the method of Folch, Lees, and Sloane-Stanley (1957). The fatty acid profile was determined by gas chromatography after previous methylation with BF₃/methanol (AOAC, 2002e). Chromatographic conditions used were as described by Muguerza, Gimeno, Ansorena, Bloukas, and Astiasarán (2001).

 0.5 μ l of the sample were injected into a gas chromatograph with a FID detector (Perkin–Elmer Autosystem XL) fitted with a capillary column SP^{TM} -2560 (100 m \times 0.25 mm \times 0.2 µm). The temperatures of the injection port and detector were both 220 °C. The oven temperature was 165 °C during 80 min, followed by an increase to 220 \degree C at a rate of 4 \degree C/min and 50 min at 220 °C. The carrier gas was hydrogen (20 psi). The identification of the peaks was done by comparison of their retention times with those of pure standard compounds (Sigma, St. Louis, MO, USA) and the quantification of individual fatty acids was based on heptadecanoic acid methyl ester as internal standard.

2.2.3. Cholesterol determination

The determination of cholesterol was done by gas chromatography, according to the method described by Kovacs, Anderson, and Ackman (1979). A Perkin– Elmer Autosystem gas chromatograph equipped with an HP1 column (30 m \times 0.25 mm \times 0.1 m) was used. The oven temperature was 265 °C. The temperatures of the injection port and detector were both 285 \degree C. The sample size was 0.5 µl. Cholesterol was identified by comparing its relative and absolute retention times with those of cholestane (Sigma, St. Louis, MO, USA) as an internal standard. A Perkin–Elmer Turbochrom programme was used for quantification.

2.2.4. Cholesterol oxides determination

Cholesterol oxides were determined using the method described by Echarte, Ansorena, and Astiasarán (2001). Cold saponification was performed, then a further extraction of the cholesterol oxides with ether, a purification with silica cartridges and a derivatization to obtain the trimethyl silyl ethers. 1μ l of sample was injected in a gas chromatograph HP 6890GC System (Hewlett-Packard) coupled to a 5973 Mass Selective Detector (Hewlett-Packard). The column used was HP-5MS (30 $m \times 250 \mu m \times 0.25 \mu m$) and helium as carrier gas (1 ml/ min). Oven temperature was initially 80 \degree C, held for 1 min, and programmed to 250 $\mathrm{^{\circ}C}$ at a rate of 10 $\mathrm{^{\circ}C/min}$, and final column temperature of 280 °C at a rate of 4 °C/ min, and held for 20 min. The injector temperature was 250 \degree C and the inlet pressure was 23.2 psig; mass range was 50/550; solvent delay was 20 min. Identification of the peaks was done by comparison of the mass spectra obtained for every pure compound with those of the samples. Quantification was done using 19-hydroxycholesterol as internal standard (Sigma, St. Louis, MO, USA).

2.3. Data analysis

Four samples were analysed from every trade of pate. Each parameter was determined four times in each sample. Data shown in the tables are the mean $(n = 16)$ with standard deviations. One-way ANOVA and a Tukeys b posteriori test were used to analyse statistical differences between samples ($p \le 0.05$). The Pearson correlation was determined among different parameters. Software used was SPSS 9.0 for Windows.

3. Results and discussion

Patés elaborated with liver and fat from pork or goose (foie-grass) are usually, according to traditional food composition tables, highly energetic products (around 300–400 kcal/100 g) with relatively high percentages of lipids (30–40%) (Mataix, 1994; McCance $\&$ Widdowson, 1992; Moreiras, Carbajal, Cabrera, & Cuadrado, 2001; Senser & Scherz, 1999). Samples from the three commercial trades containing pork liver analysed in this work showed lipid concentrations of about 24– 28%, leading to calorific values that ranged between 285 and 320 kcal/100 g (Table 1). Comparing these data with those obtained for fish pates, it can be seen that only salmon patés showed similar amounts of fat (26%) , giving rise to the highest calorific value among the fish patés. Patés made with anchovy or cod showed 10–13% less fat percentages (16.10% and 13.72%, respectively) and around 70–100 kcal less per 100 g (236 and 200 kcal/ 100 g, respectively) than the rest of the pates. These differences could be due to the different proportions of fish employed in the formulation, the different fat contents of the species and the different amounts of other fat-supplying ingredients (vegetable oils). Labels of fish patés show that they were elaborated with 40% salmon, 25% anchovy and 37% cod, respectively. Furthermore, these species differ substantially in their fat content. Composition tables show values of fat of around 12% for salmon, 5% for anchovy and 0.3% for cod (Belitz $\&$ Grosch, 1988; McCance & Widdowson, 1992).

It is known that all fish species have similar protein amounts, these being inversely correlated with their contents of fat and water. No significant differences were observed among the three types of fish pates in the protein amount, values ranging from 7.51% to 8.62%. This protein comes, not only from the fish, but also from other protein ingredients (milk and additives). Meat

Table 1

General composition (g/100 g product), cholesterol content (mg/100 g product) and calorific value (kcal/100 g) of the analysed samples

	Pork liver			Fish patés		
	Paté 1	Paté 2	Paté 3	Salmon	Anchovy	Cod
Moisture	57.06 ± 0.46^a	52.46 ± 0.31 ^a	$56.95 + 0.25^{\text{a}}$	$55.44 + 0.47$ ^a	$56.06 + 2.75^{\circ}$	$64.63 + 3.22^b$
Protein	$9.93 + 0.65^{\rm b}$	$14.50 + 1.03^c$	$8.38 + 0.45^{\circ}$	$7.51 + 80.38^a$	$8.62 + 0.46^a$	$7.99 + 0.57$ ^a
Fat	$26.18 + 0.88^{\circ}$	$28.05 + 0.52^d$	$24.61 + 0.75$ °	$26.39 + 1.55$ ^{cd}	$16.10 + 0.42^b$	13.72 ± 0.83 ^a
Ash	$2.21 + 0.21$ ^a	$2.72 + 0.24^b$	$2.46 + 0.06^{ab}$	$2.37 + 0.19$ ^{ab}	$5.02 + 0.06^{\circ}$	$2.54 + 0.16^{ab}$
$Carbo$ hydrates $(*)$	4.62	2.27	7.6	8.29	14.2	11.1
Calorific value	294	320	285	301	236	200

Different letters in the same row show significant differences among samples ($p < 0.05$).
^{*}Calculated by difference.

samples showed a greater variability in their protein content, values ranging from 8.38% to 14.5%. This last value is similar to that observed in the composition tables (14%). The water content did not show significant differences between meat and fish pates, except for the cod pate, which showed the highest amount. Carbohydrates (which were determined by difference) were present in greater amounts in fish pates.

Values found for the total mineral content (ashes) ranged between 2.21% and 2.72% in meat samples. This type of food is considered as a good source of some minerals: Na (present as a consequence of using NaCl in the elaboration), Fe (supplied by the liver as the main ingredient), Ca, Mg and Zn. The values obtained for the fish patés were similar, except for the anchovy paté, whose ash content was double. This higher content ash was also detected in other commercial anchovy pates (Aquerreta, Astiasarán, Mohino, & Bello, 2002), and could be explained by the presence of the bones of this little fish in the final product.

Together with the calorific value, the relatively high supply of cholesterol is the main drawback of the presence of meat products in the diet. Values obtained for the pork liver pates ranged between 77.6 mg/100 g and 102 mg/100 g, clearly lower than those found in the literature for this type of product (255 mg/100 g). Chizzolini, Zanardi, Dorigoni, and Ghidinim (1999), in a review about the cholesterol content of meat and meat products, established that the cholesterol content ranged from 60 to 99 mg/100 g for meat and from 37 to 110 mg/ 100 g for meat products. Fish pates did not show significant differences in their cholesterol contents, with values ranging from 31.4 to 36.9 mg/100 g. These similar results among fish pates, in spite of the different fat contents of the species, can be explained by the supply of cholesterol from other ingredients, such as milk. Salmon pate, the one with the highest fish fat supply was elaborated with skimmed milk, whereas anchovy and cod patés were elaborated with whole milk, which was obviously of supply of cholesterol. The lower amounts of cholesterol in fish patés in relation to pork liver patés, especially salmon patés, which have similar fat content, could be explained by the fact that vegetable oils contribute to the total fat content but not to the total cholesterol content. Pork liver pates did not include vegetable oils.

Recent epidemiological studies conclude that the type of fat supplied in the diet is an important factor in relation to health. It has been proved that the substitution of saturated fat by unsaturated fat is more effective in the decreased of risk of cardiovascular disease than only reduction of total fat intake (Hu et al., 2001). Furthermore, the influence of the different types of saturated fatty acids (SFA) on the cholesterol levels and risk of cardiovascular disease has been determined.

Traditionally in the nutritional evaluation of the lipid fraction of food, stearic acid (C18:0) has been excluded from the SFA fraction, because it does not raise plasma cholesterol levels as the rest of the SFA do (Candela, Astiasarán, & Bello, 1997; Tholstrup, Marckmann, Jespersen, & Sandstrom, 1994; Zapelena, Aquerreta, Astiasarán, & Bello, 1995). However, it decreases the HDL fraction, so it seems that the exclusion is not completely justified (Hu et al., 2001) and in our work it has been included in the total amount of the SFA fraction.

Palmitic acid was most abundant in the saturated fraction of every pork liver pate, followed by stearic acid (Table 2), highly contributing to the total amount of this fraction, whose values ranged between 6.61 g/100 g and 8.07 g/100 g (Table 3). Myristic and lauric acids, presumably those with the strongest influence on raising the cholesterol levels (Kris-Etherton & Yu, 1997) supplied about 0.3 g to the total SFA fraction. The values obtained in the fish pates for the SFA fraction were lower, particularly in the anchovy patés $(1.4 \text{ g}/100 \text{ g})$ and in the cod patés $(1.8 \text{ g}/100 \text{ g})$.

Oleic acid was the most abundant fatty acid in the pork liver pates. This monounsaturated fatty acid was the main contributor of MUFA, this being the most abundant fraction in these patés $(8.6 - 10.4 \text{ g}/100 \text{ g})$. Similar data was found for salmon pate (9.83 g MUFA/ 100 g), whereas cod, and especially anchovy patés, showed much lower amounts of oleic acid. The intake of MUFA has been inversely associated with risk of CHD, although the association is weaker than for polyunsaturated fat (Hu et al., 1997).

Fish pates showed higher total PUFA amounts than pork liver pates. The main reason was probably the use of seed vegetable oils, which are a good source of ω -6 PUFA, particularly linoleic acid. Great differences were found in the linoleic contents of fish and meat products. In relation to the potential ω -3 supply of fish patés, only salmon patés showed significant differences. Total ω -3 PUFA of salmon reached 0.75 g/100 g sample, in contrast with ranges between 0.09 and 0.26 found in the rest of pates. In pork liver pates, the main $ω$ -3 PUFA was linolenic acid, which is also found in high amounts in salmon patés, probably as a consequence of the use of dewlap in its formulation or even the use of a seed vegetable oil with a higher proportion of this acid. EPA and DHA showed the highest amounts in salmon $(0.63 \text{ g}/100 \text{ g})$ and anchovy $(0.21 \text{ g}/$ 100 g) pates, the ones with the highest fish fat supply. However, cod patés $(0.07 \text{ g}/100 \text{ g})$ did not show significant differences in the contents of these fatty acids in relation to pork liver patés $(0.02-0.04 \text{ g}/100 \text{ g})$. ω -6/ ω-3 ratios were high in all samples, and especially in anchovy and cod pate, which was due to the combination of a high amount of linoleic acid supplied by vegetable oils and the low amounts of ω -3 fatty acids,

Different letters in the same row show significant differences among samples ($p < 0.05$).

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

especially in the case of the cod pate. Aquerreta et al. (2002) observed that experimental fish pates elaborated without vegetable oils showed lower ω -6/ ω -3 ratios than commercial fish pates.

All these values gave rise to higher PUFA/SFA ratios for the fish pates, particularly in the anchovy pate (4.95), than for the pork liver patés, which had showed values lower than 0.45.

The influence of *trans* fatty acids on cardiovascular health has been widely demonstrated (Hu et al., 2001; Salmerón et al., 2001). High intakes of this type of fatty acid have been associated with a stronger risk of cardiovascular diseases, mediated by a number of different mechanisms (e.g. increment of LDL cholesterol, decrease of HDL cholesterol, increment of lipoprotein A levels, increment of plasma triglycerides). Neither the pork liver patés nor the fish patés showed concentrations higher than 0.3 g/100 g. This amount can be considered as low taking into account that, for example, a medium portion of French fries contains 5–6 g trans fatty acids/100 g or donuts contain 2 g trans fatty acids/ 100 g (Katan, 2000).

Technological conditions applied during the elaboration of the pates (especially heating intensity) and the different fatty acid profiles of the samples would explain the differences in the intensity of COP formation. Total COP ranged from 38 to 283 μ g/100 g (0.38 to 2.83 ppm). Percentages of oxidation ranged between 0.05 and 0.73, the last one being that of cod pate (Table 4).The amounts of COP found in the different analysed samples were not correlated with their cholesterol contents, nor with the unsaturated fatty acid contents. Not many references have been found in the literature to COP content in commercial meat products. In dry-cured ham,

Table 4

Different letters in the same row show significant differences among samples ($p < 0.05$).

%Oxidation = $[(\mu g \text{ COP} * 10^{-3}) / \text{mg cholesterol}] * 100$.

with 12–24 months of ageing, total COP ranged from 2.8 to 5.8 ppm (Vestergaard & Parolari, 1999). Zunin, Boggia, and Evangelisti (2001) found that COP values amounted to $0.3-3$ μ g/g in canned tuna. Novelli et al. (1998) found a very large range of total COP concentrations in samples of salame Milano and mortadela (0– 30.9 ppm). These authors reputed up that the most toxic COP, 25-hydroxycholesterol and cholestanetriol, were rarely observed in the analysed samples. Wang, Yang-Nian, and Chin-Wen (1995), analysing lipid and cholesterol oxidation in Chinese-style sausages, detected neither 25-hydroxycholesterol or cholestanetriol. In the analysed pates, 25-hydroxycholesterol was only detected in one of the pork liver patés, whereas cholestanetriol appeared in all of the fish samples and in one of the pork liver pate, always at amounts lower than 0.27 ppm. 7-Ketocholesterol, considered to be a good indicator of oxidation (Penazzi et al., 1995; Zunin, Calcagno, & Evangelisti, 1998), was present in all analysed samples, except anchovy pate. This compound and the 7α -hydroxycholesterol showed significant correlations (0.780 and 0.926) with total COP.

In summary, it can be stated up that the potential nutritional advantages of fish pates in relation to classical pork liver patés depends largely on the species and the rest of the ingredients. Only significant increments in EPA and DHA were observed in salmon patés in relation to pork liver patés, ω -6/ ω -3 ratio being even much higher in anchovy and cod patés than in pork liver patés. Although cholesterol amounts were lower in fish pates than in pork liver pates, no clear differences were observed in the COP amounts.

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