Oxidation Process Affecting Fatty Acids and Cholesterol in Fried and Roasted Salmon

Maider Echarte,[†] M. Angeles Zulet,[‡] and Iciar Astiasaran*[,†]

Departamento de Bromatología, Tecnología de los Alimentos y Toxicología and Departamento de Fisiología y Nutrición, Facultad de Farmacia, Universidad de Navarra, 31080 Pamplona, Spain

Salmon was processed by three different culinary techniques: pan-frying with olive oil, pan-frying with soya oil, and roasting. Roasting did not modify the fat content from that of raw samples. Frying increased the fat content 2-fold, with no difference between samples fried with different oils. Total cholesterol oxidized products (COPs) were 0.74, 2.98, 3.35, and 7.38 μg/g fat in raw, fried with olive oil, fried with soya oil, and roasted salmon, respectively, which represent 0.01, 0.08, 0.09, and 0.15% of cholesterol. A significant correlation ($r = 0.902$, ≤ 0.01) was found between acidity index and total COPs. The most abundant COPs were 7-ketocholesterol, which appeared in all the samples, and cholestanetriol (one of the most citotoxic COP), which appeared only in cooked samples (1.05−1.33 μg/g fat). All cooked samples supplied more ω-6 polyunsaturated fatty acids (PUFAs) than raw samples and showed higher ω-6/ω-3 ratios. Roasted salmon showed the lowest ω-3 content and the highest PUFAs/(SFAs)-C18:0 and MUFAs+PUFAs/(SFAs-C18:0) ratios.

Keywords: *Cholesterol oxidation products (COPs); fatty acids; salmon; roasting; frying*

INTRODUCTION

During the last few years, there have been many studies about the beneficial effects of consuming fish in the diet. These advantages have been related with the fat composition of fish which is rich in long-chain ω-3 fatty acids, particularly eicosapentaenoic fatty acid (EPA) and docosahexaenoic fatty acid (DHA). Diets with a high content of these ω-3 fatty acids (ω-3 PUFAs) may reduce not only the risk of cardiovascular diseases but also the risks of hypertension and arthritis (*1*−*3*). High-fat fish species, which are the main supply of ω-3 PUFAs in the diet, also show significant cholesterol content. The two types of compounds, ω-3 fatty acids and cholesterol, are lipids highly susceptible to oxidation giving rise to free radicals. These compounds have been related with aging and different diseases, such as cancer, diabetes, Parkinson's disease, and Alzheimer's disease (*4, 5*).

There are some research studies indicating that high intakes of cholesterol oxides (or cholesterol oxidized products, COPs) from food can increase plasma COP levels (*6*). Also, intestinal absorption of COPs from the diet (*7*−*9*) has been demonstrated in animals. The relationship between oxidized lipoproteins and atherosclerosis has been widely studied. Staprans et al. (*10*) suggested that the consumption of food with oxidized lipids may be an important factor for atherosclerosis. Staprans et al. (*11*), in a work carried out in low-density lipoprotein receptor (LDLR)- and apolipoprotein-E-deficient mice, demonstrated that oxidized cholesterol in the diet accelerates fatty streak lesion formation. There is no direct evidence yet in humans that oxysterols contribute to atherogenesis, but oxysterols are present in human atherosclerotic plaque and are suggested to play an active role in plaque development; even the oxysterol:cholesterol ratio in plaque is much higher than that in normal tissues or plasma (*12*). Moreover, numerous reviews have been written concerning other undesirable biological properties such as cytotoxicity, mutagenicity, and carcinogenesis (*13*−*16*).

Generally, heat, pH, light, oxygen, water activity, and the presence of unsaturated fatty acids are the major factors that influence COP formation during processing or storage. Although the formation pathways of certain COPs still have not been fully clarified (*17*), it is known that the main COPs are formed during cholesterol autoxidation in C7 and C5−C6 positions and in side chain (C20, C25). Amounts of COPs in foods can frequently reach 1% of total cholesterol and occasionally 10% or more (*18*). Tai et al. (*19*), in a review about the content of COP in foods, concluded that the most-processed products containing cholesterol are susceptible to form COPs, with heating being the most common causative factor. Fish included in the diet generally are subjected to some type of culinary process involving heating, as a result of which the lipid oxidation is favored. Modifications in the fatty acid profiles by different culinary technologies, such as deep fat frying or cooking with steam, have been studied in some fish species (*20*−*24*), but few references can be found in relation to COP formation in fish. Paniangvait et al. (*25*), in a study about the presence of COP in foods, concluded that this aspect should be taken into account in relation to the safety of consumption of some products.

The aim of this work was to analyze the oxidation process suffered by fatty acids and cholesterol in one of the highest fat fish species (salmon) commonly present in the diet, as a consequence of two current culinary techniques: pan frying and roasting. Also, the influence of different oils (olive and soya) was studied.

* To whom correspondence should be addressed. Phone: 948-425600. Fax: 948-425649. E-mail: iastiasa@unav.es.
[†] Departamento de Bromatología.
[‡] Departamento de Fisiología y Nutrición.

10.1021/jf010199e CCC: $20.00
Published on Web 10/02/2001

Table 1. Fatty Acids Content (g/100 g Fat) of the Olive Oil and Soya Oil Used to Cooking

fatty acid g/100 g fat	olive oil	soya oil
lauric 12:00	0.02	0.02
(0.00; 4.98)	(0.00; 2.91)	
myristic 14:00	0.12	0.16
(0.01; 4.98)	(0.06; 6.58)	
palmitic 16:00	10.74	9.72
(0.11; 0.98)	(0.06; 0.64)	
palmitoelaidic 16:00t	0.02	0.02
(0.00; 1.19)	(0.00; 2.91)	
palmitoleic 16:1	0.14	0.11
(0.01; 7.74)	(0.01; 9.51)	
stearic 18:0	2.93	4.25
(0.05; 1.83)	(0.02; 0.54)	
oleic 18:1	71.08	21.52
(0.93; 1.31)	(0.19; 1.03)	
elaidic 18:1t	0.10	0.09
(0.01; 6)	(0.02; 22.09)	
linolelaidic 18:2t	0.04	0.11
(0.00; 12.73)	(0.05; 43.23)	
linoleic 18:2	8.83	57.32
(0.14; 1.54)	(0.60; 1.04)	
arachidic 20:0	0.48	0.33
(0.03; 5.77)	(0.17; 49.82	
linolenic 18:3	0.67	8.31
(0.03; 4)	(0.12; 1.44)	
behenic 22:0	0.23	0.34
(0.02; 9.06)	(0.08; 24)	
arachidonic 20:4	0.02	0.02
(0.00; 1.19)	(0.01; 48.36)	
brassídic 22: 1t	0.04	0.04
(0.00; 1.19)	(0.00; 2.91)	
erúcic 22:1	0.16	0.17
(0.00; 1.19)	(0.04;2.91)	
eicosapentaenoic 22:5	0.53	0.18
(0.01; 1.25)	(0.00; 3.53)	
docosahexaenoic 22:6	0.04	0.03
(0.01; 11.99)	(0.00; 2.80)	

MATERIALS AND METHODS

Sample Preparation. Salmon was purchased from a local supermarket and cut into slices around 1.5 cm thick. Fish slices were divided into four portions, and one of them was analyzed immediately. Two of them were fried at 180 °C during 4 min (2 min for each side) in a conventional frying pan (17.5-cm internal diameter and 4-cm depth) with 30 mL of olive oil and 30 mL of soya oil, respectively. The samples were introduced into the frying pan when the oil had reached the specified temperature, which was controlled by a digital thermometer (Fluka 51).The fourth portion was roasted with 45 mL of olive oil in an oven at 200 °C during 30 min. Table 1 shows the fatty acid profiles of the oils used as obtained by gas chromatography (GC).

Analytical Procedures. *Moisture.* Moisture content was calculated by drying the samples according to ISO 1443 (*26*).

Total Fat. Total fat was determined with an extraction technique using petroleum ether prior to hydrolysis (*27*).

Acidity Value. Acidity value was determined using method ISO 1740 (*28*).

Peroxide Value. Peroxide value was determined using method ISO 3960 (*29*).

Qualitative Extraction of Lipids. Total lipids were extracted using chloroform/methanol (2:1, v/v) according to the Folch et al. (*30*) procedure for the analysis of fatty acids and COP.

Fatty Acids. Fatty acid composition was determined by GC. BF_3/methanol was used for the preparation of fatty acid methyl esters (*23*). An automatic Perkin-Elmer Autosystem XL model GC fitted with an SP-2560 fused silica capillary column (100m $\times$ 0.25 mm $\times$ 0.2 μm) (Supelco, Inc., Bellefonte, PA) and flame ionization detection was used. The temperature of the injection port and detector was 220 °C, and the oven temperature was increased from 165 °C to 220 °C at a rate of 4 °C/min. The carrier gas was hydrogen. Peaks were identified by comparison of their retention times with those of standard mixtures (Sigma, St. Louis, MO, 99% purity specific for GC), and their areas were automatically integrated using nonadecanoic acid methyl ester (Sigma) as the internal standard.

Cholesterol. The cholesterol content was calculated according to the method of Kovacs et al. (*30*) by GC. A Perkin-Elmer Autosystem with an HP1, (30 m $\times$ 0.25 mm $\times$ 0.1μm) packed with Supelcoport (Supelco, Inc., Bellefonte, PA) was used. The oven temperature was 260 °C. The temperature of the injection port and detector was 285 °C. Hydrogen was the carrier gas used. Samples of 0.5 μL were injected. Cholesterol standards (Sigma), with concentrations ranging from 0 to 3 mg/mL in chloroform, were used, and to each was added 1 mL of a cholestane (Sigma) solution in chloroform (2 mg/mL) as internal standard. After analysis, the cholesterol/cholestane area ratio was determined and a plot of cholesterol/ cholestane area ratio versus cholesterol/cholestane weight ratio was obtained. By referring to this standard curve, the amount of cholesterol of the unknown sample was calculated. A Perkin-Elmer Turbochrom program was used for the cholesterol quantification.

COP. Extraction of Lipids and Saponification. Saponification was made according to the method of Guardiola et al. (*33*). Approximately 1 g of fat was added to a flask containing 1 M KOH in methanol and kept at room temperature during 22 h to complete the cold saponification. The unsaponificable material was extracted with diethyl ether. The whole organic extract was washed with water and filtered through anhydrous sodium sulfate. Then it was recovered in a round-bottom flask, and the solvent was evaporated using a rotatory vacuum evaporator at 30 °C.

Identification and Quantification. Oxysterols content was determined by a Hewlett-Packard 6980 GC with 5973 mass selective detector. GC−MS analyses were performed in Rtx-5 column (20 m $\times$ 0.18 mm $\times$ 0.2 μm). Helium was used as the carrier gas, and the chromatographic conditions were as follows: initial column temperature at 80 °C, held for 1 min, and programmed to 250 °C at a rate of 10 °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 °C and the inlet pressure was 23.2 psig; mass range, m/z = 50/550; solvent delay was 20 min. Calibration curves were developed for five cholesterol oxides (7-ketocholesterol, 7-β-hydroxycholesterol, 7-α-hydroxycholesterol, 5α,6α-epoxycholesterol, and 25-hydroxycholesterol) and cholestanetriol. 19-Hydroxycholesterol was used as the internal standard (IS). All these standards were purchased from Sigma (St. Louis, MO). Figure 1 shows the chromatogram resolution of standard mixture. Figure 2 also shows the chromatogram resolution of one of the analyzed samples (roasted sample).

Data Analysis. Four samples were analyzed from each of the four portions. Each parameter was determined four times in each sample. Data shown in the tables are the means (n = 16) with standard deviations and coefficients of variations. Statistical test one-way ANOVA and a Tuckey's posteriori test were used to analyze statistical differences between samples. Correlations between different parameters were analyzed by Pearson's correlation coefficients (r). A significance level of $p \leq 0.05$ was used for the study of differences between means, and a significance level of $p \leq 0.01$ was used for Pearson's coefficients. Software used was SPSS version 9.0.

RESULTS AND DISCUSSION

In all cooked samples the sum of moisture and fat kept quite constant (71−77%) and was similar to that of raw samples (75%) (Table 2). Cooking with heating caused not only the loss of water but also a significant increase of fat in the fried samples. No significant differences were found in the absorption of fat between samples fried with olive oil and soya oil. Candela et al. (*23*) when applying deep fat frying with sunflower oil to the same fish species (salmon) did not find a significant increase in the fat content. In that paper, sardines,

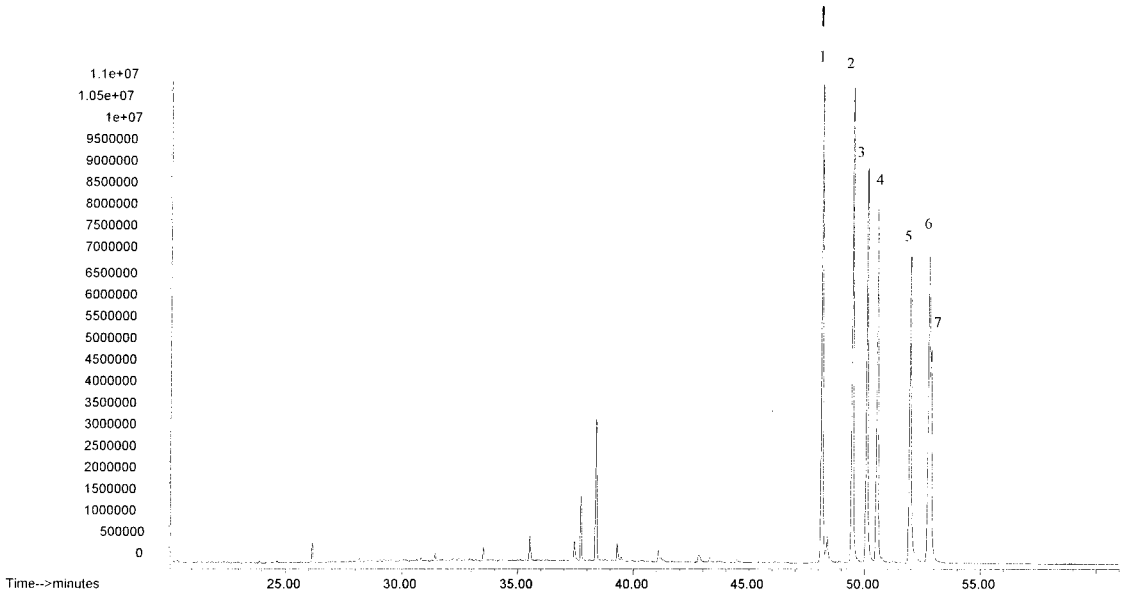

Figure 1. GC/MS chromatogram of standard mixture of TMS-ethers of cholesterol and its oxidation derivatives. Peaks: 1, 7α-hydroxycholesterol; 2, 19-hydroxycholesterol (IS); 3, 7*β*-hydroxycholesterol; 4, 5α,6α epoxycholesterol; 5, cholestanetriol; 6, 25-hydroxycholesterol; 7, 7-ketocholesterol.

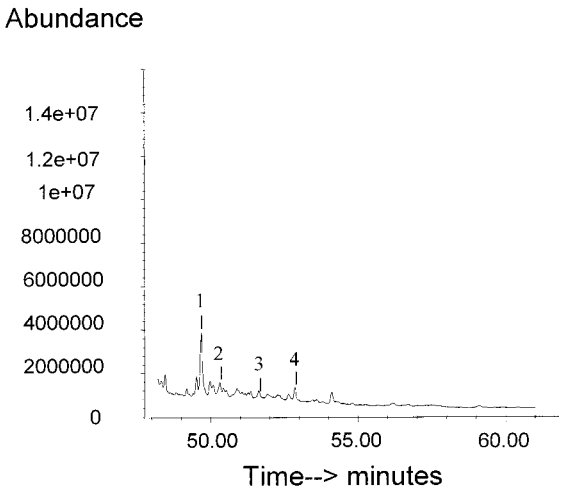

Figure 2. GC/MS chromatogram of a salmon sample roasted at 200 °C during 30 min. Peaks: 1, 19-hydroxycholesterol (I. S.; 2, 7*β*-hydroxycholesterol; 3, cholestanetriol; 4, 7-ketocholesterol.

another high-fat fish, showed a greater increment in its fat content (from 4% in raw sample to 13.3% in fried sample).

In roasted samples no significant modifications were observed in the fat content, and the moisture decrease was much less than that in fried samples. Comparing the roasted salmon with the fried salmon the difference in the fat content was about 10%, which implies a difference in the calories supplied of around 90K cal/100 g.

In relation to the cholesterol content, Table 2 shows the obtained results expressed by mg/100 g sample and mg/g fat. The cholesterol content in cooked samples, especially in the case of fried samples, when it is referred to fat (mg/g fat) was lower than that in raw salmon. Probably the decrease of cholesterol in fat with frying is due to the absorption of vegetable oil which did not contain cholesterol in its composition. The oxidation process which probably took place with frying and roasting also could contribute to the decrease of cholesterol content in these cooked samples. In roasted samples this is probably the main cause for the cholesterol decrease because the total fat content did not show significant differences with reference to raw samples. When the cholesterol content is referred to a 100 g sample the supply by fried samples was higher (without differences between samples fried with olive and soya oils) than that of roasted samples.

Table 2 shows also the results obtained for acidity index and peroxide values. Acidity index is one parameter that measures the free fatty acids produced during the lypolisis, which is one of the processes that take place during the fat alteration. The roasted samples showed the highest acidity index, probably as a result of the more drastic conditions of the process (200 °C and 30 min). Lypolysis favored the oxidation process which affects, between other compounds, fatty acids and cholesterol. The primary components from the oxidation process are peroxides: unstable compounds which disappear resulting in other oxidized products. The analysis of peroxide values showed that none of analyzed samples had detectable amounts of peroxides.

The fatty acid profiles of raw salmon, salmon fried with olive oil, salmon fried with soya oil, and roasted salmon are showed in Table 3. From the quantitative point of view the main fatty acids were palmitic, oleic, eicosapentaenoic, and docosahexaenoic acids in all the samples. Lauric, linolelaidic, arachidic, and behenic did not have their contents modified significantly with cooking. Palmitoleic and eicosapentaenoic fatty acids decreased, whereas arachidonic and linoleic acids increased, with frying and roasting. Docosahexaenoic acid showed a little but significant increase with roasting, which is difficult to explain when taking into account that the olive oil used contained scarcely any of this fatty acid (Table 1). Total PUFAs and MUFAs were 21.39 and 24.36; 21.23 and 21.43; 22.61 and 19.83; and 22.34 and 25.91, in fresh, fried with olive oil, fried with soya oil, and roasted salmon, respectively. These results showed that unsaturated fatty acids which are susceptible to the oxidation process did not oxidize in a significant way. The observed variations were probably due to the vegetable oils used during frying (*34*).

Table 2. Moisture, Fat, and Cholesterol Contents, Acidity Index, and Peroxide Values of the Different Analyzed Samples[a]

salmon	moisture %	fat %	cholesterol mg/100 g sample	cholesterol mg/g fat	acidity index	peroxide value
raw	65.49[d]	9.76[a]	53.34[b]	5.47	2.15[a]	-
(1.28; 1.96)	(0.35; 3.64)	(3.21;3.16).	(0.13; 6.19)			
fried with olive oil	55.57[a]	19.54[b]	69.96[c]	3.58	2.64[b]	-
(0.48; 0.87)	(1.13; 5.80)	(2.21; 3.16)	(0.39; 14.6)			
fried with soya oil	59.12[b]	17.97[b]	66.38[c]	3.69	3.48[b]	-
(0.62; 1.05)	(2.46; 14.24)	(1.25; 1.89)	(0.25; 7.31).			
roasted	62.51[c]	8.57[a]	40.92[a]	4.77	7.63[c]	-
(0.99; 1.59)	(0.38; 4.47)	(4.37; 10.68)	(0.32; 4.20)			

[a] Data are mean values, with standard deviations and coefficient of variation in parentheses. −, not detected. Different letters denote significant differences among samples ($p < 0.05$) for every parameter.

Table 3. Fatty Acids Content (g/100 g Fat) of the Different Samples[a]

fatty acid g/100 g fat	raw salmon	salmon fried with olive oil	salmon fried with soya oil	roasted salmon
lauric 12:00	0.08[a]	0.08[a]	0.07[a]	0.06[a]
(0.08; 2.13)	(0.01; 14.61)	(0.00; 4.66)	(0.00; 6.60)	
myristic 14:00	4.55[c]	4.13[bc]	3.87[ab]	3.46[a]
(0.19; 4.15)	(0.24; 5.79)	(0.08; 2.01)	(0.34; 9.76)	
palmitic 16:00	10.92[ab]	12.32[c]	11.77[bc]	10.25[a]
(0.48; 4.36)	(0.16; 1.30)	(0.03; 0.22)	(1.16; 11.34)	
palmitoelaidic 16:1t	0.16[a]	0.23[b]	0.16[a]	0.15[a]
(0.01; 6.13)	(0.05; 20.30)	(0.01; 7.59)	(0.01; 7.23)	
palmitoleic 16:1	5.37[c]	4.75[b]	4.43[b]	3.90[a]
(0.27; 5.11)	(0.10; 2.17)	(0.04; 0.84)	(0.43; 11.04)	
stearic 18:0	2.01[a]	2.62[b]	2.58[b]	2.09[a]
(0.08; 3.88)	(0.02; 0.75)	(0.02; 0.81)	(0.25; 11.95)	
oleic 18:1	11.41[a]	13.49[bc]	12.27[ab]	14.56[c]
(0.58; 5.07)	(0.13; 0.96)	(0.11; 0.87)	(1.86; 13.03)	
elaidic 18:1t	0.27[ab]	0.44[c]	0.34[ab]	0.21[a]
(0.19; 68.76)	(0.09; 20.169	(0.02; 5.99)	(0.02; 8.81)	
linolelaidic 18:2t	0.10[a]	0.07[a]	0.11[a]	0.08[a]
(0.01; 7.13)	(0.02; 30.99)	(0.02; 18.89)	(0.04; 56.58)	
linoleic 18:2	2.89[a]	3.80[b]	5.20[c]	4.96[c]
(0.14; 4.99)	(0.05; 1.39)	(0.04; 0.80)	(0.55; 11.15)	
arachidic 20:0	0.15[a]	0.19[a]	0.19[a]	0.15[a]
(0.01; 8.47)	(0.02; 12.19)	(0.01; 7.36)	(0.02; 15.98)	
linolenic 18:3	0.88[b]	0.55[a]	1.33[c]	1.35[c]
(0.03; 3.26)	(0.02; 3.67)	(0.01; 0.56)	(0.14; 10.56)	
behenic 22:0	0.29[a]	0.29[a]	0.32[a]	0.28[a]
(0.02; 4.66)	(0.94; 32.80)	(0.11; 34.26)	(0.09; 32.19)	
arachidonic 20:4	0.11[a]	0.15[b]	0.14[b]	0.18[c]
(0.04; 4.04)	(0.02; 12.86)	(0.01; 8.18)	(0.02; 11.69)	
brassídic 22:1t	0.43[b]	0.57[c]	0.40[b]	0.34[a]
(0.02; 4.66)	(0.02; 3.12)	(0.01; 1.46)	(0.03; 9.50)	
erúcic 22:1	5.57[b]	0.57[a]	0.55[a]	5.36[b]
(0.28; 5.10)	(0.02; 3.12)	(0.01; 1.31)	(0.84; 15.70)	
eicosapentaenoic 22:5	8.75[c]	7.83[b]	7.35[b]	5.34[a]
(0.50; 5.67)	(0.19; 2.42)	(0.06; 0.87)	(0.43; 7.99)	
docosahexaenoic 22:6	8.76[a]	8.9[a]	8.59[a]	10.51[b]
(0.47; 5.34)	(0.90; 10.10))	(0.20; 2.29)	(0.71; 6.78)	

[a] Data are mean values with its standard deviation and coefficient of variation in parentheses. Different letters denote significant differences among samples ($p < 0.05$) for every parameter.

The oxidation process affected cholesterol in a more significant way. Table 4 shows the results of the analysis of different cholesterol oxides. Total oxides increased from 0.74 μg/g fat in raw salmon to 2.98 in fried with olive oil, 3.35 in fried with soya oil, and 7.38 μg/g in roasted salmon. The highest content of COP in roasted samples was in agreement with the highest acidity index shown by these samples. A significant correlation ($r = 0.902$, $p \leq 0.01$) was found between values of acidity index and total COP in the analyzed samples. Total COP values represent 0.01, 0.08, 0.09, and 0.15% of cholesterol in raw salmon, salmon fried with olive oil, salmon fried with soya oil, and roasted salmon, respectively. Pie et al. (*35*) found that in egg powder, butter cakes, and cookies, cholesterol oxides represented 0.16% to 1.47% of cholesterol. Zanardi et al. (*36*) and Ghiretti et al. (*37*), studying different products such as mortadella, sausage, and coppa, found an average cholesterol oxidation ranged from 0.02% to 0.18%.

Roasted samples showed at the same time higher COP and cholesterol contents than those of fried samples which could be explained by two facts: the lowest proportion of fish fat in fried samples in which the vegetable oil absorbed at high level did not supply cholesterol, and the lowest intensity of cholesterol oxidation process during frying.

Oshima et al. (*38*), when analyzing cholesterol oxides in fish products popular in Japan (salted−dried, boiled−dried, and smoked), found that 7-β-hydroxycholesterol and 7-ketocholesterol were the most prominent. Jeong-Hee et al. (*39*) cooking saury (a fish with a high content

Table 4. Cholesterol Oxidized Products (μg/g fat) of the Different Analyzed Samples[a]

salmon	cholestanetriol	7-keto-cholesterol	α-epoxy-cholesterol	25-hydroxy-cholesterol	7-α-hydroxy-cholesterol	7-β-hydroxy-cholesterol	total oxides
raw	0[a]	0.74[a] (0.04; 5.51)	nd	nd	nd	0[a]	0.74
fried with olive oil	1.05[ab] no data	1.93[b] (0.45; 23.19)	nd	nd	nd	0[a]	2.98
fried with soya oil	1.76[b] (0.36; 20.7)	0.85[a] (0.06; 7.73)	nd	nd	nd	0.74[a] (0.06; 7.82)	3.35
roasted	1.33[b] (0.07; 4.91)	3.35[c] (0.20; 5.86)	nd	nd	nd	2.7[b] (1.11; 4.13)	7.38

[a] Data are mean values with its standard deviation and coefficient of variation in parentheses. Different letters denote significant differences among samples, ($p < 0.05$) for every parameter.

Table 5. Sum and Ratios between Different Types of Fatty Acids in the Different Samples

fatty acid[a]	raw salmon	salmon fried with olive oil	salmon fried with soya oil	roasted salmon
ΣSFAs−C18:0) g/100 g sample	1.62	3.32	2.92	1.22
ΣMUFAs g/100 g sample	2.38	4.19	3.56	2.20
ΣPUFAs g/100 g sample	2.09	4.02	4.06	1.91
Σω6 g/100 g sample	0.29	0.77	0.96	0.44
Σω3 g/100 g sample	1.88	3.29	3.10	1.47
ω6/ω3	0.15	0.23	0.31	0.30
MUFA+PUFA/SFA-C18:0	2.76	2.47	2.60	3.77
PUFA/SFA-C:18:0	1.29	1.21	1.39	1.57
trans fatty acid	0.09	0.25	0.01	0.06

[a] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

of eicosapentaenoic and docosahexaenoic fatty acids) by microwave found that 25-hydroxycholesterol and cholestanetriol are formed by deep-fat frying. In our study, α-epoxycholesterol, 25-hydroxycholesterol, and 7α-hydroxycholesterol were not detected. 7β-Hydroxycholesterol appeared in only roasted salmon and in salmon fried with soya oil. The most abundant COPs were 7-ketocholesterol which appeared in all samples, and cholestanetriol, one of the most citotoxic COPs (*40*), which appeared only in cooked samples.

Osada et al. (*41*) analyzed the cholesterol oxides formed by heating and suggested that the influence of coexisting fats should be taken into account, especially the degree of unsaturation of fatty acids. Nawar et al. (*42*) found that the presence of different compounds (triacylglycerols and amino acids) not only influenced the rate of cholesterol oxidation but also exerted different influences on its oxidative pathway. These authors pointed out that adding triacylglycerols (linoleate, stearate, and oleate) favored the cholesterol oxidation and increased the amount of epoxides. As we have seen in our case, cholesterol epoxides were not originated with the assayed treatments. Visiolo et al. (*43*) suggested that the generation of oxidation products by individual fatty acids is not directly related to their degree of unsaturation and indicated that the differential contribution of individual fatty acids should be taken into account. The results obtained in this work did not show a clear effect of the degree of unsaturated fatty acids in the cholesterol oxidation. In this case the heating conditions had more effect than the composition of fat.

Antioxidants content also could be taken into account because of their capacity to inhibit COP formation (*19*). In this work we did not analyze the antioxidant contents of samples. In general, salmon (fresh salmon) does not contain a significant amount of vitamin E; whereas the nutritional information given by used oils showed that the amounts of vitamin E were 20 mg/100 g for olive oil and 16 mg/100 g for soya oil. This similar content of α-tocopherol could be implicated in the similar total oxides obtained in fried samples using different oils.

Table 5 shows the sums of and ratios between different types of fatty acids with interest from the nutritional point of view. In this table the results have been expressed as fatty acids content per 100-g sample. It can be observed that samples fried with olive oil supplied higher contents of monounsaturated fatty acids than samples fried with soya oil. All cooked samples supplied more ω-6 polyunsaturated fatty acids than raw samples and showed higher ω-6/ω-3 ratios. Roasted salmon showed the lowest ω-3 content and the highest PUFA/(SFA−C18:0) and MUFA + PUFA/SFA−C18:0 ratios. The amounts of trans fatty acids were not significant in any sample.

In summary, it can be concluded that the studied culinary processes favored the COP formation. Roasted salmon showed better fatty acid ratios than fried samples (fried with olive oil and fried with soya oil), but at the same time it showed the highest formation of COP.

LITERATURE CITED

(1) Leaf, A.; Weber, P. C. Cardivascular effects on n-3 fatty acids. *N. Engl. J. Med.* **1988**, *318*, 549−557.

(2) Lee, T. H.; Hoover, R. L.; Williams, J. D.; Sperling, R. I.; Ravalese, J.; Spur, B. W.; Robinson, D. R.; Corey, E. J.; Lewis, R. A.; Austen, K. F. Effect of dietary enrichemnt with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N. Engl. J. Med.* **1985**, *312*, 1217−1224.

(3) Phillipson, B. E.; Rothrock, D. W.; Connor, W. E.; Harris, W. S.; Illinworth, D. R. Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. *N. Engl. J. Med.* **1985**, *64*, 1470−1475.

(4) Imao, K.; Wang, H.; Komatsu, M.; Hiramatsu, M. Free radical scavenging activity of fermented papaya preparation and its effect on lipid peroxide level and superoxide dismutase activity in iron-induced epileptic foci of rats. *Biochem. Mol. Biol. Int.* **1998**, *45* (1), 11−23.

(5) Hino, T.; Kawanishi, S.; Yasui, H.; Oka, S.; Sakurai, H. HTHQ (1-*O*-hexyl-2,3,5-trimethylhydroquinone), an antilipid-peroxidative compound: its chemical and biochemical characterizations. *Biochim. Biophys. Acta* **1998**, *1425* (1), 47−60.

(6) Emanuel, H.; Hassel, C. A.; Addis, P. B.; Bergmann, S. D.; Zavoral, J. H. Plasma cholesterol oxidation products (oxysterols) in human subjects fed a meal rich in oxysterols. *J. Food Sci.* **1991**, *56*, 843−847.
(7) Fornas, F.; Martínez-Sales, V.; Camañas, A.; Baguena, J. Intestinal absorption of cholesterol autoxidation products in the rat. *Arch. Farmacol. Toxicol.* **1984**, *10*, 175−182.
(8) Bascoul, J.; Domergue, N.; Crastes de Paulet, A. Intestinal absorption of cholesterol autoxidation products in dietary fats. *J. Am. Oil Chem. Soc.* **1985**, *62*, 623a.
(9) Osada, K.; Kodama, T.; Yamada, K.; Sugano, M. Lymphatic absorption of oxidized cholesterol in rats. *Lipids* **1994**, *29*, 555−559.
(10) Staprans, I.; Rapp, J. H.; Pan, X. M.; Hardman, D. A.; Feingold, K. R. Oxidized lipids in the diet accelerate the development of fatty streaks in cholesterol-fed rabbits. *Arterioscler. Thromb. Vasc. Biol.* **1996**, *16* (4), 533−538.
(11) Staprans, I.; Pan, X. M.; Rapp, J. H.; Grunfeld, C.; Feingold, K. R. Oxidized cholesterol in the diet accelerates the development of atherosclerosis in LDL receptor- and apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **2000**, *20* (3), 708−714.
(12) Brown, A. J.; Jessup, W. Oxysterols and atherosclerosis. *Atherosclerosis* **1999**, *142* (1), 1−28.
(13) Addis, P. B.; Carr, T. P.; Hassel, C. A.; Huang, Z. Z.; Warner, G. J. Atherogenic and anti-atherogenic factors in the human diet. In *Free Radicals and Oxidative Stress: Environment, Drugs and Food Additives*; Rice-Evans, C., Halliwell, B., Lunt, G. G., Eds.; Portland Press: London, 1995, pp 259−271.
(14) Addis, P. B.; Park, S. W.; Guardiola, F.; R.; Codony, R. Analysis and Health Effects of cholesterol Oxides. In *Food Lipids and Health*; McDonald, R. E., Min, D. B., Eds: Dekker: New York, 1996; pp 199−239.
(15) Addis, P. B.; Warner, G. J. The potential health aspects of lipid oxidation products in food. In *Free Radicals and Food Additives*; Aruoma, O. I., Halliwell, B., Eds.; Taylor and Francis: London, 1991; pp 77−119.
(16) Peng, S. K., Morin, R. J., Eds. *Biological Effects of Cholesterol Oxides*. CRC Press: Boca Raton, FL, 1992.
(17) Tai, C.-Y.; Chen, Y. C.; Chen, B. H. Analysis, formation and inhibition of cholesterol oxidation products in foods: an overview (part I). *J.Food Drug Anal.* **1999**, *7*(4), 243−257.
(18) Kumar, N.; Shingal, O. P. Effect of processing conditions on the oxidation of cholesterol in ghee. *J. Sci. Food. Agric.* **1992**, *58*, 267−273.
(19) Tai, C.-Y.; Chen, Y. C.; Chen, B. H. Analysis, formation and inhibition of cholesterol oxidation products in foods: an overview (part II). *J. Food Drug Anal.* **2000**, *8* (1), 1−15.
(20) Sebedio, J. L.; Ratnayake, W. M. N.; Ackman, R. G.; Prevost, J. Stability of polyunsaturated ω-3 fatty acids during deeep fat frying of atlantic mackerel (*Scomber scombrus* L.). *Food Res. Int.* **1993**, *26* (3), 163−172.
(21) Aurboug, S. P.; Medina, I.; Pérez-Martín, R. Polyunsaturated fatty acids in tuna phospholipids: Distribution in the sn-2 location and changes during cooking. *J. Agric. Food Chem.* **1996**, *44*, 585−589.
(22) Candela, M.; Astiasarán, I.; Bello, J. Effects of frying and warmholding on fatty acids and cholesterol of sole (*Solea solea*), codfish (*Gadus morrhua*) and hake (*Merluccius merluccius*). *Food Chem.* **1997**, *58* (3), 227−213.
(23) Candela, M.; Astiasarán, I.; Bello, J. Deep-fat frying modifies high-fat fish lipid fraction. *J. Agric. Food Chem.* **1998**, *46* (7), 1793−1796.
(24) Sánchez-Muñiz, F. J.; Viejo, J. M.; Medina, R. Deep fat frying of sardines in different culinary fats. Changes in the fatty acid composition of sardines and frying fats. *J. Agric. Food Chem.* **1992**, *40*, 2252−2256.
(25) Paniangvait, P.; King, A. J.; Jones, A. D.; German, B. G. Cholesterol oxides in foods of animal origin. *J. Food Sci.* **1995**, *60*, 1159−1173.
(26) ISO. Determination of moisture content, ISO 1442−1997. In International Standards Meat and Meat Products; International Organization for Standardization: Geneva, Switzerland, 1973.
(27) ISO. Determination of total fat content, ISO 1443−1973. In *International Standards Meat and Meat Products*; International Organization for Standardization: Geneva, Switzerland, 1973.
(28) ISO. Determination of acidity content, ISO 1740−1991. In International Standards Meat and Meat products. International Organization for Standardization: Geneva, Switzerland, 1980.
(29) ISO. Determination of peroxide content, ISO 1443-1973. In *International Standards Meat and Meat Products*; International Organization for Standardization: Geneva, Switzerland, 1973.
(30) Folch, J.; Lees, M.; Sloane-Stanley, G.M. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **1957**, *226*, 497−508.
(31) AOAC. Fatty acids in oils and fats, 969.33. In *Official Methods of Analysis*, 17th ed.; Association of Official Analytical Chemists: Washington, DC, 2000.
(32) Kovacs, M. I. P.; Anderson, E.; Ackman, R. G. A simple method for the determination of cholesterol and some plant sterols in fishery based food products. *J. Food Sci.* **1979**, *44*, 1299−1301, 1305.
(33) Guardiola, F.; Codony, R.; Rafecas, M.; Boatella, J. Comparison of three methods for the determination of oxysterols in spray-dried egg. *J. Chromatogr. A.* **1995**, *705*, 289−304.
(34) Nawar, W.; Hultin, H.; Li, Y. J.; Xing Y. H.; Kelleher, S.; Wilhem, C. Lipid oxidation in seafoods under conventional conditions. (Review). *Food Rev. Int.* **1990**, *6*, 647−660.
(35) Pie, J. E.; Shapis, K.; Seillan, C. Evaluation of oxidative degradation of cholesterol in food and food ingredients: identification and quantification of cholesterol oxides. *J. Agric. Food Chem.* **1990**, *38*, 973−979.
(36) Zanardi, E.; Novelli, E.; Ghiretti, G. P.; Dorigoni, V.; Chizzolini, R. Prodotti di salumeria crudi (prosciutto, salame, coppa) e ossidazione lipidica. *Riv. Sci. Aliment.* **1999**, *28*, 93−103.
(37) Ghiretti, G. P.; Zanardi, E.; Novelli, E.; Campanini, G.; Dazzi, G.; Madarena, G.; Chizzolini, R. Comparative Evaluation of some antioxidants in salame milano and mortadella production. *Meat Sci.* **1997**, *47*, 1/2, 167−176.
(38) Oshima, T.; Li, N.; Koizumi, C. Oxidation decomposition of cholesterol in fish products. *J. Am. Oil Chem. Soc.* **1993**, *70*, 595−600.
(39) Jeong-Hee, K.; Su-Jeong, J.; Yong-Ju, K. Formation of cholesterol oxides in Saury (Cololabis seira, Kongchi) during pan frying, deep fat frying, and microwave cooking. *Food Sci. Biotechnol.* **2000**, *9* (1), 48−51.
(40) Peng, S. K.; Hu, B.; Morin, R. J. Angiotoxicity and atherogenicity of cholesterol oxides. *J. Clin. Lab. Anal.* **1991**, *5*, 144−152.
(41) Osada, K.; Kodama, T.; Cui, L.; Yamada, K.; Sugano, M. Levels and formation of oxidized cholesterols in processed marine foods. *J. Agric. Food Chem.* **1993**, *41*, 1893−1898.
(42) Nawar, W. W.; Kim, S. K.; Li, Y. J.; Vadji, M. Measurement of oxidative interactions of cholesterol. *J. Am. Oil Chem. Soc.* **1991** *68*, (7), 496−498.
(43) Visioli, F.; Colombo, C.; Galli, C. Oxidation of individual fatty acids yields different profiles of oxidation markers. *Biochem. Biophys. Res. Commun.* **1998**, *245* (2), 487−489.

Received for review February 16, 2001. Revised manuscript received August 6, 2001. Accepted August 15, 2001. This work was supported by the Department of "Industria" and the Department of "Educación y Ciencia" of Gobierno de Navarra.

JF010199E