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# Effects of Different Cooking Procedures on Lipid Quality and Cholesterol Oxidation of Farmed Salmon Fish (*Salmo salar*)

SABRI AL-SAGHIR,[†] KARIN THURNER,[‡] KARL-HEINZ WAGNER,[†] GEORG FRISCH,[†] WOLFGANG LUF,[‡] EBRAHIM RAZZAZI-FAZELI,[§] AND IBRAHIM ELMADFA*,[†]

Institute of Nutritional Sciences, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria, Department of Veterinary Public Health and Food Science, Institute of Milk Hygiene, Milk Technology, and Food Science, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria, and Department of Veterinary Public Health and Food Science, Institute of Nutrition, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

Salmon fillets were steamed, or pan-fried without oil, with olive oil, with corn oil, or with partially hydrogenated plant oil. The exchange between the salmon and the pan-frying oils was marginal, but it was detectable as slight modifications in the fatty acid pattern and the tocopherol contents according to the oil used. Primary and secondary oxidation products were only slightly increased or remained unchanged, which indicated a slight lipid oxidation effect due to the heating procedures applied. The same was observed for tocopherol levels, which remained almost stable and were not affected by the oxidation process. The sum of cholesterol oxidation products (COPs) increased after the heating processes from 0.9 $\mu$g/g in the raw sample to 6.0, 4.0, 4.4, 3.3, and 9.9 $\mu$g/g extracted fat in pan-fried without oil, with olive oil, corn oil, partially hydrogenated plant oil, and steamed, respectively. A highly significant correlation was found between the fatty acid pattern and the total amount of COPs ($r^2 = 0.973$, $p < 0.001$). No change has been determined in the n-3 fatty acids content and in the polyunsaturated/saturated-ratio of the cooked salmon fillets. Moderate pan-frying (6 min total) and steaming (12 min) of salmon did not accelerate lipid oxidation but significantly increased the content of COPs. The highest increase of COPs was found through steaming, mainly due to the longer heat exposure. The used frying oils did not influence the outcome; no significant difference between heat treatment with or without oil has been determined.

**KEYWORDS: Salmon; pan-frying; steaming; lipid oxidation; cholesterol oxidation; tocopherols**

## INTRODUCTION

Pan-frying of food has become more and more popular in daily life, because it is a fast and convenient technique for food preparation with typical characteristics of color, flavor, texture, and palatability, and it is highly appreciated by consumers. However, these positive attitudes are normally accompanied by some undesirable modifications in the frying medium (*1*), as well as the fried products (*2*). Lipid oxidation, which is shown to be induced during thermal treatments, not only severely compromises the quality of some foods but also limits their shelf life (*2*−*5*). Vegetable oils have different susceptibilities toward oxidative degradation due to differences in their fatty acid unsaturation and the varying type and contents of antioxidants (*6*). Therefore, the use of different fat sources for thermal treatments results in different effects on the oxidative stability of the treated products, especially under household conditions.

In particular, fish and fish oils are highly susceptible to oxidation due to their high content of polyunsaturated fatty acids (30−40%), mainly eicosapentaenoic acid (EPA, 20:5n-3) (5−18%) and docosahexaenoic acid (DHA, 22:6n-3) (1−12%). These unique fatty acids distinguish fish lipids from other plant and animal lipids (*4*, *7*).

The consensus of evidence over the last two decades confirms the beneficial effects of longer-chain n-3 polyunsaturated fatty acids (PUFA), especially of the EPA and DHA, against various human disorders, including cardiovascular and inflammatory conditions (*7*, *8*). However, cooking and thermal treatment of n-3 PUFA is known to increase their susceptibility toward lipid oxidation in general (*9*). Moreover, changes in fish flavor induced by lipid oxidation are one of the major problems in quality deterioration during processing and storage (*5*, *10*, *11*).

Although many studies on the oxidation of fats and oils during deep-frying have been published (*12*−*16*), the information on lipid oxidation initiated by different cooking procedures when using various lipids and their impact on fatty acids and cholesterol in fish is limited.

* To whom correspondence should be addressed. Tel.: +43 1 4277 54912. Fax: +43 1 4277 9549. E-mail: karl-heinz.wagner@univie.ac.at.
[†] University of Vienna, Althanstrasse.
[‡] Institute of Milk Hygiene, Milk Technology, and Food Science, University of Veterinary Medicine, Veterinärplatz.
[§] Institute of Nutrition, University of Veterinary Medicine, Veterinärplatz.

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**Table 1.** Main Characteristics of the Pan-frying Oils Used in the Study[a]

| | olive oil | corn oil | phPO |
|---|---|---|---|
| acid value (mg KOH/g) | 1.75 ± 0.10 | 0.75 ± 0.09 | 0.67 ± 0.01 |
| peroxide value (mEqu/kg) | 11.8 ± 0.03 | 0.95 ± 0.04 | 1.4 ± 0.05 |
| *p*-anisidine value | 7.4 ± 0.10 | 3.5 ± 0.21 | 2 ± 0.11 |
| α-tocopherol (mg/100 g) | 13.8 ± 1.41 | 20.4 ± 1.44 | 17.7 ± 0.29 |
| γ-tocopherol (mg/100 g) | 1.1 ± 1.11 | 103.5 ± 4.70 | n.d. |
| SFA (% of total fatty acids) | 14 ± 0.02 | 13 ± 0.04 | 30 ± 0.01 |
| MUFA (% of total fatty acids) | 78 ± 0.10 | 30 ± 0.02 | 36 ± 0.02 |
| PUFA (% of total fatty acids) | 8 ± 0.06 | 57 ± 0.14 | 34 ± 0.04 |
| TFA (% of total fatty acids) | 0.71 ± 0.02 | 0.7 ± 0.01 | 2.2 ± 0.02 |
| P/S ratio | 0.6 | 4.4 | 1.1 |

[a] phPO, partially hydrogenated plant oil; SFA, saturated fatty acids; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids; TFA, trans fatty acids; n.d., not detectable.

The present study was aimed at evaluating (1) changes in lipid oxidation and cholesterol oxidation products (COPs) of pan-fried or steamed salmon fillets and (2) the impact of pan-frying without oil versus different frying oils.

## MATERIALS AND METHODS

**Chemicals and Reagents.** Acetonitrile LiChrosolv, methanol LiChrosolv, chloroform p.a., *n*-hexane p.a., diethyl ether p.a., and acetone p.a. were purchased from Merck (Vienna, Austria). The COPs standards cholestan-3β,5α,6β-triol, cholest-5-en-3β,25-diol, cholestan-5α,6α-epoxy-3β-ol, cholestan-5β,6β-epoxy-3β-ol, and cholest-5-en-3β-ol-7-on were purchased from Sigma Aldrich (Vienna, Austria). The standards cholest-5-en-3β,19-diol, cholest-5-en-3β,7α-diol, and cholest-5-en-3β,7β-diol were from Steraloids purchased via Szabo-Scandic (Vienna, Austria). All other chemicals were purchased from Sigma (Vienna, Austria).

**Samples and Sample Preparations.** Raw farmed salmon fish (Norwegian *Salmo salar*, about 4.5 kg/fish), bought from a special fish supplier were filleted, boned, and skinned. Three frying oils were used in this study for the pan-frying procedure − extra virgin olive oil, refined corn oil, and a partially hydrogenated plant oil (phPO). The main characteristics of the used oils are shown in **Table 1**.

A special type of stainless steel cooking device (AMC Multicooking System) was applied to perform the cooking procedures. The cooking set included the AMC Audiotherm, a signaling device, and the AMC Atmosfera, a mobile ceramic surface that can be used upside down on an AMC cookware unit. For our study it was used as a standardized heating unit.

For the cooking procedures, only the backside of every fish fillet (∼1kg) was used in this study. These fractions were shaped, uniformed, and cut into 70−75 g portions with an equal thickness of about 11−12 mm. Standardization was made in the pre-experiments for the different fillet fractions and different salmon brands. Randomization was made for the different fillet fractions prior to cooking trials.

**Cooking Procedures.** (*A*) *Pan-Frying with Olive Oil*, *Corn Oil*, *and Partially Hydrogenated Plant Oil.* The samples were inserted into the frying pan after the oil (15 g for each test) had reached the desired temperature (180 °C), which was controlled by a digital thermometer. Fish portions (70−80 g each) were fried for 6 min (3 min each side).

(*B*) *Pan-Frying without Oil.* The pan was prepared by way of reaching the desired temperature. Samples were then put in and fried for 6 min (3 min each side).

(*C*) *Steaming*. Steaming was accomplished with the AMC pot. A total of 250 g of water was poured into the bottom of the pot and heated to the boiling point, then the fish portions were put on a middle layer of the pot (not in contact with the water). The portions were then steamed for 12 min. The core temperature of the samples during cooking was 66−67 °C. After each cooking trial, the cooking residues were collected to be analyzed. During the entire time of cooking, all of the cooking containers were covered with their special Visiotherm cover. The standardization of the cooking steps have been tested and standardized in pre-experiments.

After the heat treatments were accomplished, portions were drained with soft papers and directly cooled to 5 °C for about 30 min. Then, they were minced, homogenized, vacuum packed, deep frozen (−50 °C for 3 days), freeze-dried, ground, and stored at −75 °C until analysis.

**Analytical Procedures.** Moisture content was determined gravimetrically at 105 °C for 6 h.

Fat extraction for total fat determination was accomplished with an accelerated fat extracting system (ASE 100; Accelerated Solvent Extract − DIONEX) using petroleum ether as a solvent; 1 g of the dried, ground sample was placed in a 22-mL extraction cell and then extracted. Thereafter, the solvent was evaporated, and the remaining extract was dried under a nitrogen stream and subsequently placed in an oven at 100 °C. The extract weight was obtained gravimetrically.

Fat extraction for the chemical analysis was performed with a Soxhlet extractor using a low boiling Petroleum Ether (30−50 °C boiling points) as a solvent.

*Cholesterol Oxidation Products.* About 6−8 g of the homogenized, freeze-dried sample were extracted with chloroform/methanol (2:1, v/v) at +4 °C overnight. The two phases were separated by ultracentrifugation with 5000 rpm, including two washing steps with water. The solvent was evaporated by a rotary evaporator (Büchi, Flawil, Switzerland).

About 2 g of the extracted fat were dissolved in 5 mL of *n*-hexane, 19-hydroxycholesterol was added as internal standard (4 μg 19-OH in 100 μL of *n*-hexane). The separation of interfering apolar lipids and cholesterol was done by solid-phase extraction with a silica cartridge (International Sorbent Technology, Mid-Glamorgan, UK) according to Lai et al. (*17*). Three washing steps with *n*-hexane and diethyl ether were included (95:5, v/v (10 mL); 90:10, v/v (25 mL); and 80:20, v/v (15 mL)). Finally, the COPs were eluted with 5 mL of acetone. After the evaporation of the acetone under nitrogen, the residue was dissolved in 1 mL of mobile phase (acetonitrile/methanol, 60:40, v/v).

The separation and detection of the COPs was carried out by an LC-MS-method according to Razzazi-Fazeli et al. (*18*). The LC-system consisted of a Waters inline degasser, a Waters 626 pump, a Waters 600S controller, and a Waters 717plus autosampler (Waters, Milford, MA). As an analytical column, an Aquasil $C_{18}$ column (Keystone Scientific, Bellefonte, PA) was used (250 × 4.6 mm, 5-μm, 100 Å); the isocratic elution was performed with acetonitrile/methanol (60:40, v/v) at a flow rate of 1 mL/min and 25 °C. The COPs were detected by a Quattro II mass spectrometer (Fisons Instruments, MA), with an $APcI^{+}$−Interface. Nitrogen by a nitrogen generator (Parker Balston, MA) was used as a nebulizing and carrier gas. The source temperature was maintained at 110 °C, the sample temperature was set at 500 °C, and the cone voltage was set at 20 kV. Detection was done in SIM mode (selected ion monitoring) in a dwell time of 0.3 s and a span of 0.3 Da. The applied method allowed us to analyze seven COPs: Cholestan-3β,5α,6β-triol (triol), cholest-5-en-3β-ol-7-on (7-keto), cholest-5-en-3β,7α-diol (7α-OH), cholest-5-en-3β,7β-diol (7β-OH), cholestan-5α,6α-epoxy-3β-ol (5α6α-ep), cholestan-5β,6β-epoxy-3β-ol (5β6β-ep), and cholest-5-en-3β,25-diol (25-OH). The identification of the sample peaks was based on retention times of standard substances. COPs were quantified with calibration curves of each COP standard by using an internal standard method with cholest-5-en-3β,19-diol (19-OH). **Figure 1** shows an example chromatogram of a raw salmon sample in SIM mode.

Fatty acid pattern of the extracted lipids of the investigated samples, as well as for the fresh frying oils were converted into methyl esters and analyzed with Gas Chromatography (GC) by use of an Auto-System Gas Chromatographer, (Perkin-Elmer, Vienna, Austria), equipped with a split/splitless capillar injector as described previously (*19*). FAME were separated by a 30-m × 0.25-mm ID fused silica column (RTx-2330) and detected with a flame ionization detector (FID). The FID temperature was set at 250 °C. The fatty acid pattern was analyzed in duplicate. To describe the extent of lipid oxidation the iodine value (IV) (*20*), acid value (AV) (*21*), peroxide value (PV) (*22*), *p*-anisidine value (*p*-AV) (*23*) and conjugated dienes (CD) (*24*) were determined according to the AOCS (American Oil Chemist's Society) official methods Cd 1c-85, Cd 3d-63, Cd 8-53, Cd 18-90, and Ti 1a-64,

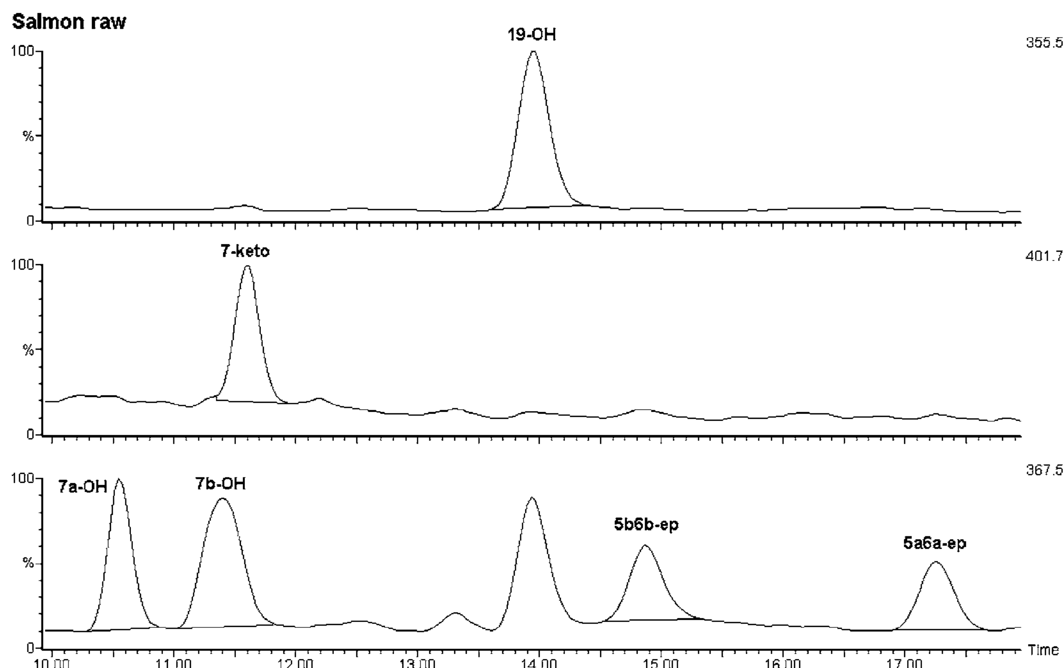


**Figure 1.** Chromatogram of a raw salmon sample in SIM mode (selected ion monitoring).

**Table 2.** Fat and Moisture Content of the Investigated Salmon Fillets (Values are mean ± SD)

| treatment[d] | fat content (% wet wt) | moisture content (% wet wt) |
|---|---|---|
| raw | 15.6 ± 0.66[a] | 64.4 ± 0.35[c] |
| P–F/without oil | 19.0 ± 0.45[b] | 59.0 ± 0.21[a] |
| P–F/olive oil | 18.0 ± 1.85[a] | 57.7 ± 1.07[a] |
| P–F/corn oil | 18.6 ± 0.13[a] | 58.0 ± 0.43[a] |
| P–F/phPO | 16.2 ± 0.47[a] | 60.2 ± 0.14[a] |
| steaming | 15.5 ± 0.27[a] | 62.3 ± 0.31[b] |

[a–c] Different letters in a column indicate significant differences ($p < 0.05$). [d] P–F, pan-frying; phPO, partially hydrogenated plant oil.

respectively. Tocopherols of the extracted fats were analyzed in duplicate with reversed-phase HPLC, as described previously (*25*). The system consisted of a L-7100 pump, L-7400 UV-detector, D-7000 Interface module, and a 250 × 4.0 mm, 5-$\mu$m LiCrospher RP-18 column, all by Merck (Vienna, Austria). Methanol/dichloromethane (85:15, v/v) was used as mobile phase, the flow rate was 0.8 mL/min, and the detection wavelength was set at 295 nm. Extracted lipids were dissolved in hexane, diluted, and evaporated until dryness under vacuum, resolved in the mobile phase, and injected.

**Data Analysis.** Each cooking procedure was done in two replicates, and the analytical parameters were determined in duplicate of each cooking replicate. The data are presented as mean ± SD. Statistical significance was determined by using the T-Test, differences were considered significant at a value of $p < 0.05$. Correlations between different parameters were analyzed by way of Pearson's correlation coefficients ($r$), a significant level of $p < 0.01$ was used. Statistical analysis was conducted using SPSS 11.0. for Windows.

## RESULTS AND DISCUSSION

**Total Lipid and Moisture Content. Table 2** illustrates the changes of the lipid and moisture content of the investigated samples according to the wet weight. After pan-frying, the lipid content of all samples increased marginally, unless it was only significant for the preparation without oil, which may refer not only to the lipid absorption of the frying medium but also to the loss of water content (dehydration) in the cooked samples. Hence, the significant increase ($p < 0.05$) of the fat content when samples were cooked without oil occurred as a consequence of the greater loss of water in this cooking trial. Pan-frying with olive oil and corn oil seemed to increase the mass transfer between the salmon fillets and frying medium more than the samples cooked with phPO did; however, it was not significant. Accordingly, pan-frying with different oils has no significant influence on the fat content of salmon fillets. Candela et al. (*14*) mentioned that deep-fat frying of fish did not significantly affect the fat content of salmon. Previously, Mai et al. (*11*) mentioned that in fish with a high fat content, few lipid changes occurred during frying.

**Changes in the Fatty Acid Pattern.** The profiles of the most important fatty acids of the salmon fish samples after the preparations are shown in **Table 3** in greater detail. Quantitatively, the most abundant fatty acids were oleic acid (18:1n-9), palmitic acid (16:0), brassidic acid (22:1n-9), gadoleic acid (20:1n-9), DHA (C22:6n-3), and EPA (20:5n-3). The latter four are specifically fish fatty acids. The changes in the content of fatty acids after the preparations were marginal, in particular when focusing on the most important n-3 fatty acids EPA and DHA. No significant differences in the total amount of SFA, MUFA, and PUFA have been observed after the different preparations, which supports the explanation that the marginal increase in the lipid content was a result of water loss. As an important nutritional index of fatty acids alteration during cooking, n-3/n-6 ratio in the prepared samples showed apparent change in the samples fried with corn oil and phPO, while frying without oil showed a slight increase to this ratio. The P/S ratio only showed a tendency but did not show significant changes in the investigated samples. Changes that took place with some of the fatty acids primarily occurred as a result of the frying medium effect, mainly oleic acid after the olive oil preparation and linoleic acid after the corn oil preparation (see **Table 3**). The most important fatty acids in fish lipids EPA (3.4−4.3% of total fatty acids) and DHA (5.7−6.8% of total fatty acids) have not been considerably influenced by the cooking procedures applied. Sebedio et al. (*26*) and Candela et al. (*14*) affirmed in their study on deep-fat frying of mackerel and salmon that there were no significant changes found in EPA and DHA content in the fish after frying.

**Table 3.** Composition of the Main Fatty Acids (% of Total Fatty Acids) in the Extracted Fat of the Investigated Salmon Fillets[a]

| fatty acid | raw | without oil | with olive oil | with corn oil | with phPO | steaming |
|---|---|---|---|---|---|---|
| 14:0 | 3.4 ± 0.04 | 4.2 ± 0.80 | 3.1 ± 0.28 | 3.3 ± 0.33 | 2.6 ± 0.37 | 3.0 ± 0.03 |
| 16:0 | 9.1 ± 0.49 | 9.4 ± 1.00 | 9.3 ± 1.14 | 9.4 ± 1.48 | 11.1 ± 0.68 | 9.2 ± 0.15 |
| 18:0 | 1.9 ± 0.11 | 2.1 ± 0.12 | 2.2 ± 0.14 | 2.2 ± 0.14 | 2.3 ± 0.14 | 2.0 ± 0.04 |
| **Σ SFA[b,e]** | **15.9 ± 0.39** | **17.2 ± 1.79** | **16.9 ± 1.56** | **16.1 ± 2.16** | **18.6 ± 0.47** | **16.1 ± 0.25** |
| 16:1n-7 | 5.3 ± 0.04 | 5.8 ± 0.10 | 4.3 ± 0.38 | 4.6 ± 0.35 | 3.8 ± 0.13 | 4.4 ± 0.02 |
| 18:1n-9 | 23.0 ± 2.89 | 22.2 ± 2.09 | 25.5 ± 2.98 | 23.4 ± 2.47 | 22.3 ± 2.60 | 23.0 ± 0.32 |
| 18:1n-7 | 3.5 ± 0.23 | 3.4 ± 0.03 | 3.5 ± 0.05 | 3.4 ± 0.08 | 3.4 ± 0.44 | 3.6 ± 0.14 |
| 20:1n-9 | 9.3 ± 0.88 | 8.9 ± 0.88 | 8.5 ± 1.00 | 8.4 ± 1.69 | 9.1 ± 1.30 | 9.7 ± 0.05 |
| 22:1n-9 | 9.3 ± 1.66 | 9.9 ± 2.27 | 6.9 ± 0.42 | 9.9 ± 2.24 | 7.0 ± 0.40 | 9.4 ± 0.88 |
| **Σ MUFA[c]** | **53.7 ± 0.63** | **52.7 ± 0.87** | **54.6 ± 1.99** | **52.0 ± 1.73** | **51.6 ± 3.20** | **54.2 ± 0.39** |
| 18:2n-6 | 8.5 ± 2.15 | 8.5 ± 2.11 | 7.4 ± 1.79 | 11.3 ± 1.36 | 8.8 ± 2.43 | 8.7 ± 0.16 |
| 20:2n-6 | 0.8 ± 0.13 | 0.9 ± 0.21 | 0.7 ± 0.15 | 0.8 ± 0.08 | 0.7 ± 0.17 | 0.8 ± 0.03 |
| 18:3n-3 | 2.9 ± 0.62 | 2.8 ± 0.57 | 2.6 ± 0.47 | 2.8 ± 0.60 | 2.6 ± 0.62 | 2.9 ± 0.01 |
| 18:4n-3 | 1.1 ± 0.12 | 1.0 ± 0.30 | 0.9 ± 0.22 | 1.0 ± 0.06 | 1.0 ± 0.12 | 1.1 ± 0.08 |
| 20:5n-3 | 4.3 ± 0.10 | 4.4 ± 0.42 | 3.4 ± 1.52 | 3.9 ± 0.18 | 3.7 ± 0.20 | 4.2 ± 0.16 |
| 22:5n-3 | 2.1 ± 0.18 | 2.2 ± 0.21 | 1.9 ± 0.23 | 2.1 ± 0.01 | 1.8 ± 0.16 | 2.0 ± 0.08 |
| 22:6n-3 | 6.7 ± 0.11 | 6.8 ± 0.70 | 5.8 ± 0.07 | 6.3 ± 0.15 | 5.7 ± 0.53 | 6.7 ± 0.23 |
| **Σ PUFA[d]** | **30.2 ± 1.75** | **29.9 ± 0.53** | **28.4 ± 0.28** | **31.7 ± 1.05** | **29.6 ± 2.79** | **29.5 ± 1.34** |
| **n-3/n-6** | **1.8 ± 0.30** | **1.9 ± 0.47** | **1.8 ± 0.47** | **1.2 ± 0.07** | **1.4 ± 0.06** | **1.6 ± 0.01** |
| **P/S ratio** | **1.9 ± 0.18** | **1.7 ± 0.23** | **1.7 ± 0.15** | **2.0 ± 0.31** | **1.6 ± 0.15** | **1.8 ± 0.02** |

[a] Values are mean ± SD. phPO, partially hydrogenated plant oil. [b] Σ SFA also includes 15:0, 17:0, 20:0, and 22:0. [c] Σ MUFA also includes 14:1, 16:1n-9, and 24:1. [d] Σ PUFA also includes 18:4n-3, 20:3n-3, 18:2n-6t, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, and 22:4n-6. [e] There is no significant difference ($p > 0.05$) between the preparations when considering the Σ SFA, Σ MUFA, Σ PUFA and the P/S ratio.

**Table 4.** Lipid Oxidation Parameters of the Extracted Fat of the Investigated Salmon Fillets (Values are mean ± SD)

| treatment[e] | AV[d] mg KOH/g | PV mequ/kg | p-AV | CD % |
|---|---|---|---|---|
| raw | 1.79 ± 0.02[c] | 2.00 ± 0.25[a] | 0.30 ± 0.03[a] | 0.39 ± 0.01[a] |
| P–F/without oil | 1.24 ± 0.01[a] | 2.35 ± 0.07[a] | 1.13 ± 0.22[b] | 0.42 ± 0.01[a] |
| P–F/olive oil | 1.43 ± 0.08[a,b] | 5.70 ± 0.70[b] | 1.69 ± 0.57[b] | 0.39 ± 0.01[a] |
| P–F/corn oil | 1.36 ± 0.01[a,] | 3.75 ± 0.35[b] | 0.97 ± 0.27[a,b] | 0.40 ± 0.01[a] |
| P–F/phPO | 1.54 ± 0.03[b] | 2.60 ± 0.09[a] | 1.60 ± 0.42[b] | 0.42 ± 0.01[a] |
| steaming | 1.53 ± 0.05[b] | 1.95 ± 0.11[a] | 0.50 ± 0.04[a] | 0.40 ± 0.01[a] |

[a−c] Different letters in a column indicate significant differences ($p < 0.05$) [d] AV, acid value; PV, peroxide value; p-AV, *p*-anisidine value; CD, conjugated dienes. [e] P-F, pan-frying; phPO, partially hydrogenated plant oil.

**Table 5.** Lipid Oxidation Parameters of Extracted Fat of the Frying Residues

| treatment[b] | AV[a] mg KOH/g | PV mequ/kg | p-AV |
|---|---|---|---|
| P–F/without oil | 1.3 | 18.2 | 34.5 |
| P–F/olive oil | 1.2 | 17.7 | 22.4 |
| P–F/corn oil | 0.9 | 11.1 | 13.7 |
| P–F/phPO | 1.2 | 13.4 | 24.1 |

[a] AV, acid value; PV, peroxide value; p-AV, *p*-anisidine value. [b] P–F, pan-frying; phPO, partially hydrogenated plant oil.

In general, pan-frying with different frying oils, as well as frying without oil, insignificantly increased the total content of saturated fatty acids (SFA) as compared to the initial raw salmon and the steaming preparation. The changes of total MUFA and PUFA were attributed mainly to the frying oils used. These results show that pan-frying does not influence the high content of n-3 fatty acids significantly, although a marginal, but detectable exchange of fats between the pan-frying medium and the sample does in fact occur. Moreover, the use of phPO, which initially contains 2.2% trans fatty acids (TFA), did not increase the TFA content in the investigated samples significantly.

**Lipid Oxidation Products.** The main issue of this study was to determine whether different cooking procedures when using various unsaturated cooking oils may change the susceptibility toward lipid oxidation and the concentration of cholesterol oxidation products of salmon, which is initially rich in n-3 fatty acids and therefore more susceptible to lipid oxidation (*2*, *7*). To consider the complexity of the lipid oxidation process, both primary and secondary oxidation products have been assessed. However, the increase in lipid oxidation due to the pan-frying process was marginal (**Table 4**). The PV significantly increased only for the pan-frying samples with olive and corn oil. The variation in the hydroperoxides formed in the pan-fried samples was attributed to the impact of the frying medium and its initial oxidation parameters as shown in **Table 1**. In particular, olive oil had a high initial PV, which provides the explanation for the increased PV of salmon after pan-frying with olive oil. Although the increase with corn oil was assessed to be significant, the absolute level of 3.75 shows no sign of extended lipid oxidation. The results are confirmed by the formation of CD, as they are primary oxidation products, which remained unchanged. The p-AV, which assesses the formation of secondary oxidation products, significantly increased in all pan-fried samples except in those fried with corn oil; however, again due to the low absolute level, this cannot be defined as increased lipid oxidation, since the values remained very low. To examine the influence of steam production during pan-frying on the content of free fatty acids, which are more susceptible to fat hydroperoxidation, the AV was determined. Its reduction in all cooked samples emphasizes that changes in fat oxidation were not substantial. Frankel (*4*) mentioned that the amount of free fatty acids produced during frying are generally small, and lipid oxidation may decrease at increasing moisture levels. Generally, it is obvious that steaming and pan-frying without oil have lower influence on fat oxidation of salmon fillets than other treatments.

To consider the oxidative development of the frying medium after cooking, the lipid phase of the frying residues, which were a mixture of sample oil-drain and frying medium, had been extracted and analyzed on AV, PV, and p-AV (**Table 5**). As expected, the lipid oxidation of the residues was advanced. The highest increase was found for the residues of cooking without oil, which revealed the highly oxidative potential of the fish oil that drained out of the sample during cooking. Furthermore, the drained oil was in direct contact with the pan temperature of 180 °C, whereas the salmons' core temperature did not exceed

**Table 6.** Content of α-, γ-, and δ-Tocopherols of Raw and Prepared Salmon Fillets (mg/100 g of Extracted Oil) (Values are Mean ± SD)

| treatment[c] | α-tocopherol (mg/100 g) | γ-tocopherol (mg/100 g) | δ-tocopherol (mg/100 g) |
|---|---|---|---|
| raw | 18.1 ± 1.1[a] | 12.7 ± 0.1[a,b] | 0.50 ± 0.01[a] |
| P–F/without oil | 16.7 ± 1.9[a] | 12.3 ± 1.4[a,b] | 0.51 ± 0.07[a] |
| P–F/olive oil | 16.6 ± 1.2[a] | 10.8 ± 0.6[a] | 0.41 ± 0.04[a] |
| P–F/corn oil | 15.9 ± 1.8[a] | 15.8 ± 1.8[b] | 0.61 ± 0.01[a] |
| P–F/phPO | 16.8 ± 1.1[a] | 10.8 ± 0.2[a] | 0.40 ± 0.06[a] |
| steaming | 16.2 ± 1.4[a] | 13.3 ± 2.1[a,b] | 0.47 ± 0.06[a] |

[a,b] Different letters in a column indicate significant differences ($p < 0.05$). [c] P–F, pan-frying; phPO, partially hydrogenated plant oil.

70 °C. Therefore, from a nutritional point of view, the consumption of the remaining residue, which is commonly used to enrich the flavor, should not be recommended due to the increased oxidation status of the residues.

The low oxidation data are supported by the salmon tocopherols (**Table 6**). Tocopherols are well-known antioxidants and are known to be reduced during increasing oxidative stress (*27*, *28*). No significant decrease in the content of α-, γ-, or δ-tocopherols was observed. The slight differences, significant only for γ-tocopherol, are due to the different tocopherol patterns of the oils used in particular, corn oil is rich in γ-tocopherol. This is a further indication for the oil exchange.

**Cholesterol Oxidation.** The formation of cholesterol oxidation products is shown in **Table 7**. The total amount of COPs significantly increased during all preparations ($p < 0.05$), as compared to the raw sample but without significant differences between the pan-frying oils. The preparation without fat resulted in an insignificantly higher mean value of total COPs when compared to frying with fats. The highest COPs formation was observed after steaming (9.88 ± 0.97 μg/g extracted fat), which may be attributed to the longer heating time of 6 min for frying versus 12 min for steaming. Steaming induced a significantly higher total COPs amount than the frying procedures with the oils ($p < 0.05$), but not more than frying without oil.

Since the literature on COPs in salmon hardly exists, an extensive comparison of the presented data is difficult. The major conformity can be found with Echarte et al. (*29*). Their approach was to fry salmon fillets with 30 mL of olive oil for 4 min (2 min each side) at 180 °C. After cold saponification, the analysis was then carried out by using GC-MS. The total COPs content rose from 0.74 μg/g fat to 2.98 μg/g fat, a relative increase of 403% as compared to the raw sample.

In the presented study, a more or less similar increase of 463% was assessed. This slightly higher increase might be due to the longer heating period of a total of 6 min. In addition, it might be influenced by the different ratio of salmon to the added oil, considering a possible dilution effect of COPs. Further publications dealing with COPs generation in salmon are scarce.

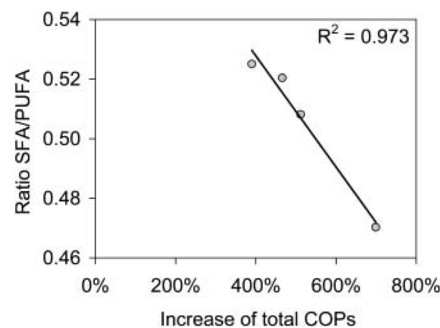


**Figure 2.** Correlation of the ratio saturated fatty acids/polyunsaturated fatty acids (SFA/PUFA) and the increase of total amount of COPs in % (fried samples).

Ohshima et al. (*30*) determined the total COPs content of smoked keta salmon by using GC-MS after cold saponification. They found mean values of 26.8 μg/g dry weight, which was about a 10-fold higher result than our findings, where the total amount of COPs of the processed salmon ranged between 1.4 and 4.1 μg/g dry matter. However, smoking is not comparable to any of the procedures that were applied here.

A very recent paper of Echarte et al. (*31*) showed a total COPs content of salmon patés of 174 μg/100 g product corresponding to 6.6 μg/g fat. This is fairly comparable with the results of the presented study, although salmon paté and pan-frying is not directly comparable.

In contrast to salmon, several papers are published dealing with the generation of COPs during the preparation of meat. Pie et al. (*32*) and Larkeson et al. (*33*) observed increases in the COPs content between 120 and 315% after the frying of beef and pork without the addition of oil. These are obviously lower increases with regard to percentage as compared to the 700% increase of total COPs due to frying without addition of oil in comparison to the raw sample (100%) reached herein. However, the total content was significantly higher than that found in salmon. According to the model system of Li et al. (*34*), this can be explained by the different fatty acid composition. The higher the degree of unsaturation, the higher the lipid oxidative processes, and the more COPs are formed. This is supported by the strong correlation between the fatty acid composition and the increase in the total amount of COPs of the fried salmon samples ($r^2 = 0.973$) shown in **Figure 2**. This strengthens the fact that salmon fillets with more polyunsaturated fatty acids are more susceptible to cholesterol oxidation. Actually, we should also expect an important effect of the degree of unsaturation of the frying fat. Indeed, there are interactions between food and the pan-frying oils as the fatty acid data above (**Table 3**) already showed. Nevertheless, we could not demonstrate any significant effect of using a certain kind of plant oil. On one hand, this can be attributed to the short time of heating. In the model system of Li et al. (*34*), the incubation time for

**Table 7.** Total Amounts of COPs in the Extracted Fat of Different Salmon Preparations (Values are Mean ± SD)

| treatment[d] | 7α-OH μg/g extracted fat | 7β-OH μg/g extracted fat | 5β6β-ep μg/g extracted fat | 5α6α-ep μg/g extracted fat | 7-keto μg/g extracted fat | total COPs μg/g extracted fat |
|---|---|---|---|---|---|---|
| raw | 0.07 ± 0.01[a] | 0.15 ± 0.02[a] | 0.07 ± 0.05[a] | 0.03 ± 0.04[a] | 0.53 ± 0.36[a] | 0.86 ± 0.27[a] |
| P–F/without oil | 0.61 ± 0.13[b] | 1.38 ± 0.39[b] | 0.84 ± 0.30[a,b] | 0.20 ± 0.07[a] | 2.96 ± 1.86[a] | 5.98 ± 2.70[b] |
| P–F/olive oil | 0.40 ± 0.04[b] | 0.88 ± 0.04[b] | 0.56 ± 0.02[b] | 0.18 ± 0.04[b,c] | 1.96 ± 0.88[a] | 3.98 ± 0.86[b] |
| P–F/corn oil | 0.44 ± 0.14[b] | 1.03 ± 0.41[b] | 0.58 ± 0.24[b] | 0.24 ± 0.18[b,c] | 2.09 ± 1.28[a] | 4.38 ± 2.24[b] |
| P–F/phPO | 0.40 ± 0.08[b] | 0.81 ± 0.13[b] | 0.51 ± 0.08[b] | 0.09 ± 0.06[b,c] | 1.53 ± 0.41[a] | 3.34 ± 0.17[b] |
| steaming | 1.48 ± 0.05[c] | 3.10 ± 0.07[c] | 1.69 ± 0.13[c] | 0.34 ± 0.04[c] | 3.27 ± 0.90[a] | 9.88 ± 0.97[c] |

[a–c] Different letters in a column indicate significant differences ($p < 0.05$). [d] P–F, pan-frying; phPO, partially hydrogenated plant oil.

cholesterol and triglycerides of a variable degree of unsaturation lasts nearly 40 days at 25 °C. Therefore, the acceleration of the cholesterol oxidation by coexisting highly unsaturated triacylglycerols has been more pronounced. However, our aim was geared toward a modern cooking style as it is applied in cuisine at present. To guarantee this demand, a professional cook was responsible for the preparations.

The distribution of the COPs showed a characteristic pattern for all preparations (**Table 7**). The most abundant COP was 7-keto (48 ± 9%, mean ± SD of all salmon samples), followed by 7β-OH (24 ± 5%). The amount of the 5,6-epoxides and 7α-OH remained below 15%. This ratio turns out to be positive in particular when considering the mutagenicity and cancerogenicity of the epoxides (*35*). Moreover, from the latter point of view, neither the triol nor the 25-OH, which were already described to be the most cytotoxic (*36*), could have been detected in any of the samples.

Our results lead to the conclusion that among the positive effects of its long chain n-3 fatty acids regarding cardiovascular diseases (*8*), salmon can also contain COPs as a potential toxicological health risk. At the moment, far too little research exists for a clear evaluation of the problem, but it seems that using fresh, shortly stored raw materials and short, mild heating conditions can keep down the amount of COPs generation.

The data obtained in this study showed that the investigated cooking procedures have little impact on the lipid oxidation and fatty acids content of the salmon fillets. All the cooking procedures applied have not really affected the content of EPA and DHA of salmon oil. Total COPs increased after each cooking procedure. In particular, steaming increased the total amount by more than 1000%, which should be considered in nutritional recommendations, although the initial COPs content of salmon is low compared to other foods such as meat.

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## LITERATURE CITED

(1) Dobarganes, C.; Marquez-Ruiz, G.; Velasco, J. Interactions between fat and food during deep-frying. *Eur. J. Lipid Sci. Technol.* **2000**, *102*, 521−528.
(2) Pokorny, J. Changes of nutrients at frying temperature. In *Frying of Food*; Boskou, D., Elmadfa, I., Eds.; Technomic Publishing CO.: Lancaster, PA, 1999; pp 69−103.
(3) Fillion, L.; Henry, C. J. Nutrient losses and gains during frying. *Int. J. Food Sci. Nutr.* **1998**, *49* (*2*), 157−168.
(4) Frankel, E. N. *Lipid Oxidation*; The Oily Press LTD: Bridgewater, UK, 1998.
(5) Khalil, A.; Mansour, E. Control of lipid oxidation in cooked and uncooked refrigerated carp fillets by antioxidant and packaging combinations. *J. Agric. Food Chem.* **1998**, *46*, 1158−1162.
(6) Kamal-Eldin, A. *Lipid Oxidation Pathway*; AOCS Books and Special Publications Committee: Champaign, IL, 2003.
(7) Kulas, E.; Ackman, R. G. Different tocopherols and the relationship between tow methods for determination of primary oxidation products in fish oil. *J. Agric. Food Chem.* **2001**, *49*, 1724−1729.
(8) GISSI−Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI−Prevenzione trial. *Lancet* **1999**, *354*, 447−455.
(9) Regulska-Ilow, B.; Ilow, R. Comparison of the effect of the microwave cooking and conventional cooking methods on the composition of fatty acids and fat quality indicators in herring. *Nahrung/Food* **2002**, *46* (*6*), 383−388.
(10) Undeland, I.; Hall, G.; Lingnert, H. Lipid oxidation in fillets of herring (*Clupea harengus*) during ice storage. *J. Agric. Food Chem.* **1999**, *47*, 524−532.
(11) Mai, J.; Shimp; J.; Weihrauch, J.; Kinsella, J. E. Lipids of fish fillets: changes following cooking by different methods. *J. Food Sci.* **1978**, *43*, 1669−1674.
(12) Saguy, S.; Dana, D. Integrated approach to deep fat frying: engineering, nutrition, health, and consumer aspects. *J. Food Eng.* **2003**, *56*, 143−152.
(13) Sanchez-Muniz, F.; Viejo, J.; Medina, R. Deep-frying of sardines in different culinary fats. Changes in the fatty acid composition of sardines and frying fats. *J. Agric. Food Chem.* **1992**, *40*, 2252−2258.
(14) Candela, M.; Astiasaran, I.; Bello, J. Deep-fat frying modifies high-fat lipid fraction. *J. Agric. Food Chem.* **1998**, *46*, 2793−2796.
(15) Hoffman, L. C.; Prinsloo, J.; Casey, N.; Theron, J. Effects of five cooking methods on the proximate, fatty acid, and mineral composition of fillets of the African sharptooth catfish. *Die SA Tydskrif vir Voedselwetenskap en Voeding* **1994**, *6* (*4*), 146−152.
(16) Boskou, D.; Elmadfa, I. *Frying of Food*. Technomic Publishing Company, Inc.: Lancaster, PA, 1999.
(17) Lai, S.-M.; Gray, J. I.; Zabik, M. E. Evaluation of solid-phase extraction and gas chromatography for determination of cholesterol oxidation products in spray-dried whole egg. *J. Agric. Food Chem.* **1995**, *43*, 1122−1126.
(18) Razzazi-Fazeli, E.; Kleineisen, S.; Luf, W. Determination of cholesterol oxides in processed food using high-performance liquid chromatography−mass spectrometry with atmospheric pressure chemical ionisation. *J. Chrom. A* **2000**, *896*, 321−334.
(19) Wagner, K−H.; Auer, E.; Elmadfa, I. Content of trans fatty acids in margarines, plant oils, fried products, and chocolate spreads in Austria. *Eur. Food Res. Technol.* **2000**, *210*, 237−241.
(20) AOCS Official Methods: Iodine value. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; AOCS Press: Champaign, IL, 1998; Official Method Cd 1c-85.
(21) AOCS Official Methods: Acid value. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; AOCS Press: Champaign, IL, 1998; Official Method Cd 3d-63.
(22) AOCS Official Methods: Peroxide value. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; AOCS Press: Champaign, IL, 1998; Official Method Cd 8−53.
(23) AOCS Official Methods: *p*-Anisidine value. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; AOCS Press: Champaign, IL, 1998; Official Method Cd 18−90.
(24) AOCS Official Methods: Spectrophotometric Determination of Conjugated Dienoic Acid. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; AOCS Press: Champaign, IL, 1998; Method Ti 1a-64.
(25) Jakob, E.; Elmadfa, I. Rapid HPLC−Assay for assessment of vitamin K1, A, E and β-carotene status in children (7−19 years). *Int. J. Vit. Nutr. Res.* **1995**, *65*, 31−35.
(26) Sebedio, J. L.; Ratnayake, W.; Ackman, R.; Provost, J. Stability of polyunsaturated omega-3 fatty acids during deep fat frying of Atlantic mackerel (*Scomber scombrus L.*). *Food Res. Int.* **1993**, *26* (*3*), 163−172.
(27) Isnardy, B.; Wagner, K.-H.; Elmadfa, I. Effects of α-, γ-, and δ-tocopherols on the autoxidation of rapeseed oil triglycerides in a system containing low oxygen. *J. Agric. Food Chem.* **2003**, *51*, 7775−7780.
(28) Wagner, K.-H.; Isnardy, B.; Elmadfa, I. Effects of α-, γ-, and δ-tocopherols on the autoxidation of a 10% rapeseed oil triglyceride-in-water emulsion. *Eur. J. Lipid Sci. Technol.* **2004**, *106*, 44−51.

(29) Echarte, M.; Zulet, M. A.; Astiasaran, I. Oxidation process affecting fatty acids and cholesterol in fried and roasted salmon. *J. Agric. Food Chem.* **2001**, *49*, 5662−5667.

(30) Ohshima, T.; Li, N.; Koizumi, C. Oxidative decomposition of cholesterol in fish products. *J. Am. Oil Chem. Soc.* **1993**, *70*, 595−600.

(31) Echarte, M.; Conchillo, A.; Ansorena, D.; Astiasaran, I. Evaluation of the nutritional aspects and cholesterol oxidation products of pork liver and fish patés. *Food Chem.* **2004**, *86*, 47−53.

(32) Pie, J. E.; Spahis, K.; Seillan, C. Cholesterol oxidation in meat products during cooking and frozen storage. *J. Agric. Food Chem.* **1991**, *39*, 250−254.

(33) Larkeson, B.; Dutta, P. C.; Hansson, I. Effects of frying and storage on cholesterol oxidation in minced meat products. *J. Am. Oil Chem. Soc.* **2000**, *77*, 675−680.

(34) Li, N.; Ohshima, T.; Shozen, K.; Ushio, H.; Koizumi, C. Effects of the degree of unsaturation of coexisting triacylglycerols on cholesterol oxidation. *J. Am. Oil Chem. Soc.* **1994**, *71*, 623−627.

(35) Morin, R. J.; Hu, B.; Peng, S.-K.; Sevanian, A. Cholesterol oxidation and cancer. In *Biological Effects of Cholesterol Oxides*; Peng, S.-H., Morin, R. J., Eds.; CRC Press: Boca Raton, FL, 1992, pp 191−202.

(36) Peng, S.-K.; Sevanian, A.; Morin, R. J. Cytotoxicity of cholesterol oxides. In *Biological Effects of Cholesterol Oxides*; Peng, S.-H., Morin, R. J., Eds.; CRC Press: Boca Raton, FL, 1992; pp 147−166.

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