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ORIGINAL PAPER

Retention of health-related beneficial components during household preparation of selenium-enriched African catfish (*Clarias gariepinus*) fillets

Sabine Mierke-Klemeyer · Rune Larsen · Jörg Oehlenschläger · Hanne Maehre · Edel O. Elvevoll · Narcisa Maria Bandarra · Raquel Parreira · Ana Margarida Andrade · Maria Leonor Nunes · Edward Schram · Joop Luten

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Abstract Industrial processing and heat treatment of fish muscle generally lead to losses of water-soluble components, some of which may have beneficial health effects. The aim of this work was to determine the retention of taurine, selenium and n-3 polyunsaturated fatty acids when preparing African catfish by three traditional household techniques: boiling in pouches, deep-frying and baking. Cooking did not significantly reduce the content of selenium, having retention between 91and 104%. Deep-frying resulted in a taurine loss of 40%, which was significantly higher than in baking where losses were 25% The fatty acid profiles were similar for baked and boiled fillets, but were significantly different from deep-fried fillets, due to absorption of vegetable frying oil. Baking was the best preparation technique with regard to retention of 20:5n-3 (eicosapentaenoic acid; EPA) and 22:6n-3 (docosahexaenoic acid, DHA), retaining above 80% for both fatty acids, whereas boiling and deep-frying were able to retain only approximately 54 and 65% of each, respectively.

Keywords African catfish · Taurine · Selenium · n-3 Polyunsaturated fatty acids · Household preparation · Losses · Retention

S. Mierke-Klemeyer (✉) · J. Oehlenschläger
Research Department for Fish Quality, Federal Research Centre for Nutrition and Food, Palmaille 9, 22767 Hamburg, Germany
e-mail: sabine.klemeyer@bfel.de

R. Larsen · H. Maehre · E. O. Elvevoll
Department of Marine Biotechnology, Norwegian College of Fishery Science, University of Tromsø, 9037 Tromsø, Norway

N. M. Bandarra · R. Parreira · A. M. Andrade · M. L. Nunes
Departamento de Inovação Tecnológica e Valorização dos Produtos da Pesca, INRB/IPIMAR, Av. Brasília, 1449-006 Lisbon, Portugal

E. Schram
Wageningen IMARES, PO box 68, 1970 AB IJmuiden, The Netherlands

J. Luten
Norwegian Institute of Fisheries and Aquaculture Research Fiskeriforskning, PO box 6122, 9191 Tromsø, Norway

Introduction

Fish contains many health-beneficial components such as vitamins, minerals and n-3 polyunsaturated fatty acids (PUFA), which makes it unique for human nutrition. Seafood is also an excellent protein source with a high nutritive value due to its favorable essential amino acid composition. The health aspects of eating seafood have mainly been linked to marine lipids. Evidence from epidemiological data based on seafood consumption and clinical trials with n-3 PUFA of marine origin are associated with a reduced risk of coronary heart disease (CHD) [1, 2]. In addition to n-3 PUFA, the micronutrients taurine and selenium have beneficial health effects.

Seafood, especially invertebrates such as molluscs and crustaceans, are high in taurine (2-aminoethanesulfonic acid) [3–5]. Fish muscle has a higher digestibility and gives increased concentration of serum taurine when compared to beef and chicken [6]. Taurine is an exclusively free amino acid (FAA), which may be regarded as conditionally essential [7]. It is regarded to be important in membrane stabilization, as an antioxidant and in the development of the central nervous system [8]. Reduced risk of CHD through the effects of taurine alone and combined with n-3 PUFA [9–11] has been suggested. Indeed, the beneficial

effects of dietary taurine on the risk of cardiovascular diseases have been observed in both animal and human studies [12–15].

Selenium is an essential micronutrient and the German Nutrition Society recommends a daily selenium intake of 30–70 μg [16]. It is incorporated into selenoproteins such as glutathione peroxidases, deiodinases and selenoprotein P. The glutathione peroxidases are involved in cell antioxidant systems and have more subtle functions in ensuring the regulation and formation of arachidonic acid metabolites that derive from hydroperoxide intermediates [17]. There are at least three selenium-dependent deiodinase enzymes (type I, II, III) that exert tight control on local and systemic availability of the active thyroid hormone (T3) [18]. The selenoprotein P, which contains ten selenocystein residues, functions as an extra-cellular oxidant defense [19]. Furthermore, selenium has been reported to act as an insulin-mimetic agent with regard to normalization of blood glucose levels and regulation of some insulin-mediated metabolic processes [20]. Significant reductions in the incidence of prostate, colon and lung cancers were found when American subjects were supplemented with 200 μg selenium per day as selenium enriched yeast [21].

Culinary treatments are responsible for important changes in the fish muscle, especially on sensory attributes, chemical characteristics and nutritional composition, which are mostly dependent on the temperature, time and culinary procedure. There are both positive and negative effects of thermal processing of food. The advantages include killing pathogens, inactivation of anti-nutrient enzymes and increased digestibility and bioavailability [22]. Heating may also provide or enhance the desired taste. Thermal degradation of components, browning reactions and isomerization of fatty acids are some detrimental effects of heat treatment [23]. Also when preparing fish and meat, water-soluble compounds may leach out during preparation and it is usually referred to as cooking losses [24, 25]. Our primary interest with regard to FAA was to determine the loss of taurine during household preparation. Glycine and alanine were also quantified as they are the primary FAA in the fish fillets and important seafood taste components.

A new approach to the development of functional seafood products includes dietary modulation of farmed fish. The content of selenium in African catfish increases via dietary modulation of feed with organic Se-compounds up to a level of 1.2 mg/kg. Fish selenium is a highly bioavailable source of dietary selenium [46]; therefore, the development of functional seafood products is very interesting. The objective of this study was to evaluate the retention of selenium, taurine, glycine, alanine and PUFA during household preparation of Se-enriched African catfish fillets.

Materials and methods

Raw material

African catfish were reared (at Wageningen IMARES, The Netherlands) in three tanks for 43 days with a diet supplemented with selenium-enriched garlic. The overall selenium content in the feed was 8.5 mg/kg. The fish ($n = 42$, length 35 cm ± 2.4 cm, approximate weight 383 g ± 82 g) were killed by a blow on the head. Immediately after gutting, the fish were stored in boxes with melting ice and transported by a covered pick-up within 7 h to Hamburg. After transportation to FRCNF in Hamburg, the fish were manually filleted and skinned.

Experimental design

From each tank, 14 fish were sampled and the fillets from two individuals were combined to make one sample. Of the seven samples from each tank, two samples were not prepared whereas the other five samples were subjected to a culinary treatment. Thus, each treatment group contained five samples from the same tank, and a total of six samples remained untreated and served as control.

The household preparation methods chosen for this experiment were boiling in pouches, baking in aluminium foil and deep-frying. For boiling, fillets were enclosed in pouches and put in water at 90–95 °C for 10 min, leading to a core temperature of 65 °C. Baking was performed by putting fillets into aluminum boxes followed by heating in an oven with circulating air at 180 °C for 27 min (core temperature: 80 °C). For both boiled and baked samples, drip was collected and fillets and drip were weighed separately. Deep-fried samples were immersed in hydrogenated vegetable fat at 160 °C for 4 min (core temperature: 65 °C) and adherent fat was removed with a paper towel. After cooling down, the fillets were weighed.

After the cooking procedures, all samples were frozen and lyophilized. The lyophilized samples were homogenized and put in glass jars with screw lids, and the headspaces were flushed with nitrogen. Furthermore, the samples were submitted to analysis of proximate composition (moisture, protein, fat and ash), fatty acid profiles, FAA and selenium content.

Analysis of proximate composition

Duplicate samples were analyzed for proximate composition. Official AOAC methods for analysis [26] were used for the determination of moisture (method 925.04), protein (method 981.10) and ash (method 938.08). The factor used

to convert nitrogen to the crude protein value was 6.25 and an in-house reference sample served as quality control. The determination of fat content was performed according to the method described by Smedes [27].

Determination of selenium content

The samples (0.30–0.35 g) were digested with 4 mL 65% nitric acid and 1 mL 30% hydrogen peroxide in a microwave high pressure system (ultraCLAVE III, MLS GmbH, Leutkirch). Initial conditions were 22 °C/40 bar, ramping to 50 °C/100 bar in 5 min, then to 110 °C/120 bar in 25 min and finally to 170 °C/110 bar in 10 min. Final conditions were held stable for 45 min, before cooling down and releasing pressure after 50 min. To ensure quality control of the results, certified reference material CRM 422 (cod muscle) was also digested and analyzed in each run.

The samples and the reference material were analyzed by a Perkin Elmer ZL 4100 graphite furnace atomic absorption spectrometer (GF-AAS) with an electrodeless discharge lamp for Se analysis (196 nm and slit 2 nm), using Pd modifier and standard addition technique. All measurements were performed with Zeeman background correction. The analytical conditions of the GF-AAS are shown in Table 1. The limit of quantification is 170 ng selenium/g fish fillet.

Determination of free amino acids

The FAA were extracted by homogenizing approximately 1 g of freeze-dried tissue with 9 mL milli-Q H_2O and 1 mL of 20 mmol/L norleucine with an Ultra Turrax T25 basic (IKA Werke GmbH, Staufen, Germany) for 15 s before adding 1 mL 35% sulphosalicylic acid and homogenizing for another 15 s. Norleucine served as an internal standard. The suspension was centrifuged at 10,000 × *g* at 4 °C for 15 min. An aliquot of the supernatant was diluted 1:5 with sample buffer (lithium citrate) and submitted to FAA analysis. The concentration of FAA was determined using a Knauer A200 amino acid analyzer (Knauer GmbH, Berlin, Germany) with a lithium citrate equilibrated column. The signal was analyzed by Knauer's ChromGate software and compared with A9906 physiological amino acids (Sigma Chemical Co., St Louis, MO., USA). The samples were analyzed in triplicate.

Table 1 Temperature- and time conditions of the GF-AAS for determination of selenium

Temperature (°C)	Ramp time (s)	Hold Time (s)
110	1	30
130	15	20
200	15	30
900	10	10
1,300	10	20
2,000[a]	0	5
2,450	1	5
1,110	10	2
100	5	5

[a] Read step

Determination of fatty acid profile

Fatty acid methyl esters (FAMEs) were prepared according to Lepage and Roy [28] as modified by Cohen et al. [29]. The FAMEs preparation was carried out using 0.3 g of freeze-dried material and 5 mL of the acetyl chloride:methanol mixture (1:19, v/v). The transesterification was carried out at 80 °C for 1 h. After cooling, 1 mL of water and 2 mL of *n*-heptane were added to the mixture, which was stirred and centrifuged at 2,150 × *g* for 10 min. The organic phase was collected, filtered and dried over anhydrous sodium sulfate. The solvent was removed under nitrogen and the FAMEs dissolved in 0.1 mL of *n*-heptane. The analyses were performed in a Varian CP-3800 (Walnut Creek, CA, USA) gas chromatograph equipped with an auto sampler and fitted with a flame ionization detector at 250 °C.

The separation was achieved using a capillary column DB-WAX (30 m length, 0.25 mm id and 0.25 µm film thickness) from Hewlett Packard (Albertville, MN, USA). After holding at 180 °C for 5 min, the temperature was ramped from 4 °C/min to 220 °C and maintained at 220 °C for 25 min with the injector at 250 °C. The split ratio was 100:1. The identification of the sample peaks was made by comparison of the retention times with the standards from Sigma. The fatty acid profile was obtained by calculating the relative area percent of the chromatographic peaks.

Absolute values were calculated based on the corrective factors presented by Weihrauch et al. [30]. Based on fatty acid results expressed in mg/100 g, thrombogenic index (TI) was calculated following the expression published in [31].

Calculation of true retention

True retention (TR) was calculated for all components as described by Murphy et al. [32]

$$\mathrm{TR}\% = \frac{(\text{nutrient content per g of prepared fish} \times \text{g of fish after preparation})}{(\text{nutrient content per g of raw fish} \times \text{g of fish before preparation})} \times 100$$

Statistical analysis

Values are presented as mean ± standard deviation (SD). Statistical analyses of FAA and selenium were performed with SPSS 12.0.1 (SPSS Inc., Chicago, IL, USA) and fatty acids were analyzed with Statistica 6.0 for Windows. Significant differences between treatments were determined by one-way analysis of variance (ANOVA) with post hoc comparison using the Dunnets T3 test. Level of significance was set to $P < 0.05$.

Results and discussions

Proximate composition and weight losses

Proximate composition of raw and prepared fillets and the weight loss during preparation is presented in Table 2. Raw fillets contained 78.8% water, 18.0% raw protein, 2.5% fat and 0.7% ash, and this matched with previously published results of African catfish [33, 34]. All cooking conditions resulted in a reduction of water content (WC) and increased protein levels. Especially, deep-fried fillets ended up with an altered composition due to uptake of fat during preparation, and thus WC was reduced to 64.1% and fat levels raised to 8.6%. Boiling, baking and deep-frying resulted in a weight loss of 32, 23 and 36%, respectively.

Selenium and FAA

This study was part of a broader experiment investigating the effect of added selenium in the feed. The fish were fed a diet with selenium enriched garlic and this resulted in relatively high selenium content in raw fillets, 0.92 mg/kg wet fillet.

The concentrations of taurine, glycine and alanine in raw fillets were 2.01, 0.68 and 0.41 mg/g, respectively, which are similar to those concentrations reported by Ip et al. [35]. The contents of selenium, taurine, glycine and alanine of raw and prepared African catfish fillets are shown in Table 3. In order to better evaluate the losses of compounds during preparation, TR was calculated, and the retention of FAA and selenium after preparation is displayed in Fig. 1.

The retention of selenium was 91, 97 and 104% for baking, boiling and deep-frying, respectively, and no significant difference was found between any of the cooking procedures. The exudate appearing during boiling and baking was collected and the selenium content was 5.6 and 8.5 μg/g dry weight, respectively. This result supported the fact that loss of Se was higher for baking compared to boiling. Other studies on seafood also report high retention of selenium during cooking. Based on dry weight calculation, baking of flounder fillets wrapped in aluminium foil caused no consistent losses of selenium [36]. The effect of cooking has been studied for tuna and no significant difference ($P < 0.05$) in total selenium concentration was found before and after cooking [35]. In an investigation dealing with the effects of cooking on the mineral content of seafood, only losses of sodium and potassium were observed, whereas the other minerals (iron, zinc, selenium and arsenic) were found to be almost stable [37].

Losses of taurine, glycine and alanine during preparation were 25–40, 30–40 and 20–30%, respectively, depending upon the cooking procedure. In general, there was no significant difference in retention of FAA between baking and boiling. However, deep-frying resulted in significantly lower TR of taurine compared to baked samples. These results generally agree with retention of taurine, glycine and alanine during household preparation of cod, although subjected to different cooking conditions [38].

Table 2 Proximate composition (mean ± SD) of raw and cooked fillets of African catfish and the weight loss during preparation

	n	WC (%)	Protein (%)	Fat (%)	Ash (%)	Weight loss (%)
Raw	6	78.8 ± 0.6[a]	18.0 ± 0.4[a]	2.5 ± 0.6[a]	0.7 ± 0.1[a]	
Baked	5	73.7 ± 1.5[b]	21.7 ± 0.9[b]	2.9 ± 0.7[a]	0.8 ± 0.2[a]	23 ± 2
Boiled	5	72.6 ± 1.2[b]	23.0 ± 0.8[b,c]	3.1 ± 0[a]	0.7 ± 0.2[a]	32 ± 5
Deep fried	5	64.1 ± 1.3[c]	24.6 ± 0.3[c]	8.6 ± 07[b]	0.9 ± 0.1[a]	36 ± 3

[a,b,c] Columns that do not share a common letter are significantly different ($P < 0.05$)

Table 3 Contents (mean ± SD) of selenium, taurine, glycine and alanine of raw and cooked African catfish fillets

	n	Selenium (mg/kg)	Taurine (mg/g)	Glycine (mg/g)	Alanine (mg/g)
Raw	6	0.92 ± 0.14[a]	2.01 ± 0.32	0.68 ± 0.18	0.41 ± 0.06[a, b]
Baked	5	1.21 ± 0.15[a]	1.91 ± 0.17	0.55 ± 0.10	0.37 ± 0.03[a]
Boiled	5	0.97 ± 0.13[a]	1.76 ± 0.19	0.62 ± 0.07	0.41 ± 0.04[a, b]
Deep fried	5	1.55 ± 0.09[b]	1.75 ± 0.11	0.56 ± 0.07	0.50 ± 0.05[b]

[a,b] Columns that do not share a common letter are significantly different ($P < 0.05$)

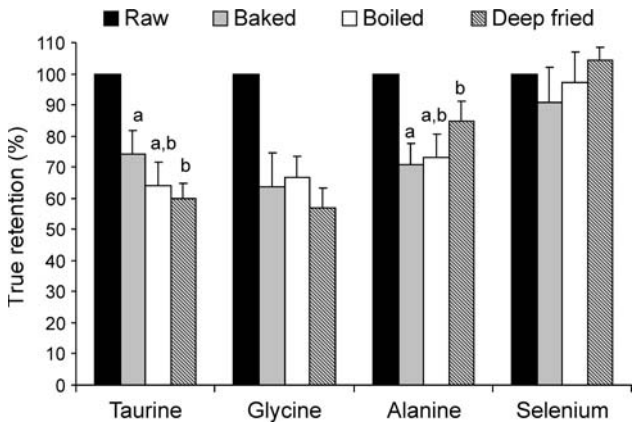

Fig. 1 True retention (mean ± SD) of taurine, glycine, alanine and selenium in African catfish fillets after baking, boiling and deep-frying. *Column sets* that do not share a common letter were significantly different ($P < 0.05$)

Losses of taurine from farmed cod during sous-vide preparation was 35 and 55% after the first and second heat treatments, respectively [39]. A mean loss of 27% taurine was found when blue mussel was steamed for 1 min [3]. Chiou et al. [40] reported a 22–35% decrease in the total amount of FAA on heating abalone to 98 °C inside vacuum packed polypropylene bags, while the same procedure at 80 °C gave insignificant differences compared to the raw product.

Fatty acids

The fatty acid profiles of raw and cooked fillets are presented in Table 4. The most abundant compounds in raw fillets were 16:0, 18:1n-9 and 22:6n-3 (DHA). Monounsaturated fatty acids (MUFA) constitute the most abundant group, followed by saturated fatty acids (SFA) and PUFA. Within the former, the amounts of 20:5n-3 (EPA) and DHA almost correspond to 50% of the total. According to the literature [41], the ratio n-3/n-6 in freshwater fish ranges between 1 and 4, in the present work was around 2.9, and such value is close to that found for most freshwater species. This ratio gives important dietetic information related to a healthy balanced synthesis of eicosanoids. The actual WHO recommendations state that the daily ratio n-3/n-6 in the human diet should be no higher than 1:5 (0.2) [42]. However, taking into account that seafood are the exclusive contributors of long chain n-3 fatty acids in diet and that n-6 fatty acids are present in a large part of other foods, the consumption of African catfish can be considered to be very advantageous in terms of a healthy balanced diet, concerning especially n-3 fatty acids.

In addition to losses of water solubles during cooking, a portion of lipids is also removed due to liquefaction and rupture of cell walls that make them more prone to being removed. In the present work, boiling and baking did not significantly affect ($P > 0.05$) the individual fatty acid profile. On the other hand, deep-frying was responsible for important changes in fatty acid individual levels. Catfish

Table 4 Levels of the main fatty acids (mean ± SD) of raw, boiled, baked and deep-fried African catfish fillets

Fatty acids	Raw (mg/100 g)	Boiled (mg/100 g)	Baked (mg/100 g)	Deep fried (mg/100 g)
Σ SFA	686 ± 69[a]	596 ± 14[a]	600 ± 18[a]	2365 ± 132[b]
Σ MUFA	766 ± 74[a]	655 ± 14[a]	698 ± 25[a]	1440 ± 62[b]
18:2n-6	141 ± 31[a]	116 ± 8[a]	119 ± 5[a]	1029 ± 94[b]
20:5n-3	105 ± 14	92 ± 3	99 ± 5	93 ± 20
22:5n-3	36 ± 6	31 ± 1	34 ± 2	32 ± 6
22:6n-3	258 ± 43	225 ± 6	244 ± 18	243 ± 58
Σ PUFA	643 ± 72[a]	554 ± 16[a]	593 ± 28[a]	1498 ± 179[b]
Σ (n-3)	470 ± 67	412 ± 8	444 ± 25	426 ± 94
Σ (n-6)	173 ± 32[a]	143 ± 8[a]	148 ± 7[a]	1072 ± 98[b]
(n-3)/(n-6)	2.8 ± 0.5[a]	2.9 ± 0.1[a]	3.0 ± 0.2[a]	0.4 ± 0.1[b]
Total	2105 ± 190[a]	1814 ± 16[a]	1900 ± 12[a]	5310 ± 27[b]

[a,b] Rows that do not share a common letter are significantly different ($P < 0.05$)

Table 5 Main fatty acids composition of vegetable frying oil

Fatty acid	Fatty acid (%)
16:0	34.3 ± 0
18:0	11.4 ± 0.1
Σ SFA	48.4 ± 0.1
18:1n-9	23.4 ± 0.1
Σ MUFA	23.7 ± 0.2
18:2n-6	27.8 ± 0.2
Σ PUFA	28.0 ± 0.1
Σ (n-3)	0.2 ± 0.1
Σ (n-6)	27.8 ± 0.2

fillets were deep-fried in highly saturated vegetable oil, the fatty acid profile of which can be seen in Table 5. Consequently, the level of saturated and PUFA n-6 fatty acids was substantially higher in the fried samples due to the absorption of frying oil, as was observed in cod and salmon by other authors [43]. Similarly, there was also an uptake of other fatty acids, especially 18:2n6, which is reflected in the substantial increase of this fatty acid. Thus, the fatty acid profile as well as the ratio of n-3/n-6 of fried catfish was significantly different.

Moreover, the retention of fatty acids was 55–61 and 78–82% for boiled and baked fish, respectively, as shown in Fig. 2. Boiling resulted in a significantly higher loss of fatty acids compared to baking, probably due to an easier leaching of lipids under these conditions. As previously mentioned, deep-frying caused a significant alteration of the fatty acid profile resulting in a TR for 18:n6 and Σ (n-6) of 407 and 358%, respectively. Compared to the other cooking procedures, the retention of 20:5n3, 22:5n3, 22:6n3 and Σ (n-3) in general was lower than in baked samples and higher than in boiled samples.

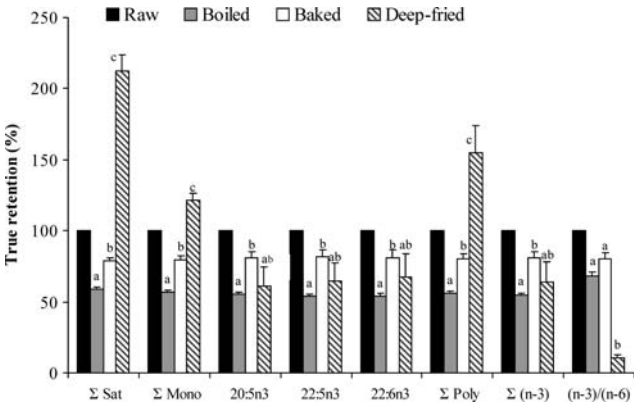

Fig. 2 True retention (mean ± SD) of the main fatty acids and n3/n6 ratio in African catfish fillets after baking, boiling and deep-frying. *Column sets* that do not share a common letter were significantly different ($P < 0.05$)

To obtain 500 mg of EPA + DHA per day, as recommended by ISSFAL [44] for the prevention of cardiovascular disease, it is necessary to consume 146 g of baked, 158 g of boiled and 149 g of fried African catfish. In fact, no significant differences were found in the absolute level of these two endogenous fatty acids after cooking. Nevertheless, it is important to take into account not only EPA and DHA, but also the level of saturated fatty acids, which was higher in deep-fried fillets. The TI reflects this statement and helps in the choice of healthy foods. Thus, fried catfish presents a TI value of 0.94 compared to 0.36 for boiling and 0.32 for baking, showing that these culinary treatments are better in the prevention of cardiovascular disease. These results agree with a human study investigating intake of fish and the effects of different cooking methods on cardiac structure, function and hemodynamics [45]. It concluded that fried fish intake was associated with structural abnormalities, whereas boiled or baked fish was associated with improved cardiac hemodynamics.

Conclusion

Household preparation of African catfish fillets caused low or no losses of selenium, no matter the cooking procedure. Inconsistent results were obtained with regard to the retention of FAA among the preparation methods, but significant losses from raw fillets were measured for taurine, glycine and alanine. Baking was the most suitable preparation method with regard to retention of EPA and DHA. Deep-frying led to an unfavorable thrombogenic index due to oil absorption, which made boiling and baking healthier culinary treatments with respect to cardiovascular diseases.

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