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Research Article

Influence of Different Cooking Procedure on the Hg Concentration in Fish

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Abstract: The effect of culinary treatment on reducing mercury (Hg) concentration in fish was studied with 35 samples of 14 the most common fish species from the South African market. An analytical determination of Hg was carried out by direct thermal decomposition (DTD) of fish samples using a Model RA-915+ Zeeman Mercury analyzer. The Hg concentration in fish had been determined before and after traditional ways of cooking (boiling, poaching, simmering, deep frying and grilling on coal fire) on a dry basis to avoid uncertainty of different water content in cooked and uncooked fish. It was found that the Hg concentration in most cooked fishes (60%) was not changed in comparison with uncooked samples. The cooking procedure, which could reduce Hg concentration by 10-26.3%, is the fish treatment with vegetable brines or lemon juice, possibly, due to formation of soluble complexes of Hg2+ ions with citric acid. The other cooking procedure, which allows lowering the amount of Hg up to 26.5% is the thermal treatment of fish by grilling. The removal of Hg can be explained by a process, which is called "steam distillation", when fish oil is evaporated at a temperature of boiling water together with methylmercury (MeHg).

Keywords: Total mercury; Decreasing of Hg content in fish by cooking; Uncooked and cooked fish.

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Introduction

Mercury (Hg) is one of the most hazardous pollutants in the environment that affects human health (ATSDR). This element is counted as a global pollutant, because when Hg is released into atmospheric air from local sources of emission, it settles down thousands kilometers away on surface of ocean (Pacyna et al. 2010; Pirrone et al. 201). In aqueous media, elemental Hg^{o} and inorganic species of Hg^{+2} are converted by microorganisms into methylmercury MeHg, the most toxic form of mercury (Mergler et al. 2007, Grandjean et al. 2010). Since the level of MeHg accumulation in fish is cumulative and normally increases at each level of the food chain, older top-predatory fish generally have the highest Hg concentration (Focardi S. 2012; Storelly et al. 2007). Mercury in fish exists in the form of inorganic Hg^{2+} and organic form as MeHg (May et al. 1987). To protect general population against hazardous exposure through the fish consumption, national agencies have set limits for Hg concentration in wet weight (raw) fish. Thus the U.S. Food and Drug Administration and World Health Organization (FDA/WHO, 2004) have set a level of 0.5 $\mu g\ g^{-1}$ of Hg and 1.0 $\mu g\ g^{-1}$ for predatory fish. Although in most cases a cooked fish is consumed, the majority of studies has been related to levels of Hg in raw fish products (US. FDA). The information concerning Hg concentration in cooked fish and the effects of cooking methods on Hg content in fish is more limited. The influence of cooking procedures (boiling for different time periods, frying and roasting) on Hg content in fish have been studied by several groups of researchers (Burger et al.2003; Perello et al. 2008; Farias et al. 2010; Ouedraogo et al.2011; Maulvault et al. 2012; Kalogeropoulos et al. 2012; Schmidt et al. 2015). Most of them draw the conclusion that while the relative concentration of Hg in cooked fish is increasing, due to the fat and water loss during cooking procedure, the total mass of Hg in fish remains unchanged. This conclusion indicates that cooking does not influence on the amount of Hg in fish.

Therefore, it was a reassurance to see an article, in which decrease of Hg, from 15 to 70%, after the roasting of some omnivorous and herbivorous fish species of Amazon region in Brazil on coal fire has been described (Farias et al. 2010). In more recent publication (Schmidt et al. 2015) it was also found that up to 33% of Hg can be removed from fish by frying. The results of these investigations are in contradiction with previously published data, but at the same time create some hope that reducing the amount of Hg in fish by cooking is possible.

The aim of this study was to test and evaluate some cooking methods for reducing Hg concentration in fish. This could be done by the removal of Hg in the form of soluble or volatile compounds before or in the process of main cooking procedure. The concentration of Hg in fish was planned to determine before and after traditional ways of cooking on a dry weight basis to avoid uncertainty of different water content in cooked and uncooked fish.

Materials and Methods

Instrumentation

A Model RA-915+ Zeeman atomic absorption mercury analyser (Lumex, St. Petersburg, Russia) was used for Hg determination (Sholupov *et al.* 2004;). The application of the instrument for Hg determination in fish is based on the direct thermal decomposition (DTD) of wet or dry fish samples with the following detection of Hg vapours by atomic absorption spectrometer (Panichev & Panicheva, 2015). Fish or fish products were analysed with RA-915+ Zeeman Mercury Analyser without any chemical pre-treatment, minimizing the risk of sample contamination.

Analytical balance (Radwag), model AS 220/C/2, 220g x 0.1 mg, were used for weighing of fish samples, pH-meter (Hanna Instruments), model HI 8519N- for pH measurements and type K chromel-alumel thermocouple, with temperature range from -270 ^{o}C. to 1372^{o}C for the measurement of internal temperature of fish.

Unsweetened 100% lemon juice, (Brookers) and sunflower oil (Excella), products of South Africa, home fermented and commercial sauerkraut, product of Germany, as well as cucumbers in brine, Buei Darom, product of Israel, were used for fish culinary treatment.

Fish samples

Fish samples, represented 14 the most common sea fish species on the South African market (Cawthorn et al. 2011) were collected at local Tshwane market in Pretoria. They include: Kingklip (*Genypterus capensis*), Norwegian salmon (*Salmo salar*), Hake and Cape Hake (*Merluccius paradoxus/Merluccius capensis),* Kabeljou (*Argyosomus spp.*), Sole large (*Bothidae*), Yellowtail (*Seriola lalandi*), Snoek (*Thyrsites atun*), Soldierfish (*Myripristinae*), Red snapper (*Lutjanus spp*.), Mackerel (*Scomber japonicus*), Cape salmon (*Atractoscion aequidens),* Slinger *(Chrysoblephus puniceus)*, Calamari *(Loliginidae)*. All fish samples were fresh, except of Snoek, some Mackerel and Calamari, which were obtained frozen. The fishes were cleaned, washed and fileted with skin-on for different cooking procedures. All studies were performed with individual specimen of fish.

Culinary treatment

A filet of fish was cooked using the main domestic cooking procedures such as boiling, poaching, simmering, deep frying and grilling on coal fire. Cooked and uncooked fish were taken for analysis without skin. Fish cooked in oil was taken out on a filter paper to eliminate oil excess. Fish cooked in aqueous medium was taken out at the end of cooking. For the evaluation of possible influence of cooking procedures on Hg content in fish, the initial and cooked samples of each fish have been analysed after 7 days of open air-drying.

Certified Reference Materials

Certified Reference Materials (CRM) were used in present work for two reasons. One - to calibrate the RA-915+ Zeeman Mercury analyzer, which has been done with CRM DC 73308 (Stream Sediments), of China National Analysis Center, certified value 280 ± 30 ng g^{-1}. The other aim of CRM application was to confirm results of Hg determination in fish samples. The SRM 1515 (Apple leaves) of the National Institute of Standards and

Technology, US. Department of commerce, certified value 44 ± 4 ng g^{-1} and CRM TORT-2 (*Lobster hepatopancreas*) of the National Research Council of Canada, certified value 270 ± 60 ng g^{-1} have been used for validation of analytical results.

Analytical procedure of Hg determination in fish

Each fish sample, taken for the analysis was weighed on an analytical balance in milligrams units and placed into a quartz boat of the instrument. After it was inserted into an evaporation tube, preheated to 350–400

°C, vapours of Hg, together with smoke, formed due to combustion of organic matter, were transported into an analytical cell, constantly heated to 800°C, for the measurements of Hg by atomic absorption method. Background absorption was eliminated by the high-frequency Zeeman correction system. The calibration curve for Hg determination plotted as absolute mass of Hg (ng) versus value of integrated analytical signal (arbitrary units) using different masses of CRM with known concentration of Hg. The concentration of Hg in fish (ng g^{-1}) was determined from the data on absolute mass of Hg (ng) and mass of the fish sample (g). The mass of samples ranges between 50 and 300 mg. The exact weight of the sample was set to the software of RA-915+Mercury Analyser. In the process of analysis, the software displays a curve of Hg evaporation, and at the end of measurements, it shows the maximum absorption value, the area of the peak and calculated concentration of Hg in the sample.

Direct thermal decomposition (DTD) analysis of fish samples affords many benefits. Eliminating wet chemistry greatly reduces chemical waste generation during acid digestion of fish samples, prevents Hg losses and sample contamination at the stage of sample preparation. The limit of detection (3s criteria) and limit of quantification (10 s criteria) for the determination of Hg in fish samples with mass of 250 mg was found to be 0.6 ng g^{-1} and 2.0 ng g^{-1}, respectively (Panichev & Panicheva, 2015). Time taken for the analysis of one sample (three replicates) is about 10 min. Validation of results were carried out by analysis of CRM TORT-2, lobster hepatopancreas (NRC Canada) with good correspondence of certified (270 ±60 ng g-1) and found values (283 ± 45 ng g-1). The SRM 1515, apple leaves (US department of commerce) was used to control the stability of calibration curve position in time. Analyses of certified reference materials (CRMs) at the beginning and end of each set of samples (typically 12-16) verified that the instrument remained calibrated. The mean value of Hg concentration was found to be 45 ± 6 ng g^{-1}(n=35) with certified value 44 ± 4 ng g^{-1}.

Results

Hg concentration in cooked and uncooked fish

The data on Hg concentration in dry samples of uncooked (X_1) and cooked (X_2) fish, prepared with traditional methods of fish cooking (boiling, poaching, frying and grilling over coal) and concentration change (ΔC) presented in Table 1. From the results of measurements follows that in most analyzed fish samples of different species the Hg concentration remains unchanged. This conclusion is confirmed by statistical treatment of analytical results, using null-hypothesis test (Miller J.N. & Miller J.C., 2005). Most of the obtained results are in an agreement with conclusions that Hg amount in fish does not changed by cooking (Burger et al.2003; Domingo J.L., 2011). The statistically meaningful decrease in Hg concentration have been observed for a limited number of fish species. They are Sole (ΔC=25 ng g^{-1} or 15.6%), Yellowtail (ΔC=49 ng g^{-1} or 19.1%), after simmering in vegetable oil and three samples of Yellowtail fishes, separately grilled over coal fire. All samples of Yellowtail show relatively high values of Hg degrease (ΔC=59 ng g^{-1} or 23.0%; ΔC=23 ng g^{-1} or 16.3% and ΔC=68 ng g^{-1} or 26.5%). Significance test for each sample reveal that the calculated value of t exp exceeds a critical value of t, taken from table of t-distribution (Miller J.N. & Miller J.C., 2005). As a result, the null hypotheses for those samples was rejected. Decrease in Hg concentration is statistically meaningful. The difference in Hg concentration can be explained by removal of Hg in the volatile form (possibly as MeHg) together with fish fat by a "steam distillation" (Atkins, 2006). The fundamental principle of "steam distillation" is that it enables some heat-sensitive, water-insoluble organic compound to be distilled with water vapors at a lower temperature than their normal boiling point. During grilling, the fish internal temperature, according to our measurement was 96°C. This value corresponds to the temperature of boiling water in Pretoria, because the city is located at the height 1265 m above the sea level. At these conditions, some amount of Hg was removed as MeHg together with fish fat. This phenomenon could be observed only for fish species enriched in fat. There were no noticeable changes of Hg concentration in fish species with low fat, like Red snapper, Cape salmon or Slinger, grilled under the same conditions over coal fire. It appears to be that the possibility to remove Hg depend not only on a cooking procedure, but also on fish species.

Hg concentration in fish with and without preliminary treatment before cooking

The possibility of Hg removal from fish before main cooking procedure is based on chemical properties of some organic acids to form soluble complexes with Hg^{2+} ions. This process could be performed by preliminary treatment of fish in vegetable brines or lemon juice. For example, sauerkraut brine contains large number of organic acids and sulfur containing compounds (Trail et al. 1996), formed during fermentation. Experimental results on Hg concentration in fish, preliminary treated with brines, lemon juice or directly boiled in them, presented in Table 2. From the results of studies follow that soaking of Slinger fish in diluted lemon juice (pH=2.7-2.9) followed by simmering for 40 min does not affect the Hg content. Soaking in sauerkraut brine (pH=3.5 -3.8) followed by simmering for 40 min has no effect on Hg content in Yellowtail tuna. The same negative result has been observed after boiling of Snoek or poaching of Cape Hake in cucumber brine (pH=4.2).

Nevertheless, direct boiling of some low in fat fish species in sauerkraut brine (pH=3.5 -3.8) for 20-30 min reduced Hg concentration in Snoek on ΔC=71 ng g^{-1} or 11.7%, Slinger - for ΔC=41 ng g^{-1} or 5.8% and Cape Hake on ΔC=23 ng g^{-1} or 10%.

Table 1: The results of Hg determination in cooked and uncooked sea fish.

Fish name	Fish weight, kg, and it's state at purchase	Concentration, C, ng g^{-1}, C ± Δ, P=0.95		Conc. change ΔC= X_2-X_1, ng/g	Some remarks
		Uncooked X_1	Cooked X_2		
Boiling in water 20 min.					
* Cape hake	0.76 fresh	229 ± 14 n=6	215 ± 9.4 n=7		Stainless steel kitchenware with a lid
* Cape hake	0.78 fresh	598 ± 6.4 n=7	595 ± 8.6 n=6		
*Yellowtail	1.74 fresh	461 ± 57 n=3	480 ± 36 n=3		
Poaching in water for 30-40 min.					
* Hake	1.25 fresh	353 ± 46 n=3	364 ± 34 n=3		Stainless steel kitchenware with a lid
* Kabeljou	0.58 fresh	291 ± 28 n=3	302 ± 30 n=3		
* Red snapper	2.19 fresh	159 ± 62 n=3	161 ± 9.4 n=3		
* Norwegian Salmon	3.1 fresh	39.7 ± 3.8 n=3	43.8 ± 8.3 n=4		
* Snoek	1.21 frozen	240 ± 24 n=3	255 ± 4.0 n=3		
Poaching in a plastic bag at 190^{0}C with a small amount of water					
* Kingklip large	1.99 fresh	464 ± 21 n=3	445 ± 77 n=3		Roasting bag
* Mackerel	0.32 frozen	283 ± 75 n=3	284 ± 20 n=3		
* Mackerel	0.33 frozen	447 ± 103 n=3	468 ± 53 n=3		
Simmering in a small amount of water with addition of sunflower oil for 20 min.					
* Calamari	steak frozen	244 ± 22 n=3	233 ± 9.4 n=3		Stainless steel kitchenware
Simmering in a small amount of sunflower oil for 30 min.					
* Sole large	0.5 fresh	210 ± 26 n=3	196 ± 8.7 n=3		Stainless steel kitchenware with a lid
Sole large	<0.5 fresh	160 ± 18 n=3	135 ± 16 n=4	-25	
Yellowtail	2.79	257 ± 9.0 n=7	208 ± 7.2 n=6	-49	
Boiling in a sunflower oil for 3 min.					
Cape Hake	0.82 fresh	708 ± 17 n=6	666 ± 26 n=6	-42	Kitchenware with a ceramic covering
Baking over an open fire 30 min.					

* Red snapper	2.19 fresh	159 ± 62 n=3	155 ± 31 n=3		Fish is not covered
* Cape salmon	1.85 fresh	575 ± 24 n=12	613 ± 21 n=3		
* Mackerel	2.06 fresh	257 ± 16 n=6	258 ± 20 n=6		
Yellowtail	2.79 fresh	257 ± 9.0 n=7	198 ± 4.9 n=7	-59	
Yellowtail	2.44 fresh	141 ± 12 n=5	118 ± 12 n=6	-23	
Yellowtail	2.22 fresh	257 ± 11 n=6	189 ± 14 n=7	-68	
* Slinger	0.75 fresh	380 ± 74 n=3	403 ± 92 n=3		Fish is wrapped in Al foil, 45min

*Cooked and uncooked samples with the same mercury results confirmed by null-hypothesis t-test, P=0.95.

Fruit and vegetable brines and juices used: sauerkraut, cucumber, lemon.

Content of Hg in sunflower oil was ≤ 0.6 ng g^{-1}

Table 2: The results of Hg determination in cooked and uncooked fish after preliminary treatment of fish in brines and juices.

Fish name and methods of cooking	**Fish weight, kg, and it's state at purchase**	**Concentration, C, ng g^{-1} C ± Δ, P = 0.95**		**Conc. change ΔC= X_2-X_1, ng/g**	**Some remarks**
		Uncooked	**Cooked**		
		X_1	**X_2**		
Soaking in a diluted lemon juice for 2 hours followed by simmering in a fresh portion of juice 40 min, pH=2.7-2.9					
* Slinger	0.73 fresh	323 ± 20 n=6	335 ± 14 n=6		Kitchenware with a ceramic covering
* Slinger	0.45 fresh	209 ± 12 n=4	207 ± 7.9 n=5		
Boiling in a diluted lemon juice 20 min, pH=2.7-2.9					
Cape Hake	0.76 fresh	229 ± 14 n=6	193 ± 10 n=5	-36	Kitchenware with a ceramic covering
Soldier fish	0.69 fresh	483 ± 16 n=5	356 ± 6.7 n=6	-127	
Soaking in a vegetable brine(sauerkraut, pH=3.5-3.8) for 2h followed by simmering 40 min					
* Sashimi tuna	stake frozen	1784 ± 17 n=5	1772 ± 69 n=5		Kitchenware with a ceramic covering
Boiling in sauerkraut brine, pH=3.5-3.8					
Snoek	2.24 frozen	607 ± 34 n=3	536 ± 16 n=4	-71	30 min
Slinger	0.58 fresh	704 ± 25 n=6	663 ± 15 n=6	-41	20 min
*Slinger	0.57 fresh	430 ± 28 n=8	400 ± 16 n=6		20 min
Cape Hake	0.76 fresh	229 ± 14 n=6	206 ± 5.0 n=5	-23	20 min
Poaching in vegetable brine(cucumber, pH=4.2) 30min					

* Cape Hake	1.1 fresh	231 ± 12 n=3	231 ± 2.9 n=3		Kitchenware with a ceramic covering
* Snoek	2.24 frozen	607 ± 34 n=3	566 ± 45 n=4		
Poaching in a plastic bag for 20 min at 190^{0}C after been marinated $^{\Delta}$ for two hours, pH=2.6					
Cape hake	0.78 fresh	598 ± 6 n=7	554 ± 12 n=6	-44	Roasting bag
Slinger	0.58 fresh	704 ± 25 n=6	673 ± 3 n=6	-31	

*Cooked and uncooked samples with the same mercury results confirmed by null-hypothesis t-test, P=0.95.

Fruit and vegetable brines and juices used: sauerkraut, cucumber, lemon.

$^{\Delta}$Original lemon & coriander marinade, product of South Africa.

The boiling of Cape Hake in lemon juice (pH=2.7- 2.9) reduced Hg concentration on ΔC=36 ng g^{-1} or 15.7% and Soldier fish on ΔC=127 ng g^{-1} or 26.3%. Poaching of Cape hake in a plastic bag for 20 minutes after been marinating for two hours decreased Hg concentration in Cape hake on ΔC=45 ng/g or 7.5% and in Slinger fish on ΔC=31 ng/g or 4.4%

Such changes of Hg concentration are statistically significant what was confirmed by t-test based on null hypothesis assumption. The loss of mercury can be explained by the formation of water-soluble mercury complex with citric acid, stability constant of which is pK=10.9 (Martell et al. 1998), and other compounds.

Discussion

It should be noticed that Hg concentration in fish used for cooking experiments have been in the range of 12-180 ng g^{-1} on a wet weight basis and the only one fish, related to predators (Yellowfin tuna), contained 540 ng g^{-1} of Hg. That is, all fish samples obtained at the local fish market, have the Hg concentrations in the range of permissible level (Panichev & Panicheva, 2015). From general consideration it follows that to reduce Hg concentration in fish is possible either by formation of soluble complexes of Hg^{2+}ions with organic acids by cooking with vegetable brines or lemon juice or by thermal removal of volatile MeHg. Due to the fact that from 73 to 99% of total Hg in fish is present in the form of MeHg (May *et al*. 1987), the treatment of fish with brines or lemon juice can remove only limited amount of Hg in the form of Hg^{2+}ions. This conclusion can be confirmed by the decrease of Hg concentration in Snoek, Slinger, Cape hake and Soldier fish in the range of 5.8-26.3% after their treatment in sauerkraut brine and lemon juice (Table 2).

The other way of Hg removal from fish connected with cooking that could release Hg in the form of MeHg. The efficiency of Hg thermal removal by grilling was in the range of 16.3-26.5%. Simmering in sunflower oil could be the other example of thermal removal of Hg from the fat fishes. We observed 19.1% loss of Hg for rich in fat Yellowtail and only 5.9% for lean Cape hake fish. Therefore, the major part of Hg remains in fish, possibly connected to protein. Boiling and poaching in water do not change Hg concentration in fish.

Some researchers observed fish Hg increase after cooking procedures as a sequence of moisture and fat losses if the measurements were conducted on a wet weight samples (Burger et al.2003; Perello et al.2008; Kalogeropoulos et al. 2012). According to the literature data frying and roasting fish processing shows that the Hg content decrease on a dry weight basis (Farias et al. 2010; Ouedraogo et al. 2011; Schmidt et al. 2015). So, Schmidt L. shows 33% decrease of Hg concentration by frying. Moisture loss during cooking may cause fish Hg concentration increase in several times and therefore the 33% decrease of Hg concentration on a dry weight basis would be masked if the results are presented on a wet weight basis.

From our previous study (Panichev & Panicheva, 2015) follows that Hg concentration in fish could change within the ratio of wet weight/dry weight of the fish. The mean value of that ratio was found to be 4.29 for sea fishes used in the present study. It appears to be that the choice of results presentation depends on a solving problem.

Conclusion

The results of this study, based on the analysis of 35 samples of 14 the most common fish species obtained at the local fish market, prepared with traditional methods of cooking (boiling, poaching, simmering, frying and grilling over coal) show that Hg concentration in most the of cooked fish samples remains unchanged.

To diminish Hg concentration in fish was possible by complexation of Hg^{2+} ions with organic acids of vegetable brines or lemon juice. Direct boiling of Snoek, Slinger and Cape Hake in sauerkraut brine (pH=3.5-3.8) for 20-30 min reduced Hg concentration in these lean fishes up to 11.7%. The boiling of Cape hake and Soldier fish in lemon juice (pH=2.7-2.9) reduced Hg concentration on 15.7 and 26.3% respectively. Such changes of Hg concentration were found to be statistically significant.

Some noticeable changes of Hg concentration have been observed for Sole (15.6%) and Yellowtail (19.1%), after simmering in oil, and for Yellowtail fishes (23.0, 16.3 and 26.5%), grilled over a coal fire. Such a change of Hg concentration has been observed only for fish species rich with fat.

From the results of this studies follows, that some domestic cooking procedures could be applied for the moderate reducing of Hg in fish.

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