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Total mercury and arsenic concentrations in edible and non-edible tissues of Iranian tuna fish

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RESEARCH ARTICLE

Abstract

The concentration of total mercury (Hg) and arsenic (As) has been measured in edible and non-edible tissues (flesh, viscera, gill, skin, fin and tail) of two species of tuna fish including 15 yellowfin (*Thunnus albacares*) and 15 skipjack (*Katsuwonus pelamis*). They were fished from Chabahar port, the Oman Sea, Iran, in April-May 2011. Atomic absorption spectrometer equipped with cold vapour and hydride generation system was used to determine total concentrations of Hg and As after microwave digestion. Based on the results, the highest and the lowest Hg contents (wet weight basis) in yellowfin and skipjack were observed in viscera (1.29±0.06 and 1.15±0.04 μg/g), and fin and tail (0.48±0.04 and 0.30±0.08 μg/g), respectively. Furthermore, the highest level of As accumulation in yellowfin and skipjack was found in skin (1.99±0.35 and 2.17±0.20 μg/g, respectively), while the lowest was measured in fin and tail (0.19±0.04 and 0.23±0.03 μg/g, respectively). Most yellowfin samples had significantly more heavy metals than skipjack samples ($P<0.05$). Total Hg and As levels of the flesh of fish were found to be lower than the maximum permissible limit set by the European commission and the United States Environmental Protection Agency. Therefore, the complete separation of edible and non-edible tissues during the process of canning tuna fish is critical.

Keywords: total mercury, total arsenic, edible and non-edible tissues, Iranian tuna fish, atomic absorption spectrometer

1. Introduction

Fish consumption as a healthful source of essential amino acids, minerals and valuable poly-unsaturated fatty acid (omega-3) is very popular among the majority of the people all over the world (Ikem and Egiebor, 2005; Tuzen and Soylak, 2007; Ruelas-Inzunza *et al.*, 2011). However, the advantages of the consumption of marine products may decrease when fish are exposed to chemicals such as heavy metals in polluted water (Burger and Gochfeld, 2004; Tuzen *et al.*, 2009). Mercury (Hg) and arsenic (As) as toxic heavy metals are very harmful and they are not biodegradable in marine environments. They may accumulate in big fish such as swordfish and different types of tuna fish such as yellowfin and skipjack through direct absorption or aquatic food chain and can then be transferred to human beings by the process of consumption. Consequently, in the long run, they may cause chronic or acute disorders (Kojadinovic *et al.*, 2007; Yilmaz *et al.*, 2010). Mercury, as a hazardous toxicant, mostly effects on the nervous system and is particularly present as a very toxic and absorbable form, methyl mercury, in fish (Saei-Dehkordi *et al.*, 2010; Tuzen *et al.*, 2009). Chronic exposure to As can lead to dermatitis, mild pigmentation keratosis of skin and lung cancer (Ikem and Egiebor, 2005). Different metal accumulation levels in various fish tissues, including flesh, viscera, gill, skin and liver, have been reported in several studies by researchers (Adam, 2004; Agah *et al.*, 2009, 2010; Atta *et al.*, 1993; Berg *et al.*, 2000; Mendil *et al.*, 2005; Saei-Dehkordi *et al.*, 2010; Tuzen, 2003, 2009; Uluozlu *et al.*, 2007; Yilmaz*et al.*, 2010). These levels are attributed with some factors such as metal bioavailability, season of sampling, size, age, sex, fish species and changes in tissue constituent (Ganjavi *et al.*, 2010; El-Moselhy, 2006; Tuzen and Soylak, 2007).

Thus far, some studies have focused on Hg and As levels in different fish tissues from the Persian Gulf (Agah *et al.*, 2009, 2010; Mortazavi and Sharifian.,2011; Saei-Dehkordi *et al.*, 2010) but such records on tuna fish species from the Oman Sea as one of the most important regions in fishery and oil trafficking, are very scarce. So the objectives of this study were to: (1) measure total Hg and As concentrations in edible and non-edible tissues (flesh, viscera, gill, skin, tail and fin) of yellowfin and skipjack which are commercially used in tuna fish canning industry; (2) evaluate which tissues have the higher heavy metals concentrations; and (3) compare the heavy metal contents with respect to maximum permissible level which has been set by the authentic international agencies or organisations.

2. Materials and methods

Sample collection

In this study, 30 samples of two species of tuna fish including skipjack (*Katsuwonus pelamis*) (n=15) and yellowfin *(Thunnus albacares*) (n=15) were collected out of the waters of southeast of Iran (25°17'28" N, 60°38'15" E), Chabahar port, the Oman sea in April-May 2011. Sampling (10±0.01 g) from different tissues including edible tissue as flesh and non-edible tissues as viscera, gill, skin, fin and tail was performed randomly during the butchery step in a canned tuna fish producing factory (Ganjavi *et al.*, 2010). All of the samples were frozen at -22 °C and were kept at this temperature for 24 h. Then, they were brought to the laboratory under controlled conditions (-18 °C) and were preserved as frozen until the beginning of the analytical work (-22 °C).

Reagents

All reagents used were of analytical grades. For preventing contamination, all glassware, containers and laboratory utensils were washed by detergent, soaked in nitric acid (10%) for 24 h and rinsed by deionised water before analysis (Saei-Dehkordi *et al.*, 2010; Tuzen and Soylak, 2007). Deionized water (Milli-Q Millipour 18.2 MΩ/cm resistivity) was used for all dilutions. Standard stock solutions of $Hg(No_3)_2$ and $AsCl_3$ (1000 mg metal/ml) were prepared by diluting the required amount of the prior standard solutions (Merck, Darmstadt, Germany) for calibration of atomic absorption spectrometer.

Digestion procedure

Before microwave digestion, each sample was defrosted by keeping it at room temperature (25 °C) over night. After that, each tissue was homogenised, using a stainless steel hand grinder. For sample digestion, 0.50±0.01 g of wet sample, 1 ml H_2O_2 (30%) (Suprapur; Merck) and 7 ml of concentrated HNO_3 (65%) (Suprapur; Merck) were added to a Teflon vial and put in the microwave system (Ethos one; MileStone Srl, Sorisole, Italy). The digestion was carried out in two periods of time, 10 min and 20 min for 500 W, 200 °C (Berg *et al.*, 2000; Uysal *et al.*, 2008). A blank digestion was carried out in the same way. The digested sample in each Teflon vial was transferred into a volumetric flask followed by addition of 3 ml H_3PO_4 as modifier and filled up to 25 ml by deionised water. This solution sonicated with ultrasonic bath (UR1; Retsch, Haan, Germany) (Ganjavi *et al.*, 2010) for 30 min and then filtered by filter paper to obtain a clear solution.

Determination of total mercury and total arsenic concentration

Measurements of total Hg and As was carried out by an atomic absorption spectrometer equipped with cold vapour (Varian Spectra AA-220-VGA; Varian, Melbourne, Australia) and hydride generation system (PerkinElmer AA-4100; PerkinElmer, Waltham, MA, USA) which adjusted on 253.7 and 193.7 nm, respectively. High purity argon was used as inert gas. The measurement was carried out in triplicate. The limit of detection for Hg and As were 4 and 0.7 ng/g (wet weight), which is the concentration of six times the standard deviation of the blanks (Storelli *et al.*,2010). The percentages of recovery (Table 1) demonstrate the method accuracy and are determined by means of spiking different amounts of Hg and As into samples (Ganjavi *et al.*, 2010).

Moisture analysis

Moisture content of the samples was determined according to the AOAC official method 950.46.B (convection, gravity method; AOAC, 1995). The mass of each sample was

Table 1. Recoveries corresponding to mercury and arsenic by means of spiking different contents of elements into the samples.

Mercury content (µg/l)	Mercury spiked (µg/l)	Recovery (%)	Arsenic content (µg/l)	Arsenic spiked (µg/l)	Recovery (%)
20.63	25	82.75	21.30	25	93.07
	50	90.20		50	86.27
	100	83.14		100	96.70

measured (2±0.01 g) before and after drying overnight in an oven that was maintained at 105 °C (Rasmussen and Morrissey, 2008). In this study, total Hg and As are presented as μg/g wet weight and dry weight.

Statistical analysis

Statistical program SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for data processing. Analysing and comparing differences between mean values ($P<0.05$) was performed by ANOVA, Duncan's new multiple range test and paired sample t-test, respectively.

3. Results and discussion

Mercury concentrations in different tissues

The mean total Hg levels in the edible tissue (flesh) and non-edible tissues (viscera, gill, skin, fin and tail) in yellowfin and skipjack are shown in Table 2. It seems that the accumulation of Hg (μg/g wet weight) slightly differs in edible and non-edible tissues of yellowfin and skipjack. Based on records, the accumulation in each part depends considerably on the accumulation capacity of the tissue (Stancheva *et al.*, 2013). The Hg concentration (μg/g wet weight) in different parts is as follows: viscera > flesh > gill ≥ skin > fin and tail. As per results, the Hg level in viscera was significantly ($P<0.05$) higher than in flesh, skin, and gill, and much higher than in fin and tail. In addition, according to Hg contents (μg/g dry weight) viscera and gill are the most contaminated tissues, which accumulate Hg more than other parts. It could be due to the important role of fish viscera, especially the liver, in the detoxification and accumulation of heavy metals rather than flesh and the other fish tissues (Moslehy, 2006; Yilmaz *et al.*, 2010).

This may also be due because of lipophilic property of Hg (especially methyl mercury chloride) which has a high affinity for accumulating in fat tissues such as liver (Ramos, 2012). As a point of permanent consumption, Hg may damage brain, kidneys and developing foetus (Stancheva *et al.*, 2013). The reported results from other surveys on different fish species taken from various places such as seas, lakes and rivers are mostly in agreement with this study. Atta *et al.* (1993) have reported that the viscera contained more lead (Pb), cadmium (Cd), zinc and copper than flesh and head of *Tilapia nilotica* which have been collected from Egypt. Considering the fact that studies related to Hg accumulation in viscera is not very accessible, comparison with literature values has been done by the observed results from records on Hg accumulation in liver. Although the Hg level in viscera was higher than in most of the reported articles on the contamination level of liver in various fish, especially those reported by Berg *et al.* (2000) (0.03-0.91 μg/g wet weight) and El-Moselhy (2006) (0.05-0.30 μg/g wet weight for male and 0.03-0.09 μg/g wet weight for female), it was in agreement with the highest level of Hg contamination in fish liver from the Persian gulf reported by Agah *et al.* (2010) (0.02-1.30 μg/g wet weight). So according to the literature, viscera (especially liver) can be considered as an important tissue in monitoring Hg contents of fish. It may mark the critical role of the butchery step in complete and accurate removal of non-edible tissues from edible tissues. The contents of Hg in gill in other studies, were in the range of 0.004-0.001 μg/g for male and 0.002-0.006 μg/g wet weight for female (El-Moselhy, 2006). According to dry weight results in the present study, it seems that the effect of moisture is considerable in edible and non-edible tissues. Based on dry weight results, the highest total Hg concentrations have been measured in gill and viscera of other aquatic organism such as hard clam (*Meretrix lusoria*; Tzong-Shean and Hon-Cheng, 1993) and edible crab (*Cancer* pagurus; Andersena and Depledgeb, 1997). As shown in Table 2, these results are in agreement with the present study.

Table 2. Total mercury (Hg) concentrations (μg/g, wet and dry weight) in edible (flesh) and non-edible tissues (viscera, gill, skin, fin and tail) of tuna fish taken from the Oman Sea (mean ± SD, n=15).

Tissues	Hg (μg/g wet weight basis)		Hg (μg/g dry weight basis)	
	Yellow fin	Skipjack	Yellowfin	Skipjack
Flesh	0.84±0.08^{bA}	0.69±0.03^{bB}	3.00±0.15^{bA}	2.56±0.01^{cA}
Skin	0.63±0.02^{cA}	0.60±0.02^{bA}	1.91±0.22^{cA}	1.27±0.12^{cB}
Viscera	1.29±0.06^{aA}	1.15±0.04^{aA}	4.96±0.01^{aA}	5.75±0.05^{aB}
Gill	0.73±0.19^{bA}	0.43±0.12^{cB}	5.21±0.25^{aA}	3.07±0.09^{bB}
Fin and tail	0.48±0.04^{cA}	0.30±0.08^{dA}	2.08±0.12^{cA}	0.68±0.11^{dB}

Means within the same column followed by different superscript lower case letter differ significantly ($P<0.05$); means within the same row (wet weight or dry weight) followed by different superscript capital letter differ significantly ($P<0.05$).

Arsenic concentrations in different tissues

The results of the mean total As concentrations in different parts of yellowfin and skipjack are given in Table 3. By considering the highest to the lowest As contents (µg/g wet weight) in edible and non-edible tissues, different parts are ranked as follows: skin> gill > viscera > flesh > fin and tail. In yellowfin, no significant difference was observed among As concentrations in gill, viscera and flesh ($P>0.05$). The results of the present study demonstrate that skin and also gill are two main organs of As accumulation.

There are limited data about the As content in tuna fish from the Oman Sea region, in the literature. The present results are in disagreement with a survey done by Yilmaz *et al.* (2010) on three fish species (*Triglia lucerna*, *Lophius budegassa* and *Solea lascaris*), which had been collected in Iskenderun Bay, Turkey. The announced results showed that As contents were lower in skin tissue (0.76-0.87 µg/g wet weight) than in liver (1.01-1.98 µg/g wet weight) and muscle (0.98-1.74 µg/g wet weight). Also, in another study on monkfish (*Lophius piscatorius*), black scabbard (*Aphanopus carbo*), blue ling (*Molva dypterygia*), blue whiting (*Micromesistius poutassou*) and hake (*Merluccius merluccius*), which had been taken from Rockall Trough west of Scotland, As was found more in muscle organs such as flesh and heart than in liver and gill tissues. Their records demonstrated that As in the muscle tissues ranged from 1.25 to 8.63 µg/g and in liver tissues from 3.04 to 5.72 µg/g wet weight (Mormede and Davies, 2001). The types of the fish, level of heavy metal pollution in water and several other factors have an important role in the bioaccumulation. As is mostly water-soluble, so in the present study, the reason of presenting high levels of As in skin may be because of its direct contact with surrounding water.

Based on the evaluation of As concentrations as µg/g dry weight, skin and gill accumulate more than other fish tissues and the comparison of the results based on dry and wet weight show that the moisture content has more effect than fish species on heavy metal levels in non-edible tissues. The results from one study determining the total amount of As in five tissues (gills, mouthpiece, intestine, liver and muscles) of 10 fish species caught from As contaminated Manchar Lake, Sindh Pakistan, have shown differences between mentioned parts. The As concentrations ranges were as follows: gills (1.01-10.4 µg/g on dried weight basis), intestine (1.01-11.2 µg/g on dried weight basis), liver (3.51-10.9 µg/g on dried weight basis) and in muscles (2.12-15.2 µg/g on dried weight basis) (Shah *et al.*, 2009).

As presented in Table 4, by comparing total Hg and As levels in flesh as edible tissue in this research with results of other studies, obviously it can be noted that the heavy metal concentrations vary in tuna specious due to the different factors such as their size and their habitats (Ganjavi *et al.*, 2010).

In this study, the Hg contents (wet weight basis) were found to be lower than the maximum permissible limit set by the European Communities (EC) and US Food and Drug Administration (1 µg/g wet weight) (EC, 2001; Ikem and Egiebor, 2005). On the other hand, these results exceeded the guidelines for Hg concentrations that have been set by the World Health Organization and the Japanese Ministry of Health (0.5 and 0.4 µg/g wet weight), respectively. In Japan, because of very high fish consumption ratio, the government has recommended that fish with Hg level of ≥0.3 (µg/g wet weight) should not be sold to consumers (Ikem and Egiebor, 2005). Besides, the United States Environmental Protection Agency has reported 1.3 µg/g of wet weight as maximum allowable level for As in fish (Sai-Dehkordi *et al.*, 2010). So, based on the results from the concerned samples and by considering low annual per capita fish consumption in Iran (7.62 kg in 2009) (Sai-Dehkordi *et al.*, 2010), tuna fish flesh consumption containing 0.69-0.84 Hg (µg/g wet weight) and 0.34-0.53 As (µg/g wet weight) does not pose a threat for Iranian people.

Table 3. Total arsenic (As) concentrations (µg/g, wet and dry weight) in edible (flesh) and non-edible tissues (viscera, gill, skin, fin and tail) of tuna fish taken from the Oman Sea (mean ± standard deviation, n=15).

Tissues	As (µg/g wet weight basis)		As (µg/g dry weight basis)	
	Yellow fin	Skipjack	Yellowfin	Skipjack
Flesh	0.53±0.05^{bA}	0.34±0.06^{dB}	1.88±0.01^{cA}	1.26±0.04^{cB}
Skin	1.99±0.35^{aA}	2.17±0.20^{aB}	6.03±0.04^{aA}	4.61±0.08^{aB}
Viscera	0.56±0.17^{bA}	0.63±0.06^{cA}	2.15±0.33^{cA}	3.15±0.06^{bB}
Gill	0.66±0.05^{bA}	1.10±0.13^{bB}	4.71±0.11^{bA}	4.58±0.02^{aA}
Fin and tail	0.19±0.04^{cA}	0.23±0.03^{dA}	0.82±0.09^{dA}	0.52±0.05^{dB}

Means within the same column followed by different superscript lower case letter differ significantly ($P<0.05$); means within the same row (wet weight or dry weight) followed by different superscript capital letter differ significantly ($P<0.05$).

Table 4. Comparison of total mercury (Hg) and arsenic (As) levels (µg/g wet weight) in tuna fish flesh as edible tissue in the present study with results of other studies.

Tuna fish species/origin	Mean of total Hg (µg/g)	Mean of total As (µg/g)	Reference
Blackfin tuna/Atlantic Ocean	1.07	–	Adams (2004)
Bluefin tuna/Mediterranean Sea	1.02	–	Storelli and Marcotrigiano (2001)
Little tunny/Atlantic Ocean	0.94	–	Adams (2004)
Yellowfin tuna/Oman Sea	0.84	0.53	Present study
Skipjack tuna/Oman Sea	0.69	0.34	Present study
Bluefin tuna/Tyrrhenian Sea	0.61	–	Storelli *et al.* (2010)
Atlantic bluefin tuna/Catalonia, Spanisch coast	0.48	1.12	Falcó *et al.* (2006)
Yellowfin tuna/Reunion Island, Western Indian Ocean	0.30	–	Kojadinovic *et al.* (2007)
Yellowfin tuna/Atlantic Ocean	0.25	–	Adams (2004)
Skipjack tuna/Reunion Island, Western Indian Ocean	0.19	–	Kojadinovic *et al.* (2007)
Albacore tuna/Pacific Ocean	0.17	–	Rasmussen (2007)
Long tail tuna/Persian Gulf	0.094	–	Mortazavi and Sharifian (2011)
Permissible level[1]			
EC, FDA	1	–	EC (2001); Ikem and Egiebor (2005)
WHO	0.5	–	Ikem and Egiebor (2005)
Japanese Ministry of Health	0.4	–	Ikem and Egiebor (2005)
EPA	–	1.3	Saei-Dehkordi *et al.* (2010)

[1] Permissible level: maximum level that has been set by authentic international organisations.

EC = European Communities; FDA = US Food and Drug Administration; EPA = United States Environmental Protection Agency; WHO = World Health Organization.

The differences of mercury and arsenic contents between tuna fish species

The statistical analysis using paired sample t-test demonstrated that the mean concentration of Hg and As in yellowfin samples were significantly higher than in skipjack samples ($P<0.05$). The obtained result could be because of the larger size of yellowfin in comparison with skipjack. The accumulation of heavy metal levels in fish depends on several factors such as catch location, pollution sources, food supply, age, size and species variation (El-Moselhy, 2006; Islam *et al.*, 2010; Rasmussen and Morrissey, 2008; Tuzen and Soylak, 2007). So yellowfin may be considered as a bioindicator for determining heavy metal accumulation in tuna fish species.

Application of non-edible tissues as animal feed

Viscera, skin, gill, fins, tail and other unusable fish body parts are considered as an important by-product during the butchery step in tuna fish canning industry. During the butchery step, all non-edible tissues that include 50-70% of the original raw material are removed from the fish flesh. They are used as fishmeal for feeding animals such as chicken (Ovissipour *et al.*, 2010; Shah *et al.*, 2010). They are a good source of proteins (58% dry matter), fat (19% dry matter which consists of highly unsaturated fatty acid) and have a high content of minerals (22% dry matter). Hence, high levels of heavy metals such as Pb, Cd as well as Hg and As in these tissues may be hazardous for animals that are feeding on them (Arvanitoyannis and Kassaveti, 2008). It has been announced that the maximum allowed Hg content of feed stuff with a moisture content of 12% is 0.5 mg/kg (EFSA, 2008) which is lower than the concentration of Hg levels (mg/kg dry weight) in different tissues especially viscera and gill in the present study. So, these fish parts are likely to pose a threat for animal feeding.

4. Conclusions

This study has been carried out to provide information on the distribution of total Hg and As in different tissues of two tuna fish species from the Oman Sea. It can be concluded that non-edible tissues contain more heavy metals than edible tissues. The accumulation order of Hg (µg/g wet weight) in different parts is as follows: viscera > flesh > gill ≥ skin > fin and tail. In addition, As levels (µg/g wet weight) in different tissues are ranked as follows: skin > gill > viscera

> flesh > fin and tail. The results present new information on the distribution of As and Hg in edible and non-edible tissues of tuna fish species from the Oman Sea region, Iran. In most samples, yellowfin contained more heavy metals than skipjack. This may be due to the bigger size of this kind of tuna fish. However, the consumption of tuna fish flesh as edible tissue when collected from the Oman Sea can be safe for human consumption, but a potential danger may emerge in the future on the Oman Sea because of oil trafficking, industrial and domestic waste waters and this suggests that regular monitoring by the authority should be implemented. Besides, the complete separation of edible and non-edible tissues (especially viscera, gill and skin) during the butchery step is critical in canned tuna fish processing factories.

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