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MERCURY AND ARSENIC CONTENT IN SEAFOOD SAMPLES FROM THE JAKARTA FISHING PORT, INDONESIA

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ABSTRACT

Mercury and arsenic are considered to be among the most toxic metals and have been associated with serious adverse health effects. These two trace metals and other contaminants that are found in fish products are therefore of public concern for food-safety reasons. Hence, we selected three marine species to study *i.e.,* yellow fin tuna, marlin and green mussels because of their economic values in the international and local markets. The objective of our study was to determine the arsenic and mercury content in these three marine species as a first step in monitoring metal content in seafood products. The tissue samples of tuna and marlin were collected from the Jakarta Fishing Port, while the green mussels was collected from aqua-culture sites in Jakarta Bay. The metal content was determined by ICP-MS and validated using CRM DORM-2 and DORM-3. The speciation of arsenic (organic and inorganic forms) was determined using HPLC-ICPMS. All measurements were based on dry weight samples. The result showed that the mercury concentration in yellow-fin tuna, marlin and green mussel samples was 0.68 ± 0.08 mg kg^{-1}, 0.56 ± 0.06 mg $kg^{-1}$and 1.51 ± 0.10 mg kg^{-1}, respectively. The total arsenic concentration in yellow-fin tuna, marlin and green mussel samples was 3.47 ± 0.21 mg kg^{-1}, 2.71 ± 0.18 mg kg^{-1}, and 6.77 ± 0.32 mg kg^{-1}, respectively. The mercury content in the fish tissue was below the maximum allowable concentration (National Standard of Indonesia 1.0 mg kg^{-1}), except for the green mussels. For total arsenic concentration, all the samples were above the national standard concentration (1.0 mg kg^{-1}). The organic arsenic species arsentobetaine (AB) found in tuna and marlin fish samples was not toxic. Inorganic and organic arsenic was found in the green mussel samples. Our results suggest that there is a need to establish a national program to regularly monitor the content of selected trace metals in fishery products.

Keywords: Mercury, arsenic, yellowfin tuna, marlin, green mussel.

INTRODUCTION

Fish and shellfish are sources of micro-nutrients and have a low saturated fatty acids content that is known to contribute to good health. However, seafood contaminated by heavy metals or metalloids such as mercury and arsenic can contribute to human health risk. Humans can be exposed directly to mercury in the form of mercury vapor (HgO), and indirectly through the inorganic mercury ion (Hg^{2+}) formed from the oxidation of vapor of mercury in the environment or through methyl mercury ($MeHg^{+}$). Methyl mercury is formed in a wet environment with the help of certain bacteria. Mercury vapor and methyl mercury is highly toxic, especially to the organs of

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the body (mercury vapor through the lungs, methyl mercury through digestive organs), and because of the lipophilic nature of the mercury it can easily be transported through the blood to the brain.

The arsenic species found in marine biota are arsenate AsO_4^{3-}, arsenite AsO_3^{3-}, mono-methyl arsenate (MMA) $CH_3AsO_3^{2-}$, dimethyl arsenate (DMA) $CH_3AsO_2^{-}$, trimethylarsin oxide (TMAO) $(CH_3)_3AsO$, tetramethyl arsonium ion (TMA^+) $(CH_3)_4As^+$, arsenocholin (AC) $(CH_3)_3As^+CH_2CH_2OH$, and arsentobetaine (AB) $(CH_3)_3As^+CH_2COO^-$. Arsensugar and arsenolipid also found in marine biota samples (Francesconi et al., 1990; Francesconi et al., 1998).

The toxicity of arsenic depends on the species, inorganic arsenic is more toxic than organic arsenic (Claudia et al., 2010). Inorganic arsenic such as As (III) is more toxic than As (V), *i.e.,* a lethal dose (LD_{50}) of As (III) = 34.5 mg kg^{-1} and LD_{50} As (V) = 41.0 mg kg^{-1}. Arsentobetaine (AB) is less toxic than other arsenic species, the LD_{50} for AB is more than 10.000 mg kg^{-1}. The risk associated with the carcinogenic effects of inorganic arsenic is expressed as the excess probability of contracting cancer over a lifetime of 70 years (Francesconi, 2010). In a sample of tuna and marlin, most of the total arsenic concentration (95%) comes from organic arsenic, arsentobetaine (AB) (Francesconi, 1985; Francesconi, 1990).

Arsenic in seafood can come from volcanic dusts, agricultural pesticides and the use of preservatives in wood and the textile industry. Marine organisms allegedly have an arsenic absorption rate that is relatively high so that the consumption of marine biota such as tuna and mussels can be dangerous for humans (Dang Q. Hung et al., 2004; Chen-Wuing Liu et al., 2006; Ching-Ping Liang et al., 2011).

Hence, the objective of our study is to determine the arsenic and mercury content in three marine species (i.e., tuna, marlin and green mussels) as a first step in monitoring the metal content in seafood products that are landed in Jakarta.

MATERIALS AND METHODS

Mercury and arsenic concentration

Sample Collection. Yellow-fin tuna and marlin were collected from the Jakarta Fishing Port. The green mussels were collected from farmers who cultured the mussels around Jakarta Bay. The samples of muscle tissue were minced, homogenized, and freeze-dried for 24 hours. Liquid nitrogen was added to the dried samples, and ground using an agate mortar. The powdered samples were sieved (100 mesh) then packed in dark glass bottles in 3 g lots.

Standard and reagent. This research used standard equipment routinely used in chemical testing laboratories, high-precision measuring equipment, grade A flasks and pipettes. Weighing was carried out using an analytical balance with an accuracy of 0.1 mg. The pH meter used was a desktop type, Orion model 420A. Acid digestion was conducted using an Ultraclave III with a temperature 400 ºC and a pressure of 60 bar. Measurement was conducted using ICPMS Agilent 7500 series. All the solvent used was AquaMillipore. The chemicals used in this research were of high purity from E-Merck, such as HNO_3, As(III), As(V), MMA, DMA, AC, TMAO and TETRA standard stock of 1000 ppm standard. CRM DORM-2 from the National Research Council Canada (NRCC, 1993), and AB from Institute for Chemie, Karl-Franszen University, Graz, Austria.

Instrumentation. ICPMS measurements were performed with an Agilent 7500ce (Agilent Technologies, Waldbronn, Germany). The ICPMS was equipped with a Burgener Ari Mist HP nebulizer (Burgener Re-search Inc, Mississauga, Canada) and a Scott double pass spray chamber. The HPLC was equipped with a binary pump, a vacuum degasser, column oven, and an autosampler with a variable 100 µL injection loop; it was connected to the ICPMS with 0.125 mm PEEK (polyether-etherketone) tubing (Upchurch Scientific, Oak Harbour, USA). The signal from the ICP-MS, measured at m/z 75 and m/z 77 using a dwell time of 300 ms, was evaluated using Chromatographic

Table 1. Mercury (Hg) and total Arsenic (As) concentration (average ± sd, mg kg^{-1}) in dry weight. n = 6 samples.

Tissues	Mercury	Total Arsenic
Tuna	0.68 ± 0.08	3.47 ± 0.21
Marlin	0.56 ± 0.06	2.71 ± 0.18
Green mussels	1.51 ± 0.10	6.77 ± 0.32
Indonesia/National Standard	1.00	1.00

Data Software Version 1.00 c. G1824C (Agilent, Waldbronn, Germany). Microwave digestions and extractions were performed with an Ultraclave III (MLS GmbH, Leutkirch, Germany). The samples were freeze-dried in a Christ Gamma I-16 freeze-dryer (GmbH, Oserode, Germany).

Determination of mercury and total arsenic. Each sample of fish and shellfish was analyzed in triplicate in the following manner. A portion of the powdered samples (about 250 mg weighed with a precision of 0.1 mg) was weighed directly into 12 mL quartz tubes, and nitric acid (2 mL) and water (2 mL) were added. The tubes were transferred to a Teflon rack in the Ultraclave microwave system and covered with Teflon caps. After closing the system, an argon\pressure of $4x10^6$ Pa was applied and the mixture was heated to 250 ºC for 30 min before being allowed to cool to room temperature. After mineralization, the samples were transferred to 15 mL polypropylene tubes (Greiner, Bio-one, Frickenhausen, Germany) and diluted with water to 10 g (based on mass). Finally 1 mL of a solution containing 50% methanol (to enhance the arsenic response) and 100 g L^{-1}each of Ge and In as internal standards were added to all the digested samples giving a final concentration of 5% methanol and 10 g $L^{-1}$of Ge and In. All standards for total arsenic determinations were prepared with 20% nitric acid and also 5% methanol for matrix matching with the digested samples. The arsenic concentrations in the digests were determined by ICPMS using helium as collision cell gas for removing polyatomic interferences from argon chloride ($^{40}Ar^{35}Cl$ on ^{75}As). This procedure was developed from previous researchers (Rodriguez et al., 2009; Raber et al., 2012; Koesmawati et al., 2013; Koesmawati et al., 2013). The method was validated against the certified reference material DORM-2 which has a certified mercury content of 4.64±0.26 g Hg kg^{-1} and arsenic content of 18.00± 1.10 g As kg^{-1}; we obtained 4.60±0.28 g Hg kg^{-1} and 17.70 ± 0.08 g As kg^{-1} (n = 3) in this study.

RESULT

Mercury and arsenic standard solution was prepared from 0 to 200 ng g^{-1}. From the calibration curve, we found the correlation coefficients (r) for mercury and arsenic were 0.9995, and 1, respectively. The line equation was y=7.764x+32.30 and y=181x, respectively. Both calibration curves were used to measure the sample concentrations. The concentrations of mercury (Hg) and total arsenic (As) are presented in Table 1. Concentrations of mercury in tuna and marlin were below the maximum allowable concentration in the national standard, but the total concentration of total arsenic was twice to triple the standard recommended concentration. In contrast, Hg and total As in the green mussel's tissue were both above the allowable concentration for consumption.

Arsenic speciation

Based on the analysis of total arsenic which was on average about three times the maximum allowable recommended concentration under the Republic of Indonesia regulations, we carried out the speciation analysis of arsenic. This speciation analysis was conducted in order to find out the potential risk associated with the carcinogenic effects of inorganic arsenic which is expressed as the excess probability

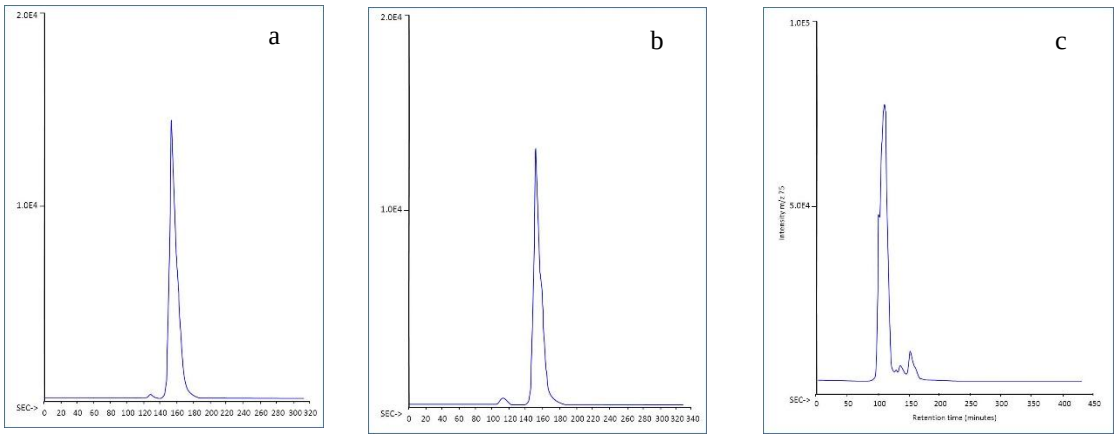

Fig 1. HPLC-ICPMS chromatogram for AB Standard (a), Tuna and Marline fish samples (b), and Green Mussel (c), using cationic exchange column Chrompack Ionosphere 5c, with mobile phase Pyridine 10 mM at pH 2.3 and flow rate of 1.0 mL min^{-1}

Table 2. HPLC-ICPMS operation

	Anion exchange column	**Cation exchange column**
Column type	Hamilton PRPX-100, 150x4.6 mm	Chrompack–Ionos-phere 5C, 100x3 mm
Temp.	40 °C	40 °C
Mobile phase	10 mM Malonic Acid pH 5.6	10 mM Pyridine pH 2.3
Flowrate	1.2 ml/min	1.5 ml/min
Injection	10 µl	10 µl
Detector	ICP-MS	ICP-MS
Standard	1,5,10,20 ppb	1,5,10,20 ppb

of contracting cancer over a lifetime of 70 years. The Arsenic speciation was conducted using HPLC-ICPMS. The instruments are presented in Table 2.

Retention time and resolution results from the speciation of arsenic species standards are shown in Table 3 and 4. The separation of arsenic species was carried out using HPLC-ICP-MS. The A Hamilton PRPX-100 anion exchange column was used with mobile phase malonic acid 10 mM at pH 5.6, and a flow rate of 1.2 mL min^{-1}. Four arsenic species was separated As(III), MMA, DMA, and As(V), the retention times were 2.5 ; 3.2 ; 3.45 and 5.3 minutes and are listed in Table 3. Using a Chrompack Ionosphere 5c cation exchange column, with mobile phase Pyridine 10 mM at pH 2.3 and a flow rate of 1.5 mL min^{-1}, four arsenic species, namely AB, AC, TMAO, and TETRA were separated nicely.

The retention times of 1.50, 2.90, 4.50 and 5.30 are listed in Table 4. The result of chromatograms of tuna fish and green mussels is presented in Figure 1.

DISCUSSION

Marine organisms, in general, accumulate contaminants from the environment and therefore have been widely used in marine pollution monitoring programs (Agusa et al. 2005; Fernandes et al. 2006). In many developing countries, industrial wastes, agricultural activities and the mining of metals, create a potential source of heavy metal pollution in aquatic environments.

Table 3. Arsenic speciation result using anion column

Arsenic species	Retention time (minutes)	Resolution
As(III)	2.50	4.3
DMA	3.20	8.0
MA	3.45	9.5
As(V)	5.30	11.5

Table 4. Arsenic speciation result using cation column

Arsenic species	Retention time (minutes)	Resolution
AB	1.50	3.8
TMAO	2.90	10.0
AC	4.50	12.0
TETRA	5.70	18.0

Due to their toxicity and tendency to accumulate, as well as the deleterious effect of metals (Hg and As) on marine ecosystems and human health, it is necessary to monitor their bioaccumulation in key species such as mussels and predator fishes.

Mercury concentrations in yellowfin tuna, marlin and green mussel samples were 0.68 ± 0.08 mg kg^{-1}, 0.56 ± 0.06 mg kg^{-1} and 1.51 ± 0.10 mg kg^{-1}, respectively. Mercury concentrations in both the tuna and marlin were below the maximum allowable concentration of total arsenic and mercury in fish, 1.0 mg kg^{-1} (SNI, 2009). In contrast mercury in the green mussels was above the maximum concentration. The high concentration of mercury in mussels can be attributed to their feeding behavior as sedentary filter-feeding biota in a contaminated coastal ecosystem, while yellowfin tuna and marlin are both migratory carnivorous species, living in the open ocean.

Previous studies showed that the mercury concentration in water and suspended particles were relatively high, 0.83 - 1.23 µg kg^{-1}, in Jakarta Bay (Arifin and Firtiati, 2006), while the mercury content in green mussels was 0.005 – 0.020 mg kg^{-1} on a dry weight basis (Arifin, 2008). The mercury concentration in green mussels has increased almost ten times in comparison to a previous result in 2008. Hence, we suggest that there is a need to carry out a study on the potential risks of green mussel consumption especially for low income communities who live around the Jakarta, Banten and West Java provinces.

The total arsenic in yellow-fin tuna, marlin and green mussel samples was 3.47 ± 0.21 mg kg^{-1}, 2.71 ± 0.18 mg kg^{-1}, and 6.77 ± 0.32 mg kg^{-1}, respectively. Marlin and tuna tissues also had total arsenic concentrations higher than 1.0 mg kg^{-1}, but the total arsenic content in the fish came from arsenobetaine, AB (95%) which was non-toxic. Therefore, the arsenic content in fish tissues was not a major concern in these two species, tuna and marlin. In contrast, the green mussels contained both organic and inorganic arsenic that potentially poses a risk to human health.

The determination of total arsenic is complicated by a number of factors in respect to these biological materials. The AB molecule does not decompose easily and can remain intact in situations where most other organic molecules have decomposed (Entwisle et al., 2006). HPLC-ICP-MS measurement method is a robust and sensitive technique, suitable for aqueous samples and for aqueous extracts of environmental and biological samples. This technique enabled us to detect all arsenic species with an essentially uniform response which greatly

Table 5. Calibration curve for arsenic species using anion column. y = area and x = concentration (ng g^{-1})

	AsO_3^{3-}	DMA	MA	AsO_3^{3-}
Equation	y=66053x +5910.2	y= 65547x +7124.2	y=74137x +5679	y=69323x -19087
Correlation coef.	0.9993	0.9998	0.9996	0.9996

Table 6. Calibration curve for arsenic species using cation column. y = area and x = concentration (ng g^{-1})

	AB	TMAO	AC	TETRA
Equation	y=46983x +5156	y= 43262x +9171	y=44306x +14480	y=45325x +2958
Correlation coef.	0.9999	0.9998	0.9999	0.9997

facilitates quantification of the various species. Spectral interference (e.g. 40Ar35Cl) can occur but this can be readily overcome by chromatography, or by the use of reaction/collision cell technology or high resolution mass analysers. The technique is generally restricted to mobile phases with low organic content and hence has not yet been applied to the determination of non-polar arsenic species (e.g. arsenolipids), assignment of arsenicals is by chromatographic comparison with standards (retention time matching), and thus the method depends on the availability of standard compounds, provides no structural information and hence is greatly restricted in its ability to identify novel arsenic compounds (Larsen et. al., 1993; Francesconi and Kuehnelt, 2004). The availability of synthetic arsenobetaine made it possible to identify this compound by comparing the chromatographic properties of the natural and the synthetic materials (Leemarker et al., 2006).

All calibration curves had a linear correlation for concentration 0-20 ng g^{-1} (Table 5 and 6). Chromatograms of tuna and marlin fish samples were identical with the AB standard (Figure 1a,b). However, the green mussels had an inorganic arsenic content (DMA) and other arsenic species (Figure 1c). Inorganic arsenic, although a minor component of the total arsenic content of each marine animal when compared to arsenobetaine, presents a larger potential toxicity problem. Although toxicity of DMA is less than As (III), we should consider the risk of toxicity to consumers (Edmond et al., 1993; Peshut et al., 2008; Mc Intyre et al., 2011).

CONCLUSION

The mercury content in the fish (tuna and marlin tissues) was below the maximum allowable concentration (National Standard of Indonesia: 1.0 mg kg^{-1}), but was not in the green mussels. The total arsenic content in all samples was higher than the maximum allowable concentration. It was between 2.5 to 6.0 times higher than the national standard. The organic arsenic species arsenobetaine (AB), which is not toxic, was found in the tuna and marlin fish samples. Inorganic and other arsenic species were found in the green mussel samples. The regular monitoring of trace metal content in marine biota is necessary for human health purposes.

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