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Determination of Total Arsenic in Indonesian Tuna Fish Sample

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ABSTRACT

Indonesia contributed about 4 percent of total global fresh and frozen tuna exports, exporting about 65.5 tones in 2007, valued at US$ 150 million. The determination of total arsenic fish and fish product has not been standardized in Indonesia. To get an accurate result of total arsenic measurement in tuna fish is very difficult, it is due to the relatively low concentration of total arsenic in the sample and the characteristic of organic arsenic species (arsenobetain). Arsenobetaine in particular, are not decomposed to arsenate, which can lead to serious error in quantification when a hydride generator step is additionally used in the analysis scheme. This research covers the performance evaluation of three methods of sample preparation. Determination of total arsenic concentration is done by using hydride generator quartz furnace atomic absorption spectrophotometry (HG-QF-AAS) and ICP-MS, and being validated using CRM DORM-2 and DORM-3. The sample preparation and measurement method obtained were expected to be used as an alternative method for determination of total arsenic in tuna fish sample.

Abbreviations: HG-QF-AAS: hydride generator quartz cell flame atomic absorption spectrophotometer, ICP-MS: inductively coupled plasma mass spectrophotometer.

INTRODUCTION

Indonesia is the biggest tuna-producer country in the world, with a 15 percent contribution of global production in 2009, followed by the Philippines, China, Japan, Korea, Taiwan, and Spain. The main commercially caught tuna species in Indonesia are skipjack (62% of total tuna landings), yellowfin (29%), bigeye (7%), albarcore (1%), and Southern bluefin (1%).

The fishing grounds for Indonesian tuna fall under two convention areas, Indian Ocean and Western Central Pacific Ocean (WCPO). The Western Central Pacific Ocean currently supports the largest industrial tuna fishery in Indonesia, contributing almost 80 percent of the total Indonesian commercial tuna production, while Eastern Indian Ocean contributes 20 percent (FAO, 2010).

Tuna products are the second biggest Indonesian fishery product export, after shrimp, contributing 14 percent of total export value, about USD 352 million, in 2009.

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The main markets for tuna exported from Indonesia are Japan (35%), the United States (20%), Thailand (12%), European Union countries (9%), and Saudi Arabia (6%) (MMAF, 2010). Tuna usually includes all of the large tunas (*Thunnus* spp.- yellowfin, bigeye, SBT, and albacore), and the tuna-like species (marlins, sailfish, swordfish). Skipjack tuna are usually reported as a separate group named “cakalang”. “Tongkol” generally includes eastern little tuna (*Euthynus* spp.), the frigate and bullet tunas (*Auxis* spp.), and longtail tuna (*Thunnus* tonggol). “Tenggiri” includes the larger species of mackerel (*Scomberomorus* spp.)- narrow barred mackerel and Indo-Pacific king mackerel. In this research the candidate reference material RM was prepared using tuna fish (*Thunnus* spp.- yellowfin). The National Standardization Agency of Indonesia (BSN) had published an Indonesian National Standard (SNI) Handbook for testing fish and fishery products: Microbiology and chemical testing method in 2007. The SNI handbook comprises of 5 SNIs related to microbiology testing including the method used to detect, isolate and confirm coliform, escherichia coli, salmonella, vibrio parahaemolyticus, vibrio cholerae bacteria as well as to define the total of aerob and an-aerob microorganism found in fishery product; and 7 SNIs related to chemical test which includes determination of ashes, water, fat and

protein contents as well as heavy metals content such as Pb, Hg, and Cd on fishery product. However, the determination of total arsenic and arsenic species in major fish and fish product has not been standardized in Indonesia, due to availability of reference material and sample preparation method. Arsenic species which is found in marine biota are : arsenate AsO_4^{3-} , arsenite AsO_3^{3-}, monomethyl arsenate (MMA) $CH_3AsO_3^{2-}$, dimethyl arsenate (DMA) $CH_3AsO_2^{-}$, trimethylarsin oxide (TMAO) $(CH_3)_3AsO$, tetramethyl arsonium ion (TMA^+) $(CH_3)_4As^+$, arsenocholine (AC) $(CH_3)_3As^+CH_2CH_2OH$, and arsenobetaine (AB) $(CH_3)_3As^+CH_2COO^-$. Arsensugar and arsenolipid also found in marine biota samples (Francesconi, *et al.* 1994; Francesconi and Edmonds, 1998; Niegel and Matysik, 2010). The toxicity of arsenic is depending on the species, inorganic arsenic is more toxic than organic arsenic (Shiomi, 1994).

Inorganic arsenic such as As(III) is more toxic than As(V), the LD_{50} As(III) = 34.5 mg kg^{-1}, and LD_{50} As(V) = 41 mg kg^{-1}. Arsenobetain (AB) is less toxic compared to other arsenic species, the LD_{50} for AB is > 10.000 mg kg^{-1}. Determination of total arsenic was strongly influenced by the properties of the species. In a sample of tuna fish, the total arsenic concentration mostly (95%) comes from organic arsenic speci, arsenobetain (AB) (Francesconi and Edmonds, 1998; Francesconi and Edmonds, 1997). Arsenic speciation analysis as well as total analysis is very important for arsenic chemistry, but very difficult. Under the above common understanding, an international comparison relating to analytical capability of arsenic and AB in fish tissue sample was carried out among the national metrology institutes in Consultative Committee for Amount of Substance-Metrology in Chemistry/the International Committee of Weights and Measures (CCQM/CIPM) under the Meter Convention in 2007. Neutron activation analysis (NAA) is one of the most useful measurement methods with multielement determination capability (Miura *et al.,* 2010). Molecule AB is very difficult to be decomposed compared to other organic species. In general, digestion using a microwave oven at low pressure and temperature will not be able to decomposed AB molecule, but at high pressure and temperatures, AB is possible to decomposed. Decomposition of AB is recommended at 250^0C and 40 bars, using microwave digestion Ultraclave III (Gomez, *et al.* 2005; Damkrogen, *et al.* 1997; Goessler and Pavkov, 2003). Due to characteristic of AB which is very difficult to decomposed, sample preparation plays an important aspect for marine biota analysis, especially fish. Dry ashing with slurry method can be used for total arsenic in organic matrix samples, but should be done very carefully to avoid contamination. The contamination will cause a fluctuation of the analysis result, so is very difficult to obtain a precise and accurate result (Francesconi and Edmonds, 1998; Gomez, *et al.* 2005; Damkrogen, *et al.* 1997; Goessler and Pavkov, 2003). Total arsenic in tuna fish is relatively found in a very low concentration of below 10 µg g^{-1}. Two major difficulties in the measurement of total arsenic in tuna fish are related to the concentration and its matrix interferences. Accuracy and precision in its measurement is mandatory for an accreditated testing laboratory, therefore the availability of a suitable reference material (RM) is necessary. RM is necessary in method development and validation, estimation of measurement uncertainty, internal quality control, proficiency testing and training (ISO Guide 30, 2006; Eurachem Guide, 1998). Both homogeneity and stability are essential in the preparation of a biological origin RM. The National Research Council Canada (NRC) developed certified reference material (CRM) DORM-2, dogfish muscle CRM for trace metals, which was replaced by CRM DORM-3, fish protein CRM for trace metals. However, in Indonesia CRM is difficult to obtain.

The objective of this study is to provide a standard method and RM which can be used as in-house reference material for quality control in the determination of total arsenic in tuna fish samples for Indonesia testing laboratories.

MATERIAL AND METHODS

Area of study

Tuna fish samples were collected from Muara Baru, Jakarta (Figure 1). The tuna muscle (*thunnus* spp) (Figure 2) was minced, homogenized, and freeze-dried for 24 hours. Liquid nitrogen was added to the dried samples, and grinded using an agate mortar. The powdered samples were sieved (100 mesh) then packed in dark glass bottles of 10 g lots.

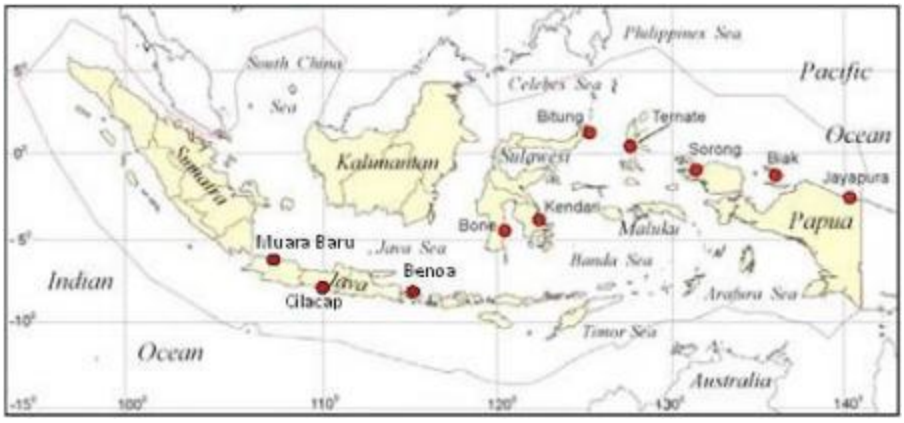

Fig. 1: Major Ports of Tuna Fisheries in Indonesia

Yellowfin tuna

marlins fish

fish meat

Fig. 2: Indonesia tuna fish.

Table. 1: Total arsenic concentration of tuna fish from different sample preparations.

Sample preparation method	HG-FAAS Cons. (µg g^{-1})	ICP-MS Cons. (µg g^{-1})
Acid digestion using Microwave Ultraclave III		3.61 ± 0.24
Acid digestion using Microwave conventional	0.09 ± 0.01	-
Dry ashing with slurry (MgO& Mg-nitrat) non-reduction	1.71 ± 0.28	-
Dry ashing with slurry (MgO& Mg-nitrat) with reduction (1)	3.08 ± 0.21	-
Dry ashing with slurry (MgO& Mg-nitrat) with reduction (2)	3.49 ± 0.08	-
Acid digestion using waterbath		3.56 ± 0.14

(1) HCl:KI:ascorbic acid mixture (5:1:1 v/v)
(2) HCl:KI:ascorbic acid mixture (5:1:2 v/v)

Tabel. 2: T-test of total arsenic measurement using ICPMS and HG-FAAS.

	ICPMS	*HG-FAAS*
Mean	3.61	3.49
Variance	0.0461	0.0405
Observations (n)	7	6
Pooled Variance	0.0435	
Hypothesized Mean Difference	0.0000	
Degrees of freedom (Df)	11	
t Stat	1.021	
P(T<=t) one-tail	0.164	
t Critical one-tail	1.795	
P(T<=t) two-tail	0.329	
t Critical two-tail	2.201	

Table. 3: Validation method results for total arsenic.

	HG-QF-AAS	ICP-MS
Concentration range	0 - 7 ng g^{-1}	0 – 100 ng g^{-1}
Linear equation	y = 0.0024 x + 0.0006	y = 293x
Correlation coefficient (r)	0.9980	0.9999
LOD (n=6; 3s)	0.15 ng g^{-1}	0.067 ng g^{-1}
LOQ (n=6; 6s)	0.23 ng g^{-1}	0.085 ng g^{-1}
RSD	4.04%	3.83%
Horwitz	13.39	13.36
Horwitz ratio (HorRat)	0.30	0.30
Recovery	86.52%	100.90%
CRM :		
DORM-2 (18.0±1.10 µg g^{-1})	17.49±1.00 µg g^{-1}	17.7 ± 0.08µg g^{-1}
DORM-3 (6.88±0.30 µg g^{-1})	(not reported)	6.77 ± 0.14µg g^{-1}

Table. 4: Results of the homogeneity test for total arsenic in the candidate reference material.

Bottle no.	ICPMS[a] Conc (µg/g)	Bottle no.	ICPMS[b] Conc. (µg/g)
2	3.36	15	3.44
23	3.50	21	3.64
11	3.42	12	3.53
14	3.58	22	3.36
13	3.86	8	3.34
3	3.93	29	3.34
17	3.61	25	3.37

ICPMS[a] : Institut fur chemie, Karl Franzens University, Graz, Austria (microwave assisted acid digestion)
ICPMS[b] : The University of Queensland, National Research Centre for Environmental Toxicology, Brisbane, Australia (water bath acid digestion)
F_{calc} and F_{crit} values were calculated results of the ANOVA.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.109829	1	0.109829	3.708635	0.078152	4.747225
Within Groups	0.355371	12	0.029614			
Total	0.4652	13				

SS = sum square, Df = degrees of freedom, MS = mean square, F = variance between treatments/variance within treatments, P-value = probability of observing a test statistic, F_{crit} = F value in F table for ANOVA (1,12) at $\alpha = 0.05$ (confidence level of 95%)

Chemical, reagents and standards

Water used throughout the investigation was Milli-Q water (18 mΩ cm^{-1}). The following chemicals, which are analytical grade were used in the study, hydrochloric acid, magnesium oxide, magnesium nitrate, sodium borohydride, sodium hydroxide, potassium iodide, ascorbic acid, and arsenic (III) standard from Merck-Germany.

Analytical procedures

The dried sample was accurately weighed (ca. 50 mg). Three different methods of preparation were conducted namely, dry ashing, microwave assisted acid digestion, and waterbath acid digestion, respectively. Dry ashing method was modified from Penrose, et.al. (Penrose, *et al.* 1975). Slurry, consisting of 3 g $Mg(NO_3)_2$ and 5.0 g MgO in 50 mL, was added to a sample of freeze dried tuna fish. A 50 mg of dried sample was weighted and mixed with 1.5 ml slurry in a crucible and dried for 15 hours at 80°C, followed by 1 hour at 200°C, 1 hour at 300°C and 8 hours at 500°C in muffle furnace. After cooling, the samples were dissolved by adding 10 ml mixture solution of 2M HCl : 15% KI : 15% ascorbic acid of 5:1:2 v/v/v, then warmed for 10 minutes at 40-50°C. The solution was put into polypropylene tube and weighted to 25 g by adding 1.5% HCl. The measurement was conducted by HG-QF-AAS [9]. A 1% sodium borohydride ($NaBH_4$) in NaOH 0.4% solution was used as the reducing agent. The reaction time was 10 seconds through quartz cell and argon gas was used as a carrier gas. A calibration curve of absorbance versus concentration of arsenic (III) was prepared by weight and the concentration range was 0.5 – 7.0 ng g^{-1} in HCl 1.5 N. Validation of method was carried out by determining the LOD, LOQ, precision. Accuracy was measured using CRM and percent recovery.

Microwave assisted acid digestion was prepared as follows, a 100 mg dried sample, 2 ml of HNO_3 and 2 ml of water was transferred into 12 ml quartz tubes. The tubes were placed in Teflon rack and covered with Teflon caps, the ranch was mounted into microwave system. The holding vessel was filled with 300 g of water and 5 g H_2SO_4, the systems was closed and loaded with argon to $4x10^5$ Pa and the mixture was heated for 30 minutes at 250°C. After mineralization, the samples were diluted to 10 g with water in polypropylene tube before analysis with ICP-MS. While water bath acid digestion was conducted by weighing a 50 mg dried sample, then put into 15 mL polypropylene tube, added 1 ml HNO_3 and heated in water bath at 70-80°C for 2 hour (Whaley-Martin, 2011). After cooled, the solution was diluted to 10g with water and diluted 5 times before measured with ICP-MS. All measurements were validated using CRM DORM-2 and DORM-3. The samples which were prepared by Ultraclave III microwave and water bath was measured using ICP-MS Agilent 7500 cs series, RF plasma power 1500W, and carrier gas 0.82 l min^{-1}.

Homogeneity test

The standard method of homogeneity testing are describes in ISO 13528 (2005), in the IUPAC Harmonized Protocol (2006) and in ISO guide 35 (2006) (ILAC ILAC Discussion Paper on Homogeneity and Stability Testing). In general, the preferred technique is to use duplicate measurements on a minimum 10 randomly chosen samples. The between sample variability is determined and compared with either a fraction of the evaluation criteria (13528), a statistical test (Guide 35), or a combination of fitness for purpose and statistical criteria (IUPAC) (ISO Guide 35, 2006).

In this study, total arsenic was assessed using different methods in two different laboratories. Twenty 10g lots were prepared for the homogeneity test. Random bottle number was applied in this analysis. Statistical analysis was carried out and the uncertainty of homogeneity for total arsenic was calculated using the one-way analysis of variance (ANOVA) (Eurachem Guide, 1998).

Stability test

Procedures for testing stability are not as well as discussed as procedures for testing homogeneity. However, the general procedure is to take a small number of samples at the end of the test period, take a measurement on each one, and compare the mean result with the average of the result determined in the homogeneity check.

In this study, the stability of total arsenic in tuna fish was evaluated over 12 months, under room temperature storage condition. Each measurement was conducted in triplicate in three different laboratories and statistical analysis was carried out using student t-test.

RESULTS AND DISCUSSION

Sample preparation techniques play an important aspect of total arsenic in marine samples for HG-QF-AAS measurements. Dry ashing method followed by reduction solution using HCl:KI:Ascorbic acid (5:5:2 v/v/v) has given the best result compared to other preparation techniques using HG-QF-AAS measurement. Table 1 showed total arsenic concentration from different sample preparations.

ICP-MS with acid digestion using Microwave Ultraclave III or water bath was the best method for determination of total arsenic in marine sample. Those measurements were validated using CRM DORM-2. Method validation was conducted for HG-QF-AAS and ICP-MS measurements. The performance parameters were linearity, accuracy, precision, recovery, LOD and LOQ. Table 2 showed the t-test statistic calculation to compare the average value of total arsenic measurement by ICPMS and HG-QF-AAS, t-stat value is less than t-critical value, the measurement result for total arsenic using ICPMS and HG-QF-AAS was similar. Table 3 showed a method validation result for HG-QF-AAS and ICP-MS measurement.

HG-QF-AAS measurement.

The concentration range was 0 - 7.0 ng g^{-1} with linear equation was $y = 0.0024x + 0.0006$ and linear coefficient (r) = 0.998. The dynamic range was 14 and found wide enough for trace

analysis concentration (ng g^{-1}). Coefficient correlation from the calibration curve was found almost 1. The LOD and LOQ was measured from 6 repeated blank solutions, and the concentration was calculated by average concentration from the graph plus 3 times standard deviation and 5 times standard deviation, respectively.

The LOD was 0.15 ng g^{-1} and LOQ was 0.23 ng g^{-1}. HG-QF-AAS was found sensitive for determination of total arsenic in tuna fish. Relative standard deviation (RSD) was calculated from standard deviation divided by average concentration multiplied by 100%. RSD value was 4.035%. Horwitz was calculated from $2^{1-0.5 \log C}$, where C is fraction concentration of the analyte (3.263 µg g^{-1} = 0.000003263) and the result was 13.39. Horwitz Ratio (HorRat) was calculated from RSD divided by Horwitz value, the HorRat value accepted was 0.3-1.3. In this research the HorRat value was 0.3. Recovery was calculated from final concentration minus sample concentration divided by standard concentration added multiply by 100%. The recovery value was found 86.52%. This value was acceptable by AOAC and Pharmacopeia for low concentration (80-115%). CRM DORM-2 was prepared and measured as samples.

The total arsenic concentration in CRM was found 17.49 µg g^{-1} ± 1.00 µg g^{-1} and certificate value was 18.0 µg g^{-1} ± 1.10 µg g^{-1}. The error of this method was 2.83%, and this value was smaller than AOAC standard error of 5%.

ICP-MS measurement.

Concentration range for this measurement was found 0-100 ng g^{-1}, with the linear equation was y = 293 x. Dynamic range was 200, and the correlation coefficient was 0.999. LOD and LOQ was 0.067 ng g^{-1} and 0.085 ng g^{-1}, respectively. The RSD value was 3.83%, CV Horwitz was 13.36 and HorRat value was 0.3. The accuracy was measured by CRM and %recovery, using CRM DORM-2 and DORM-3. Percent recovery was found 100.90%, and CRM value of DORM-2 and DORM-3 was 17.7 µg g^{-1} ± 0.08 µg g^{-1} and 6.77 µg g^{-1} ± 0.14µg g^{-1}, respectively. The certificate value of CRM DORM-2 and DORM-3 was 18.0 µg g^{-1} ± 1.10 µg g^{-1} and 6.88 µg g^{-1} ± 0.30 µg g^{-1}, respectively.

In the process for RM preparation, homogeneity is the first consideration. The homogeneity of a liquid or gases type of RM can be easily obtained by repetitive shaking. However, it is more difficult to homogenize a solid type of RM. Two different methods were conducted to confirm homogeneity of the candidate RM in this study. Table 4 showed the homogeneity test results. No significant differences were found for concentration of total arsenic by the two methods. The calculated F-values was lower than the critical F-values as shown in Tabel 4. In chemical testing laboratories, dried samples usually store at room temperature. Therefore, the stability test for this candidate RM was performed over 12 months at room temperature in three different laboratories. The statistical results showed no significant changes in stability as measured by the total arsenic concentration, because t_{calc} are lower that t_{crit} for confidence level of 95%, as shown in Table 5. CRM DORM-2 which has been periodically analysed for more than nine years and found to be both physically and chemically stable over that time period. Due to the time constraint, the candidate RM in this study was only measured to meet short term stability criteria at room temperature.

CONCLUSION

Slow heated dry ashing method with mixture of magnesium oxide and magnesium nitrate and subsequent reduction with mixture of HCl, KI, and ascorbic acid 5:1:2 (v/v/v) before measured using HG-QF-AAS was found accurate compared to non-reduction method. Digestion of the sample using conventional microwave was not recommended for the determination of total arsenic in tuna fish, because AB in the samples was not completely decomposed.

Dry ashing method was considered appropriate for tuna fish samples and can be used as a standard method for determination of total arsenic in tuna fish sample using HG-QF-AAS. The candidate reference materials developed in this study is suitable for quality assurance for the determination of total arsenic in fish tuna. It shows both satisfactory homogeneity and stability. The other marine biota samples such as mussel and green algae need to be considered for future research since mussel can accumulate all pollutant from the sea water and sediment and might consist of inorganic arsenic.

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