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RESEARCH ARTICLE

Effects of cooking methods on arsenic, cadmium, and lead in tilapia and human risk assessment

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Abstract

Objective: Effects of boiling, frying, and grilling methods on arsenic, cadmium, and lead in tilapia were studied and to estimate the potential to health risk.

Materials and Methods: Nile tilapia (n=120) were randomly purchased from the local markets in four provinces of the northeast Thailand and then the samples were boiled, fried, and grilled under specified conditions. Concentration of heavy metals levels were determined by inductively coupled plasma mass spectrometry. Human risk assessment was calculated by using the equation established by the United States Environmental Protection Agency.

Results: The results showed that heavy metals levels in fresh fish following the order: arsenic > lead > cadmium. The increased in heavy metals levels after cooking followed the order grilling > frying > boiling. Grilling and frying methods were increased the levels of three elements in tilapia samples but boiling was slightly decreased arsenic and cadmium concentrations. The average levels of heavy metals increased after cooking when compared with fresh samples was 52.74±19.16%.

Conclusion: Boiling, grilling and frying methods affected the amount of three elements in tilapia samples. However, arsenic, cadmium, and lead concentrations were below the maximum residue limits of Thailand regulations. The outputs of the target hazard quotients and the carcinogenic risk indicated that the values were safe for human health.

Keywords: Arsenic, Cadmium, Lead, Cooking, Risk assessment

Introduction

The economic growth and increasing population can lead to increasing of the emission and contamination of heavy metals. The increased of heavy metals from industries, agricultures and community wastes has been discharged into the environmental media. Fish can be accumulated heavy metals via absorbed and ingested of contaminated water, feed, and soil (Noel el al., 2013). Arsenic (As), Cadmium (Cd) and Lead (Pb) compounds are toxic elements and they are the ranks first, second and seventh in the ATSDR lists of the metals posing which the potential threat to human (ATSDR 2007a, b, 2012; Yu et al., 2017). Many literatures have been reported on the contaminations of As, Cd, and Pb in fish collected from the different part of the world with ranges not-detected to 6.24 µg/g; 0.005 to 10.67 µg/g and not-detected to 10.27 µg/g, respectively (Gill-Manrique et al., 2017; Huque et al., 2014; Nunez et al., 2018; Rajeshkumar and Li, 2018; Rahman et al., 2012; Saipan et. al., 2012, 2014). Heavy metals levels in fish can vary greatly depending on several factors such as agricultural and cultivating practices, amount atmospheric deposition and other anthropogenic contamination (Yi et al., 2013).

The elements can be affected by cooking process due to water loss during cooking that can increased or depleted of the heavy metal contents in the fish (Atta et al., 1997). Several studies have been investigated of the effects of cooking methods on heavy metal concentrations in fish and seafoods such as in seabass (Ersoy et al., 2006); sardine, hake, and tuna (Perello et al., 2008); African catfish (Ersoy, 2011); seabass, and red seabream (He et al., 2010); finfish and shellfish (Kalogeropoulos et al., 2012); *Polydactylus quadratifilis*, *Chrysichthys nigrodigitatus* and *Cynoglassus senegalensis* (Bassey et al., 2014); Caspian white fish (Hosseini et al., 2014), European seabass (Lomolino et al., 2016), and tuna (Nunez et al., 2018).

The northeastern region of Thailand has 20 provinces which the highest population approximately 21,916,034 inhabitants (NSO, 2017). According to the fishery statistics of Thailand between 2015 and 2017, the Nile tilapia (*Oreochromis niloticus*) is the freshwater fish with the highest annual production (about 176,463 tons) accounting for 34% of the total freshwater animal production and tilapia is the highest popular fish that consumed by Thai people (DOF, 2017). However, limited data are available on the effects of cooking methods on the heavy metals in Nile tilapia and there is little data regarding on As, Cd, and Pb in freshwater fish in Thailand including evaluated of health risk from consumption of contaminated tilapia (Jankong et al., 2007; Ngorpork et al., 2013; Ruangwises et al., 2012; Saipan et al., 2012, 2014). The purpose of the present study was to investigate the effects of cooking practices on the As, Cd, and Pb concentration in tilapia and potential of health risk from intake contaminated fish.

Materials and Methods

Sample collection and cooking methods

Nile tilapia (*Oreochromis niloticus*) samples (n=120) were randomly purchased in the local markets from 4 provinces (Nakorn ratchasima, Khon kaen, Udon thani and Chaiyaphum) of the northeastern region, Thailand. The sample was transported to the laboratory in polyethylene bags and placed in cold ice boxes within 12 h. All samples were washed several times with water, removed scale with plastic knife, measured of lengths and weights. Fillet of tilapia was separated from other tissues and then cut into pieces, washed with deionized water, fish fillets were divided into four groups (500 g for each fish; uncooked, boiled, fried, and grilled groups), and kept at -18 °C until next processing. Weights of each muscle sample before and after cooking was used for calculation of the moisture content.

Cooking are several methods such as grilling, boiling, frying, toasting, microwave radiation. However, the most frequency methods for preparing freshwater fish in private homes in this region are boiling, frying, and grilling (Atsawanan and Saipan, 2017). In brief, the sample was boiled on the electric stove for 25 min at 120 ºC in stainless steel pots with 500 ml distilled water. Sample was fried in a frying pan which contained 500 ml of sunflower oil with heat to 160 ºC until for 10 min or the sample turned brown. Grilling was done on a domestic electric roaster with automatic temperature control at 150 ºC for 20 min or until the sample was completed grilled. Raw and cooked fish samples were homogenized and each group was determined of heavy metals in the same way.

Determination of heavy metals

The samples were digested using a microwave digestion (Anton Paar GmbH - Multiwave 3000, Austria).

For each sample, approximately 0.5 g of sample was weighted and placed in a TFM vessel with 5 ml of 65% nitric acid (Suprapur, Merck, Germany) and 1 ml of 30% hydrochloric acid (Suprapur, Merck, Germany) in the microwave digestion system following the program: 85 ºC at 400 W for 15 min, followed by 120 ºC at 800 W for 20 min and 200 ºC at 1600 W for 10 min. The cooled solutions were washed into separate polypropylene tubes with Milli-Q water and made up to 20 mL. After cooling, the digested solutions were filtered and made up to 25 ml with 50% v/v HCl (Weich et al., 2017). Concentration of heavy metals levels were determined under specified condition according to the manufacturer as inductively coupled plasma mass spectrometry (ICP-MS, Agilgent 7500C Octopole Reaction System, Tokyo). HNO_3, HCl and other chemicals were purchased from Merck Chemicals (Darmstadt, Germany). Ultrapure water (18 MΩ · cm) was used for preparation of all solutions. All glassware was treated with 10% (v/v) HNO_3 for 24 h, after rinsing using ultrapure water. Calibration standards were prepared after successive dilutions from multielement standards stock solutions of 1,000 mg/l (Fuka, UK). Calibration standards were prepared by a manual standard addition in the four ranges of each element and blanks were analyzed for each calibration run using the same procedure. The analytical detection limits for As, Cd, and Pb were 0.050, 0.004, 0.010 µg/g, respectively. Standard reference material, oyster tissue (SRM 1566b, NIST, USA), was used to verify the accuracy of determination. Each sample has been analyzed in duplicate.

Data analysis

Normal variable distribution was tested by the Anderson-Darling's test and the Levene's test was employed to test homogeneity of variance. The differences levels of heavy metals and cooking methods were used Analysis of variance and Turkey-Kramer multiple comparison test by using SPSS Statistics version 17.0 for Windows. Statistical significance at a level of $P < 0.05$ was accepted for all comparisons.

Estimation of non-carcinogenic human risk

The non-carcinogenic risk is expressed as the target hazard quotients (THQs). THQs values were calculated by using the equation established by the USEPA (1989):

$$THQs = \frac{C \times IR \times ED \times EF}{RfD \times BW \times ATn} \times 10^{-3}$$

where, C = concentrations of heavy metals in tilapia samples (mg/kg); IR = ingestion rate of tilapia (g/day); ED = exposure duration (30 years); EF = exposure frequency (365 days/year); BW = body weight (55 kg); ATn = averaging time for non-carcinogenic effects (ED x 365 days); RfD = the oral reference dose of heavy metals (mg/kg/d); 0.0003 for inorganic As; 0.001 for Cd; and 1.5 for Pb (USEPA, 2019).

In the present study, the concentration of inorganic arsenic was assumed at 14% of total arsenic concentration referred by Saipan et al. (2012). The average of intake rate (IR) of tilapia for Thai people referred by the data of food consumption data of Thailand that reported by National Bureau of Agricultural Commodity and Food Standards, Thailand is 11.34 g/day (ACFS, 2016).

Estimation of carcinogenic human risk

Currently, Cd has been not quantitative estimated of carcinogenic slope factor values from oral exposure (CSFo), only inorganic As and Pb are reported carcinogenic slope factor (CSFo) by the Integrated Risk Information System at values 1.5 and 0.0085 $(mg/kg/day)^{-1}$ (USEPA, 2019). Carcinogenic risk (CR) was calculated by the following equation (USEPA, 1989):

$$CR = \frac{C \times IR \times ED \times EF \times CSFo}{BW \times ATc} \times 10^{-3}$$

where, C = concentration of inorganic As and Pb in tilapia (mg/kg); IR = ingestion rate of tilapia (11.34 g/day); ED = exposure duration (70 years); EF = exposure frequency (365 days/year); BW = body weight (55 kg); ATc = averaging time for carcinogenic effect (25,550 days).

Results and Discussion

The objective of the present study was analyzed of As, Cd, and Pb in tilapia and conducted to assess the effect of boiling, frying, and grilling on those metals including to estimate the target hazard quotient and carcinogenic risk from consumption of tilapia. Measured values of

As, Cd and Pb levels found in this study were agreed with the standard reference material (NIST 1556b, oyster) (Table 1) which recovery rates ranged 93.34–100.39% and the relative standard deviation 3.03–5.70%. Lengths and weights of tilapia samples were 30.54 ± 1.05 cm (range 28.68 to 31.05 cm) and 385.25 ± 6.72 g (range 378 to 398.50 g). Moisture contents were decrease following the order frying (25.68 to 32.92%) > grilling (22.50 to 35.76%) > boiling (4.02 to 10.58%). Grilling and frying methods were treated at high temperatures which could lead to significant loss of water.

Table 2 presented that the heavy metals levels in this study were generally comparable to the concentrations detected in other literatures.

The concentrations of Pb were ranges not-detected to 0.78 µg/g (Adewumi et al., 2017; Atique Ullah et al., 2017; Copat et al., 2013; Ju et al., 2017; Maria and Grazia, 2013; Noel et al., 2013; Rajeshkumar and Li, 2018; Rahman et al., 2012) which were similarity the values found in the present study. Nuapia et al. (2018) reported that an average level of Pb in tilapia collected of Democratic Republic of Congo was 1.54 ± 0.96 µg/g which higher concentration than the present study. However, it is difficult to directly compare the concentrations of elements among the studies because the heavy metals residue levels can be influenced by many factors such as: type and size of the fish, rates of exposure to the heavy metals in feed or environment (Jiang et al., 2016). Thailand set regulatory limits for general foods and seafood products at 2, 0.2, and 1 µg/g of As, Cd and Pb, respectively (ACFS, 2005; MOPH, 2003). All of three elements concentrations in the tilapia samples in the present study were lower than the permitted levels of Thailand regulation limits.

The average and ranges concentrations of As, Cd, and Pb in all samples are shown in Table 3 including the percentage of the elements were changed after cooking. The average levels of heavy metals increased after cooking when compared with fresh samples was 52.74±19.16%.

The results showed that heavy metals levels in fresh fish following the order: As > Pb > Cd. The increased in heavy metals levels after cooking followed the order grilling > frying > boiling. Grilling and frying methods were increased the levels of three elements in tilapia samples but boiling was slightly decreased As and Cd concentrations. The average concentration of heavy metals increased after cooking when compared with fresh samples was 52.74 ± 19.16% (Table 3). The average of As in fresh tilapia sample was found to be 0.106 mg/kg. In boiled, fried, and grilled samples were 0.102, 0.146, and 0.188 mg/kg, respectively. This increase in As level was significant ($P < 0.05$) for grilled sample, while it was not significant different concentrations for boiling and frying methods. Statistically analysis showed that Pb levels were difference between the grilling method and uncooked fish, fried and boiled samples ($P < 0.05$) (Table 3). The Cd concentrations were increased after frying and grilling while slightly decreased after boiled sample when compared with fresh sample.

The levels of heavy metals in fish can be affected by cooking methods due to water loss during cooking that can increased or depleted of the metal contents (Atta et al., 1997). Comparable of other studies, Okareh and Akande (2015) reported that cadmium content in catfish were decreased after boiling, grilling and frying range (2.2–5.7 µg/g) or decreasing of range 25–64.77% when compared with fresh sample (8.8 µg/g). Perello et al. (2008) presented that fried and grilled methods were increased cadmium levels in sardine sample about 50%. The finding of Wiech et al. (2017) showed that boiled crab (0.840 ± 0.660 µg/g) was higher level of cadmium than fresh carb (0.024 ± 0.012 µg/g) which increased more than 30 times. Ersoy el al. (2006) reported that As and Cd levels in seabass were increased to 1.41 and 0.741 mg/kg after microwave cooking when compared with raw samples (0.372 and

Table 1. Concentration of heavy metals in NIST 1556b (oyster) standard (mean ± SD), measurement recoveries, and relative standard deviation.

Element	Certified value (µg/g)	Measured value (µg/g)	Recovery (%)	RSD (%)
As	7.65 ± 0.65	7.68 ± 0.23	100.39	3.03
Cd	2.48 ± 0.08	2.43 ± 0.08	97.88	3.31
Pb	0.308 ± 0.009	0.29 ± 0.02	93.34	5.70

Table 2. Concentration of arsenic (As), lead (Pb), and cadmium (Cd) in freshwater fish

Countries	Concentration (µg/g)	References
China	As (not detectable - 0.039) Cd (not detectable - 2.00)	Yi et al. (2011)
	As (0.748 ± 0.651)	Jia et al. (2018)
	Cd (0.005 to 0.055)	Ju et al. (2017)
	Cd (0.042 ± 0.001)	Rajeshkumar and Li (2018)
Turkey	As (0.0646–0.0807)	Varol et al. (2017)
Congo	As (12.04 ± 2.23) Cd (2.50 ± 0.78) Pb (1.54 ± 0.96)	Nuapia et al. (2018)
Italy	Cd (0.01 to 0.15)	Copat et al. (2013)
Thailand	As (0.106 ± 0.035) Cd (0.027 ± 0.005) Pb (0.031 ± 0.007)	Present study
	As (0.140–0.286)	Ruangwises et al. (2012)
	As (0.183–0.711)	Saipan et al. (2012)

Table 3. As, Cd, and Pb concentrations (wet weight) in fresh and cooking samples (mean ± SD; range)

Elements	Fresh (mg/kg)	Cooking method (mg/kg)			Percentage of the elements change (range)
		Boiled	Fried	Grilled	
As	0.106 ± 0.035 (nd–0.562)	0.102 ± 0.022 (0.050–0.625)	0.146 ± 0.017 (0.055–0.812)	0.188 ± 0.032* (0.055–0.926)	34.82 (3.77–77.36)
Cd	0.027 ± 0.005 (0.005–0.056)	0.025 ± 0.012 (0.010–0.162)	0.030 ± 0.008 (0.012–0.180)	0.056 ± 0.016* (0.022–0.188)	50.46 (7.41–107.41)
Pb	0.031 ± 0.007 (nd–0.088)	0.040 ± 0.015 (0.020–0.584)	0.066 ± 0.018 (0.026–0.605)	0.085 ± 0.010* (0.034–0.782)	72.94 (29.03–174.19)

nd = not-determined; * significantly different from the fresh sample in each element ($P<0.05$)

0.278 mg/kg) (267–379%). The present study, average levels of heavy metals increased after cooking when compared with fresh samples was 52.74 ± 19.16%. The levels heavy metal after cooking were different changed depending on the cooked processing such as time, temperature, medium of cooking including types of fish (Joyce et al., 2016; Perello et al., 2008; Wiech et al., 2017).

The target hazard quotients (THQs) results in this study ranged 1.02×10^{-2} to 9.81×10^{-3} for As; 1.15×10^{-2} to 6.18×10^{-3} for Cd; and 1.20×10^{-5} to 9.0×10^{-6} for Pb and the carcinogenic risk (CR) values of As and Pb were ranges 4.59×10^{-6} to 8.14×10^{-6} and 1.16×10^{-7} to 7.01×10^{-8} (Table 4). The present results obtained the THQs values were comparable to those found in other studies; 1.96×10^{-1} to 9.37×10^{-3} for As, 4.89×10^{-3} to 6.60×10^{-3} for Cd, and 1.01×10^{-2} to 9.78×10^{-3} for Pb (Zhang et al., 2014); 2.11×10^{-3} for As, 1.5×10^{-4} for Cd, and 1.0×10^{-2} for Pb (Varol et al., 2017). Islam et al. (2015) reported that As, Cd and Pb in fish samples collected from Bangladesh were 0.091–0.53, 0.007–0.13, and 0.052–2.7 µg/g, respectively and THQ was followed in the descending order of As > Pb > Cd including the sum of individual metal THQ was 1.6 to 2.9. The CR of As and Pb values were 2.0×10^{-2} and 2.1×10^{-4}. Zhang et al. (2018) presented the results of THQs and

Table 4. Results of target hazard quotients (THQs) and Carcinogenic risk (CR) calculated from consumption of fresh and cooked tilapia.

Method/ Element	Concentration of total arsenic (mg/kg)	Intake rate (g/day)	RfDo (mg/kg/ day)	THQs	CSFo (mg/kg/ day)$^{-1}$	CR
Raw		11.34				
As[a]	0.106		0.0003	$1.02x10^{-3}$	1.5	$4.59x10^{-6}$
Cd	0.027		0.001	$5.57x10^{-3}$	-	-
Pb	0.031		1.5	$4.0x10^{-6}$	0.0085	$5.43x10^{-8}$
Boiled		11.34				
As	0.102		0.0003	$9.81x10^{-3}$	1.5	$4.42x10^{-6}$
Cd	0.025		0.001	$5.15x10^{-3}$	-	-
Pb	0.040		1.5	$5.0x10^{-6}$	0.0085	$7.01x10^{-8}$
Grilled		11.34				
As	0.188		0.0003	$1.89x10^{-2}$	1.5	$8.14x10^{-6}$
Cd	0.056		0.001	$1.15x10^{-2}$	-	-
Pb	0.085		1.5	$1.20x10^{-5}$	0.0085	$1.49x10^{-7}$
Fried		11.34				
As	0.146		0.0003	$1.41x10^{-2}$	1.5	$6.32x10^{-6}$
Cd	0.030		0.001	$6.18x10^{-3}$	-	-
Pb	0.066		1.5	$9.0x10^{-6}$	0.0085	$1.16x10^{-7}$

[a] The concentration of iAs was assumed at 14% of total arsenic concentration referred from Saipan et al. (2012)

CR in fish consumption form Honghu city, China were 3.5 x 10^{-2} and 1.60 x 10^{-5}. Atique Ullah et al. (2017) presented that THQs and CR values were 9.13 x 10^{-2} and 2.7 x 10^{-4} for As; 1.22 x 10^{-1} and 4.2 x 10^{-6} for Pb. Jiang et al. (2016) showed that THQ was 7.5 x 10^{-2}. Nunez et al. (2018) reported that CR value was 1.2 x 10^{-5} and Zhong et al. (2018) reported that the CR of inorganic As ranged 2.71 x 10^{-6} to 1.04 x 10^{-4} for adult in central and eastern North China from consumption of freshwater fish. USEPA (1989) recommended that CR value ranges 10^{-4} to 10^{-6} are considered acceptable. In present study, CR values were comparable to those found in other studies and were within the acceptable ranged.

Conclusions

Boiling, frying, and grilling were increased the concentrations of Pb in tilapia while As and Cd can be increased after cooking by frying and grilling methods. However, the concentrations of those were still lower than the Thai regulatory standard. It can be recommended that boiling is the most suitable cooking method for human consumption. The target hazard quotient and carcinogenic risk values were still within the acceptable ranges. Further investigation should be investigated the other heavy metals and health risk assessment for exposed to offal tissues or other visceral organs of foodstuffs from animal origin.

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Compliance with ethical standards.

Conflict of interest the authors declare that they have no conflict of interest.

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