A market-based probabilistic risk assessment of As concentration in raw and cooked rice in Bangkok

Miss Supanad Hensawang

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การประเมินความเสี่ยงแบบความน่าจะเป็นจากปริมาณสารหนูในข้าวสารและข้าวหุงสุกที่จำหน่ายในตลาดกรุงเทพมหานคร

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By	Miss Supanad Hensawang
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Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the Graduate School
(Associate Professor Thumnoon Nhujak, Ph.D.)

DISSERTATION COMMITTEE

..... Chairman
(Associate Professor Ekawan Luepromchai, Ph.D.)
..... Thesis Advisor
(Assistant Professor PENRADEE CHANPIWAT, Ph.D.)
..... Examiner
(Professor KYOUNG WOONG KIM, Ph.D.)
..... Examiner
(Assistant Professor Chantra Tongcumpou, Ph.D.)
..... Examiner
(Assistant Professor Nutta Taneepanichskul, Ph.D.)
..... External Examiner
(Assistant Professor Pensri Watchalayann, Ph.D.)

ศุภนาถ เห็นสว่าง : การประเมินความเสี่ยงแบบความน่าจะเป็นจากปริมาณสารหนูในข้าวสารและข้าวหุงสุกที่จำหน่ายในตลาดกรุงเทพมหานคร. (A market-based probabilistic risk assessment of As concentration in raw and cooked rice in Bangkok) อ.ที่ปรึกษาหลัก : ผศ. ดร.เพ็ญรดี จันทร์ภิวัฒน์

ข้าวเป็นอาหารหลักของมนุษย์และยังอาจเป็นเส้นทางการรับสัมผัสสารหนูที่สำคัญในมนุษย์ การศึกษานี้มีวัตถุประสงค์เพื่อ 1) ตรวจสอบความเข้มข้นของสารหนูทั้งหมด สารหนูอนินทรีย์ และสารหนูที่อยู่ในรูปแบบที่สิ่งมีชีวิตดูดซึมได้ที่ถูกสะสมอยู่ในเมล็ดข้าวขัดสีและข้าวไม่ขัดสี 2) ศึกษาผลกระทบของกระบวนการปรุงประกอบข้าว (การขัดสี การซาวข้าว และการหุงข้าว) เพื่อการบริโภคต่อความเข้มข้นของสารหนูทั้งหมดในเมล็ดข้าว และ 3) ประเมินความเสี่ยงทางสุขภาพของมนุษย์จากการสัมผัสสารหนูผ่านการบริโภคข้าว การศึกษาได้ดำเนินการเก็บตัวอย่างข้าวสารขัดสีและไม่ขัดสี จำนวนทั้งสิ้น 208 ตัวอย่างที่ถูกจำหน่ายในตลาดภายในพื้นที่กรุงเทพมหานครและปริมณฑล พบการศึกษาตรวจพบปริมาณความเข้มข้นของสารหนูทั้งหมดในเมล็ดข้าวขัดสีและไม่ขัดสีเท่ากับ 0.0878 ถึง 0.2949 มิลลิกรัมต่อกิโลกรัม และ 0.1187 ถึง 0.5172 มิลลิกรัมต่อกิโลกรัม ตามลำดับ สัดส่วนของสารหนูอนินทรีย์ต่อความเข้มข้นของสารหนูทั้งหมดในเมล็ดข้าวขัดสีและไม่ขัดสี มีค่าร้อยละ 58 และร้อยละ 52 ตามลำดับ การขัดสีเมล็ดข้าวพบว่าที่ระดับการขัดสีร้อยละ 8 เพื่อทำให้ได้ข้าวสีดีพิเศษนั้น สามารถทำให้ความเข้มข้นของสารหนูทั้งหมดที่สะสมอยู่ในเมล็ดข้าวลดลงอย่างมีนัยสำคัญถึงร้อยละ 25 เมื่อเทียบกับความเข้มข้นของสารหนูทั้งหมดที่สะสมอยู่ในเมล็ดข้าวไม่ขัดสี การซาวข้าวขัดสีทั้งสิ้น 3 ครั้ง พบว่าสามารถทำให้ความเข้มข้นของสารหนูทั้งหมดที่สะสมอยู่ในเมล็ดข้าวที่ถูกล้างด้วยน้ำแล้วลดลงอย่างมีนัยสำคัญถึงร้อยละ 29 ในขณะที่การซาวข้าวสารไม่ขัดสีนั้น พบว่าไม่สามารถทำให้ความเข้มข้นของสารหนูทั้งหมดที่สะสมอยู่ในเมล็ดข้าวลดลงอย่างมีนัยสำคัญได้ ในส่วนของการลดลงของปริมาณการสะสมของสารหนูในข้าวสุดที่หุงจากข้าวสารที่ถูกซาวด้วยน้ำจำนวน 3 ครั้ง พบว่าการหุงข้าวแบบเช็ดน้ำ และการหุงข้าวด้วยหม้อหุงข้าวไฟฟ้า ทำให้สารหนูในเมล็ดข้าวลดลงถึงร้อยละ 60 และร้อยละ 21 ตามลำดับ ปริมาณสารหนูที่อยู่ในรูปแบบที่สิ่งมีชีวิตดูดซึมได้ในข้าวที่ศึกษาทั้งสองชนิดนั้นลดลงอย่างมีนัยสำคัญเมื่อข้าวนั้นผ่านกระบวนการหุงสุก ผลการประเมินความเสี่ยงต่อสุขภาพจากการรับสัมผัสสารหนูที่อยู่ในรูปแบบที่สิ่งมีชีวิตดูดซึมได้ ผ่านการบริโภคข้าวที่ถูกหุงสุกด้วยหม้อหุงข้าวไฟฟ้า พบว่าประชากรส่วนใหญ่ในทุกกลุ่มประชากรศึกษา ซึ่งได้แก่ วัยเด็ก วัยรุ่น และวัยผู้ใหญ่ นั้นไม่มีความเสี่ยงต่อการเกิดผลกระทบทางสุขภาพจากการได้รับสารหนูแบบไม่ก่อให้เกิดมะเร็ง ในขณะที่ประชากรวัยผู้ใหญ่นั้นมีความเสี่ยงที่จะได้รับผลกระทบทางสุขภาพจากการได้รับสารหนูแบบที่อาจก่อให้เกิดมะเร็งได้ โดยมีค่าเฉลี่ยของความเสี่ยงต่อการเกิดมะเร็งอยู่ที่ 1.12×10^{-4} ซึ่งเกินค่าที่ยอมรับได้ที่ 1×10^{-4} ปัจจัยสำคัญที่ส่งผลต่อระดับความเสี่ยงต่อการผลกระทบทางสุขภาพ ประกอบไปด้วย ปริมาณการบริโภคข้าว น้ำหนักตัว และความเข้มข้นของสารหนูที่สะสมอยู่ในข้าวหุงสุกพร้อมบริโภค

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ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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Supanad Hensawang : A market-based probabilistic risk assessment of As concentration in raw and cooked rice in Bangkok. Advisor: Asst. Prof. PENRADEE CHANPIWAT, Ph.D.

Rice is a staple food of human and can be a main source of arsenic (As) exposure. This study was conducted to i) investigate the concentrations of total, inorganic and bioaccessible of As in raw rice, ii) investigate the effects of rice processing before human consumption on the concentrations of As in rice, and iii) evaluate the potential health impacts of As exposure through rice consumption. A total of 208 polished and non-polished samples sold in the local markets of Bangkok were collected. The total As concentrations in polished (0.0878 to 0.2949 mg kg^{-1}, n = 154) and non-polished (0.1187 to 0.51722 mg kg^{-1}, n = 54) were determined. The percentages of inorganic As to total As in rice were 58% and 52% in polished and non-polished rice, respectively. Approximately 25% of total As in 8% DOP rice grain were significantly reduced compared to the total As in non-polished rice (0% DOP) ($p < 0.01$). Only 3-time washing could significantly decrease total As concentration in polished rice ($p < 0.05$) by approximately 29%. Washing did not effectively remove As from non-polished rice grain. Rice washed for 3 times and then cooked with the traditional and modern cooking method using an aluminum pot and an electric rice cooker, respectively, had approximately 60% and 21% of total As reduction comparing to the raw rice. Cooked rice from both cooking methods had significantly lower As bioaccessibility than the raw rice. The deterministic and probabilistic risk assessment results of bioaccessible As exposure through the consumption of modern cooked rice indicated a negligible non-carcinogenic effects in the majority of children, adolescents, and adults as the hazard quotients in all groups were less than 1. Adults were susceptible population to carcinogenic effects especially from the non-polished rice consumption. The average cancer risk which was approximately 1.12×10^{-4} exceeded the acceptable cancer risk of 1×10^{-4}. Three important factors affecting the health risk levels were rice ingestion rate, individual body weight, and As concentration in cooked rice.

Field of Study:	Hazardous Substance and Environmental Management	Student's Signature .....
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LIST OF ABBREVIATION

Abbreviation	Full term
ADD	Average daily dose
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
As	Arsenic
As(III)	Arsenite
As(V)	Arsenate
AT	Averaging time
bAs	Bioaccessible arsenic
BW	Body weight
CF	Substance concentration
CR	Cancer risk
DMA	Dimethylarsenic acid
DOP	Degree of polishing
DRA	Deterministic risk assessment
EAM	Elemental analysis manual
ED	Exposure duration
EDTA	Ethylenediaminetetraacetic acid
EF	Exposure frequency
Eh	Oxidation-reduction potential
ET-AAS	Electrothermal atomic absorption spectroscopy
EWP	Extra well-polished rice
FAO	Food and Agricultural Organization
FAO/WHO	Joint Food and Agricultural Organization and World Health Organization
HG-AAS	Hydride generation atomic absorption spectroscopy
HPLC-ICP-MS	High performance liquid chromatography coupled with inductively coupled plasma-mass spectroscopy
HQ	Hazard quotient

IARC	International Agency for Research on Cancer
iAs	Inorganic arsenic
ICP-MS	Inductively coupled plasma-mass spectroscopy
IR	Ingestion rate
IRIS	Integrated Risk Information System
IRRI	International Rice Research Institute
KDML 105	Kao Dok Mali 105 rice variety
LADD	Long-term average daily dose
MMA	Monomethylarsenic acid
N/A	Not applicable
N/D	Not defined
NIST	National Institute of Standards and Technology
oAs	Organic arsenic
OECD	Organisation for Economic Co-operation and Development
OM	Organic matter
OP	Ordinarily polished rice
PBET	Physiologically based extraction test
PE	Polyethylene
PRA	Probabilistic risk assessment
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment, The Netherland)
r	Correlation coefficients
R^2	Coefficient of determination
RfD	Oral reference dose
RSD	Relative standard deviation
RWP	Reasonably well-polished rice
S-XRF	Synchrotron X-ray fluorescence
SD	Standard deviation
SE	Standard error of the mean
SF	Cancer slope factor

SHIME	Simulator of human intestinal microbial ecosystem of infants
SRM	Standard reference material
tAs	Total arsenic
TIM	TNO gastrointestinal model
US FDA	United States Food and Drug Administration
WG	Wholegrain (non-polished) rice
WHO	World Health Organization
WP	Well-polished rice
XAS	X-ray absorption spectroscopy

CHAPTER I
INTRODUCTION

1. Theoretical backgrounds

One of the metal(liod)s, which is naturally found in Earth's crust, is arsenic (As). Its predominant forms are inorganic As including arsenic sulfide, arsenite (As(III)), and arsenate (As(V)) (Flora, 2015) . Human health impacts of As exposure can be both acute and chronic effects. The health symptoms of acute exposure are vomiting, abdominal pain, and diarrhea. However, the high level of As exposure in a short term is rarely found. In contrast to adverse acute health effects, health effects of long-term As exposure in population are more frequently observed especially for the symptoms of hyperpigmentation and hyperkeratosis (Integrated Risk Information System, 1995). In addition, inorganic As has been classified as a carcinogenic substance that can cause cancer to several organs including lung, bladder, and skin (Agency for Toxic Substances and Disease Registry, 2007). Even though As is widely and naturally distributed in the environment, its release to the environment leading human exposure is usually activated by natural activities (e.g. volcanic activity, minerals dissolution, and exudates from plant cultivation) and anthropogenic activities (e.g. mining, metal smelting as well as usage of pesticide and wood preservation) (International Agency for Research on Cancer, 2012). The contamination of As in soil, surface water, and groundwater and its adverse human health effects are found in several parts of the world. For example, groundwater containing As about 5 times exceeding the World Health Organization (WHO) standard of drinking water (10 μg L^{-1}) in West Bengal, India has caused local residents' severe health problems such as skin lesion, liver and spleen enlargement, and fluid in the abdomen (Chowdhury et al., 2000; World Health Organization, 2008). Therefore, the groundwater in this area has been prohibited to be used as a source of drinking water. However, the contaminated groundwater has been used as irrigation water of several food crops. Therefore, human health effects of As toxicity are exacerbated by not only the As groundwater contamination but also As contamination in the food chain, especially, paddy rice (Islam et al., 2016).

The factors affecting As accumulation in rice are including contaminated environmental media for cultivation, rice varieties, and cultivation practices. In general, rice is grown in a flooding condition, which could cause a reduction of soil redox potential that consequently enhance the As bioavailability in the soil solution. Subsequently, high accumulation of As in rice parts, for example, grain is observed (Halder et al., 2014). Several studies have reported the total As concentration in raw rice exceeding the Joint Food and Agricultural Organization and World Health Organization (FAO/WHO) maximum level of inorganic As in polished rice (0.2 $mgkg^{-1}$) (Food and Agriculture Organization/World Health Organization, 2014), for example, 0.321 mg kg^{-1} and 0.203 mg kg^{-1} of total As in Bangladeshi rice and Thai rice, respectively (Ahmed et al., 2015; Hensawang and Chanpiwat, 2017)

Even if the Joint FAO/WHO Food Standard Programme has agreed for the use of total As concentration as a screening approach for inorganic As content in rice grain, inorganic As determination is necessary when the total As concentration in rice is higher than the standard. This is due to the fact that inorganic As is more toxic than other As species and rice is reported to be one of the inorganic As hyperaccumulating food crops (Bruce et al., 2007). Thus, rice can be an important source of inorganic As exposure in human, especially, when it is consumed on a daily basis. According to the Organisation for Economic Co-operation and Development (OECD) and the Food and Agricultural Organization (FAO), Thailand ranks fourth in the amount of per capita annual rice consumption (Zhuang et al., 2016; Organisation for Economic Co-Operation and Development/Food and Agriculture Organization, 2019) . During the last decade (2009 to 2019), each Thai individual annually consumed approximately 99 kg of raw rice. This high amount of rice consumption indicates high potential of exposure and health impacts of inorganic As in Thai population. Up to date, the study of total and inorganic As in rice that Thai population normally consumed is limited. In addition, those previous studies only focused on the grab sampling with small sample size (Ruangwises et al., 2012; Nookabkaew et al., 2013).

In reality, raw rice is normally washed and cooked before consumption. Those processes may affect As concentration in rice that human consumed. For instance, Raab et al. (2009) found that washing reduced less than 10% of total As in rice while, Gray et al. (2015) found no significant reduction of total As in rice after washing. In the case

of cooking, approximately 30% to 45% of total As in rice was reduced when cooking rice with high volume of water to rice ratio such as 6: 1 and 10: 1 (Raab et al., 2009; Gray et al., 2015). Although, there are few studies on the effects of rice preparation processes on the As concentration in rice grain, the similar study, especially on Thai rice, has never been conducted.

Even though the total and inorganic As concentrations in raw and cooked rice are determined, they, to some extent, could not represented the certain amount of As that is readily for digestion and absorption into a human body. It is believed that the bioaccessible concentration of any substances is equal to the actual amount of substance which can be digested in the gastrointestinal tract and reach human body (Pasias et al., 2013). The bioaccessible substance released from foods can be extracted through the *in vitro* digestion. This method mimics the human digestive system in the oral cavity, gastric, and intestine of human (Versantvoort et al., 2004; Omar et al., 2013) in order to breakdown the foods into the smaller sizes, until they can be absorbed and assimilated into human body.

Human health risk assessment is a process which is normally employed to estimate the risk of chemical exposure in the population (European Food Safety Authority, 2010). Even though a deterministic risk assessment (DRA) has been widely used to evaluate the level of exposure and characterize the potential risks of exposure in human. The results obtained may overestimate or underestimate the risk since this process applies only a single point value of each variable into the calculation. As a consequence, the over concerned or ignorant reaction might happen among the population. Therefore, a probabilistic risk assessment (PRA) was developed to provide more accurate results of exposure and risk level. Since the PRA includes a probability distribution function of all variables instead of using a single (average) point value into the risk calculation, more accurate levels of exposure and potential risk can be obtained (Idaho Department of Environmental Quality, 2014). In regard to the rationales mentioned earlier, this research was conducted to assess human health risk of As exposure through rice consumption in the population living in Bangkok, a capital city of Thailand.

2. Objectives

1. To determined concentrations of total As (tAs), inorganic As (iAs) and bioaccessible As (bAs) in Thai polished and non-polished rice sold in the local markets of Bangkok.

2. To study the effect of rice polishing process on tAs concentration in rice.

3. To study the effects of washing and cooking processes on tAs and iAs concentrations in rice.

4. To determine the concentration of bAs in cooked rice.

5. To assess and compare the human health risks of As exposure in Bangkok population based on the DRA and PRA approaches.

3. Hypotheses

1. Concentrations of tAs, iAs and bAs in the non-polished rice will be higher than those of polished rice.

2. Polishing process could significantly reduce tAs contained in the polished rice grain comparing to that tAs in the non-polished rice grain (wholegrain).

3. Washing and cooking processes could reduce approximately 50% of iAs and bAs concentrations comparing to those iAs and bAs contained the raw rice grain.

4. Lower As bioaccessibility will be found in the processed rice comparing to that of the raw rice grain.

5. The levels of potential non-carcinogenic and carcinogenic health risks evaluated by the DRA approach will be significantly higher than those evaluated by the PRA approach.

4. Scope of study

This study can be simply divided into 3 parts as shown in Figure 1-1 including 1) the determination of normal ranges of tAs, iAs, and bAs concentrations in raw rice, 2) the effects of rice processing on As concentrations in rice, and 3) the assessment of As exposure and its health risks through a daily rice consumption.

First, polished and non-polished rice samples which were cultivated within 3 different crop years (2015, 2017, and 2018) were collected from 12 local markets in

Bangkok and 2 wholesale markets in Pathumthani to ensure the randomness of samples throughout the 3-year period of sampling campaign. All samples collected were 100% Thai jasmine rice in which the information of rice cultivation areas (rice origin) was also obtained upon collection. In addition, samples were collected from a minimum of two stores at each sampling site (market). All raw rice samples were determined for tAs concentrations. While, the representative raw rice samples containing tAs concentration higher than the FAO/WHO maximum level of iAs in rice (> 0.2 mg kg^{-1} for polished rice and > 0.35 mg kg^{-1} for non-polished rice) were selected for the determination of iAs and bAs concentrations.

Second, the different polishing, washing, and cooking scenarios were performed to study the effects of rice processing on As concentrations in rice. A four stepwise polishing process was applied to polish the non-polished rice grain to result in the polished rice grain with different degrees of polishing. In the case of washing and cooking processes, the representative raw rice samples containing tAs equal to or greater than the 75^{th} percentiles were washed with different washing scenarios. The scenarios applied in this study are those washing methods that Thai population normally applied to cook rice before consumption. The washed rice samples resulted in the greatest tAs reduction in rice was, then, cooked under two cooking methods including the modern and traditional methods. The concentrations of tAs, iAs and bAs were determined in the cooked rice. The concentrations of bAs obtained were used as the input variables for the exposure assessment and risk characterization processes.

Lastly, the human health risk assessment was conducted using two assessment approaches including DRA and PRA. The single point value, in this case an average value, of each variable was used for DRA approach. On the other hand, a range of values with probability distribution function of each variable was used for the PRA approach. Risk results from these DRA and PRA approaches were finally compared to obtain the ultimate goal of the study.

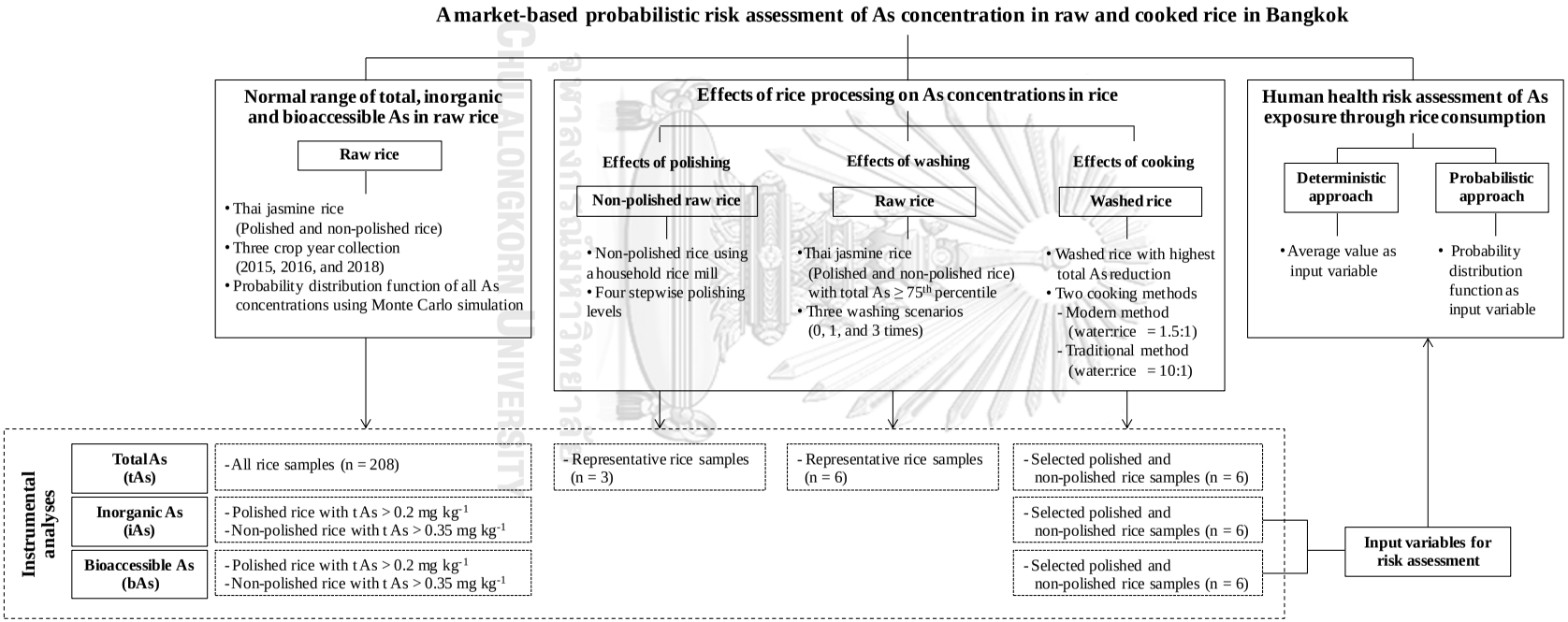


Figure 1- 1 A research conceptual framework

5. Organization of dissertation

This study aims to assess human health risk of As exposure through rice consumption. Since the study was divided into 3 parts as shown in Figure 1-1, the contents of dissertation following the conceptual framework were organized as follows.

Chapter 1 describes the background and rationale of this study, objectives, hypotheses, and scope of the study.

Chapter 2 presents the basic information of As and a summary of previous research results of As contamination in rice. The review of literature contains 7 parts including i) Arsenic in the environment and its toxicity, ii) Arsenic contamination in rice, iii) Total and inorganic As determination in rice, iv) Bioaccessible As concentration in rice, v) Effect of rice polishing on As content, vi) Effect of washing and cooking processes on As content, and vii) Deterministic and probabilistic human health risk assessment approaches.

Chapter 3 presents the results of the determinations of normal ranges of tAs, iAs, and bAs concentrations in raw polished and non-polished rice based on a market basket survey in Bangkok. Results showed in this chapter include the results from the instrument analyses as well as the Monte Carlo simulations. In addition, the relationships among those As concentrations were also addressed in this chapter.

Chapter 4 presents the effects of stepwise polishing process on tAs concentrations in raw non-polished rice. The resulting polished rice in this study is in compliance with a classification of Thai polished rice announced by the National Bureau of Agricultural Commodity and Food Standards in 2017.

Chapter 5 presents the effects of different rice washing and cooking methods on tAs, iAs and bAs concentrations. Both practical washing and cooking methods which are normally performed at each Thai household were conducted in this study. The reduction in the tAs, iAs and bAs concentrations comparing to those contained in the raw rice were reported.

Chapter 6 evaluates the level of As exposure and the potential human health impacts as a result of As exposure through rice consumption on a daily basis using the DRA and PRA approaches. The comparison of results obtained from both approaches was also examined.

Chapter 7 summarizes the key findings of each part of the study. In addition, the recommendations for future study were provided.

CHAPTER II
LITERATURE REVIEW

This chapter provides the basic information of As, a summary of previous studies of As contamination in rice in various aspects, as well as the information of health risk assessment approach. This review of literature was divided into 7 main topics including i) Arsenic in the environment and its toxicity, ii) Arsenic contamination in rice, iii) Total and inorganic As determination in rice, iv) Bioaccessible As concentration in rice, v) Effect of rice polishing on As content, vi) Effect of rice processes on As content, and vii) Deterministic and probabilistic human health risk assessment approaches.

1. Arsenic in the environment and its toxicity

1.1) General properties of As

General properties of As are including atomic weight of 74.9, the atomic number of 33, a density of 5.73 g mL^{-1}. It is a metalloid and normally presents in 3 different allotrophic forms: yellow, black, and grey. The grey form of As is important to industry as it is the only form of As that has a metallic appearance. While, the yellow As and black As are in the metastable forms. The common oxidation states of As are –III, 0, +III, and +V. These common oxidation states can be found in the major As compounds including arsine (AsH_3), arsenic trioxide (As_2O_3), sodium arsenite ($NaAsO_2$), arsenic pentoxide (As_2O_5), sodium arsenate ($NaAsO_3$), monomethylarsenic acid (MMA), and dimethylarsenic acid (DMA) (Adriano, 2001). Arsine (As_2O_3) commonly presents in the gas form. While As which present in the arsenic trioxide (As_2O_3), sodium arsenite ($NaAsO_2$), arsenic pentoxide (As_2O_5) and sodium arsenate ($NaAsO_3$) are in the form of inorganic As. The organic As (oAs) generally presents in the form of MMA and DMA (Adriano, 2001).

1.2) Arsenic in the environment

In general, As is naturally found in the Earth's crust and ranks as the 20th abundant element in the earth crust. It can be released into the environment by both natural and anthropogenic activities. In case of natural geological process, the weathering and erosion of rocks and sediments, hydrothermal activities, volcanic

eruption, sulfide minerals dissolution, wild fire, and wind-blown dust are the main natural activities that enhance the occurrences of As in the environment. On the other hand, the man-made activities which use As for several purposes (e.g. poisoning, warfare, or painting), have become the major sources of As in the environment. The current usages of As compounds for several human activities are summarized in Table 2-1. An instant increase in As contamination has been found since the industrial revolution because As compounds have been used in the production process of various products such as glass, wood preservative chemical, pesticide, and petroleum. In addition, As can be released into the environment from the landfill and hazardous waste disposal sites.

Table 2- 1 As usages and compounds

Purposes	Current uses	Compounds
Agriculture	Pesticides	Lead arsenate
		Calcium arsenate
		Sodium arsenite
	Herbicides	DMA
	Cotton desiccants	Arsenic acid
	Wood preservative	Copper chromium arsenate
Industrials	Glass manufacture	
	Sulfur removal	
	Strengthen alloys	Element As
	Semiconductor, solar cells, and space material production	

Diverse forms of As can be found in the various environmental media including ore, rock, soil, groundwater, and surface water. The predominant forms of As in minerals are realgar (As_4S_4), arsenopyrite (FeAsS), and orpiment (As_2S_3). These As-bearing minerals are the most common arsenic sulfide minerals. The concentrations of As in rock depend on rock types. For example, the As concentration in sedimentary rock (shale and phosphate) is normally higher than that in other types of rocks (0.06 to 113 mg kg^{-1}). Therefore, mining and burning of coal, one type of the sedimentary rocks, could strongly affect the concentration of As in the surrounding environment. For example, Li et al. (2014) and Cao et al. (2013) reported the As contamination in soil

around the mining and coking areas in China with the concentrations approximately 216 mg kg^{-1} and 51.51 mg kg^{-1}, respectively. In the case of unpolluted soil, the concentration of As is generally lower than 10 mg kg^{-1}. However, wide variation of As in the soil can be found and the usage of pesticides, herbicides as well as fertilizers can affect the As concentration especially in agricultural soil. For instance, a very wide range (1 to 625 mg kg^{-1}) of As in the cultivated soils in the northern United States has been reported by (Adriano, 2001). The paddy soil in Bangladesh was found with the As concentrations of 1 to 88 mg kg^{-1} (average = 8 mg kg^{-1}) (Chowdhury et al., 2017). On the other hand, a narrower range of As in the Vietnamese (Thanh Binh District) soil (6 to 20 mg kg^{-1}) was reported by Huang et al. (2016). These previous reports clearly confirmed that As concentrations in soil are highly site specific.

It is well known that soil texture is the main factor affecting As mobilization in soil. The higher concentration of As tends to be released from sandy soil (coarse-grained soil) than clay soil (fine-grained soil). In addition, the availability and mobility of As in soil can be affected by several soil characteristics such as pH, oxidation-reduction potential (Eh),organic matter (OM), and constituent minerals such as oxyhydroxide of iron (Adriano, 2001).

pH plays the importance role of As mobilization. The major ratios of As compounds in soil and sediment under the different Eh and pH levels are shown in Figure 2-1. In general, the mobility of As is declined under an aerobic condition. Regarding the pH-Eh diagram of As shown in Figure 2-1, As(V) is a predominant As specie under the aerobic condition in which $H_2AsO_4^-$ and $HAsO_4^{2-}$ are the most stable forms at pH 2 – 7 and pH > 7, respectively. Under the moderate anaerobic condition with the range of pH 4 – 9, As(V) in the form of H_3AsO_3 can be reduced to As(III).

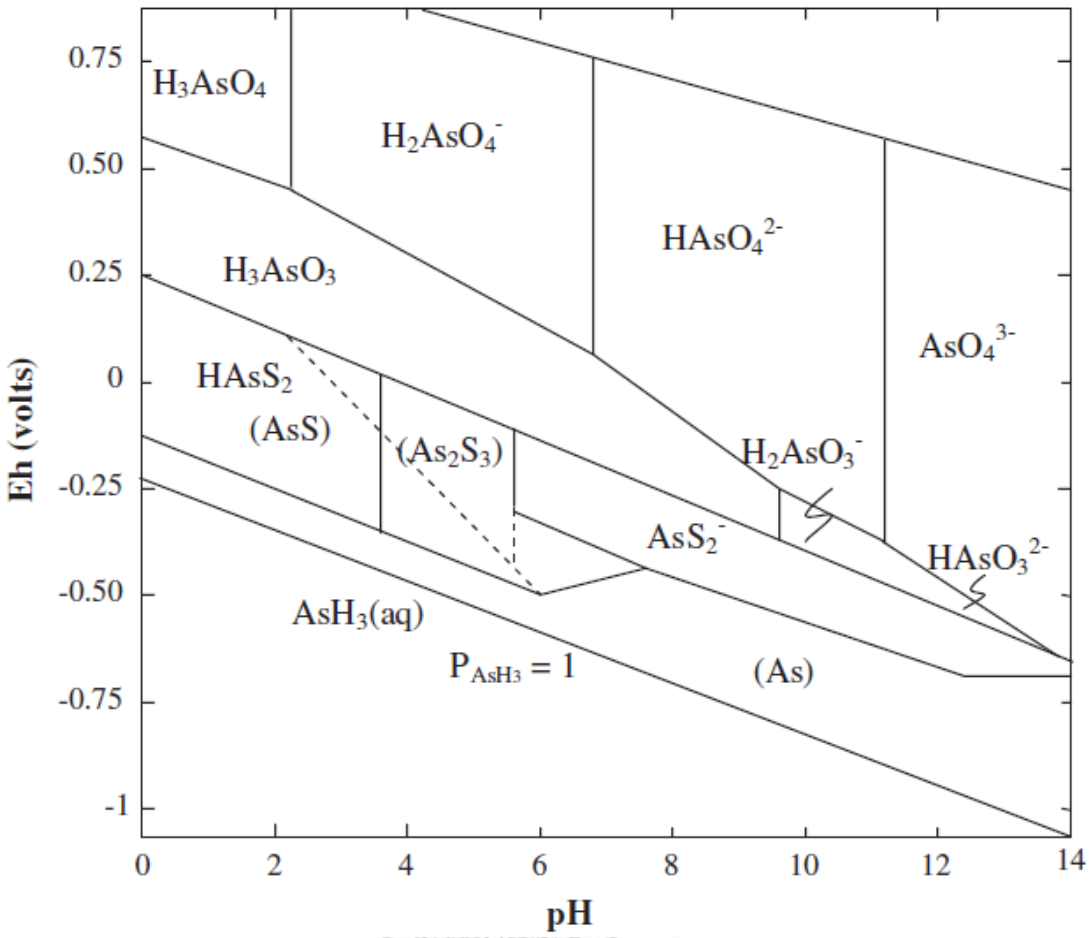


Figure 2- 1 The Eh-pH diagram of As at 25°C and 1 atm.
(Reprinted from Wang and Mulligan (2006))

An increasing in OM in soil from the addition of vermicompost and farmyard manure can decrease the mobility of As (Das et al., 2008). While, iron oxy/hydroxide (Fe(O)OH) has shown a strong capability to adsorb As onto the soil particles. However, the co-precipitation between Fe(O)OH and As normally decrease when pH is higher than 8.5 (Goldberg, 2002).

In the case of As in water environment, concentration of As in the non-contaminated areas is generally less than 10 µg L^{-1}. The worldwide surface water and groundwater As contamination have been reported by many studies. For example, the Karnaphuli River of Bangladesh was contaminated with 34.46 µg L^{-1} (winter) and 23.36 µg L^{-1} (summer) of As which was released from the upstream area (Ali et al., 2016). While, the interaction between water and soil enriched with As has caused the As contamination in groundwater with the level up to 5,000 µg L^{-1} (Smedley and

Kinniburgh, 2002). The West Bangal of India, as an example, has been recognized as the As contaminated land since 1980's. The As level in groundwater in 9 out of 19 districts was higher than 300 µg L^{-1} (Bhowmick et al., 2018). Approximately 5% of the investigated tube wells (21,155 wells) in China were found with As exceeding the former Chinese groundwater standard (50 µg L^{-1}) (Rodriǵuez-Lado et al., 2013).

In case of Southeast Asia region, As concentrations ranging from 6.64 to 1,543 µg L^{-1} were detected in Kandal Province of Cambodia. Approximately 86% of samples contained As higher than the WHO drinking water standard (10 µg L^{-1}) (Luu et al., 2009). The As-rich areas in Mekong Delta floodplains, which cover the Southern Vietnam and border of Cambodia, were contaminated by As with a range of 0.1 to 1,340 µg L^{-1} (Buschmann et al., 2008). Approximately 37% of the studied wells contained As exceeding the WHO standard. The iAs, a combination of As(III) and As(V), was the main chemical form of As in the groundwater collected from the Mekong Delta areas. Three main mechanisms that enhance As mobilization from soil and sediment to groundwater in these areas are; 1) sulfide minerals (as FeS_2) oxidation under an aerobic condition in water, 2) the acidic (pH < 7) and aerobic condition in water that cause the release of $H_3As(III)O_3$ and $HAs(III)O_4^{2-}$ from ferric arsenate and ferric arsenite into the groundwater, and 3) an anion exchange of As adsorption with phosphate from fertilizers (Singh, 2006).

1.3) Toxicity of As

The toxicity of As has become a problem of concern since As, particularly iAs has been classified as a carcinogenic substance. Inorganic As (As(III) and As(V)) can cause both acute and chronic adverse human health effects. The symptoms of short-time (acute) exposure initially begin with a metallic taste of garlicky odor notice with dry mouth and difficulty in swallowing. After that, the symptoms of severe nausea and vomiting, abdominal pain, and diarrhea are developed. Next, the extreme effects such as muscle cramping along with kidney damage inducing drowsiness and confusion may appear. Finally, shocking symptom can cause seizures, coma, and death (World Health Organization, 2010). In the case of long-term exposure of As, particularly iAs, dermal lesions (e.g. hyperpigmentation, hyperkeratosis, desquamation, and hair loss) are the most visible symptoms (Duker et al., 2005). Inorganic As can cause peripheral

neuropathy, gastrointestinal symptoms, diabetes, and renal system effects. In addition, the International Agency for Research on Cancer (IARC) has classified the iAs as human carcinogenic substance (Group 1), especially to lung, bladder, kidney, skin, and liver (International Agency for Research on Cancer, 2012). After human body absorption of iAs, iAs is enzymatically sequentially converted to the oAs including the methylated products such as MMA and DMA. The toxicity of arsenicals conforms to the following order, from highest to 1owest toxicity: arsines > inorganic arsenites > organic trivalent compounds (arsenoxides) > inorganic arsenates > organic pentavalent compounds > arsonium compounds > elemental arsenic (Adriano, 2001)

In general, ingestion is a main exposure pathway of chronic As exposure. Apart from drinking water, food is the other major source of As in human (International Agency for Research on Cancer, 2012). Approximately 40% of As in the human body comes from the food chain (Flora, 2015). Rice is a principal source of As, especially iAs exposure, in the general population living in the non-As endemic regions (Sohn, 2014; Flora, 2015; Lai et al., 2015).

2. As contamination in rice

2.1) Mobilization and transportation of As in the rice cultivation system

The As concentration in plant tissues cultivated in the non-contaminated areas is normally lower than 1.5 mg kg^{-1}. In general, plant can uptake As from the contaminated environmental media such as soil and water that it was cultivated with. The As translocation normally decreases from root to stem and leaf to edible part (Adriano, 2001). The As accumulating capabilities depend on many factors including cultivation media (i.e. soil and water), availability of As, as well as the species of cultivated plant. *Pteridaceae,* a fern family plant, is one of the As hyperaccumulating plants that can accumulate up to 20,000 mg kg^{-1} of As in its tissues (Wenzel, 2013). In addition, Farooq et al. (2016) found that the wetland plants has higher capacity to uptake As from the environment and accumulate it in their tissues than the that of the terrestrial plants. Since rice is also considered as a wetland plant, rice is therefore considered as an As hyperaccumulator. Regarding to Williams et al. (2007) and Sohn (2014), rice generally accumulates approximately 10 times of higher As concentration than the other food crops such as maize and barley.

Paddy rice (*Oryza sativa* L.), one of the food crops in the grass family, is considered as a staple food of human living in Southern and Southeast Asia. Regarding the wet and warm conditions in these regions, rice is, therefore, cultivated under the submersion condition (Gnanamanickam, 2009). The flooded paddy soil condition normally leads to the anaerobic and reducing conditions. These conditions, consequently, enhance the mobility and availability of As in soil. With these environmental conditions, As is readily to be taken up into the rice tissues.

Not only flooded condition, other soil characteristics including soil texture, OM, Fe(O)OH contents, pH, Eh, phosphate (P), silicon (Si), and soil microorganisms can also increase the As availability in the rice cultivation system (Azam et al., 2016). For instance, the presence of P(V), one of the predominant nutrient in soil and fertilizer, in the rice growing system can reduce the As uptake in rice as the transportation pathway of P(V) through rice root under the aerobic condition is similar to that of As(V) (Li et al., 2011). Similarly, the Si transport pathway in rice is also similar to that of the As(III). Therefore, the addition of silicic acid to the rice cultivation can inhibit As uptake by rice (Tripathi et al., 2014).

The As species that are readily available for plant uptake from soil solution are As(III), As(V), and methylated As (e.g. DMA and MMA). Regarding different As species, several pathways of As uptake and transportation to rice may affect the level of As accumulation in rice. For instance, As(V), the predominant form of As under the aerobic condition, is taken up through the similar transport pathway as P(V). In contrast, As(III) which is the predominant form of As under the anaerobic condition, has a similar tetrahedral size as well as high pKa value to the silicic acid and arsenious acid, thus, Si and As(III) are sharing the same pathway of transportation into the rice plant. In case of the DMA and MMA, they are normally transported through the plasma membrane at rice root, which is called aquaglyceroporins, via the glycerol transport pathway (Rahman et al., 2011).

Figure 2-2 depicts the potential sources of As as well as the transformation and translocation of As in the rice cultivation system.

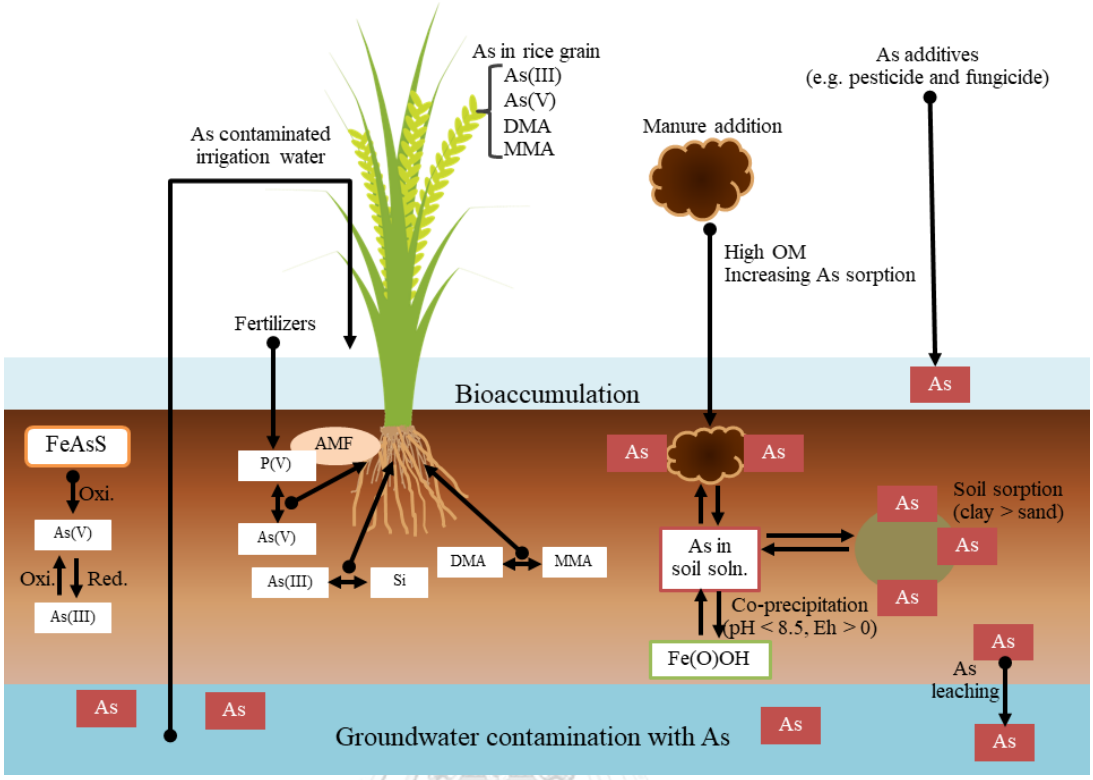


Figure 2- 2 Pathway of As uptake and accumulation in rice.
(modified from Azam et al. (2016)).

2.2) Accumulation of As in difference parts of rice

After uptaken through rice root, As can be translocated to other parts of rice. The concentration of As in rice generally decreases following the order of root > straw > husk > grain (Wang et al., 2006; Rahman et al., 2007; Smith et al., 2008; Lei et al., 2013). The concentrations of As in different parts of rice are summarized in Table 2-2. It was reported that the accumulation of As in rice normally depends on the concentrations of As in the cultivation soil and irrigation water (Islam et al., 2016).

Table 2- 2 As concentrations (mg kg^{-1}) in different parts of rice

Study area	Rice part				Reference
	Root	Straw	Husk	Grain	
West Bengal, India	7.19 – 18.63	1.2 – 4.2	0.56 – 1.35	0.25 – 0.73	Bhattacharya et al. (2010)
Brazil[a]	64.8 – 373.0	13.5 – 38.2	-	0.7 – 2.1	Batista et al. (2014)
Taiwan[b]	126 – 464	2.3 – 7.5	-	0.076 – 1.183	Lin et al. (2015)
China	19.6 – 380.5	0.43 – 6.0	-	0.065 – 0.277	Ma et al. (2017)

Remark: [a] Results obtained from the pot experiment
[b] Results obtained from the field experiment

Marin et al. (1993) found that different rice parts can accumulate different As species. Among several As species, DMA was rapidly taken up and translocated from rice root to the shoot. While As(III), As(V), and MMA were normally located and accumulated in the root. Smith et al. (2008) further reported that As(III) and As(V) were the dominant As species accumulated in the roots, stems, and leaves of rice plant. Although, the lowest As concentration was found to be accumulated in rice grain as comparing to the other parts of rice plant, high concentration of the most toxic of As form, As(III) was normally found in this edible compartment of rice. Smith et al. (2008) reported that approximately 85% to 94% of total As in rice was in the forms of As(III) and DMA. Therefore, the study on both tAs and iAs concentrations in rice grain is important to the public health and scientific aspects.

3. Total and inorganic As determinations in rice grain

3.1) Concentrations of tAs and iAs in rice

Since rice is a staple food for human consumption, human can expose to the As in rice through the ingestion pathway. Due to the fact that As, especially iAs is a carcinogenic substance, the maximum allowable concentrations of iAs in polished (0.20 mg kg^{-1}) and non-polished (0.35 mg kg^{-1}) rice grain have been recommended to protect the adverse health effects which may be developed in the general population (Food and Agriculture Organization/World Health Organization, 2014; Food and Agriculture Organization/World Health Organization, 2016). Since the determination of iAs is time

consuming and requires an advanced analytical technology, the Codex Commission which is the joint initiative of FAO/WHO, has agreed for the use of tAs concentration as a screening approach for the iAs in rice. The determination of iAs is necessary when the concentration of tAs is higher than the FAO/WHO guideline values. On the other hand, rice is assumed to be safe for consumption when tAs is lower than the FAO/WHO recommended iAs standards.

The concentrations of tAs ad iAs contained in rice from many countries are summarized in Table 2-3. As clearly shown in the table, it is possible to be concluded that the As concentration in non-polished rice was generally higher than that of the polished rice. It is because As mainly localizes at the grain surface, which is the layer between pericarp and aleurone layer, and the rice germ (Meharg et al., 2008; Lombi et al., 2009; Kramar et al., 2017). Figure 2-3 demonstrates the localization of As in non-polished (brown) and polished (white) rice grain.

Table 2- 3 Concentrations of tAs and iAs and the present of iAs in rice grain from different countries

Country	Type of rice	Concentration (mg kg^{-1})[a]		%iAs/tAs	Reference
		tAs	iAs		
Australia	Polished (n = 3)	0.283	0.165	58%	Rahman et al. (2014)
	Non-polished (n = 3)	0.438	0.276	63%	
Bangladesh	Polished (n = 3)	0.590 (0.32 – 0.75)	0.197 (0.18 – 0.22)	33%	Sun et al. (2008)
	Non-polished (n = 4)	0.735 (0.41 – 0.98)	0.313 (0.23 – 0.39)	43%	
Brazil	N/D (n = 13)	0.212 (0.06 – 0.78)	0.077 (0.02 – 0.17)	48%	Ciminelli et al. (2017)
Cambodia	N/D (n = 10)	0.255 (0.01 – 1.19)	0.204 (0.01 – 0.95)	80%[b]	Phan et al. (2013)
China	N/D (n = 43)	0.129	0.118	86%	Ma et al. (2016a)
India	N/D (n = 29)	0.283	0.194	69%	Halder et al. (2014)
South Korea	Polished (n = 2)	0.116	0.066	57%	Yim et al. (2017)
	Non-polished (n = 2)	0.200	0.139	70%	
Taiwan	Polished (n = 51)	0.117	0.065	56%	Chen et al. (2016)
	Non-polished (n = 13)	0.216	0.106	49%	
Thailand	Polished (n = 79)	0.140 (<0.01 – 0.30)	0.085 (0.01 – 0.16)	63.2%	Nookabkaew et al. (2013)
	Non-polished (n = 14)	0.239 (0.12 – 0.34)	0.124 (0.08 – 0.21)	53.6%	
United States	N/D (n = 158)	0.198 (<0.01 – 0.71)	0.103	52%	Zavala and Duxbury (2008); Zavala et al. (2008)
Vietnam	Polished (n = 10)	0.136 (0.09 – 0.20)	0.091 (0.04 – 0.16)	67%	Nookabkaew et al. (2013)
	Non-polished (n = 2)	0.299 (0.25 – 0.34)	0.213 (0.20 – 0.23)	73%	

Remark: N/D means the type of rice studied was not defined.

[a] Concentration is presented as an average value. Numbers in the parenthesis are the range of As concentrations.

[b] Number is reported based on an estimation.

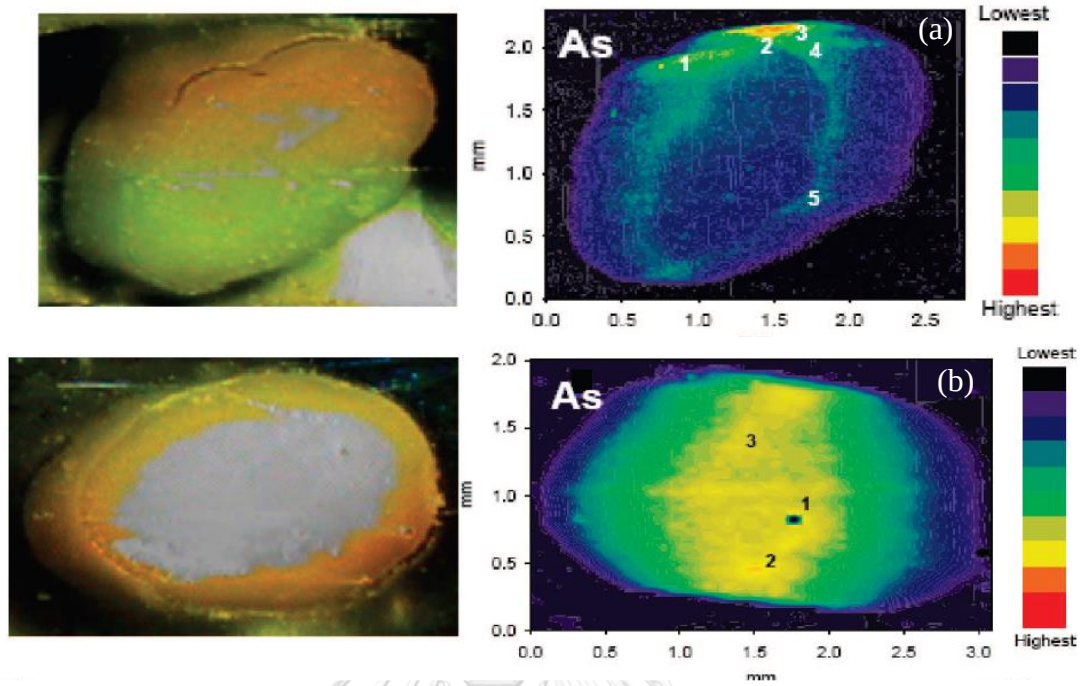


Figure 2- 3 The synchrotron X-ray fluorescence (*S-XRF*) derived elemental maps of As in (a) non-polished and (b) polished rice.
(Reprinted from Meharg et al. (2008))

3.2) Methods of tAs and iAs determinations

There are several methods that can be used to determine As concentration in rice grain. A schematic diagram on the methods of As determinations are summarized in Figure 2-4.

In order to avoid the complexity, time consuming, and high technology demanding, the determination of tAs is recommended. In general, the analysis of tAs can be divided into two steps; digestion and determination. There are several procedures that can be applied for food matrix digestion. Most of them used nitric acid (HNO_3) as a main digested chemical (Phan et al., 2013; Ciminelli et al., 2017; Roya and Ali, 2017). Some studies used a mixture of HNO_3 with hydrogen peroxide (H_2O_2) in the different ratios (Zavala and Duxbury, 2008; Ahmed et al., 2015; Jallad, 2015; Ma et al., 2016b; Shrivastava et al., 2017). Food sample is subjected to various digestion methods such as microwave digestion, opened digestion, and closed digestion. Finally, the concentration of As can be determined by various instruments such as hydride

generation atomic absorption spectroscopy (HG-AAS) and inductively coupled plasma-mass spectroscopy (ICP-MS).

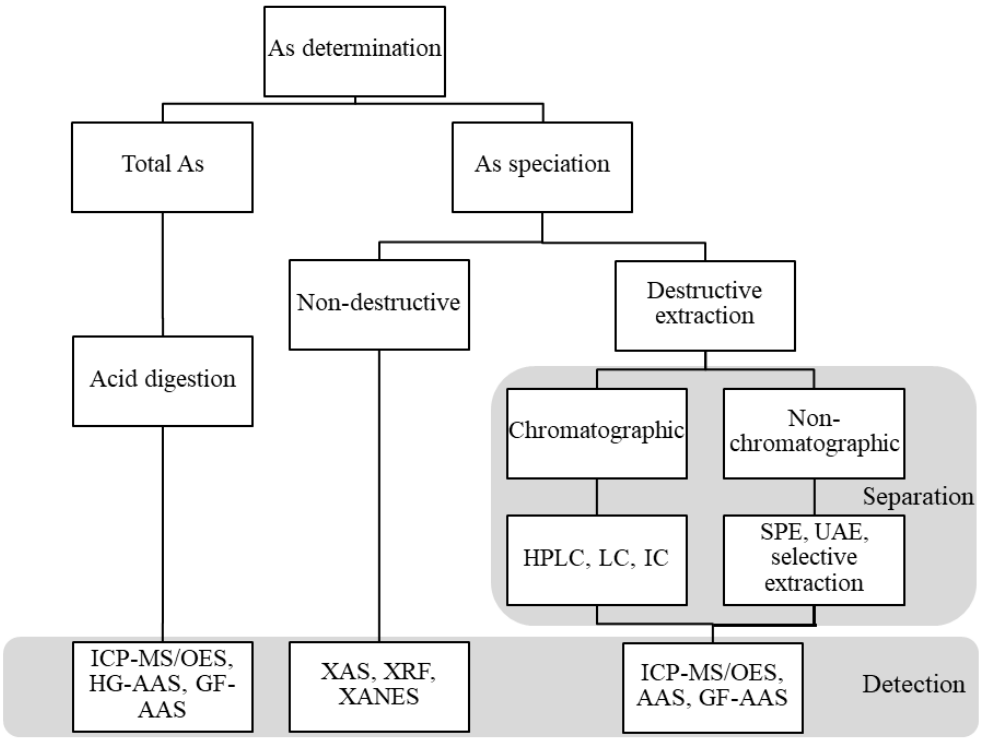


Figure 2- 4 Schematic diagrams on the methods of As determinations

Since iAs is the most toxic form of As and the FAO/WHO has set the maximum allowable concentration of iAs in rice, the determination of iAs is also necessary especially when the tAs is greater than the FAO/WHO standard. In general, iAs is a summation of As(III) and As(V). Two main procedures including non-destructive and destructive methods can be employed to extract As species contained in the sample (Figure 2-4). Though, the non-destructive method (i.e. *in situ* synchrotron X-ray absorption spectroscopy; XAS) does not change the species of As, this method which requires a very high performance instrument could provide only the qualitative data (Meharg et al., 2008; Lombi et al., 2009; Carey et al., 2010).

In contrast, the destructive method can be used to quantify the species of As in rice grain. This destructive method comprises of the step of extraction and separation. The important step for iAs determination is a separation of As species which is normally

conducted by using either the chromatography or non-chromatography technique. According to the United States Food and Drug Administration (US FDA), the chromatography separation using high performance liquid chromatography coupled with inductively coupled plasma mass spectroscopy (HPLC-ICP-MS) is recommended (Kubachka et al., 2012). Even this technique gives better sensitivity and more accurate quantification results, the expensive cost of analysis and the limitation in availability of this advanced technology are the most significant drawbacks.

Therefore, the non-chromatographic separation process which requires less laboratory facility and equipment was recommended. However, the methods of sample extraction and separation should be carefully selected according to the nature of food matrix (Welna et al., 2015). Up to date, several extraction methods including HCl back-extraction (Jorhem et al., 2008; Fontcuberta et al., 2011), Ethylenediaminetetraacetic acid (EDTA) with ultrasonic assisted extraction (Pasias et al., 2013), and the selective hydride generation (Chaney et al., 2018) were tested. The comparisons between these non-chromatographic iAs separation methods are summarized in Table 2-4.

Table 2- 4 Comparisons between different non-chromatographic iAs separation methods

	HCl acid back-extraction	**EDTA extraction**	**Selective hydride generation**
Sample extraction	Water (1 mL) was added into 2.5 g of rice sample. The sample was left overnight after 10 mL of HCl (37% v/v) was added. Afterward, 1 mL of HBr (47% v/v) and 0.5 mL of $NH_2NH_2{\cdot}H_2SO_4$ were added and the solution was shaken for 30 s. Then, 4.8 mL of $CHCl_3$ was added. After the solution was shaken for 3 min, it was centrifuged for 15 min. Finally, the $CHCl_3$ layer (lower part) was separated into the new tube. This procedure should be repeated for two more times.	Rice sample (0.5 g) was digested by 5 mL of HNO_3 (1 M). Then, sample was vortexed, ultrasonicated, and centrifuged (4,000 rpm). Each step took 15 min.	Rice sample (0.70 g) of rice sample was digested by 10 mL of HNO_3 (0.28 M). Then, the sample was heated in a water bath at 95 °C for 90 min. Next, sample was left to achieve room temperature. The sample solution was filtrated by Whatmann filter paper no. 40 and made up total solution volume to 20 mL.

Table 2-4 Comparisons between different non-chromatographic iAs separation methods *(continued)*

	HCl acid back-extraction	**EDTA extraction**	**Selective hydride generation**
Extraction of iAs	Back-extraction was done by adding 4.8 mL of HCl (1 M) into the tube containing digestion solution and shaking for 3 min. Then, the upper part (acid layer) was separated into a 10-mL volumetric flask. This process was repeated one more time. Finally, the total volume of the solution was made to 10 mL by 1 M of HCl.	Precisely 15 mL of 0.1% EDTA (w/v) was added, vertexed, and centrifuged for 15 min. Then, the supernatant was separated for analysis.	A mixture of 8 mL of the rice digestion solution, 3 mL of HCl, 6 mL of KI/AA solution (5% w/v), and 5 mL of sulfamic acid was made. Then, sample was left stand for 20 min.
Apparatus	N/A	Ultrasonic bath	N/A
Analytical instrument	High-resolution HPLC-ICP-MS	Electrothermal atomic absorption spectroscopy (ET-AAS)	HG-ICP/OES
Limit of detection	0.005 mg kg^{-1}	0.030 mg kg^{-1}	0.002 mg kg^{-1}
Reference	Jorhem et al. (2008) Fontcuberta et al. (2011)	Pasias et al. (2013)	Carney et al. (2018)

Remark: N/A means not applicable.

4. Bioaccessible As concentration in rice

4.1) Bioaccessibility of substance in food

The total level of the substance which is directly determined from the sample, may not reflect the potential amount of the substance that circulated in the blood stream. Thus, the term of bioaccessibility of substance is introduced. The bioaccessible concentration is the quantity or fraction of compound or substance from the food matrix which is readily to be absorbed into the human gastrointestinal tract. The concentration of bioaccessible fraction of substance is a very important input variable in the process of health risk assessment since it indicates the amount of substance released from food and absorbed into the blood stream and can become bioactive substance. The concentration of bioaccessible fraction can be applied as the worst case scenario of substance exposure (Cardoso et al., 2015).

The bioaccessible fraction of substance in food can be determined by either *in vivo* or *in vitro* digestion method. In principal, both processes involve the food transportation in digestive system, the absorption into intestinal system, and pre-systematic metabolism into the process (Carbonell-Capella et al., 2014). However, the main difference of these two digestion methods is the method of digestion system. The *in vivo* digestion method is normally conducted in the animal or human subject while, the *in vitro* digestion method can be simulated in the laboratory without the involvement of any living organisms. The advantages and disadvantages of *in vivo* and *in vitro* digestion methods are compared in Table 2-5.

Table 2- 5 Advantages and disadvantages of *in vivo* and *in vitro* digestion methods for the extraction of bioaccessible fraction of substance from food

	***In vivo* digestion**	***In vitro* digestion**
Methodology	- Digestion method involves either animal or human subject in the study	- Digestion method can be simulated following the human gastrointestinal digestion
Advantages	- Subject-specific study - Provide pharmacokinetic information of substance	- Cost-effective technique - Simple laboratory experiment
Disadvantages	- Complexity of functional systems - Uncertainty in the extrapolation from animal studies to human - Lacking of certified reference standard - Expensive laboratory experiment - Ethical constraints	- Uncertainty in the extrapolation from *in vitro* to *in vivo* system - Not include the homeostatic mechanism into the study - Complete dynamic conditions of gastrointestinal tract cannot be conducted - The intestinal bacteria and hepatic metabolism are not considered in the study

Due to its simple experiment, the *in vitro* digestion method was recently applied in many studies to analyze the bioaccessible concentration of substances in several environmental media, for example, sediment, soil, and foodstuffs. Several models of the *in vitro* digestion methods which were conducted in the previous studies are summarized in Table 2-6.

Table 2- 6 Summary of different in vitro digestion methods used in the previous studies to extract bioaccessible fraction

Model of *in vitro* digestion	**Digestion condition**	**Limitation of the model**	**Reference**
Physiologically Based Extraction Test (PBET)	- Static gastrointestinal model - Simulation of the mobilization of elements from the solid matrix under gastric pH conditions	- Oral and small intestine digestion are not involved - Suitable for the analysis of bioaccessible fraction in soil	Bruce et al. (2007)
Method E DIN 19738	- Static gastrointestinal model - Simulation of digestive juices (gastric and intestinal juices) in the digestion	- An oral digestion is not considered - Suitable for the analysis of bioaccessible organic compound - Suitable for the analysis of bioaccessible fraction in soil	Hack and Selenka (1996)
Simulator of Human Intestinal Microbial Ecosystem of Infants (SHIME)	- Static gastrointestinal model - Simulation of young children gut - Full configuration of 3 colon compartments with a mixed microbial community	- Not suitable to be used for adult's digestion model - Complexity of the digestion model	Van De Wiele et al. (2007)
RIVM	- Static gastrointestinal model - Simulation of oral, gastric, and small intestine digestions	-Validation of the method	Versantvoort et al. (2004)
TNO Gastrointestinal Model (TIM)	- Dynamic gastrointestinal model - Simulation of the pH profile in the digestion - Continuous addition of enzyme	- An oral digestion is not considered - Complexity of the digestion model	Minekus et al. (1995)

4.2) *in vitro* digestion methods for As in rice

The bioaccessible fraction of As in rice was previously studied using both *in vivo* and *in vitro* digestion methods. Juhasz et al. (2006) studied the bioaccessibility of As in rice using the *in vivo* digestion with the swine animal model and claimed that the iAs in Basmati rice cooking with iAs contaminated water has shown the high percentage of bioaccessibility (89.4 ± 9.4%). In contrast, it was found that only 33.1 ± 3.2% of As were found in rice cooking with As-free water and the predominant As form in rice is the organic As (DMA). Islam et al. (2017) studied the bioaccessibility of As in different genotypes of cooked rice in swine and found approximately 25% to 94% of bioaccessible As in which the predominant species was iAs.

Since the *in vivo* digestion has a limitation of the involvement of animal or human subjects in the study (Table 2-5), the *in vitro* digestion has been introduced as an alternative method for the study of the As bioaccessibility in rice. Several *in vitro* digestion models have been developed in order to simulate human gastrointestinal digestive system. In principal, *in vitro* digestion normally consists of either two or three steps of gastrointestinal digestive system including oral cavity (optional), stomach, small intestine. Different digestive juices should be synthesized to extract the bioaccessible fraction of As from food matrix (Omar et al., 2013). Up to date, 3 simplified *in vitro* digestion models including PBET, SHIME, and RIVM have been applied in the study of the As bioaccessibility in rice as summarized in Table 2-7.

To be more specific, PBET is an *in vitro* digestion method that suitable for the solid matrix sample. At the beginning, this approach was used to predict the soil metal bioaccessibility in the gastrointestinal tract (Ruby et al., 1996). The mimic digestive system focuses on two parts including gastric and small intestine. PBET was also applied to extract the bioaccessible As fraction in the rice flour. For example, Zhuang et al. (2016) determined the As bioaccessibility in raw and cooked rice in the different parts of gastrointestinal tract and found the highest percentage of As bioaccessibility (75% to 96%) in the small intestine. Moreover, they reported that approximately 72% to 80% of tAs was in the form of bAs.

Meanwhile, SHIME, another *in vitro* digestion model, has been developed to mimic the static gastrointestinal system of young children. Beside the oral capacity (optional), stomach, and small intestine digestions, the microbial community in 3 colon

compartments are incorporated into the digestion. The SHIME *in vitro* digestion scheme was also employed for the analysis of As bioaccessibility in cooked rice (Sun et al., 2012). It was found that only 55% to 57% of tAs was bAs. The bioaccessibility of As slightly increased during the small intestine digestion but decreased over the time in colon.

The RIVM, has been extensively used to assess the bioaccessibility of chemicals in food. This model was developed by the National Institute of Public Health and the Environment of the Netherlands. Even though this method was firstly developed to analyze the bioaccessibility of toxic compounds in the contaminated soil (Sips et al., 2001), it is, later on, adopted to demonstrate the bioaccessibility of chemicals in food, for example, cadmium (Cd) in vegetables (Versantvoort et al., 2004), and As in seaweed (Brandon et al., 2014) and rice (Praveena and Omar, 2017). This RIVM method is currently considered as the most appropriate *in vitro* digestion method for the assessment of bioaccessible content in rice since all digestion systems (oral, gastric, and small intestine) involved in this method are the main carbohydrate digestion processes in human (Omar et al., 2015).

Table 2- 7 Comparisons of three different of in vitro ingestion models

		PBET	SHIME	RIVM
Amount of rice sample		0.5 g	1.82 g	4.5 g
Step of gastrointestinal digestive system	Oral cavity	N/A	Incubation of 14 mL of saliva solution at 37°C for 5 min	Incubation of 6 mL of saliva solution (pH 6.8) at 37°C for 5 min
	Gastric	Addition of 50 mL of gastric solution (pH 1.5) and incubation at 37°C for 1 h	Addition of 21 mL of gastric juice (pH 2) and incubation at 37°C for 2 h	Addition of 12 mL of gastric juice (pH 2 – 3) and incubation at 37°C for 2 h
	Small intestine	Addition of 52.5 mg of bile salts and 15 mg of pancreatin (pH 7) and incubation at 37°C for 2 h	Addition of 24 mL of duodenal juice (pancreatin) and 8 mL of bile solution (pH 6) and incubation at 37°C for 4 h	Addition of 12 mL of duodenal juice, 6 mL of bile solution, and 2 mL of HCO_3^- (pH 6.5 – 7) and incubation at 37°C for 2 h
	Colon	N/A	Transfer of 35 mL of digested solution into 100 mL of serum bottles, addition of 35 mL of colon SHIME solution, flushing with N_2 gas for 30 min, and incubation at 37°C for 48 h	N/A
	Reference	Zhuang et al. (2016)	Sun et al. (2012)	Versantvoort et al. (2004)

Remark: N/A means not applicable.

5. Effects of rice polishing on As content

Regarding to the rice grain structure shown in Figure 2-5, rice hull or rice husk have to be removed from the rice grain before human consumption. After the hull is removed, the non-polished (brown) rice can be obtained. Even though, brown rice is a source of energy, protein, several antioxidants, vitamins, lipids and minerals to human, polished rice (white rice) is commonly consumed by human since it has superior cooking quality (Puri et al., 2014).

Polishing or milling is a process which is employed to remove the bran or outer layers of the non-polished (brown) rice to resulted in the polished rice grain for human consumption. Several parts of rice grain including pericarp, seed coat, nucellus, and aleurone layer are removed from the rice grain during polishing. Since several beneficial substances to human such as protein, lipids, vitamins, and nutrients are normally found in the rice bran, significant reductions of lipids, proteins, vitamins B (B1, B3, B6, and B9) and minerals were reduced after the rice passed through the polishing process (Perdon et al., 2001). The higher degree of polishing (DOP) applied to the process, the higher losses of nutrients and trace elements (e.g. As, Cr, Mn, and Sr) in rice grain were also found (Liu et al., 2009; Narukawa et al., 2014).

Not only the losses of several beneficial substances from rice grain as a result of polishing, the concentration of As was also reduced during rice polishing. According to the previous studies, non-polished rice has been found with significant tAs concentration higher than that of the polished rice since As normally localizes in the bran layer (Sun et al., 2008; Nookabkaew et al., 2013; Rahman et al., 2014; Chen et al., 2016). Narukawa et al. (2014) reported the higher concentration of tAs in the bran which was removed from the rice grain with the greater level of DOP. Comparing to the tAs concentration in non-polish rice, approximately 44.2 ± 9.4%, 70.1 ± 9.0%, and 91.7 ± 8.9% were found in the 10%, 30%, and 50% milled bran, respectively. The higher percentage of milling indicated the higher DOP level. Another study by (Yim et al., 2017) also found a significant effect of DOP on tAs in rice. Concentrations of tAs in the unit of mg kg^{-1} in the rice grain were 0.1240 ± 0.0077 for non-polished rice grain, 0.1182 ± 0.0048 for 95.3% of non-polished rice, 0.0933 ± 0.0034 for 75.2% of non-polished rice, and 0.0805 ± 0.0034 for 64.9% of non-polished rice.

In case of the iAs, similar trend of tAs reduction in the polished rice was found. Approximately 11% to 25% and 30% to 49% of iAs in 95% and 90% polished grain were reduced comparing to that of the non-polished rice grain.

These previous research results clearly confirmed the effect of DOP on the concentration of As in rice and the localization of As in the outer layer especially the bran layer of rice grain. However, the main constraint on the study of effect of polishing on As concentration in those previous studies is a failure to carry out the effect of different DOPs on As concentration from the same original sample. The polished rice used in those previous studies was not directly prepared from its own original non-polished rice. In fact, those previous studies normally collected available polished rice and non-polished rice and determine the tAs concentration without a consideration whether or not those polished rice were obtained from the same non-polished rice they have collected.

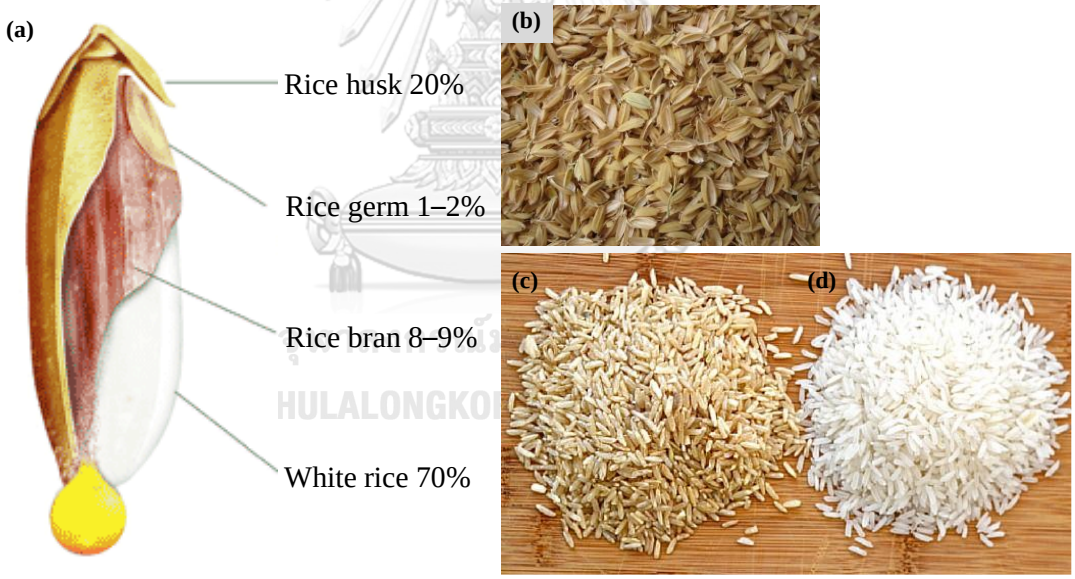


Figure 2- 5 Pictures showing (a) structure and composition of rice grain, (b) rice husk which is removed from the grain, (c) non-polished (brown) rice grain, and (d) polished (white) rice grain.

6. Effect of washing and cooking processes on As content

In practical, rice is washed and has to be cooked before consumption. Washing is recommended to remove milling dust and remaining bran. However, different rice varieties require different washing times (2 to 5 times). In case of Thailand, as the rice

that Thai population normally consumed contains medium amylase content, approximately 2 to 3 times of washing prior to cooking is recommended.

In the case of rice cooking, rice can be cooked using either traditional (cooking with excess water) or modern (cooking with enough amount of water) cooking method. The traditional process was commonly used to cook rice in the past. The modern cooking method is more popular nowadays since rice can be cooked easily using an electric rice cooker. Cooking rice using the rice cooker is more convenient for the general population. For the modern cooking method, rice is normally cooked with the optimum water to rice ratio and boiled until all water is absorbed (Perez and Juliano, 1978). While, the traditional cooking method can be done by boiling rice with the high water to rice ratio (10:1 to 20:1) (Chakkaravarthi et al., 2008). In addition, the excess water with the leached components from rice has to be discarded when rice is well cooked.

Several studies have been conducted to determine the concentrations of tAs, iAs, and bAs in the processed rice. For example, a study of Raab et al. (2009) demonstrated that approximately 13% to 15% of metal reduction when the Basmati rice, one type of extra-long grain rice, was washed. Meanwhile, only 4% of metal was washed out from the long grain rice. Thus, Raab et al. (2009) concluded that washing has minimal effect on As removal from rice grain. Similar to the previous study, approximately 8% of tAs was removed after the polished rice was washed (Gray et al. (2015). (Naito et al., 2015) reported the lowest tAs reduction (2%) even though the non-polished rice was washed for 3 times. For the reduction of iAs after washing, approximately 16% of iAs was reduced when rice was passing through the washing step (Jitaru et al., 2016).

Cooking is the rice processing step which could significantly affect the level of As in rice. This is particularly important when rice is cooked with the excess As-free water. For instance, Signes-Pastor et al. (2012) found approximately 0.7 times lower in tAs concentration in rice which was cooked with the 5:1 water to rice ratio. Gray et al. (2015) increased the water to rice ratio during rice cooking to 6:1 and 10:1 and respectively found 15% and 30%, on an average, of tAs reduction comparing to that of the raw polished long grain rice. This finding confirmed that when the water to rice ratio is increased, the higher As reduction was found. According to the high water

solubility of iAs, it might be discarded with surplus cooking water. This is particularly true for As(III) which has high mobility at neutral pH (Raab et al., 2009).

On the other hand, no significant tAs reduction in the rice grain was found when rice was cooked with the modern cooking method (Mihucz et al., 2007; Raab et al., 2009; Gray et al., 2015).

During the past decade, the concentration of bAs in cooked rice has been determined by few studies. Sun et al. (2012) digested the modern cooked rice and found the highest As bioaccessibility (38% to 57%) in the small intestine digestion. While, the different result was reported by Zhuang et al. (2016). The group found the highest percentage of As bioaccessibity in the gastrointestinal digestion (Zhuang et al. (2016). In addition, Zhuang et al. (2016) found that cooking can reduce the bioacceessibility of As by approximately 16% when compared to that of the raw rice.

7. Deterministic and probabilistic human health risk assessment

Risk assessment is a stepwise process which can be applied to identify the hazard of substance, the likelihood of adverse health effects, the magnitude of substance exposure and the presence or absence of unacceptable risk in human. Theoretically, there are four main steps of human health risk assessment (Figure 2-6) as following (International Programme on Chemical Safety, 2004);

1. Hazard identification: This step is conducted to identify the type and nature of adverse health effects of a chemical.

2. Hazard characterization: This step is conducted to analyze the relationship between total amount of an agent administered to, taken up by, or absorbed into the body and likelihood and severity of adverse health effects in response to the agent.

3. Exposure assessment: This is the process of measuring or estimating human exposure to an agent in the environment.

4. Risk characterization: This step is conducted to evaluate the nature and the presence or absence of risks from an agent exposure. The information on how the risk was assessed and the recommendation for decision making should also be provided.

The four steps of human health risk assessment are shown in Figure 2-7.

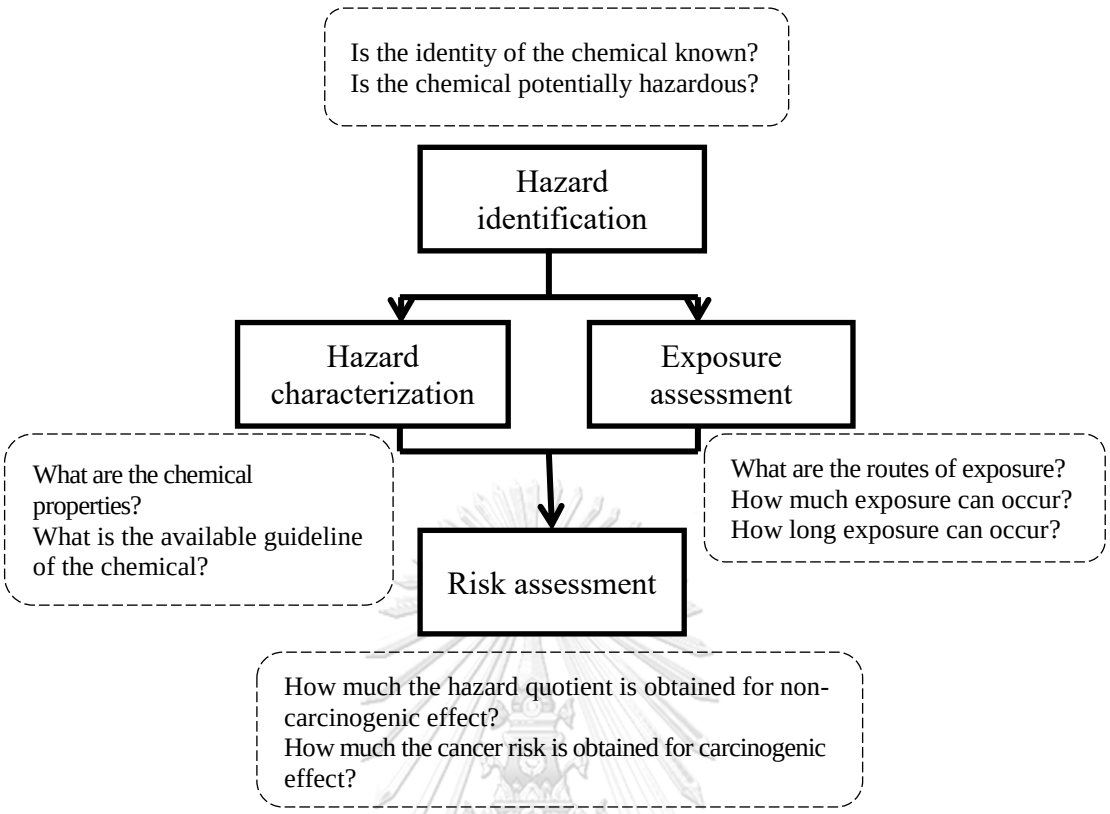


Figure 2- 6 Four steps of human health risk assessment.
(Adapted from International Programme on Chemical Safety (2010))

Two different human health risk assessment approaches including DRA and PRA can be applied to the steps of exposure assessment and risk assessment. The differences between DRA and PRA approaches including their calculations, strengths, and limitations are summarized in Table 2-8. Briefly, both DRA and PRA approaches follow the same human risk assessment steps to identify the exposure and risk of chemical in the population. The main difference between these 2 approaches is the type of statistical value used for each variable to calculate exposure and risk levels.

The DRA applies a single point value of each assessment variable such as substance concentration (CF), ingestion rate (IR), body weight (BW), exposure frequency (EF), exposure duration (ED), and averaging time (AT) into the calculation. While, the probable ranges of those variables are applied into the PRA approach. Since the DRA approach usually refers to the exact values as inputs to assess the risk without considering the uncertainty (incomplete) and variability (diversity) of data (U.S. Environmental Protection Agency, 2006; U.S. Environmental Protection Agency, 2011), results obtained from the DRA may be overestimated or underestimated.

The input variables of PRA as shown in Figure 2-7 are described by a probability distribution (e.g. CF, IR, BW). This approach was developed to reduce the impacts of the uncertainty and diversity of the data which could affect to the uncertainties in the results obtained. Therefore, the certainty degrees and the differences among exposed population (sensitive populations or life stages) can be included in the step of risk calculation (U.S. Environmental Protection Agency, 2014). As a consequence, PRA approach provides more accurate estimation in the range and possibility of a hazard, exposure, or risk.

Table 2- 8 Comparisons between two approaches (DRA and PRA) of risk assessment

	Deterministic risk assessment	**Probabilistic risk assessment**
Method of calculation	- Single point value (An average or upper percentile value is used) - Same equation for exposure assessment calculation is used	- Probability distribution (Monte Carlo simulation) is used - Same equation for exposure assessment calculation is used
	Equation for exposure assessment calculation Exposure = [CF × IR × EF × ED]/[BW × AT]	
	- Single point values applied into the equation are substance concentration (CF), ingestion rate (IR), and body weight (BW)	- Values of probable ranges applied into the equation are substance concentration (CF), ingestion rate (IR), and body weight (BW)
	- Constant values applied into the equation are exposure frequency (EF), exposure duration (ED), and averaging time (AT)	- Constant values applied into the equation are exposure frequency (EF), exposure duration (ED), and averaging time (AT)
Strengths	- Simple calculation - Conservative exposure assumptions - Relatively quick and economical method - Pre-screening approach	- More detailed analysis - Realistic exposure or best estimate assumptions - Include uncertainty and variability of data into the model
Limitations	- Not include uncertainty and variability of data - Causes overestimation or underestimation	- Complex analysis - Require more resources (e.g. software, data, trained staffs, or tools)

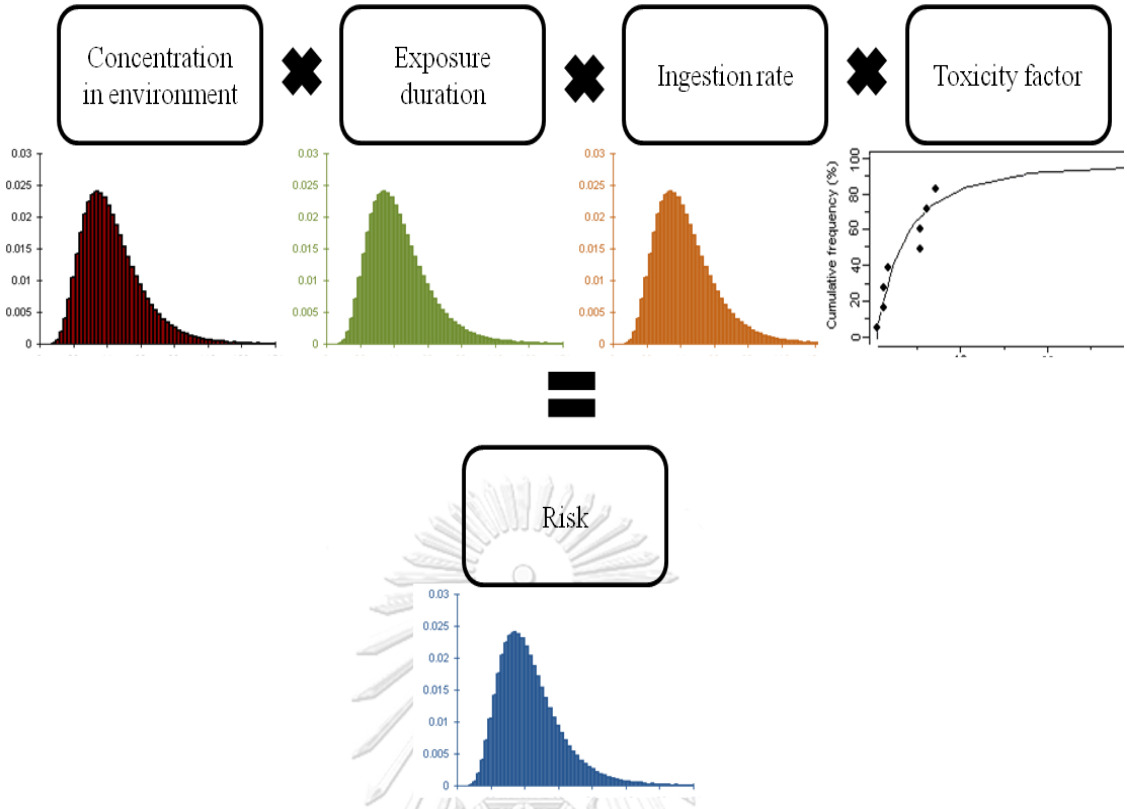


Figure 2- 7 Schematic diagram of PRA approach.
(Modified from U.S. Environmental Protection Agency (2014))

CHAPTER III

TOTAL, INORGANIC, AND BIOACCESSIBLE ARSENIC CONCENTRATIONS IN RAW POLISHED AND NON-POLISHED RICE BASED ON A MARKET SURVEY IN BANGKOK

1. Introduction

According to high carbohydrate content (approximately 79%) in its composition, rice (*Oryza Sativa*) has become a staple food to provide the main energy supply to human worldwide (Gnanamanickam, 2009). In 2017, the global polished rice production was approximately 489 million tons (Foreign Agricultural Service/U.S. Department of Agriculture, 2018). The Asia is considered to be the largest rice cultivation area (Nakaya et al., 2018). In addition, approximately 80% of total rice production was consumed by the Asian population (Foreign Agricultural Service/U.S. Department of Agriculture, 2018)

With the considerations of the high capacity of As accumulation in rice plant (Williams et al., 2007), the high rice consumption rate (Foreign Agricultural Service/U.S. Department of Agriculture, 2018) and the high toxicity of As (World Health Organization, 2010; International Agency for Research on Cancer, 2012), the concerns on public health impacts from As exposure through rice consumption have been caught in the spotlight. This leading to the announcement of the maximum allowable iAs in both polished (0.2 mg kg^{-1}) and non-polished rice (0.35 mg kg^{-1}) to ensure the safe exposure of As in the general population as well as the sensitive groups of population such as infants, toddlers and young children (Food and Agriculture Organization/World Health Organization, 2014; Food and Agriculture Organization/World Health Organization, 2016). Thailand, in particular, was in the 2nd rank of amount of rice exported (11.0 million tons) and the 7th rank of amount of domestic rice consumed (10.2 million tons). The average rice consumption during 2018 in Thailand was approximately 99 kg per capita per year (Organisation for Economic Co-Operation and Development/Food and Agriculture Organization, 2019).

To avoid a limitation in the instrument availability and the complexity of analytical procedure, the Codex Alimentarius Commission allows the country to use

the concentration of tAs as a screening level for the iAs in rice. Rice is assumed to be safe for consumption when the tAs concentration is lower than the FAO/WHO standard. The determination of iAs is required when tAs is higher than the standard. The concentrations of tAs in rice grain were determined and reported in many countries, for example, Chinese rice, Cambodian rice, and Thai rice were found to contain approximately 0.119 mg kg^{-1}(Qian et al., 2010), 0.012 to 0.578 mg kg^{-1} (Phan et al., 2013) and 0.002 mg kg^{-1} to 0.343 mg kg^{-1} (Nookabkaew et al., 2013) of tAs, respectively.

In the case of Thai rice, most studies on As contamination in rice mainly focused on the tAs concentration with a limitation of small sample size (Hensawang and Chanpiwat, 2017). For example, the ranges of tAs in polished (n = 79) and non-polished (n = 14) rice were 0.002 mg kg^{-1} to 0.304 mg kg^{-1} and 0.118 mg kg^{-1} to 0.343 mg kg^{-1}, respectively, were reported by Nookabkaew et al. in 2013. They furthered analyzed iAs and found that approximately 63% of tAs in Thai rice was the iAs (Nookabkaew et al., 2013). Though, Hensawang and Chanpiwat (2017) recently reported the levels of tAs in 4 types of most consumed rice purchased from local markets in Thailand including white jasmine rice (0.088 to 0.295 mg kg^{-1}, average = 0.203 mg kg^{-1}), white rice (0.084 to 0.265 mg kg^{-1}, average = 0.167 mg kg^{-1}), brown jasmine (0.181 to 0.489 mg kg^{-1}, average = 0.329 mg kg^{-1}), and sticky rice (0.094 to 0.174 mg kg^{-1}, average = 0.148 mg kg^{-1}), the study conducted by Nookabkaew et al. (2013) was yet the only study reported about iAs concentration in Thai rice.

Since total concentration of any substances in food could not represent the certain amount of substance that can be absorbed into the human body, the bioaccessible fraction of particular substance should be determined to ensure the actual concentration of substance which is readily available to be digested, absorbed, and metabolized in human body (Galanakis, 2017). The bioaccessible concentration of substance can be obtained after that particular substance is subjected to the digestive system. Two types of digestive systems; *in vivo* and *in vitro* digestion methods can be used to extract the bioaccessible concentration of substance from food. The *in vitro* digestion method is relatively simple comparing to *in vivo* method. The *in vitro* digestion does not require either animal or human subject in the study. In addition, the digestion can be conducted in the laboratory through the simulation of the human digestive system. In this study,

the RIVM method which comprising of 3 main human digestive systems (Versantvoort et al., 2004) was applied to extract the bioaccessible As in rice. By applying the results of bioaccessible concentration of substance into the human health risk assessment, the exact level of substance exposure in human can be obtained. In addition, the errors in the over- or under-estimation of exposure and risk results can be reduced. Many recent studies on the As exposure from the ingestion of several types of foods have been using the concentration of bAs for the risk assessment process (He et al., 2012; Sun et al., 2012; Brandon et al., 2014; Zhuang et al., 2016; Bolan et al., 2017). Yet, the scientific report on the bioaccessibility of As in rice sold in the local markets in Thailand has never been published.

Therefore, this study was conducted to i) determine concentrations of tAs, iAs, and bAs in Thai polished and non-polished rice sold in the local markets of Bangkok, ii) study the relationships among those As concentrations in rice, and iii) calculate the probable ranges of tAs, iAs, and bAs concentrations in Thai rice.

2. Materials and method

2.1) Rice sample collection and preparation

A total of 208 raw rice samples cultivated during 3 different crop years (2015 to 2018) were collected from 12 representative local markets in Bangkok and 2 central markets in Pathumtani as shown in Figure 3-1. The information of study areas and the representative markets are provided in Table 3-1. All rice samples collected can be divided into the polished (n = 154) and non-polished rice (n = 54).

At each sampling location, approximately 1 kg of each rice sample was collected in a clean zip lock bag with appropriate labeling, transferred to the laboratory and stored in a refrigerator at 4^{o}C until further sample preparation. After a delivery to the laboratory, each rice sample was blended by an aluminum blade blender. The blended sample was sieved (~420 µm), dried at 85 °C in a hot air oven to a constant weight, and stored in a clean plastic tube in a desiccator until further sample digestion.

Figure 3- 1 Locations of 14 local markets for rice sample collection.

Table 3- 1 List of representative markets

No.	Markets	Location of the study area
1.	Khlongtoei	Khlongtoei, Bangkok
2.	Or Tor Kor	Chatuchak, Bangkok
3.	Yingcharoen	Bangkhen, Bangkok
4.	Bangkapi	Bangkapi, Bangkok
5.	Minburi	Minburi, Bangkok
6.	Samyan	Pathumwan, Bangkok
7.	Ekachai	Bangbon, Bangkok
8	Thonburi	Thawiwatthana, Bangkok
9.	Sriyan	Dusit, Bangkok
10.	Bangkunsri	Bangkoknoi, Bangkok
11.	Chomthong	Chomthong, Bangkok
12.	Hataimitr	Khlongsamwa, Bangkok
13.	Simummuang	Lamlukka, Pathumthani
14.	Talaad Thai	Klongluang ,Pathumthani

2.2) Sample digestion and analysis

1) Total arsenic

All samples were digested by an acid digestion according to Phan et al. (2013). Briefly, about 0.10 g of rice sample was weighed into a clean 15 mL polyethylene tube. Next, 1 mL of concentrated nitric acid (HNO_3) (superpure grade for trace analysis) was added into the tube. After that, the tube was capped and left in a hood at room temperature for 48 h. Then, 9 mL of deionized water (DI water, 18.2 MΩ cm^{-1}) was added into a tube containing digested solution. Lastly, the final solution was filtrated through a 0.45 µm syringe filter to obtain a final solution for tAs determinations. The digested solution was kept at 4°C until analyses. All solutions were analyzed by ICP-MS (Agilent ICP-MS model 7300CE).

2) Inorganic arsenic

Two-step operating procedure for iAs extraction from rice grain was conducted. First, a high temperature (95°C) extraction method adopted from Chaney et al. (2018) was conducted. In summary, about 0.700 g of dried rice flour was weighed into a 50 mL polyethylene centrifuge tube. Then, 10 mL of 0.28 M HNO_3 was added into the

tube. The lid was tightly sealed afterward. After that, the tube was heated to reach 95°C for 90 min. Then, sample was left stand to achieve room temperature before filtering through the Whatman no. 40 filter paper which was placed on a suitable size of the polyethylene (PE) funnel and rinsing with 0.28 M HNO_3 for two more times. However, it should be noted that the total volume of rinsing solution should be kept to a level of 9 mL. Finally, the final volume of the extracted solution was made to 20 mL by 0.28 M HNO_3.

Then, the pre-reduction step should be followed. In short, 8 mL of extracted solution obtained from the first operating step, 3 mL of concentrated hydrochloric acid (36% v/v HCl), and 6 mL of the reducing agent was consecutively added into the fresh tube and mixed homogeneously. The reducing agent was prepared by dissolving 5 g of potassium iodide (KI) and 5 g of ascorbic acid in the deionized water with the final volume of 100 mL. Then, 5 mL of 1.73 M sulfamic acid (H_3NSO_4) was slowly added to the tube to avoid agitated bubbling. The tube was left still at room temperature for 20 min to achieve a complete reduction of iAs to As(III). Finally, the solution was injected to the HG-AAS for iAs determination.

The conditions of HG-AAS for iAs analysis which was adopted from De La Calle et al. (2017) are summarized in Table 3-2. In principle, the hydride generation can convert the substance of interest to become volatile compound by using a reducing agent. In this study, the sodium borohydride ($NaBH_4$) in the acid condition (3% HCl) was used as the reducing agent. In such condition (reducing and strong acid) of sample introduction into the instrument, all iAs would be reduced to form As(III). Lastly, the arsine (AsH_3) gas can be generated for the analysis. The stepwise formation of AsH_3 for the iAs analysis by HG-AAS is shown in Eq. 3.1.

$$NaBH_4 + 3H_2O + HCl \rightarrow H_3BO_3 + NaCl + 8H^+ + As(III) \rightarrow AsH_3 + H_2 \qquad \text{Eq. 3.1}$$

The 3% HCl was prepared by mixing 42 mL of concentrated HCl with the deionized water and bringing the solution to final volume of 500 mL. The $NaBH_4$ solution was prepared by dissolving 0.10 g of sodium hydroxide (NaOH) and 1.0 g of $NaBH_4$ in the deionized water. After the final volume of the solution was made to 500 mL, the solution was filtrated and 1.8 mL of Antifoam B emulsion (Sigma A5757) was

added into the solution. Antifoam solution was added to prevent an intense bubbling of sample in the HG-AAS system. It should be noted that both 3% HCl and $NaBH_4$ solution have to be prepared prior to the sample analysis by HG-AAS (Analytik Jena HG-AAS model ZEEnit 700 P).

Table 3- 2 Instrumental conditions for iAs quantification by using HG-AAS

Instrument		**Condition**
HG	Reducing agent	0.5% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH with the addition of 0.9 mL of Antifoam B emulsion (Sigma A5757) into 250 mL of reducing agent. 3% (v/v) HCl solution
AAS	Wavelength	193.7 nm
	Spectral band-pass	0.7 nm
	Lamp current setting	400 mA
	Cell temperature	950°C

3) Bioaccessible arsenic

A three-step RIVM *in vitro* digestion that mimic the human digestive system adopted from Versantvoort et al. (2004) was conducted. The conditions of the *in vitro* digestion, including the phase of digestion, pH and volume of digestive juice, and digestion time are summarized in Table 3-3. This method simulates the digestion in oral cavity by saliva, gastric phase by gastric juice, and small intestine phase by duodenal juice and bile. In brief, 4.5 g of rice sample was continuously incubated for 5 min with 6 mL of saliva, 2 h with an additional 12 mL of gastric juice, and another 2 h with 12 mL of duodenal juice, 6 mL of bile and 2 mL of HCO_3^-. Methods of all digestive juice preparations were summarized in Appendix I. The incubation was performed in a water bath with orbital shaking at 37°C. Afterward, the solution was centrifuged for 5 min at 3,000 rpm in order to completely separate the supernatant and the precipitate phase. The supernatant was filtrated through a 0.45 µm syringe filter. After the completeness of digestion procedure, concentration of As in the resulting solution was determined by a graphite furnace AAS (Varian Spectra AA240Z, Spectra AA240FS, Australia). The

concentration obtained is believed to represent the actual amount of As which is readily to be absorbed into the human body.

Table 3- 3 A summary for the conditions of the *in vitro* digestion

Step no.	Digestion phase in gastrointestinal tract	pH of the digestive solution	Digestive solution	Digestion conditions	
				Volume of digestive solution (mL)	Digestion time
1.	Oral cavity	6.8	Saliva	6	5 min
2.	Stomach	2 – 3	Gastric juice	12	2 h
3.	Small intestine	6.5 – 7	Duodenal juice	12	2 h
			Bile	6	
			HCO_3^-	2	

2.3) Statistical analyses

All statistical analyses were performed using the IBM SPSS Statistical Package version 22 (SPSS, USA). Normality of data was confirmed by the Kolmogonov-Sminov test ($n > 50$) or Shipo-Wilk test ($n < 50$). Means and standard deviation values of As concentrations among different types of rice were subjected to independent T-test to determine the significant differences in As concentrations in the different rice types. While the analysis of variance (ANOVA) and post hoc tests were analyzed to confirm the significant differences in the As concentrations in rice samples collected from the different crop years. In addition, the significant differences in the tAs, iAs, and bAs concentrations among the same type of rice were also determined. The *p* value of 0.01 was used to identify the significant of all tests.

2.4) Probable ranges of arsenic in rice grain

The Monte Carlo simulation was applied into the results of tAs concentrations, percentages of iAs/tAs, and As bioaccessibility obtained from the instrumental analyses to generate the probability distribution of tAs, iAs, and bAs concentrations which can be found in rice grain. The probable distribution functions were drawn by applying 10,000 iterations of the Monte Carlo simulation. All simulations were performed by the @Risk software (DecisionTool Suite Industrial, Palisade Corporation, USA) combined with Microsoft Excel (Serial No. 7112263). A classification of probable As

concentrations into 4 levels (low, normal range, high, and unusually high) was categorized according to the criteria proposed by Zavala and Duxbury (2008).

3. Results and discussion

3.1) Results of method validations

To ensure the accuracy and precision of both acid digestion and analytical methods, the standard reference material (SRM) 1568a (rice flour) and 1643e (trace elements in water) supplied by the National Institute of Standards and Technology (NIST) were used, respectively. The tAs concentration was analyzed by ICP-MS. The accuracy of acid digestion method and the precision of the analytical methods are shown in Table 3-4. Since the percentage recovery of both digestion and analytical method of interested metal were within ± 15% of the certified values, it can be concluded that all results in this present study were accurately obtained.

For iAs digestion, the recovered iAs concentration from the 1568a SRM was compared to the level of i As concentration recovered from the same SRM as reported by the U.S. Food and Drug Administration (Kubachka et al., 2012). The recovery percentages of iAs which equal to 111.6% was in good agreement with the Association of Official Analytical Chemists (AOAC) standard method performance requirement for heavy metals in foods of 60% to 115%.

Table 3- 4 Results of the digestion method validation and the instrumental analysis validation for tAs determination

SRM	Certified value	Analytical value	Recovery rate
1643e	58.98 µg L^{-1}	63.43 µg L^{-1}	107.6%
1568a	0.2900 mg kg^{-1}	0.2959 mg kg^{-1}	102.0%

3.2) Total As concentrations in polished and non-polished rice grains

Concentrations of tAs in both polished and non-polished rice grain are summarized in Table 3-5. Concentrations of tAs in polished and non-polished rice ranged from 0.0878 to 0.2949 mg kg^{-1} and 0.1187 to 0.5172 mg kg^{-1}, respectively. Concentrations of tAs in all rice samples were lower than the Thai standard of As in food (2 mg kg^{-1}) as regulated by the Ministry of Public Health (2003). It should be noted that Thailand does not regulate the level of As in rice. Following the Codex

Alimentarius Commission recommendation on the use of tAs as a screening level for iAs in rice, approximately 58.8% and 57.4% of polished (n = 154) and non-polished (n = 54) rice samples, respectively, contained tAs concentrations higher than the Codex standard levels (0.2 mg kg^{-1} for polished rice and 0.35 mg kg^{-1} for non-polished rice). The maximum tAs concentrations found in both polished and non-polished rice were approximately 1.5 times greater than the FAO/WHO maximum allowable iAs levels in both types of rice (Food and Agriculture Organization/World Health Organization, 2014). The results reported in Table 3-5 indicate a necessary in the determination of iAs in the rice samples.

Table 3- 5 Concentrations of tAs (mg kg^{-1}) in rice samples

Statistical value	**Rice type**	
	Polished (n = 154)	**Non-polished (n = 54)**
Minimum	0.0878	0.1187
Maximum	0.2949	0.5172
Average	0.2078	0.3511
Median	0.2073	0.3610
SE[a]	0.0028	0.0127

Remark: [a] Standard error of the mean

The sampling campaigns in this study were expanded into 3 crop years (2015, 2016, and 2018) to ensure randomness of samples. In each sampling campaign, samples were collected during June to August. It is because the cultivation period of Thai jasmine rice in Thailand normally starts in June to November (Vanavichit et al., 2018). Not only the same method of sample collection and the same period of sample collection was applied to each sampling campaign and each sampling site to ensure the randomness of samples, but the results of non-significant differences in the tAs concentrations in rice collected from different crop years also indicated the randomness of samples used in this study ($p > 0.01$). Figure 3-2 demonstrates no significant differences in tAs concentrations contained in the same type of rice collected from different crop years. Even though, the significant of tAs concentrations in three different crop years were not found, it was found that the tAs concentrations in both types of rice tend to be increase after each year of collection. Therefore, the tAs concentrations in rice grain should be monitored ensure the safe rice consumption and

to check whether the increasing trend of tAs concentration still exist in the following crop year such as 2019 crop year.

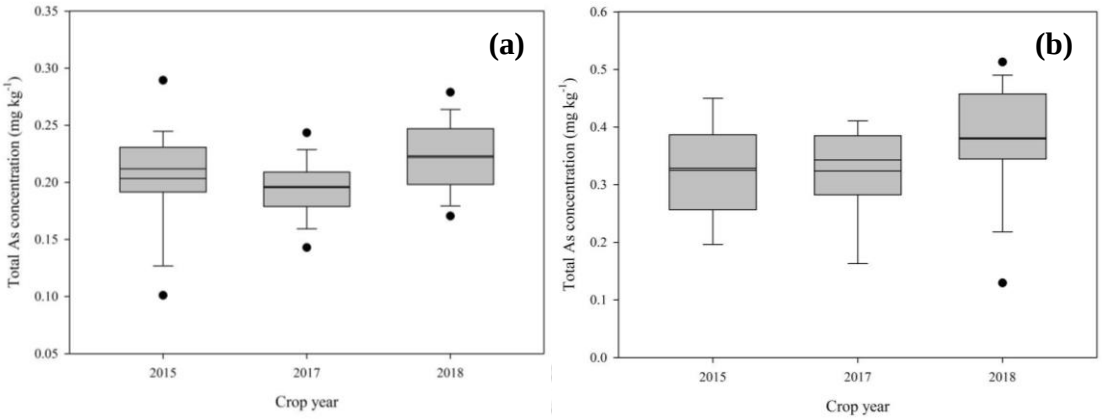


Figure 3- 2 Box plots of tAs concentrations in (a) polished (b) non-polished rice samples in different crop years.

The concentrations of tAs in rice in this study are comparable with the previous study reported by Nookabkaew et al. (2013). Nookabkaew et al. (2013) reported tAs of 0.0225 to 0.3043 mg kg^{-1} (average = 0.1395 mg kg^{-1}) in polished rice and 0.1182 to 0.3431 mg kg^{-1} (average = 0.2991 mg kg^{-1}) in polished rice. Even though, tAs concentrations in both types of rice in this study were slightly higher than the results reported by Nookabkaew et al. (2013), their concentrations were in the same magnitude of concentrations. The different genotypes of rice studied between this study and Nookabkaew et al. (2013) may be the reason of different tAs concentrations in rice. This study only focused on the jasmine (fragrance) rice with different color or different degree of polishing while Nookabkaew et al. (2013) did not consider the genotypes and varieties of rice in their study.

Several previous reports have shown that different rice genotypes have different abilities to uptake As from the environmental media to the grain. The tAs concentrations in different polished rice genotypes were in the following order: *basmati* rice (0.321 ± 0.032 mg kg^{-1}) and *indica* rice (0.0213 to 0.2965 mg kg^{-1}) (Jiang et al., 2012; Ahmed et al., 2015). The tAs concentrations in the *indica* rice in this study were in good agreement with the result reported by Jiang et al. (2012). Same genotype of rice but different in varieties also showed different levels of tAs in the grain. This can be confirmed by the results reported by Hensawang and Chanpiwat (2017) that lower tAs concentration was determined in the non-fragrance rice (typical polished white rice,

average = 0.167±0.009 mg kg^{-1}) than that of the fragrance rice (polished jasmine rice, average = 0.203±0.008 mg kg^{-1}).

When taking the different types of rice into account of statistical analysis in this study, it was found that tAs concentration in non-polished rice was significantly higher than that of the polished rice by approximately 1.7 times ($p < 0.01$). This result was in accordance with Mu et al. (2019) who also reported higher tAs concentration in non-polished rice (0.011 to 0.235 mg kg^{-1}) than those of the polished rice (0.020 to 0.326 mg kg^{-1}). Higher tAs concentration in non-polished rice than the polished rice is found because As mainly localizes between the brown part, the layer between the bran and aleurone layers, of the grain (Meharg et al. (2008). To be more specific, the thiols in the complex protein compounds contained in the rice bran and aleurone are the specific As-binding site in the rice grain (Du et al., 2019). As a result, the lower As concentration in polished rice, which contains more carbohydrate of endosperm and less protein, could be found.

3.3) Spatial variation of total As concentrations in rice grain

All rice samples in this study were Thai jasmine rice (Kao Dok Mali 105; KDML 105). The KDML105 is a photoperiod-sensitive rice variety. Its cultivation period normally starts from June and the flowering and harvesting usually take place around October and November, respectively (Boling et al., 2011). This rice variety is typically cultivated in the northern and northeastern parts of the country (Vanavichit et al., 2018). According to the topography characteristics of undulating landscape with poor condition of soil and unstable rainfall in these regions, the KDML105 is, then, cultivated under rainfed condition during the rainy season.

Regarding all rice samples in this study (Appendix II), approximately 11% and 57% of all polished rice samples were cultivated in the northern and north eastern, respectively. While, approximately 7% and 52% of all non-polished rice samples were cultivated in the northern and north eastern, respectively. Regarding to the survey at the same time of rice sample collection, rice samples grown in the northern part were cultivated in the Cheingrai and Payao (Appendix II). Meanwhile, Surin, Yasotorn, Roi Et, Srisaket, Burirum, Ubon Ratchatani, Karasin, Chaiyapum, and Nakon Phanom were the areas of rice cultivation in the northeastern part of the country (Appendix II). The

statistical analysis results confirmed no significant differences in tAs concentrations contained in the same type of rice cultivated in different areas ($p > 0.01$) and different crop years ($p > 0.01$). The spatial distribution of the cultivation area and the spatial variation in rainfall according to the changing redox condition were reported to affect the level of tAs concentration in rice grain (Brackhage et al., 2014). Even though, the increasing amount of rainfall reported in both northern and northeastern parts of the country during the period of study (Rice Department, 2018) may be related to the increasing trends of tAs concentrations in rice grain in this study, the major factors which could affect the tAs accumulation in rice grain were reported to be rice varieties, As contamination in soil, and cultivation practice (Halder et al., 2014).

3.4) Inorganic As concentrations in polished and non-polished rice grains

Since the tAs concentrations of both polished and non-polished rice samples reported in Table 3-5 exceeded the FAO/WHO maximum allowance concentrations of iAs for polished (0.2 mg kg^{-1}) and non-polished rice (0.35 mg kg^{-1}) (Food and Agriculture Organization/World Health Organization, 2014; Food and Agriculture Organization/World Health Organization, 2016). The determination of iAs was conducted in a total of 41 representative samples with tAs concentrations greater than the FAO/WHO standards and high percentage of As accessibility. Concentrations of tAs and iAs contained in the representative rice samples are summarized in Table 3-6.

Regarding to the results shown in Table 3-5, the maximum iAs concentrations in polished and non-polished rice samples were approximately 1.10 and 1.45 times lower than the FAO/WHO standards of iAs in polished (0.2 mg kg^{-1}) and non-polished polished (0.35 mg kg^{-1}) rice, respectively. All samples contained iAs lower than the FAO/WHO standards. Therefore, it can be concluded that the both polished and non-polished jasmine rice collected from the local markets in study are safe for consumption. Although both average (0.1765 mg kg^{-1}) and median (0.1965 mg kg^{-1}) iAs concentrations in the non-polished rice were higher than those of the polished rice (average = 0.1384 mg kg^{-1} and median = 0.1355 mg kg^{-1}) (Table 3-6), concentrations of those iAs in both types of rice were not significantly different ($p > 0.01$). Non-polished rice in this study averagely contained 1.23 times of more iAs than the polished rice.

When compared the concentrations of iAs in rice in this study (Table 3-6) to those reported by Nookabkaew et al. (2013), same magnitude of iAs concentrations were found in both types of rice. Though, the concentrations of iAs in rice in this study were approximately 1.4 to 1.6 times higher than those reported by Nookabkaew et al. (2013). Concentrations of iAs in the polished and non-polished by Nookabkaew et al. (2013) were 0.0163 to 0.1616 mg kg^{-1} (average = 0.0852±0.0031 mg kg^{-1}) and 0.0837 to 0.2067 mg kg^{-1} (average = 0.1244±0.01012 mg kg^{-1}), respectively.

Table 3- 6 Concentrations of iAs in rice samples

Statistical value	Concentration (mg kg^{-1})		Percentage of iAs/tAs
	tAs	iAs	
Polished rice (n = 28)			
Minimum	0.2077	0.0706	28.58
Maximum	0.2899	0.1822	78.76
Average	0.2385	0.1384	58.04
Median	0.2335	0.1355	58.89
SE[a]	0.0046	0.0047	1.65
Non-polished rice (n = 13)			
Minimum	0.1187	0.0580	31.75
Maximum	0.4999	0.2420	75.27
Average	0.3494	0.1765	51.70
Median	0.3934	0.1965	45.26
SE[a]	0.0314	0.0161	3.85

Remark: [a] Standard error of the mean

Concerning the percentages of iAs to tAs, this study found that approximately 58.0% and 51.7% of tAs in polished and non-polished rice, respectively, were iAs (Table 3-6). These results were in good agreement with the percentage of iAs in the jasmine rice (54%, n = 18) reported by Kollander et al. (2019). Comparing to the percentages of iAs in rice collected by Nookabkaew et al. (2013) in which iAs in the polished and non-polished rice were approximately 63.2% (±1.3), and 53.6% (± 3.1) to tAs, respectively, lower percentages of iAs in both types of rice were found in this study. Similar to the differences in tAs and iAs in non-polished and polished rice which were reported earlier, no significant differences in the percentages of iAs in both types of rice were found ($p > 0.01$).

Figure 3-3 shows the variations of iAs and oAs concentrations in each individual rice sample. As clearly shown in Figure 3-3, the percentages of iAs in both types of rice

varied widely. According to the percentages of iAs/tAs, 28.6% to 78.7% (average = 58.0%) and 31.8% to 75.3% (average = 51.7%) of tAs in polished and non-polished rice, respectively, were iAs. The wide percentage ranges of iAs in rice were also found in the Spanish and Brazilian rice with the values ranging between 27% to 93% and 27% to 89%, respectively (Torres-Escribano et al., 2008).

Regarding to the results presented in Figure 3-3 and the classification of rice depending on the percentages of iAs and oAs in rice, it is possible to concluded that both inorganic-dominant and organic-dominant types of rice can be found within the rice with the same variety and milling process applied. Though, several environmental conditions in the paddy field such as climate conditions, water management regime, and As speciation in the soil solution could affect to the accumulation of different As species in rice grain, Zavala et al. (2008) concluded that the genetic features of rice are the most important factor affecting the species dominant in rice.

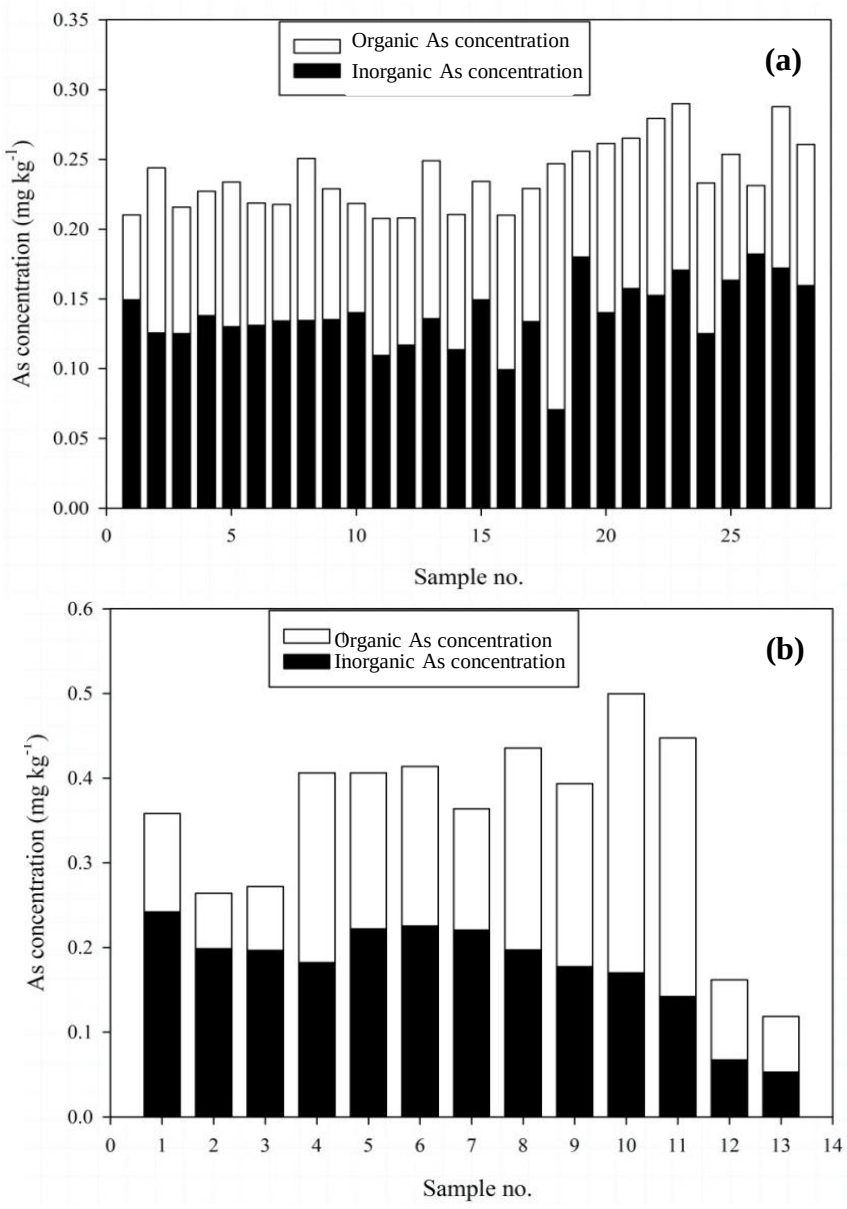


Figure 3- 3 Variations in the presences of iAs in (a) polished rice and (b) non-polished rice.

Even though the species of iAs and oAs were not analyzed in this study, it is possible to make an assumption based on the previous study on the As species in polished and non-polished Thai rice (Nookabkaew et al., 2013) that As(III) and DMA are the dominant iAs and oAs species accumulated in rice grain. Nookabkaew et al. (2013) found that approximately 88.8% to 95.6% of iAs was As(III) and almost 100% of oAs was DMA. In term of toxicity, the iAs is more toxic than the oAs.

3.5) Bioaccessible As concentrations in polished and non-polished rice grains

The concentrations of bAs in polished and non-polished rice were in the range of 0.0256 to 0.1896 mg kg^{-1} and 0.0646 to 0.2785 mg kg^{-1}, respectively (Table 3-7). Similar to the tAs concentrations, bAs concentrations in the non-polished rice were significantly 1.5 times higher than those in the polished rice ($p < 0.01$). As clearly shown in Table 3-5 and Table 3-7, the concentrations of bAs were lower than the tAs approximately 2.0 times for polished rice and 1.8 for times for non-polished rice. The results obtained clearly indicated that some levels of As (as tAs in study) can be digested, metabolized, and absorbed during the gastrointestinal digestive system before reaching the blood circulation system. When compared the results of bAs in rice to the FAO/WHO standards of iAs in rice, concentrations of bAs in all polished and non-polished rice samples were lower than the standards.

Table 3- 7 Concentrations of bAs and As bioaccessibility

Statistical value	Concentration (mg kg^{-1})		As bioaccessibility (%)
	tAs	bAs	
Polished rice (n = 89)			
Minimum	0.0878	0.0256	8.7
Maximum	0.4063	0.1896	95.2
Average	0.2133	0.1054	50.5
Median	0.2102	0.0979	46.7
SE[a]	0.0042	0.0038	1.85
Non-polished rice (n = 41)			
Minimum	0.1808	0.0646	20.6
Maximum	0.4893	0.2782	87.1
Average	0.3430	0.1531	45.9
Median	0.3530	0.1557	43.6
SE[a]	0.0103	0.0065	2.23

Remark: [a] Standard error of the mean

The bioaccessibility of As in polished and non-polished rice in this study (Table 3-7) was calculated as the ratio of bAs concentration to tAs concentration. Although the bioaccessibility of As in the polished rice (8.7% to 95.2%) varied more than that of the non-polished rice (20.6% to 87.1%), the average As bioaccessibilities in both types of rice (50.5% for polished rice and 45.9% for non-polished rice) were not significantly different ($p > 0.01$). A difference in bAs among the two types of rice was approximately 10%. This result is in accordance with He et al. (2012) who found approximately twice

the average percentage of As bioaccessibility in non-polished rice than that of the polished rice.

The wide variations of As bioaccessibilities in polished rice and non-polished rice are demonstrated in Figure 3-7(a) and Figure 3-7(b), respectively. Comparing the results of As bioaccessibilities in this study to the previous studies, less variations in the As bioaccessibilities in the polished rice collected from the United States (56.0% to 81.3%), China (70% to 93%), Bangladesh, Korean, and Indian rice (72% to 98%) as well as the non-polished rice from the United States (56.0% to 81.3%) were found (Trenary et al., 2012; Zhuang et al., 2016; Bolan et al., 2017). The main reasons that could explain the differences between the results of this study and those previous studies include the differences in chemical compositions of each rice sample and the differences in rice genotypes cultivated in each study area. In term of As toxicity, Du et al. (2019) have found a significant positive correlation between bAs and As(III).

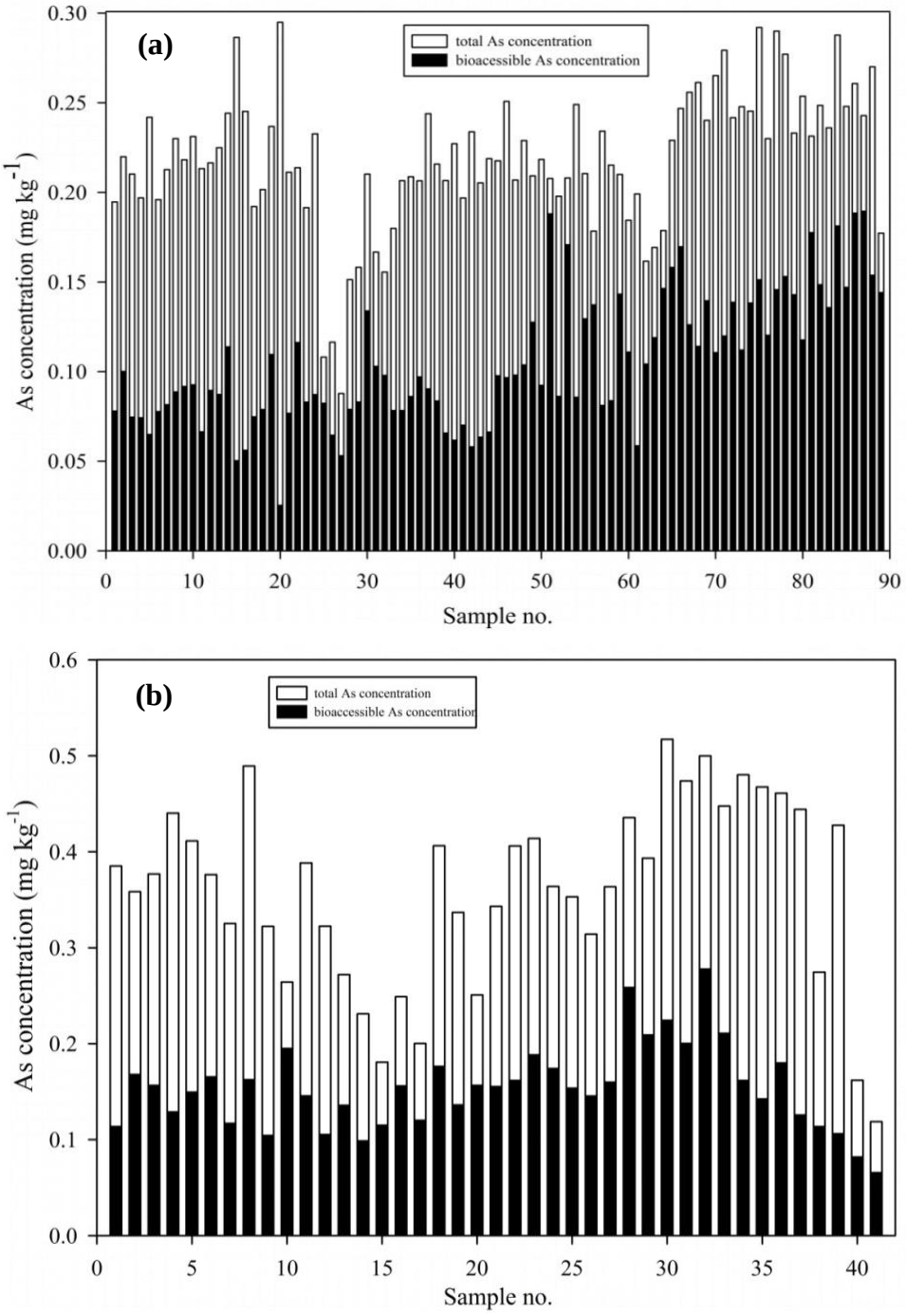


Figure 3- 4 Concentrations of tAs and bAs in (a) polished and (b) non-polished rice.

3.6) Relationships between tAs, iAs, and bAs concentrations in rice

The relationships between tAs, iAs, and bAs concentrations in rice are summarized in Table 3-8. The strong positive correlations ($p < 0.01$) were found between iAs-tAs and bAs-tAs with the correlation coefficients (r) of 0.691 and 0.732, respectively. Meanwhile, the relationship between iAs and bAs was considered as a weak positive correlation ($r = 0.475$, $p < 0.01$). Following the analyses of significant relationships among As concentrations, the analyses of simple linear regressions among tAs, iAs, and bAs were conducted to obtain the estimation equations of different As species (Table 3-9).

It can be concluded from the results shown in Table 3-8 and Table 3-9 that the higher tAs concentration in rice grain, the higher concentrations of iAs and bAs would be found in rice. In the case that only tAs concentration was determined, the concentration of tAs can be used to estimate to possible concentrations of iAs and bAs in rice by following the estimation equation as shown in Table 3-9.

Table 3- 8 Pearson correlations between tAs, iAs, and bAs concentrations in rice

As species	**tAs (n = 41)**	**iAs (n = 41)**	**bAs (n = 41)**
tAs		0.691**	0.732**
iAs			0.475**

Remark: ** Correlation is significant at the 0.01 level (2-tailed)

Table 3- 9 Estimation equations for concentrations of different As species in rice

Relationship		**Estimation equation**	R^2	***p* value**
x-axis	**y-axis**			
tAs	iAs	$y = 0.346x + 0.056$	0.478	< 0.01
tAs	bAs	$y = 0.463x + 0.014$	0.536	< 0.01
iAs	bAs	$y = 0.601x + 0.050$	0.226	< 0.01

3.7) Classification of As concentrations in rice

Figure 3-5 and Figure 3-6, respectively, demonstrate the probable distribution functions of (a) tAs, (b) iAs, and (c) bAs concentrations in polished rice and non-polished rice as a result of the Monte Carlo simulations by @Risk program. Regarding to the study of Zavala and Duxbury (2008) on the estimation of normal levels of tAs in rice grain in which the concentrations at > 25^{th} percentiles, 25^{th} to 75^{th} percentiles, 75^{th} to 95^{th} percentiles and > 95^{th} percentiles were categorized as rice with low, normal, high and unusually high concentrations of As, the classifications of As concentrations in the polished and non-polished rice in this study can also be identified as shown in Table 3-10 and Table 3-11, respectively.

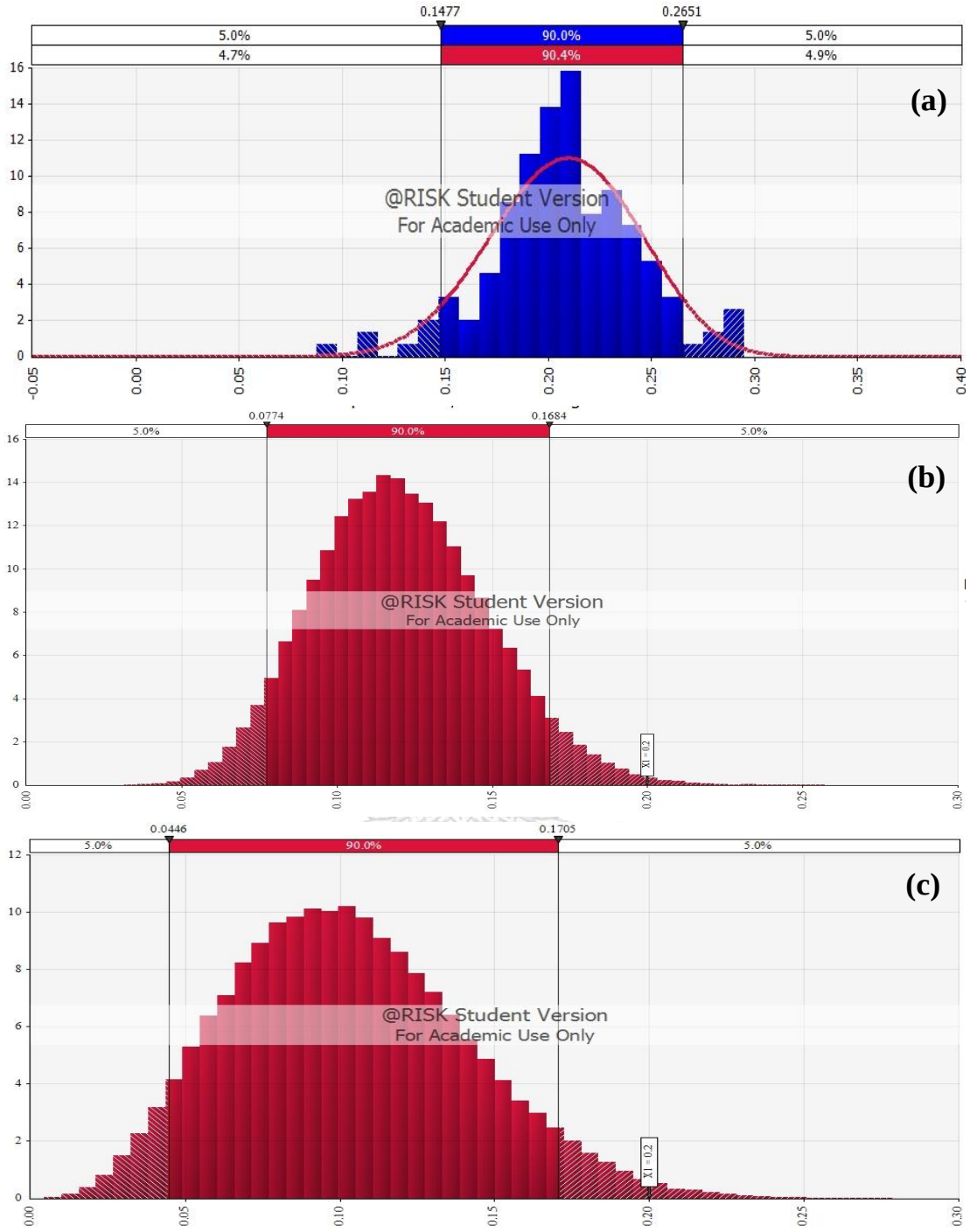


Figure 3- 5 Probable distribution functions of (a) tAs, (b) iAs, and (c) bAs concentrations in polished rice resulting from the Monte Carlo simulation by @Risk program.

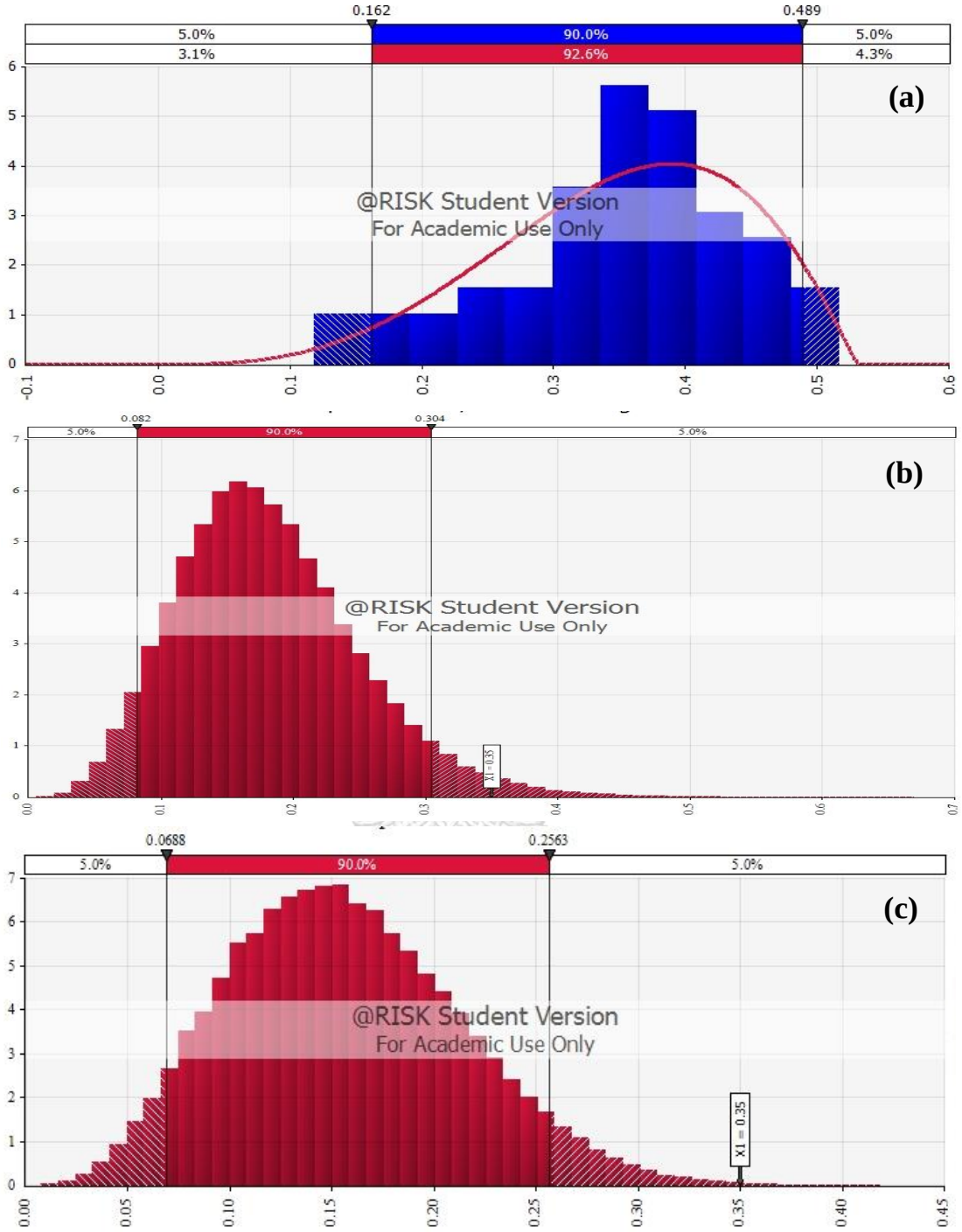


Figure 3- 6 Probable distribution functions of (a) tAs, (b) iAs, and (c) bAs concentrations in non-polished rice resulting from the Monte Carlo simulation by @Risk program.

Table 3- 10 Classifications of As concentrations (mg kg^{-1}) in polished rice

As species	**Level of classification**			
	Low	**Normal**	**High**	**Unusually high**
tAs	< 0.1837	0.1837 – 0.2324	0.2324 – 0.2664	> 0.2664
iAs	< 0.1012	0.1012 – 0.1391	0.1391 –0.1685	> 0.1685
bAs	< 0.0747	0.0747 – 0.1283	0.1283 – 0.1700	> 0.1700

Table 3- 11 Classifications of As concentrations (mg kg^{-1}) in non-polished rice

As species	**Level of classification**			
	Low	**Normal**	**High**	**Unusually high**
tAs	< 0.2879	0.2879 – 0.4227	0.4227 – 0.4859	> 0.4859
iAs	< 0.1327	0.1327 – 0.2222	0.2222 –0.3044	> 0.3044
bAs	< 0.1145	0.1145 – 0.1940	0.1940 – 0.2563	> 0.2563

When the concentrations of tAs, iAs, and bAs of all polished rice and non-polished rice samples which were obtained from the instrumental analyses and reported earlier in this chapter (in the section 3.2 to section 3.5) were overlayed with the classification of As level in rice, Figure 3-7 and Figure 3-8 were obtained. Both figures can be used to indicate the status of As concentration accumulated in polished and non-polished rice collected in this study. It was clearly shown that the As accumulated in both polished (Figure 3-7) and non-polished rice (Figure 3-8) can be categorized as either normal range of As or high level of As in rice. Though, the normal range of tAs in both polished and non-polished rice exceed the FAO-WHO standards, the concentrations of iAs even in the group of high As were lower than the standards.

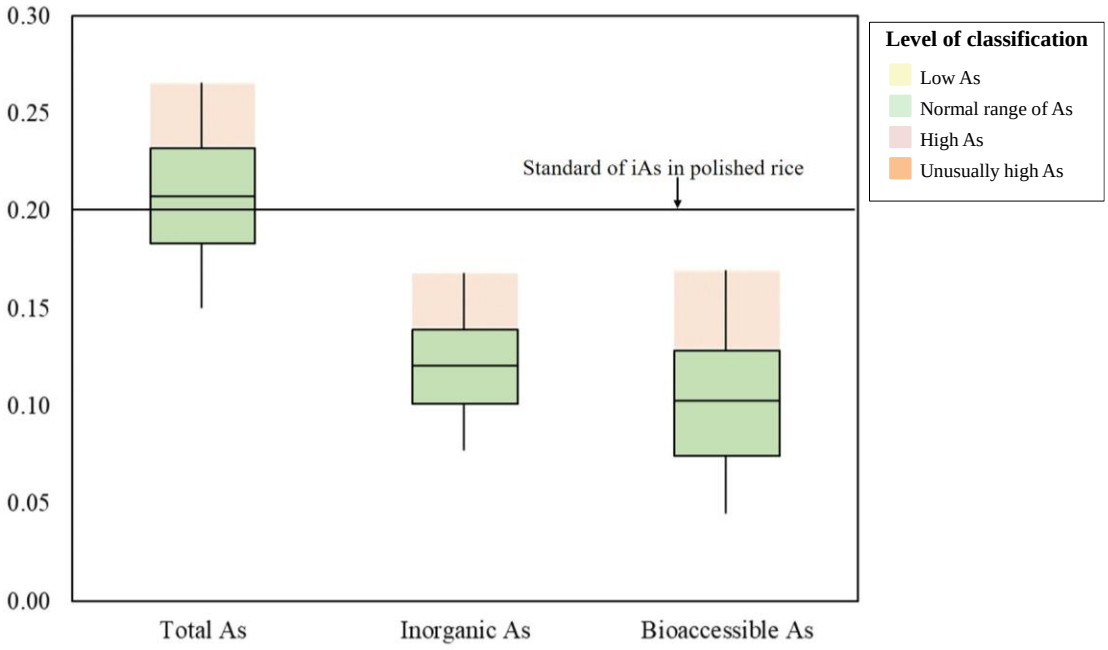


Figure 3- 7 Status of As concentration accumulated in polished rice collected in this study.

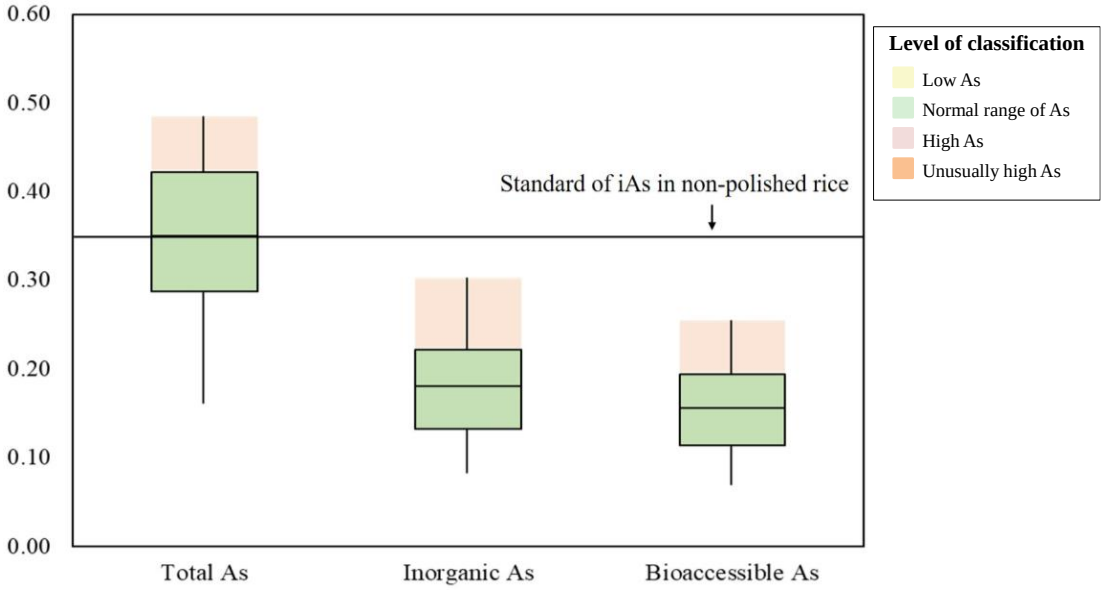


Figure 3- 8 Status of As concentration accumulated in non-polished rice collected in this study.

4. Conclusions

This study determined the concentrations of tAs, iAs, and bAs in the polished (n = 154) and non-polished (n = 54) rice which were sold in local markets in Bangkok, Thailand. Approximately 59% and 57% of all polished and non-polished rice samples were found with tAs concentrations higher than the FAO/WHO maximum allowable iAs concentrations of 0.2 mg kg^{-1} and 0.35 mg kg^{-1}. However, none of the samples tested had iAs exceeding the FAO/WHO standard. Non-polished rice contained significantly higher tAs, iAs, and bAs than those of the polished rice. The iAs concentrations in rice accounted for approximately 58.0% and 51.7% of tAs in polished and non-polished rice, respectively. Most of rice collected can be considered as the iAs rice type. The bioaccessibility of As in rice, which mean a certain amount of As in rice that is readily for human body absorption, presented in a level of 50.5% and 45.9% to tAs in polished and non-polished rice, respectively. Positive significant relationships were found between tAs-iAs, tAs-bAs, and iAs-bAs. The concentrations of tAs can be used to estimate the concentrations of iAs and bAs following the estimation equations obtained from the regression model. Status of tAs, iAs, and bAs concentrations in polished and non-polished rice were determined. The normal ranges of tAs, iAs, and bAs in polished rice were 0.1837 – 0.2324 mg kg^{-1}, 0.1012 – 0.1391 mg kg^{-1}, and 0.0747 – 0.1283 mg kg^{-1}, respectively. While the normal ranges of tAs, iAs, and bAs in non-polished rice were 0.2879 – 0.4227 mg kg^{-1}, 0.1327 – 0.2222 mg kg^{-1}, and 0.1145 – 0.1940 mg kg^{-1}, respectively. The normal ranges of tAs in both polished and non-polished rice were greater than the FAO-WHO standards. However, the concentrations of iAs even in the group of high As were lower than the standards.

CHAPTER IV

EFFECT OF POLISHING ON TOTAL ARSENIC CONCENTRATIONS IN NON-POLISHED RICE AND RESULTING POLISHED RICE

1. Introduction

Rice not only provides the dietary energy, it also provides several vitamins (vitamin B1, B2, B3 and E), essential amino acids (glutamic acid, aspartic acid, lysine), and essential elements (copper (Cu), iron (Fe), zinc (Zn), manganese (Mn)) (Rohman et al., 2014; Chaudhari et al., 2018). In some cases, rice can be an important route of some toxic elements such as As and Cd exposure (Food and Agriculture Organization/World Health Organization, 2011; Food and Agriculture Organization/World Health Organization, 2013; Hensawang and Chanpiwat, 2017; Xiao et al., 2018). It is well known that non-polished (wholegrain or brown) rice provides those beneficial nutritional compositions more than those in polished rice as they mainly exist in the outer layer (germ and bran) of the grain (Juliano, 2016).

Before human consumption, rice has to be milled to remove the husk to obtain the non-polished wholegrain rice. Afterward, it may be passed through the polishing process to result in white rice, the dominant form of rice consumed in many countries. It was found that the polishing process normally plays a key role in determining rice quality. For example, Babu et al. (2009) reported the reductions of vitamin B1 (80%), vitamin B3 (67%), vitamin B6 (90%) in rice after passing through the complete polishing process that converts wholegrain rice to white rice. Babu et al. (2009) and Chaudhari et al. (2018) reported approximate reductions of 2% to 22% of essential amino acids, as well as the essential macroelements to human body including 40% to 80% of calcium, 69% of magnesium, 65% to 84% of phosphorus and 58% of potassium. In term of essential microelements, approximately 60% of Fe, 50% of Mn, 27% of Selenium (Se) and 20% of Zn were removed during the polishing process to produce white rice from wholegrain rice.

Since most of the As accumulated in rice generally localizes in the brown part of the grain (Meharg et al., 2008), As could be removed after the wholegrain rice is

polished. Even though there are several reports on the differences in the concentration of tAs and iAs contained in non-polished rice and polished rice in which approximately 1.25 to 2.20 times more As were found in the non-polished rice (Sun et al., 2008; Nookabkaew et al., 2013; Rahman et al., 2014; Chen et al., 2016; Yim et al., 2017), the two types of rice studied in those studies were separately collected. The study on the effect of polishing on the level of As in rice with a consideration in the similarity in the characteristics and origin of rice is limited. Up to now, there is only a study of Naito et al. (2015) on the effect of polishing on the concentration of tAs and iAs in polished rice which was the obtained from the non-polished rice reported. When 10% (by weight) of rice bran was polished, approximately 34% to 39% and 30% to 49% reductions of tAs and iAs, respectively, were found in the polished rice comparing to those As found in the non-polished rice. Yet, the relationship between different DOPs and the loss of As in rice of the same origin and characteristics have never been reported. Therefore, this study was conducted to i) investigate the effects of different polishing degrees on the concentrations of tAs in rice, and ii) study the relationship between DOP and the remaining tAs concentration in polished rice grain. It should be noted that the classification of polished rice in this study was categorized according to the National Bureau of Agricultural Commodity and Food Standards (2017).

2. Materials and methods

2.1) Rice sample collection

Three sets of non-polished rice (*Oryza sativa* L.) sold in a traditional rice store in a local market and two retail supermarkets located in Bangkok were collected in 2018 (Table 4.1). Samples were collected from these sources because they are the two main sources of domestic rice consumption of the country. It was reported that approximately 60–70% of rice for domestic consumption was distributed to the households through either a sell loose in the traditional rice stores (50–55%) or a packaged rice available in the retail stores (40–45%) (Krungsri Research, 2018). At least 1 kg of sample sold in the traditional rice store was collected in a clean zip lock bag. Rice sample sold at the retail supermarket was purchased as a whole package with a minimum weight of 1 kg. After collection, all samples were stored in a refrigerator at 4°C until further preparation.

Table 4- 1 Sample information

Sample ID	Sampling site	Production area
A	Local market	Yasotorn
B	Supermarket	Kalasin
C [a]	Supermarket	Yasotorn

Remark: [a] Rice was cultivated by an organic farming method

2.2) Polishing process

Each set of the non-polished rice sample was divided into 5 different subsamples. Afterward, one subsample was kept as the original wholegrain rice sample and the remaining subsamples were subjected to the polishing process with four different steps of polishing using a portable household rice mill (Figure 4-1). Approximately 150 g of each subsample was polished for 1 min until the resulting polished rice was obtained. The weights of rice grain before and after polishing were recorded to calculate the percentage of polishing (% DOP) as shown in Eq. 4.1 (Liu et al., 2015). As a result of the polishing, five different types of rice samples were obtained as shown in Figure 4.2 and Table 4.2.

$$\% \text{ DOP} = [1 - (\text{Weight of rice grain after polishing} / \text{Weight of rice grain before polishing})] \times 100 \quad \text{Eq. 4-1}$$

The classification of %DOP to the quality of milled rice regulated by the National Bureau of Agricultural Commodity and Food Standards (2017) was done regarding to the recommendation of Naivikul (2017). Regarding the different percentage ranges of DOP, rice can be classified into 5 types namely; non-polished or wholegrain (0% DOP), (b) ordinarily polished rice (5 – 5.9% DOP), reasonably well-polished rice (6 – 6.9% DOP), well-polished rice (7 – 7.9% DOP), and extra well-polished rice (≥ 8% DOP).

In addition to the different types of polished rice obtained after each set of non-polished rice was polished, another 4 types of rice bran were also obtained and collected. They were classified as ordinarily polished bran, reasonably well-polished bran, well-polished bran, and extra well-polished bran.

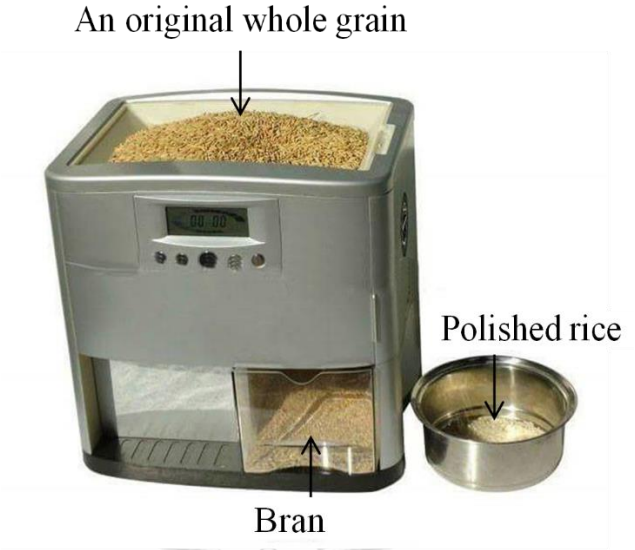


Figure 4- 1 Rice polishing machine.

Table 4- 2 Information of five subsamples from each set of non-polished rice sample

Step of polishing	% DOP			Classification of rice[a]
	Average	SD	%RSD	
	-	-	-	Wholegrain (non-polished) rice (WG)
1	5.9	0.38	2.2	Ordinarily polished rice (OP)
2	6.3	0.75	11.9	Reasonably well-polished rice (RWP)
3	7.5	0.59	7.8	Well-polished rice (WP)
4	8.1	0.33	4.1	Extra well-polished rice (EWP)

Remark: [a] Classification was made according to Naivikul (2017) and the National Bureau of Agricultural Commodity and Food Standards (2017) on the classification of rice of different polishing/milling degrees.

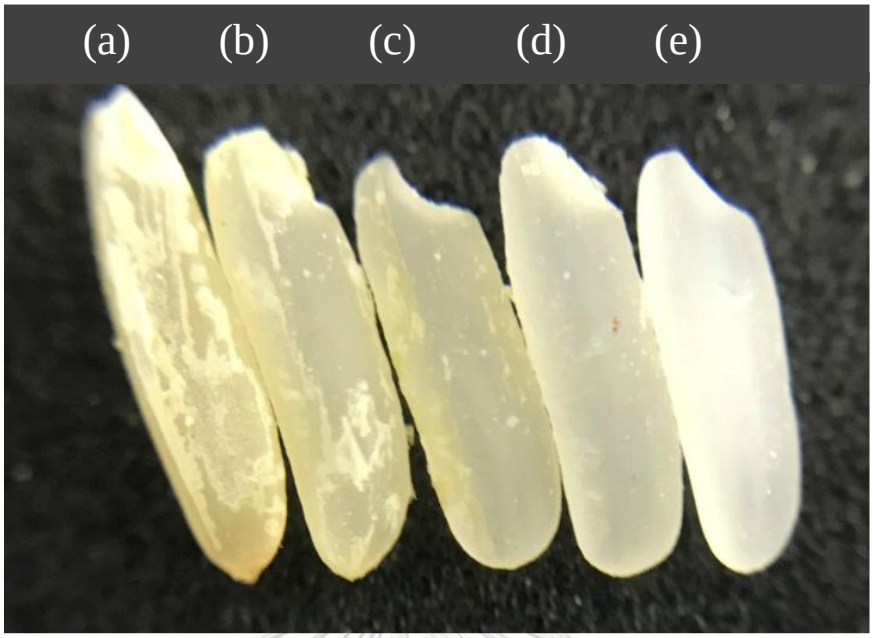


Figure 4- 2 A magnified picture of rice grains after passing through a stepwise polishing process resulting in polished rice with different %DOPs including (a) original wholegrain (WG, 0% DOP) , (b) ordinarily polished rice grain (OP, 5.9% DOP), (c) reasonably well-polished rice grain (RWP, 6.3% DOP), (d) well-polished rice grain (WP, 7.5% DOP), and (e) extra well-polished rice grain (EWP, 8.1% DOP).

2.3) Rice sample preparation and analysis

Approximately 50 g of each type of rice of all 3 sets of original non-polished rice was blended by an aluminum blade blender. The blended sample was sieved (~420 µm), dried at 85°C in a hot air oven to a constant weight and stored in a desiccator until further sample digestion.

An exact weight of 0.1 g of the sieved rice or sieved bran sample was weighed and transferred into a clean 15 mL tube for sample digestion for metal analysis following Phan et al. (2013). After that, 1 mL of a super pure and concentrated nitric acid (67–69% HNO_3, CARLO EBRA reagent, Val de Reuil, France) was added into the tube. The tube was, then, left at room temperature to allow complete digestion. After 48 h of sample digestion, the total volume of the acid digestion solution was brought up to 10 mL by the deionized water. Finally, the sample solution was filtrated through a 0.45-µm nylon syringe filter before storing at 4°C until elemental analysis using ICP-MS. In addition, a validation of the acid digestion method was also applied using the

NIST SRM 1568a (rice flour). The SRM was treated in the same manner as the sample digestion.

2.4) Statistical analyses

The IBM SPSS Statistical Package version 22 (SPSS, USA) was used to perform all statistical analyses. The Shapiro-Wilk test ($n \leq 50$) was applied to check the distribution of the data. The average and SD values of As concentration among different degrees of polishing were subjected to ANOVA test in order to confirm the differences in the concentrations of As contained in rice with different DOPs. The relationships between the reductions of tAs from non-polished rice grain and the level of DOP was performed using Pearson correlation. A level of 0.01 (*p*-value) was used to determine the significant level.

3. Results and discussion

3.1) Method validation

To ensure the accuracy and precision of both acid digestion and analytical methods, the SRM 1568a (rice flour) and 1640a (trace element in natural water) supplied by the NIST were used, respectively. The tAs concentration was analyzed by ICP-MS. The accuracy of acid digestion method and the precision of the analytical methods are shown in Table 4-3. The percentages of repeatability relative standard deviation (%RSD_r) and reproducibility relative standards deviation (%RSD_R) in this study were 3.53% and 4.9%, respectively. The ICP-MS performance were in accordance with the method performance requirements of 15% RSD_r, 15% RSD_R, and 60–115% recovery as recommended by the Journal of AOAC International (2013). Therefore, it is possible to concluded that all results in this present study were accurately obtained.

Table 4- 3 Results of the digestion method validation and the instrumental analysis validation for tAs determination

SRM	Certified value	Analytical value	Recovery rate
1640a	8.075 µg L^{-1}	8.159 µg L^{-1}	101%
1568a	0.290 mg kg^{-1}	0.3350 mg kg^{-1}	115%

3.2) Effects of polishing on tAs concentrations in rice

The average %DOP for each type of resulting polished rice after passing through the rice polishing machine were provided in Table 4-2. The differences in the physical appearances of rice grain with different DOPs are demonstrated in Figure 4-2. The concentrations of tAs in non-polished (WG), ordinarily polished (OP), reasonably well-polished (RWP), well-polished (WP), and extra well-polished (EWP) rice and their bran are summarized in Table 4-4. It was found that tAs concentration was approximately and significantly 1.33 times lower than that of the non-polished rice ($p < 0.01$). This result was corresponding well to the results reported in the earlier chapter (Chapter 3 on total, inorganic, and bioaccessible arsenic concentrations in raw polished and non-polished rice based on a market survey in Bangkok) that the significant higher tAs concentration was found in non-polished rice than that of the polished rice. As clearly shown in Table 4-4 and Figure 4.3, concentrations of tAs decreased upon the increasing step of polishing. However, it should be noted that still the tAs concentrations in all types of polished rice in this study were higher than the FAO/WHO maximum allowable As concentration in polished rice of 0.2 mg kg^{-1}.

Table 4- 4 Concentrations of tAs in rice with different DOPs and resulting bran

Sample	Rice type[a]	Total As concentration (mg kg^{-1})				
		Min	**Max**	**Average**	**Median**	**SE[b]**
Grain	WG	0.3081	0.3758	0.3310	0.3090	0.0224
	OP	0.2612	0.2728	0.2654	0.2623	0.0037
	RWP	0.2613	0.2910	0.2741	0.2701	0.0088
	WP	0.2501	0.2750	0.2640	0.2670	0.0073
	EWP	0.2200	0.2622	0.2471	0.2590	0.0136
Bran	OP	0.7118	0.8277	0.7850	0.8156	0.0368
	RWP	0.7354	0.8507	0.8097	0.8429	0.0372
	WP	0.7356	0.8530	0.8131	0.8509	0.0388
	EWP	0.7490	0.8246	0.7946	0.8102	0.0232

Remark: [a] Total number sample was 3
[b] Standard error of the mean

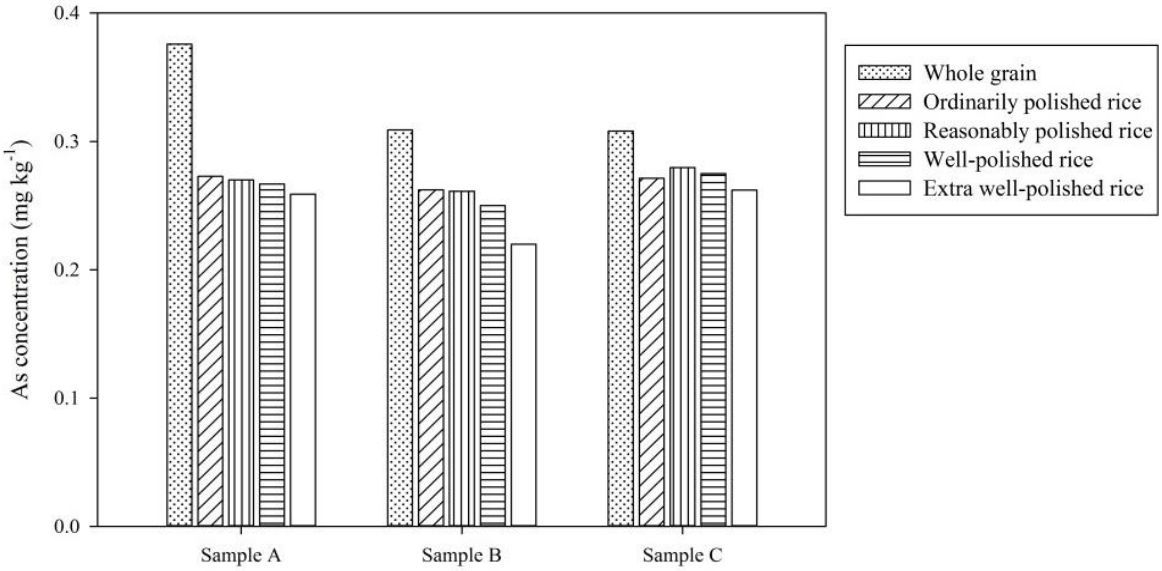


Figure 4- 3 Comparisons of tAs concentrations in the resulting polished rice grain from a stepwise polishing process.

Comparing to the tAs concentrations in original WG rice (Table 4.4), the average percentages of tAs reduction were approximately 18.7 (± 7.6) % for OP rice, 16.4 (± 11.3) % for RWP rice, 19.6 (± 9.2) % for WP rice, and 24.9 (± 8.8) % for EWP rice.

The significant percentage of As reduction (15% to 31%) comparing to the tAs content in the WG rice was found in the EWP rice (p<0.01). On average, approximately 27% of tAs concentration was decreased at the end of the fourth polishing step to give the EWP rice as the product. This level of As reduction from WG to EWP was in a similar range of that 22% As reduction which was reported by Jo and Todorov (2019). On the other hand, Pedron et al. (2019) reported up to 41% of As reduction in white rice compared to that in the brown rice as the higher mass loss (18.3%) during the polishing process was found comparing to this study (8.1%). When compared the effects of particular DOP on tAs reduction in rice grain in this study to those previous studies, similar magnitudes of As reduction were found. For example, Sun et al. (2008) and Naito et al. (2015) reported approximately 17% to 54% and 12% to 27% of tAs reductions in rice with 7% DOP and 5% DOP, respectively. While, approximately 25.7% and 25.2% in tAs reductions were determined in rice with 5.9% and 7.5% DOP in this study.

Concerning the stepwise polishing process, the highest percentage of As reduction (26%) in this study was also found in the first polishing step (Figure 4-3). This pattern was similar to the reduction of essential microelements in the stepwise polished rice. The level of tAs contained in the bran which was polished and removed in this study was approximately 12% to 20% of the tAs in WG rice. The concentration of tAs in the bran (0.7118 to 0.8530 mg kg^{-1} of bran) was approximately 2.3 to 2.8 times more concentrated than that tAs concentration contained in the WG rice (Table 4-4). This result clearly showed that As is mainly concentrated in the brown layer of the grain. This result is in accordance with the previous studies reported that As is normally localizes on the surface of the rice grain (Meharg et al., 2008; Smith et al., 2009). To be more specific, Lombi et al. (2009) found the large localization of As in the husk and the grain peripheral layer. This layer is so called the aleurone layer, the outermost layer of the endosperm. Once the layer is removed, it is called the bran and it could present approximately 3% of As higher than that of the endosperm (Pedron et al., 2019). In addition, it was also reported that germ or embryo can also accumulate a larger concentration of As comparing to the inner parts of the endosperm or the starchy endosperm (Seyfferth et al., 2011)

3.3) Concentrations of tAs concentrations in bran

On an average, concentration of tAs in bran was approximately 2.5 times higher than that of the non-polished rice (Table 4-4). To be more specific, tAs concentration in EWP bran was higher than the tAs concentration in the EWP rice by approximately 3.3 times. This instrumental analysis results clearly confirmed the localization of As in the brown part of the grain. It is also confirmed by Jo and Todorov (2019) that bran layer had higher As contents (0.86 mg kg^{-1}) than the endosperm (0.17 mg kg^{-1}). Moreover, Jo and Todorov (2019) found a gradual decrease in tAs concentration from the outer part of the endosperm (white grain) to the central part of the grain.

3.4) Mass balance of tAs concentrations in rice

The mass balance of tAs in rice samples with different DOPs was calculated by multiplying the summation of tAs concentrations in polished rice and its bran with the percentage of weight loss then, dividing by the tAs concentration in the non-polished rice. The results of mass balance of all rice samples regarding the DOPs are shown in Figure 4-4. The recoveries of tAs concentrations in rice samples were within the acceptable range of ±15 %. The average (±SD) recoveries of mass balance were 95.4 % (±8.5) for OP rice, 99.1 % (±12.0) for RWP rice, 98.9 % (±9.9) for EP rice, and 94.7 % (±9.3) for EWP rice.

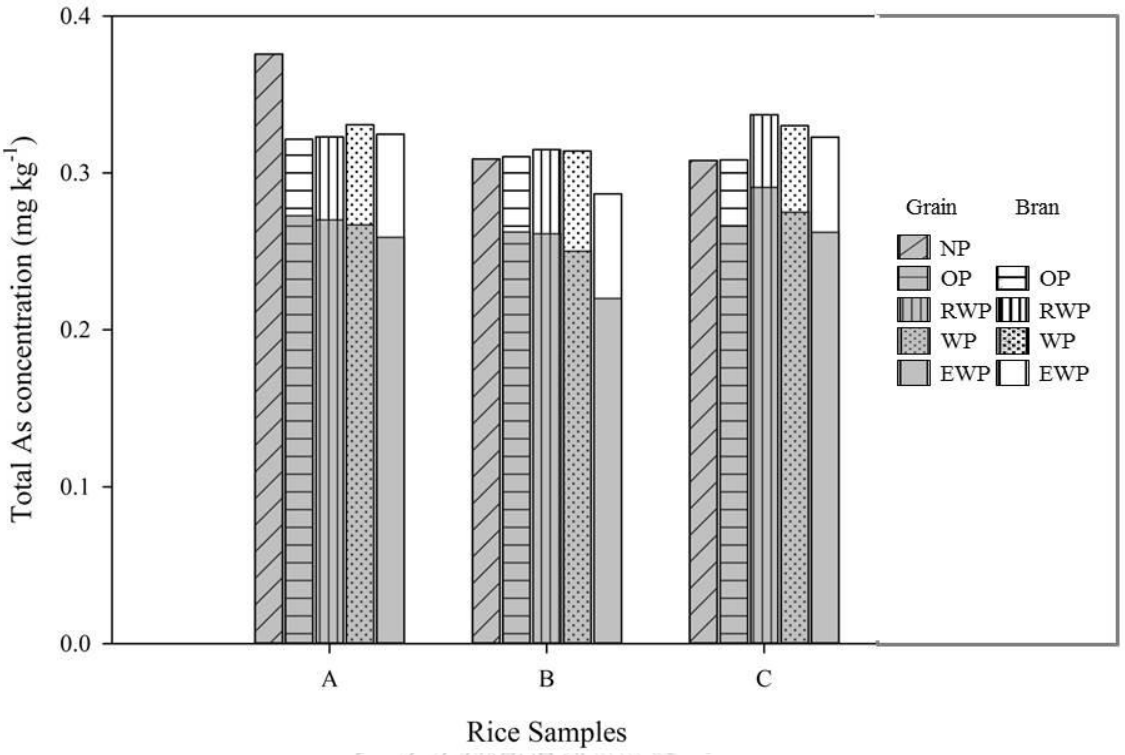


Figure 4- 4 Mass balance of tAs concentration in rice grain and bran.

3.5) Relationship between DOP and the remaining tAs concentrations in polished rice

A correlation between remaining tAs concentrations in rice and polishing degrees is demonstrated in Figure 4-5. A strong negative significant correlation between remaining tAs concentrations and DOPs (r = 0.713, p < 0.01) strongly confirmed not only the localization of As in the bran but also the effect of polishing on the reduction of As from the rice grain.

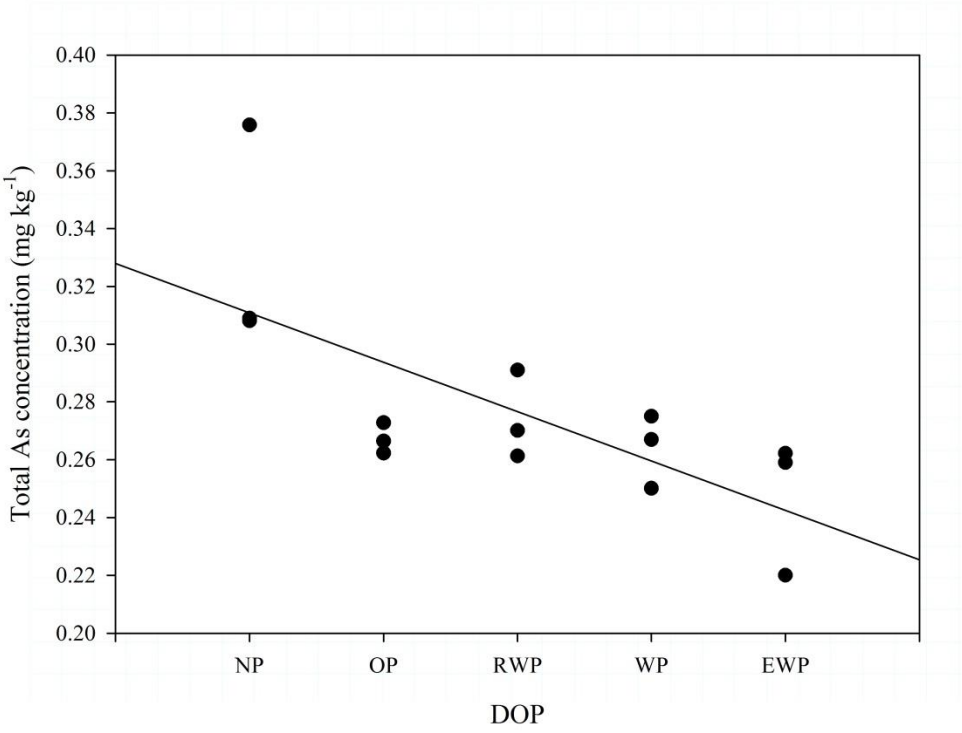


Figure 4- 5 Pearson correlation between DOPs and tAs concentrations in rice.

4. Conclusions

This study determined the effect of polishing on tAs concentration in rice that passes through different polishing levels according to the Thai national classification of polished rice which classified rice into non-polished (WG) rice, ordinarily polished (OP) rice, reasonably well-polished (RWP) rice, well-polished (WP) rice, extra well-polished (EWP) rice. The results showed that polishing process can remove As from the rice grain. The higher degree of polishing applied, the higher tAs reduction was found. When the highest degree of polishing was applied, approximately 24.9% of tAs reduction was determined comparing to the tAs concentration in the non-polished rice. While, another lower degrees of polishing could reduce tAs by approximately 16.4% to 19.6%. Even though a strong negative significant correlation between the remaining tAs concentration and DOP strongly confirmed the effect of polishing on the reduction of tAs from the rice grain, it should be noted that all types of polished rice still contained tAs concentrations exceeding the FAO/WHO maximum allowable concentration of iAs in polished rice (0.2 mg kg^{-1}).

CHAPTER V

EFFECTS OF RICE WASHING AND COOKING PROCESSES ON TOTAL, INORGANIC, AND BIOACCESSIBLE ARSENIC CONCENTRATIONS IN RICE

1. Introduction

Prior to human consumption, several home preparation processes including soaking, washing, and cooking are normally apply. The preparation process applied may be different depending on the rice genotype, rice type, cultural background as well as the sensory preference. For example, soaking rice before cooking is popular in South Asia and it is normally applied to the *basmati* rice. Some types of rice such as *indica* rice and *japonica* rice are directly washed before cooking with or without excess cooking water (Yu et al., 2017). It was recently reported by Kumarathilaka et al. (2019) that a modification to the rice cooking practices at the household kitchen level could reduce As concentration in cooked rice grain to a greater extent.

Washing of rice prior to cooking is a common practice to remove bran, dust and dirt which might be contaminated to rice during the storage of rice grain. During washing some water-soluble compositions, nutrients and elements might be leached out and removed. For example, the International Rice Research Institute (IRRI) in collaboration with the FAO have reported significant losses of several proximate compositions, essential elements, minerals, and vitamins after rice washing (International Rice Research Institute/Food and Agriculture Organization, 1993). In the case of As removal from rice after washing, washing of rice before cooking has been recommended in numerous studies to reduce tAs content in cooked rice (Naito et al., 2015). Wide variations in the percentage of As removal from washing were reported. For instance, Naito et al. (2015) reported approximately 81% to 84% and 71% to 83% of tAs reductions from the polished and non-polished rice grain after washing rice for 3 times with deionized water. While, Raab et al. (2009) found only 13% to 15% of tAs reduction after the *basmati* rice was washed. In contrast, Halder et al. (2014) reported a negligible effect of washing on tAs concentration in rice grain even though rice was

washed for 2 to 4 times. Regarding these previous reports, Kumarathilaka et al. (2019) recently concluded that both positive and negative effects of rice washing on tAs concentrations in rice can be attributed to the initial concentrations of As in both washing water and raw rice grain, mode of washing, number of washing steps, washing time, and the studied sample size.

Cooking is another rice preparation process before human consumption which may affect the concentration of As in rice. The cooking method is the most important factor influencing the concentration of As in cooked rice. Cooking rice with high water to rice ratio and subsequently discarding excess water has been reported to effectively reduce As concentration in cooked rice. This method, a popular rice cooking method in the past, is sometime called as a traditional cooking method. The traditional cooking method with the 6:1 of water (deionized water) to rice ratio could reduce approximately 30% to 35% and 45% of tAs and iAs in the rice grain, respectively, compared to raw rice (Raab et al., 2009; Mwale et al., 2018). When the water to rice ratio was increased up to 10 – 12:1, approximately 28% to 66% and 40% to 60% of tAs and iAs reductions in the cooked rice comparing to raw rice were found (Carey et al., 2015; Gray et al., 2015). Owings to the complicated cooking procedure of the traditional cooking method, the modern cooking process in which rice is normally cooked in an electric rice cooker have become very popular cooking method in the modern society. For this modern cooking method, rice is normally cooked with the optimum water to rice ratio, boiled, and steamed until all water is absorbed into the cooked rice grain and evaporated during cooking. Although this modern cooking method is a common practice worldwide, the study on the effect of this modern cooking method on tAs reduction in rice is still limited. Up to date, only the result on the non-significant tAs reduction in rice cooked by the modern cooking method was reported by Raab et al. (2009).

Therefore, this study was conducted to i) study the effects of different washing and cooking scenarios on tAs concentrations in rice, and ii) determine the concentrations of tAs, iAs, and bAs in cooked rice. It should be noted that the local Thai household washing and cooking practices were adopted in this study.

2. Materials and method

2.1) Rice sample collection and preparation

Regarding to the determination of tAs concentration in a total of 208 rice samples reported in Chapter 3, a total of 6 representative polished and non-polished rice samples containing tAs concentration equal to or greater than the 75th percentiles with low percentage of relative standard deviation (%RSD) were selected. The information of all representative samples is summarized in Table 5-1.

Table 5- 1 Information of the representative rice samples

No.	Sample code	Rice type	tAs concentration (mg kg^{-1})		
			Concentration	Average ± SD[a.]	%RSD[b]
1.	J1_215	Polished rice	0.2158	0.2174 ± 0.0015	0.69
2.	J1_231	Polished rice	0.2188		
3.	J1_232	Polished rice	0.2177		
4.	J2_217	Non-polished rice	0.4063	0.4087 ± 0.0044	1.07
5.	J2_239	Non-polished rice	0.4061		
6.	J2_240	Non-polished rice	0.4139		

Remark: [a] Standard deviation
[b] Relative standard deviation

2.2) Rice washing procedures

For each set of rice sample, two subsets of each rice sample were prepared. To obtain one subset of rice sample, approximately 50 g of raw rice sample was weighed into the beaker. Then, 300 mL of deionized water (18.2 MΩ cm^{-1}) was transferred into the beaker containing rice sample. After that, the mixture was manually stirred for 2 min and allowed to be settled for another 3 min. In this study, two different washing methods (1- and 3-time washing) were applied to the two subsets of rice sample. The washing for each scenario and each rice sample was conducted in duplicates. After washing, the washing water was discarded and the washed rice was lyophilized using a freeze dryer, sealed in a clean plastic tube, and stored in a desiccator until further sample preparation.

2.3) Rice cooking procedures

Resulting washed rice with the highest tAs removal was selected as the representative sample for the study on the effect of cooking on tAs, iAs, and bAs concentrations in cooked rice. Two different cooking methods namely; traditional and

modern cooking methods were applied to cook the washed rice. Approximately 50 g of washed rice was separately weighed and transferred into the rice cooking pot. The modern cooking method with the water to rice ratio of 1.5:1 was performed using an electric rice cooker (Figure 5-1(a)), while the traditional cooking method with excess water to rice ratio of 10:1 was conducted in an aluminum pot (Figure 5-1(b)). The water used for cooking in this study was deionized water (18.2 MΩ cm^{-1}) which can be considered as As-free water. The cooking for each scenario and each rice sample was conducted in duplicates. Once cooking was completed, all cooked rice samples were frozen at -60°C in a refrigerator for 24 h before lyophilizing in a freeze dryer for another 24 h. Then, cooked rice sample was blended by an aluminum blade blender. The blended sample was sieved (~420 µm), dried at 85 °C in a hot air oven to a constant weight, and stored in a desiccator until further sample preparation.

Figure 5- 1 Pictures of (a) an electric rice cooker for a modern cooking method and (b) an aluminum pot for a traditional rice cooking method.

2.4) Sample digestion and analysis

1) Total arsenic

All samples were digested by an acid digestion according to Phan et al. (2013). Briefly, about 0.10 g of rice sample was weighed into a clean 15 mL polyethylene tube. Next, 1 mL of concentrated HNO_3 (superpure grade for trace analysis) was added into the tube. After that, the tube was capped and left in a hood at room temperature for 48 h. Then, 9 mL of deionized water (18.2 MΩ cm^{-1}) was added into a tube containing digested solution. Lastly, the final solution was filtrated through a 0.45 µm syringe filter to obtain a final solution for tAs determinations. The digested solution was kept at 4°C

until analyses. All solutions were analyzed by ICP-MS (Agilent ICP-MS model 7300CE).

2) Inorganic arsenic

Two-step operating procedure for iAs extraction from rice grain was conducted. First, a high temperature (95°C) extraction method adopted from Chaney et al. (2018) was conducted. In summary, about 0.700 g of dried rice flour was weighed into a 50 mL polyethylene centrifuge tube. Then, 10 mL of 0.28 M HNO_3 was added into the tube. The lid was tightly sealed afterward. After that, the tube was heated to reach 95°C for 90 min. Then, sample was left stand to achieve room temperature before filtering through the Whatman no. 40 filter paper which was placed on a suitable size of the polyethylene (PE) funnel and rinsing with 0.28 M HNO_3 for two more times. However, it should be noted that the total volume of rinsing solution should be kept to a level of 9 mL. Finally, the final volume of the extracted solution was made to 20 mL by 0.28 M HNO_3.

Then, the pre-reduction step should be followed. In short, 8 mL of extracted solution obtained from the first operating step, 3 mL of concentrated hydrochloric acid (36% v/v HCl), and 6 mL of the reducing agent was consecutively added into the fresh tube and mixed homogeneously. The reducing agent was prepared by dissolving 5 g of potassium iodide (KI) and 5 g of ascorbic acid in the deionized water with the final volume of 100 mL. Then, 5 mL of 1.73 M sulfamic acid (H_3NSO_4) was slowly added to the tube to avoid agitated bubbling. The tube was left still at room temperature for 20 min to achieve a complete reduction of iAs to As(III). Finally, the solution was injected to the HG-AAS for iAs determination.

The conditions of HG-AAS for iAs analysis which was adopted from De La Calle et al. (2017) are summarized in Table 5-2. In principle, the hydride generation can convert the substance of interest to become volatile compound by using a reducing agent. In this study, the sodium borohydride ($NaBH_4$) in the acid condition (3% HCl) was used as the reducing agent. In such condition (reducing and strong acid) of sample introduction into the instrument, all iAs would be reduced to form As(III). Lastly, the arsine (AsH_3) gas can be generated for the analysis.

The 3% HCl was prepared by mixing 42 mL of concentrated HCl with the deionized water and bringing the solution to final volume of 500 mL. The $NaBH_4$ solution was prepared by dissolving 0.10 g of sodium hydroxide (NaOH) and 1.0 g of $NaBH_4$ in the deionized water. After the final volume of the solution was made to 500 mL, the solution was filtrated and 1.8 mL of Antifoam B emulsion (Sigma A5757) was added into the solution. Antifoam solution was added to prevent an intense bubbling of sample in the HG-AAS system. It should be noted that both 3% HCl and $NaBH_4$ solution have to be prepared prior to the sample analysis by HG-AAS (Analytik Jena HG-AAS model ZEEnit 700 P).

Table 5- 2 Instrumental conditions for iAs quantification by using HG-AAS

Instrument		**Condition**
HG	Reducing agent	0.5% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH with the addition of 0.9 mL of Antifoam B emulsion (Sigma A5757) into 250 mL of reducing agent.
		3% (v/v) HCl solution
AAS	Wavelength	193.7 nm
	Spectral band-pass	0.7 nm
	Lamp current setting	400 mA
	Cell temperature	950 °C

3) Bioaccessible arsenic

A three-step RIVM *in vitro* digestion that mimic the human digestive system adopted from Versantvoort et al. (2004) was conducted. The conditions of the *in vitro* digestion, including the phase of digestion, pH and volume of digestive juice, and digestion time are summarized in Table 5-3. This method simulates the digestion in oral cavity by saliva, gastric phase by gastric juice, and small intestine phase by duodenal juice and bile. In brief, 4.5 g of rice sample was continuously incubated for 5 min with 6 mL of saliva, 2 h with an additional 12 mL of gastric juice, and another 2 h with 12 mL of duodenal juice, 6 mL of bile and 2 mL of HCO_3^-. Methods of all digestive juice preparations were summarized in Appendix I. The incubation was performed in a water

bath with orbital shaking at 37°C. Afterward, the solution was centrifuged for 5 min at 3,000 rpm in order to completely separate the supernatant and the precipitate phase. The supernatant was filtrated through a 0.45 µm syringe filter. After the completeness of digestion procedure, concentration of As in the resulting solution was determined by a graphite furnace AAS (Varian Spectra AA240Z, Spectra AA240FS, Australia). The concentration obtained is believed to represent the actual amount of As which is readily to be absorbed into the human body.

Table 5- 12 A summary for the conditions of the *in vitro* digestion

Step no.	Digestion phase in gastrointestinal tract	pH of the digestive solution	Digestive solution	Digestion conditions	
				Volume of digestive solution (mL)	Digestion time
1.	Oral cavity	6.8	Saliva	6	5 min
2.	Stomach	2 – 3	Gastric juice	12	2 h
3.	Small intestine	6.5 – 7	Duodenal juice	12	2 h
			Bile	6	
			HCO_3^-	2	

2.5) Statistical analysis

All statistical analyses were performed using IBM SPSS Statistical software, version 22 (SPSS Chicago, IL, USA). Since the sample size of this study was less than 50, the Shapiro-Wilk test was performed to confirm the normality of As concentration in both raw and processed rice. The results were expressed as average and SD. The paired sample t-test was performed to determine the differences in As concentrations in raw, washed, and cooked rice. A level of 0.05 (*p*-value) was used to determine the significant level.

3. Results and discussion

3.1) Effect of rice washing on tAs concentrations in rice

The tAs reductions in polished and non-polished rice after different washing scenarios were applied were illustrated in Figure 5-2. After the polished and non-polished rice was washed for 1 time by the deionized water which can be considered as As-free water, approximately 11.1% and 12.6% of tAs reductions were found. The tAs

reductions in polished and non-polished rice were increased to approximately 29.3% and 12.7%, respectively, when 3-time rice washing was conducted. From a statistical point of view, tAs concentrations in polished rice was significantly reduced by both washing scenarios ($p < 0.05$). While, no significant reduction in tAs concentration was observed in the washed non-polished rice even up to 3 times of rice washing was applied ($p > 0.05$). Results of lower tAs reductions in the polished rice comparing to the non-polished rice may cause by the lower water absorption capacity of the non-polished rice. The outer layer (bran and aleurone layer) of the non-polished rice could prevent water absorption to the grain. As a result, lower concentration of As which may leached out during rice washing was observed.

The effects of different rice washing scenarios to tAs concentrations in rice in this study were in consistent with the study of (Jaafar et al., 2018) who found that the tAs reduction was increased in corresponding to the increasing washing step. Approximately 20% to 28% of tAs was removed when the polished rice was washed by either low As water or As-free water for 3 times were reported by several studies (Gray et al., 2015). In the case of washing of non-polished rice, a similar result of non-significant tAs reduction in 1- and 3-time washed rice was also reported by Naito et al. (2015). Naito et al. (2015) even reported several times lower tAs reductions (approximately 2% to 5%) than this study (12.7%).

Although the studies of Naito et al. (2015), Jaafar et al. (2018) and Kumarathilaka et al. (2019) reported non-significant effect of the additional washing step on the tAs removal from washed rice grain, this study found a significant tAs reduction when increasing washing step was applied to wash the polished rice. The common finding among those previous studies and this present study was the observation of highest removal of tAs after the first washing step. In addition, as the iAs is uncharged and has high mobility at neutral pH of DI water (Sun et al., 2012), it is expected that iAs would be the main As species which was removed during rice washing process.

It should be noted that as the highest tAs reductions in both polished and non-polished rice in this study were observed after both types of rice were washed for 3 times (Figure 5-3), this 3-step rice washing scenario was considered as the best rice washing scenario.

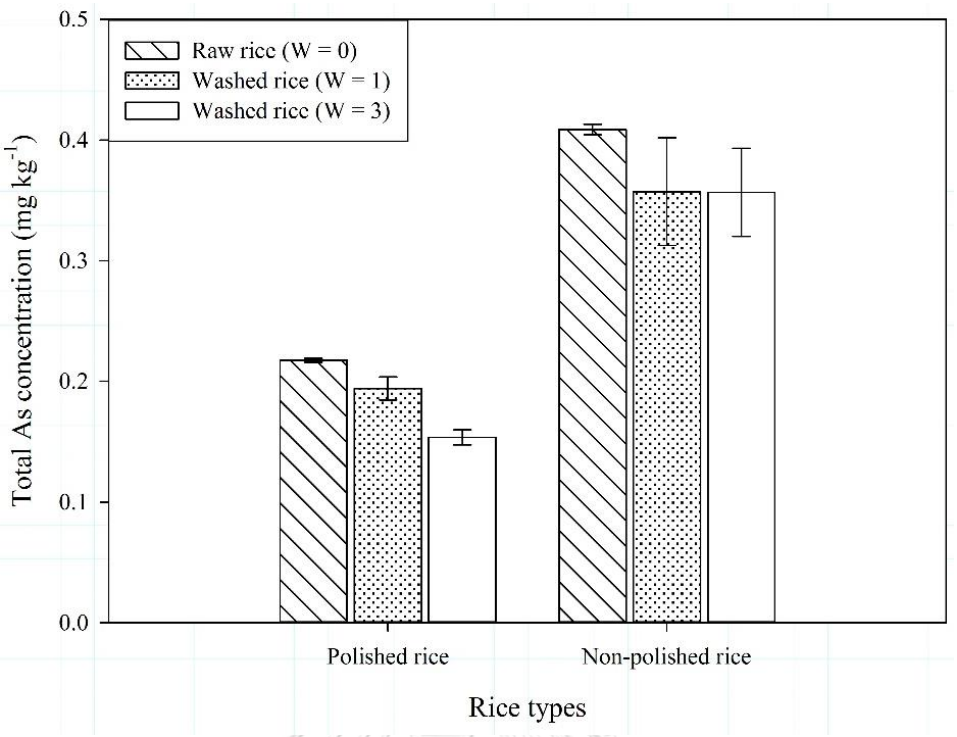


Figure 5- 2 Concentrations of tAs in raw rice and rice washed for 1 and 3 times.

3.2) Effect of rice cooking method on tAs concentrations in rice

Regarding to the best rice washing scenario (3-step rice washing) obtained from the previous section of this study (Section 3.1: Effect of rice washing on tAs concentrations in rice), raw rice grain which was washed for 3 times were cooked under 2 different cooking methods namely: traditional and modern cooking methods.

The physical appearances of the modern and traditional cooked rice after cooking are illustrated in Figure 5-3 and Figure 5-4, respectively.

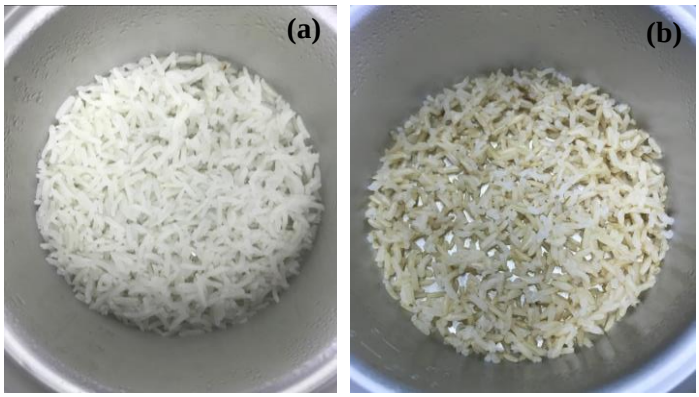


Figure 5- 3 Physical appearances of (a) polished rice and (b) non-polished rice after cooking by the modern cooking method.

Figure 5- 4 Pictures showing the traditional cooking method and Physical appearances of (a) polished rice and (b) non-polished rice after cooking by the traditional cooking method.

Table 5-4 summarizes the concentrations of tAs in rice before and after cooking with different cooking methods. It was found that approximately 17.8% of tAs in polished rice and 23.4% of tAs in non-polished rice were decreased after cooking by the modern cooking method. Meanwhile, the traditional cooking method reduced up to 60% of tAs in cooked rice compared to the raw rice. The significant tAs reductions in both polished and non-polished cooked rice were obtained from both cooking methods ($p < 0.05$) (Figure 5-5). Considering the remaining tAs concentrations in cooked rice for human consumption, all cooked rice contained tAs lower than the FAO/WHO

maximum allowable As concentration of 0.2 mg kg^{-1} for polished rice and 0.35 mg kg^{-1} for non-polished rice. It can be implied from these results (Table 5-4) that consumption of both types of rice is safe.

Table 5- 3 Concentrations of tAs in raw rice and modern and traditional cooked rice

Statistical value	**tAs concentration (mg kg^{-1})**		
	Raw rice	**Modern cooked rice**	**Traditional cooked rice**
Polished rice			
Min	0.2158	0.1738	0.0822
Max	0.2188	0.1871	0.0909
Average	0.2174	0.1787	0.0867
Median	0.2177	0.1753	0.8688
SD[a]	0.0015	0.0073	0.0043
Non-polished rice			
Min	0.4061	0.2912	0.1549
Max	0.4139	0.3304	0.1736
Average	0.4088	0.3130	0.1655
Median	0.4063	0.3173	0.1681
SD[a]	0.0044	0.0200	0.0096

Remark: [a] Standard deviation

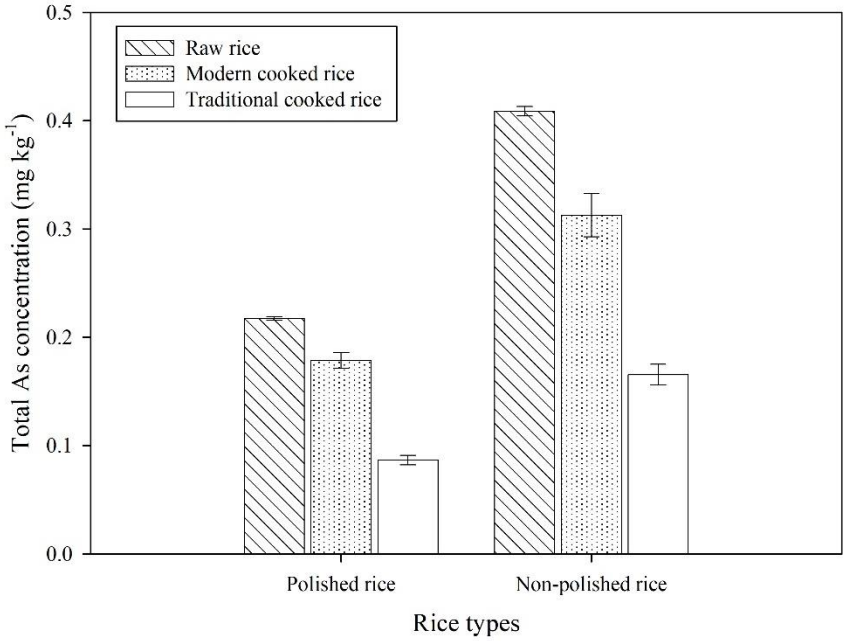


Figure 5- 5 Concentrations of tAs in modern and traditional cooked rice comparing to raw rice.

Regarding the modern cooking method, the results obtained from this study agreed well with those previous results. Comparing to the previous study of Zhuang et al. (2016) who reported approximately 6% of tAs reduction in 3-step washed and modern cooked rice, higher tAs reduction was found in this study (17.8%). On the other hand, Sun et al. (2012) also reported approximately 20.6% and 7.1% tAs reduction in the market-based rice sample and As contaminated rice sample upon washing and cooking with 3:1 water to rice ratio.

When compared the tAs concentrations in 3-time washed polished and non-polished rice with and without cooking, the tAs concentrations in those samples were not statistically significantly different ($p < 0.05$). Therefore, it can be concluded from these results that the significant reduction of tAs in modern cooked rice was mainly come from the washing step.

When compare the tAs concentrations in 3-time washed and modern non-polished cooked rice to the tAs concentrations in only washed non-polished rice, it was interesting to find that the factor that can impact tAs reduction in the modern non-polished cooked rice was the evaporation of oAs at the cooking temperature higher than the stable temperature of As species (120 ºC) (Sun et al., 2012). Technically, the

temperature in the electric rice cooker can increase up to 145ºC after all cooking water was absorbed into rice grain. In addition, the dilution of tAs concentration in non-polished cooked rice can be another factor affected to the tAs reduction as it was reported by (Bowman et al., 2011) that a conversion factor of 0.36 was required to convert the mass of cooked rice to the mass of the uncooked (raw) rice.

In the case of traditional cooking method, the polished and non-polished cooked rice approximately had 2.41- to 2.63-fold and 2.38- to 2.62-fold lower tAs concentrations than the raw rice. The tAs concentrations in polished and non-polished rice cooked with the traditional cooking method contained approximately 2.05- to 2.11-fold and 1.88- to 1.90-fold lower tAs concentrations than the same type of rice cooked with the modern cooking method. The significant As reduction in rice cooked with the traditional cooking process was found because the excess cooking water (cruel) which may leached out As from the rice grain during boiling and cooking was discarded from the cooking system. The reason behind this result was confirmed by a previous study (Mihucz et al., 2010) reported that 30% to 40% of As was removed by hot water comparing to the As removal from rice grain by the normal temperature water (5% to 20% of As removal). Increasing temperature during cooking process can increase the water penetration into the rice grain thus, higher concentration of metal accumulated in the grain could be dissolved in the boiling water (Sharafi et al., 2019b). With a consideration of As speciation, Halder et al. (2014) reported approximately 42.1% of iAs reduction in traditional cooked rice comparing to that of the raw rice. In addition, a very strong positive correlation between tAs and iAs reductions were identified. It can be implied from this previous study that the change in tAs concentration in rice grain was dominated by the change in As(III) concentration in the grain during the cooking process. Moreover, Mihucz et al. (2007) found out that As(III) was the main As species in discarded cooking water.

However, it should be noted that up to 129% of tAs concentration in cooked rice can be observed if the As-contaminated water was used for cooking (Mandal et al. (2019). Therefore, Jaafar et al. (2018) have concluded that the final As concentration in cooked rice mainly depends on the tAs concentration in the cooking water.

3.3) Concentrations of tAs, iAs, and bAs in modern cooked rice

Since up to 60% of tAs concentration in the traditional cooked rice was removed, lower As concentration than the limit of quantitation of the analytical instrument was remained in the cooked rice grain. Therefore, the analyses of tAs, iAs, and bAs concentrations in cooked rice were only conducted in the modern cooked rice. This is also in accordance to the practical consumption behavior of the general Thai population that rice consumed is normally cooked using the electric rice cooker.

Concentrations of tAs, iAs, and bAs in raw and modern cooked rice are summarized in Table 5-5. Approximately 17.8% and 23.4% of tAs in raw polished and non-polished rice, respectively, was removed by the modern cooking process. Although, the average percentage of tAs reduction in non-polished rice was slightly higher than that of polished rice, significant differences in tAs reductions from those types of cooked rice could not be observed ($p > 0.05$).

In contrast, the percentages of iAs reductions between polished and non-polished cooked rice (Figure 5-6) were significantly different ($p < 0.05$). Approximately 46.1% and 19.3% of iAs removal were reported comparing to those level in raw rice. Considering the percentages of iAs to tAs in raw and cooked rice, the significant lower percentages of 59.8% and 39.3% were found in polished and non-polished rice, respectively. This indicated that washing and cooking processes significantly reduce iAs concentration in rice. Since iAs generally localizes throughout the outer layer of rice grain, iAs can be leached out during any methods that apply water into the rice preparation process. Whereas, an increase in the percentage of iAs to tAs was found in non-polished cooked rice (54.2%) comparing to that of the raw non-polished rice (51.3%). This may be attributed to the localization of iAs under the brown part of the grain which has lower water absorption capacity. Therefore, it is not easy to leach iAs out from the grain of non-polished rice.

Table 5-5 Concentrations of tAs, iAs, and bAs in raw and modern cooked rice

Rice type	Concentration (average ± SD, mg kg^{-1})					
	tAs		iAs		bAs	
	Raw rice	Cooked rice	Raw rice	Cooked rice	Raw rice	Cooked rice
Polished	0.2174 ± 0.0015	0.1787 ± 0.0073	0.1301 ± 0.0046	0.0704 ± 0.0160	0.0827 ± 0.0157	0.0304 ± 0.0028
Non-polished	0.4088 ± 0.0044	0.3130 ± 0.0200	0.2098 ± 0.0240	0.1685 ± 0.0128	0.1760 ± 0.0134	0.0756 ± 0.0224

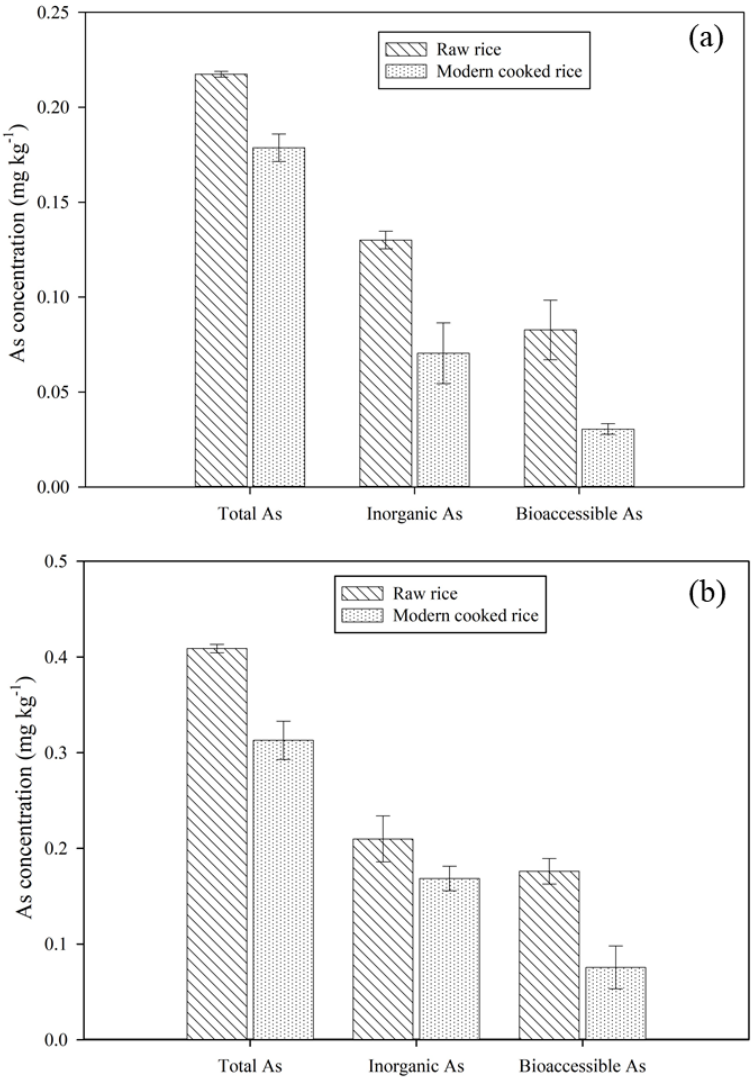


Figure 5-6 Concentrations of tAs, iAs, and bAs in (a) polished and (b) non-polished rice cooked by the modern cooking method.

The average As bioaccessibilities in raw and modern cooked polished-rice were 38.1% and 17.1%, respectively. While, the As bioaccessibilities in raw and modern

cooked non-polished rice were 43.0% and 23.9%, respectively. The bioaccessiblity of As in raw and cooked rice are illustrated in Figure 5-6. The As bioaccessibility in cooked rice significantly lower than that of the raw rice ($p < 0.05$). However, no significant differences were observed in the As bioaccessibilities ($p > 0.05$) between the different types of rice. In the case that rice type was not considered, the percentages of bioaccessibilities in raw and cooked rice were 40.6% and 20.5%, respectively. This clearly confirmed that cooking process could reduce almost a half of the bAs concentration in rice.

Concerning the concentrations of tAs, iAs, and bAs contained in modern cooked rice, concentrations of all As species of interested were lower than the FAO/WHO standards.

4. Conclusions

This study focused on the effects of rice washing and cooking processes on the concentrations of tAs in polished and non-polished rice. Different washing and cooking procedures which are normally applied in the Thai local household were conducted in this study. There were no significant differences in tAs concentrations in non-polished rice washed by As-free water either for 1 or 3 times. However, tAs concentrations were significantly reduced by approximately 29% when 3-times washing was applied to wash polished rice. In case of cooking, the traditional cooking method (10:1 of water to rice ratio) significantly decreased tAs concentration (60%) comparing to the raw rice. Whereas, the modern cooking method (1.5:1 of water to rice ratio) illustrated slight reductions of tAs in 3-time washed and cooked polished (17.8%) and cooked non-polished rice (23.4%), respectively. A significant iAs reduction can be observed only in the cooked polished-rice. Modern cooking method significantly reduced the bioaccessibility of As in both rice types. The bAs concentrations were reduced up to 2.7 and 2.3 times lower than those bAs concentrations in the raw polished and non-polished rice. It could be finally concluded from overall results obtained that 3-time washed with consequently cooked by the modern cooking method can be considered as a proper rice processing and cooking method that provide cooked rice with safe level of tAs, iAs, and bAs for human consumption.

CHAPTER VI

DETERMINISTIC AND PROBABILISTIC RISK ASSESSMENTS OF ARSENIC EXPOSURE THROUGH RICE CONSUMPTION

1. Introduction

Due to the fact that rice is considered as the human staple food, especially for the Asian population (Organisation for Economic Co-Operation and Development/Food and Agriculture Organization, 2019), rice can be a main As exposure pathway of human (Flora, 2015). Previously, the total concentration of substance was used to assess to levels of exposure and potential health effects that could be develop in human. However, the total concentration of substance is normally higher than the concentration of substance which is actually absorbed into the human body. Therefore, the use of total concentration of substance in the human health risk assessment can overestimate the exposure and toxic effects of that particular substance. An *in vitro* digestion, which is the simplified the human digestive system, could provide the more accurate approximation for the portion of contaminants which is released from the food matrix, readily to be absorbed into the intestine and finally enter the blood circulation to exert the toxic effects. This portion of contaminant is scientifically called as bioaccessibility (Versantvoort et al., 2004; Moreda-Piñeiro et al., 2011). By applying this bioaccessible concentration of substance into the human health risk assessment equation, more accurate exposure and risk results can be obtained and the uncertainties of the resulted can be reduced. In term of the exposure of As through rice consumption, several recent works have taken the bioaccessibility of As into their human health risk assessment approaches (Trenary et al., 2012; Zhuang et al., 2016; Bolan et al., 2017)

Even though the DRA has been widely used to evaluate the level of exposure and characterize the potential risks of exposure in human. The results obtained may overestimate or underestimate the risk since this process applies only a single point value of each variable into the calculation. As a consequence, the over concerned or ignorant reaction might happen among the population. Therefore, the PRA is developed to provide the more accurate results of exposure and risk level. Since the PRA includes

a probability distribution function of all variables instead of using a single point value into the risk calculation, more accurate levels of exposure and potential risk can be obtained (Idaho Department of Environmental Quality, 2014).

The objectives of this study were to i) determine bAs concentrations in cooked rice, and ii) assess the levels of exposure and health risks of As through daily rice consumption in Thai population using the DRA and PRA approaches.

2. Materials and method

2.1) Rice sample selection and preparation

Regarding to the determination of tAs concentration in a total of 208 rice samples reported in Chapter 3, a total of 6 representative raw rice samples (3 polished rice and 3 non-polished rice samples) containing tAs concentration higher than the FAO/WHO maximum As allowable standard of 0.2 mg kg^{-1} in polished rice and 0.35 mg kg^{-1} in polished rice were selected for the further rice cooking process.

Regarding to the results reported about the effects of washing and cooking processes on tAs concentrations in rice which were reported earlier in Chapter 5 of this study, each rice sample (50 g) was washed for 3 times with 300 mL of deionized water (18.2 MΩ cm^{-1}). Afterward, washed rice was cooked by the modern cooking method using the electric rice cooker with the water to rice ratio of 1.5 to 1. The processing of each rice sample was conducted in duplicates. After cooking, cooked rice was frozen at -60°C in a freezer for 24 h before lyophilizing in a freeze dryer for another 24 h. Then, cooked rice sample was blended by an aluminum blade blender. The blended sample was sieved (~420 µm), dried at 85°C in a hot air oven to a constant weight, and stored in a desiccator until further *in vitro* digestion

2.2) *in vitro* digestion of rice sample

A three-step RIVM *in vitro* digestion that mimic the human digestive system adopted from Versantvoort et al. (2004) was conducted. The conditions of the *in vitro* digestion, including the phase of digestion, pH and volume of digestive juice, and digestion time are summarized in Table 6-1. This method simulates the digestion in oral cavity by saliva, gastric phase by gastric juice, and small intestine phase by duodenal juice and bile. In brief, 4.5 g of rice sample was continuously incubated for 5 min with

6 mL of saliva, 2 h with an additional 12 mL of gastric juice, and another 2 h with 12 mL of duodenal juice, 6 mL of bile and 2 mL of HCO_3^-. Methods of all digestive juice preparations were summarized in Appendix I. The incubation was performed in a water bath with orbital shaking at 37°C. Afterward, the solution was centrifuged for 5 min at 3,000 rpm in order to completely separate the supernatant and the precipitate phase. The supernatant was filtrated through a 0.45 µm syringe filter. After the completeness of digestion procedure, concentration of As in the resulting solution was determined by a graphite furnace AAS (Varian Spectra AA240Z, Spectra AA240FS, Australia). The concentration obtained is believed to represent the actual amount of As which is readily to be absorbed into the human body.

Table 6- 1 A summary for the conditions of the *in vitro* digestion

Step no.	Digestion phase in gastrointestinal tract	pH of the digestive solution	Digestive solution	Digestion conditions	
				Volume of digestive solution (mL)	Digestion time
1.	Oral cavity	6.8	Saliva	6	5 min
2.	Stomach	2 – 3	Gastric juice	12	2 h
3.	Small intestine	6.5 – 7	Duodenal juice	12	2 h
			Bile	6	
			HCO_3^-	2	

2.3) Exposure and risk assessment of As through rice consumption

Exposure rate (Eq. 6.1) and health risk assessment of As exposure (Eq. 6.2 and Eq. 6.3) through the polished and non-polished rice consumption were conducted regarding to the Integrated Risk Information System (IRIS) of the U.S. Environmental Protection Agency (U.S. EPA) (U.S. EPA, 1989).

$$\text{Exposure rate} = [CF \times IR \times ED] / [BW \times AT] \qquad \text{Eq. 6.1}$$

where CF is bAs concentration in cooked rice (mg kg^{-1}), IR is amount of rice consumed (kg d^{-1}), ED is exposure duration or a time period the person is in contact with As in rice (years), BW is body weight (kg) over the period of exposure (AT), and AT is period of exposure (days) or the period that individual consumes rice.

The hazard quotient (HQ) of non-carcinogenic health risks of As were calculated using Eq. 6.2.

$$HQ = \text{Exposure rate} / RfD \qquad \text{Eq. 6.2}$$

where exposure rate is the estimated As exposure rate obtained from Eq. 6.1 and RfD is an oral reference dose. The RfD value for iAs is 3×10^{-4} mg kg^{-1} d^{-1} (U.S. Environmental Protection Agency, 2016)

Since As, is classified by the IARC and IRIS as a confirmed human carcinogen, the lifetime carcinogenic risk (CR) of As was calculated using Eq. 6.3.

$$CR = \text{Exposure rate} \times SF \qquad \text{Eq. 6.3}$$

where CR represents a lifetime cancer risk, exposure rate can be estimated using Eq. 6.1 with the value of AT as a result of Thai life expectancy (75 years) multiplying by 365, and SF is cancer slope factor ((mg $kg^{-1}d^{-1})^{-1}$) which equals to 1.5 (mg $kg^{-1}d^{-1})^{-1}$ (U.S. Environmental Protection Agency, 2016)

This study applied both DRA and PRA approaches to calculate exposure rate and risks of As exposure in the population. Different values of each variable shown in Eq. 6.1 were used. The input values for each variable used in Eq. 6.1 are presented in Table 6-2. In case of IR for each group of population, the value was calculated by multiplying the number of 1.77 g kg^{-1} BW d^{-1} (dry weight) to the body weight value. This constant value of 1.77 g kg^{-1} BW is the Thai rice consumption rate reported by the (National Bureau of Agricultural Commodity and Food Standards, 2016).

In the case of input values for the PRA approach, the probability distributions of C, IR and BW as shown in Table 6-2 were drawn using the 10,000 iterations of the Monte Carlo simulation. The probable ranges of IR and BW of the Bangkok population based on the secondary data obtained from the National Bureau of Agricultural Commodity and Food Standards (2016) and Health Insurance System Research Office (2011) were also drawn using the 10,000 iterations of the Monte Carlo simulation (Appendix III). While, the ED for the probabilistic exposure and risk assessment was set as point value in each age group of the population (Table 6-2). This means that the exposure duration in the same age group was assumed to be same regardless the individual. After repeated samplings from the input variables (Table 6-2), a distribution

of output variables regarding the risks and probability functions was obtained. All simulations were performed by the @Risk software (DecisionTool Suite Industrial, Palisade Corporation, USA) combined with Microsoft Excel (Serial No. 7112263).

2.4) Statistical analysis

All statistical analyses were performed using IBM SPSS Statistical software, version 22 (SPSS Chicago, IL, USA). Since the sample size of this study was less than 50, the Shapiro-Wilk test was performed to confirm the normality of As exposure and health risk levels. The results were expressed as average and SD. The paired sample t-test was performed to determine the differences in the As exposure and health risk levels evaluated by DRA and PRA approaches. A level of 0.01 (*p*-value) was used to determine the significant level.

Table 6- 2 Input variables for risk calculation as deterministic and probabilistic approaches

Input variable		Unit		Approach		Data sources
				DRA	PRA	
C	bAs concentration in cooked rice	mg kg^{-1}	Polished rice	0.0292	BetaGeneral (15.039,2.659,0, 0.0537)	This study
			Non-polished rice	0.0649	BetaGeneral (4.2257, 2.1778,0,0.0983)	
IR	Ingestion rate of cooked rice	g d^{-1}	Children	57.15	Pearson6 (1240605, 9.339,0.00038)	National Bureau of Agricultural Commodity and Food Standards (2016)
			Adolescents	93.42	Pearson6 (403851, 14.81,0.00032)	
			Adults	113.30	Pearson6 (82.876, 33.734,44.75)	
ED	Exposure duration	years	Children		10	
			Adolescents		5	
			Adults		47	
BW	Body weight	kg BW	Children	32.29	Lognorm (32.30,11.75)	Health Insurance System Research Office (2011)
			Adolescents	52.78	Lognorm (52.78,14.80)	
			Adults	64.01	Lognorm (64.01,13.46)	
AT	Averaging time	d	Non-carcinogenic risk assessment	ED × 365 d $year^{-1}$		U.S. Environmental Protection Agency (1989)
			Carcinogenic risk assessment	75 × 365 d $year^{-1}$		World Health Organization (2014)
RfD	Oral reference dose	mg kg^{-1} d^{-1}		Inorganic As = 0.0003		U.S. Environmental Protection Agency (2016)
SF	Slope factor	(mg kg^{-1} d^{-1})$^{-1}$		Inorganic As = 1.5		U.S. Environmental Protection Agency (2016)

3. Results and discussion

3.1) Bioaccessibility of As in cooked rice

The bioaccessibility of As was calculated as the ratio of the bAs concentration in cooked rice to the tAs concentration in raw rice. Concentrations of tAs and bAs and the levels of As bioaccessibility are presented in Table 6-3. The ranges of As bioaccessibility in cooked polished and cooked non-polished rice were 13.0% to 15.6% and 12.4% to 22.7%, respectively. The As bioaccessibility in cooked non-polished rice was slightly higher than those in cooked polished rice; however, significant difference could not be observed between both rice types ($p > 0.01$).

Table 6- 3 Concentrations of tAs and bAs and the levels of As bioaccessibility

Rice type	**Average concentration±SD[a] (mg kg^{-1})**		**As bioaccessibility (%)**
	tAs in raw rice	**bAs in cooked rice**	
Polished rice	0.2174 ± 0.0015	0.0304 ± 0.0028	14.0%
Non-polished rice	0.4088 ± 0.0044	0.0756 ± 0.0224	18.5%

Remark: [a] Standard deviation

According to the results of tAs, iAs, and As bioaccessibility shown in Table 6-3, the differences between tAs concentrations in raw rice and bAs concentrations in cooked rice may derived from the accessible As level in human body through different rice matrix and the As reduction during rice preparation process. The distributions of bAs concentrations in cooked rice samples which were considered as the input variable for the PRA were obtained after the Monte Carlo simulation of the results of multiplication of tAs and bioaccessiility of As of each individual rice sample. The probable distribution functions of bAs concentrations in the polished and non-polished cooked rice were presented in Figure 6-1(a) and Figure 6-1(b), respectively. Regarding Figure 6-1(a) and Figure 6-1(b), the average (± SD) concentrations of bAs in polished and non-polished cooked rice were 0.0292 ± 0.0050 mg kg^{-1} and 0.0689 ± 0.0171 mg kg^{-1}, respectively. The maximum bAs concentrations were 0.0537 mg kg^{-1} for cooked polished rice and 0.0983 mg kg^{-1} for cooked non-polished rice. The distribution type of

bAs concentrations in both rice types were BetaGeneral in which the components consist of alpha1, alpha2, minimum, and maximum values. These results are presented in Table 6-2.

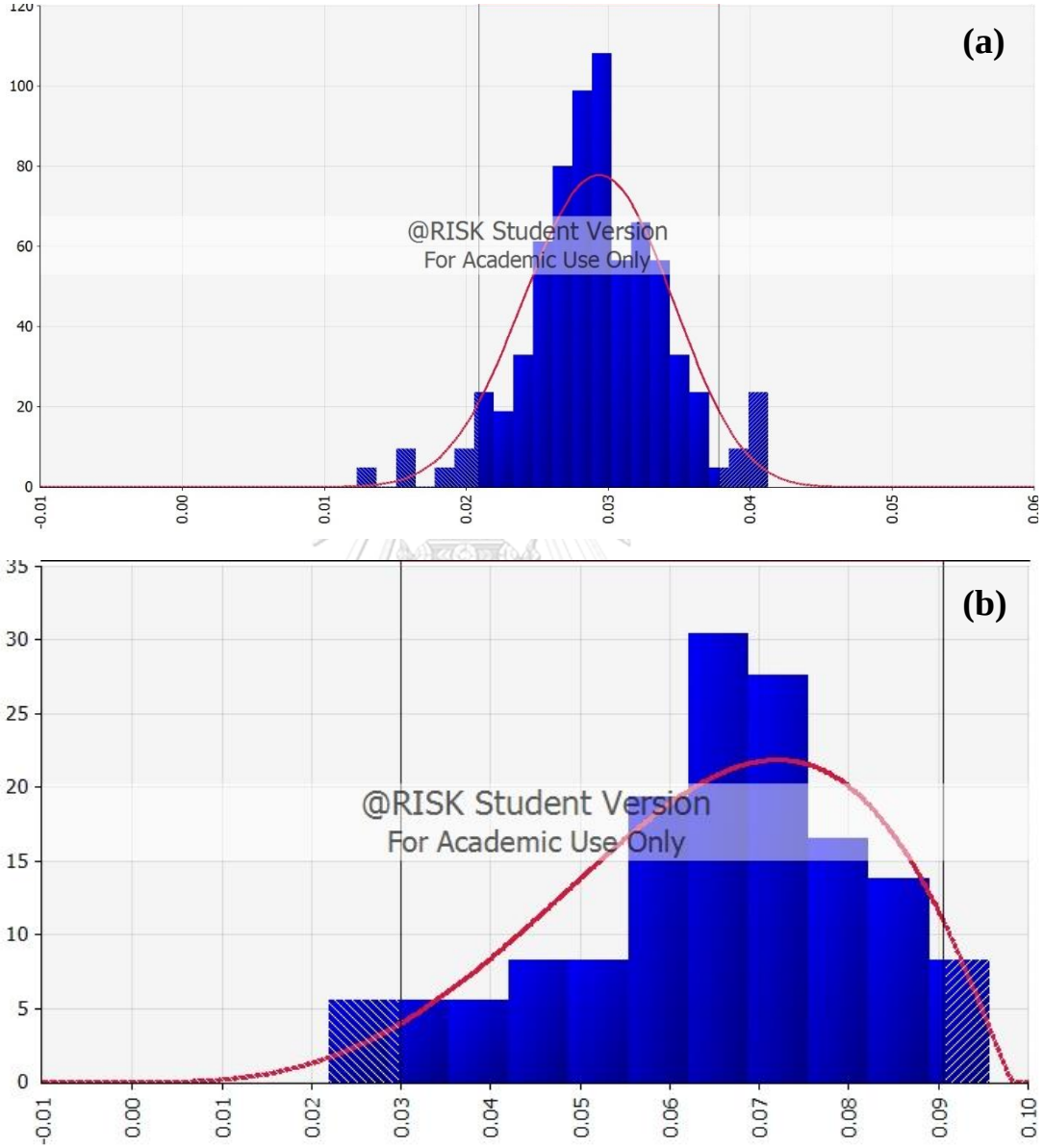


Figure 6-1 Probable distributions of bAs concentrations in (a) polished and (b) non-polished cooked rice calculating based on tAs concentrations in raw rice and the As bioaccessibility.

3.2) Exposure and risks of As via rice consumption evaluated by the DRA approach

Since the bAs concentration could represent the concentration of As that is believed to be released from the rice into the gastrointestinal tract before reaching the blood circulating system, this bAs was used as the input value of CF in Eq. 6.1. The average daily dose (ADD) and long-term average daily dose (LADD) of As exposure via rice consumption are summarized in Table 6-4. The population group in this study was divided into three groups including children (3 to 12.9 years), adolescents (13 to 17.9 years), and adults (18 to 64.9 years). According to the non-carcinogenic effect of As, the daily exposure rates of non-polished rice consumption in all age groups was higher than those of the polished rice consumption. This is because the bAs concentration in non-polished cooked rice was higher than that of the polished cooked rice for approximately 2.49 times. Therefore, the As exposure rates in children, adolescents, and adults via non-polished rice consumption were higher than those As exposure via polished rice consumption by approximately 2.24, 2.23, and 2.23 times, respectively. Children, in particular, who normally consume rice in lower rate, were found with highest ADD of As. It is because of their lowest body weight among all age groups which could resulted in the more concentration of substance per one unit of body weight. In case of ADD of As in adolescents and adults, similar results were obtained since their body weight and rice consumption rate were almost equal. Thus, it can be concluded that children was the most susceptible population group of non-carcinogenic health impacts.

The LADD is normally taken into account when the carcinogenic effects have become an issue of health concerned. The negative health effects from As exposure could be developed along the individual's lifespan. The results of LADD calculations are summarized in Table 6-4.

When compared the results of LADD to the results of ADD, the different patterns of negative health outcomes were reported. The highest LADD of As exposure to indicate the potential cancer development in population was found in adults who consume rice for a long period time. The average LADD of As in all population groups were in the following order: adults > children > adolescents. The consumption of non-

polished rice usually resulted in the greater LADD values than that of polished rice by a factor of 2.4. This result was found to be similar to that of ADD.

Table 6- 4 Exposure of As and potential non-carcinogenic and carcinogenic risks as a result of rice consumption by age group

Population group	Non-carcinogenic risk		Carcinogenic risk	
	ADD (mg kg^{-1} BW d^{-1})	HQ	LADD (mg kg^{-1} BW d^{-1})	CR (mg kg^{-1} BW d^{-1})$^{-1}$
	Polished rice consumption			
Children	6.38×10^{-5}	0.21	8.51×10^{-6}	1.28×10^{-5}
Adolescents	4.88×10^{-5}	0.16	3.25×10^{-6}	4.88×10^{-6}
Adult	4.58×10^{-5}	0.15	2.87×10^{-5}	4.31×10^{-5}
	Non-polished rice consumption			
Children	1.43×10^{-4}	0.48	1.90×10^{-5}	2.85×10^{-5}
Adolescents	1.09×10^{-4}	0.36	7.27×10^{-6}	1.09×10^{-5}
Adult	1.02×10^{-4}	0.34	6.42×10^{-5}	9.62×10^{-5}

The HQ is an index used to indicate whether the adverse non-carcinogenic health impact can be developed in the individual or population of concerned. In principle, the HQ value higher than 1 indicates that the human adverse health effect could be observed according to the particular substance exposure. The non-carcinogenic risks of bAs exposure via cooked rice consumption are shown in Table 6-4. The exceeding HQ than the threshold limit of 1 in different rice types consumption among population group were not found. Therefore, it is possible to conclude that the non-carcinogenic effects of As would not developed and observed in all population groups. Similar to the ADD results, the HQ values in children from both types of rice consumption were highest.

For the carcinogenic effects, CR was also reported for different age groups based on polished and non-polished rice consumption. Comparing with the acceptable value in a level of 1 in 10,000 individuals, all population groups who consume rice in a daily basis had the CR lying within the acceptable limit. However, the possibility of cancer

development was higher in the non-polished rice consumption than that of the polished rice consumption. Among all population groups, adults are expected to be mostly affected by cancer. Cancer might be developed in approximately 5 to 10 individuals in 100,000 adults.

3.3) Exposure and risks of As via rice consumption evaluated by the PRA approach

The probable distribution of ADD and LADD values are shown in Table 6-5. The levels lie at 5^{th} percentiles and 95^{th} percentiles were considered as extreme cases of lowest and highest ADD and LADD of As. For non-carcinogenic effect of As, the ADD of As was found in the following order: children > adolescents > adults. Meanwhile the LADD of As was found in the following order: adults > adolescents > children. These results were in accordance with the exposure and risk results obtained from the DRA approach. According to higher bAs concentration in non-polished rice comparing to that of polished rice, the consumption of non-polished rice resulted in approximately 2.2-time greater ADD and LADD values than those of polished rice consumption.

Table 6- 5 Average daily dose (ADD) and lifetime daily dose (LADD) of bioaccessible As exposure (mg kg^{-1} BW day^{-1}) through cooked rice consumption in different age groups by PRA approach

Rice type	Age group	Probabilistic estimation of As exposure (mg kg^{-1} BW d^{-1})							
		Minimum	Maximum	Mean	SD	Median	Percentile		
							5^{th}	90^{th}	95^{th}
ADD									
Polished rice	Children	8.0×10^{-6}	4.5×10^{-4}	5.7×10^{-5}	3.2×10^{-5}	5.0×10^{-5}	2.2×10^{-5}	9.6×10^{-5}	1.2×10^{-4}
	Adolescents	1.0×10^{-5}	2.3×10^{-4}	5.5×10^{-5}	2.4×10^{-5}	5.1×10^{-5}	2.6×10^{-5}	8.6×10^{-5}	1.0×10^{-4}
	Adults	1.2×10^{-5}	1.7×10^{-4}	5.4×10^{-5}	1.9×10^{-5}	5.1×10^{-5}	2.9×10^{-5}	7.9×10^{-5}	8.9×10^{-5}
Non-polished rice	Children	9.3×10^{-6}	1.2×10^{-3}	1.3×10^{-4}	7.8×10^{-5}	1.1×10^{-4}	4.3×10^{-5}	2.3×10^{-4}	2.8×10^{-4}
	Adolescents	9.3×10^{-6}	6.2×10^{-4}	1.2×10^{-4}	6.1×10^{-5}	1.1×10^{-4}	4.8×10^{-5}	2.0×10^{-4}	2.4×10^{-4}
	Adults	8.0×10^{-6}	4.7×10^{-4}	1.2×10^{-4}	4.9×10^{-5}	1.1×10^{-4}	5.2×10^{-5}	1.8×10^{-4}	2.1×10^{-4}
LADD									
Polished rice	Children	9.4×10^{-7}	5.2×10^{-5}	7.7×10^{-6}	4.3×10^{-6}	6.7×10^{-6}	2.9×10^{-6}	1.3×10^{-5}	1.6×10^{-5}
	Adolescents	7.5×10^{-7}	1.6×10^{-5}	3.7×10^{-6}	1.6×10^{-6}	3.4×10^{-6}	1.7×10^{-6}	5.8×10^{-6}	6.8×10^{-6}
	Adults	8.7×10^{-6}	1.3×10^{-4}	3.4×10^{-5}	1.2×10^{-5}	3.2×10^{-5}	1.8×10^{-5}	4.9×10^{-5}	5.6×10^{-5}
Non-polished rice	Children	1.3×10^{-6}	1.6×10^{-4}	1.7×10^{-5}	1.0×10^{-5}	1.5×10^{-5}	5.5×10^{-6}	3.0×10^{-5}	3.7×10^{-5}
	Adolescents	4.9×10^{-7}	3.6×10^{-5}	8.3×10^{-6}	4.0×10^{-6}	7.5×10^{-6}	3.2×10^{-6}	1.3×10^{-5}	1.6×10^{-5}
	Adults	6.9×10^{-6}	3.5×10^{-4}	7.5×10^{-5}	3.1×10^{-5}	7.1×10^{-5}	3.2×10^{-5}	1.2×10^{-4}	1.3×10^{-4}

The distribution of HQ values as a result of bAs exposure through polished and non-polished cooked rice consumption among different age groups are presented in Figure 6-2. The maximum HQ for children who consume non-polished rice cooked on a daily basis was 3.87. This level was higher than the criteria value of HQ of 1; however, the majority of the children population (95^{th} percentiles) had HQ equals to 0.92 (Figure 6-2(a)). Meanwhile, the maximum and the 95^{th} percentiles of HQ values of the lower risk population group were 2.05 and 0.80 for adolescents and 1.56 and 0.69 for adults, respectively. In case of the population who consumes polished rice on a daily basis, the maximum HQ values of children, adolescents, and adults were 1.49, 0.78, and 0.56, respectively. The HQ values at the 95^{th} percentiles in children, adolescents, and adults were 0.39, 0.34, and 0.30, respectively. Thus, it is also possible to conclude that non-carcinogenic effect of As exposure could not be observed in the majority of the Thai population. At the 95^{th} percentiles of all population groups, the HQ values of all population groups who consume both rice types on a daily basis were less than the threshold HQ value of 1. Although, the PRA results indicated that the HQ value of non-polished rice consumption was not exceed the threshold level, this type of rice consumption could provide more risk than the consumption of polished rice. Moreover, children were considered as the most vulnerable group of population to the non-carcinogenic human health effects from the daily rice ingestion. Thus, a special attention and recommendation should be paid to the consumption of non-polished rice consumption in children.

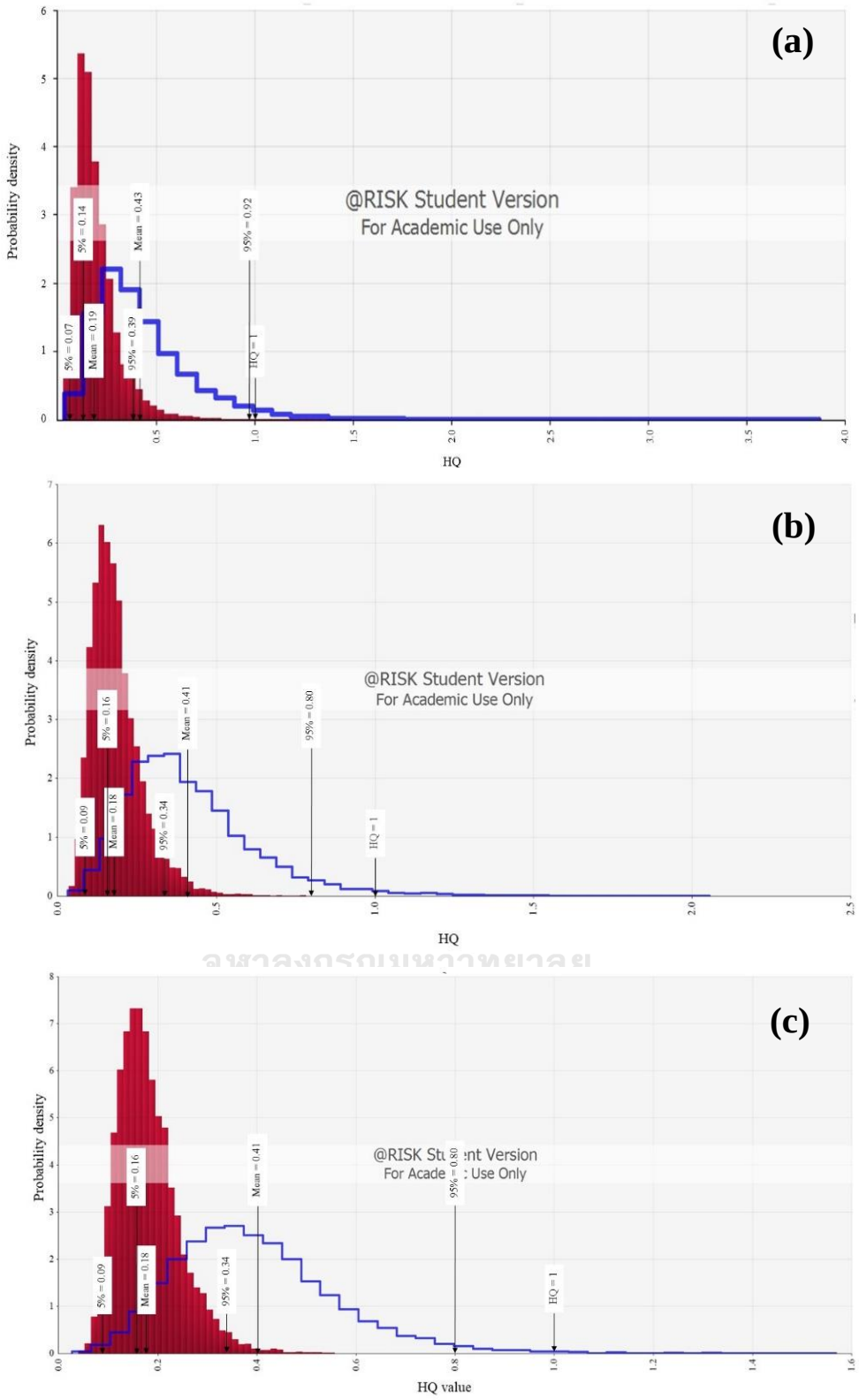


Figure 6- 2 Non-carcinogenic risks of As exposure through polished and non-polished rice consumption in (a) children, (b) adolescents, and (c) adults. The red area indicates the polished rice consumption pattern and the blue line indicates the non-polished rice consumption pattern.

Comparing to the previous study, the HQ values of As exposure via Chinese rice consumption evaluated based on the bAs concentration in rice were 0.51, 1.18, and 2.20 at the 5^{th}, 50^{th}, and 95^{th} percentiles, respectively for children. The 5^{th},50^{th}, and 95^{th} percentiles of HQ values in adult population were 0.34, 0.90, and 1.83, respectively (Li et al., 2018). Although, the HQ values in this present study were less than the previous study by approximately 5.6 and 6.1 times for children and adults, respectively, children in both studies (this study and previous study) was the group with the highest risks of non-carcinogenic effect development.

The PRA results of LADD As exposure via rice ingestion are illustrated in Figure 6-3. As illustrated in the Figure 6-3, adults were at risk of carcinogenic effects as a results of non-polished rice consumption since the average CR values were higher than the acceptable risk of 1×10^{-4} (Figure 6-3(b)). Approximately 56.9% of adult population who consumed non-polished rice were at risk cancer. While, adults who consume polished rice on a daily basis were found with the average CR value in a level of 5.05×10^{-5} in which approximately 1.4% of adult population had CR higher than the acceptable value. The maximum probabilities of cancer development in adults resulting from polished and non-polished rice consumption were 2 and 5 individuals in 10,000 individuals, respectively. Interestingly, all adolescents were considerably safe from carcinogenic risk effects since all of CR values were lower than the acceptable limit (Figure 6-3(a)). In case of children, CR values of polished rice consumption were lower than a level of 1×10^{-4}. Approximately 0.3% of children population who consumes non-polished rice on a daily basis tends to be at risk of cancer development. Since the carcinogenic effects could be developed after the long-term exposure thus, risk reduction program should be organized especially to the adults.

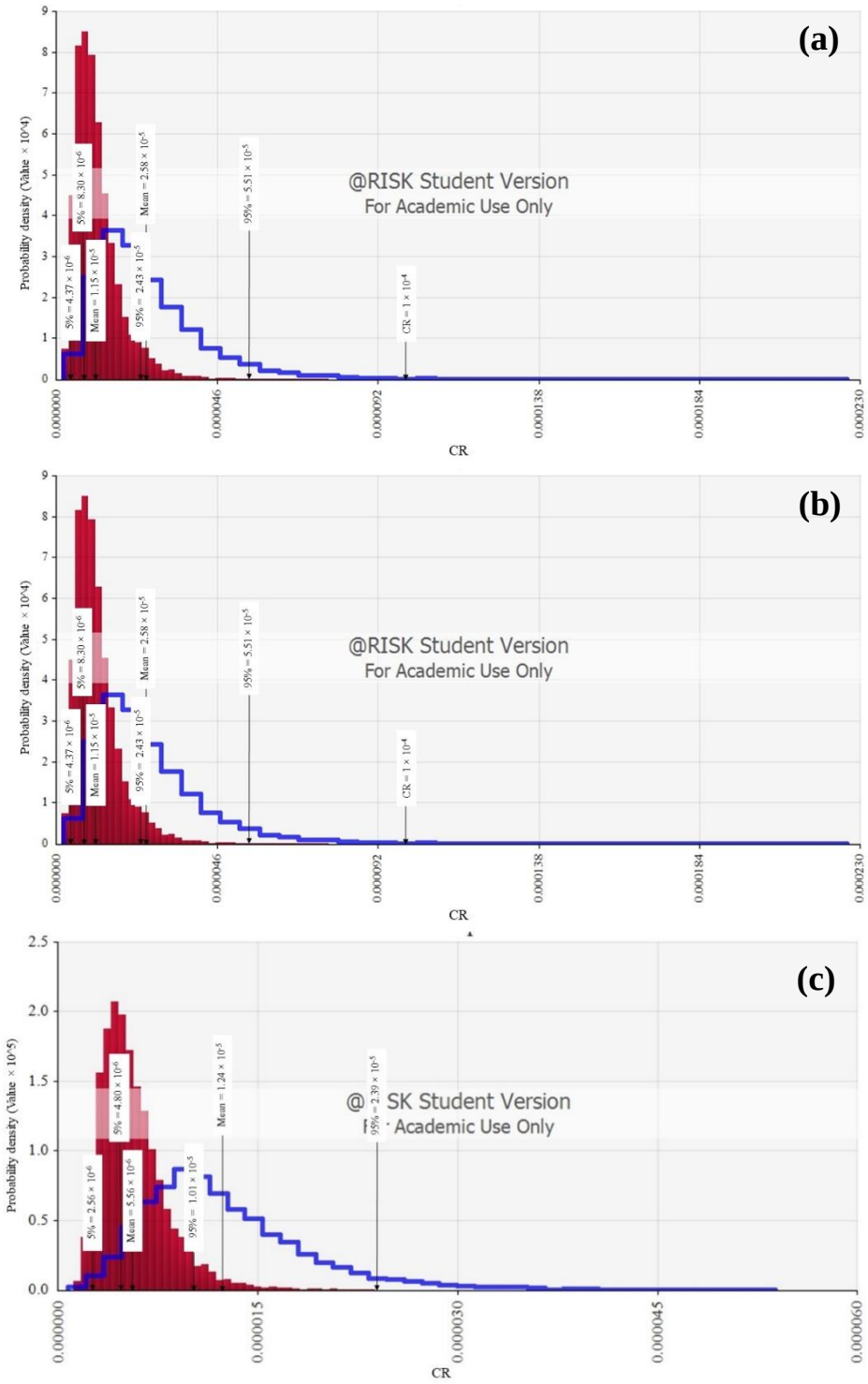


Figure 6-3 Carcinogenic risks from As exposure through polished and non-polished rice consumption in (a) children, (b) adolescents, and (c) adults. The red area indicates the polished rice consumption pattern and the blue line indicates the non-polished rice consumption pattern.

Comparing to the previous study of (Sharafi et al., 2019a) who have reported the uncertainty of CR in the range of 2.2×10^{-5} to 8.2×10^{-5} (10^{th} to 90^{th} percentiles) in Iranian population who consumed Iranian, Indian, and Pakistani rice, the similar magnitude of uncertainty was also found in this present study. However, it should be noted that the analysis in the previous study was conducted based on the As bioaccessibility in raw rice.

The sensitivity analysis for the HQ and CR calculations was also conducted in this study. The order of contributed uncertainty in the risk assessment results for children and adolescents were IR, BW, and bAs concentration in cooked rice. Meanwhile the highest contribution of HQ and CR value for adults is respectively related with the bAs concentration in cooked rice, IR, and BW.

3.4) Comparison between results obtained from the DRA and PRA approaches

Comparing between the risk assessment results obtained from the DRA and PRA approaches, the single point values of HQ in children, adolescents, and adults were 0.21, 0.16, and 0.15 respectively. Meanwhile, the median HQ results from Monte Carlo simulation was 0.17 for all groups of population. The comparison between results obtained from these two risk assessment approaches indicated approximately 1.23 times overestimation of HQ in children from the DRA approach. On the other hand, the underestimations in results obtained from the DRA approach were found in adolescents and adults. Considering the population who generally consumes non-polished rice, the median HQ value from the PRA result was 0.37 for all age group. The average HQ values obtained from the DRA were 0.40, 0.36, 0.34 for children, adolescents and adults, respectively. The similar overestimation outcomes of HQ in children were approximately 1.30 times, while the lower single point of HQ results were found in adolescents and adults.

The carcinogenic risk assessment based on the DRA and PRA approaches given the same magnitude of CR ranging from 1×10^{-6} to 1×10^{-4}. With an exception of adults who consumes non-polished rice on a daily basis, it was found that the median CR obtained from the PRA was 1.07×10^{-4} and approximately 1.11 folds lower than the CR results obtained from the DRA approach.

4. Conclusions

The purpose of this study was to assess the potential health risks of As exposure from the daily polished and non-polished rice consumption. The DRA and PRA approaches were separately applied to evaluate the As exposure and its adverse health impacts. The maximum bAs concentrations in cooked rice obtained from the Monte Carlo simulation were 0.0537 mg kg^{-1} for polished rice and 0.0983 mg kg^{-1} for non-polished rice. According to a public health risk evaluation, the non-polished rice consumption may result in higher impacts of both non-carcinogenic and carcinogenic effects to human more than those resulted from the polished rice consumption. In case of non-carcinogenic effects, children were the most susceptible group of population. On the other hand, adults were considered as the most impacted population group of carcinogenic effects. Three factors that contributing the highest impacts to the potential negative health risk outcomes were rice ingestion rate, individual body weight, and the concentration of As in cooked rice.

CHAPTER VII
A SUMMARY OF KEY FINDINGS AND RECOMMENDATIONS FOR FURTHER STUDY

1. A summary of key findings in relation to hypotheses

1. Concentrations of tAs and bAs in non-polished rice were significantly higher than those in polished rice. While, the concentrations of iAs in both rice types was not significantly different.

2. Polishing process could significantly reduce tAs contained in the extra-well polished rice comparing to that tAs concentration in the non-polished rice grain.

3. Three times rice washing and traditional cooking method can reduce approximately 60% of tAs concentration comparing to that of tAs contained in the raw rice grain. In addition, both iAs and bAs concentrations were reduced in relation to the reduction of tAs in the processed rice grain.

4. Lower As bioaccessibility was found in the modern polished and non-polished cooked rice. Approximately 1.80- and 2.24-time of lower As bioaccessibility were found in the modern non-polished cooked rice and the modern polished cooked rice than those of As bioaccessibility in the raw rice, respectively.

5. No significant difference in the exposure levels and the levels of potential non-carcinogenic and carcinogenic health risks evaluated by the DRA and PRA approaches were found.

2. A summary of key findings in relation to the research framework

A summary of key findings in this present study in relation to the research framework was focused on four chapters including 1) Chapter 3: Total, inorganic, and bioaccessible arsenic concentrations in raw polished and non-polished rice based on a market survey in Bangkok, 2) Chapter 4: Effect of polishing on total arsenic concentrations in non-polishing rice and resulting polished rice, 3) Chapter 5: Effects of rice washing and cooking processes on total, inorganic, and bioaccessible arsenic concentrations in rice, and 4) Chapter 6: Deterministic and probabilistic risk assessments of arsenic exposure through rice consumption

In chapter 3, the polished and non-polished rice (n = 208) were collected from 12 local markets in Bangkok and 2 wholesale markets in Pathumtani. The average tAs concentrations in the raw polished and non-polished rice were 0.2078 mg kg^{-1} and 0.3511 mg kg^{-1}, respectively. The significant higher tAs and iAs concentrations were found in the non-polished rice. The percentages of iAs to tAs concentration of approximately 58.0% and 51.7% were found in the polished and non-polished rice, respectively. The As bioaccessibilities in raw polished and non-polished rice were 50.5% and 45.9%, respectively. The normal ranges of tAs, iAs, and bAs in polished rice obtained from the Monte Carlo probable distribution functions at the 25th to 75th percentiles were 0.837 to 0.2324 mg kg^{-1} for tAs, 0.1012 to 0.1391 mg kg^{-1} for iAs and 0.0747 to 0.1283 mg kg^{-1} for bAs. For non-polished rice, the normal ranges were 0.2879 to 0.4227 mg kg^{-1} for tAs, 0.1327 to 0.2222 mg kg^{-1} for iAs and 0.1145 to 0.1940 mg kg^{-1} for bAs. Significant positive relationships were identified between concentrations of tAs-iAs, tAs-bAs, and iAs-bAs.

In chapter 4, the effects of a stepwise polishing on tAs concentrations in raw rice were reported. According to the National Bureau of Agricultural Commodity and Food Standards announced in 2017, rice with different polishing degrees can be classified into five groups including non-polished rice, ordinarily polished rice, reasonably well-polished rice, well-polished rice, extra well-polished rice. The highest tAs reduction (24.9%) was found in the extra well-polished rice. A strong significant negative correlation between tAs concentration and polishing degree was found. In the case of rice bran, tAs concentrations ranging from 0.7850 to 0.8131 mg kg^{-1} were approximately 3.3 times higher than those tAs in the rice grain.

In chapter 5, the effects of 1-time and 3-time rice washing on tAs concentrations in washed rice were reported. For polished rice, 3-times washed rice had significant lower tAs concentration than unwashed (raw) rice by approximately 29%. No significant differences in tAs concentrations in non-polished washed by different washing scenarios were found. Since the highest tAs reduction was observed in 3-time washed rice, this washing scenario was considered as the best washing method. When considering the effects of cooking methods on As concentrations, the traditional cooking method with high water to rice ratio (10:1 water to rice) can reduce up to 60% of tAs concentration in both rice types comparing to that of raw rice. While,

approximately 17.8% and 23.4% of tAs were reduced from rice cooked with the modern cooking method using an electric rice cooker. The reductions of iAs in cooked rice comparing to that of raw rice were approximately 46.1% for polished rice and 19.3% for non-polished rice. The bioaccessibility of As in cooked rice was also significantly reduced. It can be concluded that cooking can reduce the availability of As and its exposure in human body.

In chapter 6, the potential human health risk of bAs exposure through 3-time washed and cooked rice consumption in Bangkok population using both DRA and PRA approaches were evaluated. The non-carcinogenic effects could not be observed in the majority of the Bangkok children, adolescents, and adults population because the resulting HQs in all groups were lower than the acceptable HQ of 1. Children have higher chance to develop non-carcinogenic effects than other population group. The PRA results given the maximum HQ value, the worst-case scenario, in children of 3.87. The risk of carcinogenic effects was found in adults. Even though, the average CR values, resulted from the DRA approach, of all population groups for both rice types ingestion were within the acceptable limit (1×10^{-4}), the values from PRA approach indicated the risk of 1.4% and 56.9% of those adults consumed polished and non-polished rice having the CR higher than the acceptable limit. Approximately 2 to 5 individuals in 10,000 individuals are expected as the maximum possibility for the cancer development as a result of As exposure through rice consumption on a daily basis.

3. Recommendations for further study

For the better understanding in the issues of As accumulation in rice and its potential health impacts after consumption, the following suggestions can be taken into consideration in the further study.

1. Total number of samples in regard to the cultivation area, cultivation period, and crop year should be increased to ensure the variability and the accuracy of data obtained.

2. Localization of As species throughout the rice grain should be analyzed and studied.

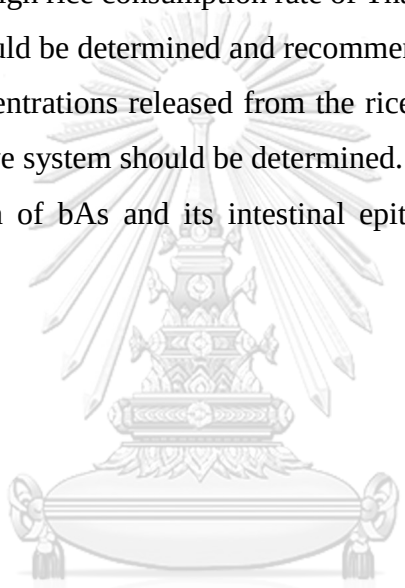
3. Concentrations of tAs, iAs, and bAs in other rice types which are normally consumed by Thai population should be determined.

4. The area of study should be expanded to the whole country level in order to complete the human health risk assessment through rice consumption in Thailand. In addition, the primary data collection including information of typical rice washing and cooking practices, rice consumption behaviors, types of rice consumed, amount and frequency of rice consumption, individual body weight should be conducted to obtain the more accurate variables and scenarios for the risk assessment process.

5. Based on the high rice consumption rate of Thai population, the Thai standard of iAs in rice grain should be determined and recommended.

6. The bAs concentrations released from the rice grain in the different parts of gastrointestinal digestive system should be determined.

7. The speciation of bAs and its intestinal epithelium cells intake should be determined.



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APPENDIX I

Preparation methods of digestive juice for the *in vitro* digestion

1) Simulation of saliva (pH 6.5)

Saliva was prepared from 1 mL of KCl (89.6 g L^{-1}), 1 mL of NaH_2PO_4 (88.8 g L^{-1}), 1 mL of Na_2HPO_4 (57 g L^{-1}), 0.17 mL of NaCl (175.3 g L^{-1}), 0.18 mL of NaOH (40 g L^{-1}) (inorganic), and 0.8 mL of urea (25 g L^{-1}) (organic). Inorganic and organic solutions of saliva were augmented to 50 mL with distilled water. Then, 14.5 mg of α-amylase, 1.5 mg of uric acid, and 5 mg of mucin were added to the simulated saliva.

2) Simulation of gastric juice (pH 1.07)

Gastric juice was prepared from 1.57 mL of NaCl (175.3 g L^{-1}), 0.3 mL of NaH_2PO_4 (88.8 g L^{-1}), 0.92 mL of KCl (89.6 g L^{-1}), 1.8 mL of $CaCl_2 \cdot 2H_2O$ (22.2 g L^{-1}), 1 mL of NH_4Cl (30.6 g L^{-1}), 0.83 mL of HCl (37% v/v) (inorganic), 1 mL of glucose (65 g L^{-1}), 1 mL of glucuronic acid (2 g L^{-1}), 0.34 mL of urea (25 g L^{-1}) as well as 1 mL of glucosamine hydrochloride (33 g L^{-1}) (organic). Inorganic and organic solutions of gastric juice were augmented to 50 mL with distilled water. Finally, 0.1 g of BSA, 0.1 g of pepsin, and 0.3 g of mucin were added to the simulated gastric juice.

3) Simulation duodenal juice (pH 7.8)

Duodenal juice was prepared from 8 mL of NaCl (175.3 g L^{-1}), 8 mL of $NaHCO_3$ (84.7 g L^{-1}), 2 mL of KH_2PO_4 (8 g L^{-1}), 1.26 mL of KCl (89.6 g L^{-1}), 2 mL of $MgCl_2$ (5 g L^{-1}), 36 μL of HCl (37% v/v) (inorganic) and 0.8 mL of urea (25 g L^{-1}) (organic). Inorganic and organic solutions of duodenal juice were augmented to 100 mL. Then, 1.8 mL of $CaCl_2 \cdot 2H_2O$ (22.2 g L^{-1}), 0.2 g of BSA, 0.6 g of pancreatin, and 0.1 g of lipase were added to the simulated duodenal juice.

4) Simulation of bile juice (pH 8.0)

Bile was prepared from 3 mL of NaCl (175.3 g L^{-1}), 6.83 mL of $NaHCO_3$ (84.7 g L^{-1}), 0.42 mL of KCl (89.6 g L^{-1}), 20 μL of HCl (37% v/v) (inorganic), and 1 mL of urea (25 g L^{-1}) (organic). Inorganic and organic solutions of bile were augmented to 50 mL with distilled water. After that, 1 mL of $CaCl_2 \cdot 2H_2O$ (22.2 g L^{-1}), 0.18 g of BSA, and 0.6 g of bile were added to the simulated bile.

For the pH adjustment, 6 M HCl and 6 M NaOH were used.

APPENDIX II

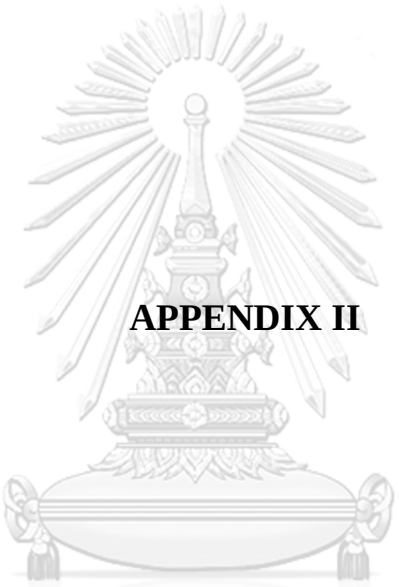

Table S-1 Sample information of polished rice and its tAs concentration (mg kg^{-1})

No.	Sample code	Crop year	Market	District	Cultivation area	tAs concentration (mg kg^{-1})			
						Rep 1	Rep 2	Ave	%RSD
1	001	1	Klongtoei	Klongtoei	Surin	0.2030	0.1864	0.1947	8.5
2	002	1	Klongtoei	Klongtoei	Phatumtani	0.2052	0.2346	0.2199	13.4
3	003	1	Klongtoei	Klongtoei	Yasotorn	0.2147	0.2057	0.2102	4.3
4	004	1	MOF	Chatuchak	Surin	0.1893	0.2046	0.1969	7.8
5	005	1	MOF	Chatuchak	Yasotorn	0.2523	0.2315	0.2419	8.6
6	006	1	MOF	Chatuchak	Cheingrai	0.1979	0.1940	0.1960	2.0
7	007	1	Yingcharoen	Bangkhen	Surin	0.2069	0.2186	0.2127	5.5
8	008	1	Yingcharoen	Bangkhen	Surin	0.2247	0.2352	0.2299	4.5
9	009	1	Yingcharoen	Bangkhen	Yasotorn	0.2280	0.2084	0.2182	9.0
10	010	1	Bangkapi	Bangkapi	Cheingrai	0.2293	0.2330	0.2312	1.6
11	011	1	Bangkapi	Bangkapi	Cheingrai	0.2095	0.2172	0.2133	3.6
12	012	1	Bangkapi	Bangkapi	Surin	0.2217	0.2112	0.2164	4.9
13	013	1	Minburi	Minburi	Surin	0.2285	0.2214	0.2249	3.1
14	014	1	Minburi	Minburi	Surin	0.2348	0.2536	0.2442	7.7
15	015	1	Minburi	Minburi	Chachengsao	0.2735	0.2993	0.2864	9.0
16	016	1	Samyan	Phatumwan	Yasotorn	0.2511	0.2394	0.2452	4.8
17	017	1	Samyan	Phatumwan	Surin	0.1786	0.2057	0.1921	14.1
18	018	1	Samyan	Phatumwan	Cheingrai	0.2111	0.1921	0.2016	9.4
19	019	1	Suksawat	Bangbon	Yasotorn	0.2332	0.2403	0.2367	3.0
20	020	1	Suksawat	Bangbon	Surin	0.3520	0.2377	0.2949	38.8
21	021	1	Suksawat	Bangbon	Surin	0.2002	0.2222	0.2112	10.4
22	022	1	Sanam Luang 2	Taweewattana	Ubon Ratchathani	0.2157	0.2119	0.2138	1.8
23	023	1	Sanam Luang 2	Taweewattana	Surin	0.1867	0.1962	0.1914	5.0
24	024	1	Phutthamonthon	Taweewattana	N/A	0.2315	0.2338	0.2327	1.0
25	079	1	Department store	N/A	Chachengsao	ND	ND	0.1081	ND
26	080	1	Department store	N/A	Yasotorn	ND	ND	0.1164	ND
27	081	1	Department store	N/A	N/A	ND	ND	0.0878	ND
28	082	1	Department store	N/A	N/A	ND	ND	0.1513	ND
29	083	1	Department store	N/A	N/A	ND	ND	0.1582	ND
30	084	1	Department store	N/A	N/A	ND	ND	0.2102	ND
31	085	1	Department store	N/A	Surin	ND	ND	0.1667	ND
32	086	1	Department store	N/A	Chaiyapum	ND	ND	0.1555	ND
33	201	2	Simummuang	Phatumthani	Yasotorn	0.1906	0.2020	0.1963	5.8
34	202	2	Simummuang	Phatumthani	Surin	0.1915	0.1984	0.1950	3.5
35	204	2	Simummuang	Phatumthani	Surin	0.1592	0.1590	0.1591	0.2
36	206	2	Simummuang	Phatumthani	Surin	0.1780	0.1819	0.1799	2.2

Table S-1 Sample information of polished rice and its tAs concentration (mg kg^{-1}) (*continued*)

No.	Sample code	Crop year	Market	District	Cultivation area	tAs concentration (mg kg^{-1})			
						Rep 1	Rep 2	Ave	%RSD
37	207	2	Simummuang	Phatumthani	N/A	0.2001	0.1925	0.1963	3.9
38	208	2	Simummuang	Phatumthani	N/A	0.2134	0.1997	0.2065	6.6
39	209	2	Simummuang	Phatumthani	N/A	0.2045	0.2129	0.2087	4.0
40	210	2	Simummuang	Phatumthani	N/A	0.2073	0.2056	0.2064	0.8
41	211	2	Simummuang	Phatumthani	Surin	0.1778	0.1717	0.1747	3.5
42	212	2	Simummuang	Phatumthani	N/A	0.2020	0.1972	0.1996	2.4
43	213	2	Simummuang	Phatumthani	N/A	0.1626	0.1617	0.1622	0.5
44	214	2	Simummuang	Phatumthani	Yasotorn	0.2498	0.2381	0.2439	4.8
45	215	2	Simummuang	Phatumthani	Surin	0.2073	0.2243	0.2158	7.9
46	221	2	Taladdtai	Phatumthani	N/A	0.2070	0.2061	0.2066	0.5
47	222	2	Taladdtai	Phatumthani	Surin	0.2246	0.2296	0.2271	2.2
48	223	2	Taladdtai	Phatumthani	N/A	0.1951	0.1987	0.1969	1.8
49	224	2	Taladdtai	Phatumthani	N/A	0.1413	0.1440	0.1426	1.9
50	225	2	Taladdtai	Phatumthani	N/A	0.1416	0.1537	0.1477	8.2
51	226	2	Taladdtai	Phatumthani	Surin	0.2009	0.1859	0.1934	7.8
52	227	2	Taladdtai	Phatumthani	N/A	0.1956	0.1891	0.1924	3.4
53	228	2	Taladdtai	Phatumthani	N/A	0.2385	0.2291	0.2338	4.0
54	229	2	Taladdtai	Phatumthani	Yasotorn	0.1972	0.2134	0.2053	7.9
55	230	2	Taladdtai	Phatumthani	N/A	0.1987	0.1830	0.1908	8.3
56	231	2	Taladdtai	Phatumthani	Ubon Ratchathani	0.2158	0.2219	0.2188	2.8
57	232	2	Taladdtai	Phatumthani	Yasotorn	0.2204	0.2150	0.2177	2.5
58	233	2	Taladdtai	Phatumthani	Surin	0.2553	0.2462	0.2507	3.6
59	234	2	Taladdtai	Phatumthani	Surin	0.1758	0.1771	0.1764	0.7
60	235	2	Taladdtai	Phatumthani	Yasotorn	0.1396	0.1316	0.1356	5.9
61	236	2	Taladdtai	Phatumthani	Yasotorn	0.2123	0.2016	0.2069	5.2
62	237	2	Taladdtai	Phatumthani	Surin	0.2332	0.2247	0.2289	3.7
63	238	2	Taladdtai	Phatumthani	N/A	0.2101	0.2083	0.2092	0.8
64	245	2	Hathaimit	Khlongsamwa	N/A	0.2206	0.2162	0.2184	2.0
65	246	2	Hathaimit	Khlongsamwa	Chiang Rai	0.1942	0.1920	0.1931	1.1
66	247	2	Hathaimit	Khlongsamwa	Chiang Rai	0.1862	0.1902	0.1882	2.1
67	248	2	Hathaimit	Khlongsamwa	N/A	0.1838	0.1888	0.1863	2.7
68	249	2	Hathaimit	Khlongsamwa	N/A	0.1700	0.1851	0.1775	8.5
69	250	2	Hathaimit	Khlongsamwa	Surin	0.2056	0.2099	0.2077	2.1
70	251	2	Hathaimit	Khlongsamwa	Yasotorn	0.1946	0.2012	0.1979	3.3
71	255	2	Sriyan	Dusit	N/A	0.2098	0.2062	0.2080	1.7
72	256	2	Sriyan	Dusit	N/A	0.2448	0.2533	0.2491	3.4
73	258	2	Sriyan	Dusit	N/A	0.1732	0.1675	0.1703	3.3

Table S-1 Sample information of polished rice and its tAs concentration (mg kg^{-1}) (*continued*)

No.	Sample code	Crop year	Market	District	Cultivation area	tAs concentration (mg kg^{-1})			
						Rep 1	Rep 2	Ave	%RSD
74	259	2	Sriyan	Dusit	N/A	0.2052	0.2157	0.2105	5.0
75	260	2	Sriyan	Dusit	Chiang Rai	0.1719	0.1792	0.1756	4.1
76	263	2	Bangkhunsi	Bangkoknoi	N/A	0.1730	0.1799	0.1764	3.9
77	264	2	Bangkhunsi	Bangkoknoi	N/A	0.1748	0.1803	0.1775	3.1
78	265	2	Bangkhunsi	Bangkoknoi	N/A	0.1783	0.1785	0.1784	0.1
79	267	2	Bangkhunsi	Bangkoknoi	N/A	0.1385	0.1453	0.1419	4.8
80	268	2	Bangkhunnon	Bangkoknoi	Chiang Rai	0.2066	0.1922	0.1994	7.2
81	269	2	Bangkhunnon	Bangkoknoi	Yasotorn	0.2048	0.1893	0.1971	7.8
82	270	2	Bangkhunnon	Bangkoknoi	Surin	0.1947	0.1832	0.1890	6.1
83	272	2	Bangkhunnon	Bangkoknoi	N/A	0.2370	0.2314	0.2342	2.4
84	273	2	Bangkhunnon	Bangkoknoi	N/A	0.2094	0.2210	0.2152	5.4
85	275	2	Chomthong	Chomthong	Chiang Rai	0.1941	0.1809	0.1875	7.0
86	276	2	Chomthong	Chomthong	Yasotorn	0.2056	0.2145	0.2100	4.3
87	278	2	Chomthong	Chomthong	Chiang Rai	0.1920	0.1829	0.1874	4.8
88	279	2	Chomthong	Chomthong	Yasotorn	0.1849	0.1841	0.1845	0.4
89	281	2	Chomthong	Chomthong	Chiang Rai	0.1514	0.1618	0.1566	6.7
90	282	2	Chomthong	Chomthong	Yasotorn	0.1907	0.1900	0.1903	0.3
91	283	2	Chomthong	Chomthong	Surin	0.1973	0.2010	0.1991	1.9
92	284	2	Chomthong	Chomthong	Nakhon Ratchasima	0.1952	0.1975	0.1964	1.2
93	301	3	MOF	Chatuchak	Surin	0.1830	0.2012	0.1921	9.5
94	302	3	MOF	Chatuchak	Chiang Rai	0.1877	0.1729	0.1803	8.2
95	304	3	MOF	Chatuchak	Surin	0.1791	0.1834	0.1812	2.4
96	305	3	MOF	Chatuchak	Yasotorn	0.1881	0.1802	0.1841	4.3
97	307	3	Klongtoei	Klongtoei	Yasotorn	0.1842	0.1830	0.1836	0.6
98	308	3	Klongtoei	Klongtoei	Surin	0.1448	0.1785	0.1616	20.8
99	310	3	Klongtoei	Klongtoei	Surin	0.1754	0.1632	0.1693	7.2
100	311	3	Klongtoei	Klongtoei	Yasotorn	0.1735	0.1838	0.1787	5.8
101	313	3	Minburi	Minburi	Surin	0.1947	0.1868	0.1907	4.2
102	314	3	Minburi	Minburi	Chiang Rai	0.1493	0.1492	0.1493	0.1
103	316	3	Minburi	Minburi	Yasotorn	0.2090	0.2797	0.2443	28.9
104	317	3	Minburi	Minburi	Chiang Rai	0.2128	0.2080	0.2104	2.3
105	319	3	Hathaimit	Khlongsamwa	Surin	0.2320	0.2262	0.2291	2.5
106	320	3	Hathaimit	Khlongsamwa	Roi Et	0.2456	0.2481	0.2468	1.0
107	322	3	Hathaimit	Khlongsamwa	Burirum	0.2143	0.2028	0.2085	5.5
108	323	3	Hathaimit	Khlongsamwa	N/A	0.2449	0.2383	0.2416	2.7
109	325	3	Yingcharoen	Bangkhen	Yasotorn	0.2555	0.2603	0.2579	1.8
110	326	3	Yingcharoen	Bangkhen	Surin	0.2670	0.2447	0.2558	8.7

Table S-1 Sample information of polished rice and its tAs concentration (mg kg^{-1}) (*continued*)

No.	Sample code	Crop year	Market	District	Cultivation area	tAs concentration (mg kg^{-1})			
						Rep 1	Rep 2	Ave	%RSD
111	328	3	Yingcharoen	Bangkhen	Yasotorn	0.2595	0.2630	0.2613	1.3
112	329	3	Yingcharoen	Bangkhen	Surin	0.1885	0.1937	0.1911	2.7
113	331	3	Simummuang	Phatumthani	N/A	0.2403	0.2401	0.2402	0.1
114	332	3	Simummuang	Phatumthani	N/A	0.2754	0.2548	0.2651	7.8
115	333	3	Simummuang	Phatumthani	N/A	0.2814	0.2771	0.2793	1.6
116	334	3	Simummuang	Phatumthani	N/A	0.2490	0.2343	0.2417	6.1
117	336	3	Simummuang	Phatumthani	Chiang Rai	0.2504	0.2452	0.2478	2.1
118	337	3	Simummuang	Phatumthani	N/A	0.2317	0.2153	0.2235	7.3
119	338	3	Simummuang	Phatumthani	N/A	0.2374	0.2533	0.2453	6.5
120	339	3	Simummuang	Phatumthani	N/A	0.2544	0.3295	0.2920	25.7
121	341	3	Taladdtai	Phatumthani	N/A	0.2419	0.2180	0.2300	10.4
122	343	3	Taladdtai	Phatumthani	Surin	0.3113	0.2685	0.2899	14.8
123	344	3	Taladdtai	Phatumthani	Surin	0.2098	0.2023	0.2061	3.7
124	346	3	Taladdtai	Phatumthani	N/A	0.2385	0.2126	0.2256	11.5
125	347	3	Taladdtai	Phatumthani	Surin	0.2654	0.2888	0.2771	8.5
126	348	3	Taladdtai	Phatumthani	Yasotorn	0.2019	0.2062	0.2040	2.1
127	349	3	Taladdtai	Phatumthani	Yasotorn	0.2229	0.2223	0.2226	0.3
128	351	3	Samyan	Phatumwan	Chiang Rai	0.2336	0.2221	0.2278	5.1
129	352	3	Samyan	Phatumwan	Yasotorn	0.2280	0.2382	0.2331	4.3
130	354	3	Samyan	Phatumwan	Yasotorn	0.1909	0.2024	0.1966	5.8
131	355	3	Samyan	Phatumwan	Surin	0.2232	0.2227	0.2230	0.2
132	357	3	Bangkapi	Bangkapi	Nakhon Ratchasima	0.2056	0.2010	0.2033	2.3
133	358	3	Bangkapi	Bangkapi	Chiang Rai	0.2221	0.2233	0.2227	0.5
134	360	3	Bangkapi	Bangkapi	Yasotorn	0.2073	0.2015	0.2044	2.8
135	361	3	Bangkapi	Bangkapi	N/A	0.1978	0.1982	0.1980	0.2
136	363	3	Bangmod	Chomthong	Nakhon Ratchasima	0.2481	0.2592	0.2536	4.4
137	364	3	Bangmod	Chomthong	Surin	0.2320	0.2307	0.2313	0.5
138	365	3	Bangmod	Chomthong	Yasotorn	0.2120	0.2119	0.2120	0.0
139	366	3	Bangmod	Chomthong	Surin	0.2035	0.1931	0.1983	5.2
140	368	3	Suksawat	Bangbon	Surin	0.2306	0.2261	0.2284	2.0
141	369	3	Suksawat	Bangbon	Yasotorn	0.2148	0.2108	0.2128	1.9
142	371	3	Suksawat	Bangbon	Yasotorn	0.2613	0.2356	0.2485	10.3
143	372	3	Suksawat	Bangbon	Yasotorn	0.2342	0.2378	0.2360	1.5
144	374	3	Sanam Luang 2	Taweewattana	Yasotorn	0.2762	0.2992	0.2877	8.0
145	375	3	Sanam Luang 2	Taweewattana	Yasotorn	0.2191	0.2065	0.2128	5.9
146	376	3	Sanam Luang 2	Taweewattana	Srisaket	0.2034	0.2032	0.2033	0.1

Table S-1 Sample information of polished rice and its tAs concentration (mg kg^{-1}) (*continued*)

No.	Sample code	Crop year	Market	District	Cultivation area	tAs concentration (mg kg^{-1})			
						Rep 1	Rep 2	Ave	%RSD
147	378	3	Bangkhunnon	Bangkoknoi	Surin	0.2092	0.1990	0.2041	5.0
148	379	3	Bangkhunnon	Bangkoknoi	N/A	0.2516	0.2443	0.2480	3.0
149	380	3	Bangkhunnon	Bangkoknoi	Yasotorn	0.2554	0.2660	0.2607	4.1
150	381	3	Bangkhunnon	Bangkoknoi	Yasotorn	0.2452	0.2405	0.2428	1.9
151	383	3	Sriyan	Dusit	N/A	0.2250	0.2278	0.2264	1.3
152	384	3	Sriyan	Dusit	N/A	0.2701	0.2700	0.2700	0.0
153	385	3	Sriyan	Dusit	N/A	0.1760	0.1819	0.1789	3.3
154	386	3	Sriyan	Dusit	Yasotorn	0.1835	0.1711	0.1773	7.0

Remark: * N/A means data is not available
**Rep 1 and Rep 2 is duplicate analyses results

Table S-2 Sample information in non-polished rice and its tAs concentration (mg kg^{-1})

No	Sample code	Crop year	Market	District	Cultivation area	tAs concentration (mg kg^{-1})			
						Rep 1	Rep 2	Ave	%RSD
1	065	1	Klongtoei	Klongtoei	Burirum	0.3825	0.3878	0.3851	1.4
2	066	1	Klongtoei	Klongtoei	N/A	0.3566	0.3602	0.3584	1.0
3	067	1	MOF	Chatuchak	Yasotorn	0.3763	0.3774	0.3769	0.3
4	068	1	MOF	Chatuchak	Yasotorn	0.3846	0.4959	0.4403	25.3
5	069	1	Yingcharoen	Bangkhen	Yasotorn	0.4016	0.4209	0.4112	4.7
6	070	1	Yingcharoen	Bangkhen	Surin	0.3772	0.3751	0.3761	0.6
7	071	1	Bangkapi	Bangkapi	Nakorn Ratchasima	0.3274	0.3233	0.3253	1.3
8	072	1	Bangkapi	Bangkapi	N/A	0.4950	0.4837	0.4893	2.3
9	073	1	Minburi	Minburi	Surin	0.3224	0.3222	0.3223	0.0
10	074	1	Minburi	Minburi	Surin	0.1721	0.3562	0.2641	69.7
11	075	1	Samyan	Phatumwan	Yasotorn	0.3559	0.4207	0.3883	16.7
12	076	1	Samyan	Phatumwan	Payao	0.3872	0.2578	0.3225	40.1
13	077	1	Suksawat	Bangbon	Surin	0.2520	0.2920	0.2720	14.7
14	078	1	Sanam Luang 2	Taweewattana	Srisaket	0.2830	0.1790	0.2310	45.0
15	095	1	Department store		Srisaket	ND	ND	0.1808	ND
16	096	1	Department store		Karasin	ND	ND	0.2489	ND
17	097	1	Department store		N/A	ND	ND	0.2002	ND
18	217	2	Simummuang	Phatumthani	Yasotorn	0.4022	0.4104	0.4063	2.0
19	218	2	Simummuang	Phatumthani	N/A	0.3417	0.3319	0.3368	2.9
20	219	2	Simummuang	Phatumthani	N/A	0.2484	0.2532	0.2508	1.9
21	220	2	Simummuang	Phatumthani	N/A	0.3375	0.3486	0.3431	3.2
22	239	2	Taladdtai	Phatumthani	N/A	0.3947	0.4175	0.4061	5.6
23	240	2	Taladdtai	Phatumthani	N/A	0.4241	0.4037	0.4139	4.9
24	242	2	Taladdtai	Phatumthani	Surin	0.3661	0.3614	0.3638	1.3
25	243	2	Taladdtai	Phatumthani	Nakhon Phanom	0.3191	0.3180	0.3186	0.4
26	252	2	Hathaimit	Khlongsamwa	Surin	0.3494	0.3566	0.3530	2.1
27	257	2	Sriyan	Dusit	N/A	0.3184	0.3100	0.3142	2.7
28	262	2	Sriyan	Dusit	N/A	0.1373	0.1305	0.1339	5.1
29	274	2	Bangkhunnon	Bangkoknoi	Sa Kaeo	0.2121	0.2016	0.2068	5.1
30	280	2	Chomthong	Chomthong	N/A	0.3652	0.3619	0.3636	0.9
31	303	3	MOF	Chatuchak	Yasotorn	0.3773	0.3888	0.3831	3.0
32	306	3	MOF	Chatuchak	Yasotorn	0.4719	0.3995	0.4357	16.6
33	309	3	Klongtoei	Klongtoei	Burirum	0.3585	0.3934	0.3760	9.3
34	312	3	Klongtoei	Klongtoei	N/A	0.3497	0.3507	0.3502	0.3
35	315	3	Minburi	Minburi	Surin	0.4036	0.3832	0.3934	5.2

Table S-2 Sample information in non-polished rice and its tAs concentration (mg kg^{-1}) (*continued*)

No.	Sample code	Crop year	Market	District	Cultivation area	tAs concentration (mg kg^{-1})			
						Rep 1	Rep 2	Ave	%RSD
36	318	3	Minburi	Minburi	Burirum	0.5366	0.4979	0.5172	7.5
37	321	3	Hathaimit	Khlongsamwa	Surin	0.4699	0.4777	0.4738	1.6
38	324	3	Hathaimit	Khlongsamwa	Sa Kaeo	0.5086	0.4911	0.4999	3.5
39	327	3	Yingcharoen	Bangkhen	Chiang Rai	0.3108	0.3572	0.3340	13.9
40	330	3	Yingcharoen	Bangkhen	N/A	0.4383	0.4568	0.4475	4.1
41	335	3	Simummuang	Phatumthani	N/A	0.4769	0.4835	0.4802	1.4
42	340	3	Simummuang	Phatumthani	N/A	0.3718	0.3722	0.3720	0.1
43	345	3	Taladdtai	Phatumthani	N/A	0.4641	0.4710	0.4675	1.5
44	350	3	Taladdtai	Phatumthani	N/A	0.4235	0.4987	0.4611	16.3
45	353	3	Samyan	Phatumwan	Yasotorn	0.3763	0.3754	0.3758	0.2
46	356	3	Samyan	Phatumwan	Phayao	0.3460	0.3418	0.3439	1.2
47	359	3	Bangkapi	Bangkapi	N/A	0.3980	0.4905	0.4442	20.8
48	362	3	Bangkapi	Bangkapi	N/A	0.2810	0.2682	0.2746	4.6
49	367	3	Bangmod	Chomthong	N/A	0.4172	0.4382	0.4277	4.9
50	370	3	Suksawat	Bangbon	Surin	0.1582	0.1657	0.1620	4.7
51	373	3	Suksawat	Bangbon	Burirum	0.1163	0.1210	0.1187	4.0
52	377	3	Sanam Luang 2	Taweewattana	Srisaket	0.3103	0.3279	0.3191	5.5
53	382	3	Bangkhunnon	Bangkoknoi	Ubon Ratchathani	0.3281	0.3668	0.3474	11.1
54	387	3	Sriyan	Dusit	Chiang Rai	0.3421	0.3590	0.3505	4.8

Remark: * N/A means data is not available
**Rep 1 and Rep 2 are duplicate analyses results

Table S-3 iAs concentration in raw rice (mg kg^{-1})

No.	Sample code	Rice type	iAs concentration (mg kg^{-1})			
			Rep 1	Rep 2	Ave	%RSD
1	084	Polished	0.1390	0.1595	0.1493	13.7
2	214	Polished	0.1278	0.1234	0.1256	3.5
3	215	Polished	0.1260	0.1242	0.1251	1.4
4	222	Polished	0.1364	0.1396	0.1380	2.3
5	228	Polished	0.1309	0.1293	0.1301	1.2
6	231	Polished	0.1325	0.1293	0.1309	2.4
7	232	Polished	0.1355	0.1329	0.1342	1.9
8	233	Polished	0.1413	0.1275	0.1344	10.3
9	237	Polished	0.1314	0.1390	0.1352	5.6
10	245	Polished	0.1339	0.1463	0.1401	8.9
11	250	Polished	0.0990	0.1199	0.1095	19.1
12	255	Polished	0.1235	0.1101	0.1168	11.5
13	256	Polished	0.1458	0.1258	0.1358	14.7
14	259	Polished	0.1076	0.1196	0.1136	10.6
15	272	Polished	0.1512	0.1474	0.1493	2.5
16	276	Polished	0.1007	0.0976	0.0992	3.1
17	319	Polished	0.1422	0.1250	0.1336	12.9
18	320	Polished	0.0661	0.0750	0.0706	12.6
19	325	Polished	0.1849	0.1869	0.1859	1.1
20	326	Polished	0.1739	0.1859	0.1799	6.7
21	328	Polished	0.1389	0.1413	0.1401	1.7
22	332	Polished	0.1554	0.1594	0.1574	2.5
23	333	Polished	0.1241	0.1809	0.1525	37.2
24	343	Polished	0.1602	0.1810	0.1706	12.2
25	352	Polished	0.1305	0.1195	0.1250	8.8
26	363	Polished	0.1602	0.1666	0.1634	3.9
27	364	Polished	0.1817	0.1827	0.1822	0.5
28	374	Polished	0.1647	0.1795	0.1721	8.6
29	066	Non-polished	0.2445	0.2394	0.2420	2.1
30	074	Non-polished	0.1878	0.2098	0.1988	11.1
31	077	Non-polished	0.1907	0.2023	0.1965	5.9
32	217	Non-polished	0.1788	0.1854	0.1821	3.6
33	239	Non-polished	0.2192	0.2247	0.2220	2.5
34	240	Non-polished	0.2214	0.2291	0.2253	3.4
35	242	Non-polished	0.2240	0.2170	0.2205	3.2
36	306	Non-polished	0.1964	0.1980	0.1972	0.8
37	315	Non-polished	0.1791	0.1756	0.1774	2.0
38	324	Non-polished	0.1688	0.1714	0.1701	1.5
39	330	Non-polished	0.1389	0.1453	0.1421	4.5
40	370	Non-polished	0.0629	0.0721	0.0675	13.7
41	373	Non-polished	0.0547	0.0516	0.0531	5.8

Remark: *Rep 1 and Rep 2 are duplicate analyses results

Table S-4 bAs concentration in polished raw rice (mg kg^{-1})

No.	Sample code	bAs concentration (mg kg^{-1})			
		Rep 1	**Rep 2**	**Ave**	**%RSD**
1	001	0.0665	0.0899	0.0782	29.9
2	002	0.1017	0.0988	0.1003	2.9
3	003	0.0638	0.0857	0.0747	29.4
4	004	0.0712	0.0776	0.0744	8.6
5	005	0.0611	0.0690	0.0651	12.1
6	006	0.0715	0.0843	0.0779	16.3
7	007	0.0726	0.0909	0.0817	22.4
8	008	0.1008	0.0768	0.0888	27.0
9	009	0.0969	0.0868	0.0919	11.0
10	010	0.0939	0.0916	0.0928	2.5
11	011	0.0772	0.0559	0.0666	32.0
12	012	0.0887	0.0907	0.0897	2.3
13	013	0.0868	0.0881	0.0874	1.4
14	014	0.1255	0.1024	0.1140	20.3
15	015	0.0498	0.0511	0.0505	2.6
16	016	0.0534	0.0592	0.0563	10.3
17	017	0.0749	0.0751	0.0750	0.4
18	018	0.0700	0.0881	0.0790	22.9
19	019	0.1105	0.1089	0.1097	1.5
20	020	0.0292	0.0219	0.0256	28.6
21	021	0.0669	0.0870	0.0769	26.1
22	022	0.1034	0.1293	0.1164	22.2
23	023	0.0923	0.0740	0.0832	22.0
24	024	0.0808	0.0938	0.0873	14.8
25	079	0.0786	0.0865	0.0825	9.5
26	080	0.0709	0.0584	0.0646	19.4
27	081	0.0603	0.0461	0.0532	26.6
28	082	0.0752	0.0830	0.0791	9.9
29	083	0.0944	0.0720	0.0832	26.9
30	084	0.1296	0.1384	0.1340	6.5
31	085	0.1070	0.0992	0.1031	7.6
32	086	0.1038	0.0923	0.0980	11.8
33	206	0.0688	0.0882	0.0785	24.8
34	208	0.0717	0.0852	0.0784	17.2
35	209	0.0809	0.0916	0.0863	12.4
36	210	0.0970	0.0975	0.0972	0.5
37	214	0.0896	0.0914	0.0905	2.0
38	215	0.0875	0.0801	0.0838	8.8

Table S-4 bAs concentration in polished raw rice (mg kg^{-1}) (*continued*)

No.	Sample code	bAs concentration (mg kg^{-1})			
		Rep 1	**Rep 2**	**Ave**	**%RSD**
39	221	0.0674	0.0643	0.0659	4.7
40	222	0.0645	0.0594	0.0619	8.1
41	223	0.0719	0.0687	0.0703	4.5
42	228	0.0572	0.0592	0.0582	3.3
43	229	0.0632	0.0641	0.0636	1.4
44	231	0.0573	0.0756	0.0665	27.5
45	232	0.0938	0.1019	0.0979	8.2
46	233	0.0932	0.1004	0.0968	7.5
47	236	0.0999	0.0965	0.0982	3.5
48	237	0.0937	0.1140	0.1039	19.5
49	238	0.1210	0.1343	0.1277	10.4
50	245	0.0924	0.0927	0.0926	0.3
51	250	0.1842	0.1921	0.1882	4.2
52	251	0.0921	0.0805	0.0863	13.4
53	255	0.1896	0.1524	0.1710	21.8
54	256	0.0846	0.0870	0.0858	2.7
55	259	0.1281	0.1310	0.1296	2.2
56	265	0.1412	0.1336	0.1374	5.6
57	272	0.0834	0.0792	0.0813	5.1
58	273	0.0839	0.0840	0.0839	0.1
59	276	0.1372	0.1496	0.1434	8.7
60	279	0.1144	0.1077	0.1110	6.0
61	283	0.0621	0.0554	0.0588	11.4
62	308	0.0926	0.1162	0.1044	22.7
63	310	0.1107	0.1275	0.1191	14.1
64	311	0.1733	0.1198	0.1465	36.5
65	319	0.1139	0.2027	0.1583	56.1
66	320	0.1752	0.1645	0.1699	6.3
67	326	0.1454	0.1072	0.1263	30.2
68	328	0.1036	0.1249	0.1142	18.7
69	331	0.1348	0.1446	0.1397	7.0
70	332	0.1002	0.1213	0.1108	19.1
71	333	0.1220	0.1180	0.1200	3.3
72	334	0.1432	0.1346	0.1389	6.2
73	336	0.1052	0.1192	0.1122	12.5
74	338	0.1345	0.1424	0.1385	5.7
75	339	0.1558	0.1470	0.1514	5.8
76	341	0.0932	0.1477	0.1205	45.2

Table S-4 bAs concentration in polished raw rice (mg kg^{-1}) (*continued*)

No.	Sample code	bAs concentration (mg kg^{-1})			
		Rep 1	**Rep 2**	**Ave**	**%RSD**
77	343	0.1118	0.1800	0.1459	46.7
78	347	0.1273	0.1792	0.1533	33.9
79	352	0.1420	0.1441	0.1431	1.5
80	363	0.1167	0.1189	0.1178	1.9
81	364	0.2104	0.1451	0.1778	36.7
82	371	0.1479	0.1495	0.1487	1.1
83	372	0.1579	0.1137	0.1358	32.5
84	374	0.1934	0.1695	0.1815	13.2
85	379	0.1490	0.1453	0.1472	2.5
86	380	0.1892	0.1880	0.1886	0.6
87	381	0.1783	0.2010	0.1897	12.0
88	384	0.1617	0.1462	0.1540	10.1
89	386	0.1405	0.1480	0.1443	5.2

Remark: * Rep 1 and Rep 2 are duplicate analyses results

Table S-5 bAs concentration in non-polished raw rice (mg kg^{-1})

No	Sample code	bAs concentration (mg kg^{-1})			
		Rep 1	Rep 2	Ave	%RSD
1	065	0.1097	0.1186	0.1142	7.8
2	066	0.1605	0.1762	0.1684	9.4
3	067	0.1591	0.1550	0.1570	2.6
4	068	0.1509	0.1078	0.1294	33.3
5	069	0.1345	0.1654	0.1500	20.6
6	070	0.1720	0.1596	0.1658	7.4
7	071	0.1160	0.1188	0.1174	2.4
8	072	0.1826	0.1432	0.1629	24.2
9	073	0.1147	0.0949	0.1048	18.9
10	074	0.2034	0.1876	0.1955	8.1
11	075	0.1347	0.1575	0.1461	15.6
12	076	0.0928	0.1187	0.1058	24.4
13	077	0.1382	0.1342	0.1362	2.9
14	078	0.1074	0.0909	0.0991	16.7
15	095	0.1213	0.1098	0.1156	9.9
16	096	0.1628	0.1501	0.1564	8.1
17	097	0.1380	0.1033	0.1206	28.7
18	217	0.1733	0.1804	0.1768	4.0
19	218	0.1368	0.1366	0.1367	0.2
20	219	0.1519	0.1623	0.1571	6.6
21	220	0.1438	0.1676	0.1557	15.3
22	239	0.1622	0.1622	0.1622	0.0
23	240	0.1932	0.1845	0.1889	4.6
24	242	0.1557	0.1933	0.1745	21.6
25	252	0.1522	0.1559	0.1540	2.3
26	257	0.1462	0.1456	0.1459	0.3
27	280	0.1625	0.1582	0.1603	2.7
28	306	0.2996	0.2185	0.2590	31.3
29	315	0.2186	0.2006	0.2096	8.6
30	318	0.2030	0.2466	0.2248	19.4
31	321	0.1960	0.2056	0.2008	4.8
32	324	0.2111	0.3454	0.2782	48.3
33	330	0.2239	0.1986	0.2112	12.0
34	335	0.1448	0.1794	0.1621	21.3
35	345	0.1431	0.1427	0.1429	0.3
36	350	0.1685	0.1924	0.1805	13.2
37	359	0.1096	0.1427	0.1261	26.2
38	362	0.1137	0.1143	0.1140	0.5
39	367	0.1166	0.0967	0.1066	18.6
40	370	0.0832	0.0814	0.0823	2.2
41	373	0.0533	0.0790	0.0661	38.8

Remark * Rep 1 and Rep 2 are duplicate analyses results

Table S-6 tAs concentration in raw rice with different degrees of polishing (mg kg^{-1})

Type of sample	DOP	Rep 1	Rep 2	Ave	%RSD
Sample A					
Rice grain	Non-polished	0.3763	0.3754	0.3758	0.2
Rice grain	Ordinarily polished	0.2786	0.2669	0.2728	4.3
Rice grain	Reasonably well-polished	0.2692	0.2710	0.2701	0.7
Rice grain	Well -polished	0.2692	0.2648	0.2670	1.6
Rice grain	Extra well- polished	0.2537	0.2643	0.2590	4.1
Rice bran	Ordinarily polished	0.8548	0.8006	0.8277	6.6
Rice bran	Reasonably well-polished	0.8700	0.8157	0.8429	6.5
Rice bran	Well-polished	0.8577	0.8441	0.8509	1.6
Rice bran	Extra well- polished	0.8356	0.7847	0.8102	6.3
Sample B					
Rice grain	Non-polished	0.3001	0.3180	0.3090	5.8
Rice grain	Ordinarily polished	0.2547	0.2699	0.2623	5.8
Rice grain	Reasonably well-polished	0.2541	0.2684	0.2613	5.5
Rice grain	Well -polished	0.2488	0.2515	0.2501	1.1
Rice grain	Extra well- polished	0.2148	0.2251	0.2200	4.7
Rice bran	Ordinarily polished	0.7980	0.8333	0.8156	4.3
Rice bran	Reasonably well-polished	0.8502	0.8511	0.8507	0.1
Rice bran	Well-polished	0.8080	0.8979	0.8530	10.5
Rice bran	Extra well- polished	0.8381	0.8111	0.8246	3.3
Sample C					
Rice grain	Non-polished	0.3059	0.3103	0.3081	1.4
Rice grain	Ordinarily polished	0.2560	0.2768	0.2664	7.8
Rice grain	Reasonably well-polished	0.2797	0.3023	0.2910	7.8
Rice grain	Well -polished	0.2705	0.2795	0.2750	3.3
Rice grain	Extra well- polished	0.2441	0.2803	0.2622	13.8
Rice bran	Ordinarily polished	0.6783	0.7454	0.7118	9.4
Rice bran	Reasonably well-polished	0.7541	0.7167	0.7354	5.1
Rice bran	Well-polished	0.7255	0.7457	0.7356	2.7
Rice bran	Extra well- polished	0.7383	0.7598	0.7490	2.9

Remark: * Rep 1 and Rep 2 are duplicate analyses results

Table S-7 tAs concentration in different rice washing scenarios (mg kg^{-1})

Rice samples	Rep 1	Rep 2	Ave	%RSD
Washing time = 1				
J1_215	0.1975	0.2121	0.2048	7.1
J1_231	0.1876	0.1860	0.1868	0.9
J1_232	0.1863	0.1944	0.1904	4.3
J2_217	0.3459	0.2790	0.3125	21.4
J2_239	0.3367	0.3795	0.3581	11.9
J2_240	0.4000	0.4028	0.4014	0.7
Washing time = 3				
J1_215	0.1546	0.1581	0.1564	2.3
J1_231	0.1614	0.1545	0.1579	4.4
J1_232	0.1466	0.1465	0.1466	0.0
J2_217	0.3164	0.3334	0.3249	5.2
J2_239	0.3804	0.4130	0.3967	8.2
J2_240	0.3432	0.3535	0.3484	3.0

Remark: * Rep 1 and Rep 2 are duplicate analyses results

Table S-8 tAs concentration in modern and traditional cooked rice after 3-times washing (mg kg^{-1})

Rice samples	Rep 1	Rep 2	Ave	%RSD
Modern cooked rice				
J1_215	0.1780	0.1725	0.1752	3.2
J1_231	0.1802	0.1673	0.1738	7.5
J1_232	0.1923	0.1819	0.1871	5.5
J2_217	0.3241	0.3366	0.3304	3.8
J2_239	0.3221	0.2603	0.2912	21.2
J2_240	0.3309	0.3038	0.3173	8.6
Traditional cooked rice				
J1_215	0.0789	0.0855	0.0822	8.1
J1_231	0.0832	0.0906	0.0869	8.5
J1_232	0.0946	0.0871	0.0909	8.3
J2_217	0.1793	0.1678	0.1736	6.6
J2_239	0.1509	0.1589	0.1549	5.2
J2_240	0.1714	0.1648	0.1681	3.9

Remark: * Rep 1 and Rep 2 are duplicate analyses results

Table S-9 iAs and bAs concentration in modern cooked rice (mg kg^{-1})

Sample code	Rep 1	Rep 2	Ave	%RSD
iAs concentration (mg kg^{-1})				
J1_215	0.04965	0.05553	0.0526	11.2
J1_231	0.07343	0.07695	0.0752	4.7
J1_232	0.08131	0.08573	0.0835	5.3
J2_217	0.14864	0.16258	0.1556	9.0
J2_239	0.18114	ND	0.1811	ND
J2_240	0.17451	0.1633	0.1689	6.6
bAs concentration (mg kg^{-1})				
J1_215	0.0339	0.0334	0.0336	1.4
J1_231	0.0227	0.0362	0.0294	45.7
J1_232	0.0306	0.0261	0.0283	15.6
J2_217	0.0673	0.0975	0.0824	36.5
J2_239	0.0505	ND	0.0505	ND
J2_240	0.1131	0.0745	0.0938	41.2

Remark * Rep 1 and Rep 2 are duplicate analyses results

APPENDIX III

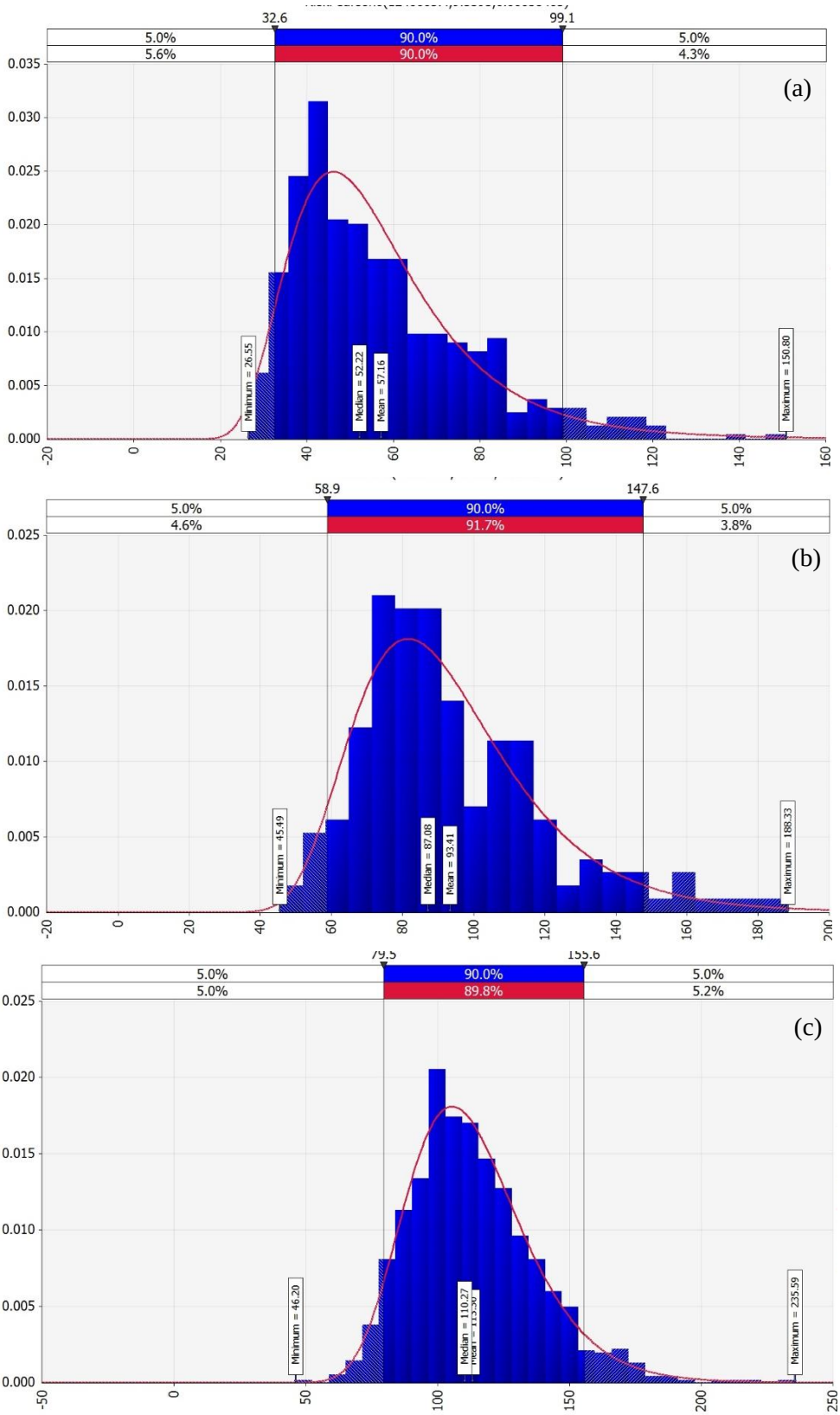

Figure S-1 Distribution functions of ingestion rates in (a) children, (b) adolescents and (c) adults.

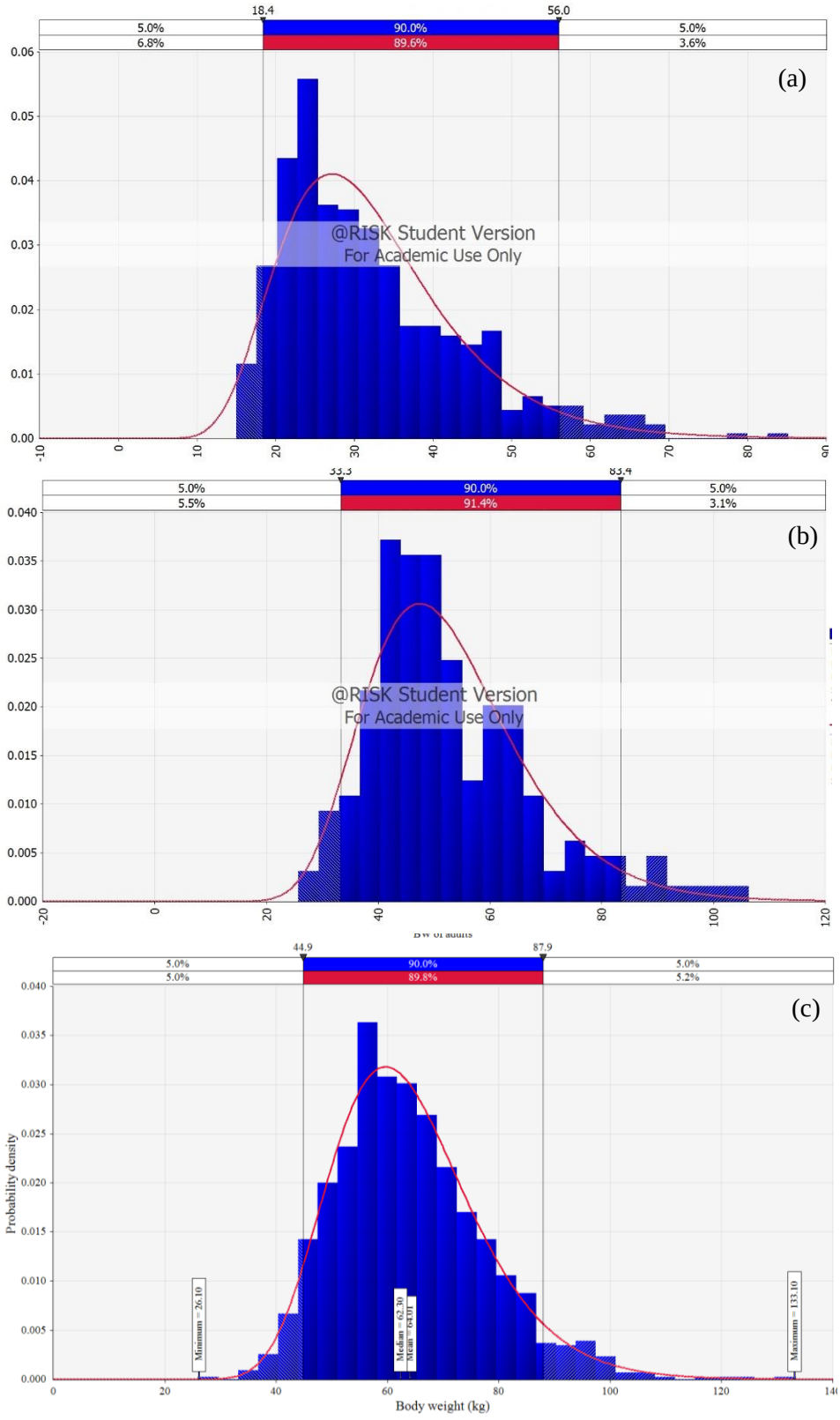

Figure S-2 Distribution functions of body weight in (a) children, (b) adolescents and (c) adults.

VITA

NAME	Supanad Hensawang
DATE OF BIRTH	26 June 1992
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	Bachelor Degree of Science (Chemistry), Department of Chemistry, Faculty of Science, Chulalongkorn University Master Degree of Science (Hazardous Substance and Environmental Management), Graduated School, Chulalongkorn University
HOME ADDRESS	58/628 Ramintra Road Kannayao Bangkok 10230 Thailand
PUBLICATION	Hensawang, S. and Chanpiwat, P. 2018. Analysis and probabilistic risk assessment of bioaccessible arsenic in polished and husked jasmine rice sold in Bangkok. Chemosphere 207: 637-648. (ISI database, IF = 4.95, Citations = 1) Chanpiwat, P., Hensawang, S., Suwatvitayakorn, P. and Ponsin, M. 2018. Risk assessment of bioaccessible arsenic and cadmium exposure through rice consumption in local residents of the Mae Tao Sub-district, Northwestern Thailand. Environmental Geochemistry and Health 41(1): 343-356.(ISI database, IF = 2.99, Citations = 4) Hensawang, S. and Chanpiwat, P. 2017. Health impact assessment of arsenic and cadmium intake via rice consumption in Bangkok, Thailand. Environmental Monitoring and Assessment 189: 599 (ISI database, IF = 1.959 , Citations = 7)