VALIDATION OF INORGANIC ARSENIC DETECTION METHODS USING HPLC-ICPMS AND ITS CONTAMINATION IN MARKET RICE IN MALAYSIA

By

ZULIANA BINTI ZAKARIA

A thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Faculty : Food Science and Technology

The contamination of inorganic arsenic in rice has become worldwide concerned due to its associated with cancer effect. The demand for rice has forced farmers to apply pesticides to meet demand and prevent production loss especially on a large scale. The pesticide residue, particularly arsenic pesticides, has the potential to leach into the ground, leading to soil contamination and the presence of inorganic arsenic (iAs) in paddy seeds. However, reports on iAs contamination in Malaysian rice remain limited. Therefore, this study was performed to (1) to validate a reliable analysis for iAs (As (III), As (V)) in rice by using HPLC-ICP-MS and (2) to determine the iAs levels in local market rice using the established and validated method. The sample was grinded, sieved and grinded again until completely transformed to fine powder before analyse for total Arsenic (As) and iAs. The coefficient of determination (R2) calculation for the analytes was above 0.99. Also, the analytes exhibited a limit of detection at 0.002 mg/kg, including the limit of quantification of 0.005 mg/kg. Calibration standards were established in

solution at 1.00µg/kg and 10.00 µg/kg. Recovery studies showed 93.74±6.43 (low concentration), 90.59±3.49 (medium concentration) and 89.63±3.42 (high concentration). The method was tested on the Food Analysis Performance Assessment Scheme (FAPAS) Proficiency Test sample and obtained a z-score of 1.09. Thus, the proposed method proved satisfactory for determining iAs in rice. The validated method was then tested on 23 rice brand variants, comprising of 15 imported and 8 domestic rice types. The convenience sampling approach was used as sampling technique. The recorded data was between 0.01 mg/kg and 0.102 mg/kg, where brown and coloured rice displayed 2 to 5 times higher iAs than white rice. None of the samples violated the Codex Alimentarius Commission (CAC) permissible level (0.20 mg/kg to 0.35 mg/kg) or the maximum permitted limit of European Union (EU) regulation (0.10 mg/kg to 0.30 mg/kg). In short, this study summarised that the level of iAs in rice available in the Malaysian market is generally low.

Keywords: Inorganic arsenic, HPLC-ICPMS, Method validation, Rice

SDG: Goal 2: Zero Hunger

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

VALIDASI KAEDAH PENGESANAN ARSENIK BUKAN ORGANIK MENGGUNAKAN HPLC-ICPMS DAN PENCEMARANNYA DALAM BERAS DI PASARAN MALAYSIA

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Pencemaran inorganik arsenik dalam beras telah menjadi kebimbangan di seluruh dunia kerana ia dikaitkan dengan kesan penyakit kanser. Permintaan terhadap beras menyebabkan petani terpaksa menggunakan racun perosak bagi memenuhi permintaan dan mengelakkan kerugian yang besar. Sisa racun perosak, terutamanya racun perosak yang berasaskan arsenik, berpotensi untuk meresap ke dalam tanah, menyebabkan pencemaran tanah tanaman dan seterusnya pencemaran inorganik arsenik (iAs) dalam benih padi. Walaubagaimanapun, laporan kajian mengenai pencemaran iAs dalam beras di Malaysia masih terhad. Oleh sebab itu, kajian ini dilakukan untuk (1) menjalankan validasi kaedah analisis yang sesuai dan berpotensi untuk penentuan iAs (As (III), As (V)) dalam beras dengan menggunakan HPLC-ICP-MS dan (2) untuk menentukan tahap iAs dalam beras di pasaran tempatan menggunakan kaedah yang telah di validasi. Sampel beras di kisar, di ayak dan di kisar semula sehingga menjadi serbuk halus sebelum di analisis untuk jumlah Arsenik (As) dan iAs. Pengiraan pekali penentuan (R2) yang diperolehi

untuk analit iAs adalah melebihi 0.99. Seterusnya, had pengesanan (LOD) diperolehi pada 0.002 mg/kg, manakala had kuantifikasi (LOQ) adalah 0.005 mg/kg. Piawaian penentukuran kalibrasi ditetapkan dalam larutan pada kadar 1.00µg/kg dan 10.00µg/kg. Kajian pemulihan (recovery) menunjukkan 93.74%±6.43 (kepekatan rendah), 90.59%±3.49 (kepekatan sederhana) dan 89.63%±3.42 (kepekatan tinggi). Kaedah tersebut telah diuji pada sampel Ujian Kecekapan Skim Penilaian Prestasi Analisis Makanan (FAPAS) dan memperoleh z-skor 1.09. Oleh itu, kaedah yang di validasi terbukti berjaya untuk menentukan iAs dalam beras. Kaedah yang di validasi ini kemudiannya diuji pada 23 varian jenama beras, terdiri daripada 15 jenis beras import dan 8 jenis beras domestik. Pendekatan persampelan mudah (convenience sampling) digunakan sebagai teknik persampelan. Kandungan inorganik arsenik yang direkodkan pada sampel yang diuji adalah antara 0.01 mg/kg dan 0.102 mg/kg, di mana beras perang dan berwarna menunjukkan iAs 2 hingga 5 kali lebih tinggi daripada beras putih. Tiada sampel yang didapati melanggar tahap dibenarkan Codex Alimentarius Commission (CAC) (0.20 mg/kg hingga 0.35 mg/kg) atau had maksimum yang dibenarkan oleh peraturan Kesatuan Eropah (0.10 mg/kg hingga 0.30 mg/kg). Secara ringkasnya, kajian ini merumuskan bahawa tahap iAs dalam beras yang terdapat di pasaran Malaysia secara amnya adalah rendah.

Kata kunci : Inorganik arsenik, HPLC-ICPMS, Validasi Kaedah, Beras

SDG : Goal 2: Zero Hunger

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LIST OF ABBREVIATIONS

AOCS	American Oil Chemist's Society
As(V)	Arsenate
As(III)	Arsenite
AOAC	Association of official Agricultural Chemists
ANZFA	Australia New Zealand Food Authority
BMDL	Benchmark dose level
COA	Certificate of Analysis
CRM	Certified Reference Material
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants
CCCF	CODEX Committee on Contaminants in Food
DMA	Dimethylarsinic Acid
ET-AAS	Electrothermal Atomic Absorption Spectrometry
EDTA	Ethylenediaminetetraacetic Acid
EC	European Council
EU	European Union
FAO	Food and Agriculture Organization
FSQP	Food Safety and Quality Programme
HDPE	High Density Polyethylene
HPLC-ICPMS	High Performance Liquid Chromatography – Inductively Coupled Plasma Mass Spectrometry
HG-AAS	Hydride Generation Atomic Absorption Spectrometer
iAs	Inorganic Arsenic
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry

IC-ICPMS	Ion Chromatography – Inductively Coupled Plasma Mass Spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additive
KADA	Kemubu Agricultural Development Authority
LC	Liquid Chromatography
MR	Malaysian Rice
MA	Malonic Acid
MHC	Ministry of Health, China
MMA	Monomethylarsonic Acid
MADA	Muda Agriculture Development Authority
NAP	National Agriculture Policy
HNO3	Nitric Acid
NTA	Nitrilotriacetic Acid
ODS	Octadecylsilane
OSA	Octanesulfonic Acid
OLS	Ordinal Least Square
BERNAS	PadiBeras Nasional Berhad
PTFE	Polytetrafluoroethylene
PVDF	Polyvinlidene Fluoride
PRSD	Predictive Relative Standard Deviation
PT	Proficiency testing
RF	Radio frequency
RM	Reference material
RSD	Relative Standard Deviation
SSL	Self-sufficient Level
SPE	Solid-phase extraction
SMPR®	Standard Method Performance Requirements

TMAH	Tetramethylammonium Hydroxide
TFA	Trifluoroacetic acid
U.S FDA	US Food and Drug Administration
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

The World Health Organisation (WHO) has classified iAs derivatives, particularly arsenite (As III), among the top 10 chemicals or chemical groups that pose a substantial impact on public health (Nguyen et al., 2019). Direct exposure to arsenic can negatively influence the human body, leading to various illnesses (U.S. Government Accountability Office, 2018). The effect of consuming excessive amounts of iAs can lead to cancer in the lungs, skin, bladder and skin lesions (Hughes et al., 2011). These detrimental effects are based on the report by (Khazanah Research Institute Malaysia, 2018).

Based on research by several researchers, as reported by (Zakaria et al., 2021), the heavy metals in paddy plants are cadmium, arsenic, lead, chromium, copper and zinc. From the list, arsenic (As) is the most concerning. Since arsenic is a geogenic contaminant, the soil containing arsenic has emerged as rice's most hazardous heavy metal contamination (Fytianos et al., 2001). The inorganic form of arsenic could occur naturally in soil, sediments, and groundwater of paddy fields (Hang et al., 2009). Moreover, the anaerobic conditions in flooded rice fields have led to the increase of As levels in rice compared to other plantations, particularly arsenate (AsV), due to its hydrophilic characteristics (Williams et al., 2007).

The inorganic arsenic (iAs) posed detrimental effects to humans, especially in countries that eat rice as a staple food, including Malaysia.

Rice makes up fifty percent of the world's essential food sources, particularly in Southeast Asia (Majumder et al., 2021). Rice and rice products have become a national necessity in Malaysia. Therefore, it is unsurprising that the Khazanah Research Institute Malaysia (2018) discovered that in 2016, Malaysians consumed an average of 80 kg of rice per person per year. This accounted for roughly 26% of the daily caloric intake and cost an average of Ringgit Malaysia (RM) 44 per month per household (Khazanah Research Institute Malaysia, 2018). In a report by Institute for Public Health (IPH) 2014, the prevalence rate of rice consumption among the adult population in Malaysia varied between 6.61% and 98.37%, with brown rice showing the lowest consumption rate and white rice being the most commonly consumed. The properties of rice, especially its low allergenic effect, make it the ideal choice for introducing babies to solid foods (Mennella et al., 2006).

The rising trend of rice consumption worldwide has created a demand for manufacturers to accelerate rice production and avoid trade rejection and profit losses (Qian et al., 2019). Thus, using arsenic pesticides and phosphate fertilizer (TSP, triple super phosphate) has opened the way for iAs contamination in rice. The chemicals applied to the plants will eventually accumulate in the soil and be re-absorbed by the plant (Jayasumana et al., 2015). Besides pesticides and fertilizers, soil contamination can originate from natural sources in the earth's crust due to mining and ore smelting activities (Hoang et al., 2021) .

In Malaysia, the increased national demand for rice has required the country to enhance rice availability through elevated domestic production and imports from other countries (Makhtar et al., 2022). Data indicated that 67% of rice in Malaysia was produced locally, and the rest was imported (Omar et al., 2019). The rice production in Malaysia is from the species of *Oryza sativa* cultivated under flooded soil conditions. This cultivation method is a significant contributor to heavy metal contamination due to the utilisation of contaminated water for irrigation (Payus et al., 2015).

Both iAs and dimethylarsinic acid (DMA) are the dominant species found in rice grains (Abedin et al., 2002). The iAs compound can convert into various methylated metabolites and become more cytotoxic and genotoxic (Fatoki & Badmus, 2022). When rice consumption is high across civilizations or subpopulations, iAs levels in rice will significantly affect dietary intake. Hence, evaluating the bioavailability of iAs found in rice is essential for understanding human exposure and mitigating the risks associated with iAs-related ailments (Gundert-Remy et al., 2015). As a result, the global concern regarding the contamination of rice by iAs has increased (Zhu et al., 2008).

The issue raised significant concern within the CODEX Committee on Contaminants in Food (CCCF) during its 5th meeting session in March 2011. In response, the committee decided to advocate for the adoption of a validated method for enforcement purposes (FAO/WHO, 2011).

Due to the potential non-linearity of cancer risk, determining a safe level cannot solely rely on using high-dose exposures to estimate potential risks; instead, it requires a different approach (Cubadda et al., 2017).

1.2 Problem statement

For years, there has been an expectation of iAs contamination in drinking water, especially after fatal arsenic poisoning caused by contaminated drinking water from well water in Virginia (Armstrong et al., 1984). The outbreak involving 9 family members was an eye-opener to the nation on the side-effect of arsenical pesticides and fertilizer. Starting from that, researchers began to trace the toxic effect of iAs along the chain.

The level of iAs in soil, fish and seaweed was first investigated (Heikens, 2006), Roychowdhury, 2008). However, a revelation by (Zhu et al., 2008) on the exposure of iAs in rice was critical. The presence of iAs in rice poses potential hazards to human health. Arsenic exposure, especially in its inorganic form, could affect almost every organ and develop various health effects, such as skin lesions, cancer, diabetes and lung diseases (U.S. Government Accountability Office, 2018)

The CODEX Committee of Contaminants in Food (CCCF) addressed the above issues further at its 8th session in March 2014, stating that a field study should be conducted for iAs uptake in rice crops. In response, several countries have conducted investigations regarding the presence of iAs contamination in rice within their territories, such as the United Kingdom

(Menon et al., 2020), Switzerland (Guillod-Magnin et al., 2018), China (Zeng et al., 2015), and the Iberian Peninsula Europe (Signes-Pastor et al., 2016). However, the exposure to iAs in rice remains underreported in Malaysia.

Currently, the regulation on iAs in rice is nowhere stated in the Malaysia Food Regulations 1985, even though it has been established since 2016 by the U.S. FDA and European Union (EU). Consequently, no established analytical method for detecting iAs contamination in rice available in Food Safety and Quality Programme (FSQP) laboratories.

Hence, a reliable and accurate analytical method is needed to overcome this issue. The method must adhere to ISO/IEC 17025:2005 requirements to demonstrate fitness for purpose (Peris-Vicente et al., 2015). The verified technique will be employed to analyse the presence of iAs in rice and present preliminary data on iAs contamination in rice found in the Malaysian market.

1.3 Research question

1) What is the practical and reliable technique to determine iAs content in rice using the facilities available at the enforcement laboratory in Malaysia?
2) What is the level of As and iAs contamination in rice varieties sold in the market?

1.4 Research hypotheses

1) The study is able to validate analytical method for iAs that suits the facilities and instruments available in the Food Safety Enforcement Laboratories under the Ministry of Health.
2) The contamination of iAs in rice sold in Malaysian market significantly posed risk to human health.

1.5 Research objectives

1.5.1 General objectives

Based on the problem stated the main objective of this study is to develop a reliable analytical method for determining iAs, specifically Arsenate (AsV) and Arsenite (AsIII) in rice, and to evaluate the contamination of iAs in different types of rice varieties in Malaysia market.

1.5.2 Specific objectives

The specific objectives of this study were as follows:

a) To validate a reliable and rapid method for enforcement laboratory of the Ministry of Health, Malaysia through method validation for Inorganic Arsenic in Rice by using HPLC-ICP-MS;
b) To determine the baseline data on contamination of Inorganic Arsenic in Rice available in Malaysia market.

1.6 Research Scope

The scope of this study is to determine iAs derivatives, specifically Arsenite (As III) and Arsenate (As V), in both method validation and real sample analysis. The analysis of the organic arsenic components, Dimethylarsinic acid (DMA) and Monomethylarsonic Acid (MMA), could not be performed due to the unavailability of the reference standards in liquid form.

In regards to objective 2, the explicit design was for pilot-scale analysis of real samples, specifically raw rice available in the market for general consumption. The study includes locally produced and imported rice, covering nine distinct rice categories defined by PadiBeras Nasional Berhad (BERNAS).

1.7 Significance of study

This study showed the extent of iAs contamination in rice within the Malaysian market, elucidating the modified analytical method employed through the available in-house facilities. As a result, the validated method can be employed by laboratories under the FSQP of the Ministry of Health, specifically for the analytical process. The Food Safety and Quality Laboratories can apply the validated method to analyse routine samples for surveillance or enforcement purposes, aligning with the function of the FSQP, which aims to protect Malaysians from the risks and fraudulent practices associated with food consumption. Moreover, routine enforcement activities may significantly contribute to community food safety control. This involves continuous and regular testing for the safety and quality of consumed rice, especially imported varieties.

Additionally, this study has complemented the National Agriculture Policy 2.0 (NAP 2.0), which focuses on four key subsectors aimed at promoting the production of healthier rice and incorporates five essential policy trusts. This initiative will drive the progress of sustainable agricultural practices and enhance the overall development of the food system.

Furthermore, the study can become a key factor in the increment of the regulating performance to increase the safety and quality of local rice sold in Malaysia, thereby significantly contributing to the economy's stability. This commitment will foster confidence in the domestic rice market and facilitate international trade opportunities.

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