DETERMINATION OF LEAD, ARSENIC AND NICKEL IN COMMERCIAL RICE BY FLAME ATOMIC ABSORPTION SPECTROSCOPY WITH CHEMOMETRICS FOR FORENSIC COMPARISON

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by

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LIST OF ABBREVIATIONSss

AAS	Atomic Absorption Spectroscopy
ATR-FTIR	Attenuated Reflectance Fourier Transform Infrared
BERNAS	Beras Nasional
JECFA CODEX	the Joint FAO/WHO Expert Committee on Food Additives Codex Alimentarius Collection of Food Standards
DMA	Dimethylarsanic
DNA	Deoxyribonucleic acid
EPA	Environment Protection
FTIR	Fourier Transform Infrared
FOA	Food and Agricultural Organization
GI	Glycemic Index
GC-TOF-MS	Gas Chromatography-Time Of flight Mass Spectrometry
HNO_3	Nitric Acid
H_2O_2	Hydrogen Peroxide
ICP-MS	Induced Coupled Plasma Mass Spectrometry
IADA PP	Integrated Agricultural Development Area
ICP-OES	Induced Coupled Plasma Optical Emission Spectroscopy
IRL	Interim Reference Level
KADA	Lembaga Kemajuan Pertanian Kemubu
MSMA	Monosodium Methanosornate

MMA	Monomethylarsonic
MADA	Muda Agricultural Development Authority
NMR	Nuclear Magnetic Resonance
NIR	Near Infrared
PTTI	Provisional Total Tolerable Intake
PCA	Principle Component Analysis
SIMCA	Class modelling technique
SVD	Singular value Decomposition
SSL	Self Sufficiency Level
SPSS	Statistical Package for the Social Sciences

PENENTUAN PLUMBUM, ARSENIK DAN NIKEL DALAM BERAS KOMERSIAL MENGGUNAKAN SPEKTROSKOPI PENYERAPAN ATOM NYALAAN DENGAN KEMOMETRIK UNTUK PERBANDINGAN FORENSIK

ABSTRAK

Beras, sebagai suatu makanan penting, sentiasa dikaitkan dengan keselamatan makanan dunia. Kehadiran logam berat dalam beras komersial berkemungkinan membawa risiko kesihatan kepada pengguna, dan dengan itu perlu ditentukan dan diukur. Tambahan pula, profil elemen pada beras juga boleh membantu perbandingan antara sampel. Justeru, kajian ini bertujuan untuk menentukan kandungan plumbum (Pb), arsenik (As) dan nikel (Ni) dalam beras komersial dengan spektroskopi penyerapan atom nyalaan (FAAS) diikuti dengan pengkelompokan dan/atau pembezaan sampel beras melalui kemometrik. Sejumlah 65 sampel beras komersial telah dikumpul dan diperiksa secara fizikal. Seterusnya, sampel tersebut telah dianalisis menggunakan AAS dan ditentukan dengan analisis komponen utama (PCA). Pemeriksaan fizikal termasuk bentuk dan warna telah membolehkan pembezaan sampel beras masing- masing antara beras giling and beras pulut serta beras putih dan beras warna. Profil elemen yang mengkhususkan Pb, As dan Ni telah didapati berbeza antara sampel beras tanpa mengira kumpulan beras. Sehingga 84% sample beras yang diuji dalam kajian ini telah didapati mengandungi Pb. Secara perbandingan, pengesanan positif untuk kehadiran As dan Ni adalah lebih rendah

peratusannya, masing-masing pada 21% dan 46% bawah keadaan kajian ini. Data elemen kemudiannya telah diubah secara logaritma dan dianalisis secara statistik dengan PCA. Ujian tersebut telah membenarkan pembentukan lapan kelompok. Sampel dalam setiap kelompok boleh dibezakan daripada yang lain, berkemungkinan disebabkan oleh perbezaan dalam profil elemen mereka. Kesimpulannya, aplikasi FAAS bersama dengan PCA dalam kajian ini telah berjaya menentukan kehadiran Pb, As dan Ni dalam sebahagian sampel beras, dan seterusnya dikelompokkan ke dalam kelompok masing-masing. Kehadiran logam berat tersebut dalam beras komersial perlu diberi perhatian bagi penyiasatan seterusnya untuk mengawal keselamatan makanan selain daripada perbandingan forensik antara sampel.

DETERMINATION OF LEAD, ARSENIC AND NICKEL IN COMMERCIAL RICE BY FLAME ATOMIC ABSORPTION SPECTROSCOPY (FAAS) WITH CHEMOMETRICS FOR FORENSIC COMPARISON

ABSTRACT

Rice, being an important food, is always associated with world food security. The presence of heavy metals in the commercial rice would poses potential health risks to the consumers, deserving determination and quantification. Furthermore, the elemental profiles of the rice could also aid in the sample-to-sample comparison. Therefore, this study was aimed to determine the contents of lead (Pb), arsenic (As), and nickel (Ni) in commercial rice by flame atomic absorption spectroscopy (FAAS) followed by the clustering and/or discrimination of rice samples through chemometrics. A total of 65 commercial rice samples were collected and physically examined. Subsequently, the samples were analysed using FAAS and decomposed by principal component analysis (PCA). The physical examination, including the shape and colour, allowed for the differentiation of rice samples between milled and glutinous rice, as well as white and colour rice, respectively. The elemental profiles, focusing on Pb, As, and Ni, were found different among the rice samples regardless of the rice groups. Up to 84% of the rice samples tested in this study were found to have contained Pb. Comparatively, the positive detection for the presence of As and Ni were in lower percentages, at 21% and 46% respectively under the experimental conditions. The elemental data was then logarithm transformed and statistically analysed by PCA, allowing for the formation of eight clusters. The samples in each

cluster could be discriminated from the others, probably due to the differences in their respective elemental profiles. To conclude, the application of FAAS combined with the PCA in this study had successfully determined the presence of Pb, As, and Ni in certain rice samples, and subsequently grouped into respective clusters. The presence of such heavy metals in the commercial rice shall be given attention for further investigation to safeguard the food security, in addition to the forensic sample-to-sample comparison.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Rice serves as the staple food for over half of the global population in Asia alone for everyday meal, social rites, festivals, and rituals. The consumption of rice globally goes to Asians with 84% followed by Africa with 7%, 3% from the South America and 2% from the Middle East according to Bhandari on the Global Rice Production, Consumption and Trade. In detail, the rice is consumed by over 2 billion people in Asia alone to go through their day with the energy from such food (Chaudhari, 2018).

Rice is the main source of carbohydrate from the food pyramid as it contains an average of 80% carbohydrate, 3% fats, 7-8% protein and 3% fibre as well as vitamins such as vitamin B including other minerals such as zinc, iron, copper, and manganese (Liew et al., 2020). According to Britannica (2019), rice farms in hilly areas are planted in terraced for the paddies to be flooded at various elevations. The successful rice production depends on the construction of dams and waterwheels, as well as the quality of soil. Different productions of rice are indicated by different type of harvesting processes, such as the production of brown rice, white rice, or parboiled white rice. Today, modern milling techniques have been employed to control losses from having broken grain by relying on controlled drying plants (Britannica, 2019).

The population in Malaysia continues to grow linearly and expecting to reach approximately 43 million by the end of 2050. It was noted that currently our country has reached 32.7 million compared to 32.6 million in 2020 of an annual growth of 0.2 percent as stated in the Official portal of the Department of Statistics Malaysia (2021). Being the staple food for Malaysian, the quality of the rice must be ensured to safeguard the health and safety of our population. The quality of rice is defined through the determinations of the physical and chemical properties, and the rice must meet the needs of its users (Gummert, 2010).

As rice is a widely grown crop and diverse, the high production from the traders" exportation might lead to rice adulteration, in order to gain more profits from the consumers. According to Śliwińska-Bartel (2021), adulteration on food referred to adding the chemicals, substituting the ingredient, and misleading statements that may pose threats to public health. Food safety includes ensuring the food's quality before it is distributed to markets and retail establishments (FDA, 2011). Various pollutant or contaminant could be originated from the environment and subsequently enter the food chain, contributing to potential health and safety effects. The term "pollution" or "contamination" refers to a change in the physical, chemical, radiological, or even biological quality of a source, such as the air, land, or water, that may be brought on by the human activity and pose a threat to life (Kabir, 2019).

Among the source of pollution and contamination, heavy metals are very common encountered. Heavy metal pollution could be brought on by anthropogenic causes such industrial waste, sewage sludge, burning of fossil fuels, and organic and inorganic fertilisers. Industrialisation nowadays had contributed to high level of heavy metal contamination, including the food, or more specifically our rice (Ahmed et al., 2019). Due to their toxicity, non-biodegradability, and persistent natures, heavy metals are the main environmental pollutants that needs attention. They can build up in the food chain and have an impact on human health, particularly if they are consumed often. Therefore, the testing of rice samples, if they contained heavy metal which exceeding the permissible levels set by the Food and Drug Administration (FDA) shall be initiated and carried out in this study. Such information could determine whether the consumption of certain rice could have posed long-term health exposure to the consumers (Yang et al., 2004). Besides that, the elemental profiles of the rice could also be useful to compare among the rice samples whether the composition of heavy metals, if any, could be related to the different rice groups or the common source. Therefore, this study was to determine the levels of heavy metals such as Pb, As, and Ni in commercial rice using analytical method and chemometrics.

1.2 Problem Statement

Rice production in Malaysia can only support approximately 70% of our population, and the remaining must be filled by the imported rice. It was also reported recently that our country might need to increase the rice import due to the continuous increase of consumption as well as limited resources for paddy cultivation throughout the country (Che Omar *et al*., 2019). It was noted that Thailand, Vietnam, and Pakistan are the main sources where our imported rice originated from (Giraud, 2013). Regardless of local rice or imported rice, traders might adulterate or substitute the products for economical profits (Galvin-King et al., 2017; Ch et al., 2021). Specifically, the rice product might be mixed or substituted with lower-quality or contaminated products. Reports had been established where premium rice such as Basmati rice was adulterated with non-basmati rice, and fragrant rice was replaced with lower grade aromatic rice. There were also cases where the rice products from other regions was mis-labelled as premium or high-quality rice (Anami et al., 2019; Ch et al., 2021). It was also unclear if the replaced rice contained heavy metals, dangerous to human‟s health, and therefore, the determination of any presence of heavy metals shall be conducted.

In Malaysia, there are lack of data representing the elemental profiles of rice. During the forensic food analysis, physical examination could provide a direct information of the types of rice and analytical studies were seldom conducted. The possibility of rice contamination, specifically the heavy metals which could be arisen from the soil cultivation, processing and manufacturing, as well as the packaging and distribution were underestimated. Therefore, the establishment and comparison of element profiles by flame atomic absorption spectroscopy (FAAS) on the diverse types of rice gathered in the local market could aid in investigating the elemental contents. Coupling with chemometric methods, sample-to-sample comparison could also be carried out to track the possible source of origin and distribution channels.

1.3 Aim and Objectives

This study was aimed to determine the contents of Pb, As, and Ni in the commercial rice using FAAS coupled with chemometric methods. To achieve the aim, the objectives of the study are set as follows:

1. To physically examine the various types of rice collected from the local market.
2. To compare the content of Pb, As, and Ni in the rice samples using FAAS.
3. To classify and discriminate rice samples using chemometric methods based on the elemental profiles.

1.4 Significance of Study

This study would provide information on the elemental profiles of various types of rice, purchased within the boundary of Malaysia. To certain extent, it could assist the food and agriculture department to detect if the rice products sold in the markets could have contained non-permissible level of heavy, specifically the Pb, As, and Ni. Apart from that, the resulted elemental profiles among the rice samples would also aid in the forensic comparison whenever any food fraud case was detected. Similar profiles might suggest that the two rice samples could have share certain degree of similarities, such as their source of origin or the distribution chain.

CHAPTER 2

LITERATURE REVIEW

2.1 The Concept of Rice (Oryza Sativa L)

Rice is the major food source for more than 100 countries worldwide, especially in the South Asia, the East, Middle East, the West Indies, and Latin America (Fukagawa and Ziska, 2019). It continues to be the most widely consumed food in a daily basis contributing to a person"s nutritional needs. The cultivated rice plant, *Oryza sativa L*., belongs to the family Gramineae's tribe Oryzeae, which falls within the subfamily Pooideae (Poaceae). Recently, the biosystematists classified the genus into various sections, with *O. sativa* falling under Sativa under the section of Sativae (Weerakoon et al., 2018). In addition to *O. sativa*, there is also another common species of rice known as African Rice or *Oryza glaberrima* (Awan et al., 2017). Among the Asian rice or *O. sativa*, more than 40,000 species are available in Asia, and they can be divided into four subspecies including the indica, japonica, aromatic, and glutinous (Randhawa, 2021).

There are various species of rice available in the market where the morphological characteristics of rice plant include spherical, hollow, jointed culms, relatively flat, sessile leaf blades, and a terminal panicle (Weerakorn et al., 2018). The rice plant can grow up to 1.2 metres in height and with broad and spreading fibrous roots system. The grain produced and collected from the plant is ensigned from the panicle or inflorescence (flower cluster) which made up of spikelet bearing with flowers that produces fruit. The rice is produced as the milled white rice is removed from the outer hull and its bran layer of rough rice through the process of milling and dehulling (Chen et al., 2010).

Globally, rice supply is cultivated from 144 million farms from 100 countries, where 163 million harvested areas have produced about 745 million tonnes of paddy in 2018. Amongst, 90% of the total rice produced from Asia (Bhandari, 2019). In term of rice consumption, China has the most people worldwide who consumes up to 154.9 million metric tonnes of rice per year, followed by India, Vietnam, Bangladesh, and Indonesia (Statistica, 2022).

Rice that retains its husk after threshing is known as standard paddy rice or rough rice. Husked rice, such as brown rice or cargo rice, is rice from which only the husk has been removed. Milled rice, also known as white rice, is husked rice from which all of the bran and gem or the embryo have been removed. Other than that, parboiled rice is made from husked or milled rice that has been processed from paddy or husked rice that has been soaked in water, heated to fully gelatinise the starch, and then dried. Glutinous rice or waxy rice are the kernels of unique varieties of rice that have a waxy texture. The kernels of some rice cultivars known as sticky rice or waxy rice have an opaque and white look. Nearly all of the sticky starch is made up of amylopectin (Malawi, 2016).

2.1.1 The Morphology of Rice

The rice fruit is a caryopsis where the single seed is fused with the wall of ripened ovary or called as the pericarp forming a seed-like grain which is the rice. As seen in the Figure 2.1, rice grains can be divided into three components, namely the bran, white rice, and germ. According to a Trinkley‟s study (2003) on rice cultivation, the grain's core is the germ that emerges once the seed is sown. The germ is said to contain vitamins B, E, protein, unsaturated fat, carbs, dietary fibres, and minerals. The greatest portion of the grain is the endosperm, where the white rice is primarily consumed. It includes some starchy carbs, some incomplete protein, and a little amount of trace minerals and vitamins. Finally, there is the bran component, which is the outer layer and primarily consists of the carbohydrate cellulose, with minor amounts of minerals, such as iron, phosphorus, magnesium, and potassium, vitamins including thiamine, niacin, and B-6, as well as the unfinished proteins (Trinkley and Fick, 2003).

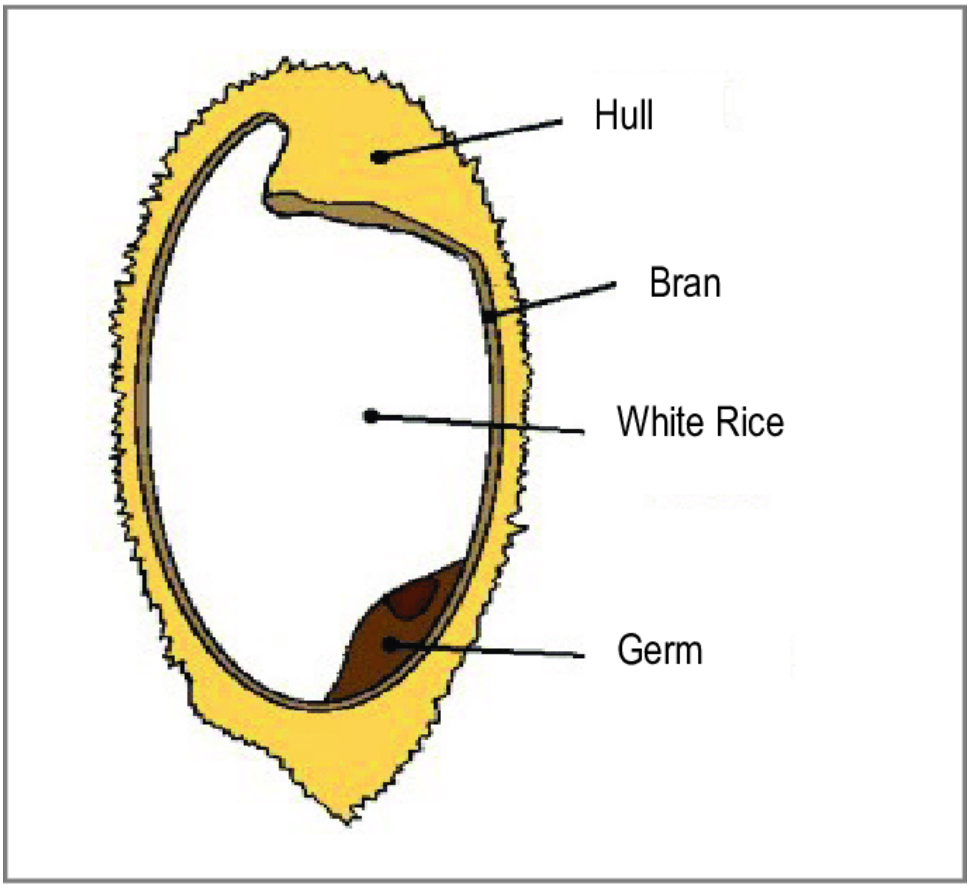

Figure 2. 1: The morphology of rice (Garba et al., 2019)