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Original Article

# Elemental analysis of commercially available rice samples in Malaysia by using ICP-MS and SEM-EDX

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## Abstract

Rice is the staple food in Malaysia. There are limited literature about study on the elemental levels and observation on ultrastructure of commercially available white rice and brown rice in Malaysia. Therefore, the objectives of this study were to determine the concentration of elements in three uncooked long rice grain, *i.e.* two brown rice and one white rice varieties that are commercially available in Malaysia using inductively coupled plasma mass spectrometry (ICP-MS) and compare the structure and elements of uncooked and cooked rice grain using Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDX). The ICP-MS results of the three uncooked rice grains showed high concentration of copper, manganese and zinc, followed with potassium, magnesium and calcium. Besides, the element contaminant such as plumbum, antimony and cadmium in the rice grains were under Malaysian permissible limit (Malaysian Food Act, 1983) and international standard (FAO/WHO, 2002). Arsenic was below Malaysian permissible limit but higher than safe limit by international standard. Silicon was observed in brown rice after viewed by using SEM-EDX. Besides, brown rice showed higher concentration of elements than white rice

**Keywords**: Brown rice, Elements, ICP-MS, SEM-EDX, White rice

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## Introduction

Almost half of the world populations particularly those who lived in East Asia, Southeast Asia, the Middle East, and the West Indies usually consume rice (*Oryza sativa*) as one of the most precious cereal crops and staple food (Jain et al., 2012; Rohman et al., 2014). In Malaysia, rice is staple food with 97% of the population eating rice twice daily at an average of 2½ plates per day with white rice as preference compared to brown rice (Kumar et al., 2011; Norimah et al., 2008). The Codex Standard 198-1995 defined rice as whole and broken grains achieved from the species

*Oryza sativa* L (Codex Standard, 1995). The intact rice kernels consisted of three major layers, *i.e.* endosperm, brans, and germ. The endosperm of the rice kernel consists of the major constituents of rice, *i.e.* starch and protein whereas the bran and germ layers consisted of non-starchy constituents such as fat, fiber, minerals, and vitamins (Rosniyana et al., 1995). The bran layers consist of 80% of the minerals, whereas the germ layers consisted of vitamin E, minerals, unsaturated fats, antioxidants, and phytochemicals (Jain et al., 2012). Brown rice is converted to white rice by removing the bran layers and germ in the milling and polishing process. As a result, it causes a total reduction of vitamins, minerals and dietary fiber. Although many new varieties of white rice are added with nutrients, the processed rice is different from the original nutritious brown rice (Babu et al., 2009).

Based on the health benefit and importance, elements can be classified as essential, namely sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo), barium (Ba), nikel (Ni) and non-essential elements, namely arsenic (As), aluminium (Al), silver (Ag), cadmium (Cd), antimony (Sb), thalium (Tl), plumbum (Pb), thalium (Th), uranium (U). Essential elements are further divided into macro- and trace mineral according to the daily dietary requirement. Essential macro-elements include Na, Mg, K, Ca, while trace elements include Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Ba, and Ni (FAO/WHO, 2002; Ministry of Health, 2017). A few non-essential elements particularly Ag, As, Pb, and Cd are categorised as metal contaminants which are regulated by Malaysian permissible limit (Malaysian Food Act, 1983) and international standard (FAO/WHO, 2002). There are standard rules that were enforced in many region worldwide to control the emission of elements and the maximum safety limit in food or drinks in order to reduce toxic heavy metal build-up in cereals for crop production, especially in rice, which is one of the most frequently consumed cereals (Liu et al., 2007). Currently, there are little literature that focused on elemental levels and comparison of ultrastructure on commercially available white rice and brown rice in Malaysia. Hence, the objective of this study was to compare the concentration of elements and ultrastructure in three cooked and uncooked long rice grain (two brown rice and one white rice) that commercially available in Malaysia. The results will help in better understanding of nutrition composition of rice grain and give better insight of food choice for food consumer, particularly in Malaysia.

## Material and Methods

### Sample collection

Two long grain brown rice and one long grain white rice samples were purchased from local market in Kota Bharu, Kelantan. One white rice variety was chosen as comparison to two brown rice varieties. The rice samples were kept in vacuum container before sample preparation and analysis.

### Reagents and standards

For ICP-MS analyses, the reagent water was deionised water with resistivity of 18.2 M Ohm. All acid, include acid nitric ($HNO_3$), peroxide acid ($H_2O_2$) used are suprapure reagent grade. The standard reagents of elements were obtained from environmental calibration standard (Agilent Technology, United States).

### Sample preparation

For sample preparation of ICP-MS analysis, the purchased uncooked rice samples were grounded into rice powder and samples were kept in 4℃ prior analysis.

Moreover, to prepare sample for SEM-EDX analysis, the uncooked samples were directly subjected to analysis, whereas the cooked samples was prepared using rice cooker (Panosonic, Malaysia). The uncooked rice was washed with water until the washing water is clear. The water was drained off before cooking. The rice: water ratio for white rice and brown rice kernel are 1:2 (w/w), respectively and cooked until the electric cooker will automatically turned off and simmered for 5 min to obtain completely cooked rice (Daomukda et al., 2011; Zhang et al., 2011).

### Analytical determination

The concentration of the elements were analysed using ICP-MS method (Suzuki, 2006) with slight modification. Approximately 0.5 g of rice powder, 6 mL $HNO_3$ and 2 mL $H_2O_2$ were digested in PTFE vessel by microwave system. A blank and control were carried out in the same method. The concentration of 23 elements were analysed with inductively coupled plasma mass spectrometry (Agilent technologies 7700 Series) equipped with a concentric Nebuliser, a quartz spray chamber and torch. Quality control of the analysis was checked by analysing two levels (1 and 50 ppb) of the standard solution using ICP-MS. Standard solutions (1-50 g/mL) were analysed to develop a calibration curve (Suzuki, 2006). Each element was read three times with relative standard deviation (RSD) were less than 10%, while the correlation coefficient for all the element analysis was between 0.99-1.00.

**Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDX)**
Cooked and uncooked rice samples were fractured using a razor blade. All the samples underwent critical point dry and mount onto round aluminium stubs with the help of double-sided adhesive tape. The samples were then be coated with approximately 19.3 µm silver by high vacuum evaporator (Leica EM SCD005) and scanned. The structure and elements of the selected region of the surfaces were observed and photographed for both cross-sectional and longitudinal view using SEM-EDX (Quanta FEG450) (Deepa et al., 2008; Ogawa et al., 2003). Each SEM-EDX photograph was captured in single spot for element analysis. Values are presented in percentage.

## Results and Discussion

**Determination of element concentration of uncooked rice samples using ICP-MS**
In general, the concentration of elements in rice was decreased in the following descending order:
Uncooked Brown rice variety 1 (BR 1R):
(i) Essential elements:
Cu>Zn>Mn>K>Mg>Ca>Cr>Ba>Mo>N>Na>Fe>Se>Co.
(ii) Non-essential elements:
Al>Pb>Cd>Ag>Co>Sb>U>Th>Tl.
Uncooked Brown rice variety 2 (BR 2R):
(i) Essential elements:
Cu>Zn>Mn>K>Mg>Ca>Cr>Ni>Ba>Mo>Na>Fe>Se>Co.
(ii) Non-essential elements:
Al>As>Sb>Pb>Ag>Cd>U>Tl>Th.
Uncooked White rice variety 1 (WR 1R):
(i) Essential elements:
Mn>Cu>Zn>K>Ca>Mg>Ni>Cr>Ba>Na>Mo>As>Fe>Se>Co.
(ii) Non-essential elements:
Al>As>Cd>Ag>Pb>Sb>U>Tl>Th.

**Essential elements**
Table 1 shows the element concentration of uncooked rice samples per serving (30 g). Three of the uncooked rice samples presented Cu, Zn, Mn as the highest concentration of essential elements, followed with K, Mg and Ca. Among the highest concentration of elements are Cu (10.86 mg and 41.07 mg per serving) for brown rice varieties, 1.86 mg per serving for white rice), Zn (7.86 mg and 8.36 mg per serving) for brown rice and 0.58 mg per serving for white rice variety), Mn (3.87 mg and 5.04 mg for brown rice varieties and 2.18 mg for white rice; followed with K (0.71 and 0.6 mg for brown rice varieties and 0.25 mg for white rice), Mg (0.35 and 0.28 mg for brown rice varieties and 0.04 mg for white rice), and Ca, *i.e.* 0.07 mg per serving for three uncooked rice samples.

**Table 1. Determination of elements concentration of uncooked rice grain using ICP-MS**

| Element | Elements concentration of rice varieties (mg per serving, 30g) | | |
|---|---|---|---|
| | BR 1R | BR 2R | WR 1R |
| Na | 0.01 | 0.02 | 0.02 |
| Mg | 0.35 | 0.28 | 0.04 |
| K | 0.71 | 0.60 | 0.25 |
| Ca | 0.07 | 0.07 | 0.07 |
| Fe | 0.01 | 0.01 | <0.01 |
| Al | 0.18 | 0.33 | 0.16 |
| Cr | 0.06 | 0.07 | 0.03 |
| Mn | 3.87 | 5.04 | 2.18 |
| Co | <0.01 | <0.01 | <0.01 |
| Ni | 0.02 | 0.04 | 0.04 |
| Cu | 10.86 | 41.07 | 1.86 |
| Zn | 7.86 | 8.36 | 0.58 |
| As | 0.01 | 0.01 | <0.01 |
| Se | <0.01 | <0.01 | <0.01 |
| Mo | 0.03 | 0.03 | 0.01 |
| Ag | <0.01 | <0.01 | <0.01 |
| Cd | <0.01 | <0.01 | <0.01 |
| Sb | <0.01 | <0.01 | <0.01 |
| Ba | <0.01 | 0.04 | 0.03 |
| Tl | <0.01 | <0.01 | <0.01 |
| Pb | <0.01 | <0.01 | <0.01 |
| Th | <0.01 | <0.01 | <0.01 |
| U | <0.01 | <0.01 | <0.01 |

BR 1R, Uncooked brown rice variety 1; BR 2R, uncooked brown rice variety 2; WR 1R, uncooked brown rice variety 1.

Essential elements especially K and Mg which usually present in rice samples are important for normal cellular metabolism, cell division and growth, and immunity. Tolerable upper intake level (UL) was set by national and international organisation worldwide according to life stage group for each elements intake to ensure safety food consumption limit (Ministry of Health, 2017). Previous study conducted in Malaysia showed low concentration of bioavailable Cu in the paddy plant (Khairiah et al., 2013) which seemed to be in contrast with current finding. Copper (Cu) level of uncooked brown rice variety 1 and 2 were high, *i.e.* 41.07 mg and 10.86 mg per serving as compared to recommended UL (10 mg per day), maybe due to different soil origin and environment of plantation (Khairiah et al., 2013) and unwashed nature of rice samples. Besides, the interactive effects of soil bioavailable elements on transportation in rice grain (Xiao et al., 2017), low level of anthropogenic activities into the paddy fields, the acidic soil and high usage of specific metal-containing fertilisers and pesticides (Khairiah et al., 2013) might be the main contributors of the high concentration of elements particularly Cu, Zn and Mn in the present study. Previous finding also showed that a few Australian grown and Bangladeshi rice on sale in Australia showed high element content, especially Mn and Zn (Rahman et al., 2014). Ibrahim (2013), our previous colleague, revealed that the level of Cu, Zn and Mn level of uncooked rice grains varieties available in Malaysia were under safety food consumption limit using Atomic Absorption Spectroscopy. According to Ibrahim (2013), the concentration of Cu of brown rice varieties in Malaysia was between 0.0031±0.0003 and 0.0035± 0.0035 (mg/kg), whereas white rice variety was approximately 0.003 ±0.0003 (mg/kg); the concentration of Mn was between 0.0203 ± 0.003 and 0.0322 ± 0.002 (mg/kg), whereas white rice was approximately 0.016 ±0.0003 (mg/kg); the level of Zn of brown rice varieties were between 0.0598± 0.0003 and 0.0825 ±0.0014 (mg/kg), whereas the level of Zn in white rice was 0.1017 ±0.0012 (mg/kg). Since ICP-MS analyses were not conducted among cooked rice samples, we could not estimate the maximum permissible limit of Cu element in cooked rice in present study. Despite health benefits obtained from rice grain, the consumers should take safety precaution measurement, such as washing rice before cooking to remove potentially excessive elements concentration in rice grain.

**Metal contaminant**

The dietary exposure of high concentration of metal contaminant such as plumbum, arsenic, cadmium and antimony might cause irreversible health condition. The concentration of plumbum in white rice was 0.0579 $mgkg^{-1}$, whereas the concentration of brown rice variety 1 and 2 were 0.0961 and 0.1149 $mgkg^{-1}$. The level of plumbum in all the samples were below Malaysian standard limit , 2 $mgkg^{-1}$, which are regulated by Malaysian Food Act (1983) and below FAO/WHO (2002), *i.e.*, 0.2 $mgkg^{-1}$.The concentration of arsenic of brown rice varieties were within 0.2226 and 0.2610 $mgkg^{-1}$, whereas white rice 0.1425 $mgkg^{-1}$. The level of arsenic in three of the sample was below safe limit regulated by Malaysian Food Act (1983) that is 1 $mgkg^{-1}$, however brown rice varieties were slightly above FAO/WHO (2002) safety limit, *i.e.* 0.2 $mgkg^{-1}$.The concentration of cadmium of brown rice varieties were within 0.0709 and 0.071 $mgkg^{-1}$, whereas the concentration of white rice was 0.0933 $mgkg^{-1}$. The level of cadmium of all the samples was below safe limit regulated by Malaysian Food Act, that is 1 $mgkg^{-1}$ and FAO/WHO (2002) safety limit, *i.e.*, 0.4 $mgkg^{-1}$.The concentration of antimony (Sb) of brown rice varieties were 0.0098 and 0.2497 $mgkg^{-1}$, whereas the concentration of white rice was 0.0132 $mgkg^{-1}$. The level of antimony of the samples was below safe limit regulated by Malaysian Food Act (1983) that is 1 $mgkg^{-1}$. The finding is parallel with compositional analyses of white and brown rice of Malaysian popular Malaysian rice. The concentration of metal contaminant in the rice samples were below maximum permissible limit. White rice was significantly lower in its metal contaminant concentration compared with brown rice (Abubakar et al., 2018).

The limitation of this study was the samples were measured only once in this study since ICP-MS was a costly analyses. However, spiking test and recovery analysis were performed to ensure the reliability of the test. Furthermore, each element was read three times and the RSD value were less than 10%. Bigger sample size, replication of measurement of the rice samples, and elemental analyses of cooked rice instead of uncooked rice were recommended for future study.

**Comparison of element concentration and ultrastructure difference of uncooked and cooked rice using SEM-EDX**

As shown in Table 2, for SEM-EDX analysis, carbon (C) and oxygen (O) were present in all samples of cooked and uncooked rice. Besides, magnesium was detected in three uncooked rice (0.09-0.3%); potassium (K) was found in three uncooked rice samples (0.09-0.46%), cooked brown rice variety 1 (0.08%) and cooked white rice (0.17%), whereas silicon (Si) was detected in uncooked brown rice variety 1 and 2 (0.29% and 0.16%). SEM-EDX analysis showed lesser element detection as compared to multi-element analysis of rice samples using ICP-MS since SEM have lower instrumental detection limit (Suzuki, 2006). Besides, the nature of rice sample is less conductive and only one spot was captured for analysis in this study which might cause under-reported element detection. Silicon was detected in brown rice varieties in this study, partly due to the unpolished bran layer. The presence of silicon play roles in controlling rice blast diseases and reduces abiotic stress such as heavy metal stress (Ashtiani et al., 2012). Multi-spot capture of SEM-EDX photograph for elemental analysis and bigger sample size were recommended in future study to increase validity of the study.

**Table 2. Percentage weight of elements in uncooked and cooked rice grain using SEM-EDX**

| Element | Percentage weight of elements (%) | | | | | |
|---|---|---|---|---|---|---|
| | **BR1 R** | **BR 2R** | **WR 1R** | **BR 1C** | **BR 2C** | **WR 1C** |
| C | 16.18 | 15.71 | 8.4 | 8.35 | 12.3 | 12.81 |
| O | 11.17 | 11.65 | 6.21 | 9.2 | 14.96 | 16.01 |
| Mg | 0.09 | 0.2 | 0.1 | ND | ND | ND |
| Si | 0.29 | 0.16 | ND | ND | ND | ND |
| K | 0.36 | 0.46 | 0.09 | ND | 0.08 | 0.17 |

BR 1R, Uncooked brown rice variety 1; BR 2R, uncooked brown rice variety 2; WR 1R, uncooked brown rice variety 1; BR 1C, Cooked brown rice variety 1; BR 2C, cooked brown rice variety 2; WR 1C, Cooked white rice variety 2; ND, Not Detectable.

For ultrastructure analyses of rice samples which were viewed under SEM, the appearance of white rice variety showed smooth surface as compared to brown rice varieties since white rice undergo higher degree of polishing and milling than brown rice in 100x magnification, longitudinal sections in Figure 1(a-c). Three cooked grain section showed rupture cell walls as shown in Figure 1 (d-f). Cooked brown rice varieties showed two different layers of cell layers, as pointed by two arrows in longitudinal section and cross section as showed in Figure 1(d,e) and Figure 1(j,k), whereas no distinctive layer was observed in cooked white rice as shown in Figure 1 (f,i).

The longitudinal (Figure 2a,b,d,e) and cross-sections (Figure 2 g,h,j,k) of two types of uncooked and cooked brown rice varieties showed slightly different ultrastructure, which consist of two distinct layer of outer layer (bran layer) and inner layer (endorsperm) in 1000x magnification. The bran layer is composed of pericarp, seed coat, nucellus, and aleuron layer. Uncooked and cooked white rice showed no distinct layers, only endosperm is observed in either longitudinal (Figure 2 c,f) or cross-section (Figure 2 i, l) in 1000x magnification. Brown rice generally has thick pericarp or parenchyma cell walls and aleuron layer compared to white rice, which delay gelatinisation and water absorption into grain during cooking (Deepa et al., 2008). The cooked rice samples are freshly prepared, without undergo any drying process under SEM observation in this study. Therefore, it showed less porosity than dried and processed cooked rice undergo vary temperature (Luangmalawat et al., 2008).

Figure 3 shows longitudinal section of uncooked and cooked rice grain in 5000x magnification. Fissure was observed between two different layers, *i.e*., pericarp and endosperm, of the uncooked brown rice variety (Figure 3a). The fissure and crack under SEM observation were less obvious in uncooked rice as compared cooked rice grain which undergo drying process. The presence of fissure and cracks indicated the water flow channel inside the rice grain during cooking process (Ogawa et al., 2003). Parenchyma cell walls enclosed aleuron grains (ag) in uncooked brown rice (Figure 3a, c) and cooked brown rice (Figure 3 b, d). However, only endosperm layer was observed in raw and cooked white rice (Figure 3 e, f). Rice undergo several structural changes during gelatinisation process, i.e., from endosperm cell, to development of amyloplast and starch (honeycomb structure) (Tomita et al., 2014). Rice consists of polygonal starch granules and several polyhedral compound in a single amyloplast which might be caused by the compression of starch granules during cooking process (Watson and Dikeman, 1977). Water penetration into rice grain causes the increase of dimension of granules starch and asymmetrical starch granule folding during gelatinisation process (Deepa et al., 2008).

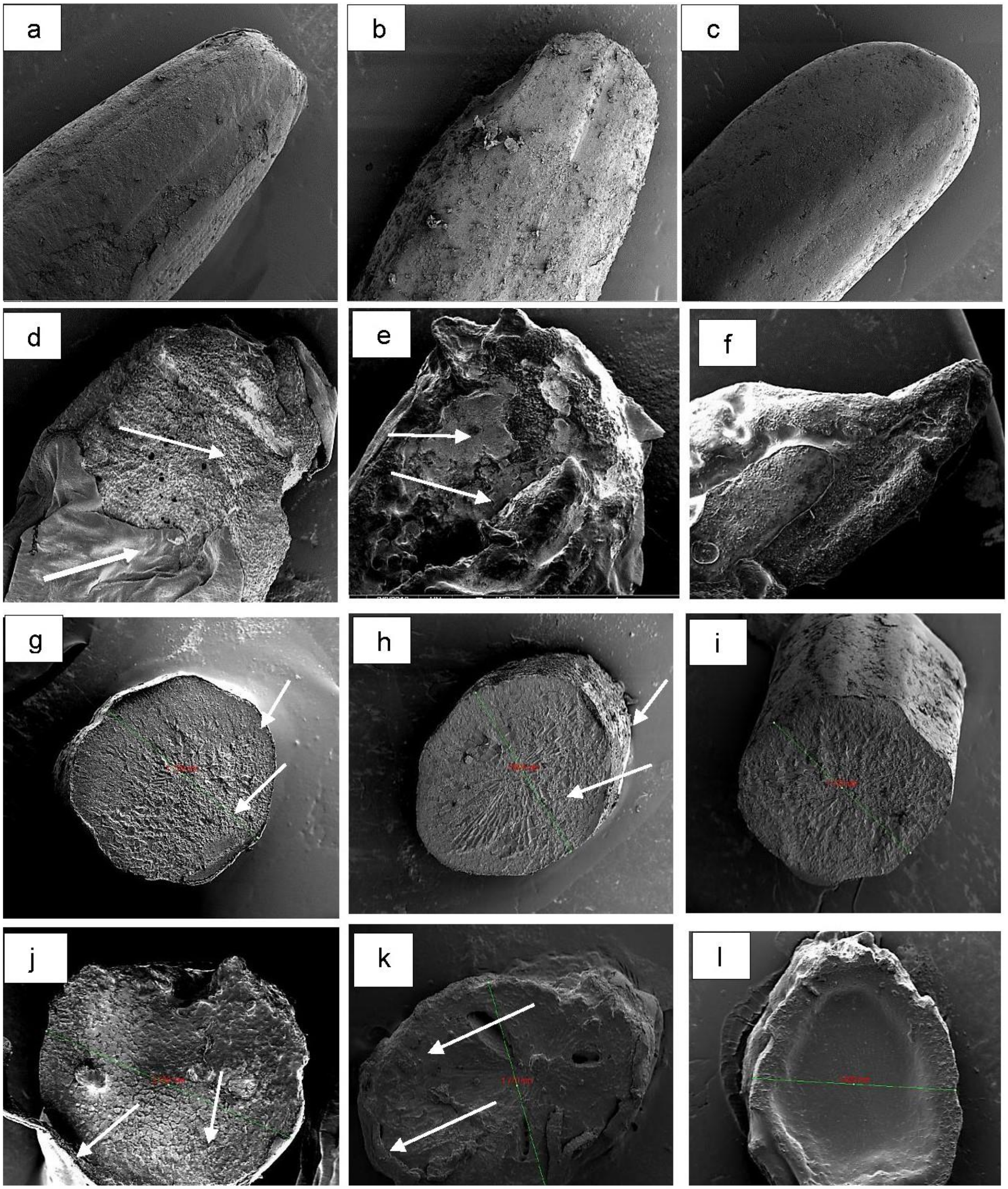


**Figure 1. Scanning electron microscope photographs of longitudinal section and cross sections of raw and cooked brown rice and white rice in 100x magnification. Longitudinal section of uncooked brown rice variety 1 and 2 (a, b), white rice variety 1 (c); longitudinal section of cooked brown rice variety 1 and 2 (d,e), white rice variety 1 (f); Cross section of uncooked brown rice variety 1 and 2 (g, h), white rice variety 1 (i); cross section of cooked brown rice varieties 1 and 2 (j,k), white rice variety 1 (l).**

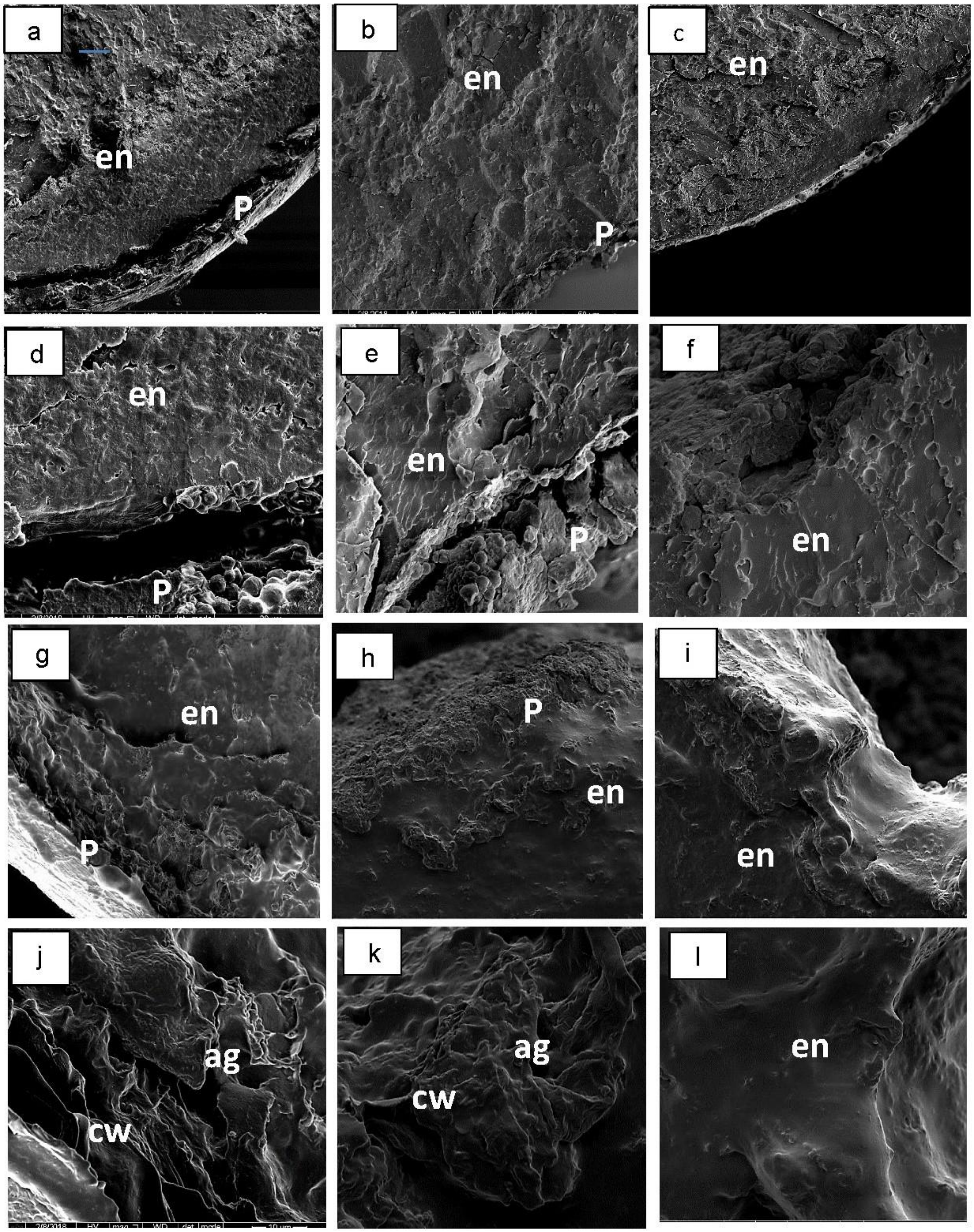


**Figure 2. Scanning electron microscope photographs of cross sections of uncooked and cooked brown rice and white rice. Uncooked brown rice variety 1 and 2(a,b), white rice variety 1 (c) in 1000x magnification; cooked brown rice variety 1 and 2(d, e) and white rice variety 1(f) in 1000x magnification; Uncooked brown rice variety 1 and 2(g, h) and white rice variety 1 (i) in 5000x magnification; cooked brown rice variety 1 and 2 (j, k) and white rice variety 1(l) in 5000x magnification.**

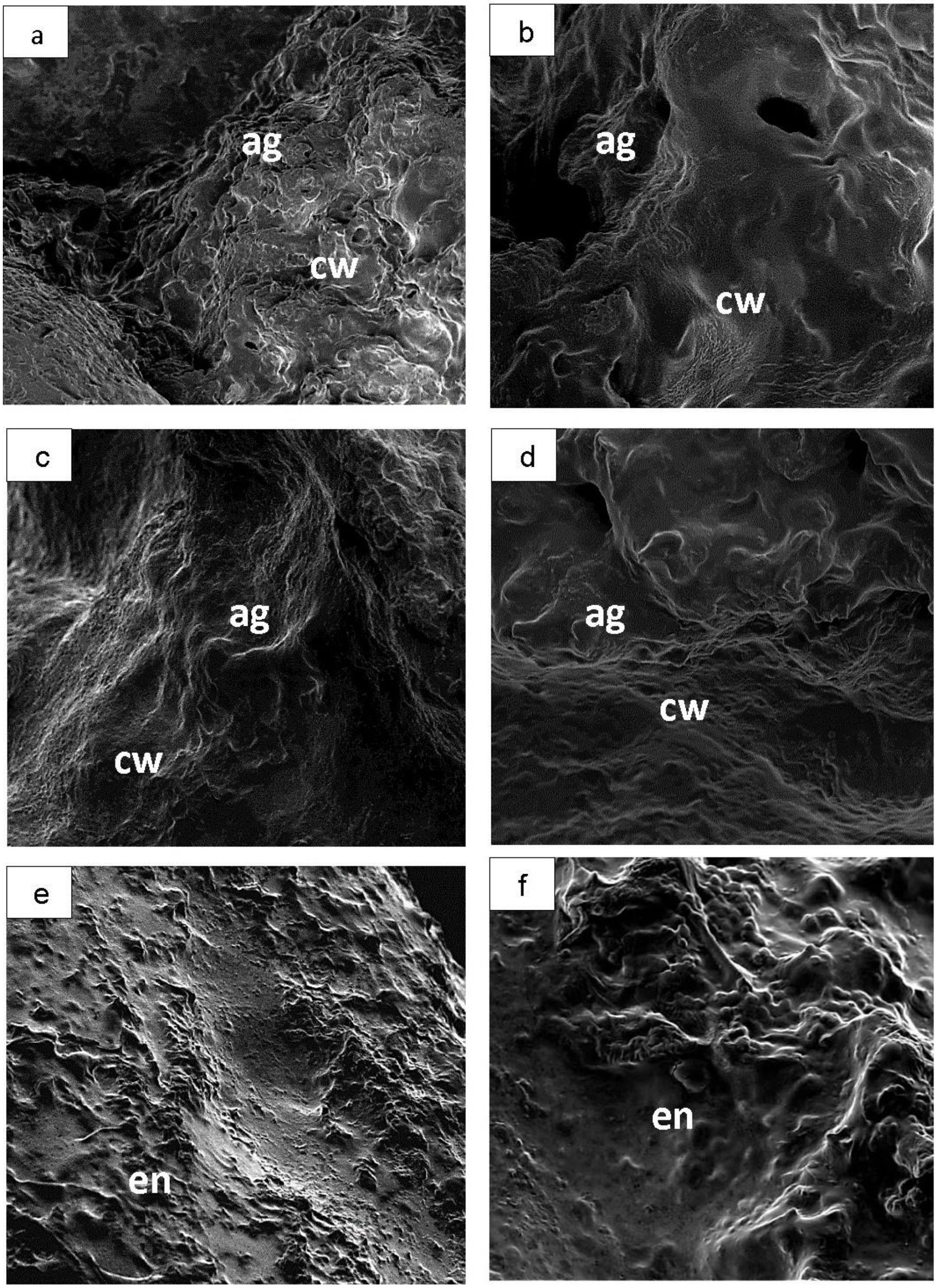


**Figure 3. Scanning electron microscope photographs of longitudinal section of uncooked brown rice variety 1 and 2(a, c) and white rice variety 1(e); cooked brown rice variety 1 and 2(b, d) and white rice variety 1(f) in 5000x magnification.**

## Conclusion

Our current findings showed that brown rice has higher element concentration than white rice. Some of the elements are essential for human (Cu, Mn, Zn); however, these elements might cause health hazards if their concentration exceed tolerable upper intake levels limit set by national and international organisation globally. The metal contaminants in the uncooked rice samples were below maximum permissible limit, except arsenic which is higher than the safe limit determined by FAO/WHO (2002). Besides, uncooked brown rice showed higher element concentration (mg per 30g carbohydrate portion size) than white rice in ICP-MS finding. Since ICP-MS analyses were not conducted among cooked rice samples, we could not compare element concentration between cooked and uncooked rice and unable to estimate the maximum permissible limit of elements in cooked rice in present study.

For SEM-EDX analyses, silicon was detected in two uncooked brown rice samples, *i.e.* 0.16 and 0.29%. The presence of outer layer of brown rice before cooking and cell wall disruption after cooking process as observed under SEM indicate that there might be more element content for cooked brown rice compared to white rice located in the outer layer. However, further study was recommended to compare the different degree of polishing of rice grain available in Malaysia to know the exact percentage of loss element after outer layer being removed. The present study provides better understanding of the potential benefit and health hazards of elements in rice to Malaysian consumers.

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## Contribution of Authors

Gee OM: First author and wrote the full article.
Jalil RA: Principal Investigator and grant holder who also conceived idea, designed the study, and proofread the article.
Ishak WRW: Conceived idea, designed the study, and proofread the article.
Hamid NA: Assisted in English proofread of the article.
Aziz CBA: Assisted in proofread of the article.
Nik WSW: Helped in data collection.
Hamid NF: Helped in data collection.
Malik V: Assisted in study design.
Willet W: Assisted in study design.
Hu F: Assisted in study design.

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