

Physical Properties and Glycemic Index of Organic and Conventional Black Rice Grown in Bali, Indonesia

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Abstract— Similar variety of black rice was grown by organic and conventional methods in Jatiluwih and Penatih districts of West Bali, respectively, separated by 46 km distance. Each rice is very similar, having black pericarp and white rice inside. In this study, we found that the degree of crystallinity of the two rice is about 24%. The Bragg parameters of the conventional rice are very close to dehydrating α -amylose, whereas the organic rice exists in polymorphic form. The crystallites in the endosperm area are dominated by polyhedral shapes in contact with each other at the edges and formed a cyclic geometric environment. The size of the crystallites was between three and six microns. Overall, the crystallites are packed in rectangular blocks connected radially from the center towards the pericarp. XRF experiments show each rice contain P, K, S, Si, Mg, Cl, Al, Zn, Fe, Cu, and Rb. However, ruthenium and manganese were detected in organic rice. Both organic and conventional black rice possesses a low glycemic index of about 31 ± 5.0 compared to 50 ± 5.8 for commercial Basmati rice. Thermogravimetric investigation showed that the black rice was stable up to about 300°C , and the gradual mass loss between 80 and 210°C is due to water molecules and additional components present in the rice. After the major decomposition at 310°C , the mass gradually decreased and reached 18% residual at 600°C .

Keywords— black organic Bali rice; crystallinity; polymorphism; glycemic index percentage; endosperm and thermogram.

I. INTRODUCTION

Colored rice is beginning to receive acceptance due to its health properties despite its slight differences in taste, physical, and chemical properties compared to the normal white rice. High carbohydrate rice has become the subject of concern due to the increase in diabetes as a significant chronic disease in many countries, including Japan, where type-2 diabetic has been associated with the traditional dietary pattern of Japanese white rice as staple food [1]–[4]. The presence of anthocyanin in the colored rice played an important role not only to the physical and chemical properties of the rice but also to the health-promoting aspect as it is known as a good antioxidant. Besides, there are polyphenols, flavonoids, and vitamins also present [5], [6]. Black rice was reported to contain the highest total anthocyanin of 327.60mg per 100 g compared to other colored rice varieties [7]. Anthocyanin extract of black rice has been shown to inhibit cancer cell proliferation in vitro [8]

and reduced significantly improves lipid profile and inflammatory status [9]. It also provides endless opportunities for diversification of rice-based food products in the beverage industries. Today, we can find some colored rice in 1 to 5 kg of packaging are being sold in the hypermarket and mostly came from Thailand, China, Sri Langka, India, and Indonesia. In Indonesia, there are more than 50 accession numbers for germplasm collections [10]. However, there are only 24 names of local colored cultivars [11]–[14] being grown mainly in Java and Sulawesi. Recently colored rice is also grown in Sumatra. One thing for sure is that all the rice is given a name based on the location or name of the farmers (accession number) and that it needs further analysis of their genotypes. Black and brown rice are also cultivated in Bali and sold for local consumption. No detailed information or specification is given unless it is sold in proper packaging as in the hypermarket. Therefore, we decided to carry out the detailed physical and chemical studies on our local black rice

cultivated by both conventional and organic methods. In this paper, the physical and morphological properties and elemental analysis, including a glycemic index of our locally grown black rice, are presented.

II. MATERIALS AND METHODS

A. Chemicals and instrumentation

All the chemicals purchased from Merck were Reagent grade and used without further purification. Glycemic index study was carried out using the Gluco blood glucose test apparatus Dr. AGM-2100 type produced by Medicus production Co., Ltd. Perkin Elmer Infrared red spectrophotometer model spectrum400 FT-IR with a wavenumber range of 650-4000 cm^{-1} was used. All the samples were ground into a fine powder before the spectral acquisition.

Scanning electron microscope model FE-SEM, ZEISS Merlin operating at 0.2-30kV beam current up to 400nA was used to obtain the morphological image of rice grain. The energy-dispersive X-ray unit is attached to the equipment for elemental determination. In many situations, a 15kV accelerating electron was applied. The transversal cross-section of the rice grain was mounted on a circular aluminum stub and platinum-coated in a vacuum using a sputter coater.

X-ray diffractometer Bruker D8 Advance was used to study the crystallinity of the rice. The rice in powdered form and was tightly packed in the smallholder. Each sample was exposed to X-Ray beams with a generator running at 20 mA and 30kV. The scanning diffraction angle (2θ) ranged from 5° to 80° . was used applied. The overall degree of crystallinity was calculated by taking the ratio of the area of crystalline reflections to the overall diffraction area. X-Ray Fluorescence experiments were performed on Bruker S8 Tiger spectrometer running at 1kW, 50kVmax, 50mAmax, Tube (Rh, Be:75 μm).

A thermogravimetric study on the rice sample was performed using a thermogravimetric analyzer (Mettler-Toledo with small furnace S.F.). Approximately 4-7 mg samples and references were placed in the 140 μL standard platinum crucibles. The samples were subjected to heat with a constant heating rate of $10^\circ\text{C min}^{-1}$ in a room environment under the flow of 50 mL/min air in the temperature range of 25-600 $^\circ\text{C}$. The whole process was running in nitrogen gas (99.999%).

B. Rice Samples

The conventional black rice was grown in Penatih, East Denpasar, Bali, Indonesia, while organic was obtained from Jatiluwih, Tabanan Regency, Bali Indonesia, about 46 km away in the west of Bali. For organic farming, composted fertilizer prepared from the farm and plant waste was used. Organic materials and biological control methods were applied for plant protection. In the conventional method, the main fertilizer was urea and commercial pesticide for plant protection. So far, conventional black rice is sold to a limited customer with a price of 1.46 USD- 1.75 USD per kg. Only organic black rice is sold in the hypermarket with nice packaging, and the price is higher than the conventional one between 1.96 USD – 2.90 USD per kg. Both rice has not yet

followed the S.N.I. or premium standard; probably, the amount available is still low. All standard properties measurements were carried out in our laboratory. Rice grain sizes were measured using Vernier clipper.

C. Amylose Content analysis

The iodine method reported by Juliano [15] was applied to determine the amylose content in the rice samples. The mixture of 100 mg fat-free rice flour, 1 ml of ethanol (95%), and 9 mL of 1 N NaOH in a volumetric flask (100 ml) was placed in a sonic bath for 15 minutes to ensure thorough mixing. The starch was gelatinized by heating on a boiling water bath for 20 minutes. After cooling, 5 mL of the gelatinized starch solution was transferred into a 100 ml volumetric flask. 1 mL of 1N acetic acid and 2 ml of iodine solution were added into the flask, and the volume was adjusted to 100 ml by distilled water. The solution was shaken and left for 20 minutes to become homogenous. The absorbance measurement was made at 620 nm using a UV-Spectrophotometer (Model AA-6650, Shimadzu Co. Japan). The amylose content in samples was determined based on the standard curve prepared using potato amylose

D. Preparation a Standard Curve

The standard amylose solution was prepared by weighing 40 mg of pure amylose into a 100 ml measuring flask. One ml of 95% ethanol and 9 ml of 1N sodium hydroxide were added into the flask. The standard solution was heated in a water bath for 10 minutes and added distilled water to the mark. A series of 1, 2, 3, 4, and 5 ml of the standard solution were pipetted into a 100 ml measuring flask and added 0.2, 0.4, 0.6, 0.8 and 1.0 ml of 1N CH_3COOH respectively, were added to the flask followed by 2 ml iodine solution into each flask and finally filled with distilled water to the mark and left for 20 min. The absorbance of the blue intensity formed was then measured using a spectrophotometer at a wavelength of 620 nm. The standard curve was drawn by plotting the absorbance versus amylose concentration. Amylose content in the sample was calculated based on the following formula:

$$\text{Amylose Content} = \frac{CxVxF}{W} \times 100 \quad (1)$$

information:

C = amylose concentration from the standard curve (mg / ml)

V = final volume of sample (ml)

F = dilution factor

W = sample weight (mg)

Amylopectin content can be calculated simply by subtracting the amylose content, $100 - \text{amylose content}$.

E. Study Protocol and Criteria

The study protocol and ethics approval was granted by the University Hospital of Warmadewa University Ethics Committee and conducted under its rules and regulations. The standard glycemic index testing protocol, as described by Wolever et al. [16], [17] was adopted in this study. Glucose was used as the reference food with a G.I. score of 100, tested in the subject at baseline, midway, and the end of the study. Only candidates who are non-diabetic, non-smoking, healthy, active lifestyle, without any diagnosed

diseases, and not on prescribed medication were selected for the study. They were asked to maintain their daily activity.

F. Rice Preparation for Postprandial Screening

Rice was thoroughly washed before cooked in an electronic rice cooker (model SHARP) with 2mL water g-1 rice at cooked control temperature and time [18]. A few portions of the rice were weighed after cooled according to the carbohydrate content considering dietary fiber content.

G. Glycemic index experimental design

Under certain constrained conditions and large population, randomization can become problematic nonprobability purposive sampling technique can still be useful in the design for glycemic index determination [19], [20]. Six well and healthy male students with an excellent medical record, such as no diabetic and cardiac problems with ages between 20 and 30 years old, were selected for the investigation. After 10 hours of overnight fasting (from 2200 until 0800H), the students were given 50 g available carbohydrate portions of the test rice followed by 250 mL of water. The sugar level in their blood was tested at 0, 30, 90, and 120 minutes after the administration. The students could see their regular academic activity in a comfortable room for the next 2 hours or so. The blood glucose levels were determined by using an automatic glucose device model Easy Touch ® G.C.U., which can be purchased from the pharmacy shop. The device can also determine the cholesterol and uric acid level as well. To avoid the bias of each test food, the duplicating treatment was spaced at four days apart [21]. From the sugar level data, the incremental areas under the curve (IAUC), including the area beneath the fasting level was calculated geometrically [16]. The glycemic index (G.I.) of the tested rice was finally calculated from the graph and expressed as a percentage of the mean response area of the glucose (reference food) taken by the same subject.

III. RESULTS AND DISCUSSION

A. Physical appearance and Rice quality

Both rice samples looked very similar (Figure 1) with the outer most layer (pericarp) appeared black and inside is white. Removing the black layer by polishing is not recommended because it will lose the healthy or medicinal component.

The length and width ratios of the rice are also about the same (Table 1). The ratio of length to width for conventional and organic rice is quite the same of 2.7-3.0, which, according to the International Rice Research Institute classification, is under medium class and very much related to the local consumer tastes. The weight of 1000 organic rice seeds was slightly higher than conventional rice. The broken and head rice between the two rice is not much different

compared to their lime grains. The lime grain of conventional rice is 4.7%, whereas in organic rice is zero.



Fig 1. Conventional (on the left) and organic (on the right) black rice

TABLE I
LENGTH AND WITH A DEPTH OF BLACK RICE SAMPLES

Rice sample	Length (cm)	Width (cm)	Weight of 1000 grains (g)	Ratio length/width
Conventional	6.89 ±0.17	2.33 ±0.27	1.86±0.10	3.00±0.47
Organic	6.75 ±0.49	2.46 ±0.20	2.07±0.04	2.75±0.15

Unlike white rice, organic black rice (Jatiluwi) are sold in the supermarket in a well-documented packaging. Typical packaging for organic black rice is, as shown in Figure 2. The detailed nutritional and health parameters such as elements, vitamins, sugar, and cholesterol are given. For health-promoting marketing strategy, some statements like superfood (new cancer-fighting), antioxidants without anthocyanin data seem to be quite an exaggeration or questionable. Conventional black rice is not readily available because of the unwillingness of many farmers to grow it unless there is a demand by interested customers.



Fig 2. A well-documented Indonesian Organic black rice packaging.

However, for marketing and sale purposes, each country has developed the same standard of rice grading, which is very much physical in nature but still follow the F.A.O. Guidelines.

TABLE II
RESULT OF THE S.N.I. 6128:2015 INDONESIA GRADING TEST ON THE BLACK RICE

Rice sample	Moisture content (%)	Rice head (%)	Broken rice (%)	grain Menir (%)	Yellow/broken rice (%)	Lime grains (%)	Foreign body (%)	Grain (%)
Conventional	15.10±0.002	88.08±0.002	5.27±0.002	0.90±0.003	0.82±0.001	4.77±0.0003	0.000±0.000	0.000±0.000
Organic	14.04±0.002	95.17±0.003	4.03±0.002	1.13±0.002	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000

B. Infrared spectroscopy

The infrared spectra of both kinds of rice are similar with broad intense peak between 3200 and 3600 cm^{-1} due to the stretching of free and hydrogen-bonded O.H. and a phenolic hydroxyl group (Figure 3). The peak at 2930 cm^{-1} is due to aliphatic C-H stretching [22]. The region between 1600 and

1716 cm^{-1} can be due to C=C and C=O functional groups [23]. The symmetric C-O stretching of cellulose, hemicellulose, and lignin is most likely shown by the 1078 cm^{-1} wavenumber [24]. Other functionalities, including the peaks in the fingerprint region are presented in Table 3.

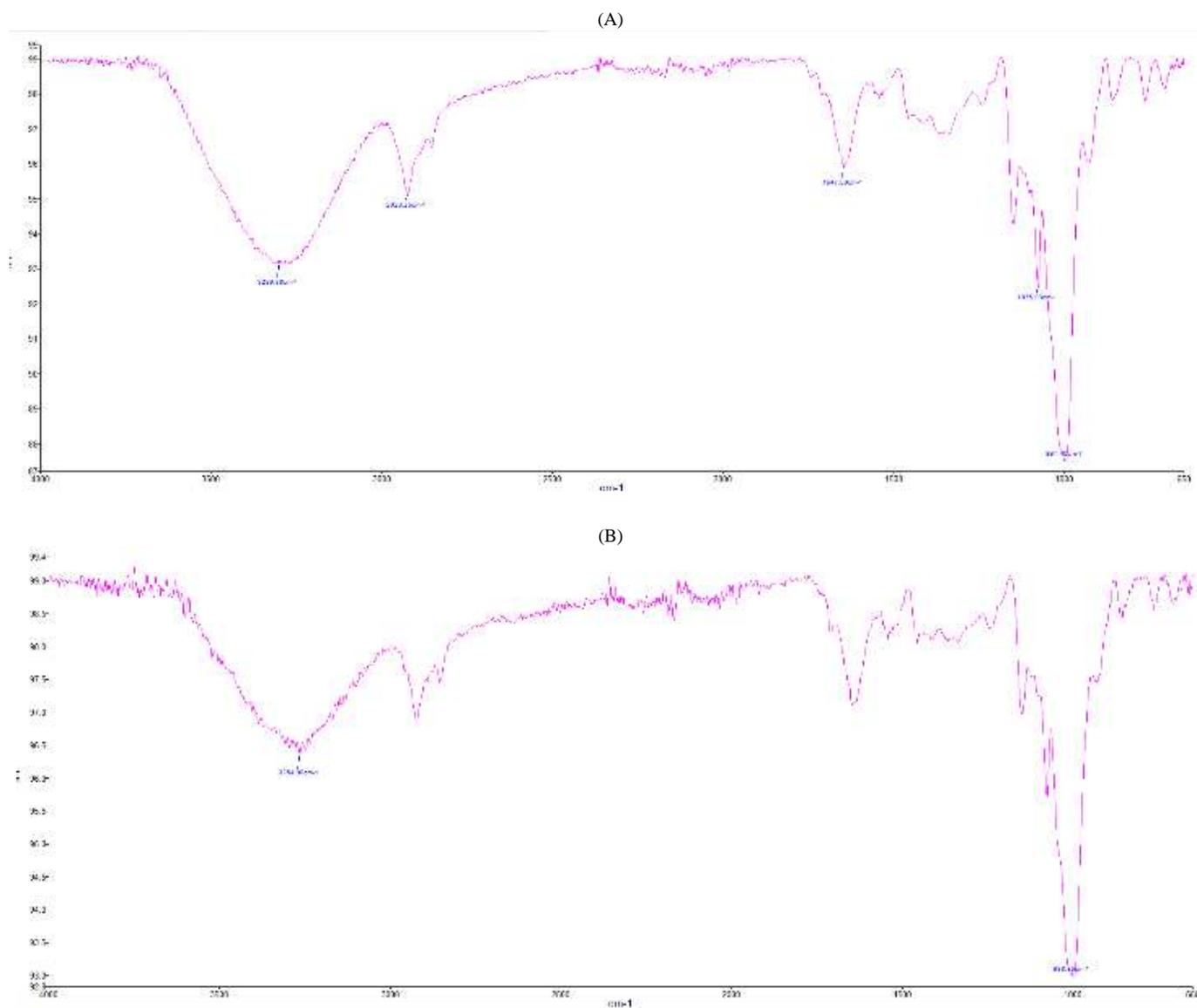


Fig 3. The infrared spectrum of conventional (A) and organic (B) black rice

TABLE III
SOME IMPORTANT INFRARED FUNCTIONALITIES FOR THE RICE (cm^{-1})

Functionalities	Organic rice/ cm^{-1}	Conventional rice/ cm^{-1}
v(O-H)	3284.9	3306.7
v(C-H) aliphatic	2930.3	2923.6
C=C or C=O	1636.6	1647.3
Ester, ether, 1 st and 2 nd C-OH	1151.3	1149.5
sp ³ carbon or sym C-O	1078.2	1076.5
	999.3	998.7
	924.7	926.7
	858.4	858.4
	764.3	762.3
	708.1	706.8

The peaks below 1000 cm^{-1} are normally due to aromatic rings. It is not easy to identify peaks due to anthocyanin from the bulk unless an extraction to isolate it is done.

C. Crystallinity

Each rice showed a similar XRD diffractogram with 2θ angles between 10 and 30°. The reflection d values and 2θ angles of the conventional black rice was close to those in the starch α -amylose dihydrate ($\text{C}_{18}\text{H}_{39}\text{O}_{15} \cdot 2\text{H}_2\text{O}$) of corn as given in the library. However, organic rice shows slightly different Bragg parameters (Table 4). The d and 2θ angles values at [100] surface of 100% intensity were very much different.

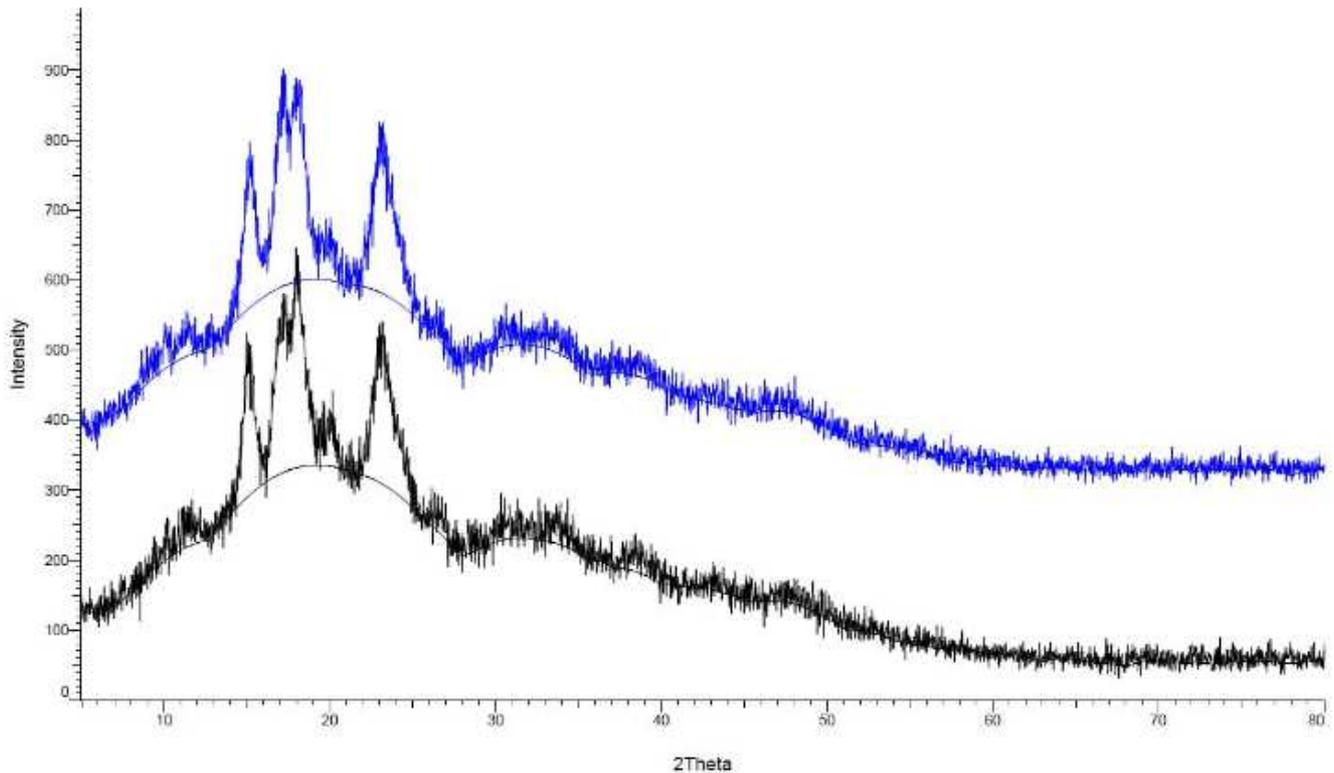


Fig 4. Diffractogram of conventional (blue color) and organic black rice (black color).

The presence of diffractions with 2θ about 15, 17, 17.8, 19, and 23° indicates that each rice maintains the typical A-type crystalline structure [25], [26]. The tendency of organic rice to show polymorphism certainly desired further investigation.

TABLE IV
XRD REFLECTION PARAMETERS OF BLACK RICE

Rice sample	Reflection/diffraction angles (d, $2\theta^\circ$, I%)
Conventional rice	1.(7.59727,11.639,18.3),2.(5.8847,15.043,62.8), 3.(5.168,17.142,79.6),4.(4.88008,18.164,100), 5.(4.39113,20.206,23),6.(3.8566,23.043,65.4), 7.(3.36024,26.505,12.7),8.(2.312, 38.93, 12.1)
Organic rice	1.(8.69649,10.163,15.6),2.(7.59727,11.693,9.6), 3.(5.84090,15.157,78.0),4.(5.219,16.972,98.6), 5.(4.89524, 18.107, 100),6. (4.4910,19.753,16.8),7.(3.84718,23.100,90.0), 8.(3.38157,26.334,1.3.6),9.(2.893, 30.874, 15.5), 10.(2.327,38.647, 8.9)
α -amylose dihydrate	1.(7.926,11.15),2.(5.9300,14.927),3.(5.1623,17.163), 4.(4.9859,17.775),5.(4.4287,20.033),6. (3.859,23.028), 7.(3.3613,26.17)

D. Elemental Determination by XRF

Although all rice show similarity in term of macronutrient composition, their percentage may vary depending on the variety, agronomic factors, and method of farming. Several elements such as Al, Ca, Cu, S, N, Fe, K, Mg, Mn, Na, P, Ti, Si, Cl, Ni, Mo, and Zn have been detected in rice [27]–[29]. The presence of toxic elements like As and Hg have also been reported [30]. Recently, ruthenium (Ru) has been detected in white rice by XRF technique [31]. Similarly, in the present study, Ru was detected in the organic black rice

but not in conventional rice. Surprisingly rubidium (Rb) was detected in both conventional and organic rice. The percentage of elements present is given in Table 5. No manganese was detected in conventional rice. The presence of Zn and Fe is always desired and good for health. However, the detection of Rb and Ru certainly created concern. The experiment was repeated twice, and the elemental analysis on the spectrum by using P.E.T., LIF200 AND XS-55 consistently showed the presence of the metals. The percentage of elements after normalization by XRF technique for each rice is given in Table 5.

TABLE V
PERCENTAGE OF ELEMENTS PRESENT IN CONVENTIONAL AND ORGANIC BLACK RICE

Element	Conventional (%)	Organic (%)
P ₂ O ₅	39.21	38.71
K ₂ O	27.19	26.30
SO ₃	14.44	14.06
MgO	10.68	10.22
SiO ₂	4.87	6.26
Cl	1.64	1.55
Al ₂ O ₃	1.15	1.53
Fe ₂ O ₃	0.34	0.35
ZnO	0.28	0.32
CuO	0.12	0.13
Rb ₂ O	0.07	0.10
Ru	-	0.22
Mn	-	0.25

In the present study the percentage of an element in the rice is decreasing in the following sequence;

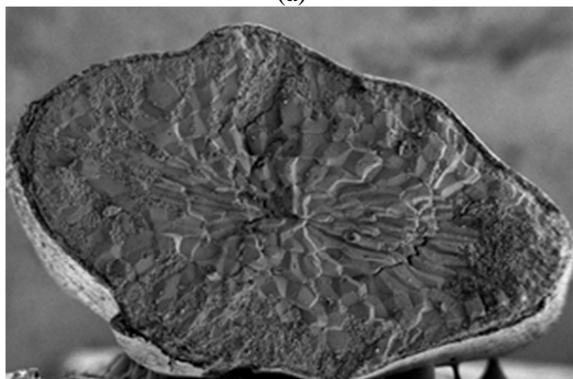
Conventional: P>K>S>Mg>Si>Cl>Al>Fe>Zn>Cu>Rb
Organic: P>K>S>Si>Mg>Cl>Al>Fe>Zn>Mn>Ru>Cu>Rb

E. Morphology of the rice grain by Scanning Electron Microscopy

The transversal cross-section micrograph of each rice shows the presence of the black pericarp at the outermost layer (Figure 5). The starch crystallites are packed in blocks connected radially toward the pericarp.



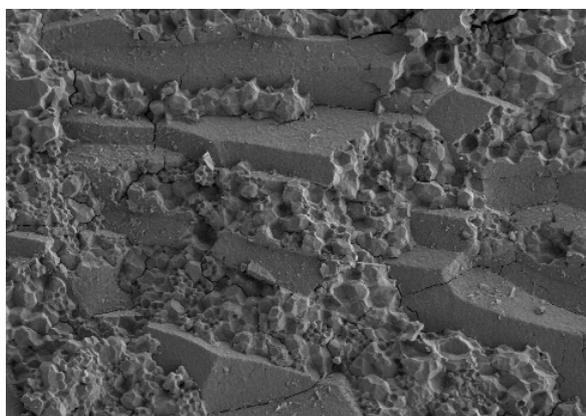
(a)



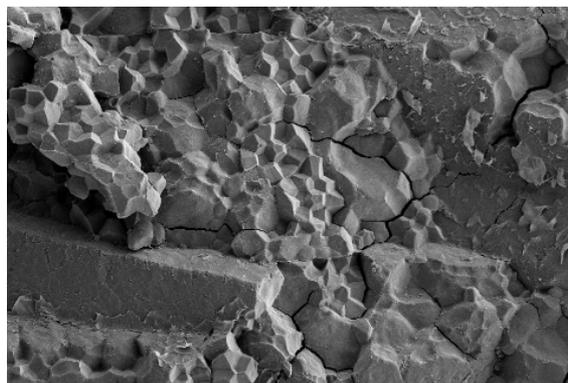
(b)

Fig 5. Transversal cross-section morphology of conventional (a) and organic (b) black rice at 45 times magnification

A closer look at the endosperm area, we can see the blocks and some aggregated crystallites that were broken from the block during the cutting (Figure 6). Most of the crystallites have polyhedral shapes, and they are connected or packed at the faces forming a cyclic or ring type of geometric formation. The length of the block on the transversal cross-section can reach $2,700\mu\text{m}$.



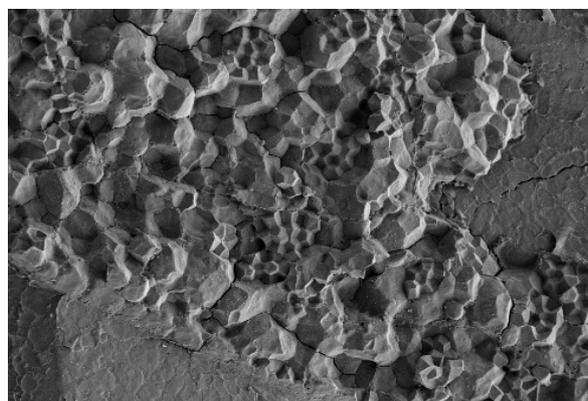
(a)



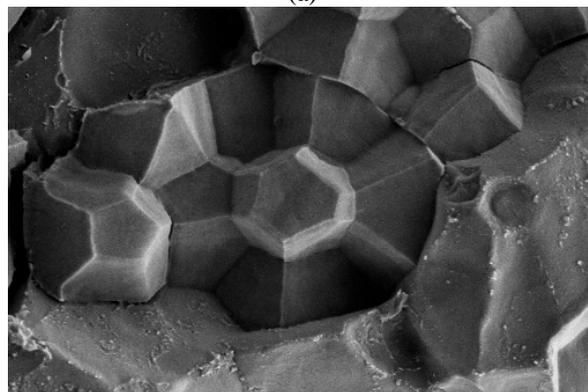
(b)

Fig 6. Transversal cross-section photomicrograph of conventional (a) and organic (b) black rice at and 1000 times magnification, respectively.

Similar crystallites and packing were also observed in organic rice (Figure 7). The length of the pentagonal edge is about $4.57\mu\text{m}$ and formed a six-coordination type of environment. The driving force that leads to the kind of packing is something interesting to be explored.



(a)

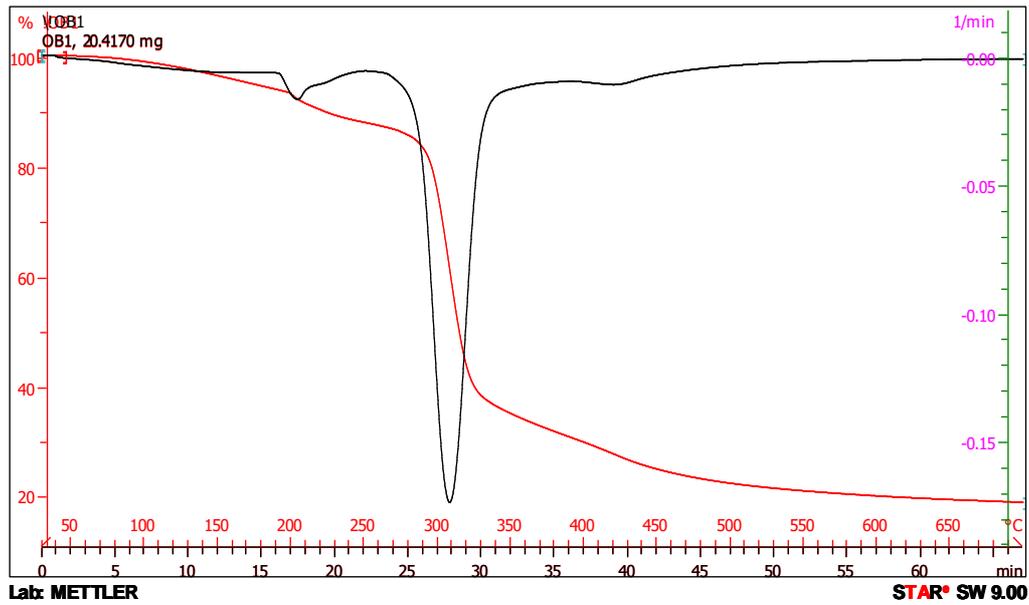


(b)

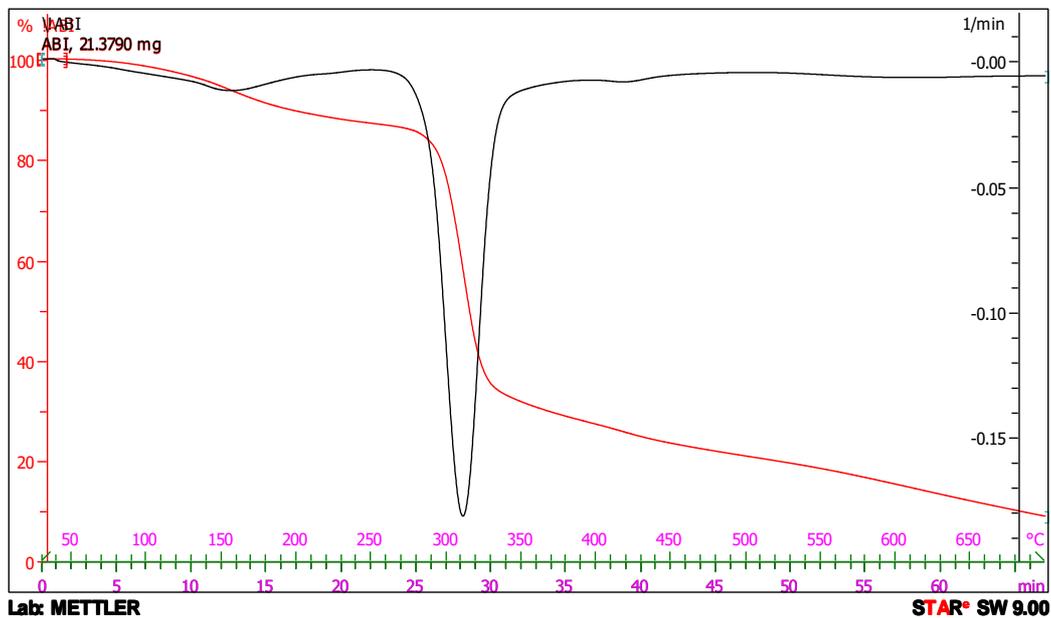
Fig 7. Transversal cross-section photomicrograph of organic black rice at 648 (a) and 5000 (b) times magnification respectively.

F. Thermogravimetric Studies

The thermograms of both kinds of rice are very similar, involving two mass loss steps (Figure 8) and, in fact, quite close to white rice [31]. The first step is a gradual loss of water from 80°C toward 250°C , together with a small loss at about 210°C , which was not observed in white rice previously reported. The second significant loss is at 310°C , leaving finally the carbon residue of about 18%.



(a)



(b)

Fig 8. Thermogram of organic (a) and conventional (b) black rice.

G. Amylose Content

The amylose content of the organic and conventional black rice was found to be 1.89 and 1.29 %, respectively, very much lesser than the generally reported for black rice of about 3% [32]. Low amylose content usually gives sticky rice after boiling or cooking. However, it depends on the amount of water used and the time of boiling to soften the pericarp layer. High amylopectin that is soluble in water will have a high degree of digestibility. Therefore, white rice has lower digestibility than black rice.

H. Glycemic index

Figure 9 shows a typical glycemic response plot for rice showing an increase in blood glucose concentration at 30 minutes and then slowly decreasing toward 120-minute time.

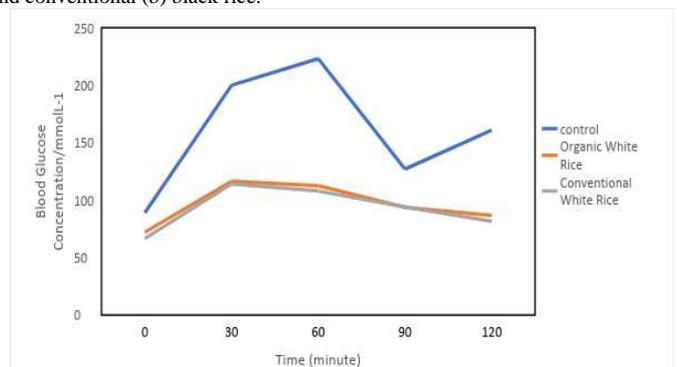


Fig 9. Mean glycemic response 2h for 50 g of boiled conventional and organic black rice.

Both conventional and organic rice possess were found to have a low glycemic index of 30.84 ± 4.90 and 32.54 ± 5.42 , respectively. These values are, in fact, lesser than those for

Thai red, Basmati, and Jasmine rice of 55.10 ± 8.6 , 50 ± 5.8 and 77 ± 7.3 , respectively [30], [31]. It seems that the low G.I. agrees with the low amylose content in the black rice. G.I. values were categorized as low (less than 55%), intermediate (55-70%), and high (more than 70%) by the FAO/WHO [37].

IV. CONCLUSION

The glycemic index of each black rice is lower than the white rice reported so far. The starch in the organic rice tends to exist as polymorph. Based on the glycemic index and the presence of zinc and iron are indicative of health quality of the rice. However, the presence of rubidium and niobium certainly raised concerns and need further investigation. Black rice varieties showed a higher amount of minerals than ordinary white rice.

ACKNOWLEDGMENT

We would like to thank the Agriculture Faculty of Warmadewa University for providing the facilities to conduct the research, Safe network for a special grant. No 321/SAFE-NETWORK /Bali-Green/2018 and the Research and Instrumentation Centre of Universiti Kebangsaan Malaysia for S.E.M., XRD and XRD services for the analysis works. Thanks to Prof Idris B.A. Ghani of Universiti Kebangsaan Malaysia for making some comments to improve the paper.

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