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Physical Properties and Glycemic Index Studies of Singaraja White Rice Grown by Conventional and Organic Methods in Denpasar Bali Indonesia

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Abstract*— Both Singaraja white rice grown by organic and conventional method showed similar quality and posses glycemic index of 46.8±4.03 and 48.2±4.65 respectively. These values are lesser than the commercial Basmati rice of 50±5.8 which is in the low GI category. The scanning electron macroscopy photomicrograph of the transversal section of both rices show the array of crystalline blocks from the central endosperm area toward the outer part. The blocks are surrounded by polyhedral crystallites of the size between 3-6 microns. The polyhedral crystalline seem to be more dominant in the conventional rice. X-ray difrractograms of the rice indicate about 24% crystallinity with amylose dihydrate exist as polymorph in the organic rice. Elemental analysis data by XRF indicate organic rice contains slightly higher Magnesium and Phosphorus. Other elements detected were K, Al, S, Cl,O, Si, Zn, Ru, Ni, Pd and Fe. However, no iron was detected in the conventional rice . On the other hand no calcium was detected in the organic rice. Thermogravimetric study showed the solid rice is stable up to about 200°C with the loss of water molecules at about 90°C.

***Keywords*— organic conventional rice; Singaraja; electron microscope; crystallite; glycemic index.**

I. INTRODUCTION

Most countries in Asia rice is included in the government list of controlled items. A standard based on physical and chemical characterization is introduced to grade the rice to determine the quality and consequently the price. However, customers choice is quite complex. In rice producing countries like Japan, Korea and Taiwan foreign rice has difficulty to get acceptance by the general public because they are used to the local rice or rice that they have enjoyed for such a long time . Government policy on importing rice also has an important role. Therefore in some supermarkets rices are sold with detailed nutritional data but quite a number of rice are packed in beautiful packaging without any detailed analysis or specification at all. Among the popular rice in the global market are the long grain Basmathi for making briyani, Chinese black rice, Jasmine rice, Paella rice, Risotto rice and sushi rice. In Asia China, India, Indonesia ,Thailand, Vietnam, Pakistan, Bangladesh and Komboja are well known major exporters of rice. Generally the quality of rice is determined by both physical and chemical characteristic. The physical characteristic may include whole and broken grains, shape and size of the grains, colour of the grain, chalkiness, weights, damaged and discolored kernels, foreign materials like dirt, stones, moisture content of the grain before cooking. The chemical characteristic may consist of how the grain look after the cooking and taste when eating, gelitinization temperature, amylase content, gel consistency, texture(how it feels when eaten), and aroma (smell). However such information is not necessarily be available in the standardization or stated in the rice packaging. Several discussions about the determinants to improve the current standardisation in many countries including Malaysia, Indonesia and Philippine have been reported [1-5]. The current interest on health aspect of rice or functional foods has attracted attention to many researchers globally from multi-disciplinary approach. Prior to that several high carbohydrate rich staple foods such as potatoes and rice have become the subject of concern due to the increase in diabetic as a major chronic desease in Asia

[6-8]. Infact high prevalence of type-2 diabetic in Japan has been associated to the traditional dieatry pattern of Japanese white rice a staple food [9]. The effect of glycemic index and glycemic load on type-2 dibeatic has been established by Millet et al [10]. New development on rice cultivation especially the genetically modified rice and also organically grown rice will require the need for more detailed study and analysis. Such a detailed analysis are still lacking even for locally popular rices. Therefore we decided to carry out the detailed physical and chemical studies on our local rice cultivated by both conventional and organic methods. In this paper, the physical and morphological properties and elemental analysis including glycemic index of our local white Singaraja rice grown by conventional and organic method in Bali were investigated.

II. Materials And Methods

A. Chemicals and instrumentation

All the chemicals such as eosin (0.5%) , methylene blue and alcohol purchased from Merck were AnalaR grade and used without further purification. Glycemic index test was carried out using Gluco blood glucose test apparatus Dr, AGM-2100 type, Medicus production Co., Ltd.

B. Rice samples

Both Singaraja organic and conventional rice can easily be obtained from the ordinary shops or supermarket. The rices were grown in Jatiluweh village,Tabanan Regency. The organic farmers used their farm and plant waste composted as fertilizer and organic materials and biological control to protect the rice from the plant-disturbing organism or insects. The conventional farmers used mainly urea as fertilizer and the commercial pesticides for the plant protection. In the supermarket both rices are sold as grade medium with the price of Rp13,000.00 per kg for conventional and almost doube the price of Rp23,000.00 for organic.

C. Physical characterization methods

All standard properties measurements were caried out I our laboratory. Rice grain sizes were measured using vernier cliper.

D. Scanning Electron Microscopy

The grain rice was cutted longitunally or transversally and mounted on a circular aluminium stub and platinum-coated in a vacuum using a sputter coater. The rice morphology was examined by using scanning electron microscope energy-dispersive X-ray spectrometry FE-SEM, ZEISS Merlin operating at 0.2-30kV beam current up to 400nA and the lowest vacuum of few pA-300nA. In the present study 15kV accelerating electron was applied.

E. Infrared spectroscopy

Infrared spectroscopy was carried out on Perkin Elmer Model Spectrum 400 FT-IR with imaging system and resolution pixel of 6.25, 25 or 50 microns. The measurements were collected over the range 4000-650 cm-1 and 50 co-added scans. All samples were grounded into powders prior to spectral acquisation. All spectra were in Transmittance units.

F. X-Ray Diffraction Analysis

X-ray diffractogram of rice samples were obtained by using X-ray diffractometer Bruker D8 Advance. The rice were grinded into a powdered form and was tightly packed in small holder. Each sample was exposed to X-Ray beams with generator running at 20 mA and 30kV. The scanning diffraction angle (2θ) ranged from 5° to 80°.0 The overall degree of crystallinity was quantified as the ratio of the area of crystaline reflections to the overall diffraction area.

G. X-Ray Flouresecnce

X-Ray Flouresence experiments were performed on Bruker S8 Tiger spectrometer running at 1kW , 50kVmax, 50mAmax, Tube (Rh, Be:75μm).

H. Thermogravimetry experiments

Nonisothermal thermogravimetry measurements were performed using a thermogravietric analyzer (Mattler-Toledo with small furnace SF). Standard platinum crucibles (140μL) were used for both the samples and reference.The sample with mass approximately 4-7 mg were scanned at constant heating rate of 10°C min-1 in room environment under the flow of 50 mL/min air in the temperature range of 25-600°C. Nitrogen gas (99.999%) was used as protective gas in the instrument.

I. Study protocol and criteria

The study protocol and ethics approval was granted by University Hospital of Warmadewa University Ethic Committee and conducted in accordance with its rules and regulations. The study was carried out by using standard glycemic index testing protocol as described by Wolever et al [11,12].. Glucose was used as the reference food with a GI score of 100, tested in the subject at base line, midway and at the end of the study. Only nondiabetic , nonsmoking, healthy, active lifestyle, without any diagnosed diseases and not on prescribed medication were selected for the study.They are asked to maintain their daily activity.

J. Rice preparation for postprandial screening

The rice was throughly washed and cooked in an electronic rice cooker (model SHARP) with 2mL water g-1 rice at cooked control temperature and time [13]. The rice portion was weighed after cooled according to the carbohydrate content taking into account for dietry fibre content.

K. Glycemic index experimental design

Nonprobability puporsive sampling technique [14] was adopted in the study. Although this technique has the limitation because of the subjective nature of choosing the samples and not a good representative of the population it is still useful when randomization is impossible like the population is so large and selection is restricted to certain conditions [15]. Six healthy nondiabetic subjects (all males) with age range of 20-30 years,were carefully selected. 50 g available carbohydrate portion of the test rice were administered to the subject on separate mornings after 10 hour overnight fast (2200 until 0800H). After taking the rice they were given 250 mL water . The subjects were then tested for blood at minute 0, 30, 60, 90, and 120, where each treatment was spaced at 4 days apart to avoid the bias of each tested food.[16]. The targets were alowed to do their daily academic activities. An automatic blood glucose/cholestrol/uric acid device model Easy Touch ®GCU was used directly to determine the blood glucose. The increamental areas under the curve (IAUC), excluding the area beneath the fasting level, was calculated geometrically [11]. Finally, the GI was calculated by expressing the glycemic response area for the rice as a percentage of the mean response area of the reference food (glucose) taken by the same subject.

III. Results and Discussion

A. *Physical appearance and Rice quality*

Under ordinary optical microscope both rice samples looked very similar and the length and width ratios are about the same (Table 1).

TABLE I
LENGTH AND WITH DEPTH OF RICE SAMPLES

Rice sample	Length (cm)	Width (cm)	Weght of 1000 grains (g)	Ratio length/width
Conventional	0.515 ±0.037	0.245 ±0.051	21.5 – 22.0	2.192 ±0.481
Organic	0.570 ±0.057	0.275 ±0.044	23.1 – 24.0	2.133 ±0.448

Both the ratio of length and width for conventional and organic rice are quite the same of 2.1 which is under medium class according to the International Rice Research Institute classification and very much related to our local consumer tastes. The weight of 1000 organic rice seeds is higher than conventional rice. This is related to broken rice and head rice, where organic rice (5%) is less than conventional rice (15%).

TABLE II
THE RESULT OF THE SNI 6128:2015 INDONESIAN GRADING TEST ON THE RICE

Rice sample	Conventinal white rice	Organic white rice
Moisture content (%)	13	13
Milling degree (%)	100	100
Rice head (%)	79	84
Broken rice (%)	15	5
grain Menir (%)	3	3
Yellow/broken rice (%)	0	0
Lime grains (%)	1,5	8
Foreign body (%)	0	0
Grain (%)	0	0

Today rice quality can include many aspects of studies not only physical and chemical characterization but also nutritional and health benefit as well. However, for marketing and sale purposes each country has developed more or less the same standard of rice grading which is very much physical in nature and still follow the FAO guidelines. There are also many studies to improve the grading system which include other determinants that are important in determining the quality of the rice [17, 18].

B. *Infrared spectra*

As expected the infrared spectra of both rices are similar with broad intense peak between 3200 and 3600 cm-1 due to the strecthing of free and hydrogen bonded OH and phenolic hydroxyl group (Figure 1). The peak at 2930cm-1 is due to aliphatic C-H stretching[19]. The region btween 1600 and 1716 cm-1 can be due to C=C and C=O functional groups[20].The symmetric C-O stretching of cellulose, hemicelulose and lignin is most likely shown by the 1078cm-1 wave number [21]. Other functionalities icluding the peaks in the finger print region are shown in Table 3.

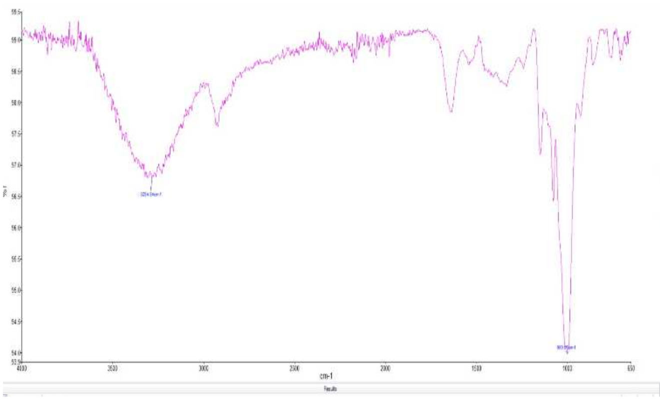


Fig.1. Infrared spectrum of Singaraja organic white rice

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

Functionalties/cm^{-1}	Organic rice	Conventional rice
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,1and 2nd 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 due to aromatic rings. It is known that the fingerprint region (878, 979,989. 1100, 1210 cm-1) has displayed a reliable peaks for chemometric study on rice samples [22]. A better resolution spectrum was obtained by isolating the starch or acetylated the starch where all the carbonyl and other functionalies are well resolved[23]. The combination of physicochemical properties with infrared spectra of rice enable an assesment of rice grain quality be easily carried out[24].

C. *Crystallinity*

The XRD diffractogram of both white rices showed the same crystallinity behaviour (Figure 2) of about 24%. However the Bragg parameters are slightly difference (Table 4) which may be due to polymorphism of the starch crystal.

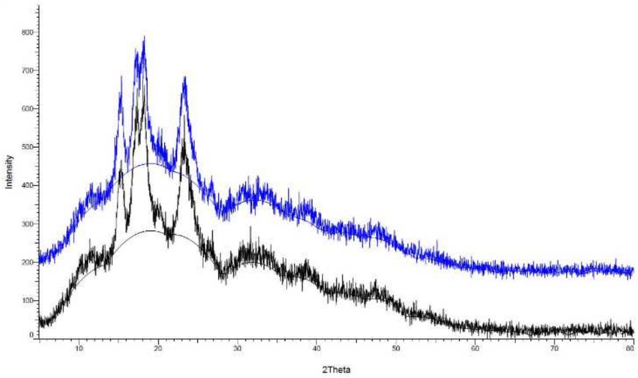


Fig.2 Diffractogram of conventional(blue colour) and organic white rice (black colour).

The reflection d values and 2θ angles of the conventional white rice is close to those in the starch α-amylose dihydrate ($C_{18}H_{39}O_{15}.2H_2O$) extracted from the corn as given in the library. The d and 2θ angle at (100) with 100% intensity and other reflections are significantly different. However, both have diffractions with 2θ about 15, 17, 17.8, 19 and 23° indicating a typical of A-type crystalline structure [25].

TABLE IV
XRD REFLECTION PARAMETERS OF RICE

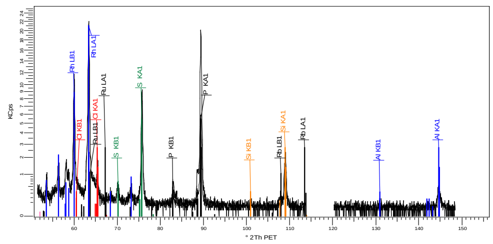
Rice sample	Reflection/diffraction angles(d, 2θ°,I%)
Conventional rice	1.(7.8254,11.298,15.9), 2.(5.819,15.213,75.1), 3.(5.152,17.199, 100), 4.(4..865,18.221,91.2), 5.(3.828,23.214,85.2), 6.(3.6,39,26.75,8.0), 7. (2.728, 32.803, 8.8), 8.(2.312., 38.93, 12.1)
Organic rice	1.(8.648,10.22,13.2), 2.(7.709,11.46,9.7), 3.(5.797,15.270, 56.3), 4.(5.1684,17.142,90.3), 5.(4.865, 18.221, 100), 6. (4.453,19.923, 20.9), 7.(3.828,23.214,61.7), 8.(3.381,26.334,1.3), 9.(3.382, 29.12, 1.3), 10.(2.652,33.77, 8.2).
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)

* relevent reflections selected from the library

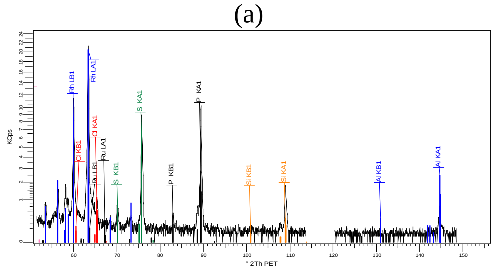
The difference in the Bragg parameters of the rices indicate that the amylose in the organic rice exist in polymorphic form. Similar A-type crystallinity was also observed by Wani et al [26] and Yu, et al [27]. Detailed study on the factors that caused the polymorphism other than the basic rate of evaporation of the milk will be quite interesting.

D. Elemental Determination by XRF

The distribution pattern of micronutrients or metals in the whole grain is quite important because a considerable amount of it will be removed during the milling process before consumption. On the other hand the knowledge of mineral delocalization within the grain is also important for understanding of their role in the seed development. The detection of metals or elements in the rice grain has also been investigated from nutritional and safety point of views. A total of about 26 essential 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 by several techniques[28] including X-ray flouresence and ICP. The presence of toxic elements and pesticide residues [29] is an other important and serious aspect of health. Atomic Absorption Spectrometry, inductively coupled plasma atomic emission spectrometry (ICP-OES), energy dispersive X-ray spectrometry and X-ray fluorescence techniques (XRF) are commonly used for the detection of the elements[30]. The use of XRF and EDXRF are quick, direct and does not require digestion or extraction of sample. Metallic elements with atomic weight higher than sodium such as Al, Ca,Cu,S, Fe, K, Mg, Mn,, P, Ti, Si ,Cl, Ni, Mo and Zn were observed in rice by EDXRD [31]. However, in the present study 11 elements were observed in the conventional rice whereas in organic rice 12 elements were detected (Figure 3) by XRF technique.



(a)



(b)

Fig. 3. Flourescence spectra of white convention rice (a) and organic rice (b)

The presence of ruthenium in both rice and in particular palladium in the organic rice is unusual. The experiment was repeated twice and the elemental analysis on the spectrum by using PET, LIF200 AND XS-55 consistantly showed the presence of the metals. The percentage of elements after normalization by XRF technique for both rice is given in Table 5.

The percentage of Mg, Cl and P, are significantly higher in the organic rice compared to conventional rice. The chloride content in organic rice is less by more than half of the conventional. No nickel was observed in the organic rice. On the other hand no iron was detected in conventional rice. The presence of palladium in organic rice is quite suprising and deserve further investigation. It is quite common that Cr,

Cu, Mn, Se, Br, I and As present in rice husk [32]. However, it seem that none of these elements were detected the rice. Even if they were present in the Sigaraja rice none of them have migrated into the rice grain.

TABLE V
PERCENTAGE OF ELEMENTS PRESENCE IN THE RICE SAMPLES

Element	Conventional rice (%)	Organic rice (%)
K	13.69	13.33
Mg	4.82	7.09
P	11.96	14.45
Al	2.11	1.46
S	11.94	10.05
Cl	5.00	2.04
O	45.75	46.82
Si	3.89	3.72
Zn	0.37	0.29
Ru	0.30	0.22
Ni	0.17	-
Pd	-	0.28
Fe	-	0.30

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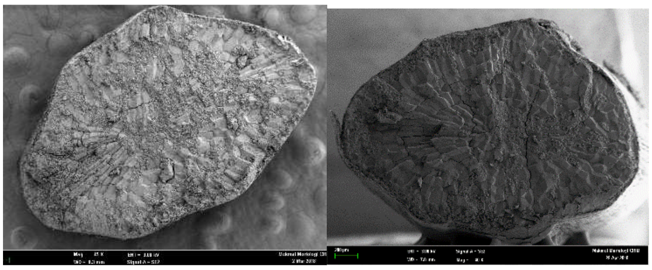
E. Morphology by Scanning Electron Microscopy

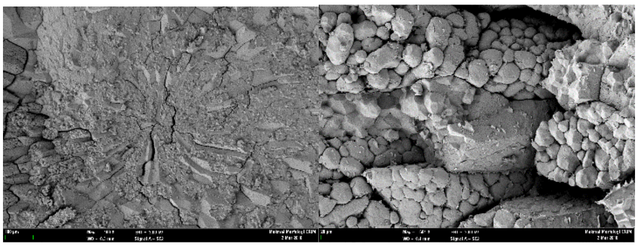
Unlike black rice no pericap covering the outer part of the white rice. Figure 4 shows the transversal cross section photomicrograph of both rices. The surface of the cross section is not smooth but consists of rectangular blocks in the endosperm area spreading toward the outer part. It is also possible that crystallization started from the outer part towards the center of the andosperm even by a small temperature gradient or variation. At the center the crystallites are aggregated but not enough force to pack them together into a block form. Andosperm is an important area where starch, lipids, protein and fibre are located. The nutritional value of the rice will also depend on the composition and structure of it. The starch crystals in sweet potatoes are well defined and distributed through out the bulk potato because in 77% water content crystallization is much more at ease in soft environment in the tuber below the ground. On the other hand crystallization of starch in rice involved solidification of milky white latex in the silica cage (husk) that is exposed to hot open air environment.



(a) (b)

Fig. 4. Transversal cross section photomicrograph of organic (a) and conventional white rice (b) at 45Xand 40 X magnification respectively.

A closed look at the endosperm area the blocks are arranged like a terrace houses with crystalline solids in between. There are irregular crystalline solids that are packed in the blocks (Figure 5)



(a) (b)

Fig. 5. Endosperm area of organic white rice at 500X(a) and 1000X (b) times magnifcation respectively. The blocks are well observed at 1000X magnification.

The crystalline solids beween the blocks have polyhedral shape (Figure 6)

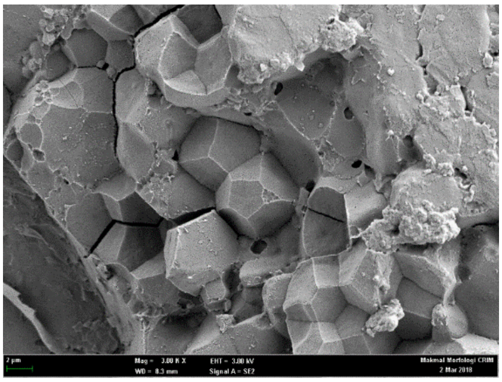
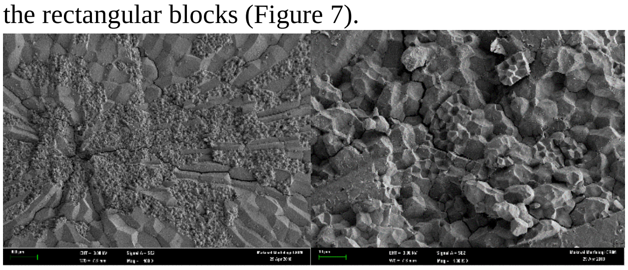


Fig. 6. Polyhedral crystalline solids between the rectangular blocks at 3000X magnification.

In conventional rice the polyhedral crystalline solids seem quite dominantly spreading from the central part along with the rectangular blocks (Figure 7).



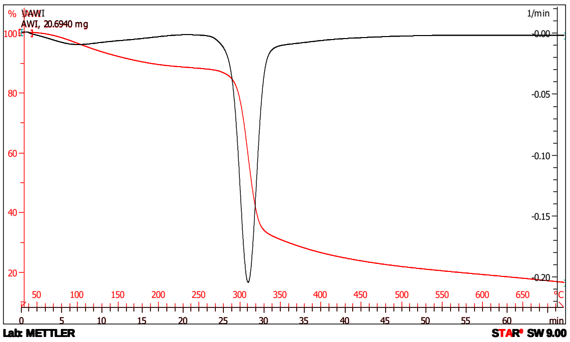
(a) (b)

Fig. 7. Endosperm section at 3K magnification (a) and polyhedral crystals at 45K magnification for conventional rice

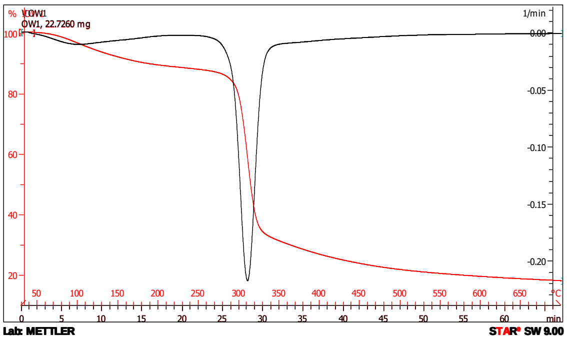
It seem that the packing of cyrstallites in the block of conventional rice is much stronger than in the organic and the dominancy of polyhedral crystals are more obvious. In both rice the crystallites are dominating at the central part of the endosperm. Slightly different morphological pattern was observed in cracked and chalkiness grain in japonica rice [33]. The variation of granule morphology is said due to biological origin and physiology of rice [34]. The environmental factor during transformation of milky latex into solid rice in the silica cage certainly also has a role on the crystallization of starch as well.

F. Thermogravimeric studies

The thermograms of both rice are very similar involving two mass loss steps (Figure 8)



(a)



(b)

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

The first step is the removal of water molecules at about 90°C followed by a major loss at 210°C and leaving finally a carbon residue of about 18%. Therefore boiling the rice at temperature below 150°C will not cause any loss or thermaldegradation of the important nutrients but may change the physical nature of the rice due to the removal of starch and gelatinisation.

G. Glycemic index

The mean glycemic response plot is quite typical (Figure 9) showing an increase in blood glucose concentration at 30 minutes time and then slowly decreasing toward 120 minute time.

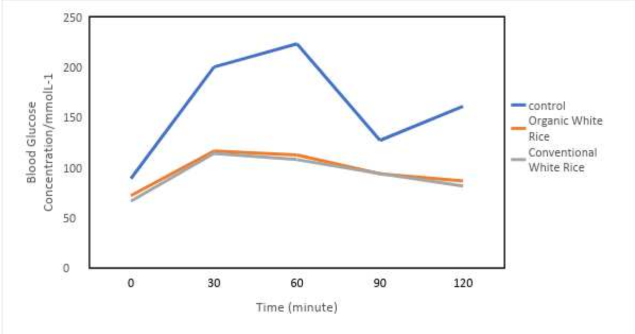


Fig. 9. Mean glycemic response 2h for 50 g of boiled Singaraja conventional and organic rice .

Both conventional and organic rices posses low glycemic index of 48.2±4.60 and 46.8±4.03 respectively. These values are infact lesser than those for Thai red, Basmati and Jasmine rice of 55.± 8.6, 50±5.8 and 77±7.3 respetively [35, 36]. GI values were categorized as low (less than 55%), intermediate (55-70%) and high (more than 70%) by the FAO/WHO [37].

IV. CONCLUSION

The physical properties of the rice have been studied and the length-width ratio of about 2 for both rice placed them as medium class that match well with the local taste. The starch in the organic rice exist as polymorph. The glycemic index of both conventional and organic Singaraja rices are classified as low (<55%) and safe for daily consumption

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