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REGULAR ARTICLE

Ferulic acid, gamma oryzanol and GABA content in whole grain rice and their variation with bran colour

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ABSTRACT

The health advantages of whole grain rice are largely associated with the bioactive food components namely, ferulic acid (FA), gamma oryzanol (GO) and gamma aminobutyric acid (GABA) in the germ and bran of grain kernel. This study was aimed to determine the content of these health-promoting compounds among 53 local rice cultivars and their relation to bran colours. There was remarkable amount of health-promoting compounds in the rice collection, especially "Segerit", "Merah" from Rumah Ulat and "Keladi" from Menitam. FA was the highest with an average of 1034.0 μg/g followed by GO 757.90 μg/g and GABA 191.16 μg/g. Rice with pigmented bran stored more FA and GO than their non-pigmented counterparts. An analysis of variance and Pearson's student correlation showed that FA responded significantly with rice bran colour (r = 0.75) but no significant relations was found for GO and GABA. This result indicated that bran colour could be a quick indicator of FA content in whole grain rice. Whole grain rice rich in bioactive compounds is a good material for the development of nutraceutical products and functional food.

Keywords: Bran colour; Ferulic acid; Gamma oryzanol; Gamma aminobutyric acid (GABA); Whole grain rice

INTRODUCTION

Rice (*Oryza sativa L.*) is consumed as a staple carbohydrate and energy source by over one-half of the world's population with more than 90% of production in Asia. The consumption of whole grain rice may reduce the risk of many chronic diseases than pearled rice (Mira et al., 2008; Liu, 2007). This is mainly owed to the presence of beneficial bioactive compounds and micronutrients in their husk and bran layer (Butsat and Siriamornpun, 2010; Liu, 2007).

Whole grain rice with different colour pigments has been reported to associate with antioxidant properties. This phytochemical-rich pigments layer provides a protective effect on cell constituents against oxidative damage. Such antioxidant properties may help in preventing cancer, cardiovascular and nerve diseases (Liu, 2007; Kehrer, 1993). Moreover, coloured rice has also been used as a natural food colouring agent and aromatic agent due to its unique fragrance (Bradbury et al., 2005). The pigmented rice has gained popularity and value as functional food in local markets by offering many nutrients and health benefits through diets.

The health promoting phytochemicals of whole grain rice comprise of phenolic compounds, sterols, tocopherols, tocotrienols and amino acids. They have noteworthy antioxidant characteristics and pharmaceutical functions including the potential for preventing cancer, cardiovascular and nerve diseases, as well as functional food applications (Saikia et al., 2012; Nam et al., 2006). Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a phenolic compound found covalently bound to the plant cell wall polysaccharides and localized mainly in bran fraction (Fry, 1982; Fulcher et al., 1972). Ferulic acid is present predominantly in grains in free form, soluble and insoluble conjugate form (Sosulski et al., 1982; Bunzel et al., 2001; Harukaze et al., 1998). Gamma oryzanol is primarily composed of trans-ferulic acid esters of lipophilic phytosterols (triterpene alcohols and plant sterols) that is found in rice bran oil

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and exhibits antioxidative activity and cholesterol-lowering effect (Miller and Engel, 2006). Gamma aminobutyric acid is a non-protein amino acid which is produced mainly by the decarboxylation of L-glutamic acid in the embryo of a seed and the production of GABA can be catalysed by the glutamate decarboxylase enzyme during the grain germination process (Bown et al., 1999). These three compounds are known as the major bioactive chemical compounds in whole grain rice.

Sarawak is endowed with ample rice varieties, including many specialty rice cultivars (Wong et al., 2009; Teo, 2010; Lee et al., 2011). These rice varieties may contain a significant amount of natural phytochemicals in their pigmented bran layers (Chee et al., 2009; Sing et al., 2011). In this study, a quantitative analysis of ferulic acid, gamma oryzanol and gamma aminobutyric acid and their relation to bran colour was investigated for fifty three rice cultivars collected from northern area of Sarawak, Malaysia. This relationship profile could provide a rapid estimation of total bioactive chemical compounds in rice cultivars and would be useful for cultivars selection in rice improvement programs.

MATERIALS AND METHODS

Rice samples collection

A total of fifty three rice cultivars (Table 1) were collected from northern divisions (Fig. 1) of Sarawak, Malaysia. The paddy samples were oven dried at 35 °C for 2 days, dehusked manually with mortar and pestle and ground with a laboratory grinder. The powdery rice samples were sieved through a 355 µm sieve and then stored in an air-tight container at 4 °C.

Classification of bran colours

The bran colour of whole grain rice was determined and grouped into seven colour groups according to the rice standard evaluation system (IRRI, 2002). The white colour is coded as 1, light brown is coded as 2, speckled brown is coded as 3, brown is coded as 4, red is coded as 5, variable purple is coded as 6 and purple colour is coded as 7 for further statistical analysis.

Determination of ferulic acid (FA)

FA content of northern Sarawak rice cultivars were analysed using UV-Vis method according to the method developed by Jankovska et al. (2001) with minor modification. One gram of rice flour was weighed into a conical flask and added with 100 mL of 0.2 mol/L HCl. The flask was kept for 30 minutes at room temperature and then heated in 85 °C water bath for 60 minutes. The warm suspension was filtered to remove the sediment and 0.2 mol/L NaOH was added into the extracts until the pH reached to the value of

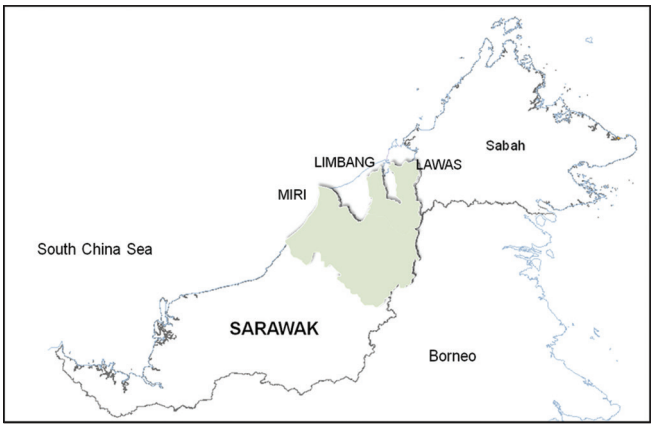

Fig 1. Sampling areas - Miri, Limbang and Lawas divisions in northern Sarawak.

12.5. The hydrolyzed extracts were kept for 60 minutes at room temperature to facilitate the saponification reaction. The reaction was ended by adding 0.2 mol/L HCl drop by drop until the pH value reached 10. FA was measured in UV-visible spectrometer (Scinco Co. LTD) at the wavelength of 345 nm. FA calibration curve was constructed with 2x serial dilution method of pure FA standard hydrolized with 0.2 mol/L HCl and 0.2 mol/L NaOH.

Determination of gamma oryzanol (GO)

GO content in rice samples was analysed using a simple spectrometry method as described by Lilitchan et al. (2008). One gram of rice flour was weighed into two centrifuge tubes respectively. Rice samples were extracted with 4 and 8 mL of hexane (95%) and well mixed using vortex mixer (Velp Scientifica) for 1 minute. The organic solvent layer was separated by 2,500 rpm centrifugation (Kubota 3110) for 10 minutes. The absorbance spectra of the supernatants were measured using UV-Vis spectrometer at 314 nm. Standard calibration curve was plotted with 2x serial dilution method of pure GO standard in hexane (95%). Total GO content per gram of rice (µg/g) was calculated by solving following equation where x_1 indicates the amount (µg) of GO in the 4 mL extract and x_2 indicates the amount (µg) of GO in the 8 mL extracts respectively (Lilitchan et al., 2008).

$$y = \frac{x_1 x_2}{2x_1 - x_2}$$

Determination of gamma aminobutyric acid (GABA)

GABA was analysed using High Performance Liquid Chromatography (HPLC) according to Varanyanond et al. (2005) with minor modification. One-half gram of rice flour was weighed into a centrifuge tube and added with 2mL of deionized water. The sample solution was mixed with vortex mixer (Velp Scientifica) and separated by 4,500 rpm centrifugation (Allegra™ X-22R, Beckman Coulter) for 10 minutes. GABA was derivatized through the

Table 1: FA, GO and GABA content (µg/g) among the northern Sarawak rice cultivars

Cultivars/colour	Location	District	FA (µg/g)	GO (µg/g)	GABA (µg/g)
Code 6: Variable purple					
Hitam	Batu Niah	Miri	1254.0^{f}	776.55^{hijklmno}	257.60^{gh}
Hitam Keladi	Batu Niah	Miri	1354.0^{e}	1222.48^{a}	56.0^{o}
Keladi	Kpg. Menitam	Limbang	2034.0^{a}	1132.32^{ab}	336.0^{d}
Keladi	Rh. Sabang	Limbang	1004.0^{ijklmn}	583.74^{opqrstuv}	81.20^{klmno}
Selasih Hitam	Rh. Tinggang	Miri	1420.0^{d}	509.02^{rstuv}	LLOQ
Telasih/Hitam	Rh. Ekom, Sepangah	Limbang	1651.0^{b}	961.48^{bcdefgh}	112.0^{ijkl}
Code 5: Red					
Bario Merah	Batu Niah	Miri	1236.0^{f}	411.02^{v}	78.40^{lmno}
Merah	Kpg. Merasam Ulu	Limbang	1516.0^{c}	846.39^{efghijk}	67.20^{no}
Merah	Rh. Ulat, Batu Niah	Miri	1429.0^{d}	1007.28^{bcdef}	442.40^{c}
Pusu Merah	Lpg. Merasam Ulu	Limbang	1057.0^{h}	712.48^{jklmnopq}	72.80^{mno}
Rengut Merah	Batu Niah	Miri	988.0^{jklmno}	1010.54^{bcdef}	LLOQ
Code 2: Light brown					
3A	Paya Mengeliang	Limbang	823.0^{wx}	881.79^{defghij}	LLOQ
Adan	Rh. Jubang, Tanah Merah	Limbang	973.0^{mnop}	683.09^{klmnopqr}	89.60^{jklmn}
Adan	Paya Mengeliang	Limbang	874.0^{tuv}	853.20^{efghijk}	LLOQ
Adan Kelabit	Bario		983.0^{klmno}	690.06^{jklmnopqr}	207.20^{h}
Bandul	Rh. Sabang	Limbang	791.0^{xy}	576.13^{pqrstuv}	81.20^{jklmn}
Bario	Kpg. Menitam		967.0^{nopq}	791.92^{ghijklmn}	LLOQ
Bario Panjang	Rh. Jubang, Tanah Merah	Limbang	1147.0^{g}	525.80^{qrstuv}	89.60^{jklmn}
Bario Selepin	Batu Niah	Miri	873.0^{tuv}	680.88^{klmnopqr}	LLOQ
Bario (A)	Rh. Tinggang	Miri	1026.0^{hijklm}	830.21^{fghijkl}	117.60^{ijk}
Beras Sederhana	Batu Niah	Miri	1050.0^{hi}	642.06 ^{lmnopqrst}	89.60^{jklmn}
Biris	Batu Niah	Miri	873.0^{tuv}	645.89^{lmnopqrst}	100.80^{ijklm}
Biris	Paya Mengeliang	Limbang	980.0^{lmnop}	731.68^{ijklmnop}	100.80^{ijklm}
Biris	Kpg.Lubok Lasas	Limbang	838.0^{vw}	620.09^{mnopqrstu}	112.0^{ij}
Dari	Kpg. Belunad	Lawas	1032.0^{hijkl}	708.98^{jklmnopq}	252.0^{efg}
Ensuluai	Rh. Tinggang	Miri	1033.0^{hijk}	802.50^{ghijklmn}	LLOQ
Code 2: Light brown					
Gupung	Rh. Ekom, Sepangah	Limbang	1060.0^{h}	1078.51^{abc}	128.80^{i}
Jepun	Batu Niah	Miri	907.0^{rstu}	584.74^{opqrstuv}	LLOQ
Kubok	Rh. Ekom, Sepangah	Limbang	897.0^{stu}	745.89^{ijklmnop}	78.40^{lmno}
Mayang	Rh. Sabang	Limbang	1002.0^{ijklmn}	977.44^{bcdefg}	78.40^{lmno}
Meet	Rh. Sabang	Limbang	790.0^{xy}	689.55^{jklmnopqr}	78.40^{lmno}
Minyak	Paya Mengeliang	Limbang	714.0^{z}	646.75^{lmnopqrst}	67.20^{no}
Pandan	Kpg. Menitam	Limbang	862.0^{uvw}	710.18^{jklmnopq}	285.60^{e}
Pandan	Batu Niah	Miri	979.0^{mnop}	674.23	LLOQ
Pasir	Kpg. Menitam	Limbang	930.0^{pqrs}	479.19^{tuv}	308.0^{d}
Pulut Emas	Kpg. Merasam Ulu	Limbang	962.0^{nopq}	924.19^{cdefghi}	84.0^{klmno}
Pusu	Kpg. Lubok Lasas	Limbang	930.0^{pqrs}	1042.80^{bcd}	LLOQ
Pusu	Rh. Sabang	Limbang	937.0^{opqrs}	709.92^{jklmnopq}	100.80^{ijklm}
Rengut Putih	Batu Niah	Miri	907.0^{rstu}	770.76^{hijklmnop}	70.0^{mno}
Roti	Batu Niah	Miri	988.0^{jklmno}	603.85^{nopqrstu}	LLOQ
Roti	Kpg. Belunad	Lawas	907.0^{rstu}	806.56^{ghijklm}	LLOQ
Salleh	Batu Niah	Miri	989.0^{jklmn}	801.38^{ghijklmn}	229.60^{gh}
Sarau	Batu Niah	Miri	921.0^{qrst}	436.20^{uv}	LLOQ
Segerit	Batu Niah	Miri	1036.0^{hijk}	804.19^{ghijklm}	800.80^{b}
Seluai	Batu Niah	Miri	1051.0^{hi}	742.62^{ijklmnop}	LLOQ
Semanyok Merah	Batu Niah	Miri	771.0^{y}	645.27^{lmnopqrst}	95.20^{jklmn}
Sentra	Paya Mengeliang	Limbang	956.0^{nopqr}	766.45^{ijklmnop}	92.40^{jklmn}
Seratus	Kpg. Menitam	Limbang	990.0^{jklmn}	1219.24^{a}	1176.0^{a}
Siam	Batu Niah	Miri	997.0^{jklmn}	483.61^{stuv}	100.80^{ijklm}
Thomas	Kpg. Menitam	Limbang	922.0^{qrst}	777.38^{hijklmno}	291.20^{ef}
Tiga A	Batu Niah	Miri	1040.0^{hij}	1030.78^{bcde}	263.20^{fgh}
Tit	Batu Niah	Miri	1140.0^{g}	449.94^{uv}	LLOQ
Tit	Kpg. Belunad	Lawas	1023.0^{hijklm}	729.39^{ijklmnop}	LLOQ

Means (n = 5) with different letters within the same column are significantly different (DNMRT, P<0.05). The values lower than detection limits were indicated as LLOQ

reaction of adding 1mL of supernatant with 200 µL 0.4 M $NAHCO_3$ and 200 µL of 6 mM DABSYL-Cl acetonitrile solution. Then the sample was heated in 70° C water bath for 20 minutes to complete the derivatization reaction. The warm suspension was filtered through 0.45 µm nylon filter membrane before injecting into HPLC system. GABA content was quantified using HPLC Isocratic System (Waters). Ten microliters of filtrate was injected into a reversed phase C18 column and determined with UV detector at 436 nm. The column temperature was maintained at 30 °C. The composition of the optimized mobile phase A was 25 mM potassium dihydrogen phosphate (35%) at pH 6.8, mobile phase B was acetonitrile (45%) and mobile phase C was methanol (20%). The flow rate was set at 0.5 mL/min. The GABA content was calculated with an external standard calibration curve which was plotted by 2x serial dilution of GABA standard in DABSYL-Cl acetonitrile solution.

Statistical analysis

The data obtained from the analysis were then analysed using Statistical Analysis Software (SAS) version 9.2. Analysis of variance (ANOVA) was used to analyse the variations of FA, GO and GABA content among the rice cultivars and bran colours. Duncan's New Multiple Range Test (DNMRT) was used for mean comparisons. Pearson's student correlation was performed to test on the relations between bran colour and bioactive chemical compounds.

RESULTS AND DISCUSSION

Rice bran colour

Majority of the rice cultivars showed rice bran in light brown colour (79.24 %) followed by variable purple colour (11.32 %) and red colour (9.43 %). These showed that dark coloured rice is less commonly cropped by local farmers in northern Sarawak. The dark coloured rice could be less appealing in appearance than light coloured rice for the consumers in northern Sarawak region. The distribution of the rice cultivars with different rice bran colour were shown in Fig. 2.

Ferulic acid content

FA rarely occurs in free form in plants. It is usually found as ester covalently conjugated with cell wall polysaccharide, proteins, lignin and other insoluble carbohydrate biopolymers (Barnerousse et al., 2008). FA was extracted in this study by chemical hydrolysis which breaks the ester linkages with polysaccharides and lignin and followed by saponification with diluted sodium hydroxide solutions. The amount of FA was measured as sodium ferulate under UV-Vis light (Mathew and Abraham, 2004).

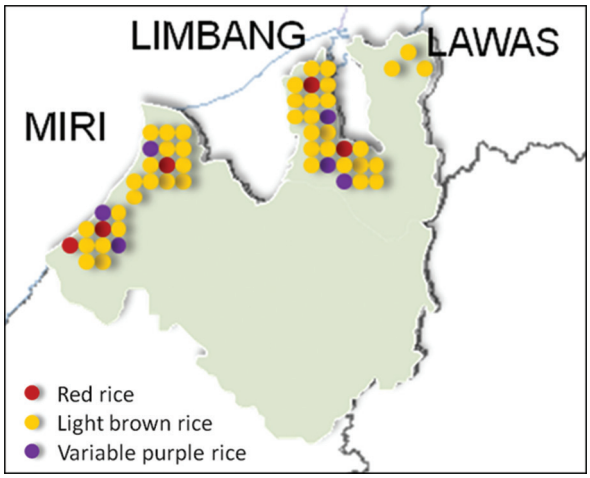

Fig 2. The distribution of rice cultivars collected according to divisions.

Table 2: The mean of the FA, GO and GABA content grouped according to sampling districts

Districts	N	FA (µg/g)		GO (µg/g)		GABA (µg/g)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Lawas	3	13.42a	23.25	680.77a	67.35	8.33a	14.43
Miri	25	34.25a	171.26	735.32a	202.86	28.80a	34.30
Limbang	25	58.71a	293.53	789.73a	191.39	42.28a	35.04

Means with different letters within the same column are significantly different (DNMRT, $P<0.05$). S.D. indicates standard deviation of the respective population

The FA content in fifty three northern Sarawak rice cultivars was significantly different between cultivar and ranged from 714.0 to 2034.0 µg/g. The FA content in the rice collection is relatively high compared to Japanese brown rice (0.032 to 1.96 µg/g), Chinese brown rice (350.0 to 400.0 µg/g) and Australian brown rice (255 to 362 µg/g) (Tian et al., 2004; Zhou et al., 2003). The rice cultivars showed the mean of 1034.2 µg FA/g samples, with exceptionally high FA content detected in cultivar Keladi from Kpg. Menitam, Limbang (2034.0 µg FA/g). The FA content in these rice cultivars was not significantly different between cultivation locations (Table 2) but significantly different between bran colour (Table 3). FA content was found to be higher in whole grain rice with rice bran in variable purple colour, followed by red colour and light brown colour. This trend often reflected in antioxidant activity of whole grain rice, where darker pigmented rice possessed higher amount of phenolics and antioxidative activities (Shen et al., 2009; Goffman and Bergman, 2002; Nam et al., 2006). These suggest that FA is the major phytochemical responsible for the antioxidant activity of dark coloured rice.

Gamma oryzanol content

GO contains an alcohol group (-OH) in its ferulate portion, which makes it relatively high polarity and likely to be soluble in both polar and non polar solvents (Xu and Godber, 2000). The amount of GO in the rice collection

Table 3: The mean of the FA, GO and GABA content grouped according to rice bran colour

Bran colour	N	FA (µg/g)		GO (µg/g)		GABA (µg/g)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Light brown	42	949.31a	94.27	737.99a	168.84	30.12a	35.45
Red	5	1245.20b	228.46	797.54a	249.19	66.20a	13.05
Variable purple	6	1452.83c	354.64	864.27a	290.60	34.33a	29.86

Means with different letters within the same column are significantly different (DNMRT, $P<0.05$). S.D. indicates standard deviation of the respective population

was significantly different between cultivar and ranged from 411.02 to 1222.48 µg/g, with the mean of 757.9 µg/g. GO content in present work was within a narrow range while compared to southern Sarawak rice cultivars (370 to 1290 µg/g) as reported by Chee et al. (2009). The highest content of GO was recorded in Hitam Keladi from Batu Niah, Miri with 1222.48 µg GO/g rice. The GO content in these rice cultivars was not significantly different between cultivation locations (Table 2) and bran colour (Table 3).

Gamma aminobutyric acid content

GABA content in the rice collection was significantly different between rice cultivars with the range from non-detectable level to 1176.0 µg GABA/g rice. Majority of the rice cultivars showed GABA content below 500 µg/g while 30 % of the rice cultivars showed non-detectable level of GABA. The GABA content in these rice cultivars was not significantly different between cultivation locations (Table 2) and bran colour (Table 3). Most of the cultivars in present study showed relatively higher GABA content than Thailand brown rice (30.80 to 58.80 µg/g) (Karladee and Suriyong, 2012). Exceptionally high GABA content was recorded in Segerit (800.80 µg/g) and Seratus (1176.0 µg/g), which could be associated with their high amylose content. Varanyanond revealed that GABA content varies with amylose content due to the embryo size of the rice grains. High amylose rice provides larger germ portion and higher GABA content which accumulates only in the germ of a grain (Varanyanond et al., 2005).

Phytochemicals profile and bran colour

Although 70 % of the rice collection showed low level of FA, GO and GABA, the remaining rice cultivars showed good profiles of bioactive chemical compounds, either rich in FA, GO, GABA or all of the bioactive compounds measured. These rice cultivars mainly consisted of dark pigmented rice with bran in red or variable purple colour, which are associated with high FA and GO content. There are few rice cultivars with bran in light brown colour showing high GABA content. Rice cultivars with notable high content in these bioactive compounds includes Seratus from Kpg. Menitam, Limbang (1176.0 µg GABA/g; 1219.24 µg GO/g; 990.0 µg FA/g), Segerit (800.80 µg GABA/g, 804.19 µg GO/g and 1036.0 µg FA/g), Merah from Rh. Ulat, Batu Niah (442.40 µg GABA/g, 1007.28 µg GO/g and 1429.0 µg FA/g) and Keladi from Menitam (336.0 µg GABA/g, 1132.32 µg GO/g and 2034.0 µg FA/g).

Table 4: Correlation matrix between bran colour and the level of bioactive compounds

	GO	FA	GABA	Colour
GO				
Pearson correlation	1	0.328*	–0.029	0.214
Sig. (2-tailed)		0.016	0.838	0.124
N	53	53	53	53
FA				
Pearson correlation	0.328*	1	0.000	0.748**
Sig. (2-tailed)	0.016		0.999	0.000
N	53	53	53	53
GABA				
Pearson correlation	–0.029	0.000	1	0.185
Sig. (2-tailed)	0.838	0.999		0.184
N	53	53	53	53
Colour				
Pearson correlation	0.214	0.748**	0.185	1
Sig. (2-tailed)	0.124	0.000	0.184	
N	53	53	53	53

*Indicates correlation is significant at the 0.05 level (2-tailed). **Indicates correlation is significant at the 0.01 level (2-tailed)

Coloured rice varieties, same as other coloured plants such as red grape, tomato and blueberry are known to be strong in antioxidative activities (Kaneda et al., 2006). In this study, there are strong relationship between bran colour and FA content but not in GO and GABA content (Table 4). These were in congruence with previous studies reported on higher levels of total phenolic and antioxidant capacity in pigmented genotypes (Sompong et al., 2011; Shen et al., 2009; Mira et al., 2008; Kaneda et al., 2006; Nam et al., 2006; Goffman and Bergman, 2002). No relationship was found between rice bran colour and GO content although correlation was found between GO and FA. This could be due to difference in their chemistry including pigmentation and solubility behaviours. GABA, present abundantly in embryo of rice grains is also not associated with rice bran colour, possibly due to low GABA in bran layer.

CONCLUSION

Present study revealed that bioactive compounds in whole grain rice had different association with bran colour. The level of FA was significantly correlated with rice bran colour, but no correlation was found for GO and GABA. The level of FA followed the trend of variable purple > red > light brown, which are commonly associated with antioxidant activity. Therefore, bran colour could serve as an indicator of FA content in whole grain rice. This information could assist rice producers or food technologists in rice cultivar

selection for nutraceutical food developments and also targeted compounds extraction.

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Author Contributions

S. X. S. made the major contribution in data collection and writing of the manuscript. All other authors made equal contributions, edited and approved the manuscript.

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