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Corresponding author:
Parisut Chalermchaiwat,
E-mail: fagprsch@ku.ac.th

Research article

Principal Component Analysis Application on Nutritional, Bioactive Compound and Antioxidant Activities of Pigmented Dough Grain

Junthanee Teravecharoenchai¹, Parisut Chalermchaiwat^{1,*} and Ratiya Thuwapanichayanan²

¹ Food and Nutrition Program, Department of Home Economics, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand
² Department of Farm Mechanics, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

Abstract The dough stage of rice plant is a critical stage during the grain development. The rice endosperm continues to expand and the color of the seed coat develops in accordance with rice variety, so it is called as dough grain. This research was carried out to examine nutritional value, bioactive compound and antioxidant activity of Thai dough grain. Ten varieties of pigmented dough grain including non-glutinous and glutinous rice were studied. Principal component analysis (PCA) was conducted to visualize the difference in nutrition amongst ten rice varieties. Hierarchical cluster analysis was used to identify the unique characteristics of each rice variety. Three principal components (PC1-PC3) showed 82.04% of the total variance and could divide pigmented dough grain varieties into 3 groups. Group 1 consisted of a red dough grain (Red Hom Mali). It had the highest total phenolic content as well as high protein and antioxidant activities. Group 2 consisted of two red dough grains (Thabthim Chumphae and Niao Daeng) and two black dough grains (Riceberry and Niaodum Kumbaikeaw), which had moderate antioxidant activities. Group 3 consisted of two green dough grains (Khao Dawk Mali 105 and Kor Khor 6) and three black dough grains (Black Hom Mali, Mali Black Sulin and Niaodum Kumnoi), exhibiting high gamma oryzanol value but, low total phenolic content. Hence, red and black dough grains had higher nutritional value, total phenolic content, total anthocyanin and antioxidant activities than green dough grains. In addition, dough grains from non-glutinous rice had higher protein and lower fat than those from glutinous rice. Therefore, the data from this research could benefit the utilization of these pigmented dough grain as functional food ingredients and healthy food product.

Keywords: Antioxidant activity, Bioactive compound, Nutrition, Pigmented dough grain, Principal component analysis

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INTRODUCTION

Recently, pigmented rice varieties have received more attention from consumers due to their high bioactive compounds that present potential nutraceutical benefits to health. It's also well known that these compounds are primarily located in the outer layer of the rice grain along with the rice hull which protects the rice seed during growth. Rice grain gradually starts developing from flowering to fully ripe stage, during each stage the content of bioactive compounds changed differently depending on rice genotypes and cultivation environment (Jiamyangyuen et al., 2017). Dough stage of rice (dough grain) is the stage after milk grain and can be harvested within 14 days after pollination, which is faster than fully mature that can be harvested within 30 days after pollination (Jiamyangyuen et al., 2017; Naivikul, 2017). Different stages of rice are used for producing different products. For example, milk stage is used for producing rice milk and fully mature stage is used for producing steamed rice and snacks. However, dough stage is still not widely used. The majority of Thai people normally use the dough grain from glutinous rice varieties for producing 'Khao Mao' (Pramchinnawong, 2004). There are various varieties of dough grain in Thailand with different colors both in non-glutinous and glutinous rice, such as green, red and black. Moreover, the research was found that dough grain had some bioactive compounds (total phenolic content, total flavonoid content and total proanthocyanidin content) (Jiamyangyuen et al., 2017). Therefore, it should be used as a raw material due to its shorter harvesting time. This research used principal component analysis (PCA) to distinguish the samples as well as to identify important variables in a multivariate data matrix (Bourneow and Toontam, 2019). There have been few studies focused on finding bioactive compounds from the pigmented dough grain in Thailand. For example, Jiamyangyuen et al. (2017) studied bioactivity and chemical components of Thai rice (Mali white, Mali Red and Riceberry) in five stages of grain development and Junin (2016) studied bioactive compound of dough stage from glutinous rice (Kor Khor 6 and E-tie). Therefore, this research applied the principal component analysis (PCA) to find correlation between nutrition, bioactive compound and antioxidant activity of pigmented dough grain from non-glutinous and glutinous rice. This is a way to support usage of dough grain rice varieties by using statistical technique to classify dough stage of rice and to select suitable dough grain for developing the healthy food products. There is also the prospect of additional practical implications, not only for agriculture expansion but also for the food industry.

MATERIALS AND METHODS

Materials

Ten pigmented dough grain varieties that are green rice (Khao Dawk Mali 105 : KDML105 and Kor Khor 6: RD6), red rice (Red Hom Mali: MLR, Thabthim Chumphae: TCP and Niao Daeng: NR) and black rice (Black Hom Mali: MLB, Riceberry: RBR, Mali Black Sulin: MSL, Niao dum Kumbaikeaw: NBK and Niao dum Kumnoi: NBN) from Sakon Nakhon Rice Research Center and Plang na sa-at Community Enterprise harvested in November-December 2018 were used in this study. Dough grain in this study was harvested at twenty days after flowering and then sun-dried before threshing. Thereafter, the samples were dried at 50°C in a hot air oven for 6 hours until their moisture contents were less than 14% (National Bureau of Agricultural Commodity and Food Standards, 2010). The rice samples were then de-husked to obtain the unpolished rice, packed in vacuum aluminium foil and stored at 4°C, until further quality analysis.

Physical qualities of pigmented dough grain

The color of samples was measured in the L*, a* and b* mode of CIE by using a Hunter Lab colorimeter (ColorFlex, USA). L*, a* and b* indicate lightness, redness/greenness and yellowness/blueness, respectively. Three samples were tested and average value was reported. Water activity (aw) determination was performed in triplicate using a water activity meter (AQUA LAB, USA).

Nutritional properties of pigmented dough grain

Nutritional properties of dough stage of rice were analyzed in triplicate. Protein was determined by the Kjeldahl method and percentage of protein was calculated by multiplying %N with a factor of 5.95 (Bulletin of the international dairy federation, 2006), moisture content, crude fat, ash and crude fiber were determined according to the AOAC (2012). Total carbohydrates content (%) was determined by total percentage of solids minus protein, fat and ash based on dry basis.

Bioactive compounds of pigmented dough grain

Gamma aminobutyric acid (GABA) was analyzed using In-house method based on TAS 4003-201 by the Central Laboratory (Thailand) Co., Ltd. The results were expressed in milligram gamma aminobutyric acid per 100 g dry weight (mg/100 g dw)

Gamma oryzanol was analyzed using Food Anal. Method (2015) 8:649-655 by the Central Laboratory (Thailand) Co., Ltd. The results were expressed in milligram gamma oryzanol per 100 g dry weight (mg/100 g dw)

Extraction of pigmented dough grain

Extract was prepared from ground dough stage of rice (2.0 g). The samples were extracted at room temperature with methanol under agitation using a magnetic stirrer for 30 min. The mixtures were centrifuged at 2,500 xg for 10 min. and the supernatants were collected. The residues were re-extracted twice under the same conditions, resulting finally volume adjustment in 50 ml extract by methanol. All extracts were analyzed for total phenolic content, total anthocyanin and antioxidant activity by the methods of Sompong et al. (2011) and Songtip et al. (2013) with some modifications.

Total phenolic content (TPC) of pigmented dough grain

TPC of extracts was determined using the Folin-Ciocalteu colorimetric method (Ayoola et al., 2008). Gallic acid was used as a standard. The absorbance was read at 760 nm using a spectrophotometer (GeneQuant, Sweden). The results were expressed as gallic acid equivalents in milligram per 100 g of dry weight (mg GAE/100 g dw)

Total anthocyanin (TAC) of pigmented dough grain

TAC was determined using pH-Difference method according to the method proposed by Jiamyangyuen et al. (2017) with some modifications. Briefly, the extract 1 ml was dissolved in 1 ml of two different pH buffer solution (potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5)). Absorbance of the solutions was determined at 510 nm and 700 nm using a spectrophotometer. The results were reported as mg/100 g dry weight.

Antioxidant activity of pigmented dough grain

DPPH radical scavenging activity was based on a decrease in methanolic DPPH in the presence of a hydrogen-donating antioxidant. DPPH solution, an intense violet color, which showed absorption band at 517 nm, was measured in terms of electric transfer reaction using the stable free radical DPPH method proposed by Sompong et al. (2011). The absorbance was measured at 517 nm using a spectrophotometer. The results were expressed as Trolox equivalents in milligram per 100 g of dry weight (mg Trolox/100 g dw)

Ferric reducing antioxidant power (FRAP) assay is based on a decrease in the Fe^{3+} -TPTZ complex to the ferrous form at low pH according to a modified method of Sompong et al. (2011) and Jiamyangyuen et al. (2017). This decrease is monitored by measuring the absorption change at 595 nm using a spectrophotometer. All determinations were performed in triplicates. The results were expressed in milligram FeSO_4 per 100 g of dry weight (mg FeSO_4 /100 g dw)

Statistical analysis

Analysis of variance (ANOVA), followed by the Duncan's multiple range test, was used to determine the significant differences among samples using the IBM SPSS® version 23 software (IBM SPSS Inc., USA). XLSTAT® version 2018 (Addinsoft, New York, USA) was used to perform principal component analysis (PCA) with classification analysis using hierarchical cluster analysis (HCA) and biplots were also generated.

RESULTS

Physical quality

Physical quality is presented in table 1. The color parameters (L^* , a^* , and b^*) of the samples were significantly different ($P \leq 0.05$). The lightness (L^*) was more dominant in the green dough grains followed by the red dough grains, while the black dough grains had the lowest L^* value. All the red dough grains showed the highest a^* value and all the black dough grains showed the lowest b^* value. Water activities (a_w) of all samples were in the range of 0.40 to 0.64.

Table 1. Physical quality of pigmented dough grain.

Dough stage of rice		Color			a_w
		L^*	a^*	b^*	
Non-glutinous rice	KDML105	53.03 $\pm$ 1.55 ^b	-1.52 $\pm$ 0.27 ^f	20.16 $\pm$ 0.63 ^c	0.54 $\pm$ 0.00 ^d
	MLR	38.46 $\pm$ 0.48 ^d	11.94 $\pm$ 0.51 ^a	15.98 $\pm$ 0.29 ^d	0.59 $\pm$ 0.00 ^b
	TCP	42.09 $\pm$ 0.81 ^c	12.30 $\pm$ 0.84 ^a	24.54 $\pm$ 0.74 ^a	0.40 $\pm$ 0.00 ^g
	MLB	18.84 $\pm$ 0.83 ^g	5.54 $\pm$ 0.70 ^c	-1.98 $\pm$ 0.52 ^h	0.64 $\pm$ 0.00 ^a
	RBR	16.27 $\pm$ 0.68 ^h	1.37 $\pm$ 0.27 ^e	2.97 $\pm$ 0.34 ^g	0.44 $\pm$ 0.00 ^f
	MSL	19.71 $\pm$ 0.48 ^g	5.21 $\pm$ 0.26 ^{cd}	5.04 $\pm$ 0.48 ^f	0.45 $\pm$ 0.00 ^f
Glutinous rice	RD6	57.56 $\pm$ 1.36 ^a	-2.95 $\pm$ 0.26 ^g	21.89 $\pm$ 0.14 ^b	0.52 $\pm$ 0.00 ^e
	NR	34.76 $\pm$ 1.69 ^e	12.53 $\pm$ 1.10 ^a	15.02 $\pm$ 0.98 ^e	0.64 $\pm$ 0.00 ^a
	NBK	20.21 $\pm$ 1.23 ^g	4.51 $\pm$ 0.60 ^d	-1.98 $\pm$ 0.33 ^h	0.59 $\pm$ 0.01 ^b
	NBN	25.93 $\pm$ 1.34 ^f	6.51 $\pm$ 0.44 ^b	2.97 $\pm$ 0.13 ^f	0.55 $\pm$ 0.00 ^c

Note: ^{a-h} Means within the same column with different letters are significantly different ($P \leq 0.05$).

Nutritional value

Nutritional values of ten varieties dough grain were significantly different ($P \leq 0.05$) shown in Table 2. These dough grains have many important nutrients like fully mature grain. Carbohydrates are the majority of the nutrients found in the dough grain (82-86%). MLR, MLB, NR and NBN had the highest value of moisture content. RBR had the highest protein content. MLB, RD6, NR and NBK had the highest fat content. MLB and NR had the highest crude fiber content. NBN had the highest ash. KDML105, TCP and NR had the highest carbohydrates.

Table 2. Nutritional of pigmented dough grain.

Dough stage of rice		Moisture (%)	Protein (% dw)	fat (% dw)	Crude fiber (% dw)	Ash (% dw)	Carbo hydrate (% dw)
Non-glutinous rice	KDML105	11.72 $\pm$ 0.07 ^c	8.81 $\pm$ 0.01 ^e	3.93 $\pm$ 0.02 ^c	2.61 $\pm$ 0.26 ^{cd}	1.34 $\pm$ 0.01 ^f	85.89 $\pm$ 0.12 ^{ab}
	MLR	13.10 $\pm$ 0.08 ^a	10.06 $\pm$ 0.05 ^c	3.22 $\pm$ 0.13 ^d	2.59 $\pm$ 0.64 ^{cd}	1.64 $\pm$ 0.02 ^{cd}	85.11 $\pm$ 0.21 ^{cd}
	TCP	9.25 $\pm$ 0.07 ^f	9.11 $\pm$ 0.09 ^d	2.55 $\pm$ 0.10 ^e	1.67 $\pm$ 0.09 ^e	1.52 $\pm$ 0.06 ^e	86.42 $\pm$ 0.57 ^a
	MLB	13.20 $\pm$ 0.14 ^a	9.28 $\pm$ 0.02 ^d	4.12 $\pm$ 0.18 ^{abc}	3.73 $\pm$ 0.23 ^a	1.71 $\pm$ 0.06 ^{bc}	84.89 $\pm$ 0.19 ^{de}
	RBR	10.04 $\pm$ 0.07 ^e	12.67 $\pm$ 0.14 ^a	3.37 $\pm$ 0.13 ^d	1.52 $\pm$ 0.15 ^e	1.74 $\pm$ 0.01 ^b	82.21 $\pm$ 0.02 ^f
	MSL	10.33 $\pm$ 0.15 ^d	10.58 $\pm$ 0.04 ^b	3.39 $\pm$ 0.09 ^d	1.67 $\pm$ 0.02 ^e	1.74 $\pm$ 0.06 ^b	84.40 $\pm$ 0.15 ^e
Glutinous rice	RD6	11.63 $\pm$ 0.07 ^c	8.35 $\pm$ 0.11 ^f	4.41 $\pm$ 0.13 ^a	2.34 $\pm$ 0.15 ^d	1.61 $\pm$ 0.01 ^d	85.61 $\pm$ 0.10 ^{bc}
	NR	13.30 $\pm$ 0.16 ^a	8.23 $\pm$ 0.09 ^f	4.36 $\pm$ 0.07 ^{ab}	3.48 $\pm$ 0.17 ^{ab}	1.32 $\pm$ 0.10 ^f	86.07 $\pm$ 0.03 ^{ab}
	NBK	13.07 $\pm$ 0.05 ^a	8.89 $\pm$ 0.01 ^e	4.30 $\pm$ 0.11 ^{ab}	3.07 $\pm$ 0.30 ^{bc}	1.77 $\pm$ 0.05 ^b	85.02 $\pm$ 0.17 ^d
	NBN	12.71 $\pm$ 0.26 ^b	8.79 $\pm$ 0.09 ^e	4.08 $\pm$ 0.23 ^{bc}	3.00 $\pm$ 0.01 ^{bc}	1.88 $\pm$ 0.02 ^a	85.25 $\pm$ 0.49 ^{cd}

Note: ^{a-f} Means within the same column with different letters are significantly different ($P \leq 0.05$).

Bioactive compound and antioxidant activity

Antioxidant activities of pigmented dough grains as determined by DPPH and FRAP assays were significantly affected by rice colors. Red (MLR, TCP and NR) and black (MLB, RBR, MSL, NBK and NBN) dough grains had higher TPC and antioxidant activities than

the green dough grains (KDML105 and RD6). The total anthocyanin content (TAC) was found in red and black dough grains but not determined in the green dough grains. The highest antioxidant activities as determined by both DPPH and FRAP assays were found in MLR (non-glutinous rice) and NR (glutinous rice), respectively, which corresponded to their highest TPC as shown in Table 3.

Table 3 also shows GABA and Gamma oryzanol. It was found that GABA and Gamma oryzanol contents of pigmented dough grains were in the range of 0.49-4.84 mg/100 g. and 29.49-62.33 mg/100 g., respectively. In this study, MLB had the highest GABA, gamma oryzanol and fat, and also had high antioxidant determined by DPPH assay.

Table 3. Bioactive compounds and antioxidant activity of pigmented dough grain.

Dough stage of rice		TPC (mg GAE/ 100g)	DPPH (mg TE/100g)	FRAP (mmol FeSO ₄ /100g)	TAC (mg/100g)	GABA (mg/100g)	Gamma Oryzanol (mg/100g)
Non-glutinous rice	KDML105	95.87 ± 4.71 ^a	35.57 ± 0.24 ^e	157.59 ± 6.44 ^f	ND	0.68 ± 0.01 ^f	43.16 ± 0.32 ^f
	MLR	478.22 ± 3.22 ^a	50.70 ± 0.39 ^b	1613.27 ± 13.70 ^a	1.19 ± 0.12 ^d	0.49 ± 0.01 ^g	45.29 ± 0.52 ^e
	TCP	166.24 ± 5.96 ^e	47.88 ± 0.17 ^c	975.41 ± 9.13 ^b	0.11 ± 0.01 ^e	0.64 ± 0.02 ^f	29.49 ± 0.21 ^j
	MLB	162.03 ± 3.81 ^e	50.77 ± 0.07 ^b	317.51 ± 3.11 ^e	3.86 ± 0.07 ^b	4.84 ± 0.05 ^a	62.33 ± 0.83 ^a
	RBR	78.56 ± 2.86 ^f	43.90 ± 0.07 ^d	763.57 ± 6.98 ^c	4.61 ± 0.97 ^a	0.92 ± 0.03 ^d	38.52 ± 0.19 ^h
Glutinous rice	MSL	267.88 ± 4.91 ^c	47.65 ± 0.04 ^c	327.04 ± 4.47 ^e	4.08 ± 0.05 ^{ab}	0.95 ± 0.03 ^d	60.07 ± 0.58 ^b
	RD6	107.77 ± 2.92 ^f	30.63 ± 2.48 ^f	172.23 ± 2.18 ^f	ND	0.79 ± 0.01 ^e	39.59 ± 0.43 ^g
	NR	301.35 ± 5.86 ^b	56.21 ± 0.17 ^a	974.06 ± 12.18 ^b	0.16 ± 0.01 ^e	0.74 ± 0.01 ^{ef}	35.82 ± 0.26 ⁱ
	NBK	264.48 ± 1.72 ^c	47.52 ± 0.04 ^c	751.43 ± 13.82 ^c	1.93 ± 0.05 ^c	1.30 ± 0.02 ^b	48.47 ± 0.12 ^d
	NBN	200.65 ± 4.90 ^d	48.61 ± 0.33 ^c	489.28 ± 13.30 ^d	0.51 ± 0.11 ^e	1.12 ± 0.02 ^c	53.64 ± 0.43 ^c

Note: ^{a-j} Means within the same column with different letters are significantly different ($P \leq 0.05$).

ND Means Not determine

Hierarchical cluster analysis (HCA)

Three groups of dough grain samples were determined as shown in Figure 1. Group 1 consisted of a red dough grain (MLR). It had the highest total phenolic content as well as high protein and antioxidant activities. Group 2 consisted of two red dough grains (TCP and NR) and two black dough grains (RBR and NBK), which had moderate antioxidant activities. Group 3 consisted of two green dough grains (KDML105 and RD6) and three black dough grains (MLB, MSL and NBN), exhibiting high gamma oryzanol value but, low total phenolic value.

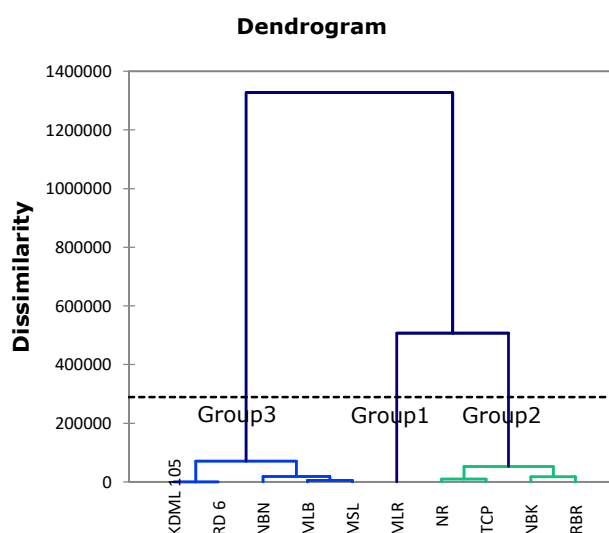


Figure 1. The dendrogram of hierarchical cluster analysis (HCA) of pigmented dough grain.

Principal component analysis (PCA)

The biplot from the principal component analysis of pigmented dough grains is presented in Figure 2. It's a useful tool to obtain a common pattern from complex data set presenting the relationship between pigmented dough grains and nutritional value, bioactive compounds and antioxidant activities. Three PCs could explain up to 82.52%

of the total variance (PC1 36.07%, PC2 24.55% and PC3 21.42%). The first principal component (PC1) was closely related to L^* , b^* , carbohydrates and adverse to ash, anthocyanin, GABA and gamma-oryzanol. The second principal component (PC2) was associated with a^* , antioxidant activities (DPPH and FRAP) and phenolic content. The third principal component (PC3) was closely related to crude fibers, fat and adverse to protein.

Dough grain samples projected in different positions were different in characteristics. Pigmented dough grains load in PC1, which were KDML105, RD6, TCP, had high L^* , b^* and carbohydrates but low ash, anthocyanin, GABA and gamma-oryzanol. However, MLB, RBR, MSL and NBK were opposite. Pigmented dough grains, which were in PC2, MLR and NR, were red dough grain. They had high phenolic content and antioxidant activities. NBN and NBK had high crude fibers and fat but low protein. However, RBR were adverse.

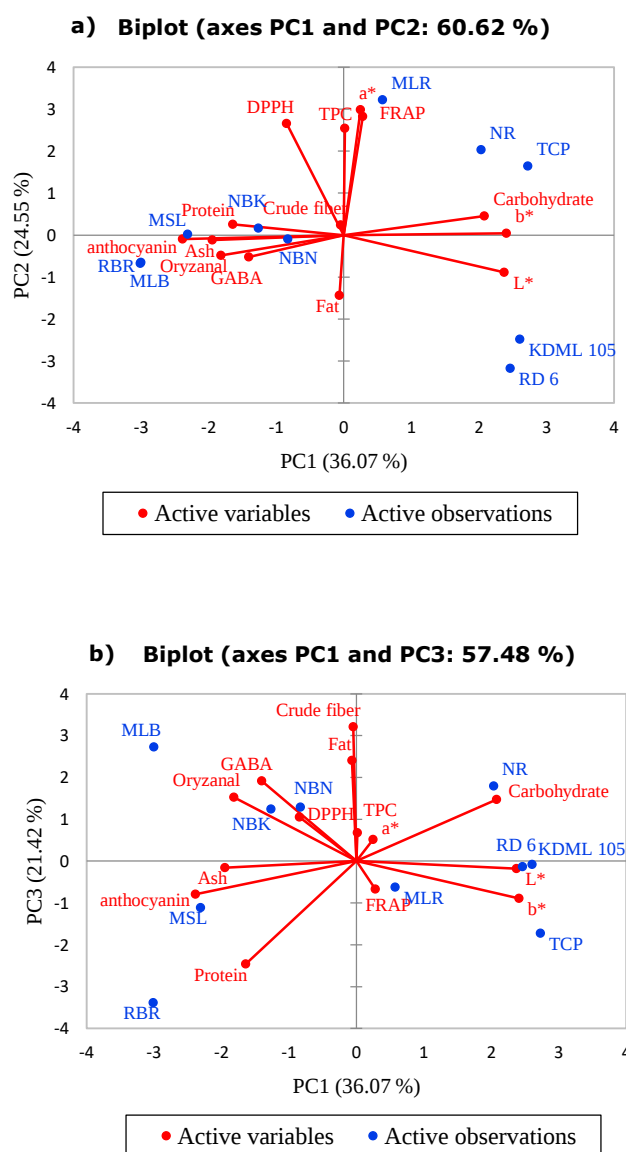


Figure 2. Biplots of pigmented dough stage of rice on nutritional, bioactive compound and antioxidant activities, a) PC1 and PC2, b) PC1 and PC3

DISCUSSION

The highest a^* value of the red dough grains and the lowest b^* value of the black dough grains as seen in Table 1 were due to the bioactive compounds in red and black

rice such as anthocyanin (blueness and purpleness), proanthocyanidins (redness) and carotenoids (yellowness) (Kraithong et al., 2018). Main color of black rice is affected by anthocyanin and main color of red rice is affected by proanthocyanidins and carotenoids (Limtrakul et al., 2019). The aw of pigmented dough grains in this study seemed to be lower than the study of Junin (2016), who reported that aw of RD6 dough grain paddy after harvest was 0.99 and Jongskulsri (2010) reported that aw of Khao Mao that produced by RD6 dough grain was 0.67. This might be due to the differences in rice varieties, growing area and drying process. In addition, lower values of aw in this study is due to the fact that the pigmented dough grain was dried in a hot air oven until their moisture contents (9.25-13.30%) were less than 14% followed by National Bureau of Agricultural Commodity and Food Standards (2010). The high value of aw correlated to the high moisture content.

Different quantities of moisture, protein, fat, crude fiber, ash, carbohydrate shown in Table 2 are based on rice varieties. These results are in agreement with Kraithong et al. (2018), who reported that the proximate composition of Thai organic rice flours depended on rice varieties. Most of ash, protein, fat, and crude fiber in pigmented rice are in rice bran and germ. However, higher values of those components contributed to lower carbohydrate content (Wang et al., 2016). This is due to the fact that percent total solids content includes total carbohydrates, protein, fat and ash. It is worth noting that dough grains from non-glutinous rice had higher protein and lower fat than dough grains from glutinous rice. Similar to the fully mature grain reported by Sompong et al. (2011), who studied nine red and three black rice varieties, found that fat value was significantly different amongst the varieties and black glutinous rice had higher fat value. But this result contrasted with that of Charoenthaikij et al. (2012), who found that fully mature grain of non-glutinous rice (KDML105) had protein of 6.7%, fat of 2.7%, fiber of 0.6% and glutinous rice (RD6) had protein of 7.1%, fat of 2.9%, fiber of 0.6%. It might be due to the rice varieties and the chemical changes during seed growth. Most of the proteins contained in the starch are enzymes that synthesize starch. The starch synthase family is highly expressed in the developing rice endosperm (Hayashi et al., 2018). So it might affect protein in dough grains from non-glutinous and glutinous rice due to non-glutinous rice had high amylose than glutinous rice. Protein, fat, fiber, ash and carbohydrate found in dough grain were not much different from those found in fully mature rice. Kraithong et al. (2018) reported that KDML105, MLR and RBR had protein of 8.0-8.3%, fat of 2.0-3.9%, fiber of 1.0-1.8%, ash of 1.2-1.7% and carbohydrate of 85.2-86.6%. In this study dough grain of KDML105, MLR and RBR had protein of 8.8-12.7%, fat of 3.2-3.9%, fiber of 1.5-2.6%, ash of 1.3-1.7% and carbohydrate of 82.2-85.89%. Therefore, the pigmented dough grain can use instead of fully mature stage of rice, which can save time and cost of cultivation.

Red and black dough grains had higher antioxidant activity, TPC and TAC than green dough grains as seen in Table 3. The color of rice are related to the quantity of phenolic compounds, proanthocyanidins (redness), anthocyanin (blueness and purpleness) and antioxidant activity in rice (Kraithong et al., 2018; Shao et al., 2018). TAC was prominent in the black rice varieties, followed by red rice varieties (Pramai and Jiamyangyuen, 2016), in this study it's not found in green dough grains.

Gamma aminobutyric acid (GABA) is a free amino acid which could help relieving or preventing non-communicable diseases in human. It is the most important inhibitory neurotransmitter in the brain, acting like a break during time of runaway stress. It can improve relaxation and enhance sleep (Karladee and Suriyong, 2012). GABA is produced primarily by the decarboxylation of L-glutamic acid, catalyzed by the enzyme, glutamate decarboxylase. The different amounts of GABA content among rice varieties are mainly due to their genetic constitution and environment. Wattanakul et al. (2011) studied the effect of harvesting period (30, 37 and 44 days after flowering) on GABA of Sung yod rice. It was found that the harvesting period had no significant effect on the GABA content of Sung yod rice. However, Charoenthaikij et al. (2012) found that the GABA contents of KDML105 and RD6 brown rice were 2.11 mg/100 g and 2.41 mg/100 g, respectively. While the GABA contents of KDML105 and RD6 dough grains in this study were 0.68 and 0.79, respectively. Such lower GABA contents of dough grains as compared with brown rice at fully mature stage is probably due to the different cultivation area.

Gamma oryzanol is a family of ferulic acid esters of unsaturated triterpenoid alcohols, has been characterized in rice bran. It's a class of non-saponifiable lipid and help in the regulation of elevated LDL (Samyot et al., 2017). Pramai and Jiamyangyuen (2016) and Laokuldilok et al. (2011) reported that gamma oryzanol and phenolic acids were the major antioxidants for pigmented rice bran. A similar trend was observed by Jiamyangyuen et al. (2017) who found that red rice in milk stage and dough stage had the highest antioxidant activity as they had high quantities of phenolic compounds, anthocyanin, proanthocyanidin content. Furthermore, Chadsuwan and Areekul (2010), who studied phenolic compounds in 51 different rice species, reported that phenolic compounds in colored rice (red rice, purple rice and black glutinous rice) were higher than those in white rice. This is due to the fact that dark purple aril consists of anthocyanin, proanthocyanidin, flavonoid and vitamin E, which are the group of phenolic compounds, having antioxidant activity (Dejkriengkraikul, 2014).

Hierarchical cluster analysis (HCA) is an algorithm that groups similar objects into groups called clusters as shown in Figure 1. The objects within each cluster are broadly similar to each other. Thus, hierarchical clustering is easy to implement and the dendrogram produced is very useful in understanding the data (Rani and Rohil, 2013). From this research, the result of HCA supported the result of PCA (Figure 2). In PCA, pigmented dough grains load in PC1, PC2 and PC3 were different in characteristics. PC2 was associated with a*, antioxidant activities and phenolic content. MLR and NR in PC2 had high phenolic content and antioxidant activities. Jiamyangyuen et al. (2017) also found that red dough grain had high antioxidant activities, determined by both FRAP and DPPH assays, due to high contents of TPAC, TPC and TFC. Therefore, the obtained PCA result was good for classification pigmented dough grain varieties and it was more obviously distinguished using HCA to classified between red, black, and green dough grain varieties.

CONCLUSION

Significant nutrition properties of dough grain were different amongst rice varieties and colors. Bioactive compounds, i.e., TPC and TAC as well as antioxidant activities (assessed by DPPH and FRAP assays) in pigmented dough grains (red and black) were significantly higher than those in green dough grains. The highest value of TPC and antioxidant activity by FRAP assay found in MLR. The pigmented dough stage of rice is a good alternative for functional food ingredient and also for other health food products due to its shorter harvesting period and saver cost of cultivation. Moreover, the finding of this research may help food and beverage industrial to apply dough grains instead of fully mature stage of rice in some food application.

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