

## Relationship among Starch Digestibility, Antioxidant, and Physicochemical Properties of Several Rice Varieties using Principal Component Analysis

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### Abstract

Seven rice samples were investigated for *in vitro* starch digestibility, total phenolic content (TPC), total anthocyanin content (TAC), antioxidant activities and physicochemical properties such as moisture, color, total starch (TS), digested starch (DS) and resistance starch (RS) contents. The relationship between these parameters and the samples was investigated by principal component analysis (PCA). All of the samples exhibited predicted glycemic index (*pGI*) 47.87 – 52.22, TS 65.69 – 80.60 g/100 g d.b., and DS 65.69 – 80.22 g/100 g d.b., ( $p < 0.05$ ). RS content was generally lower than 1 g/100 g d.b. The TPC and TAC varied in the range of 0.15 – 2.25 mg gallic acid equivalent (GAE)/g and 0.51 – 68.70 mg cyanidin -3- glucoside (CG)/g, respectively ( $p < 0.05$ ). The PC1 (50.51%) explained the digestion parameters with reference to polished-waxy rice (KorKhor 6 and Sanpatong) and low-amylose milled rice (KhaoDawkMali 105), which is opposite to yellowness and RS which refer to unpolished rice (Homnil, brown DawkMali, and red Jasmine). The PC2 (40.89%) explained the antioxidant properties with reference to unpolished-black rice (KumDoiSaket), which is opposite to lightness which refer to polished-waxy rice and low-amylose milled rice. The PCA plot clearly differentiated the dissimilarity between PC1 and PC2. The high RS and TPC improve strong antioxidant property and low starch digestion. It can be used a combination of these parameters to classify rice properties better than one individual parameter.

**Keywords:** rice varieties, starch digestibility, antioxidant properties, physicochemical properties, principal component analysis

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## 1. Introduction

Rice, being one of the primary dietary sources of carbohydrates, is a staple food for over half of the world's population [1–3]. It is a good source of thiamine, riboflavin, niacin, and dietary fiber [4]. The world's rice is produced and consumed in Asia, North and South America and Africa with thousands of varieties [4–5]. Specialty rice varieties, such as aromatic rice (Thai Jasmine rice), local rice varieties of different pigments (red/purple/ black) and glutinous rice (very low amylose content and superior processing quality), are recently being increasingly cultivated and consumed with the growing of its health benefits [5–6]. Several bioactive compounds are present in rice, such as anthocyanins: cyanidin-3-glucoside and peonidin-3-glucoside, anthocyanidin, quercetin, tocopherol, and phenolic acids: ferulic acid, 4-hydroxycinnamic acid, 4,7-dihydroxy vanillic acid, protocatechuic acid methyl ester, syringaldehyde, vanillin [7–9]. These compounds have shown antioxidant properties [10] and aldose reductase inhibitory activity [7], which are beneficial in oxidative stress and diabetes prevention, respectively.

The GI has been recommended as a helpful guideline as regards food choices, especially starchy food [11] because it has been reported that low GI foods improve blood glucose control [12–13] and increase insulin sensitivity [14]. Miller *et al.* [15] reported that the GI values of foods are grouped into low GI (< 55), medium GI (55–70), and high GI (> 70). Rice is known to have a relatively high glycemic response compared to other starchy foods [1]. Rice has been given a wide range of GI values, ranging from 54 to 121, with white bread as the reference [15]. The consumption of rice and diets with a high GI are associated with an increased risk of developing type 2 diabetes as the report of Indrasari *et al.* [13] and Hu *et al.* [16]. A better understanding on starch digestibility of rice grain varieties is important for diet-related health complications, especially type 2 diabetes. Differences in GI value of rice have been ascribed to various factor including physicochemical properties and food processing. Amylose content had an impact on the estimated GI value and resistant starch (RS) content [1, 17–19]. As the research of Hu *et al.* [17], they reported that the high amylose rice cultivar was obviously higher in RS content and lower in GI value. There is no published data available to help with understanding the effects of either RS or antioxidant capacity on starch digestibility of rice at this present. The aim of this work was to investigate the beneficial rice varieties which have high potential in resistant starch and antioxidant activity but low starch digestibility. This study covered these aspects in terms of the physicochemical properties and the antioxidant activities of seven different rice varieties. Starch digestibility as a digestogram and as mathematical model parameters was also described. The present study will consequently help to guideline the consumption of rice by increasing consumer awareness of the health benefits of rice grains, especially type 2 diabetes population. In this respect, it will be recommended for the selected properties in food industry application.

## 2. Materials and Methods

### 2.1 Rice preparation

Rice was harvested in 2013 and provided by Chiang Mai Rice Research Center, Chiang Mai, Thailand. Selected rice varieties used in this study are shown in Table 1. Rough rice grains were dehusked (C.L.P. Engineering Co., Ltd., Thailand), milled with an Ultra Centrifugal Mill (ZM200, Retsch, Germany) to achieve uniform powder (100–300 µm), vacuum packed in aluminum bag, and stored at –18°C until use.

**Table 1.** Selected rice samples used in this study

Cultivar	Process	Rice variety	Amylose content	Color	Abbreviation
<i>Oryza sativa</i> L.	Milled	KhaoDawkMali 105	15-17%	White	KDML
	Un-milled	DawkMali	18-20%	Brown	BHML
		Jasmine	17-22%	Red	RHML
		Homnil	19-25%	Purple	HN
<i>Oryza sativa</i> var. glutinosa	Milled	KorKhor 6	4-7%	White	RD6
	Un-milled	Sanpatong	5-8%	White	GST
		KumDoiSaket	2.5-5%	Black	KDSK

## 2.2 Physicochemical properties

Moisture content (%) of the rice sample was investigated by using a hot air oven (Binder R3 Controller, Binder GmbH, Germany) at 105°C [20]. Color quality was investigated on the rice sample by using a color measurement (Hunter Lab Color Quest XE, Color Global Co., Ltd, USA). The color data of ground rice sample were expressed as tri-stimulus parameters: L\*, a\* and b\*. L\* values indicate lightness (100=white and 0=black), while a\* and b\* values indicate redness–greenness and yellowness–blueness, respectively [21].

Total starch (TS), resistant starch (RS), and digested starch (DS) contents of the rice powder sample were determined using a glucose oxidase kit (K-RSTAR 08/11) from Megazyme (Megazyme International, Ireland) by the modified method of Englyst *et al.* [22], which is based on controlled enzymatic hydrolysis of starch and measurement of released glucose. RS refers to the starch that is not digested following 16 h of exposure to enzymes (pancreatic  $\alpha$ -amylase and amyloglucosidase) at 37°C. The absorbance was measured at a wavelength of 510 nm using a Thermo Spectronic Biomate 5 UV/Vis spectrophotometer (Scintek Instruments, USA), and the glucose concentration was converted into starch content by applying the factor 0.9. The TS content in g/100 g d.b. was calculated by estimating the composition of DS and RS.

## 2.3 Starch digestion analysis

The time-course starch digestion was determined using a rapid *in vitro* digestibility assay based on glucometry [23]. Ground sample (0.5 g) was treated with artificial saliva containing porcine  $\alpha$ -amylase (250 U/mL, E.C.3.2.1.1.) before the addition of pepsin (1 mg/mL, E.C.3.4.23.1.), and incubated at 37°C for 30 min in a reciprocating water bath (Mettmert, Germany) at 85 rpm. The digesta was neutralized with 0.02 M NaOH before adjusting the pH to 6.0 (sodium acetate buffer) prior to the addition of pancreatin (2 mg/mL, Sigma-P1750) and amyloglucosidase (28 U/mL, E.C. 3.2.1.3.). The mixture was incubated for 3 h, during which the glucose concentration in the digesta was measured with an Accu-Check® Performa® glucometer at specific periods (0, 30, 60, 90, 120, 150, 180 min). The digested starch (DS) as g/100 g d.b. starch was calculated as,  $(0.9 \times G_G \times Mw \times V) / (W \times S \times [100 - M])$ , where  $G_G$ =glucometer reading (mM/L),  $V$ =volume of digesta (mL),  $Mw$ =molecular weight of glucose (180 g/mol),  $W$ =weight of sample (g),  $S$ =starch content of sample (g/100 g sample),  $M$ =moisture content of sample (g/100 g sample), and 0.9=stoichiometric constant for starch from the glucose content.

The digestogram (digested starch at a specific time period) of each sample was modeled using a modified first-order kinetic model as discussed in the method of Mahasukhonthachat *et al.* [24]. From the report of Goni *et al.* [25], single-point measurement of starch digestion at 90 min in the sample (H90) was also used to calculate the predicted glycemic index ( $pGI$ ) as,  $39.21 + (0.803 \times H90)$ .

## 2.4 Total phenolic content (TPC)

Rice extract was prepared by the procedure of Abru *et al.* [26] in the following steps. Ground rice (10 g) and distilled water (200 mL) were mixed and heated at 100°C for 15 min. The rice solution was then separated by a centrifuge at 1,500 rpm for 10 min before antioxidant analysis. The total phenolic content was measured using the Folin–Ciocalteu method with slight modifications [9] where gallic acid (GA) (in the concentration range of 0–100 mg/mL) was used as the standard. A volume of 200 µL of the sample solution or gallic acid standard (0–100 mg/L) was added into a test tube with 1.0 mL of diluted 10-fold Folin–Ciocalteu's reagent, and it was mixed thoroughly. The mixture was allowed to stand at room temperature for 5 min. Then, 800 µL of 7.5% (w/v) Na<sub>2</sub>CO<sub>3</sub> was added to the mixture and mixed gently. After leaving the sample at room temperature in darkness condition for 30 min for the stabilization of the blue color formed, the absorbance was measured by a UV/Vis spectrophotometer at 765 nm. The absorbance of each reference standard was plotted as a linear graph and the calculation was carried out to obtain a linear equation. The content of phenolic acid, evaluated using the obtained linear equation, was expressed as mg gallic acid equivalent (GAE)/ g of dried sample.

## 2.5 Total anthocyanin content (TAC)

The anthocyanin content was determined by the pH differential method [8]. The absorbance was measured at 500 nm and 700 nm for the elimination of interference from the background. The rice extract which was diluted with the potassium chloride buffer, pH 1.0, was left at rest for 15 min before measurement, whereas the rice extract diluting with the sodium acetate buffer pH 4.5 was measured after 5 min. The corresponding pure buffer was used as the reference sample in the spectrophotometer. The absorbance shift ( $\Delta A$ ) was formulated as,  $(A_{500} - A_{700})_{pH\ 1.0} - (A_{500} - A_{700})_{pH\ 4.5}$ . The total anthocyanin content (TAC) in mg of cyanidin-3-glucoside (CG)/g of dried sample was calculated as,  $(\Delta A \times Mw \times Df \times 10^3) / (Me \times L)$ , where Mw=molecular weight of cyanidin-3-glucoside (449.2 g/mol), Df = dilution factor (10), Me = molar extinction coefficient of cyanidin-3-glucoside (29,600 L/mol cm), and L=width of cuvette (1 cm).

## 2.6 Antioxidant activities

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity: This activity was measured spectrophotometrically according to the DPPH assay [27]. The volume of 60 µM of the DPPH solution in ethanol was prepared fresh daily. The DPPH radical stock solution (2000 µL) was added to 200 µL of the rice extract. The mixture was placed in a dark room (30°C) for 30 min and monitored at 517 nm of a UV/Vis spectrophotometer.

ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) free radical scavenging activity: This activity was assessed by the method of Re *et al.* [28] with slight modifications. The ABTS radical cation (ABTS<sup>+</sup>) was generated by making 1 portion of ABTS stock aqueous solution (7 mM) react with 1 portion of K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (2.45 mM) after incubation in darkness at room temperature for 12–16 h. The freshly prepared ABTS<sup>+</sup> solution was diluted with distilled water to obtain absorbance of 0.70±0.05 verified by a spectrophotometer at 734 nm before use. The ABTS<sup>+</sup> solution (2,000 µL) was added into the sample solution (200 µL). The absorbance reading at 734 nm was recorded exactly 6 min after initial mixing and standing in darkness at room temperature using a UV/Vis spectrophotometer.

The decreasing in the absorbance of sample solution after incubation was measured and computed in the %inhibition of DPPH and ABTS radical scavenging activity as the following

equation,  $[(AB-AS)/AB] \times 100$ , where AB=absorbance of blank (no rice extract) and AS=absorbance of sample. Distilled water was used as a blank.

## 2.7 Statistical analysis

The results are presented as mean  $\pm$  standard deviation from three replicate. The analysis of variance (ANOVA), significant differences among means using Duncan's multiple range test (DMRT), and the principal component analysis (PCA) were performed using SPSS (Version 16, SPSS Inc., Chicago, USA) at a 95% significance level.

## 3. Results and discussion

### 3.1 Physicochemical properties

As demonstrated in Table 2, the moisture contents of the seven rice samples were in the range of 9.35%–10.52% ( $p < 0.05$ ). There were significant differences in the moisture content,  $L^*$ ,  $a^*$  and  $b^*$  values ( $p < 0.05$ ). The  $L^*$  value of the rice samples, which expresses as the brightness, varied between 26.40 and 52.82. The pigmented rice samples, KDSK, HN and RHML, exhibited much lower  $L^*$  values than the non-pigmented rice. The positive  $a^*$  values of redness were also high for all of the three rice varieties (1.58–4.67),  $p < 0.05$ . The  $b^*$  value of yellowness was the highest for KDSK. As far as this result is concerned, the different rice colors were dark purple, light purple, and red brown, respectively. The color of pigmented rice could be derived from the mixture of anthocyanins, which are located on a rice seed coat or aleurone layer [3, 7, 26]. The difference in color could depend on the form of anthocyanin content and rice genotype [7, 20]. As for the non-pigmented rice varieties, KDML and GST, they gave higher  $L^*$  values, nearer to BHML and RD6. The negative  $a^*$  values (redness) of KDML, GST and RD6 were also very low (–0.13 to –0.07),  $p \geq 0.05$ . The white rice genotypes had no the presence of proanthocyanidin which were typically observed in the red kernels [8].

The starch content is given in Table 3. The TS content ranged between 65.98% and 80.60% d.b. with slightly significant differences, except for KDSK, which stood out with a lower TS content (65.98% d.b.),  $p < 0.05$ . Generally, the RS content was very low (lower than 1% d.b.). As this result, the DS content was high in all of the rice samples and varied from 65.69% to 80.22% d.b.,  $p < 0.05$ . In agreement with the study of Frei *et al.* [1] and Juliano [5], they described that TS and DS contents were high in all rice varieties. As far as this results are concerned, the RS content of the three waxy rice varieties (RD6, GST, and KDSK) which refer to glutinous and low-amylose rice varieties, were significantly lower than the RS content of the non-waxy rice varieties, except KDML 105 ( $p < 0.05$ ). It is possible due to the compact linear structure of high- and intermediate-amylose in non-waxy rice was more resistant to starch digestion than the molecule of amylopectin in waxy rice [17, 29].

**Table 2.** Moisture content and color of different rice samples

Rice sample	MC (%)	L* value	a* value	b* value
KDML	9.46 ± 0.06 <sup>c</sup>	51.01 ± 1.43 <sup>a</sup>	-0.07 ± 0.02 <sup>c</sup>	4.36 ± 0.21 <sup>bc</sup>
BHML	10.44 ± 0.11 <sup>ab</sup>	48.20 ± 0.74 <sup>b</sup>	0.41 ± 0.03 <sup>d</sup>	7.92 ± 0.46 <sup>a</sup>
RHML	9.65 ± 0.11 <sup>bc</sup>	37.28 ± 0.83 <sup>d</sup>	4.67 ± 0.11 <sup>a</sup>	7.69 ± 0.11 <sup>a</sup>
HN	9.36 ± 0.16 <sup>c</sup>	30.46 ± 1.26 <sup>c</sup>	1.58 ± 0.07 <sup>c</sup>	4.34 ± 0.03 <sup>bc</sup>
RD6	10.52 ± 1.16 <sup>a</sup>	45.86 ± 1.49 <sup>c</sup>	-0.13 ± 0.03 <sup>c</sup>	4.13 ± 0.22 <sup>c</sup>
GST	9.61 ± 0.24 <sup>bc</sup>	52.82 ± 1.10 <sup>a</sup>	-0.11 ± 0.01 <sup>c</sup>	4.62 ± 0.14 <sup>b</sup>
KDSK	9.35 ± 0.17 <sup>c</sup>	26.40 ± 0.27 <sup>f</sup>	3.14 ± 0.04 <sup>b</sup>	2.28 ± 0.02 <sup>d</sup>

<sup>a-f</sup> Values with different letters in the same column are significantly different ( $p < 0.05$ ).

**Table 3.** Total starch, digestive starch, and resistant starch content (g/100 g d.b.) of different rice samples

Rice sample	Total starch	Digestive starch	Resistant starch
KDML	80.60 ± 1.92 <sup>a</sup>	80.22 ± 1.96 <sup>a</sup>	0.38 ± 0.04 <sup>b</sup>
BHML	80.41 ± 0.59 <sup>a</sup>	79.64 ± 0.58 <sup>a</sup>	0.77 ± 0.01 <sup>a</sup>
RHML	79.19 ± 1.62 <sup>ab</sup>	78.39 ± 1.68 <sup>ab</sup>	0.80 ± 0.07 <sup>a</sup>
HN	76.59 ± 1.46 <sup>ab</sup>	75.81 ± 1.44 <sup>ab</sup>	0.78 ± 0.03 <sup>a</sup>
RD6	75.73 ± 1.27 <sup>b</sup>	75.14 ± 1.27 <sup>b</sup>	0.39 ± 0.00 <sup>b</sup>
GST	77.98 ± 3.11 <sup>ab</sup>	77.61 ± 3.13 <sup>ab</sup>	0.37 ± 0.02 <sup>b</sup>
KDSK	65.98 ± 0.48 <sup>c</sup>	65.69 ± 0.47 <sup>c</sup>	0.29 ± 0.01 <sup>c</sup>

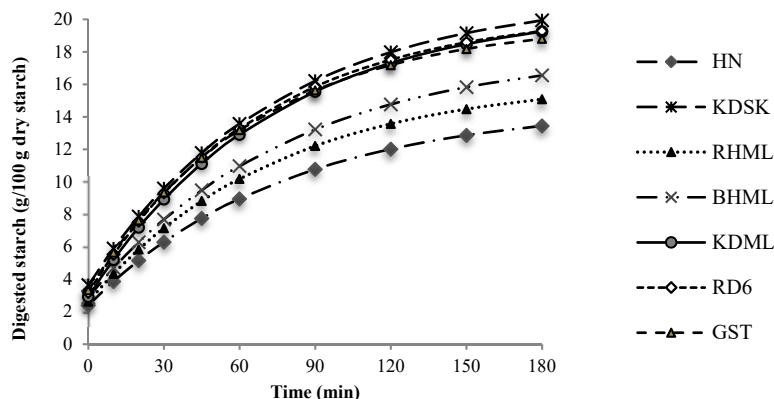
<sup>a-b</sup> Values with different letters in the same column are significantly different ( $p < 0.05$ ).

### 3.2 Starch digestibility

Seven rice samples were digested for different hydrolysis periods of time (0–180 min), and the glucometer was able to differentiate between the digestogram of the different samples. Figure 1 shows that all of the three glutinous rice varieties (KDSK, GST, RD6) and the milled white rice (KDML) had higher starch digestibility than the colored and non-waxy rice varieties (BHML, HN, RHML). The starch digestibility of the unmilled purple rice (HN) was the lowest in comparison to the other rice varieties. The finding also shows that the starch digestibility values of the unmilled red rice (RHML) and the unmilled rice (BHML) presented themselves into the second and third order, respectively.

The estimated parameters,  $D_0$ ,  $D_n$ , and  $k$ , from the starch hydrolysis model, H90 and  $pGI$ , are shown in Table 4. The results indicate substantial differences between the rice samples in all of the parameters. Our comparison of the results of the seven rice samples indicates that their  $pGI$  decreased in the following order: KDSK > RD6 > GST > KDML > BHML > RHML > HN. The  $pGI$  value of all the seven rice grains varied in the range of 47.87–52.22. All of the seven rice grains could be grouped into the category of low GI food material [19]. As this result, it can be concluded that the glutinous rice which is a low-amylose rice had higher  $pGI$  than the non-glutinous rice which is an intermediate-amylose rice. This was consistent with previous studies on rices [1, 17] and rice flours [19, 30]. The digestion rate ( $k$ ) of the non-waxy and intermediate-amylose colored rice varieties (BHML, RHML, HN) presented the lowest value (0.013 min<sup>-1</sup>) in comparison with the low-amylose rice and waxy rice varieties (RD6, 0.015 min<sup>-1</sup>; GST, 0.015 min<sup>-1</sup>; KDSK, 0.013 min<sup>-1</sup>) and KDML (0.014 min<sup>-1</sup>). The comparative  $k$  value of the selected rice samples within the group of the waxy rice, KDSK which was a colored waxy rice, showed lower  $k$  values than RD6 and GST which were a milled white waxy rice. In similar results, the  $k$  value of the rice samples within the

group of the non-waxy rice, the unpolished rice varieties (BHML, RHML, HN) exhibited lower  $k$  values than the milled white rice (KDML). The low  $pGI$  value of rice grains is mainly contributed by the high RS value and amylose content present in them [1, 17, 19, 30]. However, the RS content is not the only factor that affects starch digestibility. Many studies have reported that the starch digestibility of rice and rice flour are influenced by the genetic cultivar [17], the starch granule morphology [19], including other components such as the formation complexes between amylose and lipid [1], protein [23], and phytochemical compounds [31].



**Figure 1.** The starch digestograms of different rice sample. KDSK, KumDoiSaket; HN, Homnil; RHML, red Jasmine; BHML, brown DawkMali; KDML, KhaoDawkMali 105; RD6, glutinous rice KorKhor 6; GST, Sanpatong

**Table 4.** Model parameters and predicted glycemic index of different rice samples

Rice sample	$D_0$	$D_t$	$k$ ( $\text{min}^{-1}$ )	H90	HI	$pGI$
KDML	29.30	178.60	0.014	15.54	19.24	51.69
BHML	28.90	152.50	0.013	13.23	16.56	49.84
RHML	26.20	137.00	0.013	12.22	15.09	49.02
HN	24.00	123.00	0.013	10.78	13.45	47.87
RD6	31.80	173.80	0.015	15.84	19.28	51.93
GST	33.30	166.00	0.015	15.62	18.81	51.75
KDSK	36.40	178.70	0.014	16.21	19.94	52.22

$D_0$  = the digested starch at starting time (g/100 g d.b.),  $D_t$  = the digestion at infinite time (g/100 g d.b.),  $k$  = the rate constant ( $\text{min}^{-1}$ ), H90 and HI (hydrolysis index) are the mean predicted digestibility starch at 90 and 180 min, respectively, using the modified first-order kinetic model of Mahasukhonthachat *et al.* [24], and the  $pGI$  using the equation of Goni *et al.* [25].

### 3.3 Antioxidant property

The phytochemicals, namely total phenolic content and anthocyanin content, that were studied in seven rice samples are reported in Table 5. The results showed that milled white rice and low-amylose rice varieties (KDML, RD6, GST) contained significant lower amounts of TPC (0.15–0.18 mg GAE/g) as compared to unmilled rice and colored rice varieties, BHML (0.56 mg GAE/g); RHML (1.51 mg GAE/g); and purple rice, HN and KDSK (1.81–2.25 mg GAE/g);  $p < 0.05$ . In similar results, TAC was found to be significantly high in purple rice (KDSK, HN, 10.83–68.70 mg CG/g)

as compared to red rice (RHML, 2.43 mg CG/g), brown rice (BHML, 0.91 mg CG/g) and milled white rice (KDML, RD6, GST, 0.51–0.71 mg CG/g);  $p < 0.05$ . From this result, it is evident that the group of the purple rice group had higher polyphenolic and anthocyanin content than the red, brown, and white rice groups, respectively, in agreement with the study of Pongjanta *et al.* [29]. It can be noticed that TAC had higher values in the pigmented rice than in the white rice [3, 8, 29, 31]. This is related to their color parameter, especially the  $a^*$  and  $b^*$  values [3, 20, 26]. The high amount of anthocyanin content has been found in dark purple rice, with the possible phytochemicals identified as cyanidin-3-glucoside and peonidin-3-glucoside [7, 9]. Moreover, TPC of the unpolished rice varieties had higher than those of the polished rice varieties. Previous works evidenced that phenolic compounds present in the kernel of rice [8].

The antioxidant activity of the seven rice water extracts was determined by DPPH $^{\cdot}$  and ABTS $^{+}$  assays, as presented in Table 5. There were significant differences in their antioxidant activity (in both the DPPH $^{\cdot}$  and the ABTS $^{+}$  assays);  $p < 0.05$ . The white rice water extracts (KDML, RD6, GST) had DPPH radical inhibition activity in the range of 1.40–5.76%, and the values were lower than those of the brown-colored rice (BHML, 23.32%), the red-colored rice (RHML, 49.54%), and the purple-colored rice (61.44–86.05%);  $p < 0.05$ , respectively. Similar results were observed in the ABTS $^{+}$  radical inhibition activity: the pigmented rice water extracts (KDSK, HN, RHML) had higher ABTS $^{+}$  radical inhibition activity than those of the brown rice and the white rice ( $p < 0.05$ ). The first three high values of the rice water extracts were from the KDSK, HN and RHML varieties, and the values were 96.91%, 94.63% and 83.31%, respectively.

**Table 5.** Antioxidant property of different rice samples

Rice sample	TPC (mg GAE/g)	TAC (mg CG/g)	%Antioxidant activity*	
			DPPH	ABTS
KDML	$0.18 \pm 0.01^e$	$0.71 \pm 0.18^d$	$1.40 \pm 0.61^e$	$17.60 \pm 1.28^d$
BHML	$0.56 \pm 0.01^d$	$0.91 \pm 0.06^d$	$23.32 \pm 3.83^d$	$46.55 \pm 1.45^c$
RHML	$1.51 \pm 0.03^c$	$2.43 \pm 0.05^c$	$49.54 \pm 1.63^c$	$83.31 \pm 3.83^b$
HN	$1.81 \pm 0.05^b$	$10.83 \pm 0.63^b$	$61.44 \pm 4.46^b$	$94.63 \pm 0.65^a$
RD6	$0.15 \pm 0.01^e$	$0.51 \pm 0.18^d$	$5.76 \pm 2.26^e$	$18.55 \pm 0.78^d$
GST	$0.17 \pm 0.01^e$	$0.71 \pm 0.18^d$	$2.64 \pm 0.25^e$	$20.72 \pm 1.97^d$
KDSK	$2.25 \pm 0.02^a$	$68.70 \pm 1.53^a$	$86.05 \pm 0.61^a$	$96.91 \pm 0.13^a$

\*It was performed at a concentration of 0.05 mg/mL of rice solution.

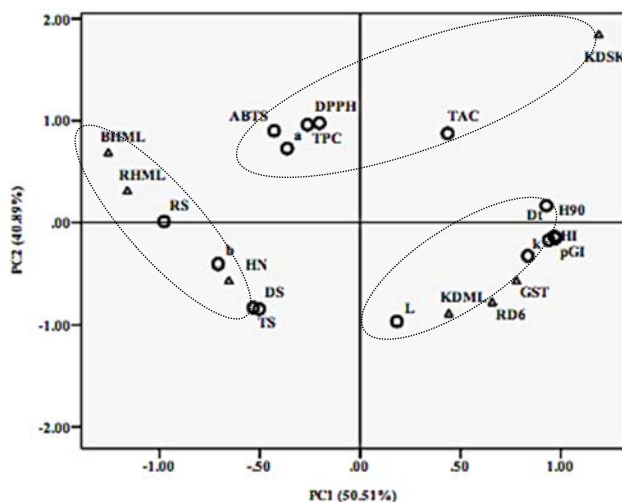
<sup>a-e</sup>Values with different letters in the same column are significantly different ( $p < 0.05$ ).

It is known from previous studies that pigmented rice varieties and grains are a good source of antioxidant compounds associated with pigmentation in rice and grains, and show extremely strong antioxidant capacity [8–9]. This was consistent with the studies on pigmented rice varieties [9, 20, 26, 31]. Rice is well known to contain phenolic and flavonoid compounds, especially the mixture of anthocyanins which have been found as a major active component for antioxidant property in rice [7]. Rice phenol compounds exist in free, esterified, and insoluble bound forms which can be released by water, base, acid, or enzymatic treatments prior to extraction, leading to higher phenolic contents, thus occurring in antioxidant activity [3, 8]. This finding summarizes that the pigmented and unmilled rice varieties were the most effective in anti-oxidative reactions, confirming in the pigmented rice had higher the DPPH $^{\cdot}$  and ABTS $^{+}$  scavenging activities than the milled white rice.



### 3.4 Principal component analysis (PCA)

PCA was applied to the data set of seven different rice samples after standardization (the mean of values for each variable is subtracted from each variable value and the result is divided by the standard deviation of values for each variable). After standardization, each parameter contributes equally to the data set variance and carries equal weight in the principal component (PC) calculation. The PCA plot of the seven rice samples based on their physicochemical properties, antioxidant capacity, and starch digestibility is presented in Figure 2. The sample score plot for PC1 vs PC2 is shown with 91.40% of the total variance explained. PC1 explained 50.51% of the total variance in the data set while PC2 explained 40.89% of the total variance. PC1 is generally better correlated with the variables than PC2. This is to be expected because PCs are extracted successively, with each one accounting for as much of the remaining variance as possible. Based on the relationships between their properties as studied in the present work, PC1 could be used to group the starch digestion parameters ( $D_0$ ,  $D_t$ ,  $k$ , H90, HI,  $pGI$ ) which are negatively related to the RS content with a highly significant correlation (with  $r$  ranging from  $-0.942$  to  $-0.857$ ). PC2 could be used to combine the antioxidant capacity, DPPH, ABTS, TPC and TAC, with  $a^*$  value which are opposite to the  $L^*$  value and the TS and DS contents. TPC,  $a^*$  value and antioxidant activity (both DPPH and ABTS) are clustered together on the top left-hand side of the loading plot ( $r=0.809-0.993$ ). The TAC values correlate significantly negatively with the  $L^*$  and the  $b^*$  values based on their Pearson correlation coefficients ( $r=-0.738$  and  $-0.617$ , respectively). At the same time, TS and DS occupied a unique location at the very low position of the loading plot, with a strong positive correlation ( $r=0.999$ ).



**Figure 2.** The principal component analysis (PCA) plots of seven rice samples and different properties (starch content, color, antioxidant property, and starch digestibility).

By using the PCA, it is possible to suggest reasons for the location of the rice varieties on the basis of their physicochemical properties, starch digestibility, and antioxidant properties. The location of KDML, RD6 and GST, which are the milled white rice and waxy white rice varieties, exhibited nearness on the right-hand side of the PC1 zero point. They confirmed their high starch digestibility (Table 4) but low RS content (Table 3), antioxidant capacity (Table 5) and a\* value (Table 2). On the other hand, the intermediate-amylose content and pigmented rice varieties, HN, RHML and BHML were clearly at a differentiable distance away from the PC1 zero point to the left-hand side due to the RS content (Table 3) and the pigmentation (Table 2), resulting in their antioxidant capacity (Table 5). The high positive contribution on PCs of KDSK, which was the low-amylose waxy and purple-colored rice varieties showed that it had high TAC and starch digestibility as a negative RS property.

Therefore, it can be noticed that it was the antioxidant activity related to the polyphenol and anthocyanin contents that caused the colored rice pigmentation. This relationship of TPC, TAC, antioxidant properties (DPPH and ABTS) and color is consistent with other previous reports on various rice varieties [3, 9, 20, 29, 31]. They reported that the antioxidant properties were strongly correlated with total phenolic and anthocyanin contents in rice [3, 9, 20, 31]. As for the color parameters, L\* and b\* values were negatively correlated with TPC and TAC, and a\* value was positively correlated with TPC [3, 20, 29]. Moreover, the starch digestibility for the waxy rice and low-amylose varieties were relatively high, especially for the waxy rice varieties, in agreement with the study of Hu *et al.* [17]. Similarly, the lower starch digestibility exhibited in the intermediate-amylose and relatively high RS rice varieties [17, 30]. Pongjanta *et al.* [29] reported that the predicted glycemic index had highly positive association with RS content and TPC. A combination of parameters is better than one individual parameter to classify rice properties. However, Thailand has many rice varieties from spread of cultivar areas, leading to their different properties. Therefore, it might be a useful data if further more investigation in other varieties was studied.

#### **4. Conclusions**

Seven rice samples were investigated in this study for their properties, especially color, antioxidant activity, and starch digestibility. The pigmented rice varieties had higher antioxidant capacity, in both the content and the activity, than that of the groups of non-pigmented rice varieties. The principal component analysis showed the relationship of their color, antioxidant capacity and starch digestibility with PC1 (50.51%) and PC2 (40.89%). The high resistant starch contained in the pigmented and non-waxy rice was shown to have strongly affected the reducing of starch digestibility, particularly the predicted glycemic index. This useful piece of information provides the knowledge to help consumers for the healthy rice consumption and the increasing of consumer awareness of the health benefits of rice varieties, especially in relation to the risk of type 2 diabetes. As this result, rice varieties which has high resistant starch and antioxidant capacity, Homnil, suitable for antidiabetes. Moreover, the alternative properties of rice varieties will be promoted for various value-added product development in food industry such as, ready-to-eat rice, ready-to-cook rice, rice noodle, instant rice, and protein-based flour bread and cookies in order to give the optimal properties.

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