

Original article

Physicochemical, antioxidant properties and *in vitro* digestibility of wheat–purple rice flour mixturesWarinporn Klunklin*  & Geoffrey Savage 

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Summary The physicochemical characteristics, antioxidant properties and *in vitro* digestibility of high-antioxidant content flours made from different combinations of Thai purple rice flour and refined wheat flour from 25%, 50% to 75% (w/w) were investigated and these were compared with whole flour from purple rice and refined wheat flour. The increase in substitution levels of purple rice flour affected all the functional properties of flours, at the same time the levels of dietary fibre, protein digestibility and antioxidant compositions were also changed. The purple rice flour exerted a particularly strong effect on starch digestibility as the purple rice increased to 50% in the mixture. Moreover, purple rice flour showed lower amounts of rapidly digested starch (RDS), whereas slowly digested starch (SDS) of whole flour from purple rice and 75% substitution purple rice flour was found to be the highest for all samples. The *in vitro* starch digestibility of all samples also showed a positive correlation between dietary fibre and antioxidant compounds. Overall, the addition of purple rice flour improved the final nutritional properties, notably a lower predicted glycaemic index, and a higher antioxidant potential, which are two important nutritional properties for human health.

Keywords Antioxidants, flour functional properties, *in vitro* digestibility, purple rice flour, wheat flour.

Introduction

Thai purple rice (*Oryza sativa* L.) is grown in Northern Thailand and it has been widely recognised as a potential cereal grain that contains high amounts of bioactive compounds, which are as usual located in the bran layer (Hosseinian *et al.*, 2008; Jang & Xu, 2009). The two major anthocyanins found in the purple rice pericarp and aleurone layers, are cyanidin-3-*o*-glucoside and peonidin-3-*o*-glucoside. Moreover, phenolic acids are also found in the outer layers of purple rice grains (Jang & Xu, 2009). The bioactive compounds of purple rice exhibit high antioxidant activities due to the presence of antioxidant compounds (Yawadio *et al.*, 2007; Jang & Xu, 2009). Several reports have focused on the extraction and identification of phenolic compounds and antioxidant activity in different pigmented rice cultivars (Yawadio *et al.*, 2007; Loypimai *et al.*, 2016; Shao *et al.*, 2018).

Rice flour is one of the alternative flours recommended to produce gluten-free products because it contains no gluten (Chung *et al.*, 2014). Many commercial gluten-free products are made from white rice

because it is a cheap raw material. However, the characteristics and use of flours to produce acceptable sensory characteristics products depend on the functional properties of the flours (Oladale & Aina, 2007).

Because they have high glycaemic indexes, rice and rice-based products are concern as they can increase insulin resistance for individuals who consume large amounts of these products (An *et al.*, 2016). The glycaemic index is commonly used to classify dietary carbohydrates and this method is based on the blood glucose response, which can be measured using the area under curve when test subjects are fed test amounts of carbohydrate foods, compared to a reference food or a 50 g sample of glucose (Willett *et al.*, 2002). The predicted glycaemic index (pGI) uses an *in vitro* method based on a simulated gastric digestion method, together with a small intestine digestion step, to predict the glycaemic index that would be otherwise determined using human subjects (Monro *et al.*, 2010). With the concern about the increasing number of people with diabetes worldwide, there is an important need to produce rice-based products with a low glycaemic indexes (low starch digestibility) using pigmented rice flour. Purple rice also contains high dietary fibre in the bran and germ fractions, which

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delays the starch granules' accessibility to digestive enzymes in the human digestive system (Jang & Xu, 2009; An *et al.*, 2016). Therefore, understanding the digestibility of flour mixtures is important to achieve or increase product quality.

Colour is an important factor for initial acceptability by consumers who select food products based on their emotional expectation. Making the flours' colour similar to consumers' expectations can affect the food acceptance and liking scores (Sozer *et al.*, 2014). The colour of wheat flour can be a reliable indicator of the quality of the flour. The pigmentation of purple rice is mainly from anthocyanins that accumulate in a single layer of cells (Jang & Xu, 2009). Moreover, Shao *et al.* (2018) reported that there are correlation between L^* , a^* , b^* colours and antioxidant compounds of pigmented rice.

The effect of wheat–purple rice protein on the rate of starch digestion, together with *in vitro* protein digestibility of raw flours, has not been undertaken so far. Moreover, few studies have reported the effects of wheat–purple rice flour mixtures on the nutritional properties, *in vitro* digestibility and antioxidant compounds, such as phenolic acids and anthocyanins. Therefore, this study investigated the physicochemical characteristics, protein and starch digestibility, antioxidant compounds and antioxidant activities of purple rice blend flours by adding wheat flour at different levels to produce a series of biscuits for further study.

Materials and methods

Raw materials

Blended flours were prepared with refined wheat flour (Pams Products Ltd., Auckland, NZ) and whole grain purple rice (*Oryza sativa* L. *Var.* Sanpatong) (Big T. supermarket, Upper Riccarton, Christchurch, NZ). The whole rice grains were imported from Thailand in 5 kg bags. Whole purple rice grains were milled into a fine flour using a Whisper Mill (Grote Molen Inc., USA). Flours were then prepared by the substitution of purple rice flour into the wheat flour at levels of 25%, 50% and 75% (w/w) of total flour sample weights by comparing with wheat flour and whole flour from purple rice.

Functional properties of flours

Water and oil absorption capacity

Beuchat (1977)'s method was used to determine the water and oil absorption capacity. Distilled water or soybean oil (Goodman Fielder, Auckland, NZ) (10 mL) was added to 1.0 g of flour sample in a centrifuge tube. The suspension was stirred using a vortex

mixer. The suspension was then centrifuged at 2200 g for 25 min. The separated water and oil was then removed with a pipette and weighed. The water or oil absorption capacities were expressed as g of water or oil absorbed per g of the sample.

Bulk density

The bulk density was determined using the method of Okaka & Potter (1977). 50 g of each flour sample was weighed into a 100 mL graduated cylinder and then tapped continuously 20–30 times. The bulk density (g mL^{-1}) was calculated as weight per unit volume of sample after the flour had settled.

Swelling power

Swelling power was determined by the method of Oledale & Aina (2007). One gram of each flour sample was mixed with 10 mL of distilled water in a test tube. The suspension was heated in a shaking water bath at 80 °C for 30 min. The tube was cooled to room temperature and then centrifuged at 870 g for 15 min. The paste was weighed to determine the swelling power.

Foaming capacity and stability

The foaming capacity and stability were measured using the method of Coffman & Garcia (1977). Sample (2 g) was added to 50 mL distilled water in 100 mL graduated cylinder. The suspension was shaken vigorously for 5 min to form a foam. The volume of the foam (mL) after shaking for 30 s was expressed as the foam capacity. The volume after 1 h was used as an indicator of foam stability and this was expressed as a percentage of the initial foam volume.

Least gelation concentration

The least gelation concentration was determined by the method of Coffman & Garcia (1977). Flour sample was mixed with 5 mL distilled water in test tubes to make flour dispersions at concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v). The suspensions were then heated at 90 °C for 1 h in a water bath, then cooled rapidly under running tap water (12 °C) and further cooled for 2 h at 4 °C. The least gelation concentration was regarded as the concentration at which the inverted sample did not slip down the side of the test tube.

All functional properties of the flour mixtures were carried out in triplicate.

Proximate analysis

The moisture content was measured using the oven drying method at 105 °C for 18 h (AOAC, 2000). Total nitrogen was determined using the Rapid Max N exceed[®] method of Dumas (Elementar, Hanau,

Germany) and the factor 6.25 was used to calculate crude protein. Fat and ash were evaluated using AOAC methods 920.176 and 900.029, respectively (AOAC, 2000). Total starch content was determined using the Megazyme starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland-approved method 76-13) (Reed *et al.*, 2013). Total dietary fibre was determined using a total dietary fibre assay kit (Sigma-Aldrich, MO, USA). All proximate composition measurements were carried out in triplicate.

Colour

The colour of samples was determined using a Minolta Chroma Meter CR-410 (Konica Minolta, Osaka, Japan) using the CIE $L^*a^*b^*$ system (Ruangchakpet & Sajjaanantakul, 2007). The L^* , a^* and b^* parameters represent lightness–darkness, redness–greenness and yellowness–blueness of flour mixtures, respectively. Six replicates were performed for each analysis.

In vitro digestibility

Protein digestibility

Protein digestibility of the flour mixtures was investigated using the method of Akeson & Stahmann (1964). This method uses a two-stage *in vitro* digestion using pepsin from porcine gastric mucosa (Sigma Aldrich, USA, 66 units mg^{-1} protein) followed by pancreatin from porcine pancreas (AppliChem Chemica Synthesis, Germany, 30.315 units mg^{-1} protein). The stimulated solutions were centrifuged at 1600 g for 10 min. The supernatants were analysed for their digested protein contents using an Elementar (Hanau, Germany) Vario TOC cube instrument fitted with a chemi-luminescence detector for determining total bound nitrogen (TNb). The analyses were performed in triplicate.

Starch digestibility

Flours were tested *in vitro* to determine a simulated gastric digestion, and a small intestine digestion, using the method from Goni *et al.* (1997) and Rosin *et al.* (2002). Samples (0.5 g) were digested in 60 mL specimen pots on a multi-magnetic stirrer (IKA-Werke RT 15 Power, Staufen/Germany) at the steady speed at 37 °C 30 mL of water and 0.8 mL of 1 M HCl was added to adjust the pH of the samples. Then, 1 mL of 10% pepsin (Sigma, P 7000; 800–2500 U mL^{-1}) solution in 0.05 M HCl was added immediately. The solutions were digested for 30 min to accomplish gastric digestion. The sample was neutralised by the gastric HCl with 2 mL of 1 M NaHCO_3 and 5 mL of 0.1 M sodium maleate buffer pH 6. Starch digestion was commenced adding 5 mL of 2.5% pancreatin (EC: 232-468-9, CAS: 8049-47-6,

activity: 42362 FIP-U g^{-1} , AppliChem GmbH, Darmstadt, Germany) in 0.1 M sodium maleate buffer pH 6 and the pots were immediately filled up to 53 mL with distilled water. Triplicate of 1.0 mL samples were added to 4 mL ethanol, before ($T = 0$), and at 20, 60, 120 and 180 min after adding the pancreatin solution. The digested samples were centrifuged at 180 g for 5 min. A 0.05 mL aliquot of ethanolic sample, standard glucose (5 and 10 mg mL^{-1} glucose) and reagent blank (distilled water) were added with 0.25 mL of enzyme solution A (1% enzyme invertase and 1% amyloglucosidase (Megazyme, E-AMGDF; 3260 U mL^{-1}) in acetate buffer pH 5.2) and incubated at room temperature for 20 min. Reducing sugars were determined by adding 0.75 mL dinitrosalicylic mixture (0.5 mg mL^{-1} glucose, 4 M NaOH and DNS reagent were mixed in ratio 1:1:5) and heated at 100 °C for 15 min. The solution was cooled before 4 mL distilled water was added and read at 530 nm using a UV-Vis spectrophotometer (V-1200 spectrophotometer, Global Science, Auckland, New Zealand). The measured glucose released from the hydrolysed samples was expressed as the area under curves (AUCs) of digested starch of each sample for up to 180 min (Goni *et al.*, 1997). The AUCs were calculated for each sample from the area under the glucose release curve. The levels of rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured at 20 and after intestinal digestion between 20 and 180 min, respectively (Rosin *et al.*, 2002).

Predicted glycaemic index (pGI)

The hydrolysis index (HI) was the AUC of each sample from the *in vitro* starch digestibility divided by the AUC of a standard (50 g white bread). The predicted glycaemic index (pGI) was calculated based on the HI values using the equation of Goni *et al.* (1997). Predicted glycaemic index = $39.71 + 0.549 \times \text{HI}$.

Extraction and determination of antioxidant compounds and antioxidant activities

The samples were extracted using a method of Jang & Xu (2009). The extracted solution was kept at -20 °C until analysis. The antioxidant determinations were carried out in triplicate. The total phenolic contents were determined according to the method of Kaneda *et al.* (2006). Total flavonoid contents were determined using the method of Zhishen *et al.* (1999). Hosseinian *et al.* (2008)'s method was used to analyse the anthocyanin contents. The anthocyanin concentration (mg L^{-1}) of samples were calculated according to the following formula and expressed as Cyanidin-3-glucoside (C3G) equivalents:

$$\text{C3G}(\text{mg L}^{-1}) = \frac{\left[(A_{\lambda 700-\lambda 520})_{\text{pH}1.0} \right] - \left[(A_{\lambda 700-\lambda 520})_{\text{pH}4.5} \right] \times \text{MW of Cy-3-glu} \times \text{DF}}{\varepsilon \times l} \times 1000$$

Where MW = the molecular weight of C3G = 449.2 g mol⁻¹, DF = the dilution factor and ε = the extinction coefficient (L × cm⁻¹ × mol⁻¹) = 26 900 for C3G.

The ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] assay was performed using the method of Re *et al.* (1999). The samples (0.1 mL) were mixed with 2.9 mL of ABTS solution and incubated for 6 min before reading at 734 nm. A standard curve of trolox was set up in the range of 0–100 mg/100 mL. The results were expressed as trolox equivalents (μmol trolox per g DM of sample). The DPPH (2,2-diphenyl-2-picrylhydrazyl) contents of the sample extracts were analysed according to the method of Mahakunakorn *et al.* (2004). The extracts were mixed with DPPH (2,2-diphenyl-2-picrylhydrazyl) solution (1:1, v/v) and then incubated for 30 min at room temperature. The absorbance of the resulting yellow colour was measured at 520 nm with a spectrophotometer (UV-Vis). MeOH (4 mL of 80%) was added as a control. All determinations were conducted in triplicate.

Statistical analysis

All results generated in this work were statistically analysed by one-way ANOVA using a complete randomised design. The mean values were analysed by Duncan's multiple range test for a multi-comparison of means. The level of significance was assigned at $P < 0.05$. Pearson correlation coefficients were also performed. All statistical analyses were undertaken using the SPSS Statistics (v. 22.0, SPSS Inc., Chicago, IL, USA).

Results and discussion

Functional properties of flours

Water absorption capacity (WAC) and oil absorption capacity (OAC)

The WAC of the flour played an important role in the functional and sensory properties during food preparation. Moreover, WAC represents the ability of a product to mix with water under conditions where water is limited (Du *et al.*, 2014). The WACs of the flours ranged from 98.10% to 135.81%, where the WAC of refined wheat flour was the highest and the whole flour from purple rice was the lowest (Table 1). The difference in WAC of flour mixtures is due to the difference in fibre

and carbohydrate contents. Moreover, the amylopectin/amylose ratio can cause a lower solubility of the flours (Chandra *et al.*, 2015). It is reported that low WAC corresponds to high amylopectin contents of rice (Ihegwuagu *et al.*, 2009). The OAC of flours was another important factor for improving the mouth feel and maintaining the flavour of food products. The OAC of the refined wheat flour was significantly ($P < 0.05$) higher than those of the other flours. The carbohydrate content in the mixtures is also responsible for the elevated OAC. The differences in OAC have been attributed to the variations in the amylose/amylopectin ratio as well as their chain length distribution (Chandra *et al.*, 2015). Hydrophobic proteins play an important role in water and oil absorption, and this is important for the development of a stable product (Du *et al.*, 2014).

Bulk density

Bulk density plays an important role in food preparation. Significant differences ($P < 0.05$) were observed among the bulk densities of the different flours (Table 1). The bulk density of the flours varied from 0.52 to 0.79 g mL⁻¹, where the lowest and the highest values were obtained from the purple rice flour and wheat flour, respectively. It was clear that the proportion of wheat flour increased the bulk density of 50% purple rice flour substitution compared with whole flour from purple rice. Joshi *et al.* (2015) reported that the bulk density of different flours ranged from 0.59 g mL⁻¹ for rice grains to 0.80 g mL⁻¹ for refined wheat flour; these were comparable to the results in this study. The bulk density had a direct influence on the protein content, preparation, treatment and storage of the sample.

Swelling power

The swelling power represented the degree of starch granules in the flour that can absorb water. Swelling is, essentially, a property of the whole amylopectin molecule, rather than parts of it, and amylose alone appeared to be a diluent, while lipids (as complexes with amylose) strongly inhibited swelling. Table 1 shows that the swelling power of the flours ranged from 4.42 to 11.56 g g⁻¹. Purple rice flour had high swelling power due to its higher amylopectin content compared with wheat flour (Chan *et al.*, 2009).

Foaming properties (foam capacity and foam stability)

The foam properties of flour mostly depend on proteins and the interfacial protein film trapping air

Table 1 Functional properties of the five different flour mixtures*

Functional properties	Wheat flour	Purple rice flour substitution (%) [†]			Whole flour from purple rice
		25	50	75	
Water absorption capacity (%)	135.81 ± 1.55 ^a	120.93 ± 1.69 ^b	116.25 ± 1.59 ^c	106.58 ± 0.60 ^d	98.10 ± 0.59 ^e
Oil absorption capacity (%)	159.97 ± 1.07 ^a	149.05 ± 1.27 ^b	145.28 ± 0.28 ^c	132.68 ± 0.30 ^d	119.13 ± 0.53 ^e
Bulk density (g mL ⁻¹)	0.79 ± 0.01 ^a	0.73 ± 0.01 ^b	0.68 ± 0.01 ^c	0.58 ± 0.01 ^d	0.52 ± 0.01 ^e
Swelling power (g g ⁻¹)	4.42 ± 0.06 ^e	5.38 ± 0.01 ^d	7.17 ± 0.04 ^c	9.44 ± 0.15 ^b	11.56 ± 0.16 ^a
Foaming capacity (%)	17.33 ± 1.76 ^a	14.00 ± 1.15 ^b	10.67 ± 0.67 ^b	10.67 ± 1.33 ^b	6.67 ± 0.67 ^c
Foaming stability (%)	1.82 ± 0.01 ^a	1.76 ± 0.07 ^a	1.60 ± 0.06 ^a	1.39 ± 0.06 ^b	0.96 ± 0.02 ^c
Least gelation concentration (%)	8.67 ± 0.67 ^b	9.33 ± 0.67 ^{ab}	10.00 ± 0.00 ^{ab}	10.67 ± 0.34 ^{ab}	11.33 ± 0.67 ^a

*Values represent mean ± standard error.

[†]In each row, sample means not having the same letter are significantly different (Duncan's multiple range test, $P < 0.05$).

bubbles in liquid or solid suspensions and decelerating the rate of accumulation of bubbles in flours (Chandra *et al.*, 2015). The highest foam capacity was observed for refined wheat flour (17.33%), the 50% and 75% purple rice flour substitutions (10.67%), and the lowest foam capacity was the whole flour from purple rice (6.67%). A lower value of foaming capacity for white rice flour (3.52%) had previously been reported by Chandra & Singh (2013) who stated that this was due to differences in protein concentrations in the flours. Foam stability represents the capacity of the foam to retain its structure over time, and can be described as the ability of the proteins to form strong cohesive films around air vacuoles that then resist air diffusion from the vacuoles (Chandra *et al.*, 2015). The foam stability decreased as increasing amounts of purple rice flour were added to the wheat flour mixture. The low foam capacity and stability were desirable attributes for flours intended for the production of a variety of baked products, such as biscuits and crackers (Chandra *et al.*, 2015).

Least gelation concentration

The least gelation concentration measured the lowest concentration of proteins to maintain a gel that formed in the inverted tube, and was defined as an index of gelation capacity. This depended on the relative ratios of their proximate contents, such as protein, carbohydrates and lipids (Chandra *et al.*, 2015). Purple rice flour quickly formed a gel at the highest concentration (11.33%), whereas wheat flour formed a gel at a very low concentration (8.67%). The increasing substitution levels of purple rice flour in the flour mix reduced interactions among the binding forces which, in turn, decreased the gelling ability of the flour. It can be implied that whole flour from purple rice needed a higher quantity of flour substituted to form a gel; however, refined wheat flour was better in terms of gelling ability.

Proximate composition of the flours

The proximate compositions of the different levels of purple rice flour are critical factors in the development of new baked products and were used to adjust the formulations are shown in Table 2 (Varastegani *et al.*, 2015). The moisture content of the refined wheat flour (12.53%) was slightly higher compared with the substituted flour samples (11.98%) and the whole flour from purple rice samples (11.57%). The whole flour from purple rice had the highest total dietary fibre contents compared to the other flours. The fibre and ash contents of the refined wheat flour were related to the amounts of bran in the wheat flour (Varastegani *et al.*, 2015). The refined wheat flour contained higher starch and protein contents than the whole flour from purple rice. Increasing purple rice flour substitution significantly reduced ($P < 0.05$) the protein content and at the same time increased the total dietary fibre content, these affected the functional properties such as foaming and least gelation concentration of the flour mixtures (Table 1). The starch and protein contents were the major compositions in foods, affecting the physico-chemical characteristics of food products and play an important role in the final quality of bakery products (Varastegani *et al.*, 2015).

Table 2 shows the $L^*a^*b^*$ colour parameters of the raw flours. The lightness (L^*) showing that all flours had significant colour differences among them. The factor affecting the L^* value of the flour mixtures was wheat protein, which showed a positive relationship with the b^* values (Beuchat, 1977). Okaka & Potter (1977) stated that the brightness of the flours tended to decrease with the increasing protein content; however, the lightness of these flour mixtures decreased due to the dark colour of the purple rice flour. The addition of purple rice flour, at all levels, increased the redness (a^*) and lowered the yellowness (b^*) in the mixture due to the predominance of phenolic acids in rice hulls and bran layers (Jang & Xu, 2009). The

Table 2 Proximate composition and $L^*a^*b^*$ colour values of the five different flour mixtures*

Nutritional properties	Wheat flour	Purple rice flour substitution (%) [†]			Whole flour from purple rice
		25	50	75	
Moisture content (%)	12.53 ± 0.23 ^a	12.29 ± 0.04 ^a	11.88 ± 0.04 ^b	11.78 ± 0.06 ^b	11.57 ± 0.14 ^b
Crude protein (%)	15.41 ± 0.06 ^a	14.25 ± 0.03 ^b	12.93 ± 0.19 ^c	12.88 ± 0.04 ^c	12.97 ± 0.12 ^c
Protein digestibility (%)	24.80 ± 0.50 ^e	33.33 ± 0.20 ^d	50.48 ± 0.14 ^c	60.33 ± 0.20 ^b	66.46 ± 0.86 ^a
Total dietary fibre (%)	2.31 ± 0.15 ^e	2.82 ± 0.01 ^d	3.32 ± 0.09 ^c	4.54 ± 0.17 ^b	5.56 ± 0.10 ^a
Ash (%)	0.88 ± 0.02 ^d	1.03 ± 0.01 ^c	1.06 ± 0.01 ^{bc}	1.09 ± 0.01 ^{ab}	1.10 ± 0.02 ^a
Total fat (%)	1.97 ± 0.05 ^c	1.97 ± 0.01 ^c	2.09 ± 0.01 ^c	2.51 ± 0.12 ^b	2.77 ± 0.07 ^a
Colour values L^*					
a^*	-1.42 ± 0.02 ^e	0.96 ± 0.01 ^d	2.13 ± 0.03 ^c	3.14 ± 0.01 ^b	3.91 ± 0.07 ^a
b^*	13.08 ± 0.16 ^a	10.83 ± 0.14 ^b	3.96 ± 0.02 ^c	3.51 ± 0.02 ^d	2.16 ± 0.01 ^e

*Values represent mean ± standard error.

[†]In each row, sample means not having the same letter attached to them are significantly different (Duncan's multiple range test, $P < 0.05$).

colour of purple rice flour is derived from the accumulation of anthocyanins and proanthocyanidins in the outer surface of the sheath (Jang & Xu, 2009).

In vitro starch digestibility

Although all flours had similar starch contents, the starch digestion rates were significantly different ($P < 0.05$) (Table 3). The total starch contents of all flours were not significantly different from each other ($P > 0.05$). The area under the starch hydrolysis curve (AUC) was evaluated after the digestion of each sample to explain the glycaemic response of the selected foods compared to a reference sample of white bread (An *et al.*, 2016). As described, the delay in starch hydrolysis observed in the whole flour from purple rice led to a decrease in the rapidly digestible starch and an increase in the slow digestible starch fraction compared to the wheat flour (473.21 mg min dL⁻¹). The measurement of reducing sugar released using the *in vitro* method can be divided into RDS (glucose released in the first 20 min of digestion) and SDS (the amount of glucose released from 20 to 180 min of digestion) based on the amount of glucose released. RDS was the predominant fraction in flour mixtures that were measured as glucose at 20 min of digestion, to reflect the rate of absorption in the small intestine. From Table 3, it can be seen that the substitution of purple rice flour in the mixture has significantly lower RDS than wheat flour, whereas the substituted purple rice flour also had a higher SDS compare to wheat flour by 25%. Flours with higher amounts of SDS are

required as they are able to reduce the prevalence of health problems such as heart disease and diabetes (An *et al.*, 2016). Raw starch is generally not very digestible since many intrinsic and extrinsic factors can affect the digestibility of starch. Purple rice starches typically contain high levels of amylopectin, which may be retrograded during the hydrolysis to make the flour mixtures less sensitive to the digestion (Sumczynski *et al.*, 2016). This may be the case with purple rice flour, which shows minimal enzyme digestibility over 120 min of enzyme incubation. The same results were also reported by Sumczynski *et al.* (2016). Before the purple rice can be used in products, it has to be milled. This processing may modify the amylose content of the purple rice starch, which in turn will affect the digestibility. In addition, it is possible that starch digestibility is related to granule size. Larger granules tend to have a lower digestibility (Reed *et al.*, 2013). The correlation between the antioxidant compounds and *in vitro* starch digestibility is also reported in this study.

Glucose release curves in Fig. 1 show the rate of reducing sugars released from starch in a digestive enzymatic method. Among all the flour mixtures, the digestion rates of the whole flour from purple rice samples were the lowest, indicating that purple rice flour had a stronger resistance to digestion than wheat flour after 120 min of enzymatic hydrolysis, which resulted in slower and lower increases in glucose in the *in vitro* method. An *et al.* (2016) showed that different cultivars may have different nature of starch, protein and dietary fibre contents

Table 3 *In vitro* starch digestion profile and amylolytic digested starch fractions (rapidly digestible starch, RDS and slowly digestible starch, SDS) of flour mixtures*

Nutritional properties	Wheat flour	Purple rice flour substitution (%) [†]			Whole flour from purple rice
		25	50	75	
Total starch (%)	74.50 ± 0.71 ^{NS}	73.60 ± 0.38	72.30 ± 0.12	73.23 ± 0.35	73.00 ± 1.04
RDS (mg g ⁻¹ sample)	42.70 ± 0.07 ^a	34.12 ± 0.17 ^b	33.74 ± 0.11 ^b	30.08 ± 0.03 ^c	22.66 ± 0.28 ^d
SDS (mg g ⁻¹ sample)	13.74 ± 0.14 ^c	12.55 ± 0.07 ^d	15.92 ± 0.31 ^b	20.05 ± 0.34 ^a	20.35 ± 0.41 ^a
AUC of digested starch (mg min dL ⁻¹) [‡]	473.21 ± 0.10 ^a	389.26 ± 0.31 ^b	266.32 ± 0.17 ^c	235.64 ± 0.98 ^d	178.90 ± 0.28 ^e
pGI	63.11 ± 0.01 ^a	58.96 ± 0.03 ^b	55.29 ± 0.03 ^c	52.88 ± 0.01 ^d	48.56 ± 0.02 ^e

*Values represent mean ± standard error; NS, not significant difference.

[†]In each row, sample means not having the same letter attached to them are significantly different (Duncan's multiple range test, $P < 0.05$).

[‡]Area under the curve (AUC) of digested starch can predict the glycaemic response of each flour.

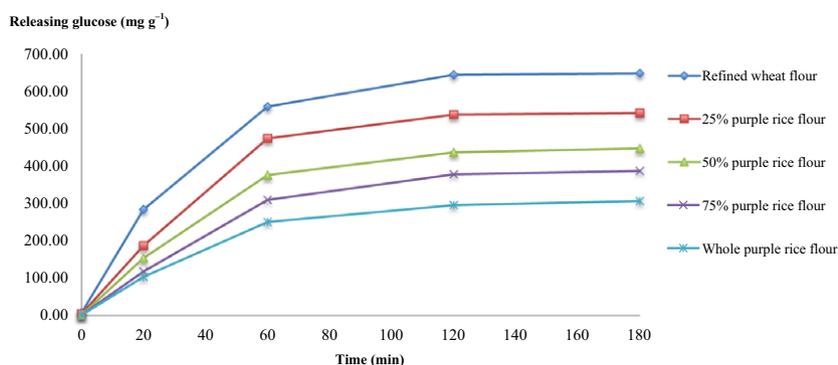


Figure 1 Amount of glucose released (mg g⁻¹ of starch) of each flour mixture during *in vitro* starch digestion using different enzymatic hydrolysis for up to 180 min. [Colour figure can be viewed at wileyonlinelibrary.com]

and changes in these may affect the digestibility of starch. Protein can restrict the rate of starch granules swelling and gelatinisation, which was partially responsible for the low digestibility. Moreover, the purple rice flour, which was rich in dietary fibre and protein, showed a significant decrease in starch digestibility. Based on the results of An *et al.* (2016), the starch digestibility of black rice flour substituted in wheat flour is related to the phenolic content rather than dietary fibre with decreasing rate of glucose released in the *in vitro* digestion method. All samples digested rapidly within the first 20 min of the hydrolysis, and the digestion rate from 20 to 180 min increased slowly thereafter (Fig. 1).

The pGI values of the flours were calculated based on HI derived from the starch digestibility of each flour. As shown in Table 3, the calculated pGI values significantly decreased ($P < 0.05$) with increasing purple rice flour substitution levels. Thus, whole flour from purple rice had the lowest amount of digested starch released using the *in vitro* method. The suppression effects of the substituted purple rice flour in refined wheat flour on starch digestibility were based on the phenolic contents and dietary fibre (An *et al.*,

2016). Moreover, the variation in the proportions of rapidly digestible starch, slowly digestible starch, as well as the content of other macronutrients, such as fat, fibre and protein, had a distinct impact on the pGI of food products (An *et al.*, 2016). As stated previously, purple rice flour, which was rich in dietary fibre, had lower amounts of glucose released over time compared to wheat flour. A similar result was reported by An *et al.* (2016) who stated that the pGI values of black rice flour were significantly lower than wheat flour during an *in vitro* starch digestion. Another study reported that high amylose rice varieties also showed higher pGI values compared to purple rice, which has a lower amylose content (Frei *et al.*, 2003). Brand-Miller (1994) showed that brown rice with a low glycaemic index reduced the insulin resistance and adjusted the blood glucose levels in both Type 1 and Type 2 diabetic patients. Therefore, the substituted purple rice flour can be said to have a low glycaemic index (GI < 60), which is good for both diabetics and healthy people (Brand-Miller, 1994). Moreover, the control flour (refined wheat flour) can be regarded as an intermediate GI (60–85) ingredient (Brand-Miller, 1994).

Antioxidant compounds and antioxidant activities of different flour mixtures

The antioxidant characteristics and activities of all flour samples are shown in Table 4. The whole flour from purple rice obviously had higher total phenolic, anthocyanin and flavonoid contents than the other flours. Large differences in the total phenolics were identified; the total phenolics ranged from 28.66 mg GAE/100 g DW in refined wheat flour to 318.28 mg GAE/100 g DW in whole flour from purple rice. The total phenolic contents of the whole flour from purple rice in this study were similar to the results of Shao *et al.* (2018). In contrast, the results of the whole flour from purple rice in this study were higher than reported by Rocchetti *et al.* (2017). These results may be due to the differences in the extraction methods used by Rocchetti *et al.* (2017). The major anthocyanin of purple rice analysed in this study was cyanidin-3-o-glucoside (C3G). The whole flour from purple rice was the richest in phenolic compounds and these were mainly ascribed to anthocyanins (Shao *et al.*, 2018). Shao *et al.* (2018) also stated that black rice from different cultivars had C3G, which ranged from 12.03 to 1106.00 mg kg⁻¹ DW; these values were similar to the values for whole flour from purple rice in this study. Therefore, it could be expected that purple rice flour had higher flavonoid contents than wheat flour. The total flavonoid contents of the refined wheat flour (29.67 mg catechin (CE)/100 g) were significantly lower ($P < 0.05$) than those of the whole flour from purple rice (149.83 mg CE/100 g). These findings were similar to report from Kim & Kim (2017). The variations of the antioxidant compounds were due to the different levels of purple rice flour added to the wheat flour. Whole flour from purple rice had the highest ABTS and DPPH radical scavenging activity compared to substituted flour and refined wheat flour. The effectiveness in scavenging ability on ABTS and DPPH

radicals were in a range of the results reported by Sumczynski *et al.* (2016) that varied from 10.0 to 23.6 mmol trolox per kg and ranged from 7.2 to 12.5 mmol trolox per kg in black rice, respectively. In addition, Sumczynski *et al.* (2016) also stated that results from ABTS scavenging method were consistent with the DPPH scavenging method.

Correlation among selected proximate composition, a^* colour parameter, antioxidants and *in vitro* starch digestibility

Pearson's correlations among selected proximate composition, a^* colour parameter, antioxidant compounds and antioxidant capacity are shown in Table 5. Among all flour accessions, the colour parameter a^* value had significantly positive correlation with anthocyanins ($r = 0.975$ at $P < 0.01$). However, negative correlation of whole rice grain a^* with antioxidant capacity has been reported by Shen *et al.* (2009). Regarding the correlation among colour parameter a^* value, dietary fibre and protein content, significant correlations were observed. It was also found that dietary fibre and total phenolics significantly correlate with pGI. It can be suggested that the higher the fibre content in flour mixtures the lower the digestibility values

Table 5 Pearson correlation among selected proximate composition, a^* colour value, antioxidant compounds, antioxidant activities and *in vitro* starch digestibility

	Colour a^*	pGI	DPPH assay	ABTS assay
Dietary fibre	0.916**	0.196*	0.901**	0.958**
Protein content	-0.931**	0.190	-0.956**	-0.622**
Total starch	-0.414	0.007	-0.418	-0.070
Total phenolics	0.946**	0.241*	0.944**	0.917**
Flavonoids	0.946**	0.241	0.944**	0.880**
Anthocyanins	0.975**	0.211	0.977**	0.917**

*, ** indicate significant at $P < 0.05$ and $P < 0.01$, respectively.

Table 4 Antioxidant compounds and antioxidant activities (DPPH and ABTS assays) of the five different flour mixtures*

Antioxidant properties	Wheat flour	Purple rice flour substitution (%) [†]			Whole flour from purple rice
		25	50	75	
Total phenolics (mg GAE/100 g DW)	28.66 ± 0.09 ^e	76.46 ± 0.55 ^d	153.57 ± 0.46 ^c	211.84 ± 2.54 ^b	318.28 ± 1.51 ^a
Anthocyanins (C3G, mg kg ⁻¹ DW)	0.22 ± 0.05 ^e	118.49 ± 0.61 ^d	249.69 ± 0.35 ^c	388.73 ± 1.23 ^b	492.59 ± 0.95 ^a
Flavonoid contents (mg CE/100 g DW)	29.67 ± 1.23 ^c	58.74 ± 0.66 ^d	95.92 ± 5.55 ^c	119.92 ± 1.30 ^b	149.83 ± 2.17 ^a
DPPH (μmol Trolox per g DW) ^h	3.72 ± 0.14 ^e	16.97 ± 0.46 ^d	35.46 ± 0.17 ^c	41.00 ± 0.04 ^b	46.75 ± 0.08 ^a
ABTS (μmol Trolox per g DW) ⁱ	6.73 ± 0.21 ^d	8.28 ± 0.09 ^d	10.48 ± 0.19 ^c	77.84 ± 0.38 ^b	102.77 ± 1.11 ^a

GAE, gallic acid equivalent; C3G, cyanidin-3-glucoside; CE, catechin equivalent; DPPH, 2,2-diphenyl-2-picrylhydrazyl radical scavenging activity; ABTS, [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging activity.

*Values represent mean ± standard error.

[†]In each row, sample means not having the same letter attached to them are significantly different from each other (Duncan's multiple range test, $P < 0.05$).

could be observed (Table 3). *In vivo* study also reported by Jenkins *et al.* (1977) that dietary fibre increases the viscosity of intestinal contents in the intestinal tract and reduces the absorption of carbohydrates. The significant correlation between antioxidant compounds and antioxidant activity has also been observed. From an analytical point of view, more purple rice content in the flour mixtures gives a higher content of antioxidant compounds. Although the data obtained from ABTS and DPPH scavenging activities (Table 4) were different, both ABTS and DPPH had highly positive correlations with antioxidant compounds among all flour mixtures. Nearly, all other studies found similar results in the correlations between antioxidant compounds and antioxidant activity in rice grains (Sumczynski *et al.*, 2016; Shao *et al.*, 2018).

Conclusion

The purple rice flour with high antioxidant contents can be used as an effective flour to substitute wheat flour to produce healthy bakery products to meet consumers' demand. This study is the first report on the substitution of purple rice flour for wheat flour where the functional properties, antioxidant characteristics of the flours and their correlations have been measured. The functional properties of flour mixtures were changed after substituted purple rice flour into the mixture, which will affect the quality of the products. The substitution of purple rice flour varied the nutritional properties, *in vitro* digestibility, with only small changes in the $L^*a^*b^*$ colour values. Inclusion of increasing purple rice flour in the mixture had a significant effect on increasing the *in vitro* digestibility of protein, which can slow the starch digestion rate on human metabolism. The whole flour from purple rice showed a stronger resistance to starch digestion and a higher SDS fraction compared to wheat flour. *In vitro* starch digestion of whole flour from purple rice has a significantly positive correlation with dietary fibre and antioxidant compounds. This study revealed that the purple rice flour could be an effective alternative flour with a low starch digestibility, low pGI and high antioxidant contents which needs further study to understand behaviour of these flour mixtures in a real food product.

Conflict of interest

The authors declare no conflict of interest.

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