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Research article

Optimization of flour mix from three pigmented rice varieties to produce blended instant mixed rice using response surface methodology

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Abstract

Importance of the work: Pigmented rice has been proven to have superior nutrition, nutraceutical and hypoallergenic characteristics. The development of specialty food products from pigmented rice is of high interest in response to people's shifting lifestyles and the increasing trends toward improved health and nutrition.

Objectives: To optimize the formulation of three pigmented rice flour types to produce blended instant mixed rice using response surface methodology to evaluate its quality attributes.

Materials & Methods: The pigmented rice was processed and prepared into flour using a drum dryer and formulated using a mixture design (d-optimal) with three center points, namely, Hom Hua Bon (18.2–54.5%), Hom Chai Ya (18.2–54.5%) and Riceberry (18.2–36.4%), representing red rice, brown rice and purple rice, respectively.

Results: The regression analysis indicated that the mix of the three pigmented rice varieties significantly affected the bulk density, tapped density and peak viscosity. The optimum amounts of Hom Hua Bon, Hom Chai Ya and Riceberry were 54.5%, 18.2% and 27.3%, respectively, with the optimum response values being 0.55 g/cm^3 for bulk density, 0.61 g/cm^3 for tapped density and 1,886.34 cp for peak viscosity.

Main finding: Hom Hua Bon (red rice) provided an acceptable bulk density, tapped density and peak viscosity making it suitable as an ingredient in instant products, such as a cereal beverage that is desirable in the market.

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Introduction

Nowadays, people's habits in consuming food are highly dependent on their lifestyles that have become more dynamic due to higher job demands (Kirtanayasa and Maliarta, 2021). However, public awareness for healthy living is also rising, which directly affects the rapidly increasing need for nutritious food. For example, in the last decade, food items have not only been used in response to hunger but also have provided major nutrients for human life which can have health benefits by preventing and controlling diseases (Wirivutthikorn, 2020). With the shifting lifestyles and increasing trends in health and nutrition, the innovative development of nutritious food products has become the most important strategy in the food industry (Swaminathan and Guha, 2018). Some agricultural products, such as oat, corn and soybean, have been successfully processed into instant cereal beverage powders (Lee et al., 2018).

Rice contributes around 60–70% of the energy needed in Asian countries (Swaminathan and Guha, 2018). Furthermore, rice is consumed as a staple food by more than one-half of the world's inhabitants, indicating that it is widely available, including pigmented rice, such as red, brown and purple rice (Tangsrianugul et al., 2019). The popularity of rice is continuously increasing due to its nutritional, nutraceutical, gluten-free and hypoallergenic characteristics, which makes it particularly suitable for the preparation of ready-to-use products (Swaminathan and Guha, 2018). Red rice contains 10.5% protein, 3.5% dietary fiber and 25% amylose; brown rice contains 2.6% protein, 3.7% dietary fiber and 20.5% amylose; and purple rice contains 9.3% protein, 1.4% dietary fiber and 23.3% amylose (Ascheri et al., 2012; Settapramote et al., 2018; Veni, 2019; Thongkaew and Singthong, 2020; Farooq et al., 2021). Several studies have suggested that intake of pigmented rice has profound health benefits due to the presence of various antioxidants, such as polyphenols, flavonoids, anthocyanins, vitamin E, phytic acid and γ–oryzanol (Settapramote et al., 2018; Maulani et al., 2019; Veni, 2019). These antioxidant compounds eliminate reactive oxygen species, such as lipid peroxide, superoxide and anion radicals and lower the cholesterol content (Nam et al., 2008).

The development of specialty food products from pigmented rice is the "need of the hour" for superior nutritional and nutraceutical benefits and to widen its utilization. Though reports on various rice products are available in the literature, there is only meager information on the development and quality assessment of instant rice beverages using only rice as the grain source. The use of pigmented rice in the development of instant cereal beverages will provide an additional advantage to the product due to its color and intrinsic aroma. Hence, the objective of the current study was to optimize the formulation of a pigmented rice mixture using response surface methodology (RSM) by evaluating its quality attributes (physical, chemical, pasting and nutritional properties) as a part of a research project aiming to develop an instant cereal beverage from pigmented rice flour.

Materials and Methods

Materials

Different colored rice grains—red rice (Hom Hua Bon), brown rice (Hom Chai Ya) and purple rice (Riceberry)—were purchased from a local market in Hat Yai, Songkhla province, Thailand.

Chemicals

Ethanol (C_2H_5OH), Folin-Ciocalteu, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol (CH_3OH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate ($K_2S_2O_8$), acetate buffer, 2,3,5-triphenyltetrazolium chloride (TPTZ), hydrochloric acid (HCl), iron (III) chloride ($FeCl_2 \cdot 6H_2O$), potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$), copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), sulfuric acid (H_2SO_4) and acetone (C_3H_6O) were analytical grade and purchased from Chemipan Corporation Co. Ltd (Thailand) and Sigma-Aldrich Corporation (USA).

Preparation of mixed pigmented rice flour

The formulation was done using the Design Expert 13.0.0 software (Stat Ease Inc.; USA) with a mixture as the study type, D-optimal as the initial design and quadratic as the design model. Three mixture components were investigated: HHB (low: 18.2%, high: 54.5%), HCY (low: 18.2%, high: 54.5%) and RB (low: 18.2%, high: 36.4%) with a total of 100%. The runs were set to have three center points, one lack of fit and one replication only, resulting in 9 runs (Table 1).

Table 1 Nine formulations resulting from mixture design (d-optimal)

Formula	HHB	HCY	RB
1	54.5	18.2	27.3
2	54.5	27.3	18.2
3	45.4	18.2	36.4
4	27.3	54.5	18.2
5	18.2	54.5	27.3
6	36.4	36.4	27.2
7	36.4	36.4	27.2
8	36.4	36.4	27.2
9	18.2	45.4	36.4

HHB = Hom Hua Bon; HCY = Hom Chai Ya; RB = Riceberry

The mixed pigmented rice was prepared following the method from Wiriyawattana et al. (2018) with slight modification. Each rice sample was cleaned of dust and cooked with water (rice-to-water ratio of 1:15 weight per volume, w/v) at 80 °C for 2 h with occasional stirring. The porridge was processed through a twin drum drying machine (TDD300; PSA21 Limited Partnership; Thailand). The roller diameter and length were 300 mm and 400 mm, respectively. The drum drying conditions were set at 120 °C, with 344737.86 pounds per square inch (Pa) steam pressure and a roller speed of 5 revolutions per minute (rpm). Then, the powder was collected from the tray below the roller drum, ground and passed through a 100-mesh sieve, after which the rice powder was measured and mixed evenly according to Table 1. The physical, chemical, pasting and nutritional properties were analyzed and evaluated to achieve the optimal level of rice mixture.

Physical properties determination

Color measurement

The L* (lightness), a* (red intensity) and b* (yellow intensity) of the mixed rice were measured using a Colorimeter (ColorFlex EZ; HunterLab; USA) with the CIELAB measuring system, illuminant D65 and an observation angle of 108°.

Water solubility index

The water solubility index (WSI) of the mixed rice was determined following the method from Jafari et al. (2017) with slight modification. A sample (2.5 g) of powder was suspended in 30 mL of distilled water (DW) in a centrifuge tube at 25 °C. The suspension was vortex mixed for 1 min and placed in a water bath for 30 min at 37 °C. Then, the tube was centrifuged at 5,500×*g* for 20 min at 4 °C using a refrigerated centrifuge (CR22G III; Hitachi Koki Co., Ltd.; Japan). The supernatant was placed in a tared moisture can and dried at 105 °C in a hot-air oven. Then, the WSI was calculated using Equation 1:

$$\%\text{WSI} = \frac{\text{Weight of dried solids in supernatant}}{\text{Weight of dried solids}} \times 100 \quad (1)$$

Dispersibility

The dispersibility was determined by stirring vigorously 1 g of mixed rice into 10 mL DW for 15 s. Then, the mixed rice was filtered and transferred to a Petri dish and dried in a hot-air oven overnight at 105 °C (modified from Swaminathan and Guha, 2018). The percentage dispersibility was calculated using Equation 2:

$$\%\text{Dispersibility} = (10 + \text{a}) \times \frac{\%\text{TS}}{\text{a}} \times \frac{100\text{b}}{100} \quad (2)$$

where *a* is the amount of powder, *b* is the moisture content of the powder and *TS* is the dry matter of powder.

Bulk and tapped density

The bulk and tapped density were measured following the method of Swaminathan and Guha (2018) with modification. The mixed rice was loaded until it reached the 10 mL mark in a 10 mL graduated cylinder and weighed. The volume was used to calculate the bulk density (BD) based on the mass per volume formula. Then, the cylinder was tapped 25 times with an amplitude of 3 ± 0.33 mm. Based on the mass per final tapped volume formula, the volume read after the tapping process was used for the tapped density (TD) calculation.

Flowability and cohesiveness

The flowability and cohesiveness were measured using the Carr index (CI) and Hausner ratio (HR) by calculating the BD and TD using Equations 3 and Equation 4, respectively (Swaminathan and Guha, 2018):

$$\%\text{CI} = \frac{\text{TD - BD}}{\text{TD}} \times 100 \quad (3)$$

$$\%\text{HR} = \frac{\text{TD}}{\text{BD}} \times 100 \quad (4)$$

where *TD* is the tapped density and *BD* is the bulk density.

Chemical properties determination

Moisture content

The moisture content was measured as described in the standard methods of Association of Official Analytical Chemists (2019).

Total phenolic content, total flavonoid content and antioxidant activity

The sample extractions for the total phenolic content (TPC), the total flavonoid content (TFC) and antioxidant activity (AA, based on DPPH, ABTS and ferric ion reducing antioxidant power (FRAP) analyses, respectively, were performed as described by Samakradhamrongthai et al. (2022) with slight modifications. Each mixed rice formulation (1 g) was extracted with ethanol (10 mL) under 30 min of agitation using a magnetic stirrer and left at room temperature (25 ± 5 °C) overnight. The mixture was filtered using Whatman paper in a Buchner funnel and the extract was stored in an amber-colored bottle at 4 °C until further analyses (TPC, TFC, and AA) utilizing a spectrophotometer (LiRBa S22; Biochrom; UK).

For the TPC, extracted samples (each 0.2 mL) were mixed with 1 mL of 10% Folin-Ciocalteu and 0.8 mL of 7.5% Na_2CO_3 under dark conditions. The mixture was left at room temperature for 45 min and then, the absorbance was read at 765 nm. Gallic acid was used as the standard and the results were expressed as milligrams gallic acid equivalent (GAE)/100 g dried sample.

For the TFC, extracted samples (each 1 mL) were mixed with 300 µL of 5% $NaNO_2$ and 300 µL of 5% $AlCl_3$ under dark conditions. The mixture was left at room temperature for 5 min and directly added with 1 mL of NaOH and DW until it reached 10 mL of solution. Then, the absorbance was read at 510 nm. Catechin was used as the standard and the results were expressed as milligrams catechin equivalent (CE)/100 g dried sample.

The AA was measured using the DPPH, ABTS and FRAP methods where Trolox was used as the standard. The results were expressed as milligrams Trolox equivalent (TE)/100 g dried sample.

In the DPPH radical scavenging assay, the extracted samples (each 150 µL) were mixed with a 3.85 mL DPPH solution made from 2.5 mg of DPPH powder with 100 mL of CH_3OH under dark conditions. Then, the mixture was left for 30 min at room temperature and the absorbance was read at 517 nm.

In the ABTS radical scavenging assay, 7 mM of ABTS in water was mixed with 2.45 mM of $K_2S_2O_8$ and left for 16 h at room temperature. The ABTS solution was diluted with DW (ABTS solution-to-DW ratio 1:10 volume/volume, v/v). Then, 3.85 mL of ABTS solution was mixed with 150 µL of extracted sample and the absorbance was read at 734 nm.

In the FRAP radical scavenging assay, 300 mM acetate buffer solution was mixed with 10 mM of TPTZ in 40 mM of HCl and 20 mM of $FeCl_2 \cdot 6H_2O$ with a ratio of 10:1:1 (v/v/v). The mixture was heated at 37 °C in the water bath for 30 min. Then, 3.85 mL of the solution was mixed with 150 µL of extracted sample and heated again at 37 °C in a water bath for 30 min, after which the absorbance was read at 593 nm.

Pasting properties

The pasting profile was determined using 4 g of flour and 25 mL distilled water using a rapid visco analyzer (RVA; RVA-4 SA, Newport Scientific Pty Ltd.; Australia) with constant stirring at 160 rpm, where the temperature was increased to 50 °C for 60 sec, raised to 93 °C for 5 min, held at 93 °C for 7 min, then decreased to 50 °C in 4 min and held at 50 °C for 3 min. The peak viscosity, breakdown and final viscosity pasting properties were recorded in centipoise (cP) units, while the peak time and pasting temperature were recorded in minutes and degrees celsius, respectively.

Nutritional properties analysis

Gross energy

The gross energy (GE) of the mixed rice was measured using a ballistic bomb calorimeter (Yoshida Seisakusho Co. Ltd; Japan). Each sample (±0.3 g) was placed into a nickel crucible and ignited in the bomb filled with oxygen. The bomb was fired and the galvanometer was stabilized for ±3 min before the result was read.

Crude protein

The protein value was determined using the Lowry method by utilizing ultraviolet absorbance spectroscopy. Each sample was extracted using ethanol following the method from Sadaiah et al. (2018). The 0.1 mL extracted sample was hydrolyzed with 0.1 mL of 2N NaOH at 100 °C for 10 min in a water bath. Reagent I (48 mL of 2% Na_2CO_3, 1 mL of 1% $KNaC_4H_4O_6 \cdot 4H_2O$, 1 mL of 0.5% of $CuSO_4 \cdot 5H_2O$) was added while waiting for the mixture to cool for 10 min. Then, 0.1 mL of Folin reagent was added and vortex mixed. After 30 min at room temperature, the absorbance was read at 550 nm using a spectrophotometer (LiRBa S22; Biochrom; UK).

Crude fiber

The crude fiber content was analyzed using an ANKOM^{200} Fiber Analyzer (ANKOM Technology; USA). The measured fiber bag was filled with 1 g of sample and sealed and the fiber bag was arranged in the container and placed inside the machine. Then, 2 L of 1.25% H_2SO_4 was poured in, agitated and heated at 100 °C for 40 min after which the H_2SO_4 was discarded and the sample was rinsed with hot water three times.

Then, 2 L of 1.25% NaOH was poured in, agitated and heated at 100 °C for 40 min after which the NaOH was discarded and the sample was rinsed with water three times. The sample was removed and rinsed again with acetone before drying overnight in a hot-air oven at 105 °C after which the sample was ashed in a muffle furnace at 600 °C for 4 h.

Estimated glycemic index

The glycemic index was analyzed using a Glucose Assay Kit (GAGO20-1KT; Sigma-Aldrich; USA). The sample was extracted by mixing it with deionized water and heating at 75 °C and stirring magnetically, followed by filtering using Whatman paper to clarify the extracted solution. The extracted sample was mixed with 2 mL assay reagent (0.8 mL of o-dianisidine reagent and 39.2 mL of glucose oxidase/peroxidase reagent) in test tubes. The test tubes were placed in a water bath for 30 min at 37 °C. Then, 2 mL of 6M H2SO4 was added to each test tube to stop the reaction. The absorbance of each samples was read at 540 nm.

Data analysis

All measurements were conducted in triplicate and results were expressed as mean ± SD. The statistical analysis was carried out using the SPSS 11.0 software (IBM Corp.; USA) whereby analysis of variance was performed. Then Duncan's multiple range test was applied. All tests were considered significant at $p < 0.05$. The regression analysis to indicate the optimal content of mixed pigmented rice was identified using the Design Expert 6.0 software.

Results and Discussion

Physical properties

The color (L*, a*, b*), dispersibility, bulk density and tapped density were significantly different among the formulations, in contrast to the WSI, flowability and cohesiveness that were not significantly different (Table 2). The L* value increased significantly by decreasing the amount of RB and increasing the amount of HCY. A similar trend was observed for the a* and b* values that increased along with increasing amounts of HHB and HCY. This occurred due to the quantities of anthocyanin pigment in each pigmented-rice variety. Purple rice has the most cyanidin 3-glucoside (C3G) compared to red and brown rice, which is associated with the blacker color (Maulani et al., 2019; Yamuangmorn and Prom-u-Thai, 2021).

Table 2 Physical properties of nine formulations of mixed pigmented rice

Formula	L*	a*	b*	Water solubility index (%)	Dispersibility (%)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Flowability (%CI)	Cohesiveness (%HR)
1	66.33 ± 0.04^{f}	6.47 ± 0.01^{b}	6.39 ± 0.03^{a}	3.15 ± 0.34	23.43 ± 0.73^{cd}	0.50 ± 0.03^{ab}	0.59 ± 0.03^{cd}	15 ± 1.00	1.18 ± 0.01
2	70.83 ± 0.00^{b}	5.76 ± 0.02^{f}	6.14 ± 0.03^{b}	3.21 ± 0.24	23.06 ± 0.68^{cd}	0.48 ± 0.02^{bc}	0.59 ± 0.01^{cd}	19 ± 1.73	1.23 ± 0.03
3	61.67 ± 0.01^{i}	7.11 ± 0.04^{a}	6.38 ± 0.09^{a}	3.54 ± 0.03	23.94 ± 1.09^{bc}	0.46 ± 0.01^{c}	0.57 ± 0.01^{c}	19.33 ± 1.15	1.24 ± 0.02
4	73.85 ± 0.00^{a}	4.73 ± 0.05^{h}	5.06 ± 0.06^{f}	3.33 ± 0.26	25.71 ± 0.21^{a}	0.50 ± 0.01^{ab}	0.63 ± 0.01^{a}	20.33 ± 0.57	1.26 ± 0.01
5	68.59 ± 0.02^{c}	5.62 ± 0.03^{g}	5.29 ± 0.07^{de}	3.20 ± 0.64	25.11 ± 0.15^{ab}	0.51 ± 0.01^{ab}	0.62 ± 0.01^{a}	18.67 ± 3.21	1.23 ± 0.05
6	67.45 ± 0.01^{e}	6.24 ± 0.04^{c}	5.88 ± 0.05^{c}	3.38 ± 0.05	22.19 ± 0.19^{de}	0.51 ± 0.01^{ab}	0.62 ± 0.01^{a}	18.67 ± 0.58	1.23 ± 0.01
7	67.75 ± 0.0^{d}	6.04 ± 0.08^{d}	5.97 ± 0.10^{c}	3.88 ± 0.23	22.49 ± 0.89^{de}	0.51 ± 0.01^{a}	0.63 ± 0.01^{a}	18.33 ± 0.58	1.22 ± 0.01
8	65.80 ± 0.01^{h}	5.96 ± 0.03^{e}	5.40 ± 0.02^{d}	3.56 ± 0.28	21.47 ± 0.62^{e}	0.50 ± 0.01^{ab}	0.62 ± 0.01^{a}	18.67 ± 1.53	1.23 ± 0.02
9	66.16 ± 0.02^{g}	6.22 ± 0.04^{c}	5.20 ± 0.08^{e}	4.02 ± 0.54	24.10 ± 1.09^{bc}	0.51 ± 0.03^{a}	0.61 ± 0.01^{ab}	16.67 ± 3.05	1.20 ± 0.04
p value	< 0.001	< 0.001	< 0.001	0.061	< 0.001	0.012	< 0.001	0.064	0.069

L* = lightness; a* = red intensity; b* = yellow intensity of mixed rice; CI = Carr's index; HR = Hausner Ratio.
Values (mean ± SD) in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

The dispersibility values were in the range 21.47–15.11%. The increasing amount of HCY increased the dispersibility, indicating that HCY had the best dispersibility among the pigmented rice samples. The dispersibility determines the tendency of flour to distribute in water molecules and to reconstitute (Ashogbon and Akintayo, 2012; Joy and Ledogo, 2016). According to Goalard et al. (2006), the dispersibility of powdered products is highly correlated with the wettability, where the fat content is one of the main factors. A higher amount of fat negatively affects the wettability as fat is hydrophobic, which was consistent with the current findings, as HCY had the lowest fat content (Veni, 2019). The bulk and tapped densities were in the ranges 0.46–0.51 g/cm^3 and 0.59–0.63 g/cm^3, respectively. Higher amounts of HCY caused the formula to have higher bulk and tapped density values due to HCY (brown rice) having the highest fiber content compared to HHB and RB (see Table 5). In addition, both the dispersibility and density were highly affected by the particle size, indicating that HCY had a finer particle size compared to the other pigmented-rice flour samples. The mixed rice could be classified with a good level of flowability and an intermediate level of cohesiveness (around 15–20% and 1.2–1.26%, respectively, according to Jinapong et al. (2008)), except for the formula with the highest HHB content, which only reached 1.18% in cohesiveness.

Chemical properties

The moisture contents of the samples ranged were in the range 2.23–2.62%, with no significant differences, as all the formulas had the same thermal treatment during the drum drying. However, the total phenolic, flavonoid and antioxidant activities differed significantly between the formulations (Table 3). The total phenolic contents were in the range 388.90–547.04 mg GAE/100g sample and the total flavonoid contents were in the range 48.81–89.72 CE/100g sample. Based on the results, the formula with the highest phenolic and flavonoid contents (54.5% HHB, 18.2% HCY, 27.3% RB) had a higher HHB content and the formula with the lowest phenolic content (18.2% HHB, 45.4% HCY, 36.4% RB) and lowest flavonoid content (18.2% HHB, 54.5% HCY, 27.3% RB) had the lowest HHB, indicating that the HHB content in the formula greatly affected the total phenolic and flavonoid contents. Regarding the antioxidant activity of the nine tested formulations, the DPPH results were in the range 74.88–58.35 mg TE/100 g sample, the FRAP results were in the range 258.76–382.08 mg TE/100g sample and the ABTS results were in the range 114.37–162.79 mg TE/100g sample.

Table 3 Chemical properties of nine formulations of mixed pigmented rice

Formula	Moisture content (%)	Total phenolic content (mg GAE/100 g sample)	Total flavonoid content (mg CE/100 g sample)	Antioxidant activities (mg TE/100 g sample)		
				DPPH	FRAP	ABTS
1	2.43 ± 0.51	547.04 ± 10.12^{a}	89.72 ± 6.23^{a}	62.33 ± 1.33^{cd}	382.08 ± 24.09^{a}	158.62 ± 2.39^{a}
2	2.45 ± 0.08	539.42 ± 2.0^{ab}	88.54 ± 0.89^{a}	58.35 ± 1.26^{d}	364.9 ± 10.23^{ab}	162.79 ± 9.00^{a}
3	2.55 ± 0.19	528.79 ± 23.7^{ab}	86.95 ± 4.42^{ab}	67.08 ± 2.58^{b}	368.25 ± 13.63^{ab}	155.04 ± 15.42^{a}
4	2.61 ± 0.59	440.58 ± 8.36^{c}	68.09 ± 2.04^{c}	61.73 ± 2.07^{cd}	295.18 ± 7.74^{c}	120.66 ± 3.03^{bc}
5	2.59 ± 0.27	416.22 ± 15.82^{d}	48.81 ± 1.33^{d}	65.08 ± 2.43^{bc}	258.76 ± 19.40^{d}	118.99 ± 3.09^{c}
6	2.29 ± 0.57	517.66 ± 19.31^{b}	84.74 ± 0.95^{ab}	67.34 ± 3.09^{b}	353.62 ± 6.65^{b}	130.80 ± 1.44^{b}
7	2.23 ± 0.39	520.01 ± 14.37^{b}	81.29 ± 3.62^{b}	69.40 ± 3.11^{b}	358.09 ± 4.07^{ab}	132.10 ± 5.79^{b}
8	2.24 ± 0.21	514.73 ± 10.57^{b}	83.81 ± 3.37^{ab}	65.67 ± 2.07^{bc}	358.19 ± 6.95^{ab}	131.36 ± 0.82^{b}
9	2.62 ± 0.56	388.90 ± 3.5^{e}	52.90 ± 1.35^{d}	74.88 ± 3.44^{a}	270.26 ± 12.71^{d}	114.37 ± 2.45^{c}
p value	0.889	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

GAE = gallic acid equivalent; CE = catechin equivalent; TE = Trolox equivalent; DPPH = 2,2-diphenyl-1-picrylhydrazyl assay; FRAP = Ferric Reducing Antioxidant Power Assay; ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid assay.

Values (mean ± SD) in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

These results indicated that a higher HHB content produced higher FRAP and ABTS results, whereas, in contrast, the DPPH result was the lowest in the formulation with the highest HHB content. Thus, HHB was also high in FRAP and ABTS activity, while HCY and RB were high in DPPH activity, with the formula with the highest DPPH (18.2% HHB, 45.4% HCY, 36.4% RB) having high amounts of HCY and RB. The contradictory results from the ABTS, FRAP and DPPH assays were due to the different types of antioxidants contained in each rice. Brown and purple rice have an abundant amount of anthocyanidin, while red rice is rich in proanthocyanidin (Francavilla and Joye, 2020; Maulani et al., 2019). According to Chen et al. (2014), proanthocyanidin has a significant correlation with DPPH scavenging activity, which was consistent with the current results, where only DPPH was high in the high HHB formula.

Pasting properties

The peak viscosity, breakdown and final viscosity were significantly different among the formulations, with ranges of 1,861.67–2,558 cP, 343.33–764.33 cP and 3,390.67–4,187.67 cP, respectively (Table 4). The highest viscosity (54.5% HHB, 27.3% HCY, 18.2% RB) was from the formula with a higher content of HHB and a lower content of RB, while the lowest viscosity (18.2% HHB, 45.4% HCY, 36.4% RB) was from the formula with a lower content of HHB and a higher content of RB. These results were due to the higher amylopectin content in red rice compared to the other pigmented rice samples because amylopectin, a highly branched starch molecule, is responsible for the viscosity of a product (Juhász and Salgó, 2008; Ascheri et al., 2012; Thilakarathna et al., 2017). There results were consistent with Wiriyawattana et al. (2018) who reported that foods with a high amylose content tended to retrograde rapidly during the cooling process. Xu et al. (2019) reported that proanthocyanidins have a strong starch-binding capacity, thereby increasing the paste viscosity as is common in red rice.

The peak time and pasting temperature were significantly different for the formulations within the ranges 6.94–7.53 min and 89.94–90.78°C, respectively (Table 4). The formulation with the highest peak time (18.2% HHB, 45.4% HCY and 36.4% RB) had the highest HHB, while the formulation with the lowest peak time (54.5% HHB, 18.2% HCY, 27.3% RB) had the highest HHB value. This was related to the amylopectin content in the rice with the higher the amylopectin, the lower the peak time as the branched molecules have a weaker hydrogen bond (Juhász and Salgó, 2008). Similar to the peak time result, the formulation with the highest pasting temperature (10 HHB, 25 HCY, 20 RB) had the lowest HHB content, in contrast to the formulation with the lowest pasting temperature (54.5% HHB, 27.3% HCY, 18.2% RB) that had the highest HHB content. A high pasting temperature indicates that the starches have high resistance to swelling and rupture which is related to the amylose content of the rice. A high pasting temperature reflects the higher the amylose content with greater hydrogen bonding interactions (Biduski et al., 2018; Sadimantara et al., 2019).

Nutritional properties

The gross energy and crude protein were not significantly different among the formulations, in contrast with the crude fiber and estimated glycemic index that were significantly different among the samples (Table 5). The crude fiber content varied in the range 3.70–4.85%, with the highest crude fiber content in the formula (27.3% HHB, 54.5% HCY, 18.2% RB), while the highest amount of HCY and the lowest crude fiber content was in the formula (54.5% HHB, 18.2% HCY, 27.3% RB) with the lowest amount of HCY. Thus, HCY appeared to have a greater amount of fiber among the pigmented rice samples, as the red rice (HHB), brown rice (HCY) and purple rice (RB) had 0.7%, 1.8% and 0.7% fiber content respectively.

Table 4 Pasting properties of nine formulations of mixed pigmented rice

Formula	Peak viscosity (cP)	Beakdown (cP)	Final viscosity (cP)	Peak time (min)	Pasting temperature (°C)
1	1,946.33 ± 1.53[f]	412.00 ± 2.65[f]	3,717.33 ± 14.15[g]	6.94 ± 0.06[d]	90.63 ± 0.23[a]
2	2,558.00 ± 33.15[a]	764.33 ± 4.04[e]	4,187.67 ± 11.50[a]	6.98 ± 0.40[d]	89.94 ± 0.34[b]
3	2,350.33 ± 13.32[b]	589.00 ± 8.18[c]	4,060.00 ± 40.73[b]	7.30 ± 0.10[bc]	90.55 ± 0.33[ab]
4	2,363.33 ± 8.08[b]	581.33 ± 5.03[c]	3,959.00 ± 22.54[d]	7.13 ± 0.06[bcd]	90.13 ± 0.06[ab]
5	2,260.33 ± 35.13[d]	504.00 ± 1.00[d]	3,838.67 ± 19.29[f]	7.08 ± 0.07[cd]	90.33 ± 0.75[ab]
6	2,122.33 ± 14.19[e]	448.00 ± 16.37[e]	3,745.67 ± 3.055[g]	7.32 ± 0.07[b]	90.50 ± 0.39[ab]
7	2,311.33 ± 2.08[c]	576.33 ± 7.02[c]	3,900.67 ± 9.61[e]	7.14 ± 0.05[bcd]	90.75 ± 0.17[a]
8	2,376.00 ± 33.78[b]	687.00 ± 4.58[b]	4,022.00 ± 27.18[c]	7.13 ± 0.20[bcd]	90.22 ± 0.08[ab]
9	1,861.67 ± 17.24[g]	343.33 ± 2.08[g]	3,390.67 ± 4.16[h]	7.53 ± 0.31[a]	90.78 ± 0.25[a]
p value	0.023	0.018	0.023	0.012	0.012

Values (mean ± SD) in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 5 Nutritional properties of nine formulations of mixed pigmented rice

Formula	Energy (Kcal/ 100g)	Crude protein (%)	Crude fiber (%)	Estimated glycemic index
1	510.93 ± 9.89	7.03 ± 0.85	3.70 ± 0.19^{g}	40.91 ± 1.13^{f}
2	489.24 ± 11.13	7.29 ± 0.29	3.91 ± 0.09^{efg}	43.39 ± 0.56^{f}
3	504.85 ± 32.52	6.77 ± 0.02	3.86 ± 0.14^{fg}	52.15 ± 2.30^{d}
4	506.27 ± 6.09	6.76 ± 0.67	4.85 ± 0.14^{a}	62.22 ± 6.77^{c}
5	505.60 ± 17.07	7.26 ± 0.28	4.44 ± 0.05^{bc}	55.34 ± 3.45^{d}
6	491.68 ± 6.05	7.05 ± 0.33	4.33 ± 0.16^{cd}	75.42 ± 1.15^{a}
7	496.00 ± 20.67	7.31 ± 0.18	4.21 ± 0.21^{cde}	73.18 ± 1.15^{ab}
8	494.45 ± 49.79	7.18 ± 0.22	4.12 ± 0.08^{def}	70.27 ± 2.21^{b}
9	486.85 ± 28.93	7.24 ± 0.10	4.64 ± 0.28^{ab}	48.50 ± 2.88^{e}
p value	0.921	0.604	< 0.001	< 0.001

Values (mean ± SD) in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

The estimated glycemic index (GI) was in the range 75.42–40.91, with an increase in HHB resulting in a lower GI, while an increase in HCY produced an increase in the GI. HCY had the highest GI compared to the other pigmented rice samples. Kabir et al. (2021) stated that there is an inverse relationship between GI and amylose, which was supported by the current results as the amylose contents of the red rice (HHB), brown rice (HCY) and purple rice (RB) were 25%, 20.5% and 23.3% respectively (Ascheri et al., 2012; Thongkaew and Singthong, 2020; Farooq et al., 2021).

Regression model fitting for mixed pigmented rice optimization

The optimization of mixed pigmented rice flour was generated using a regression equation and contour plots for the significant responses (see Table 6), with A, B and C as HHB, HCY and RB, respectively. The regression equation of significant responses was generated from the contour plots as shown in Fig. 1. The analysis indicated that only the bulk density, tapped density and peak viscosity were involved in the optimization of the mixed pigmented rice based on fitting a quadratic model.

Table 6 Regression equations for responses of mixed pigmented rice based on response surface methodology

Response	Regression equation	R squared	*p* value
Bulk density (g/cm^3)	+0.007×A+0.009×B-0.01×C-0.00002×AB+0.0006×C+0.0005×BC	0.9212	0.0168
Tapped density (g/cm^3)	+0.007×A+0.009×B-0.0009×C+0.0002×AB+0.0005×AC+0.0003×BC	0.9131	0.0194
Peak viscosity (cP)	-3.86×A+37.48×B+343.28×C+4.29×AB-7.35×AC-9.99×BC	0.9289	0.0144

A = Hom Hua Bon (HHB); B = Hom Chai Ya (HCY); C = Riceberry (RB)

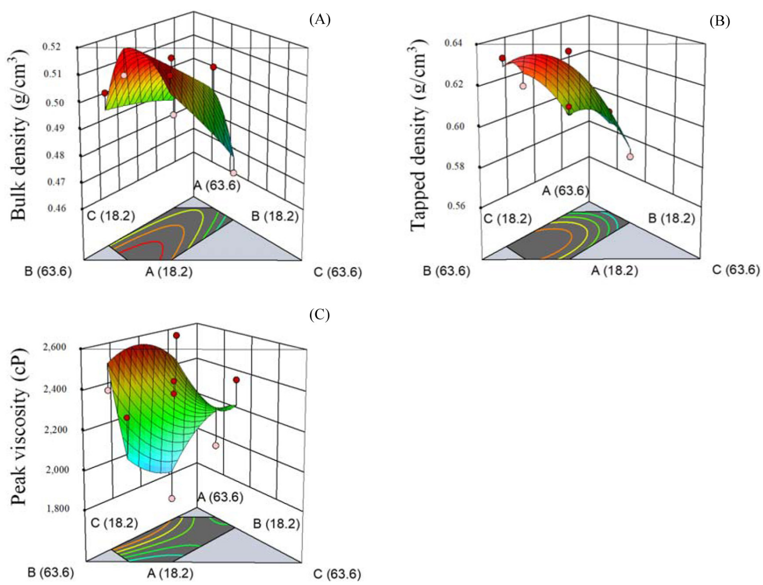

Fig. 1 Response surface showing regression model between HHB: HCY and RB: (A) bulk density; (B) tapped density; (C) peak viscosity; Red and yellow dots in the axis show the maximum and minimum levels of factors used in the present study.

Increasing the HHB and HCY contents increased the bulk density and tapped density (see Figs. 1A and 1B). Both interactions of HHB with RB and HCY with RB increased the bulk density. The mixture of HHB, HCY and RB influenced the tapped density. The peak viscosity increased by increasing the amount of HCY and RB; however, the combination of HHB and HCY also increased the peak viscosity (see Fig. 1C).

The RSM of all significant responses was overlaid to optimize the content of mixed pigmented rice. The optimized contents of HHB, HCY and RB were 54.5%, 18.2% and 27.3%, respectively. The approximation error from the observation value from the optimized mixed pigmented rice was in the range 2.89–9.70%, which showed the optimization prediction could provide validation for optimizing the mixed pigmented rice as the percentage of error was lower than 10% (Table 7).

Table 7 Validation of mixed pigmented rice from responses with approximation error

Response	Prediction value	Observation value	Approximation error (%)
Bulk density (g/cm^3)	0.55	0.47 ± 0.02	9.70
Tapped density (g/cm^3)	0.61	0.60 ± 0.03	2.89
Peak viscosity (cP)	1,886.34	1,946.33 ± 1.53	3.08

The observation values expressed as mean ± SD of three replicates.

In conclusion, this research confirmed that it was possible to develop an instant cereal beverage with satisfactory quality properties using pigmented rice. The optimization process showed the optimal amounts of HHB (red rice), HCY (brown rice) and RB (purple rice) to create an acceptable product were 54.5%, 18.2% and 27.3%, respectively. The mixed rice made from the optimized formula had a bulk density of 0.47g/cm^3 a tapped density of 0.60 g/cm^3 and peak viscosity of 1946.33 cP. Instant cereal beverages made from red rice, brown rice and purple rice could be potentially novel food product offering desirable physical, chemical, pasting and nutritional properties.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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