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Comparison of bioactive compounds, chemical properties and physical properties of Dawk Kha rice flour and wheat flour

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Abstract

Thailand has produced less wheat flour than ever before. The reason behind this is the increased manufacturing costs of approximately 3.6%, making wheat flour prices go up even higher. In order to address this problem, this research aimed to replace wheat flour with Dawk Kha rice flour as a more inexpensive alternative. Thus, the objectives of this research study were to determine and compare the bioactive compounds, chemical properties, and physical properties of Dawk Kha rice flour and wheat flour to determine if Dawk Kha rice flour is an acceptable flour alternative. The chemical properties of Dawk Kha rice flour possessed a higher content of ash, carbohydrates, dietary fiber, and fat when compared to wheat flour. However, wheat flour tended to contain higher protein content, amylose, and water activity. While measurement of moisture content revealed that the moisture of Dawk Kha rice flour (11.1%) was not significantly different compared with wheat flour (11.4%). Regarding the physical properties of wheat flour and Dawk Kha rice flour, wheat flour seemed to be lighter in color, ranging from white to light yellow, contain fine powder, have agreeable odor characteristics, and free of any impurities. To investigate the starch pasting properties of the two types of flour, the rapid visco analyser (RVA)method was applied. Dawk Kha rice flour began to be cooked at 87°C, whereas wheat flour was at 81°C, making Dawk Kha rice flour more retrogradable than wheat flour. Finally, there was a statistically significant difference ($P<0.05$) between bioactive compounds (phenolic compounds and anthocyanin compounds) between Dawk Kha rice flour and wheat flour. Based on this research, Dawk Kha rice flour can be used as an alternative flour source within the food industry, in bakery products, rice-flour noodles, nutritional enrichment for functional food and to serve as basic information for further application.

1. Introduction

Pigmented rice varieties (Hom-Mali Dang or red jusmin rice, Sang Yod, Riceberry, Sri-Nin, Khao Dawk Kha, and Kullakar) grow in Da, especially Thailand, and Tamil Nadu, India. These are grown in sandy clay, except Khao Dawk Kha is a traditional upland rice of Southern Thailand. Colored rice has a distinctive kernel color, including purple, black, red, and reddish-brown, which are also scattered inside its rice bran. The pigmented rice contains high levels of anthocyanin, oryzanol, vitamins, minerals, and lower levels of sugar, fat, and gluten. Furthermore, they contain dietary precursors of bioactive compounds that function in biological activities with health benefits (Ghasemzadeh *et al*., 2018; Kowsalya *et al*., 2022).

“Khao Dawk Kha” rice, also known as “Khao Rai Dawk Kha” rice, has been widely known in Thailand as one of the best upland rice breeds, which originated in Phangnga province, as a geographical indication (GI) product. The fragrant aroma, tender texture, and delicious flavor of Khao Dawk Kha" rice are indicative of its exceptional cooking quality. According to Thailand’s Rice Department (2020), the recommended basic water to “Khao Dawk Kha” rice ratio was 1 cup of water to 1.2 cups of rice. Khao Dawk Kha is a photoperiod-sensitive variety yielding 332–400 kg per rai, which is mainly produced in Phangnga province, specifically in Bang Thong sub-district of Thai Mueang district, and Tark Dad sub-district of Mueang district. Harvesting is typically expected to begin between November and December each year. Because the native genotypes of “Khao Dawk Kha” rice has the ability to be resistant to plant diseases and produce long grains of

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high quality, rice producers in the area want to grow it. Khao Dawk Kha's exceptional cooking qualities are distinguished by their enticing scent, smooth texture and mouthwatering flavor.

Wheat flour is a powder essential for making most baked goods. Interestingly, there are no other types of flour that can substitute for wheat flour, which is distinguished by the two types of proteins it contains, including gluten and gliadin. When wheat flour is mixed with water, the two proteins combine to form gluten, which leaves it unique in terms of its viscoelastic and adhesive properties. In addition, when heated, gluten properties tend to give the dough its elasticity, help it rise, keep its shape, and frequently leave the final product with a spongey structure, as mentioned in the study by Jammek and Naivikul (2011).

Dawk Kha rice flour possesses an important role in the development of new value-added products that tend to raise income for farmers and reduce the dependence on rice cultivation; thus, the objectives of this research were to determine and compare the bioactive compounds, chemical properties, and physical properties of Dawk Kha rice flour and wheat flour.

2. Materials and methods

2.1 Materials

Samples of Dawk Kha rice were obtained from members of the Baan Rae Village Fund and Urban Community in Bang Thong Subdistrict, Thai Mueang District, Phangnga Province. Wheat flour purchased from UFM Food Centre Co., Ltd., located in Samut Prakarn province, was also used in the experimental trials.

2.2 Preparation of Dawk Kha rice flour

The Dawk Kha rice was processed into flour using the standard method of dry milling in a multi-purpose milling machine of the Huangcheng EP200 model. After completing the procedure, Dawk Kha rice flour would be sieved in a 100-mesh flour sifter and packed in a polyethylene (PE) bag and stored at room temperature (4°C) until the time of use, as described by Boonying *et al*. (2009).

2.3 Determination of chemical and physical properties of Dawk Kha rice flour and wheat flour

2.3.1 Moisture content

For the determination of moisture content, a crucible with its cover was preheated (105°C) in a hot air oven, cooled in desiccators and weighed. Sample (2 g) was placed in the crucible, loosely covered and heated in the hot air oven at 105°C until a final constant weight. The crucibles were cooled down in the desiccators and the final weight was noted. Moisture content (%) was calculated using the following equation.

Moisture content (%) = (Initial weight of sample-weight of dried sample)/(Initial weight of sample) × 100

2.3.2 Protein content

Protein content was determined by calculating the total nitrogen content of the sample using the Kjeldahl method. In the Kjeldahl digestion tube, sample (0.5 g), concentrated H_2SO_4 (10 mL) and catalyst mixture ($CuSO_4$:K_2SO_4, 0.5:1 w/w) (1 g) were added. The sample was then digested in a digester in three settings (1.5 hrs at 150°C, 1 hr at 250°C and 2 hrs at 450°C) until a light green solution was obtained. The digested mixture was allowed to cool down and distilled water (10 mL) was added to it. It was then taken to the distillation unit, and distilled with NaOH solution (40%, 85 mL). The distillate was collected into boric acid solution (4, 25 mL) along with mixed indicator (mixture of bromocresol green and methyl red of concentration 0.1% w/v). Finally, the distillate was titrated with HCl (0.1 N) to the final pink-red color change. The total nitrogen content and protein content were calculated using equations.

N (%) = (14.01 × mL of titrant of sample-mL of blank × M of std.HCl acid)/(g of sample × 10)

Crude protein (%) = % N × factor

2.3.3 Fat content

The fat content of the sample was determined using the Soxhlet system (Model HT6, Tecator, Sweden). Sample (2 g) was packed in Whatman No. 1 filter paper and placed inside the thimble. Petroleum ether (60 mL) was kept in extraction cup and the Soxhlet system was set in 3 stages i.e. immersion stage (110°C and 30 mins), washing stage (30 mins) and recovery stage (60 mins). After the completion of extraction time, the residual petroleum ether was removed by drying the extraction cup in the oven (100°C). The cup was cooled in a desiccator and the final weight was noted. The fat content of the sample was determined using the following equation.

Fat content (%) = (Weight of sample of fat)/(Initial weight of sample)×100

2.3.4 Crude fiber

For crude fiber determination, the mixture of sample (2 g) and con. H_2SO_4 (200 mL) was heated (100°C for 30 mins) with shaking. The heated mixture was cooled to room temperature (25°C), filtered to remove the residue and mixed with NaOH solution (1.25% w/v, 200 mL).

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The process of heating, cooling and residue separation was repeated. Finally, the residue was washed by hot water and ethanol (95%) and dried in a hot air oven at 130°C to remove the remaining moisture. The residue was cooled in a desiccator, weight was noted and then incinerated at 400°C. The final weight of incinerated sample was noted, and crude fiber content was determined using the following equation.

Crude fiber (%) = (Weight loss in incineration)/(Initial weight of sample)×100

2.3.5 Carbohydrate content

The carbohydrate content of sample determined by subtracting ash, protein, fat, and crude fiber from 100.

2.3.6 Amylose content

The amylose content of the sample was determined by following the method as described by Juliano (1971), which is based on the calorimetry determination of formation of complex between amylose and iodine. The mixture of the sample (100 mg), ethanol (1 mL) and 1N sodium hydroxide (9 mL) was heated (100°C for 10 mins), cooled to room temperature (25°C) and adjusted to final volume 100 mL with distilled water. To this starch solution (5 mL), 1N acetic acid (1 mL) and iodine solution (2 mL) were added and adjusted to final volume 100 mL with distilled water. The mixture was incubated (25°C, for 20 mins) and the absorbance was measured at 620 nm with a spectrophotometer (Gene Quant 1300, USA) against the diluted iodine reagent (1 mL iodine reagent diluted to 50 mL) as blank solution. The potato amylose at different concentrations was treated the same as the sample and the standard curve of absorbance versus amylose concentration was plotted for the interpolation of the amylose content of the sample.

2.3.7 Water activity

Water activity (aw) of the samples was measured at 25°C using a water activity meter (Aqua Lab, 4TEV, USA). Approximately 0.5 g of each sample was transferred to a sample holder equipped with a relative humidity probe. Water activity values were recorded at relative humidity equilibration.

2.3.8 Color

Color spectra of the sample were determined by using a Hunter-Lab spectrophotometer (Color Flex: 45/0, Reston, USA). Sample (10 g) was placed in the glass sample container put kept over the slit of the instrument. The color was measured in terms of CIELab system where L* = 0 indicates black, while 100 indicates white, a* = negative value indicates greenness and positive value indicates redness and b* = negative value indicates blueness and positive value indicates yellowness (Nasrin and Anal, 2014).

2.3.9 Scanning electron microscopy

Scanning electron microscope (SEM) (JSM 6310F, Tokyo, Japan) was used to determine the microstructural image of Dawk Kha rice flour and wheat flour samples. The sample particles were sprayed over the adhesive carbon tape and placed in a vacuum chamber maintained at a vacuum of 8 Pa, plasma current of 15 mA and then splattered with the gold particles (90 s). The stub with gold splattered samples was then kept inside the SEM chamber for imaging and the images were analyzed at 6000× resolution.

2.3.10 Rapid visco analyser analysis

A rapid visco analyser (RVA) NEWPORT, Model RVA-4, USA) was used for determining the pasting properties. The samples (3 mg) were placed in a canister and added with 25 mL distilled water. Viscosity profiles were recorded under setting conditions as holding temperature at 50°C, for 1 min; then raised to 95°C at the rate of 1.5°C/min and kept for 20 mins. The sample was then cooled down to 50°C at the rate of 1.5°C/min. Peak viscosity, trough viscosity, final viscosity, setback, and pasting temperature were recorded.

2.4 Determination of bioactive compound profiles of Dawk Kha rice flour and wheat flour

Total phenolic was extracted following the method of Waterman and Mole (1994). Approximately 1 g of Dawk Kha rice flour and wheat flour were separately extracted with 10 mL of 80% methanol in distilled water. The mixture was continuously shaken for 30 mins at room temperature, and the extracted mixture was passed through the filter paper (Whatman No. 4). Following the general procedure, 1 mL of the filtered solution was mixed with 5 mL of 10% Folin-Ciocalteu reagent in distilled water and shaken. After 10 mins, 4 mL of 7.5% Na_2CO_3 solution was added. The mixture was mixed thoroughly and in the dark at room temperature for 2 hrs. The absorbance was measured by spectrophotometer at 765 nm. The data were expressed as mg gallic acid equivalents (GAE)/g dry matter, based on the calibration curve of gallic acid.

Total anthocyanin content in Dawk Kha rice flour and wheat flour was determined by the spectrophotometric method with slight modification according to Abdel-Aal and Hucl (1999). Anthocyanins were extracted from 0.5 g of Dawk Kha rice flour and wheat flour using acidified methanol (methanol and 1.0 N HCl, 85:15, v/v). Extracts were centrifuged at 27,200×*g* for 15 mins and repeated three more times,

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then the supernatants were combined. The extracts were filtered through a 0.45 μm filter and absorbance was measured at 535 nm. The total anthocyanin contents of the samples were calculated as mg of cyanidin-3-glucoside equivalent (Cy-3-G) per 100 g of sample.

2.5 Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the SPSS statistics program for Windows Version 21 (SPSS Inc., USA). Duncan's new multiple range tests were used to identify differences between treatments at a 5% significance level ($p<0.05$).

3. Results

3.1 Chemical properties of Dawk Kha rice flour and wheat flour

The chemical compositions of Dawk Kha rice flour and wheat flour, such as moisture content, protein content, fat content, ash, fiber, carbohydrate content, water activities, and amounts of amylose, are presented in Table 1. Statistically significant differences ($P<0.05$) are found in the chemical compositions between Dawk Kha rice flour and wheat flour (Table 1). Dawk Kha rice flour had a higher fat content, carbohydrate content, and ash. In contrast, wheat flour contained a higher protein content, moisture content, amounts of amylose, fiber, and water activities when compared. These findings were in comparable to the previous report of Thailand's Rice Science Center (2016) describing the chemical properties of Dawk Kha rice flour having 2.75% db of fat, 7.82% db of protein content, 73.73% db of carbohydrate and 3.90% db of fiber. Interestingly, the carbohydrate content found in the study was similar to that of the Sinin rice flour substituted bread dough at 78.13% (Phuphechr *et al*., 2009) and glutinous purple rice at 76.85% (Chokchaithanawiwat *et al*., 2019). The ash and moisture content in Dawk Kha rice flour were similar to those found in previous work (Sirichokworrakit *et al*., 2015) at 1.13% and 10.94%, respectively. Dawk Kha rice flour's protein content was found to be higher than in wheat flour (Han *et al*., 2011; Mamat and Hill, 2018). Protein content in each variety of rice is variable (Sukhonthara, 2016). Protein extracted from rice flour with lower protein contents had a lower ability to absorb moisture, which created a dryer texture that lacked firmness (Narkrugsa, 1992).

Table 1. Chemical properties of Dawk Kha rice flour and wheat flour.

Quality	Dawk Kha rice flour	Wheat flour
Fat	$2.34±0.33^a$	$1.26±0.11^b$
Protein	$7.21±0.60^b$	$10.5±0.89^a$
Carbohydrate	$78.3±2.04^a$	$76.4±1.70^b$
Ash	$1.01±0.04^a$	$0.49±0.03^b$
$Moisture^{ns}$	11.10±0.26	11.40±0.40
Amylose	$24.00±0.50^b$	$26.9±1.01^a$
Fiber	$1.71±0.02^b$	$2.46±0.10^a$
a_w^{ns}	0.53±0.03	0.54±0.01

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$). ^{ns} not significant

Rice starch generally contains 60 to 90% amylopectin, however, the study by Juliano (1971) found the amylose content was 15-30% in Dawk Kha variety and 23.33% amylose in Riceberry rice flour (Thongkaew and Singthong, 2020). Thus, Dawk Kha rice flour comprising 24% amylose was an ideal ingredient for wheat flour substitute. There is a possibility to combine wheat flour with Dawk Kha rice flour to further improve the flour texture of the product and to meet the consumer's expectations (Phuphechr *et al*., 2009).

The moisture content between Dawk Kha rice flour and wheat flour is similar ($P<0.05$) having a moisture content of 11.1% and 11.4%, respectively. These results are in accordance with the Industrial Product Standards Act 375-B.E. 2560 describing the standard optimum moisture content of wheat flour being no higher than 14% by weight.

The low moisture content of the flour is significant in terms of lowering the activity of microorganisms, increasing its shelf life, and improving baking quality. Phuphechr *et al*. (2009) revealed that Dawk Kha rice flour and Sri-Nin rice flour tended to have similar moisture content and water activity of 11.46% and 0.57%, respectively.

3.2 Physical properties of Dawk Kha rice flour and wheat flour

Table 2 presents the color values of the flours L* a* b* obtained, while Figure 1 illustrates their results. In Figure 2, the differences between the particle size and shape of the flours obtained by Scanning Electron Microscope (SEM) are shown, whereas the results of the pasting properties of the flours are shown in Table 3. Based on Table 2 and Figure 1, using the CIE L* a* b* scale, the results of the color analysis of Dawk Kha rice flour and wheat flour are shown as follows.

3.2.1 L a* b* color values of flour*

Color differences between flour types were determined using the value L*a*b. Dawk Kha rice flour tended to possess a slightly darker color compared to wheat flour. The wheat was required to be yellowish white with a neutral odor and without extraneous materials, which is in accordance with Thailand Industrial Standard (TIS). Dawk Kha rice flour, on the other hand, gave it a darker, lighter brown color and a neutral odor. When their textures were compared, wheat flour was likely to be finer than Dawk Kha rice flour.

The parameter L* a* b* values of Dawk Kha rice flour and wheat flour Table 2. Regarding the lightness analysis of wheat flour, L* values of 93.52 were found, which were similar to those in the work of (Panya, 2002), while the a* value and the b* value was 0.28 and 6.78, respectively. When compared to wheat flour, Dawk Kha rice flour had a tendency for lower L* values (P<0.05) but seemed to possess higher a* and b* values (P<0.05). This indication may result from the existence of anthocyanin when the a* values of Dawk Kha rice flour were higher.

Table 2. Color parameters of Dawk Kha rice flour and wheat flour.

Quality	Dawk Kha rice flour	Wheat flour
Color values		
L*	79.08±0.05^{b}	93.52±0.43^{a}
a*	5.77±0.33^{a}	0.28±0.03^{b}
b*	9.27±0.01^{a}	6.78±0.17^{b}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different (p<0.05).

3.2.2 Scanning electron microscope of particle and size of Dawk Kha rice flour and wheat flour fractions

Dawk Kha rice flour showed the presence of an uneven surface of starch granules with a high-density of small crystalline starch granules attached. The cause of this event is likely its low moisture content. The particle size is important to the physical properties of flours. Dawk Kha rice flour shows small round, polygonal and truncated shapes, that it is similar to maize flour, unlike wheat flour, which is round and bimodal in shape (Zobel and Stephen, 2006; Mamat and Hill, 2018). Wheat flour contained two types of starch granules: a smaller size with 0.5–10 microns of diameter and a larger size with 10-45 microns of diameter. The larger starch granules accounted for 90% of the total weight of wheat flour. Wheat flour granules were circular and occasionally oval in shape, whereas rice flour granules were flatter with edges (Maningat and Seib, 1992). The structure and orientation of Dawk Kha rice flour differ from wheat flour by texture composition, which has high fiber and

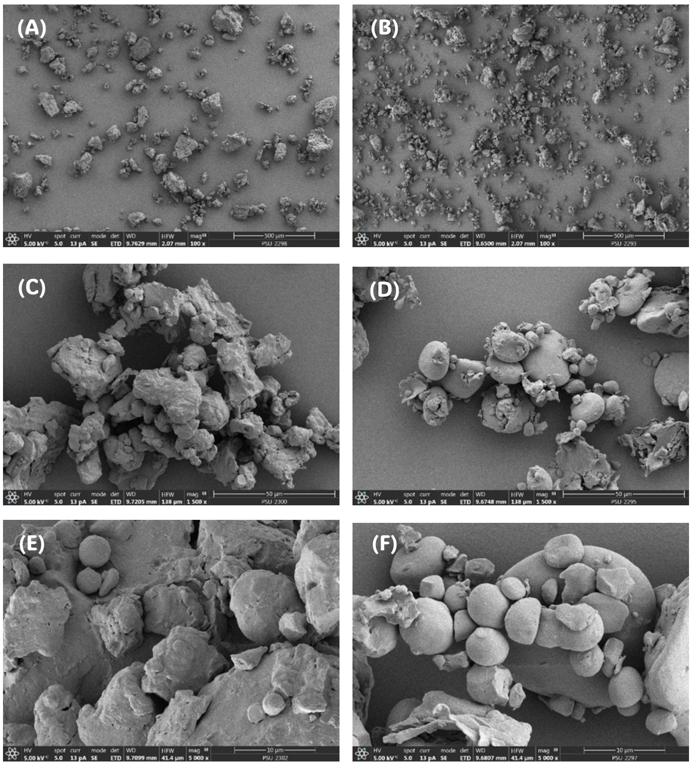

Figure 1. Scanning Electron Microscope (SEM) of Dawk Kha riceflour magnified (A)100×, (B)1,500× (C) 5,000× and wheat flour magnified (D)100×, (E)1,500×,(F)5,000×.

phytochemical constituents within brown rice flour and the study in Riceberry flour by Thongkaew and Singthong (2020). Physical and chemical characteristics such as particle size by milling method, endogenous protein, lipid content, amylose and amylopectin composition can affect the functionality of rice flour (Kim, 2013; Anugrahati *et al*., 2017; Hasmadi *et al*., 2020; Ronie and Hasmadi, 2022).

3.2.3 Pasting properties of Dawk Kha rice flour and wheat flour

In Table 3, it was found that Dawk Kha rice flour had the tendency for faster and higher optimum pasting properties, as shown in comparative pasting curves compared to wheat flour. When higher temperatures were applied to the starch granules, the water absorption activity of flour increased, as did swelling and viscosity (Sriroth and Piyajomkwan, 2003). In the case of Dawk Kha rice flour and wheat flour, they tended to start being cooked at 80.80 and 86.98°C, respectively. Interestingly, Dawk Kha rice flour was more likely to prefer a higher temperature in order to make its starch granules absorb water and swell before starting the pasting process. However, at peak pasting temperature values, Dawk Kha rice flour tended to swell highly and rapidly when compared to the pasting temperature of wheat flour, which was higher. In addition, the peak pasting properties of the Dawk Kha rice flour solution seemed to be much higher than those of wheat flour. In the event of uneven baking performance of flour during cooking,

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Dawk Kha rice flour began cooking at 5.67 mins and wheat flour began cooking at 6.03 mins. The test results for peak pasting properties were similar (Thiranusornkij *et al*., 2019). At peak viscosity during heat treatment, starch reached complete gelatinization, indicating peak viscosity at high temperatures is related to the stickiness of cooked rice, while the lowest trough viscosity was found during the cooled-down process (Sriroth and Piyajomkwan, 2003). Regarding the breakdown pasting properties, Dawk Kha rice flour tended to possess lower thermal stability and mixing parameters than wheat flour.

Table 3. Results of pasting properties of Dawk Kha rice flour and wheat flour obtained from Rapid Visco Analyzer (RVA).

Quality	Dawk Kha rice flour	Wheat flour
Pasting temperature (°C)	80.80±0.78[b]	86.98±1.21[a]
Peak time (min)	5.67±0.08[b]	6.03±0.14[a]
Peak viscosity (cP)	2441.67±16.92[a]	1953.00±26.29[b]
Trough (cP)	1527.00±54.58[a]	1244.00±33.78[b]
Breakdown viscosity (cP)	914.67±38.55[a]	709.00±26.46[b]
Final viscosity (cP)	3927.00±70.74[a]	2318.33±8.14[b]
Setback viscosity (cP)	2400.00±23.43[a]	1074.33±37.61[b]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

Concerning the breakdown, if the value was low, this could be an indication that the starch granules were able to withstand high temperatures, making it easier to stir after the gelatinization process (Sriroth and Piyajomkwan, 2003). Moreover, the indication of higher final pasting properties and setback pasting properties somehow unveiled the possibility of higher retrogradation in Dawk Kha rice flour compared to wheat flour. Flour retrogradation appeared to have a direct influence on texture quality, with lower retrogradation producing a softer texture and higher retrogradation producing a dry and rough texture (Sriroth and Piyajomkwan, 2003; Tulayatan, 2006). For final pasting properties, the results indicated that the gel properties of starch were related to the hardness value of cooked rice. If the final pasting properties were higher, it could be assumed that the rice was hard, or in other words, contained a high amylose content (Sriroth and Piyajomkwan, 2003). Regarding the setback pasting properties, the difference between peak viscosity values and trough viscosity values could be indicating the capability of rearranging amylopectin and amylopectin structures, or so-called "retrogradation". Unless the value of flour setback properties is high, better starch structure rearrangement may occur.

3.3 Bioactive compounds of Dawk Kha rice flour and wheat flour

Total phenolic content (TPC) and total anthocyanin content (TAC) of Dawk Kha rice flour and wheat flour are presented in Table 4. Dawk Kha rice flour obtained 446.37 mg GAE/g of TPC and 295.16 mg/kg of TAC, whereas wheat flour contained 10.37 mg GAE/g of TPC and 4.30 mg/kg of TAC, respectively. TPC found in Dawk Kha rice flour was 436.37 times higher than that of wheat flour; black glutinous rice tended to possess the highest TAC, followed by red rice and brown rice, respectively (Hu *et al*., 2003). Rice with black or red pigment in the pericarps tends to possess very high antioxidant values when compared to white rice (Walter *et al*., 2013). Nonetheless, antioxidant capacity also depends on several factors, including the amount of phenolic content found in the rice.

Table 4. Bioactive compounds of Dawk Kha rice flour and wheat flour.

Quality	Dawk Kha rice	Wheat flour
Total Phenolic content	446.37±1.67[a]	10.37±0.55[b]
Total Anthocyanin	295.16±0.78[a]	4.30±0.01[b]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

4. Conclusion

Dawk Kha rice flour showed higher contents of fat, carbohydrate, ash, and fiber than wheat flour, though wheat flour exhibited higher contents of protein, moisture content, and amylose. Regarding both types of physical and texture characteristics, Dawk Kha rice flour was characterized by a darker color when compared to wheat flour, although wheat flour exhibited a powdery texture with a yellowish-white color and a neutral odor without extraneous materials. The retrogradation degree in Dawk Kha rice flour was found to be higher than that of wheat flour, directly affecting the texture of the flour. TPC was found in greater quantities in Dawk Kha rice flour than in wheat flour. Based on the physical and chemical characteristics of Dawk Kha rice flour in this study, this rice variety can be used as an alternative to wheat flour in food products such as noodles, bread, cookies, cakes, muffins, surimi, and artificial meat.

Conflict of interest

The authors declare no conflict of interest.

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