

## Physicochemical and antioxidant properties of gluten-free chiffon cake prepared with riceberry rice flour as replacement for rice flour

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### Article history

Received:

12 October 2023

Received in revised form:

7 May 2024

Accepted:

10 June 2024

### Keywords

riceberry rice,  
chiffon cake,  
gluten-free

### Abstract

The present work aimed to examine the effects of replacing rice flour (RF) with riceberry flour (RBF) on the physicochemical and antioxidant properties of gluten-free batter and chiffon cake. The ratios of RBF to RF at 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0 was investigated and labelled 0RBF, 20RBF, 40RBF, 60RBF, 80RBF, and 100RBF, respectively. The pasting properties of the composite flours revealed that increasing the RBF ratio resulted in a lower peak, final, breakdown viscosity, and setback than RF. As the amount of RBF increased, the colour parameters ( $L^*$ ,  $C^*$ , and  $h^\circ$ ) of the gluten-free batter, crust, and crumb of the chiffon cake decreased significantly ( $p \leq 0.05$ ). The viscosity and density of the gluten-free batter, as well as the density of the gluten-free chiffon cake (GFC), increased as the proportion of RBF increased, while the specific volume decreased significantly ( $p \leq 0.05$ ). Increasing the proportion of RBF also increased the hardness and chewiness, but decreased the cohesiveness and springiness index of the cake crumbs significantly ( $p \leq 0.05$ ). The bioactive compounds, total phenolic and anthocyanin contents, and ferric-reducing antioxidant power of the chiffon cakes increased significantly ( $p \leq 0.05$ ) with increasing RBF. Baking decreased the total phenolic content and ferric-reducing antioxidant power of the batter by more than 50%, and anthocyanin by almost 50%. The findings suggested that RBF could be utilised as a gluten-free replacement in cake and bakery products.

### DOI

<https://doi.org/10.47836/ifrj.31.4.07>

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## Introduction

Celiac disease, an autoimmune disorder caused by the gliadin and associated prolamins found in gluten from wheat, barley, and rye grains (Radlović, 2013), makes the small intestine unable to absorb food, potentially resulting in malnutrition. Most bakery products are produced with wheat flour or gluten-containing cereal flour. As a result, celiac disease sufferers must permanently exclude gluten from their diet for the rest of their lives because finding high-quality gluten-free products is very difficult. Gluten proteins contribute to viscoelasticity, which is a major characteristic of bakery products. Glutenin, a polymeric gluten protein, is elastic in contrast to gliadin, a monomeric gluten protein (Veraverbeke and Delcour, 2002). Therefore,

developing gluten-free products is a very difficult challenge for researchers.

Chiffon cakes are low-fat cakes with a distinct form and texture, created by meticulously folding an egg yolk-vegetable oil emulsion into foamed egg white. Kim *et al.* (2014) describes the batter as a sophisticated dispersion with multiple air bubbles and fat globules scattered in the same continuous phase. Rice flour has been traditionally and extensively used as a cereal grain flour for preparing gluten-free bakery products because it is inexpensive, easily digested, and hypoallergenic (Capriles and Arêas, 2014).

Riceberry rice is a popular rice variety used in bakery products. It is a cross of Hom Nil rice (Thai black rice) and Hom Mali 105 (Thai jasmine rice), also known as Thai fragrant rice. Riceberry rice is

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high in various nutritious components, and a rich source of antioxidants, such as phenolic acids and anthocyanins. These are beneficial for anaemia and diabetes sufferers because of their high iron and low glucose contents, and anti-inflammatory and hepatoprotective effects. However, using rice flour to make cakes may reduce the quality of the finished product. According to Laokuldilok *et al.* (2021), the amount of riceberry flour in the recipe also affected the textural attributes. Chueamchaitrakun *et al.* (2011) studied the use of glutinous rice flour and Hom-Mali rice flour in bread, with Thiranusornkij *et al.* (2019) reporting that increasing the content of Hom-Mali flour increased the specific volume and hardness of butter cake. Unfortunately, there have been few investigations into the use of RBF in GFC products. As a consequence, developing a new recipe for manufacturing GFC would be desirable.

Therefore, the purpose of the present work was to prepare GFC with 0 to 100% (w/w) substitution of RF with RBF to investigate its effects on the physicochemical and antioxidant activities of gluten-free batter and chiffon cakes.

## Materials and methods

### Raw materials

Sunfood Corp Ltd. (Samutprakan, Thailand) provided refined riceberry flour (RBF), and BS Starch Chemical Co., Ltd. (Bangkok, Thailand) provided the rice flour (RF). The other ingredients for making GFC were purchased from the local market (Bangkok, Thailand).

### Determination of pasting properties of composite flour

A Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, NSW, Australia) was used to measure the pasting properties of the flours with a standard analysis 1 profile. A 2.5-g flour sample with a corrected moisture content between 10 and 14% was used. The samples were heated to 50°C for 1 min, then to 95°C for 4.42 min, and then held at this temperature for 2.30 min, followed by cooling to 50°C for 3.48 min, then held at this temperature for 2 min. During the procedure, the rotational speed stayed at 960 rpm for 10 s, then was reduced to 160 rpm. The pasting temperature, peak viscosity, breakdown, final viscosity, and setback were all measured. The viscosity was measured in centipoise units (cP).

### Preparation of gluten-free chiffon cake

Different formulations of gluten-free chiffon cake were obtained by making composite flours with RBF and RF, which were formulated by replacing RF with 0, 20, 40, 60, 80, and 100% RBF. The formulas, shown in Table 1, were developed following the Lee and Lin (2008) method. The gluten-free chiffon cake batter was placed into a cake pan (20.3 cm in diameter and 7 cm in height), then cooked at 160°C for 20 min in a preheated oven. Each treatment was prepared in a random sequence. The gluten-free chiffon cakes were taken out of the pans after cooling for 30 min at room temperature before being placed in polypropylene bags for physical and chemical analyses.

**Table 1.** Formulations of GFC.

Ingredient	Formulation (g)					
	0RBF	20RBF	40RBF	60RBF	80RBF	100RBF
RF	100	80	60	40	20	0
RBF	0	20	40	60	80	100
Sugar	79	79	79	79	79	79
Yolk	37	37	37	37	37	37
Egg white	80	80	80	80	80	80
Rice bran oil	54	54	54	54	54	54
Milk	42	42	42	42	42	42
Baking powder	4	4	4	4	4	4
Salt	1	1	1	1	1	1

### Gluten-free batter characteristics

After mixing, the gluten-free batter (500 g) was immediately put into a 600-mL beaker. The rotational viscometer L4 spindle was used to measure the

viscosity at 30 rpm (DV-II+ Viscometer, Ametek Brookfield, Middleboro, MA, USA). The density of the batter was measured using an Elcometer 1800 (Elcometer, Manchester, UK), which was a cup with

a 50-mL cylindrical receptacle and a cover with a hole for removing extra liquid. The cup was first weighed unfilled for calibration, and then filled with batter. The density was estimated by dividing the weight by the volume (Gularte *et al.*, 2012). The colour of the batter was evaluated using a spectrophotometer (Color Quest XE, Hunter Lab, Reston, VA, USA) equipped with a D65 illuminant and a 2° standard observer using the CIE L\* C\* h° system. Each formulation was measured three times to ensure the accuracy and consistency of the results.

#### *Gluten-free chiffon cake characteristics*

##### *Specific volume*

The specific volume of the GFC samples was calculated as the ratio of the apparent volume by millet seed displacement and weight (g) after baking using the AACC (2000) method.

##### *Weight loss*

The batter was weighed before (A), and after baking (B). The percentage of weight loss was calculated as  $((A - B) \times 100)/A$ .

##### *Texture analysis*

A texture analyser (TA plus, Lloyd Instruments, Bognor Regis, UK) was used for evaluating the texture characteristics with a maximum force of 50 N to imitate the chewing action of the teeth. The GFC samples were measured after 60 min of cooling. Using an adaptor, a cylinder with a diameter of 50 mm, they were pressed at the centre to 50% of the initial height at a test speed of 1.0 mm/s. The texture criteria, hardness, cohesiveness, springiness index, and chewiness, were measured.

##### *Proximate analysis*

The chemical proximate compositions of GFC samples were analysed following the AOAC (2005) methods.

##### *Water activity ( $a_w$ )*

The  $a_w$  was determined at 25°C using an Aqua Lab Series 3 TE water activity meter (Decagon Devices Inc., Pullman, WA, USA). The analysis used a ground sample of approximately 2 g.

##### *Crust and crumb colour value*

The crust of the chiffon cake was placed directly on the reflectance port of the spectrophotometer (Hunter Lab). To measure the

colour values of the cake crumb, the crust of the chiffon cake was first removed as follows: the crust surface of the chiffon cake was removed to create a 3 × 3 cm cake slice (Akesowan, 2007). The colour of the chiffon cake samples was then measured using a spectrophotometer equipped with a D65 illuminant and the 2° standard observer, following the CIE L\*, C\*, h° system.

#### *Evaluation of total phenols, total anthocyanins, and antioxidant activity of gluten-free chiffon cake extracts*

The method for extracting the antioxidants from the GFC was adapted from Mau *et al.* (2017). The GFC sample was soaked in 80% (v/v) methanol (100 mL) using an Elmasonic P ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) at 37 kHz and 40°C for 10 min. A Whatman No. 1 filter paper was used to filter the extract. The collection of extracts was stored at -18°C until further use. The total phenolic content (TPC) of the GFC extract was evaluated using the Folin-Ciocalteu technique as modified by Wolfe *et al.* (2003). The samples (125 µL) were mixed in a test tube with 500 µL of distilled water, followed by the addition of Folin-Ciocalteu reagent (125 L), then left to stand at room temperature for 6 min. After adding 7% Na<sub>2</sub>CO<sub>3</sub> (1,250 µL) followed by distilled water (1,000 µL), it was left to stand at room temperature for 90 min. The absorbance was measured using a spectrophotometer (UV mini-1240, Shimadzu, Columbia, MD, USA) at 760 nm. The TPC was expressed as mg gallic acid equivalent/g sample in the extract.

The total anthocyanin content (TAC) analysis technique was modified as described by Giusti and Wrolstad (2001). The extract was adjusted in 25-mL volumetric flasks with potassium chloride buffer (0.03 mol/L, pH 1.0) and sodium acetate buffer (0.4 mol/L, pH 4.5). Each flask was placed in the dark for 15 min at room temperature. The absorbance of the samples was measured using a spectrophotometer at 510 and 700 nm. The TAC of the sample (mg/g sample) was determined using Eq. 1, and expressed as cyanidin-3-glucoside equivalents:

$$\text{TAC (mg/100 g sample)} = \frac{(A \times \text{MW} \times \text{DF} \times V \times 100)}{(\epsilon \times l \times G)} \quad (\text{Eq. 1})$$

where, A = (absorbance at 700 nm - absorbance at 550 nm) at pH 1.0 - (absorbance at 700 nm - absorbance at 550 nm) at pH 4.5; MW = molecular weight of

cyanidin-3-glucoside (449.2 g/mol); DF = dilution factor (20 µL sample was diluted to 2 mL, DF = 100); V = solvent volume (mL);  $\epsilon$  = extinction coefficient ( $L\ cm^{-1}\ mol^{-1}$ ) = 26,900 for cyanidin-3-glucoside where L (path length) = 1 cm; l = volume of solvent; and G = weight of the sample.

The ferric-reducing antioxidant power (FRAP) assay was modified from Butsat and Siriamornpun (2010). The extracts (60 µL) were mixed with the FRAP reagent (1.8 mL) and distilled water (180 µL). After 4 min of incubation at 37°C, the absorbance of the mixture was measured at 593 nm. The FRAP reagent was made up of 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tris(2-pyridyl)-s-triazine in 40 mM HCl, 20 mM FeCl<sub>3</sub>, and distilled water in the ratio of 10:1:1:1.2 (v/v/v/v). The FRAP value was calculated by comparing the absorbance change in the test mixture to doses obtained from increasing concentrations of Fe(III), and was expressed in micromoles of Fe(II) per g of sample.

#### Scanning electron microscopy (SEM) analysis

Before the SEM examination using a JSM-6610LV scanning electron microscope (JEOL, Tokyo, Japan), the GFC samples were frozen in liquid nitrogen and freeze-dried, then sputter-coated with gold-palladium before inspection using the HUMMER VII Sputter Coating Machine (Anatech Electronics, Garfield, NJ, USA) to make them electrically conductive. The crumb sections of the cakes were examined at a magnification of 30×

#### Ethics statement

The present work was considered and approved

by the Ethics Committee of Suan Sunandha Rajabhat University (approval no.: COE. 2-294/2023).

#### Statistical analysis

A completely randomised design (CRD) was used for the experiment. The physical and chemical data from each sample were examined using analysis of variance (ANOVA) for significant variables ( $p \leq 0.05$ ). Differences between means were determined with Duncan's new multiple range test (DMRT).

## Results and discussion

#### Pasting properties

The changes that occur in flour-based food products by applying heat in the presence of moisture are known as the pasting properties. These changes affect the texture, digestibility, and final usage of the food product (Ocheme *et al.*, 2018). Table 2 shows the pasting properties of RF, RBF, and the composite flours. Increasing the proportion of RBF significantly decreased peak viscosity, breakdown, final viscosity, setback, and pasting temperature. The RBF and their composite flours exhibited significantly higher pasting temperatures than RF. The higher gelatinisation temperatures for RBF and its composite flour may have caused the formation of the cake structure to be delayed, enabling air being retained in the batter during mixing to expand and escape, resulting in a smaller volume. These results agreed with those of Singh *et al.* (2015), who reported that the cake volume decreased due to the use of navy bean flour, which had a greater gelatinisation temperature than wheat flour.

**Table 2.** Pasting properties of RF, RBF, and composite flours.

Sample	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Pasting temperature (°C)
RF	4633.67 ± 16.80 <sup>a</sup>	1593.33 ± 16.80 <sup>a</sup>	4849.33 ± 9.01 <sup>a</sup>	1812.33 ± 6.81 <sup>a</sup>	74.72 ± 0.43 <sup>f</sup>
20RBF	3076.33 ± 14.84 <sup>b</sup>	750.67 ± 14.84 <sup>b</sup>	4301.33 ± 7.09 <sup>b</sup>	1982.67 ± 3.05 <sup>b</sup>	87.25 ± 0.05 <sup>e</sup>
40RBF	2005.67 ± 3.79 <sup>c</sup>	292.63 ± 15.09 <sup>c</sup>	3425.33 ± 2.52 <sup>c</sup>	1713.33 ± 12.34 <sup>c</sup>	89.70 ± 0.00 <sup>d</sup>
60RBF	1371.67 ± 6.43 <sup>d</sup>	61.55 ± 2.55 <sup>d</sup>	2544.33 ± 4.36 <sup>d</sup>	1307.33 ± 13.58 <sup>d</sup>	91.68 ± 0.44 <sup>c</sup>
80RBF	816.67 ± 2.89 <sup>e</sup>	55.37 ± 5.29 <sup>d</sup>	1746.67 ± 8.23 <sup>e</sup>	992.33 ± 18.77 <sup>e</sup>	93.18 ± 0.45 <sup>b</sup>
RBF	419.33 ± 4.16 <sup>f</sup>	41.67 ± 4.72 <sup>d</sup>	1065.06 ± 4.18 <sup>f</sup>	631.02 ± 9.54 <sup>f</sup>	94.43 ± 0.03 <sup>a</sup>

Means in the same columns followed by different lowercase superscripts are significantly different ( $p \leq 0.05$ ).

The higher peak viscosity indicated a better ability to form a gel. Amylopectin, which has a high-water holding capacity, predominantly contributes to a high peak viscosity (Wang *et al.*, 2016). This suggested that RF contained more amylopectin than

the composite flours, including RBF. Generally, a higher amylopectin content enhances the swelling power of flour. RBF was probably more resistant to swelling and rupturing than RF (Rakkhumkaew *et al.*, 2019). The breakdown viscosity indicates the fragility

of the gelatinised starch or its susceptibility to disintegration during prolonged stirring and heating as a result of variations in the stiffness of the swollen starch granules. The results showed that RBF and their composite flours had a lower breakdown value than RF, implying that they could have higher paste stability under heating conditions than RF. The final viscosity while cooling indicates the aggregation or rearrangement of amylose molecules, which gives stability and stiffness to the swollen granule structure.

The setback value is a measure of the propensity of starch paste to retrograde, thus can be used to estimate the shelf life of products made from flour (Thiranusornkij *et al.*, 2019). In the present work, RBF and the composite flours exhibited lower final viscosity and setback values than RF, indicating that they had less tendency to retrograde, and might be effective in foods that need extended storage times. The pasting properties of RBF and its composite flours may also be related to the structure of the phenolic compounds in RBF comprising OH groups, which can bind water (Lang *et al.*, 2020). According to Zhu (2015), phenolic chemicals in RBF may interact with water and the hydroxyl groups of starch *via* hydrogen bonding, thus inhibiting the hydrophobic contact of starch chains for

retrogradation.

#### Colour values of gluten-free batter, crust, and crumbs of chiffon cake

The CIE L\*, C\*, and h° values are related to lightness, chroma, and hue angle (0° is red, 90° is yellow, 180° is green, and 270° is blue), respectively. The colour value results are shown in Table 3. The L\*, C\*, and h° values of cake batters decreased significantly with increasing RBF levels ( $p \leq 0.05$ ), as shown by the darkening in colour. Substituting RBF for RF also significantly affected the colour of the crust and crumb of the GFC; GFC added with RBF were darker than the RF cake. RBF contains dark purple pigments, the main ingredients of which are phenolic compounds and anthocyanins (Ranok and Kupradit, 2020). These results agreed with those of Thongkaew and Singthong (2020), who showed that rice noodles with RBF exhibited higher redness values than noodles without RBF. This was supported by Mau *et al.* (2017), who observed that the substitution using RBF in the product resulted in the dark hue of the chiffon cake. The colour change of the chiffon cake might be caused by the oxidation of the pigments and phenolic compounds of RBF.

**Table 3.** Colour values of gluten-free batter, crust, and crumb chiffon cakes with different composite flours.

Colour value	Sample					
	0RBF	20RBF	40RBF	60RBF	80RBF	100RBF
Batter						
L*	83.70 ± 0.21 <sup>a</sup>	37.66 ± 1.31 <sup>b</sup>	32.19 ± 1.74 <sup>c</sup>	28.42 ± 0.88 <sup>d</sup>	22.78 ± 0.58 <sup>e</sup>	23.32 ± 0.33 <sup>e</sup>
C*	18.45 ± 0.14 <sup>a</sup>	4.86 ± 0.09 <sup>b</sup>	3.41 ± 0.37 <sup>c</sup>	2.51 ± 0.33 <sup>d</sup>	2.48 ± 0.02 <sup>d</sup>	2.69 ± 0.11 <sup>d</sup>
h°	82.11 ± 0.14 <sup>a</sup>	69.29 ± 1.06 <sup>b</sup>	42.87 ± 1.68 <sup>c</sup>	30.26 ± 1.84 <sup>d</sup>	16.79 ± 1.42 <sup>e</sup>	17.68 ± 1.60 <sup>e</sup>
Crust						
L*	52.06 ± 0.16 <sup>a</sup>	43.67 ± 0.21 <sup>b</sup>	35.11 ± 0.01 <sup>c</sup>	31.55 ± 0.04 <sup>d</sup>	29.07 ± 0.08 <sup>e</sup>	27.55 ± 0.12 <sup>f</sup>
C*	39.57 ± 0.21 <sup>a</sup>	29.89 ± 0.77 <sup>b</sup>	19.64 ± 0.07 <sup>c</sup>	13.92 ± 0.01 <sup>d</sup>	10.41 ± 0.39 <sup>e</sup>	9.44 ± 0.57 <sup>f</sup>
h°	65.27 ± 0.20 <sup>a</sup>	64.33 ± 0.39 <sup>a</sup>	57.75 ± 0.28 <sup>b</sup>	52.52 ± 0.18 <sup>c</sup>	49.54 ± 0.21 <sup>d</sup>	47.75 ± 1.20 <sup>e</sup>
Crumb						
L*	71.58 ± 0.34 <sup>a</sup>	36.11 ± 0.70 <sup>b</sup>	27.86 ± 0.01 <sup>c</sup>	26.39 ± 0.09 <sup>d</sup>	24.72 ± 0.13 <sup>e</sup>	23.28 ± 0.05 <sup>f</sup>
C*	21.25 ± 0.91 <sup>a</sup>	10.75 ± 0.45 <sup>b</sup>	9.39 ± 0.02 <sup>c</sup>	7.24 ± 0.02 <sup>d</sup>	6.48 ± 0.03 <sup>e</sup>	4.52 ± 0.02 <sup>f</sup>
h°	89.72 ± 0.16 <sup>a</sup>	68.82 ± 0.20 <sup>b</sup>	45.97 ± 0.44 <sup>c</sup>	40.55 ± 0.64 <sup>d</sup>	37.12 ± 1.26 <sup>e</sup>	32.37 ± 1.25 <sup>f</sup>

Means in the same rows followed by different lowercase superscripts are significantly different ( $p \leq 0.05$ ).

#### Physical characteristics of gluten-free batters and chiffon cakes

The cake volume is a measure of cake size because it represents the amount of air captured during mixing, as well as the amount of air, moisture, and CO<sub>2</sub> entrapped, and the enlargement during baking. A low cake volume indicates a heavy, less

preferable crumb. The physical characteristics of gluten-free batters and chiffon cakes in terms of viscosity, density, weight loss, and specific volume are shown in Table 4. The results showed that a high level of RBF tended to increase the viscosity and density of the gluten-free batters. The increase in batter viscosity with increasing RBF was caused by

the higher fibre content of RBF compared with that of RF (Table 5). The fibre in RBF absorbed more moisture while mixing, resulting in a thicker consistency, and an increased viscosity of the batter.

The density of the cake usually refers to the air content of the cake. This means that a decrease in the air volume added to the cake can be associated with an increase in the density of the samples. The results revealed that by increasing the level of RBF in chiffon cake flour, the density of GFC successively increased to 0.45 g/mL for 100RBF, which was significantly ( $p \leq 0.05$ ) higher than that of the 100RF GFC. While

there was no difference between the specific volumes of 0RBF, 20RBF, and 40RBF, increasing the amount of RBF tended to decrease the specific volume. Similar results were also reported by Mau *et al.* (2017), who found that the volume of chiffon cakes containing 60 - 100% black rice was lower than that of those containing 0 - 50% black rice.

There was also no difference in weight loss in the chiffon cake samples. Therefore, the cooked cakes containing more RBF had a decreased capacity to hold air, resulting in an unsatisfactory aerated structure with a lower volume.

**Table 4.** Physical characteristics of gluten-free batters and chiffon cakes with different composite flours.

Sample	Cake batter			Chiffon cake	
	Viscosity (cP)	Density (g/mL)	Weight loss <sup>ns</sup> (%)	Density (g/mL)	Specific volume (cm <sup>3</sup> /g)
0RBF	4968.33 ± 2.89 <sup>e</sup>	0.50 ± 0.02 <sup>c</sup>	8.41 ± 0.25	0.29 ± 0.00 <sup>e</sup>	3.45 ± 0.05 <sup>a</sup>
20RBF	4722.67 ± 2.52 <sup>f</sup>	0.50 ± 0.02 <sup>c</sup>	8.37 ± 0.13	0.31 ± 0.01 <sup>d</sup>	3.32 ± 0.03 <sup>a</sup>
40RBF	5720.67 ± 1.15 <sup>d</sup>	0.51 ± 0.01 <sup>c</sup>	8.36 ± 0.21	0.32 ± 0.01 <sup>d</sup>	3.28 ± 0.02 <sup>ab</sup>
60RBF	6052.33 ± 2.52 <sup>c</sup>	0.52 ± 0.02 <sup>c</sup>	8.37 ± 0.29	0.35 ± 0.00 <sup>c</sup>	2.61 ± 0.51 <sup>b</sup>
80RBF	7644.00 ± 4.00 <sup>b</sup>	0.65 ± 0.03 <sup>b</sup>	8.44 ± 0.11	0.38 ± 0.00 <sup>b</sup>	2.58 ± 0.03 <sup>b</sup>
100RBF	8380.67 ± 1.15 <sup>a</sup>	0.71 ± 0.03 <sup>a</sup>	8.40 ± 0.22	0.45 ± 0.01 <sup>a</sup>	2.26 ± 0.02 <sup>b</sup>

Means in the same columns followed by different lowercase superscripts are significantly different ( $p \leq 0.05$ ).

**Table 5.** Proximate composition and water activity ( $a_w$ ) of RF, RBF, and GFC with different composite flours.

Sample	$a_w$	Moisture (%)	Protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)
0RBF	0.916 ± 0.005 <sup>b</sup>	29.94 ± 0.11 <sup>a</sup>	6.77 ± 0.23 <sup>b</sup>	13.59 ± 0.21 <sup>b</sup>	0.44 ± 0.00 <sup>f</sup>	1.34 ± 0.12 <sup>d</sup>
20RBF	0.917 ± 0.002 <sup>b</sup>	29.83 ± 0.07 <sup>a</sup>	6.83 ± 0.11 <sup>b</sup>	14.39 ± 0.23 <sup>ab</sup>	0.75 ± 0.05 <sup>e</sup>	1.42 ± 0.10 <sup>cd</sup>
40RBF	0.927 ± 0.004 <sup>a</sup>	29.28 ± 0.61 <sup>b</sup>	6.83 ± 0.19 <sup>b</sup>	15.44 ± 0.12 <sup>a</sup>	0.92 ± 0.02 <sup>d</sup>	1.49 ± 0.13 <sup>bc</sup>
60RBF	0.914 ± 0.008 <sup>b</sup>	28.89 ± 0.06 <sup>bc</sup>	6.98 ± 0.15 <sup>b</sup>	14.59 ± 0.13 <sup>ab</sup>	1.20 ± 0.02 <sup>c</sup>	1.53 ± 0.13 <sup>b</sup>
80RBF	0.923 ± 0.001 <sup>ab</sup>	28.78 ± 0.11 <sup>bc</sup>	8.05 ± 0.16 <sup>a</sup>	14.42 ± 0.32 <sup>ab</sup>	1.49 ± 0.03 <sup>b</sup>	1.54 ± 0.12 <sup>b</sup>
100RBF	0.920 ± 0.005 <sup>ab</sup>	28.63 ± 0.16 <sup>c</sup>	8.20 ± 0.10 <sup>a</sup>	15.57 ± 0.19 <sup>a</sup>	1.89 ± 0.03 <sup>a</sup>	1.76 ± 0.14 <sup>a</sup>
RF	0.397 ± 0.011	10.77 ± 0.09	6.05 ± 0.15	1.45 ± 0.03	0.25 ± 0.05	0.52 ± 0.02
RBF	0.469 ± 0.001	10.99 ± 0.13	8.06 ± 0.16	2.68 ± 0.12	2.73 ± 0.03	1.18 ± 0.02

Means in the same columns followed by different lowercase superscripts are significantly different ( $p \leq 0.05$ ).

#### Texture profile analysis of gluten-free chiffon cakes

Texture properties are an important factor influencing the consumer's acceptance of a product. In the present work, texture profile analysis was used to determine the texture of the GFC, *i.e.* hardness, chewiness, cohesiveness, and springiness indices (Table 6). The results revealed that increasing the level of RBF tended to enhance the hardness of the chiffon cake. The GFC with 100RBF exhibited the highest crumb hardness, which was not significantly different from 80RBF, whereas GFC with 0RBF exhibited the lowest crumb hardness. The increasing hardness of the cake might be caused by the higher

crude fibre content of RBF resulting in a stronger crumb structure. This result agreed with that of Mau *et al.* (2017), who found that the hardness of chiffon cake increased when it was replaced with black rice flour. The increasing hardness may also be related to the density and specific volume of the chiffon cake.

Cohesiveness indicates the characteristic of the extent of the sample adhering between the first and second compressions, and is measured on a scale of 0 to 1. A value close to 1 means that the food sample is well-agglomerated, and needs a great deal of energy to disintegrate. Based on our results, the cohesiveness of chiffon cake containing 20RBF was not

significantly different from that of 0RBF. However, there was no significant difference in cohesiveness when RF was replaced with 40 - 100RBF.

The springiness index is a measure of the elasticity of recovery between the first and second compressions. The springiness index of the GFC tended to decrease as the proportion of RBF increased (Table 6). This decrease could be associated with the destruction of the cake structure resulting in its inability to recover.

Chewiness is the product of hardness, cohesiveness, and springiness. Thus, a strong increase in the hardness would enhance the chewiness of the RBF chiffon cake. However, GFC where RF was replaced by 20 - 80RBF exhibited no significant differences in chewiness. From the experimental results, hardness appears to be a critical parameter because it is related to chewiness, specific volume, and density, all of which affect the consumer's acceptance of chiffon cake.

**Table 6.** Texture profile analysis of GFC with different composite flours.

Sample	Hardness (N)	Cohesiveness	Springiness index	Chewiness (Nmm)
0RBF	6.56 ± 1.36 <sup>c</sup>	0.39 ± 0.03 <sup>a</sup>	0.90 ± 0.07 <sup>a</sup>	50.96 ± 4.96 <sup>b</sup>
20RBF	7.46 ± 0.67 <sup>c</sup>	0.42 ± 0.01 <sup>a</sup>	0.85 ± 0.02 <sup>ab</sup>	62.38 ± 5.80 <sup>ab</sup>
40RBF	10.59 ± 1.34 <sup>b</sup>	0.31 ± 0.02 <sup>b</sup>	0.80 ± 0.01 <sup>bc</sup>	60.01 ± 7.80 <sup>ab</sup>
60RBF	11.15 ± 1.47 <sup>b</sup>	0.30 ± 0.01 <sup>b</sup>	0.77 ± 0.04 <sup>cd</sup>	60.53 ± 6.95 <sup>ab</sup>
80RBF	11.78 ± 1.04 <sup>ab</sup>	0.33 ± 0.04 <sup>b</sup>	0.77 ± 0.03 <sup>cd</sup>	68.07 ± 6.62 <sup>ab</sup>
100RBF	13.61 ± 0.55 <sup>a</sup>	0.30 ± 0.01 <sup>b</sup>	0.72 ± 0.02 <sup>d</sup>	72.47 ± 19.21 <sup>a</sup>

Means in the same columns followed by different lowercase superscripts are significantly different ( $p \leq 0.05$ ).

#### Proximate composition of gluten-free chiffon cakes

Table 5 shows the results of the analysis of the proximate composition and  $a_w$  of the GFC. In the present work, the moisture contents and  $a_w$  ranged from 28.63 - 29.94% and 0.914 - 0.927, respectively. When the RBF level in the GFC increased, the protein, crude fat, crude fibre, and ash content increased because RBF contained more of these components than RF. These results were related to the chemical composition of RF and RBF (Table 5), and in agreement with those of Kraithong *et al.* (2018), who found that the protein, crude fat, crude fibre, and ash contents of RF were lower than those of RBF. Thongkaew and Singthong (2020) observed that the protein and crude fat contents of RBF were 9.50 and 6.38%, respectively, whereas those of RF were 6.50 and 2.09%. This suggested that increasing the RBF

level in chiffon cake would increase the crude fibre and ash contents which have a beneficial impact on health.

#### TPC, TAC, and FRAP values of gluten-free batter and chiffon cake

Table 7 presents the TPC, TAC, and free radical scavenging activity (FRAP) values of batter and GFC with different composite flours. Adding RBF resulted in higher TPC, TAC, and FRAP values in the batter. No TAC was detected in the RF batter cake because RF contained no anthocyanin-related pigments. The FRAP assay is a method for measuring the antioxidant capacity of a sample. Antioxidants can reduce ferric ions ( $Fe^{3+}$ ) to their ferrous ( $Fe^{2+}$ ) form so those in the sample can either directly reduce the ferric ions or donate electrons to other compounds

**Table 7.** TPC, TAC, and FRAP of gluten-free batter and chiffon cake with different composite flours.

Sample	Cake batter			Chiffon cake		
	TPC (mg GAE/100 g)	TAC (mg/100 g)	FRAP ( $\mu$ mole/g)	TPC (mg GAE/100 g)	TAC (mg/100 g)	FRAP ( $\mu$ mole/g)
0RBF	42.39 ± 6.78 <sup>f</sup>	n.d.	4.55 ± 0.08 <sup>f</sup>	14.32 ± 0.58 <sup>f</sup>	n.d.	1.70 ± 0.01 <sup>f</sup>
20RBF	66.62 ± 2.42 <sup>e</sup>	1.02 ± 0.01 <sup>e</sup>	8.60 ± 0.04 <sup>e</sup>	34.91 ± 0.10 <sup>e</sup>	0.63 ± 0.01 <sup>e</sup>	3.56 ± 0.041 <sup>e</sup>
40RBF	152.06 ± 3.57 <sup>d</sup>	3.58 ± 0.18 <sup>d</sup>	17.64 ± 0.14 <sup>d</sup>	71.50 ± 0.47 <sup>d</sup>	1.19 ± 0.18 <sup>d</sup>	7.81 ± 0.13 <sup>d</sup>
60RBF	180.83 ± 2.97 <sup>c</sup>	4.46 ± 0.08 <sup>c</sup>	26.67 ± 0.67 <sup>c</sup>	86.20 ± 0.20 <sup>c</sup>	2.46 ± 0.08 <sup>c</sup>	10.00 ± 0.24 <sup>c</sup>
80RBF	228.37 ± 3.39 <sup>b</sup>	7.20 ± 0.18 <sup>b</sup>	34.80 ± 0.67 <sup>b</sup>	116.07 ± 1.25 <sup>b</sup>	3.51 ± 0.18 <sup>b</sup>	12.37 ± 0.39 <sup>b</sup>
100RBF	302.14 ± 3.15 <sup>a</sup>	8.73 ± 0.01 <sup>a</sup>	46.01 ± 0.13 <sup>a</sup>	128.58 ± 1.29 <sup>a</sup>	4.41 ± 0.01 <sup>a</sup>	14.63 ± 0.16 <sup>a</sup>

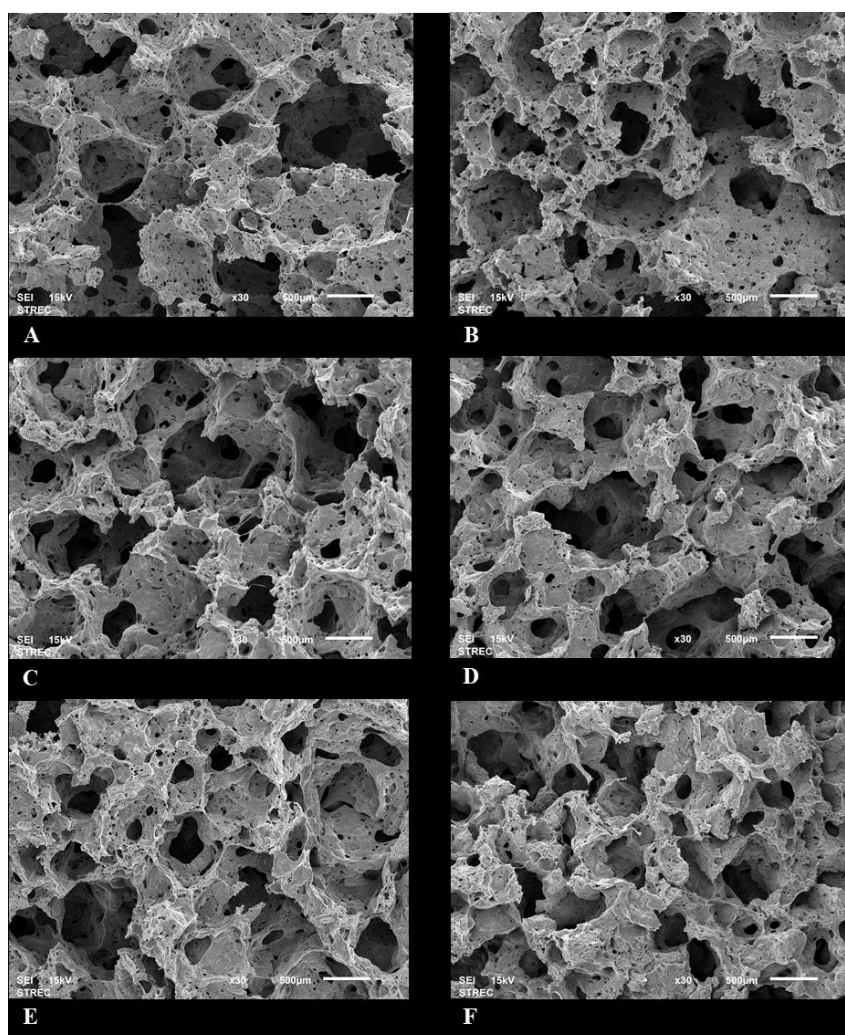
Means in the same columns followed by different lowercase superscripts are significantly different ( $p \leq 0.05$ ). n.d.: not detected.

that can then reduce the ferric ions (Gulcin, 2020). Table 7 shows that the FRAP value of the batter increased as the TPC and TAC increased. The TPC, TAC, and FRAP values of GFC behaved in a similar way to those of the batter. These results were supported by Weenuttranon *et al.* (2023), who found that antioxidant activity was correlated with TPC. The results showed that GFC with 100RBF exhibited the highest TPC, TAC, and FRAP values of 128.58 mg GAE/100 g cake, 4.41 mg/100 g cake, and 14.63  $\mu\text{mol/g}$  cake, respectively. TPC, TAC, and FRAP values were found to be considerably higher in the batter than in the chiffon cake. When RBF was used, the TPC, TAC, and FRAP values of the GFC were lower than its batter. The temperature has a significant influence on phenolic compounds during food thermal processing and storage (Zafrilla *et al.*, 2003). Murakami *et al.* (2004) have also reported that the radical-scavenging activity in foods can persist

more than the initial polyphenolic compound content throughout heating and processing.

#### SEM analysis

The influence of RBF on the structure of GFC was investigated using a scanning electron microscope with a magnification of 30 $\times$  as shown in Figure 1. A high specific volume and low density normally indicate that baked cakes have a more porous structure. The 0RBF chiffon cake had a more porous and larger structure than cakes with RF replaced with RBF, especially for the 60 - 100RBF chiffon cakes. The images of the chiffon cakes could be correlated with their specific volume and density (Table 4). These findings were also consistent with the hardness value of chiffon cakes. Since chiffon cakes with a more aerated structure had lower hardness values, the hardness of 0RBF chiffon cake was lower than that of the others (Table 5).



**Figure 1.** Scanned images of different GFCs with different composite flours. (A) GFC with 0RBF; (B) GFC with 20RBF; (C) GFC with 40RBF; (D) GFC with 60RBF; (E) GFC with 80RBF; and (F) GFC with 100RBF.



## Conclusion

The chemical and physical properties of gluten-free batter and chiffon cake were significantly affected by including RBF in the formulations. The RBF gluten-free batter and chiffon cake contained more bioactive compounds than those with no RBF. The RBF gluten-free batter and chiffon cakes exhibited high antioxidant activity. Although the physical characteristics of the RBF cake tended to deteriorate, the texture of the 20RBF and the specific volume of the 40RBF GFC were not significantly different from those with no RBF. Therefore, RBF could be used as a substitute for RF to develop alternative healthy gluten-free bakery products.

## Acknowledgement

The Research and Development Institute of Suan Sunandha Rajabhat University provided funding for the completion of the present work.

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