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Development of cookies fortified with defatted rice bran flour derived from dough grain stage: nutritional, phytochemical, and antioxidant properties

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Abstract Defatted Jasmine rice bran flour (DRBF) had higher phytochemical content and antioxidant activity than wheat flour. The addition of 15%-25%DRBF significantly improved the phytochemical content and antioxidant activity of the cookies, with the highest activity observed at 25%DRBF. However, sensory evaluation showed that scores for all sensory attributes decreased significantly at 20%-25%DRBF. The cookies with 15%DRBF achieved the best balance between consumer acceptability and antioxidant activity. Although higher DRBF levels (20%-25%DRBF) increased antioxidant activity, they led to reduced consumer preference. Additionally, substitution with 15%DRBF increased fiber and protein content of the cookies while decreasing their carbohydrate and calorific values. These findings suggested that incorporating 15%DRBF into cookies enhanced both sensory attributes and health benefits, making it an ideal functional ingredient.

Keywords: Antioxidant activity, Jasmine rice bran, Nutritional value, Rice bran cookies

Introduction

Jasmine rice bran, particularly from Thung Kula Ronghai in Roi Et province, is well-known for its outstanding quality because of the region's peculiar natural characteristics, such as its distinctive soil composition and climate.

Rice bran, a by-product of the rice milling process that accounts for approximately 10% of the grain's weight, is an abundant source of bioactive compounds such as phenolics, dietary fiber, sterols, vitamins, tocotrienols, tocopherols, and γ-oryzanol (Manzoor *et al.*, 2023; Tan *et al.*, 2023). The extraction of rice bran oil generates additional by-products, such as rice bran

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wax, defatted rice bran, and filter cake. Traditionally, rice bran has been used as fuel, fertilizer, or an ingredient in animal feed (Anthina *et al*., 2021), and in many cases, it is discarded as waste. Rice bran is produced during various stages of rice grain development, including the dough grain stage, which occurs late in the maturation process. Rice bran from this stage has a higher concentration of phytochemicals and antioxidant activity, particularly γ-oryzanol (Duangsa *et al*., 2025). These compounds have potent antioxidant and anti-inflammatory properties that contribute to numerous health benefits, such as lowering cholesterol, improving heart health, and combating oxidative stress (Phunikhom *et al*., 2021; Francisqueti-Ferron *et al*., 2021). Additionally, these bioactive compounds are thought to offer protection against cancer, vascular diseases, and type 2 diabetes (Tan *et al*., 2023). Rice bran flour (RBF), derived from this valuable by-product, has gained increasing recognition for its potential in the development of functional foods, including pasta enriched with anthocyanin-rich black rice bran (Sethi *et al*., 2020), porridge fortified with rice bran (Calvo-Castro *et al*., 2019), and biscuits incorporating soluble dietary fiber from defatted rice bran (Jia *et al*., 2020). The growing interest in bioactive substances like those found in RBF has increased due to the growing demand for functional foods, which provide nutritional value and improve health by lowering the risk of chronic diseases (Martirosyan and Singh, 2015).

Recent research has focused on using underutilized and non-conventional food resources to boost the nutritional value of food products. Defatted jasmine rice bran (DRB), which is obtained after oil extraction, remains a rich source of fiber, protein, and phytochemicals, making it highly nutritious. The removal of oil extends the shelf life of defatted rice bran, addressing one of the primary challenges in utilizing rice bran—its susceptibility to rancidity. Due to its high protein and fiber content, along with bioactive compounds, DRB serves as a valuable addition to various foods, including bread, cookies, cakes and noodles, without negatively impacting their texture or functionality (da Rocha Lemos Mendes *et al*., 2021; Mishra, 2017; Pakhare *et al*., 2016). Among widely consumed bakery products, cookies offer an excellent platform for incorporating functional ingredients into the diet without requiring significant changes in eating habits (Kucerova *et al*., 2013; Sudha *et al*., 2007). As cookies are compact, convenient, and resistant to microbial spoilage due to low moisture, DRB can effectively enhance their nutrient availability and storage stability. Although previous studies have explored the enrichment of cookies with whole grains, fibers, and fruit by-products to enhance their nutritional profiles (Kohajdová *et al*., 2018; Cheng and Bhat, 2016), there is limited research on the use of DRB flour from dough grain stage of rice in cookie formulations. This gap highlights the need to investigate the effects of DRB flour at varying concentrations on the antioxidant activity and sensory qualities of cookies. Developing functional bakery products requires finding the ideal balance between increased nutritional content and customer acceptance. Therefore, this research was aimed to develop and evaluate cookie formulations containing different levels of DRBF (5%, 10%, 15%, 20%, and 25%) by evaluating their physicochemical, phytochemical, antioxidant, and sensory qualities.

Materials and methods

Rice bran sample from the dough grain stage of organic Jasmine rice was provided by the Community Enterprise Banmao Career Promotion Group Limited Partnership in Suwannaphum, Roi Et, Thailand. Stabilization was carried out through heat treatment in a hot air oven (Memmert, UF110, Germany) at 70°C for 3 h, followed by defatting using a screw press machine (Model FEA-101ss-M-H-Tc-2015, Friend Energy Limited Partnership, Thailand) at 80-85°C. All chemicals and reagents used in this study were of analytical grade.

Preparation of defatted rice bran flour (DRBF)

The DRB from the dough grain stage was finely ground and dried in a hot air oven at 70°C for 5 h or until the moisture content dropped below 5%. The dried sample was sieved through a 60-mesh sieve. The resulting sample, referred to as defatted rice bran flour (DRBF), was stored at -20°C for further analysis.

Chemical composition analysis

The chemical composition of the DRBF, including moisture, ash, protein, fat, dietary fiber, and carbohydrates was analyzed following AOAC methods (AOAC, 2000).

Water activity (a_w) and color measurement

The water activity (a_w) of DRBF was measured using a water activity meter (AQUA LAB 4TE, USA). The color (L^*, a^*, b^*) was measured using a colorimeter (Hunter Lab Color Flex 4510, USA), and the results were compared to commercial wheat flour.

Phytochemical analysis

To assess the phytochemical content, 0.1 g of DRBF was mixed with 2.5 mL of water and vortexed for 1 min. The mixture was centrifuged (HERMLE Labortechnik GmbH, Wehingen, Germany) at 10,000 rpm for 10 min, and the supernatant was collected for further analysis of phytochemical and antioxidant properties.

Total phenolic content

The total phenolic content (TPC) of the samples was determined using the Folin-Ciocalteu method, as described by Khongla *et al*. (2024). TPC was measured by mixing 100 μL of the sample with 2 mL of 2% Na_2CO_3, followed by the addition of 100 μL of Folin-Ciocalteu reagent (1:1 (v/v) in ethanol). After 30 min of incubation at room temperature, absorbance was measured at 750 nm, and the results were expressed as mg gallic acid eq./g of sample.

Total flavonoid content

The total flavonoid content (TFC) of the samples was measured using the method of Liu *et al*. (2002). TFC was determined by mixing 250 μL of the sample with 1,250 μL of distilled water and 75 μL of 5% $NaNO_2$. After 6 min, 150 μL of 10% $AlCl_3.6H_2O$ was added, followed by 500 μL of 1M NaOH after 5 min. The absorbance was measured at 510 nm, and the results were expressed as mg catechin eq./g of sample.

Gamma-oryzanol content

The gamma-oryzanol content of DRBF was determined following the method of Duangsa *et al*. (2025). The absorbance of a clear sample extract was measured at 315 nm using a UV-VIS spectrophotometer (Thermo Fisher, Scientific Genesys 10 UV scanning, USA). The gamma-oryzanol concentration was calculated by comparing the absorbance with a standard curve ranging from 0.0025 to 0.050 mg/mL, and the results were expressed as mg gamma-oryzanol/g of sample.

Antioxidant activity analysis

The DPPH radical scavenging activity of the sample was evaluated following the method of Musika *et al*. (2021). A 50 μL aliquot of the sample was mixed with 1,950 µL of DPPH solution (40 mg/L in methanol). The mixture was shaken and incubated in the dark for 20 min, and the absorbance was measured at 517 nm. The results were expressed as mg Trolox eq./g of sample.

The ABTS radical scavenging activity of the sample was measured according to the method of Khongla *et al*. (2022). A 20 µL aliquot of the sample was mixed with 1,980 µL of ABTS solution. The mixture was incubated in the dark for 5 min, and the absorbance was measured at 734 nm. The results were expressed as mg Trolox eq./g of sample.

The ability of RBF to reduce Fe^{3+} to Fe^{2+} was determined using the method of Benzie and Strain (1996). A 100 µL aliquot of the sample was mixed with 1 mL of FRAP reagent, prepared by mixing 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride, and 10 mM TPTZ in a 10:1:1 ratio (v/v/v). The mixture was incubated at 37°C for 15 min, and the absorbance was measured at 593 nm. The results were expressed as mg Trolox eq./g of sample

Metal chelating activity was done by evaluating the antioxidant capacity of the samples which was assessed by measuring their ability to chelate ferrous ions, following the method of Decker and Welch (1990). A 100 µL aliquot of the sample was mixed with 2,400 µL of deionized water, followed by the addition of 50 µL of 2 mM $FeCl_2$ solution and 100 µL of 5 mM Ferrozine solution. The mixture was incubated at room temperature for 20 min, and the absorbance was measured at 562 nm. The chelating ability of the sample was expressed as mg EDTA eq./g of sample.

Development of cookies supplemented with DRBF

Wheat flour was partially substituted with DRBF at levels of 0%, 5%, 10%, 15%, 20%, and 25% (w/w), as shown in Table 1. To prepare the cookies, wheat flour and DRBF were sifted together. Butter and sugar were blended until smooth, then eggs were added and mixed at medium speed for 30s. The flour mixture, baking powder, and salt were gradually incorporated and mixed for 1 min. A butter-milk aroma was then added as the final ingredient. The dough was then portioned into 5-6 g pieces, baked at 150°C for 25 min, cooled on a wire rack, and then stored in a sealed plastic bag.

Table 1. Ingredient composition of cookies with wheat flour replaced by DRBF at ratios of 0%, 5%, 10%, 15%, 20%, and 25% (w/w)

Ingredients	Content (g)					
	F0	F1	F2	F3	F4	F5
Wheat Flour	100	95	90	85	80	75
Rice Bran Flour	0	5	10	15	20	25
Butter	84	84	84	84	84	84
Granulated Sugar	40	40	40	40	40	40
Eggs	25	25	25	25	25	25
Baking Powder	0.8	0.8	0.8	0.8	0.8	0.8
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Total	250	250	250	250	250	250

F0, Cookies made entirely from 100% wheat flour; F1, Cookies containing 5% DRBF; F2, Cookies containing 10% DRBF; F3, Cookies containing 15% DRBF; F4, Cookies containing 20% DRBF; F5, Cookies containing 25% DRBF. Approximately 2.5 mL of butter-milk aroma was added to all formulations.

Sensory evaluation of cookies

Sensory evaluation was conducted using a 9-point hedonic scale to assess appearance, color, odor, taste, texture, and overall acceptability. Thirty-five untrained panelists participated in the sensory evaluation. The study was approved by the Human Research Ethics Committee of Rajamangala University of Technology Isan (HEC-01-67-073). All participants confirmed that they had no dietary allergies.

Phytochemical and antioxidant properties of cookies

Each cookie formulation (0.1 g) was mixed with 2.5 mL of water and homogenized using an IKA T25 Digital Ultra Turrax (Staufen, Germany). The mixture was then filtered and centrifuged at 10,000 rpm for 10 min. The supernatant was collected for phytochemical and antioxidant property analysis, as previously described.

Physical properties and chemical composition of cookies

Water activity (a_w) and color (L^*, a^*, b^*) of the cookies were measured using the same methods as previously described. In addition, the chemical composition of the most preferred cookie formulation, including moisture, ash, protein, fat, dietary fiber, and carbohydrates, was analyzed using AOAC methods.

Statistical analysis

The data were analyzed through analysis of variance (ANOVA) at a 95% confidence level. Mean comparisons were conducted using Duncan multiple range test (DMRT), with significance determined at $p \leq 0.05$. An independent samples t-test was also applied to evaluate differences between two experimental groups at the same confidence level ($p \leq 0.05$).

Results

Chemical Composition of DRBF

The chemical analysis of DRBF is summarized in Table 2. The results revealed that DRBF contained 4.78±0.04% moisture, 9.72±0.00% ash, 13.77±0.47% crude fat, 6.73±0.05% crude fiber, 12.64±1.93% crude protein, and 52.36±0.00% carbohydrates. The relatively high levels of dietary fiber, fat, and protein in DRBF suggest its potential as a nutritionally beneficial ingredient for food product development.

Table 2. Chemical composition of DRBF

Component	Defatted rice bran flour (%w.b.)
Moisture	4.78±0.04
Ash	9.72±0.00
Crude fat	13.77±0.47
Crude fiber	6.73±0.05
Crude protein	12.64±1.93
Carbohydrate	52.36±0.00

Carbohydrate content was calculated using formula 100 - (moisture + ash + protein + fat + dietary fiber).

Physical qualities of DRBF

The physical properties of DRBF were compared with those of commercial wheat flour, and the results are summarized in Table 3. The moisture content of DRBF was 4.78±0.04%, which was slightly higher than that of wheat flour (3.37±0.16%). The water activity (a_w) of DRBF was 0.5657±0.0067, while wheat flour had a lower a_w of 0.4947±0.0178. However, a_w of both flours were below 0.60, indicating microbiological stability.

Regarding color properties, wheat flour exhibited a higher lightness (L^*) value of 93.46±0.56 compared to 63.18±0.65 for DRBF, indicating that wheat flour is visibly lighter due to its refined nature. DRBF, being less processed, retained more pigments, resulting in a darker appearance. The a^* value, which measures green-red tones, was negative for both flours, with wheat flour at -0.54±0.11 and DRBF at -0.12±1.00, indicating a subtle green hue in both samples. The b^* value, representing blue-yellow tones, was higher in DRBF (30.33±2.16) compared to wheat flour (6.22±0.19), signifying a stronger yellow color in rice bran due to its natural pigments.

Table 3. Physical properties of DRBF compared to commercial wheat flour

Physical property	Wheat flour	Defatted rice bran flour
Moisture	3.37±0.16^b	4.78±0.04^a
a_w	0.4947±0.0178^b	0.5657±0.0067^a
Color		
L^*	93.46±0.56^a	63.18±0.65^b
a^{*ns}	-0.54±0.11	-0.12±1.00
b^*	6.22±0.19^b	30.33±2.16^a

Different letters within the same row indicate statistically significant differences at the 95% confidence level ($p \leq 0.05$), as determined using an independent samples t-test. ns denotes no significant difference.

Phytochemical properties and antioxidant activities of DRBF

A comparison of the phytochemical and antioxidant properties between DRBF and commercial wheat flour revealed notable differences in several bioactive compounds, as summarized in Table 4. DRBF showed a significantly higher total phenolic content of 3.54±0.14 mg gallic acid eq./g compared to 0.89±0.02 mg gallic acid eq./g in wheat flour. Similarly, its total flavonoid content was 2.01±0.19 mg catechin eq./g, which was much higher than the 0.15±0.02 mg catechin eq./g found in wheat flour. Although gamma oryzanol content was not analyzed in wheat flour, DRBF contained 0.30±0.02 mg/g.

Antioxidant activity, was evaluated using four different methods, and the results revealed that DRBF exhibited significantly stronger activity than wheat flour (Table 4). DPPH radical scavenging activity in DRBF was 2.54±0.15 mg Trolox eq./g, while wheat flour showed no activity. For ABTS radical scavenging activity, DRBF exhibited 8.01±0.79 mg Trolox eq./g, which was significantly higher than the 1.68±0.26 mg Trolox eq./g observed in wheat flour. Similarly, the ferric reducing antioxidant power of DFBF was 3.66±0.25 mg Trolox eq./g, which was significantly higher than the 0.19±0.09 mg Trolox eq./g found in wheat flour. Additionally, the metal chelating activity in DRBF was 5.91±0.20 mg EDTA eq./g, which was significantly higher than the 1.36±0.07 mg EDTA eq./g observed in wheat flour. These results indicated that DRBF exhibits superior antioxidant capacity and higher levels of bioactive compounds, making it is promised the functional ingredient for food applications.

Table 4. Phytochemical and antioxidant activities of DRBF compared to 100% commercial wheat flour (w/w)

Phytochemical and Antioxidant Activities	Wheat Flour	Defatted Rice Bran Flour
Total phenolic compounds (mg Gallic acid eq./g sample)	0.89±0.02[b]	3.54±0.14[a]
Total flavonoid content (mg Catechin eq./g sample)	0.15±0.02[b]	2.01±0.19[a]
Gamma oryzanol content (mg/g sample)	not performed	0.30±0.02
DPPH radical scavenging activity (mg Trolox eq./g sample)	0.00[b]	2.54±0.15[a]
ABTS radical scavenging activity (mg Trolox eq./g sample)	1.68±0.26[b]	8.01±0.79[a]
Ferric reducing antioxidant power (mg Trolox eq./g sample)	0.19±0.09[b]	3.66±0.25[a]
Metal chelating activity (mg EDTA eq./g sample)	1.36±0.07[b]	5.91±0.20[a]

Different letters within the same row indicate statistically significant differences at the 95% confidence level ($p \leq 0.05$), as determined using an independent samples t-test.

Development of cookies supplemented with DRBF

The physical quality of cookies supplemented with varying concentrations of DRBF is presented in Table 5. Water activity (a_w) of all six cookie formulations (F0, F1, F2, F3, F4, and F5) was not significantly different, with values ranging from 0.3148-0.3864.

Table 5. Physical properties of cookies supplemented with DRBF at varying substitution levels of 0%, 5%, 10%, 15%, 20%, and 25% (w/w)

Cookie formulation	a_w [ns]	Color		
		L^*	a^*	b^*
F0	0.3238±0.0574	58.20±0.36[a]	11.20±0.35[d]	57.00±1.73[a]
F1	0.3173±0.0474	52.87±0.67[b]	13.83±1.50[c]	58.23±0.45[a]
F2	0.3148±0.0271	51.37±0.95[c]	15.27±0.23[bc]	55.73±1.14[a]
F3	0.3537±0.0100	48.40±1.00[d]	16.47±0.40[c]	51.90±2.07[b]
F4	0.3864±0.0267	45.17±0.45[e]	19.30±0.10[b]	52.67±0.90[b]
F5	0.3309±0.0303	38.47±1.17[f]	23.70±1.40[a]	58.33±2.70[a]

Different letters within the same column indicate statistically significant differences ($p \leq 0.05$). ns denotes no significant difference, F0, Cookies made entirely from 100% wheat flour; F1, Cookies containing 5% DRBF; F2, Cookies containing 10% DRBF; F3, Cookies containing 15% DRBF; F4, Cookies containing 20% DRBF; F5, Cookies containing 25% DRBF

The color assessment of cookies enriched with different concentrations of DRBF is summarized in Table 5. The lightness (L^*) values decreased with increasing concentrations of DRBF. The F5 (25%DRBF) formulation demonstrated the lowest L^* value (38.47±1.17), indicating the darkest appearance. In contrast, the redness (a^*) increased with higher concentrations of DRBF. The F5 (25%DRBF) formulation exhibited the highest a^* value (23.70±1.40), which resulted in a deeper red tone. The yellow hue (b^*) of all cookies ranged from 51.90-58.33.

Phytochemical and antioxidant activity of cookies supplemented with DRBF

Phytochemical properties

The total phenolic and flavonoid content of cookies supplemented with DRBF (F0-F5) is displayed in Figure 1. The phenolic content ranged from 0.91-1.56 mg gallic acid eq./g. The F5 (25%DRBF) formulation exhibited the highest phenolic content at 1.56±0.03 mg gallic acid eq./g, followed by F4 (20%DRBF) and F3 (15%DRBF) with 1.36±0.14 and 1.32±0.03 mg gallic acid eq./g, respectively, with no significant difference between them (p>0.05). F2 (10%DRBF) showed a phenolic content of 1.09±0.02 mg gallic acid eq./g. Meanwhile, F1(5%DRBF) and the control (F0) showed similar values of 0.96±0.06 and 0.91±0.06 mg gallic acid eq./g (Figure 1A). These results indicate that increasing the DRBF content from 10%-25% significantly enhanced the phenolic content.

The total flavonoid content in cookies supplemented with varying concentrations of DRBF ranged from 0.50-0.75 mg catechin eq./g (Figure 1B). The F5 (25%DRBF) formulation exhibited the highest flavonoid content at 0.75±0.01 mg catechin eq./g, followed by F4 (20%DRBF) and F3 (15%DRBF), which had values of 0.74±0.05 and 0.74±0.09 mg catechin eq./g, respectively. Formulation F2 (10%DRBF) displayed a flavonoid content of 0.67±0.02 mg catechin eq./g, while F1 (5%DRBF) contained 0.60±0.02 mg catechin eq./g. The control formulation (F0) had the lowest flavonoid content at 0.50±0.05 mg catechin eq./g. These findings indicated that increasing the proportion of DRBF from 5%-25% significantly enhanced the total flavonoid content in the cookies.

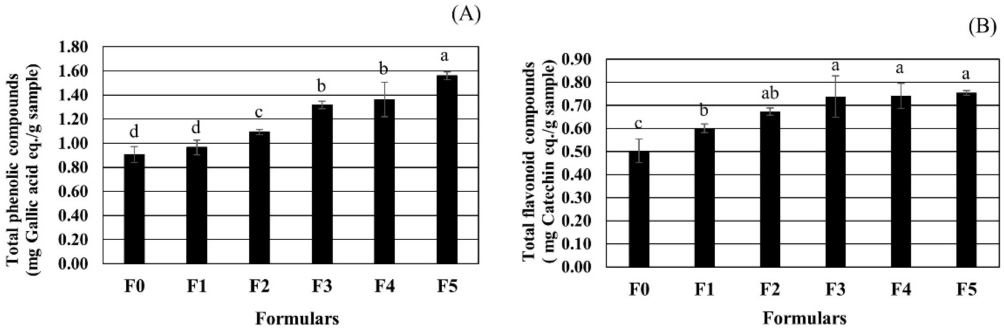

Figure 1. Phytochemical properties of cookies supplemented with DRBF: (A), total phenolic compounds; (B), total flavonoid compounds; F0, Cookies made entirely from 100% wheat flour; F1, Cookies containing 5%DRBF; F2, Cookies containing 10%DRBF; F3, Cookies containing 15%DRBF; F4, Cookies containing 20%DRBF; F5, Cookies containing 25%DRBF. Different letters above the bars indicate statistically significant difference ($p \leq 0.05$)

Antioxidant activity

The antioxidant activity of cookies supplemented with DRBF (F0-F5) is illustrated in Figure 2. Based on the DPPH antioxidant analysis, the DPPH radical scavenging activity ranged from 0.45-3.95 mg Trolox eq./g. The F5 cookie formulation (25%DRBF) exhibited the highest antioxidant capacity, with a value of 3.95±0.03 mg Trolox eq./g. The antioxidant activities of the F4, F3, and F2 cookie formulations (containing 20%, 15%, and 10%DRBF, respectively) were 3.57±0.15, 2.44±0.24, and 1.00±0.05 mg Trolox eq./g, respectively. The F1 and F0 cookies (with 5%DRBF and the control) showed DPPH radical scavenging activities of 0.64±0.03 and 0.45±0.10 mg Trolox eq./g, which was not a statistically significant difference (Figure 2A). These results indicated that incorporating DRBF in the cookies enhanced their antioxidant capacity.

ABTS antioxidant activity showed that the F5 (25%DRBF) exhibited the highest ABTS antioxidant activity at 1.35±0.12 mg Trolox eq./g. This was followed by F4 (20%DRBF) and F3 (15%DRBF), with values of 0.81±0.14 and 0.42±0.16 mg Trolox eq./g, respectively. No detectable ABTS activity was observed in the control, F1 (5%DRBF), or F2 (10%DRBF) formulations, likely due to their lower phenolic and flavonoid contents. These findings suggested that supplementing cookies with 15%-25%DRBF significantly enhanced ABTS antioxidant activity (Figure 2B).

The ferric reducing antioxidant power (FRAP) analysis showed an increasing trend with higher DRBF concentrations, ranging from 0.18-0.83 mg Trolox eq./g. The F5 formulation (25%DRBF) exhibited the highest ferric reducing power, while the control had the lowest. These findings suggested that supplementing cookies with 5%-25%DRBF effectively enhanced their ferric reducing antioxidant capacity (Figure 2C).

The metal chelating ability of the cookies increased with higher levels of DRBF. The F5 (25%DRBF) had the highest activity at 2.47±0.16 mg EDTA eq./g, followed by F4 (20%DRBF) and F3 (15%DRBF) with values of 1.50±0.09 and 1.04±0.17 mg EDTA eq./g, respectively. The F2 (10%DRBF) and F1 (5%DRBF) showed values of 0.40±0.22 and 0.33±0.09 mg EDTA eq./g, which was not significant differed between them. These findings indicated that incorporating DRBF at levels of 5%-25% enhanced the ferrous ion chelating capacity of cookies (Figure 2D).

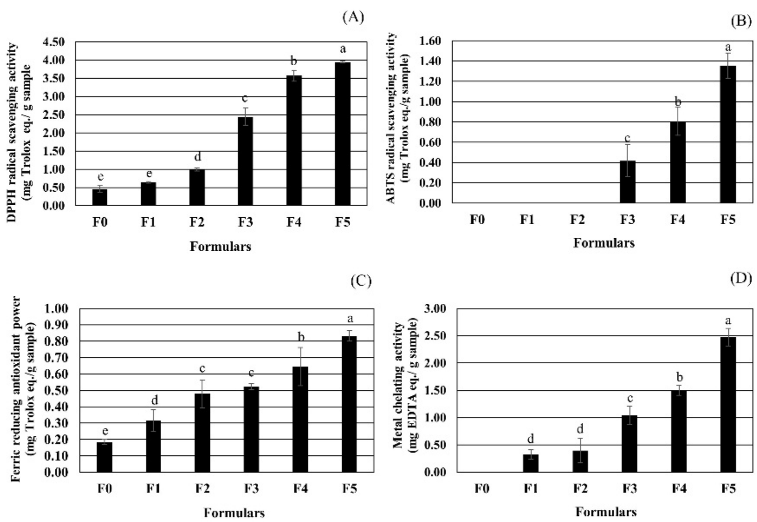

Figure 2. Antioxidant activity of cookies supplemented with DRBF: (A), DPPH radical scavenging activity; (B), ABTS radical scavenging activity; (C), Ferric reducing antioxidant power; (D), Metal chelating activity; F0, Cookies made entirely from 100% wheat flour; F1, Cookies containing 5%DRBF; F2, Cookies containing 10%DRBF; F3, Cookies containing 15%DRBF; F4, Cookies containing 20%DRBF; F5, Cookies containing 25%DRBF. Different letters above the bars indicate statistically significant difference ($p \leq 0.05$)

Sensory evaluation

The sensory evaluation of cookies supplemented with various levels of DRBF was conducted using a 9-point hedonic scale, with 35 untrained panelists assessing appearance, color, odor, taste, texture, and overall acceptability (Table 6). The sensory attributed scores of cookies supplemented with 10% (F2) and 15%DRBF (F3) which was not significantly different from those supplemented with 5%DRBF (F1), except for the color score of F2 cookie formulation. However, cookies with higher levels of DRBF, 20% (F4) and 25% (F5), exhibited lower scores for all sensory attributes compared to F1. These results indicate that supplementing cookies with 20%-25%DRBF negatively affected sensory attributes compared to 5%. The F5 formulation exhibited the lowest scores in appearance (6.63±1.55), aroma (6.11±1.55), taste (5.63±1.68), texture (6.54±1.29), and overall acceptability (6.09±144).

These findings indicated that adding 5%-15% DRBF to cookies that helped to preserve favorable sensory qualities, while higher levels (20%-25%) diminished the taste and overall consumer preference. Based on phytochemical content, antioxidant activity, and sensory attributes, the F3 cookie (15%DRBF) demonstrated the highest levels of phytochemicals content and antioxidant activity compared to F1 and F2 cookies. Additionally, F3 cookie exhibited higher scores of appearance, color, odor, and overall acceptability compared to F4 and F5 cookies. Therefore, the F3 cookie formulation was selected for further analysis of its chemical composition compared to the cookie without DRBF.

Table 6. The sensory quality attributes of cookies supplemented with DRBF at various concentrations (5%, 10%, 15%, 20%, and 25% w/w)

Cookie Formulation	Appearance	Color	Odor	Taste	Texture
F1	7.91±1.22^{ab}	8.14±0.88^{a}	7.34±1.16^{a}	7.74±0.82^{a}	7.91±1.04^{a}
F2	7.49±1.22^{bc}	7.46±1.24^{bc}	7.40±1.19^{a}	7.69±1.28^{a}	7.40±1.61^{ab}
F3	8.17±0.86^{a}	7.66±1.14^{ab}	7.49±1.42^{a}	7.45±1.20^{ab}	7.54±1.17^{ab}
F4	7.26±1.38^{c}	6.97±1.46^{c}	6.80±1.47^{b}	7.00±1.31^{b}	7.23±1.03^{b}
F5	6.63±1.55^{d}	6.97±1.46^{c}	6.11±1.55^{c}	5.63±1.68^{c}	6.54±1.29^{c}

Different letters within the same column indicate statistically significant differences ($p \leq 0.05$). F1, Cookies containing 5%DRBF; F2, Cookies containing 10%DRBF; F3, Cookies containing 15%DRBF; F4, Cookies containing 20%DRBF; F5, Cookies containing 25%DRBF. N = 35.

Chemical composition of cookies supplemented with 15%DRBF and without DRBF

The chemical compositions of the F0 cookie formulation (without the DRBF) and F3 cookie formulation (containing 15%DRBF) are presented in Table 7. The main components observed in both F0 and F3 cookie formulations were carbohydrates, followed by fat content. The moisture, ash, and fat content of F0 and F3 showed no significant differences, with values ranged from 2.07%-2.79%, 1.28%-1.56%, and 31.33%-31.66%, respectively. F3 cookie formulation contained higher fiber and protein than F0 cookie. However, F3 cookie formulation contained lower carbohydrates than F0 cookie. The calorific value of F0 cookie (538.77 Kcal) was higher than that of F3 cookie (531.30 Kcal).

Table 7. The chemical composition of cookie formulation supplemented with 15% DRBF (F3) compared to cookie without DRBF (F0)

Chemical composition	F0 Formulation	F3 Formulation
Moisture (%w.b.)^{ns}	2.07±0.54	2.79±0.27
Ash (%w.b.)^{ns}	1.28±0.16	1.56±0.23
Crude fat (%w.b.)^{ns}	31.33±0.40	31.66±0.20
Crude fiber (%w.b.)	1.08±0.12^{b}	2.40±0.09^{a}
Crude protein (%w.b.)	10.28±0.27^{b}	11.21±0.30^{a}
Carbohydrates (%w.b.)	53.96±0.00^{a}	50.38±0.00^{b}
Energy (Kcal)	538.77±0.00^{a}	531.30±0.00^{b}

Carbohydrate content was calculated using the formular 100 - (moisture + ash + protein + fat + crude fiber). Different letters within the same row indicate statistically significant differences ($p \leq 0.05$). ns denotes no significant difference.

Discussion

The chemical composition of DRBF in this study was significantly differed from that reported in prior research. Our findings indicate 4.78% moisture, 13.77% fat, 12.64% protein, and 6.73% dietary fiber. In comparison, Kumari *et al*. (2018) observed a similar moisture content (4.80%) but reported a notably lower fat content (0.04%) and a significantly higher fiber content (13.10%). Similarly, Abdul-Hamid and Luan (2000) and Alauddin *et al*. (2019) described rice bran as being rich in diverse nutrients, including protein, fiber, lipids, carbohydrates, oryzanols, and a wide array of fatty acids, vitamins, and minerals. Mishra (2017) reported further variation in DRBF composition, with 11.3% moisture, 15.6% protein, 34.9% fiber, 2.2% fat, and 9.86% ash. Additionally, Kumari *et al*. (2018) reported 13.8% protein, 13.1% fiber, and 11.6% ash. The observed variability in protein, fat, and fiber content across studies may be influenced by factors such as rice variety, cultivation conditions, and processing methods. In addition, rice bran is rich in various minerals including aluminum, calcium, chlorine, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc, as well as vitamins such as vitamin E, thiamine, and niacin (Devi *et al*., 2021). The rich nutrient profile of DRBF, particularly its protein content, emphasizes its potential as a valuable ingredient for food fortification. These findings highlight versatility and its potential to enhance the nutritional quality of various food products.

DRBF exhibited distinct physical characteristics compared to commercial wheat flour. Its moisture content was higher (4.78±0.04%) than that of wheat flour (3.37±0.16%), though still well within the general limit of 14% for general-purpose wheat flour (TIS 375-2017). This is comparable to previously reported moisture values in laboratory-defatted (13.3%) and commercial-defatted rice bran (11.1%) (Sairam *et al*., 2011), and falls within the range of 2.39%-10.22% reported by Saidi *et al*. (2019) for stabilized rice bran. The increased moisture content in DRBF was likely due to its hygroscopic nature, allowing it to absorb moisture from the environment until reaching ambient humidity equilibrium. The water activity (a_w) of DRBF was also higher (0.5657±0.0067) than that of wheat flour (0.4947±0.0178), although it remained below the threshold for microbial growth (a_w<0.6), suggesting acceptable stability for shelf life. In terms of color, DRBF showed lower lightness (L^*=63.18±0.65) and higher yellowness (b^*=30.33±2.16) compared to wheat flour. This difference in color is likely due to the presence of natural pigments and phenolic compounds abundant in rice bran (Devi *et al*., 2021). Such color properties may influence the visual appeal of final products and are important considerations when developing DRBF-enriched foods, especially in bakery and snack applications.

Comparisons of phytochemical properties and antioxidant activities between wheat flour and DRBF showed that DRBF exhibited significantly higher phytochemical and antioxidant activities than wheat flour. DRBF contained total phenolic content of 3.54 mg gallic acid eq./g and flavonoid content of 2.01 mg catechin eq./g, as well as gamma-oryzanol content of 0.30 mg/g. In antioxidant assays, DRBF showed significantly higher DPPH and ABTS radical scavenging activities (2.54 and 8.01 mg Trolox eq./g, respectively), along with enhanced ferric reducing power and metal chelating activity. Hexane extracts of commercial and laboratory defatted rice bran exhibited higher oryzanol concentrations (0.013 g and 0.006 g/100 g, respectively) than methanol extracts (0.003 g and 0.001 g/100 g, respectively), likely due to extraction method differences and the slight oil content retained in hexane extracts (Sairam *et al*., 2011). Both samples showed DPPH scavenging abilities, at 59.69% and 58.93%, respectively, with increasing concentrations enhancing their reducing power (Sairam *et al*., 2011). Significant phytochemical and antioxidant variations were also found among pigmented rice bran, with black rice bran showing the highest levels of phenolic and flavonoid compounds, followed by red and brown rice bran (Ghasemzadeh *et al*., 2018). Major bioactives in rice bran, such as flavonoids (apigenin, quercetin), phenolic acids (ferulic, p-coumaric), and phytosterols, contribute to their antioxidative and health-promoting properties. These bioactive compounds provide benefits, including anti-inflammatory, anti-cancer, hypotensive, anti-diabetic, cardioprotective, and cholesterol-lowering effects (Ghasemzadeh *et al*., 2018; Tan *et al*., 2023). These results suggested that DRBF could serve as a valuable ingredient in health-focused food formulations.

Cookies were developed with varying concentrations of DRBF, ranging from 0%-25% (F0-F5 formulations), and evaluated for their physical, phytochemical, antioxidant, and sensory properties. The results showed that a_w of all cookies ranged from 0.3148-0.3864, classifying them as dry foods (a_w<0.6). This low a_w minimizes the risk of microbial growth, thereby improving shelf stability. Color is a crucial component of food appearance and one of the key determinants of consumer approval. The results showed that the b^* values of all cookies in our experiment were relatively high, ranging from 51.90-58.33, indicating that the cookies had an intense yellow color. These could be due to the presence of yellow pigments from both the rice bran and butter in the cookies. The lightness (L^*) values of cookies decreased with increasing DRBF levels, consistent with increasing redness (a^*) values. It indicated that the addition of higher concentrations of DRBF resulted in darker (lower L^*) and redder (higher a^*) color compared to the cookies with lower DRBF concentrations. In addition, the Maillard and caramelization reactions occuring during the heating process of cookies might have contributed to their darker color. Darker colors in bakery products, such as bread and cookies, are often due to caramelization, starch dextrinization, or the Maillard reaction- a non-enzymatic process between reducing sugars and lysine in proteins (Sudha *et al*., 2007). Similar findings in other studies showed that incorporating rice bran in baked products resulted in a crust that showed generally darker, and more reddish (da Rocha Lemos Mendes *et al*., 2021; Mishra, 2017; Sharif *et al*., 2009).

The sensory evaluation indicated that the addition of DRBF at high levels (20%-25%) in cookies negatively affected the appearance, color, odor, taste, texture, and overall acceptability score of the panelists. It might be undesirable flavor and dark color of the rice bran incorporated into cookies. Based on the sensory evaluation results in the current study, the appropriate concentration of DRBF incorporated in cookies should not exceed 20% of the total flour. It has been reported that incorporating DRBF into baked products, such as cakes and biscuits, boosted fiber, phenolic content, and antioxidant capacity, with 30%DRBF in cakes yielding high acceptance (da Rocha Lemos Mendes *et al*., 2021). In bread, adding 5%-10%DRBF increased firmness and reduced loaf volume, with sensory ratings comparable to market-available high-fiber breads (Abdul-Hamid and Luan, 2000). Sharif *et al*. (2009) found that supplementing cookies with 10%-20%DRBF enhanced dietary fiber and mineral content without compromising quality. Mishra (2017) confirmed that adding up to 15%DRBF enhanced nutritional value with minimal sensory impact. The concentration of DRBF used in food products varies based on factors like product type, sensory qualities, nutritional enhancement, and processing methods. Higher levels of DRBF increased fiber and antioxidants but can be negatively affected the sensory attributes, requiring a careful balance to maintain product appeal. Based on the sensory attributes and antioxidant activity results, the addition of 15%DRBF to the cookies was found to be optimal. Therefore, the F3 cookie formulation with 15%DRBF was selected chemical composition analysis, in comparison to cookies without DRBF.

The F3 cookie formulation contained higher crude fiber (2.40%), and crude protein (11.21%), but lower carbohydrates (50.38%) compared to the F0 cookie (0%DRBF). These findings suggested that the addition of 15%DRBF to cookies not only improved phytochemical content and antioxidant activity but also increased fiber and protein content. Consistent with previous studies, it was found that the protein and fiber content of cookies increased after supplementation with 15%DRBF compared to cookies without DRBF (Mishra, 2017). The increase in protein and fiber content may be ascribed to the higher protein and fiber content in rice bran compared to wheat flour (Mishra, 2017). The fat content of the F0 cookie (31.33%) was not significantly differed from that of the F3 cookie (31.66%), likely due to the use of defatted rice bran in this experiment. In addition, the high fat in the cookies might be due to the high fat content in the butter. There was no significant effect of the defatted rice bran on the moisture content of the cookies, possibly because the moisture content in rice bran (4.78%) is close to that of wheat flour (3.37%). The calorific value of the cookies decreased from 538.77 Kcal to 531.30 Kcal with the addition of 15%DRBF. From a nutritional perspective, cookies with a reduced calorific value are found to be preferable due to their potential health benefits. The lower calorific value of the F3 cookie is attributed to its lower carbohydrate content. Mishra (2017) reported that the composition of fortified cookies with 15%DRBF was as follows: moisture (7.19%), fiber (7.88%), fat (16.25%), ash (2.75%), protein (13.77%), carbohydrate (52.67%), and calorific value (410.39 Kcal). Younas *et al*. (2011) also reported the composition of fortified cookies with 15%DRBF as moisture (6.2-6.3%), fat (16.17-16.27%), and protein content (12.56-12.64%). The difference in chemical compositions of the cookies in our work and previous works may be due to different cookie formulations and sources of defatted rice bran.

In conclusion, DRBF is rich in phytochemicals and antioxidants, which contribute to its health benefits. Cookies supplemented with 15%DRBF were well-accepted by consumers and showed strong antioxidant activity. These findings suggested that DRBF, particularly at the 15% level, could serve as a valuable ingredient for improving the overall quality and health appeal of cookies. Future research should explore additional applications of DRBF in different bakery products to further capitalize on its health-promoting properties.

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Conflicts of interest

The authors declare no conflict of interest.

References

Abdul-Hamid, A. and Luan, Y. S. (2000). Functional properties of dietary fibre prepared from defatted rice bran. Food Chemistry, 68:15-19.

Alauddin, M., Rahman, S., Islam, J., Shirakawa, H., Komai, M. and Howlader, M. Z. H. (2019). Development of rice bran functional food and evaluation of its healthful properties. Rice Bran and Rice Bran Oil, 183-206.

Anthina, L., Costas, B. and Irakli, M. (2021). Comparative evaluation of the nutritional, antinutritional, functional, and bioactive attributes of rice bran stabilized by different heat treatments. Foods, 10:57.

AOAC. (2000). Official methods of analysis. 17th eds. Association of Official Chemists, Gaithersberg, Maryland.

Benzie, I. F. F. and Strain, J. J. (1996). The ferric reducing ability of plasma as a measure of antioxidant power: The FRAP assay. Analytical Biochemistry, 239:70-76.

Calvo-Castro, L. A., Sus, N., Schiborr, C., Bosy-Westphal, A., Duran, M. L., Fesenmeyer, D., Frank, J. and Rimbach, G. (2019). Pharmacokinetics of vitamin E, γ-oryzanol, and ferulic acid in healthy humans after the ingestion of a rice bran-enriched porridge prepared with water or with milk. European Journal of Nutrition, 58:2099-2110.

Cheng, Y. F. and Bhat, R. (2016). Functional, physicochemical, and sensory properties of novel cookies produced by utilizing underutilized jering (*Pithecellobium jiringa* Jack.) legume flour. Food Bioscience, 14:54-61.

da Rocha Lemos Mendes, G., Souto Rodrigues, P., de las Mercedes Salas-Mellado, M., Fernandes de Medeiros Burkert, J. and Badiale-Furlong, E. (2021). Defatted rice bran as a potential raw material to improve the nutritional and functional quality of cakes. Plant Foods for Human Nutrition, 76:46-52.

Decker, E. A. and Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. Journal of Agricultural and Food Chemistry, 38:674-677.

Devi, R., Veliveli, V. L. and Devi, S. S. (2021). Nutritional composition of rice bran and its potentials in the development of nutraceuticals rich products. Journal of Pharmacognosy and Phytochemistry, 10:470-473.

Duangsa, B., Rodpon, P., Khongla, C., Musika, S., Rattanajun, P., Khumpumuang, P., Jangkloy, U., Jampatech, S., Thiamjite, P. and Sawisit, A. (2025). Gamma-oryzanol, physicochemical, and antioxidant properties of stabilized rice bran oil from dough and mature grain stages. Current Applied Science and Technology, 25:e0260012.

Francisqueti-Ferron, F. V., Ferron, A. J. T., Altomare, A., Garcia, J. L., Moreto, F., Ferreira, A. L. A., Minatel, I. O., Aldini, G. and Corrêa, C. R. (2021). Gamma-oryzanol reduces renal inflammation and oxidative stress by modulating AGEs/RAGE axis in animals submitted to high sugar-fat diet. Brazilian Journal of Nephrology, 43:460-469.

Ghasemzadeh, A., Karbalaii, M. T., Jaafar, H. Z. and Rahmat, A. (2018). Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran. Chemistry Central Journal, 12:1-13.

Jia, M., Yu, Q., Chen, J., He, Z., Chen, Y., Xie, J., Nie, S. and Xie, M. (2020). Physical quality and in vitro starch digestibility of biscuits as affected by the addition of soluble dietary fiber from defatted rice bran. Food Hydrocolloids, 99:105349.

Khongla, C., Chuaingan, J., Siadkhunthod, T., Somnam, P., Musika, S. and Sangsawad, P. (2022). Physicochemical properties of rice bran hydrolysate prepared in a pilot scale process and its application in milk tablets. Trends in Sciences, 19:2316.

Khongla, C., Yuwang, P., Yuwang, T. and Musika, S. (2024). Physicochemical, phytochemical, and antioxidant properties of organic sweet potato flour and its application in breadstick. Trends in Sciences, 21:8162.

Kohajdova, V. K. Z., Karovicova, J. and Lauková, M. (2018). Physical, textural, and sensory properties of cookies incorporated with grape skin and seed preparations. Polish Journal of Food and Nutrition Sciences, 68:309-317.

Kucerova, J., Sottikova, V. and Nedomova, S. (2013). Influence of dietary fiber addition on the rheological and sensory properties of dough and bakery products. Czech Journal of Food Sciences, 31:340-346.

Kumari, N., Vinita, N. K. and Rani, P. (2018). Nutrient composition of full fat and defatted rice bran. Asian Journal of Dairy and Food Research, 37:77-80.

Liu, M., Li, X. Q., Weber, C., Lee, C. Y., Brown, J. and Liu, R. H. (2002). Antioxidant and antiproliferative activities of raspberries. Journal of Agricultural and Food Chemistry, 50:2926-2930.

Manzoor, A., Pandey, V. K., Dar, A. H., Fayaz, U., Dashe, K. K., Shams, R., Fayaz, R., Ahmad, S., Bashir, I., Fayaz, J., Singh, P., Khan, S. A. and Ganaie, T. A. (2023). Rice bran: Nutritional, phytochemical, and pharmacological profile and its contribution to human health promotion. Food Chemistry Advances, 2:100296.

Martirosyan, D. M. and Singh, J. (2015). A new definition of functional food by FFC: what makes a new definition unique? Functional Foods in Health and Disease, 5:209-223.

Mishra, N. (2017). Utilization of waste defatted rice bran in formulation of functional cookies and its effect on physiochemical characteristic of cookies. International Journal of Advanced Science and Research, 2:64-68.

Musika, S., Pokratok, N., Pliankratoke, J., Khongla, C., Kupradit, C., Ranok, A. and Mangkalanan, S. (2021). Antioxidant, antityrosinase, and antibacterial activities of fruit peel extracts. International Journal of Agricultural Technology, 17:1447-1460.

Pakhare, K. N., Dagadkhair, A. C., Udachan, I. S. and Andhale, R. A. (2016). Studies on preparation and quality of nutritious noodles by incorporation of defatted rice bran and soy flour. Journal of Food Process Technology, 7.

Phunikhom K., Sattayasai J., Tiamkao S. and Gaysonsiri, D. (2021). A randomized, double blind clinical study to assess the effects of a gamma-oryzanol-enriched rice bran oil on lipid profile in the hypercholesterolemic patients. Journal of the Medical Association of Thailand, 104:64-69.

Saidi, N., Fitriani, D. and Syamsuddin, M. (2019). Quality of rice bran submitted to different stabilization procedures during storage periods. IOP Conference Series: Journal of Physics: Conference Series, 1232:012046.

Sairam, S., Krishna, A. G. G. and Urooj, A. (2011). Physico-chemical characteristics of defatted rice bran and its utilization in a bakery product. Journal of Food Science and Technology, 48:478-483.

Sethi, S., Nanda, S. K. and Bala, M. (2020). Quality assessment of pasta enriched with anthocyanin-rich black rice bran. Journal of Food Processing and Preservation, 44:e14952.

Sharif, M. K., Butt, M. S., Anjum, F. M. and Nawaz, H. (2009). Preparation of fiber and mineral enriched defatted rice bran supplemented cookies. Pakistan Journal of Nutrition, 8:571-577.

Sudha, M. L., Vetrimani, R. and Leelavathi, K. (2007). Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. Food Chemistry, 100:1365-1370.

Tan, B. L., Norhaizan, M. E. and Chan, L. C. (2023). Rice bran: From waste to nutritious food ingredients. Nutrients, 15:2503.

Thai Industrial Standards Institute (TISI). (2017). TIS 375-2560: Wheat flour: general purpose flour. Ministry of Industry, Bangkok, Thailand.

Younas, A., Bhatti, M. S., Ahmed, A. and Randhawa, M. A. (2011). Effect of rice bran supplementation on cookie baking quality. Pakistan Journal of Agricultural Sciences, 48:129-134.

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