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Assessment of functional, sensory, and bioactive properties of crackers enriched with Hom Pathum and black glutinous rice flour

Paponpat Pattarathitiwat[1]
Teerapap Panklai[2]
Nattapas Yousawang[3]
Apidech Pongprajak[4]
Pornpachen Chuchird[5+]

[1,4,5]*Department of Food and Nutrition, Faculty of Home Economics Technology, Rajamagala University of Technology Thanyaburi, Pathum Thani, Thailand.*
[1]*Email: Paponpat_p@rmutt.ac.th*
[4]*Email: Apidech_p@rmutt.ac.th*
[5]*Email: Pornpachen_c@rmutt.ac.th*
[2]*Faculty of Science, Chulabhorn Royal Academy, Bangkok, 10210, Thailand.*
[2]*Email: Teerapap.pan@cra.ac.th*
[3]*Chulabhorn Hospital, Chulabhorn Royal Academy, Bangkok, Thailand.*
[3]*Email: Nattapas.you@cra.ac.th*

(+ Corresponding author)

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ABSTRACT

Crackers are widely consumed snack foods known for their thin, dry, and crisp texture. Traditionally, they are produced using soft wheat flour, which is nutritionally limited, as it contains low amounts of vitamins, minerals, and fiber while also having a high glycemic index (GI). To address these shortcomings, this study explored the development of crackers enriched with Hom Pathum (HP) rice and black glutinous (BG) rice flour to enhance their nutritional value. Sensory evaluation results indicated no significant difference among the formulations, suggesting consumer acceptability. The optimal formulation was identified as crackers supplemented with a 40:25 ratio of HP to BG rice flour. Color analysis (L^*, a^*, b^*) revealed statistically significant differences ($p < 0.05$), showing that higher BG rice flour content produced darker crackers. Microbiological quality met safety standards, with mold and bacterial counts below 1×10^4 and 100 cfu/g, respectively. Nutritional analysis demonstrated considerable bioactive compounds, with total phenolic, flavonoid, and anthocyanin contents recorded at 2.95, 5.70, and 79.05 mg/g (DW), respectively. Functional properties were also promising, as antioxidant activity showed 14.33% and 8.77% inhibition by ABTS and DPPH assays, while α-glucosidase inhibition reached 28.44%. These findings indicate that supplementing crackers with HP and BG rice flour not only improves their nutritional and functional value but also provides consumers with a healthier snack alternative that may contribute to better dietary management and disease prevention.

Contribution/Originality: This study introduces a new formulation by enriching crackers with Hom Pathum and black glutinous rice flours, thereby enhancing bioactive compounds, antioxidant activity, and α-glucosidase inhibition compared to wheat-based crackers. The combination of local rice varieties results in a functional, consumer-acceptable snack that supports healthier diets and the prevention of chronic diseases.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a vital energy source and staple food in many countries. Thailand is a significant producer of both domestic consumption and exports, consistently maintaining the highest export volume for over a decade (Attaviroj & Noomhorm, 2014). It is crucial for global food security, especially in developing nations. In 2018, Thailand was ranked as the sixth-largest rice producer worldwide, offering a wide variety of rice types, including local and market varieties, and Thai people consume both unpolished or non-colored rice and polished or colored rice (Chinvongamorn & Sansenya, 2020). Like other cereal grains, rice is a rich source of essential nutrients, such as carbohydrates, proteins, fatty acids, vitamins, and trace minerals. Additionally, it contains bioactive non-nutrient compounds like antioxidants, including phenolic compounds (Vichapong, Sookserm, Srijesdaruk, Swatsitang, & Srijaranai, 2010).

Hom Pathum (HP) Rice, also known as Pathum Thani 1 Rice, possesses characteristics such as tenderness and a fragrance reminiscent of Jasmine Rice (Suan Sunandha Rajabhat University Samut Songkhram Campus, 2022). The nutritional profile consists of the following values per 100 g: 79.00 g of carbohydrates, 7.25 g of protein, 1.52 g of fat, 14.30 g of amylose, and 0.20 g of fiber (Udomkun, Innawong, & Niruntasuk, 2018). The bioactive compounds of HP consist of γ-oryzanol (Chinvongamorn & Sansenya, 2020) and various phenolic compounds, such as *p*-coumaric acid, ferulic acid, guaiacol, *p*-cresol, *o*-cresol, and 3,5-xylenol (Vichapong et al., 2010). Black glutinous (BG) rice, often called black sticky rice, is a specific type of short-grain rice known for its stickiness and sweet flavor. This rice variety is characterized by uneven pigmentation and is primarily used in Asian desserts (Rahim et al., 2022). Notably, BG rice contains several bioactive compounds, including phenolic compounds such as protocatechuic acid, protocatechuic aldehyde, vanillic acid, vanillin, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, caffeine, and chlorogenic acid (Setyaningsih, Saputro, Palma, & Barroso, 2016; Sompong, Siebenhandl-Ehn, Linsberger-Martin, & Berghofer, 2011) and anthocyanins (Sivamaruthi, Kesika, & Chaiyasut, 2018). These compounds have been recognized for their advantageous contributions to human health.

Crackers are prominent baked goods, known for their nutritional qualities and adaptability. Their unique textures and flavors have gained immense popularity as a favored snack (Nurhanan, Xin, & Tham, 2021). At the same time, the global snack market has experienced substantial growth, with an annual increase of 6.2%. Projections suggest an impressive rise, with the market expected to reach an astonishing valuation of USD 639 billion by 2023. Crackers have seen even faster adoption within the broader landscape of snack foods. Crackers, as a category of food, are typically characterized by their thin, dry, and crisp qualities. They are primarily made from soft wheat flour, which is considered a poor source of vitamins, minerals, and fiber and has a high glycemic index (GI). Their composition contains minimal sugar, fat, and moisture, making them appealing as lighter and lower-calorie snacks (Giannoutsos et al., 2023). These savory treats have achieved the status of beloved bakery products with global appeal. Furthermore, crackers are appropriate for enhancing their nutritional value with bioactive compounds, catering to the increasing desire for health-promoting dietary choices. In today's world, snacks are anticipated to impact a person's general health favorably. This has led researchers to investigate inventive approaches for improving the nutritional value of crackers by including bioactive compounds (Lee, Lim, Kim, & Hwang, 2022). The objectives of this study were as follows: (i) to investigate the optimal ratio of Hom Pathum (HP) rice and Black Glutinous (BG) rice for the production of crackers, (ii) to examine the physicochemical properties, microbiological qualities, and sensory acceptability, and (iii) to explore the bioactive compounds and biological activities of the crackers.

2. MATERIAL AND METHODS

2.1. Materials

Cake flour, salts, sugar, butter, baking powder, milk powder, ammonium bicarbonate, all-purpose flour, rice bran oil, corn flour, corn syrup, Hom Pathum (HP) rice flour, and black glutinous (BG) rice flour were purchased from a local market in Pathum Thani province (Thailand). Ethanol, copper sulfate ($CuSO_4$), potassium sulfate (K_2SO_4), sulfuric acid (H_2SO_4), boric acid (H_3BO_3), sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium bicarbonate ($NaHCO_3$), sodium acetate (CH_3COONa), aluminum chloride ($AlCl_3$), quercetin, gallic acid, potassium chloride buffer, acetate buffer, Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were obtained from Sigma Aldrich (St. Louis, MO, USA).

2.2. Preparation of Cracker Supplemented with Hom Pathum and Black Glutinous Rice Flour

2.2.1. Preparation of Basic Formulas

The basic formula of the cracker was obtained by sensory evaluation from four formulas. All ingredients consist of cake flour, all-purpose flour, corn flour, butter, salts, sugar, corn syrup, baking powder, ammonium bicarbonate, rice bran oil, milk powder, and water (Table 1).

Table 1. Four basic formulas of cracker.

Raw materials (g)	Formula 1	Formula 2	Formula 3	Formula 4
Cake flour	200	-	-	-
All-purpose flour	-	170	170	150
Corn flour	-	-	-	24
Butter	40	40	40	67
Salts	2	2	2	3
Sugar	15	4	4	4
Corn syrup	-	-	-	9
Baking powder	1	6	6	-
Ammonium bicarbonate	10	-	3	8
Rice bran oil	-	40	40	-
Milk powder	10	-	-	4
Water (ml)	75	50	50	40

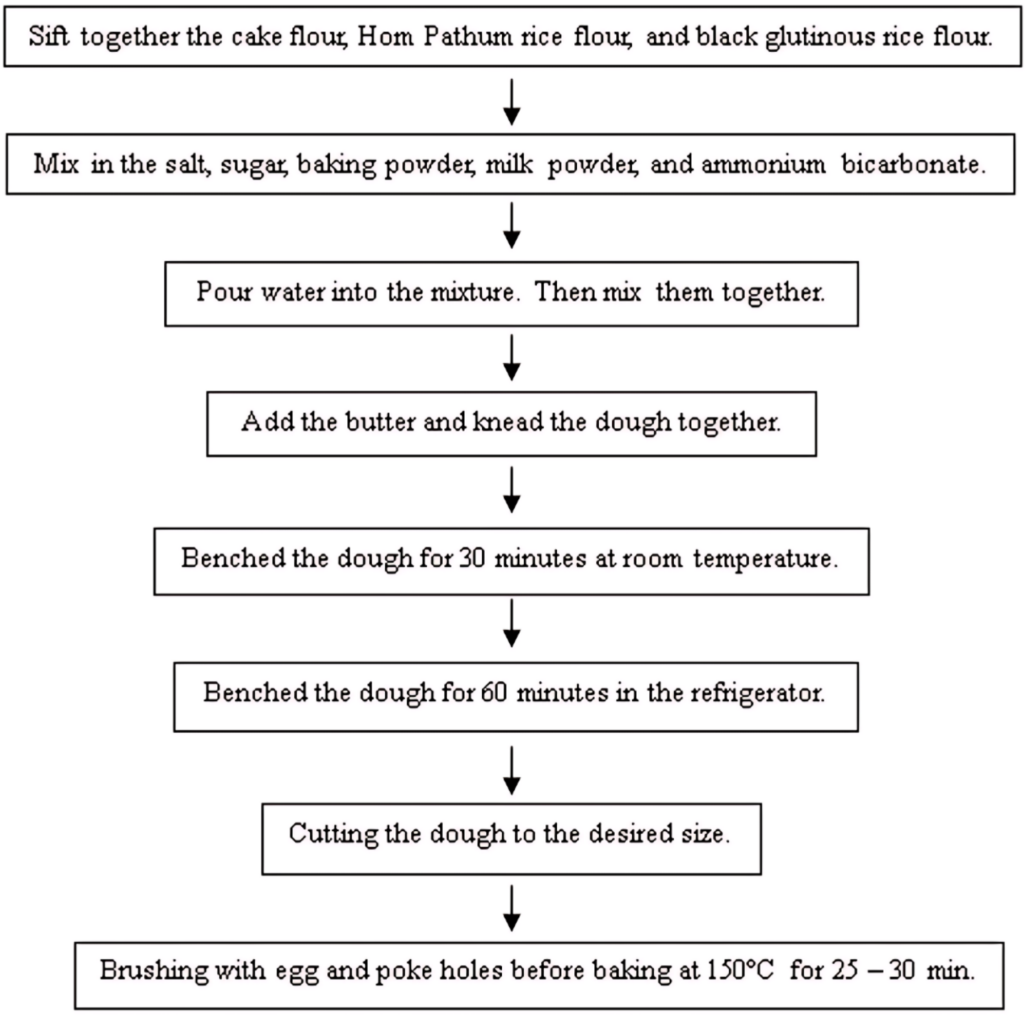

Figure 1. The procedure for making crackers.

2.2.2. Preparation of Crackers from Hom Pathum and Black Glutinous Rice Flour

The basic formula for crackers, obtained from 2.2.1, was used to determine the proper ratio of cake flour, HP rice flour, and BG rice flour in the ratios of 55:40:5, 50:40:10, 45:40:15, 40:40:20, and 35:40:25, respectively. The procedure for making crackers is demonstrated in Figure 1.

2.3. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was determined using a modified method from Nanok and Sansenya (2021). Briefly, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was dissolved in methanol and prepared at a concentration of 0.2 mM. The sample was prepared as a stock solution at 10 mg/mL with methanol and diluted within the concentration range of 0.1 mg/mL. The sample was then mixed with DPPH, vortexed, and left to stand at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm. The percentage of DPPH radical inhibition was calculated using the following equation:

$$\text{Radical scavenging (\%)} = (1 - (A_s / A_c)) \times 100$$

Where A_c and A_s are the absorbances of the control and sample, respectively. The percent inhibition of radical scavenging activity was compared with BHT.

2.4. ABTS Assay

The ABTS radical cation scavenging activity was evaluated following a modified method of Nanok and Sansenya (2021). A 7 mM solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was mixed with 2.45 mM potassium persulfate in a 1:0.5 ratio and incubated in the dark at room temperature for 12–16 hours. The solution was then diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 100 µL of the sample (1 mg/mL) was combined with 10 mL of ABTS, incubated for 6 minutes in darkness, and the absorbance was measured at 734 nm using Trolox as the standard.

2.5. Total Flavonoid Content

The total flavonoid content was determined according to a modified method of Paponpat (Pattarathitiwat, Chinvongamorn, & Sansenya, 2021). Briefly, a 300 mL sample solution (1 mg/mL in methanol) was combined with 200 mL deionized water and 30 mL of 5% $NaNO_2$ and shaken for 5 min. 30 mL of 10% $AlCl_3$ was added after that, and the mixture was shaken for 5 min. 200 mL of 1 M NaOH was then added to the process. Deionized water was used to adjust the final volume to 1 mL, and it was allowed to stand for 15 min at room temperature. The absorbance was then determined at 415 nm. The total flavonoid content was expressed as mg of Quercetin Equivalent (QE) per gram of dry weight (DW).

2.6. Total Phenolic Content

The total phenolic content was analyzed using a modified method of Paponpat (Pattarathitiwat et al., 2021). A sample solution (1 mg/mL) was prepared by dissolving 1 mg of crude extract in 1 mL of deionized water. Then, 1 mL of the sample was mixed with 200 µL of 10% (v/v) Folin-Ciocalteu reagent and shaken for 3 minutes. After adding 800 µL of 20% (w/v) sodium carbonate, the mixture was incubated in the dark for 60 minutes. The absorbance was recorded at 765 nm, and results were expressed as mg gallic acid equivalents (GAE) per gram of dry weight (DW).

2.7. Total Anthocyanin Content

The total anthocyanin content was measured using a modified method of Sompong (Sansenya, Chumanee, & Sricheewin, 2019). The sample was diluted 1:9 in either 0.025 M potassium chloride buffer (pH 1.0) or 0.4 M acetate buffer (pH 4.5). Absorbance was recorded at 520 nm and 700 nm, and the total anthocyanin content was calculated using the corresponding equation.

$$\text{Total anthocyanin content} = (\text{Acorrection} \times \text{MW} \times \text{DF} \times 1000) / (\varepsilon \times \text{L})$$

$$A_{correction} = (A_{520} - A_{700})_{pH=0.1} - (A_{520} - A_{700})_{pH=4.5}$$

Where A_{520} and A_{700} are the absorbances at 520 nm and 700 nm, respectively. MW is the molecular weight of anthocyanin (compared to cyanidin-3-glucoside; 449.2 g/mol). DF is the dilution factor. L is the path length of the light through the solution (1 cm). ε is the molar extinction coefficient (26,900).

2.8. Physicochemical Properties

The ColorFlex EZ Spectrophotometer was used to measure the color (L^*, a^*, and b^*) values. Proximate analysis, including carbohydrate, protein, fat, ash, and fiber, was determined using standard methods described by the Association of Official Analytical Chemists (2000). All measurements were repeated three times to produce the average values.

2.9. α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was assessed using a modified method of Nanok and Sansenya (2021). p-Nitrophenyl-α-glucopyranoside (4-pNPG) served as the substrate. The enzymatic reaction consisted of 0.1 mg/mL of sample, 0.25 mM 4-pNPG, and 2.5 µg/mL α-glucosidase, incubated at 37°C for 20 minutes. The reaction was stopped by adding 0.5 M Na_2CO_3, and absorbance was measured at 405 nm using a UV/Vis spectrophotometer. The percentage inhibition was calculated using the standard equation.

$$[(A_b - A_s)/A_b] \times 100$$

Where A_b = absorbance without sample and A_s = absorbance with sample

2.10. Microbiological Evaluation

Microbiological analysis was conducted for total plate count, yeast, and mold using the Pour Plate method.

2.11. Sensory Evaluation

Sensory evaluation was conducted using a 9-point hedonic scale, where panelists rated the crackers' appearance, color, flavor, taste, crispiness, crumbliness, and overall acceptability. Scores ranged from 9 (like extremely) to 1 (dislike extremely).

2.12. Statistical Analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation (SD). Significant differences among means were determined by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at $p < 0.05$, using statistical software.

3. RESULTS AND DISCUSSION

3.1. Sensory Characteristics and Color of Basic Formulas

To evaluate the sensory attributes of the appropriate basic formulas, panelists are used to obtain data on appearance, color, flavor, crispiness, crumbliness, and overall acceptance (Table 2). Regarding color, flavor, crispiness, and overall acceptance, it was determined that the four basic formulations had statistically significant differences ($p<0.05$). When evaluations for appearance, flavor, crispiness, and overall acceptance were taken into consideration, Formula 1 obtained the highest scores. Consequently, it was determined to be the best formula for a future development product. The color of the four basic cracker formulas was identified in terms of lightness (L^*), redness (a^*), and yellowness (b^*). All of the parameters were statistically significantly different ($p<0.05$). The greatest values of lightness (L^*) and yellowness (b^*) were observed in Formula 1 (Table 2).

Table 2. Sensory evaluation and color of four basic formulas.

Parameters	Formula 1	Formula 2	Formula 3	Formula 4
Sensory scores				
Appearance ns	7.20±1.56	6.87±1.38	7.13±1.52	6.47±1.33
Color	7.13±1.65^{ab}	7.27±0.94^{a}	6.87±1.38^{ab}	6.47±1.22^{b}
Flavor	6.83±1.87^{a}	6.60±1.61^{ab}	6.60±1.69^{ab}	5.80±1.58^{b}
Crispiness	7.97±1.12^{a}	7.00±1.11^{b}	5.93±2.08^{c}	5.40±2.22^{c}
Crumbliness ns	6.63±1.84	7.13±1.33	6.93±1.08	7.20±1.34
Overall acceptance	7.97±0.96^{a}	7.40±0.89^{ab}	7.13±1.22^{b}	6.33±1.32^{c}
Color				
*L**	70.84±2.26^{a}	69.48±0.71^{a}	63.01±3.80^{b}	55.70±3.76^{c}
*a**	12.96±1.42^{ab}	11.69±3.47^{b}	10.60±3.72^{b}	14.89±2.33^{a}
*b**	40.84±2.54 ^{a}	39.70±4.93^{a}	35.43±6.17 ^{b}	37.08±2.66^{ab}

Note: Mean values with different superscripts in the same row (a, b, c) are significantly different (p<0.05).
ns = not significantly different.

3.2. Sensory Characteristics, Color, and Microbiological Evaluation of Crackers Supplemented with Hom Pathum and Black Glutinous Rice Flour

The appearance, flavor, color, crispiness, crumbliness, and overall acceptance of crackers supplemented with HP rice flour and BG rice flour in various ratios were assessed (Table 3), and Figure 2 presents the visual appearance of these crackers was evaluated. No statistically significant difference existed between their sensory scores. It is indicated that increasing the amount of black glutinous rice flour in the mix does not change the sensory qualities, which is similar to findings reported for the substitution of black glutinous rice flour for wheat flour in batters and cakes (Itthivadhanapong & Sangnark, 2016). Thus, black glutinous rice flour could be effectively used as an ingredient to enhance healthy cracker products. The color was evaluated using the following parameters: lightness (*L**), redness (*a**), and yellowness (*b**). These parameters showed statistically significant differences ($p < 0.05$). The color of the cracker became darker with an increasing amount of black glutinous rice flour, which is similar to the results of Itthivadhanapong and Sangnark (2016) and Utami and Fitriani (2023). The microbiological measurement did not exceed the THAI SMEs STANDARD of BISCUITS AND CRACKERS (Thai Industrial Standards Institute, 2020) (Mold count and bacterial count were less than 1 x 10^4 and 100 cfu/g, respectively).

Table 3. Sensory evaluation, color, and microbiological evaluation of crackers supplemented with Hom Pathum and black glutinous rice flour.

Parameters	The ratio of cake flour: HP: BG				
	55:40:5	50:40:10	45:40:15	40:40:20	35:40:25
Sensory scores					
Appearance ns	6.77±1.38	6.70±1.14	6.77±1.07	7.03±0.92	6.33±1.60
Color ns	6.87±1.38	6.50±1.19	6.83±1.59	6.77±1.13	6.23±1.71
Flavor ns	6.93±1.01	6.90±1.15	6.73±1.70	6.60±1.24	6.43±1.52
Crispiness ns	7.37±1.15	7.20±1.12	7.13±1.07	7.33±1.12	7.47±1.35
Crumbliness ns	7.00±1.28	6.93±1.01	6.87±1.57	6.83±1.34	7.13±1.27
Overall acceptance ns	7.47±1.10	7.00±1.20	6.80±1.75	7.47±1.10	7.13±1.47
color					
*L**	69.71±0.16^{a}	62.68±0.13^{b}	59.42±0.02^{c}	51.01±0.29^{e}	53.59±0.13^{d}
*a**	2.44±0.02^{e}	4.43±0.02^{d}	5.55±0.00^{c}	6.79±0.03^{a}	6.13±0.04^{b}
*b**	14.05±0.15^{b}	16.60±0.10^{a}	13.39±0.09^{c}	10.00±0.13^{e}	11.38±0.08^{d}
Microbiological evaluation					
Bacterial count (cfu/g)	81.5 x 10^{-4}	38.5 x 10^{-4}	< 25	< 25	< 25
Mold count (cfu/g)	28.5 x 10^{-4}	< 25	< 25	< 25	< 25

Note: Mean values with different superscripts in the same row (a, b, c, d) are significantly different. (p<0.05).
ns = not significantly different.

3.3. Bioactive Compounds, Biological Activities, and Proximate Analysis of Crackers Supplemented with Hom Pathum and Black Glutinous Rice Flour

Table 4 presents the bioactive compounds, biological activities, and proximate composition of crackers supplemented with Hom Pathum and black glutinous rice flour in a 40:25 ratio. The total phenolic, flavonoid, and anthocyanin contents were 2.95, 5.70, and 79.05 mg/g (DW), respectively. Antioxidant activity, measured by ABTS and DPPH assays, showed 14.33% and 8.77% inhibition, respectively, while α-glucosidase inhibition was 28.44%. Previous studies have indicated that black glutinous rice is rich in various compounds, including phenolic compounds. These compounds offer several health benefits, such as antioxidant, antiproliferative activity, and antidiabetic (Hu, Chen, & Sun, 2022; Setyaningsih et al., 2016; Sivamaruthi et al., 2018; Sompong et al., 2011). Proximate analysis revealed 72.85% carbohydrates, 5.19% protein, 8.63% fat, 1.59% ash, and 5.08% fiber. The study by Ito and Lacerda (2019) shows that black rice is a good source of both fiber and protein.

Table 4. Bioactive compounds, biological activities, and proximate analysis of crackers supplemented with Hom Pathum and black glutinous rice flour.

Parameters	**The ratio of cake flour: HP: BG**
	35:40:25
Total phenolic, mg/g (DW)	2.95 ± 0.05
Total flavonoid, mg/g (DW)	5.70 ± 0.14
Total anthocyanin, mg/g (DW)	79.05 ± 7.28
ABTS (% inhibition)	14.33 ± 2.21
DPPH (% inhibition)	8.77 ± 0.34
α-glucosidase (% inhibition)	28.44 ± 0.64
Proximate analysis (%)	
Carbohydrate	72.85
Protein	5.19
Fat	8.63
Ash	1.59
Fiber	5.08

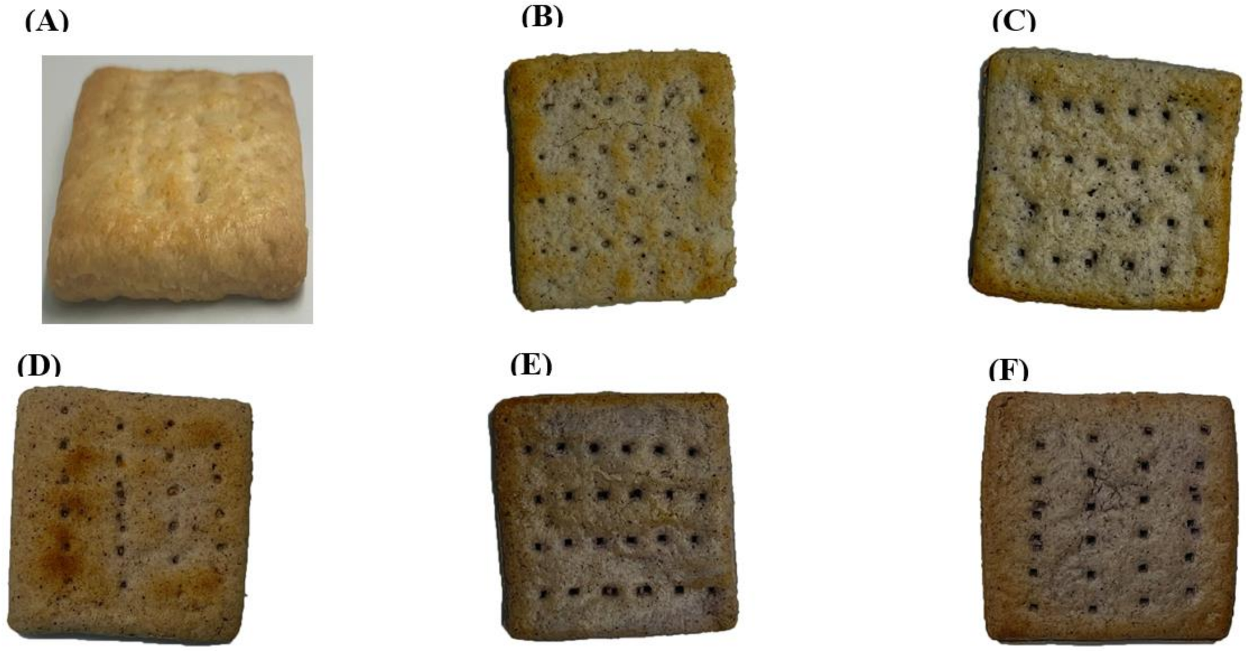

Figure 2. Crackers made from different ratios of cake flour: Hom Pathum (HP) rice flour: black glutinous (BG) rice flour. **(A)** 55:40:0, **(B)** 55:40:5, **(C)** 50:40:10, **(D)** 45:40:15, **(E)** 40:40:20, and **(F)** 35:40:25.

4. CONCLUSIONS

The present study showed that the best formula chosen was a cracker supplemented with a 40:25 ratio of Hom Pathum rice and black glutinous rice flour. Sensory evaluation showed that while the amount of black glutinous rice flour did not affect the sensory qualities, higher amounts did darken the cracker. Our data demonstrated that cracker supplemented with black glutinous rice flour contained bioactive compounds such as phenolic compounds, flavonoids, and anthocyanins, and exhibited antioxidant and α-glucosidase inhibitory activities. Thus, black glutinous rice flour is a promising functional ingredient that enhances the cracker's value and health benefits.

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