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Enhancing Antioxidant Properties of Ice Cream with Broken-Milled Riceberry Rice Extract

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Abstract

This study investigated the potential of broken-milled Riceberry rice extract as a natural bioactive ingredient for ice cream enrichment. Extracts were prepared using ethanol-water solutions at various concentrations (10–50%), with 50% ethanol yielding the highest levels of anthocyanins (20.84 mg cyanidin-3-glucoside/100 g DW), total phenolics (143.15 mg GAE/100 g DW), and strong antioxidant activity (78.36% DPPH scavenging activity). High-performance liquid chromatography (HPLC) revealed a phenolic profile rich in vanillic, protocatechuic, and sinapic acids. The extract was freeze-dried and incorporated into ice cream at 0.5%, 1.0%, and 1.5% (w/w). Fortification significantly increased total phenolic content and antioxidant activity, with the 1.5% formulation reaching 84.97% DPPH scavenging activity. Physical characteristics such as viscosity and color were also affected, while pH remained stable. These results highlight the extract's potential for use in functional frozen desserts, providing a sustainable way to valorize agricultural byproducts while enhancing nutritional quality.

Introduction

Riceberry rice (*Oryza sativa* L.), a Thai hybrid cultivar, is distinguished by its deep purple pigmentation and rich composition of bioactive compounds, particularly anthocyanins. These water-soluble pigments not only contribute to the rice's striking color but also exhibit potent antioxidant and anti-inflammatory properties. Beyond their physiological roles, anthocyanins are associated with various health benefits, including cardiovascular protection, neuroprotection, anti-diabetic effects, and potential anti-cancer activities (Tena et al., 2020; Hair et al., 2021; Kumar et al., 2021; Gao et al., 2021). With the rising global awareness of the link between diet and health, the demand for functional foods—those offering benefits beyond basic nutrition—has increased markedly (Temple, 2022). Incorporating anthocyanin-rich ingredients into familiar food products,

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such as dairy-based items, presents a practical approach to improve the population's dietary intake of health-promoting phytochemicals in an appealing and accessible manner (Essa, 2023).

Among the promising yet underutilized sources of these compounds are broken-milled Riceberry rice, an agricultural byproduct generated during rice milling. Despite often being discarded as waste, broken-milled Riceberry rice retains a considerable amount of nutritional and phytochemical constituents. Luang-In (2018) demonstrated that Riceberry broken rice extract possesses significant antioxidant activities, as evidenced by its values in the DPPH (6.36 mg TEAC/g), FRAP (1.33 mg $FeSO_4$/g), and ABTS (5.82 mg TEAC/g) assays. The extract also contained high levels of total phenolic content (9.94 mg GAE/g) and total flavonoid content (67.2 mg CE/g). Notably, the aqueous extract exhibited approximately 45% tyrosinase-stimulating activity, underscoring its potential applicability in both functional foods and cosmeceutical products.

Optimizing the extraction of bioactive compounds is key to their effective incorporation into food matrices. Ethanol, recognized as a green and food-safe solvent, is particularly suitable due to its low toxicity, biodegradability, and high efficiency in extracting polar compounds (Chemat et al., 2012). Previous studies have shown that ethanol-water mixtures not only enhance extraction yields but also help preserve the bioactivity of compounds from plant materials, including pigmented rice (Martins et al., 2023; Garcia-Salas et al., 2010; Azmir et al., 2013).

Ice cream, as a widely enjoyed and versatile food product, serves as an excellent delivery system for incorporating bioactive compounds (Wangcharoen, 2012). Its overall quality is influenced by a range of physicochemical parameters such as overrun, melting rate, viscosity, and hardness. For instance, overrun directly affects texture and mouthfeel, while a slower melting rate is often associated with greater consumer satisfaction. The viscosity of the mix and the hardness of the final product also play crucial roles in consumer perception (Goff & Hartel, 2013). In addition to these core properties, several formulation-related factors, such as the type and concentration of stabilizers, emulsifiers, sweeteners, and incorporated bioactive ingredients, which significantly influence the sensory and structural attributes of ice cream (Muse & Hartel, 2004). Therefore, enriching ice cream with anthocyanin-rich extracts aligns with current consumer preferences for healthier indulgences, offering a palatable and functional dessert option (Mohammed et al., 2022; Arslaner & Salik, 2020; Soodbar et al., 2024; Soukoulis et al., 2014).

The highlights of this study include the utilization of broken-milled Riceberry rice, an underused agricultural byproduct, as a novel source of bioactive compounds for food application. By optimizing ethanol-based extraction, the study achieved high recovery of anthocyanins and phenolic compounds, which were then successfully incorporated into ice cream. The extract significantly enhanced antioxidant activity and modified key physicochemical properties of the product. These findings contribute to both nutritional improvement of frozen desserts and sustainable byproduct utilization in food innovation.

Materials and methods

1. Materials

Broken-milled Riceberry rice was obtained from a local supplier in Suphan Buri Province, Thailand. Upon receipt, the rice was stored in airtight plastic bags at room temperature (25±2°C) in a dry, dark environment until use (Yamuangmorn et al., 2018). All reagents and solvents used in this study were of analytical grade, and distilled water was used for all experimental procedures.

2. Extraction of bioactive compounds from broken-milled Riceberry rice

Bioactive compounds were extracted from broken-milled Riceberry rice using a modified protocol based on Shen et al. (2009). In brief, ground Riceberry rice was mixed with ethanol-water solutions containing 10%, 20%, 30%, 40%, and 50% (v/v) ethanol at a solid-to-solvent ratio of 1:3 (w/v). The extraction was carried out at room temperature (25±2°C) for 24 h, under light-protected conditions.

After extraction, the mixture was centrifuged at 4,000 ×*g* for 15 min at 25°C to separate the supernatant from the solid residue. The resulting crude extract was then transferred to amber glass bottles to protect it from light-induced degradation and stored at -20°C until further analysis (Chen et al., 2019). A flowchart illustrating the extraction process of broken-milled Riceberry rice is shown in Fig. 1.

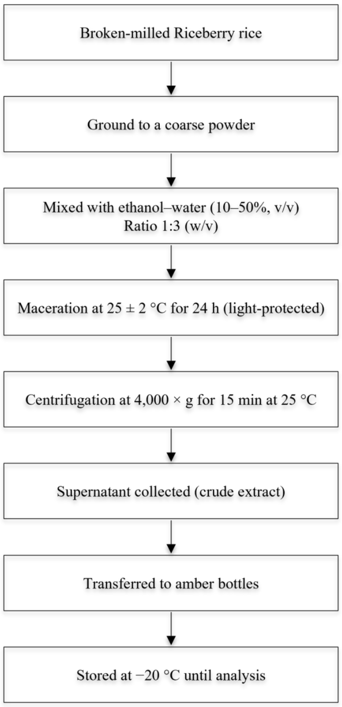


Fig. 1 Flowchart of extraction process of broken-milled Riceberry rice

3. Analysis of bioactive compounds and antioxidant activity of extracts

3.1 Total phenolic content

The total phenolic content of the rice extracts was measured using the Folin-Ciocalteu method, as described by Maizura et al. (2011), with slight modifications. Briefly, 0.4 mL of the rice extract was mixed with 2 mL of 10% Folin-Ciocalteu reagent and allowed to stand for 4 min. Afterward, 1.6 mL of 5% sodium carbonate (Na_2CO_3) was added, and the mixture was incubated in the dark for 30 min. The absorbance at 765 nm was determined using a spectrophotometer (UV-2401PC, Shimadzu, Japan). Results were compared against a standard curve of gallic acid at concentrations of 0, 20, 40, 60, 80, and 100 µg/mL. The total phenolic content was expressed as mg of gallic acid equivalents/100 g of dry weight (mg GAE/100 g dry weight).

3.2 DPPH free radical scavenging activity

The antioxidant activity of the rice extracts was determined using the DPPH assay, as modified by Zhang et al. (2015). A 2 mL sample of the rice extract was reacted with 2 mL of 200 mM DPPH solution and incubated in the dark for 30 min. The absorbance was then measured at 515 nm using a spectrophotometer (UV-2401PC, Shimadzu, Japan). Results were compared with a standard curve of Trolox at concentrations of 0, 10, 20, 30, 40, and 50 µM. The antioxidant activity was expressed as DPPH scavenging activity in mM of Trolox equivalents/100 g of dry weight (mM Trolox equivalents (TE)/100 g dry weight).

3.3 Anthocyanin content by pH-differential method

The measurement of anthocyanin content using the pH-differential method was adapted from Bangsiri (2014). The extract was diluted with potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5). The absorbance was then measured at wavelengths of 510 nm and 700 nm, ensuring that the values fell within the range of 0.2–0.8. The results were calculated and reported as the total anthocyanin content in mg of cyanidin-3-glucoside/g of dry weight.

The absorbance values obtained were used to calculate the anthocyanin content, expressed as cyanidin-3-glucoside, using the following formula:

$$\text{Anthocyanin (mg/L)} = A \times MW \times DF \times 1000/\varepsilon \times l$$

Where:
A = (A510–A700) pH 1.0 – (A510–A700) pH 4.5
MW = 499.2 g/mol (molecular weight of cyanidin-3-glucoside)
DF = Dilution factor
ε = Molar extinction coefficient (26,900 L/mol.cm for cyanidin-3-glucoside)
l = Path length of the cuvette (cm)

Then, a suitable solvent for extracting bioactive compounds from broken-milled Riceberry rice was selected based on the total phenolic content, antioxidant activity using the DPPH method, and total anthocyanin content. The extraction was then scaled up using the selected solvent, and the extract was concentrated by evaporating the solvent with an evaporator at 40°C. The resulting crude extract was further dried using freeze-drying to obtain broken-milled Riceberry rice extract powder in a larger quantity for use in the subsequent product development process.

4. HPLC analysis of phenolic profile

Phenolic compounds, particularly phenolic acids and flavonoids, are key bioactive constituents that contribute significantly to antioxidant activity. These compounds are abundant in pigmented rice varieties such as Riceberry rice. To characterize these constituents, the phenolic profile of broken-milled Riceberry rice extract was analyzed using High-Performance Liquid Chromatography HPLC system (Thermo Separation Products, USA).

The analysis was conducted using an HPLC pump (Spectra System P4000, Thermo Separation Products, USA) coupled with a C18 column (Luna 5 µm, 4.6×150 mm, Phenomenex, USA) and a guard column. Detection was performed with a Spectra System UV-2000 detector at 280 nm, and data acquisition was processed using ChromeQuest software. The mobile phase consisted of solvent A (1% acetic acid in water) and solvent B (acetonitrile). Gradient elution was programmed as follows: 0–5 min, 5–9% B; 5–15 min, hold at 9% B; 15–22 min, 9–11% B; 22–38 min, 11–18% B; 38–43 min, 18–23% B; and 43–44 min, 23–90% B. Each run was followed by a 6-min wash with 80% B and a 15-min re-equilibration at 5% B. The flow rate was maintained at 1 mL/min.

Samples were injected using a Spectra System AS3500 autosampler at a volume of 10 µL (n=3). Prior to injection, all samples were filtered through 0.45 µm syringe filters. Phenolic acids were identified by comparing retention times with those of authentic standards

5. Preparation of powdered broken-milled Riceberry rice extract

To prepare the powdered extract, the broken-milled Riceberry rice extract obtained using the solvent that yielded the highest amount of bioactive compounds (as determined by total phenolic content, DPPH antioxidant activity, and total anthocyanin content) was selected. This extract was then concentrated by solvent evaporation using a rotary evaporator at 45°C.

Following concentration, an appropriate amount of maltodextrin (DE 10–12) was added as a carrier agent to support powder formation and enhance the physical stability of the extract during the freeze-drying process.

The quality parameters of the resulting powder, including its bioactive compound content and antioxidant activity, were analyzed according to the procedures detailed in Section 3. This powdered extract was then utilized for further studies on its application in developing ice cream products.

6. Preparation of ice cream

The most suitable solvent for extracting bioactive compounds from broken-milled Riceberry rice was selected based on the total phenolic content, antioxidant activity (assessed using the DPPH method), and total anthocyanin content. The resulting extract was then freeze-dried (lyophilized) to obtain a powdered broken-milled Riceberry rice extract. This powder was analyzed for its bioactive compound content and antioxidant activity using the earlier methods. Subsequently, the powdered extract was incorporated into developing ice cream formulations.

The ice cream formulation used in this study was slightly modified form Kaothien (2012). The control recipe included the following ingredients: 8% sugar, 1% dextrose, 4% maltodextrin, 0.4% stabilizer and emulsifier (S/E), 6% skim milk powder, 9.6% hot water, 0.1% salt, 60% fresh milk, and 10% whipping cream. Broken-milled Riceberry rice extract powder was incorporated at 4 levels (0%, 0.5%, 1.0%, and 1.5% w/w).

The dry ingredients were combined in a container to prepare the ice cream and then mixed with pasteurized milk and whipping cream. The mixture was homogenized, pasteurized, and allowed to mature before being processed in an ice cream maker (Gelato Chef 5L Automatic, Nemox, Italy) until it reached the desired consistency. The resulting ice cream was then packaged and stored at -18°C for at least 24 h before evaluation.

7. Ice cream analyses

7.1 Analysis of antioxidant activity and total phenolic compounds in ice cream

The determination of antioxidant activity and total phenolic compounds was conducted based on the method proposed by Limsuwan et al. (2014), with some minor modifications. In brief, 10 g of ice cream were mixed with 50 mL of 50% ethanol and allowed to stand at room temperature (approximately 28°C) for 18 h. The mixture was then centrifuged at 5,000 rpm for 10 min. The clear supernatant was collected and analyzed for total phenolic compounds using the Folin-Ciocalteu colorimetric method and for antioxidant activity using the DPPH (2,2-diphenyl-1-picryl-hydrazyl) method described previously.

7.2 Melting rate

The melting rate was determined using a modified method based on Rizk et al. (2014). Ice cream samples weighing 50 g were placed in plastic cups and stored at -18°C for 24 h. After storage, the samples were carefully removed and set on a wire mesh (1 cm^2) positioned over a pre-weighed beaker to collect the melted ice cream. The setup was maintained at a temperature of 25±1°C, and the weight of the melted ice cream was recorded every 5 min. The linear portion of the resulting curve was analyzed to calculate the best fit straight line, with the slope representing the melting rate (g/min).

7.3 Overrun

The overrun of ice cream (%) was calculated using the equation from Rizk et al. (2014), as follows:

$$\%\ \text{Overrun} = \frac{\text{Weight of mix - Weight of the same volume of ice cream}}{\text{Weight of the same volume of ice cream}} \times 100$$

7.4 Color

The color of the ice cream samples was measured using a Konica Minolta Colorimeter (Model CR-400, Konica Minolta, Japan) based on the CIE Lab* color scale. The measurements were expressed as L* (lightness), +a* (redness), and +b* (yellowness).

7.5 Viscosity

The viscosity of the ice cream mixture was measured after aging, using a Brookfield Digital Viscometer (Model LVDV-I Prime, AMETEK Brookfield, USA), following the method of Rizk et al. (2014). The viscosity was measured at 4±1°C after 4 h of aging. The readings were taken using spindle no. 1, after the motor had run for 30 sec, with the liquid temperature controlled at 20±1°C.

7.6 Analysis of proximate composition

The ice cream's proximate composition, including moisture, total ash, crude protein, crude fat, crude fiber, and carbohydrate content, was determined using standard methods for proximate analysis (AOAC International, 2019). The carbohydrate content was calculated by subtracting the sum of the percentages of crude protein, ash, fat, and crude fiber from 100.

8. Statistical analysis

All analyses were performed in triplicate. The data were analyzed statistically using SPSS Statistics 23 (IBM, USA). Differences between values were considered significant at P≤0.05, and averages were compared using Duncan's new multiple-range test.

Results and discussion

This study demonstrates the significant influence of solvent type on the extraction efficiency of anthocyanins, total phenolic content (TPC), and antioxidant capacity (DPPH) from broken-milled Riceberry rice. Additionally, incorporating broken-milled Riceberry rice extract powder into ice cream formulations highlights its potential as a bioactive ingredient for enhancing bioactive properties. The findings are discussed below.

1. Bioactive compound content and antioxidant activity of broken-milled Riceberry rice extracts

Table 1 summarizes the impact of different solvent types on broken-milled Riceberry rice extraction, TPC, and antioxidant capacity. The variations observed are primarily attributed to differences in solvent polarity, which affects the ability to extract bioactive compounds from rice.

1.1 Anthocyanin content

Ethanol-based solvents, particularly those with 30% or higher ethanol concentration, significantly improved broken-milled Riceberry rice extraction compared to water or lower ethanol concentrations. The highest total anthocyanin content (20.84 mg cyanidin-3-glucoside/100 g dry weight) was obtained with 50% ethanol, supporting previous findings that moderate ethanol concentrations enhance anthocyanin solubility by interacting with both polar and non-polar molecular structures (Thao et al., 2015).

In contrast, water extraction yielded the lowest anthocyanin content (4.51 mg cyanidin-3-glucoside/100 g dry weight). This is likely due to anthocyanins being bound to the rice cell walls, requiring a less polar solvent like ethanol for efficient extraction (Taghavi et al., 2023).

1.2 Total phenolic content (TPC)

A similar trend was observed for total phenolic content (TPC), with ethanol-based solvents yielding the highest values, particularly at 40% and 50% ethanol. The 50% ethanol extract contained 143.15 mg GAE/100 g, significantly higher than the water extract (120.83 mg GAE/100 g). These results align with previous studies indicating that ethanol enhances the extraction of a broad spectrum of phenolic compounds, including flavonoids and phenolic acids, which contribute to antioxidant properties (Özbek et al., 2020).

1.3 Antioxidant capacity (DPPH scavenging activity)

Antioxidant capacity, as measured by DPPH scavenging activity, followed a similar pattern. The highest activity was recorded for extracts obtained with 30% and 40% ethanol (82.50% and 81.15%, respectively). However, a slight decline was observed with 50% ethanol (78.36%), possibly due to anthocyanin degradation at higher ethanol concentrations or reduced solubility of certain phenolic compounds (Nour et al., 2013).

In contrast, the water extract exhibited the lowest DPPH scavenging activity (38.39%), further confirming the role of phenolic compounds in antioxidant activity. These findings support previous research by Alothman et al. (2009), which established a strong correlation between phenolic content and antioxidant potential in plant-based extracts.

Although the extract obtained using 50% ethanol exhibited the highest total phenolic and anthocyanin contents, the 30% ethanol extract demonstrated the greatest antioxidant capacity. This discrepancy suggests that there is no strict linear correlation between total phenolic content and antioxidant activity in the studied samples. Similar findings have been reported by Bajpai et al. (2005), who observed a lack of correlation between phenolic concentration and antioxidant efficacy in various medicinal plant extracts. This inconsistency may be attributed to the presence of other antioxidant compounds such as ascorbic acid, tocopherols, or pigments within the extracts, which may enhance the overall antioxidant activity. Additionally, synergistic interactions among multiple phytochemicals can influence antioxidant responses beyond the effect of phenolics alone. It should also be noted that the Folin–Ciocalteu assay may react with various reducing substances, not exclusively phenolics, potentially leading to overestimation. (Rahiman et al., 2012).

Table 1 Bioactive compound content and antioxidant activity of broken-milled Riceberry rice extracts using varying ethanol concentrations

Solvents	Total anthocyanin (mg cyanidin-3-glucoside/100 g extract)	Total phenolic content (mg GAE/100g extract)	Antioxidant capacity (%DPPH scavenging activity)
Water	4.51±0.14^{e}	120.83±1.09^{d}	38.39±1.60^{d}
10% Ethanol	7.41±0.21^{d}	123.85±1.02^{d}	52.03±1.07^{c}
20% Ethanol	12.54±0.74^{c}	128.77±1.22^{c}	77.60±0.83^{b}
30% Ethanol	16.27±1.06^{b}	133.63±2.59^{b}	82.50±0.88^{a}
40% Ethanol	20.61±0.75^{a}	139.93±2.64^{a}	81.15±0.64^{a}
50% Ethanol	20.84±0.63^{a}	143.15±3.74^{a}	78.36±0.98^{b}

Remark: Different letters (a-e) in the same column indicate significant differences ($p \leq 0.05$).

2. Phenolic profile of extracts by HPLC analysis

Phenolic acids represent a group of versatile bioactive dietary components known for their potential roles in the prevention and management of chronic diseases. Their health benefits are largely associated with their favorable oral bioavailability and broad spectrum of biological activities, including antioxidant, anti-inflammatory, and anti-aging properties (Heleno et al., 2018).

This study used HPLC to analyze the phenolic profile of a broken-milled Riceberry rice extract obtained with 50% ethanol, identifying key bioactive compounds with antioxidant and anti-inflammatory properties. Table 2 shows the results, with Fig. 2 depicting chromatograms of (a) the external standard mixture and (b) the broken-milled Riceberry rice extract, highlighting major phenolic acids such as gallic, protocatechuic, vanillic, caffeic, coumaric, ferulic, and sinapic acids.

The phenolic composition observed in this study aligns with findings from previous research on rice and cereal grains (Shahidi et al., 2021; Zhang et al., 2019). Among the identified compounds, vanillic acid was the most abundant at 452.4 µg/g, followed by protocatechuic acid (156.5 µg/g) and sinapic acid (127.5 µg/g). Vanillic acid, a stable phenolic commonly found in plant-based foods, exhibits strong antioxidant and antimicrobial activities. Its stability under processing conditions and role as a polyphenol metabolite support its contribution to long-lasting bioactivity, particularly in functional food applications (Magiera et al., 2025). Protocatechuic acid has demonstrated anti-inflammatory properties, making it a potential therapeutic agent for chronic inflammatory diseases (Semaming et al., 2015), while sinapic acid contributes to neuroprotective and cardioprotective benefits, supporting its role in functional food applications.

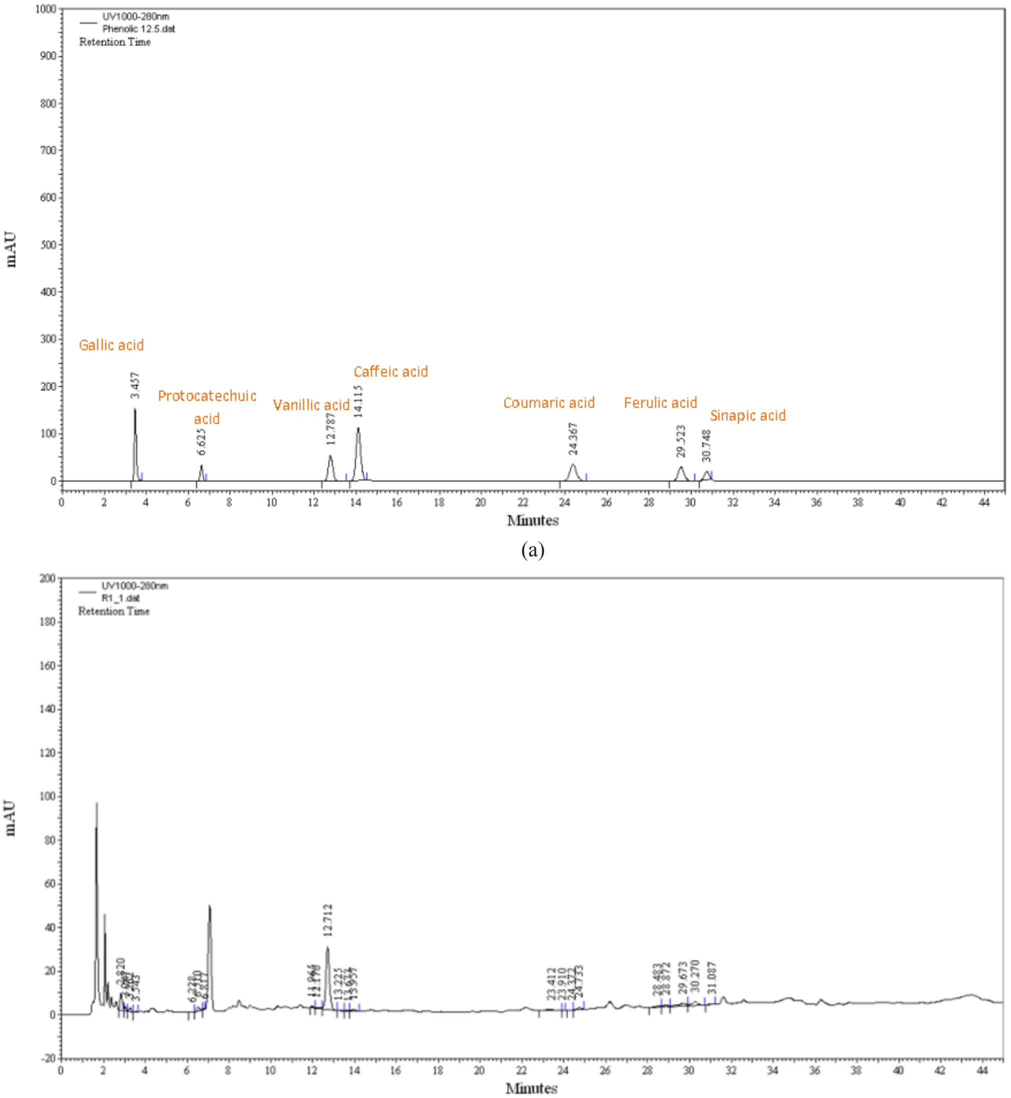
In addition to these major phenolic acids, other minor phenolic acids were identified at lower concentrations, each offering unique biological functions. Ferulic acid (90.02 µg/g) is particularly noteworthy for its ability to protect against degenerative diseases and enhance the bioavailability of other nutrients (Giacomo et al., 2022). Similarly, coumaric acid (43.64 µg/g) and caffeic acid (28.75 µg/g) have been associated with anticancer and antioxidative effects, further supporting the health benefits of Riceberry rice (Giacomo et al., 2022).

Riceberry rice distinguishes itself from other grains due to its rich phenolic composition, including anthocyanins, flavonoids, and phenolic acids. Studies have shown that its total phenolic content is significantly higher than other rice varieties, such as jasmine rice (Petchlert & Sangprathum, 2020).

Identifying these phenolic compounds highlights the potential of broken-milled Riceberry rice as a valuable source of bioactive compounds. These phenolic acids enhance its nutritional value and reinforce its application as a bioactive ingredient in health-promoting food products.

Table 2 Phenolic profile of broken-milled Riceberry rice extract

Phenolic compounds	µg/g
Gallic acid	17.02 ± 1.21
Protocatechuic acid	156.5 ± 25.1
Vanillic acid	452.4 ± 16.4
Caffeic acid	28.75 ± 2.63
Coumaric acid	43.64 ± 4.89
Ferulic acid	90.02 ± 18.95
Sinapic acid	127.5 ± 19.0



(a)

(b)

Fig. 2 HPLC chromatograms of phenolic compounds (a) chromatogram of an external standard mixture containing 7 phenolic compounds. Peaks correspond to gallic acid (1), protocatechuic acid (2), vanillic acid (3), caffeic acid (4), coumaric acid (5), ferulic acid (6), and sinapic acid (7) (b) chromatogram of the broken-milled Riceberry rice extract obtained with 50% ethanol

3. Characteristics of powdered broken-milled Riceberry rice extract

3.1 Physical appearance

Broken-milled Riceberry rice was first processed using 50% ethanol extraction, followed by freeze-drying to obtain a powdered extract. As shown in Fig. 3, the broken-milled Riceberry rice (Fig. 3a) is visibly deep purple in color. After extraction and lyophilization, the resulting product (Fig. 3b) was a fine, dark purplish powder, consistent with the presence of anthocyanins known to occur in Riceberry rice. The fine texture of the powder facilitates homogeneous dispersion when incorporated into food matrices or functional product formulations.

3.2 Bioactive compound content and antioxidant activity of powdered extract

The bioactive compound content and antioxidant activity of the powdered extract were re-evaluated to

(a) (b)

Fig. 3 Appearance of broken-milled Riceberry rice and its powdered extract (a) broken-milled Riceberry rice (b) powdered extract of broken-milled Riceberry rice produced through freeze-drying

assess the retention of these valuable components after the freeze-drying process. The results are presented in Table 3.

3.2.1 Anthocyanin content

Anthocyanins are the primary pigments responsible for the deep purple color of Riceberry rice and are known for their potent antioxidant activity. These compounds are key in neutralizing free radicals, reducing oxidative stress and lowering the risk of chronic diseases such as cardiovascular disease and cancer (Rattanachaisit & Kongkiattikajorn, 2015; Chen et al., 2006).

In this study, the anthocyanin content of the extract was 22.48 mg cyanidin-3-glucoside/100 g, comparable to other anthocyanin-rich extracts. This finding underscores the potential of broken-milled Riceberry rice as a functional food ingredient. Previous studies have reported that Riceberry rice contains significant levels of anthocyanins, particularly cyanidin-3-glucoside and peonidin-3-glucoside, which contribute to its antioxidant properties (Chumanee & Yunchalard, 2015).

3.2.2 Total phenolic content (TPC)

The total phenolic content (TPC) of the extract was 570 mg GAE/100 g dry weight, further emphasizing its antioxidant potential. Phenolic compounds act as antioxidants by donating hydrogen atoms or electrons to neutralize free radicals, stabilizing them (Alothman et al., 2009). Previous research has shown that Riceberry rice bran is particularly rich in phenolic compounds, with reported levels reaching up to 55.45 mg GAE/g (Sirichokworrakit et al., 2020).

3.2.3 Antioxidant activity

The antioxidant activity of the extract was assessed using DPPH scavenging activity, which was recorded at 72%. This result highlights the strong free radical neutralization ability of the extract, making it comparable to other well-established antioxidant-rich food sources. These findings align with previous studies on rice bran extracts, which have also demonstrated high antioxidant potential (Luang-In et al., 2018).

Overall, broken-milled Riceberry rice extract is a promising bioactive ingredient with its high anthocyanin, phenolic, and antioxidant content. It compares favourably with other widely recognized antioxidant-rich foods, such as those containing gamma-oryzanol, further supporting its application in functional food development (Khawsuk et al., 2018).

Table 3 Bioactive compound content and antioxidant activity of powdered broken-milled Riceberry rice extract

Sample	Total anthocyanin (mg cyanidin-3-glucoside/100 g DW)	Total phenolic content (mg GAE/100g DW)	%DPPH Scavenging activity
Broken-milled Riceberry rice extract powder	22.48±0.89	570±0.09	72±0.94

Remark: DW = dry weight, GAE = gallic acid equivalent.

4. Quality characteristics of ice cream incorporating broken-milled Riceberry rice extract

4.1 Ice cream formulation and characteristics

Two representative ice cream samples are shown in Fig. 4 the control ice cream (without extract) and the sample formulated with Riceberry rice extract powder at a concentration of 1.5% (w/w), which demonstrated the highest antioxidant activity among all tested levels. The control sample displayed a smooth, creamy white appearance, typical of plain dairy-based ice cream without added pigments or plant-derived compounds.

In contrast, the ice cream enriched with 1.5% Riceberry rice extract powder exhibited a noticeably darker tone with a pale purplish-brown hue, reflecting the presence of anthocyanins naturally found in Riceberry rice. Despite the difference in color, the texture of the fortified ice cream remained smooth and creamy, comparable to that of the control formulation.

(a) (b)

Fig. 4 Representative images of ice cream samples (a) control ice cream (0% extract) (b) ice cream containing 1.5% (w/w) Riceberry rice extract powder

4.1 Antioxidant activity and total phenolic compounds in ice cream

The results presented in Table 4 demonstrate the beneficial effects of increasing broken-milled Riceberry rice extract powder content in ice cream on its total phenolic content, DPPH scavenging activity, and overall antioxidant capacity. As the concentration of extract powder increased, the antioxidant properties of the ice cream also improved, indicating a dose-dependent relationship between the amount of extract powder and its bioactive properties.

At baseline (0% extract), the ice cream showed a relatively low total phenolic content of 23.37 mg GAE/100g, alongside a DPPH scavenging activity of 38.79% and a modest antioxidant capacity of 13.26 mM TE/100g. However, incorporating broken-milled Riceberry rice extracted powder led to significant improvements across all measured parameters. The total phenolic content increased substantially, reaching 37.39 mg GAE/100g at 1.5% anthocyanin content. This increase aligns with previous studies highlighting that anthocyanins are rich in phenolic compounds known for their antioxidant properties (Nour et al., 2013).

The DPPH scavenging activity, which measures antioxidant potential, also significantly increased with higher anthocyanin content, rising from 38.79% in the control group to 84.97% in the 1.5% anthocyanin treatment. This finding is consistent with similar research showing that anthocyanin-rich extracts from various fruits and plants enhance antioxidant activity (Radovanović & Radovanović, 2010).

Table 4 Total phenolic content and antioxidant capacity of ice cream samples fortified with broken-milled Riceberry rice extract powder

Ice cream formulation	Total phenolic content (mg GAE/100g ice cream)	DPPH scavenging activity (%)	Antioxidant capacity by DPPH method (mM TE/100g ice cream)
Control ice cream	23.37±0.72^{d}	38.79±0.13^{d}	13.26±0.05^{d}
Ice cream with 0.5% broken-milled Riceberry rice extract powder	29.76±1.48^{c}	56.58±1.19^{c}	19.57±0.42^{c}
Ice cream with 1.0% broken-milled Riceberry rice extract powder	33.24±0.62^{b}	79.94±1.32^{b}	27.85±0.47^{b}
Ice cream with 1.5% broken-milled Riceberry rice extract powder	37.39±2.09^{a}	84.97±0.20^{a}	29.64±0.07^{a}

Remark: Different letters in the same column indicate significant statistical differences ($p \leq 0.05$).

Moreover, the antioxidant capacity of the ice cream was substantially enhanced by adding broken-milled Riceberry rice extract powder, with values reaching 29.64 mM TE/100g at 1.5% anthocyanin content.

The findings of this study are significant for the development of functional foods, such as ice cream a widely consumed product particularly when enhanced with natural antioxidants like anthocyanins. The results suggest that increasing anthocyanin content in ice cream could potentially provide consumers with a healthier product that offers increased antioxidant capacity, which may aid in the prevention of oxidative stress-related diseases (Tena et al., 2020).

4.2 Physical properties of ice cream

The addition of broken-milled Riceberry rice extract powder at different concentrations (0.5%, 1.0%, and 1.5%) significantly influenced the physical properties of the ice cream formulations (Table 5).

The pH values of all ice cream samples ranged between 5.72 and 5.75, with no significant differences among most treatments except for a slightly lower pH in the 0.5% treatment. The slightly acidic pH is characteristic of dairy-based ice cream, and the addition of Riceberry extract did not substantially alter acidity, indicating good compatibility of the extract with the ice cream matrix (Muse & Hartel, 2004).

A significant change in color was observed with increasing concentrations of Riceberry extract. The L* values (lightness) decreased significantly, indicating darker coloration in the ice cream as the extract level increased. Meanwhile, the a* values (redness) increased, and b* values (yellowness) decreased, which reflects the presence of anthocyanins, —naturally occurring pigments in Riceberry rice known for their purplish-red hues (Laokuldilok et al., 2011). These changes are consistent with prior studies on anthocyanin-enriched dairy products (Cavalcanti et al., 2011).

The viscosity of the ice cream mix increased significantly with 0.5% extract addition (131.80±0.36 cP), which could be attributed to the presence of polyphenolic compounds and residual fibers in the extract that increase water-holding capacity and network formation. However, viscosity slightly declined at higher concentrations (1.0% and 1.5%), possibly due to phase separation or particle interference within the matrix (Adapa et al., 2000).

The highest overrun was observed in the 0.5% treatment (71.88%), while the 1.5% treatment showed a markedly lower overrun (42.84%). This could be due to the high solid content and pigment interference at higher extract levels, which reduce the ability of the mixture to entrap air (Goff & Hartel, 2013). A similar trend has been reported in ice cream fortified with plant-based extracts (Soukoulis et al., 2009).

The control ice cream had the slowest melting rate (2.09 g/min), while the 0.5% extract ice cream melted faster (2.32 g/min). This may be attributed to the destabilization of the fat-protein network by extract components or changes in ice crystal size and structure (Akin et al., 2007). Interestingly, the melting rate in 1.0% and 1.5% formulations was slightly lower than the 0.5% formulation, possibly due to higher solid content enhancing thermal stability (Muse & Hartel, 2004).

Table 5 Physical properties of ice cream with varying percentages of broken-milled Riceberry rice extract powder

Physical attributes	Control ice cream	0.5% Broken-milled Riceberry rice extract powder Ice Cream	1.0% Broken-milled Riceberry rice extract powder Ice Cream	1.5% Broken-milled Riceberry rice extract powder Ice Cream
pH value	5.74 ± 0.01^{a}	5.72 ± 0.01^{b}	5.75 ± 0.01^{a}	5.74 ± 0.01^{a}
L* value	57.86 ± 0.40^{a}	51.46 ± 0.75^{b}	50.93 ± 0.30^{b}	47.03 ± 0.90^{c}
a* value	-1.63 ± 0.28^{d}	0.46 ± 0.12^{c}	1.33 ± 0.15^{b}	2.83 ± 0.23^{a}
b* value	6.96 ± 0.05^{a}	4.90 ± 0.10^{b}	4.20 ± 0.45^{c}	2.66 ± 0.15^{d}
Viscosity (centipoise)	127.23 ± 0.05^{c}	131.80 ± 0.36^{a}	129.50 ± 0.65^{b}	128.63 ± 0.55^{b}
Overrun (%)	69.46 ± 1.11^{b}	71.88 ± 0.45^{a}	69.84 ± 0.95^{b}	42.84 ± 0.95^{c}
Melting rate (g/min)	2.09 ± 0.03^{c}	2.32 ± 0.02^{a}	2.17 ± 0.01^{b}	2.16 ± 0.01^{b}

Remark: Different letters in the same row indicate significant statistical differences ($p \leq 0.05$).

6. Proximate composition of ice cream

The ice cream formulation containing 1.5% broken-milled Riceberry rice extract powder was selected due to its high total phenolic content and strong antioxidant activity. The proximate composition of this formulation, as presented in Table 6, provides meaningful insights into its nutritional and functional attributes.

The product exhibited a protein content of 5.92±0.17%, fat content of 4.13±0.07%, and carbohydrate content of 19.96±0.91%. Additionally, it contained 1.44±0.04% crude fiber, 0.93±0.01% ash, and 67.73±0.49% moisture.

Protein is essential in ice cream for structural stability, emulsification, and texture development. Milk proteins, in particular, enhance overrun, contribute to emulsion stability, and improve meltdown resistance (Goff & Hartel, 2013). The observed protein level is within the typical range for standard formulations and reflects a balanced contribution from dairy sources.

Fat plays a central role in delivering creaminess, promoting flavor release, and stabilizing air bubbles. The fat content of 4.13% is lower than that of conventional full-fat ice cream, which typically contains 10–16% fat (Goff & Hartel, 2013). However, this aligns with current trends favoring reduced-fat or functional ice cream formulations for health-conscious consumers.

Carbohydrates not only provide sweetness but also influence texture by increasing total solids, lowering freezing point, and enhancing body and mouthfeel (Goff & Hartel, 2013). The carbohydrate level of 19.96% is in line with conventional ice cream recipes, reflecting the combined effect of added sugars and other carbohydrates.

Importantly, the ice cream contains 1.44 g of crude fiber/100 g, a value significantly higher than that of typical commercial ice cream, which often lacks dietary fiber. This elevated fiber content enhances the functional value of the product and may offer health benefits, particularly in supporting digestive health (Akbari et al., 2016).

The ash content of 0.93% indicates the presence of minerals, primarily derived from milk solids and the broken-milled Riceberry rice extract, and falls within the expected range for dairy-based frozen desserts (El-Nagar et al., 2002).

Finally, the moisture content of 67.73% is appropriate for ice cream, which generally contains 60–70% water. Moisture level is a critical factor affecting ice cream texture, overrun, and freezing behavior, as it determines the extent of ice crystal formation (Goff & Hartel, 2013).

Table 6 Proximate composition of ice cream with 1.5% broken-milled Riceberry rice extract powder

Proximate composition	Ice cream with 1.5% broken-milled Riceberry rice extract powder
Protein	5.92 ± 0.17
Fat	4.13 ± 0.07
Carbohydrates	19.96 ± 0.91
Crude fiber	1.44 ± 0.04
Ash	0.93 ± 0.01
Moisture	67.73 ± 0.49

Conclusion

This study demonstrates that broken-milled Riceberry rice extract, particularly that obtained using 50% ethanol, is a rich source of anthocyanins and phenolic compounds with potent antioxidant activity. When incorporated into ice cream at concentrations up to 1.5% (w/w), the extract significantly enhanced antioxidant capacity without compromising key physicochemical properties. The most enriched formulation exhibited a DPPH scavenging activity of 84.97% along with improved total phenolic content, viscosity, and color characteristics, supporting its application as a natural bioactive ingredient. Overall, these findings highlight the feasibility of using broken-milled Riceberry rice extract to improve the nutritional profile of frozen desserts, while also demonstrating a sustainable strategy for valorizing agricultural byproducts in food innovation. This aligns with current trends toward health-oriented and environmentally responsible food development. Nevertheless, certain limitations should be acknowledged. Variability in raw material composition due to seasonal and agricultural factors may influence the consistency of bioactive compound yields, potentially affecting reproducibility. Future studies should employ compound-specific profiling of anthocyanins using advanced techniques such as high-performance liquid chromatography (HPLC) to identify major constituents, including cyanidin-3-O-glucoside and peonidin-3-O-glucoside. In addition, in vivo studies on bioavailability and antioxidant efficacy, as well as assessments of storage stability under varying conditions, are recommended to address compound degradation over time and support potential functional claims in commercial applications.

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