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Effect of Germination on Bioactive Compounds and Antioxidant Properties of Riceberry Rice for Functional Ice Cream

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Abstract

This study investigates the effects of germination time on the bioactive compounds, chemical composition, and antioxidant properties of Riceberry rice, along with the incorporation of germinated Riceberry milk (GRM) into ice cream. Germination significantly increased gamma-aminobutyric acid (GABA) levels, reaching a peak at 20 h (19.49 mg/100 g dry basis), while total phenolic content (TPC) and antioxidant capacity (DPPH assay) showed a slight decline after 18 h. At 20 h, germinated rice exhibited lower carbohydrate content and higher levels of fat, fiber, and ash compared to non-germinated rice. GRM, containing 1.74% protein, 1.10% fat, and an antioxidant capacity of 172.06 mmol Trolox/100 g, was incorporated into ice cream formulations at concentrations ranging from 0% to 50%. Increasing GRM content improved the ice cream's antioxidant capacity and TPC, with the highest levels observed at 50% GRM (14.47 mg gallic acid/100 g and 31.33 mmol Trolox/100 g, respectively). GRM addition also affected physical attributes such as apparent viscosity and overrun. Sensory evaluation revealed that high GRM concentrations (≥30%) negatively impacted texture, taste, and overall acceptability. In contrast, the 20% GRM formulation provided a favorable balance between enhanced nutritional properties and consumer acceptance, showing no significant difference from the control. These findings support the potential of GRM as a functional ingredient in health-oriented frozen desserts, offering nutritional and antioxidant benefits without compromising sensory quality when used at optimal levels.

Introduction

Riceberry rice (*Oryza sativa* L.) is a cross between Jao Hom Nin (a non-glutinous purple rice) and Khao Dawk Mali 105 (a fragrant white rice). It is characterized by its deep purple colour, which is attributed to its high anthocyanin content and its rich nutritional profile, including fiber, vitamins, and minerals (Caceres et al.

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2017). However, the potential of Riceberry rice can be further enhanced through germination, a process that not only improves its nutritional value but also increases the bioavailability of its bioactive compounds. Among these bioactive compounds, gamma-aminobutyric acid (GABA) has emerged as one of the most studied (Cornejo et al. 2015). GABA is a non-protein amino acid that acts as an inhibitory neurotransmitter in the brain, and its consumption has been associated with several health benefits, including stress reduction, improved sleep quality, and potential antihypertensive effects, the inhibition of cancer cell proliferation, and blood pressure control (Cornejo et al., 2015; Oh & Oh, 2004)

The process of germination has been shown to enhance the levels of GABA in rice, as it activates enzymes responsible for converting glutamic acid to GABA (Komatsuzaki et al., 2007; Zhang et al., 2006; Oh, 2003). Germination also influences rice's overall chemical composition, including its carbohydrate, protein, fat, and fiber content, making it a potential source of functional food ingredients. However, the optimal germination time required for maximizing bioactive compounds, particularly GABA, while maintaining the nutritional integrity of the rice remains a topic of interest.

Recently, interest has increased in incorporating functional ingredients into popular food products to enhance their health benefits without compromising sensory appeal. Ice cream, a widely consumed dessert, presents an excellent opportunity to incorporate functional ingredients due to its popularity and versatility. However, developing functional ice cream requires careful consideration of the added ingredients' physical, chemical, and sensory properties to ensure consumer acceptance. Germinated Riceberry milk (GRM), derived from germinated Riceberry rice, is a novel ingredient that combines the nutritional benefits of germination with the convenience of a liquid form, making it suitable for incorporation into several food products, including ice cream.

Despite the potential of germinated Riceberry rice and GRM, there is limited research on their application in food products, particularly ice cream. This study aims to fill this gap by investigating the effects of germination on the GABA content, total phenolic content (TPC), and antioxidant capacity of Riceberry rice, as well as the potential of GRM as a functional ingredient in ice cream. The study also evaluates the physical, chemical, and sensory properties of GRM-containing ice cream to determine the optimal level of incorporation that balances health benefits with consumer acceptability. By exploring the potential of germinated Riceberry rice and GRM, this research contributes to developing innovative functional foods that meet the growing demand for health-promoting products.

Materials and methods

1. Chemical reagents

All chemicals used were of analytical grade. Ethanol was obtained from RCI-Labscan Ltd., Thailand. The Folin-Ciocalteu reagent and anhydrous sodium carbonate (Na_2CO_3) were sourced from Loba Chemie Pvt. Ltd., India. Other chemicals and standards, including 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, and gallic acid, were purchased from Sigma-Aldrich, USA.

2. Raw materials

Riceberry rice (*Oryza sativa* L.) used in this study was purchased from a local supplier, with the crop cultivated in Suphan Buri Province, Thailand, and harvested in October 2015. Riceberry is a Thai rice variety officially registered in 2002. It was developed by the Rice Science Center at Kasetsart University through a cross between Jao Hom Nin (JHN), a local non-glutinous purple rice, and Jasmine rice (KDML105), also known as Thai Hom Mali Rice. Freshly dehulled Riceberry grains were used for germination to ensure optimal sprouting performance. Following dehulling, the rice grains were sealed in plastic bags and stored at room temperature until further use.

3. Germination of rice

The germination process followed the method described by Jiamyangyuen and Ooraikul (2008) with minor modifications. Briefly, 100 g of rice grains were soaked in distilled water at a ratio of 1:2 (w/v) at room temperature for 4 h, a duration determined through prior experimentation. After soaking, the water was drained, and the grains were washed. The soaked grains were placed on moist cloth and germinated in a hot air oven at 35°C for varying durations of 0, 16, 18, 20, 22, and 24 h. After germination, the rice grains were dried in a tray dryer at 50°C for 4 h, reducing moisture content to below 10%. The dried grains were then ground using a blender, as described by Komatsuzaki et al. (2007), and sieved through a 60-mesh sieve. The ground samples were collected and stored at -18°C for further analysis.

4. Analysis of germinated rice

4.1 Rice extraction

Rice extraction was performed using a modified method from Shen et al. (2009). Briefly, 50% ethanol was used to extract the ground rice at a 1:25 (w/v) ratio for 18 h. The mixture was centrifuged at 4000×g for 15 min at room temperature to separate the supernatant and pellet. The crude extract was transferred to brown glass bottles and stored at -18°C for later use.

4.2 Determination of total phenolic content

The rice extracts' total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Maizura et al., 2011) with slight modifications. In brief, 0.4 mL of the rice extract was mixed with 2 mL of 10% Folin-Ciocalteu reagent and allowed to react for 4 min. Then, 1.6 mL of 5% Na_2CO_3 was added, and the mixture was incubated in the dark for 30 min. The absorbance was measured at 765 nm using a spectrophotometer (UV-2401PC, Shimadzu, Japan). The TPC was determined by comparing the absorbance to a standard curve of gallic acid with concentrations ranging from 0 to 100 µg/mL. Results were expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g dry weight).

4.3 Determination of DPPH free radical scavenging activity

The antioxidant activity of the rice extract was assessed using the DPPH method, adapted from Zhang et al. (2015). A 2 mL aliquot of the rice extract was mixed with 2 mL of 200 mM DPPH solution and incubated in the dark for 30 min. The absorbance was measured at 515 nm using a spectrophotometer. The antioxidant activity was determined by comparing the absorbance with a standard curve of Trolox at concentrations ranging from 0 to 50 µM. The results were expressed as DPPH scavenging activity in millimoles of Trolox equivalents per 100 g of dry weight (mM TE/100 g dry weight).

4.4 Determination of gamma-aminobutyric acid (GABA)

The content of gamma-aminobutyric acid (GABA) in the rice grains was determined using a modified method from Sarkar et al. (1997). The procedure involved the following steps:

Sample Extraction: 1 g of rice sample was placed into a 50 mL test tube, and 25 mL of 70% ethanol was added. The sample was homogenized for 2 min and then centrifuged at 10,000 rpm for 20 min at 20°C. The supernatant was evaporated to dryness using a rotary evaporator and then dissolved in 2 mL of borate buffer for derivatization.

Derivative Preparation: A 200 µL aliquot of the sample was mixed with 200 µL of 9-fluorenyl methyl chloroformate. After 90 sec, 120 µL of cleavage reagent was added, and the mixture reacted for another 3 min. Finally, 200 µL of quenching reagent was added and the sample was prepared for HPLC analysis.

GABA quantification by HPLC: The derivatized samples were analyzed using a high-performance liquid chromatography (HPLC) system (SCL-10AVP, Shimadzu, Japan) equipped with a fluorescence detector (RF-10AXL), with excitation at 263 nm and emission at 313 nm. Separation was achieved using an Ultra C18 column (5 µm, 250 × 4.6 mm) at 40°C with a 1 mL/min flow rate. The mobile phases were 20 mM ammonium dihydrogen orthophosphate in 15% (v/v) methanol (mobile phase A) and 90% (v/v) acetonitrile in water (mobile phase B). GABA standards from Sigma were used for calibration.

4.5 Determination of proximate composition

The proximate composition of Riceberry rice and germinated Riceberry rice, including moisture, total ash, crude protein, crude fat, and crude fiber, was determined using standard methods for proximate analysis (AOAC, 2007). The carbohydrate content was estimated by difference, calculated as 100 minus the sum of crude protein, crude fat, crude fiber, moisture, and ash contents.

5. Preparation and chemical characterization of germinated Riceberry milk

5.1 Preparation of germinated Riceberry milk (GRM

Germinated Riceberry rice at its optimal germination stage was used to produce Riceberry milk. The milk was prepared using 80 g of germinated Riceberry rice and blended with 1 L of water then heated using a Philips automatic soy milk maker (model HD2079/07, Philips Electronics Co., Ltd., Thailand). The germinated Riceberry milk was analyzed for several quality parameters.

5.2 Determination of proximate composition, total phenolic content, and antioxidant activity of GRM

The chemical and bioactive properties of germinated Riceberry milk (GRM) were evaluated by determining its proximate composition, total phenolic content (TPC), and antioxidant activity, following the procedure described in Section 4.

6. Development of ice cream containing germinated Riceberry milk

6.1 Preparation of ice cream

Ice cream was prepared based on a modified recipe from Kaothien (2012), with germinated Riceberry milk (GRM) substituted for fresh milk at 0%, 20%, 30%, 40%, and 50%. The ingredients included: 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. The preparation process was as follows: The dry ingredients (skim milk powder, sugar, dextrose, maltodextrin, and salt) were mixed in a bowl. The stabilizer and emulsifier (S/E) were dissolved in a pot of hot water heated to 60°C, with rapid stirring until fully dissolved, and then removed from heat. The liquid ingredients (fresh milk, extracted germinated Riceberry milk, and dissolved S/E) were combined. The dry ingredients were added gradually to the mixture, stirring until fully dissolved. Whipping cream was incorporated into the mixture and blended at high speed for 2 min to ensure a uniform texture. The mixture was pasteurized at 80±1°C for 2 min, then rapidly cooled. The ice cream mixture was aged at 4±2°C for 4 h. After ageing, the mixture was churned in an ice cream maker for 30-40 min at room temperature (25°C). The final ice cream was transferred to plastic containers with lids and frozen at -20±2°C for 24 h before quality evaluation.

6.2 Ice cream analysis antioxidant activity and total phenolic compounds analysis

The antioxidant activity (DPPH) and total phenolic content in the ice cream were determined following the method of Limsuwan et al. (2014) with slight 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 separated and analyzed for total phenolic content using the Folin-Ciocalteu method and antioxidant activity using the DPPH method, as described earlier.

6.2.1 Melting rate

The melting rate of ice cream was determined using a method adapted from Rizk et al. (2014). Ice cream samples were weighed in plastic cups and stored at -18°C for 24 h before evaluation. Then 50 g of ice cream was removed from the cups and placed on a wire mesh (1 cm^2) with a pre-weighed beaker beneath to collect melted ice cream. The setup was maintained at 25±1°C and the weight of the melted ice cream was recorded every 5 min. The melting rate (g/min) was calculated from the slope of the linear portion of the melting curve.

6.2.2 Overrun

The overrun of the ice cream was calculated as a percentage using the formula outlined by Rizk et al. (2014):

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

6.2.3 Color

The color of the ice cream was measured using a colorimeter (Model CR-400, Konica Minolta, Japan) based on the CIE Lab* color scale. Color parameters L* (lightness), +a* (redness), and +b* (yellowness) were recorded.

6.2.4 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 number 1, after the motor had run for 30 sec, with the liquid temperature controlled at 20±1°C.

6.2.5 Sensory quality evaluation

Sensory evaluation of the ice cream was conducted with 50 untrained panellists (both male and female), aged between 19 and 45 years. The panellists assessed several sensory attributes, including colour, aroma, flavour, creaminess, smoothness, texture, and overall liking. Each panellist received approximately 30 g of each sample, which was coded with a three-digit random number. The ice cream was stored at -18°C and thawed to -10°C before tasting. The samples were evaluated using a 9-point Hedonic scale, where 1 represented "dislike extremely" and 9 represented "like extremely.

All sensory evaluations were performed with informed consent in accordance with standard ethical practices applicable to non-invasive human studies.

7. Statistical analysis

Statistical analyses were conducted using IBM SPSS statistics software (version 23.0). When significant variation was detected through analysis of variance (ANOVA) at a significance level of 0.05, Duncan's multiple range test was used to compare the means.

Results and discussion

The present study aimed to investigate the variations in the bioactive compounds, chemical composition, and antioxidant properties of germinated Riceberry rice at different germination times and the effects of germinated Riceberry milk (GRM) on ice cream formulation. The findings highlight significant changes in gamma-aminobutyric acid (GABA), total phenolic content (TPC), and antioxidant capacity with germination time, as well as the impact of GRM content on ice cream's physical, chemical, and sensory properties.

1. Effect of germination on bioactive compounds in Riceberry rice

The results presented in Table 1 demonstrate that germination time significantly affects the levels of gamma-aminobutyric acid (GABA), total phenolic content (TPC), and antioxidant capacity (measured by the DPPH method) in Riceberry rice. These bioactive compounds are crucial indicators of the functional and health-promoting properties of germinated rice, a product increasingly recognized for its enhanced nutritional profile compared to non-germinated rice.

1.1 Gamma-aminobutyric acid (GABA)

GABA content increased markedly during germination, reaching a peak at 20 h (19.49±1.44 mg/100g db), which represents a more than tenfold increase compared to the initial level at 0 h (1.81±0.20 mg/100g db). The increase was statistically significant ($p \leq 0.05$), with the highest value observed between 20 and 24 h. Although GABA levels slightly decreased after 20 h, the differences among 20, 22, and 24 h were not statistically significant, indicating that the biosynthesis of GABA reaches a plateau beyond this point. This trend aligns with previous studies by Moongngarm and Saetung (2010), who reported enhanced GABA accumulation during rice germination. The increase in GABA is attributed to the activation of glutamate decarboxylase (GAD), which catalyzes the decarboxylation of glutamic acid into GABA under germination conditions (Komatsuzaki et al., 2007; Bown & Shelp, 1997). Germinated Riceberry rice after 20 h of germination is presented in Fig. 1.

1.2 Total phenolic content (TPC)

In contrast to GABA, TPC showed a significant decline after germination. The highest TPC was recorded in the ungerminated sample at 0 h (994.35±7.55 mg GAE/100g db) and significantly lower values were observed at all germinated time points ($p \leq 0.05$). For example, TPC decreased to 856.18±6.47 mg GAE/100g db at 16 h and remained between 883.66±2.15 and 891.29±8.63 mg GAE/100g db during 20–24 h. This reduction is likely due to the leaching of water-soluble phenolic compounds during the soaking and washing steps of germination, as previously reported by Chanphrom (2007) and Sompong et al. (2011). Despite the decline, phenolic content remained relatively high, contributing to the antioxidant potential of the germinated product.

1.3 Antioxidant capacity (DPPH)

The antioxidant capacity, as determined by the DPPH assay, significantly increased with germination time ($p \leq 0.05$), peaking at 18 h (414.94±3.36 mg TE/100g db), representing a 1.5-fold increase compared to the initial value at 0 h (276.55±3.92 mg TE/100g db). High values were also observed at 22 and 24 h (411.76±5.60 and 404.63±4.48 mg TE/100g db, respectively), with no significant differences among the top three time points. The enhancement in antioxidant capacity is likely associated with increased levels of bioactive compounds such as tocopherols and tocotrienols, as reported by Jiamyangyuen and Ooraikul (2008) and Kayahara and Tsukahara (2000), who found a rise in vitamin E derivatives during rice germination. These compounds play vital roles in preventing lipid peroxidation and promoting overall health benefits (Xu et al., 2001).

Germination significantly enhances the functional quality of Riceberry rice by increasing GABA and antioxidant activity, while slightly reducing phenolic content. The optimal germination time appears to be between 18 and 20 h for maximizing GABA and antioxidant levels without substantial loss of phenolic compounds. These findings support the potential of germinated Riceberry rice as a functional food ingredient with health-promoting properties.

Table 1 Bioactive compounds and antioxidant capacity of germinated Riceberry rice at varying germination times

The germination time (h)	Gamma-aminobutyric acid (mg/100g db)	The total phenolic content (TPC) (mg GAE/100g db)	The antioxidant capacity (DPPH) (mg TE/100g db) DPPH
0	1.81±0.20[c]	994.35±7.55[a]	276.55±3.92[c]
16	11.56±1.45[b]	856.18±6.47[d]	395.11±4.49[b]
18	10.89±1.02[b]	885.18±2.15[bc]	414.94±3.36[a]
20	19.49±1.44[a]	883.66±2.15[c]	397.49±3.35[b]
22	18.97±0.56[a]	891.29±8.63[bc]	411.76±5.60[a]
24	17.82±1.45[a]	889.92±4.32[b]	404.63±4.48[ab]

Remark: Different letters (a, b, c) in the same column indicate statistically significant differences at $p \leq 0.05$.

Fig. 1 Germinated Riceberry rice at 20 h of germination

2. Proximate composition changes in germinated Riceberry rice

Table 2 presents the comparative analysis of the proximate composition between non-germinated and 20-h germinated Riceberry rice. Germination induced several statistically significant changes ($p \leq 0.05$) in fat, carbohydrate, fiber, and ash contents, whereas protein and moisture contents showed no significant differences.

Specifically, the fat content increased significantly from 3.18±0.01% in non-germinated rice to 3.84±0.03% in germinated rice ($p \leq 0.05$). This increase may be attributed to enhanced lipase activity during germination, which hydrolyzes stored triacylglycerols into free fatty acids and glycerol for energy production and membrane biosynthesis. In some seeds, this lipid mobilization process also triggers structural changes that may increase extractable lipid content (Ali & Elozeiri, 2017).

Conversely, carbohydrate content significantly decreased from 71.76±0.09% to 70.08±0.19% ($p \leq 0.05$), likely due to enzymatic hydrolysis of starch into simpler sugars for use as energy in metabolic processes (Chinma et al., 2015).

Fiber content increased markedly and significantly ($p \leq 0.05$) from 1.86±0.09% to 2.58±0.19%, suggesting an enhancement in dietary fiber levels during germination, potentially through cell wall remodeling and synthesis of non-digestible oligosaccharides (Mudgil & Barak, 2013). This improvement could offer functional benefits such as enhanced digestive health and improved glycemic response (Slavin, 2013).

Additionally, ash content significantly increased from 1.55±0.01% to 1.74±0.05% ($p \leq 0.05$), indicating a rise in total mineral content during germination. This finding is consistent with the study by Chinma et al. (2015), which reported that germination enhanced the quantities of magnesium, phosphorus, and potassium in flour samples, while levels of calcium, iron, sodium, and zinc were not significantly affected.

No significant differences ($p > 0.05$) were found in protein content (11.90±0.12% vs. 11.76±0.18%) and moisture content (9.71±0.10% vs. 9.97±0.14%), indicating stability of these components during the early germination stage.

These compositional changes suggest that germination enhances the nutritional quality of Riceberry rice by increasing beneficial components such as fat, fiber, and minerals, reinforcing its potential as a functional food.

Table 2 Proximate composition (%) of non-germinated and 20-h germinated Riceberry rice

The proximate composition (%)	Non germinated Riceberry rice	20-h germinated Riceberry rice
Protein^{ns}	11.90±0.12	11.76±0.18
Fat	3.18±0.01^{b}	3.84 ±0.03^{a}
Carbohydrate	71.76±0.09^{a}	70.08±0.19^{b}
Fiber	1.86 ±0.09^{b}	2.58±0.19^{a}
Ash	1.55±0.01^{b}	1.74±0.05^{a}
Moisture^{ns}	9.71±0.10	9.97±0.14

Remark: Different letters (a, b) within the same row indicate statistically significant differences ($p \leq 0.05$).
ns indicates a non-significant difference.

3. Proximate and bioactive properties of germinated Riceberry milk (GRM)

Germinated Riceberry rice at its optimal germination stage was used to produce germinated Riceberry milk (GRM). The proximate composition and bioactive properties of GRM, summarized in Table 3, revealed relatively low protein (1.74±0.04%) and fat (1.10±0.04%) contents compared to conventional dairy products. The carbohydrate content was moderate (2.50±0.01%), accompanied by a notable dietary fiber level (1.17±0.01%). Ash content (0.15±0.01%) indicates the presence of essential minerals, which are vital for metabolic and physiological functions. The moisture content was high (93.32±0.05%), typical of milk-like beverages. Importantly, GRM exhibited a high total phenolic content (731.75±6.87 mg GAE/100 g) and strong antioxidant capacity (172.06±6.26 mmol TE/100 g), underscoring its potential as a functional ingredient with health-promoting properties. These bioactive compounds, especially phenolics, are well recognized for their antioxidant activity and likely contribute significantly to the nutritional and functional value of the product.

The visual appearance of GRM, shown in Fig. 2, demonstrates its distinct purple coloration, which are characteristic of Riceberry rice and reflect the presence of natural pigments such as anthocyanins.

Table 3 Proximate and bioactive properties of germinated Riceberry rice milk

Proximate and bioactive properties	Germinated Riceberry rice milk
Protein (% wet weight)	1.74±0.04
Fat (% wet weight)	1.10±0.04
Carbohydrate (% wet weight)	2.50±0.01
Fiber (% wet weight)	1.17±0.01
Ash (% wet weight)	0.15±0.01
Moisture (% wet weight)	93.32±0.05
The total phenolic content (TPC) (mg GAE/100g wet basis)	731.75±6.87
The antioxidant capacity (DPPH) (mmol TE/100g wet basis)	172.06± 6.26

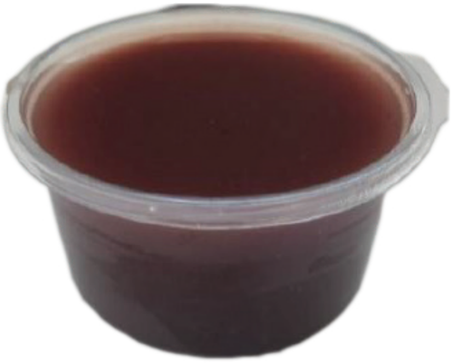

Fig. 2 Germinated Riceberry milk (GRM)

4. Development of ice cream containing germinated Riceberry milk

4.1 Ice cream formulation and characteristics

Ice cream was formulated by substituting fresh milk with germinated Riceberry milk (GRM) at levels of 0% (control), 20%, 30%, 40%, and 50%, following a modified recipe based on Kaothien (2012). The substitution of GRM was found to influence the physical characteristics of the final product, particularly in terms of color, aroma, and texture.

The control sample (0% GRM) exhibited typical ice cream characteristics, including a creamy white color, a pronounced dairy aroma, and a smooth, uniform texture (Fig. 3a). Ice cream containing 20% and 30% GRM began to display a light purple hue, attributed to the natural pigments in Riceberry rice, along with a mild, pleasant aroma specific to germinated Riceberry. The texture in these groups remained smooth and comparable to the control (Fig. 3b and 3c).

In contrast, samples with 40% and 50% GRM showed a markedly deeper purple coloration and a more intense Riceberry aroma. The texture became slightly coarse, and increased ice crystal formation was visually observed, suggesting a potential impact on the product's stability and mouthfeel compared to formulations with lower GRM content (Fig. 3d and 3e).

All formulations were subsequently subjected to comprehensive physical, chemical, and sensory evaluations to further assess their quality attributes.

4.2 Physical properties of ice cream

The incorporation of germinated Riceberry milk (GRM) into ice cream formulations significantly influenced several physical properties, as summarized in Table 4. Statistically significant differences ($p \leq 0.05$) were observed across all evaluated parameters, except for the melting rate.

The apparent viscosity increased significantly ($p \leq 0.05$) with higher GRM concentrations, rising from 105.86±2.44 cP in the control (0% GRM) to 328.96±5.57 cP at 50% GRM. This trend is likely due to the elevated levels of fiber and carbohydrates in GRM, which contribute to a more viscous texture. This result is consistent with previous studies indicating that fiber-rich ingredients, such as barley, increase viscosity through the water-binding ability of gelatinized starch and soluble fibers (Gab-Allah et al., 2023).

Concomitantly, overrun decreased significantly ($p \leq 0.05$) from 43.86±1.67% at 0% GRM to 27.83±1.73% at 50% GRM. This inverse relationship between viscosity and overrun is well documented; higher viscosity reduces air incorporation during churning, leading to a denser ice cream matrix (Alvarez et al., 2005).

Colour parameters (L*, a*, b*)** also exhibited statistically significant changes ($p \leq 0.05$). Lightness (L* value) decreased from 53.00±1.47 at 0% GRM to 32.90±0.10 at 50% GRM, while the a* value (redness) increased significantly from –3.13±0.15 to 2.60±0.56, reflecting the reddish-purple pigment of Riceberry rice. The b* value (yellowness) decreased from 7.76±0.45 to 3.26±0.25, supporting the visual shift in ice cream colour.

Interestingly, the melting rate did not differ significantly across the formulations ($p > 0.05$), remaining within a narrow range (2.12–2.36 g sample/min). This suggests that despite changes in viscosity and overrun, the thermal stability of the mix remained relatively consistent. This result aligns with findings from

Fig 3. Representative images of ice cream samples: (a) control (0% GRM), (b) ice cream with 20% GRM, (c) ice cream with 30% GRM, (d) ice cream with 40% GRM, and (e) ice cream with 50% GRM

Table 4 Physical properties of ice cream with varying GRM content (%)

GRM content (%)	Physical properties					
	Colour			Apparent viscosity (centipoise)	Overrun (%)	Melting rate (g sample/min)
	L*	a*	b*			
0	53.00±1.47[a]	-3.13±0.15[d]	7.76±0.45[a]	105.86±2.44[e]	43.86±1.67[a]	2.33± 0.09[a]
20	39.93±1.03[b]	0.50±0.44[c]	4.53±0.28[b]	186.80±3.17[d]	34.47±1.45[b]	2.12±0.04[b]
30	38.63±0.23[b]	0.60±0.61[bc]	4.73±0.15[b]	231.46±4.42[c]	31.29±1.08[c]	2.23±0.06[ab]
40	36.53±1.28[c]	1.40±0.30[b]	4.46±0.15[b]	284.73±6.69[b]	29.39±1.68[cd]	2.22±0.03[ab]
50	32.90±0.10[d]	2.60±0.56[a]	3.26±0.25[c]	328.96±5.57[a]	27.83±1.73[d]	2.36±0.05[a]

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

Choi & Shin (2014), which reported that ingredient modifications might alter physical properties like density and aeration, yet have minimal impact on melting behaviour due to inherent thermal properties of the base mix.

Overall, these results demonstrate that GRM significantly alters viscosity, aeration, and colour, while maintaining thermal performance, indicating its potential for functional ice cream development with distinct sensory and textural attributes.

4.3 Total phenolic content and antioxidant capacity of ice cream

Table 5 presents the effects of varying extracted germinated rice milk (GRM) concentrations on the total phenolic content (TPC) and antioxidant capacity of ice cream. The results demonstrate a statistically significant increase ($p \leq 0.05$) in both parameters as the GRM concentration increased.

4.3.1 Total phenolic content (TPC)

The TPC of the control sample (0% GRM) was 12.09±0.07 mg GAE/100g, which increased significantly with higher GRM incorporation. At 50% GRM, the TPC reached its peak at 14.47±0.47 mg GAE/100g ($p \leq 0.05$), representing a 19.7% increase compared to the control. This progressive increase is attributed to the phenolic compounds inherently present in germinated rice. Similar findings were reported by Moolwong et al. (2023), who observed increased TPC values with higher avocado pulp concentrations in ice cream. Likewise, Wangcharoen (2012) reported a notably higher TPC in ginger-flavored soya milk ice cream, suggesting that both the type and concentration of plant-derived ingredients significantly influence phenolic content.

4.3.2 Antioxidant capacity (DPPH Assay)

A significant enhancement in antioxidant activity ($p \leq 0.05$) was also observed with increasing GRM content. The control sample exhibited an antioxidant capacity of 16.75±1.54 mM TE/100g, which steadily increased across formulations, reaching 31.33±0.43 mM TE/100g at 50% GRM — an 87% increase compared to the control. The strong positive correlation suggests that phenolic compounds in GRM contribute significantly to the radical scavenging activity measured by the DPPH assay. These results emphasize the functional potential of GRM as a natural antioxidant booster in ice cream formulations.

Overall, the incorporation of GRM enhances the nutritional profile of ice cream by significantly increasing its total phenolic content and antioxidant capacity, thereby potentially offering additional health benefits.

Table 5 Total phenolic content and antioxidant capacity of ice cream with varying GRM content (%)

GRM content (%)	Total phenolic content (mg GAE/100g sample)	Antioxidant capacity (mM TE/100g sample) DPPH•
0	12.09±0.07[d]	16.75±1.54[d]
20	12.50±0.11[cd]	21.10±2.84[c]
30	12.89 ±0.29[bc]	26.35±2.55[b]
40	13.36±0.18[b]	29.65 ±1.33[ab]
50	14.47±0.47[a]	31.33± 0.43[a]

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

4.4 Sensory evaluation of ice cream

The sensory evaluation results (Table 6) revealed statistically significant differences ($p \leq 0.05$) in various sensory attributes of ice cream as the concentration of extracted germinated rice milk (GRM) increased. Although all samples received moderately high acceptance, increasing levels of GRM led to a gradual decline in sensory scores, particularly for smoothness, flavor, and texture.

Color: The color scores remained relatively stable across treatments, ranging from 6.90±1.37 to 7.46±1.03,

Table 6 Sensory attributes of ice cream with varying GRM content (%)

GRM content (%)	Sensory attribute					
	Color	Smoothness	Flavor	Taste	Texture	Overall liking
0	7.46±1.03[a]	7.72±0.83[a]	7.22±1.05[a]	7.64±0.96[a]	7.50±1.13[a]	7.80±1.12[a]
20	7.32±1.18[ab]	7.46±1.15[a]	6.96±1.38[ab]	7.56±1.26[a]	7.40±1.21[a]	7.62±1.03[a]
30	6.90±1.37[b]	6.28±1.34[c]	6.40±1.48[bc]	6.54±1.44[b]	6.52±1.31[b]	6.76±1.22[b]
40	7.06±1.31[ab]	6.96±1.34[b]	6.58±1.44[bc]	6.66±1.68[b]	6.98±1.36[ab]	6.90±1.60[b]
50	6.96±1.24[ab]	6.76±1.36[bc]	6.30±1.53[c]	6.36±1.68[b]	6.70±1.60[b]	6.62±1.71[b]

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

with no significant difference between most formulations. However, the 30% GRM sample scored significantly lower than the control ($p \leq 0.05$).

Smoothness: A clear decreasing trend in smoothness was observed as GRM content increased. The control sample (0%) scored highest (7.72±0.83) while the 30% sample recorded the lowest value (6.28±1.34, $p \leq 0.05$), indicating that higher levels of GRM may negatively affect creaminess.

Flavor and Taste: Flavor and taste scores also decreased significantly ($p \leq 0.05$) at higher GRM concentrations. The control sample received a flavor score of 7.22±1.05, which declined to 6.30±1.53 at 50% GRM. Similarly, taste ratings dropped from 7.64±0.96 to 6.36±1.68.

Texture: Texture scores followed a similar pattern, with the control and 20% samples scoring above 7.40, whereas the 30–50% GRM samples showed lower texture acceptability (6.52–6.98), significantly different from the control group ($p \leq 0.05$).

Overall Liking: Despite the declines in individual attributes, overall liking remained relatively high across all formulations. The control sample had the highest score (7.80±1.12), while the 50% GRM formulation still received a reasonably acceptable score of 6.62±1.71, suggesting that while sensory qualities were impacted, the product was still generally well-received.

The results demonstrated that increasing the proportion of germinated rice milk (GRM) in the ice cream formulation significantly enhanced the total phenolic content and antioxidant capacity ($p \leq 0.05$). The 50% GRM formulation exhibited the highest values for both antioxidant activity (31.33±0.43 mM TE/100g) and total phenolic content (14.47±0.47 mg GAE/100g).

Nevertheless, sensory evaluation indicated that high levels of GRM (≥30%) negatively affected sensory attributes, particularly smoothness, taste, flavor, and overall liking, with statistically significant differences from the control group ($p \leq 0.05$).

In contrast, the 20% GRM formulation maintained favorable sensory characteristics, with no significant differences from the control in overall liking (7.62±1.03) or in other key attributes such as smoothness, flavor, and taste. Although its antioxidant capacity was not the highest, it was significantly improved compared to the control (21.10±2.84 vs. 16.75±1.54 mM TE/100g, $p \leq 0.05$).

These findings suggest that the 20% GRM formulation offers an optimal balance between enhanced nutritional properties and acceptable sensory quality, making it a promising candidate for further product development.

Conclusion

This study highlights the beneficial effects of germination on Riceberry rice, particularly the enhancement of gamma aminobutyric acid (GABA) levels, and demonstrates the potential of incorporating germinated Riceberry milk (GRM) into ice cream formulations. While germination for 20 h significantly increased GABA content, slight reductions in total phenolic content (TPC) and antioxidant activity were observed after 18 h. Nevertheless, GRM retained substantial levels of bioactive compounds.

Incorporating GRM into ice cream significantly improved antioxidant capacity and TPC. However, higher concentrations (≥30%) negatively impacted sensory attributes such as texture, taste, and overall acceptability. In contrast, the 20% GRM formulation achieved a desirable balance between enhanced nutritional value and favorable sensory properties, showing no significant difference from the control in consumer acceptance.

These findings support the use of GRM as a promising functional ingredient in the development of health-oriented frozen desserts. Future studies should examine stability tests of germinated Riceberry milk (GRM), and product stability to support commercial application.

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