Effect of Black Rice Enrichment on the Quality of Fermented Milk Produced with *Lactiplantibacillus plantarum*

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Abstract. The incorporation of plant-based ingredients into fermented dairy products can enhance their functional value while maintaining product quality. This study investigated the effects of black rice powder enrichment (0–12% w/v) on the key characteristics of fermented milk produced using *Lactiplantibacillus plantarum*. Increasing the black rice concentration resulted in a significant decrease in pH from 4.98 to 3.81, indicating enhanced acidification. Viscosity and water-holding capacity showed a significant decrease from 2869.33 to 241.33 cP and from 100% to 50%, respectively, reflecting the disruption of the milk protein gel structure. Colour attributes were significantly affected, with lightness (L*) decreasing from 84.09 to 38.15 and a shift toward reddish-purple hues associated with anthocyanin pigments. The probiotic viability remained consistently high across all formulations (8.45–8.81 log CFU/mL), confirming successful fermentation. Sensory evaluation revealed significant differences among treatments, with moderate black rice enrichment (3–6% w/v) achieving the highest overall acceptability, whereas higher substitution levels negatively affected texture and flavour. These findings demonstrate, for the first time, that black rice powder can be effectively incorporated into fermented milk to enhance its functional properties while preserving probiotic viability and consumer acceptance.

1 Introduction

Black rice (*Oryza sativa* L.), historically referred to as "emperor's rice" or "forbidden rice," is traditionally cultivated in Asia, especially in Indonesia and China. It is usually reserved for royalties in China and Indonesia because of its rarity, high value, and perceived therapeutic properties [1]. This ancient grain has gained popularity own to its distinctive dark pigment and unique nutritional profile, often classifies it as a superfood. Black rice contains high nutritional values such as protein and essential amino acids, such as lysine and tryptophan, while also being naturally low in fat and gluten-free, making it suitable for easier digestion and wider dietary needs. Notably, black rice has significantly higher levels of antioxidant levels. Previous research has shown that black rice contains higher levels of phytochemical compounds, such as phenolics and flavonoids, than other varieties of rice, such as red rice and brown rice [2]. It also provides essential minerals, including calcium, magnesium, potassium, copper, zinc, phosphorus, manganese, iron, and B-complex vitamins, although their levels may differ among cultivars and cultivation conditions [3]. Owing to its abundant nutritional value and health-promoting properties, it has great potential to be utilized as a functional ingredient in the development of food products such as beverages.

Processing technology called fermentation has long been an essential aspect of human life millennia ago. Evidence of fermented grain beverages exists from as early as 13,000 years ago, while ancient Egyptians utilized fermentation for foods like beer, bakery, and dairy. Among these, fermented milk products became particularly prominent. However, the increasing prevalence of dairy-related adverse reactions and the growing adoption of plant-based lifestyles have driven interest in dairy alternatives.

Plant-based milks produced by extracting the soluble nutrients from plant materials, have gained widespread popularity for both their health benefits and consumer appeal. Although the concept of plant-based milk is not new, surging consumer demand has inspired innovations in raw materials and processing technologies, including lactic acid fermentation. This technique enhances nutritional properties and extends product shelf life by acidifying the environment, suppressing spoilage organisms, and creating microbial growth-inhibitory properties, such as bacteriocins, organic acids, and hydrogen peroxide. These actions preserve the product stability and safety [4]. Current research on plant-based yogurt alternatives explores diverse plant substrates and probiotic lactic acid bacteria (LAB) strains, which contribute positively to human health when consumed in sufficient quantities.

Lactiplantibacillus plantarum (formerly *Lactobacillus plantarum*) is one of the most adaptable LAB used for the fermentation of both dairy and plant-based beverages. It enhances product quality through acidification, flavour formation, and probiotic functionality, making it a key microorganism in the development of functional and health-promoting drinks

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[5]. Considering its nutritional richness, functional potential, and growing consumer interest, developing a fermented beverage using black rice and lactic acid bacteria offers a promising innovation in plant-based functional food production. To date, there is a lack of comprehensive studies evaluating the effects of black rice powder substitution on the physicochemical and sensory attributes of milk fermented by *Lactiplantibacillus plantarum*. Accordingly, this study aimed to assess how incorporating black rice powder into fermented milk produced using *L. plantarum* affects its characteristics.

2 Material and methods

2.1 Preparation of *L. plantarum* inoculum

A two-stage approach was used to produce the *Lactiplantibacillus plantarum* starter culture, consisting of primary culture activation and the subsequent development of the working culture. Previously grown on MRS agar, a colony of *L. plantarum* was inoculated into sterile MRS broth using a sterile loop. After incubation at 37 °C for 24 h, 10 mL of heated whole milk was inoculated with 0.5 mL of the MRS-grown bacterial culture. The mixture was incubated again for 24 h at 37 °C to obtain a working starter culture for fermentation trials.

2.2 Formulation of black rice into fermented milk

The preparation of black rice–fermented milk followed multiple stages of processing. Initially, all ingredients were accurately weighed based on the formulations presented in Table 1.

Table 1. Ratio of black rice in fermented milk

Formula (black rice)	Black Rice Powder (% w/v)	Milk Powder (% w/v)
Control (0%)	0	12
1 (3%)	3	9
2 (6%)	6	6
3 (9%)	9	3
4 (12%)	12	0

First, full-cream milk powder (0-12% w/v) and black rice powder (0-12% w/v), were mixed at the ratios listed in Table 1. The mixture was then dissolved in water. Then, other dried ingredients such as carboxymethyl cellulose (CMC) (0.5%) and sugar, (3.75%) were added and stirred until a uniform suspension was obtained. The suspension was subsequently homogenized and heated at 80 °C for 10 min to eliminate unwanted microorganisms and establish favourable conditions for lactic acid fermentation. After pasteurization, the mixtures were cooled to ambient temperature to prevent the thermal inactivation of the probiotic inoculum. After cooling, an activated culture of *L. plantarum* was inoculated at a concentration of 5% (v/v) (inoculum concentration approximately 10^{12} CFU/mL). Inoculated samples were incubated at 37 °C for 24 h to facilitate fermentation. Upon completion of fermentation, a series of black rice–enriched fermented milk beverages were obtained and subsequently evaluated for their physicochemical properties, bacterial viability, and sensory liking.

2.3 Determination of total acid and acidity (pH)

A calibrated portable pH meter was used to determine the acidity of fermented milk containing black rice. To evaluate the total titratable acidity (TAT), 10 mL of the sample was pipetted into an Erlenmeyer flask and supplemented with a phenolphthalein (PP) indicator. The sample was subsequently titrated with 0.1 N sodium hydroxide (NaOH) until the emergence of a subtle pink hue, marking the titration endpoint.

2.4 Analysis of physical properties

The physical properties of the fermented drink were examined by analysing total soluble solids, water-holding capacity, colour, and viscosity. A colorimeter NH-200 was used to measure colour. A Brookfield viscometer DV-1 was set to 50 RPM and spindle no. 3 was used to measure the viscosity of liquid product, in a 400 mL beaker glass. A refractometer was used to measure the total soluble solids (TSS). To determine the amount of water the fermented beverages could hold, 10 g of the sample was centrifuged at 5000 ×g for 10 min. The water holding capacity was measured by calculating the volume of the supernatant that was left over.

2.5 Analysis of the viability of lactic acid bacteria

The microbial serial dilution method was used to test the survival of lactic acid bacteria (LAB) in the beverages. The procedure began by transferring 1 mL of yogurt to a test tube containing 9 mL of sterile 0.85% NaCl solution. After vortexing to ensure complete mixing, 0.1 mL of the suspension was taken to prepare a series of tenfold dilutions up to 10^{-6}. From each dilution, 2 mL was dispensed into Petri dishes and subsequently layered with molten MRS agar until the plates were half-filled. The plates were kept warm (37 °C) for 48 h to allow bacterial growth. The viability results were obtained by counting the colonies that grew on the medium and were expressed as log CFU/mL.

2.6 Sensory analysis

The samples were assessed using a hedonic rating test employing a 9-point hedonic scale, where 1 signifies extreme dislike and 9 indicates excessive liking. Prior to participation, all panelists were informed of the purpose and procedures of the sensory evaluation and provided informed consent. The analysis included 42 untrained panelists without lactose intolerance who evaluated their preferences regarding various aspects, including taste, colour, texture, scent, and overall impression.

2.7 Statistical analysis

For each parameter, the mean values from triplicate samples and standard deviations were determined. Variability among samples was assessed using ANOVA, followed by post-hoc analysis to identify significant differences at the 5% level ($p < 0.05$).

3 Results and discussion

3.1 Physicochemical characteristics and microbial viability of black rice-substituted fermented milk

Fermentation has been regarded as an appealing alternative to improve the quality of dairy and plant-based milk products. The substitution of black rice powder with milk powder is a viable option for improving the characteristics of the products. Table 2 shows the microbial viability and physicochemical properties of the products.

Table 2. Physicochemical characteristics and microbial viability of fermented milk substituted with black rice

Parameters	Control	1	2	3	4
pH	4.98 ± 0.02^{a}	4.93 ± 0.02^{b}	4.22 ± 0.01^{c}	4.06 ± 0.03^{d}	3.81 ± 0.03^{e}
TAT (%)	0.35 ± 0.04^{c}	0.46 ± 0.05^{a}	0.46 ± 0.01^{a}	0.37 ± 0.03^{b}	0.35 ± 0.02^{c}
TSS (°Brix)	10.33 ± 0.29^{b}	10.17 ± 0.29^{b}	10.50 ± 0.00^{b}	11.83 ± 0.29^{a}	10.00 ± 0.50^{b}
WHC (%)	100.00 ± 0.00^{a}	60.00 ± 0.00^{b}	45.00 ± 0.00^{e}	30.00 ± 0.00^{c}	50.00 ± 0.00^{d}
Viscosity (cP)	2869.33 ± 15.14^{a}	1544.00 ± 6.93^{b}	466.67 ± 8.33^{c}	404.0 ± 31.24^{d}	241.33 ± 12.86^{e}
Viability (log CFU/mL)	8.48	8.62	8.81	8.65	8.45
L^{*}	84.09 ± 0.36^{a}	62.43 ± 0.30^{b}	53.02 ± 0.41^{c}	44.00 ± 0.47^{d}	38.15 ± 0.33^{e}
a^{*}	-0.53 ± 0.13^{e}	10.45 ± 0.01^{c}	12.16 ± 0.13^{a}	10.91 ± 0.13^{b}	8.99 ± 0.17^{d}
b^{*}	9.41 ± 0.40^{a}	2.73 ± 0.16^{b}	2.46 ± 0.13^{b}	-0.09 ± 0.06^{c}	-1.17 ± 0.39^{d}
ΔE^{*}	-	23.66 ± 0.47^{d}	32.42 ± 0.55^{c}	39.55 ± 0.46^{b}	46.44 ± 0.79^{a}

Notes: Significant differences ($P < 0.05$) are indicated by different superscript letters.

The physicochemical characteristics of fermented milk are generally affected by substitution with black rice powder. A gradual decrease in pH from 4.98 to 3.81 was observed when black rice powder concentration substituted the milk powder, accompanied by an initial increase up to sample 2, followed by a subsequent decline in titratable acidity (TAT). This pattern suggests that black rice provides additional fermentable carbohydrates that enhance lactic acid production by *L. plantarum* at moderate substitution levels of up to 6% of black rice powder, but excessive inclusion may reduce the acidity due to altered lactic acid concentration, as well as an increase in phenolic content. As reported previously, the acidity of the final product can be decreased when milk is substituted with other ingredients [6]. The increase in total soluble solids (TSS) up to 11.83 °Brix also indicates a contribution of soluble nutrients from black rice, whereas the slight decline at the highest concentration might be attributed to microbial utilization of sugars during fermentation. Aligning with these findings, a similar study reported an increase in the TSS [7].

In contrast, the water-holding capacity (WHC) decreased across all black rice-enriched formulations. The control sample exhibited the highest WHC, while the lowest value was observed in sample 3. This reduction is likely attributable to interference in the protein gel network by black rice components, which weakens the matrix structure, reduces moisture retention, and consequently increases the likelihood of syneresis during storage [8]. Therefore, the reduction in milk powder concentration also decreased the viscosity of the product, where sample 4 with full substitution of black rice powder (12%) demonstrated the lowest viscosity (241.33 cP).

Colour analysis of fermented milk samples showed that black rice addition had a pronounced effect on the visual characteristics of the product. The lightness (L^{*}) of the sample dropped notably, in line with the increment of black rice concentration, resulting in a darker appearance relative to the control. In contrast, the redness (a^{*}) value rose sharply, while the yellowness (b^{*}) value decreased, indicating a clear transition from the original pale, milky colour to a reddish-purple tone. Black rice pigment, such as anthocyanin, may be responsible for the colour changes (ΔE^{*}), where the higher substitution level of black rice increases the anthocyanin concentration. Under incubation at 37 °C in acidic conditions, anthocyanins predominantly exist in the flavylium cation form, which is the most stable molecular structure and is responsible for maintaining intense red–purple coloration [9]. This structural stability likely contributed to the pronounced and progressive increase in total colour difference (ΔE^{*}) observed with increasing black rice concentration in the fermented milk.

Although some differences were observed, all samples maintained lactic acid bacteria viability above 8 log CFU/mL, which is well above the acceptable limit for fermented milk products (>6 log CFU/mL). The highest viability, 8.81 log CFU/mL was found in sample 2, suggesting that a moderate concentration of black rice offers favourable substrate conditions for *L. plantarum* and may act as a prebiotic, such as dietary fibre [10]. Nonetheless, viability somewhat diminished at higher substitution concentrations of black rice powder, probably attributable to the reduced lactose content required for growth.

3.2 Acceptability of fermented milk substituted with black rice powder

Sensory evaluation using the hedonic rating method was conducted to determine the acceptability of fermented milk beverages (Table 3). Generally, the sample with 0% substitution of black rice was perceived by the panelists as the most acceptable formula for all parameters. The substitution of 3% black rice powder in sample 1 gave the relatively acceptable scores, particularly for texture, colour, taste, aroma, and overall. Higher concentration of black rice substitution compared to samples with a higher concentration of black rice.

Table 3. Rating hedonic results of fermented black rice milk beverages

Attributes	Control	1	2	3	4
Colour	8.24 ± 1.43^{a}	6.81 ± 1.63^{b}	6.55 ± 1.74^{b}	6.24 ± 2.01^{b}	6.48 ± 1.99^{b}
Aroma	7.76 ± 1.79^{a}	6.86 ± 1.54^{b}	6.14 ± 1.79^{bc}	6.10 ± 1.76^{bc}	5.43 ± 1.99^{c}
Taste	8.31 ± 1.52^{a}	6.38 ± 1.96^{b}	6.24 ± 1.86^{b}	5.93 ± 1.80^{b}	5.55 ± 2.07^{b}
Texture	8.21 ± 1.55^{a}	6.24 ± 1.66^{b}	6.48 ± 1.37^{b}	6.14 ± 1.59^{b}	6.07 ± 1.88^{b}
Overall	8.43 ± 1.11^{a}	6.60 ± 1.47^{bc}	6.67 ± 1.32^{b}	6.21 ± 1.49^{bc}	5.90 ± 1.97^{c}

Notes: Different superscript letters indicate the significant differences ($p < 0.05$).

Formulas 3 and 4 showed the decrease in taste and aroma acceptability. This phenomenon could be explained by the strong earthy and grain-like notes inherent to black rice, which tend to overshadow the subtle flavour nuances of fermented milk. Black rice contains volatile phenolic compounds such as ferulic acid, vanillic acid, and various aromatic aldehydes [11], which impart distinctive sensory characteristics. Although these compounds contribute valuable antioxidant and health-promoting effects, their pronounced aroma and mildly bitter undertones can create an unfamiliar flavour profile when present in high amounts. Thus, although the inclusion of black rice enhances the functional value of the beverage, excessive levels may negatively affect its sensory acceptability.

Imparting a visually pleasing purplish tint typically associated with antioxidant-rich foods, the anthocyanins in black rice likely contributed to the acceptable colour observed in all formulations. Research indicates that anthocyanins boost aesthetic appeal and offer practical advantages, potentially favourably affecting consumer perception [12].

Texture scores declined greatly in beverages containing black rice formulations. The diminished scores aligned with the decreased viscosity and water-holding capacity, which likely resulted in a thinner or less creamy mouthfeel. These results differ from those of previous reports, indicating that rice-based ingredients may increase the WHC and viscosity [13]. Overall, fermented milk-substituted black rice gave an acceptable score, especially in samples 1 and 2. This suggests that, although incorporating black rice modifies the sensory characteristics of the product, moderate substitution levels remain applicable without surpassing the panelist's threshold for acceptability.

4 Conclusion

Black rice powder enrichment significantly influenced fermented milk by enhancing acidification, maintaining the high viability of *Lactiplantibacillus plantarum*, intensifying colour through stable anthocyanins, and modifying texture-related properties. A black rice powder substitution level of 3–6% (w/v) was identified as optimal, providing the best balance among functional enhancement, physicochemical stability, and sensory acceptability. These findings demonstrate the potential application of black rice powder as a functional ingredient in the development of naturally coloured fermented dairy products. Further improvements in product quality may be achieved by optimizing fermentation time, applying appropriate black rice pretreatments, and reducing particle size to enhance texture, colour uniformity, and overall consumer acceptance.

Data availability statement

The datasets obtained in this study are fully reported within the article.

Contributorship statement

The roles included formal analysis, investigation, and data curation by Akbar Wiradamas, and conceptualization along with manuscript drafting, writing, and editing by Andreas Romulo.

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