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From Grain to Gut Health: Ragi Milk Extraction and Probiotic Product Development

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study aimed to optimize ragi milk extraction and develop probiotic-rich dairy alternatives at the Department of Food Science and Nutrition, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur. Ragi grains were pre-treated through soaking, germination, and drying to enhance milk extraction efficiency. Probiotic products, including ragi curd, ice cream, and *ambli*, were formulated and evaluated using sensory and nutritional analyses. The physical properties of ragi, such as uniform size, high density, and sphericity, underscored its suitability for food processing. Proximate analysis showed that ragi grains, compared to ragi malt, had lower moisture and higher fat and fibre content, enhancing storage stability. Sensory evaluations indicated that ragi curd was well-received, while ragi ice cream and one version of ragi ambli were particularly favoured for taste and overall acceptability. This research contributes to the development of nutritious, sustainable plant-based dairy alternatives, promoting millet consumption.

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1. INTRODUCTION

The term "*millet*" is derived from the French word "mille," signifying that a small quantity of millet consists of thousands of tiny seed grains (Meena *et al.*, 2021). In India, millets have a rich history dating back thousands of years, playing a crucial role in the nation's agriculture, culture, and cuisine (Singh, 2023). Globally, millets rank sixth in crop productivity, known for their pest and disease resistance, short growing seasons, and resilience to drought (Devi *et al.*, 2014). These grains are often referred to as "miracle grains" due to their ability to thrive in the most impoverished soils with minimal or no external inputs and without irrigation (Baranwal and Singh, 2014).

Among the various types of millets, ragi or finger millet (Eleusine coracana) is one of the most widely cultivated. India is the leading producer of ragi, accounting for approximately 41% of global production, followed by Africa. Globally, ragi ranks as the fourth most important millet after sorghum, pearl millet, and foxtail millet (Chandra et al., 2016). Ragi boasts a nutritional profile comparable to other millets and cereals. containing 81.5% carbohydrates, 18% to 20% dietary fiber, 65% to 75% starch, 9.8% protein, 1% to 1.7% fat, 2.7% minerals, and 4.3% crude fiber (Saleh et al., 2013). It is also rich in protein, dietary fiber, B vitamins, zinc, potassium, magnesium. and essential fattv acids. contributing to various health benefits such as regulating blood sugar levels, managing blood pressure, and aiding in the prevention of thyroid. cardiovascular, and celiac diseases (Rao et al., 2017).

Processing methods like soaking, malting, and fermentation enhance the nutritional benefits of ragi. Soaking ragi grains in water, salt solution, or sodium bicarbonate reduces phytic acid content, thereby increasing mineral bioavailability (Chauhan and Sarita, 2018).

Malting, which involves controlled germination and drying, not only improves nutritional availability and digestion but also enhances protein quality and efficiency. Malted ragi flour is versatile and can be incorporated into various foods, including cakes and milk-based drinks (Shobana *et al.*, 2013). Fermentation further boosts the nutritional value of ragi by enhancing protein and starch digestibility while reducing antinutrients such as tannins and phytic acid. This process also improves amino acid balance and carbohydrate accessibility. Fermented ragi products like porridge, pancakes, and beverages are traditional favorites that have stood the test of time (Singh and Raghuvanshi, 2012). Recent advancements have linked ragi fermentation to the development of probiotic-rich foods, which enhance immune response and support gastrointestinal health (Nithya et al, 2023). These probiotics can be incorporated into a variety of foods, including yogurt, ice cream, and other fermented products, providing numerous health benefits.

Ragi milk, a plant-based milk alternative, has recently gained popularity as a nutritious and substitute for sustainable dairv milk (Dhanushkodi et al., 2023). This has opened the door to the creation of innovative probiotic products such as radi curd, radi ice cream, and ragi ambli. Ragi curd, a reinvention of a traditional dairy product, combines ragi milk with probiotic microorganisms. The fermentation process not only adds a desirable texture and flavor but also increases the probiotic content, promoting digestive health. Similarly, ragi ice cream offers a healthy twist on a beloved treat, blending the nutritional benefits of ragi with the enjoyment of ice cream, making it an appealing choice for health-conscious consumers.

Ragi *ambli*, a traditional fermented beverage, is celebrated for its cooling properties and nutritional benefits. The fermentation process enhances the probiotic content of the drink, making it a potent functional beverage that supports gut health and hydration. The development of ragi curd, ragi ice cream, and ragi *ambli* underscores the versatility of ragi milk and the potential for incorporating probiotics into a wide range of food products. This research aims to study the physical and nutritional properties of ragi grain, extraction of ragi milk, and preparation and organoleptic evaluation of probiotic products.

2. MATERIALS AND METHODS

Ragi was sourced from Big Basket, an online grocery store. Buttermilk and curd were obtained from Namaste India Dairy. Other ingredients, including bananas, dates, cashews, cocoa powder, vanilla essence, jaggery, black salt, and cumin seed powder, were purchased from the local market in Kanpur. All materials were carefully selected to ensure quality and integrity for the study.

Pre-processing of ragi: Malting of finger millet was carried out according to the procedure suggested by (Chilakawar and Pawar, 2015) with necessary modifications. Finger millet grains were weighed, dust, dirt were removed and washed well. The grains were kept for steeping in water for 24 hours at room temperature. The grains: water ratio was kept at 1:2. During steeping, the water was changed after every 4 hours. After soaking, the grains with grain to water ratio 1:2 were left out at room temperature (38°C) with continuous sprinkling of water to support germination over a period of three days. The seeds were then spread uniformly and the sprouted grains were then sun-dried for one day at 39°C 18 hours. Finally, the malted grains were ground into flour, completing the pre-processing of ragi.

Physical and functional properties of ragi:

Geometric mean diameter (Dg): The geometric mean diameter (mm) was calculated using Eq. (1) based on the length (L), width (W), and thickness (T) of the ragi grains (Mpotokwane *et al.*, 2008). This parameter assesses the uniformity of grain size, crucial for consistent processing conditions such as grinding and milling.

$$(L \times W \times T)^{1/3}$$
 (1)

Arithmetic mean diameter (Da): The arithmetic mean diameter (mm) was determined (Mpotokwane *et al.*, 2008). This measure is essential for calibrating processing equipment and influencing the texture and sensory properties of ragi-based products.

$$Da = L + W + T/100$$
 (2)

One thousand sample weight: The weight of 1000 grains was measured using a digital balance, as per the method described by Sangamithra *et al.* ,2016. This metric serves as an indicator of grain quality and nutrient content.

Bulk density: Bulk density (kg/m³) was calculated using Eq. (3), measuring the sample's mass divided by its volume (Varnamkhasti *et al.*, 2008). Bulk density influences storage, packaging, and transportation logistics.

Bulk density = Sample weight /Volume (3)

True Density: True density (kg/m³) was determined using the liquid displacement method (Karababa and Cokuner, 2013). It provides insights into grain purity and quality.

$$\mathsf{Pt} = \frac{{}^{30\mathrm{g}}}{{}^{\mathrm{V2-V1}}} \tag{4}$$

where: Pt = true density, V1 = initial volume and <math>V2 = final volume.

Porosity: Porosity (%) was calculated based on true density and bulk density (Sangamithra *et al.*, 2016). Porosity is crucial for assessing storage aeration and moisture management.

$$\varepsilon = \frac{Pt - Pb}{Pt} \times 100$$
 (5)

where ε = porosity, pt = true density and pb = bulk density

Sphericity: Sphericity (%) was calculated using Eq. (6), representing the shape uniformity of the grains, which impacts processing efficiency (Hamdani et al., 2014).

$$\phi = \frac{(LWT)1/3}{L} \times 100 \tag{6}$$

Proximate analysis of Ragi grains and Ragi Malt:

Near-Infrared Reflectance Spectroscopy (NIRS) is a rapid, non-destructive analytical technique used to determine the chemical composition of food products. It works by measuring the reflectance of near-infrared light (700-2500 nm) that penetrates the sample. In this study, the NIRS 2500 (Foss Analytical) instrument was employed to analyze the nutritional properties of both ragi grains and ragi malt. Parameters such as protein, fat, crude fiber, ash, and moisture content were measured. The NIRS 2500 was calibrated using standard reference materials. Approximately 5 grams of the sample was scanned in the 1100-2500 nm range, with multiple scans averaged for accuracy. The use of NIRS allowed for accurate and efficient analysis of multiple samples with minimal sample preparation, providing reliable data for comparing the nutritional profiles of different ragi-based products.

Extraction of ragi Milk: Malted ragi flour and water were combined in a 1:1 ratio to form a slurry, which was mixed thoroughly for 5 minutes to ensure a uniform consistency. The slurry was then cooked for an additional 5 minutes.

Following cooking, the mixture was filtered through muslin cloth to separate the liquid from any remaining solids. The resulting ragi milk was collected in a clean container for further use.

Development of probiotic products:

Ragi curd: Ragi milk was first extracted from ragi malt, serving as the base for the preparation of ragi fortified curd. To enhance the flavor, jaggery was incorporated into the ragi milk. Curd was then added as an inoculum or culture to initiate the fermentation process. The mixture was allowed to ferment at room temperature for 8-9 hours, during which the ragi curd was formed. Once the fermentation was complete, the ragi curd was collected and subsequently mixed with regular curd, resulting in the production of ragi fortified curd.

Ragi ice cream: Ripe bananas were first frozen for 24 hours to prepare the base for the ice cream. Ragi milk was then added to a mixer grinder, followed by the addition of cocoa powder, dates, and vanilla essence. The ingredients were blended together in the mixer grinder until a smooth paste was achieved. The resulting batter was then placed in a refrigerator and allowed to freeze for 10 hours. Once frozen, the ice cream was served, garnished with cashew nuts for added texture and flavor.

Ragi ambli: To prepare the ragi ambli, 250 g of malted ragi flour was combined with 250 ml of water to form a slurry. Simultaneously, 250 ml of water was boiled in a pan for 10 minutes. The prepared slurry was then gradually added to the boiling water while stirring continuously to prevent lumps from forming. This mixture was cooked for 15 minutes until thickened. Once cooked, it was transferred into an earthen pot and covered with a muslin cloth to allow fermentation. The pot was kept undisturbed for 8-9 hours to ferment. After fermentation, the fermented ragi was mixed with buttermilk. To enhance the flavor, jaggery was added to sweeten the beverage, and cumin seed powder and black salt were incorporated to create a Savory taste. The ragi ambli, a traditional fermented ragi beverage, was then ready to be served.

Sensory evaluation: Sensory evaluation of the probiotic products—ragi curd, ragi ambli, and ragi ice cream—was conducted using a 9-point hedonic scale, assessing appearance, aroma, flavor, texture, and overall acceptability. A trained panel of 25 judges evaluated the products, with

samples presented in random order to minimize bias, and palate cleansing with water between samples.

Statistical analysis: Statistical analysis of the sensory data included calculation of mean scores and analysis of variance (ANOVA) to determine significant differences among products and attributes by using OPSTAT. This evaluation provided valuable insights into the sensory attributes and consumer acceptance of the probiotic ragi-based products developed in this study.

3. RESULTS AND DISCUSSION

Pre processing treatment of ragi grains: The optimal 1:2 grain-to-water ratio enhances hydration, ensuring more effective soaking Steeping the grains for 24 hours provides sufficient time for water absorption, activating the enzymes necessary for successful germination. Soaking at 38°C with a 1:2 ratio further optimizes enzymatic activity, promoting efficient hydrolysis. Ragi grains soaked with a 1:2 grain-to-water ratio germinated efficiently in 72 hours, enabling optimal metabolic transformations and nutrient development. Ragi grains soaked with a 1:2 grain-to-water ratio underwent drying at 39°C for 24 hours, a process that ensured gradual moisture removal while preserving their nutritional value and minimizing mould risk. The slower drving method effectively balanced moisture loss with nutrient retention.

Effect of grain to water ratio on time, temperature and drying of ragi grains: The grain-to-water ratio played a pivotal role in determining the quality, efficiency, and nutritional integrity of ragi grain processing. With a 1:2 grain-to-water ratio, ragi grains gained 10% of demonstrating substantial weight, water absorption vital for enzyme activation and successful germination. The slight weight loss in ragi grains with a 1:2 grain-to-water ratio during germination indicates optimal metabolic activity and efficient nutrient utilization without substantial mass loss.

Ragi grains with a 1:2 grain-to-water ratio achieved a notable 8% of weight loss during drying, reflecting superior moisture reduction that effectively extends shelf life and prevents spoilage. This process optimally prepares the grains for storage and further processing.

Physical properties of ragi: The physical properties of ragi grains as mentioned in Table 1,

paint a vivid picture of their potential in food processing. With geometric and arithmetic mean diameters of 1.49 ± 0.13 mm and 1.50 ± 0.06 mm, these grains boast a uniform size, ensuring consistency in processing and packaging. The weight of a thousand grains, at 496.8 ± 5.00 g, is not just a statistic but a key to unlocking precise processing and planting strategies. Their bulk density of 993.6 ± 11.44 kg/m³ hints at optimal packing efficiency, making storage and distribution more streamlined. The true density of 1515.6 ± 34.88 kg/m³ offers a glimpse into the grains' robust internal structure, essential for milling and other processing tasks. With a porosity of 32.41 ± 5.40%, these grains hold just the right amount of air, balancing flowability and compaction for smooth handling. And with a sphericity of 83.21 ± 0.08, their near-perfect roundness makes them a dream to process. ready to roll through the machinery with ease.

Table 1. Physical properties of Ragi grains

Physical properties	Value
Geometric mean diameter	1.49 ± 0.13
Arithmetic mean diameter	1.50 ± 0.06
One thousand (1000)	496.8 ± 5.00
sample weight (wt.g)	
Bulk density (kg/m ³)	993.6 ± 11.44
True density (kg/m ³)	1515.6 ± 34.88
Porosity (%)	32.41 ± 5.40
Sphericity (%)	83.21 ± 0.08

Nutritional Evaluation of Ragi grains and Ragi malt:

Ragi grains: Ragi grains are a nutritional powerhouse, offering a compelling blend of health benefits that position them as a superior choice in plant-based diets. With a moisture content of 7.48%, they maintain stability and resist microbial growth, outperforming other millets like those studied by Desai et al. (2010). Their protein content is a remarkable 14.49%, making ragi a standout in vegetarian and vegan nutrition, far surpassing the levels reported by Desai et al. (2010). Ragi's fat content, at 4.09%, not only boosts energy density but also enhances the absorption of vital fat-soluble vitamins, offering a richer profile than traditionally noted. The 11.5% crude fiber content is a digestive champion, promoting gut health and blood sugar stability, significantly higher than previous findings. The ash content of 3.57% reflects a wealth of essential minerals, fortifying the grain's role in bone health and metabolic support, as highlighted by Gupta (2014). Even with a carbohydrate content of 58.87%, slightly

below previous reports, ragi ensures sustained energy release, cementing its place as a nutritional staple and a cornerstone for developing health-forward food products.

Ragi malt: Ragi malt undergoes notable changes during the malting process, enhancing its nutritional profile while retaining key benefits. The moisture content rises slightly to 7.62% from 7.48% in raw ragi, crucial for stability and spoilage prevention. Protein content in ragi malt increases to 16.1%, from 14.49% in raw grains, enhancing its value as a protein source, particularly for vegetarians. The fat content decreases marginally to 3.9% from 4.09%, reflecting the utilization of fats during sprouting (Desai et al. 2010). Crude fiber also slightly reduces to 10.24% from 11.50%, yet remains substantial for digestive health. The ash content stays relatively stable at 3.50%, ensuring the retention of essential minerals, unlike the lower levels reported by. Carbohydrate content dips to 57.75% from 58.87% due to the conversion of complex carbohydrates into simpler sugars, yet ragi malt continues to offer sustained energy, confirming its suitability for health-conscious diets. The comparison between the nutritive values of ragi grains and ragi malt is shown in Fig. 1.

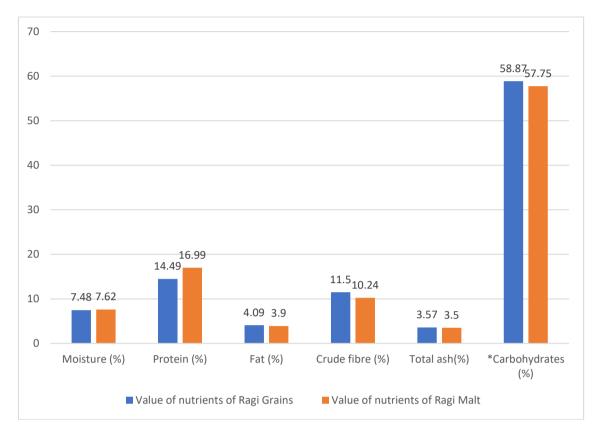
Organoleptic acceptability of probiotic products prepared from Ragi milk: The sensory evaluation of ragi curd as revealed in Table 2 shows that the control sample achieved the highest scores in all attributes, with taste, appearance, flavour, texture, colour. and aftertaste/mouthfeel scores of 9.48±0.58, 9.24±0.52, 9.28±0.45, 9.44±0.50, 9.32±0.47, and 9.32±0.47, respectively. Among the ragi curd variants, T3 emerged as the most favorable, with taste (8.24±0.59), colour (8.32±0.55), appearance (8.32±0.55), flavour (8.48±0.50), texture (8.32±0.55), and aftertaste/mouthfeel (7.56±0.58) scores significantly higher than T1 and T2. Despite T3's superior performance compared to T1 (taste: 6.76±0.66, colour: appearance: 8.04±0.67, 6.84±0.74, flavour: 7.00±0.64, texture: 6.84±0.74, aftertaste: 6.48±0.50) and T2 (taste: 7.60±0.64, colour: 7.04±0.67, appearance: 8.16±0.80, flavour: 7.40±0.57, texture: 7.04±0.67, aftertaste: 7.00±0.64), it still did not match the control sample's overall acceptability. The statistical significance of these differences, as confirmed by critical difference (CD) and standard error of mean (S.E(m)) values, underscores T3's potential for further development.

Sample	Parameter						
	Taste	Colour	Appearance	Flavour	Texture	After taste/ mouth feel	Overall acceptability
Т0	9.48± 0.58	9.24±0.52	9.28±0.45	9.44±0.50	9.32±0.47	9.32±0.47	9.48±0.58
T1	6.76±0.66	6.84±0.74	8.04±0.67	7±0.64	6.84±0.74	6.48±0.50	6.76±0.66
T2	7.6±0.64	7.04±0.67	8.16±0.80	7.4±0.57	7.04±0.67	7±0.64	7.6±0.64
Т3	8.24±0.59	8.32±0.55	8.32±0.55	8.48±0.50	8.32±0.55	7.56±0.58	8.24±0.59
CD (5%)	0.351	0.355	0.357	0.316	0.350	0.314	0.351
S.E(m)	0.125	0.126	0.127	0.113	0.124	0.112	0.125

Table 2. Organoleptic evaluation of controlled curd (T0) and ragi curd (T1, T2, T3)

Table 3. Organoleptic evaluation of fermented ragi ambli (T1 and T2)

Name of	Parameter						
sample	Taste	Colour	Appearance	Flavour	Texture	After taste/ mouth feel	Overall acceptability
T1	7.36±0.81	8.88±0.66	8.96±0.73	7.32±0.69	8.76±0.59	7.4±0.91	7.36±0.81
T2	9.04± 0.73	9.08±0.64	9.08±0.57	8.72±0.67	9.04±0.61	8.52±0.71	9.04±0.73
CD (5%)	0.441	-	-	0.390	-	0.468	0.441
S.E(m)	0.155	0.131	0.132	0.137	0.121	0.164	0.155



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Fig. 1. Proximate analysis of Ragi grains and Ragi malt

The sensory evaluation of ragi ice cream revealed that it is well-received overall. The taste of the ice cream earned a high mean score of 9.08±0.70, indicating that it is highly enjoyable. though there was some variation in participants' opinions. The color was rated at 8.88±0.66, reflecting a positive visual appeal, with slight differences in individual preferences. The appearance received an excellent score of 9.04±0.67, highlighting the product's attractive presentation. Flavour was particularly praised with a score of 9.20±0.57, showing strong consensus on its pleasing profile. Although texture was rated slightly lower at 8.56±0.50, it remains favorable. suggesting room for improvement to enhance creaminess. The aftertaste/mouthfeel scored 8.80±0.70, indicating general satisfaction with the lingering flavor and texture, despite some variability in responses. Overall, the ice cream achieved a high acceptability score of 9.08±0.70, demonstrating strong potential for future development and commercialization.

Sensory evaluation of fermented ragi ambli: The sensory evaluation reveals significant differences between the ragi ambli variants T1 and T2 across multiple attributes, with T2

consistently outperforming T1. For taste, T2 scored 9.04±0.73, notably higher than T1's 7.36±0.81, with a critical difference (CD) value of 0.441 and a low standard error (S.E(m)) of 0.155, indicating a genuine and consistent preference for T2. In terms of color, both variants scored highly-T1 with 8.88±0.66 and T2 with 9.08±0.64-though the difference was not statistically significant, as indicated by a significance value of 0.28. Appearance ratings also favored T2 slightly at 9.08±0.57 compared to T1's 8.96±0.73, with a significance value of 0.52, showing no substantial difference. Flavor saw a more pronounced distinction, with T2 scoring 8.72±0.67 versus T1's 7.32±0.69, supported by a CD value of 0.390 and S.E(m) of 0.137, highlighting a strong preference for T2, also represented graphically in Fig. 1. Texture ratings were similarly close, with T2 at 9.04±0.61 and T1 at 8.76±0.59, but the difference did not statistical significance (p=0.10784). reach Aftertaste and mouthfeel favored T2 with a score of 8.52±0.71, compared to T1's 7.4±0.91. confirmed by a CD value of 0.468 and S.E(m) of 0.164. Overall acceptability strongly favored T2, scoring 9.04±0.73 against T1's 7.36±0.81, with a CD of 0.441 and S.E(m) of 0.155, making T2 the superior choice in nearly all sensory aspects.

These results suggest that T2 offers a more favorable sensory profile, positioning it as a more promising candidate for consumer acceptance and potential commercialization.

4. CONCLUSION

The study successfully enhanced the efficiency and quality of ragi milk extraction through the optimization of critical bioprocessing parameters, including soaking at a 1:2 grain-to-water ratio and 38°C, germination at 41°C, and drying at 39°C. The nutritional analysis confirmed that ragi is rich in protein and dietary fiber, making it a valuable addition to gut health. The improved physical properties of ragi support efficient processing, preservation, and storage, ensuring the integrity of its nutritional benefits. The organoleptic evaluation of probiotic productsragi curd, ragi ice cream, and ragi amblihighlighted their strong consumer acceptability. Among the variants, ragi curd T3 was highly favored, while ragi ice cream was universally preferred for its sensory attributes, offering a healthier alternative for summer desserts. Ragi ambli (T2) stood out for its exceptional taste and overall quality. Given the rising demand for nutritious, natural, and healthy foods, there is substantial potential to develop diverse probiotic products from ragi milk. This study underscores ragi's potential as a beneficial dairy substitute, contributing to gut health while supporting the expanding field of plant-based foods. These findings provide a robust foundation for the advancement and commercialization of ragibased products, emphasizing their role in promoting overall gut health.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. ChatGPT 3.5 and ChatGpt 4.0 For editing abstract and final editing for checking grammatical mistakes and shortening unnecessary information.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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