JOURNAL OF CURRENT RESEARCH IN FOOD SCIENCE

**E-ISSN:** 2709-9385
**P-ISSN:** 2709-9377
JCRFS 2025; 6(2): 35-39

www.foodresearchjournal.com
Received: 18-05-2025
Accepted: 22-06-2025

**Anees Khan**
Department of Life Sciences and Nutrition, Capital Degree and PG College, Shapur Nagar, Hyderabad, Telangana, India

**Dr. Anil Bukya**
School of Nutrition and Dietetics, Symbiosis Skills and Professional University, Pune, Maharashtra, India

**Correspondence**
**Dr. Anil Bukya**
School of Nutrition and Dietetics, Symbiosis Skills and Professional University, Pune, Maharashtra, India

# Development and evaluation of a plant-based flavored milk alternative from *Euryale ferox* seeds

**Anees Khan and Anil Bukya**

**DOI:** https://www.doi.org/10.22271/foodsci.2025.v6.i2a.243

**Abstract**
The present study focused on the development and comprehensive evaluation of a plant-based flavored milk alternative derived from *Euryale ferox* (foxnut) seeds. Three formulations ($T_1$, $T_2$, and $T_3$) with varying seed concentrations were prepared and assessed for sensory attributes, nutritional composition, mineral content, antioxidant activity, and microbial safety. Among the variants, $T_1$ (10 g seeds/100 ml) was identified as the most acceptable in terms of sensory attributes. Proximate analysis revealed that $T_1$ provided a low-calorie profile (36.5 kcal/100 ml) with modest protein (0.8 g/100 ml) and carbohydrate (7.2 g/100 ml) content. Mineral analysis indicated the presence of calcium (42 mg/100 ml), potassium (50 mg/100 ml), magnesium (13.5 mg/100 ml), and iron (0.4 mg/100 ml). The product exhibited promising antioxidant properties, with significant levels of gallic acid, chlorogenic acid, and epicatechin, contributing to its functional food potential. Microbiological evaluation confirmed the product's safety and stability under refrigeration. The findings suggest that *Euryale ferox* seed milk can serve as a nutritionally viable, microbiologically safe, and sensory-acceptable alternative to conventional dairy milk, particularly for health-conscious and lactose-intolerant consumers.

**Keywords:** *Euryale ferox* seed milk, plant-based dairy alternative, sensory evaluation, antioxidant activity, mineral composition, functional beverage

## 1. Introduction
The growing shift toward plant-based diets is reshaping the global food industry, particularly in the dairy sector, where alternatives to conventional milk are increasingly in demand. Factors such as rising lactose intolerance, ethical concerns regarding animal welfare, increasing prevalence of milk allergies, and a broader awareness of the environmental footprint of dairy farming have led consumers to seek sustainable and health-promoting alternatives. In this context, plant-derived beverages from legumes, nuts, cereals, and seeds have emerged as viable options, offering varying degrees of nutritional, sensory, and functional benefits (Rybicka *et al*., 2020; FAO, 2013) [12, 3].

Among these, seed-based milk alternatives are gaining attention due to their unique bioactive profiles and relatively lower allergenic potential. *Euryale ferox* Salisb., commonly known as foxnut or makhana, is an aquatic plant belonging to the family Nymphaeaceae and traditionally consumed across parts of South and Southeast Asia. While extensively used in roasted or puffed form for snacks, the unpopped seeds of this plant remain underutilized despite their dense nutritional profile and functional attributes (Verma & Mishra, 2020). These seeds are rich in complex carbohydrates, particularly resistant starch, alongside modest amounts of protein, dietary fiber, and essential minerals such as calcium, potassium, and magnesium (Gupta *et al*., 2020) [5].

Recent studies have highlighted the pharmacological and nutraceutical potential of *Euryale ferox*, reporting antioxidant, anti-inflammatory, and antidiabetic properties attributed to its polyphenolic compounds such as gallic acid, chlorogenic acid, and epicatechin (Jain *et al*., 2019; Singh & Dhawan, 2021) [6, 14]. These properties position foxnut seeds as an ideal functional ingredient in health-oriented food formulations. However, despite their promising bioactive profile, limited efforts have been made to incorporate them into plant-based milk formulations. Developing a milk alternative from foxnut seeds not only leverages their nutritional and therapeutic potential but also aligns with the current demand for clean-label, low-fat, lactose-free beverages.

The use of jaggery as a natural sweetener and vanilla extract as a flavor enhancer in the formulation further contributes to consumer acceptability by offering a familiar taste and avoiding artificial additives. As consumer preference continues to evolve toward minimally processed, nutrient-dense plant products, exploring novel seed-based beverages is timely and relevant.

While previous studies have focused on puffed makhana as a snack or flour ingredient, few have investigated its application in liquid dairy alternatives using its resistant starch-rich, unpopped seed kernels. Additionally, comprehensive evaluation of such formulations encompassing sensory properties, proximate composition, mineral content, antioxidant capacity, and microbial safety-remains limited in peer-reviewed literature. The study was framed to develop and evaluate a dairy alternative flavored milk beverage from foxnut (*Euryale ferox*) seeds with a focus on its nutritional composition, antioxidant activity, sensory acceptability, and microbial safety.

## 2. Materials and Methods

### 2.1. Ingredient Procurement

Un-popped foxnut (*Euryale ferox*) seeds were procured from a reputable FSSAI-certified supplier in Hyderabad, ensuring food-grade quality and traceable origin. Jaggery powder and vanilla extract were obtained from established retail brands from a certified local supermarket. All ingredients were handled under hygienic conditions and stored at ambient temperature until use.

### 2.2 Preparation of Foxnut Seed Milk Base

Fully matured foxnut seeds were washed thoroughly under potable water and then soaked for approximately 6-8 h at room temperature to soften the seed coat. After soaking, seeds were boiled in clean water for 10 minutes to enhance extractability and reduce microbial load. Boiled seeds were allowed to cool to ambient temperature before subsequent processing.

### 2.3 Formulation of $T_1$, $T_2$ and $T_3$ Samples

Three experimental formulations were prepared, designated $T_1$, $T_2$, and $T_3$, differing only by foxnut seed concentration:

Procedure for each formulation:

- The boiled and cooled seeds were ground with 100 ml of potable water in a high-speed blender to form a fine slurry.
- The slurry was filtered 3-4 times through muslin cloth or fine mesh sieve to extract a smooth liquid free of coarse residues.
- Jaggery (5 g) was dissolved into the filtrate under continuous stirring.
- Vanilla extract (1 g) was added as a flavor enhancer.
- The homogenized product was transferred into sterile, airtight containers and stored under refrigeration (4±2 °C) pending analyses.

**Table 1:** Formulation of preparation of Fox nut seed milk

| Ingredients | $T_1$ sample | $T_2$ sample | $T_3$ sample |
|---|---|---|---|
| Fox Nut seed | 10 g | 15 g | 20 g |
| Jaggery | 5g | 5 g | 5 g |
| Vanilla extract | 1g | 1 g | 1 g |
| Water | 100 ml | 100 ml | 100 ml |

### 2.4 Sensory Evaluation (Hedonic Scale)

A panel of ~20 trained assessors evaluated the three samples using the 7-point Hedonic scale (1 = "dislike extremely", 7 = "like extremely"). Attributes assessed included colour/appearance, texture/mouthfeel, aroma, taste, and overall acceptability. Samples were anonymized with three-digit codes and served in randomized order to minimize bias. Scores were recorded individually and later compiled for statistical analysis to identify the most preferred formulation.

### 2.5 Nutritional (Proximate) Analysis

**Standard AOAC methods were employed for proximate determinations:**

- **Moisture content:** Oven drying at ~100 °C until constant weight (AOAC 925.09)
- **Ash:** Muffle furnace incineration at 550 °C (AOAC 942.05)
- **Crude fat:** Soxhlet ether extraction (AOAC 985.29)
- **Protein:** Kjeldahl digestion and nitrogen conversion (AOAC 981.10).

  Energy was calculated from macronutrient values via Atwater factors.

### 2.6 Vitamin and Mineral Analysis

Mineral quantification (calcium, potassium, magnesium, iron) was performed using wet digestion followed by as per AOAC protocols. Vitamin content (if analyzed) followed appropriate AOAC with calibration against certified standards.

### 2.7 Antioxidant Activity Measurement

**Functional antioxidant activity was assessed using:**

- DPPH radical scavenging assay (AOAC 2015.06) to determine DPPH value
- Gallic acid, chlorogenic acid, and epicatechin quantification using validated AOAC methods (2020.05 and 2020.07) calibrated with appropriate phenolic standards.

### 2.8 Microbiological analysis and shelf-life study

Microbial safety and shelf stability were assessed using AOAC Official Methods: Aerobic plate count (APC) by AOAC 990.12; Yeast and mold count via AOAC 997.02; Enterobacteriaceae including coliforms (AOAC 991.14); Staphylococcus aureus detection (AOAC 991.14).

## 3. Results and Discursion

### 3.1 The sensory evaluation of *Euryale ferox* seed milk

The sensory evaluation of *Euryale ferox* seed milk samples revealed notable differences across the tested formulations ($T_1$, $T_2$, and $T_3$). Among the three, $T_1$ consistently received the highest scores across all sensory attributes, including colour (7.00±0.00), texture (7.00±0.00), smell (6.90±0.31), taste (6.85±0.37), and overall acceptability (6.95±0.22), indicating superior sensory appeal. $T_2$ scored the lowest in

all parameters, particularly in taste (6.00±0.00) and overall acceptability (6.00±0.00), suggesting a less favorable consumer perception. $T_3$ showed moderate acceptability, with scores ranging from 6.20±0.62 to 6.50±0.61, indicating potential for improvement. These findings suggest that the formulation used in $T_1$ may offer the most desirable combination of sensory properties, likely due to optimal ingredient ratios or processing conditions. Similar studies on plant-based milks have also emphasized the importance of sensory quality in consumer acceptance (Mäkinen *et al.*, 2016; McClements & Grossmann, 2021) [10, 11]. The results underscore the necessity of balancing nutritional enhancement with sensory appeal in the development of functional plant-based beverages.

**Table 2:** Sensory parameters of *Euryale ferox* seed milk samples $T_1$, $T_2$ and $T_3$

| Attribute | $T_1$ (Mean ± SD) | $T_2$ (Mean ± SD) | $T_3$ (Mean ± SD) |
|---|---|---|---|
| Colour | 7.00±0.00 | 6.25±0.44 | 6.50±0.51 |
| Texture | 7.00±0.00 | 6.25±0.44 | 6.50±0.61 |
| Smell | 6.90±0.31 | 6.15±0.37 | 6.35±0.59 |
| Taste | 6.85±0.37 | 6.00±0.00 | 6.25±0.55 |
| Overall acceptability | 6.95±0.22 | 6.00±0.00 | 6.20±0.62 |

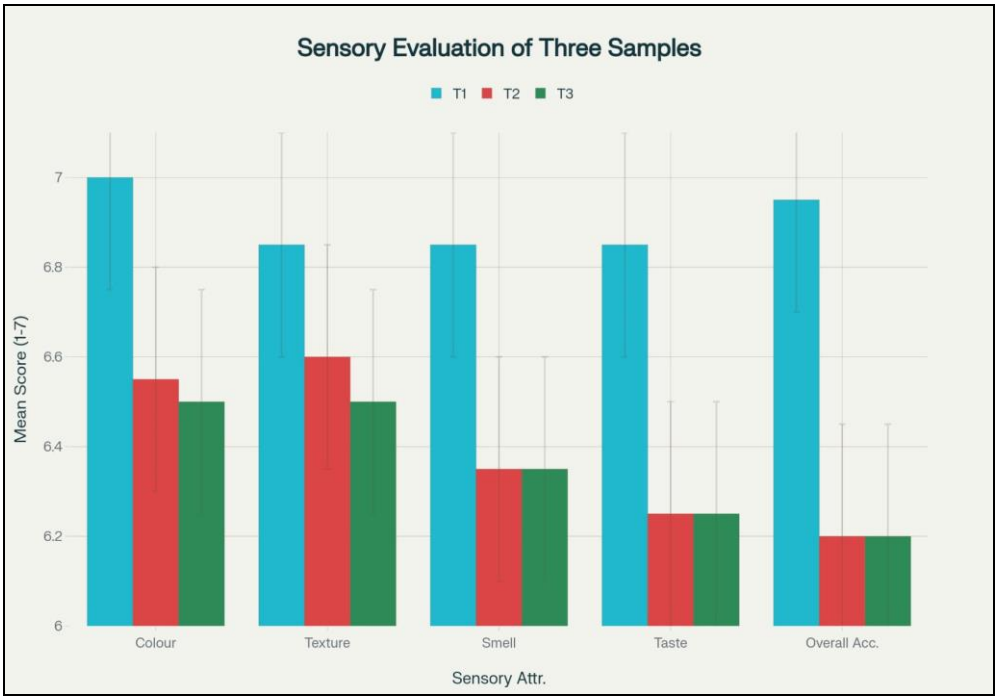


**Fig 1:** Sensory parameters of tested samples $T_1$ $T_2$ $T_3$

### 3.2 The Nutrient Analysis of The Optimized *Euryale ferox* Seed Milk Sample ($T_1$)

The nutrient analysis of the optimized *Euryale ferox* seed milk sample ($T_1$) indicates its potential as a low-calorie, plant-based beverage with modest nutritional value. The sample provided an energy content of 36.5 kcal per 100 ml, which is relatively low compared to conventional dairy milk, making it suitable for calorie-conscious consumers. The protein content was 0.8 g/100 ml, as determined by AOAC 992.23, aligning with typical values reported for nut- and seed-based milk alternatives (Sethi *et al.*, 2016) [13]. Carbohydrates were present at 7.2 g/100 ml, suggesting that the product could serve as a mild source of energy from natural sugars or starches inherent in *Euryale ferox* seeds. Total fat content was minimal (0.5 g/100 ml) based on AOAC 996.06, indicating a lean nutritional profile. Moisture content was 9.1% (AOAC 925.10), and ash content, representing total mineral matter, was 0.4% (AOAC 923.03), both within acceptable ranges for plant-based beverages. The overall composition suggests that *Euryale ferox* milk can be positioned as a functional drink with potential health benefits, especially for individuals seeking low-fat and plant-derived alternatives to dairy. These findings are consistent with prior research highlighting the nutritional viability of non-dairy milk substitutes derived from seeds and legumes (Mäkinen *et al.*, 2016; McClements & Grossmann, 2021) [10, 11].

**Table 3:** Nutrient Analysis of The Optimized *Euryale ferox* Seed Milk Sample ($T_1$)

| S. No | Test parameter | Unit | Results | Test method |
|---|---|---|---|---|
| 1. | Energy | k/cal | 36.5 | - |
| 2. | Protein | g/100ml | 0.8 | AOAC 992.23 |
| 3. | Carbohydrates | g/100ml | 7.2 | - |
| 4. | Total fats | g/100ml | 0.5 | AOAC 996.06 |
| 5. | Moisture | % | 9.1 | AOAC 925.10 |
| 6. | Ash | % | 0.4 | AOAC 923.03 |

### 3.3 The Mineral Composition of The Optimized *Euryale ferox* Seed Milk Sample ($T_1$)

The mineral composition of the optimized *Euryale ferox* seed milk sample ($T_1$), analyzed using AOAC 984.27, demonstrates its potential as a supplementary source of essential micronutrients. The sample contained 0.4 mg/100 ml of iron, which, while modest, may contribute to dietary iron intake, particularly in plant-based diets where iron

bioavailability is often limited. Notably, calcium was present at 42 mg/100 ml, highlighting *Euryale ferox* milk as a potential non-dairy calcium source, albeit lower than that of cow's milk, which typically contains around 120 mg/100 ml (Mäkinen *et al*., 2016) [10]. Magnesium and potassium were detected at 13.5 mg/100 ml and 50 mg/100 ml, respectively, both of which are important for neuromuscular function and electrolyte balance. These findings suggest that while *Euryale ferox* seed milk may not fully substitute dairy milk in terms of mineral density, it does provide a natural alternative with beneficial mineral content, especially suitable for consumers seeking plant-based, lactose-free options. The results align with existing literature on the mineral profiles of plant-based beverages, which often emphasize their lower but functionally relevant mineral levels (Vanga & Raghavan, 2018; McClements & Grossmann, 2021) [15, 11].

**Table 4:** Minerals Composition of The Optimized *Euryale ferox* Seed Milk Sample ($T_1$)

| Test parameter | Unit | Results |
|---|---|---|
| Iron | mg/100ml | 0.4 |
| Calcium | mg/100ml | 42 |
| Magnesium | mg/100ml | 13.5 |
| Potassium | mg/100ml | 50 |

### 3.4 The Antioxidant profile of the optimized *Euryale ferox* Seed Milk ($T_1$)

The antioxidant profile of the optimized *Euryale ferox* seed milk ($T_1$) highlights its potential as a functional beverage with notable free radical scavenging properties. The DPPH radical scavenging activity was recorded at 175 mg/100 ml, indicating substantial antioxidant capacity. Specific phenolic compounds quantified using validated AOAC methods included gallic acid (20 mg/100 ml; AOAC 2015.06), chlorogenic acid (13 mg/100 ml; AOAC 2020.05), and epicatechin (5.6 mg/100 ml; AOAC 2020.07). These polyphenols are well-documented for their health-promoting properties, including anti-inflammatory, cardioprotective, and neuroprotective effects (Chen *et al*., 2020) [2]. Gallic acid, a potent antioxidant, contributes significantly to the oxidative stability and therapeutic potential of plant-based products, while chlorogenic acid and epicatechin are also known for their ability to modulate oxidative stress and metabolic pathways (Liu *et al*., 2017) [8]. The presence of these bioactive compounds in *Euryale ferox* milk reinforces its value as a plant-derived beverage with added health benefits, aligning with the growing consumer demand for functional foods rich in natural antioxidants.

**Table 5:** Antioxidants profile of the optimized *Euryale ferox* seed milk ($T_1$)

| Test parameter | Unit | Results |
|---|---|---|
| DPPH | mg/100ml | 175 |
| Gallic acid | mg/100ml | 20 |
| Chlorogenic acid | mg/100ml | 13 |
| Epicatechin | mg/100ml | 5.6 |

### 3.5 The Microbiological analysis of the optimized *Euryale ferox* seed milk sample ($T_1$)

The microbiological analysis of the optimized *Euryale ferox* seed milk sample ($T_1$) confirmed its compliance with food safety standards, indicating its microbiological stability and suitability for consumption. The aerobic plate count (APC), a general indicator of microbial load, was found to be <10 CFU/ml, well below the permissible limit of $1 \times 10^6$ CFU/ml as per AOAC 990.12, suggesting effective hygiene and processing conditions. Similarly, yeast and mold counts were also < 10 CFU/ml (AOAC 997.02), indicating minimal fungal contamination and a low risk of spoilage under standard storage conditions. Importantly, pathogenic bacteria such as *Enterobacteriaceae* and *Staphylococcus aureus* were absent in 25 ml of the sample, as determined by AOAC 991.14, meeting the microbiological safety criteria for ready-to-consume beverages. These results underscore the microbiological integrity of the product and support its shelf-stability when manufactured under good hygienic practices. Comparable findings in other plant-based beverages have shown that proper thermal processing and aseptic handling play critical roles in ensuring microbial safety and the current findings align with such evidence, affirming the sanitary quality of *Euryale ferox* milk.

**Table 6:** Microbiological Analysis of The Optimized *Euryale ferox* Seed Milk Sample ($T_1$)

| Test parameter | Unit | Results | Limits |
|---|---|---|---|
| Aerobic plate count | CFU/ml | <10 | $1x\ 10^6$ |
| Yeast and molds | CFU/ml | <10 | $1x\ 10^6$ |
| Enterobacteriaceae | CFU/ml | Absent | Absent/25ml |
| *S. aureus* | CFU/25ml | Absent | Absent/25ml |

## Conclusion

The study successfully demonstrated that *Euryale ferox* seed milk, particularly the $T_1$ formulation, offers a promising alternative to conventional dairy milk in terms of sensory appeal, nutritional balance, functional bioactives, and microbial safety. The incorporation of jaggery and vanilla not only enhanced palatability but also supported clean-label formulation trends. Rich in antioxidants and essential minerals, the product holds potential for positioning as a health-oriented, plant-based beverage suitable for lactose-intolerant individuals and those seeking low-fat, minimally processed alternatives. Further research may explore shelf life extension, fortification strategies, and consumer market acceptance at scale.

## References


1. Biswas P, *et al*. Enzyme mediated resistant starch production from Indian fox nut and study on functional properties. Carbohydr Polym. 2020;247:116741.
2. Chen GL, Chen SG, Zhao YY, Luo CX, Li J, Gao YQ. Total phenolic contents of 33 fruits and their antioxidant capacities before and after in vitro digestion. Ind Crops Prod. 2020;143:111907.
3. Food and Agriculture Organization (FAO). Dietary protein quality evaluation in human nutrition. FAO Food Nutr Pap; 2013, p. 92.
4. Association of Official Analytical Chemists (AOAC). Official methods of analysis. 21st/22nd Ed. Gaithersburg (MD): AOAC International.
5. Gupta N, Raj D, Sharma A. Nutritional and therapeutic potential of makhana (*Euryale ferox* Salisb.): A review. J Food Sci Technol. 2020;57(6):2067-75.
6. Jain R, Jain S, Singh R. Phytochemical and pharmacological profile of *Euryale ferox* Salisb.: A review. Asian J Pharm Clin Res. 2019;12(1):10-5.
7. Liaquat M, *et al*. Roasted fox nuts: Phenolics, mineral,

antioxidant content and glycemic index. Food Prod Process Nutr. 2022;4:12.
8. Liu RH, Finley J. Potential health benefits of phytochemicals in functional foods: A review. J Food Sci. 2005;70(9):R134-R138. https://doi.org/10.1111/j.1365-2621.2005.tb09935.x
9. Ma Y, Song Y, Jin J. Polyphenolic compounds in health and disease: From molecular mechanisms to therapeutic applications. J Funct Foods. 2019;62:103520.
10. Mäkinen OE, Wanhalinna V, Zannini E, Arendt EK. Foods for special dietary needs: Non-dairy plant-based milk substitutes and fermented dairy-type products. Crit Rev Food Sci Nutr. 2016;56(3):339-349.
11. McClements DJ, Grossmann L. The science of plant-based foods: Constructing next-generation meat, fish, milk and egg analogs. Compr Rev Food Sci Food Saf. 2021;20(4):4049-4100.
12. Rybicka I, Świgło GA, Górecka D. Plant-based milk substitutes: Nutritional and technological challenges. Food Rev Int. 2020;36(3):267-289.
13. Sethi S, Tyagi SK, Anurag RK. Plant-based milk alternatives: An emerging segment of functional beverages. Crit Rev Food Sci Nutr. 2016;56(3):339-49.
14. Singh A, Dhawan S. Functional properties and therapeutic applications of foxnut (*Euryale ferox*): A review. J Ethnopharmacol. 2021;273:113961.
15. Vanga SK, Raghavan V. How well do plant-based alternatives fare nutritionally compared to cow's milk? J Food Sci Technol. 2018;55(1):10-20.
16. Verma DK, Mishra A. Nutritional evaluation and processing of fox nut (*Euryale ferox*): A review. J Pharmacogn Phytochem. 2020;9(3):1662-1666.