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Research Article

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Bioprocess Optimization and Functional Characterization of Peanut Milk-based Vegan Fermented Products as Sustainable Dairy Alternatives

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Abstract

The present study was conducted to develop and characterize plant-based fermented curd and kefir from peanut milk as potential dairy alternatives for lactose-intolerant individuals and consumers following plant-based diets. Peanut milk was fermented with *Lactobacillus acidophilus* to produce curd and with a commercial mixed kefir starter culture, mainly containing *Lactobacillus kefiri*, to produce kefir. The developed peanut curd had 82.7% moisture, 7.9% fat, 8.93% protein, and 119.25 mg/100 mL carbohydrate. It had a total flavonoid content (TFC) of 285.47 ± 5.87 mg QE/100 mL, an anthocyanin content of 4.2 mg cyanidin-3-glucoside (C3G) eq/L, and moderate *in vitro* antioxidant activity, with 54% 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity. Fermentation increased the titratable acidity to 3.543 ± 0.586 mEq/g, while the final pH was 4.19, indicating successful lactic acid fermentation. Microbiological analysis showed a viable bacterial count of 2.13×10^5 colony-forming units per milliliter (CFU/mL) at the countable dilution. Peanut milk kefir had 88.7% moisture, 3.9% fat, 6.76% protein, and 126.75 mg/100 mL carbohydrate, with a TFC of 225.13 ± 3.55 mg QE/100 mL, an anthocyanin content of 4.4 mg C3G eq/L, and 52% DPPH radical-scavenging activity. The jaggery-supplemented peanut kefir showed a high viable microbial count of 1.9×10^9 CFU/mL, indicating successful fermentation and good microbial viability. The jaggery-supplemented kefir also showed good starter-culture survival during fermentation, as indicated by the high viable microbial count. Sensory evaluation revealed that the jaggery-supplemented formulations had better flavor, taste, and overall acceptability. The results indicate that peanut milk is a suitable substrate for the production of fermented plant-based products with desirable nutritional, phytochemical, microbiological, and sensory characteristics. Further research on shelf-life stability, textural improvement, probiotic efficacy, and the bioavailability of bioactive compounds is needed before wider health, or commercial applications can be considered.

Keywords

Peanut milk, Vegan, Dairy alternatives, Peanut milk curd, Peanut milk kefir, Plant-based products, Fermented products

Introduction

Milk is a nutritious, white liquid produced by mammals and has been a staple component of the human diet for centuries because of its nutritional value and versatility [1]. Cow's milk is mainly composed of water and macronutrients, including carbohydrates (mainly lactose), fat, and protein, as well as micronutrients such as vitamins (A, D, E, and K) and minerals (calcium, phosphorus, and zinc) [2]. Cow's milk proteins, such as casein and whey proteins, are sources of essential amino acids [3, 4], while milk fat contains various types of fatty acids, including saturated and unsaturated fatty acids [5]. Milk also contains

immunoglobulins, lactoferrin, lysozyme, lactoperoxidase, growth factors, oligosaccharides, and peptides, which exhibit specific physiological activities [6, 7]. These components contribute to various functional properties of milk, including immunomodulatory, antibacterial, antioxidant, and prebiotic activities [8, 9]. Research findings have demonstrated the positive effects of milk on human health, including improved bone health due to its calcium content, enhanced immune function, and potentially reduced risks of diseases such as cardiovascular disease, type II diabetes, and certain forms of cancer [10-12].

The global milk supply is under increasing pressure due to several factors. Climate change, including weather events such as droughts and floods, has adversely affected agricultural activities and dairy cattle feed availability [13, 14]. These impacts include feed shortages and heat stress, which have affected milk production and quality [15]. In addition, political instability and trade barriers have affected global supply chains, limiting the transportation of milk from areas with excess production to areas with high demand [16, 17]. Furthermore, the growing global population has increased the demand for dairy products, placing considerable pressure on milk availability [18]. This has resulted in increased milk prices, reduced availability, and concerns about shortages in some areas, as consumer demand continues to rise due to population growth, climate change, and geopolitical issues [19].

The increase in milk prices further intensifies this problem, making it difficult for vulnerable groups to afford dairy products, which may lead to poor health outcomes and health inequalities [20]. Milk shortages can affect both the availability and overall consumption of milk, particularly in developing countries and among vulnerable populations. Poor households may have no choice but to reduce milk consumption or switch to less nutritious alternatives, potentially leading to malnutrition [20]. For instance, children and adolescents are particularly vulnerable because they require adequate calcium and vitamin D for proper physical development and growth, and reduced milk intake may negatively affect their health [21].

Lactose intolerance is a condition characterized by low lactase enzyme activity. Consumption of dairy products containing lactose can cause gastrointestinal discomfort. To address this issue, lactose-free products have been developed from both animal and plant sources. Plant-based milk alternatives (PBMAs) made from nuts, cereals, seeds, legumes, and pseudocereals are nutrient-rich and environmentally friendly alternatives [11]. Among these, oat milk is rich in dietary fiber and has cholesterol-lowering properties; soy milk is high in high-quality protein and isoflavones; peanut milk contains significant amounts of antioxidants and essential nutrients; almond milk is rich in healthy fats and vitamins; coconut milk has unique antimicrobial properties; sesame milk is rich in high-quality protein and lignans; hemp milk is rich in essential fatty acids; and rice milk is a simple, high-calorie, nutrient-fortified option [11, 12, 21]. Despite technological challenges and variations in consumer acceptance due to differences in taste and texture, an increasing number of consumers are choosing plant-based milks because of their potential health benefits and environmental sustainability. However, these alternatives often require fortification to achieve a nutritional quality comparable to that of cow's milk, particularly in terms of protein, zinc, and potassium levels [22].

For centuries, cow's milk has been considered a vital component of the human diet because of its high nutritional value. However, in recent years, the demand for PBMAs has increased due to conditions such as lactose intolerance and lifestyle choices such as veganism [20]. PBMAs are liquid products produced by breaking down plant materials (e.g., cereals, pseudocereals, legumes, oilseeds, and nuts) and homogenizing them with water. Their particle size distribution ranges from 5 to 20 μm, which is similar to the appearance and texture of cow's milk [12]. Plant-based milk and dairy alternatives represent a growing niche in food product development, particularly within the functional and specialty beverage markets worldwide. Nowadays, consumers choose alternatives to cow's milk for various reasons, including cow's milk allergy, lactose intolerance, calorie concerns, high cholesterol levels, or preference for a vegan diet. This shift in consumer preferences has contributed to the increasing adoption of plant-based dairy alternatives.

Although numerous studies have investigated plant-based fermented products derived from soy, oat, almond, and coconut milk, studies on peanut milk-based fermented products remain limited, particularly those involving the simultaneous development and comprehensive characterization of peanut milk curd and peanut milk kefir. Previous studies have mainly focused on the physicochemical properties or sensory evaluation of peanut-based fermented beverages, while relatively few studies have reported on their nutritional composition, phytochemical properties, antioxidant activity, microbial viability, and consumer acceptability [8]. Therefore, the focus on developing and evaluating peanut milk curd and peanut milk kefir as alternatives to conventional dairy products is based on several important considerations. First, the increasing prevalence of lactose intolerance and dairy allergies worldwide has increased the demand for non-dairy fermented products such as curd and kefir. Second, peanuts are widely available and relatively inexpensive in many developing countries, particularly in regions where dairy production is constrained by climatic and infrastructural limitations. Using peanuts as a substrate for curd and kefir fermentation not only adds value to a locally available crop but also aligns with food security and value-addition objectives in low-income countries [23]. Furthermore, consumers are increasingly interested in functional, non-dairy fermented beverages. The international market for plant-based dairy alternatives is expected to expand rapidly, with fermented plant-based products potentially playing an important role [24]. The development of peanut milk curd (Figure 1) and peanut milk kefir (Figure 2) could contribute to this growing trend while potentially providing probiotic benefits similar to those of fermented dairy products [25]. Hence, the present study aimed to prepare peanut milk-based vegan curd and kefir and evaluate their proximate



Figure 1: Peanut curd.



Figure 2: Peanut Kefir.

composition, phytochemical profile, antioxidant potential, microbial quality, and sensory attributes to investigate their potential as sustainable plant-based alternatives to dairy products.

Material and Methods

Sample collection

A total of 20 kg of peanuts was purchased from a local vendor at an open-air market in Doimukh, Arunachal Pradesh, India. The peanuts were selected based on their size and quality. These peanuts were used as the primary sample for the present study on nutritional analysis and product formulation. As an inoculum, a bottle containing 60 capsules of in life probiotic *L. acidophilus*, with 5 billion CFU per capsule, was purchased. The product was manufactured by Live Stamin Healthcare, Hyderabad, India, under manufacturing license No. 10017047000694. A kefir starter culture (Feel Good Kefir), manufactured by Feel Good, 19/442/D, Krishi Bhavan, Vadakode, Ernakulam, was also used, along with plant-based gelatin.

Chemicals and reagents

All chemicals used in the study were obtained from Sisco Research Laboratories Pvt. Ltd., India, and HiMedia Laboratories Pvt. Ltd., India. The instruments and tools used included a measuring cylinder, muslin cloth, desiccator, deep freezer, Soxhlet apparatus, Philips Classic 500 W domestic mixer, round-bottom flask, burette, conical flask, centrifuge (R-8C Plus, Remi Elektrotechnik Ltd.), pH meter, volumetric flask (amber-colored) with a glass stopper, aluminum foil, vortex mixer, autoclave, biosafety cabinet, weighing balance, tissue paper, beaker, micropipette, micropipette tips, mask, gloves, Petri dishes, cotton plug, L-shaped spreader, Bunsen burner, ultraviolet-visible (UV-Vis) spectrophotometer (Thermo Scientific, Singapore), cuvette, hot air oven, analytical weighing balance, test tubes, 96-well microtiter plate, biochemical oxygen demand incubator (Prolab, India), glass bottle, paper cup, and spoon.

Peanut milk preparation

Peanut milk was prepared according to the method described by Bensmira and Jiang [26], with slight modifications (Figure 3 and figure 4). Raw peanuts (*A. hypogaea*) were soaked in water for 12 h before peeling. Water was then added to the peeled peanuts at a ratio of 1:5 (w/v) to produce peanut milk with a total solids content of 10 g/100 g, and the mixture was blended for 10 min. The blended mixture was then filtered through a muslin cloth. The resulting milky liquid was used for the production of curd and kefir. The extracted peanut milk was then subjected to pasteurization at 100 °C and subsequently allowed to cool.

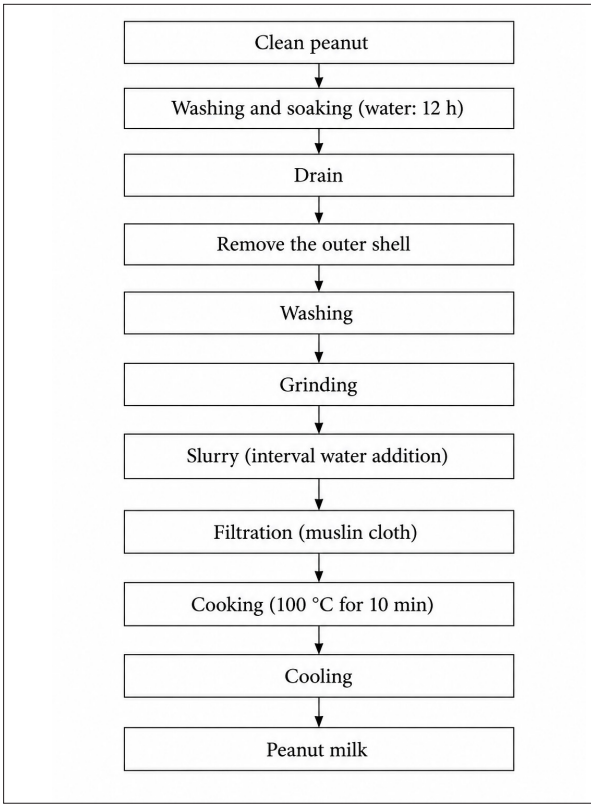


Figure 3: Flowchart for the preparation of peanut milk.

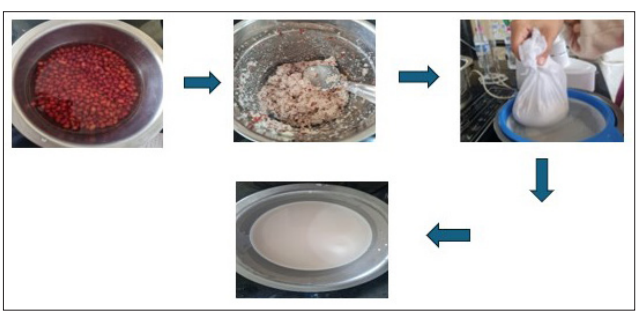


Figure 4: Pictorial flowchart for preparation of peanut milk.

Preparation of the sample

Peanut curd

Heat treatment of peanut milk at 100 °C for 10 min reduced the native microbial load, inactivated spoilage microorganisms, and denatured endogenous enzymes (Figure 5). This heat treatment, which is commonly applied to plant-based milks, helps improve microbial safety and reduce antinutritional factors present in legumes. After cooling to 45 °C, the starter culture was added, which is within the optimum inoculation temperature range for *L. acidophilus*. The inoculated milk was shaken vigorously to ensure uniform dispersion of the bacteria and to facilitate subsequent fermentation. The bacteria fermented the sugars present in the peanut milk into lactic acid, resulting in curd formation. The samples were incubated under hygienic conditions until coagulation occurred.

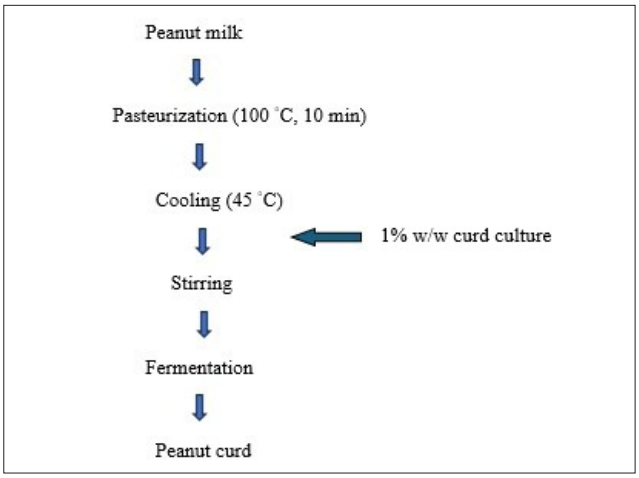


Figure 5: Flow chart for the preparation of peanut curd.

Peanut kefir

Peanut milk kefir was prepared with slight modifications to the method described by Arshad et al. [27] (Figure 6). Briefly, peanut milk was heated at 100 °C for 10 min to ensure microbial safety and enhance product stability, followed by cooling to 45 °C. A 100 mL portion of the cooled milk was inoculated with 0.5 g of kefir starter culture. The kefir starter culture (Feelgood Kefir, Ernakulam, Kerala, India; powdered form) contained a mixed microbial consortium characteristic of traditional kefir fermentation. The bacterial fraction was primarily composed of *L. kefiri*, *L. acidophilus*, *Lactococcus lactis*, and *Leuconostoc mesenteroides*. The culture also contained yeasts, such as *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and *Candida humilis*, which are important for kefir fermentation. The combination of lactic acid bacteria and yeasts contributes to acidification and flavor generation, which are responsible for the characteristic properties of the final product. The inoculated mixture was stirred to ensure uniform distribution of the starter culture and was then incubated until fermentation was complete to obtain peanut milk kefir.

Sensory evaluation of the developed product

Sensory evaluation of peanut milk curd and kefir was carried out using a 9-point hedonic scale following the methodology of Stone et al. [28], where 1 indicated "dislike extremely" and 9 indicated "like extremely." Twenty trained panelists aged 18 - 45 years from Rajiv Gandhi University, India, comprising faculty members and research scholars, participated in the study after providing informed consent. The panelists were trained in the sensory attributes and evaluation procedures prior to the assessment. Three types of peanut milk curd were evaluated: A (sugar-sweetened curd), B (plain curd), and C (jaggery-sweetened curd). Similarly, three kefir samples, S (dairy milk kefir), S1 (peanut milk kefir), and S2 (jaggery-supplemented peanut milk kefir), were also evaluated. The samples were assessed for appearance, color, aroma, flavor, texture, taste, and overall acceptability. Sensory scores were summarized using descriptive statistics and expressed as mean ± standard deviation (SD) based on the responses of the 20 panelists.

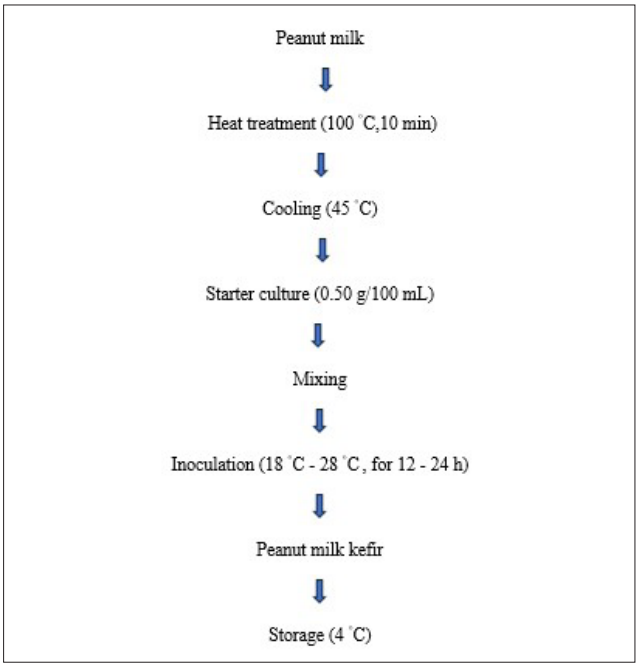


Figure 6: Flow chart for the preparation of peanut milk kefir.

Proximate analysis

Moisture content

The moisture content of the samples was determined using a hot air oven. Five grams of each sample were analyzed according to the Association of Official Analytical Chemists (AOAC) 1999 standard [29]. The samples were dried at 105 °C in a hot air oven for 6 h until a constant weight was obtained. The average moisture content of the samples was calculated using equation 1.

$$\text{Moisture content } (\%) = \frac{W-d}{W} \times 100 \quad (1)$$

Where, W is the wet weight of sample and d is the weight of dried sample.

Fat content

A minimum sample weight of 5 g was used, and 150 mL of petroleum ether was added as the extraction solvent. The extraction was carried out in a Soxhlet apparatus for 6 h [30]. The crude fat content was calculated using equation 2.

$$\text{Crude fat} = \frac{(W_2 - W_1)}{W_3} \times 100 \quad (2)$$

Where, W_1 is the weight of the empty flask, W_2 is the weight of the flask containing the extracted fat, and W_3 is the initial weight of the sample.

Protein estimation

The protein content of the peanut-based vegan product samples was determined using the Kjeldahl method [30]. Following acid digestion with a copper catalyst and concentrated sulfuric acid (H_2SO_4), the samples were distilled, and the released ammonia was collected in a 4% boric acid solution containing a mixture of methylene blue and methyl red as indicators. The ammonia content, and hence the nitrogen content, was determined by titration against a standardized 0.1 N hydrochloric acid (HCl) solution. As peanuts are plant-based materials, a nitrogen-to-protein conversion factor of 6.25 was used to calculate the crude protein content. The nitrogen and protein contents of the peanut-based vegan products were calculated using equation 3 and 4.

$$\text{Nitrogen \%} = \frac{14.01 \times 0.1 \times (TV - BV)}{W \times 1000} \times 100 \quad (3)$$

$$\text{Protein \%} = N \times 6.25 \quad (4)$$

Where, 0.1 is the normality of the titration solution, BV is the blank value, W is the sample weight, 14.01 is the atomic mass of nitrogen, and TV is the titer value.

Carbohydrates estimation

The Anthrone method was used to determine the carbohydrate content of the peanut milk-based vegan samples [31]. Briefly, the samples were hydrolyzed with 2.5 N HCl, neutralized with sodium carbonate, and then treated with Anthrone reagent. The resulting green to dark-green colored complex was measured spectrophotometrically at 630 nm. A standard curve prepared using different concentrations of glucose was used to quantify the carbohydrate content by comparing the absorbance values.

Ash content

The ash content was determined using the AOAC method [30]. Pre-weighed crucibles containing 5 g of ground peanut product powder were placed in a muffle furnace and heated at 550 ˚C for 6 h. The crucibles were then transferred to a desiccator and allowed to cool. The crucibles containing the ash were weighed, and the ash content was calculated using equation 5.

$$\text{Ash content \%} = \left(\frac{w_1}{w_2}\right) \times 100 \quad (5)$$

Where, W_1 is the weight of ash and W_2 is the weight of sample.

Crude fiber estimation

Crude fiber was determined using the method described by Ranganna [32], with a hot plate. Samples free from moisture and fat were used for the determination of crude fiber. The samples were first boiled with 1.25% H_2SO_4 and then filtered. The residue was collected and subsequently boiled with 1.25% sodium hydroxide (NaOH) and filtered. The residue was then collected and dried in a hot air oven. The crude fiber content was calculated using equation 6.

$$\text{Crude fiber \%} = \frac{(W_2 - W_1)}{S} X100 \quad (6)$$

Where, W_1 is the weight (g) of the sample (free from moisture and fat), W_2 is the weight (g) of the residue, and S is the weight (g) of the sample.

pH

The pH of the peanut milk samples was measured using a duly calibrated pH meter [33]. The pH meter was calibrated using standard buffer solutions at pH 4, 7, and 10 before the measurements to ensure accuracy. The samples were allowed to equilibrate to a stable temperature, after which the electrode was immersed in the sample. The pH was recorded after the reading had stabilized. Between measurements, the electrode was rinsed with distilled water to prevent interference from the previous sample and ensure accurate pH determination.

Acidity

The titratable acidity of the peanut milk curd and kefir samples was determined by titration with 0.1 N NaOH solution [33]. Each sample (10 g) was accurately weighed and transferred into separate Erlenmeyer flasks. A few drops of phenolphthalein indicator solution were added to each flask. The samples were then titrated with 0.1 N NaOH solution until a faint pink color persisted for 30 s, indicating the endpoint of the titration. The volume of NaOH solution used was recorded. The acidity of the samples was expressed as milliequivalents of NaOH per gram of sample (mEq/g) and calculated using equation 7.

$$\text{Titratable acidity (mEq/g)} = \frac{N \times V}{W} \quad (7)$$

Where, N is the normality of NaOH, V is the volume of NaOH used (mL), and W is the sample weight (g).

Phytochemicals analysis

Total anthocyanin content (TAC)

The pH differential method was used to determine the TAC of the peanut milk samples [34]. Anthocyanin pigments exhibit reversible changes in their molecular structure in response to changes in pH, resulting in different absorption spectra. The colored oxonium form is predominant at pH 1.0, whereas the colorless hemiketal form is predominant at pH 4.5.

Briefly, 1 mL of the extracted solution was transferred into a 10 mL volumetric flask to prepare two different sample dilutions. One dilution was adjusted to pH 1.0 using potassium chloride buffer, while the other was treated with sodium acetate buffer at pH 4.5. The two dilutions were allowed to equilibrate for 15 min, after which the absorbance of each dilution was measured at 510 and 700 nm using a UV-Vis spectrophotometer against a distilled water blank. All measurements were performed between 15 min and 1 h after preparation. The anthocyanin content was calculated using equation 8 - 10.

The absorbance of the diluted sample (A) was calculated using equation 8:

$$A = (A510 - A700)\,pH\,1.0 - (A510 - A700)\,pH\,4.5 \quad (8)$$

The monomeric anthocyanin pigment concentration was calculated using equation 9:

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1) \quad (9)$$

The value was subsequently converted to mg TAC/100 g of sample.

The anthocyanin pigment concentration, expressed as C3G equivalents, was calculated using equation 10:

$$\frac{AMWDF10}{\varepsilon 1} \quad (10)$$

Where, A is the difference between the absorbance values at 510 and 700 nm at pH 1.0 and pH 4.5; MW is the molecular weight of C3G (449.2 g/mol); **l** is the path length (cm); ε is the molar extinction coefficient (26,900 L × mol^{-1} × cm^{-1}); and DF is the dilution factor used during sample preparation.

TFC

TFC was determined using the aluminum chloride ($AlCl_3$) colorimetric method described by Jahan et al. [35], with slight modifications. Briefly, 20 µL of the peanut curd or peanut kefir sample was mixed with 1.5 mL of 95% ethanol, 20 µL of 10% aluminum chloride ($AlCl_3$), 20 µL of 1 M potassium acetate, and 0.56 mL of distilled water. The reaction mixture was incubated at room temperature for 40 min. The absorbance was measured at 415 nm using a UV-Visible spectrophotometer (Thermo Scientific, Singapore, Model 1550) against a distilled water blank. A calibration curve was prepared using quercetin as the standard, and the TFC was calculated from the regression equation and expressed as milligrams of quercetin equivalents per 100 mL of sample (mg QE/100 mL).

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined according to the method described by Bensmira and Jiang [26], with slight modifications. A DPPH stock solution (0.101 mM) was prepared by dissolving 2 mg of DPPH in 50 mL of 80% methanol. For the assay, 1 mL of DPPH solution was mixed with 1 mL of sample extract and incubated in the dark for 30 min at room temperature [36]. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition of DPPH was calculated for each sample using equation 11.

$$\text{DPPH inhibition} = \frac{A\ blank - A\ sample}{A\ blank} \times 100 \quad (11)$$

Where, A_{blank} is the absorbance of the blank and A_{sample} is the absorbance of the sample.

Microbiological analysis

The microbial quality of the peanut curd and kefir samples was analyzed using the serial dilution and pour plate techniques [37]. One gram of each curd and kefir sample was aseptically transferred to a sterile diluent to prepare a 1:10 dilution. Subsequent serial dilutions were prepared to obtain a wide range of concentrations (1:100, 1:1,000, etc., up to 10^6). From each dilution, 1 mL aliquots were aseptically transferred and mixed with molten plate count agar, a non-selective medium that supports bacterial growth. The mixture was then poured into sterile Petri dishes and allowed to solidify.

The plates were subsequently incubated at 37 °C for 24 - 48 h to facilitate colony growth. After incubation, plates containing 30 - 300 colonies were considered suitable for enumeration. For samples with higher microbial loads, dilutions such as 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} were used. To ensure that the colony counts were within the appropriate enumeration range, 1 mL aliquots from the respective dilutions were plated [38]. The number of colony-forming units per gram (CFU/g) or per milliliter (CFU/mL) of the original sample was determined based on the dilution factor and volume plated, providing an estimate of the viable microbial load of the samples. The standard formula used to calculate CFU/g or CFU/mL is given in equation 12.

$$\text{CFU/g or CFU/mL} = \frac{\text{Mean colony count} \times \text{Dilution factor}}{\text{Volume plated (mL)}} \quad (12)$$

Where, mean colony count is the average number of colonies observed on the plate, dilution factor is the reciprocal of the dilution (e.g., for 10^{-3}, the dilution factor = 10^3), and volume plated (mL) is the volume of the diluted sample inoculated.

Experimental replication and statistical analysis

All experiments were carried out in triplicate (n = 3), and the results are expressed as mean ± SD. Statistical analysis was performed using IBM SPSS Statistics v20.0 (IBM Corporation, Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to evaluate differences among the samples, followed by Tukey's post hoc test, where appropriate. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Sensory evaluation of peanut milk products

The sensory analysis of three peanut acidophilus curd products (control, sucrose-sweetened, and jaggery-sweetened), one control kefir (dairy milk kefir), and two peanut milk kefir products (plain and jaggery-sweetened) revealed differences in product acceptability, and the areas requiring improvement are presented in table 1. The jaggery-sweetened curd received the highest overall liking score of 7.09. In contrast, the sugar-sweetened curd received the lowest score of 5.36, primarily due to complaints regarding its excessive nutty flavor. The jaggery-sweetened kefir received the highest overall liking score of 7.01, which may be attributed to its sweetness, which balanced the traditional sour taste. The plain kefir received a relatively low score of 4.86 because it was not sufficiently sweet, indicating that the addition of fruits or spices could improve its taste. These observations emphasize the importance of sensory evaluation in product development, as it helps assess consumer preferences and facilitates modifications to product formulations [28]. The samples with the highest overall acceptability scores, namely, jaggery-sweetened curd (C) and jaggery-sweetened kefir (S2), were subsequently selected for further analysis.

Table 1: Sensory evaluation of peanut curd and kefir.

Product		Color	Aroma	Taste	Texture	After taste	Overall acceptance
Peanut curd	A	6.18 ± 0.52^{a}	6.09 ± 0.61^{a}	5.73 ± 0.48^{a}	5.91 ± 0.56^{a}	5.05 ± 0.63^{a}	5.36 ± 0.47^{a}
	B	6.45 ± 0.43^{a}	7.09 ± 0.58^{b}	6.64 ± 0.51^{b}	4.64 ± 0.67^{b}	4.46 ± 0.71^{b}	5.91 ± 0.54^{b}
	C	7.36 ± 0.39^{b}	6.64 ± 0.46^{ab}	7.27 ± 0.42^{c}	7.27 ± 0.51^{c}	6.23 ± 0.49^{c}	7.09 ± 0.45^{c}
Peanut kefir	S	6.45 ± 0.44^{a}	5.39 ± 0.59^{a}	6.30 ± 0.47^{a}	5.20 ± 0.53^{a}	4.70 ± 0.62^{a}	5.60 ± 0.50^{a}
	S1	5.40 ± 0.58^{b}	5.18 ± 0.64^{a}	4.73 ± 0.68^{b}	4.09 ± 0.61^{b}	4.90 ± 0.55^{a}	4.86 ± 0.57^{b}
	S2	7.14 ± 0.36^{c}	6.18 ± 0.42^{b}	7.09 ± 0.40^{c}	7.20 ± 0.45^{c}	7.00 ± 0.38^{b}	7.01 ± 0.41^{c}

Note: Values are presented as mean ± SD of sensory scores based on a 9-point hedonic scale. Within each column, means with different superscript letters differ significantly ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's HSD post hoc test. Means sharing one or more common superscript letters are not significantly different. For example, a mean denoted by "^{ab}" does not differ significantly from means labelled "^{a}" or "^{b}" but differs significantly from means with no common superscript letter.

Similar observations have been reported in previous studies on plant-based fermented beverages, including peanut milk yogurt and other probiotic fermented products, which showed that the addition of natural sweeteners such as jaggery, honey, or fruit pulp significantly improved the texture, flavor, aroma, and overall acceptability of the products by masking the characteristic beany flavor of legumes [39, 40]. Fermented peanut- and soy-based beverages generally develop pronounced acidic and nutty flavors during fermentation, which may reduce sensory acceptance unless balanced with sufficient sweetness [41]. Moreover, studies on non-dairy kefir prepared from plant-based milks have reported that sweetened formulations received higher sensory scores than plain formulations due to better taste balance and reduced perception of sourness [42]. These findings are consistent with the present study, in which jaggery-sweetened peanut curd and kefir exhibited higher sensory acceptance than their unsweetened or sugar-sweetened counterparts.

Proximate analysis

The results of the proximate analysis of the developed samples are presented in figure 7.

Moisture content

The moisture content of the developed peanut curd and peanut milk kefir was found to be 82.7% and 88.7%, respectively. Previous studies have reported similar moisture contents, including 84.8% for peanut milk-based fermented curd [43] and 85.1 - 89.4% for kefir [25]. Similarly, Milanda and Kusuma [44] reported a moisture content of 87.5% in cow's milk kefir. However, slight differences were observed compared with the results reported by Arslan [45], who found moisture contents ranging from 89% to 90%.

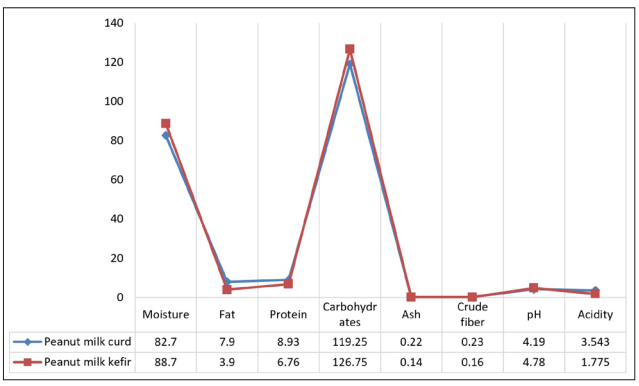


Figure 7: Proximate analysis of peanut milk-based developed products.

Fat content

The fat content of peanut curd (7.9%) was higher than the 3.97 - 7% reported for peanut milk-based *misti doi*. This difference could be attributed to variations in peanut varieties, processing methods (e.g., roasting) [46], or the ratio of peanuts to water used during milk preparation. In contrast, the fat content of jaggery-supplemented peanut milk kefir was 3.9%, which was slightly higher than that of dairy milk kefir (3.4%) [47]. This difference may be attributable to variations in the production process or potential errors during fat extraction. These results highlight the influence of raw materials and processing methods on the fat content of peanut-based dairy substitutes. Furthermore, the use of live bacteria and kefir cultures may have influenced lipid metabolism during fermentation, potentially affecting fat retention or microbial lipid synthesis.

Protein estimation

The average titration value for peanut curd was 0.3, which corresponds to an estimated protein content of 8.93%. This value was higher than the protein content typically reported for dairy curd (3 - 4%) [48]. This finding is consistent with previous research indicating that fermentation of plant-based products can enhance protein levels through microbial activity [49]. In peanut milk kefir, the average titration value was 0.2 mL, resulting in an estimated protein content of 6.76%. This value was higher than the protein content reported for commercial peanut milk, which ranges from 2.5% to 3.3% [50]. The findings of the present study support the potential of peanut-based products as nutrient-dense foods and potential protein sources, particularly for individuals following meat-free or dairy-free diets. These results indicate that both peanut milk curd and peanut milk kefir may contribute to dietary protein intake due to their relatively high protein content.

Carbohydrate estimation

The carbohydrate contents of peanut curd and peanut milk kefir were 119.25 mg/100 mL and 126.75 mg/100 mL, respectively. The carbohydrate content of peanut milk kefir (126.75 mg/100 mL) was significantly lower than that of dairy milk kefir (4 - 5 g/100 mL) [48] and peanut milk (8000 mg/100 mL) reported by Ramesh [46]. Similarly, the

carbohydrate content of peanut curd (119.25 mg/100 mL) was lower than that reported for dairy products. These findings suggest that peanut-based alternatives may be suitable for individuals seeking low-carbohydrate food options and for those who are lactose intolerant.

Ash content

The ash content of peanut curd (0.22%) was higher than that of peanut-based kefir (0.14%). In comparison, the ash content of other plant-based kefirs has been reported to be higher. For example, the ash content of a fermented beverage prepared from soy milk was reported to be 0.41% [51]. Similarly, the ash content of kefir samples prepared from coconut milk was reported to be 0.54% and 0.59% [52].

Crude fiber

Peanut-based curd exhibited a crude fiber content of 0.23 ± 0.05%, which may be attributed to the naturally occurring fiber in peanuts. A slight reduction in crude fiber content (0.16 ± 0.05%) was observed in peanut kefir following the addition of jaggery, which may be attributed to dilution effects or interactions between jaggery components and fiber during fermentation. These results are consistent with previous studies indicating that plant-based substrates can contribute to higher fiber content in fermented beverages. For example, studies on kefir prepared from mixtures of cow's milk and almond milk reported higher dietary fiber content, highlighting the contribution of plant-based ingredients to fiber enrichment [52].

pH

The pH of peanut curd (4.19) was slightly lower than that of peanut kefir (4.78), consistent with findings from previous studies on peanut curd [53], in which pH values ranged from 3.62 to 4.29. This observation is consistent with previous studies showing that, during fermentation, the pH of fermented products decreases as fermentable sugars are converted into organic acids [53]. The decrease in pH contributes to the characteristic sour flavor and may enhance the shelf stability of fermented products [54].

Acidity test

The peanut kefir samples showed an average acidity of 1.775 ± 0.188 mEq/g (mean ± SD), whereas the peanut curd samples showed a considerably higher average acidity of 3.543 ± 0.586 mEq/g. The comparatively higher acidity of peanut curd is consistent with the expected outcome of lactic acid fermentation. This may reflect the ability of *L. acidophilus* to utilize complex sugars such as raffinose and stachyose [55], thereby increasing total acidity. These findings indicate that the addition of jaggery to peanut milk kefir may influence the acidity and pH of the fermented product compared with dairy milk kefir, which exhibits pH values ranging from 4.0 to 4.6 after fermentation [56].

Phytochemicals analysis

Figure 8 presents the phytochemical properties and antioxidant activity of the fermented products prepared from peanut milk.

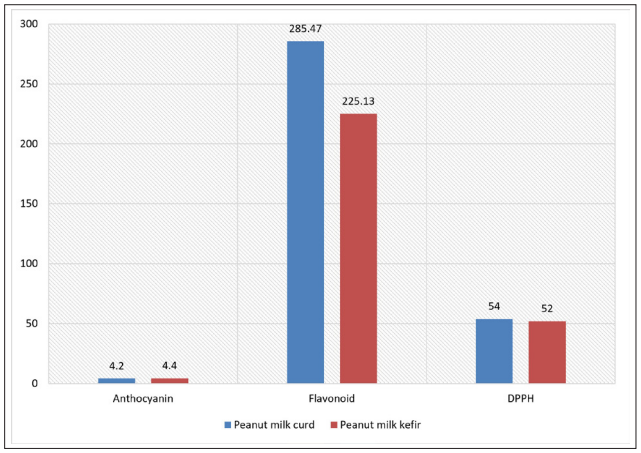


Figure 8: Phytochemical analysis of peanut milk-based products.

TAC

The absorbance values measured at pH 1.0 (A510 = 0.207, A700 = 0.1566) and pH 4.5 (A510 = 0.168, A700 = 0.1326) were used to calculate the TAC. The resulting TAC was estimated to be 4.2 mg C3G eq/L in peanut curd and 4.4 mg C3G eq/L in peanut kefir. The peanut milk was prepared from peeled peanuts; therefore, the seed coat, which is the main source of anthocyanins in peanuts, was removed prior to processing. Consequently, the low TAC values obtained in this study are likely attributable to trace levels of anthocyanin-like compounds detected by the pH differential method. Therefore, the contribution of anthocyanins to the antioxidant activity of the products is expected to be minimal. Other phenolic compounds and flavonoids are more likely to be responsible for the observed antioxidant activity [57]. Future studies using chromatographic techniques, such as high-performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry, are recommended to confirm the presence and quantify the anthocyanin compounds.

TFC

The TFC of peanut curd and kefir prepared with unpeeled peanuts (including the skins) was determined to be 285.47 ± 5.87 mg QE/100 mL and 225.13 ± 3.55 mg QE/100 mL, respectively. These values were considerably higher than the TFC typically reported for peanut peel alone (10 - 50 mg QE/100 g) [58], where most flavonoids are concentrated. The fermentation process may also increase the flavonoid content of curd and kefir [59]. However, the high oil content of peanuts may interfere with TFC determination, potentially leading to an overestimation of the actual flavonoid content [60, 61].

DPPH radical scavenging activity

The DPPH radical inhibition percentages of methanolic extracts were 54% and 52% for peanut curd and peanut kefir, respectively. These findings indicate that the peanut-based fermented products exhibited lower antioxidant activity than traditional dairy milk kefir [62]. However, the high oil content of peanuts can interfere with DPPH determination using spectrophotometry, potentially leading to an underestimation of the actual inhibition [60]. Although spectrophotometry is a commonly used method for antioxidant determination,

more precise techniques, such as HPLC, may be required to accurately quantify antioxidant compounds in high-oil peanut samples and to minimize potential interference from lipids [63]. Further investigation of the bioavailability and bioactivity of these compounds *in vivo* is also warranted.

Microbiological analysis

Colony counts at dilutions of 10^0, 10^1, and 10^2 for curd and 10^0 to 10^6 for kefir were too numerous to count (TNTC), indicating a very high microbial load, similar to that reported for commercial curd [7] (Figure 9). The mean colony count of 212.67 ± 4.73 colonies at the 10^{-3} dilution, which was within the countable range (30 - 300 colonies), was used to estimate the total viable bacterial count of peanut curd at 2.13 × 10^5 CFU/mL. Similarly, 190 colonies were counted at the 10^{-7} dilution for peanut kefir, corresponding to a total viable bacterial count of 1.9 × 10^9 CFU/mL. The microbial counts at different dilution levels are presented in table 2.

The high viable counts observed in both fermented products indicate successful microbial fermentation. Similar microbial populations have been reported in commercial fermented dairy products. Curd generally contains 10^6 - 10^9 CFU/g, whereas kefir generally contains 9.23 - 9.55 log CFU/mL of viable microorganisms [7]. The observed microbial counts are also consistent with the acidic environment of the fermented products (pH 4.19 - 4.78), which is favorable for the growth of *L. acidophilus* and other lactic acid bacteria [64]. Furthermore, the incorporation of jaggery as a natural sweetener may have provided additional fermentable carbohydrates, thereby supported microbial growth and improved the viability of the starter cultures during fermentation.

According to international recommendations, probiotic foods should contain a minimum viable count of approximately 10^6 CFU/mL or CFU/g at the time of consumption to confer potential health benefits [65-68]. The microbial counts obtained in the present study exceeded this recommended threshold, suggesting that the developed peanut curd and peanut kefir possessed adequate viable microbial populations. However, microbial enumeration alone does not confirm probiotic efficacy, and further studies involving strain identification, survival under simulated gastrointestinal conditions, and functional characterization are required to establish their probiotic potential.

The use of boiling (100 °C for 10 min) before inoculation helped reduce the indigenous microbial population and improve the microbial safety of the peanut milk without affecting starter culture viability, as the cultures were added only after the peanut milk had cooled to the optimum inoculation temperature (approximately 45 °C). This heat treatment also helps improve the stability and safety of plant-based milk prior to fermentation.

Conclusion

The present study successfully formulated peanut milk-based vegan curd and kefir using *L. acidophilus* and a commercial

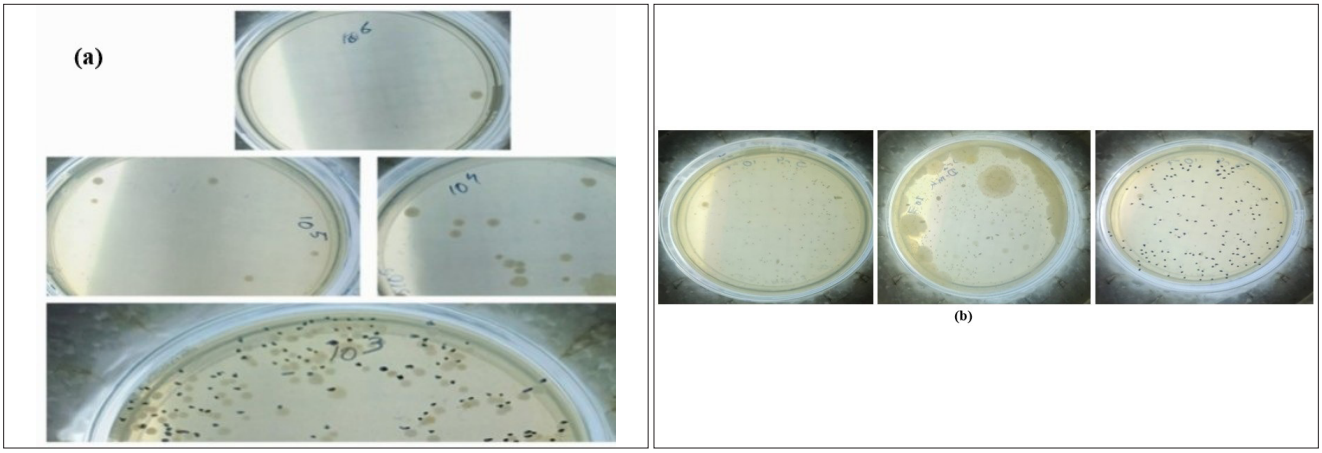


Figure 9: Total plate count of **(a)** peanut curd, and **(b)** peanut kefir at different dilutions.

Table 2: Microbial counts (CFU/ml) of peanut curd and kefir at different dilutions.

Sample	Dilution	Mean colony count	CFU/mL
C	10^{-2}	TNTC	TNTC
	10^{-3}	212.67	2.13 × 10^5 CFU/mL
	10^{-4}	131.67	1.32 × 10^6 CFU/mL
	10^{-5}	15.00	1.50 × 10^6 CFU/mL
	10^{-6}	2.67	2.67 × 10^6 CFU/mL
S2	10^{-1} - 10^{-6}	TNTC	TNTC
	10^{-7}	190.95	1.91 × 10^9 CFU/mL
	10^{-8}	110.00	1.10 × 10^{10} CFU/mL
	10^{-9}	24.00	2.40 × 10^{10} CFU/mL

Note: C = peanut milk-based curd with jaggery and S2 = peanut milk kefir with jaggery.

kefir starter culture. Sensory evaluation revealed that the jaggery-supplemented formulations achieved the highest acceptability scores, indicating strong consumer preference and potential commercial viability. Proximate analysis confirmed appreciable levels of protein and fat. Phytochemical assays showed notable flavonoid concentrations and moderate antioxidant activity, with 54% DPPH inhibition in the curd and 52% in the kefir. The antioxidant potential was mainly attributed to phenolic compounds and flavonoids, as peeled peanuts were used, thereby minimizing the contribution of anthocyanins. Microbiological analysis showed high viable counts after fermentation, indicating successful microbial growth and survival of the starter cultures. Overall, peanut milk proved to be a suitable substrate for producing fermented plant-based products with desirable nutritional, physicochemical, microbiological, and sensory properties. These findings highlight the potential of peanut curd and kefir as functional foods and sustainable dairy alternatives, particularly for lactose-intolerant and vegan populations. However, further investigations into shelf-life stability, texture optimization, probiotic functionality, and the bioavailability of bioactive compounds are warranted to strengthen their commercialization prospects and global acceptance.

Future Prospects

Future investigations should focus on optimizing fermentation parameters by exploring diverse starter cultures and assessing their probiotic properties and sensory attributes in peanut curd and kefir, particularly with regard to the beany aftertaste associated with peanut-based products. Additionally, different natural and artificial sweeteners could be evaluated, and the products could also be fortified with different fruits and berries to enhance their functional properties. Furthermore, evaluating the shelf life under different storage conditions and conducting comprehensive microbiological studies could further strengthen the findings of the present study. Further research could help establish peanut milk curd and kefir as sustainable, nutritious, and commercially viable dairy alternatives, thereby catering to the rising demand for plant-based probiotic products.

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Conflict of Interest

None.

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