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**FUNCTIONAL PROPERTIES OF YOGURT PREPARED USING A
BLEND OF ALMOND MILK (*Prunus dulcis*) AND PEANUT MILK
(*Arachis hypogaea L.*)**

ANGELINE BC* AND NORA VK

1: M. Sc, Foods and Nutrition, Women's Christian College, Chennai – 600 004, Tamil Nadu, India

2: Associate Professor, Department of Home Science, Women's Christian College, Chennai – 600 006, Tamil Nadu, India

***Corresponding Author: Ms. Angeline Brenda. C: E Mail: angelinebrenda.c@gmail.com**

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ABSTRACT

In response to the growing demand for plant-based alternatives, yogurt blends prepared from almond milk (*Prunus dulcis*) and peanut milk (*Arachis hypogaea L.*) were developed and assessed. Two yogurt blends were formulated: Yogurt Blend 1 (25 mL almond milk: 75 mL peanut milk) and Yogurt Blend 2 (75 mL almond milk : 25 mL peanut milk). Phytochemical analysis demonstrated higher phenol and flavonoid content in Yogurt Blend 1, attributed to its higher peanut milk content. Antioxidant potential, measured by DPPH assay, showed strong radical scavenging activity in both blends, with Yogurt Blend 2 displaying a lower IC₅₀ value. Probiotic counts were higher in Yogurt Blend 2, meeting FSSAI standards, and physicochemical assessment showed that Yogurt Blend 2 had a lower pH indicating increased fermentation and the titratable acidity range was lower in both the yogurts compared to commercially available yogurts. The study concludes that both yogurt blends offer significant antioxidant and probiotic benefits, serving as plant-based alternatives for consumers seeking functional and health-promoting products.

Keywords: Plant based milk, yogurt, probiotic, sporlac, nuts, functional foods

INTRODUCTION

Plant-based milks are becoming increasingly popular, particularly for individuals with dietary preferences such as veganism, lactose intolerance, or allergies. These products are made from water extracts of plants like nuts, seeds, and beans, which resemble the appearance and consistency of dairy milk due to sufficient breakdown and homogenization [1]. Fermentation of plant-based milks activates bioactive compounds, providing functional food benefits [2].

Almond milk is rich in antioxidants that protect against ailments like cancer and heart disease, and homemade versions provide calcium [3]. Similarly, peanut milk is a good source of antioxidants like p-coumaric acid, and is rich in protein, minerals, and essential fatty acids such as linoleic and oleic acids, making it valuable for human health [4, 5].

Almond and peanut milk are suitable for fermentation, but care must be taken to prevent aflatoxin contamination, commonly associated with nuts under poor pre-harvest and storage conditions [6]. Lactic acid fermentation can significantly reduce mycotoxins, including aflatoxins and fumonisins [7, 8].

Yogurt is a fermented dairy product containing live lactic acid bacteria, produced through lactic acid fermentation using strains of *Streptococcus* and *Lactobacillus*, either individually or in

mixed cultures [9, 10]. Yogurt is a widely consumed probiotic food with significant health benefits [9].

Probiotics and Prebiotics are the best-known functional compounds [11]. The Food Agricultural Organization/World Health Organization (FAO/WHO) has defined Probiotics as “Live microorganisms which when administered in adequate amounts confer a health benefit to the host”. Probiotics have been shown to aid in the treatment of diarrhea and bowel disorders, while also benefiting the immune system and enhancing intestinal integrity [12].

Probiotics can be consumed as live cells in pill form, but incorporating them into food offers greater benefits, they help in improving the biological and functional value of the food [13].

Strains are selected based on their specific health-promoting effects, but the safety aspects are also carefully considered to make sure that the probiotics enriched should not cause health risks to the consumer [14]. In 1949, a Japanese physician, Dr. Nakayama, isolated *B. coagulans* from green malt. It is marketed in India under the brand name Sporlac and has been designated as strain SNZ 1969. *Lactobacillus sporogenes*, a previous name of *B. coagulans*. Its consumption as an added food ingredient from its intended use up to 2×10^9 cfu/serving and estimated daily

intakes of 36.4×10^9 cfu of Sporlac spores/day is considered safe. Based on a critical evaluation of the publicly available data, described and summarized herein, the Expert Panel members have individually and collectively concluded that *Bacillus coagulans* SNZ 1969 spore preparation, when used as a food ingredient in infant formula at use levels of 2×10^8 cfu per 100 ml of formula, as ready for consumption, resulting in a maximum estimated daily intake of 4.27×10^8 cfu *B. coagulans* SNZ 1969 spores/kg bw, is considered safe and Generally Recognized As Safe (GRAS) [15].

The species *L. sporogenes* was originally isolated and described in 1933 by Horowitz- Wlassowa and Nowotelnow and subsequently reclassified as *Bacillus sporogenes*. Even if some commercial products are still labelled as “*L. sporogenes*”, it is well known that *L. sporogenes* is to be renamed as *B. coagulans* [16]. *B. coagulans* is considered a probiotic by FSSAI [17].

MATERIALS AND METHODS

Procurement of ingredients

Almond (*Prunus dulcis*) and peanut (*Arachis hypogaea* L.) were purchased from

local supermarket, nuts were FSSAI-certified. Sporlac DS tablet was purchased from a local pharmacy.

Standardization and preparation of Almond-Peanut yogurt blends

Preparation of almond milk and peanut milk

Fifty grams of almonds and roasted peanuts (without skin) were soaked in water for 8 hours. After 8 hours of soaking, the skin of the almonds was removed. The soaked almonds and roasted peanuts were blended separately until smooth by adding 150 ml of water to procure the milk. Finally, the almond milk and peanut milk were strained using a muslin cloth.

Preparation of Almond-Peanut yogurt blends

Two blends of yogurt were prepared using two different proportions of almond milk and peanut milk blended. The starter culture used to prepare the yogurt is a probiotic tablet named Sporlac DS.

Yogurt Blend 1 – 25ml of almond milk will be mixed with 75ml of peanut milk

Yogurt Blend 2 – 75ml of almond milk will be mixed with 25ml of peanut milk



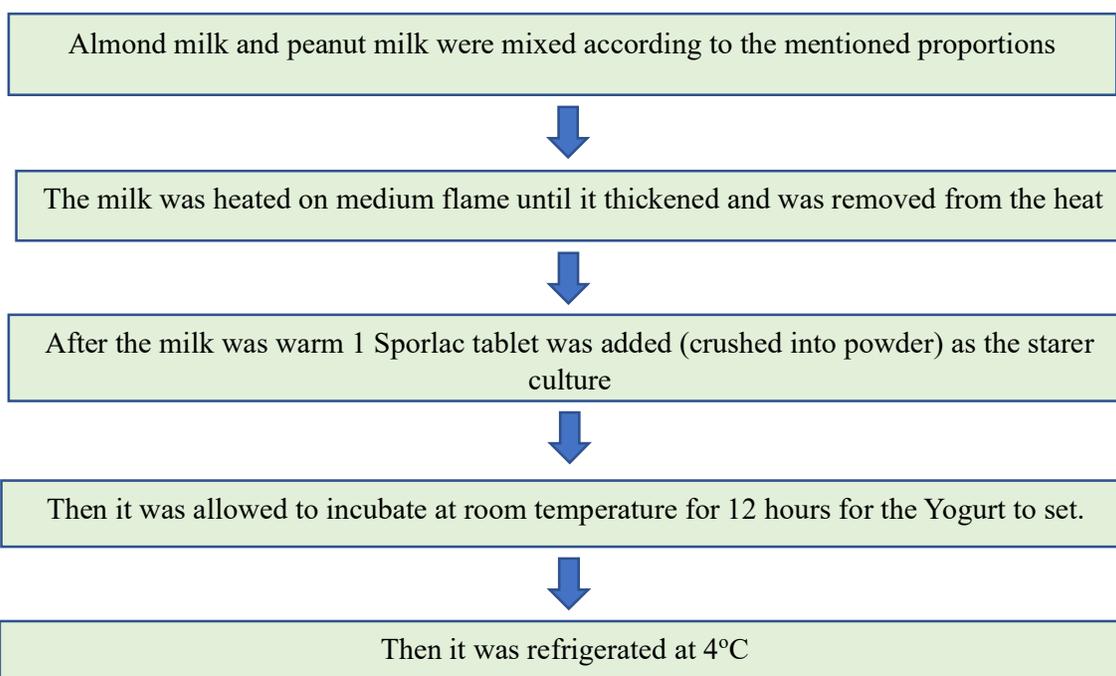
Plate 1: Yogurt Blend 1– 25ml:75ml



Plate 2: Yogurt Blend 2 – 75ml:25ml

Figure 1

Method of yogurt preparation



Antioxidant potential of Almond-Peanut yogurt blends

DPPH radical scavenging assay

The radical scavenging capacity of the extract was measured based on DPPH (1, 1- diphenyl 2-picrylhydrazyl) radical scavenging activity. One mL of 0.1 mM DPPH solution in methanol was mixed

with 1 mL of various concentrations (50-300 µg/mL) of samples. The mixture was then allowed to stand for 30 min incubation in dark. One mL methanol mixed with 1 mL DPPH solution was used as the control The decrease in absorbance was measured at 517 nm [18]. The percentage of inhibition was calculated as

$$\% \text{ of DPPH } \cdot \text{ radical inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Phytochemicals in the Almond-Peanut yogurt blends

Total phenol content

Folin-Ciocalteu reagent method was used with slight modifications. To 100 µL of extract (1mg/mL), 900 µL methanol was added. Then 1 mL of Folin Ciocalteu reagent was added (1:10 diluted with distilled water). To this 1 mL of 20 per cent sodium carbonate solution was also added, shaken well and incubated at room temperature for 30 mins in dark. Absorbance of supernatant was taken at 765 nm in UV-Vis spectrophotometer. The total phenol content was expressed in terms of gallic acid equivalent, which is a reference compound [19].

Total flavonoids content

Aluminum chloride reagent method was used with slight modifications. To 500µL of extract from 1mg/mL solution,

500µL methanol was added. To this 1 mL of 5 per cent sodium nitrite solution is added. Then 1 mL of 10 per cent aluminum chloride solution was added and incubated for 5 min at room temperature. To this 1 mL of 1 M NaOH solution was added and then incubated for 30 mins in room temperature. The absorbance of the supernatant was taken at 510 nm in UV-Vis spectrophotometer. The result was expressed as (µg/mg of extract) quercetin equivalent [20].

Total count of probiotic bacteria present in Almond-Peanut milk and yogurt blends

The test sample was prepared in decimal dilutions and transferred into the Petri dishes using a sterile pipette. MRS agar was prepared at pH 5,7 and was poured into sterile Petri dishes containing the sample in different dilutions. The Petri dishes were incubated at 30 °C for 24 h. The colony

counter was used to count the colonies. From the number of colonies obtained and possibly confirmed, the number of mesophilic lactic acid bacteria per millilitre of test sample was calculated [21].

Physicochemical properties of Almond-Peanut yogurt blends

A pH meter is an electronic instrument used to measure acidity or alkalinity of a solution - also known as pH [22].

In yogurt blends, the titratable acidity is expressed as the percentage of lactic acid present. The titratable acidity of the two yogurt blends was found out using the titration method according to Bureau of Indian Standards [23].

Statistical analysis

The software used for statistical analysis was SPSS version 21

**RESULTS AND DISCUSSION
SPECIFIC PHYTOCHEMICALS IN ALMOND-PEANUT YOGURT BLENDS**

Phytochemicals in yogurt blends 1 and 2 were estimated and the findings are presented in Table 1 and Figure 2 and 3.

From Table 1, the results have shown that the phenol content in 100 grams of yogurt blend 1 and 2 was estimated to be 219.940 ± 2.615 GAE (µg/ml) and 192.620 ± 1.376 GAE (µg/ml) respectively. Out of the two yogurt blends the phenol content was higher in the yogurt blend that contained higher amount of peanut milk.

Table 1: Quantity of phenols and flavonoids in yogurt blends 1 and 2

PHYTOCHEMICALS	Yogurt Blend 1 25ml:75ml (Mean ± SD)	Yogurt Blend 2 75ml:25ml (Mean ± SD)	't' value	'p' value
Phenols GAE (µg/ml)	219.940 ± 2.615	192.620 ± 1.376	16.016	0.000***
Flavonoids QE (µg/ml)	33.240 ± 0.453	27.807 ± 0.723	11.031	0.000***

NS – Not significant, *** - Significant at p<0.05 level

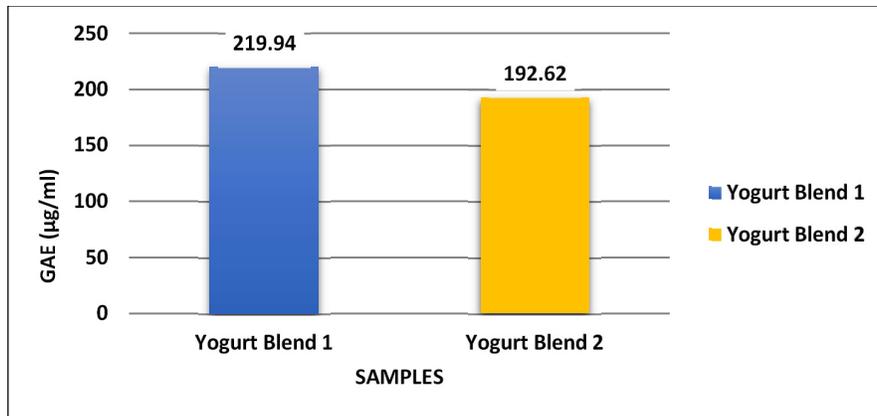


Figure 2: Comparison of total phenol content in yogurt blends 1 and 2

From **Table 1**, 100 grams of yogurt blend 1 and 2 contained 33.240 ± 0.453 QE ($\mu\text{g/ml}$) and 27.807 ± 0.723 QE ($\mu\text{g/ml}$) of flavonoids. Out of the two yogurt blends the flavonoid content was higher in the yogurt blend that contained a higher amount of peanut milk.

Thus it was noted that the yogurt blend that contained higher amount of peanut milk had an increased amount of phenols and flavonoids as a study done by [24] show that peanuts are an excellent source of compounds like phenolic acids and flavonoids.

Kumar and Goel (2019) reported that phenolic acids, which are readily absorbed

through the intestinal tract, provide health benefits due to their antioxidant properties that reduce cell damage from free radicals. Regular consumption of phenolic acids also promotes anti-inflammatory activity [25].

Patel (2008) noted that flavonoids' key functions include acting as antioxidants and preventing coronary heart diseases, along with their effects against ulcers, viruses, inflammation, arthritis, osteoporosis, and diarrhoea [26]. Pietta, (2000), highlighted that flavonoids prevent free radical damage through direct scavenging, forming more stable, less-reactive radicals [27].



Figure 3: Comparison of total flavonoid content in yogurt blends 1 and 2

ANTIOXIDANT POTENTIAL OF ALMOND-PEANUT YOGURT BLENDS

Antioxidant potential of yogurt blends was estimated and the findings are presented in **Table 2, 3 and Figure 4.**

Table 2: Radical scavenging activity of yogurt blends 1 and 2

Concentration ($\mu\text{g/mL}$)	Yogurt Blend 1 25ml:75ml (Mean \pm SD)	Yogurt Blend 2 75ml:25ml (Mean \pm SD)
50	65.23 ± 0.907	4.63 ± 0.053
100	72.23 ± 0.551	22.04 ± 0.036
150	81.23 ± 0.321	31.25 ± 0.031
200	83.23 ± 0.451	68.21 ± 0.032
250	92.53 ± 0.306	69.72 ± 0.020
300	95.23 ± 0.252	81.04 ± 0.032

Yogurt blends 1 and 2 were analysed for their antioxidant activity using DPPH assay. DPPH radical scavenging method was the most common and widely used method for screening the free radical ability of a substance. This assay method was sensitive, easy to do and provided a quick way to filter the activity of radicalization that indicates the presence of an antioxidant substance [28].

From **Table 2**, it can be observed that the percentage of DPPH radical inhibition for yogurt blend 1 and 2 was found to be 95.23 ± 0.252 and 81.04 ± 0.032 at 300 $\mu\text{g/mL}$ concentration.

Figure 4 shows the comparison of the radical scavenging activity of the yogurt blend 1 and 2. In the yogurt blend 2 the percentage of inhibition drastically increased in a concentration dependant manner whereas the percentage of inhibition of yogurt blend 1 was observed to have only a gradual increase as the concentration increased. Therefore, the radical scavenging activity of yogurt blend 1 and yogurt blend 2 was dose dependent indicating that an increase in the concentration of the sample lead to an increase in the antioxidant activity.

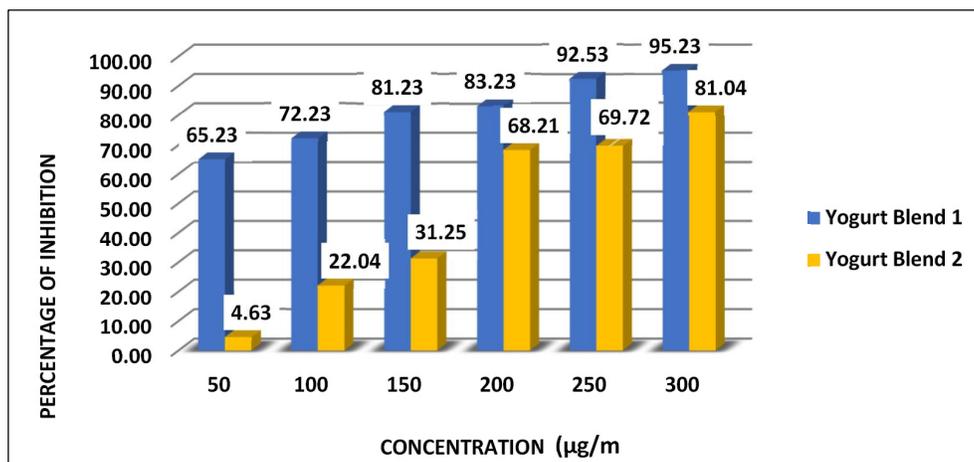


Figure 4: Comparison of radical scavenging activity of yogurt blends 1 and 2

Table 3: The half maximal inhibitory concentration (IC_{50}) of yogurt blends 1 and 2

Yogurt Samples	IC_{50} Value ($\mu\text{g/mL}$)
Yogurt Blend 1	38.32
Yogurt Blend 2	36.65

IC_{50} (inhibitory concentration) is the concentration of the sample required to inhibit half the amount of DPPH radicals. A lower IC_{50} value represents higher

antioxidant potential of the sample. In a study done by Phongpaichit *et al.* (2007) IC_{50} value ranging between 10 to 50 $\mu\text{g/mL}$ is considered to possess strong antioxidant

acitivity. In the present study, the IC₅₀ of the yogurt blends 1 and 2 was 38.32 and 36.65 (µg/mL) respectively [29].

According to a study done by Chen *et al.* (2006) almond has high antioxidant activity owing to the α-tocopherol and polyphenolic constituents [30]. In the present study, it can be concluded that yogurt blend 2 that contained higher amount of almond milk had a good radical scavenging potential with low IC₅₀ value when compared to yogurt blend 1 that contained higher amount of peanut milk. A low IC₅₀ value, as discussed earlier, represents higher antioxidant activity. However, the IC₅₀ value of the yogurt blends 1 and 2 have only a marginal difference, indicating that both the yogurt blends have a good radical scavenging activity.

TOTAL COUNT OF PROBIOTIC BACTERIA IN ALMOND-PEANUT MILK AND YOGURT BLENDS

Total count of probiotic bacteria in milk and yogurt blends was estimated and the findings are presented in Table 4 and plates 3, 4, 5 and 6.

From Table 4, the result for total count of probiotic bacteria in milk blend and

yogurt blend can be interpreted. The total count of probiotic bacteria in the milk blend and yogurt blend 1 was 1.25X10⁶ CFU/ml and 1.95X10⁶ CFU/ml. Out of the milk blend and yogurt blend it was observed that the yogurt blends had a slightly higher count of probiotic bacteria. Similarly, the total count of probiotic bacteria in the milk blend and yogurt blend 2 was 6.5X10⁵ CFU/ml and 7.650X10⁶ CFU/ml. In comparison of milk blends with yogurt blends, the yogurt blends had increased number of probiotic bacteria.

According to a study done by Robinson, (1987) and Salminen *et al.* (1998) the suggested concentration for probiotic bacteria is in the range 10⁶-10⁷ CFU/g of the product [14, 31]. Lourens-Hattingh and Viljoen, (2001) in their review of several reports have shown that survival and viability of probiotic bacteria is often low in yoghurt and result in less than 10⁸-10⁹ cells daily recommended intake [32]. Thus, in the present study it was observed that both the yogurt blends have 10⁶ CFU/ml, indicating it as a good probiotic product meeting the required range specified by the FSSAI.

Table 4: Total count of probiotic bacteria in milk and yogurt blends 1 and 2

TOTAL COUNT OF PROBIOTIC BACTERIA	Blend 1 25ml:75ml	Blend 2 75ml:25ml
Milk Blend	1.25X10 ⁶ CFU/ml	6.5X10 ⁵ CFU/ml
Yogurt Blend	1.95X10 ⁶ CFU/ml	7.650X10 ⁶ CFU/ml

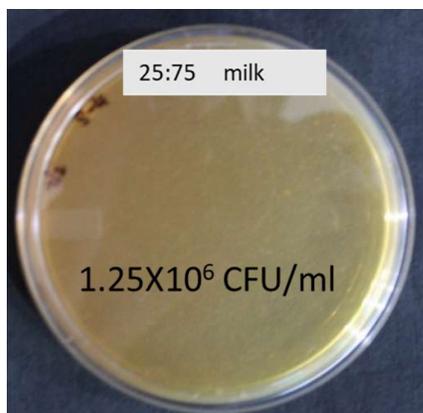


Plate 3: Total count of probiotic bacteria in milk blend 1

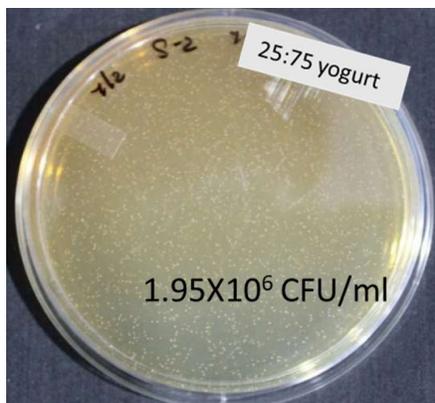


Plate 4: Total count of probiotic bacteria in yogurt blend 1

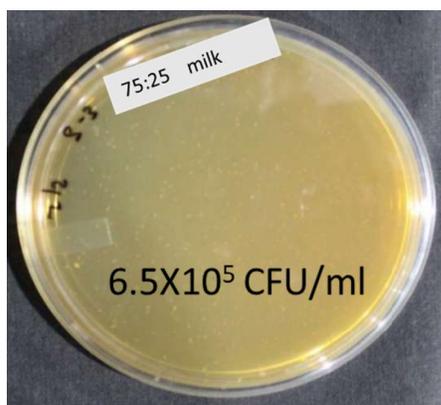


Plate 5: Total count of probiotic bacteria in milk blend 2

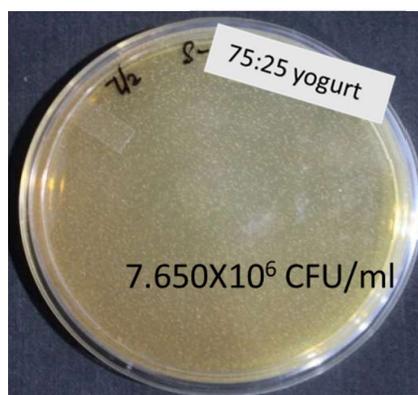


Plate 6: Total count of probiotic bacteria in yogurt blend 2

PHYSICOCHEMICAL PROPERTIES (PH AND TITRATABLE ACIDITY) OF ALMOND-PEANUT YOGURT BLENDS

The pH of milk blends and yogurt blends were estimated and the findings are presented in **Table 5** and **Figure 5**.

From **Table 5**, it was observed that the pH of the milk blend and yogurt blend 1 was 7.023 ± 0.0252 and 6.623 ± 0.021 . The

pH of the milk blend and yogurt blend 2 was 6.747 ± 0.031 and 6.440 ± 0.021 respectively. According to a study done by Lomer *et al.* (2008) if the pH is below 7 it is an advantage as it will discourage the growth of pathogens that may cause gastrointestinal problems [33]. Thus, in the present study, pH of the two yogurt blends is below 7 indicating that the pathogen growth is controlled.

Table 5: pH of milk and yogurt blends 1 and 2

pH		(Mean ± SD)	't' value	'p' value
Blend 1 25ml:75ml (Mean ± SD)	Milk blend	7.023 ± 0.0252	69.282	0.000***
	Yogurt blend	6.623 ± 0.021		
Blend 2 75ml:25ml (Mean ± SD)	Milk blend	6.747 ± 0.031	34.773	0.001***
	Yogurt blend	6.440 ± 0.021		

NS – Not significant, *** - Significant at $p < 0.05$ level

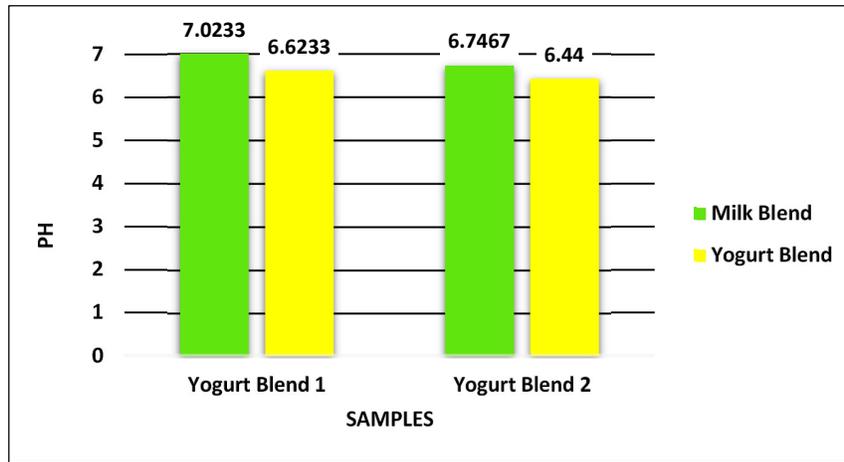


Figure 5: Comparison of pH of milk blend and yogurt blend

Table 6: Titratable acidity of the yogurt blends 1 and 2

Titratable acidity	Yogurt Blend 1 25ml:75ml (Mean % ± SD)	Yogurt Blend 2 75ml:25ml (Mean % ± SD)	't' value	'p' value
	0.0353 ± 0.0025	0.065 ± 0.0036	11.686	0.000***

NS – Not significant, *** - Significant at p<0.05 level

The titratable acidity of yogurt blends were estimated and the findings are presented in **Table 6** and **Figure 6**.

From **Table 6**, it can be observed that the titratable acidity of yogurt blends 1 and 2 was $0.0353 \pm 0.0025\%$ and 0.065 ± 0.0036 per cent respectively.

In comparison, the titratable acidity was higher in the yogurt blend that contained high amounts of almond milk. According to a study done by Rai *et al.*

(2018) acid production in the medium depends on the growth of microorganisms and their ability to ferment the available carbohydrates [34]. In a study done by Matela *et al.* (2019) the titratable acidity of commercially available diary yogurts ranges from 0.6 – 1.8 per cent [35]. Therefore, in the present study it was observed that the acidity range was lower compared to diary yogurt which indicates a reduced development of lactic acid.

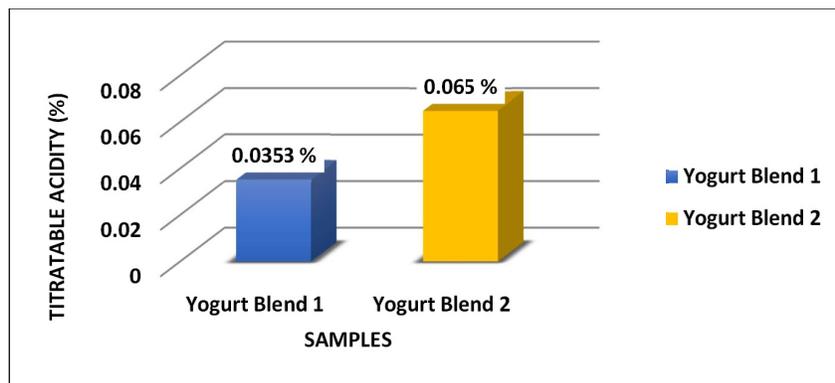


Figure 6: Comparison of titratable acidity of yogurt blends 1 and 2

CONCLUSION

The present study aimed to assess the yogurt blends prepared using almond milk and peanut milk. From the results obtained, it can be concluded that the yogurt blend 1 that contained a high amount of peanut milk had higher amounts of phenols and flavonoids. Yogurt Blend 2 which contained a high amount of almond milk had greater antioxidant potential, a higher count of total probiotic bacteria and a slightly higher acidic range compared to Yogurt Blend 1.

Future Prospects

The total count of probiotic bacteria can be increased by using a probiotic capsule/tablet with a different strain. The other bioactive components present in the yogurt blend can be identified and quantified.

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