

Original Research Article

Formulation and Quality Analysis of Yoghurts Fortified with *Menthaspicata*, *Moringaoleifera*, and *Murrayakoenigii* Leaf Extracts

ABSTRACT

The aim of this study was to investigate the impact of incorporating *Menthaspicata* (mint), *Moringaoleifera* (*moringa*), and *Murrayakoenigii* (curry) leaf extracts into yoghurt to enhance its nutritional and therapeutic benefits. The study assessed the sensory, physicochemical, microbiological, and antibacterial properties of the fortified yoghurts along with control. Among the four yoghurt samples, curry leaf extract-fortified yoghurt has the fastest setting time (4.5 h), highest protein content (3.42%) and lowest syneresis (8%) and was the most preferred in sensory analysis, excelling in color, texture, and overall acceptability ($p=0.05$). It also showed the strongest antibacterial activity against *Salmonella* and *Staphylococcus aureus*. All yoghurt samples were found to be safe for consumption. The incorporation of leaf extracts significantly improved the growth of lactic acid bacteria, sensory qualities and functional benefits of yoghurt, making them promising ingredients for developing health-promoting beverages. However, there is a need for optimization when using these extracts to reduce its adverse taste effects, particularly at higher concentrations.

Keywords: Yoghurt fortification, Functional yoghurt, Mint, Moringa, Curry leaves extract, Phytochemicals, Antimicrobial activity

1. INTRODUCTION

Fermented milk products, such as yoghurt, are among the most widespread and nutritionally valuable foods available. Yoghurt, manufactured from milk by lactic acid fermentation enabled by symbiotic yoghurt cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*, is renowned for its increasing global consumption and popularity. This popularity is attributed to yoghurt's significant nutritional benefits and health advantages. Yoghurt offers several health benefits, including improved lactose tolerance and physiological advantages such as antimicrobial and anticancer properties. Additionally, it has been shown to combat gastrointestinal infections, stimulate the immune system, and reduce serum cholesterol levels [1, 2, 3].

The incorporation of botanical extracts into yoghurt has garnered significant interest due to their potential to enhance the nutritional, sensory, and therapeutic properties of this widely consumed dairy product. Recent studies have highlighted the benefits of integrating natural

extracts, such as those from mint, moringa, and curry leaves, into yoghurt, leveraging their diverse phytochemical profiles for improved health benefits and sensory appeal [4, 5, 6]. These plant extracts are known for their rich content of bioactive compounds including alkaloids, flavonoids, and phenols, which have been associated with various pharmacological and nutritional advantages, such as antimicrobial, antioxidant, and anti-inflammatory activities [4, 7].

Mint (*Mentha spicata*), moringa (*Moringa oleifera*), and curry leaves (*Murrayakoenigii*) each offer unique phytochemical compositions that may influence the sensory and functional properties of yoghurt. Mint is praised for its robust flavor and potential health benefits due to its high content of essential oils and phenolic compounds [8]. Moringa, often referred to as a "superfood," is noted for its high nutritional value and medicinal properties, though its taste can be a limiting factor when used in high concentrations [9]. Curry leaves, known for their distinctive flavor and therapeutic properties, have shown potential in improving the sensory quality and stability of yoghurt [10].

This study aims to explore the impact of these extracts on yoghurt's sensory attributes, physicochemical properties, and microbiological quality. By evaluating their effects, this research seeks to identify the most effective and acceptable natural ingredient for yoghurt fortification, ultimately enhancing its nutritional and therapeutic value while meeting consumer preferences.

2. MATERIAL AND METHODS

2.1 Plant material collection and preparation of leaves extracts

Samples of mint (*Mentha spicata*), moringa (*Moringa oleifera*) and curry leaves (*Murrayakoenigii*) were collected from local markets of Hebbal. Upon arrival at the laboratory, the leaves were separated from leaf stalks and cleaned to remove all foreign matter adhered. The leaves were then washed with potable water followed by salt water and again rinsed with potable water. The leaves were ground finely by using a mechanical blender and filtered using a muslin cloth. The extracts obtained were heat treated at 85°C for 30 min to ensure food safety and used for the addition into yoghurt.

2.2 Preliminary phytochemical screening of secondary metabolites of leaves extracts

Preliminary phytochemical screening for alkaloids (Wagners test), carbohydrates (Molisch's test), cardiac glycosides (Kellar -Kelian test), flavonoids (Alkaline reagent test), polyphenols (Ferric chloride test), saponins (Foam test), tannins (Braymer test) and terpenoids (Salkowski test) was carried out for leaf extracts following standard methods previously reported for their identification and confirmation [11,12,13,14,15].

2.3 Preparation of yoghurt incorporated with leaves extract

Leaf extract incorporated yoghurt was prepared using cow milk subjected to heat treatment at 90°C for 10 minutes and yoghurt starter bacteria (*Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus*). Starter culture (1%) was inoculated at 42°C and mixed. Afterwards, 5% sterilized leaf extract of mint (Y1), moringa (Y2) and curry leaves (Y3) were added to the mixture. The inoculated milk was then transferred to polyethylene terephthalate cups and incubated at 42°C until the coagulum was formed and was followed

by rapid cooling. Yoghurt sample without any extracts served as control (Y). Time taken to get the milk set was noted.

2.4 Physicochemical analysis of prepared yoghurt- Titratable acidity, pH and syneresis

Titrateable acidity was determined using the method described by [16]. Exactly 1g of yoghurt sample was mixed with 9 ml of distilled water. Three drops of phenolphthalein were added to the mixture and titrated against 0.1 M NaOH. Titration end point was indicated by the appearance of a pink colour and titrateable acids (lactic acid percentage equivalent) was calculated from the titre values. For determination of pH, 10 g of yoghurt sample was dissolved in 100 mL of distilled water. The mixture was allowed to equilibrate at room temperature and pH was measured using calibrated digital pH meter (Esico pH meter). Syneresis was measured by spreading 10 g of each yoghurt sample in to a thin layer to cover the surface of Whatman No.1 filter paper and was allowed to rest on the top of a funnel. The yoghurt was filtered under vacuum for 10 min. The quantity of remaining yoghurt was weighed and the percentage of syneresis was calculated as per the equation described by [17].

$$\text{Free whey (\%)} = \frac{\text{Initial weight of sample} - \text{Weight of sample after filtration}}{\text{Initial weight of sample}} \times 100$$

2.5 Analysis of proximate composition of prepared yoghurt

All yoghurt samples were analyzed for their proximate compositions such as moisture, total solids, fat, protein, and lactose according to the AOAC procedures[18].

2.6 Microbiological analysis of prepared yoghurt

For microbial analysis 10 g of sample was aseptically transferred to a flask containing 90 ml sterile phosphate buffer to make the 10^{-1} dilution. Serial dilutions were made and total lactic acid bacteria count, yeast and mold count and coliform count were determined according to standard methods and the results were expressed as log CFU/ml [19]. Total lactic acid bacteria, coliforms and Yeast and molds were enumerated on de Man, Rogosa and Sharpe (MRS) agar incubated anaerobically at 37°C for 24 - 48 hours, Violet Red Bile Agar (VRBA) at 37°C for 16-18 hours and malt extract agar (MEA) with pH adjusted to 3.5 using 10% filter sterile lactic acid at 25 °C for 5-7 days respectively. All the media were sterilized prior to inoculation by autoclaving at 121°C for 15 minutes.

2.7 Antibacterial activity

Agar well diffusion method was used to determine the antibacterial activity of leaf extract incorporated yoghurt samples against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella*. 20 ml of sterilized Muller Hinton agar medium was poured into each sterile petriplate and allowed to solidify. The active test bacteria cultures optimized at concentration of 10^8 CFU/ml, were evenly spread over the media with the aid of a sterile swab stick. Then wells of 7 mm were made in the medium using a sterile cork borer. Yoghurt samples were centrifuged at 8000 rpm for 10 minutes and supernatant collected was filtered and transferred into separate wells (100 μ l), followed by the incubation of the plates at 35 °C for 24 h. After the incubation period, the zones measuring the diameters of zone of inhibition (ZI) were observed and measured in millimeter [14].

2.8 Antibiotic Resistance Analysis

Antibiotic resistance pattern of *Streptococcus thermophilus* and *L.delbrueckii subsp bulgaricus* was checked according to the simple disk diffusion method (Kirby Baur). Briefly, active bacteria at concentrations (10^6 CFU/mL) were cultured on Mueller-Hinton agar. Different antibiotic disks, including Ampicillin (AMP10), Penicillin (P10), Streptomycin (S25), Bacitracin (B10) and Cefoperazone (CPZ75) were placed on the media contained bacteria. The plates were incubated at 37°C (for *S. thermophilus*) and 42°C (for *L. bulgaricus*) for 24–48 h. The diameter of the growth inhibition zone surround each disk was measured [20].

2.9 Sensory evaluation of prepared yoghurt

The organoleptic characteristics such as colour, flavor, texture, appearance and overall acceptability of prepared yoghurt samples were evaluated by 10 trained panelists from the Department of Dairy Microbiology, Dairy Science College, KVAFSU. The samples were evaluated using a nine-point hedonic scale. Score = 1 has been assigned for “like extremely” and score = 9 has been assigned for “dislike extremely” [21].

Statistical Analysis

The data obtained were analyzed using R studio (version 4.2.2). Data was expressed as means \pm standard deviation. Differences among means were analyzed using analysis of variance (ANOVA) in the agricolae package. Significant differences between treatments were evaluated using Tukey’s HSD test at $p = 0.05$.

3. RESULTS AND DISCUSSION

3.1 Preliminary phytochemical screening of secondary metabolites of leaves extracts

The phytochemical profiles of mint, moringa, and curry leaves are depicted in Table 1. All the three extracts contain alkaloids, flavonoids and phenols suggesting a commonality in their potential medicinal, pharmacological, antimicrobial and antioxidant properties. Mint and curry leaves extract shows the presence of most critical phytoconstituents like cardiac glycosides, tannins and terpenoids whereas moringa leaves were devoid of them. The data are in accordance with results mentioned by [9] in which aqueous extract of moringa leaves showed the moderate abundance of flavonoids, least availability of phenols, alkaloid, saponins and glycosides and absence of triterpenoids and tannins. However in another research conducted by [22] documented the presence of flavonoids, glycosides, terpenoids and tannins and absence of alkaloids, phenols and saponins. Carbohydrates were present only in curry leaves, while saponins are absent in curry leaves but present in mint and moringa leaves. Previous research has also noted the absence of saponins in the curry leaf species examined in the study [23]. However, presence of flavonoids (1.717), alkaloids (0.086), glycoside (0.030), sterol (0.006) and saponins (0.769) was reported in curry leaves in another study [24]. In a related study conducted by [15], showed that alcoholic extract of mint leaves contains alkaloid, flavonoids, phenols, glycoside, protein, tannins, and saponin. Several other studies also had previously reported the presence of flavonoids, phenols, cardiac glycosides, tannin, terpenoids and absence of saponins in *Mentha spicata* [25]. The presence, concentration and bioactivity of phytochemicals in leaf extracts can vary due to several factors like plant species and varieties, plant growth stage, age and maturity, environmental conditions, harvesting conditions, extraction methods etc.

Understanding these factors is crucial for optimizing the extraction and use of these leaf extracts for medicinal, nutritional, or industrial purposes.

Table 1. Preliminary phytochemical screening of secondary metabolites of leaves extracts

Secondary metabolite	Mint	Moringa	Curry leaves
Alkaloids	+	+	+
Carbohydrates	-	-	+
Cardiac glycosides	+	-	+
Flavonoids	+	+	+
Polyphenols	+	+	+
Saponins	+	+	-
Tannins	+	-	+
Terpenoids	+	-	+

Note: (+) = Present, (-) = Absent

The phytochemical compounds found in these extracts are known for their significant psychological and medicinal effects. Alkaloids play crucial roles in analgesic, neuroprotective, antimicrobial, and antimalarial activities. Flavonoids, derived from plants, offer antidiarrheal, antimicrobial, antioxidant, and anti-inflammatory benefits, with polyphenolic flavonoids specifically interacting with bacterial cell walls. Phenols and tannins also exhibit strong antimicrobial properties. Terpenes serve as antimicrobial agents by weakening microbial cell walls and additionally provide anticancer and antidiabetic effects[23]. Overall, these phytochemicals presents in these leaves extracts under study possess a range of biological activities, including antimicrobial, antioxidant, anti-inflammatory, antiplasmodial, and anticancer properties, which could contribute to their varying uses and benefits in pharmaceutical and nutraceutical sectors.

3.2 Physicochemical analysis of prepared yoghurt

Effect of leaf extracts on setting time pH, titratable acidity and syneresis of yoghurt samples were studied and the results are shown in Table 2. Setting time refers to the duration it takes for the sample to solidify or reach a desired consistency. Sample Y takes the longest time (8 h) to set, which could indicate a slower reaction or interaction of components within the sample. On the other hand, Sample Y3 sets the fastest (4.5 h), which might be advantageous in industrial processes where time efficiency is crucial.

Table 2. Physicochemical properties of yoghurt samples

Samples	Setting Time (h)	pH	Titratable Acidity (% LA)	Syneresis (%)
Y	8.0 ± 0.5 ^a	4.6 ± 0.05 ^a	0.90 ± 0.04 ^a	16 ± 0.1 ^a
Y1	5.5 ± 0.5 ^{bc}	4 ± 0.11 ^b	0.63 ± 0.02 ^d	14 ± 0.5 ^{ab}
Y2	6.5 ± 0.5 ^b	4 ± 0.05 ^b	0.81 ± 0.01 ^c	10 ± 0.1 ^{ab}
Y3	4.5 ± 0.5 ^c	4.5 ± 0.05 ^a	0.85 ± 0.01 ^b	8 ± 0.1 ^b

*Values are expressed as mean ± SD of triplicate determination. Different alphabet superscript within the same column are significantly different (p=0.05).

The pH of a product can influence its texture, taste, and microbial stability. Samples Y1 and Y2 have the lowest pH (4.0), suggesting they are more acidic than the other samples. No significant difference was observed in pH of sample Y and Y3 (p=0.05). The pH of all yoghurt samples containing leaf extracts met the Food and Drug Administration (FDA) guidelines, which require yoghurt to have a maximum pH of 4.5.

Titratable acidity, expressed as a percentage of lactic acid, measures the total acidity in the product. It's an important quality parameter in fermented products, influencing taste and shelf-life. Sample Y has the highest acidity (0.90%) adhering to the FDA's minimum titratable acidity requirement of 0.9% [26]. Lower acidity in Sample Y1 (0.63%) suggests a milder taste and potentially less impact on the structural integrity of the product. The moderate acidity levels in Samples Y2 (0.81%) and Y3 (0.85%) suggest a balance between taste and product stability. All the samples complied with FSSAI standards, which mandate a minimum titratable acidity of 0.6% lactic acid for yoghurt. Low pH values and increase in acidity could be attributed to the fermentation of lactose in milk to lactic acid by the starter culture bacteria.

Syneresis refers to the expulsion of liquid from a gel-like structure, an undesirable trait in many products like yoghurt or gel-based pharmaceuticals. A higher percentage indicates more liquid separation, which can affect texture, appearance, and consumer acceptability. Sample Y shows the highest syneresis (16%), indicating it is the least stable, likely due to its longer setting time and higher acidity. Sample Y3 with curry leaf extract has the lowest syneresis (8%), suggesting it is the most stable and likely to maintain a smooth, cohesive texture over time.

3.3 Analysis of proximate composition of prepared yoghurt

The result of the proximate compositions of all the four yoghurt samples are summarized in Table 3.

Table 3. Proximate compositions of yoghurt samples

Samples	Moisture (%)	Total Solids (%)	Fat (%)	Protein (%)	Lactose (%)
Y	87.44 ± 0.20 ^c	12.56 ± 0.03 ^a	3.36 ± 0.01 ^a	3.12 ± 0.01 ^b	3.42 ± 0.01 ^a
Y1	87.62 ± 0.01 ^{bc}	12.38 ± 0.01 ^b	3.3 ± 0.01 ^a	3.23 ± 0.01 ^{bc}	3.43 ± 0.01 ^a

Y2	87.84 ± 0.01 ^b	12.16 ± 0.02 ^c	3.33±0.02 ^a	3.31±0.10 ^{ab}	3.41 ± 0.01 ^a
Y3	88.18 ± 0.02 ^a	11.82 ± 0.01 ^d	3.35±0.01 ^a	3.42 ± 0.01 ^a	3.44± 0.01 ^a

*Values are expressed as mean ± SD of triplicate determination. Different alphabet superscript within the same column are significantly different (p=0.05).

The obtained results showed that Sample Y3 has the highest moisture content (88.18%) and sample Y has the lowest moisture content (87.44%). While the differences in moisture content are relatively small, the slightly higher moisture levels in Y3 made it smoother than other samples. However, the presence of higher moisture content affect the texture and mouth feel of yoghurt. Moisture content for all treatments was close to the results of [27], who attributed the reason behind the high moisture content was the use of low-fat milk (1-2 %) and incorporation of leaf extracts in yoghurt manufacturing. Although, all the samples have comparable total solids, Sample Y has the highest total solids content (12.56%), suggesting it may have a slightly thicker consistency compared to the other samples. Sample Y3 has the lowest total solids (11.82%), which align with its higher moisture content, indicating a lighter texture. Our findings indicated that all yoghurt samples have a lower total solids content compared to what was reported in previous studies[17] and may be attributed to several factors including low fat and SNF content in milk and inadequate standardization of milk before fermentation. No significant differences were noticed in fat and lactose content across all the four samples. Sample Y3 has the highest protein content and this can be associated with the higher protein levels in curry leaves compared to moringa and mint leaves[28].

3.5 Microbiological analysis of prepared yoghurt

Total lactic acid bacteria in samples Y, Y1, Y2 and Y3 were found to be 4.34, 5.41, 5.49, and 5.46 Log CFU/ml respectively. Lactic acid bacteria showed better growth and viability in samples fortified with mint, moringa, and curry leaf extracts compared to the control with no extracts. The increase in lactic acid bacteria growth could be linked to the polyphenolic compounds present in mint, moringa and curry leaf extracts, which might boost fermentation rates and improve the metabolism and survival of lactic acid bacteria [29]. No coliforms, yeast, or mold were found in any of the treatments, indicating that both the control and treatment yoghurt samples were safe for consumption. Additionally, the absence of coliforms confirms that the yoghurt was produced under hygienic conditions. The results were consistent with the findings of [27, 29,30,31].

3.4 Antibacterial activity

Agar well diffusion method was adopted for the evaluation of the antibacterial activity of the leaf extracts incorporated yoghurt samples and the results are recorded in Table 4.

Table 4. Zone of inhibition (mm) produced by yoghurt samples

Samples	<i>Escherichia coli</i>	<i>Salmonella</i>	<i>Staphylococcus aureus</i>
Y	11± 0.10	13.5±0.10	16.1±0.10

Y1	11.6± 0.10	13.8±0.10	17.5±0.05
Y2	11.6± 0.05	14.2±0.10	16.4±0.20
Y3	11.5± 0.11	15.6±0.11	18.5±0.10

*Values are expressed as mean ± SD of triplicate determination

Sample Y3 with curry leaf extract shows the highest antibacterial activity against both *Salmonella* and *S.aureus*. Y2 yoghurt with moringa extract has a slightly higher antibacterial activity against *Salmonella* compared to Y1 with mint extract, whereas sample Y1 was found more effective against *S. aureus* than Y2. Against *E.coli*, the zones of inhibition are relatively similar across all yoghurt samples, with values ranging from 11 mm to 11.6 mm.

Antibacterial activity of yoghurts against *E.coli*, *Salmonella* and *S.aureus* was reported previously by [32] and [33] and the effect is also thought to be caused by metabolites like lactic acid, H₂O₂ and bacteriocins produced by the lactic acid bacteria. Various researchers have also documented the antibacterial properties of moringa, mint, and curry leaves against gram positive and gram negative bacteria which might be the reason for the amplified antibacterial effect in yoghurt samples enriched with mint, moringa, and curry leaves extract leading to greater inhibition of bacterial growth compared to the control yoghurt [34, 35, 36, 37]. The enhanced antibacterial activity observed is strongly linked to the phytochemicals present in these plants. These compounds, including essential oils, flavonoids, alkaloids, and phenolic acids, exhibit various mechanisms of antibacterial action, such as disrupting bacterial cell membranes, inhibiting protein and DNA synthesis, and causing oxidative stress [38, 39]. Thus incorporation of such phytochemical-rich ingredients into yoghurt can enhance its potential as a functional food with added health benefits, particularly in terms of antimicrobial properties.

3.5 Antibiotic Resistance Analysis

The antibiotic resistance profile of *Streptococcus thermophiles* and *Lactobacillus delbrueckii subsp. bulgaricus* used for yoghurt production is given in Table 5.

Table 5. Antibiotic resistance profile of yoghurt cultures

Antibiotics	Zone of inhibition (mm)	
	<i>Streptococcus thermophilus</i>	<i>Lactobacillus delbrueckii subsp. Bulgaricus</i>
Ampicillin (AMP10)	18±0.10	17.1±0.10
Penicillin (P10)	17.1 ±0.10	20.7 ±0.87
Streptomycin (S25)	2.8±0.26	4.9±0.30
Bacitracin (B10)	9 ± 0.15	10.9±0.30
Cefoperazone (CPZ75)	14.8±0.20	13.2±0.2

*Values are expressed as mean ± SD of triplicate determination

Streptococcus thermophilus and *Lactobacillus delbrueckii subsp. bulgaricus* were found to be sensitive to ampicillin and penicillin, as shown by the larger zones of inhibition. Both strains exhibited moderate resistance to cefoperazone and bacitracin, while high resistance was demonstrated to streptomycin (Fig. 1).

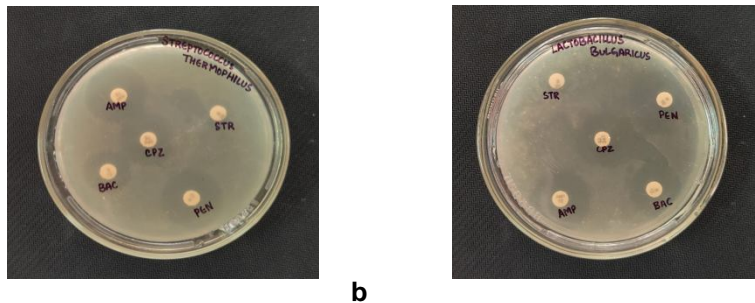


Fig.1. Antibiotic resistance of a) *Streptococcus thermophilus* and b) *Lactobacillus delbrueckii subsp. bulgaricus* against AMP10, P10, S25, B10 and CPZ75

The results were in agreement with the findings of [40], where the greatest antibiotic susceptibility was observed with antibiotics like ampicillin (25 µg), and erythromycin (15 µg).

3.6 Sensory evaluation of prepared yoghurt

All four yoghurt samples were also evaluated for their sensorial profiles such as appearance, flavour, taste, aroma and overall acceptance. The results obtained are summarized in Table 6.

Table 6. The average point score of various sensorial attributes of yoghurt samples

Samples	Colour and Appearance	Body and Texture	Flavour	Overall Acceptability
Y	7 ± 0.10 ^c	8.2 ± 0.20 ^{ab}	7.4 ± 0.26 ^b	7.7 ± 0.26 ^b
Y1	7.9 ± 0.35 ^{ab}	7.5 ± 0.40 ^{bc}	9.0 ± 0.45 ^a	8.3 ± 0.26 ^{ab}
Y2	7.4 ± 0.10 ^{bc}	7.2 ± 0.30 ^c	6.5 ± 0.15 ^b	6.8 ± 0.25 ^c
Y3	8.6 ± 0.36 ^a	8.6 ± 0.17 ^a	8.5 ± 0.37 ^a	8.5 ± 0.11 ^a

^aValues are expressed as mean ± SD of triplicate determination. Different alphabet superscript within the same column are significantly different (p<0.05)

The sensory evaluation results reveal that yoghurts fortified with mint (Y1) and curry leaf (Y3) extracts were the most preferred treatments overall, with Y3 scoring the highest in color and appearance (8.6), body and texture (8.6), and overall acceptability (8.5), while Y1 excelled in flavor (9). The control treatment showed moderate performance, particularly in body and texture (8.2), but was less impressive in appearance (7) and flavor (7.4). Moringa leaf extract incorporated yoghurt sample (Y2), however, received the lowest scores in flavor (6.5) and overall acceptability (6.8), making it the least preferred treatment in this sensory evaluation. The strong or potentially bitter taste of moringa leaves at a 5% concentration might be overpowering, making it less appealing to consumers. Similar findings have been reported, where the control yoghurt sample received significantly higher ratings (p<0.05) for appearance, flavor, texture, and overall quality compared to the yoghurt with 1.7% dried

moringa leaves [41],[42] also noted that adding moringa extract to yoghurt led to a significant decrease in flavor scores compared to control yoghurt. Additionally, [43] observed that plain yoghurt had the highest body and texture scores, while those fortified with *Moringa oleifera* seed flours showed a decline in these attributes as the *Moringa oleifera* leaf powder concentration exceeded 1.5%, likely due to the fiber content in the powder.

4. CONCLUSION

The study highlights the impact of incorporating mint, moringa, and curry leaf extracts into yoghurt on its sensory, physicochemical, microbiological, and antibacterial properties and concluded that yoghurt fortified with curry leaf extract was the most preferred, showing the best sensory attributes, fastest setting time, and lowest syneresis, making it the most stable option. Mint-fortified yoghurt also performed well, particularly in flavor. In contrast, moringa leaf extract yoghurt was the least favored due to its strong and bitter taste, negatively impacting its flavor and overall acceptability. While all fortified yoghurts were microbiologically safe and showed enhanced lactic acid bacteria growth, curry leaf extract also provided the strongest antibacterial activity, making it a promising functional ingredient in yoghurt production, enhancing both its nutritional and therapeutic benefits.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

REFERENCES

1. Oyeniran A, Gyawali R, Aljaloud SO, Krastanov A, Ibrahim SA. Probiotic characteristics and health benefits of the yogurt bacterium *Lactobacillus delbrueckii sp. bulgaricus*. Current issues and challenges in the dairy industry 2020 May 27. IntechOpen.
2. Thapa D, Kumar V, Naik B, Kumar V, Gupta AK, Mohanta YK, Mishra B, Rustagi S. Harnessing probiotic foods: managing cancer through gut health. Food Sci. and Biotechnol. 2024 Jun 28:1-20.
3. Ağagündüz D, Yılmaz B, Şahin TÖ, Güneşliol BE, Ayten Ş, Russo P, Spano G, Rocha JM, Bartkiene E, Özogul F. Dairy lactic acid bacteria and their potential function in dietetics: The food–gut–health axis. Foods. 2021 Dec 14;10(12):3099.
4. El-Gammal RE, Abdel-Aziz ME, Darwish MS. Utilization of aqueous extract of *Moringa oleifera* for production of functional yogurt. Journal of Food and Dairy Sciences. 2017 Jan 21;8(1):45-53.
5. Wajs J, Brodziak A, Król J. Shaping the physicochemical, functional, microbiological and sensory properties of yoghurts using plant additives. Foods. 2023 Mar 17;12(6):1275.
6. Kaur G, Singla N, Jain R. Nutritional and health attributes of herbs. Chemical Science Review and Letters. 2018;25(7):250-5.
7. Fan X, Li X, Du L, Li J, Xu J, Shi Z, Li C, Tu M, Zeng X, Wu Z, Pan D. The effect of natural plant-based homogenates as additives on the quality of yogurt: A review. Food Bioscience. 2022 Oct 1;49:101953.

8. Zaidi S, Dahiya P. In vitro antimicrobial activity, phytochemical analysis and total phenolic content of essential oil from *Mentha spicata* and *Mentha piperita*. International Food Research Journal. 2015 Nov 1; 22(6):2440.
9. Khalid S, Arshad M, Mahmood S, Ahmed W, Siddique F, Khalid W, Zarlisht M, Asar TO, Hassan FA. Nutritional and phytochemical screening of Moringa oleifera leaf powder in aqueous and ethanol extract. International Journal of Food Properties. 2023 Sep 22;26(1):2338-48.
10. Paswan VK, Rose H, Singh CS, Yamini S, Rathaur A. Herbs and spices fortified functional dairy products. Herbs and Spices-New Processing Technologies. 2021 Jul 20.
11. Cox-Georgian D, Ramadoss N, Dona C, Basu C. Therapeutic and medicinal uses of terpenes. Medicinal plants: from farm to pharmacy. 2019:333-59.
12. Kancherla N, Dhakshinamoothi A, Chitra K, Komaram RB. Preliminary analysis of phytoconstituents and evaluation of anthelmintic property of Cayratiaauriculata (in vitro). Maedica. 2019 Dec;14(4):350.
13. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020 Mar 1;8(2):603-8.
14. Ojah EO, Oladele EO, Chukwuemeka P. Phytochemical and antibacterial properties of root extracts from Portulaca oleracea Linn.(Purslane) utilised in the management of diseases in Nigeria. Journal of Medicinal Plants for Economic Development. 2021 Jan 26;5(1):103.
15. Paikara D, Pandey B. Phytochemicals from leaves of *Menthaspicata* and *Artemisia pallens*. Indian Journal of Scientific Research. 2018;9(1):111-4.
16. Adjei ML, Boakye A, Deku G, Pepra-Ameyaw NB, Jnr AS, Oduro IN, Ellis WO. Development of yoghurt incorporated with beetroot puree and its effect on the physicochemical properties and consumer acceptance. Heliyon. 2024 Feb 15;10(3).
17. Matela KS, Pillai MK, Thamae T. Evaluation of pH, titratable acidity, syneresis and sensory profiles of some yoghurt samples from the kingdom of Lesotho. Food Research. 2019 Dec;3(6):693-7.
18. AOAC [Association of Official Analytical Chemists]. The Official Methods of Analysis of AOAC International Association of Official Analytical Chemists. 2005 :18th Ed.. Association of Official Analytical Chemists, Washington D.C.
19. AOAC [Association of Official Analytical Chemists]. The Official Methods of Analysis of AOAC International Association of Official Analytical Chemists. 2000: 17th Ed.. Association of Official Analytical Chemists, Washington D.C.
20. Moghimi B, Ghobadi Dana M, Shapouri R, Jalili M. Antibiotic resistance profile of indigenous *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains isolated from traditional yogurt. Journal of Food Quality. 2023;2023(1):4745784.
21. Yilmaz-Ersan L, Ozcan T, Akpınar-Bayizit A, Delikanli-Kiyak B. The characterization of the textural and sensory properties of buffalo milk yogurts. International Journal of Advances in Science Engineering and Technology. 2017;5(3):37-42.
22. Tambe BD, Gadhave PD. Phytochemical screening and in vitro antibacterial activity of Moringa oleifera (Lam.) leaf extract. International Journal of Science and Research Archive. 2024;12(1):125-30.
23. Abeysinghe DT, Kumara KA, Kaushalya KA, Chandrika UG, Alwis DD. Phytochemical screening, total polyphenol, flavonoid content, in vitro antioxidant and antibacterial activities of Sri Lankan varieties of *Murrayakoenigii* and *Micromelum minutum* leaves. Heliyon. 2021 Jul 1;7(7).
24. Ike, Chiamaka J, Eze-Steven Peter E, Ani Anita Chikaodinaka. Comparative Study of the Phytochemical Composition of Aqueous Extracts of *Ocimum gratissimum* (Scent Leaves) and *Murrayakoenigii* (Curry Leaves). J. Appl. Sci. 2018: 3(3): 87-94, 2018
25. Ojewumi ME, Adedokun SO, Omodara OJ, Oyeniyi EA, Taiwo OS, Ojewumi EO. Phytochemical and antimicrobial activities of the leaf oil extract of *Mentha spicata* and its

- efficacy in repelling mosquito. International Journal of Pharmaceutical Research & Allied Sciences. 2017 Jan 1;6(4):17-27.
26. Weerathilake WA, Rasika DM, Ruwanmali JK, Munasinghe MA. The evolution, processing, varieties and health benefits of yogurt. International Journal of Scientific and Research Publications. 2014 Apr;4(4):1-0.
 27. Al-Shawi SG. The possibility of producing synbiotic yogurt containing mint extracts. EurAsian Journal of BioSciences. 2020 Jan 1;14(1).
 28. Longvah, T, Anantan, I, Bhaskarachary, K, Venkaiah, K, Longvah, T. *Indian food composition tables*. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research. 2017: 2-58
 29. Elkazaz A, Allam MA, Ayad EH, Darwish SM, Gomaa MA. Viability of Probiotic Strains in Moringa Aqueous Extract-Fortified Low-Fat Yogurt. Journal of the Advances in Agricultural Researches. 2024 Mar 1;29(1):49-63.
 30. Joung JY, Lee JY, Ha YS, Shin YK, Kim Y, Kim SH, Oh NS. Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts. Korean journal for food science of animal resources. 2016;36(1):90.
 31. Bhatt S, Singh B, Gupta M. Antioxidant and prebiotic potential of *Murrayakoenigi* and *Brassica oleracea var. botrytis* leaves as food ingredient. Journal of Agriculture and Food Research. 2020 Dec 1;2:100069.
 32. Yesillik S, Yildirim N, Dikici A, Yildiz A, Yesillik S. Antibacterial effects of some fermented commercial and homemade dairy products and 0.9% lactic acid against selected foodborne pathogens. Asian Journal of Animal and Veterinary Advances. 2011;6(2): 189-195
 33. Patel JJ, Antony Kathayat B, Patel KT. Antibacterial activity of dahi prepared from dairy industry, cottage and house hold against food borne pathogens. International Journal of Current Microbiology and Applied Sciences. 2018;7(1):2173-9.
 34. Najeeb AP, Mandal PK, Pal UK. Efficacy of leaves (drumstick, mint and curry leaves) powder as natural preservatives in restructured chicken block. Journal of Food Science and Technology. 2015 May;52:3129-33.
 35. Al Harbi H, Irfan UM, Ali S. The antibacterial effect of curry leaves (*MurrayaKoenigii*). EJPMR. 2016;3:382-7.
 36. Bardaweel SK, Bakchiche B, ALSalamat HA, Rezzoug M, Gherib A, Flamini G. Chemical composition, antioxidant, antimicrobial and Antiproliferative activities of essential oil of *Mentha spicata L.*(Lamiaceae) from Algerian Saharan atlas. BMC complementary and alternative medicine. 2018 Dec;18:1-7.
 37. Scherer R, Lemos MF, Lemos MF, Martinelli GC, Martins JD, da Silva AG. Antioxidant and antibacterial activities and composition of Brazilian spearmint (*Mentha spicata L.*). Industrial crops and products. 2013 Oct 1;50:408-13.
 38. Khameneh B, Eskin NM, Iranshahy M, FazlyBazzaz BS. Phytochemicals: a promising weapon in the arsenal against antibiotic-resistant bacteria. Antibiotics. 2021 Aug 26;10(9):1044.
 39. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms. 2021 Sep 27;9(10):2041.
 40. Yerlikaya O, Saygili D, Akpınar A. Evaluation of antimicrobial activity and antibiotic susceptibility profiles of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains isolated from commercial yoghurt starter cultures. Food Science and Technology. 2020 Oct 26;41:418-25.
 41. Kuikman M, O'Connor C. Sensory evaluation of Moringa-probiotic yogurt containing banana, sweet potato or avocado. Journal of Food Research. 2015 Sep 21;4(5):165-71.
 42. Zhang T, Jeong CH, Cheng WN, Bae H, Seo HG, Petriello MC, Han SG. Moringa extract enhances the fermentative, textural, and bioactive properties of yogurt. Lwt. 2019 Mar 1;101:276-84.

43. Dhawi F, El-Beltagi HS, Aly E, Hamed AM. Antioxidant, antibacterial activities and mineral content of buffalo yoghurt fortified with fenugreek and Moringa oleifera seed flours. *Foods*. 2020 Aug 21;9(9):1157.

UNDER PEER REVIEW