Journal of Advances in Biology & Biotechnology

Volume 27, Issue 11, Page 117-127, 2024; Article no.JABB.126115
ISSN: 2394-1081

Formulation and Quality Analysis of Yoghurts Fortified with *Mentha spicata*, *Moringa oleifera*, and *Murraya koenigii* Leaf Extracts

Anjali M.K [a++], Malashree L [a#*] and Anushree Y.K [a#]

[a] *Department of Dairy Microbiology, Dairy Science College, Bangalore, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i111597

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/126115

Original Research Article

Received: 24/08/2024
Accepted: 27/10/2024
Published: 31/10/2024

ABSTRACT

The aim of this study was to investigate the impact of incorporating *Mentha spicata* (mint), *Moringa oleifera (moringa),* and *Murraya koenigii* (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

[++] *PhD scholar;*
[#] *Assistant Professor;*
**Corresponding author: E-mail: malashree.ndri@gmail.com;*

Cite as: *M.K, Anjali, Malashree L, and Anushree Y.K. 2024. "Formulation and Quality Analysis of Yoghurts Fortified With Mentha Spicata, Moringa Oleifera, and Murraya Koenigii Leaf Extracts". Journal of Advances in Biology & Biotechnology 27 (11):117-27. https://doi.org/10.9734/jabb/2024/v27i111597.*

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 S*treptococcus thermohilus 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 (Oyeniran et al. 2020, Thapa et al. 2024, Ağagündüz et al. 2021).

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 (El-Gammal et al. 2017, Wajs et al. 2023, Kaur et al. 2018). 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 (El-Gammal et al. 2017, Fan et al. 2022).

Mint (*Mentha spicata*), moringa (*Moringa oleifera*), and curry leaves (*Murraya koenigii*) 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 (Zaidi and Dahiya 2015). 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 (Khalid et al. 2023). Curry leaves, known for their distinctive flavor and therapeutic properties, have shown potential in improving the sensory quality and stability of yoghurt (Paswan et al. 2021).

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. MATERIALS AND METHODS

2.1 Plant Material Collection and Preparation of Leaves Extracts

Samples of mint (*Mentha spicata*), moringa (*Moringa oleifera*) and curry leaves (*Murraya koenigii*) 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 -

Keliani 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 (Cox-Georgian et al. 2019, Kancherla et al. 2019, Shaikh et al. 2020, Ojah et al. 2021, Paikara et al. 2018).

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

Titratable acidity was determined using the method described by Adjei et al. 2024. 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 titratable 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 Matela et al. 2019.

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

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 2005.

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 (AOAC 2000). 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 µL), 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 (Ojah et al. 2021).

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 Baeur). 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 (Moghimi et al. 2023).

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” (Yilmaz-Ersan et al. 2017).

2.10 Statistical Analysis

The data obtained were analyzed using R studio (version 4.2.2). Data were expressed as means ± 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 (Khalid et al. 2023) 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 Tambe et al. 2024 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 (Abeysinghe et al. 2021). 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 (Ike et al. 2018). In a related study conducted by Paikara 2018, 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* (Ojewumi et al. 2017). 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.

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 (Abeysinghe et al. 2021). 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.

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*

Table 2. Physiochemical 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)*

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.

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% (Weerathilake et al. 2014)**.** 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 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)*

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

Samples	*Escherichia coli*	Salmonella	*Staphylococcus aureus*
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*

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 Al-Shawi 2020, 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 (Matela et al. 2019) 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 (Longvah et al. 2017).

3.4 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 (Elkazaz et al. 2024). 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 Al-Shawi 2020, Elkazaz et al. 2024, Joung et al. 2016, Bhatt et al. 2020.

3.5 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.

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 Yesillik et al. 2011 and Patel et al. 2018 and the effect is also thought to be caused by metabolites like lactic acid, H_2O_2 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 (Najeeb et al. 2015, Harbi et al. 2016, Bardaweel et al. 2018, Scherer et al. 2013). 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 (Khameneh et al. 2021, Vaou et al. 2021). 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.

3.6 Antibiotic Resistance Analysis

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

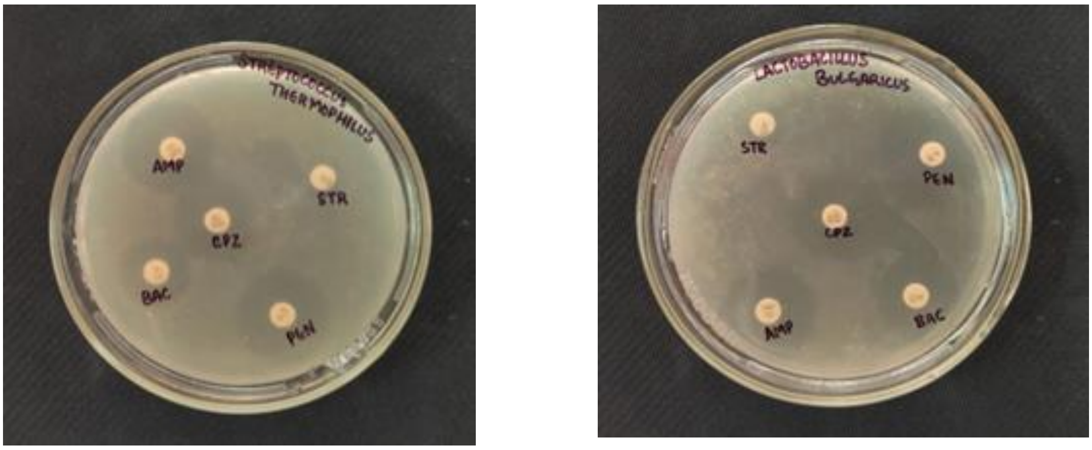
Streptococcus thermophilus and *Lactobacillus delbrueckii subsp. bulgaricus* were found to be sensitive to ampicillin and penicillin, as shown by the larger zones of inhibition. Both strains exhibited moderate resistance to cefoperazone and bacitracin, while high resistance was demonstrated to streptomycin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

The results were in agreement with the findings of Yerlikaya et al. 2020, where the greatest antibiotic susceptibility was observed with antibiotics like ampicillin (25 μg), and erythromycin (15 μg).

Table 5. Antibiotic resistance profile of yoghurt cultures

Antibiotics	Zone of inhibition (mm)	
	Streptococcus thermohilus	***Lactobacillus delbrueckii subsp. Bulgaricus***
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*



a b

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

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]

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

3.7 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.

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 Kuikman 2015. Zhang et al. 2019 also noted that adding moringa extract to yoghurt led to a significant decrease in flavor scores compared to control yoghurt. Additionally, Dhawi et al. 2020 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.

ACKNOWLEDGEMENTS

The authors are thankful to the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, for providing all necessary facilities and financial support for the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Abeysinghe, D. T., Kumara, K. A., Kaushalya, K. A., Chandrika, U. G., & Alwis, D. D. (2021). Phytochemical screening, total polyphenol, flavonoid content, in vitro antioxidant and antibacterial activities of Sri Lankan varieties of *Murraya koenigii* and

Micromelum minutum leaves. *Heliyon*, 7(7).

Adjei, M. L., Boakye, A., Deku, G., Pepra-Ameyaw, N. B., Jnr, A. S., Oduro, I. N., & Ellis, W. O. (2024). Development of yoghurt incorporated with beetroot puree and its effect on the physicochemical properties and consumer acceptance. *Heliyon*, 10(3).

Ağagündüz, D., Yılmaz, B., Şahin, T. Ö., Güneşliol, B. E., Ayten, Ş., Russo, P., Spano, G., Rocha, J. M., Bartkiene, E., & Özogul, F. (2021). Dairy lactic acid bacteria and their potential function in dietetics: The food–gut-health axis. *Foods*, 10(12), 3099.

Al-Shawi, S. G. (2020). The possibility of producing synbiotic yogurt containing mint extracts. *EurAsian Journal of BioSciences*, 14(1).

AOAC [Association of Official Analytical Chemists]. (2000). *The Official Methods of Analysis of AOAC International* (17th ed.). Association of Official Analytical Chemists.

AOAC [Association of Official Analytical Chemists]. (2005). *The Official Methods of Analysis of AOAC International* (18th ed.). Association of Official Analytical Chemists.

Bardaweel, S. K., Bakchiche, B., ALSalamat, H. A., Rezzoug, M., Gherib, A., & Flamini, G. (2018). Chemical composition, antioxidant, antimicrobial and antiproliferative activities of essential oil of *Mentha spicata* L. (Lamiaceae) from Algerian Saharan atlas. *BMC Complementary and Alternative Medicine*, 18, 1-7.

Bhatt, S., Singh, B., & Gupta, M. (2020). Antioxidant and prebiotic potential of *Murraya koenigii* and *Brassica oleracea* var. *botrytis* leaves as food ingredient. *Journal of Agriculture and Food Research*, 2, 100069.

Cox-Georgian, D., Ramadoss, N., Dona, C., & Basu, C. (2019). Therapeutic and medicinal uses of terpenes. In *Medicinal plants: from farm to pharmacy* (pp. 333-359).

Dhawi, F., El-Beltagi, H. S., Aly, E., & Hamed, A. M. (2020). Antioxidant, antibacterial activities and mineral content of buffalo yoghurt fortified with fenugreek and *Moringa oleifera* seed flours. *Foods*, 9(9), 1157.

El-Gammal, R. E., Abdel-Aziz, M. E., & Darwish, M. S. (2017). Utilization of aqueous extract of *Moringa oleifera* for production of functional yogurt. *Journal of Food and Dairy Sciences*, 8(1), 45-53.

Elkazaz, A., Allam, M. A., Ayad, E. H., Darwish, S. M., & Gomaa, M. A. (2024). Viability of probiotic strains in Moringa aqueous extract-fortified low-fat yogurt. *Journal of the Advances in Agricultural Researches*, 29(1), 49-63.

Fan, X., Li, X., Du, L., Li, J., Xu, J., Shi, Z., Li, C., Tu, M., Zeng, X., Wu, Z., & Pan, D. (2022). The effect of natural plant-based homogenates as additives on the quality of yogurt: A review. *Food Bioscience*, 49, 101953.

Harbi Al, H., Irfan, U. M., & Ali, S. (2016). The antibacterial effect of curry leaves (*Murraya Koenigii*). *EJPMR*, 3, 382-387.

Ike, C. J., Eze-Steven, P. E., & Ani, A. C. (2018). Comparative study of the phytochemical composition of aqueous extracts of *Ocimum gratissimum* (Scent Leaves) and *Murraya koenigii* (Curry Leaves). *Journal of Applied Sciences*, 3(3), 87-94.

Joung, J. Y., Lee, J. Y., Ha, Y. S., Shin, Y. K., Kim, Y., Kim, S. H., & Oh, N. S. (2016). Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts. *Korean Journal for Food Science of Animal Resources*, 36(1), 90.

Kancherla, N., Dhakshinamoothi, A., Chitra, K., & Komaram, R. B. (2019). Preliminary analysis of phytoconstituents and evaluation of anthelminthic property of *Cayratia auriculata* (in vitro). *Maedica*, 14(4), 350.

Kaur, G., Singla, N., & Jain, R. (2018). Nutritional and health attributes of herbs. *Chemical Science Review and Letters*, 25(7), 250-255.

Khalid, S., Arshad, M., Mahmood, S., Ahmed, W., Siddique, F., Khalid, W., Zarlasht, M., Asar, T. O., & Hassan, F. A. (2023). Nutritional and phytochemical screening of *Moringa oleifera* leaf powder in aqueous and ethanol extract. *International Journal of Food Properties*, 26(1), 2338-2348.

Khameneh, B., Eskin, N. M., Iranshahy, M., & Fazly Bazzaz, B. S. (2021). Phytochemicals: A promising weapon in the arsenal against antibiotic-resistant bacteria. *Antibiotics*, 10(9), 1044.

Kuikman, M., & O'Connor, C. (2015). Sensory evaluation of Moringa-probiotic yogurt containing banana, sweet potato or avocado. *Journal of Food Research*, 4(5), 165-171.

Longvah, T., Anantan, I., Bhaskarachary, K., Venkaiah, K., & Longvah, T. (2017). *Indian* *food composition tables*. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research.

Matela, K. S., Pillai, M. K., & Thamae, T. (2019). Evaluation of pH, titratable acidity, syneresis and sensory profiles of some yoghurt samples from the kingdom of Lesotho. *Food Research, 3*(6), 693-697.

Moghimi, B., Ghobadi Dana, M., Shapouri, R., & Jalili, M. (2023). Antibiotic resistance profile of indigenous *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains isolated from traditional yogurt. *Journal of Food Quality*, 2023, Article 4745784.

Najeeb, A. P., Mandal, P. K., & Pal, U. K. (2015). Efficacy of leaves (drumstick, mint and curry leaves) powder as natural preservatives in restructured chicken block. *Journal of Food Science and Technology*, 52, 3129-3133.

Ojah, E. O., Oladele, E. O., & Chukwuemeka, P. (2021). Phytochemical and antibacterial properties of root extracts from *Portulaca oleracea* Linn. (Purslane) utilized in the management of diseases in Nigeria. *Journal of Medicinal Plants for Economic Development*, 5(1), 103.

Ojewumi, M. E., Adedokun, S. O., Omodara, O. J., Oyeniyi, E. A., Taiwo, O. S., & Ojewumi, E. O. (2017). 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*, 6(4), 17-27.

Oyeniran, A., Gyawali, R., Aljaloud, S. O., Krastanov, A., & Ibrahim, S. A. (2020). Probiotic characteristics and health benefits of the yogurt bacterium *Lactobacillus delbrueckii* sp. *bulgaricus*. In *Current issues and challenges in the dairy industry*. IntechOpen.

Paikara, D., & Pandey, B. (2018). Phytochemicals from leaves of *Mentha spicata* and *Artemisia pallens*. *Indian Journal of Scientific Research*, 9(1), 111-114.

Paswan, V. K., Rose, H., Singh, C. S., Yamini, S., & Rathaur, A. (2021). Herbs and spices fortified functional dairy products. In *Herbs and Spices-New Processing Technologies*.

Patel, J. J., Antony Kathayat, B., & Patel, K. T. (2018). Antibacterial activity of dahi prepared from dairy industry, cottage and household against food borne pathogens. *International Journal of Current Microbiology and Applied Sciences*, 7(1), 2173-2179.

Scherer, R., Lemos, M. F., Martins, J. D., & da Silva, A. G. (2013). Antioxidant and antibacterial activities and composition of Brazilian spearmint (*Mentha spicata* L.). *Industrial Crops and Products*, 50, 408-413.

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603-608.

Tambe, B. D., & Gadhave, P. D. (2024). Phytochemical screening and in vitro antibacterial activity of *Moringa oleifera* (Lam.) leaf extract. *International Journal of Science and Research Archive*, 12(1), 125-130.

Thapa, D., Kumar, V., Naik, B., Kumar, V., Gupta, A. K., Mohanta, Y. K., Mishra, B., & Rustagi, S. (2024). Harnessing probiotic foods: Managing cancer through gut health. *Food Science and Biotechnology*, 1-20.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, 9(10), 2041.

Wajs, J., Brodziak, A., & Król, J. (2023). Shaping the physicochemical, functional, microbiological and sensory properties of yoghurts using plant additives. *Foods*, 12(6), 1275.

Weerathilake, W. A., Rasika, D. M., Ruwanmali, J. K., & Munasinghe, M. A. (2014). The evolution, processing, varieties and health benefits of yogurt. *International Journal of Scientific and Research Publications*, 4(4), 1-0.

Yerlikaya, O., Saygili, D., & Akpinar, A. (2020). 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*, 41, 418-425.

Yesillik, S., Yildirim, N., Dikici, A., Yildiz, A., & Yesillik, S. (2011). 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*, 6(2), 189-195.

Yilmaz-Ersan, L., Ozcan, T., Akpinar-Bayizit, A., & Delikanli-Kiyak, B. (2017). The characterization of the textural and sensory properties of buffalo milk yogurts. *International Journal of Advances in Science Engineering and Technology*, 5(3), 37-42.

Zaidi, S., & Dahiya, P. (2015). In vitro antimicrobial activity, phytochemical analysis and total phenolic content of essential oil from *Mentha spicata* and *Mentha piperita*. *International Food Research Journal*, 22(6), 2440.

Zhang, T., Jeong, C. H., Cheng, W. N., Bae, H., Seo, H. G., Petriello, M. C., & Han, S. G. (2019). Moringa extract enhances the fermentative, textural, and bioactive properties of yogurt. *LWT*, 101, 276-284.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/126115