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PHYSICOCHEMICAL, ANTIOXIDANT, AND ANTIMICROBIAL PROPERTIES OF A SYNBIOTIC DRINK BASED ON MALABAR MELASTOME *(MELASTOMA MALABATHRICUM* L.*)* AND SOYMILK

Jomarie C. Salar[*1,2] , Mary Ann Jilly R. Ramirez[3]

[1]Southern Leyte State University, Faculty of Technology, Doctor of Philosophy in Technology Management Program, Sogod, Southern Leyte, Philippines
[2]Southern Leyte State University, Faculty of Hospitality and Tourism Management, Sogod Southern Leyte, Philippines
[3]Southern Leyte State University, Department of Food Science and Technology Sogod Southern Leyte, Philippines

Abstract: Malabar melastome (*Melastoma malabathricum* L.) is widely recognized for its pharmacological properties, particularly its rich antioxidant and antimicrobial content. This study examines the development and evaluation of a synbiotic drink formulated from Malabar melastome fruit juice and soy milk as a healthier alternative to conventional dairy-based probiotic beverages. A 3×3 full factorial experimental design generated nine treatment combinations, which were assessed for physicochemical characteristics (density, viscosity, pH, total soluble solids, and titratable acidity), bioactive compounds (total phenolic content, DPPH radical scavenging activity, total monomeric anthocyanin, saponin, and tannin), microbial viability, and antimicrobial activity. The drink incorporated a mixed culture of *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *L. bulgaricus*. Results indicated that the synbiotic drink exhibited desirable viscosity, balanced sweetness and acidity, and exceptional antioxidant activity, largely attributed to the bioactive compounds in Malabar melastome. Viable counts of *L. acidophilus* and *L. bulgaricus* ranged from 7.85 log CFU/mL to 8.13 log CFU/mL. In comparison, *S. thermophilus* ranged from 4.11 log CFU/mL to 4.38 log CFU/mL after 72 hours of incubation, confirming sufficient probiotic viability for synbiotic classification. Antimicrobial assay showed inhibitory effects only against *Staphylococcus aureus* (Gram-positive). Overall, the findings suggest that Malabar melastome enhances both the functional and microbial quality of LAB-enriched drinks, offering promise as a natural, functional ingredient in synbiotic beverage development.

Key words: *lactic acid bacteria (LAB), antioxidant activity, DPPH scavenging activity, total phenolic content, total monomeric anthocyanin, antimicrobial activity*

INTRODUCTION

The current health landscape today causes concern, as numerous people are grappling with a variety of health issues, including cancer, gastrointestinal diseases, respiratory diseases, neurological diseases, and cardiovascular diseases. Unfortunately, some of these condi-

Corresponding author Phone: +639 53356 5959
Email address: jsalar@southernleytestateu.edu.ph

tions lack effective medications to halt their progression, posing great problems for those affected. Thus, many turn back to natural and organic remedies in search of hope for healing. In the absence of conventional medicine, plants become the source of medication. Since ancient times, medicinal plants have been employed to treat a wide range of health conditions, largely due to their abundance of bioactive compounds (Halberstein, 2005).

One of the medicinal plants used in traditional medicine is the Malabar melastome (*Melastoma malabathricum* L.*)*, a plant that belongs to the Melastomaceae family. This plant has become one of the important weeds among the tribes in Indo-Pacific countries due to its therapeutic benefits. Various parts such as roots, flower, stem, leaves, and fruits were used for treating diarrhea, dysentery, ulcers, wounds, prevention of wounds from smallpox, postpartum treatment, hemorrhoids, leukorrhea, toothache, skin disease, and jaundice (Kumar & Gupta, 2013; Apridamayanti, Sar, Rachmaningtyas & Aranthi, 2021; Tiwari, Barooah & Bhuyan, 2023).

Various scientific studies have demonstrated that Malabar melastome contains substantial levels of phytochemicals (Wong, Hag Ali & Boey, 2012, Chen et al., 2022). These bioactive compounds play a significant role in neutralizing free radicals, mitigating oxidative stress, reducing inflammation, and enhancing immune function (Ahmed et al., 2019, Kasunmala, Navaratne & Wickramasinghe, 2020; Lestari et al., 2022; Fiardilla, Putri & Sundari, 2023). Owing to these properties, Malabar melastome represents a promising candidate for incorporation into functional drink formulations.

As the demand for functional drinks continues to rise, incorporating Malabar melastome (*Melastoma malabathricum* L.) into these products can provide numerous health benefits, particularly when combined with other beneficial ingredients, such as probiotics and prebiotics. Probiotics are microorganisms which, if consumed in substantial amounts, bring health benefits to the host (Maftei et al., 2023), and prebiotics are non-digestible food components that serve as a source of energy for probiotics (Bevilacqua et al., 2024). The combination of probiotic and prebiotic is what is known as synbiotic, which improves the growth of human microflora (Ranjan, 2022). Several clinical studies have found that synbiotics can prevent and address illnesses caused by gut microbiome dysbiosis (Devi et al., 2023; Al-Habsi et al., 2024; Giancola et al., 2024; Yao, Wei & Zhang, 2024). Dysbiosis, or imbalance of the gut microbiota, has been linked to several diseases, including cancer, diabetes, obesity, and cardiovascular disorders (Mahdavi-Roshan, Salari, Kheirkhah & Ghorbani, 2022).

Recent studies highlight the importance of restoring host microbiome homeostasis, underscoring the gut's central role in overall human health (Olteanu et al., 2024). In this context, formulating a synbiotic drink with Malabar melastome (*Melastoma malabathricum* L.) and soymilk opens up a new avenue for health-conscious and lactose-intolerant consumers. Hence, this study aimed to produce a synbiotic drink from Malabar melastome fruit juice, soymilk and a lyophilized non-dairy yogurt starter composed of *Lactobacillus acidophilus, Streptococcus thermophilus,* and *L. bulgaricus.* Moreover, the physicochemical properties, probiotic viability, biofunctional characteristics, and antimicrobial activity were also examined.

MATERIALS AND METHODS

Raw materials, chemicals, reagents, and standards

The plant bearing fully ripe fruits and flowers was brought to the Department of Biological Sciences at Visayas State University and identified by the taxonomist as *Melastoma malabathricum* L. The fully ripe fruits of Malabar melastoma (*Melastoma malabathricum* L.) were collected from their natural habitat in the Province of Southern Leyte between March and April 2024. Distilled water, soy milk, sugar, and inulin were sourced from local markets. The non-dairy yogurt starter, Belle+Bella (containing *Streptococcus thermophilus, Lactobacillus bulgaricus,* and *Lactobacillus acidophilus*), was procured from Belle and Bella in Canada. Culture media were used for microbial analysis: MRS Agar (Hi-Media, USA) for enumerating *L. bulgaricus* and *L. acidophilus*, M17 Agar (Basebio, China) for *S. thermophilus*, Potato Dextrose Agar (TM Media, India) for detecting yeast and molds and MHA Agar (Hi-media, India) for culturing

and susceptibility testing of *Escherichia coli* and *Staphylococcus aureus*. Analytical reagents and standards includes Folin's Phenol Reagent (Scientific Phygene, China), DPPH reagent (1,1-diphenyl-2-picrylhydrazyl, Scientific Phygene, China), gallic acid (≥99% purity, Scientific Phygene, China), sodium carbonate monohydrate (Hi-Media, India), L-ascorbic acid (Sigma Aldrich, Missouri, USA), deionized water (Jeedy Essentials, Manila, Philippines), malic acid (USP grade), peptone (Hangwei, China), DNS chromogenic reagent solution (3,5-dinitrosalicylic acid, Scientific Phygene, China), 2,4-dichlo-rophenoxyacetic acid (98.0% purity, Scientific Phygene, China), potassium tartrate solution (Scientific Phygene, China), quercetin (≥98% purity, Cool Chemical Science and Technology Co., Ltd., China), aluminum chloride hexahydrate (≥99% purity, Xilong, China), 0.1% bromothymol blue indicator (Scientific Phygene, China), bromocresol green (Tianjin Dengfeng Chemical Test, China), D^{+}glucose monohydrate (Sinopharm, China), 95% ethanol, monosodium phosphate (USP grade), anhydrous sodium acetate (≥99.0% purity, Sinopharm, China), potassium chloride (≥99.0% purity, Sinopharm, China), premium ferric chloride (Jeedy Essentials, Manila, Philippines), disodium hydrogen phosphate (≥99% purity, Xilong, China), methanol, sodium hydroxide (Scharlau, Spain), calcium carbonate (Scharlau, Spain), potassium hydrogen phthalate (≥99.9% purity, HiMedia, USA), sodium nitrite titrated solution (1.004 mol/L, Shandong Puhuifen Chemical and Technology Co., Ltd., China), chloramphenicol, ferric acid, and hydrochloric acid.

Experimental design

A three-level, two-factor full factorial design was employed in this study. This design involves two factors, each evaluated at three levels, resulting in nine experimental runs that represent all possible combinations (Jankovic, Chaudhary & Goia, 2021). The factors, "fruit powder" (in grams) and "sugar" (in grams), were inputted into the statistical software Minitab to generate the experimental study design. The levels for the factors ranged from the lowest (1.0% for fruit powder and 10.0% for sugar) to the highest (2.0% for fruit powder and 20.0% for sugar) as shown in Table 1.The preparation steps of experimental symbiotic drink are schematically presented in Fig. 1.

Density determination

The 50 mL pycnometer glass was calibrated using distilled water at 0.4 °C. The empty pycnometer was weighed first in the analytical balance (Aczet, Germany). Then, it was filled with distilled water to full until the water flowed up to the capillary tube. The filled pycnometer was weighed again. The density of the samples was determined using the formula:

Mass of yogurt drink = Mass of yogurt (M_{yogurt}) – Mass of empty pycnometer (M_{empty})

$$\textbf{Density} = \frac{Mass\ of\ yogurt}{Volume\ of\ pycnometer}$$

Viscosity determination

The thickness of the fermented drinks was measured using a viscometer (IKA Rotavisc hi-vi, Germany) and follows the method of Gonzales, Adhikari and Sancho-Madriz et al. (2010).

Total soluble solids determination

Three drops of fermented drinks were placed in the digital refractometer. The result is expressed in $Brix^{0}$.

Table 1.
Three-level two-factor full factorial design for the development of synbiotic drink based on Malabar melastome fruit

Experimental runs (Treatments)	**Fruit powder** (% w/v)	**Sugar** % (w/v)
1	1.0	10
2	1.5	10
3	2.0	10
4	1.0	15
5	1.5	15
6	2.0	15
7	1.0	20
8	1.5	20
9	2.0	20

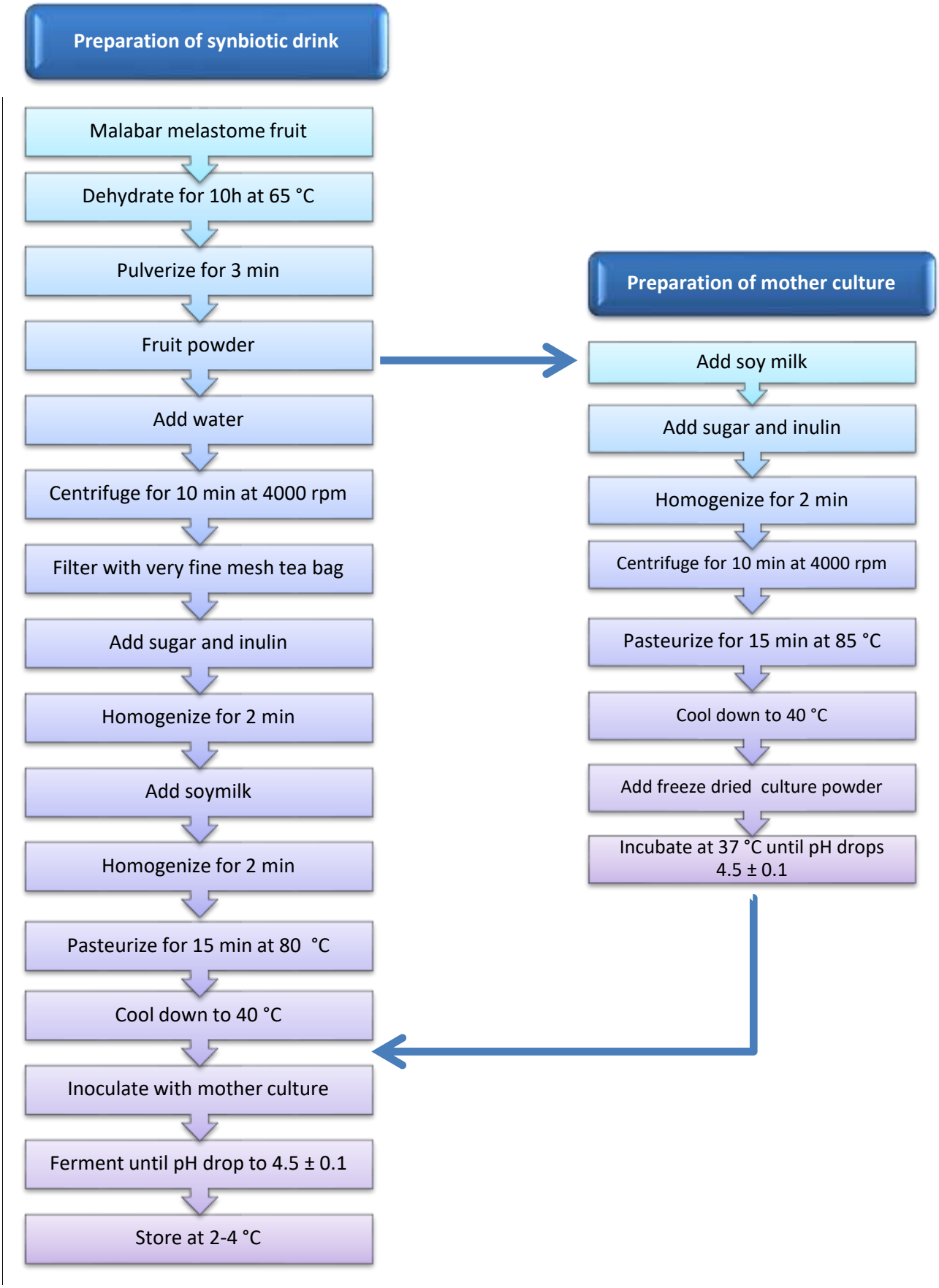

Figure 1. Process flow of the development of synbiotic drink

pH Level determination

The pH of the fermented drinks from treatment 1 to 9 and the optimized formulation was assessed using a digital benchtop pH meter (Biobase 920 Precision OP/pH Meter, China). Before measurement, the pH meter was calibrated with buffers at pH 4.0, 7.0, and 10. Determination was performed in triplicates for each fermented drink sample (20 mL) and optimized formulation per week until the last week of storage.

Titratable acidity determination

Fifty mL of the fermented drinks were filtered with Whatman filter paper no. 1. The 10 mL of the filtrate were taken and placed in a clean 50 mL Erlenmeyer flask. The filtrate was added with 10 mL distilled water and three drops of 0.1% bromothymol blue indicator and titrated in 0.97 M NaOH. Titration will continue until the green color appears and stays for 30 minutes. The titratable acidity in lactic acid equivalent was used.

$$\% \text{ Acid} = \frac{N\ x\ (V)\ x\ Equivalent\ weight\ of\ the\ acid}{W\ x\ 1000} \text{ x } 100$$

where:
N= normality of NaOH
V= volume of NaOH
W= volume of the sample.

Antioxidant assay

The antioxidant activity of the samples was evaluated using the DPPH free radical scavenging assay described by Brand-Williams, Cuvelier and Berset (1995) with slight modifications. A 5 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol. The absorbance of this solution was checked using a UV–VIS spectrophoto-meter (Shimadzu 1900i, Japan) to ensure it fell between 0.9 and 1.0 at 515 nm, with methanol as the blank. This solution served as the control for the assay. For the standard preparation, 10 mg of L-ascorbic acid was dissolved in 100 mL of methanol. Serial dilutions were prepared from this stock to obtain concentrations down to 6.25 µg/mL. From each dilution, 1 mL was transferred into labeled test tubes and mixed with 4 mL of DPPH solution. The mixtures were incubated in the dark for 30 minutes. After incubation, 3 mL from each tube was transferred into cuvettes, and absorbance was measured at 515 nm. The inhibition ratio was calculated, and a standard curve was constructed. For the sample analysis, fermented drink samples from Treatments 1 to 9 were filtered through Whatman No. 1 filter paper.

One milliliter of the filtrate was mixed with 3 mL of DPPH solution and centrifuged at 4,000 rpm for 10 minutes. The mixtures were then incubated in the dark for 30 minutes, and absorbance was measured at 515 nm.

Determination of total phenolic content

Determination of the total phenolic content follows the methods described by Kupina, Fields, Roman and Brunelle (2019) with a slight modification. One g of gallic acid was weighed in the analytical balance added with 750 mL of deionized water and sonicated in the water bath for 10 minutes until the solids were dissolved. Then, it was diluted to 1000 mL volume in the volumetric flask. This solution serves as the gallic acid stock solution.

From this, standard solutions of 25 mL from 40-200 mg/L of gallic acid concentrations were made. A 20% sodium carbonate solution was prepared by adding 30 mL of deionized water to 20 g of sodium carbonate (Na_2CO_3) in a 100 volumetric flask and filled to volume until the powder was fully dissolved. Samples of the fermented drinks from Treatment 1 to 9 were filtered the Whatman no.1 filter paper for 3 hours. The filtrate was used in the determination of the Total phenolic content using UV-VIS (Shimadzu 1900i, Japan). Seven test tubes were prepared containing 15 distilled water and 1 mL of Folin & Ciocalteu's phenol reagent (Phygene, China). Into each test tube, one of the following was added (a) 1 mL filtrate sample, (b) calibration standards (c) distilled water as the blank. The mixture was mixed using a vortex mixer (Ohaus, USA) and sat for 6 minutes. Then once 6 minutes passed, 3 mL of 20% sodium carbonate solution was added into the mixture and mixed using a vortex mixer, and incubated using a laboratory oven (Heraeus, Germany) at 32ºC for 2 hours for colorimetric reaction. Once the reaction time concluded, 3ml of each mixture was taken and filled in a quartz cuvette for spectrophotometric analysis UV-Vis (Shimadzu 1900i, Japan) at 765 nm. The calibration curve of the standards was established first then the sample.

Total monomeric anthocyanin content

This assay employed the methods of Lee, Durst and Wrolstad (2005) with slight modification. For pH 1.0 buffer 1.86 g of potassium chloride was dissolved in 980 mL distilled water then the pH was adjusted to 1.0 with hydrochloric acid. For the pH 4.5 acetate buffer, 54.43 g of sodium acetate was dissolved in 960 mL distilled water and the pH was adjusted to 4.5 using glacial acetic acid. 1 mL of filtrates from Treatment 1-9 and the optimum were mixed with 4mL pH 1.0 buffer and another 1 mL was mixed with 4 mL pH 4.5 buffer. Both pH buffer solutions (1.0 and 4.5) containing sample underwent spectral and photometric analysis to determine the maximum absorbance at 520 nm and 700 nm.

Saponin test

Determination of the presence of saponins in the samples follows the method of Mir, Parihar, Tabasum, Kumari and Mir (2016) with slight modification. Five mL of the whey was heated at 100 °C for 5 minutes and shaked vigorously for1-2 minutes, once the froth appears. Then, the frothed sample was left to sit for 10 minutes. If the froth remains after 10 minutes then there are saponins present in the sample.

Tannin test

Determination of the presence of tannins in the samples follows the method of Mir et al. (2016) with slight modification. One mL of each run (1-9) of the fermented drink was diluted in 4 mL distilled water and added with 5 drops of 1% ferric chloride solution. A green color marks the presence of tannins.

Microbiological analyses

Determination of viable yogurt bacteria was carried out using the plate count technique. One mL of each fermented drink was diluted in 9 mL of sterile peptone water and mixed uniformly using a vortex mixer (Ohaus, USA). Subsequent serial dilutions up to 10^{-6} dilutions were carried out. All media, reagents, petri plates, pipettes, and tips were sterilized using an autoclave for 15 minutes at 15 psi before the conduct of microbial methods to ensure sterility and prevent contamination.

Lactic acid bacteria count

The pour plate method was used for the enumeration of *L. acidophilus* and *L. bulgaricus*. A 1 mL aliquot of the diluted fermented beverage was pour-plated with molten De Man, Rogosa, and Sharpe (MRS) agar (HiMedia, India) in sterile Petri plates, done in tri-plicate. The plates were incubated at 37 °C for 72 hours in a laboratory oven (Heraeus, Germany). The enumeration of *Streptococcus thermophilus* was performed using the spread plate method, where 0.1 mL of the diluted sample (second dilution) was inoculated on solidified M17 agar (Basebio, China) and incubated at 37°C for 72 hours. Colony-forming units (CFU) were manually counted at 24, 48, and 72 hours, and the results were expressed as CFU/mL.

Yeast and molds count

Yeast and mold counts were conducted according to the BAM method for Yeast, Molds, and Mycotoxins. Potato Dextrose Agar (TiMedia, India) was prepared as per the manufacturer's instructions. Chloramphenicol was used to inhibit bacterial growth and was aseptically added to molten PDA agar at a ratio of 0.1 g per 40 mL of distilled water. This solution was then mixed with 960 mL of molten PDA.

The final mixture was plated into sterile Petri plates and allowed to solidify in triplicate. A 1 mL aliquot of the homogenized fermented beverage was diluted in 9 mL sterile peptone water and mixed using a vortex mixer (Ohaus, USA) for 1 minute. A 0.1 mL aliquot of the diluted sample was inoculated onto solidified PDA agar with chloramphenicol and incubated at room temperature for 5 days. Manual enumeration of yeast and molds was performed, and the results were expressed as CFU/mL.

Antimicrobial assay

The antimicrobial assay followed the well-diffusion method. The turbidity of two nutrient broths, each containing *Escherichia coli* and *Staphylococcus aureus*, was adjusted to the 0.5 McFarland standard. After adjusting the turbidity, the plates containing sterile solidified Mueller Hinton Agar (MHA) were swabbed with the pathogens to create a lawn, allowing the agar to sit for 5–10 minutes to absorb the swabbed broth. After the agar was dried, a sterile borer was used to create equidistant wells in the MHA. Each well was filled with 80 µL of the corresponding treatment, numbered 1–9. Positive controls included Penicillin G for *S. aureus* and Streptomycin for *E.*

coli, while distilled sterile water served as the negative control.

Viability of lactic acid bacteria strains

Viability screening of lactic acid bacteria was performed using a non-dairy yogurt starter containing *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus*, obtained from Belle and Bella (Canada). The lyophilized, freeze-dried microorganisms were hydrated in peptone water up to the 5^{th} dilution. The 5^{th} dilution was plated in triplicate, stored in an anaerobic chamber, and incubated at 37°C for 24 to 72 hours. Colony-forming units (CFUs) were manually counted and recorded as CFUs.

Statistical analysis

The results were analyzed statistically using MINITAB 17.0 software. One way analysis of variance (ANOVA) was utilized to compare the differences between treatments and to determine if there were statistically significant differences among treatments. The Tukey's Honestly Significant Difference (HSD) post hoc test was employed following the ANOVA analysis to make pairwise comparisons between group means, identifying which specific groups differed.

RESULTS AND DISCUSSION

TSS and pH of synbiotic drink before and after fermentation

In this study, significant observations regarding the effect of fermentation on the color, pH level and TSS in degree brix of the synbiotic drink formulations. After the fermentation process, the synbiotic drink exhibited a color change from gray to pinkish brown (Fig. 2), the pH level, on the other hand, decreased across all formulations from a range of 6.23 to 6.47 to a range of 4.44 to 4.54 as indicated in Table 2. The decrease in pH further confirms the active fermentation of the lactic acid bacteria present in the synbiotic drink. In fermented drinks, pH is a critical parameter for ensuring microbiological stability and protects the product from harmful pathogens (Mohammadi, Nouri & Mortazavian, 2021). A previous study have observed that lactic acid bacteria fermentation reduces the pH level of the drink made from sea buckthorn, soymilk and inulin, reducing the pH from 6.0 to nearly the same final level of pH after six hours of fermentation period (Maftei et al., 2023).

Furthermore, the degree Brix of the samples declines from a range of 16.00 to 23.00 to a range of 13.00 to 19.23. Degree brix reflects the sugar concentration present in the samples (Magwaza & Opara, 2015). The decline of TSS in degree brix and the pH level can be attributed to the presence of *L. acidophilus*, *L. bulgaricus* and *S. thermophilus*, which consume the sugar present in the drink and convert this into organic acid, primarily lactic acid (Ngwenya, Nkambule & Kidane, 2023). The decline of pH was almost similar across samples of the synbiotic drink formulations, even when the extent of sugar reduction differed, suggesting good buffering capacity of the drinks. The fermentation of fruits and vegetables by lactic acid bacteria is linked to improved health benefits, primarily because it leads to a reduction in their sugar content (Pinto,Vilela & Cosme, 2022). Similar findings have been reported in research exploring the fermentation of different fruit juices, such as *Opuntia ficus*-indica fruit juice (Wang et al.,2024), and jackfruit juice (Muhialdin, Hussin, Kadum, Hamid & Jaafar, 2021).

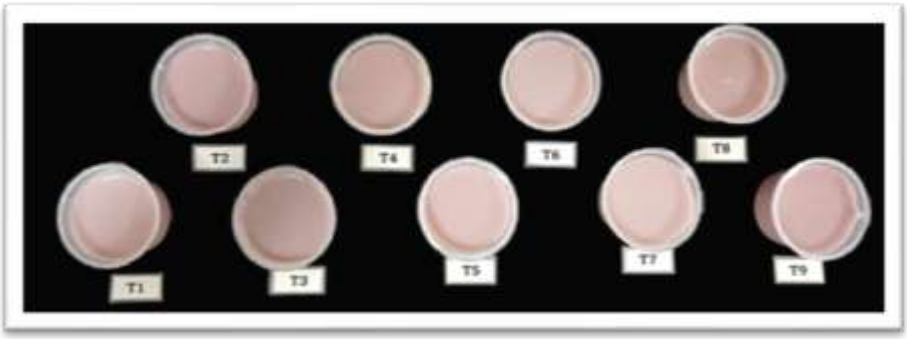

Figure 2. Appearance of the symbiotic drinks from Treatments 1-9.

Table 2.
The pH and Total soluble solids (^{0}Brix) of the synbiotic drinks before the fermentation process

Treatment	Before fermentation		After fermentation	
	TSS (^{0}Brix)	**pH**	**TSS** (^{0}Brix)	**pH**
1	16.00 ± 0.00^{i}	6.38 ± 0.00^{d}	13.40 ± 0.52^{d}	4.53 ± 0.00^{a}
2	18.00 ± 0.00^{h}	6.35 ± 0.02^{e}	13.00 ± 0.00^{d}	4.51 ± 0.00^{a}
3	18.00 ± 0.00^{g}	6.23 ± 0.01^{f}	13.03 ± 0.05^{d}	4.53 ± 0.00^{a}
4	19.00 ± 0.00^{f}	6.36 ± 0.00^{a}	15.16 ± 0.11^{bc}	4.54 ± 0.01^{a}
5	20.00 ± 0.00^{e}	6.47 ± 0.02^{bcd}	15.06 ± 0.05^{c}	4.48 ± 0.01^{a}
6	21.00 ± 0.00^{d}	6.43 ± 0.01^{bcd}	15.73 ± 0.40^{b}	4.44 ± 0.00^{a}
7	22.00 ± 0.00^{c}	6.45 ± 0.00^{b}	19.33 ± 0.05^{a}	4.46 ± 0.00^{a}
8	23.00 ± 0.00^{b}	6.45 ± 0.01^{bc}	19.20 ± 0.00^{a}	4.50 ± 0.00^{a}
9	23.00 ± 0.00^{a}	6.43 ± 0.00^{dcd}	19.23 ± 0.05^{a}	4.53 ± 0.01^{a}
F-value		142.62**	415.96**	415.96**

The values are means values n=3 ± SD.
*Values with a different letter are significantly different (p < 0.05) according to Tukey's Honestly Significant Difference (HSD) post hoc test. Uppercase superscript represents a statistically significant effect within the column. * Symbol represents p value (*p < 0.05) and (**p<0.01) highly significant*

Table 3.
The viscosity, density and titratable acidity of the synbiotic drink

TRT	Density (g/ml)	Viscosity (mpa's)	TA (%)
1	1.06 ± 0.00^{a}	102.4 ± 0.00^{b}	0.096 ± 0.023^{a}
2	1.06 ± 0.00^{a}	204.8 ± 0.00^{ab}	0.102 ± 0.018^{a}
3	1.05 ± 0.00^{a}	153.6 ± 0.00^{ab}	0.108 ± 0.013^{a}
4	1.07 ± 0.00^{a}	153.6 ± 0.00^{ab}	0.102 ± 0.013^{a}
5	1.08 ± 0.00^{a}	153.6 ± 0.00^{ab}	0.122 ± 0.000^{a}
6	1.07 ± 0.00^{a}	153.6 ± 0.00^{b}	0.108 ± 0.018^{a}
7	1.08 ± 0.00^{a}	204.8 ± 0.00^{ab}	0.099 ± 0.013^{a}
8	1.08 ± 0.00^{a}	256 ± 0.00^{a}	0.087 ± 0.009^{a}
9	1.09 ± 0.00^{a}	204.8 ± 0.00^{ab}	0.099 ± 0.010^{a}
F-value	0.60	3.48*	1.31

The values are means values n=3 ± SD.
*Values with a different letter are significantly different (p < 0.05) according to Tukey's Honestly Significant Difference (HSD) post hoc test. Uppercase superscript represents a statistically significant effect within the column. * Symbol represents p value (*p < 0.05) and (**p<0.01) highly significant*

Titratable acidity, viscosity and density of synbiotic drink samples

The presence of *L. acidophilus, L. bulgaricus*, and *S. thermophilus* influences the viscosity and density of the drink samples, as these bacteria produce exopolysaccharides during fermentation (Jurášková, Ribeiro & Silva, 2022). The viscosity values, which range from 102.4 ± 0.00 to 204.8 ± 0.00 mPa·s, are considered acceptable for a synbiotic drink. This increase in viscosity is a characteristic result of protein coagulation that occurs as the pH of the drink decreases (Li et al., 2014). Furthermore, the samples exhibited higher density, indicating greater viscosity compared to non-probiotic beverages.

The titratable acidity of the synbiotic drink samples ranges from 0.087 to 0.122, which shows that the acid content in the samples is low even if the pH of the sample reaches between 4.4 and 4.5, which is the ideal pH for a synbiotic drink. This is due to the high buffering capacity of soymilk present in the drinks. Soymilk contains a large amount of proteins, mainly glycinin and B-conglycinin (Giri & Mangaraj, 2012), that act as natural buffers that prevent rapid changes in pH. Moreover, the juice of the Malabar melastome has low acidic content (Barman & Barooah, 2016), which is natural for fruit-based probiotic beverage (Luckow, Sheehan, Fitzgerald & Delahunty, 2006). In this study, samples with a moderate level of sugar and fruit powder have a

higher percentage of lactic acid equivalent. This shows that a moderate level of sugar increases the metabolic activity of lactic acid bacteria, which leads to an increase in acidity formulation; however, formulations which contain a high level of sugar decrease the titratable acidity. Likely, this is due to the osmotic stress of lactic acid bacteria at high sugar concentration, which limits their microbial activity (Sunny-Roberts & Knorr, 2008), hindering their metabolic processes and reducing lactic acid production in the samples.

Total phenolic content, total monomeric anthocyanin, antioxidant capacity, tannin and saponin of synbiotic drink

Phenolic compounds are most common on plant based drinks (Marafon, Prestes, Carvalho, De Souza & Prudencio, 2025). In this study, the concentration of phenolic compounds reacting with the Folin–Ciocalteu reagent in the synbiotic drink samples ranged from 1.72 ± 0.00 to 4.27 ± 0.05 mg GAE/mL, with Treatment 7 (4.27 ± 0.05 mg GAE/mL), followed by Treatment 4 (4.19 ± 0.02 mg GAE/mL) as presented in Table 4. In contrast, Treatment 3 (1.72 ± 0.00 mg GAE/mL) exhibited the lowest phenolic content. These findings suggest that the phenolic compound levels were significantly influenced by the sugar and fruit powder concentrations in the drink. The trend in the data indicates that formulations with lower fruit powder levels exhibited higher measurable phenolic content, as revealed by the Folin–Ciocalteu assay. This behavior may be explained by the biochemical transformation of phenolics during fermentation (Gaur & Gänzle, 2023). Phenolic compounds can undergo degradation in the presence of fermenting microbes (Liang, Huang, Zhang & Fang, 2023). In this study, *L. acidophilus, L. bulgaricus* and *S. thermophilus* present in the synbiotic drink metabolizes phenolic compounds and thus reduce the content of phenolic compounds detectable by Folin-Ciocalteu reagent. Lactobacillus species have the ability to metabolize phenolic acids, which can sometimes be harmful or act as antinutritional factors. The breaking down of phenolic compounds by Lactobacilli is a predominant mechanism for detoxifying plant substrates (Sánchez-Maldonado, Schieber & Gänzle, 2011). This finding indicates that phenolic content in the synbiotic drink is not only dependent on the concentration of raw materials but also influenced by bacterial metabolism and fermentation.

The presence of flavonoid content in Malabar melastome has been reported to be high (Hosni, Gani, Orsat, Hassan & Abdullah, 2023), and among these compounds are the anthocyanins, water-soluble pigments known for their beneficial effects on human health (Turturică, Oancea, Râpeanu & Bahrim, 2015; Lu et al., 2021). Anthocyanins are known to exhibit antioxidant capacity by scavenging free radicals (Khoo, Azlan, Tang & Lim, 2017). According to Bakuradze et al. (2019), anthocyanin-rich fruit juice demonstrated strong antioxidant effects against free radicals. Consistent with these findings, this study reveals that the synbiotic samples exhibit strong scavenging activity against DPPH free radicals, which ranges from 87.18% to 94.44%. The antioxidant activity of the synbiotic drinks did not differ significantly among formulations 1 to 8; however, formulation 9 exhibited a statistically significant difference as revealed by a post hoc test. This variation may be attributed to the interaction between the higher levels of sugar and fruit powder in formulation 9. Previous studies have reported that higher sugar concentrations can reduce antioxidant capacity (Shalaby, Mahmoud & Shanab, 2016). Consistent with this finding, the present study observed a decrease in antioxidant activity in the formulation containing higher sugar and fruit powder levels. This reduction may be due to condensation reactions involving the hydroxyl groups of phenolic compounds from soymilk and Malabar melastome fruit powder with the hydroxyl groups of sucrose molecules.

Overall, the synbiotic drink formulation possesses strong antioxidant potential, which is primarily attributed to Malabar melastome, containing substantial amounts of phytochemicals that contribute to its excellent free radical scavenging capacity (Hosni et al., 2023). This finding is consistent with previous reports that non-dairy probiotic beverages are excellent sources of antioxidants (Zahrani & Shori, 2023). The presence and stability of anthocyanin pigments in the synbiotic drink formulations were confirmed via total monomeric anthocyanin assay. The results of the assay revealed that treatments containing higher levels

Table 4.
Biofunctional properties of the synbiotic drink samples

TRT	TA %	TPC (mg GAE/mL)	TMA (mg/L)	DPPH scavenging assay (% IR)	Tannin test	Saponin test
1	0.096 ± 0.023^{a}	3.63 ± 0.00^{c}	42.093 ± 0.31^{c}	94.02 ± 0.86^{a}	+	+
2	0.102 ± 0.018^{a}	2.37 ± 0.00^{f}	68.120 ± 1.67^{bc}	93.72 ± 0.00^{a}	+	+
3	0.108 ± 0.013^{a}	1.72 ± 0.00^{i}	70.20 ± 0.84^{ab}	94.17 ± 0.07^{a}	+	+
4	0.102 ± 0.013^{a}	4.19 ± 0.02^{b}	47.94 ± 0.32^{d}	94.88 ± 0.07^{a}	+	+
5	0.122 ± 0.000^{a}	2.65 ± 0.00^{e}	65.40 ± 0.63^{c}	93.92 ± 0.14^{a}	+	+
6	0.108 ± 0.018^{a}	1.96 ± 0.00^{h}	73.19 ± 0.63^{a}	93.77 ± 0.78^{a}	+	+
7	0.099 ± 0.013^{a}	4.27 ± 0.05^{a}	47.94 ± 1.25^{d}	94.28 ± 0.64^{a}	+	+
8	0.087 ± 0.009^{a}	2.78 ± 0.00^{d}	69.09 ± 1.88^{b}	94.28 ± 0.35^{a}	+	+
9	0.099 ± 0.010^{a}	2.09 ± 0.00^{g}	69.99 ± 0.63^{b}	87.18 ± 1.93^{b}	+	+
F-Value	1.31	10075.92**	385.00*	17.38**		

*The values are means values n=3 ± SD. Values with a different letter are significantly different (p < 0.05) according to Tukey's Honestly Significant Difference (HSD) post hoc test. Uppercase superscript represents a statistically significant effect within the column. * Symbol represents p value (*p < 0.05) and (**p<0.01) highly significant.*
(+) indicates the presence of tannins and saponins; (-) indicates the absence of tannins and saponins

of fruit powder (2 g) exhibited greater anthocyanin concentrations, regardless of sugar content (10 g, 15 g, or 20 g), as shown in Table 4. The enhanced stability of anthocyanins in formulations with increased fruit powder may be attributed to the higher pigment load present in these samples and the presence of sugar. Sugar concentrations of up to 20% have been shown to exert a protective effect on anthocyanin, thereby promoting its stability (Nikkhah, Khaymayu, Heidari, & Jamee et al., 2007). Further, it has been observed that the anthocyanin content in the drinks was preserved even during the fermentation carried out by lactic acid bacteria. This observation is consistent with the findings of Palencia-Argel, Rodríguez-Villamil, Bernal-Castro, Díaz-Moreno and Fuenmayor (2022), who reported that anthocyanin levels remained stable in synbiotic drinks containing viable cell counts above the minimum dose required to confer a probiotic effect.

Phytochemical screening further revealed that all formulations tested positive for tannins and saponins, indicating the presence of additional bioactive compounds derived from Malabar melastome. Tannins, polyphenolic compounds with astringent properties, contribute to neutralize free radicals (Cosme et al., 2025) while saponins possess emulsifying properties and exhibit cholesterol-lowering and antimicrobial activities (Timilsena, Phosanam & Stockmann, 2023). The presence of these compounds after fermentation suggests that *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* were able to release them from the plant matrix. This is consistent with previous findings that fermentation decreases the levels of total phenolic compound attributed to hydrolysis and degradation, while a corresponding increase in some bioactive compounds (Adebo, Njobeh, Adebiyi & Kayitesi, 2018).

Viability of *S. thermophilus*, *L. acidophilus,* and *L. bulgaricus* in the synbiotic drink samples

The viability of *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* were evaluated across 9 samples of synbiotic drink incubated at 40 ºC for 72 hours. The viability of lactobacillus species after the incubation period reached to 7-8 log10 CFU/ml, which is the ideal microbial count for a synbiotic drink to have a probiotic effect. This result is in consistent with the guidelines of the World Gastroenterology Organization on Probiotic and Prebiotic (Guarner et al., 2024), which states that the minimum concentration of Lactic Acid Bacteria in the food should be around 8 log CFU/mL at the time of consumption to ensure that reductions in viability during gastrointestinal tract (GIT) exposure do not impair the probiotics' functional effects on the host. Food and Agriculture Organization and World Health Organization (FAO/WHO, 2002) also emphasized that drinks with probiotics should contain at least 10^6 to 10^7 CFU/mL of microorganisms to be labeled as probiotics, of which

this study demonstrates that the viable count of lactic acid bacteria (LAB) exceeds the standards established by the FAO/WHO.

According to Castillo-Escandón, Fernández-Michel, Wong and Montfort (2019), Lactobacillus species are autochthonous to fruits and are therefore more resistant to the physicochemical properties of plant-based matrices. The inclusion of inulin and soymilk in the synbiotic drink formulated with Malabar melastome further enhances the growth performance of *L. acidophilus* and *L. bulgaricus*. Soymilk has been reported to promote the proliferation of lactic acid bacteria even without additives (Ismaiel, El-Wahed, Khalifa, Baky & Ashor, 2018). Similarly, soymilk combined with fruit juice, such as apple juice, has been shown to support the growth of *L. acidophilus* (İçier, Gündüz, Yılmaz & Memeli, 2015), emphasizing the suitability of the soymilk-fruit matrix used in this study. The presence of inulin further supports this soymilk-fruit matrix, given its established protective effect on lactic acid bacteria, thereby enhancing its survival under stressful condition (Iraporda et al., 2022) and stimulate their metabolic activity as probiotics (De Souza Oliveira, Perego, De Oliveira & Converti, 2017). This combination likely contributes to the substantial counts of Lactobacillus in the synbiotic drink samples.

On the other hand, *S. thermophilus* showed minimal variation from 48 h to 72 h of incubation period, with the count of 4 log10 CFU/mL across all samples. The lower CFU/mL of *S. thermophilus* in the samples can be attributed to the presence of anthocyanin compounds. Anthocyanin-rich juices have been reported to exert antimicrobial effects against both Gram-negative and Gram-positive bacteria (Cisowka, Wojnicz & Hendrich, 2011). In the present study, *S. thermophilus*, a gram-positive bacterium, showed minimal counts throughout the incubation period, reaching only 4 log10 CFU/mL. This number of viability is associated with the inhibitory effects of anthocyanins present in the samples, which may suppress the growth of *S. thermophilus*. Gamage, Goh and Choo (2024) described that the presence of an anthocyanin-rich fruit juice in yogurt reached to an acceptable range of 7 log10 CFU/mL; however, this finding contrasts those observed in the present study. This may be attributed to the antimicrobial properties of the fruit powder when incorporated into food systems. The addition of plant derivatives has been reported to negatively affect microorganisms due to their inherent antimicrobial activity (Buriti, Komatsu & Saad, 2007), particularly *S. thermophilus*.

In addition, microbial viability across samples significantly differs, as revealed by the post hoc test. These findings can be attributed to the different levels of sugar and fruit powder present within each sample. Sugar serves as food of the lactic acid bacteria; however, at higher concentrations, sugar can create osmotic pressure to these bacteria preventing their metabolic functions. Therefore, varying sugar levels would exhibit different lactic acid bacteria counts. Further, the combination of sugar and fruit powder creates a unique matrix for each sample. The presence of sugar as a food or osmotic stressor and the potential inhibitory or protective effects of anthocyanin results in the observed differences in microbial viability of lactic acid bacteria present in the samples. Moreover, no yeast or mold colony-forming units were detected across all samples, which was further confirmed by the PDA control plates without a sample.

Antimicrobial assay

The results of the antimicrobial activity of the synbiotic drinks, as evaluated by the agar well diffusion assay, are presented in Table 6. Zones of inhibition were measured; however, the minimum inhibition concentration (MIC) values were not included in this study. The assay shows that the three synbiotic drink formulations demonstrated antimicrobial activity against *S. aureus,* a gram-positive bacterium. The zone of inhibition of the different treatments of the synbiotic drinks showed that Treatment 1, 2, and 5 inhibited the growth of *S. aureus* at around 10.5 mm to 11.00 mm of which Treatment 5 exhibited the highest zone of inhibition with an average of (11.0 ± 1.41 mm), followed by Treatment 1 and 2 with the same zone of inhibition of 10.5 ± 0.71 mm, however, the remaining treatments showed no inhibitory activity against *S. aureus*. On the other hand, none of the drink formulations exhibited antimicrobial activity against *E. coli*, a gram-negative bacterium. The absence of antimicrobial activity in some treatments can be attributed to the presence of different an-

thocyanin concentrations in the samples. Anthocyanin efficacy is dose dependent (Mohammadi et al., 2024), which means the higher the dose of anthocyanin in the drink matrices, the higher its capacity to inhibit pathogenic bacteria. Anthocyanins can reduce the presence of pathogenic bacteria that pro-duce toxins (Ma et al., 2019) and are further known to exert stronger antibacterial effects on Gram-positive bacteria due to their ability to penetrate and disrupt cell membranes (Cisowska et al., 2011). Anthocyanins execute their antimicrobial activities by stimulating cell damage, affecting bacterial respiratory metabolism, and encouraging bacterial cell death (Deng, Meng, Xue & Li, 2024). Furthermore, lactic acid bacteria (LAB) present in the synbiotic formulations may also contribute to antagonistic activity via bacteriocin production (Imade, Omonigho, Babalola & Enagbonma, 2021; Chen, Jia, Wu & Ren, 2024). Additionally, the presence of Malabar melastome in the drink formulation may have contributed to the drink's ability to inhibit *S. aureus* but not *E. coli*. These findings are consistent with those of Mayasari, Murti, Sudarsono and Pratiwi (2021), who reported that Malabar melastome extract was more effective against *S. aureus*, a Gram-positive bacterium, than against *E. coli*, a Gram-negative bacterium. Similarly, a study by Alnajar, Abdulla, Ali, Alshawsh and Hadi (2012) found that the extract of *Malabar melastome* exhibited stronger activity against two Gram-positive bacteria: *S. aureus* and *Streptococcus agalactiae.*

Table 5
Streptococcus thermophilus and Lactobacillus strain count (Log_{10} CFU/mL)

Treatment	**Incubation period**				
	Streptococcus thermophilus		**Lactobacillus strain**		
	48 h	**72 h**	**24 h**	**48 h**	**72 h**
1	4.11 ± 0.21^{a}	4.11 ± 0.21^{a}	7.86 ± 0.13^{ab}	7.94 ± 0.08^{ab}	7.96 ± 0.07^{ab}
2	4.25 ± 0.06^{a}	4.27 ± 0.08^{a}	8.06 ± 0.04^{a}	8.12 ± 0.10^{a}	8.13 ± 0.11^{a}
3	4.23 ± 0.11^{a}	4.24 ± 0.09^{a}	8.01 ± 0.10^{a}	8.08 ± 0.06^{a}	8.08 ± 0.06^{a}
4	4.24 ± 0.04^{a}	4.29 ± 0.04^{a}	7.99 ± 0.03^{a}	8.06 ± 0.03^{ab}	8.07 ± 0.03^{ab}
5	4.18 ± 0.10^{a}	4.19 ± 0.09^{a}	7.72 ± 0.09^{b}	7.85 ± 0.07^{b}	7.85 ± 0.06^{ab}
6	4.37 ± 0.53^{a}	4.38 ± 0.52^{a}	7.97 ± 0.01^{ab}	8.05 ± 0.05^{ab}	8.07 ± 0.07^{ab}
7	4.41 ± 0.07^{a}	4.41 ± 0.06^{a}	7.88 ± 0.07^{ab}	7.94 ± 0.10^{ab}	7.95 ± 0.10^{ab}
8	4.19 ± 0.04^{a}	4.19 ± 0.04^{a}	7.82 ± 0.13^{ab}	7.95 ± 0.07^{ab}	7.96 ± 0.06^{ab}
9	4.35 ± 0.07^{a}	4.35 ± 0.07^{a}	7.89 ± 0.09^{ab}	7.94 ± 0.10^{ab}	7.97 ± 0.10^{ab}
F-value	0.49	0.49	4.30**	3.90**	3.92**

*The values given above are reported as means and standard deviations. Values with a different letter are significantly different (p < 0.05) according to Tukey's Honestly Significant Difference (HSD) post hoc test. Uppercase superscript represents a statistically significant effect within the column. * Symbol represents p value (*p < 0.05) and (**p<0.01) highly significant.*

Table 6.
Zone of inhibition of the synbiotic drink samples against *E. coli* and *S. aureus*

TRT	**Zone of inhibition** (mm)	
	E. coli	***S. aureus***
1	0.00	10.5 ± 0.71^{a}
2	0.00	10.5 ± 0.71^{a}
3	0.00	0.00^{a}
4	0.00	0.00^{a}
5	0.00	11.0 ± 1.41^{a}
6	0.00	0.00^{a}
7	0.00	0.00^{a}
8	0.00	0.00^{a}
9	0.00	0.00^{a}
F-value		2.96*

*The values given above are reported as means and standard deviations. Values with a different letter are significantly different (p < 0.05) according to the Tukey's Honestly Significant Difference (HSD) post hoc test. Uppercase superscript represent a statistically significant effect within column.*Symbol represents p value (p < 0.05) and (**p<0.01) highly significant*

Furthermore, ethanol extracts of Malabar melastome showed significant inhibition against *S. aureus* (Novelni, Yupelmi, Agustina, Putri & Minerva, 2023).

CONCLUSIONS

This study examined the novelty of developing a synbiotic drink based on Malabar melastome (*Melastoma malabathricum* L.) fruit and soymilk, supplemented with inulin and fermented with a mixture of lyophilized *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* strains. The formulated drinks exhibited high antioxidant activity and contained beneficial bioactive compounds such as phenolics, tannins, and saponins. Furthermore, the drinks showed good viscosity and density, an acceptable pH level for synbiotic beverages, and appropriate total soluble solids, as revealed by physico-chemical analysis. The microbial viability of the lactic acid bacterial strains over a 72-hour incubation period demonstrated that the drinks have a robust capacity to support LAB growth. Moreover, anti-microbial activity was detected in the different formulations against gram-positive *S. aureus*; however, no antimicrobial activity was observed against *E. coli*, as revealed by the well-diffusion method. Biofunctional assays revealed that the drink contains tannin, saponin, anthocyanin, and phenolic compounds, which contributes to its antioxidant capacity to inhibit free radicals.

Based on the findings, Malabar melastome shows potential as an ingredient in the formulation of synbiotic drinks. However, further studies using experimental designs that explore three or more variables are recommended. Future research should also include a broader range of gram-negative and gram-positive pathogens, as well as pathogenic fungi, for *in vitro* analysis. Additionally, *in vivo* studies should be conducted to assess the safety and health efficacy of the formulated synbiotic drinks. Standardized broth dilutions methods are recommended to be studied to quantitatively establish the MIC values and confirm the inhibitory activity of the formulations. Also, further studies are needed to assess the sensory acceptability and long-term stability of the synbiotic drink formulations.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, investigation, validation, formal analysis, writing—original draft, J.C.S; Supervision, writing—review and editing, conceptualization, methodology, writing—review and editing, supervision, M.A.J.R.R.
All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

To the best knowledge of the researchers, there is no conflict of interest in our research work entitled “Physico-chemical, antioxidant activeties and antimicrobial properties of synbiotic drink from Malabar melastome (*Melastoma malabathricum* L.) and soymilk”.

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FIZIČKO-HEMIJSKE, ANTIOKSIDATIVNE I ANTIMIKROBNE OSOBINE SINBIOTIČKOG NAPITKA ZASNOVANOG NA MALABARSKOM MELASTOMU *(MELASTOMA MALABATHRICUM* L.*)* I SOJINOM MLEKU

Jomarie C. Salar[*1,2] , Mary Ann Jilly R. Ramirez[3]

[1]Državni univerzitet Južni Lejte, Fakultet za tehnologiju, Doktorske studije iz menadžmenta tehnologije, Soged, Južni Lejte, Filipini
[2]Državni univerzitet Južni Lejte, Fakultet za ugostiteljstvo i menadžment u turizmu, Soged, Južni Lejte, Filipini
[3]Državni univerzitet Južni Lejte, Departman za nauku o hrani i tehnologiju, Soged, Južni Lejte, Filipini

Sažetak:: Malabarski melastom *(Melastoma malabathricum* L.*)* široko je prepoznat po svojim farmakološkim svojstvima, naročito zbog bogatog sadržaja antioksidanasa i antimikrobnih jedinjenja. Ova studija ispituje razvoj i evaluaciju sinbiotičkog napitka formulisanog od soka ploda malabarskog melastoma i sojinog mleka kao zdravije alternative konvencionalnim probiotičkim napicima na bazi mleka. Potpuni faktorski eksperimentalni dizajn (3×3) generisao je devet kombinacija tretmana. Efekat tretmana je ocenjen određivanjem fizičkohemijskih karak-teristika (gustina, viskoznost, pH, ukupne rastvorljive materije i titrabilna kiselost), sadržaja bioaktivnih jedinjenja (ukupni fenolna jedinjenja, sposobnost uklanjanja DPPH radikala, ukupni monomerni antocijanini, saponini i tanini), mikrobiološkoj održivosti i antimikrobnoj aktivnosti napitka. Napitak je u svom sastavu uključivao mešovitu kulturu *Lactobacillus acidophilus, Streptococcus thermophilus* i *L. bulgaricus*. Rezultati su pokazali da sinbiotički napitak poseduje poželjnu viskoznost, uravnotežen odnos slatkoće i kiselosti, kao i izuzetnu antioksidativnu aktivnost, uglavnom zahvaljujući bioaktivnim jedinjenjima iz malabarskog melastoma. Broj živih ćelija *L. acidophilus i L. bulgaricus* kretao se u rasponu 7,85 - 8,13 log CFU/mL. Za poređenje, brojživih ćelija *S. thermophilus* se kretao od 4,11 log CFU/mL do 4,38 log CFU/mL nakon 72 sata inkubacije, što potvrđuje dovoljnu probiotičku održivost za sinbiotičku klasifikaciju. Antimikrobni test pokazao je inhibitorne efekte samo protiv *Staphylococcus aureus* (Gram-pozitivna bakterija). Sveukupno, nalazi sugerišu da malabarski melastom poboljšava i funkcionalni i mikrobiološki kvalitet napitka obogaćenog mlečnokiselinskim bakterijama, nudeći potencijal kao prirodnog, funkcionalnog sastojka u razvoju sinbiotičkih napitaka.

Ključne reči: *mlečno-kisele bakterije, antioksidaciona aktivnost, DPPH aktivnost, ukupni fenoli, ukupni monomerni antocijani, antimikrobna aktivnost*

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