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## Thermosonication and Pasteurisation Effects on the Quality Attributes of Roselle Drink, and its Application as a Non-dairy Carrier for *Lacticaseibacillus rhamnosus* G.G

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### Abstract

This study aimed to assess the suitability of roselle drink as a non-dairy carrier for probiotic *Lacticaseibacillus rhamnosus* GG (LGG), and compare the effects of thermosonication versus pasteurisation as well as probiotic inoculation mode (free or encapsulated) on the probiotic viability, microbiological safety, chemical, physical, and sensory properties of Roselle drink with free and encapsulated LGG. Roselle drink produced was either pasteurised (72 °C, 15 s) or thermosonicated (25kHz frequency, 40 °C for 30 minutes) before inoculation for fermentation (37 °C, 12 h) with free or encapsulated *L. rhamnosus* GG, respectively, while the untreated roselle drink served as the control. All roselle drink samples were evaluated for chemical [pH, Total Titratable acidity (TTA) and anthocyanin content], microbiological [Total Viable Count (TVC) of *L. rhamnosus* GG, enumeration of fungi, coliforms, and *Salmonella* and *Shigella* spp. using the pour plating method] properties. The microorganisms enumerated were expressed in Colony Forming Units per mL (CFU/mL). Sensory evaluation was conducted on a 9-point hedonic scale. Data were analysed using ANOVA at  $p < 0.05$ . The pH of all the fermented roselle drinks significantly ( $p < 0.05$ ) decreased from the initial 3.4 (0 h) to 2.7 - 2.9 (12 h). The TTA of thermosonicated roselle drink with free (UF) or encapsulated *L. rhamnosus* GG (UE) was significantly higher ( $p < 0.05$ ) than the pasteurised counterpart by the end of the 12-h fermentation. Anthocyanin and redness attribute ( $a^*$ ) were highest in UF ( $16.84 \pm 0.06$  mg/100 mL and  $19.90 \pm 0.35$ , respectively). Total Viable Count (TVC) of free or encapsulated *L. rhamnosus* GG in pasteurised or thermosonicated roselle maintained above 10 Log CFU. Fungi, coliforms, and *Salmonella* and *Shigella* spp. were not observed in all the treated samples, whereas the control had significantly higher coliforms ( $1.1 \times 10^3$  CFU/mL). The taste and overall acceptability of sensory qualities in all LGG-fermented samples ranked above 7 on a 9-point hedonic scale, higher in the thermosonicated sample with encapsulated *L. rhamnosus* GG. Thermosonicated roselle drink exhibited improved physicochemical properties, anthocyanin

content, and sensory attributes compared with pasteurised samples, while supporting probiotic viability and ensuring microbiological safety. Roselle drink is a suitable carrier for probiotic *Lacticaseibacillus rhamnosus GG* and is a promising health-promoting beverage.

Keywords: Thermosonication, Pasteurisation, Roselle drink, Probiotic zobo drink, *L. rhamnosus GG*, Anthocyanin.

## 1.0 Introduction

Roselle (*Hibiscus sabdariffa*) drink, produced by hot water extraction from the dried calyces of the red hibiscus plant, is relished in Sub-Saharan Africa as a non-alcoholic beverage (Aboagye *et al.*, 2020). In Nigeria, it is referred to as *zobo* (Ehirim *et al.*, 2022). The red calyx variety of the hibiscus plant has significant dietary importance as it is rich in nutrients and antioxidant properties due to its anthocyanin and polyphenol contents (Abidoye *et al.*, 2022). There is growing health awareness among consumers, thus increasing the interest in healthy foods (Topolska *et al.*, 2019, Anusha-Siddiqui *et al.*, 2023). This has made roselle drink popular as a street-vended drink in Nigeria and Sub-Saharan Africa due to its health benefits, such as antioxidant properties offered by the presence of anthocyanin and other bioactive constituents (Idowu-Adebayo *et al.*, 2021).

This increasing health awareness of consumers has also popularised functional foods and other health foods such as products containing probiotics (Birch and Bonwick, 2019), which are live microorganisms that confer several health benefits- including improving gut microflora balance and stability, when administered in adequate amounts (Mbye *et al.*, 2020). These microorganisms are mainly members of the Lactic Acid Bacteria, especially those of the genera *Lactobacillus* and *Bifidobacterium*. Therefore, the inclusion of probiotics such as *Lacticaseibacillus rhamnosus GG*, in roselle drink will further enhance its health benefits, offering a product with bioactive compounds and probiotic functions. *L. rhamnosus GG* is acknowledged for its effectiveness in addressing various forms of diarrhoea and irritable bowel syndrome, a common primary symptom of gastrointestinal tract disorders (Capurso, 2019; Mekonnen *et al.*, 2020). The consumer requirements for probiotic products specify that it is essential to maintain the viability of probiotic bacteria at a level exceeding  $10^6$  Colony Forming Units (CFU) per mL or gram of food product from the time of production until consumption, to achieve favourable therapeutic outcomes (Sarowska *et al.*, 2013).

In the food industry, there is a growing interest in researching new and innovative non-dairy alternatives as carriers for probiotics. This trend is driven by the increasing demand from consumers seeking non-dairy probiotic options (Min *et al.*, 2019). Recent studies have explored various non-dairy food matrices, such as vegetable juices and cereal gruels, as potential carriers for probiotic microorganisms (Valero-Cases, 2019). However, limited information is available regarding the use of roselle drink as a carrier for probiotic *L. rhamnosus GG*. For this organism to withstand standard processing, storage and gastrointestinal conditions, it is important to apply probiotic encapsulation, which offers enhanced protection to these beneficial organisms through processing conditions of high temperatures, and also through human gastrointestinal transit (GIT) (Koh *et al.*, 2022). There have been several reports on the protective effect of

encapsulation on probiotics through the GIT, characterised by low pH and high bile concentration (And and Kailasapathy, 2005; Shi *et al.*, 2013; Sathyabama and Vijayabharathi, 2014; Lai *et al.*, 2020; Oguntaye *et al.*, 2021; Ali *et al.*, 2023).

Further, as health awareness among consumers is increasingly growing towards the consumption of healthy and beneficial foods, it is important to pay attention to the processing steps involved in such foods to prevent foodborne diseases. The choice of processing method determines the microbial properties and safety of ready-to-drink products. Roselle drink is usually handled with a low standard of hygiene and this could pose significant public health hazards to roselle drink consumers if the hygiene practices during its production are not improved (Aboagye *et al.*, 2020). Salami and Afolayan (2020) reported that roselle calyces contain high microbial load, which is a source of contamination in the drink, with other major sources of contamination reported to be the unhygienic practices and materials in the handling and preparation of roselle drink. This includes the equipment/utensils, water, packaging materials, and sweeteners. Several species of bacteria and fungi, including *Bacillus* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger*, *Fusarium oxysporum*, etc. have also been reported to be found in roselle drink (Bayoï *et al.*, 2021). These are spoilage and pathogenic organisms that are potential health risks to consumers. Therefore, the hygiene practices during roselle drink production need to be improved by High-Temperature Short Time pasteurisation thus yielding a product with an extended shelf life (Aboagye *et al.*, 2020). However, pasteurisation tends to impart negatively on the heat-sensitive constituents such as vitamins, carotenoid content and sensory quality of food products. Therefore, an improved technology such as thermosonication may be used.

Thermosonication is a promising non-thermal or mild-thermal processing technique that combines heat with high-intensity ultrasound (>20 kHz) to enhance microbial inactivation, enzyme denaturation, and quality retention in liquid foods. Unlike conventional pasteurisation, which relies solely on thermal effects, thermosonication generates pressure variations that induce cavitation—a phenomenon where microscopic bubbles rapidly form and collapse, releasing localised high-intensity energy. This process disrupts microbial cell structures, accelerates enzyme inactivation, and facilitates the breakdown of large molecular aggregates, potentially improving product stability and homogeneity. The synergy between ultrasound and heat in thermosonication enhances microbial and enzymatic inactivation while minimising thermal damage to sensitive bioactive compounds, vitamins, and volatile flavour compounds. Studies have shown that thermosonication can effectively reduce spoilage microorganisms and pathogenic bacteria, such as *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp., while preserving desirable physicochemical and sensory properties (Alcántara-Zavala *et al.*, 2021). Additionally, this method has been reported to improve the rheological characteristics and functional properties of beverages by promoting better solubility and dispersion of suspended particles (Sotelo-Lara *et al.*, 2024).

Compared to conventional pasteurisation, thermosonication offers advantages such as reduced energy consumption, shorter processing times, and lower thermal degradation of heat-sensitive nutrients. However, its effectiveness depends on factors such as ultrasound frequency, temperature, processing time, and the physicochemical composition of the liquid food matrix (Ozyurt *et al.*, 2019). Further research is needed to optimise process parameters for different

beverage formulations and assess the long-term impact on microbial stability, nutritional value, and consumer acceptance.

This research, therefore, aimed to evaluate the suitability of roselle drink as a carrier for *Lacticaseibacillus rhamnosus* GG under thermosonication or pasteurisation conditions, and determine the effects of probiotic inclusion and alginate encapsulation on its physicochemical properties, microbiological safety, anthocyanin content, sensory attributes, and probiotic viability during fermentation.

## 2.0 Materials and Methods

Dried red roselle calyces were purchased from a local market in Ibadan, Oyo state, Nigeria (7.3775°N, 3.9470°E). The lyophilised *L. rhamnosus* GG was obtained from Valio Ltd., Helsinki, Finland. All reagents used are of analytical grade.

### 2.1 Production of Roselle Drink

Roselle drink was produced according to the standard method described by Idowu-Adebayo *et al.* (2021). The dried roselle calyces were cleaned, sorted, washed in potable water and drained. The drained calyces were subjected to hot water extraction in the ratio 1: 62 (w/v) of calyces to hot water (100° ± 2°C) for 15 min, to obtain the roselle drink. The drink was packaged in sterile glass bottles for storage prior to probiotic inoculation and further analyses.

### 2.2 Cultivation and Encapsulation of *Lacticaseibacillus rhamnosus* GG

Free and alginate-encapsulated *L. rhamnosus* GG cells were prepared using the methods described by Ding and Shah (2008). *L. rhamnosus* GG was cultivated and encapsulated according to the method described by Oguntaye *et al.* (2021). Briefly, lyophilised *L. rhamnosus* GG pellet was reactivated by repeatedly culturing in De Mann, Rogosa and Sharpe (MRS) broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India) under anaerobic conditions for 48 hours in an incubator shaker at 37 ° ± 2 °C. After 48 h, the cells were harvested by centrifugation using a refrigerated (4 °C) centrifuge (Model K2015R, Centurion Scientific Ltd., Chichester, UK) at 5,000 x g for 30 min. The harvested cells were washed twice with sterile water (pH 7.0) before encapsulating using 3% (w/v) sterile sodium alginate solution, which was mixed with 25 mL of probiotic organisms suspended in physiological saline. The cells were uniformly distributed in the mixture by agitation. Thereafter, the mixture was dispensed into 0.1M CaCl<sub>2</sub> with a syringe of needle size 23G. The beads were washed with sterile deionised water and then stored at 4°C until further use (Krasaekoop and Kitsawad, 2010).

### 2.3 Thermosonication and Pasteurisation of Roselle Drink with Free or Encapsulated *L. rhamnosus* GG

The roselle drink was separated into four batches; two of which were either treated by pasteurisation or thermosonication in 500 mL amber-coloured glass bottles. The bottles containing roselle drink samples were pasteurised at 72 °C for 15 s in an electric-heated thermostatic water bath (Precision GP20, Thermo Scientific, USA) (Etchepare *et al.*, 2020). Based on preliminary studies (data not shown), the other batches were thermosonicated at 25kHz frequency, 40 °C, and for 30 minutes. After the treatments, the samples were rapidly cooled,

inoculated with free ( $1 \times 10^{10}$  Log CFU/mL) or encapsulated *L. rhamnosus* GG ( $1 \times 10^{10}$  Log CFU/g), and incubated for fermentation at 37 °C for 12 h. The untreated, unfermented roselle drink served as a control for subsequent analysis.

#### 2.4 Determination of pH and Total Titratable Acidity

The pH of the roselle samples was determined using a digital pH meter (Hanna H196107 pH/ORP meter) with a glass electrode (Oguntoye and Ezekiel, 2022). Total titratable acidity was determined using the titration method (Tyl and Sadler, 2017). A 10 mL volume of the sample was pipetted into an Erlenmeyer flask, two drops of phenolphthalein indicator were added, and it was titrated with 0.1 M NaOH to a faint pink colour, which disappeared after one minute when compared against a white background. The titre volume obtained was noted and used for calculations of Total titratable acidity expressed as percentage lactic acid. Total titratable acidity was calculated using equation 1 as follows:

$$\% \text{ Lactic acid} = \left( \frac{M \text{ NaOH} \times A \times 90.08}{V} \right) \times 100 \quad \text{Equation 1}$$

Where:

M = Molarity of NaOH used

A = volume of NaOH used (in Litres)

V = volume of sample used (in Litres)

90.08 = Equivalent weight of lactic acid

#### 2.5 Determination of Colour

The colour of the fermented and unfermented roselle drink samples was measured in triplicates according to the CIELab standard methods, using a Chroma meter (Konica Minolta Sensing Inc., Osaka, Japan; Model CR-410 with data processor DP 400) for parameters Lightness L\*, (0-100, where 0=black, 100= white), a\* (red-green components; +a\* = red, -a\* = green), and b\* (yellow-blue components; +b = yellow, -b\* = blue).

#### 2.6 Microbiological Analysis

Microbiological analyses were conducted using standard procedures. The total viable counts of free and alginate-encapsulated *L. rhamnosus* GG in roselle drinks were enumerated using the pour plating technique on MRS agar. For enumeration of spoilage and pathogenic organisms, *Salmonella* sp. was cultured on *Salmonella-Shigella* agar, fungi were enumerated on potato dextrose agar, and total coliforms were cultured on MacConkey agar, all using the standard pour plating method (Liu *et al.*, 2023).

#### 2.7 Determination of Total Monomeric Anthocyanin Content

The anthocyanin content of fermented and unfermented roselle drinks was evaluated using the pH differential method as described by Toktaş *et al.* (2018). Briefly, roselle drink samples were diluted by 0.4 mol/L sodium acetate buffer (pH 4.5) and 0.25 mol/L potassium chloride buffer (pH 1.0). Absorbance was measured at 512 and 700 nm. Anthocyanin content was calculated using equation 2 as follows:

$$\text{Anthocyanin concentration (mg/100 mL)} = \frac{A \times MW \times DF \times 100}{\varepsilon} \quad \text{Equation 2}$$

Where:

A = Absorbance (pH<sub>1.0</sub> - pH<sub>4.5</sub>)

MW = molecular weight (449.2 g/mol Cy-3-gly)

DF dilution factor,  $\varepsilon$  = molar absorptive coefficient ( $\varepsilon = 26.900$ )

## 2.8 Sensory Evaluation

A consumer preference sensory evaluation was carried out by 30 panellists, who voluntarily gave informed consent. The panellists, comprising 15 males and 15 females, aged 18 – 35 years old, were students of the Faculty of Technology, University of Ibadan, Nigeria. A 30 mL volume of freshly prepared pasteurised or thermosonicated roselle drink with free or alginate encapsulated *L. rhamnosus* GG, adjusted to 13 °Brix with sucrose, at 8° ± 2 °C was presented to the panellists in transparent polypropylene cups labelled with random three-letter alphabets. Potable water was provided to the panellists for mouth rinsing between samples. The panellists evaluated the roselle drink samples for attributes of colour, aroma, taste, turbidity, consistency, and overall acceptability using a 9-point hedonic scale where 9 represents “like extremely” and 1 represents “dislike extremely” (Larmond, 1977).

## 2.9 Statistical Analysis

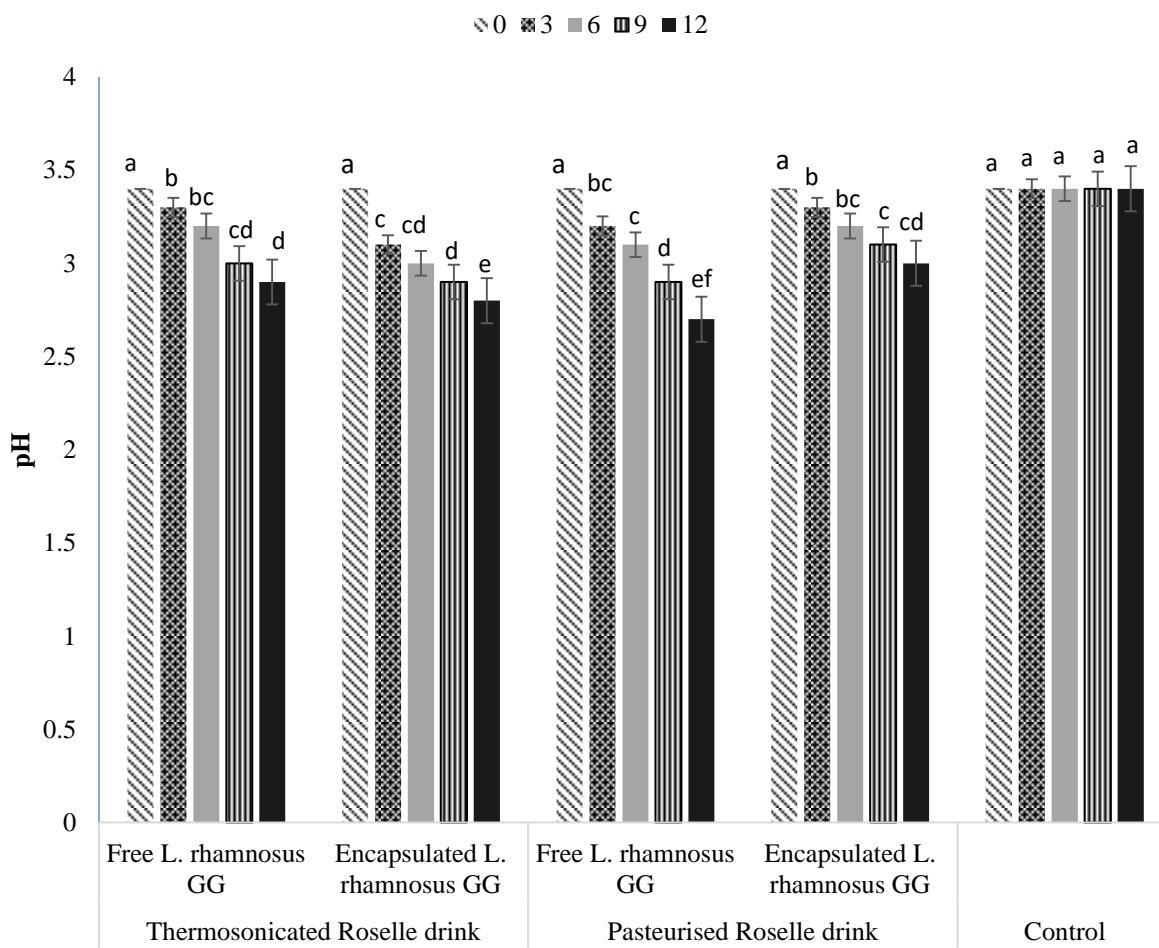
Means ± standard deviation and Analysis of Variance (ANOVA) were computed using SPSS 23.0 (SPSS Inc., Chicago, USA). For the sensory evaluation data, samples were treated as fixed effects while panellists were treated as random effects. A post hoc test of significance was performed using Duncan’s multiple range test. Statistical significance was accepted at  $p < 0.05$ .

## 3.0 Results

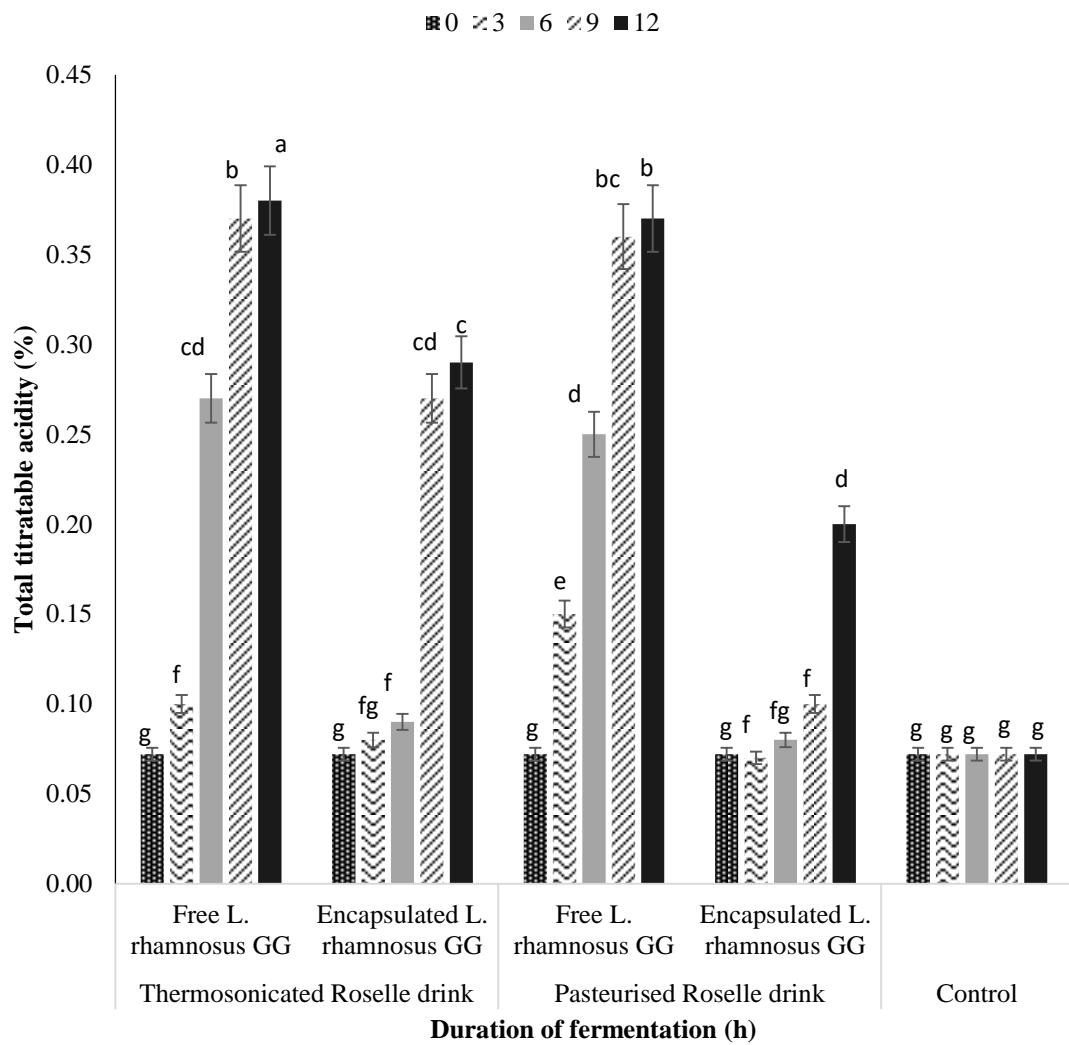
### 3.1 Effect of Pasteurisation or Thermosonication on pH, Total Titratable Acidity (TTA) and Probiotic Viability in Roselle Drink Inoculated with Free or Encapsulated *L. rhamnosus* GG

The changes in the pH, TTA, and Total Viable Counts of *L. rhamnosus* GG in pasteurised or thermosonicated roselle drink fermented with free or encapsulated *L. rhamnosus* GG at 37 °C for 12 h are presented in Figures 3.1, 3.2, and 3.3, respectively. The pH decreased significantly ( $p < 0.05$ ) between 0 and 12 h, in roselle drink pasteurised and fermented with free *L. rhamnosus* GG (PF), roselle drink pasteurised and fermented with encapsulated *L. rhamnosus* GG (PE), roselle drink thermosonicated and fermented with free *L. rhamnosus* GG (UF) and roselle drink thermosonicated and fermented with encapsulated *L. rhamnosus* GG (UE), while the untreated roselle drink (Control) showed no significant ( $p > 0.05$ ) pH change by the end of the fermentation period. However, the pH of PF was significantly ( $p < 0.05$ ) lower than all the other groups by the end of the 12-h fermentation period. The same pattern was also observed in the TTA, which showed a concurrent increase throughout the fermentation period in all the samples

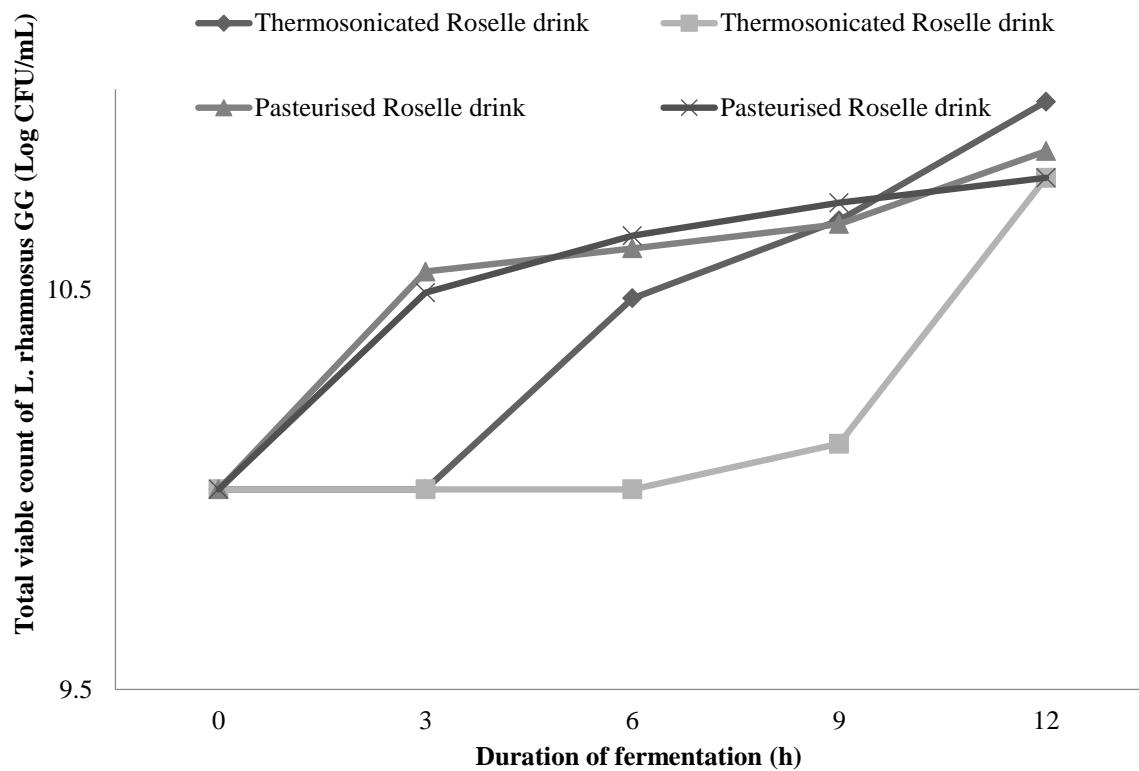
except the control, which was statistically unchanged ( $p > 0.05$ ) by the end of the fermentation period. It was



**Figure 3.1: Changes in the pH of thermosonicated or pasteurised roselle drink with free or encapsulated *L. rhamnosus* GG during fermentation at 37°C for 12 h. Vertical error bars represent the standard deviation of means ( $n = 3$ ). Vertical error bars with different lowercase alphabets indicate significant differences ( $p < 0.05$ ).**



**Figure 3.2: Changes in the lactic acid concentration of thermosonicated or pasteurised roselle drink with free or encapsulated *L. rhamnosus GG* during fermentation at 37°C for 12 h. Vertical error bars represent the standard deviation of means ( $n = 3$ ). Vertical error bars with different lowercase alphabets indicate significant differences ( $p < 0.05$ ).**



**Figure 3.3: Changes in the total viable count of *L. rhamnosus GG* in thermosonicated or pasteurised roselle drink with free or encapsulated cells during fermentation at 37 °C for 12 h.**

also significantly ( $p < 0.05$ ) lower than all the other treatments. There was a slower lactic acid production in samples PE and UE, translating to 42.85 and 257.14%, respectively, compared to a more rapid rate of lactic acid production with a 414.29 and 428.5% increase in samples PF and UF, respectively, within the first 9 h of fermentation. By the end of the 12-h fermentation, lactic acid production in thermosonicated roselle drink with free or encapsulated *L. rhamnosus GG*, which showed 442.89 and 314.29% increase, was significantly higher ( $p < 0.05$ ) than their pasteurised counterparts, with 428.57 and 185.71%, respectively (Figure 2).

The free *L. rhamnosus GG* in the roselle drink showed a significant increase in total viable counts, with the highest observed in the thermosonicated sample (Figure 3). This increase translated to 9.68 and 8.45% in the free *L. rhamnosus GG* in both the thermosonicated and pasteurised roselle drink. This was higher than the 7.73% increase in cell growth exhibited by the encapsulated *L. rhamnosus GG* in both the thermosonicated and pasteurised roselle drink samples. The total viable counts of *L. rhamnosus GG* in all the samples were above 10 log CFU by the end of the 12-h fermentation period.

### 3.2 Effect of Thermosonication or Pasteurisation on Fungi, Coliforms, and *Salmonella* and *Shigella* spp. in Roselle Drink

The microbial population of total fungi, coliforms, and *Salmonella* and *Shigella* spp., enumerated before and after fermentation at 37 °C for 12 h in thermosonicated or pasteurised Roselle drink with free or encapsulated *L. rhamnosus* GG is presented in Table 3.1. Fungi, coliforms, *Salmonella*, and *Shigella* spp. were not observed in any roselle drink samples before and after fermentation with free or encapsulated. The unfermented sample (control) had no fungi, *Salmonella*, and *Shigella* spp., but had significantly ( $p < 0.05$ ) higher counts ( $1.1 \times 10^3$  CFU/mL) of coliforms compared to the treated samples by the end of the fermentation period. This, however, could be due to possible post-processing contamination, as there was no coliform growth before fermentation.

**Table 3.1: Microbial population in thermosonicated or pasteurised roselle drink with free or encapsulated cells during fermentation at 37 °C for 12 h**

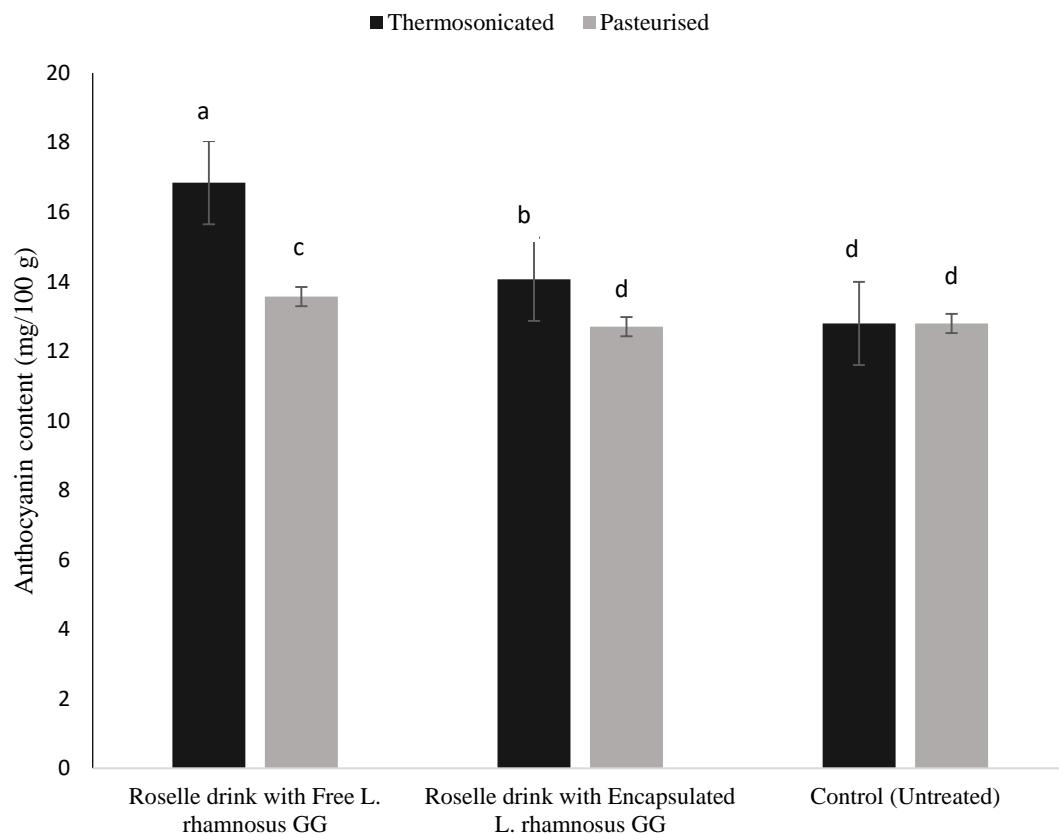
	Thermosonicated roselle drink		Pasteurised roselle drink		Control
	Free <i>L. rhamnosus</i> GG	Encapsulated <i>L. rhamnosus</i> GG	Free <i>L. rhamnosus</i> GG	Encapsulated <i>L. rhamnosus</i> GG	
Before Fermentation.	0	0	0	0	0
<i>Salmonella</i> & <i>Shigella</i> spp	After Fermentation	0	0	0	0
	Before Fermentation	0	0	0	0
Total fungi	After Fermentation	0	0	0	0
	Before Fermentation	0	0	0	0
Total Coliforms	After Fermentation	0	0	0	1.1

### 3.3 Effect of Thermosonication or Pasteurisation and Probiotic Inclusion on Anthocyanin and L, a, b Colour Attributes of Roselle Drink

The anthocyanin content of thermosonicated or pasteurised roselle drink fermented with free or encapsulated *L. rhamnosus* GG is presented in Figure 3.4. Anthocyanin content was significantly higher in thermosonicated roselle drink with free *L. rhamnosus* GG ( $16.84 \pm 0.06$  mg/100 mL) than in pasteurised roselle drink with free *L. rhamnosus* GG ( $13.57 \pm 0.01$  mg/100 mL), with each treatment significantly higher than the counterpart with encapsulated *L. rhamnosus* GG

( $14.06 \pm 0.02$  mg/100 mL). *L. rhamnosus GG* inclusion significantly improved the anthocyanin content of roselle drink compared to control ( $12.7 \pm 0.69$  mg/100 mL).

The L, a, b colour attributes of thermosonicated or pasteurised roselle drink fermented with free or encapsulated *L. rhamnosus GG* is presented in Table 3.2. The L\* (lightness) attribute was significantly ( $p < 0.05$ ) different among the various treatments, in the order PF > PE > UE > Control > UF. The highest a\* (redness) was observed in sample UF, followed by PF and these were significantly higher than the control. The pasteurised samples had significantly higher b\* values than the thermosonicated and control samples, with the lowest b\* observed in sample UF.



**Figure 3.4: Total monomeric anthocyanin content of thermosonicated or pasteurised roselle drink fermented with free or encapsulated *L. rhamnosus GG* at  $37^\circ\text{C}$  for 12 h. Vertical error bars represent the standard deviation of means ( $n = 3$ ). Vertical error bars with different lowercase alphabets indicate significant differences ( $p < 0.05$ ).**

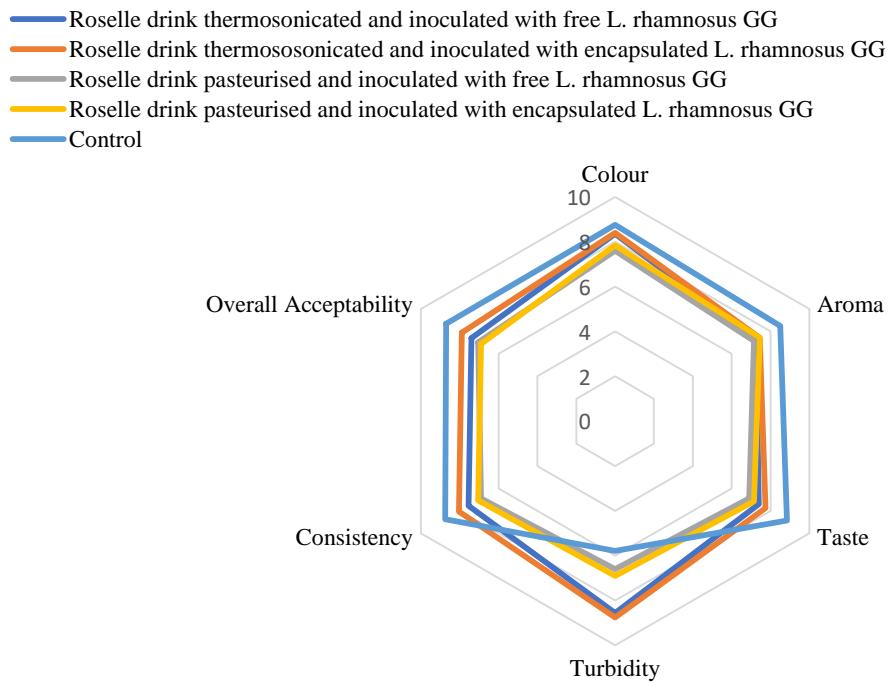
**Table 3.2: Colour attributes [(lightness (L\*), redness (a\*) and yellowness (b\*)] of pasteurised or thermosonicated roselle drink fermented with free or encapsulated *L. rhamnosus* GG**

	L*	a*	b*
Control	19.82 ± 0.03 <sup>d</sup>	9.76 ± 0.02 <sup>c</sup>	2.36 ± 0.01 <sup>d</sup>
Pasteurised Roselle drink with free <i>L. rhamnosus</i> GG	45.24 ± 0.94 <sup>a</sup>	10.86 ± 1.30 <sup>b</sup>	9.30 ± 0.39 <sup>b</sup>
Pasteurised Roselle drink with encapsulated <i>L. rhamnosus</i> GG	30.65 ± 0.08 <sup>b</sup>	5.70 ± 0.04 <sup>d</sup>	10.57 ± 0.06 <sup>a</sup>
Thermosonicated Roselle drink with free <i>L. rhamnosus</i> GG	18.60 ± 0.02 <sup>e</sup>	19.90 ± 0.35 <sup>a</sup>	0.81 ± 0.02 <sup>e</sup>
Thermosonicated Roselle drink with encapsulated <i>L. rhamnosus</i> GG	22.73 ± 0.02 <sup>c</sup>	5.14 ± 0.04 <sup>d</sup>	4.64 0.03 <sup>c</sup>

NB: Data is presented as mean ± standard deviation (n = 3). means with different alphabet superscripts within the same column indicate significant differences (p < 0.05)

### 3.4 Effect of Thermosonication or Pasteurisation and Probiotic Inclusion on the Sensory Attributes of Roselle Drink

Sensory attributes of thermosonicated or pasteurised roselle drink fermented with free or encapsulated *L. rhamnosus* GG are presented in Figure 3.5. The sensory attributes of colour, aroma, taste, turbidity, consistency, and overall acceptability were significantly (p < 0.05) higher in the control than all the treatments, followed by sample UE. Colour ranked significantly higher in the thermosonicated than in the pasteurised roselle drink, and comparable to the control. Aroma was not significantly (p > 0.05) different among the treatments but significantly (p < 0.05) ranked lower than the control. However, control had the lowest (5.79 ± 0.42) significant (p < 0.05) turbidity among all the samples. There was a significant (p < 0.05) influence of the probiotic inoculation mode – free or encapsulated – on the turbidity of all the *L. rhamnosus* GG-containing roselle drink. Consistency and overall acceptability ranked significantly highest in the control roselle drink, while these two attributes were ranked highest in sample UE. Taste of all *L. rhamnosus* GG-fermented roselle drink ranged from 6.9 ± 1.9 (PE) to 7.8 ± 1.2 (UF) on a 9-point hedonic scale, translating to 76.7 – 86.7%.



**Figure 3.5: Sensory attributes of thermosonicated or pasteurised roselle drink fermented with free or encapsulated *L. rhamnosus* GG at 37 °C for 12 h.**

## 4.0 Discussion

This study presents the suitability of roselle drink as a carrier for probiotic *Lacticaseibacillus rhamnosus* GG. It also presents the comparative effects of thermosonication or High-Temperature Short Time pasteurisation of roselle drink before the inclusion of free or encapsulated probiotic *Lacticaseibacillus rhamnosus* GG, while the untreated roselle drink served as the control. This was to establish the comparative effects of thermosonication and pasteurisation on the microbial population of fungi, coliforms, *Salmonella*, and *Shigella*, physicochemical attributes, anthocyanin content, and sensory properties of roselle drink. Over time, thermal treatments such as pasteurisation have been used for beverage processing to reduce pathogens and extend shelf-life. However, it comes with associated negative impacts on product quality, leading to a recently growing demand for minimally processed foods instead of thermal treatments (Khan et al., 2021). The protective ability of alginate encapsulation on the probiotics during the processing of roselle drink, as evidenced by probiotic viability during the 12-hour fermentation in this study, was also established.

The results showed that pH significantly decreased, while the total titratable acidity concurrently increased in roselle drinks with free and encapsulated *L. rhamnosus* GG compared to the control. All the roselle drink samples with alginate-encapsulated *L. rhamnosus* GG showed a slower rate of pH decrease during fermentation, indicating a slower acidification rate than the roselle drink inoculated with free *L. rhamnosus* GG. The higher acidification rate in the samples with free *L.*

*rhamnosus* GG could be explained by the effect of the unrestricted activity of the cells during fermentation. By the end of the fermentation period, the pH of all the samples was significantly lower than that of the control. The lower pH and higher acidity of the *L. rhamnosus* GG-fermented samples could be attributed to the acidifying activity of *L. rhamnosus* GG as evidence of active metabolism of the probiotic (Karbasi *et al.*, 2015). This is consistent with the observation of Mokhtari *et al.* (2019) during the fermentation of grape juice with free and encapsulated probiotics. Also, Oguntoye and Ezekiel (2022) fermented provitamin A cassava hydrolysate using free and encapsulated probiotics and reported the same observation. The effect of thermosonication or pasteurisation on the pH of the roselle drink was not observed.

The foregoing is corroborated by the total viable counts of *L. rhamnosus* GG in all the samples. The consistent increase in the total viable counts of free *L. rhamnosus* GG in the roselle drink compared to the encapsulated counterparts was due to the unrestrained metabolism of the free cells. The observed higher influence of thermosonication on cell viability was probably due to the cavitation effects of the process. The cavitation effect improves the physicochemical properties of the roselle drink (Gavahian *et al.*, 2022). It also enhances the availability of the prebiotic components, phenolic compounds, and other nutrients, which could support and improve probiotic viability in the samples, resulting in products with a higher quality due to lesser exposure to heat (Jadhav *et al.*, 2024). The total viable counts of *L. rhamnosus* GG in all the fermented samples were above 10 Log CFU by the end of the 12-h fermentation period, greater than the  $10^6$  cell viability recommended by WHO for a probiotic product to deliver therapeutic benefits (Lacroix and Yildirim, 2007). The absence of fungi, coliforms or *Salmonella* and *Shigella* spp., which are spoilage and pathogenic organisms in both the thermosonicated and pasteurised roselle drink samples before and after the 12-hour fermentation with free or encapsulated *L. rhamnosus* GG, indicate that the thermosonication was comparable to the pasteurisation process in the adequacy of microbial inactivation and eradication of pathogens particularly the aerobic mesophilic heterotrophic bacteria, coliforms, yeast and molds. For food safety considerations, instead of thermally treating roselle drink by pasteurisation, which may detrimentally impact quality, microbiological safety can be ensured with thermosonication (Guimarães *et al.*, 2018), which minimally exposes it to heat. While no significant influence of the mode of probiotic inoculation (free or encapsulated) with regard to the microbial load of pathogens was observed, the effect of probiotic inclusion was also observed, as the un-inoculated sample had higher levels of pathogens, which increased by the end of the fermentation period. Although, the levels recorded in this study are within the ICSMF acceptable limit (Buchanan, 2023). Probiotics have the tendency to suppress the growth of pathogens through mechanistic activities, which may include competitive exclusion, exploitative competition or interference competition (Knipe *et al.*, 2021). Probiotics have been established through many studies to possess the ability to suppress the growth of pathogens through the production of metabolites such as lactic acid and bacteriocins (Moradi *et al.*, 2020, Kumari *et al.*, 2022).

There was a significant improvement in the anthocyanin content, significantly higher in thermosonicated than in pasteurised roselle drink, attributed to reduced exposure to thermal treatment. This is consistent with the findings of Wu *et al.* (2021), which reported anthocyanin content higher in thermosonicated than pasteurised jussara pulp. A significant influence of probiotic inclusion, with a prominent influence of the mode of probiotic inoculation on the anthocyanin content of roselle drink, was also observed in this study. Anthocyanin was significantly higher in all the *L. rhamnosus* GG-fermented roselle drinks than the unfermented,

with significantly ( $p < 0.05$ ) higher values in the roselle drink fermented with free *L. rhamnosus* GG compared to the encapsulated cells. Chen *et al.* (2019) also reported that *L. brevis* and *L. acidophilus* enhanced the anthocyanin contents of Bog Bilberry juice. This could be linked to the lower pH in the fermented samples, as it was observed that pH changes also influenced the anthocyanin content, which increased as pH decreased due to acidification. The observed increase in the anthocyanin content could be due to the regeneration of anthocyanin as a result of acidification by the probiotic during fermentation. Thermal treatment and acidification have been suggested to result in the regeneration of anthocyanins (Toktaş *et al.*, 2018). However, this finding is contrary to the loss of anthocyanin in Mulberry juice reported by Cheng *et al.* (2016) due to biotransformation by probiotics and some lactic acid bacteria into other bioactive phenolic compounds. Anthocyanins are water-soluble pigments of the phenolic group, responsible for the red colour pigment of roselle drink. They are reported in the literature to exert important physiological functions, such as antioxidant and antimicrobial properties, and also have potential applications as pigments in food (Liu *et al.*, 2019, Zhao *et al.*, 2021).

In this study, fermentation by *L. rhamnosus* GG was observed to significantly improve the red colour profile of the roselle drinks, as higher  $a^*$  values, enhanced by thermosonication, were observed in the fermented roselle drink compared to the unfermented. The  $b^*$  (yellowness) attribute was increased by pasteurisation compared to the thermosonicated roselle drink and the control. This suggests that pasteurisation could introduce some degree of browning into the samples, although ameliorated in the free *L. rhamnosus* GG-fermented sample. This suggests that the probiotic, being a lactic acid bacterium, or its metabolites, may contribute to the colour profiles of roselle drink, perhaps through a mechanism involving low pH. The decrease in pH during fermentation may result in the conversion of some of the anthocyanin to flavylium cation, increasing the  $a^*$  values of the roselle drink. This observation is consistent with the study of Chen *et al.* (2023). This is also consistent with Zhu *et al.* (2023), who reported that the  $a^*$  colour attribute of Chinese bayberry pomace beverages were significantly improved by lactic acid fermentation. In this study, higher L (lightness) values were observed in the pasteurised compared to the thermosonicated samples, which could be a result of thermal degradation of the anthocyanin pigments. Fermented roselle drink samples also had higher L values compared to the unfermented, more obvious in roselle drink with free *L. rhamnosus* GG than the encapsulated counterparts. Higher L values indicate lighter samples, and it could also be attributed to the turbidity created in the samples by the presence of the probiotic cells, which was more prominent in the roselle drink with free probiotic cells. This was also confirmed by the result of the sensory evaluation.

The sensory attributes of colour, aroma, taste, turbidity, consistency, and overall acceptability of roselle drink were more significantly influenced by thermosonication than pasteurisation. The *L. rhamnosus* GG-fermented roselle drink samples had higher turbidity than the unfermented sample. There was also a significant influence of the mode of probiotic inoculation (free or encapsulated) on the turbidity and consistency of the fermented roselle drink. The turbidity was higher in the roselle drink containing free *L. rhamnosus* GG than in the encapsulated counterparts, but significantly lower in all the treatments than in the control. The observed higher turbidity and consistency may be associated with the tendency of *L. rhamnosus* GG to produce exopolysaccharides, which could increase the bulk of the medium (Maia *et al.*, 2023). Yang *et al.* (2021) also reported that the turbidity of gels fermented by lactic acid bacteria, including *L. rhamnosus*, significantly increased during fermentation, and attributed the turbidity to the

denaturation of protein during fermentation as a result of the unfolding of  $\alpha$ -helical structures, leading to aggregation. The sensory attributes of taste and overall acceptability were significantly lower in the pasteurised fermented roselle drinks (either with free or encapsulated *L. rhamnosus* GG) than in the thermosonicated counterparts and control. This could be due to the increased acidity in the pasteurised fermented roselle drink, imparting astringency to the taste attribute, which was probably more controlled in the thermosonicated samples. The sensory attributes of colour, aroma, taste, consistency, and overall acceptability of roselle drink were significantly higher, except for turbidity, which was lowest in the control than in any of the treatments.

## 5.0 Conclusion

This study established the suitability of roselle drink as a non-dairy carrier for probiotic *L. rhamnosus* GG, as it supported probiotic viability, maintaining above 10 Log CFU. Roselle drink thermosonicated before probiotic inclusion compared favourably with pasteurisation, in improving the microbiological safety of roselle drink. Thermosonication improved the physicochemical and sensory attributes of roselle drink better than pasteurisation.

Thermosonication with free *L. rhamnosus* GG inclusion preserved the anthocyanin content of roselle drink better than pasteurisation and/or encapsulated *L. rhamnosus* GG inclusion. This study also showed that thermosonication or pasteurisation using the HTST regime, in combination with probiotic inclusion employed in this study, did not negatively impact the sensory qualities of the roselle drink. The roselle drink with probiotic inclusion, regardless of the mode of inoculation, was able to maintain a significant ranking above 7, on a 9-point hedonic scale, in the attributes of taste and overall acceptability. For food safety considerations, instead of thermally treating this product by pasteurisation, which may detrimentally impact quality, microbiological safety can be ensured with thermosonication.

## Conflict of Interest

The authors confirm that there are no conflicts of interest with respect to the work described in this manuscript.

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