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Leuconostoc mesenteroides and *Pediococcus pentosaceus* Non-Alcoholic Pearl Millet Beverage Enriched with *Moringa oleifera* Leaf Powder: Nutritional and Sensory Characteristics

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Abstract: Non-alcoholic cereal beverages (NACB) are usually produced through uncontrolled fermentation driven by a cocktail of bacteria resulting in final product variability. Hence, to commercialise fermented traditional cereal beverages bioburden microbial cultures are required. This investigation aimed to evaluate the physicochemical, nutritional, and sensory characteristics of NACB produced using pure cultures of *Leuconostoc mesenteroides* and *Pediococcus pentosaceus*. Pearl millet extract (PME) pasteurised at 85 °C for 15 min and cooled to 40 °C was inoculated with *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* at 0.050% and 0.025% (1:0.5), respectively, and fermented at 37 °C for 18 h, referred to as plain non-alcoholic pearl millet beverage (PNAPMB). Moringa supplemented non-alcoholic pearl millet beverage (MSNAPMB) was produced following the same method as PNAPMB but a 4% moringa leaf extract powder was added before hydration of the pearl millet powder. The traditional non-alcoholic pearl millet beverage (TNAPMB) was prepared by mixing water and pearl millet flour (1:1.25; PMF:Water) and hydrated for 3 h at 25 °C. The mixture was divided into ¼ slurry which was mixed with sprouted rice flour (SRF) and ¾ portion that was gelatinised with 1 L of boiling water and cooled to 40 °C. The two portions were mixed and fermented at 37 °C for 18 h, followed by sieving, dilution with water (1:0.5, filtrate:water), and pasteurization for 15 min at 85 °C. The growth of lactic acid bacteria, pH, total titratable acidity (TTA), and sugar in PNAPMB and MSNAPMB were determined at 3 h intervals during fermentation. The final beverages were also analysed for proximate, colour and metabolites. The lactic acid bacteria were significantly ($p < 0.05$) affected by the fermentation period and increased from 3.32 to 7.97 log CFU/mL (pH 4.14) and 3.58 to 8.38 log CFU/mL (pH 3.65) for PNAPMB and MSNAPMB, respectively. The total titratable acidity significantly ($p < 0.05$) increased from 0.14 to 0.22% and from 0.17 to 0.38% in PNAPMB and MSNAPMB, respectively. The protein, total fat, moisture total sugar, and carbohydrates differed significantly ($p < 0.05$) among the samples. PNAPMB was preferred by a consumer panel followed by MSNAPMB and TNAPMB. Volatile compounds with beneficial anti-inflammatory and anti-pathogenic properties were identified in the beverages. Innovative fermentation of pearl millet extract using purified bioburden cultures was possible and the added *Moringa oleifera* leaf powder improved the nutritional quality of the resulting beverage.

Keywords: pearl millet extract; *Leuconostoc mesenteroides*; *Pediococcus pentosaceus;* non-alcoholic pearl millet beverage; *Moringa oleifera* leaf powder; sensory quality

1. Introduction

Pearl millet supplies basic nutrition as well as valuable compounds such as fibre, antioxidants, vitamins, minerals and essential amino acids [1]. Fermented non-alcoholic cereal beverages (NACB) are popular in African countries [1,2] as part of tradition and culture. These beverages are produced through spontaneous fermentation involving mixed microflora. However, the spontaneous fermentation of the beverage is difficult to control especially in mass production. Furthermore, unwanted microorganisms that may be in the beverage can speed up spoilage after fermentation particularly if the periods between product preparation and consumption are long, resulting in premature spoilage. The beverages are traditionally not pasteurised after fermentation and thus are considered functional beverages containing high levels of lactic acid bacteria (LAB) [3]. Consequently, the health benefits of fermented cereal beverages like pearl millet are combined into a unique synergistic effect from: (1) the beneficial content of pearl millet grains; (2) the probiotic properties of the lactic acid bacteria strains involved in the fermentation; and (3) the biochemical changes that occur in the grain substrates during fermentation and the genetic and enzymatic capabilities of LAB strains that allow them to utilise the grain carbohydrates [1].

Lactic acid fermentation is the oldest and most popular way to improve the functionality, nutritional value, taste, appearance, and safety of cereal foods and reduce the energy required for cooking [1,3]. The antimicrobial effect of LAB is mainly related to the production of organic acids and some synthesised bacteriocins, antimicrobial substances. Such application of LAB for non-alcoholic pearl millet beverage production provides new opportunities for the development of functional fermented products.

Although numerous technologies [4–10] have been developed to improve the traditional process, the production of these beverages on a large scale is still limited. Moringa oleifera leaves is reported as a fortificant in different food products resulting in overall nutritional and sensory quality [11–13]. We had reported an innovative non-alcoholic pearl millet beverage by fermenting pearl millet extract with *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* (isolated from the indigenous beverage process) for 18 h, thereby reducing the production time from the traditional 24 h [14]. Our objective was to characterise the nutritional and sensory qualities of the beverage as affected by *Moringa oleifera* leaf powder.

2. Materials and Methods

2.1. Sources of Raw Materials and Equipment

Pearl millet was purchased from Agricol in Brackenfell, Cape Town. Ground ginger was supplied by Deli Spices in Epping, Cape Town. Moringa leaf powder was purchased from Moringa South Africa, Garden Route. All reagents were analytical grade from Merck. All the equipment and sprouted rice flour were obtained from the Department of Food Science and Technology and the Agrifood Technology Station, Cape Peninsula University of Technology, South Africa.

2.2. Production of Pearl Millet Flour

Pearl millet flour was produced as reported by [14]. Dry pearl millet grains were manually cleaned to remove any seeds, broken grains, sand, twigs, and other foreign objects and washed. Excess water was drained off by spreading the grains on a stainless-steel sieve. The grains were steamed for 15 min at 110 °C in a Butcherquip junior cooker. This steaming was necessary to inactivate the hydrolytic enzymes that could cause off-flavour. The grains were washed in tap water and soaked for 6 h (1:2, grains:water). The soaked grains were dried at 50 °C for 45 h in a cabinet dryer (Geiger & Klotzbucher, Cape Town, South Africa) and milled into 0.8 mm (Falling number/Kjeldahl analysis size) powder using a Perten 3100 hammer mill. The resulting pearl millet flour was kept in a clear zipper bag at 4 °C until required.

2.3. Preparation of Moringa Leaf Powder Extract

Moringa (*Moringa oleifera*) leaf powder was blended with water at 1:12 ratio (moringa:water) using a Silverson L4R homogeniser at 7000 rpm for 15 min. The mixture was soaked for 30 min and sieved through 850, 355, 250, and finally 125 μm sieves. The extract was spread on freeze-dryer trays and frozen for 48 h at −76 °C using an Ultra-freezer (Glacier, −86 °C ultralow temperature freezer) and freeze-dried for 72 h using Virtics SP Scientific 35 XL pilot freeze dyer (freeze mode set to −50 °C). The resulting flakes with 96% moisture loss were placed into a stomacher bag and milled using an AES Smasher for 80 s. The resulting powder referred to as moringa leaf powder extract (MLPE) was kept in zipper bags until required.

2.4. Production of Plain Non-Alcoholic Pearl Millet Beverage and Moringa Supplemented Non-Alcoholic Pearl Millet Beverage

Pearl millet flour (PMF) (400 g) was mixed with 15% sprouted rice flour, 12% ground ginger, and water at 1:2.5 (PMF:Water) ratio [14]. The slurry was homogenised using Silverson L4RT blender for 15 min at 7000 rpm. The slurry was left to hydrate for 1 h at 25 °C. The supernatant was decanted and the sediments discarded. The liquid was filtered through a sterile cheesecloth followed by a 5 μm filter bag. The extract was mixed with sunflower lecithin (0.1%), sodium citrate (0.1%), pectin (0.6%), and white sugar (5%). The mixture was blended using Silverson L4RT blender at 7800 rpm for 7 min. The resulting pearl millet extract (PME) was pasteurised for 15 min in a pot at 85 °C, followed by bottling in sterile 500 mL Schott bottles and cooled immediately in cold water (≤37 °C). The cooled samples were inoculated with *L. mesenteroides* (0.05%) and *P. pentoseaceus* (0.025%) and fermented in an incubator at 37 °C for 18 h. During fermentation, samples were drawn at 3 h interval for lactic acid bacteria, pH, total titratable acidity, and sugars assays. After fermentation, the beverage was chilled at 4 °C.

Following this method, moringa supplemented non-alcoholic pearl millet beverage (MSNAPMB) was produced by adding 4% MLPE to the pearl millet extract before pasteurisation. Both beverages were chilled at 4 °C and subjected to sensory, colour, and metabolite assay.

2.5. Production of Traditional Non-Alcoholic Pearl Millet Beverage

The production process for the traditional non-alcoholic pearl millet beverage (TNAPMB) as reported by Jideani et al. [14] was followed. Pearl millet flour (PMF) was mixed with water at 1:1.5 (PMF:Water) and left to hydrate for 3 h. The paste was divided into ¼ and ¾ portions. To the ¼ portion, 48% sprouted rice flour, 16% ground ginger, and 160% water were added and mixed with a plastic spoon. The ¾ portion was gelatinised with boiling water at 1:2.7 (paste:water) ratio and cooled to 40 °C in cold water. The two portions were mixed and bottled in sterile 500 mL Schott bottles. The slurry was fermented at 37 °C for 18 h and sieved through a 106 μm stainless sieve. The filtrate was mixed with at 1:0.5 (filtrate:water) ratio, sugar (6%), and citric acid (0.25%) using Silverson L4RT at 2300 rpm for 5 min. The resulting traditional non-alcoholic pearl millet beverage (TNAPMB) was chilled at 4 °C for sensory, colour and metabolites assays.

2.6. Enumeration of Lactic Acid Bacteria in Pearl Millet Extract during Fermentation of Plain and Moringa-Supplemented Non-Alcoholic Pearl Millet Beverages

An aliquot (45 mL) of pearl millet extract (PME) in 100 mL Schott bottles during fermentation were thoroughly mixed by shaking for 1 min [14]. Mixed PME (10 mL) was transferred to a 100 mL Schott bottle containing 90 mL sterile ¼ strength of Ringer solution [15,16] to give 1:10 dilutions followed by a 10 fold serial dilution from 10^{-1} to 10^{-10}. Each dilution was sub-cultured in triplicate. A portion of the sample dilution (1 mL) was added into a 15 × 100 mm plastic Petri plates containing cooled molten deMan Rogosa and Sharpe (MRS) agar (Merck HG00C107.500) using a pipette and left to solidify [17–19]. The plates

were incubated under anaerobic conditions using an Anaerobic Gas-Pack system and anaerobic indicator strips at 30 °C for 48 h [18,20]. All microbiological data were expressed in logarithms of numbers of colony-forming units per mL (log CFU·mL^{-1}).

2.7. Physicochemical Analysis of Pearl Millet Extract (PME) during Fermentation

The pH of pearl millet extract (PME) (10 mL) during fermentation was measured at 3 h intervals in triplicates using Hanna Edge (HI-11310) glass electrode pH meter standardised with pH buffer solutions 4, 7, and 10.

Total titratable acidity (TTA) was assessed at 3 h intervals by titrating 10 mL of the sample with 0.1 N NaOH using phenolphthalein as an indicator until a light pink colour appears. The TTA was expressed as a percentage of lactic acid. Equation (1) was used to calculate the percentage acidity, with each 0.1 N NaOH equivalent to 90.08 mg lactic acid.

$$\text{TTA (\% lactic acid)} = \frac{\text{ml NaOH} \times \text{N NaOH} \times \text{M.E}}{\text{volume of sample used} \times 1000} \times 100 \quad (1)$$

where mL NaOH = volume of NaOH (mL), N NaOH = molarity of NaOH, M.E = the equivalent factor of lactic acid being 90.08 mg, 1000 = factor used to convert the M.E, which is normally in mg to grams, and 100 used to express the lactic acid concentration in percentage. All determinations were in triplicate.

2.8. Determination of Soluble Sugars in Pearl Millet Extract during Fermentation

The method of AOAC 982.14 as described by [21] was used to determine the soluble sugars in pearl millet extract (PME) during fermentation for plain and moringa supplemented non-alcoholic beverages. Sugar extraction was achieved by mixing 5 g (W_1) of the sample with 100 mL (W_2) of 50% ethanol. The mixture was heated in a water bath for 25 min at 85 °C while shaking at 25 rpm. The mixture was cooled to 25 °C and ethanol (95%) was used to bring the sample weight to the original weight (W_2). The sample was filtered through a 0.45 μm nylon syringe into a 1.5 mL clear screw neck high-performance liquid chromatography (HPLC) sample vial. The total sugar content of the extracts was determined in triplicates using HPLC (Agilent 1100 HPLC-RID system) equipped with Zorbax carbohydrates column (4.6 × 150 mm, 5 μm) and Zorbax NH_2 guard column (4.6 × 12.5 mm, 5 μm). The mobile phase used was acetonitrile mixed and de-gassed with Millipore distilled water at 75:25 (acetonitrile:water) ratio. The sugar standards were prepared by mixing sucrose (6 mg·mL^{-1}), fructose (6 mg·mL^{-1}), glucose (6 mg·mL^{-1}), maltose (6 mg·mL^{-1}), lactose (6 mg·mL^{-1}), and sucrose (30 mg·mL^{-1}) in a water/ethanol (50:50) solution. The resulting stock solution was used to prepare concentration solutions for the calibration curve. The concentration used to draw a standard curve were 0.375 (1.875) mg·mL^{-1}, 0.75 (3.75) mg·mL^{-1}, 1.5 (7.5) mg·mL^{-1}, and 3.0 (15.0) mg·mL^{-1}. The values in parenthesis show the sucrose concentration in each solution.

2.9. Proximate Analyses of Plain, Moringa-Supplemented and Traditional Non-Alcoholic Pearl Millet Beverages

The moisture and ash content in plain, moringa-supplemented, and traditional cereal beverages were determined using the oven and muffle furnace method, respectively. The protein was determined using a nitrogen analyser (Leco-TruSpec-N) with a furnace set at 950 °C. The protein factor used was 6.31 for millet. The fat content was determined using AOAC 996.06 (2005). Total dietary fibre (TDF) was determined using the Fibertec system method. The carbohydrates were determined by difference.

2.10. Colour Measurement of Plain, Moringa-Supplemented, and Traditional Non-Alcoholic Pearl Millet Beverages

A Konica Minolta spectrophotometer (CM-5) was used to measure the colour of plain, moringa-supplemented, and traditional non-alcoholic pearl millet beverages. The

equipment was set at 10° standard observer and D65 illuminant. Prior to any measurement the instrument was zero calibrated using the black tile (L * = 5.49, a * = −7.08, b * = 4.66) and white tile (L * = 93.41, a * = −1.18, b * = 0.75). The beverage (10 mL) was poured into a 30 mm diameter sample glass cup and the reflectance was measured in terms of L *, a * and b *. Each measurement was taken three times by doing a quarter-turn of the sample and each sample was measured in triplicates. L * indicates the lightness of the beverage, a * represents the redness/greenish of the beverage and b * shows the yellowness/blueness of the beverages. Chroma (C) shows the quality that differentiates a pure hue from a grey shade and describes the hue saturation or purity. The total colour difference (ΔE) in the beverage was calculated using Equation (2). The colour change is the numerical comparison of the PNAPMB and MSNAPMB to the control (TNAPMB). A colour difference of 1 is defined as a just-noticeable difference at which a trained evaluator will notice the colour differences. If the ΔE is between 4 and 8 the samples are deemed acceptable (Murevanhema, 2012).

$$\Delta E = \sqrt{\left[\left(\Delta L^{*}\right)^2 + \left(\Delta a^{*}\right)^2 + \left(\Delta b^{*}\right)^2 \right]} \quad (2)$$

L = lightness; a * = redness/greeness and b * = yellowness/blueness

2.11. Extraction and Identification of Volatile Compounds in the Non-Alcoholic Pearl Millet Beverages

Aliquot samples (200 mL) of plain non-alcoholic pearl millet beverage (PNAPMB), moringa-supplemented non-alcoholic pearl millet beverage (MSNAPMB) and traditional non-alcoholic pearl millet beverage (TNAPMB) were separately poured in 600 mL freeze-dry jars and frozen at −76 °C for 12 h in an ultra-freezer (Glacier −86 °C ultralow temperature freezer) and freeze-dried using BenchTop-Pro with omnitronics (VirTis SP Scientific) for 120 h. The dried samples were then milled using AES Smasher XL homogeniser for 40 s and stored in zipper bags. The method of Azlan Azizan et al. [22] was used with slight modification to extract and determine the volatile compounds. Volatile compounds were extracted by mixing 1 g of the beverage powder with cold (5 °C) methanol/mili-Q water (MeOH/H_2O, 80/20 *v*/*v*). The mixture was sonicated for 5 min in ice water using Sonicator cell disruptor (Heat system-ultrasonic INC). Sonicator cell disruptor, (Model W-225R) with H-1 probe set at 7 output, duty cycle at 50 and set on continuous mode. The mixture was then mixed by vortex at high speed for 1 min and filtered through a 0.45 μm nylon syringe into a 1.5 mL clear screw neck Gas-Chromatography Mass-Spectrometry (GC-MS) sample vials.

The volatile compounds of the extracts were determined in duplicates using Agilent Technologies 7890B GC-MS system equipped with HP-5 MS column (5% Phenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm × 0.25 μL). The carrier gas (helium) was set at 0.6 $mL.min^{-1}$ flow rate, pressure at 3.5105 psi, the average velocity at 28.502 $cm{\cdot}s^{-1}$, hold time at 1.7542 min, and the oven temperature at 70 °C. The temperature was set to increase at 1 $°C.min^{-1}$ to 76 °C after that at 6 $°C.min^{-1}$ to 300 °C. The scanning mode was set at a mass range of 50–500 *m*/*z*, with a solvent delay of 7 min. The spit-splitless inlet was set at a temperature of 250 °C, pressure at 3.5105 psi, total flow at 33.6 $mL.min^{-1}$, and septum purge flow at 3 $mL.min^{-1}$. The samples were injected at 50:1 ratio and split flow at 30 $mL.min^{-1}$. The peaks were identified using the National Institute of Standard and Technology 14 (NIST-14) mass spectra library.

2.12. Sensory Evaluation of the Non-Alcoholic Pearl Millet Beverages

A total of 50 consumer panellists above 18 years of age were drawn from the Cape Peninsula University of Technology (staff and students). The sensory evaluation was carried out in the sensory laboratory at 25 °C. Plain non-alcoholic pearl millet beverage (PNAPMB), moringa-supplemented non-alcoholic pearl millet beverage (MSNAPMB), and traditionally non-alcoholic pearl millet beverage (TNAPMB) were prepared and

chilled at 4 °C 24 h before the evaluation. Aliquots (40 mL) of the beverages (PNAPMB, MSNAPMB and TNAPMB) were each served chilled in a white polystyrene foam cup (250 mL) coded with a three-digit random number. The panellists were instructed to rate each beverage without comparing for appearance, colour, aroma, taste, mouthfeel, and overall acceptability and rate their likeness on a 9-point hedonic scale (1–dislike extremely to 9 like extremely).

2.13. Data Analysis

The results reported are mean ± standard deviation of three independent trials. Multivariate analysis of variance (MANOVA) was used to determine differences between treatments at $p = 0.05$. Duncan's multiple range test was used to separate means where differences existed using IBM SPSS v. 23 (IBM, 2015). Principal component analysis (PCA) was used to summarise and uncover patterns in the fermentation data set by reducing the complexity of the data (The Unscrambler X10.4).

3. Results

3.1. Effect of Fermentation Time on the Viability of Lactic Acid Bacteria in Pearl Millet Extract during Fermentation

The effect of fermentation time on the survival of *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* as lactic acid bacteria (LAB) is shown in Figure 1 ranging from 3.32 to 7.97 log CFU·mL^{-1} in plain non-alcoholic pearl millet beverage (PNAPMB) and 3.58 to 8.38 log CFU/mL in moringa supplemented pearl millet beverage. Initial LAB in plain non-alcoholic pearl millet beverage (PNAPMB) was 3.32 log CFU·mL^{-1} and significantly increased to 7.96 log CFU·mL^{-1} in 12 h. The lag phase was not visible as the cells immediately grew exponentially. The cells did not take long to adapt to the new environment. Thereafter, the growth of LAB was not significant between 12 and 15 h. At this point, the available nutrients were being depleted and bacteria started to compete for remaining nutrients. *Leuconostoc mesentoroides* which stops growing at a pH of 4.0–4.5 could also have been halted as the pH was between 4.71 and 4.13. The cells decreased from 8.16 (15 h) to 7.97 CFU·mL^{-1} (18 h).

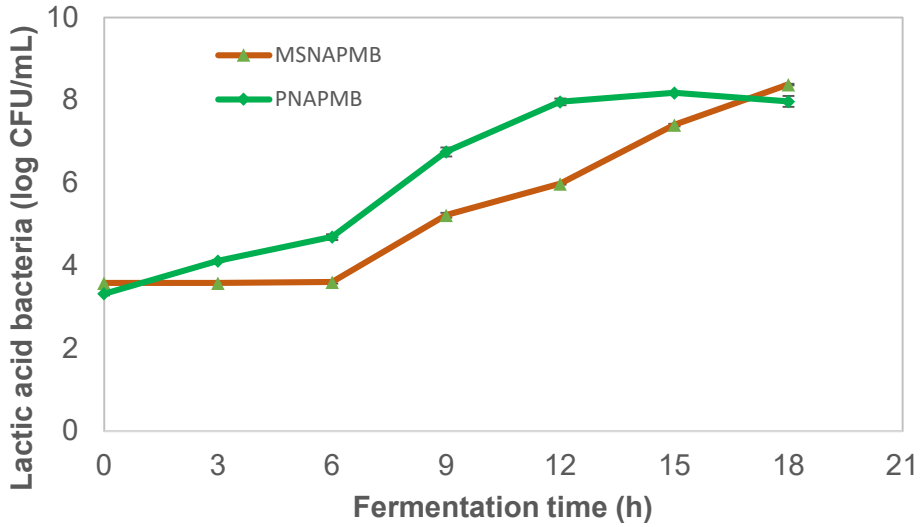

Figure 1. Changes in the lactic acid bacteria (*Leuconostoc mesenteroides* and *Pediococcus pentosaceus*) during the fermentation of pearl millet extract for the production of plain non-alcoholic pearl millet beverage (PNAPMB) and moringa supplemented non-alcoholic pearl millet beverage (MSNAPMB).

In moringa-supplemented non-alcoholic pearl millet beverage (MSNAPMB), the LAB started at 3.58 log CFU·mL^{-1} and remained stationary for 6 h followed by exponential growth ($p < 0.05$) to 8.38 log CFU·mL^{-1}. The cells could have been in the lag phase adapting to the new environment during the first 6 h. Thereafter, the cells multiplied at a maximal rate utilizing the available nutrients in the beverage. This is contrary to Simango [23] who reported a sharp increase in LAB in the first 6 h and thereafter remained the same during the fermentation of *Mahewu*, a non-alcoholic fermented cereal beverage. The total LAB cells after 18 h were 10^8 and 10^7 CFU·mL^{-1} in MSNAPMB and PNAPMB, respectively, which is ideal for organisms to confer health benefits to hosts [24]. Since no death phase was visible in MSNAPMB, this could mean that *Moringa oleifera* leaf extract supported the growth of LAB, which is in agreement with Hekmart et al. [24] who reported positive effects of *Moringa oleifera* seed on the survival of *Lactobacillus rhamnosus* GR-1 in yoghurt with added sugar. The report suggested that added sugar could have acted as a food source to the LAB. In addition, this could mean that MSNAPMB can be fermented beyond 18 h should a sour beverage be desired.

3.2. Effect of Fermentation Time on the pH and Total Titratable Acidity of Pearl Millet Extract during Fermentation

The effect of fermentation time on the pH and total titratable acidity (TTA) of pearl millet extract for the production of plain and moringa supplemented non-alcoholic pearl millet beverages is shown in Figure 2. There was a significant decrease in the pH for plain non-alcoholic pearl millet beverage (PNAPMB) during the 18 h fermentation period from 5.05 to 4.14. The pH did not decrease significantly from the onset of fermentation until after 12 h due to an increase in the activity of the lactic acid bacteria (LAB) growth breaking down the starch in pearl millet extract into simple sugars. The released monomeric sugars were utilised in the production of lactic acid which depressed the pH. Similarly, there was a significant decrease in pH of MSNAPMB after 12 h to 3.65. However, the pH was lower in MSNAPB compared to PNAPMB, which could be because the moringa leaf extract powder supported the growth of LAB.

The total titratable acidity (expressed as % lactic acid) during the 18 h fermentation period ranged from 0.14 to 0.22% for PNAPMB and 0.17 to 0.38% for MSNAPMB.

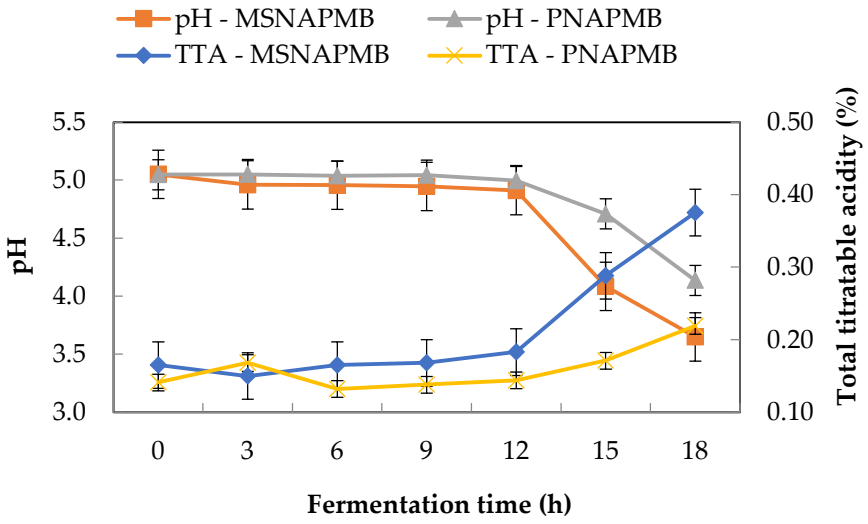

Figure 2. Changes in the pH and total titratable acidity of fermentation of pearl millet extract for the production of plain-(PNAPMB) and moringa-supplemented (MNAPMB) pearl millet non-alcoholic beverages.

The total titratable acidity (TTA) did not significantly change from the onset of fermentation until 12 h has elapsed. At this point, the lower amount of lactic acid was produced by LAB. After 12 h, the TTA of PNAPMB and MSNAPMB increased significantly to 0.22% (18 h) and 0.38% (18 h), respectively. This was caused by the decrease in pH which increased the acid content of the beverages. The decrease in pH and increase in TTA agrees with [25] who reported a decrease in pH range 5.04–5.17 to 3.74–4.35 and an increase in TTA from 1.28 to 2.59 $g.L^{-1}$ during the fermentation of rice flour with various lactic acid bacteria. In summary, there was an inverse relationship between the pH and TTA with the pH decreasing from 5.05 to 4.14 while the TTA increased from 0.14 to 0.22%.

3.3. Effect of Fermentation Time on the Sugar Content of the Pearl Millet Extract during Fermentation

Sucrose was the main sugar identified in pearl millet extract during fermentation. The sucrose significantly decreased from 5.48 to 4.93% and 5.33 to 4.65% in plain and moringa supplemented non-alcoholic pearl millet beverages, respectively (Figure 3). Wilson et al. [26] also reported a decrease in sugar (Brix) from the onset during the fermentation of *Urwangwa*, a Rwandanese banana beer. The decrease in sucrose could be caused by the utilisation of sugars by lactic acid bacteria (LAB) to produce lactic acid in line with the pH and total titratable acidity, which did not significantly change from 0 to 12 h during the fermentation period. This indicated that the LAB were still adjusting to the new environment after inoculation. In addition, the sucrose content was higher in MNAPMB than in PNAPMB, which also indicated that *Moringa oleifera* leaf powder extract favoured the growth of LAB.

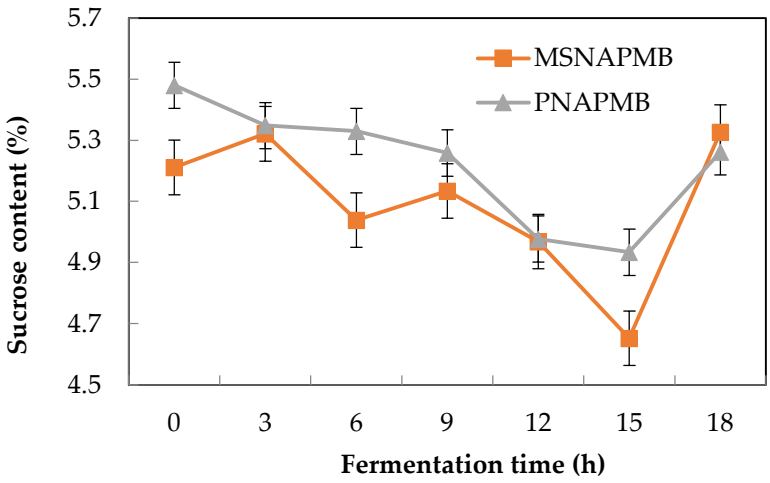

Figure 3. Effect of fermentation time on the sucrose content in pearl millet extract during the preparation of plain and moringa supplemented non-alcoholic pearl millet beverages. PNAPMB—plain non-alcoholic pearl millet beverage. MNAPMB—moringa non-alcoholic pearl millet beverage.

3.4. Proximate Composition of Plain, Moringa-Supplemented, and Traditional Non-Alcoholic Pearl Millet Beverages

The proximate composition of plain non-alcoholic pearl millet beverage (PNAPMB), moringa supplemented non-alcoholic pearl millet beverage (MSNAPMB), and traditional non-alcoholic pearl millet beverage (TNAPMB) is shown in Table 1. The moisture content differed significantly ($p < 0.05$) between the beverages and was 91.74, 91.03, and 87.59% in PNAPMB, MSNAPMB, and TNAPMB, respectively. This is due to the volume of water added during the production of the beverages. The ash content was 2.00, 1.56, and 1.18% in PNAPMB, MSNAPMB, and TNAPMB, respectively. The lower ash content of MSNAPMB could be due to the utilization of mineral elements by *L. mesenteroides* and *P. pentosaceus* during fermentation. The lower ash content could also be due to the lactic acid

bacteria whose enzymatic activity resulted in the breakdown of the beverage components into absorbable forms caused by enrichment of the beverage with *Moringa oleifera* leaf extract powder. It was reported by [27] that the decrease in ash content was caused by the supplementation of beverage with moringa leaf powder.

The protein content differed significantly ($p < 0.05$) between PNAPMB (1.62%) and MSNAPMB (2.17%). TNAPMB was comparatively significantly lower in protein (1.50%). The higher proteins in MSNAPMB could be attributed to moringa leaf extract powder which contains about 19.95% protein as reported by [28]. However, the protein content of 2.17% in MSNAPMB was lower than that reported by [29] (4.63%) at 5% moringa seed flour. This could be due to the level of moringa used and/or moringa seed flour instead of moringa leaf powdered extract as in this study. This is also an indication that the increase in moringa levels caused an increase in protein. This is supported by [27] and [30] who reported that the addition of moringa seed flour and moringa leaf flour increased the protein content of pearl millet flour, and maize-*ogi*, respectively.

Table 1. Proximate composition (g/100 mL beverage) of PNAPMB, MSNAPMB, and TNAPMB.

Nutrient	Proximate Composition * (%)		
	PNAPMB	**MSNAPMB**	**TNAPMB**
Moisture	91.74 ± 0.10 [a]	91.03 ± 0.04 [b]	87.59 ± 0.06 [c]
Ash	2.00 ± 1.55 [a]	1.56 ± 0.67 [a]	1.18 ± 0.49 [a]
Protein	1.62 ± 1.62 [a]	2.17 ± 0.02 [a]	1.50 ± 1.17 [b]
Total fats	0.92 ± 0.01 [a]	0.65 ± 0.01 [a]	1.54 ± 0.09 [a]
Saturated fats	0.23 ± 0.00 [a]	0.16 ± 0.02 [a]	0.48 ± 0.01 [a]
Palmitic acid (C_{16})	0.19 ± 0.01	0.14 ± 0.00	0.41 ± 0.03
Stearic acid (C_{18})	0.05 ± 0.00	0.12 ± 0.03	0.07 ± 0.02
Monounsaturated fats	0.24 ± 0.00 [a]	0.17 ± 0.01 [a]	0.45 ± 0.03 [a]
Oleic acid (C18: 1n9c)	0.237 ± 0.00	0.17 ± 0.01	0.45 ± 0.03
Polyunsaturated fats	0.45 ± 0.00 [a]	0.32 ± 0.01 [b]	0.61 ± 0.05 [a]
Linolelaidic acid (C_{18}: 2n6t)	0.45 ± 0.00	0.32 ± 0.01	0.61 ± 0.05
Total sugars	5.06 ± 0.03	5.31 ± 0.02	6.11 ± 0.06
Sucrose	5.06 ± 0.03 [a]	5.31 ± 0.02 [b]	3.78 ± 0.08 [a]
Glucose	0.00	0.00	2.05 ± 0.03
Fructose	0.00	0.00	0.28 ± 0.02
Carbohydrates	4.31 ± 1.42 [a]	5.03 ± 0.66 [a]	9.41 ± 0.39 [b]
Energy (kJ/100 mL)	113.23 ± 25.36 [a]	130.23 ± 12.61 [a]	197.48 ± 8.07 [b]

* Values are mean ± standard deviation of triplicates. PNAPMB—plain non-alcoholic pearl millet beverage, MSNAPMB—moringa supplemented non-alcoholic pearl millet beverage, and TNAPMB—traditional non-alcoholic pearl millet beverage. Values with different superscripts in each row are significantly ($p < 0.05$) different from each other.

In addition, the increase in protein content could be related to the solubilisation of insoluble proteins of raw pearl millet, rice flour, and the synthesis of protein by lactic acid bacteria during fermentation (Nour and Ibrahim, 2014; Nour et al., 2016). Non-soluble proteins aggregate and settle depending on the pH of the beverage. If these proteins become soluble in the beverage during fermentation the protein content could increase considerably.

The total fat content was 0.92, 0.65, and 1.54% in PNAPMB, MSNAPMB, and TNAPMB, respectively. Saturated fats were high in TNAPMB (0.48%) in comparison to PNAPMB (0.23%) and MSNAPMB (0.16%). The polyunsaturated fats in PNAPMB (0.45) and TNAPMB (0.60%) differed significantly from that of MSNAPMB (0.32%). The fatty acids identified in the beverages were palmitic acid and stearic acid (saturated fats (SFA)), oleic acid (monounsaturated fats (MUFA)), and linolelaidic acid (polyunsaturated fats (PUFA)). Oleic acid and palmitic acid were the highest in TNAPMB followed by

PNAPMB. MSNAPMB had the highest amount of stearic acid followed by TNAPMB. These fatty acids (SFA, MUFA, and PUFA) are prime in pearl millet and occur naturally [31]. The presence of palmitic acid was low in PNAPMB (0.19%) and MSNAPB (0.14%) in comparison to the TNAPMB (0.41 ± 0.03%) and stearic acid was also low in PNAPMB (0.05 ± 0.00%) and MSNAPMB (0.02 ± 0.03%) when compared to TNAPMB (0.07 ± 0.02%). Palmitic acid is associated with an increased risk of coronary heart diseases and tumours while stearic acid is associated with a neutral effect on blood total and low-density lipoprotein (LDL) cholesterol levels. However, these saturated fatty acids are lower in cereal beverages in comparison to yoghurt with palmitic and stearic acid accounting for 16.54% and 11.73%, respectively [32]. Oleic acid (omega-9) was 0.45% in TNAPMB, 0.24% in PNAPMB, and 0.17% in MSNAPMB. Linolelaidic acid is an essential omega-6 fatty acid important in the prevention of diseases related to cardiovascular and cancer [33]. The increase in fat during fermentation could be due to the transformation of carbohydrates to fat; meanwhile, the decrease could be caused by the utilization of fat by lactic acid bacteria present in the beverage during fermentation [27]. In contrast, [29] reported an increase in fat content from 1.67 to 2.20% when 5% moringa seed flour was added to the beverage. However, [27] reported a decrease in the oil content in fermented sorghum with 5% moringa seed flour. Fermentation decreases the long-chain fatty acid content in finger millet [31].

The beverages differed significantly ($p < 0.05$) in sucrose content 5.06, 5.31, and 3.78% in PNAPMB, MSNAPMB, and TNAPMB, respectively. Glucose (2.05%) and fructose (0.28%) were also present in TNAPMB. The sucrose could be from the added sugar during the production and available free sucrose found in millet [34]. The fibre in the beverages could have been utilised by fermenting LAB [27].

The carbohydrate content differed significantly ($p < 0.05$) between PNAPMB (4.31%), MSNAPMB (5.02%), and TNAPMB (9.4%). The energy content in PNAPMB, MSNAPMB, and TNAPMB was 113.23, 130.23, and 197.48 kJ·100 mL^{-1}, respectively, and differed significantly ($p < 0.05$). There was no significant increase in carbohydrate and energy as a result of supplementation with 4% moringa leaf extract. The lack of an increase in carbohydrate and energy was expected as moringa leaf extract is not a source of carbohydrate and also due to complete utilisation of the carbohydrate by the LAB. The higher energy in TNAPMB could be due to the presence of carbohydrates in the beverage due to insufficient breakdown of carbohydrates by the spontaneous LAB fermentation.

3.5. Colour Characteristics of Pearl Millet Non-Alcoholic Beverages

The colour of plain non-alcoholic pearl millet beverage (PNAPMB), moringa supplemented non-alcoholic pearl millet beverage (MSNAPMB), and traditional non-alcoholic pearl millet beverage (TNAPMB) is shown in Table 2. The beverages differed significantly ($p < 0.05$) in lightness, redness, yellowness, chroma, and hue. Pearl millet non-alcoholic beverage with moringa leaf extract (MSNAPMB) was darker, less red, and yellower than the TNAPMB while the one without the extract (PNAPMB) was lighter, greener, and yellower than the traditional beverage (TNAPMB). The added moringa leaf extract powder made the beverage darker, redder, and yellower than the one without the extract (PNAPMB). The colour of the beverages in terms of hue can be described as light yellowish-green, unsaturated for PNAPMB; grey yellowish-red, saturated for MSNAPMB; and light yellowish-red, saturated for TNAPMB (Figure 4).

Table 2. Colour of PNAPMB, MSNAPMB, and TNAPMB.

Attribute	PNAPMB	MSNAPMB	TNAPMB
L *	58.44 ± 0.05 [a]	52.70 ± 0.07 [b]	59.67 ± 0.02 [c]
a *	−1.03 ± 0.03 [a]	0.64 ± 0.03 [b]	2.45 ± 0.19 [c]
b *	15.11 ± 0.02 [a]	27.48 ± 0.03 [b]	19.71 ± 0.05 [c]
Chroma (C *)	15.14 ± 0.02 [a]	27.49 ± 0.03 [b]	19.86 ± 0.08 [c]
Hue (h *)	93.91 ± 0.13 [a]	88.66 ± 0.06 [b]	82.91 ± 0.52 [c]
Δ*E*	5.91	10.60	

* Values are mean ± standard deviation of triplicates. PNAPMB—plain non-alcoholic pearl millet beverage, MSNAPMB—moringa supplemented non-alcoholic pearl millet beverage, and TNAPMB—traditional non-alcoholic pearl millet beverage. Values with different superscripts in each row are significantly ($p < 0.05$) different from each other.

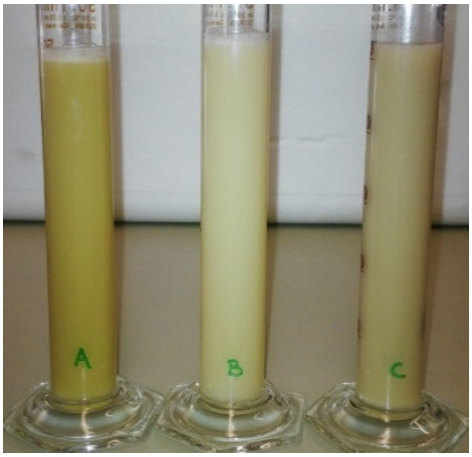

Figure 4. Non-alcoholic pearl millet beverages: A: MSNAPMB—moringa supplemented non-alcoholic pearl millet beverage. B: PNAPMB—plain non-alcoholic pearl millet beverage, and C: TNAPMB—traditional non-alcoholic pearl millet beverage.

The total colour difference (Δ*E)* between the TNAPMB and MSNAPMB (10.60) and PNAPMB (5.91) indicate a perceivable difference in colour between the novel beverages and the traditional since the colour difference is greater than one, the just-noticeable difference. However, the PNAPMB could be acceptable by the consumers since the Δ*E* falls between 4 and 8. The MSNAPMB may not be accepted due to the dominant greenish colour from the moringa leaf extract. A similar decrease in consumer acceptability with an increase in *Moringa oleifera* leaf powder (4% and 6%) in mahewu (a fermented maize beverage) compared to 2% was reported [13].

3.6. Characterisation of Chemical Composition and Colour of Non-Alcoholic Cereal Beverages Using Principal Component Analysis (PCA)

The proximate composition and colour parameters of the plain non-alcoholic pearl millet beverage (PNAPMB), moringa supplemented non-alcoholic pearl millet beverage (MSNAPMB), and traditional non-alcoholic pearl millet beverage (TNAPMB) were subjected to principal components analysis (PCA) (Figure 5).

The variations in the data could be explained by two principal components (PC1 and PC2). The cumulative variation was 82% with many variations (51%) contributed by PC1 and 31% by PC2. PC1 was highly correlated to MSNAPMB high in moisture, total sugar, proteins, hue, and yellowish/blue (b *). Furthermore, PC2 was highly correlated to PNAPMB having high total fat, saturated fat, total sugar, ash, moisture, and chroma. Figure 5 shows the scores (beverages) and loadings (proximate composition and colour parameters) of the non-alcoholic pearl millet beverages. This indicated that the variations in

characteristics of PNAPMB and MSNAPMB could be explained using total fat, saturated fat, total sugar, ash, moisture, proteins, chroma (C), hue, and yellowness (b *).

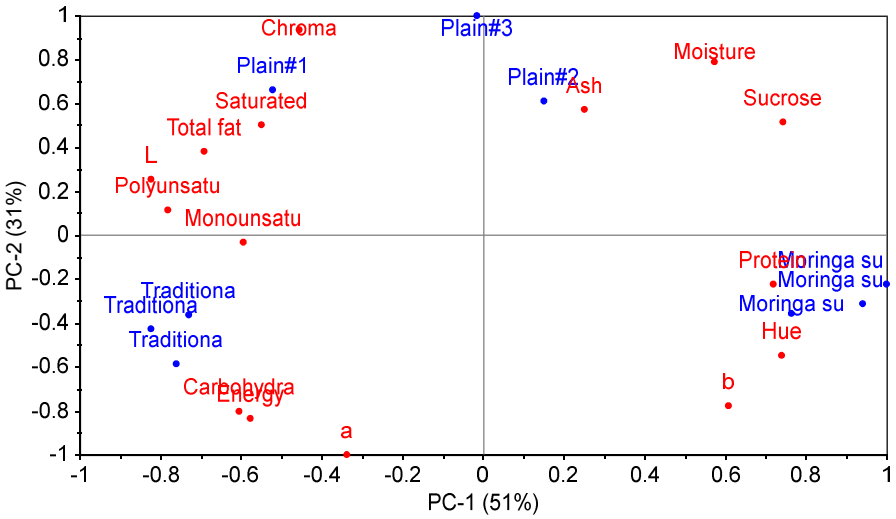

Figure 5. Principal component analysis (PCA) score plot for non-alcoholic pearl millet beverages in terms of their chemical composition and colour. PC-1 first principal component and PC-2, second principal component.

3.7. Volatile Compounds in the Non-Alcoholic Pearl Millet Beverages

The 42 compounds identified in the non-alcoholic pearl millet beverages included sugars, alcohols, alkanes, ketones, esters, fatty acids, carbonyl compounds, and organic acids are outlined in Table 3. The distribution of the metabolites among the beverages is outlined in Figure 6. The node sizes are ranked according to betweenness centrality. More metabolites were identified from the TNAPMB compared to the other beverages. The important metabolites with high betweenness centrality were melezitose (129.71), 3-deoxy-d-mannoic lactone (129.71), D-mannopyranose (64.86), 5-Hydroxymethylfurfural (49.73), Methyltris(trimethylsiloxy)silane (49.73), 2 Ethyl-oxetane (15.12), and lactose (15.12). All the beverages contained melezitose, 3-deoxy-d-mannoic lactone, and D-mannopyranose. Melezitose is a non-reducing trisaccharide produced by many sap eating insects like *Cinara pilicornis* by enzyme action. It is part of honeydew, which acts as an attractant for ants and also as a food for bees. 3-deoxy-d-mannoic lactone has been reported for antimicrobial activity. D-mannopyranose is d-mannose in its six-membered ring form, a sugar monomer of the aldohexose carbohydrates. It is important in the glycosylation of certain proteins in human metabolism. TNAPMB and PNAPMB contained 5-Hydroxymethylfurfural and Methyltris(trimethylsiloxy)silane. 2 Ethyl-oxetane and lactose were found in PNAPMB and MSNAPMB. 5-hydroxymethylfurfural is a flavouring substance in a wide variety of heat-processed products. The identified compounds contribute to the taste, aroma, and biological and medicinal potential of the beverage. For instance, the ester (3-deoxy-d-mannoic lactone) contributes to the flavour of the beverage during fermentation and has an antibacterial effect which results in a safer product [35]. The N, N′-dibutyl-N, N′-dimethyl- have immune-modulating properties while 5-Hydroxymethylfurfural have antioxidant and antiproliferative properties [35]. n-Hexadecanoic acid (palmitic acid) is a fatty acid with anti-inflammatory activities [36]. Lactose that is present in PNAPMB and MSNAPMB could be from the skim milk used during the freeze-drying of isolated lactic acid bacteria. This is supported by its absence in TNAPMB, which was not inoculated with isolated probiotics.

Table 3. Compounds tentatively identified in methanol extract of the non-alcoholic pearl millet beverages

Compound	Chemical Formula	Retention Time (min)	Molecular Weight	Category
3H-pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl	$C_6H_{10}N_2O$	10.78	126.079	alcohol
DL-arabinose	$C_5H_{10}O_5$	12.80	150.053	sugar
Melezitose	$C_{18}H_{32}O_{16}$	15.37	504.169	sugar
Lactose	$C_{12}H_{22}O_{11}$	21.18	342.116	sugar
3-deoxy-d-mannoic lactone	$C_6H_{10}O_5$	24.47	162.053	Cyclic ester
3,4-Furandiol, tetrahydro-, trans-	$C_4H_8O_3$	12.76	104.047	
5-Hydroxymethylfurfural	$C_6H_6O_3$	15.33	126.032	Aldehyde
N,N′-dibutyl-N,N′-dimethyl-		20.79	200.189	
2-Ethyl-oxetane	$C_5H_{10}O$	21.16	86.073	
Sucrose		32.25	342.116	
Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$	42.18	310.127	Organosilicon
Methyltris(trimethylsiloxy)silane	$C_{10}H_{30}O_3Si_4$	42.33	310.127	Organosilicon
Clindamycin	$C_{18}H_{33}ClO_5S$	10.81	424.18	
D-mannopyranose	$C_6H_{12}O_6$	12.74	180.063	
D(+)-talose	$C6H_{12}O_6$	12.80	180.063	sugar
6,10,13-trimmeltetradecanol	$C_{17}H_{36}O$	18.55	256.277	fatty alcohol
4,5-Diamino-2-hydroxypyrimidine	$C_4H_6N_4O$	10.78	126.054	
1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	16.20	126.065	
beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	32.24	342.116	Sugar
3,4-furandiol, tetrahydro-,trans	$C_4H_8O_3$	13.08	104.047	alcohol
Isosorbide dinitrate	$C_4H_8N_2O_8$	14.36	236.028	
D-fructose, 1,3,6-trideoxy-3,6-epithio	$C_6H_{10}O_3S$	20.54	162.035	sugar
Hexadecanoic acid	$C_{16}H_{33}O_2$	29.51	256.24	saturated fatty acid
3-(prop-2-enoloxy) dodecane	$C_{11}H_{14}O$	31.51	240.209	alkeny
Tetradecane, 2,6,10-trimethyl	$C_{17}H_{36}$	33.21	336.303	
Undec-10-ynoic acid, undercyl ester	$C_{22}H_{40}O_2$	32.23	240.282	Ester
Methoxyacetic acid, 2-tetradecyl ester	$C_{17}H_{34}O_3$	33.21	286.251	Ester
Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	34.39	697.0	
2-hexyl-1-octanol	$C_{14}H_{30}O$	34.64	214.23	fatty alcohol
Eicosane	$C_{20}H_{42}$	35.09	282.329	alkane

Eicosane, 7-hexyl	$C_{26}H_{54}$	36.01	366.423	
Octasiloxane	$C_{16}H_{50}O_7Si_8$	36.38	578.171	
Di-n-decylsulfone	$C_{20}H_{42}O_2S$	36.77	346.291	
Benzoic acid, 4-methyl-2-trimethylsilyloxy-,trimethylsilyl ester	$C_{14}H_{24}O_3Si_2$	38.06	296.126	
Cyclotrisiloxane, 2,4,6--trimethyl-2,4,6-triphenyl	$C_{21}H_{24}O_3Si_3$	40.45	408.103	
4,4,6-Trimethyltetrahydro-1,3-thiazin-2-one	$C_7H_{13}NOS$	12.72	159.072	
2-Thiophenecarboxylic acid, 5-(1,1-dimethylethoxy)-	$C_9H_{12}O_3S$	13.00	200.051	Carboxylic acid
Propanal, 2-methyl-, 2-propenylhydrazone	$C_7H_{14}N_2$	16.35	126.116	N-alkylated hydrazones
Lethane		20.59	203.098	
3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	23.85	162.053	
d-Glycero-d-ido-heptose	$C_7H_{14}O_7$	26.10	210.074	
n-Hexadecanoic acid	$C_{16}H_{32}O$	29.52	256.24	Fatty acid
Tetracosane	$C_{24}H_{50}$	34.39	338.391	Alkane

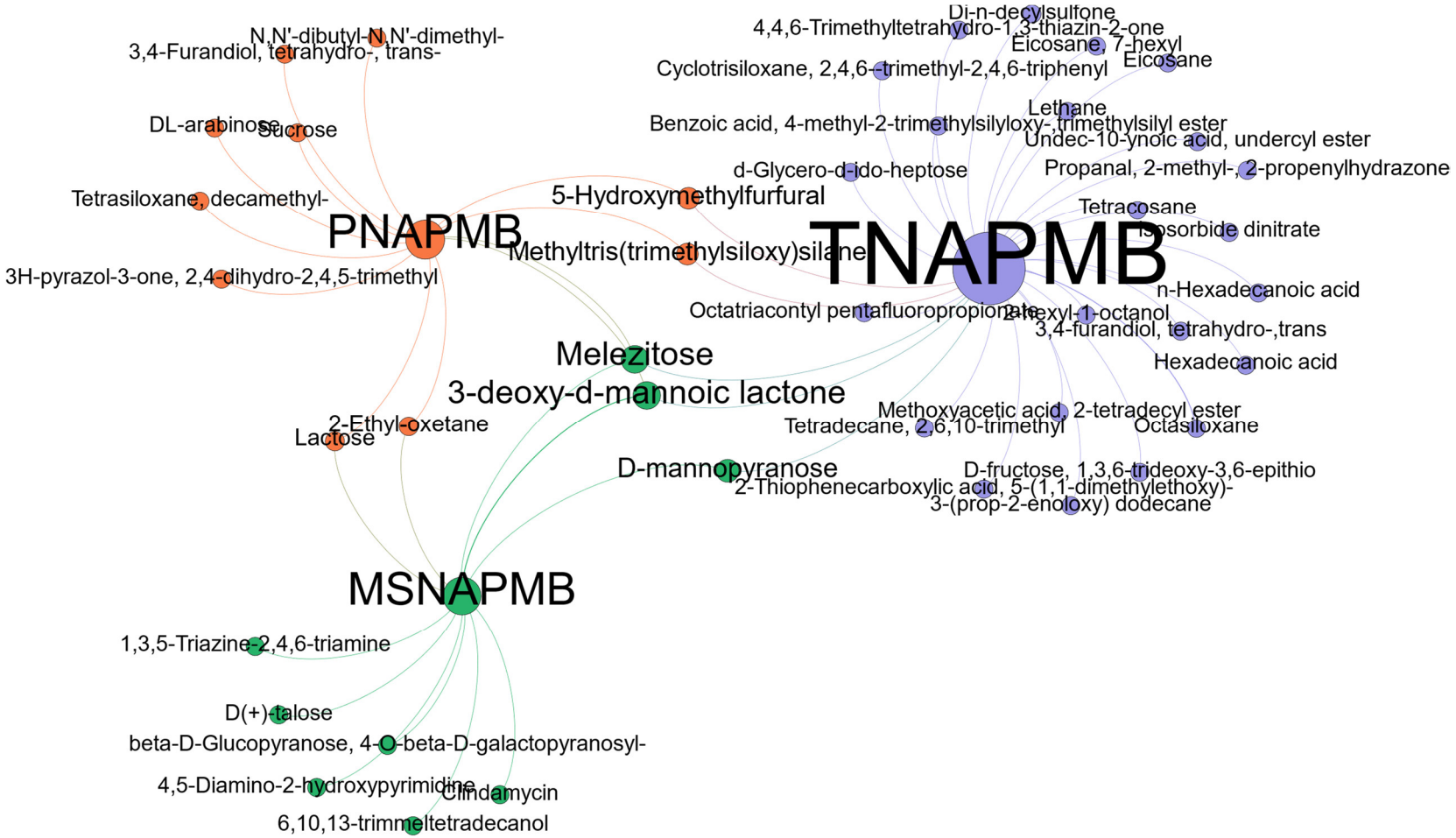

Figure 6. Network of metabolites in non-alcoholic pearl millet beverages PNAPMB = plain; MSNAPMB = moringa oleifera leaf extract powder supplemented; and TNAPMB = traditional.

The organic acids produced may preserve the beverage through the inhibition of pathogenic microorganisms. The nutritional content of the beverage is also improved. The identified compounds with their biological and medical uses prove that the beverages are not meant for refreshing only but have many benefits to consumers.

3.8. Sensory Characteristics of the Non-Alcoholic Pearl Millet Beverages

The demography of the panellists who evaluated the non-alcoholic pearl millet beverages (NAPMBs) is shown in Table 4. There were 50 panellists made-up of 24 and 71% of males and females, respectively, of which 52% were black, 10% coloured, and 2% white; 16% were staff members and 82% were students; 52% were South African citizens, and 24% were international students; and 77% less or equal to 29 years, 10% between the age of 30–39, and 10% were 40 years old or above.

Table 5 shows the sensory parameters of NAPMBs. The mean rating for appearance for the plain non-alcoholic pearl millet beverage (PNAPMB), moringa-supplemented non-alcoholic pearl millet beverage (MSNAPMB), and traditional non-alcoholic pearl millet beverage (TNAPMB) were 5.9, 5.5, and 4.5, respectively. The beverages differed significantly ($p = 0.037$) in appearance with PNAPMB being the most preferred. The mean ratings for the colour of PNAPMB, MSNAPMB, and TNAPMB were 5.8 (liked slightly), 5.6 (liked slightly), and 4.8 (neither liked nor disliked), respectively, and differed significantly ($p = 0.007$). The PNAPMB was golden brown, MSNAPMB was greenish-and-golden in colour while TMNAPMB was milky in colour and appearance. PNAPMB and MSNAPMB made using pearl millet extract appeared similar to commercial soft drinks; hence, they were preferred. In contrast, TNAPMB contained particles of starch, proteins, and minerals, which could have affected the colour. TNAPMB beverage was made with no stabilisers and the sedimentation of particles could be attributed to the lower scores.

The mean score for the aroma of PNAPMB was 5.3 (neither liked nor disliked), 4.7 (neither liked nor disliked) for MSNAPMB, and 4.2 (disliked slightly) for TNAPMB; hence, the beverages differed significantly ($p = 0.020$). The organic acids and metabolites produced during fermentation by *Leuconostoc mesentoroides* and *Pediococcus pentosaceus* could be responsible for the aroma of the beverages. Indigenous cereal slurries lack flavour but develops flavour during fermentation when volatile substances (diacetyl, acetic acid, butyric acid, amino acids, aldehydes, etc.) are developed.

Table 4. Demography of panellists used in the evaluation of the beverages.

Item	Frequency (Percentage) *
Gender	
Male	38 (24)
Female	106 (71)
No response	8 (5)
Race	
Black	126 (85)
Coloured	15 (10)
White	3 (2)
Other	3 (2)
No response	4 (1)
Status	
Staff	24 (16)
Student	123 (82)
No response	3 (2)
Nationality	
National	78 (52)
International	36 (24)

No response	36 (24)
Age group	
Less than or equal to 29 years	115 (77)
30–39 years	15 (10)
40 and above	15 (10)
No response	5 (3)

* Numbers shows frequency and percentage in the bracket.

Table 5. Acceptability ratings of sensory attributes of pearl millet beverages.

Sensory Attribute	Non-Alcoholic Pearl Millet Beverage [1]		
	Plain	Moringa Supplemented	Traditional
Appearance	5.9 ± 2.5 [b]	5.5 ± 2.0 [b]	4.5 ± 2.5 [a]
Colour	5.8 ± 2.3 [b]	5.6 ± 2.0 [a,b]	4.8 ± 2.3 [a]
Aroma	5.3 ± 2.5 [b]	4.7 ± 2.1 [a,b]	4.2 ± 2.3 [a]
Taste	5.3 ± 2.1 [a]	4.9 ± 2.1 [a]	5.44 ± 2.4 [a]
Mouthfeel	6.0 ± 2.1 [a]	5.8 ± 1.9 [a]	6.0 ± 2.2 [a]
Overall acceptability	5.8 ± 2.0 [b]	4.9 ± 2.0 [a]	5.4 ± 2.1 [a,b]

[1] Values are mean ± standard deviation of 50 panellists. Values with a different superscript in the same row differ significantly ($p \leq 0.05$).

The unique development of aromas and/or flavour depends on the chemical composition of the substrate (type of cereal, sprouted, etc.), an environmental condition during fermentation (pH, temperature, and anaerobic/aerobic) and starter culture [37]. TNAPMB was carried out by chance fermentation made up of a diversity of lactic acid bacteria and other bacteria which could have resulted in the unacceptable aromas, and thus lower rating, unlike PNAPMB and MSNAPMB, which were fermented using known purified cultures. The slight differences between PNAPMB and MSNAPMB could be due to moringa extract powder which could have released other volatile compounds during fermentation. The beverage was fermented in a closed vessel, unlike traditional beverages, which are simply covered with a cloth to exclude foreign matters. During closed fermentation CO_2 is not allowed to escape and is dissolved in the beverage, this may be ideal for anaerobic lactic acid bacteria but it may lead to spoilage and the creation of unwanted flavours or aromas. The closed system could also have caused all the released metabolites to be contained within the beverage. The level of diacetyl compound (2,3-butandione) could be high due to the lack of aeration. Hence, when citric acid was used in the beverage the 'off-like' flavour became intense because diacetyl is synthesised during utilization of citric acid. *Pediococcus pentosaceus* could also be responsible for the production of diacetyl at high levels [38]. Some of the panellists related TNAPMB to mahewu and porridge since the beverage is fermented as a whole and the flour was cooked through gelatinization.

The beverages differed significantly ($p = 0.001$) in taste. The mean rating for taste was 5.3 for PNAPMB, 4.9 for MSNAPMB, and 5.4 for TNAPMB, meaning the beverages were neither liked nor disliked in taste. TNAPMB was rated high followed by PNAPMB then MSNAPMB. The cocktail of bacteria could be responsible for the sweet–sour taste profile of TNAPMB, whereas only selected lactic acid bacteria (*L. mesentoroides* and *P. pentosaceaus*) were used in PNAPMB and MSNAPMB. The preference for TNAPMB could have been due to cultural preferences by people who are familiar with the natural fermented ethnic beverages. The lower rating of MSNAPMB could be due to the fresh leaf earthy flavour of moringa leaf extract in the beverage. The majority (77%) of the panellists were youth (≤29 years old) and are loyal supporters of carbonated drinks in South Africa. According to StatsSA [39], the South African population reported an increase in the growth rates of the elderly people meaning the beverages have the potential for growth among

the older generation who are familiar with non-alcoholic cereal beverages such as '*magewu*'.

The mean score for the mouthfeel of the beverages did not differ significantly (p = 0.094). The similarity could be because the beverages were all fermented. Phytates, phenols, and tannins found in pearl millet could be responsible for the mouthfeel of the beverages. Murevanhema and Jideani [40] also reported the influence of tannin on the mouthfeel of fermented Bambara groundnut milk. However, lactic acid bacteria during fermentation resulted in low pH (3.65–4.14) and built-up of lactic acid (0.22–0.42%) and pasteurisation of the beverages at high temperature could have resulted in the reduction in these antinutrients.

PNAPMB, MSNAPMB, and TNAPMB had a mean score for overall acceptability of 5.8, 4.9, and 5.4, respectively. The beverages differed significantly ($p < 0.05$) in overall acceptability. PNAPMB was rated high followed by TNAPMB then MSNAPMB. The overall acceptability was influenced by all the other attributes of appearance, colour, aroma, and taste. PNAPMB had a bright golden-brown appearance resembling most grape flavoured beverages; hence, it was preferred. TNAPMB had a creamy-milk appearance the panellist could have related to '*umgqomothi*' (African beverage) with which they are familiar. MSNAPMB was scored lower, which could be explained by the greenish colour and fresh earthy leaf aroma from moringa leaf powder. The taste of the beverages could be improved in future work. Possible approaches will be flavoured, carbonated, and blended with other cereals.

3.9. Conclusions

The successful use of isolated and purified cultures of lactic acid bacteria (*Leuconostoc mesenteroides* and *Pediococcus pentosaceus)* from the traditional fermentation of pearl millet for fermentation of pearl millet extract for 18 h at 37 °C to produce a stable acceptable beverage is reported. The production time was reduced to 18 h compared to the 24 h for the traditional process. Furthermore, the beverages were stable to sedimentation. Two formulations of non-alcoholic pearl millet beverages were produced, namely, plain non-alcoholic beverage (PNAPMB) and moringa supplemented plain non-alcoholic pearl millet beverage (MSNAPMB). The beverages may have beneficial anti-inflammatory and anti-pathogenic properties due to the present metabolites. Overall, the beverages were accepted by the consumers; however, the taste of the beverage could be improved.

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