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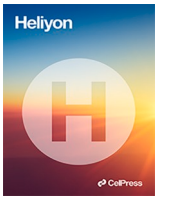

Physicochemical attributes and acceptability of marula wine fermented with natural *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae*

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ABSTRACT

The aim of the study was to test the acceptability and physico-chemical characteristics of marula wine fermented with known cultures of natural *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae*. The LAB *Lactobacillus* and *Saccharomyces* dominate the fermentation of marula wine throughout the fermentation period. These were isolated and identified from the spontaneously fermented marula wine and re-inoculated as single cultures and as mixed cultures to ferment marula juice into wine. The *Saccharomyces cerevisiae* combined with *Lactiplantibacillus plantarum* (PYL) and *Saccharomyces cerevisiae* (PY) fermented wines were not significantly different ($p \geq 0.05$) in all the physico-chemical characteristics and acceptability. The single culture of *Lactiplantibacillus plantarum* had the lowest pH of 2.8. The alcohol content of marula wine fermented with *Saccharomyces cerevisiae* was 6.10 ± 0.17, while the alcohol content of the spontaneously fermented wine was 3.33 ± 2.49. The ^{o}Brix of wine fermented with *Saccharomyces cerevisiae* only and as mixed culture was 2.07 ± 0.21 and 2.00 ± 0.00, respectively, while the control and *Lactiplantibacillus plantarum* had an ^{o}Brix of 6.23 ± 2.77 and 8.67 ± 0.06, respectively. The *Lactiplantibacillus plantarum* fermented sample and the control had significantly higher overall acceptability scores of 7.60 and 6.98, respectively. *Saccharomyces cerevisiae* is capable of producing ethanol as a single culture and co-cultured with *Lactobacillus plantarum*. The most preferred wine was that fermented by *Lactiplantibacillus plantarum* only because of its sweetness.

1. Introduction

Fermentation is a traditional low-cost biotechnology that involves the use of microorganisms or enzymes to produce food and beverages with specific desirable characteristics [1]. Marula wine is traditionally prepared by spontaneous fermentation, with microorganisms present in the fruits and depending on the duration of the fermentation, the beverage can be consumed as an alcoholic or non-alcoholic [2].

Fruits and vegetables can be fermented spontaneously by natural surface lactic acid bacteria such as *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp., [3]. Several authors have reported changes in organoleptic properties as a result of fermentation in fruits and vegetables, [3,4]. The fermentation of fruits also results in changes in physicochemical properties of fruits which includes decrease

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in pH, increase in titratable acidity, and reduction in °Brix, [5–8]. During lactic acid fermentation, the lactic acid bacteria (LAB) convert the carbohydrates in fruits into lactic acid which lowers the pH to below 4.0. This environment stabilises the shelf life of the food by restricting the growth of spoilage and pathogenic bacteria [9]. Fruits are a conducive environment for the growth of LAB and yeasts because they are rich in sugars, vitamins and minerals [9], hence the easy spontaneous fermentation.

The marula fruit has found to contain some useful nutrients for the human body. These include ascorbic acid, calcium, iron, zinc, fibre, proteins, and some B vitamins [10,11]. This fruit also has some phytochemicals such as polyphenols, flavonoids, tannins and pectin, which are good for the prevention of chronic and degenerative diseases [11]. The fruits can be eaten fresh or processed into other products. The marula fruits can be fermented into an alcoholic or non-alcoholic beverage, thanks to its skin having about 29 yeasts [11]. Other products include jam, chutney and pie fillings, owing it to the high pectin content [11]. This puts the marula plant among plants with prospects of commercialisation. The prospects of commercialising marula comes with a hub of opportunities. However, sustainability of resources and cultural relations must be considered [12]. Hal et al. [13], reported that the marula wine retains its antioxidant activity even after fermentation, especially at temperatures between 30° and 40 °C. These studies indicate that marula wine is a healthy beverage.

In the Southern African region, including Eswatini, marula (*Sclerocarya birrea* subsp. *caffra)* fruits are spontaneously fermented to make an alcoholic beverage [14]. The profile of microorganisms present in the spontaneous fermentation of marula wine have been reported in previous studies [15,16]. Phiri et al. [15]found that the LAB *Lactobacillus* and *Saccharomyces* dominated the fermentation of marula throughout the fermentation period. Dlamini & Dube [2],found that during spontaneous fermentation, the pH and sugar content decreases as the alcohol content increases.

Spontaneous fermentation of fruits can provide a platform to select highly performing microorganisms for starter cultures [17]. These starter cultures increase the efficiency of the fermentation process, especially when considering commercialisation of the wine [18]. Starter strains must be selected based on a number of factors such as ability to acidify juice, eliminate pathogenic microorganisms, acceptable sensory characteristics and nutrient enhancement or retention [17]. However, the physicochemical properties and the acceptability of marula wine fermented using isolated and identified *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae* have not been reported so far. The choice of yeast used as starter culture can determine the quality and acceptability of the wine [16]. The aim of this study was to determine the physiochemical properties and acceptability of marula wine inoculated with natural *Saccharomyces cerevisiae* and *Lactiplantibacillus plantarum*.

2. Materials and methods

2.1. Spontaneous fermentation

A traditional method for the spontaneous fermentation of the marula wine was followed. Marula fruits were collected from the lowveld of Eswatini, at Mphofu from several trees within close proximity. The marula fruits were allowed to ripen at room temperature. The pulp and juice were manually extracted using a stainless-steel table knife. To the 5L of seeds, juice and pulp, 5L water were added. About 500 g of white sugar was added to the mixture which was allowed to ferment at room temperature for 72 h. The seeds and foam were removed. On the fourth day, 2.4 L of freshly extracted marula juice with pulp was added to the ferment. The mixture was allowed to ferment for two more days. On the sixth day, another 500g of white sugar was added and the marula wine was sampled on the seventh day. Samples were kept at 4 °C until required for microbial analysis.

2.2. Isolation, characterisation and identification of Lactiplantibacillus plantarum and Saccharomyces cerevisiae

2.2.1. Isolation and characterisation of Saccharomyces cerevisiae

Samples of marula wine were serially diluted and isolated using the spread plate method on Rose Bengal Chloramphenicol agar (RBCA) (Merck, Darmstadt, Germany). Colonies were isolated based on their with different morphology and were purified by streaking onto fresh Sabouraud dextrose Agar (SDA).

Yeast isolates were screened for shape using the methylene blue staining as described by Moemen, Bedir, Awad, & Ellayeh, [19]. Cells with round shape were considered as presumptive yeast cells.

The carbohydrate fermentation was conducted using a modified method by Kechkar et al., [20]. The fermentation broth was prepared by dissolving 4g (2 %) of the test carbohydrate, 2g peptone, 1g yeast extract in 250 mL distilled water, sterilised, cooled and then inoculated with yeast strains in test tubes with Durham tubes inserted upside down. The tubes were incubated at 37 °C for 24 h and gas bubbles and colour changes were observed. Methyl red drops were added to the incubated broth and colour change was observed. A blank (uninoculated) fermentation was used as a negative control.

2.2.2. Isolation and characterisation of Lactiplantibacillus plantarum

The marula wine sample was serially diluted and poured onto liquid De Man Ragosa (MRS) agar (Merck, Darmstadt, Germany) using the pour plate method Wang et al., [21]. Colonies were picked for further characterisation based on morphology.

The pure isolates were screened for identification as LAB by conducting catalase test, and gram staining. The catalase test was done by placing a drop of 3 % hydrogen peroxide on a colony on a glass slide [22]. The gram staining was done according to the procedure described by Ref. [23]. Gram-positive rods and catalase negative isolates were considered as presumptive LAB (*Lactiplantibacillus plantarum*).

The fermentation test was conducted using a modified method by Schreckenberger & Blazevic (1976) using a test tube.

Fermentation broth was prepared by dissolving 1 g of the carbohydrate, 0.04 g disodium phosphate, 0.01 sodium dihydrogen phosphate, 0.8 g sodium chloride and 0.2 mL of 1 % aqueous solution of bromocresol green colour indicator in 250 mL distilled water. Each broth was inoculated with the bacteria culture and incubated at 30 °C for 12 h and the colour changes were observed. Fermentation broth without any test carbohydrate was used a negative control.

2.2.3. Identification of Saccharomyces cerevisiae and Lactiplantibacillus plantarum

The DNA extraction of bacteria and yeast was carried out using a modified procedure of the CTAB method by Minas, Mcewan, Newbold, & Scott, (2011).

The Polymerase Chain Reaction and sequencing of both yeast and lactic acid bacteria was done by Inqaba Biotechnical Industries, Pretoria, South Africa. The SLC6A3 polymorphism analysis was performed by polymerase chain reaction (C1000 Touch Thermal Cycler, BioRad, USA) with a final reaction volume of 20 μL approximately 30 ng of DNA, OneTaq ® Hot Start Quick-Load® 2X Master Mix with Standard Buffer (#M0488S, NEB) and 0.2 μM of each primer: F 5′-TGTGGTGTAGGGAACGGCCTGAG-3′ and R 5′-CTTCCTGGAGGTCACGGCTCAAGG-3′. Thermal cycling consisted of a 2 min initial denaturation phase at 94 °C followed by 35 cycles of 30 s at 94 °C, 60 s at the annealing temperature of 60 °C, 45 s at 68 °C. The integrity of the PCR amplicons was visualized on a 1 % agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye.

Fragments were sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000. The contents of the kit were mixed thoroughly and centrifuged briefly to collect the liquid at the bottom of the tube. The loading mix was prepared by mixing 0.5 μl, 0.5 μl Size Standard and 9 μl Super-DI. The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053). The cleaned products were then injected on the Applied Biosystems ABI 3500XL Genetic Analyser with a 50 cm array, using POP7. Sequence chromatogram analysis was performed using FinchTV analysis software. The sequences were viewed and cleaned using Chromas version 2.6 Copyright © 1998–2016 Technelysium Pty Ltd and aligned in BioEdit Sequence Alignment Editor software version 7.2. Consensus sequences were then compared to existing sequences in Genebank using the online NCBI BLASTn (U. S. National Library of Medicine) National Centre for Biotechnology Information.

2.3. Fermentation of marula wine using isolated and identified Lactiplantibacillus plantarum and Saccharomyces cerevisiae

Marula juice was mixed with water at a 1:1 ratio to reduce the viscosity of the juice [24]. Marula juice was then filtered using fibreless cloth for all samples. Then further filtered through 0.45 μm under a vacuum. Fifty (50) grams sugar was added to 1 L of juice and then fermented (the control). In three other 1 L clear glass bottles, 50 g of sugar were added to the juice and then pasteurised in a water bath at 90 °C for 10 min [25]. These were then cooled to room temperature. The cooled juice was inoculated as follows; 1) *Saccharomyces cerevisiae* combined with *Lactobacillus plantarum*, 2) *Saccharomyces cerevisiae* only and 3) *Lactiplantibacillus plantarum* only with 0.5 mL 0.1OD_{600} culture of each treatment. The juice was fermented anaerobically at 25 °C for 7 days.

2.4. Physicochemical characteristics of fermented marula juice

2.4.1. Titratable acidity and pH

Total acidity was determined using the titration method where 10 mL of each sample was mixed with 6 drops of phenolphthalein colour indicator and then titrated using 0.1 N sodium hydroxide. Total acidity was calculated using the formula:

$$Total\ acidity = \frac{(0.1xNaOH\ volume\ (L)\ x\ 90.08\ (molecular\ weight\ of\ lactic\ acid)}{Sample(mL)}$$

The pH was taken using portable pH meter (Hanna, Romania) by immersing the electrode into the sample and directly taking readings [26].

2.4.2. ^{o}Brix determination

A small aliquot of the sample was placed on a Pocket refractometer (Atago, PAL-1, Tokyo). ^{o}Brix readings were taken directly form the display screen and recorded. The ^{o}Brix measurement was used to estimate amount of dissolved sugars in the samples.

2.4.3. Determination of alcohol content

Twelve millilitres of each sample were distilled in round bottom flasks and the distillate collected into another round bottom flask. The distillate was then transferred into 15 mL clean centrifuge tubes and distilled water added to 12 mL mark. The distillates' ethanol content was measured using an alcohol meter (Anton Paar DMA 35, Graz) [2].

2.5. Consumer acceptability tests

The consumer acceptability test was conducted according to Sharif, Butt, Sharif, & Nasir, [27]. The 9-point hedonic scale was used to measure the degree of acceptability of colour, aroma, taste and mouthfeel for each of the treatments, where 9 was the extremely liked and 1 was extremely disliked. Sixty panellists were selected randomly based on their familiarity of the product and had to be of consenting age. Most of the panellists were of the youth category and were both male and female with an age range between 18 and 35 years. Before participation, the sensory evaluation exercise was explained to the panellists who then signed a consent form. The tests

were conducted in individual booths where four 3-digit coded samples were presented to the 60 untrained panellists in random order in 25 mL glass tot cups. The panellists were briefly given instructions before the start of the sensory evaluation exercise. The panellists were required to evaluate samples in a left to right order and were provided with water to rinse off their palates in between samples. Instructions were given to evaluate each attribute in the order; colour, aroma, taste, mouthfeel and overall acceptability.

3. Data analysis

Data for the physicochemical analysis and consumer tests were analysed using Statistica 8 Software. Analysis of Variance (ANOVA) was used to determine any significant differences between the measured parameters. The outliers from the sensory data were handled using outlier box plot method and those observations were removed. Means were compared using Fischer's Least Square Difference. All tests were carried out at a 95 % significant level.

4. Results and discussion

4.1. Isolation and characterization of lactic acid bacteria

The LAB colonies were observed to grow in three sites on the agar, that is, at the bottom, embedded in the agar and on the surface of the agar. The colonies that grew at the bottom of the plate were white, circular and flat with a smooth margin whereas those embedded in the media were concave and white with sharp tapering at the ends. The colonies that grew on the surface of the media were smooth, circular, raised and bigger than the others in the different sites of the media. The average CFU/mL for the LAB was 1.4×10^7.

All colonies were gram stained to determine whether they were gram-positive or gram-negative. Gram-positive bacteria were identified by retention of the violet colour which was observed under microscope. The shape of the bacterial cells of interest were rods which retained the violet colour. Three isolates were identified and labelled as B_{11}, B_{13} and B_{02} and were all gram-positive rods (*bacillus*). *Lactiplantibacillus plantarum* is a gram-positive bacterium [28]. Gram-positive rods are described as possible lactic acid bacteria in Ni, Wang, Li, Cai, & Pang [29] and hence these were selected for further purification and determinative tests. These were the colonies which had initially grown embedded or at the bottom of the agar. Anaerobic bacteria will grow well away from the surface in a semi solid media [30].

In the catalase test, all three isolates (B_{11}, B_{13} and B_{02}) were found to be catalase negative, meaning that there was no bubbling on addition of 3 % hydrogen peroxide on the culture on a slide. The catalase test ascertains the presence of the enzyme catalase which is able to break hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2), which is then emitted as a gas and form a cloud of bubbles within 10 s of contact with the hydrogen peroxide. The lactic acid bacteria *Lactiplantibacillus plantarum* lacks the catalase enzyme, which makes them unable to produce the oxygen from the hydrogen peroxide which forms the cloudy gas [31].

Fermentation tests were conducted to test the ability of the suspected LAB strains to produce acid in a carbohydrate medium which is indicated by a colour change.

As shown in Table 1, all the isolated strains were able to ferment fructose and glucose to form a stable acid indicating that the three strains are the same in their ability to ferment sugar. *Lactiplantibacillus plantarum* produces lactic acid from glucose, and ferments fructose and other sugars to produce a stable lactic acid [32,33] However, contrary to the results presented in Table 1, other studies found that *Lactiplantibacillus plantarum* was able to fermented sucrose. to produce lactic acid but at a slower rate compared to glucose (Puntillo et al., 2020; Bonestroo et al., 1992). The utilisation of sucrose by the *Lactobacillus* strain in this study might have been slow and so that the lactic acid produced was too low to be detected.

4.2. Isolation and characterisation of yeast isolates

Yeast colonies were isolated from Rose Bengal Chloramphenicol agar plates and their average concentration was found to be 1.5×10^8 CFU/mL. The isolated yeast colonies were white to creamy in colour with a higher convex palate elevation and smooth margin [20]. When grown on Sabouraud Dextrose Agar (SDA), the colonies were comparatively larger than those that grew on MRS agar. The colonies were spherical when observed under microscope, similar to those described by Sulieman, Esra, & Abdelgadir, (2015) of the *Saccharomyces* genus. The yeast colonies were labelled as; Y1–Y5 for bigger colonies and smaller colonies were labelled as Ys1-Ys4.

The carbohydrate fermentation was conducted to test the ability of yeast strains to ferment carbohydrates as well as their ability to produce gas, which is presumably carbon dioxide. The gas production was indicated by the accumulation of a gas bubble in the Durham tubes that were inserted upside down in the test tubes. The results are shown in Table 2. All strains fermented glucose and fructose, which are simple sugars with gas production with glucose as a carbon source. These strains were assumed to be of the

Table 1
Ability of bacterial isolates from marula wine to ferment carbohydrates.

Bacteria strain	Sucrose	Fructose	Glucose	Starch	No sugar
B_{11}	−	+	+	−	−
B_{13}	−	+	+	−	−
B_{02}	−	+	+	−	−

* -No fermentation observed,+fermentation was observed.

Saccharomyces genera as stated by Endoh, Horiyama, & Ohkuma, [34]. *Saccharomyces* ability to metabolise sucrose is through the secretion of an enzyme that allows the sucrose molecule to enter the yeast so that it can be metabolised [35]. Starch as a large sugar is not metabolised by yeast [35].

Acid production was indicated by a red colour change when drops of methyl red were added to the medium after anaerobic incubation for 12 h. The colour change is an indication that the strains can utilise that carbohydrate to produce acid. Organic acids are produced as by-products in yeast fermentations and play a major role in the flavour attributes of the final product [36]. As shown in Table 2, all strains fermented glucose and fructose, which are simple sugars with gas production. In a study on characterisation of yeast, *Saccharomyces cerevisiae* utilised sucrose and glucose [37]. The Y_3 strain did not produce acid with sucrose as a carbon source and was assumed not to belong to the *Saccharomyces* genera. Strain Y_4 and Y_{S2} displayed the same carbohydrate fermentation characteristics, being the only strains that were able to produce acid from starch. This could mean that these strains might not be *Saccharomyces* yeast strains and they were not considered for further studies.

4.3. Molecular identification of yeast and LAB

The lactic acid bacterial strains were identified as *Lactiplantibacillus plantarum*. Percent identity ranged from 97.77 to 99.89 %. The yeast strains were identified as *Saccharomyces cerevisiae* and the percentage identity ranged from 97.8 to 99.58 % Fig. 1 shows the identified microorganisms in relation with other strains.

The *Lactiplantibacillus plantarum* was isolated from the marula wine at 7 days, a time when other competing bacteria were assumed dead. *Lactiplantibacillus plantarum* is tolerant to low pH of 3.2–3.6 and ethanol of 13 % [38]. These levels are comparable to those displayed by *Oenococcus oeni* widely used in malolactic fermentation of grape wine, [39]. Since *Lactiplantibacillus plantarum*is tolerant to low pH and high alcohol content, it has a potential to play a role in malolactic fermentation and improve the flavour quality of marula wine. The *Lactiplantibacillus plantarum* has stress response pathways that are induced under high alcohol and acidic conditions [21]. Sieuwerts, Bron, & Smid [40], reported that *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae* stimulated each other only in the presence of glucose, fructose and lactose as carbon source. This means that there is a synergistic relationship in these two species of microorganisms during fermentation. This relationship is also shown where the presence of *Lactiplantibacillus plantarum* increased the ethanol tolerance of *Saccharomyces cerevisiae* by promoting or inhibiting various metabolic processes in the yeast cells [41]. The yeast *Saccharomyces cerevisiae* dominates the alcohol fermentation of many traditionally fermented beverages [42]. It was reported that a co-culture of *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae* in a mango slurry improved its total phenolic content [43].

4.4. Physicochemical attributes of fermented marula wine

The marula wine fermented with *Saccharomyces cerevisiae* together with *Lactiplantibacillus plantarum* (PYL) and *Saccharomyces cerevisiae* (PY) were not significantly ($p \geq 0.05$) different in all the physico-chemical characteristics. They both had the lowest ^{o}Brix 2.00 ± 0.00 and 2.07 ± 0.2, respectively, highest pH of 3.0 and highest alcohol content of 5.90 % and 6.10 % (Table 3).

Saccharomyces cerevisiae only and *Lactiplantibacillus plantarum*only treatments had a significantly different total acidity ($p \leq 0.05$). However, their total titratable acidity was not significantly ($p \geq 0.05$) different when compared to PYL and control. The PL treatment had the lowest pH of 2.80 while the other treatments had statistically similar pH. Salari & Salari [44], in a 12-h study, found that *Saccharomyces cerevisiae* grew well in a medium of pH 4.0 and 5 % dissolved oxygen. This means that *Saccharomyces cerevisiae* can survive and grow even at pH conditions lower than 4.0. In a fermentation of a mixed culture *Saccharomyces cerevisiae* and *Lactiplantibacillus plantarum*, the pH of the beer dropped within the first five days of fermentation and both microorganisms were still thriving [45]. In the same study, it was also found that single cultures *Saccharomyces cerevisiae* had a higher pH compared to the single cultures of *Lactiplantibacillus plantarum*. This clearly indicates that fermentation would reduce the pH of the medium, even though *Lactiplantibacillus plantarum* reduces it faster than *Saccharomyces cerevisiae* because of its ability to produce lactic acid [13].

Inoculation by *Lactiplantibacillus plantarum* was found to acidify faster and reduce pH faster when compared to spontaneous

Table 2
Gas and acid production ability of yeast strains isolated from marula wine on different carbohydrates.

Strain	Sucrose		Fructose		Glucose		Starch	
	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid
Y_1	+	+	+	+	+	+	–	–
Y_2	+	+	+	+	+	–	–	–
Y_3	–	–	+	+	+	+	–	–
Y_4	+	+	+	+	+	+	–	+
Y_5	+	+	+	+	+	+	–	–
Y_{s1}	+	+	+	+	+	+	–	–
Y_{s2}	+	+	+	+	+	+	–	+
Y_{s3}	+	+	+	+	+	+	–	–
Y_{s4}	+	+	+	+	+	+	–	–
Control	–	–	–	–	–	–	–	–

* *-Negative, + positive.*

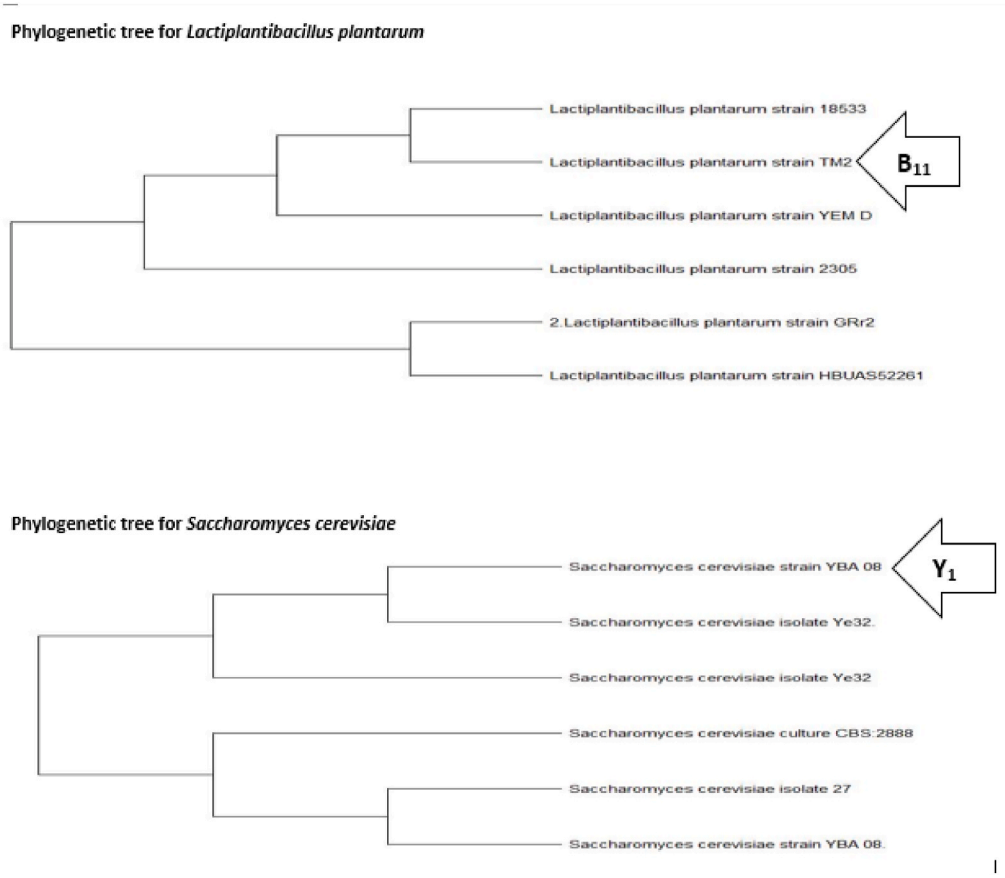

Fig. 1. The phylogenetic trees for *Lactiplantibacillus plantarum* (top) and *Saccharomyces cerevisiae* (bottom).

Table 3
Physicochemical characteristics of the marula wine.

Treatment	°Brix	pH	Total acidity (%)	Total EtOH (%, v/v)
UPO	6.23 ± 2.77^{a}	2.97 ± 0.06^{a}	0.33 ± 0.06^{ab}	3.33 ± 2.49^{b}
PYL	2.00 ± 0.00^{b}	3.00 ± 0.00^{a}	0.33 ± 0.03^{ab}	5.90 ± 0.17^{a}
PY	2.07 ± 0.21^{b}	3.00 ± 0.00^{a}	0.31 ± 0.02^{b}	6.10 ± 0.17^{a}
PL	8.67 ± 0.06^{a}	2.80 ± 0.00^{b}	0.43 ± 0.08^{a}	0.77 ± 0.25^{c}

*UPO – Control.
PYL – Inoculated with *Saccharomyces cerevisiae* + *Lactiplantibacillus plantarum*.
PY – Inoculated with *Saccharomyces cerevisiae* only.
PL – Inoculated with *Lactiplantibacillus plantarum* only.
[x]Results are means and standard deviations from three observations (N = 3).
[abc]Means followed by the same letters in the same column show no significant difference ($p \geq 0.05$).

fermentation [46]. Mango wine inoculated with *Saccharomyces cerevisiae* had an acidy of 0.63 % at 96 h [47]. In the current study, the single culture of *Saccharomyces cerevisiae* had statistically the same amount of total acidity with the mixed culture of *Saccharomyces cerevisiae* and *Lactiplantibacillus plantarum*. Co-culturing, *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae* can possibly manage the acidity of a wine [48]. The mixed culture in the current study had total acidity that was not significantly ($p \geq 0.05$) different under to the single of *Saccharomyces cerevisiae* at the end of the fermentation. Lactic and acetic acids are two of the organic acids produced during alcohol fermentation using *Saccharomyces cerevisiae* and *Lactiplantibacillus plantarum*. Chidi et al. (2018) state that production of organic acids is dependent on the genetic composition of the strain that is employed in the fermentation. This, therefore, would mean that different strains may produce different types and amounts of organic acids under different conditions. The balance in the amount of acetic and lactic acids are essential for the acceptable organoleptic qualities.

The °Brix of other fruit wines were higher than the °Brix of the marula wine in the current study [49] and they show that, the higher the °Brix, the lower the ethanol content of the wine [50]. The negative association between °Brix and alcohol content was observed in the marula wine as well where the wine samples with higher °Brix had lower ethanol content (Table 3). The results also indicated that

the *Saccharomyces cerevisiae* utilise sugar faster than the *Lactiplantibacillus plantarum* and the control, while the *Lactiplantibacillus plantarum* produces acid faster and lowers the pH. In the current study, °Brix was used as a measure of the amount of dissolved sugar. The drop in the °Brix is a measure of the used up sugars as the *Sacharomyces cerevisia* and *Lactiplantibacillus plantarum* ferments the marula juice into wine [13] The sugars are broken down by enzymes secreted by the microorganisms into simple sugars which are fermentable by the fermentation microorganism [51]. Yeast needs sugar to grow and when it grows, it produces ethanol as a by-product. *Saccharomyces cerevisiae* readily ferments simple sugars into ethanol and carbon dioxide [52]. In batch fermentations, the sugars that are present in the media at the start of the fermentation are eventually all used up and that the °Brix is decreased.

The ethanol content in the treatments were significantly ($p \leq 0.05$) different except between the PYL and PY treatments. The PL treatment recorded the lowest ethanol content of 0.77 %. Ethanol is the primary metabolite of interest in wine making. Yeast in wine making is chosen based on its ability to produce and tolerate the alcoholic and acidic medium [53,54]. The *Saccharomyces cerevisiae* strain used in this study has the tolerance for acidity and the ability to produce high amount of ethanol in the presence and absence of *Lactiplantibacillus plantarum*.

4.5. Consumer acceptability test

In terms of colour, the most acceptable marula wine was the PL sample, which had a hedonic intensity of *like moderately* with scores 7.23 (Table 4). The colour in the control was equally liked as the PYL and PY wines. This means that the colour of marula wine was not significantly ($p > 0.05$) affected by the PYL and PY treatments. The PL sample had a significantly high colour score. Colour affects the expectations of other attributes such as taste and flavour of the consumer [54]. Colour is one of the food attributes that creates expectations regarding taste and flavour of food and beverages [55].

The aroma in all the marula wine samples had a hedonic intensity of *slightly like* (5.53) and *like moderately* (6.85). There was no significant difference between the control and the PYL and PY wines ($p > 0.05$). The PYL wine had relatively the lowest score in aroma (5.53. The PL wine had the highest acceptability score. 6.85 and was significantly ($p < 0.05$) different from the control. Aroma and fruity flavour are drivers of consumer's preference in wine [56]. The colour of food or beverage can influence the perception of flavour and taste intensity [55,57,57]. In the current study, the colour and aroma were significantly different from the control, which was spontaneously fermented marula wine. This might mean that the colour did indeed influence the perception of the aroma [55]. Carvalho et al. [54], found that beer of the same flavour but different colours had different flavour ratings as they were influenced by their colours. In the current study, the sample with a more intense colour scored less in the sensory attributes. Consumers in another study associated the colour of rice with specific ingredient aroma attributes [58].

The taste of PL sample scored highest (7.83) making it *liked very much* while the PYL and PY samples were *disliked slightly* with 4.0 and 4.72 scores, respectively. The PYL and PY wines had the least °Brix, which might have contributed to the low acceptability score. The taste score (6.36) for the control was significantly ($p \leq 0.05$) different from all the treatments. The results on taste preference show that consumers preferred the wine that was more sweet than the wine with less sugar in it. °Brix as a measure of total soluble solids including sugars, affects the organoleptic quality of fruits and their products [50]. The high °Brix in the sample fermented by PL might have influenced the preference by the consumers. The marula wine fermented by *Lactoplantibacillus plantarum* only also had the highest acidity. Ezeronye [50],reports that high acidity improves palatability in fermented food and beverages, which might have also influenced the consumer preference for the sweet and sour PL wine.

The compounds that conferred Godello wine's flavour were said to be the ethyl esters and acetates which greatly influenced the aroma by imparting a fruity flavour [59]. Such compounds are important in the sensory properties of a wine, especially how they combine in the wine matrix to formulate an acceptable flavour [60]. Ethyl esters are known to impart a fruity aroma in wines [61]. In another study, some younger panellists preferred wines with more fruity flavour compared to the their older counterparts, [62]. The panellists in the current study were mostly youth, which would possibly explain their preference for the sweeter sample. Co-culturing of yeast and LAB produced profile flavour compounds close to that of spontaneous fermentation in cereal fermentation [25]. The current study also recorded same aroma preference among consumers in the co-cultured samples and the single cultured sample of PY when compared to the control.

The results in Table 4 show that the sample fermented with single culture of *Lactiplantibacillus plantarum* was *liked moderately* (7.30 score) in terms of mouthfeel and had no significant different with the score of the control (6.53). Table 4 also shows that the PL wine

Table 4
Consumer acceptability of marula wine.

Sample	Colour	Aroma	Taste	Mouthfeel	Overall acceptability
Control	6.28 ± 2.2^{b}	6.00 ± 1.7^{b}	6.36 ± 2.1^{b}	6.53 ± 2.3^{a}	6.98 ± 1.7^{a}
PYL	5.68 ± 2.2^{b}	5.53 ± 2.1^{b}	4.00 ± 2.4^{c}	4.28 ± 2.2^{b}	4.43 ± 2.2^{b}
PY	6.31 ± 1.7^{b}	6.04 ± 1.8^{b}	4.72 ± 2.3^{c}	4.94 ± 2.2^{b}	5.31 ± 2.3^{b}
PL	7.23 ± 1.7^{a}	6.85 ± 2.0^{a}	7.83 ± 1.4^{a}	7.30 ± 1.9^{a}	7.60 ± 1.2^{a}

[z]1 = dislike extremely, 9 = like extremely.
Control-uninoculated sample.
PYL-mixed culture of *Saccharomyces cerevisiae* and *Lactiplantibacillus plantarum*.
PY-single culture of *Saccharomyces cerevisiae*.
PL-single culture of *Lactiplantibacillus plantarum*.
[abc]Values with same letter in the same column are not statistically different ($p > 0.05$).

had the least sugar fermented as shown by the high ^{o}Brix, which could have possibly influenced the high acceptability in taste and mouthfeel. PYL and PY showed no significant ($p \geq 0.05$) difference in mouthfeel and were *disliked slightly* with scores of 4.28 and 5.94, respectively.

The PYL and PY samples' taste and mouthfeel were least accepted because of low ^{o}Brix which could imply that the level of sweetness was very low in both samples. Low sugar would mean that the sourness brought by the acidity is dominant and therefore results in a less acceptable taste and mouthfeel. A juice made of cashew nuts that had higher sugar content scored higher in an acceptability test when compared to those which had low sugar content [63]. This means consumers prefer sweeter beverages than less sweet beverages. The high ^{o}Brix in the control and PL samples meant that they had more sugar and were sweeter than the other samples which were inoculated with single culture of *Saccharomyces cerevisiae* and mixed culture of *Saccharomyces cerevisiae* and *Lactiplantibacillus plantarum*. However, the PL sample had a high acidy (0.43 %) and the lowest pH (Table 4) which could have possibly made the sample sourer in taste than the others but because of the sweetness brought by the higher sugar content, it was preferred.

The PL sample and the control had significantly higher overall acceptability scores of 7.60 and 6.98, respectively, than the PYL and PY samples. The overall acceptability corresponds to the level of liking for the sensory attributes. The less preferred attributes of the samples were also less preferred in the overall acceptability. The results show that sensory attributes played a role in the overall acceptability of the marula wine. In a consumer acceptance on fermented rice flour beverage, the sample which was fermented with a single culture of *Lactiplantibacillus plantarum* had high scores in the sensory attributes, like it was the case in the current study [64]. This mean that the *Lactiplantibacillus plantarum* as a single culture can impart desirable attributes in a fermented beverage when used as a starter culture.

5. Conclusions

Co-culturing *Saccharomyces cerevisiae* and *Lactiplantibacillus plantarum* produces more ethanol than the single culture of *Saccharomyces cerevisiae* and the spontaneous fermentation. Single culture of *Lactiplantibacillus plantarum* produced the least ethanol. The *Lactiplantibacillus plantarum* single cultured marula wine had the highest overall liking, followed by the spontaneously fermented wine (control) in the consumer tests. However, *Lactiplantibacillus plantarum* single cultured marula wine had the least ethanol content, which is the key component in wine production. The yeast-containing cultured wine had the highest ethanol content. Co-culturing provides for the commensalism, which benefits the *Lactiplantibacillus plantarum* as the *Saccharomyces cerevisiae* breaks down sucrose into simple sugars; fructose and glucose, which the lactic acid bacteria *Lactiplantibacillus plantarum* can easily ferment.

Ethics statement

We, the authors of this manuscript confirm that we have abided by the Human Ethics policy of the University and that we obtained prior informed consent from participants of the sensory evaluation experiment.

Data availability statement

The dataset supporting the current study have not been deposited in a public repository because the data associated with the study has some intellectual property that needs to be protected. There are other studies that are ongoing on the same product that was studied. However, data will be made available from the corresponding author on request.

CRediT authorship contribution statement

Menzi P. Ngwenya: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Thabile P. Nkambule:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Solomon W. Kidane:** Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors of the paper declare no conflict of interest.

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