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Modernizing mageu fermentation through the use of defined *Lactobacillaceae* starter cultures with potential functional applications

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Abstract

Mageu is a non-alcoholic fermented cereal beverage widely consumed in Southern Africa. It is traditionally produced via spontaneous fermentation using various inocula, but is increasingly manufactured using defined starter cultures to improve product consistency, safety, and shelf life. As part of ongoing efforts to modernize traditional fermented foods while retaining their characteristic qualities, this study evaluated the use of selected *Lactobacillaceae* starter cultures for mageu production. The strains investigated are isolates from healthy South African women identified to have promise as probiotics for women's health, thereby providing an opportunity to produce a mageu with targeted functional application. Five isolates were tested alone and with *Saccharomyces cerevisiae*, a yeast associated with mageu fermentation. Fermentation performance was evaluated using pH, titratable acidity, total solids, lactate and ethanol concentrations, viable cell counts, shelf-life, and consumer acceptability. All strains successfully facilitated mageu fermentation, achieving final pH values below 4.0 while maintaining viable cell counts in the finished product, an essential requirement for probiotic functionality. Mixed starter cultures containing yeast reduced fermentation time, increased acid production, and enhanced microbial growth compared to single-strain fermentations. All products met national standards for ethanol and solids content. Consumer acceptability testing indicated a preference for mixed-culture products over the single-strain fermentations, although the commercial flavored mageu remained the most preferred. These findings demonstrate that non-traditional *Lactobacillaceae* starter cultures can be used to produce mageu with acceptable sensory characteristics, supporting the functional diversification of this culturally significant fermented beverage.

Keywords Mageu, Fermentation, Lactobacilli, Lactic acid bacteria, Yeast, Starter culture, Functional foods, Probiotics

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Introduction

Mageu is a fermented, non-alcoholic cereal beverage commonly produced in Southern African countries [1, 2]. Multiple millions of liters of mageu are consumed in the region each year [1, 3, 4], and the product has been commercialized due to its popularity [5]. Mageu is traditionally produced by fermenting maize meal using inoculum sources of wheat flour, maize bran, or millet malt, depending on regional practices and the country of origin [2, 6, 7]. The dominant microorganisms identified in traditionally produced mageu are lactic acid bacteria (LAB) and yeasts, such as *Saccharomyces cerevisiae* [6]. In commercial production, the South African National Standard for mageu (SANS1199) allows the use of any suitable bacterial starter, provided that the final product has characteristic lactic acid fermentation flavor and odor [8].

Beyond its role as a staple fermented beverage, mageu's cultural acceptability, wide consumption, and affordability have prompted interest in its potential adaptation for functional food applications, particularly as a source of probiotics. For fermented foods to be probiotic, evidence of strain-specific health benefits is required, along with the presence of live, nonpathogenic microorganism(s) in sufficient quantities at the point of consumption [9]. Among potential functional applications, of particular interest in Southern Africa is women's health, including alternative or adjunctive treatments for bacterial vaginosis (BV), a common vaginal dysbiosis characterized by a disruption of the optimal *Lactobacillus* spp.-dominated microbiota to a diverse community of obligately anaerobic bacteria [10]. BV is highly prevalent in Southern Africa and is of particular concern due to its association with increased risk of HIV acquisition and adverse reproductive health outcomes [11–18]. In this context, Happel et al. [19] selectively isolated *Lactobacillaceae* from healthy, HIV-negative South African women with the aim of identifying strains with probiotic potential against BV. Of the 57 isolates identified, ten were prioritized for having desirable probiotic characteristics.

Building on these strain selection efforts, the objective of this study was to evaluate whether *Lactobacillaceae* strains isolated by Happel et al. [19] could be used as starter cultures to ferment mageu, thereby producing mageu with a targeted functional application. This included evaluation of the mageu products using chemical and microbial analyses as well as consumer acceptability testing, all while ensuring that the mageu production adhered to the South African mageu product specifications from the South African Bureau of Standards (SABS) and Codex Alimentarius Commission Guidelines, set out by the Joint FAO/WHO Food Standard Programs [20].

Methods

Culture collection and maintenance

Lactobacillaceae

Five *Lactobacillaceae* isolates characterized by Happel et al. [19] were used:

- *Lactobacillus crispatus* 70.6
- *Lactobacillus crispatus* 73.55
- *Limosilactobacillus mucosae* 90.13
- *Lactobacillus gasseri* 94.98
- *Lactobacillus jensenii* 95.1

These strains were from the Women's Initiative in Sexual Health (WISH) study, approved by the Human Research Ethics Committees of the University of Cape Town (UCT HREC #267/2013), followed by further isolation and characterization research (UCT HREC #319/2016).

The isolates were cultivated anaerobically at 37 °C in de Man–Rogosa–Sharpe (MRS) medium (Merck) supplemented with 0.072 g/L L-cysteine monohydrate (Merck).

Yeast

S. cerevisiae (dried Anchor Yeast) was cultivated aerobically using yeast extract–peptone–dextrose (YPD) media (10 g/L yeast extract, 20 g/L peptone, and 22 g/L glucose monohydrate; Merck) at 30 °C for 24–48 h. Bacteriological agar (15 g/L; Merck) was added where necessary for solid media.

Inoculum preparation

Lactobacillaceae preparation

The *Lactobacillaceae* isolates were incubated anaerobically in MRS broth at 37 °C for 18 h. Optical density (OD) of each culture was tracked at an absorbance of 600 nm (Thermo Scientific GENESYS 10S UV–Vis spectrophotometer). The cells were subsequently harvested by centrifugation (Eppendorf Centrifuge 5418 R at 13,000 rpm for two minutes), washed, and resuspended in PBS to a total volume of 2 mL, for addition to the maize–water mixture at a concentration of approximately 10^7 cfu/mL.

Yeast inoculum preparation

For the mageu samples with *Lactobacillaceae* strains and yeast, 0.895 g (approximately 10^6 cfu/mL) of dried yeast was added to each 500 mL bottle.

Mageu preparation

Maize-meal preparation and heating

A 12% (m/m) mixture was prepared by adding 60 g maize meal to 500 mL of sterile (autoclaved) municipal water. The maize–water mixture was heated in a water bath at 90 °C for 15 min, stirring occasionally [3]. The bottles

were subsequently left to cool between 35 °C and 40 °C before inoculating.

Inoculum addition

Individual *Lactobacillaceae* strains were added to the maize–water mixtures to achieve a starting concentration of approximately 10^7 cfu/mL. Dried yeast was also added for the mixed-culture experiments, at a concentration of 10^6 cfu/mL. No inoculum was added to the maize–water mixture for the negative control. For the positive control, 5% (m/m maize meal) wheat flour, a common natural inoculant in traditionally made mageu, was added as the inoculum [3, 6, 21]. During inoculation, the bottles were mixed thoroughly to ensure good distribution of the inoculum. The bottles were not mixed again to facilitate an anaerobic environment.

Fermentation monitoring

Before inoculating the maize–water mixture, a sample was drawn to measure pH (Lasec benchtop 50 + DHS pH meter). pH was measured again immediately after inoculation. pH was then measured hourly from hours 12 to 24 (based on the critical times identified in a trial run with continuous pH monitoring) and then every 12 h until 144 h or until a pH of 3.5 was reached, whichever occurred first. At the end of fermentation, the samples were stored at 4 °C until analysis.

Mageu analysis

Titratable acidity

After fermentation, 10 mL of mageu was pipetted into an Erlenmeyer flask and weighed, followed by dilution with 5 mL of deionized water. Three drops of phenolphthalein indicator solution were added to the mixture and mixed through swirling. The phenolphthalein indicator was prepared as 1% (w/v) phenolphthalein (Merck) to 75% ethanol absolute (Merck) and 25% deionized water. The mageu sample was titrated against a 0.1 M sodium hydroxide solution (≥ 97%, Merck) until a pink color change was observed and persisted for 30 s [3, 6]. The titratable acidity (TA) was recorded as a weight percentage (grams lactic acid per grams of sample).

Total solids content

The total solids content was measured at the end of each fermentation experiment. A known mass of mageu was placed in an evaporating dish and dried in an 80 °C oven overnight. After drying, the mass of the evaporating dish containing the dried mageu sample was measured to determine the total solids.

Lactate and ethanol concentration

Sample preparation

Lactate and ethanol concentrations in the mageu were quantified using high-performance liquid chromatography (HPLC). The method of Pswarayi and Gänzle [22] was used to prepare the samples. First, solids were removed by centrifugation (Eppendorf centrifuge 5418 R) at 13,000 rpm for 15 min. The supernatant was then mixed in a volumetric ratio of 1:1 with 7% perchloric acid (70%, Merck). This mixture was vortexed before being stored at 4 °C overnight to allow the proteins to precipitate. The mixture was then centrifuged using the same conditions, the proteins removed, and the supernatant stored at − 20 °C. From storage, the samples were mixed in a volumetric ratio of 1:1 with mobile phase for a dilution factor of 2. The diluted samples were vortexed before being filtered through a 0.22 µm syringe filter into the HPLC vials. The samples were stored at 4 °C until analysis.

HPLC operation

Organic acids and sugars were analyzed using the Bio-Rad Aminex HPX-87H ion exclusion column (300 mm × 7.8 mm) with 5 mM sulfuric acid (Kimix) as the mobile phase [22]. The operating conditions for the column were a flow rate of 0.5 mL/min and a temperature of 40 °C. The organic acids and sugars were quantified through refractive index and UV detection, operating at a wavelength of 210 nm. Standards of lactate and ethanol with concentrations varying from 0.1 g/L to 10 g/L were prepared using sodium lactate (Merck) and ethanol absolute (Merck) diluted with mobile phase.

Cell enumeration

Viable cell counts were performed through spread plating on MRS and YPD plates for the *Lactobacillaceae* strains and yeasts, respectively. The MRS plates were placed in a container with an AnaeroPak (Davies Diagnostics, MGC-PACK-10–01) to create an anaerobic environment and incubated at 37 °C. The YPD plates were grown aerobically at 30°C.

Shelf-life testing

At the end of the fermentations (shelf-life day 0), each mageu product was split into two sterile glass bottles, to be stored at 4 °C and 21 °C. Post-fermentation microbial activity in the mageu was determined by measuring the pH (Lasec benchtop 50 + DHS) after seven days.

Pathogens testing

The mageu products analyzed for consumer acceptability were tested for *E. coli* and *Clostridium* presence, as legally required for mageu production [8]. A tenfold dilution

series was performed using sterile PBS (Sigma). The diluted cultures were plated on MacConkey agar containing crystal violet, sodium chloride, and 0.15% (w/v) bile salts (Merck) to test for the presence of *Enterobacteriaceae*. The plates were incubated aerobically at 37 °C for 48 h. To test contamination by anaerobic spore forming organisms, the mageu dilutions were heat-treated at 80 °C for 12 min, before being plated on brain heart infusion (BHI) (Sigma) agar plates. The BHI plates were incubated anaerobically (85 N_2, 10% CO_2, 5% H_2) at 37 °C for 48 h in an anaerobic workstation (Concept 1000, Baker Ruskinn). Where growth on the BHI plates was observed, single colonies were subcultured into fresh BHI agar and incubated aerobically at 37 °C for an additional 24 h to determine oxygen susceptibility.

Sensory analysis

Consumer acceptability tests were conducted with an untrained consumer panel. Ethics approval for this was given by the University of Cape Town's Engineering and Built Environment Ethics Committee and the Department of Student Affairs (Ref no. HRTELL001). The requirement for participation was either current or previous regular consumption of mageu, defined as consuming mageu a minimum of once every two months. The participants were required to give informed consent in the form of a signed document before taking part. The mageu products (and hence starter cultures) were de-identified during the testing. The different starter culture origins were not revealed during testing, with the panelists only informed that the products had been prepared according to South African food safety standards and that all cultures used in the fermentation were classified as GRAS (generally recognized as safe) and thus safe for consumption. The anonymity of the consumer panel was ensured, with only age, gender, and frequency of consumption information collected.

Products tested were two single-strain-fermented samples (*L. crispatus* 70.6 and *L. mucosae* 90.13), two mixed-culture-fermented samples (*L. crispatus* 70.6 + yeast and *L. mucosae* 90.13 + yeast), a naturally fermented sample (wheat flour inoculum), and one commercially available product (Number 1 Mageu cream flavor, RCL Foods). The commercial mageu was presented last as it contained additives (sugar and flavoring) which would have skewed the other sample tasting.

Participants completed a questionnaire using a 9-point Hedonic scale to analyze the degree of likeness and likelihood of purchasing the mageu. For the degree of likeness, participants scored each sample on a scale of "like extremely" (9) to "dislike extremely" (1). For the degree of likelihood to purchase, participants scored each sample from "definitely would buy" (5) to "definitely would not buy" (1).

Statistical analysis

At least two independent duplicate runs (minimum four samples produced) were performed and analyzed for each formulation, with results presented as means ± standard deviation. For parametric tests, two-tailed *t*-tests were used to determine statistical significance with a significance level of $p < 0.05$. For nonparametric comparisons, Mann–Whitney tests were used to analyze data at a significance level of $p < 0.05$. Where multiple comparisons were performed, a Bonferroni correction factor was used to avoid Type 1 error. All plots containing error bars represent a single standard deviation for the repeat experiments.

Results and discussion

Fermentation pH profiles and duration

Lactobacillaceae fermentations

The *Lactobacillaceae* isolates as single fermentation cultures were compared by investigating pH over fermentation time, as presented in Fig. 1. All samples showed a rapid pH decrease from 6.10–6.20 to less than 5 over the first 12 h, except for the negative control. This confirms that the *Lactobacillaceae* isolates facilitated mageu fermentation similar to the positive control (traditional starter). After this initial drop, the pH reduction rate declined, indicating that microbial growth and associated metabolite production were no longer occurring at a maximum rate. This is consistent with LAB inhibition at lower pH, with the final pH of 3.5 for mageu being notably lower than the optimal pH between 4 and 4.5 for the isolates [19]. Of interest is that the *L. crispatus* 73.55 sample had consistently lower pH values over 24–72 h. However, from hour 36 until the end of fermentation, the positive control had the steepest decrease in pH. By hour 72, all the fermentations were approximately complete. This included the negative control, despite its longer lag phase. This indicates that cooking of the maize meal porridge did not remove all viable naturally occurring microorganisms. The negative control also had the largest variance—expected for an uncontrolled, spontaneous fermentation with natural variation in the starting concentrations of fermenting microorganisms. Only two single-strain fermentations reached a final pH of 3.5 by the end of the 144 h fermentation (*L. crispatus* 73.55 and *L. jensenii* 95.1).

Viable cell counts for each fermentation were determined at inoculation and at the end (Fig. 2). The counts at the end of fermentation were all higher than the initial

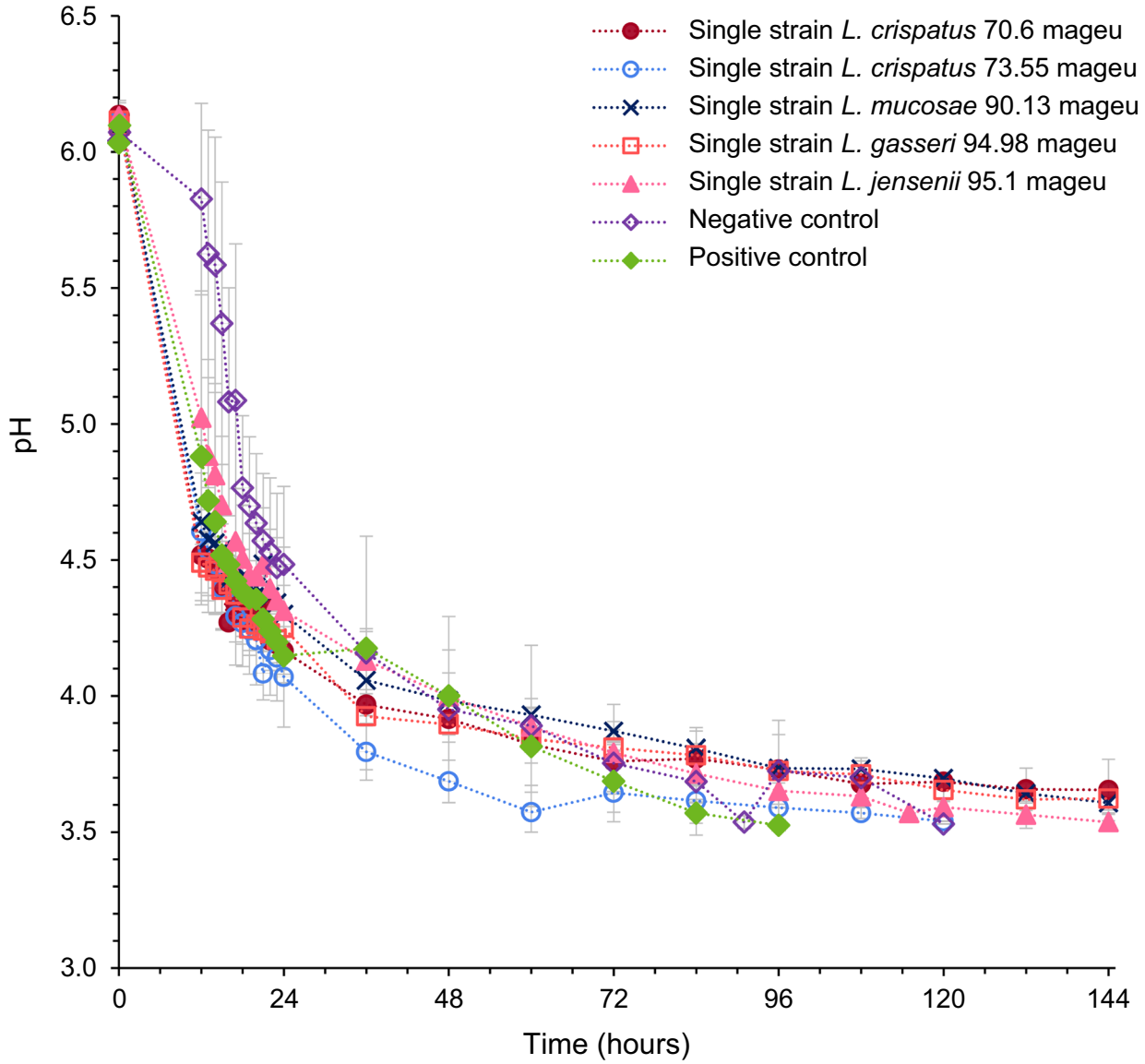

Fig. 1 Plot of pH over mageu fermentation time for the five *Lactobacillaceae* single strains and the controls

inoculum, confirming that cells were still viable despite the relatively low pH of the product.

Lactobacillaceae with yeast fermentations

The pH fermentation profiles of the mageu produced with a single *Lactobacillaceae* isolate with yeast are presented in Fig. 3. An instantaneous drop in pH was recorded when the inoculum was added, from 6 to around 5.5, likely due to components present in the commercial lyophilized yeast mixture. This drop was not observed in the single-strain experiments nor the controls. The mageu produced with the *Lactobacillaceae* and yeast combination reached their pH end point earlier than the *Lactobacillaceae*-only equivalents, due to the pH reduction rate remaining high throughout fermentation. Between hours 12 and 24, all plots including the negative and positive controls had similar gradients. After hour 24, the *L. crispatus* 73.55 and *L. gasseri* 94.98 samples both had a steep pH drop, reaching the lowest pH of all the mixed-culture mageu. The addition of yeast allowed a pH of 3.5 to be reached within 72 h in all cases.

The viable cell counts were found to have increased following fermentation (Fig. 4). These increases were more pronounced than for the single-strain fermentations, with final cell counts ranging from 7.14 to 7.97 $\log_{10}$ cfu/mL.

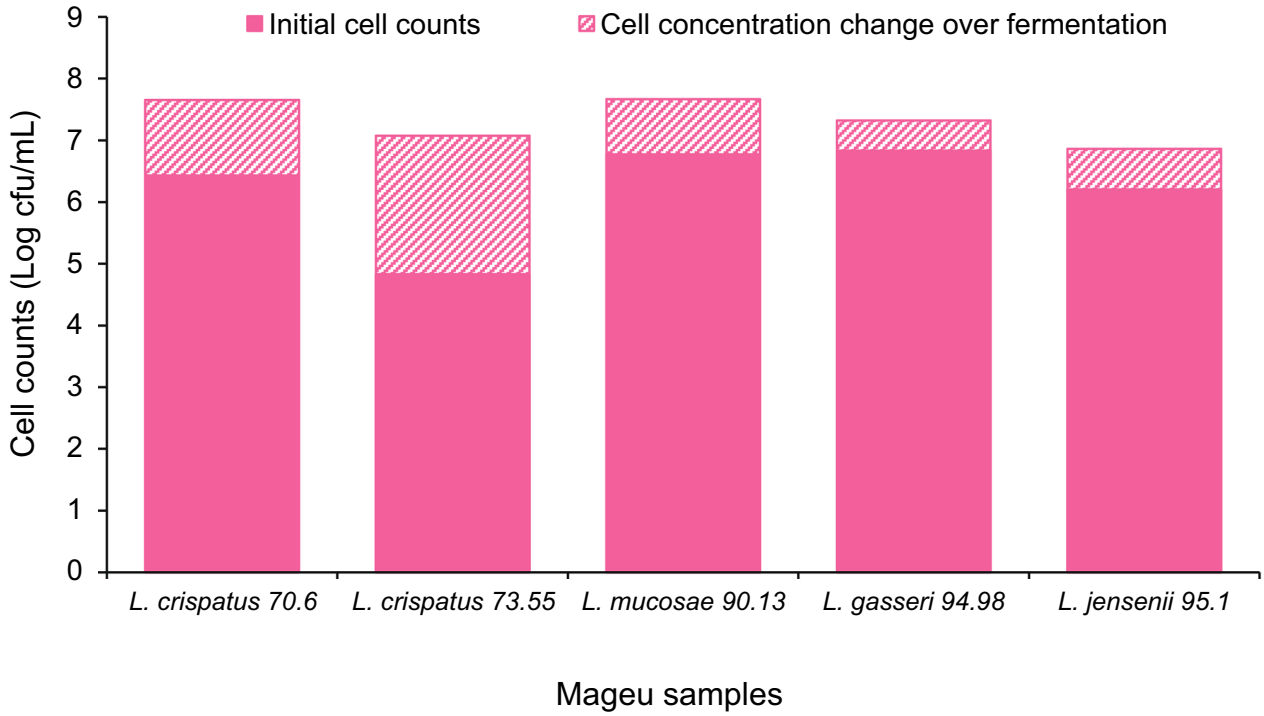

Fig. 2 Change in MRS plate cell counts over the mageu fermentation for the five *Lactobacillaceae* single strains

Fermentations time comparison

The total fermentation times of the *Lactobacillaceae* fermentations, both as single strains and with yeast, are summarized in Fig. 5. The time to reach the final pH (3.5) or hour 144, whichever occurred first, dropped with the addition of yeast compared to the single-strain samples. The decrease in fermentation time was significant for all samples with yeast addition, bar *L. crispatus* 73.55 (*t*-test, $p < 0.05$).

Titratable acidity

The final mageu products were analyzed for acid content, measured as percentage lactic acid (Table 1). All single-strain mageu products had similar titratable acidities (TAs), and inclusion of yeast consistently resulted in higher TAs, though to different degrees for the different strains. *T*-tests confirmed that the increase in TA with the addition of yeast was significant ($p < 0.05$) for all strains, except for *L. crispatus* 70.6. Yeast addition had the most pronounced impact on TA for the *L. mucosae* 90.13 mageu, which increased by 138%. Of the five LAB, all are homofermentative except for *L. mucosae* 90.13, which is heterofermentative [23]. While homofermentative bacteria primarily produce lactate, heterofermentative bacteria produce lactate and other compounds such as ethanol and carbon dioxide [24]. Heterofermenters produce less lactic acid for the same mass of glucose consumed, and this is likely why the single *L. mucosae* 90.13 mageu had the lowest TA of the bacterial strains. Since TA is a measure of lactic acid equivalence, further investigations using HPLC were conducted to determine more accurate lactic acid concentrations.

Lactate and ethanol concentrations

Lactate concentration

Lactate, the primary metabolite produced by LAB, was quantified at the end of the fermentations (Fig. 6). Post-fermentation concentrations were significantly higher than at the beginning of fermentation (pre-inoculation) (0.045 ± 0.028 g/L) for all samples. Interestingly, the positive control had the lowest overall lactate concentration (0.122 ± 0.087 g/L), which was not significantly higher than the lactate concentration present at baseline, suggesting that the drop in pH in this fermentation was due to the production of alternative acids like acetic acid, as observed by Pswarayi and Gänzle [22] in their mageu fermentations. While having the lowest TA, the single-strain *L. mucosae* 90.13 mageu lactate results indicate that this was not solely due to lactate production, as the *L. crispatus* 70.6 sample had the lowest lactate concentration. Instead, the lower TA could be due to lower production of other acids. The lactate levels in the mageu were generally higher with the added yeast than for the single strains, corresponding with the TA results.

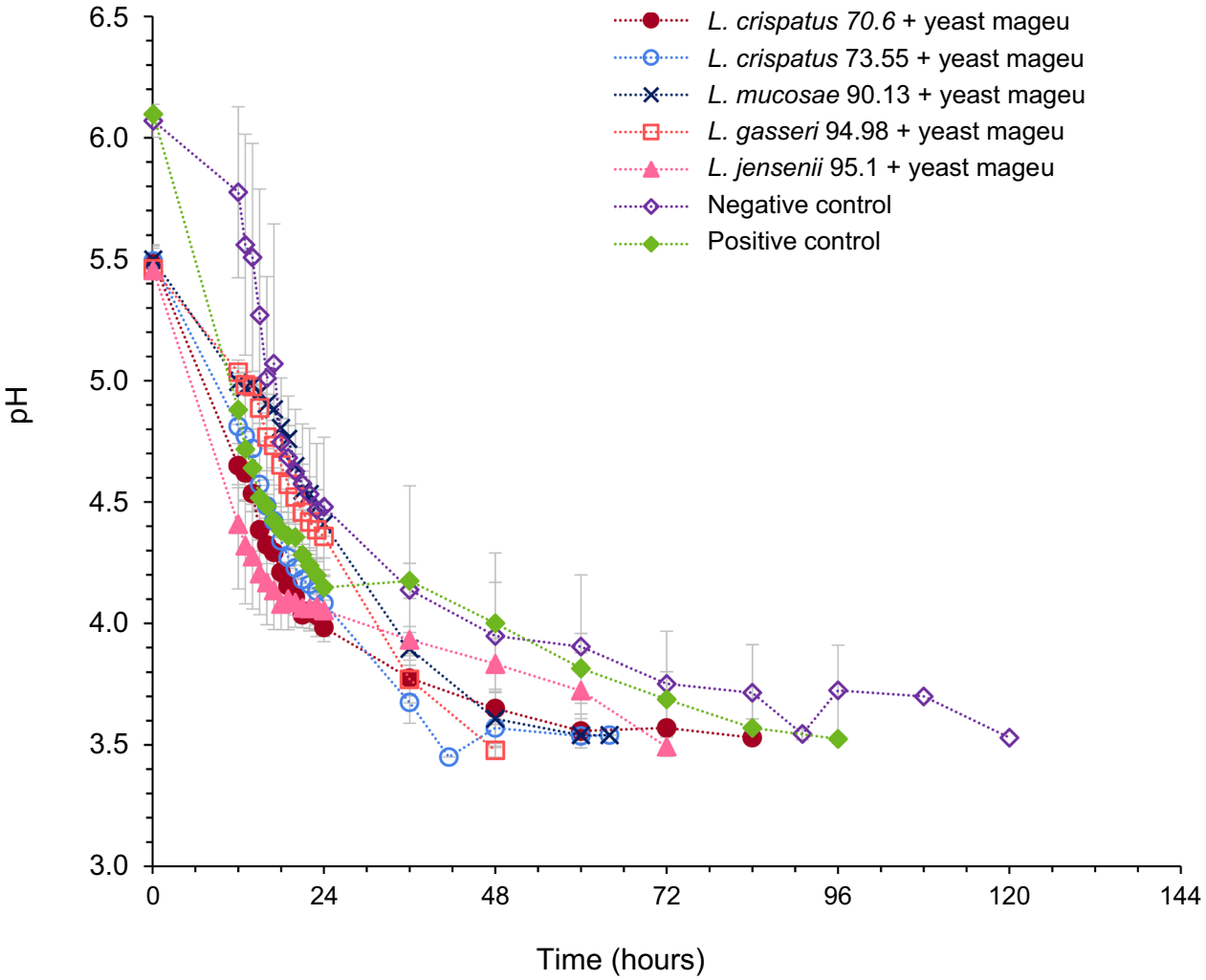

Fig. 3 Plot of pH over mageu fermentation time for the five *Lactobacillaceae* single strains with yeast and the controls

Ethanol concentration

The mageu products' ethanol concentrations were checked to ensure that they did not exceed the mageu production regulations of 2.5 g/L (0.25%(m/m)) [8]. The results are displayed in Fig. 7.

The ethanol concentrations before inoculation of the maize–water mixture were negligible, measured as zero and 0.00419 g/L in the two samples tested (run in duplicate). Therefore, the ethanol concentrations reported for all mageu samples were because of the fermentation action of the microorganisms. In all final products, the average ethanol concentration was below the limit. The mageu produced using the single strains had lower ethanol concentrations than both the negative and positive controls. Despite *L. mucosae* 90.13 being a heterofermentative isolate, its mageu did not have higher ethanol concentrations than the other (homofermentative) single-strain products. This suggests that its metabolism was primarily directed toward the production of lactic and acetic acid rather than ethanol [25]. All mageu produced with yeast had higher ethanol concentrations than the single-strain counterparts—expected as *S. cerevisiae* produces ethanol and carbon dioxide from glucose. This was most pronounced for *L. mucosae* 90.13, possibly due to a different extent of synergistic metabolism between it and the yeast.

Total solids content

The South African National Standard (SANS1199) for mageu production requires a minimum total solids content of 8% (m/m). To ensure that this requirement was met in the final fermented product, a higher concentration (12%) of maize meal to water was used in the mageu preparation. The concentrations post-fermentation are

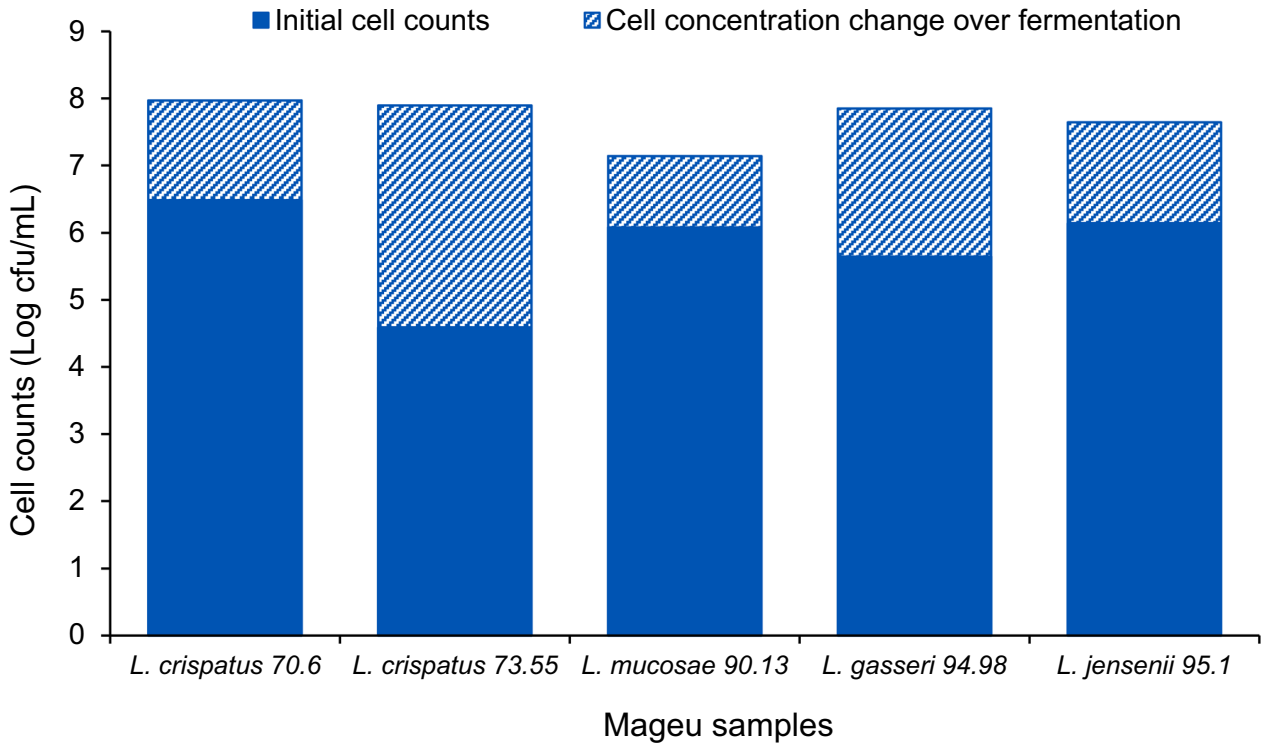

Fig. 4 Change in MRS plate cell counts over the mageu fermentation for the five *Lactobacillaceae* single strains with yeast

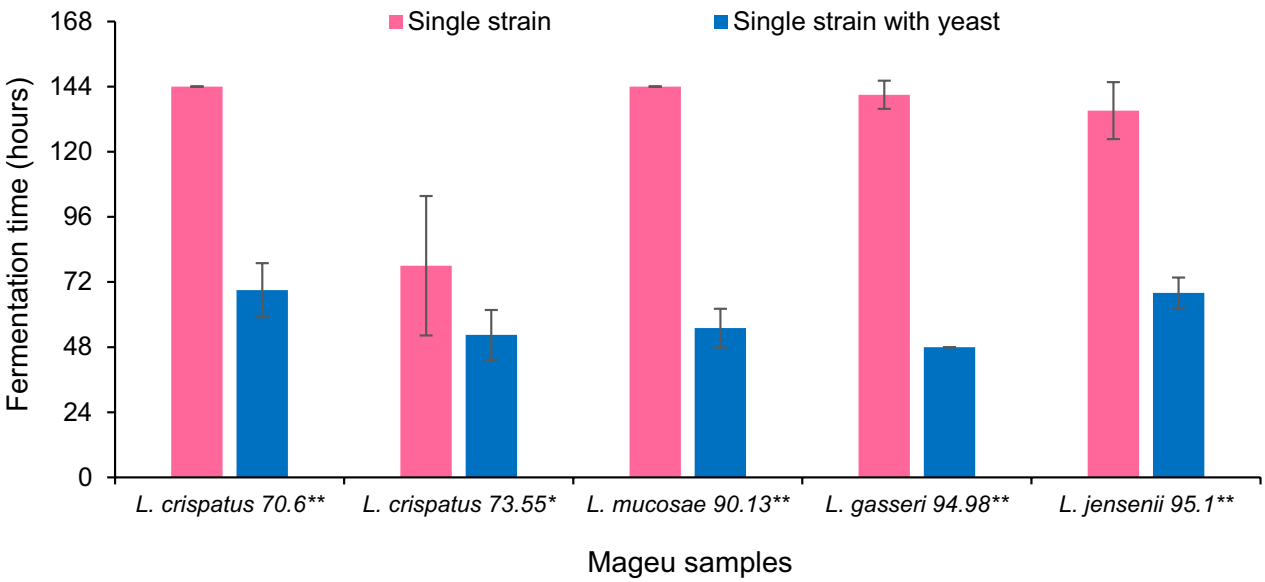

Fig. 5 Average fermentation times for the mageu samples produced with *Lactobacillaceae* isolates as single strains and with yeast (*indicates a significance level of $p < 0.1$ and **indicates a level of $p < 0.001$)

presented in Fig. 8. Though the solids contents decreased during the mageu fermentation, all the products had final solids contents above the minimum requirement, with only small variations observed across the samples.

Shelf-life analysis

Shelf-life was analyzed by measuring the pH of mageu kept refrigerated and at room temperature after seven days. The results of the single-strain samples are

Table 1 Average titratable acidity for fermentations using single *Lactobacillaceae* isolates and mixed cultures with yeast

	***L. crispatus* 70.6 (% m/m)**	***L. crispatus* 73.55 (% m/m)**	***L. mucosae* 90.13 (% m/m)**	***L. gasseri* 94.98 (% m/m)**	***L. jensenii* 95.1 (% m/m)**
Single strain	0.200 ± 0.039	0.159 ± 0.020	0.131 ± 0.010	0.140 ± 0.007	0.140 ± 0.009
Single strain with yeast	0.263 ± 0.009[a]	0.255 ± 0.019[a,b]	0.311 ± 0.018[a,c]	0.290 ± 0.021[a,b,c]	0.234 ± 0.008[b]

Means ± Standard Deviation. Average values with matching letters are not significantly different from one another ($p > 0.005$), with the Bonferroni correction

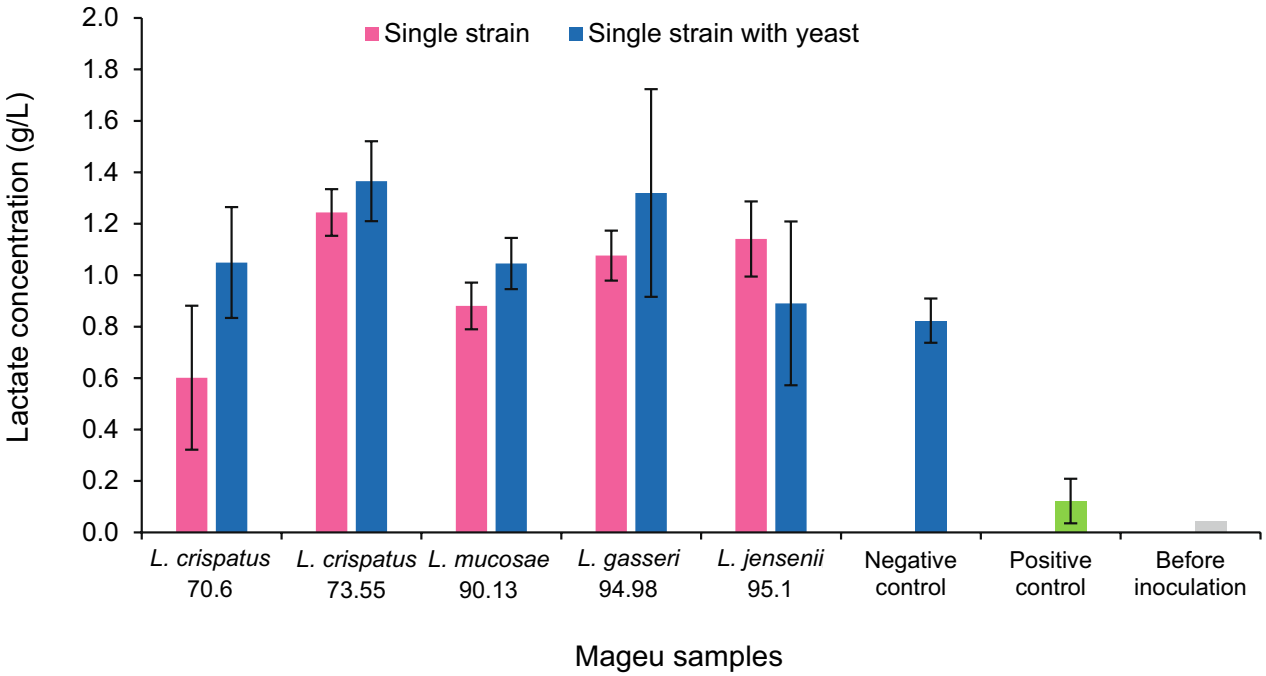

Fig. 6 HPLC determined lactate concentrations for all the mageu samples

presented in Fig. 9. The pH of all the single-strain mageu and the negative control products dropped when stored at room temperature, except for the *L. jensenii* 95.1 mageu, which saw no change. The *L. jensenii* 95.1 may have reached its pH tolerance level and therefore did not continue growing and acidifying the mageu. Comparatively, the refrigerated samples saw only slight decreases in pH.

The shelf-life results of the mixed-culture mageu samples are presented in Fig. 10. The pH drop for the mixed-culture samples was more pronounced, both at room temperature and refrigerated, than for the single-strain samples. All room-temperature samples saw a pH drop of at least 0.25, whereas the pH of mixed-culture refrigerated samples had smaller reductions. The microorganisms in the mixed-culture samples therefore continued to ferment the maize meal after reaching the pH end point. This was either due to the increased inoculum size (bacteria and yeast) or favorable co-fermentation, which increased microbial activity.

While there is no requirement for the final pH of mageu, studies suggest an ideal pH between 3.5 and 3.9 [3, 6, 26, 27]. The refrigerated samples (both single-strain and mixed-culture samples) were closer to the ideal final pH than the samples kept at room temperature after seven days. However, all samples had acceptable pH values after seven days.

Sensory evaluation of mageu samples

The mageu samples were analyzed for their consumer acceptability through a sensory analysis. An untrained consumer panel ($n = 21$) rated the samples on "liking" and "likelihood to purchase" scales [28].

Samples for the sensory evaluation were selected to allow a comparison between the flavor profiles and acceptability of mageu fermented with homofermentative and heterofermentative strains and with and without yeast. Since *L. crispatus* is the species most associated with optimal female health outcomes, *L. crispatus* 70.6 was selected as the homofermentative strain. This isolate

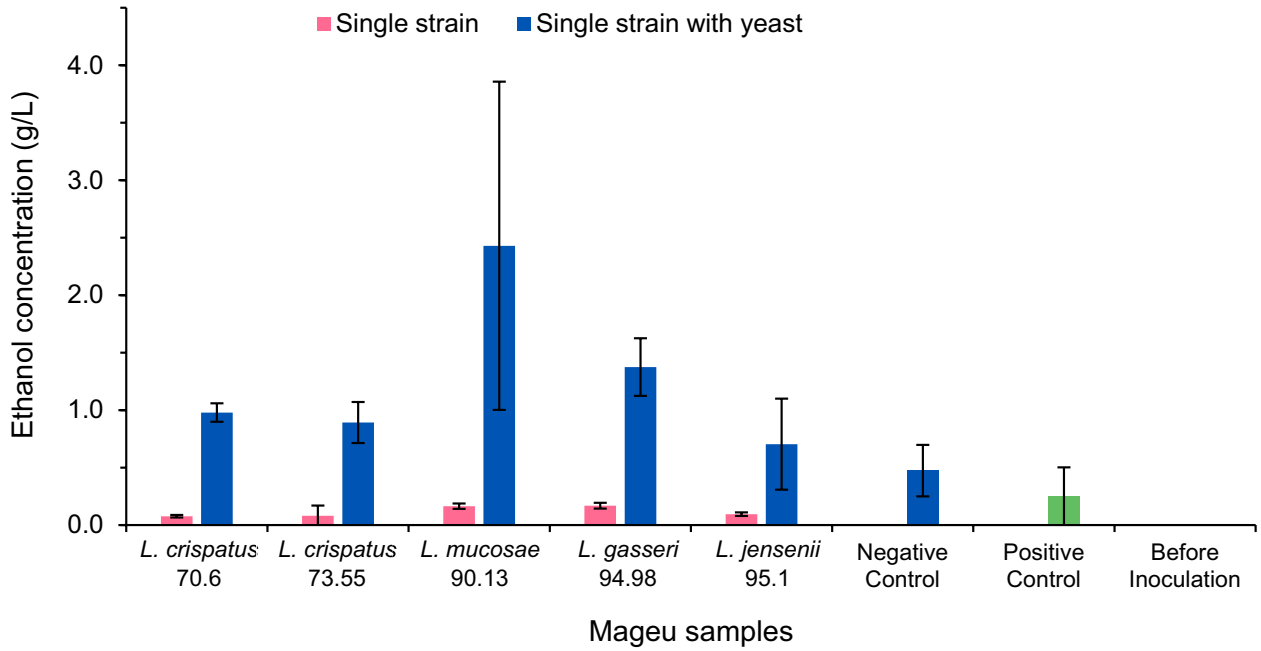

Fig. 7 HPLC determined ethanol concentration for all the mageu samples

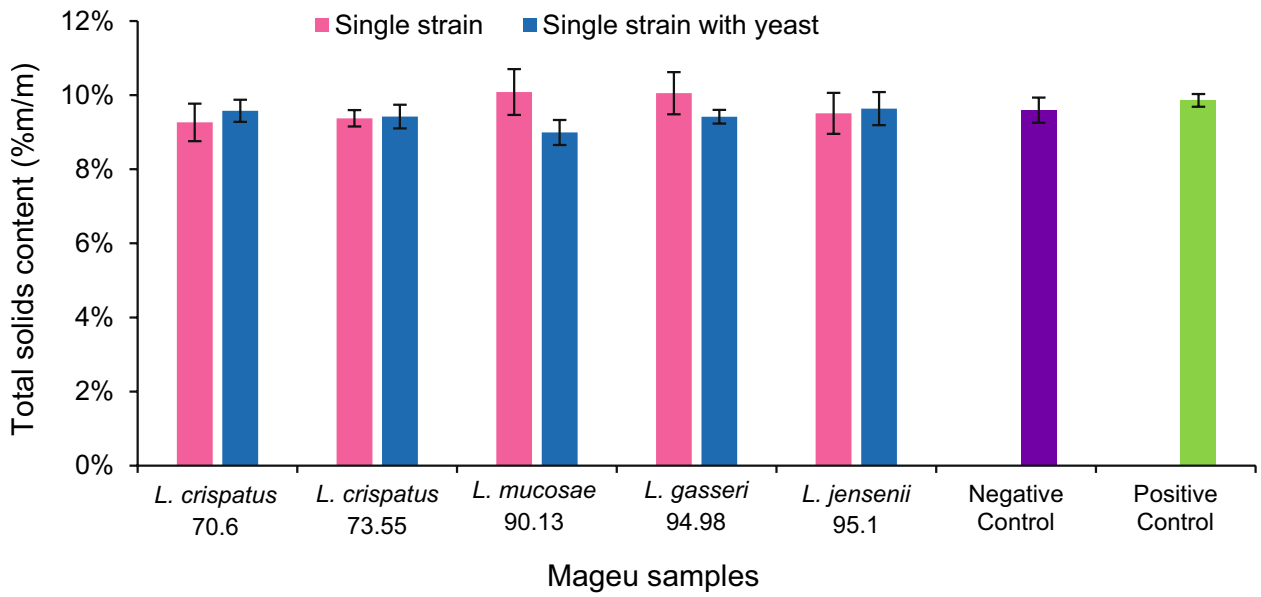

Fig. 8 Total solids content of the final mageu products

had more consistent growth and acid production than the other *L. crispatus* isolate (73.55). *L. mucosae* 90.13 was selected as a heterofermentative representative. The mageu samples produced using these strains with yeast were also included. The remaining two products tested were the positive control (prepared using a traditional wheat flour starter) and a commercially available mageu. All mageu were confirmed to be free of *E. coli* and *Clostridium* prior to tasting.

The degree-of-liking results are displayed in Fig. 11. Higher scores indicate a greater degree of liking. For all samples except the commercial mageu, there was a large spread across the degree of liking, suggesting that individual preferences played a role in how

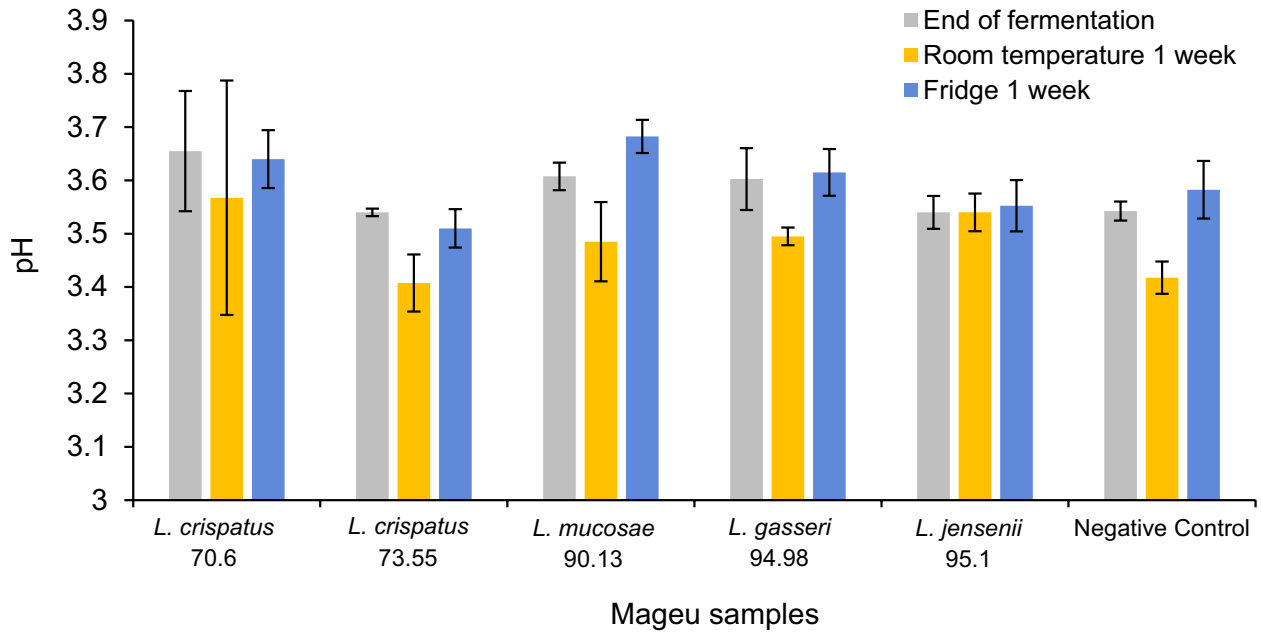

Fig. 9 The pH values of the mageu samples produced using single *Lactobacillaceae* isolates, before storage and after being kept at room temperature or refrigerated for one week

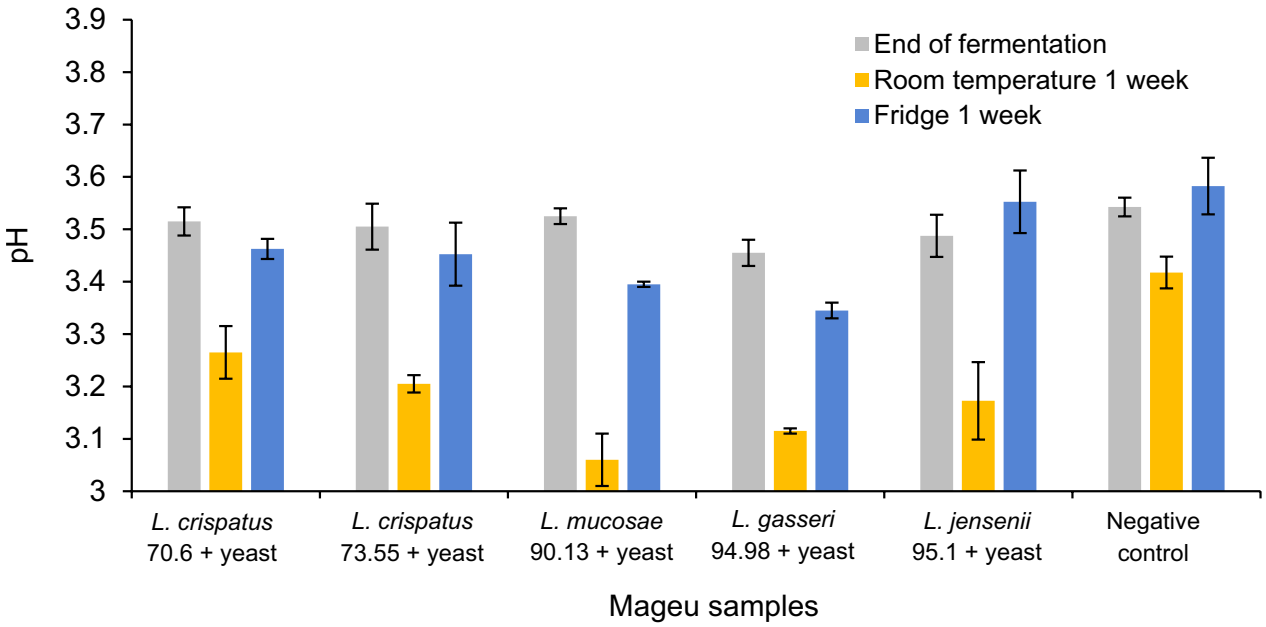

Fig. 10 The pH values of the mageu samples produced using single *Lactobacillaceae* isolates with yeast, before storage and after being kept at room temperature or refrigerated for one week

the panelists scored the samples. The positive control received the lowest score. On average (indicated by the horizontal lines), the two single-strain samples with yeast scored slightly higher than the single-strain ones. The higher levels of lactate and ethanol in the samples with yeast, supported by their lower final pH, may have contributed to more favorable flavor compounds, which could explain their slightly higher liking scores compared with the single-strain samples. Additionally, the samples with yeast had a thinner consistency than

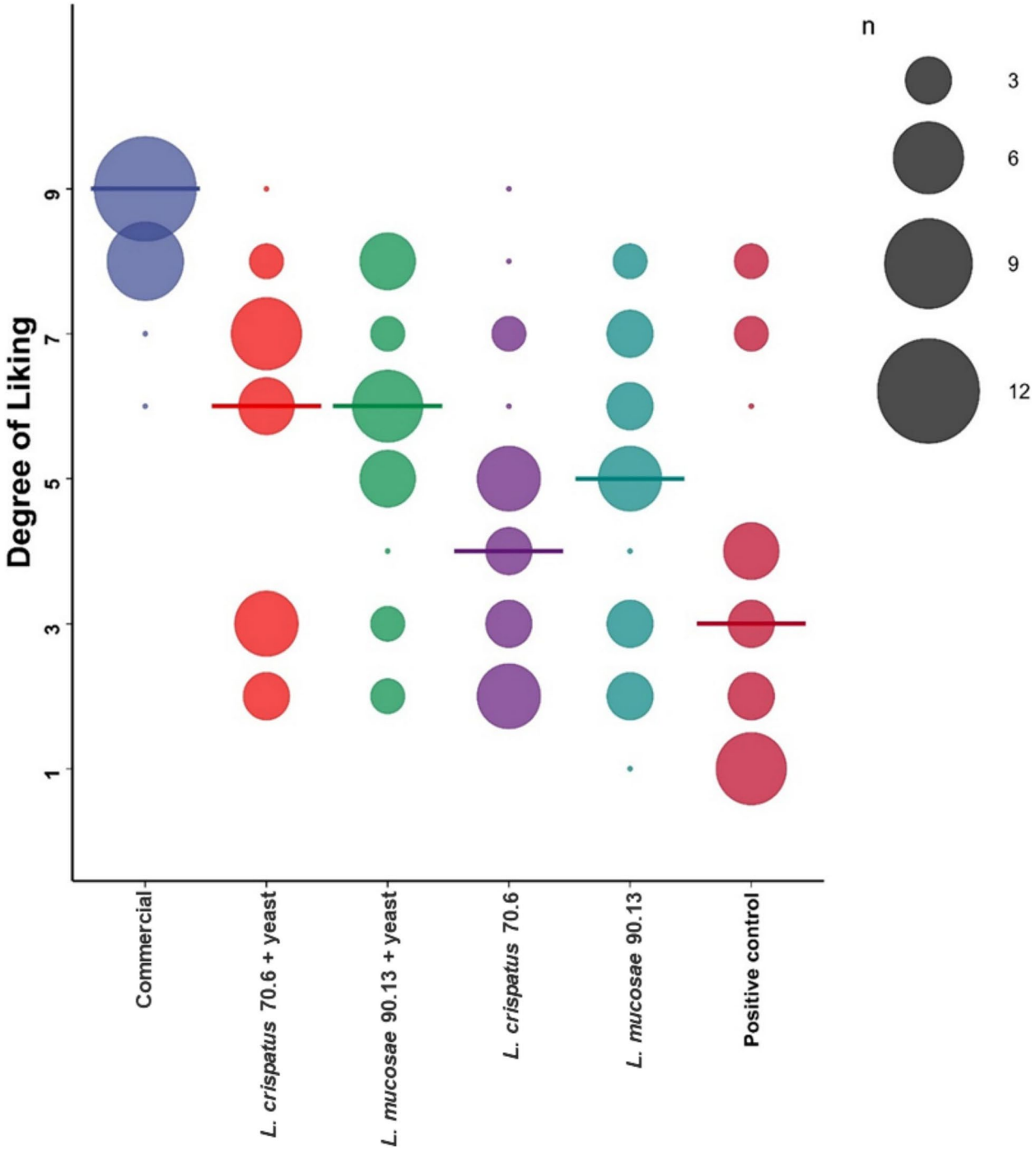

Fig. 11 Bubble plot of degree of liking for the mageu tasting samples [1 = dislike extremely, 5 = Neither like nor dislike, 9 = like extremely]. The horizontal lines represent the medians

the single-strain samples, closer to that of commercially available products, with which participants may have been familiar.

Further emphasizing the influence of individual preference, none of the LAB samples received significantly different scores from one another ($p < 0.0083$, with Bonferroni correction factor). However, the two *L. crispatus* 70.6 mageus were given a score of 9 ("like extremely") by one or more panelists. Overall, the commercial sample had the highest scores. This was

anticipated since it was the only mageu with sugar and flavoring additives. No sugar was added to the laboratory-produced mageu, not to influence the flavor nuances produced by the *Lactobacillaceae* isolates and *S. cerevisiae*.

The likelihood-to-purchase results are given in Fig. 12. Higher scores indicate a preference to purchase, and an average score of 3 shows neither preference nor dislike. Again, the spread of the likelihood-to-purchase results was high. Reflecting the taste results, the commercial sample received the highest scores. The single strain with

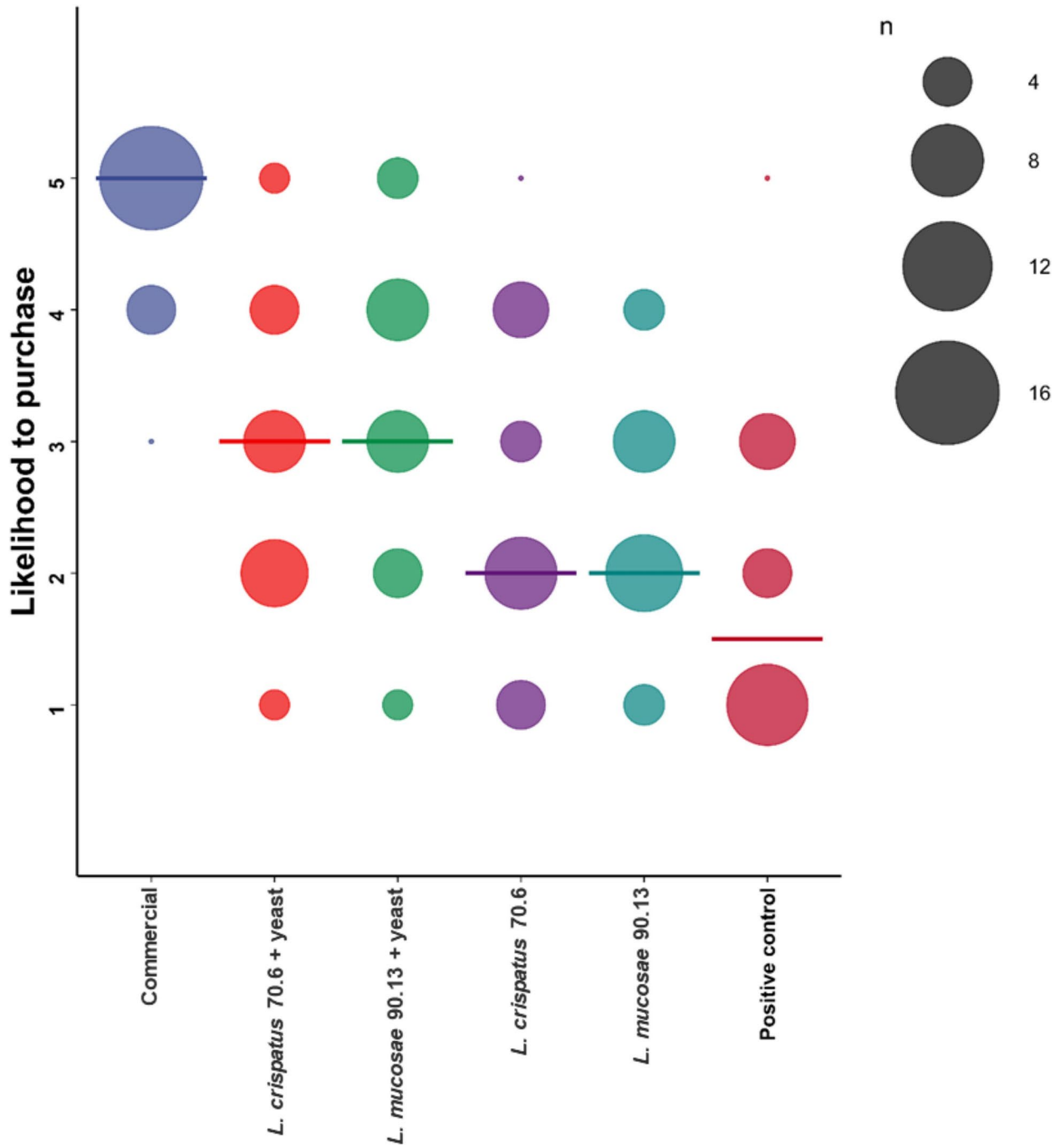

Fig. 12 Bubble plot of likelihood of purchase for the mageu tasting samples [1 = definitely would not buy, 3 = maybe/maybe not, 5 = definitely would buy]. The horizontal lines represent the medians

yeast samples scored slightly higher average scores than their single-strain counterparts, but no significant difference in likelihood-to-purchase scores was observed across the *Lactobacillaceae* samples ($p < 0.0083$, with the Bonferroni correction factor).

Study limitations

The presented research was designed as a proof-of-concept study, focused on fermentation performance and product characteristics. Thus, consideration should be given to certain limitations during interpretation of the findings.

Only a subset of previously identified isolates was assessed, which may limit the generalizability of the results to other strains with potentially different fermentation characteristics or functional properties. The negative control experiments also confirmed that strains native to the mageu ingredients persisted and thus may have affected the fermentations. In addition, evaluation of the in vivo probiotic efficacy and functional health benefits of the mageu products was beyond the scope of this project and remains to be validated.

With respect to the sensory tests, a relatively small panel drawn from a single population group was used. This was constrained by COVID-19 restrictions at the time of the study. The wide variation in scores suggests that individual preferences strongly influenced outcomes. Furthermore, the laboratory-produced mageu samples were not sweetened or flavored, unlike the commercial comparator, which may have influenced consumer preference outcomes.

Conclusions

All *Lactobacillaceae* isolates investigated in this study were able to ferment maize meal into mageu and achieved fermentation performance comparable to a traditionally inoculated control. The addition of yeast to the starter cultures reduced fermentation time, enhanced acid production, and supported increased microbial growth relative to single-strain fermentations, reflecting the mixed microbial ecology typically associated with traditional mageu production. Viable bacterial cell counts were maintained despite the low pH of the final product, critical for functional food applications. All mageu products complied with South African regulatory requirements for ethanol concentration and total solids content.

Mixed-culture fermentations showed greater post-fermentation acidification during storage than the single-strain fermentations, particularly at room temperature, highlighting the importance of starter composition and storage conditions in managing shelf-life stability. Consumer acceptability testing indicated a general preference for mixed-culture mageu over single-strain laboratory products, although commercially flavored mageu remained the most preferred.

Overall, these findings demonstrate that non-traditional *Lactobacillaceae* starter cultures can be incorporated into mageu fermentation to support controlled and consistent production while retaining acceptable sensory characteristics. This approach contributes to the modernization of mageu and highlights its potential as a culturally relevant fermented beverage that can support functional applications while adhering to established production standards.

Abbreviations

BHI	Brain heart infusion
BV	Bacterial vaginosis
GRAS	Generally recognized as safe
HPLC	High-performance liquid chromatography
LAB	Lactic acid bacteria
MRS	De Man–Rogosa–Sharpe medium
OD	Optical density
PBS	Phosphate-buffered saline
SABS	South African Bureau of Standards
SANS	South African National Standards
TA	Titratable acidity
YPD	Yeast extract–peptone–dextrose medium

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Author contributions

KHA: Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing—original draft; Writing—review & editing BK & MAFE: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing—review and editing JAP & AUH: Conceptualization; Resources HG: Resources.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The *Lactobacillaceae* isolates were derived from the Women's Initiative in Sexual Health (WISH) study, approved by the Human Research Ethics Committees of the University of Cape Town (UCT HREC #267/2013), followed by further isolation and characterization research (UCT HREC #319/2016). These studies and the relevant ethical considerations and associated approvals are fully described in Happel et al. [19], Happel [23], and Barnabas et al. [29]. Critically, the women (16–22 years old) provided written informed consent (if ≥ 18) or written assent with consent obtained from their parent(s) or legal guardian(s) (if < 18), permitting collection and study of any isolated strains and that selected bacteria could be chosen for the development of a probiotic. Ethics approval was approved for the sensory testing of the mageu products by the University of Cape Town's Engineering and Built Environment Ethics

Committee and the Department of Student Affairs (Ref no. HRTELL001). The panelists were students from the University of Cape Town, recruited via email. The participants were required to give informed consent in the form of a signed document before taking part in the panel and consuming the mageu products. The mageu products (and hence starter cultures) were de-identified during testing. Information as to the different starter culture origins were not revealed during testing, with the panelists only informed that the products had been prepared according to South African food safety standards and that all cultures used in the fermentation were classified as generally recognized as safe (GRAS), and thus safe for consumption. The anonymity of the consumer panel was ensured, with only age, gender, and frequency of consumption information collected.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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