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The impact of lactic acid bacteria and yeasts ratio on fermentation and taste of kvass

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Abstract. Kvass, a non-alcoholic beverage derived from rye malt or special rye bread through natural fermentation, traditionally involves yeast in the production process. However, the introduction of various lactic acid bacteria (LAB) accelerates fermentation and imparts a distinctive taste and aroma to the kvass. This research aimed to optimize the ratios of LAB to baker’s yeast in kvass to enhance its fermentation, sensory qualities, and physicochemical properties, thereby improving its acidic flavour and overall acceptability. Baker's yeast and three commercial LAB strains were used for fermenting the kvass wort made of dried rye bread. The experimental design focused on four distinct inoculation ratios: 100% LAB, 50% LAB:50% yeast, 80% LAB:20% yeast, and 100% yeast. Key parameters such as pH, dry matter content, and titratable acidity were monitored over 12, 14, and 16 hours of fermentation, with a detailed sensory analysis conducted on the 80:20 LAB to yeast ratio kvass samples that were fermented for 14 hours and then cooled. It was found that varying the ratios of LAB and yeast significantly affected the fermentation process. Extended fermentation times, particularly with higher LAB ratios, led to more pronounced acidity and sensory characteristics. Optimal microbial balances, notably the 80% LAB to 20% yeast ratio, enhanced kvass's flavour profile and physicochemical properties, suggesting a tailored approach to fermentation can improve kvass's quality and consumer acceptance. These variations, alongside significant strain- and species-related differences, highlight the importance of microbial balance in enhancing kvass's acidic flavour and overall acceptability.

Key words: sour kvass, lactic acid bacteria, non-alcoholic beverage, *Saccharomyces cerevisiae*.

INTRODUCTION

The popularity of traditionally fermented alcoholic and non-alcoholic beverages has varied across different historical periods. Kvass is a non-alcoholic drink traditionally produced in eastern European countries through the natural fermentation of rye malt or dried rye bread, typically involving yeast (Lidums et al., 2015). Since the 19th century, traditional sour kvass was a common beverage in Estonian farmsteads, produced by fermenting a mixture of bread and water, where often were involved various lactic acid bacteria, contributing to the unique taste and aroma variations found in different regions of the country (Taari, 1940; Moora, 2007). However, with the emergence of Soviet Union influence, standardized kvass production using commercial yeasts became widespread throughout Estonia, leading to a decline in the popularity of homemade varieties (Jaagosild, 1967). In the 1990s, as borders opened to free trade, kvass faced tough competition from a numerous of new carbonated soft drinks, resulting in a sharp drop in consumption (Jargin, 2009). Currently, kvass is experiencing a renewed popularity, although the product offerings in the Baltic countries' markets still rely heavily on the standard recipes established during the Soviet era. On an industrial scale, kvass is mainly manufactured through the dilution of malt concentrate and is frequently marketed in a pasteurized form and contains a considerable amount of additives (Lidums et al., 2015; Gambuś et al., 2015).

Naturally fermented kvass is produced through a traditional method that involves preparation of rye mash or soaking dried rye bread in hot water for several hours (Basinskiene at al., 2016). The liquid part is then separated from solids, and fermentation is initiated by adding sucrose and baker's yeast *Saccharomyces cerevisiae* (Lidums & Kaklina, 2014). Studies have demonstrated that kvass can spontaneously contain a diverse array of lactic acid bacteria (LAB), originating possibly from the rye bread or additives like juniper berries or raisins, including *Lactobacillus delbrueckii*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*, *Lacticaseibacillus paracasei*, etc. (Albuquerque et al., 2013; Bati & Boyko, 2016; Wang et al., 2022). The lactic-ethanol fermentation process with bread yeast, which is halted prior to the ethanol concentration reaching 1.2% alcohol by volume (Lidums et al., 2015), contributes to the distinctive aroma profile of kvass. This results in a foaming beverage with a complex bitter-sweet flavour and a characteristic brown-yellow colour. Additionally, fermentation process enriches kvass with minerals, vitamins, and antioxidants (Gambuś et al., 2015).

The quality of fermented kvass is greatly influenced by a range of factors, including the type of raw materials utilized, the starter microbes selected, and the specific conditions of the fermentation process. Recently, there has been growing interest in the incorporation of various raw materials (e.g. black chokeberry fruit, peppermint leaves and carob kibbles) and strains of LAB in the production of probiotic kvass, aiming to enhance its market potential (Polanowska et al., 2021; Wang et al., 2022; Kaszuba et al., 2024).

The aim of this study was to determine the optimal microbial ratios of LAB and baker's yeast that improve the fermentation process, sensory appeal, and physicochemical properties of kvass. The focus was on refining its quality and acceptability by enriching it with an acidic flavour.

MATERIALS AND METHODS

The study was carried out in laboratories of Chair of Food Science and Technology in Estonian University of Life Sciences.

Preparation of kvass

The process of kvass preparation is described on Fig. 1. The basic process was adapted from previous studies by Lidums & Karklina (2014) and Dlusskaya at al. (2008) and tested and fine-tuned in bachelor thesis experiments by Taimalu (2022).

For the production of kvass the following materials were used: special dried rye bread, designated for kvass or beer production ('Õlle-kaljateo leib', JSC Lõuna Pagarid, Estonia), baker`s yeast *Saccharomyces cerevisiae* (Nordwise Biotech Ltd, Estonia), sugar (Nordic Sugar A/S, Denmark); and a selection of lactic acid bacteria comprising *Lactobacillus plantarum* TAK59 (Nordwise Biotech Ltd, Estonia), *Lactobacillus plantarum* Sour Pitch (WildBrew^{TM} Sour Pitch, Lallemand S.A.S., France) and *Lactobacillus helveticus* Helveticus Pitch (WildBrew^{TM} Helveticus Pitch, Lallemand S.A.S., France).

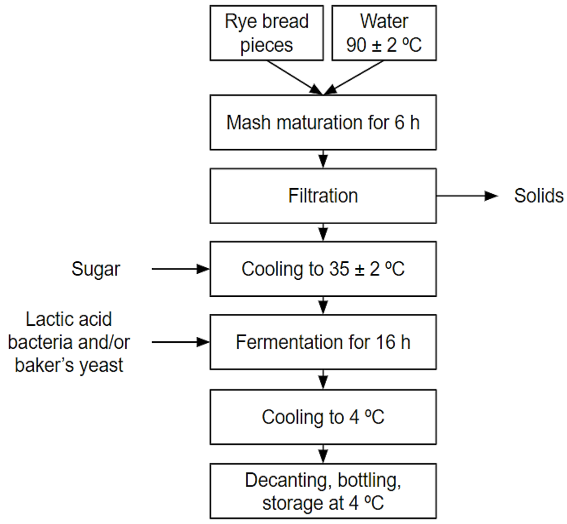

Figure 1. Scheme of kvass preparation.

First, the bread was cut into pieces and soaked in hot water (~90 °C) for approximately 6 hours, using a ratio of 10 litres per 1 kg of bread. Subsequently, the bread pieces were removed and sugar was added to the liquid fraction in a quantity of 50 grams per 1 litre. Next, the kvass wort was transferred into ten class carboys. In each carboy a specific quantity of lactic acid bacterium culture, either alone or in combination with yeast, was added The total measure of the culture(s) in each carboy was maintained at 1 gram per 1 litre of liquid. The exact amounts and specifications of the added cultures can be found in Table 1.

Table 1. Bacterial and yeast ratios per litre of kvass wort and corresponding sample designations

LAB and yeast quantities per 1L of kvass wort	Sample designation*
L. helveticus 0.5 g L^{-1}+ *S. cerevisiae* 0.5 g L^{-1}	HP 50/50
L. helveticus 0.8 g L^{-1} + *S. cerevisiae* 0.2 g L^{-1}	HP 80/20
L. helveticus 1 g L^{-1}	HP 100
L. plantarum 0.5 g L^{-1} + *S. cerevisiae* 0.5 g L^{-1}	SP 50/50
L. plantarum 0.8 g L^{-1} + *S. cerevisiae* 0.2 g L^{-1}	SP 80/20
*L. plantarum*1 g L^{-1}	SP 100
L. plantarum TAK59 0.5 g L^{-1}+ *S. cerevisiae* 0.5 g L^{-1}	NW 50/50
L. plantarum TAK59 0.8 g L^{-1}+ *S. cerevisiae* 0.2 g L^{-1}	NW 80/20
L. plantarum TAK59 1 g L^{-1}	NW 100
S. cerevisiae 1 g g L^{-1}	Y100

*abbreviations of LAB referring to brand or company names: HP – WildBrew^{TM} Helveticus Pitch; SP – WildBrewTM Sour Pitch, and NW – Nordwise Biotech Ltd.

Fermentation conditions

Fermentation of kvass was conducted in a Panasonic MIR-154 incubator (Japan). The incubation temperatures were set according to the specifications provided by the manufacturer of LAB cultures. Kvass worts containing either *L. plantarum* strains (NW and SP, see Table 1) were fermented at 30 °C, while *L. helveticus* (HP) was incubated at 38 °C. Samples containing only baker's yeast underwent fermentation at room temperature (22–24 °C). Fermentation lasted up to 16 hours.

Physicochemical analysis

Samples were analysed at 12, 14, and 16 hours of fermentation. The parameters assessed were active acidity (pH) using a Mettler Toledo pH-meter (Switzerland), dry matter content quantified as degrees of Brix (°Bx) via Atago 1311 DR-A1-Plus Abbe digital refractometer (Japan), and total titratable acidity (TTA), indicative of the sample's total acid content, was measured in grams per litre of citric acid equivalent, using a Titroline 7000 titrator (SI Analytics, Germany). All analyses were conducted in triplicate to ensure reliability.

Sensory analysis of kvass

The sensory analysis of kvass was conducted to evaluate the organoleptic properties of three selected fermentation combinations, designated as NW 80/20, SP 80/20, and HP 80/20. The choice of these combinations based on preliminary sensory analysis results from first two series of kvass wort fermentations (data not presented). The sensory evaluation involved three separate kvass batches (fermented for 14 hours and cooled to 4 °C). The assessment was carried out by total of 34 randomly selected, untrained assessors, whose ages ranged from 20 to 70 years, employing a blind test methodology.

The sensory analysis was carried out using a descriptive method, with modifications, based on the approach outlined by Abel & Andreson (2020). This method involved assigning scores in intensity on a linear scale from 0 to 10 for attributes such as aroma, acidity, taste, and aftertaste. Furthermore, the overall acceptability of the kvass was rated on a scale from 1 to 5, with 1 indicating 'dislike extremely' and 5 denoting 'like very much'. The average scores for each attribute were calculated for each three combinations of microbial cultures to ascertain their impact on kvass sensory profiles.

Statistical analysis

The data collected during the experiments were analysed using Microsoft Office Excel 2016. The statistical assessment of variations in the parameters across the samples was conducted through ANOVA (Analysis of Variance) and the Student's *t-test*. A *P*-value of less than 0.05 was indicative of statistical significance.

RESULTS AND DISCUSSION

Traditionally, kvass fermentation is completed within 9–10 hours (Lidums & Karklina, 2014); however, preliminary experiments suggested that in the presence of a high concentration of lactic acid bacteria an extended fermentation period would be beneficial, otherwise resulting in under-fermented kvass (Taimalu, 2022). This study, therefore, evaluated the physicochemical parameters (pH, TTA, and °Bx) at the 12^{th}, 14^{th}, and 16^{th} hours of fermentation to assess the impact of this extended duration.

Physicochemical parameters

pH: Initially, the kvass wort had a pH of 5.58 ± 0.13. During fermentation, the kvass samples exhibited notable variations in pH, depending on the batch, with standard deviations ranging from 0.02 to 0.58, as well as on which fermenting culture was used (Fig. 2).

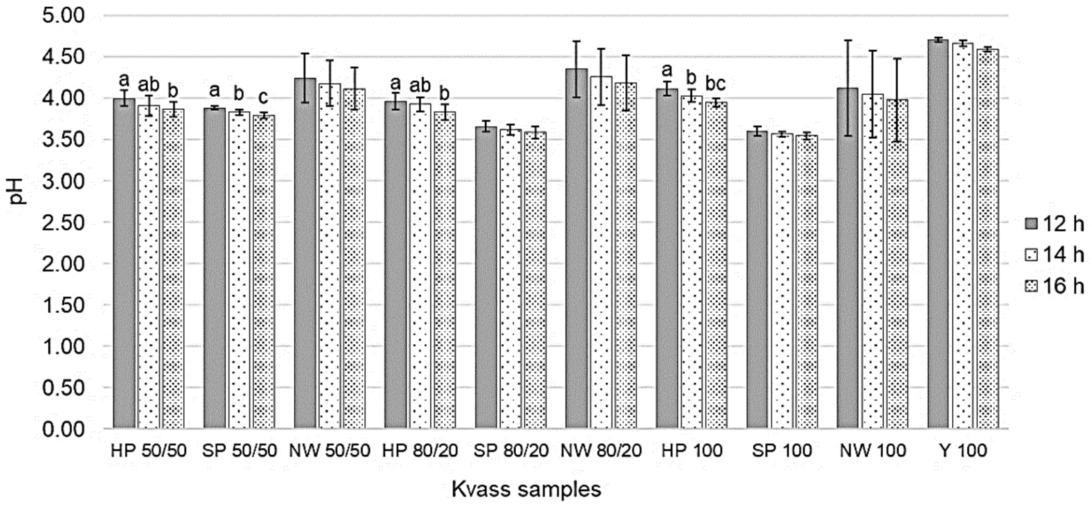

Figure 2. Changes in mean pH values with standard deviation error bars for the kvass samples at 12, 14, and 16 hours of fermentation. Means with different letters within the same group indicate statistically significant differences between the measurement times ($p < 0.05$). Sample designations are explained in Table 1.

The samples containing only yeast (Y 100) maintained the highest pH levels across all kvass samples, with notable differences ($P < 0.05$). The decline in pH for Y 100 samples was modest and not statistically significant ($P > 0.05$), with values at 4.71 ± 0.03 at 12 hours, 4.67 ± 0.04 at 14 hours, and 4.59 ± 0.02 at 16 hours of fermentation. In contrast, the SP 50/50 samples showed the most significant pH reduction at each time point, with values decreasing from 3.88 ± 0.02 to 3.83 ± 0.03, and then to 3.79 ± 0.04 ($P < 0.05$). Among the samples inoculated with LAB, those with *L. plantarum* TAK59 (NW) exhibited the slowest decrease in pH, where the mean values with considerable fluctuation remained between 3.98 ± 0.50 and 4.19 ± 0.34 at the 16-hour mark, depending on the sample type (NW 50/50, NW 80/20, or NW 100). This variation clearly highlights the strain-specific characteristics of *L. plantarum* TAK59, particularly when compared to SP samples inoculated with a different *L. plantarum* strain. Interestingly, already a study by Pepe et al. (2004), which examined *L. plantarum* strains isolated from sourdoughs, noted that the technological properties of dough are influenced more by the specific strain used rather than the species as a whole.

A comparative analysis of mean pH values across all fermentation time points for samples with identical LAB and yeast ratios (50/50 or 80/20), revealed significant strain- and species-related differences ($P < 0.05$). NW samples consistently exhibited the highest mean pH levels (4.18 ± 0.26 to 4.27 ± 0.32), while SP samples had the lowest (3.62 ± 0.07 to 3.84 ± 0.05). Notably, among single-culture samples, the only pair that did not show a significant difference ($P > 0.05$) in mean pH levels was between HP 100 and NW 100.

TTA: The TTA values, indicative of the fermentation's progression and the acidity developed by microbial activity, were monitored alongside pH measurements. Throughout the fermentation period, starting from a baseline of 0.77 ± 0.14, the TTA levels increased in most samples, reflecting the accumulation of organic acids, primarily lactic acid, produced by the LAB cultures (Fig. 3, a). This is consistent with the drop in pH values, which also indicates increasing acidity.

In some previous research works (Kobelev, 2011; Landis et al., 2022), it was claimed that yeasts inhibit LAB function, which was manifested in lower acid production compared to samples fermented only by bacteria. In our experiment, such a strong dependency was not detected.

The SP 80/20 samples exhibited the most pronounced increase in TTA at the 14th hour compared to the 12th hour time-point ($P < 0.05$), suggesting that the SP culture mix is more vigorous in acid production than the other cultures. This observation aligns with previous studies that have noted different bacteria strains produce varying amounts of acids (Kobelev, 2011; Basinskiene et al., 2016; Lidums et al., 2017). The NW 80/20 and Y 100 samples present an interesting case where the TTA actually decreases between the 12th and 16th hours, which might indicate a plateau in the fermentation process, possibly due to nutrient depletion or other metabolic factors that slow down acid production by these cultures (Landis et al., 2022). Samples with equal ratios of LAB and yeast showed no statistical difference ($P > 0.05$) between each other in their TTA values. Among samples inoculated with a single culture, HP 100 revealed statistically the lowest ($P < 0.05$) TTA values at each time-point compared to SP 100 and Y 100 samples. This finding is consistent with results presented by Basinskiene et al. (2016), highlighting the distinct contributions of different microbial agents to the fermentation process.

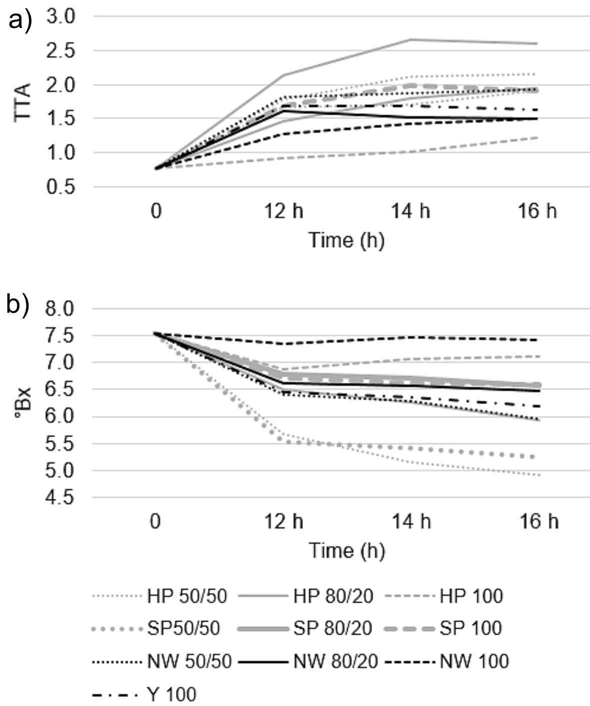

Figure 3. Dynamics in values of total titratable acid (TTA, a) and total soluble solids (°Bx, b) in kvass samples fermented for 12, 14 and 16 hours. Sample designations are explained in Table 1.

The findings indicate that a balance of LAB and yeast might be preferable for a controlled fermentation process, as seen in the NW and HP samples with mixed cultures, where the changes in TTA and pH are more gradual and could potentially lead to a more balanced flavour profile in the final kvass product. This is supported by Lidums et al. (2015; 2017), who noted the role of over 20 compounds in kvass flavour development, influenced by both yeasts and bacteria. Basinskiene et al. (2016) also emphasize the significant impact of these fermentation parameters on kvass's flavour and acidity quality. The SP cultures could result in a sharper, more acidic kvass, while the HP and NW cultures, particularly in mixed ratios, might produce a kvass with a milder acidity and potentially more nuanced flavour profile. The progression of fermentation from 12 to 16 hours shows different trajectories for each sample type, indicating that fermentation time can be optimized based on the desired acidity level and flavour profile, with some cultures reaching their optimal point earlier than others.

Brix: The °Bx measurements, representing the soluble solids content, primarily sugars, offer insight into substrate utilization by fermenting cultures. Initially, all kvass samples began with similar °Bx values (7.53 ± 0.08), indicating a uniform wort composition. Notably, at the 12-hour fermentation point, SP 50/50 and HP 50/50 samples exhibited the lowest °Bx values (5.54 ± 1.26 and 5.68 ± 0.80, respectively). As fermentation progressed, a decline in °Bx was statistically similar across all samples ($P > 0.05$), reflecting the consumption of sugars by yeast and LAB for metabolic activities, including the production of ethanol and lactic acid (Fig. 3, b). Conversely, HP 100 and NW 100 samples demonstrated an increase in °Bx values over time (from 6.88 ± 1.57 to 7.12 ± 1.03 and from 7.34 ± 0.91 to 7.42 ± 0.86, respectively), which could indicate the production of compounds that increase the refractive index of the solution. This phenomenon aligns partially with findings by Kobelev et al. (2011), who noted that the dry matter content in samples fermented solely by LAB remained nearly unchanged throughout the experiment. Thus, the distinctive rise in °Bx values in current study necessitates further research to uncover the underlying mechanisms.

In contrast to cultures with different LAB to yeast ratios, SP 50/50 samples displayed notably lower °Bx values than SP 80/20 samples during the 12 to 16-hour fermentation period ($P < 0.05$). Meanwhile, Y 100 samples consistently exhibited a decrease in °Bx values, suggesting an active yet less acidic fermentation process when viewed in conjunction with pH and TTA data.

Sensory analysis

Based on the physicochemical parameters and preliminary sensory analysis, the 14-hour fermented and cooled samples NW 80/20, SP 80/20, and HP 80/20 were chosen for detailed sensory evaluation. The sensory panel focused on assessing various attributes of the kvass samples, including the intensity of aroma, acidity, taste, and aftertaste, as illustrated in Fig. 4.

While the panellists rated the aroma and aftertaste of all three samples consistently, the perceived acidity varied significantly ($P < 0.05$) among the samples. The SP 80/20 sample was noted for its pronounced acidity. In contrast, the NW 80/20 sample was perceived to have the least acidity, with some consumers remarking on its similarity to commercially available, regular kvass. The latter also received the lowest taste ratings, being described as either tasteless or overly sweet and significantly different ($P < 0.05$) from the HP 80/20 sample that had the most intense taste, although a subset of assessors noted a slight bitterness. The variation in sensory

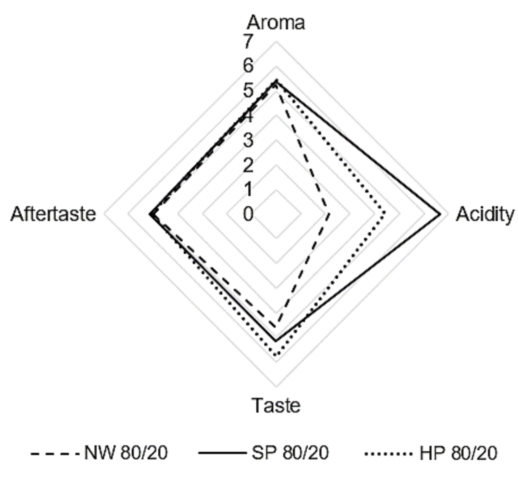

Figure 4. Evaluation of sensory properties of kvass samples. Sample designations are explained in Table 1.

experiences, notably with respect to acidity and taste, emphasizes the significance of microbial configurations in adapting kvass to suit a range of consumer tastes, pointing to the possibility of adjusting fermentation techniques to secure the sought-after sensory qualities.

On a scale from 1 (dislike extremely) to 5 (like very much), the overall acceptability ratings for the kvass samples showed no statistically significant differences ($P > 0.05$). The NW 80/20 sample scored 2.9 ± 1.1, SP 80/20 rated at 3.1 ± 1.2, and HP 80/20 at 3.3 ± 1.3. Notably, preferences varied significantly ($P < 0.05$) with assessors' age. Individuals aged 20–39 ($n = 19$) showed a preference for the acidic SP 80/20 sample, while those aged 40–70 ($n = 15$) showed higher acceptance towards the slightly bitter HP 80/20. This division may be due to the decline in the ability to detect bitter tastes with age (Barragán at al., 2018; Braun at al., 2022), leading older individuals to find the HP 80/20 kvass sample more palatable, while the NW 80/20 and SP 80/20 samples may appear less flavourful to them.

CONCLUSIONS

The study shed light on the complex processes involved in kvass fermentation, emphasizing the critical importance of the proportions and specific microbes on both the physicochemical and sensory characteristics of the drink. The key conclusions include:

- The interaction between LAB and yeast, particularly with higher LAB ratios, significantly influences kvass's acidity and flavour complexity. This underscores the importance of achieving an optimal microbial balance, where elevated LAB levels can harness the full potential of fermentation to enrich the beverage's organoleptic qualities.
- Extended fermentation times and specific microbial ratios, especially those favouring LAB, lead to notable variations in pH, TTA, and °Bx. These changes were directly correlated with the beverage's taste profile and overall quality, highlighting how increased LAB concentrations can contribute to a more desirable fermentation outcome.
- Sensory evaluations indicated age-related differences in taste preferences, emphasizing the need for targeted formulation strategies to attract diverse consumer groups.
- The distinct substrate utilization patterns of single versus mixed microbial cultures underscore the complexity of fermentation interactions and their consequential impact on kvass's characteristics.

Investigating the impact of additional microbial strains and their combinations could further enhance the understanding of kvass fermentation, potentially leading to novel flavour profiles and improved health benefits. The research significantly advances understanding in kvass manufacturing by demonstrating how careful management of microbial cultures, especially by optimizing LAB proportions, can elevate the drink's quality and appeal to a broader audience.

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