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Chemical and sensory characteristics of lingonberry (*Vaccinium vitis-idaea*) alcoholic beverages produced using *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Metschnikowia pulcherrima* yeasts

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ABSTRACT

Even though lingonberry (*Vaccinium vitis-idaea*) is categorised as a superfruit due to its bioactive composition, its challenging flavour profile leads to difficulty in valorisation. In addition, fermentation of lingonberry is limited due to presence of the antimicrobial benzoic acid. This study employed baker's yeast mediated benzoic acid decrease, followed by conventional (*Saccharomyces cerevisiae*) and non-conventional (*Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) fermentation to produce alcoholic lingonberry beverages. Profiling of the lingonberry alcoholic beverages was done through characterisation of volatile compounds with GC-MS and of sensory properties with a semi-trained panel. The benzoic acid decrement step was successful in reducing the content from 0.66 g/L to 0.04 g/L. Alcoholic beverages were created with an average alcohol content of 7.34% (±0.26). There were increments in the ester- and alcohol- odours alongside a decrease in the original lingonberry flavour. This was supported by higher contents of esters and higher alcohols along with a decrease of certain terpenes in alcoholic beverages. All alcoholic beverages were perceived as notably sour and thus, only minor differences were observed between the used yeasts. In particular, *M. pulcherrima* yeast produced a sweeter and less sour alcoholic beverage compared to the original juice. Overall, benzoic acid reduction facilitates in yeast fermentation to improve market potential of the underutilised berry in alcoholic beverage production.

1. Introduction

Lingonberry (*Vaccinium vitis-idaea*) is a wild shrub bearing small red berries with a tart, bitter, sour, and astringent flavour. Lingonberry (also referred to as partridgeberry, cowberry, redberry, and red whortleberry) is often classified as a superfruit due to the presence of high contents of phenolic compounds, such as tannins, anthocyanins, fibres, minerals, and vitamins (Kowalska, 2021). These bioactive compounds confer many potential health effects including antioxidative, anti-inflammatory, anticancer, antiseptic, antiproliferative, anti-obesity, hepatoprotective, and antimicrobial properties (Vilkickyte & Raudone, 2021). The presence of a natural antimicrobial agent, benzoic acid, increases the shelf life of lingonberry and makes it a potential dietary source of primary anthocyanins and other phenolic compounds for Nordic citizens (Dróżdż et al., 2017).

Despite the abundance and health benefits, usage of lingonberry is limited in the food and beverage industry due to intense levels of sourness, bitterness, and astringency caused by phenolic compounds and benzoic acid (Laaksonen et al., 2016; Visti et al., 2003). Many lingonberry products available in the markets, such as juices, jams, and syrups, are prepared with the addition of sugars to make them palatable. However, the European Food Safety Authority panel has recommended that a nutritionally adequate diet should minimise sugar intakes to as low as possible. This recommendation was formulated after a direct relation was found between added or free sugar consumption and chronic metabolic disease risk (Turck et al., 2022). Since lingonberry without extra sugar was perceived to have lower consumer likeability, alternative strategies to improve familiarity and mask strong flavours were suggested (Laaksonen et al., 2016).

The potential for the creation of berry alcoholic beverages is attributed to a low industrial set-up cost, unique flavour procurement, reduced post-harvest loss, conformity with the low-alcohol alcoholic beverage trend, and presence of health promoting compounds in berries (Liu et al., 2022). The natural occurrence of excessive levels of microbial

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inhibitor benzoic acid in lingonberry (0.6–1.3 g/L) acts as a limiting factor in the formulation of non-diluted additive-free alcoholic beverages (Viljakainen & Laakso, 2002). Laboratory-scale malolactic fermentations by Viljakainen and Laakso (2002) using *Oenococcus oeni* and by Markkinen (2021) using *Lactiplantibacillus plantarum* have been unsuccessful due to high benzoic acid levels. However, some successful fermentations of lingonberry have been conducted. Bergentall et al. (2024) developed a malolactic fermentation protocol that substantially decreased the sensory perception of sourness and astringency in *L. plantarum*-incubated lingonberry juice. Since the focus was on microbiological assessment, chromatographic assessment for the generated alcohol was not presented. Pärnänen (2017) performed sugar removal using a patented direct seven-day fermentation of 5.4%–6.6% *Saccharomyces cerevisiae* in cold pressed lingonberry juice, followed by a filtration method to obtain an alcohol content of 11%. Viljanen et al. (2014) effectively processed diluted lingonberry juice after heating to 80 °C for 5 min, ice-bath cooling, manually crushing, and adjusting pH to 5 with sodium hydroxide. The cultures were pre-grown in general edible medium (*Lactobacillus plantarum)* and wort sucrose broth (*Hanseniaspora uvarum)*, followed by Ringer's solution washing. The fermentations were performed using *H. uvarum* and/or *L. plantarum*, which resulted in 'fermented' and 'off-taste' odour perceptions.

Visti et al. (2003) successfully created a three step process with yeast biomass increment, benzoic acid decrement, and conventional yeast mediated lingonberry fermentation. First, 10% yeast was added to media with 10% glucose and 0.1% Vitamon Combi-yeast nutrient, incubated, centrifuged, and washed with 0.9% NaCl solution. Then, 15%–20% of single batch or consequetive 1%–3% batches of yeast biomass were inoculated in lingonberry juice (stirring, 10 min, room temperature), followed by centrifugation (5860 g, 10 min, room temperature). This was done to obtain a non-inhibitory final benzoic acid content of 0.25 g/L in the supernatent, through its uptake by yeast cells before fermentation (Warth, 1988). Finally, this bioprocessed juice was inoculted with 0.06% prehydrated dry *S. cerevisiae* (stirring, 7 days, room temperature) to obtain an alcoholic beverage with 3.5% ethanol. In particular, the uptake of benzoic acid depends upon pH, dominant benzoic acid form, and temperature of the solution (Macris, 1975). A low pH supports formation of the molecular form of benzoic acid that shows affinity for the lipophilic cell membrane. Additionally, heat inactivation of protein carrier compounds in the cell membrane at varied solution temperatures leads to a reduction in the molecular benzoic acid uptake rate (Macris, 1975). The protocol of present research was based on the study by Visti et al. (2003) with certain modifictions. These alterations included the elimination of yeast biomass increment, utilisation of non-*Saccharomyces* yeasts, and removal of the requirement for constant stirring during the incubation period. In addition, emphasis was placed on the volatile profiling to ascertain the potential of using varied non-convential strains for modulation of the inherent challenging lingonberry flavour, and hence, to discern the consumer acceptance prospects of developed lingonberry alcoholic beverages.

There is a dearth in lingonberry fermentation studies but other *Vaccinium* species' alcoholic beverages have been explored extensively. Amongst these studies, numerous different volatile compounds have been reported in other *Vaccinium* alcoholic beverages. Volatile compounds, such as ethyl hexanoate, ethyl octanoate, ethyl decanoate, and terpineol, were detected in alcoholic beverages made from cranberry (Zhang et al., 2019, 2020), blueberry (Yuan et al., 2018), bilberry (Liu et al., 2019, 2020), and bog bilberry (Lin et al., 2022; Wang et al., 2017).

Apart from traditional yeasts, non-*Saccharomyces* cultures are being used increasingly for alcoholic beverage creation due to greater variation in strains, related flavour impartment, reduced ethanol production, control of spoilage microflora, and colour stabilisation. This is a contrasting development compared to the initial perception of these yeasts as spoilage genera (Padilla et al., 2016). *Torulaspora delbrueckii* shows high fermentability and minimal off-flavour generation, such as lower acetic acid and hydrogen sulphide (Belda et al., 2015; Bely et al., 2008). *Metschnikowia pulcherrima* has a positive impact on the volatile composition of alcoholic beverages through release of α-arabinofuranosidase and β-glucosidase enzymes; and antimicrobial action against wild spoilage yeasts (Jolly et al., 2014; Oro et al., 2014). An example of successful implementation is the low production of undesirable acetoin, acetaldehyde, and acetic acid using *T. delbrueckii* along with the generation of higher alcohols using *M. pulcherrima* in bilberry (*Vaccinium myrtillus*) alcoholic beverage (Liu et al., 2019, 2020). Mixed inoculation of *M. pulcherrima* with *S. cerevisiae* enhances production of preferred compounds, such as acetate esters, ethanol, and glycerol (Canonico et al., 2019; Contreras et al., 2014; Varela et al., 2017). For instance, an increase in total acetate content was reported for sequential fermentation with *M. pulcherrima* and *S. cerevisiae* in black currant (*Ribes nigrum*) alcoholic beverage (Kelanne et al., 2022).

The objective of this study was to develop lingonberry alcoholic beverages using *Saccharomyces* and non-*Saccharomyces* yeasts after benzoic acid decrement through baker's yeast incubation of initial juice. Thereafter, *S. cerevisiae* and two non-*Saccharomyces* yeasts were used in pure and mixed inoculation during fermentation. Further, a comparative multivariate analysis was performed for evaluation of the probable relation between inoculum type, bioanalytical composition, and sensory perception to characterise key quality factors for valorisation of lingonberries in the beverage industry. The relation between sensory perception and sensory active compounds was deciphered through descriptive sensory profiling and HS-SPME-GC-MS volatile analysis. Yeast fermentation was generally expected to have a significant impact on the sensory and volatile properties of lingonberry juice, whereas the selected non-*Saccharomyces* yeasts were hypothesised to produce different outcomes compared to the traditional *S. cerevisiae*.

2. Materials and methods

2.1. Raw materials, strains, and reagents

2.1.1. Berry material and microbiological cultures

Frozen lingonberries (*Vaccinium vitis-idaea*; Marjex, Arctic International Oy, Sotkamo and Pakkasmarja Oy, Suonenjoki, Finland) were purchased from a local supermarket and stored at −20 °C until further use. Baker's yeast (Tuorehiiva, Suomen Hiiva Oy, Lahti, Finland) was bought for benzoic acid decrement. Freeze dried active granular *S. cerevisiae* (Vinoferm Bioferm Rouge, Brouwland, Beverlo, Belgium), *T. delbrueckii* (BIODIVA™TD291, Level, Edwardstown, Australia), and *M. pulcherrima* (FLAVIA®MP346, Level, Edwardstown, Australia) were used in fermentations.

2.1.2. Standard compounds and reagents

Benzoic acid, acetic acid, 4-methyl-2-pentanol, hexanoic acid, decanoic acid, 1-hexanol, 3-(methylthio)-1-propanol, 1-octen-3-ol, 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, 1-hexanal, ethyl 3-methyl butanoate, ethyl butanoate, ethyl 2-methylbutanoate, 3-methylbutyl ethanoate, ethyl octanoate, ethyl decanoate, 6-methyl-5-hepten-2-one, ethyl benzoate, 2,3-butanedione, cymene, ethyl propionate, terpineol, terpinene-4-ol, linalool, n-alkanes from C7–C30, and yeast peptone dextrose (YPD media) were purchased from Sigma Aldrich (Burlington, MA, USA). 2-Methyl-butanoic acid, 3-methyl-1-butanol, phenylethyl alcohol, eucalyptol, methanol, and acetonitrile were purchased from Fluka Honeywell (Charlotte, NC, USA). 2-Hexenal, sodium chloride, glycerol, sucrose, citric acid, caffeine, and aluminium sulphate were purchased from Thermo Fisher Scientific (Waltham, MA, USA). 1-Phenylethanone was purchased from Chem Service (West Chester, PA, USA). Ethyl 2-methylpropanoate was purchased from H&R GmbH & Co. KgaA (Hamburg, Germany). Formic acid was purchased from VWR (PA, USA). Fermentation Stopper (potassium metabisulphite, E224, and potassium sorbate, E202) were purchased from Bacchus Viiniaine Viinitalo Melkko Oy (Lahti, Finland). Ethanol was purchased from Altia Oy (Helsinki, Finland). All standards had a purity of ≥95%.

2.2. Processing of lingonberry juice

The frozen lingonberry (LB) was pooled and thawed overnight in a refrigerator at +4 °C, followed by pressing to obtain juice using the fruit press adjustment of a food processor (Chef Titanium XL, Kenwood, Havant, UK). The obtained juice was divided into batches in glass bottles and frozen at −20 °C until further use. Homogenisation of the baker's yeast for benzoic acid removal was performed by a method developed by Visti et al. (2003) with some modifications. Briefly, fresh baker's yeast was used at a 12.5% inoculation rate for a 30-min incubation period. Herein, baker's yeast was washed with 0.9% sodium chloride (normal saline), and centrifuged at 3180 g for 10 min at +4 °C (Eppendorf, Centrifuge 5810 R, Hamburg, Germany). The residual yeast mass was dispersed with the LB juice, mixed thoroughly using a sterile loop, incubated using a magnetic stirrer at 100 rpm for 30 min in room temperature (22 °C–25 °C), and separated via centrifugation at 6000 g for 10 min at +18 °C (Beckman Coulter, Avanti J-20XP Centrifuge, CA, USA).

2.3. Fermentation

Lingonberry juice (~900 mL) with reduced benzoic acid concentration was diluted using ultrapure water (Elga, Veolia Water Solutions and Technologies, Woodridge, IL, USA) in 1:1 ratio and the total soluble solids (TSS) were determined with a Brix meter (Atago Co. Ltd., Tokyo, Japan). °Brix was adjusted to 14 using sucrose after benzoic acid removal and dilution. In brief, the original TSS of bioprocessed lingonberry juice after benzoic acid removal was 11 °Brix. After dilution to minimise the effect of fluctuating inherent benzoic acid concentrations in lingonberry, the TSS dropped to 5.5 °Brix. Through chaptalisation, 20 g/100 mL sucrose was added to reach the target TSS of 14 °Brix. As 1 mol of sucrose gives 4 mol of ethanol, the given mass of sucrose (i.e., 140 g) was used to estimate ethanol volume through moles intermediate calculation.

The freeze-dried active yeast cultures were revived as 1% yeast in YPD broth. This was followed by pre-growth at 300 rpm for 48 h at 30 °C using a magnetic stirrer. The cfu/mL was estimated using dilution series-spread plating and incubation for 48 h at 30 °C in an incubator (IF-110 Plus, Memmert GmbH + Co. KG, Schwabach, Germany). The yeasts were inoculated in 1% v/v rate (10^6–10^8 cfu/mL). Three single yeast fermentations were prepared with *S. cerevisiae* (SC), *T. delbrueckii* (TD), *M. pulcherrima* (MP), and one mixed fermentation with *M. pulcherrima-S. cerevisiae* (MIX). All the fermentations were prepared in triplicates in dark conditions inside a fume hood containing a constant air flow, to ensure minimised effect of repeated volume alterations and potential light-induced changes in the samples. The samples were incubated for 21 days at room temperature (22 °C–25 °C). Fermentations were terminated when a TSS of ~5 °Brix was obtained, preceding the yeast death phase ascribed to the complete release of residual benzoic acid. Duplicates of lingonberry juice without yeast inoculation were used as control. Carbon dioxide build-up was released every other day to prevent high pressure in the fermentation bottles. The benzoic acid content estimation monitored the release of residual benzoic acid in the solution, ensuring yeast viability until it remained below satisfactory limits of 0.25 g/L (Warth, 1988). The progress of the fermentations was determined using aliquots taken on alternate days for °Brix (sugar conversion rate) and colony counts (cfu/mL). The aliquots were stored in centrifuge tubes inside a deep freezer at −80 °C until analyses.

2.4. Chemical analyses

2.4.1. Determination of benzoic acid

Benzoic acid was analysed using a method by Kelanne et al. (2020) for phenolic acids, with slight modifications. Briefly, triplicate alcoholic beverage samples were diluted with methanol (1:1, v/v) and shaken vigorously for 2 min. Diluted samples were micro centrifuged at 2012 g for 10 min at room temperature (22°C–25 °C) (Clover Lab, SD110/110VAC Centrifuge, Taiwan). This was done to separate pectin that precipitates out after solvent addition and interferes with the next step of filtration. Supernatants were filtered with 0.45 μm PTFE Membrane filters and the content of benzoic acid was determined using an ultra-high performance liquid chromatography (UHPLC) instrument (Nexera 30 Series, Shimadzu Corp., Kyoto, Japan) coupled with an SPD-M20A diode array detector (DAD), as described before by Visti et al. (2003) with slight modifications. The mobile phases were water (A) and acetonitrile (B), both containing formic acid (0.1%, v/v). The following gradient was used for the mobile phase B: 0–14 min, 2%–18%; 14–16.5 min, 18%; 16.5–17.5 min, 18%–20%; 17.5–18.5 min, 20%–60%; 18.5–20 min, 60%–2%. The flow rate of mobile phase was set to 0.5 mL/min and compounds were separated using a bioZenTM column (Peptide XB-C18, 150 mm × 2.1 mm × 1.7 μm, Phenomenex, Torrance, CA, USA). Oven was set at 30 °C and injection volume to 4 μL. UV–vis absorption spectra was recorded at 225 nm. For quantification, a standard curve was prepared using exponentially increasing benzoic acid standard dilutions.

2.4.2. Determination of ethanol

Ethanol concentration was determined as previously described by Liu et al. (2020), with slight modifications. Briefly, GC (coupled with a flame ionization detector (FID; GC-2010Plus, Shimadzu Corp., Kyoto, Japan) was used. Triplicate alcoholic beverage samples were centrifuged at 4500 g for 5 min at room temperature (22 °C–25 °C) (Eppendorf, 5804 Centrifuge, Hamburg, Germany) and filtered using 0.45 μm RC membrane filters. 0.2 mL of the samples were injected through the auto-sampler port into an HP-Innowax column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness, HewlettPackard, Avondale, PA, USA). Temperature of the column oven increased at a rate of 40 °C/min from 40 °C to 240 °C with a hold of 5 min. Injector was set at 230 °C and detector at 280 °C. Carrier gas was helium with a flow rate of 3 mL/min and a 1:25 split ratio. Calibration curve ($R^2 = 0.99$) was computed using 1%–4% external standard solutions of ethanol.

2.4.3. Determination of volatile compounds

Volatile compounds were determined as previously described by Liu et al. (2019), with slight modifications. Briefly, fermented samples were analysed in triplicate using headspace solid phase microextraction coupled with gas chromatography mass spectrometry (HS-SPME-GC-MS). Each sample was centrifuged at 4500 g for 5 min at room temperature (22 °C–25 °C) (Eppendorf, 5804 Centrifuge, Hamburg, Germany) before sample preparation. Two millilitres of each sample and 0.2 g of NaCl were placed in a 20 mL glass vial, and 10 μL of 4-methyl-2-pentanol solution (802 μg/mL in methanol) was added as an internal standard (ISTD). The volatile compounds were extracted from the headspace with a 2 cm DVB/CAR/PDMS fibre (50/30 μm, Supelco, Bellefonte, PA, USA) at 45 °C for 30 min after 10 min of incubation. The fibre was conditioned at 240 °C prior to sample extraction. After the extraction, the SPME fibre was immediately transferred to the injection port of a Trace 1310 gas chromatograph equipped with a TSQ 8000 EVO mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) to be thermally desorbed in the splitless mode at 240 °C for 3 min. A DB-WAX polar capillary column (60m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was used to separate the volatile compounds of the samples. Helium was used as the carrier gas at a flow rate of 1.6 mL/min. The initial column temperature was set at 50 °C and held for 3 min. Next, the temperature was increased to 220 °C at a rate of 5 °C/min and held at 220 °C for 8 min. Mass spectra were detected in electron impact (EI) mode at 70 eV with a scan range from *m/z* 33 to *m/z* 300. The temperatures of the MS transfer line and the ionisation source were 220 °C and 240 °C, respectively. Chromeleon 7.3.1 CDS (Sunnyvale, CA, USA) was used to control the GC-MS instrument, to perform peak integration across all the samples, and to identify the peaks with odour characteristics. $NIST_{14}$ (National Institute of Standards and

Technology) database aided in probability-predicted conformity of the mass spectra while external standard compounds helped in verification of the identified compounds. As a second criterion for the identification, Kovats retention indices (RIs) were calculated using an n-alkane mixture (C7–C30). Finally, semi-quantification of the identified volatiles was performed by division of areas of target compound with that of the ISTD, according to Elmore (2015).

2.5. Sensory evaluation

Study was approved by the Ethics Committee for Human Sciences, Humanities and Social Sciences Division, University of Turku, Finland; in relation to the ethicality of the proposed research. Twelve healthy panellists with prior experience in sensory evaluation and familiarity to alcoholic beverages were recruited after obtaining their participation consent. The privacy notice was disclosed according to Articles 13 and 14 of the EU (European Union) General Data Protection Regulation; and participation was voluntary with no monetary compensation or advertised incentives.

During the training sessions, panellists were introduced to the study aim, familiarised with the intensities of taste standards (Supplementary Table 1), introduced to the ten odour references chosen from the Alcoholic beverage Aroma Wheel (University of California Davis; https://www.alcoholic beveragearomawheel.com/), and performed mock intensity determination for the odour and taste standards. The sensory evaluations of the samples were carried out in triplicate sessions. Samples were presented as 5 mL in a tulip-shaped standard alcoholic beverage glasses covered with glass lids. Samples were labelled with random three-digit codes and presented in a balanced partially randomised order (Williams Design model where the LB juice was fixed as last sample in all sessions). The test contained two separate sections: odour section evaluated first and taste section evaluated next, using intensity line scales (0–10). Water, crackers (for tastes), and ground coffee (for odours) were served for palate cleansing to conform with the laboratory requirements set under ISO 8589. The trainings and evaluations were conducted using Compusense20 (version 23; Compusense Inc., Guelph, ON, Canada).

2.6. Data processing and statistical analyses

Panel performance was evaluated using PanelCheck 1.4.2 (Nofima, Tromsø, Norway). None of the panellists showed notably separable performance and hence, all were included in further data analysis. Three-way ANOVA test was conducted in SPSS (version 24, IBM Corp., Armonk, NY, US) to analyse the sensory evaluation data, with sample as a fixed factor, and replicate and panellist as random factors. Unsupervised multivariate PCAs and PLS-regression were constructed with Unscrambler X (version 10.3, Camo Software, Oslo, Norway). For Scores model creation, n = 15 samples were used (triplicates). For PCA Correlation loadings creation, X-data corresponded to chemical variables (n = 68) in Fig. 2 and sensory attributes (n = 10) in Fig. 3 (to be visualised together with mean values of Table 2), for the five sample types as downweighed variables (SC, TD, MP, MIX, C). For PLS Correlation loadings creation, interations were shown between chemical variables (X-data; n = 68) and sensory attributes (Y-data; n = 10), in the five sample types as downweighed variables. All statistical analyses were performed with $p < 0.05$ as the cut-off level for statistical significance.

3. Results and discussions

3.1. Benzoic acid removal

The benzoic acid content estimation checked strain viability by ensuring the content remained below 0.25 g/L throughout the fermentation (Warth, 1988). Benzoic acid has been reported to be mildly sour and pungent (Otero-Losada, 1999), thereby acting as an indicator for unwanted sensory perceptions. The benzoic acid content decreased 94% from the initial 0.66 g/L (±0.00; Fig. 1D) to 0.04 g/L, after bioprocessing of the juice with the baker's yeast. This result corresponds with the benzoic acid decrement of 75%–91% in literature (Visti et al., 2003). The average content of benzoic acid increased by 88% during the incubation period, showing a trend similar to previously reported 53%

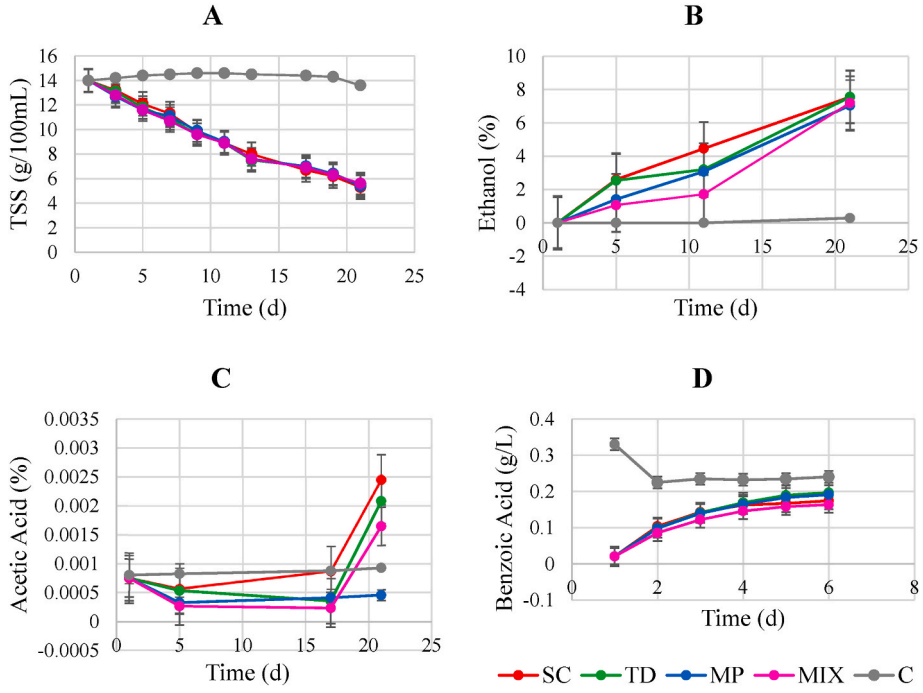

Fig. 1. Progress of TSS (A), Ethanol (B), Acetic acid (C), and Benzoic acid (D) during fermentation of lingonberry along with percentage error bars (5% for B-D). SC: *S. cerevisiae* (red), TD: *T. delbrueckii* (green), MP: *M. pulcherrima* (blue), and MIX: mixed inoculation of *S. cerevisiae* and *M. pulcherrima* (magenta), C: uninoculated non-bioprocessed lingonberry juice control.

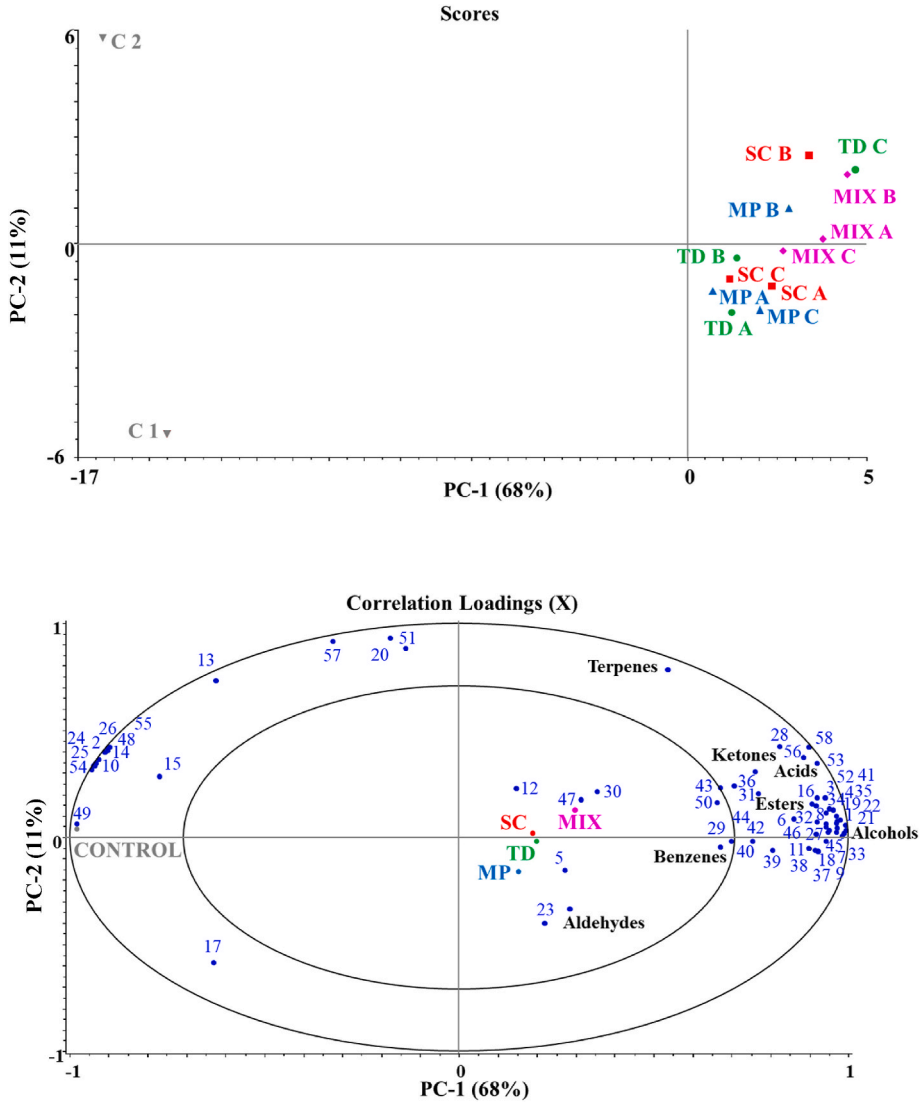

Fig. 2. Scores and loadings plots after principal component analysis (PCA) of volatile compounds in lingonberry wine samples. Scores plot of lingonberry juice (control; grey inverted triangle) and wine fermented with varied yeasts; SC: *S. cerevisiae* (red square), TD: *T. delbrueckii* (green circle), MP: *M. pulcherrima* (blue triangle), MIX: *S. cerevisiae* with *M. pulcherrima* (magenta diamond). A, B, and C as replicates of each fermentation inocula analysed thrice. Correlation loadings plot depicting contribution of each volatile compound (from Table 1) to the differences between the samples.

increment upon *H. uvarum* fermentation (Viljanen et al., 2014). Despite of the notable increase during the fermentation period, the benzoic acid values stayed below 0.25 g/L throughout the sample incubations, indicating limited antimicrobial action to enable lingonberry alcoholic beverage production. In addition, since the composition of lingonberries varies significantly due to their growth in wild uncontrolled conditions (Amundsen et al., 2023), sucrose was added after benzoic acid decrement. This was done to optimise yeast growth (MacNeil, 2001) and thus, support alcohol generation in lingonberry alcoholic beverages.

3.2. Fermentation progress

The progress of fermentation was monitored by a decrease of TSS (°Brix; Fig. 1A) and yeast growth (cfu/mL; Supplementary Table 2). The fermentation showed a steady decrease in TSS from 14.0 (±0.00) to 5.45 (±0.13). The cfu/mL illustrated that the strains were viable throughout the process and entered the death phase when TSS approached ~5 °Brix. Ethanol contents increased from 0% to an average of 7.34% (±0.26) in all alcoholic beverage samples (Fig. 1B), which was in accordance with the theoretically predicted value of 9.54% (after accounting for the acetic acid production). All the different strains proliferated in a similar time frame. Despite the inherent phenomenon of slower fermentation ability of non-*Saccharomyces* yeasts, our beverages showed similar fermentation kinetics for all strain inoculations (SC, TD, and MP), potentially ascribed to the fermentation matrix. For instance, Kelanne et al. (2020) used the same non-*Saccharomyces* yeasts in the production of black currant beverages and the results were similar with minimal kinetics difference between the *T. delbrueckii*, *M. pulcherrima*, and *S. cerevisiae* strains. However, Liu et al., 2019 also used the same non-*Saccharomyces* yeasts for bilberry wine production that showed a slower fermentation ability compared to *S. cerevisiae* and speculated the unavailability of sufficient Nitrogen in the fermentation matrix (required for non-*Saccharomyces* strains) as a cause. At the same time, acetic acid was detected in the samples (Fig. 1C) resulting from oxidative damage. Interestingly, MP sample had the significantly lowest content of acetic acid.

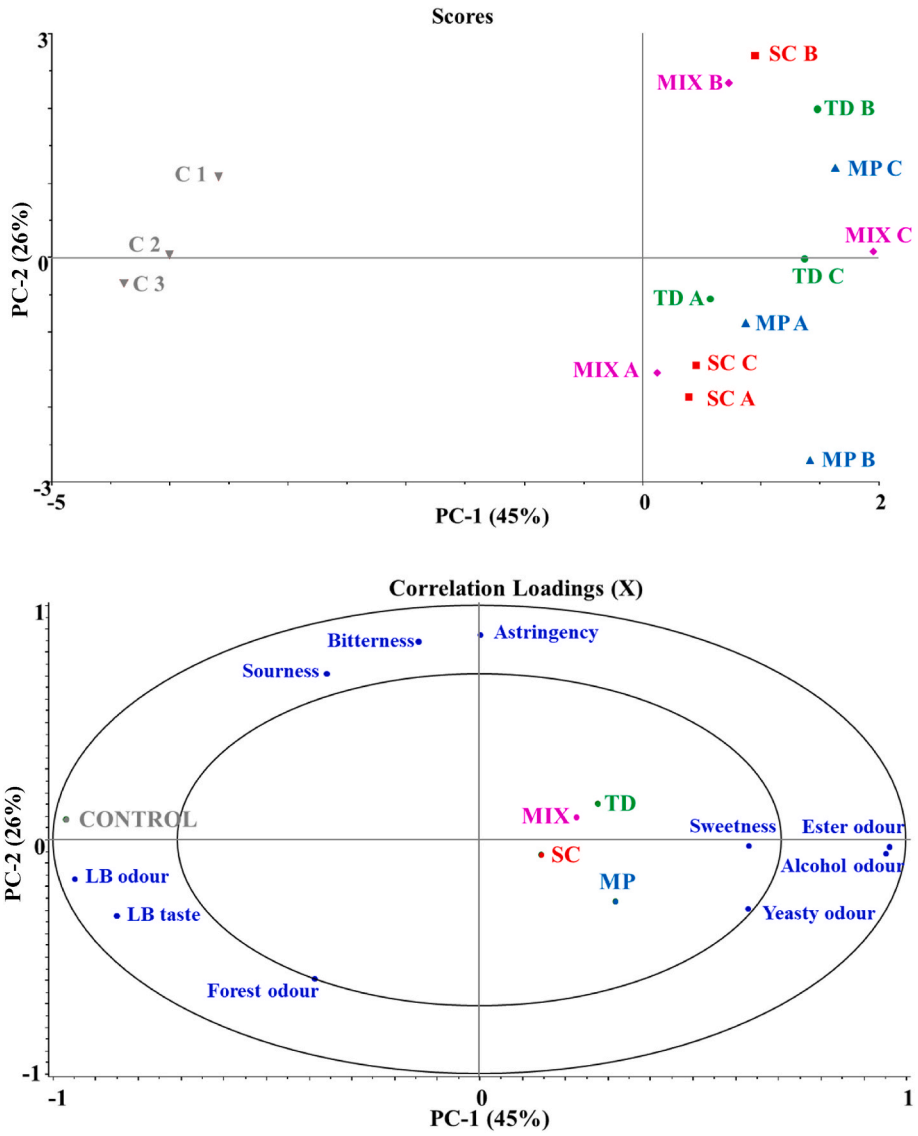

Fig. 3. Scores and loadings plots after principal component analysis (PCA) of sensory attributes in lingonberry wine samples. Scores plot of lingonberry juice (control; grey inverted triangle) and wine fermented with varied yeasts; SC: *S. cerevisiae* (red square), TD: *T. delbrueckii* (green circle), MP: *M. pulcherrima* (blue triangle), MIX: *S. cerevisiae* with *M. pulcherrima* (magenta diamond). A, B, and C as replicates of each fermentation inoculate analysed once. Correlation loadings plot depicting contribution of each sensory attribute to differences between the samples.

3.3. Volatile compounds

3.3.1. Overview of sample composition

A total of fifty-eight volatile compounds were identified in LB juice and fermented samples, of which there were 17 esters (RCOOR), 16 higher alcohols (ROH), 7 ketones (RCOR), 6 volatile acids (RCOOH), 6 aldehydes (RCHO), 5 terpenes, and 1 benzene. Compound identifications along with the semi-quantified concentrations are presented in Table 1. Out of these, 11 compounds (3 higher alcohols, 2 ketones, 3 aldehydes, 2 terpenes, and 1 volatile acid) were present only in non-fermented juice while 30 compounds (16 esters, 9 higher alcohols, 2 volatile acid, 2 aldehyde, and 1 ketone) were detected only after fermentation.

Alcoholic beverage odour is categorised under three groups, i.e., primary (*varietal*; corresponds to type of raw material), secondary (*fermentation*; corresponds to process parameters), and tertiary (*bouquet*; corresponds to ageing transformation; Padilla et al., 2016). The primary volatile compounds relating to the raw material type and hence, lingonberry odour were found as terpenes (eucalyptol, cymene, linalool, terpinen-4-ol, and α-terpineol; Table 1). Further, bulk of the composition was made up of volatile compounds categorised as secondary odour that arise during the fermentation process (e.g., decanoic acid, 4-penten-2-ol, 2-methyl-benzaldehyde, ethyl benzoate, and 3-hydroxy-2-butanone). The major volatile compounds present in their respective volatile groups were 3-methyl-1-butanol in the higher alcohol group for all alcoholic beverages samples, ethyl ethanoate in ester group for all samples, and 3-hydroxy-2-butanone in the ketone group for all pure fermented alcoholic beverages whereas 1-phenylethanone for control and mixed inoculation alcoholic beverage. In the acid and aldehyde group, 2-methyl-propanoic acid and acetaldehyde, respectively, were the most abundant volatiles in the alcoholic beverage samples. In the terpene group, linalool, and in the benzene group, styrene, was present in all samples.

The volatile composition of LB juice has been previously studied only in a few studies (Amundsen et al., 2023; Marsol-Vall et al., 2021; Anjou & von Sydow, 1967; Viljanen et al., 2014). In present study, styrene, ethyl ethanoate, 2,3-butanedione, 6-methyl-5-hepten-2-one, eucalyptol, cymene, and linalool, were also detected in the lingonberry juice

Table 1
Semi-quantitative concentrations of volatile compounds identified in uninoculated non-bioprocessed lingonberry juice controls and lingonberry alcoholic beverage samples fermented at room temperature.

#	Compound	Test RI	Lit. RI [y]	Ref. match [z]	SC	TD	MP	MIX	C	Potential odour
Acids (ACI)										
1	2-methyl-propanoic acid	1573	1554		20.15 ± 2.45	20.19 ± 2.08	19.35 ± 1.58	20.19 ± 1.28	0.23 ± 0.33 a	Acidic
2	2-methyl-butanoic acid	1679	1664	1,3,4; S	ND	ND	ND	ND	6.37 ± 3.57	Acidic
3	Hexanoic acid	1863	1860	1; S	4.76 ± 0.75 b	4.06 ± 0.80 b	3.70 ± 0.16 b	4.43 ± 0.82 b	0.93 ± 0.27 a	Fatty
4	Octanoic acid	2069	2070	1	16.69 ± 2.45	13.89 ± 2.02	13.49 ± 1.64	17.36 ± 2.50	ND	Fatty
5	Nonanoic acid	2166	2169	1	11.42 ± 2.08	14.21 ± 2.01	7.92 ± 3.47	8.03 ± 7.52	6.68 ± 2.49	Waxy
6	Decanoic acid	2259	2278	S	8.73 ± 1.20	4.80 ± 0.49 b	6.82 ± 1.58	8.38 ± 0.07	ND	Fatty
Total ACI					61.75 ± 8.93	57.15 ± 7.40	51.29 ± 8.44	58.39 ± 12.18	14.22 ± 6.67	
Higher Alcohols (HA)										
7	4-penten-2-ol	1011	1011		0.26 ± 0.02 b	0.24 ± 0.04 b	0.26 ± 0.05 b	0.25 ± 0.02 b	ND	Fruity
8	2-methyl-1-propanol	1103	1094		17.87 ± 0.50	19.78 ± 4.99	15.01 ± 1.32	17.15 ± 0.44	ND	Ethereal
9	3-methyl-1-butanol	1215	1211	1,3,4; S	360.89 ± 23.42	351.48 ± 45.43	328.54 ± 16.11	365.22 ± 10.52	ND	Fermented
10	1-pentanol	1255	1255	1,3	ND	ND	ND	ND	0.33 ± 0.17 b	Fermented
11	3-methyl-1-pentanol	1334	1338		2.29 ± 0.27 c	1.57 ± 0.17 b	1.78 ± 0.19 bc	2.29 ± 0.13 c	ND	Fermented
12	1-hexanol	1358	1359	1,3; S	1.20 ± 0.10 a	1.20 ± 0.09 a	2.72 ± 2.63 a	1.29 ± 0.05 a	1.14 ± 0.55 a	Herbal
13	3-hexen-1-ol	1388	1389	1,3,4	0.84 ± 0.07 a	0.78 ± 0.10 a	0.70 ± 0.07 a	0.85 ± 0.04 a	1.34 ± 0.83 a	Green
14	1-octen-3-ol	1451	1456	1,3; S	ND	ND	ND	ND	0.89 ± 0.56 b	Earthy
15	2-ethyl-1-hexanol	1490	1481	1	0.71 ± 0.16 a	0.71 ± 0.05 a	0.57 ± 0.08 a	0.62 ± 0.08 a	1.05 ± 0.03 b	Citrus
16	2,3-butanediol	1585	1523		1.53 ± 0.34 b	2.24 ± 0.66 b	1.88 ± 0.32 b	1.93 ± 0.23 b	ND	Creamy
17	2,4-hexadien-1-ol	1135	1570		ND	ND	ND	ND	0.06 ± 0.08 a	Green
18	3-(methylthio)-1-propanol	1732	1708	S	1.16 ± 0.09 b	1.22 ± 0.36 b	1.15 ± 0.16 b	1.35 ± 0.22 b	ND	Meaty
19	3,7-dimethyl-6-octen-1-ol	1776	1759		1.11 ± 0.09 b	1.18 ± 0.16 b	1.01 ± 0.02 b	1.24 ± 0.20 b	ND	Green
20	Benzyl alcohol	1893	1886	1,3,4	3.06 ± 0.27 a	3.29 ± 0.35 a	3.23 ± 0.53 a	3.40 ± 0.23 a	3.61 ± 2.13 a	Floral
21	Phenylethyl alcohol	1932	1923	3,4; S	91.48 ± 6.19	94.54 ± 13.42	89.12 ± 2.63	99.17 ± 4.55	ND	Floral
22	(E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	2042	2042	S	1.59 ± 0.27 b	1.68 ± 0.18 b	1.45 ± 0.23 b	1.71 ± 0.09 b	ND	Waxy
Total HA					483.99 ± 31.78	479.91 ± 66	447.41 ± 24.37	496.46 ± 16.82	8.42 ± 4.35	
Aldehydes (ALD)										
23	Acetaldehyde	680	714	1,2	3.12 ± 5.41 a	4.97 ± 5.34 a	4.32 ± 3.20 a	0.79 ± 1.37 a	ND	Ethereal
24	Hexanal	1079	1083	1-4; S	ND	ND	ND	ND	0.71 ± 0.36 b	Green
25	(E)-2-hexenal	1221	1219	1,3; S	ND	ND	ND	ND	0.31 ± 0.15 b	Green
26	α,4-dimethyl-3-cyclohexene-1-acetaldehyde/*p*-menth-1-en-9-al	1628	1620	1,3	ND	ND	ND	ND	0.35 ± 0.24 b	Herbal
27	2-methyl benzaldehyde	1662	1622		1.62 ± 0.08 b	1.70 ± 0.36 b	1.85 ± 0.31 b	1.86 ± 0.20 b	ND	Cherry
28	5-methyl-2-(1-methylethyl)-4-hexenal	1699	1699		0.35 ± 0.08 b	0.37 ± 0.03 b	0.41 ± 0.09 b	0.44 ± 0.02 b	0.15 ± 0.12 a	Herbal
Total ALD					5.10 ± 5.58	7.04 ± 5.73	6.58 ± 3.60	3.08 ± 1.59	1.52 ± 0.86	
Benzenes (BEN)										
29	Styrene/Phenylethene	1258	1254	1–4	55.97 ± 35.36	117.25 ± 24.40	126.11 ± 68.74	125.33 ± 36.10	0.31 ± 0.15 a	Balsamic
Esters (EST)										
30	Methyl ethanoate	827	827	1	2.80 ± 2.82 a	2.17 ± 2.62 a	1.22 ± 0.77 a	1.03 ± 1.37 a	ND	Ethereal

(*continued on next page*)

Table 1 (*continued*)

#	Compound	Test RI	Lit. RI [y]	Ref. match [z]	SC	TD	MP	MIX	C	Potential odour
31	Ethyl ethanoate	887	887	1–4	125.59 ± 52.86	99.79 ± 25.66	81.42 ± 20.41	105.91 ± 48.84	0.45 ± 0.26 a	Ethereal
32	Ethyl 2-methylpropanoate	963	954	S	19.49 ± 3.61	19.26 ± 6.23	17.06 ± 2.49	20.60 ± 0.41	ND	Fruity
33	Ethyl butanoate	1036	1025	S	4.82 ± 0.33 b	4.78 ± 0.93 b	4.50 ± 0.84 b	5.33 ± 0.25	ND	Fruity
34	Ethyl 2-methylbutanoate	1054	1042	S	1.19 ± 0.01 b	1.15 ± 0.16 b	1.14 ± 0.27 b	1.40 ± 0.16 b	ND	Fruity
35	Ethyl 3-methylbutanoate	1067	1060	S	1.72 ± 0.06 b	1.70 ± 0.43 b	1.72 ± 0.26 b	2.07 ± 0.27 b	ND	Fruity
36	3-methylbutyl ethanoate	1122	1126	4; S	20.05 ± 9.62	14.25 ± 3.88	11.79 ± 4.13	18.49 ± 10.63	ND	Fruity
37	Ethyl hexanoate	1236	1220		8.22 ± 1.44	7.32 ± 0.97	6.92 ± 0.56	9.21 ± 1.35	ND	Fruity
38	Ethyl 2-hydroxypropionate	1349	1331		0.59 ± 0.08 b	0.66 ± 0.09 b	0.90 ± 0.06 c	0.77 ± 0.08 bc	ND	Fruity
39	Ethyl octanoate	1434	1421	S	20.45 ± 7.52	20.61 ± 7.58	20.16 ± 11.23	26.83 ± 3.37	ND	Waxy
40	Ethyl decanoate	1642	1637	S	10.88 ± 0.60	9.98 ± 4.27	14.93 ± 10.95	14.56 ± 3.41	ND	Waxy
41	Ethyl benzoate	1679	1666	3,4; S	74.82 ± 9.57	77.40 ± 12.12	65.88 ± 8.11	82.17 ± 18.83	ND	Minty
42	Ethyl 9-decenoate	1696	1688		3.46 ± 0.72 a	3.53 ± 1.81 a	3.67 ± 2.69 a	4.42 ± 0.86 a	ND	Fruity
43	Phenylethyl acetate	1833	1826		1.36 ± 0.73 a	1.23 ± 0.45 a	0.90 ± 0.33 a	1.57 ± 1.02 a	ND	Floral
44	Ethyl dodecanoate	1852	1829		0.73 ± 0.12 b	0.53 ± 0.05 b	0.61 ± 0.19 b	0.75 ± 0.08 b	ND	Waxy
45	Ethyl 3-phenyl-2-propenoate	2137	2156		1.06 ± 0.03 b	1.14 ± 0.23 b	1.06 ± 0.13 b	1.26 ± 0.06 b	ND	Floral
46	Ethyl hexadecanoate	2232	2255		0.73 ± 0.05 b	0.72 ± 0.06 b	0.78 ± 0.20 b	0.83 ± 0.07 b	ND	Waxy
Total EST					297.95 ± 90.17	266.23 ± 67.53	234.65 ± 63.62	297.21 ± 91.03	0.45 ± 0.26	
Ketones (KET)										
47	Acetone	814	814	2	0.92 ± 0.07 a	0.35 ± 0.41 a	0.64 ± 0.46 a	1.11 ± 0.75 a	0.34 ± 0.20 a	Solvent
48	2,3-butanedione	975	977	1-4; S	ND	ND	ND	ND	0.60 ± 0.40 b	Buttery
49	4-methyl-2-pentanone	1011	1008		ND	ND	ND	ND	0.44 ± 0.01 b	Green
50	3-hydroxy-2-butanone/acetoin	1298	1287	1	2.40 ± 0.82 ab	4.45 ± 2.00 b	2.61 ± 0.98 ab	2.52 ± 0.78 ab	ND	Buttery
51	6-methyl-5-hepten-2-one	1341	1341	1-4; S	0.18 ± 0.02 a	0.18 ± 0.02 a	0.17 ± 0.00 a	0.18 ± 0.01 a	0.19 ± 0.08 a	Citrus
52	1-phenylethanone/acetophenone	1665	1670	1,3,4; S	2.38 ± 0.26 b	2.62 ± 0.20 b	2.57 ± 0.51 b	2.63 ± 0.16 b	0.76 ± 0.27 a	Floral
53	1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	1842	1829		0.18 ± 0.02 b	0.19 ± 0.02 b	0.18 ± 0.03 b	0.20 ± 0.01 b	0.08 ± 0.04 a	Floral
Total KET					6.07 ± 1.19	7.80 ± 2.64	6.17 ± 1.98	6.64 ± 1.71	2.41 ± 1.00	
Terpenes (TER)										
54	Eucalyptol/1,8-cineole	1212	1212	1-4; S	ND	ND	ND	ND	3.59 ± 1.67 b	Herbal
55	Cymene	1293	1298	1-4; S	ND	ND	ND	ND	0.24 ± 0.16 b	Terpenic
56	Linalool	1549	1549	1-4; S	9.75 ± 1.11	11.19 ± 2.39	9.70 ± 0.83	10.15 ± 1.22	3.71 ± 2.48 a	Floral
57	Terpinen-4-ol	1611	1601	1,3	0.96 ± 0.20 a	0.83 ± 0.10 a	0.88 ± 0.16 a	0.93 ± 0.03 a	1.13 ± 0.75 a	Spicy
58	α-terpineol	1707	1697	1,3; S	1.33 ± 0.15 b	1.34 ± 0.15 b	1.29 ± 0.14 b	1.43 ± 0.19 b	0.52 ± 0.37 a	Terpenic
Total TER					12.03 ± 1.45	13.37 ± 2.63	11.86 ± 1.13	12.50 ± 1.45	9.19 ± 5.42	

The values depict the means and standard deviations for triplicates. These are multiplied by 100 for easy visualisation and ND acts as a symbol for not detected. SC, TD, MP, and MIX are representatives of *S. cerevisiae, T. delbrueckii, M. pulcherrima*, and mixed simultaneous inoculation of *S. cerevisiae* with *M. pulcherrima*, respectively. C represents uninoculated non-bioprocessed lingonberry juice controls. Potential odour descriptors obtained from the database of Good Scents Company (http://www.thegoodscentscompany.com/index.html).

Letters a-c represent the grouping of samples based on ANOVA and post hoc Tukey's HSD Test.

S: Volatile identification using authentic standard compound.

Lingonberry juice volatiles reference matches as 1: Amundsen et al., 2023, 2: Marsol-Vall et al., 2021, 3: Anjou & von Sydow, 1967; and 4: Viljanen et al., 2014. y: Kovat's RI for DB Wax column (60m) from NIST.

z: Reference matched volatile compounds.

Table 2
A mixed two-way ANOVA depicting main effects and interactions in the descriptive sensory evaluation of the lingonberry samples.

Attribute	Effect	F-value	p-value	Mean sample ratings and Tukey's test[a]				
				SC	TD	MP	MIX	Control
Lingonberry odour	Sample	6.13	0.002	4.34a	3.93a	3.99a	4.29a	6.58b
	Panellist	1.92	0.069					
	Replicate	2.22	0.136					
	P*S	2.69	<0.001					
	S*R	2.24	0.032					
	P*R	1.47	0.106					
Yeasty odour	Sample	7.34	0.001	3.77b	3.49b	3.60b	3.63b	1.66a
	Panellist	5.22	<0.001					
	Replicate	12.6	<0.001					
	P*S	2.48	<0.001					
	S*R	1.96	0.062					
	P*R	1.58	0.069					
Alcohol odour	Sample	32.6	<0.001	4.53b	4.50b	4.75b	4.97b	1.06a
	Panellist	2.24	0.041					
	Replicate	0.71	0.505					
	P*S	1.76	0.012					
	S*R	1.45	0.188					
	P*R	3.41	<0.001					
Ester odour	Sample	14.3	<0.001	4.17b	4.23b	3.97b	3.93b	1.64a
	Panellist	1.39	0.228					
	Replicate	0.04	0.960					
	P*S	1.96	0.004					
	S*R	0.39	0.924					
	P*R	2.26	0.004					
Forest odour	Sample	0.47	0.760	2.16	2.02	2.08	1.96	2.31
	Panellist	12.2	<0.001					
	Replicate	3.43	0.080					
	P*S	1.10	0.351					
	S*R	0.95	0.482					
	P*R	1.42	0.127					
Sweetness[b]	Sample	3.12	0.106	3.90	4.15[b]	4.15[b]	4.29[b]	3.44
	Panellist	20.7	<0.001					
	Replicate	2.84	0.147					
	P*S	0.95	0.573					
	S*R	1.06	0.396					
	P*R	0.93	0.555					
Sourness[b]	Sample	2.21	0.204	6.56	6.30	5.78[b]	6.51	6.72
	Panellist	7.37	<0.001					
	Replicate	1.25	0.326					
	P*S	0.70	0.900					
	S*R	1.61	0.132					
	P*R	1.56	0.074					
Bitterness	Sample	0.65	0.642	4.40	4.73	4.41	4.76	4.74
	Panellist	11.0	<0.001					
	Replicate	0.24	0.789					
	P*S	1.15	0.289					
	S*R	1.08	0.384					
	P*R	1.47	0.105					
Astringency	Sample	0.13	0.966	4.56	4.62	4.37	4.35	4.51
	Panellist	10.3	<0.001					
	Replicate	0.60	0.567					
	P*S	1.18	0.255					
	S*R	2.06	0.048					
	P*R	1.87	0.021					
Lingonberry taste[b]	Sample	2.52	0.079	5.24[b]	4.91[b]	4.88[b]	5.18[b]	6.25
	Panellist	1.48	0.185					
	Replicate	1.57	0.251					
	P*S	2.41	<0.001					
	S*R	2.18	0.037					
	P*R	1.69	0.045					

SC, TD, MP, and MIX are representatives of *S. cerevisiae, T. delbrueckii, M. pulcherrima*, and mixed simultaneous inoculation of *S. cerevisiae* with *M. pulcherrima, respectively*.

[a] Letters a-c represent significant differences, if observed, between samples (Tukey's post hoc test; $p < 0.05$) rated on scale 0–10.

[b] Significant differences observed in LSD post hoc test ($p < 0.05$) between Control and the marked fermented samples.

(Table 1). However, numerous volatile compounds reported previously in the lingonberry juice were absent in our study. This gap in detected volatile compounds was attributed to a difference in bioprocessing steps and a huge lapse amongst previous studies. Marsol-Vall et al. (2021) observed some characteristic volatile compounds in lingonberry odour with GC-O. In our study, hexanal, 2,3-butanedione, eucalyptol, and linalool were also detected, but 2-methyl propanoate and methyl benzoate were absent in present lingonberry juice. Of these, hexanal, 2, 3-butanedione, and eucalyptol content decreased while the linalool content increased after fermentation. 53% of volatiles were previously reported by Viljanen et al. (2014), where volatile compounds in lingonberry beverages produced after *H. uvarum* and *L. plantarum*

fermentations were studied. Our study showed the same trend of total volatile group transformation during the fermentation of lingonberry juice. However, 2-methylbutyric acid, benzyl alcohol, 3-hexen-1-ol, styrene, ethyl benzoate, 2,3-butanedione, and 6-methyl-5-hepten-2-one showed different trends between lingonberry alcoholic beverage literature (Viljanen et al., 2014) and our alcoholic beverages. For instance, 2, 3-butanedione dissipated and benzyl alcohol decreased in present study after fermentation while both were reported to have increased by Viljanen et al. (2014). This contrast is suspected to arise due to the usage of different yeast strains, an additional pH modulation step in literature, and natural composition variation in lingonberry that is usually grown unsupervised in the wild.

Some of the compounds absent in the lingonberry alcoholic beverage literature, but present in our study, have been reported in other *Vaccinium* species berry alcoholic beverages. Of the primary volatile compounds, terpinen-4-ol and 3-(methylthio)-1-propanol were detected exclusively in literature of bog bilberry alcoholic beverage (Lin et al., 2022) and bilberry alcoholic beverage (Liu et al., 2020). It is important to mention that a few secondary volatile compounds (e.g., 4-penten-2-ol and 5-methyl-2-(1-methylethyl)-4-hexenal) in our study were undetected in all the *Vaccinium* berry alcoholic beverage literature. The overlapping of these volatile compounds within the same genus is an interesting revelation that should be explored further in cohesion with botanical research. Despite a difference in raw material, our lingonberry alcoholic beverages followed the same trends for transformation of higher alcohols, esters, and sulphur compounds, as those reported in non-conventional yeast strain fermented grape alcoholic beverage literature (for instance in Bely et al., 2008, González-Royo et al., 2015, Varela et al., 2017; Padilla et al., 2016). Oxidation mediated acetic acid generation via acetaldehyde intermediate formation resulted in opposite trend for those compounds.

3.3.2. Comparisons between samples

The volatile composition was compared between juice control (C) and developed lingonberry alcoholic beverages (pure inoculation, SC, TD, MP, and mixed inoculation, MIX). In general, the concentrations of all volatile groups increased after fermentation (Table 1). Before fermentation, the most abundant volatile compound group was volatile acids but after fermentations, it was higher alcohols. Further, before fermentation, the least abundant group(s) was benzene but after fermentations, they were aldehyde and ketone. The highest increment was observed for the ester (~610 times; highest amount in SC and MIX), benzene (~340 times; highest amount in MP and MIX), and higher alcohol (~55 times; highest amount in MIX) groups in averaged lingonberry alcoholic beverages compared to control. For ester group, ethyl ethanoate in SC and ethyl benzoate (absent in control) in MIX showed largest variation; while for higher alcohol group, 3-methyl-1-butanol and phenylethyl alcohol were the varying factors since both were absent in control. Some minor differences were observed between varied inoculations for certain compounds, for instance, 3-methyl-1-pentanol was lowest in TD but highest in pure and mixed SC, while ethyl 2-hydroxypropionate was lowest in SC but highest in MP. A large number of new esters were formed during the fermentations. Oxidation mediated acetic acid generation via acetaldehyde intermediate occurred due to frequent bottle opening for sampling.

In the PCA model for the scaled data matrix to analyse the relation between volatile attributes and samples, the first two principal components explained 79% of data variance (Fig. 2). In the PCA, the control (C) was positively correlating with 1-octen-3-ol (14), hexanal (24), 2-hexenal (25), *p*-menth-1-en-9-al (26), 4-methyl-2-pentanone (49), eucalyptol (54), and cymene (55) but negatively related with all alcoholic beverages on PC1. All the alcoholic beverage triplicates were scattered devoid of any pattern to establish relations with exact compounds on PC2, but collectively contained volatiles from alcohols such as 3-methyl-1-pentanol (11), esters such as ethyl ethanoate (31) and 3-methylbutyl ethanoate (36), acids such as decanoic acid (6), and ketones such as 1-phenylethanone (52). This scatter was attributed to oxidation amongst replicates and in coherence with the volatile data in original dimensions for occurrence of a larger proportion of secondary volatile compounds after fermentation. The PCA model did not show significant differences in the volatile composition between the lingonberry alcoholic beverages made from varied strains.

Several studies on grape alcoholic beverage have detected differences in flavour profile between the samples made from conventional (SC) versus non-conventional (TD or MP) strains. Pure TD lingonberry fermentation contained lower contents of acetic acid and ethyl ethanoate than conventional strain (Table 1), as has been previously reported by González-Royo et al. (2015) for TD mixed sparkling wines and Puertas et al. (2017) for Palomino-Chardonnay wines. However, the contents of total esters, acetaldehyde, and acetoin in our TD alcoholic beverage showed an opposing trend to aforementioned studies along with Liu et al., 2022. Pure MP lingonberry fermentation showed lower contents of acetic acid, total esters, and fatty acids (octanoic acid and decanoic acid) than conventional strain, akin to MP related-literature (Francis & Newton, 2005; Liu et al., 2022; Welke et al., 2014). Whereas acetaldehyde, higher alcohols, and terpene contents in our MP alcoholic beverage displayed opposite trends compared to Liu et al., 2022; Padilla et al., 2016. Simultaneous MIX lingonberry fermentation exhibited lower acetic acid along with a slight increase in higher alcohol content than conventional strain, conforming to mixed MP studies by Comitini et al., 2011; Varela et al., 2017. However, the ethanol and ester content did not vary based on strain type, as stated in previous studies (Contreras et al., 2014; Escribano-Viana et al., 2019). Overall, the minor, yet significant differences between the strains highlight potentially different end-products made using the non-*Saccharomyces* yeast strains, thus indicating their future potential in making different lingonberry alcoholic beverages.

3.4. Sensory perception

3.4.1. Panel performance and important sensory variables

Three main types of plots were used to check performance of the panel for sensitivity, reproducibility and anomalies as follows: 3-way ANOVA plot for identifying significant attributes, Tucker-1 plot for detecting outlier panellists (data not shown), and p-MSE plot to ascertain sensitivity (data not shown). The overall 3-way ANOVA overview plot for F values under sample effect indicated that primarily the odour attributes, lingonberry odour, yeasty odour, alcohol odour, and ester odour, were statistically significant ($p < 0.05$; Table 2) whereas the sample main effect was not significant in the taste attributes. In general, all samples had intense flavour profiles with high intensities of sourness (means 5.78–6.72) accompanied with notable levels of lingonberry flavour (4.91–6.25), bitterness (4.40–4.74), and astringency (4.35–4.62).

3.4.2. Comparison between samples

A PCA model for scaled sensory data matrix was constructed to compare relations between sensory attributes and samples. First two principal components explained 71% of data variance (Fig. 3). The lingonberry juice control was clearly separated from the fermented samples on PC1. The controls were positively correlating with lingonberry flavour and forest odour on PC1. The lingonberry alcoholic beverage samples were positively correlating with alcohol, ester, and yeasty odours on PC1. However, the fermentation replicates were scattered on PC2 showing no pattern in relation with the sensory attributes on PC2. The PCA constructed only with the berry alcoholic beverages lacked any enhanced patterns for further relations (plot not shown). It is important to visualise the data for Table 2 (shows the actual mean ratings and their statistical differences) together with Fig. 3 (illustrates the correlations and the similarities/differences of samples).

The study by Viljanen et al., 2014 on the lingonberry alcoholic beverages depicted the relation of lingonberry juice controls with fresh

and lingonberry flavour while yeast fermentations were related to fermented flavour, sourness, bitterness, and off-taste. This was in accordance with the present study's perceived lingonberry flavour in controls along with fermented (alcohol, ester, and yeasty) odours in alcoholic beverages (Table 2). Since these flavour characteristics are desirable in alcoholic beverages, they should align with the expectations of consumers. Additionally, minimal sensory perception differences were observed between the alcoholic beverages fermented using varied yeast strains. Interestingly, the alcoholic beverages made with non-*Saccharomyces* yeasts were perceived as sweeter than the initial juice (Table 2). Moreover, the alcoholic beverage made using *M. pulcherrima* strain (pure as well as simultaneous) was also perceived less sour than the juice. As similar trends were not observed using pure *S. cerevisiae*, results highlight the prospects of non-*Saccharomyces* yeasts (in particular, *M. pulcherrima*) in berry alcoholic beverage making.

3.4.3. Relation between volatile and sensory evaluation data

By comparing the two PCA models for volatile compounds (Fig. 2) and sensory attributes (Fig. 3) and their first PCs, the following conclusions were drawn: 1-octen-3-ol (14; with potential earthy properties; Table 1), hexanal (24; green), 2-hexenal (25; green), *p*-menth-1-en-9-al (26; herbal), eucalyptol (54; herbal), and cymene (55; terpenic) in Fig. 2 corresponding to control replicates were related to the lingonberry odour-taste and forest odour in Fig. 3. This is in accordance with their odour descriptors as earthy, green, and herbal despite the usage of different controls for sensory evaluation and volatile analysis from different batches. The RHS volatiles such as 3-methyl-1-pentanol (11; fermented), ethyl ethanoate (31; ethereal), and 3-methylbutyl ethanoate (36; fruity) corresponding to alcoholic beverages in Fig. 2, were related to alcohol and ester odour in Fig. 3. These are in accordance with the odour descriptors for the RHS volatiles with fruity, ethereal, and fermented odours.

From the Correlation loadings plot of PLS-regression model (Fig. 4), same correlations were drawn as previously described while comparing the two PCAs. Along the first factor, alcohol odour (87.3% of Y-variable) is explained by an incidence of ethanol and various higher alcohols (alongside esters and ketones). Ester odour (85.7%) is explained by more esters (along with ketones, ethanol, and higher alcohols). Lingonberry odour (58.9%) is explained by the volatile cluster on the RHS, with the likes of 1-octen-3-ol, 2-hexenal, *p*-menth-1-en-9-al, and 2,3-butanedione (and benzoic acid). Along the second factor, acetic acid contributes to astringency and sourness. Therefore, a decrease in the perceived intensities of lingonberry flavour and forest odour along with an increase in alcohol- and ester- odour occurred upon fermentation. These were in accordance with the decrement in total terpenic content along with increased concentration of total esters and higher alcohols after fermentation. Consequently, the significant loss of the key volatiles attributing to the initial lingonberry aroma and the simultaneous increase in fruity esters could make the end product generally 'fruity' devoid of the particular 'lingonberry' notes. Nonetheless, this research aided in the development of palatable alcoholic beverages from an inherently intense-flavoured lingonberry. This was accompanied by the generation of new flavour information through extension of the latest non-traditional yeast trend to a neglected realm of food bioscience research, the lingonberry.

4. Conclusions

To our knowledge, this is the first promising scientific endeavour at non pH modulated lingonberry alcoholic beverage development. The research was conducted at room temperature (22 °C–25 °C) conditions to study the impact of conventional and non-conventional yeasts on the flavour profile of lingonberry juice. The goal of the research was achieved by development of medium alcohol content (7.34%) lingonberry alcoholic beverages using pure and simultaneous mixed yeast cultures. This was executed through preliminary bioprocessing to decrease benzoic acid value below 0.25 g/L and TSS adjustment to 14 °Brix to ensure proper yeast nutrition for the fermentations.

As expected, yeast fermentation had a significant impact on the sensory and volatile profiles of lingonberry juice. The volatile composition illustrated an increase in total ester and higher alcohol content, along with a decrease in terpene composition (with the exception of linalool and α-terpineol) after fermentation. These coincided with increase in alcohol- and ester- odours along with a decrease in lingonberry flavour perceived intensities after fermentation, compared to the lingonberry juice. At the same time, minimal differences were noted between the used strains due to notably large variations between the biological replications. Interestingly, the non-*Saccharomyces* yeasts, especially *Metschnikowia pulcherrima*, enabled production of sweeter and less sour alcoholic beverages. Although we enable the yeast fermentation of lingonberry in this study and therefore support the utilisation of lingonberry in the beverage industry, the final products may not

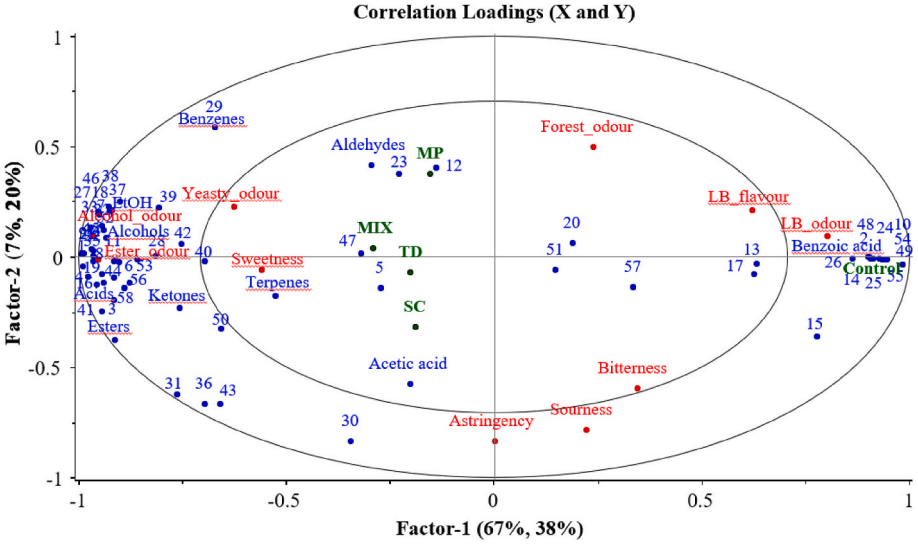

Fig. 4. Correlation loadings plot of PLS-regression model showing interactions between chemical variables (X-data; blue font; n=68, including volatiles, ethanol, acetic acid and benzoic acid) and sensory attributes (Y-data; red font; n=10) in the five sample types as downweighed variables (green): SC *S. cerevisiae*, TD *T. delbrueckii*, MP *M. pulcherrima*, and MIX mixed inoculation of *S. cerevisiae* and *M. pulcherrima*. Numbers of the volatile compounds refer to Table 1.

necessarily encapsulate the initial and unique 'lingonberry' aroma. Thereby, imparting a flavour to the lingonberry alcoholic beverage that resembles any other berry alcoholic beverage. Future studies should focus on fermentation optimisation to eliminate oxidation through usage of carbon dioxide stoppers or bioreactor alongside pathogen elimination trials. Next, specific areas for further research include phenolics, total titratable acidity, and total sugar content analyses to comprehend its impact on colour and flavour, alongside large-scale consumer tests to ensure proper representation of the consumer product preference. Fermentation of lingonberry after benzoic acid decrement led to procurement of lingonberry alcoholic beverages devoid of unpalatable flavours. This has potential as a constructive method for valorisation of the underutilised lingonberry, especially in the Nordic countries.

CRediT authorship contribution statement

Saini Shania: Writing – original draft, Visualisation, Methodology, Investigation, Formal analysis, Data curation, Conceptualisation. **Laaksonen Oskar:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualisation. **Liu Shuxun:** Writing – review & editing. **Yang Baoru:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Kelanne Niina:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualisation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.105393.

Data availability

Data will be made available on request.

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