# Development of Lingonberry Wines with a Sensomics approach

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Albeit categorization of lingonberry as a superfruit courtesy of its bioactive composition, valorisation of the superfruit has remained sub-par ascribed to the challenging flavour profile. In addition, lingonberry fermentation is non-existent due to presence of an antimicrobial benzoic acid.

The study employed baker's yeast mediated benzoic acid decrease, followed by conventional (*Saccharomyces cerevisiae*) and non-conventional (*Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) fermentation to concoct lingonberry wines. Sensomics profiling was conducted through volatile compounds' semi-quantification and sensory evaluation.

After benzoic acid decrement from 0.71g/L to 0.1g/L, wines with an average alcohol content of 7.7% (incubator; IB) and 7.3% (room temperature; RT) were created. A decrease in lingonberry odour and taste with an increase in astringency, bitterness, estery odour, and alcohol odour was detected. This was supported by an elevation in ester and higher alcohol content, along with a decrease in terpenes (except linalool and alpha-terpineol) composition in wines. Minimal statistical difference was observed in sensomics profile between varied yeast strain wines.

Therefore, benzoic acid reduction facilitates fermentation with a decrement in undesirable flavours; despite prolonging processing time. Subsequent studies should optimize RT fermentations to minimize time and eliminate oxidation.

Keywords: benzoic acid, fermentation, lingonberry wine, sensory evaluation, volatiles

# Content

## Abbreviations

| | |
|---|---|
| ACI | Acids |
| ACN | Acetonitrile |
| ALD | Aldehydes |
| ANOVA | Analysis of Variance |
| BEN | Benzenes |
| EST | Esters |
| FA | Formic Acid |
| F-IB | Fermentation in an incubator |
| F-RT | Fermentation at room temperature |
| GC-MS | Gas chromatography–Mass spectrometry |
| HA | Higher Alcohols |
| HS-SPME | Headspace Solid-phase Microextraction |
| IB | Incubator |
| ISTD | Internal Standard |
| KET | Ketones |
| LB | Lingonberry |
| P1 | Pure *Saccharomyces cerevisiae* culture sample |
| P2 | Pure *Torulaspora delbrueckii* culture sample |
| P3 | Pure *Metschnikowia pulcherrima* culture sample |
| PCA | Principal Component Analysis |
| RHS | Right Hand Side |
| RT | Room Temperature |
| S | Sequential *M. pulcherrima- S. cerevisiae* culture sample |
| SPSS | Statistical Package for Social Sciences |
| TER | Terpenes |
| TSS | Total Soluble Sugars |
| UHPLC | Ultra-High-Performance Liquid Chromatography |
| YPD | Yeast Peptone Dextrose |

# 1. Introduction

## 1.1. Berries

In botanical terms, berries refer to stone-less and seed-bearing fruits which are fleshy, develop from a single ovary, and have three layered division of pericarp (Hickey & King, 2000). In layman language, berries may also be referred to as bright, juicy, and soft fruits that are tangy, sour, or sweet (Salo et al., 2021). While the former description may include soft fruits that are not commonly recognized as berries, such as tomatoes, banana, and oranges; the latter may include soft fruits that forego the botanical characteristics of berries like blackberry, strawberries and raspberries. A third category refers to fruits that fall under both categories, with the likes of grapes, blueberry, and lingonberry.

Berries are versatile plant derived foods that bestow a myriad of health benefits due to high vitamin, carotenoid, dietary fibre, and phenolic compound composition (Beattie et al., 2005; Jimenez-Garcia et al., 2013; Olas, 2018). A diet with ample amounts of berries is related to decreased chronic disease and cancer development risk (Baby et al., 2018; Joseph et al., 2014; Kristo et al., 2016; Seeram, 2008).

Berries are a perishable food item because of the high porosity and skin thinness combined with a higher moisture content. This leads to a decrement in barrier properties of berries and an increment in the water activity. These conditions along with a high sugar content makes them a suitable target for microbial attack; thereby decreasing their shelf life. A popular method to store berries efficiently is through immediate freezing after harvest. However, long-term storage of berries requires the application of continuous freezing power source in the supply chain. This could be a limiting factor in outsourcing of berries to countries with a sub-par freezer chain. Further, certain cooking techniques enables shelf life extension of berries. These led to development of products such as jams, juices, and compotes. Alternatively, fermentation of berries to produce fruit wines has gained momentum recently. This is due to an increased interest in sustainable product development, post-harvest loss reduction, and potential health benefits (Liu., 2020).

## 1.2. Lingonberries

Lingonberries (*Vaccinium vitis-idaea*) are small red berries with a tart, bitter, sour, and astringent flavour that are quite popular in the Nordic diet culture. Lingonberries can be classified as superfruits due to the presence of antioxidants, anthocyanins, fibres, minerals, polyphenols, and vitamins (Kowalska et al., 2021). These bioactive compounds confer a lot of potential health effects including antioxidative, anti-inflammatory, anticancer, antiseptic, antiproliferative, anti-obesity, hepatoprotective, and antimicrobial properties (Vilkickyte et al., 2021).

Lingonberries are present in abundance in the Nordic forests as a shrub and are an integral part of the berry foraging season. It is a versatile berry that is used raw or processed such as pressed to form juice, simmered with sugar to make jams, and mashed to develop a compote; amongst other dishes. The presence of natural antimicrobial agent, benzoic acid, increases the shelf life of lingonberries and makes it a preferred primary anthocyanins and phenolics dietary source for Nordic citizens (Dróżdż et al., 2017). Lingonberries account for a majority of the wild berry Finnish yield with an estimated average annual yield of 257 million kg (Turtiainen et al. 2007). Turtiainen evaluated the range of variation in total lingonberry yields using the MASI inventory in 2021. Data from 1997 to 2018 was used and yields ranging from 103 to 412 million kg were calculated.

Despite the health benefits, usage of lingonberries is limited in the food manufacturing industry due to intense levels of sourness, bitterness, astringency, and acidity due to their phenolic compounds and benzoic acid (Laaksonen et al., 2011; Visti et al. 2003). To counter the palatability issue, a plethora of commercially available- lingonberry based products, such as juices, jams, and syrups, are manufactured with the addition of sugar. However, a scientific report on dietary sugars and related health issues by the European Food Safety Authority (EFSA. 2022) demonstrated the detrimental cause effect relation that exists between consumption of added/free sugars and risk of chronic metabolic diseases like obesity, non-alcoholic fatty liver disease, type 2 diabetes, high LDL levels, and hypertension with a certainty level of >50–75% (moderate) for obesity and dyslipidaemia, > 15–50% (low) for non-alcoholic fatty liver disease and type 2 diabetes, and 0-15% (very low) for hypertension. Consequently, the panel recommends that a nutritionally adequate diet must minimize sugar intakes to as low as possible.

Yet, it was observed that lingonberries without added sugars were categorized under the less liked, yet divisive group while sweeter berries like strawberries, bilberries and raspberries were most liked. The former berries were familiar to the Finnish panellists but not extensively used in abundance attributed to sour, bitter and strong flavour perception profile. This illustrates a decreasing trend in consumer acceptability of lingonberry derived products with no added sugars. A solution to this dilemma may be the development of berry wines (Laaksonen et al., 2016).

## 1.3. Berry wines

Berry wines are fruit wines prepared from berries that contain alcohol levels by volume between 12% and 14% (Matei, 2017). Whereas fruit wines with alcohol content typically up to 8% are called ‘ciders’ and those between 8-12% (prepared using honey) generally fall under the wider category of ‘melomels’. The term is inclusive of the fruits that do not fall under the botanical terminology for berry but are perceived as such (layman berries) along with fruits that fall under both definitions (botanical and layman). Bigger botanical berries that are not recognized by common man as berries are excluded from the berry wine classification and referred to as ‘fruit wines.’ Additionally, grape wines are excluded as well are referred to simply as ‘wines.’ Liu (2020) listed five key factors that demonstrated the potential for berry wines’ development as follows:

First, they require a low industrial set-up cost due to similar processing technology used in basic grape wine production. A pre-existing established production set-up could allow efficient and quick industrial transformation of berries using minimal capital investment. Second, they function as a means to create unique wines with varied flavours because of abundance in berry varieties. This could facilitate in meeting the growing consumer demand for novel wines. Third, they help in sustainable valorisation of oversupplied perishable berries while decreasing post-harvest losses. Economic loss courtesy of harvested berries’ spoilage due to poor processing facilities and postharvest management could be solved via wine processing mediated value addition.

Fourth, they follow a rising trend of low-alcohol content wines that were popularized due to a WHO strategy from 2010 to reduce harmful high alcohol level usage. Since berries contain a relatively lower amount of sugar along with higher acid content compared to grapes, they produce lower levels of ethanol. This is desirable since a 2007 World Cancer Research Fund International (WCRF) study stated that a decrease of 7% in breast and bowel cancer risk occurs when alcohol content decreases from 14.2% to 10%. Fifth, a myriad of bioactive compounds present in berries are transformed into the berry wine products after fermentation. This provides an edge over basic grape wine fermentations due to bioactivity.

## 1.4. Lingonberry wines

### 1.4.1. Commercial availability

A few lingonberry wines are commercially available, but they are often diluted with huge volumes of water and fermented with added sugars to ensure yeast viability. This leads to dilution of significant sugars and aromatic compounds in berry wines (Visti et al., 2003). Also, natural occurrence of excessive levels of microbial inhibitor benzoic acid in lingonberries (close to 0.6–1.3 g/L of unbound benzoic acid; active at low pH) acts as a limiting factor in the non-diluted additive-free formulation (Viljakainen & Laakso., 2002).

### 1.4.2. Prior development attempts

While lab-scale attempts at malolactic LAB fermentations by Viljakainen & Laakso using *Oenococcus oeni* in 2002, and by Markkinen using *Lactiplantibacillus plantarum* in 2021 have been unsuccessful due to high benzoic acid levels. Visti et al. (2003) successfully incubated 0.06% *Saccharomyces cerevisiae* in lingonberry juice to decrease benzoic acid levels and obtain a non- inhibitory final benzoic acid content of 0.25 g/L (Warth, 1988). However, a low alcohol content of just 3.5% was obtained. Furthermore, Pärnänen (2017) focussed on sugar removal using direct seven-day fermentation of 5.4 - 6.6% *S. cerevisiae* in cold pressed lingonberry juice, followed by filtration to obtain an alcohol content of 11%.

Additionally, Viljanen et al. (2014) effectively bioprocessed diluted lingonberry juice after pH adjustment 5 to perform *Hanseniaspora/ Lactobacillus- Hanseniaspora* fermentations that resulted in fermented odour and off taste perceptions. Potential modifications in future research, such as reduction in amount of generated ethanol, were suggested to improve the incurred benefits. A pH adjustment step was included since residual benzoic acid post yeast incubation can be inactivated by increasing the pH to values where benzoate ions become dominant (Macris, 1975). A low pH supports formation of protonated benzoic acid ions that have a lipophilic cytoplasmic membrane penetrative effect; once inside the cell, the protons are driven out via benzoic acid ionization; cycling of the protons and benzoic acid ions due to electrochemical gradient through the membrane leads to energy starvation, and gradual death of fermentative yeast. Inhibition of glycolysis via phosphofructokinase inactivation by the benzoate ions is another reason for fermentation failure (Warth, 1988). However, addition of alkalis had an undesirable effect on organoleptic properties, as mentioned above. An overview of varied alcoholic and malolactic fermentation attempts for lingonberry (also referred to as partridgeberry/ cowberry/ redberry/ red whortleberry) are shown in Table 1.

*Table 1. A chronological overview of prior lingonberry fermentation attempts with methods and results using different yeast strains.*

| Strain | Protocol | Observations | Reference |
|---|---|---|---|
| *Oenococcus oeni* | 2-step inoculation of 10% (a) in (b) diluted LBJ- 0.5% yeast extract adaptation medium for 3d/27°C (a: supplemented MRS general medium; 30 °C/3d/X stirring). Fermentation: 25°C/X stirring/14d. | No pH change<br>Failed attempt despite dilution and added nutrients | Viljakainen & Laakso. 2002 |
| *Saccharomyces cerevisiae- Double inoculation* | Yeast biomass↑: 10% yeast in 10% yeast nutrient- glucose media (200rpm/30ºC/3h incubation, 5860g/10min centrifugation, & 0.9% NaCl washing. BA removal: 15-20% single or several 1–3% batch in LBJ, 10min/RT/stirring incubation & 5860g/10min centrifugation. Fermentation: 0.06% yeast (RT/7d/100rpm). | Alc. 3.5%<br>59% ↓ in sugars<br>BA <0.1%<br>↑pre- treatment time; difficulty in commercial production | Visti et al. 2003 |
| *Hanseniaspora uvarum VTT C-11885* | Diluted frozen LJ heated to 80ºC/5 min, ice bath cooled, manual crushed, & pH adjusted. Microbes pre grown in GEM (LAB/ 24h/ 30ºC/anaerobic) & wort sucrose broth (24 h/25ºC/100rpm); cells washed with Ringer´s solution. Fermentation:3d/30ºC/130rpm/ anaerobic (LAB pure) & 7d/25ºC/130rpm (sequential). | ↑ OA (AA), BA, alc., & mannitol<br>↓ glucose & fructose<br>Undesirable fermented flavour | Viljanen et al. 2014 |
| *Lactobacillus plantarum VTT E-78076* | | Minute volatile/ flavour change<br>Poor attempt | |
| *Saccharomyces cerevisiae- Single inoculation* | 500mL cold-pressed LBJ+ E491 fermented anaerobically with 27-33 grams lyophilized yeast to remove sugars for 7 days. | Alc. 11%<br>↑ Cost for non-Baker's strains | Pärnänen. 2017 |
| *L. plantarum DSM 20174T/DSM104/DSM10 0813 & L. argentoratensis DSM16365T* | Microwaved, thawed, blended, pressed, and pasteurized LBJ inoculated with 1% LAB (72h/ 30°C). | Failed attempt attributed to ↑ BA | Markkinen. 2021 |

*↓ & ↑ as symbols for decrease & increase, respectively.*

### 1.4.3. Concept of chaptalization

The composition of lingonberries varies significantly due to their growth in wild uncontrolled natural conditions, as opposed to berries, which are predominantly cultivated under strict supervision, such as grapes and strawberries. Therefore, some modifications are necessary in the method by Visti et al. (2003) to optimize lingonberry wine fermentations. This is to account for the inter batch variability and generate wines with a satisfactory alcohol content. Optimizations including tests to determine effect of yeast biomass increment exclusion, variation in inoculation plus centrifugation rates, usage of unconventional yeasts, mixed inoculations, and impact of dilution with chaptalization should be conducted. Chaptalization is the addition of sugars in unfermented juice (potentially diluted) to increase the alcohol content generation and make-up the total suspended solid (TSS) content to original value. This sucrose addition is meant to nourish the yeast and facilitate in an easier fermentation rather than production of a sweeter wine (MacNeil, 2001). Finally, the added sugars must be consumed entirely by the end of the fermentations. In relation to this study, chaptalization is an integral part of the fermentations as the juices shall be diluted to minimize effect of fluctuating concentrations of inherent antimicrobial compounds in lingonberries; thereby requiring TSS adjustment.

## 1.5. Wine fermentation

### 1.5.1. General definition

Wine fermentation is defined as a biochemical metabolic process that involves the conversion of organic substrate (sugars) into desirable products (ethanol) through the action of microorganisms (usually yeasts). The following equation is a simple depiction of the process.

$C_6H_{12}O_6+H_2O \rightarrow 2CH_3CH_2OH+2CO_2$ – Equation 1 for alcoholic fermentation

### 1.5.2. Fermentation chemistry

Sugars in the must/juice are metabolized during alcoholic fermentation via combination of two pathways (glycolysis and Kreb's/ tricarboxylic acid- TCA) and an intermediate pyruvate breakdown steps. Yeasts are unicellular eukaryotic microorganisms that play a vital role in fermentations by providing certain enzymes that are essential for sugar metabolism. A brief breakdown of the process follows:

In glycolysis, a single six-carbon glucose molecule is split into two three-carbon pyruvate molecules. This pathway is conducted in ten steps via the action of enzymes and separated in two parts i.e., investment phase (steps 1 to 5) and pay-off phase (steps 6-10). Overall ATP (adenosine triphosphate) generation in the latter phase is higher than consumption in the former phase. During intermediate pyruvate breakdown, pyruvate undergoes decarboxylation to form acetaldehyde (intermediate) that reduces to form ethanol. By-products such as glycerol and volatile compounds are generated during the mentioned processes (Liu et al., 2020). Alternatively, pyruvate is dehydrogenated to form acetyl coenzyme A that enables participation in the TCA cycle for organic acid production.

Figure 1 provides an overview of the sugar metabolism process that occurs during fermentation. Focus is majorly on the glycolysis pathway and intermediate pyruvate breakdown.

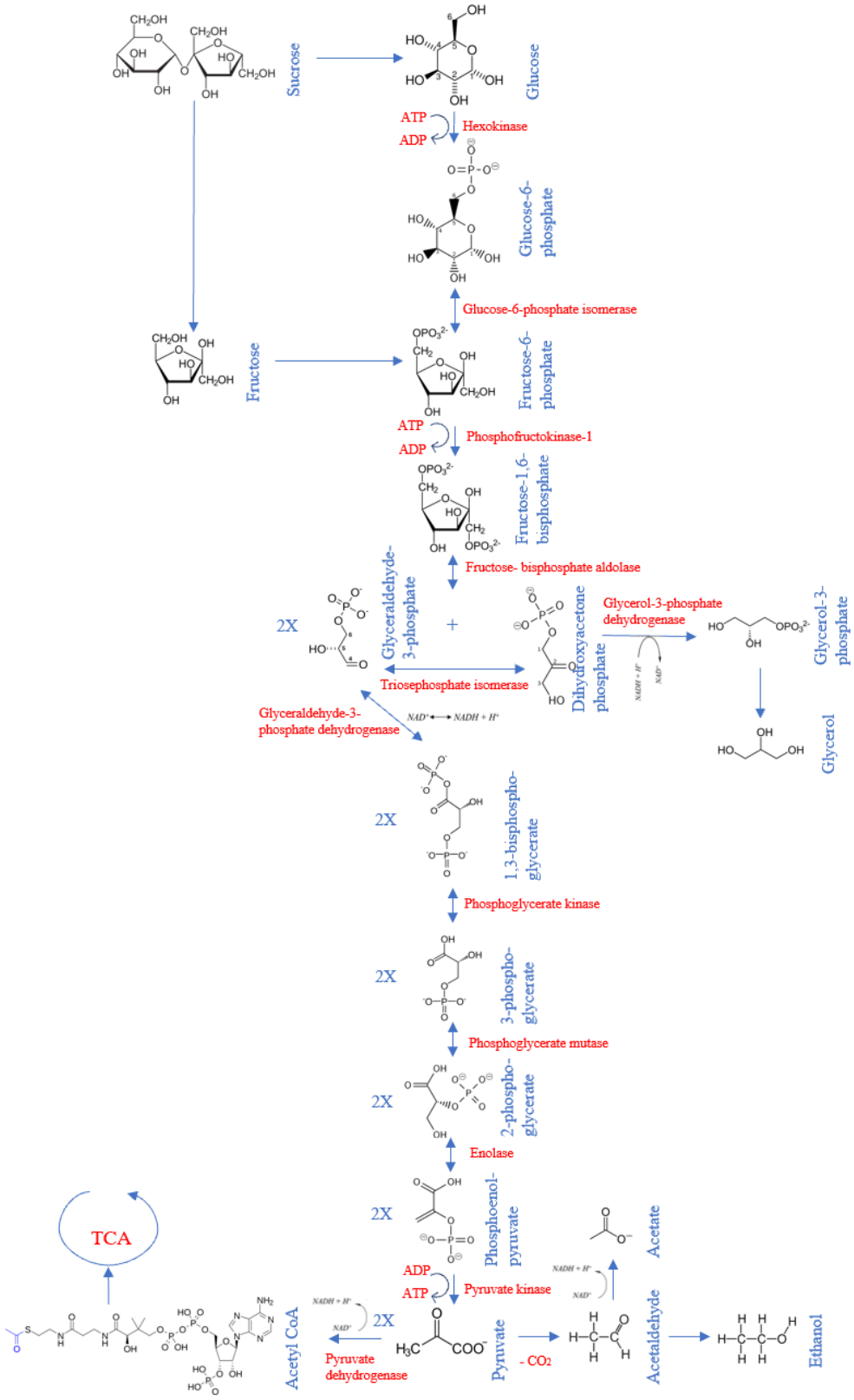



*Figure 1. Fermentation chemistry depicting conversion of sugar to ethanol along with chemical structures. It is a modified version of sugar metabolism from Liu (2020).*

### 1.5.3. *Saccharomyces* yeasts

*Saccharomyces* yeasts are one of the oldest yet most relevant yeasts in wine making. The development of commercial active dry *Saccharomyces* yeasts in the 1960s revolutionized the vinification field (Ciani et al., 2019 and Romano et al., 2019). The advent of this commercial yeasts enabled standardization, improved efficiency, and ensured control over the final product. A total of eight species are present under this genus. Out of these, *Saccharomyces* species are an ideal fermentative yeast due to the high fermentability, high tolerance to harsh growth conditions, fast exponential growth rate, ease of parameter manipulation, effective yields and low spoilage risks (Albergaria & Arneborg, 2016).

### 1.5.4. Non- *Saccharomyces* yeasts

Traditionally, *Saccharomyces* yeast strains have been extensively utilized in berry wine production as discussed in section 1.4.3. However, Non-*Saccharomyces* yeasts are increasingly being used for novel wine development due to greater variation in strains, related flavour profile impartment (via varied enzyme/ metabolite generation), reduced ethanol production, control of spoilage microflora, and colour stabilization. This is a contrasting development compared to initial perception of these yeasts as a spoilage genera (Padilla et al., 2016). The non-conventional yeasts relevant to this study are discussed below:

*Torulaspora delbrueckii*: It is the first non-conventional yeast that was utilized at industry level (Benito, 2018). First, compared to other non-*Saccharomyces* yeasts, it falls under the most popular strains due to high fermentability along with minimal off-flavour generation with lower acetic acid generation in hyperosmotic solutions (Bely et al., 2008). Second, compared to *S. cerevisiae*, it improves sensory quality of wines via decreased undesirable volatile compounds' production like hydrogen sulphide (Belda et al., 2015). Third, it enhances wine aromatics and palate feel by enhancing wated esters' perception sans development of a revolting flavour profile (Lallemand, 2013). Mixed inoculations of *T. delbrueckii* and *S. cerevisiae* ensure maximal ethanol production along with modification of flavours. Sensory evaluation comparison between sequential and pure *S. cerevisiae* wines demonstrated a better overall perception in fermentation using the former method (Loira et al., 2014).

*Metschnikowia pulcherrima:* It is also a commercialized and popular non-*Saccharomyces* strain due to its positive impact on wines' volatile profile. It possesses a specific property through which it releases α-arabinofuranosidase and β-glucosidase enzymes. This has an impact on desirable varietal aroma generation, such as terpenes and volatile thiols (Lallemand, 2013; Jolly et al., 2014). Furthermore, it has a strong antimicrobial defence versus wild spoilage strains (Oro et al., 2014) and mixed inoculations with *S. cerevisiae* are recommended to enhance preferred compounds' production, such as acetate esters, ethanol, and glycerol (Varela et al., 2017; Contreras et al., 2014; and Canonico et al., 2019).

### 1.5.5. Impact of fermentation on flavour

The organoleptic perception of wines is determined by numerous components including the acid, sugar, anthocyanin and volatile content. Wine aroma/ odour is categorized under three groups, i.e., varietal/ primary (corresponds to type of raw material), fermentation/ secondary (corresponds to process parameters), and bouquet/ tertiary (corresponds to ageing transformation) (Padilla et al., 2016). The primary and secondary aromas can be manipulated via usage of independent non-*Saccharomyces* strains (pure cultures) and/or through association of *S. cerevisiae* with the former strains (mixed cultures) for a symbiotic relation of stuck fermentation avoidance and unique aroma development, respectively. Further, mixed fermentation inoculation may be performed either through simultaneous or sequential inoculation. In simultaneous inoculation, high cell concentration inoculum of selected unconventional yeasts is inoculated alongside *S. cerevisiae*, but in sequential inoculation, unconventional yeast (e.g. *T. delbrueckii* or *M. pulcherrima)* is first inoculated in high-level and after 24-48 hours, *S. cerevisiae* is inoculated. The impact of mixed culture inoculations along with unconventional yeast on grape wines' aroma compounds' precursors are presented in Tables 2 and 3, respectively.

*Table 2. Changes in secondary (2º) aroma compounds' precursors after mixed culture fermentations in grape wines.*

| **Mixed Strains** | **Type** | **Effect on 2º Aroma Compounds' Precursors** |
|---|---|---|
| *M. pulcherrima/ S. cerevisiae* | Simultaneous | ↓ $CH_3COOH$<br>↑ $C_2H_5OH$, $CH_3CO_2R$ & higher alcohols |
| | Sequential | ↑ $C_2H_5OH$, $CH_3CO_2R$, α-Terpineol & glycerol |
| *T. delbrueckii/ S. cerevisiae* | Simultaneous | ↓ $CH_3COOH$ & $CH_3CHO$<br>↑ $C_2H_3O_2^-$ (acetate ion), $C_6H_{10}O_2$ (ethyl ester), $C_2H_5OH$ |
| | Sequential | ↓ Fatty acids & higher alcohols<br>↑$C_2H_3O_2^-$, $C_6H_{10}O_2$, $C_2H_5OH$, α-terpineol, linalool, overall perception & anthocyanins |

↓ & ↑ as symbols for decrease & increase, respectively. Summarized from Bely et al. 2008, Benito. 2018, Canonico et al. 2019, Contreras et al. 2014, Escribano-Viana et al. 2019, Francis et al. 2005, González-Royo et al. 2015, Jolly et al. 2014, Loira et al. 2015, Puertas et al. 2017, Renault et al. 2015, Varela et al. 2017, Welke et al., 2014, Zhang et al. 2018, and Zhang et al. 2022 & Padilla et al. (2016) Table 3.

*Table 3. Changes in primary (1º) & secondary (2º) aroma compounds' precursors upon unconventional fermentations in grape wines from literature.*

| **Enzyme/1º Aroma Precursor** | | | | | |
|---|---|---|---|---|---|
| β-D-glucosidase | α-L-arabinofuranosidase | α-L-rhamnosidase | β-D-xylosidase | | Carbon-sulphur lyase |
| ✓ ✓ | X X | X X | ✓ X | | ✓ ✓ |
| Rosi et al., 1994; Fernández-González et al., 2003; Rodríguez et al., 2004, 2010a; González-Pombo et al., 2008; Zott et al., 2011; Hernández-Orte et al., 2008; Maturano et al., 2012; Cordero-Bueso et al., 2013; Cuˆ s and Jenko, 2013; Data accumulated from Padilla et al. (2016) Table 1 | | | | | |
| **Hydrocarbon/2º Aroma Precursor** | | | | | |
| Acetic Acid | Higher Alcohols | Acetaldehyde | Esters | Volatile phenols | Sulphur compounds |
| - ↓ | ↑ - | ↓ - | - ↑ | ↓ ↓ | - ↑ |
| Data accumulated from Padilla et al. (2016) Table 2 | | | | | |
| *M. pulcherrima* & *T. delbrueckii* depicted in green & red fonts, respectively. | | | | | |

*✓, X, ↓, ↑ & - as symbols for presence, absence, decrease, increase & neutral aroma changes in wines, respectively.*

## 1.6. Sensomics

### 1.6.1. General introduction

Sensomics is an integrated approach to uncover hidden relations between sensory perception and variation in raw materials, sensory active compounds plus technological procedures (Vrzal & Olšovská, 2019). Odour receptors (OR) act as the interface between volatile compounds and sensory perception. They involve seven transmembrane helix receptors that are coupled with G-protein and translate external stimuli into internal data, suitable for the neural circuit (Dunkel et al., 2014). It is usually conducted via multivariate statistical analyses of data from bioanalytical test(s) and sensory evaluation results. This enables the researcher to determine whether a plausible relationship exists between these variables. Further, the field of sensomics was established to decipher the most intense-key taste inducers to establish the sensometabolome. Upon sensometabolome decoding, the most active bitter compounds are determined and used as a stimuli for hTAS2R/ligand pairs' deorphanization (i.e., highly selective ligand identification for orphan receptors). Polymorphism of hTAS2R (genetic variation amongst individuals) is speculated to be related with bitterness imparting compounds that impacts food preference of consumers (Hofmann, et al. 2009).

### 1.6.2. Sensory evaluation

Sensory evaluation is a scientific technique that determines and interprets the response to target product(s) based upon sensory perceptions (Anonymous., 1975). It is used to perform a myriad of roles in a product's life cycle for decision making (Kemp et al., 2009). First, during product conceptualization stage, it helps to identify sensory attributes that enable market acceptability, determine target consumer segment, and evaluate innovative ideas. Second, during composition check stage, the combination of sensory and instrumental analyses aids in determination and modification of the chemical plus physical properties that influence sensory profile. Third, during scale-up stage, it assists in discerning the impact of raw ingredient and production protocol modification on sensory acceptability. Fourth, during quality control stage, together with microbiological tests, it is used to detect raw material/ product variability and estimate shelf life. Fifth, in marketing stage, sensory data provides evidential support to marketing claims.

Gustation is the perception of taste via dissolution of non-volatile food compounds and subsequent detection by taste receptors of the mouth cavity. While olfaction is the perception of smell/odour via transfer of volatile food compounds (from air to nose) and sensing of these compounds by the olfactory receptors on the nasal epithelium's cilia. Further, the volatile molecules enter the nose either by sniffing (orthonasal) or from throat's back when tasting (retronasal). Furthermore, sensory profiling, also known as descriptive sensory analysis, is a method to describe products and their differences by a trained sensory panel of 10-15 trained assessors that act as an analytical tool (Naes et al.,

2010). In this method, the panel meets to decide on about 10-20 attributes depending upon study goal (generated using a wine flavour wheel in regard to this study) along with calibration of standards' scales. During the final test, a blind tasting is conducted via randomized sample order presentation where the panel is oblivious of inter-sample variability. Intensity scores are chosen by the panel on the line scale with numerical pointers (e.g. from 0 to 10) to simplify sensory data processing for statistical analyses. In relation to this study, emphasis shall be on intensity determination of attributes generated during sensory profiling panel training. These could include sweet, sour, bitter, astringent, and lingonberry taste sensations along with odour descriptors such as lingonberry, yeasty, and alcohol odours, typically associated with lingonberries and wines. There is a lack in sensory studies of lingonberry wines. Therefore, research on sensorial perception of the whole *Vaccinium* genus wines developed using various yeasts is presented in Table 4.

*Table 4. An overview of sensory evaluation studies of Vaccinium genus wines.*

| # | Common name | Scientific name | Sensory evaluation protocol | Inference | Reference |
|---|---|---|---|---|---|
| 1 | Lingonberry | *Vaccinium vitis-idaea* | Descriptive sensory analysis with 11 trained assessors.<br><br>2 replicate sessions using line scale.<br><br>Samples were evaluated for taste, odour, colour, viscosity, and off-taste. | *Hanseniaspora uvarum* VTT C-11885 fermentation led to fermented flavour, sourness, bitterness, and off-taste. | Viljanen et al., 2014 |
| 2 | Bog bilberry | *Vaccinium uliginosum* | Check-all-that-apply (CATA) and hedonic scaling tests with 93 untrained volunteer assessors.<br><br>Samples were evaluated for appearance, odour, and flavour based on the Wine Aroma Wheel®. | All commercially available samples showed fruity-, blueberry-, floral- odours and sour-, mouth puckering- and sweet-flavours.<br><br>Panel preferred fruity and floral samples. Ginger, chilli, Chinese herbs, and liquorice led to lower rating/dislike. These sensory attributes corresponded with the volatile composition of samples. | Lin et al., 2022 |
| 3 | Highbush blueberry | *Vaccinium corymbosum* | Colour, aroma, and taste evaluated by expert tasters. | Partial *S. cerevisiae* fermentation led to better colour and flavour compared to completely fermented wines. Both had high acidity. | Angeles Varo et al., 2022 |
| 4 | Rabbiteye blueberry | *Vaccinium ashei* | Ranking/ ordering test with 20 wine consuming assessors. | *S. cerevisiae* fermented wines that were $CaCO_3$ deacidified (↓ acids) and glucose syrup chaptalized (↑ sugars) were most preferred. Higher phenolics were related | Santos et al., 2016 |

| | | | | | |
|---|---|---|---|---|---|
| | | | Ranking of samples from most to least preferred depending upon colour and flavour. | to improvement of desirable colour, astringency, and bitterness. | |
| 5 | Miscellaneous blueberry | *Vaccinium sect. Cyanococcus* | Descriptive sensory analysis with 19 trained assessors.<br><br>Samples were evaluated for colour, aroma, taste, typicality. | Ultrasonic treatment led to significant promoting of age effect/ improvement in wine quality. | Zhao et al., 2023 |

↓ & ↑ as symbols for decrease & increase, respectively.

After the sensory evaluation sessions, screening of panel sensory data for errors is imperative. It aids in panel reliability determination, panel rectification opportunity, outlier identification, and data handling improvement. Sensory data errors occur due to individual differences in scale usage (variation in scores' mean and range), panel disagreement (lack of consensus in object ranking), repeatability (difference between independent replicates i.e. low precision), and discrimination (gap in discriminability between products). Use of scale errors may arise due to three effects, namely, level (refers to usage of different parts of scale for assessing product differences), range (refers to a very different scale usage for products), and variability (refers to extremely varied replicate error) effects. An illustration depicting use of scale errors is shown in Figure 2. Further, methods employed in panel checking and their tools are listed in Table 5.

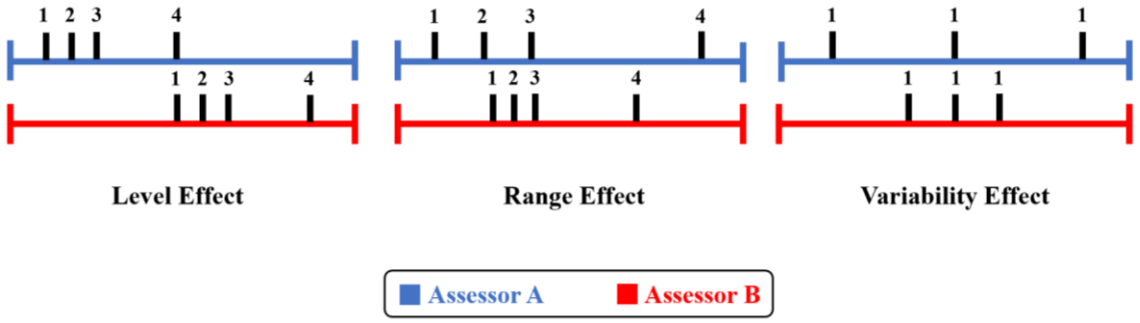



*Figure 2. Effects leading to difference in use of scale sensory data error; where 1,2,3, and 4 depict the samples. Redrawn from Naes et al. (2010).*

*Table 5. Tools for quality control of sensory profiling data with advantages and disadvantages depicted using + and – symbols, respectively.*

| # | Method | Tools | Description | + | - |
|---|---|---|---|---|---|
| 1 | Visual inspection of raw data | Means and standard deviations plot | Tells about the variation in average scores and sample replicate repeatability deviations. | Simple and gives overview of entire raw data. | Not suitable to detect outliers |
| | | Box plots | Illustrates the distribution of the entire data in a single plot. | Detects individual outlier values. | Mean is tricky to locate. |
| | | Histograms | Tells about data distribution for just one attribute and one assessor. | Detects extremely different outliers. | Large number of plots required to analyse whole data. |
| | | Line plots | Provides product profiles averaged across assessors and replicates with individual assessor data superimposed in the same plot. | Spots large replicate differences, highly relevant, and familiar shape. | It is a busy plot. |
| 2 | Mixed model ANOVA | 2-way ANOVA | Used when random replicates with no specific structure are employed. | Aids in elimination of insignificant attributes. | Unreliable assessors may erroneously make attributes insignificant. |
| | | 3-way ANOVA | Used when replicate structure is systematic. | | |
| 3 | Multivariate analyses | Tucker-1: Correlation loadings plot | Presents simultaneous information about importance of different variables. | Helps in dimensionality reduction of the 3-dimensional sensory data for easy visualization. | Low interpretability, not robust against outliers, and information loss. |
| | | Manhattan plot | Provides information about differences between assessors. Used to visualize explained variances for different attributes (from PCA) and each assessor-attribute combination (from Tucker-1). | Easy to interpret and determine explained variances. | No information on sample ranking by each assessor. |
| 4 | Perception ability | F-value column plot | Demonstrates the sorting of F-values according to assessors and the attributes under the assessor on a single plot. | A single plot gives quick overview of assessors' ability to detect differences between products. | Congested plot leads to lower clarity. |
| | | p-MSE plot | Tells about discriminability between samples and score reproduction for a particular attribute. Best assessors have low p-value and low MSE value. | Aids in sensitivity determination of the assessors. | Requires separate plots for each attribute. Not suitable for high number of attributes. |
| 5 | Individual performance versus panel average | Profile plot | Used to visualise level and range differences between the assessors when number of samples is less than 10. | Allows comparison between single assessor and panel scores. | Large number of plots required for each attribute. |
| | | Eggshell plot | It visualizes individual cumulative ranks versus consensus panel ranks. Area between assessor and | Can be used for high number of samples (>10). | Not applicable for non-ranking information. |

| | | | | | |
|---|---|---|---|---|---|
| | | | consensus ranks is inversely proportional to their correlation. | | |
| | | Individual line plots for single samples | Depicts panel average, individual average, and individual replicates for a particular assessor. | Versatile use for any type of design and reveals level effects. | Large number of plots required to represent whole dataset. |

### 1.6.3. Volatile compounds

Volatile compounds are small molecules that are responsible for aroma (odour in sensomics) in wines. Aroma is determined by a plethora of compounds that work cumulatively (Liu, 2020). Further, the perceived intensity of aroma depends on the odour detection threshold and the concentration of compounds. Odour detection threshold is referred to as a compound's lowest concentration detectable to human nose (Ilc et al., 2016). Volatile compounds evolve throughout the development of wines, with a majority of precursors originating from the initial grape variety and dynamically changing post final barrel maturation. Corresponding to section 1.5.5, volatile compounds related to grape wines are generally classified depending on their generation step. Primary or varietal volatile compounds depend on cultivation factors like raw material cultivar, irrigation, and weather (Rapp, 1998). Secondary or fermentation volatile compounds are related to fermentation process parameters. Tertiary or bouquet aroma volatile compounds are linked to ageing transformation. A few details of volatile compounds found in grape wines, their origin, and sub-types are presented in Table 6 according to their classification.

*Table 6. A brief overview of the primary, secondary, and tertiary volatile compounds related to grape wines along with the origin step as compiled from Liu (2020), Ilc et al. (2016), and Styger et al. (2011).*

| # | **Compound Class** | **Sub-Class** | **Origin** |
|---|---|---|---|
| 1 | Primary aroma volatile | Monoterpenes | Products of action of terpene synthase enzyme on mevalonate (synthesized from Acetyl CoA). |
| | | $C_{13}$-norisoprenoids | Products of oxidative cleavage action of carotenoid cleavage dioxygenases enzymes on carotenoids. |
| | | Polyfunctional thiols | Released from non-volatile precursors in bound form. |
| 2 | Secondary aroma volatile | Ethanol | Produced via breakdown of sugars from a combination of glycolysis and an intermediate pyruvate breakdown step (refer to figure 1). |
| | | Higher Alcohols | Produced via two pathways, namely, sugar anabolism and amino acid catabolism. |
| | | Esters | Products of action of alcohol acyltransferase enzymes on alcohol and acyl-CoA. Acetate esters derived from alcohols (ethanol/ higher alcohol) and acetic acid. Fatty acid ethyl esters derived from fatty acids and ethanol. |
| | | Volatile Acids | Acetic acid, short and branched aliphatic acids, and long aliphatic acids are obtained from acetaldehyde |

| | | | |
|---|---|---|---|
| | | | oxidation, amino-, and fatty- acid metabolism, respectively. |
| | | Aldehydes | Products of action of pyruvate decarboxylase enzyme on pyruvate. Act as intermediates for alcohol, acetic acid, and acetoin production. |
| | | Ketones | Exposure to oxygen, congruency with fermentation temperature, and high levels of sulphur dioxide may led to ketone formation. |
| 3 | Tertiary aroma volatile | Phenols | Derived through a microbiological process from ferulic acid/ wood or their precursors (like hydroxycinnamic acid, HCA). |
| | | Acetals | Fused form of aldehyde and alcohols in 1:2 ratio. |

The volatile composition of lingonberries has been researched upon four times till date, namely by, Marsol-Vall et al., 2014, Anjou & von Sydow, 1967, Viljanen et al., 2014, and Amundsen et al., 2023. Additionally Viljanen et al., 2014, studied yeast fermentation's effect on volatile compounds' transformation. An overview of volatile compounds identified in lingonberry juice with odour descriptions that fall under specific functional groups are listed in Table 7 in random retention indices' order.

*Table 7. Volatile compounds in lingonberry juice from different literature references along with their odour descriptions obtained from Good Scents Company.*

| **Volatile Compounds in LB Juice** | **Odours** | **Lit-A** | **Lit-B** | **Lit-C** | **Lit-D** |
|---|---|---|---|---|---|
| **Aldehydes** | | | | | |
| Acetaldehyde | Ethereal | o | | | o |
| Pentanal | Fermented | o | | o | |
| Hexanal | Green | o | | o | o |
| 2-Hexenal | Green | | | | o |
| 2-Furaldehyde/ Furfural | Bready | | o | o | |
| E-2-Heptenal | Green | | | o | o |
| Benzaldehyde | Fruity | o | o | o | o |
| 4-Methoxy benzaldehyde | Anise | | o | | |
| Octanal | Aldehydic | o | | o | o |
| Nonanal | Aldehydic | | | o | o |
| 2-Methyl propanal | Aldehydic | | | | o |
| 2-Methyl butanal | Cocoa | | | | o |
| 3-Methyl butanal | Aldehydic | | | | o |
| 3-Hexenal | Green | | | | o |
| Heptanal | Green | | | | o |
| 2,4-Hexadienal | Green | | | | o |
| E-2-Octenal | Fatty | | | | o |
| 2,4-Heptadienal | Fatty | | | | o |
| E-2-Nonenal | Fatty | | | | o |
| p-Menth-1-en-9-al | Spicy | | | | o |
| 2-Furaldehyde/ Furfural | Bready | | o | o | o |
| **Higher Alcohols** | | | | | |
| 1-Butanol | Fermented | | o | | |
| 1-Pentanol | Fermented | | o | | o |
| 1-Hexanol | Herbal | | o | | o |
| 1-Octanol | Waxy | | o | | |

| | | | | | |
|---|---|---|---|---|---|
| 1-Nonanol | Floral | | o | | |
| 2-Methyl-1-butanol | Ethereal | | o | o | |
| 3-Methyl-1-butanol | Fermented | | o | o | o |
| 2-Pentanol | Fermented | | o | | |
| Benzyl alcohol | Floral | | o | o | o |
| 2-Phenyl ethanol | Floral | | o | | |
| 3-Pentanol | Herbal | | o | | |
| 2-Methyl-2-butanol | Pungent | | o | | |
| 2-Methyl-3-buten-2-ol | Herbal | | o | | o |
| 3-Hexen-1-ol | Green | | o | o | o |
| 1-Octen-3-ol | Earthy | | o | | o |
| 2-Ethyl-1-hexanol | Citrus | | | | o |
| Z-2-Penten-1-ol | Green | | | | o |
| **Ketones** | | | | | |
| Acetone | Solvent | o | | | |
| 2,3-Butanedione/ Diacetyl | Buttery | o | o | o | o |
| 1-Phenylethanone/ Acetophenone | Floral | | o | o | o |
| 3-Hydroxy-2-butanone/ Acetoin | Buttery | | | o | o |
| 4-Octen-3-one | Coconut | | | o | |
| 2-Pentanone | Fruity | o | | o | |
| 6-Methyl-5-hepten-2-one | Citrus | o | o | o | o |
| 2-Octadecanone | Green | | o | | |
| 1-Penten-3-one | Spicy | | | | o |
| 1-Octen-3-one | Earthy | | | | o |
| **Volatile Acids** | | | | | |
| Acetic acid | Acidic | o | | | o |
| Benzoic acid | Balsamic | | o | | |
| 3-Methylbutyric acid | Cheesy | | o | o | o |
| 2-Methylbutyric acid | Acidic | | | o | o |
| Pentanoic acid | Cheesy | | | | o |
| Hexanoic acid | Fatty | | | | o |
| Heptanoic acid | Cheesy | | | | o |
| Octanoic acid | Fatty | | | | o |
| Nonanoic acid | Waxy | | | | o |
| **Esters** | | | | | |
| Methyl acetate | Ethereal | | | | o |
| Ethyl ethanoate/ Ethyl acetate | Ethereal | o | o | o | o |
| 2-Methylpropanoate | Fruity | o | | | |
| Methyl butanoate | Fruity | o | | | o |
| Methyl benzoate | Phenolic | o | o | o | o |
| Ethyl benzoate | Minty | | o | o | |
| Hexyl benzoate | Balsamic | | o | | |
| cis-3-Hexenyl benzoate | Green | | o | | |
| Benzyl acetate/ Benzyl ethanoate | Floral | | o | | o |
| Benzyl benzoate | Balsamic | | o | | |
| Ethyl propionate | Fruity | | | o | |
| Isoamyl acetate/ 3-Methylbutyl ethanoate | Fruity | | | | |
| Hexyl acetate | Fruity | | | | o |
| 3-Hexen-1-yl acetate | Green | | | | o |
| (E)-3-Hexen-1-ol acetate | Fruity | | | | o |
| (E)-2-Hexenyl acetate | Green | | | | o |
| **Terpenes** | | | | | |
| alpha-Pinene | Herbal | o | o | o | o |
| beta-Elemene | Herbal | | | o | |
| Myrcene | Spicy | | o | | |
| Limonene | Citrus | | o | o | o |
| gamma-Terpinene | Terpenic | | o | | o |
| beta-Pinene | Herbal | | o | | o |

| | | | | | |
|---|---|---|---|---|---|
| 3-Carene | Citrus | | o | | |
| Camphene | Woody | | o | | o |
| Verbenone | Camphoreous | | o | | |
| 1-p-Menthene-9-al | Spicy | | o | | |
| Camphor | Camphoreous | | o | | o |
| 4-Terpinenol | Spicy | | o | | o |
| Terpineol | Spicy | | o | | o |
| Perilla alcohol | Green | | o | o | |
| Borneol | Balsamic | | o | | |
| 1,8-Cineole/ Eucalyptol | Herbal | o | o | o | o |
| Longifolene | Woody | | o | | |
| delta-Cadinene | Herbal | | o | | |
| Cymene | Terpenic | o | o | o | o |
| Linalool | Floral | o | o | o | o |
| Carvacrol | Spicy | | o | | |
| para-Cymene-8-ol | Herbal | | o | | |
| beta-Selinene | Herbal | | | | |
| α-Terpinene/ p-Mentha-1,3-diene | Woody | | | | o |
| Terpinolene | Herbal | | | | o |
| trans-Linalool oxide/ Furanoid | Floral | | | | o |
| **Benzenes** | | | | | |
| Styrene | Balsamic | o | o | o | o |
| Toluene | Sweet | | o | | |
| Xylene | Plastic | | o | | |
| Naphthalene | Pungent | | o | | |
| Eugenol | Spicy | | o | | |
| 4-Isopropylbenzyl alcohol | Spicy | | o | | |
| Phenol | Phenol | | o | | |
| Thymol | Herbal | o | | | |

*Lit-A, Lit-B, Lit-C, and Lit-D correspond to studies by Marsol-Vall et al., 2014, Anjou & von Sydow, 1967, Viljanen et al., 2014, and Amundsen et al., 2023. 'o' as symbol for presence.*

Table 8 depicts the transformative trend of volatile compounds in lingonberry juice during fermentation in random retention indices' order, according to Viljanen et al., 2014. To account for a dearth in lingonberry wine literature, the volatile composition of various *Vaccinium* wines is also illustrated. These include studies by Zhang et al., 2019 and Zhang et al., 2020 about cranberry wines (*Vaccinium macrocarpon*); Yuan et al., 2018 about blueberry wines *(Vaccinium sect. Cyanococcus);* Liu et al., 2019 and Liu et al., 2020 about bilberry wines (*Vaccinium myrtillus*); and Wang et al., 2016 and Lin et al., 2022 about bog bilberry wines (*Vaccinium uliginosum*). Lingonberries, also referred to as mountain and lowbush cranberry, are closely related to cranberries. Upon literature comparison, it was observed that many compounds including pentanal, nonanal, 2-methyl-1-butanol, isoamyl alcohol, benzyl alcohol, phenylethyl alcohol, styrene, ethyl acetate, methyl benzoate, ethyl benzoate, isoamyl acetate, acetoin, limonene, eucalyptol, and linalool overlapped in cranberry and lingonberry, as expected. While secondary volatiles overlapped between the *Vaccinium* berries to a great extent, the primary volatiles related majorly to raw material variety showed a varied presence. For instance, limonene was only found in cranberry and LB, eucalyptol in cranberry, blueberry, and LB, p-cymene just in bog bilberry and LB, and linalool in all *Vaccinium* berry literature.

*Table 8. Summation of the volatile composition of Vaccinium genus wines. Fermentative volatile compound transformation is depicted exclusively for the lingonberry study.*

| ***Vaccinium* Berry Type** | **LB** | **Cranberry** | | **Blueberry** | **Bilberry** | | **Bog bilberry** | |
|---|---|---|---|---|---|---|---|---|
| **Volatile compounds** | **Lit-C** | **Lit-E** | **Lit-F** | **Lit-G** | **Lit-H** | **Lit-I** | **Lit-J** | **Lit-K** |
| **Aldehydes** | | | | | | | | |
| E-2-Butenal | + | | | | | | | |
| Pentanal | ↓ | ✓ | ✓ | | | | | |
| Hexanal | ↓ | | | | ✓ | | | ✓ |
| E-2-Heptenal | ↓ | | | | | | | |
| Benzaldehyde | ↑ | | ✓ | ✓ | ✓ | ✓ | | |
| Octanal | - | | | | | | | ✓ |
| Nonanal | ↓ | | ✓ | | ✓ | | ✓ | ✓ |
| 2,4-Dimethyl benzaldehyde | | | | ✓ | | | | |
| 4-Hydroxy-3,5-dimethoxybenzaldehyde/ Syringaldehyde | | | | ✓ | | | | |
| 4-Hydroxy-3-methoxybenzaldehyde/ Vanillin | | | | ✓ | | | | |
| Acetaldehyde | | | | | ✓ | ✓ | | |
| 3-Methylbutanal | | | | | ✓ | ✓ | | |
| (E)-2-Hexenal | | | | | ✓ | | | |
| 2-Methylbutanal | | | | | | ✓ | | |
| Decanal | | | | | | | ✓ | |
| Dodecanal | | | | | | | ✓ | |
| 2-Phenylacetaldehyde | | | | | | | | ✓ |
| **Higher alcohols and Phenols** | | | | | | | | |
| 2-Methyl-1-butanol | ↑ | | ✓ | ✓ | ✓ | ✓ | | |
| 3-Methyl-1-butanol/ Isoamylalcohol | ↑ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ |
| Phenylmethanol/ Benzyl alcohol | ↑ | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| 2-Phenyl ethanol/ Phenylethyl alcohol | + | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 3-Hexen-1-ol | = | | | ✓ | ✓ | ✓ | ✓ | ✓ |
| 2-Ethyl-1-hexanol | + | | | ✓ | ✓ | ✓ | | ✓ |
| 2-Methyl-1-propanol/ Isobutanol | | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ |
| 1-Hexanol | | | ✓ | | ✓ | ✓ | ✓ | ✓ |
| 1-Octen-3-ol | | | ✓ | | | | | ✓ |
| 3-Octanol | | | ✓ | | | | | |
| 3,7-Dimethyl octanol-3-ol | | | ✓ | | | | | |
| 1-Decanol | | | ✓ | | | | ✓ | ✓ |
| 9-Decen-1-ol | | | ✓ | | | | | |
| 1-Pentanol/ Amyl alcohol | | | | ✓ | | | | |
| 2-Hexen-1-ol | | | | ✓ | ✓ | ✓ | | |
| 3-Phenylpropan-1-ol/ Benzene propanol | | | | ✓ | | | | |
| 4-Isopropylbenzyl alcohol/ Cuminic alcohol | | | | ✓ | | | | |
| 3-Phenyl-2-propen-1-ol/ Cinnamyl alcohol | | | | ✓ | | | | |
| Styrene | ↓ | | ✓ | | | | | ✓ |
| 4-Ethylphenol | | ✓ | ✓ | | | | | ✓ |
| 4-Ethyl-2-methoxyphenol/ 4-Ethylguaiacol | | ✓ | ✓ | | | | | ✓ |
| Eugenol | | | ✓ | ✓ | | | | ✓ |
| 4-Methylphenol/ p-Cresol | | | | ✓ | | | | ✓ |
| 2-Methoxy-4-vinylphenol/ 4-Vinylguaiacol | | | | ✓ | | | | ✓ |
| Methyl eugenol | | | | ✓ | | | | |
| Isoeugenol | | | | ✓ | | | | |
| Methyl isoeugenol | | | | ✓ | | | | |
| 1-Propanol | | | | | ✓ | ✓ | | |
| 1-Butanol | | | | | ✓ | ✓ | ✓ | |
| 4-Methyl-1-pentanol | | | | | ✓ | ✓ | ✓ | ✓ |
| 3-Methyl-1-pentanol | | | | | ✓ | | ✓ | ✓ |
| 1-Heptanol | | | | | ✓ | ✓ | ✓ | ✓ |
| 1-Octanol | | | | | ✓ | ✓ | ✓ | ✓ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2,3-Butanediol | | | | | ✓ | | ✓ | ✓ |
| 2-Pentanol | | | | | | ✓ | | |
| 2-Heptanol | | | | | | ✓ | | ✓ |
| 3-Methyl-2-butanol | | | | | | ✓ | | |
| 3-Methyl-1-pentanol | | | | | | ✓ | | |
| 3-(Methylthio)-1-propanol | | | | | | ✓ | | |
| 2-Nonanol | | | | | | | ✓ | ✓ |
| 1-Nonanol | | | | | | | ✓ | ✓ |
| 2-Undecanol | | | | | | | ✓ | ✓ |
| 1-Dodecanol | | | | | | | ✓ | ✓ |
| 2-(2-Ethoxyethoxy) ethanol/ Carbitol | | | | | | | | ✓ |
| Guaiacol | | | | | | | | ✓ |
| 4-Methylguaiacol | | | | | | | | ✓ |
| Maltol | | | | | | | | ✓ |
| Phenol | | | | | | | | ✓ |
| o-Cresol | | | | | | | | ✓ |
| m-Cresol | | | | | | | | ✓ |
| 4-Propylguaiacol | | | | | | | | ✓ |
| 3-Ethyl phenol | | | | | | | | ✓ |
| Syringol | | | | | | | | ✓ |
| trans-Isoeugenol | | | | | | | | ✓ |
| 4-Vinylphenol | | | | | | | | ✓ |
| **Acids** | | | | | | | | |
| 3-Methylbutanoic acid/ Isovaleric acid | ↓ | | | ✓ | | | ✓ | ✓ |
| 2-Methylbutanoic acid | ↑ | | | | | | ✓ | |
| Hexanoic acid | | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| Octanoic acid | | ✓ | ✓ | | | ✓ | ✓ | ✓ |
| Decanoic acid | | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| Benzoic acid | | ✓ | ✓ | | | | | |
| Nonanoic acid | | | ✓ | | | | | |
| 2-Methylpropanoic acid/ Isobutyric acid | | | | | | ✓ | ✓ | ✓ |
| Pentanoic acid | | | | | | ✓ | | |
| Heptanoic acid | | | | | | ✓ | | |
| Acetic acid | | | | | | | ✓ | ✓ |
| Butanoic acid | | | | | | | ✓ | ✓ |
| 9-Decenoic acid | | | | | | | ✓ | |
| **Esters** | | | | | | | | |
| Ethyl ethanoate/ Ethyl acetate | ↑ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Methyl benzoate | ↑ | ✓ | ✓ | ✓ | | | | |
| Ethyl benzoate | ↑ | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| Ethyl propionate | ↑ | | | | | ✓ | | |
| 3-Methylbutyl ethanoate/ Isoamyl acetate | + | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Methyl butanoate | | ✓ | ✓ | ✓ | | | | |
| 2-Methylpropyl ethanoate/ Isobutyl acetate | | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ |
| Ethyl butanoate | | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ |
| Ethyl 2-methylbutanoate | | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| Methyl hexanoate | | ✓ | ✓ | | | ✓ | | |
| Ethyl hexanoate | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Ethyl octanoate/ Ethyl caprylate | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 2-Phenyl ethyl acetate | | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ |
| Ethyl decanoate/ Ethyl caprate | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Ethyl 3-phenylprop-2-enoate/ Ethyl cinnamate | | ✓ | ✓ | | | | | ✓ |
| Ethyl 2-methylpropanoate/ ethyl isobutyrate | | | ✓ | | | ✓ | | |
| Ethyl 3-methylbutanoate/ ethyl isovalerate | | | ✓ | ✓ | ✓ | ✓ | | ✓ |
| Methyl octanoate | | | ✓ | | | | | ✓ |
| Ethyl dodecanoate | | | ✓ | | ✓ | ✓ | ✓ | ✓ |
| Methyl 2-methylbutanoate | | | ✓ | | | | | |
| Heptyl acetate | | | ✓ | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Benzyl acetate | | | ✓ | | | | | |
| Methyl 3-methylbutanoate/ Methyl isovalerate | | | | ✓ | | | | |
| 2-Phenylethyl formate | | | | ✓ | | | | |
| Diethyl butanedioate/ Diethyl succinate | | | | ✓ | ✓ | | ✓ | ✓ |
| Methyl 2-hydroxybenzoate/ Methyl salicylate | | | | ✓ | | | | ✓ |
| Methyl 4-hydroxy-3-methoxybenzoate/ Methyl vanillate | | | | ✓ | | | | |
| 3-Methylbutyl 3-methylbutanoate/ Isoamyl isovalerate | | | | | ✓ | | | |
| Ethyl heptanoate | | | | | ✓ | | ✓ | ✓ |
| Ethyl 2-hydroxypropanoate/ Ethyl lactate | | | | | ✓ | ✓ | ✓ | ✓ |
| Methyl decanoate | | | | | ✓ | | | ✓ |
| Ethyl 9-decenoate | | | | | ✓ | ✓ | ✓ | ✓ |
| Ethyl 4-hydroxybutanoate | | | | | ✓ | | | |
| Methyl acetate | | | | | | ✓ | | |
| Hexyl acetate | | | | | | ✓ | | |
| Ethyl hex-3-enoate | | | | | | ✓ | | ✓ |
| Octyl acetate | | | | | | | ✓ | |
| Ethyl 3-hydroxybutanoate | | | | | | | ✓ | |
| Ethyl nonanoate | | | | | | | ✓ | |
| Ethyl 2-hydroxy-4-methylpentanoate | | | | | | | ✓ | ✓ |
| Ethyl furoate | | | | | | | ✓ | ✓ |
| Ethyl phenylacetate | | | | | | | ✓ | ✓ |
| Ethyl myristate/ Ethyl tetradecanoate | | | | | | | ✓ | ✓ |
| Diethyl malate | | | | | | | ✓ | |
| Ethyl pentadecanoate | | | | | | | ✓ | |
| Ethyl hexadecanoate/ Ethyl palmitate | | | | | | | ✓ | ✓ |
| 2-Methylpropyl hexanoate/ Isobutyl caproate | | | | | | | ✓ | |
| 3-Methylbutyl hexanoate/ Isoamyl hexanoate | | | | | | | ✓ | ✓ |
| 2-Methylpropyl octanoate/ Isobutyl octanoate | | | | | | | ✓ | ✓ |
| 3-Methylbutyl octanoate/ Isoamyl octanoate | | | | | | | ✓ | ✓ |
| 3-Methylbutyl decanoate/ Isopentyl decanoate | | | | | | | ✓ | |
| Ethyl 2-methylprop-2-enoate | | | | | | | | ✓ |
| Ethyl 2-hexenoate | | | | | | | | ✓ |
| Ethyl 2-hydroxyisovalerate | | | | | | | | ✓ |
| Ethyl 2,4-hexadienoate | | | | | | | | ✓ |
| Isoamyl lactate | | | | | | | | ✓ |
| Diethyl malonate | | | | | | | | ✓ |
| Ethyl (E)-4-decenoate | | | | | | | | ✓ |
| Ethyl 2-hydroxybenzoate | | | | | | | | ✓ |
| 2-Phenylethyl 2-methylpropanoate | | | | | | | | ✓ |
| **Ketones** | | | | | | | | |
| 2,3-Butanedione | ↑ | | | | | ✓ | | |
| 1-Phenylethanone | ↑ | | | | | | | |
| 3-Hydroxy-2-butanone/ Acetoin | ↑ | | ✓ | ✓ | ✓ | ✓ | ✓ | |
| 4-Octen-3-one | ↓ | | | | | | | |
| 2-Pentanone | ↑ | | | | ✓ | | | |
| 6-Methyl-5-hepten-2-one | ↑ | | | | ✓ | ✓ | | |
| 3-Octanone | | ✓ | ✓ | | | | | |
| 2-Heptanone | | | ✓ | | | | | |
| 2-Nonanone | | | ✓ | | | | ✓ | ✓ |
| 4-Methyl-2-pentanone | | | | | ✓ | | | |
| 2,3-Pentanedione | | | | | | ✓ | | |
| 2-Undecanone | | | | | | | ✓ | |
| 1-Octen-3-one | | | | | | | | ✓ |
| **Terpenes** | | | | | | | | |
| alpha-Pinene | ↓ | | | | | | | |
| beta-Elemene | ↓ | | | | | | | |
| Limonene | ↓ | ✓ | ✓ | | | | | |
| 1,8-Cineole/ Eucalyptol | ↓ | | ✓ | ✓ | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| para-Cymene | ↓ | | | | | | ✓ | ✓ |
| 3,7-Dimethylocta-1,6-dien-3-ol/ Linalool | ↑ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| beta-Selinene | + | | | | | | | |
| 2-(4-Methylcyclohex-3-en-1-yl)propan-2-ol/ Terpineol | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| beta-Damascenone | | ✓ | ✓ | | | | ✓ | ✓ |
| 2,6-Dimethyl-2,6-octadien-8-ol/ Geraniol | | ✓ | ✓ | | | | | |
| Linalool 3,7-oxide | | | ✓ | | | | | |
| 1,4-Cineole | | | ✓ | | | | | |
| β-Phellandrene | | | | ✓ | | | | |
| Terpinolene | | | | ✓ | | | | ✓ |
| Borneol | | | | ✓ | | | | |
| Myrtenol | | | | ✓ | | | | |
| (E)-Carveol | | | | ✓ | | | | |
| β-Citronellol | | | | ✓ | ✓ | | ✓ | ✓ |
| p-Menth-8-en-3-ol/ 1-Terpinenol | | | | ✓ | | | ✓ | ✓ |
| β-cis-Farnesene | | | | | | | ✓ | |
| α-Farnesene | | | | | | | ✓ | |
| Naphthalene | | | | | | | ✓ | |
| 1,1,6-Trimethyl-1,2-dihydronaphtalene | | | | | | | ✓ | ✓ |
| β-Bisabolene | | | | | | | ✓ | |
| α-Caryophyllene | | | | | | | ✓ | |
| Geranylacetone | | | | | | | ✓ | |
| trans-Nerolidol | | | | | | | ✓ | |
| 2,3-Dihydrofarnesol | | | | | | | ✓ | |
| Farnesol | | | | | | | ✓ | |
| Rose oxide | | | | | | | | ✓ |
| Linalool oxide (furanoid) | | | | | | | | ✓ |
| Nerol oxide | | | | | | | | ✓ |
| 2-Methylnaphthalene | | | | | | | | ✓ |
| Myrcenol | | | | | | | | ✓ |
| Terpinen-4-ol | | | | | | | | ✓ |
| **Others** | | | | | | | | |
| Diethylacetal | ↑ | | | | | | | |
| 2,5-Dimethylfuran | ↑ | | | | | | | |
| 3-(Methylthio)propanol /Methionol | | | ✓ | ✓ | | | ✓ | ✓ |
| 2-Furaldehyde/ Furfural | ↑ | | | | | | ✓ | ✓ |
| β-Ionol | | | | ✓ | | | | |
| 4-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-butanone | | | | ✓ | | | | |
| Dihydro-β-ionol | | | | ✓ | | | | |
| Acetovanillone | | | | ✓ | | | | |
| 1,1-Diethoxyethane/ Acetal | | | | | ✓ | ✓ | | |
| 5-Methyl furfural | | | | | | | ✓ | ✓ |
| 2-Acetylfuran | | | | | | | ✓ | ✓ |
| Butyrolactone | | | | | | | ✓ | ✓ |
| Hexalactone | | | | | | | | ✓ |
| Whiskey lactone | | | | | | | | ✓ |
| Octalactone | | | | | | | | ✓ |
| Nonalactone | | | | | | | | ✓ |
| Decalactone | | | | | | | | ✓ |
| Sotolon | | | | | | | | ✓ |
| C-10-Massoia lactone | | | | | | | | ✓ |
| γ-Undecalactone | | | | | | | | ✓ |
| Dodecalactone | | | | | | | | ✓ |

*Lit-C, Lit-E/ Lit-F, Lit-G, Lit-H/ Lit-I, and Lit-J/ Lit-K corresponds to lingonberry (Viljanen et al., 2014), cranberry (Zhang et al., 2019/ Zhang et al., 2020), blueberry (Yuan et al., 2018), bilberry (Liu et al., 2019/ Liu et al., 2020), and bog bilberry (Wang et al., 2016/ Lin et al., 2022) wines, respectively. ✓, +, -, =, ↑, and ↓ as symbols for presence, addition, removal, no change, increase, and decrease.*

## 1.7. Aim of the study

Utilization of lingonberries in the manufacture of fruit wines is scarce and research on fermentations with non-*Saccharomyces* strains in lingonberry wine formulations is non-existent. Therefore, overall goal of the research is to develop an effective protocol for lingonberry wine production for omission of the necessity to add sugars; followed by sensomics and multivariate studies to determine the best concoction. An illustration of the major goals is presented in Figure 3. The summarized objectives of this thesis are:

i) To develop medium alcohol content- non clarified lingonberry wines with no added sugars, using strains of *Saccharomyces* and non-*Saccharomyces* yeasts.

ii) To conduct chromatographic, spectrophotometric, and sensory evaluations to study the impact of variation in microbial cultures on sensomics profile of developed products.

iii) To perform comparative multivariate analyses for three-way evaluation of probable relation between inoculum type, bioanalytical composition, and sensory characteristics for lingonberry beverages' marketability improvement.

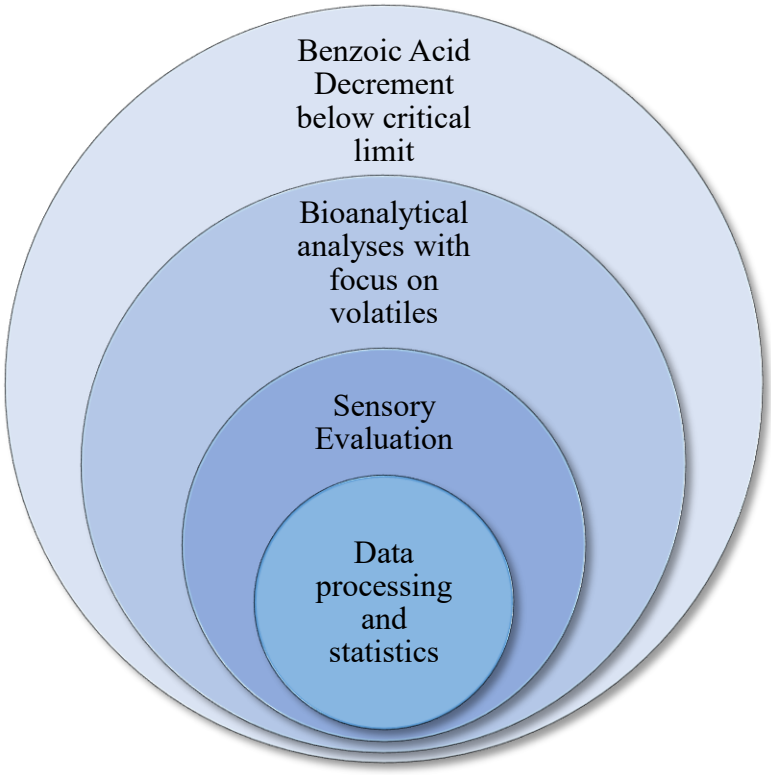



*Figure 3. An illustration depicting the four major concentric goals of present study.*

# 2. Materials and methods

## 2.1. Raw materials, strains, and reagents

### 2.1.1. Berry material

Frozen commercially available lingonberries (*Vaccinium vitis-idaea*) originating from Finland were purchased and stored at -20° C till further use in a freezer, They were from Arctic International Oy Marjex, Arctic International Oy Nurmijärvi, and Pakkasmarja Oy Suonenjoki for fermentation at room temperature (F-RT), fermentation in an incubator (F-IB), and sensory evaluation control I, respectively.

### 2.1.2. Microbiological cultures

Freeze dried active granular *Saccharomyces cerevisiae* Vinoferm Bioferm Rouge (Brouwland, Beverlo, Belgium), and *Torulaspora delbrueckii* BIODIVA™TD291 and *Metschnikowia pulcherrima* FLAVIA®MP346 (Level, Edwardstown, Australia) were used, respectively.

### 2.1.3. Standard compounds and reagents

Pure standards of benzoic acid, ethanol, acetic acid, internal standard (ISTD), 802 µg/mL in methanol of 4-methyl-2-pentanol, for volatile analysis along with the external standards hexanoic acid, decanoic acid, 1-hexanol, 3-(methylthio)-1-propanol, 1-octen-3-ol, phenylethyl alcohol, 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, 1- hexanal, ethyl 3-methylbutanoate, ethyl butanoate, ethyl 2-methylbutanoate, 3-methylbutyl ethanoate, ethyl octanoate, ethyl decanoate, 6-methyl-5-hepten-2-one, ethyl benzoate, 2,3-butanedione, cymene, a-terpineol; and the n-alkane mixture from $C_7$-$C_{30}$ were bought from Sigma-Aldrich Merck KgaA, Darmstadt, Germany. 2-methyl-butanoic acid, 3-methyl-1-butanol, and eucalyptol were bought from Fluka Honeywell, North Carolina, US. 2-hexenal, 1-phenylethanone, and ethyl 2-methylpropanoate were bought from Acros Organics Thermo Fisher Scientific, Massachusetts, US; Chem Service, Pennsylvania, US; and H&R GmbH & Co. KgaA, Hamburg, Germany; respectively. All standards had a purity of ≥ 95 %. Sodium chloride, glycerol, methanol, formic acid, acetonitrile, yeast peptone dextrose (YPD media; Sigma-Aldrich Merck KgaA, Darmstadt, Germany), and Fermentation Stopper (potassium metabisulphite (E224) and potassium sorbate (E202); Bacchus Viiniaine, Viinitalo Melkko Oy, Lahti) were used.

## 2.2. Lingonberry juice

### 2.2.1. Preparation of juice

The frozen lingonberry (LB) were pooled and thawed overnight in a refrigerator at +4 °C, followed by pressing to obtain the juice using the fruit press adjustment of a food processor (Chef Titanium XL, Kenwood, Havant, UK). A 65% yield was recovered post juicing, and the obtained solution was divided into batches in separate glass bottles and frozen until further use at -20 °C.

### 2.2.2. Preliminary trials

Preliminary trials were used to determine the optimal bioprocessing method for LB juice fermentations via benzoic acid decrement. First trial was the initial Baker's yeast biomass increment trial at different concentrations and time periods. The concentrations assessed were 10%, 20%, 30%, 40% and 50% in YPD broth, while time was 0, 1, 2, 3, 4 and 5 hours. Incubations were conducted at 200 rpm and 30 °C. Weights were checked via centrifugation at 3200 rcf/ 10 minutes/ 4 °C for samples incubated for 5 hours. Second trial was the baker's yeast benzoic acid decrease conducted for different pitching rates (8.33% and 12.5%) in 10 minutes (for biomass incubated and non-biomass incubated samples). Third trial was the fungicidal step post benzoic acid removal by sterilization at 121 °C for 15 minutes at 1 bar pressure or pasteurization at 95-97 °C for 30 seconds followed by an ice bath shock. Fourth trial was the pH modification trial where pH was modified to be ~6.82.

### 2.2.3. Chosen bioprocessing method for juice

Trial one lead to negligible weight increment of yeast biomass at varied concentrations and incubation periods. Trial two suggested a higher benzoic acid decrease efficacy at higher pitching rates for non-biomass incubated samples. Trial three demonstrated the negative effect of sterilization on colour and release of residual benzoic acid; along with null pasteurization related benefits. Trial four illustrated the development of off-flavours and undesirable colour change post pH modulation. Hence, aforementioned pre-processing was not performed; cue insignificant removal efficacy in trial. Baker's yeast was added at a 12.5% inoculation rate for a 30-minute incubation period. Baker's yeast was washed with 0.9% normal saline solution and centrifuged at 3200 g for 5 minutes to homogenize the yeast. The residual yeast mass was dispersed with the LB juice, mixed using a sterile loop, incubated at 100 rpm using a magnetic stirrer at room temperature, and separated via centrifugation at 6000 rcf for 10 minutes at +18 °C. This workflow was based on the work of Visti et al. (2003) upon the mechanism devised by Macris on benzoic acid uptake by *Saccharomyces cerevisiae* (1975).

## 2.3. Fermentations

### 2.3.1. Pre-growth of cultures

Freeze dried active granular yeast culture, defrosted stock cultures over ice, or cultured broths/ agar colonies were revived by dissolving 1 gram, 1 vial, or 1 loopful in 100 mL of YPD broth. This was followed by pre-growth at 300 rpm for 24-48 hours at 30 °C. The cfu/mL was estimated using dilution series-spread plating and incubation for 24-48 hours at suitable temperatures in Memmert IF-110 Plus incubator. Dilution plates with 30–300 colonies were chosen for enumeration.

### 2.3.2. Small-scale fermentation

Bioprocessed juice post benzoic acid reduction was diluted using milli-Q water in 1:1 ratio and the total soluble solids (TSS) were made-up to be 14 °Brix using sucrose. Then, it was inoculated 1% v/v with pure cultures of *S. cerevisiae* (P1), *T. delbrueckii* (P2), *M. pulcherrima* (P3), and simultaneously with *M. pulcherrima*- *S. cerevisiae* (S); ensuring that the inoculum had $10^6$ – $10^8$ cfu/mL. For fermentation in room temperature (F-RT), all the samples were prepared in triplicates and kept for 21 days at room temperature. For fermentation in an incubator (F-IB), S was prepared in duplicates and kept at $30^o$ Celsius for 14 days. The process was performed in replicates in dark conditions to ensure minimized effect of repeated volume alterations and potential light oxidation on the samples. The fermentations were performed till a TSS of ~5 $^o$Brix was obtained to ensure consumption of the sugar dissolved initially to assist yeast proliferation. Non-bioprocessed-uninoculated-diluted juice was used as a control fermentation for both the fermentation types as follows: duplicates for F-RT and singlet for F-IB. Carbon dioxide built up was released every alternate day to prevent pressure build in the fermentation bottles. Progress of the fermentations was determined using aliquots taken on alternate days for $^o$Brix (sugar conversion rate), colony counts (cfu/mL), and benzoic acid concentration content estimation. The acetic acid and alcohol content was analysed for first, last, and 2-mid points of fermentation. The headspace volatiles were analysed for the first and last day of fermentation.

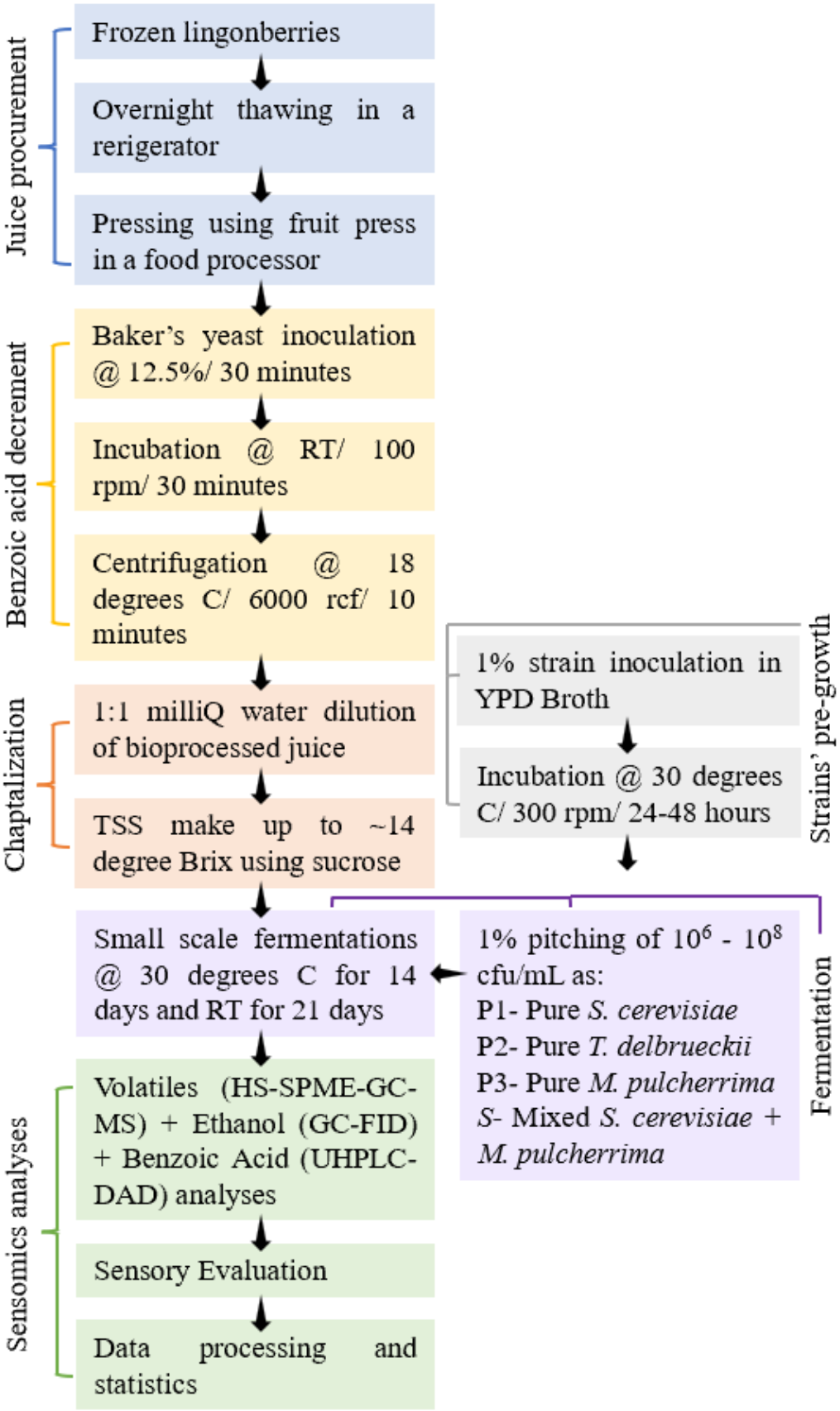



*Figure 4. Flowchart of key processing steps in lingonberry wine development.*

## 2.4. Sensomics analyses

### 2.4.1. Bioanalytical samples' preparation

Benzoic acid, anthocyanins, alcohol, and headspace volatiles were estimated via Kelanne et al. and Liu's (2020) protocols. All analytical samples were prepared in triplicates, apart from F-IB singlets for volatiles analysis.

For benzoic acid, 1:1 (v/v) sample and methanol were dissolved by vortexing for 2 minutes. This was followed by micro centrifuging at 6000 rpm for 10 minutes (Clover Lab, SD110/ 6,000rpm/ 110VAC Centrifuge, Taiwan) to separate pectin and filtering with 0.45 µm PTFE Membrane filters. For ethanol, samples were centrifuged at 3000 rcf for 10 minutes and filtered using 0.45µm RC Membrane filters. For headspace volatiles, 2 mL of centrifuged juice sample (3000 rcf for 10 minutes), 10% (w/v) NaCl, and 10 µL of 802 µg/mL of 4-methyl-2-pentanol in methanol (ISTD) were mixed in a 20 mL HS vial.

### 2.4.2. Bioanalytical analyses

The UHPLC-DAD and GC-FID was analysed using LabSolutions Workstation software and the HS-SPME-GC-MS was operated using Chromeleon.

Benzoic acid were analysed using UHPLC (Nexera 30 Series, Shimadzu Corp., Kyoto, Japan) and the detector was an SPD-M20A diode array detector (DAD). 0.1% (v/v) formic acid in milli-Q water (A) and in acetonitrile (B) were the mobile phases. B solvent's gradient program was: 2−18% increase during 0−14 min, 18% hold during 14−16.5 min, 18-20% increase during 16.5−17.5 min, 20-60% increase during 17.5−18.5 min, and 60-2% decrease during 18.5-20 min. Mobile phase's flow rate was 0.5 mL/min and compounds were separated using a bioZen$^{TM}$ column (Peptide XB-C18, 150 mm × 2.1 mm × 1.7 µm, Phenomenex, Torrance, CA, US). Oven was set at 30 °C, B pump concentration was 2%, and injection volume to 4 µL. UV–vis absorption spectra was measured at a wavelength of 225 nm.

Ethanol level was checked using GC (GC-2010Plus, Shimadzu Corp., Kyoto, Japan) and the detector was a flame ionization detector (FID). 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, US). Temperature of the column oven increased at a rate of 40°C/ minute 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.

For volatile analysis, prepared samples were incubated for 10 minutes at 45 °C in an agitator and a 2 cm DVB/CAR/PDMS fibre (50/30 µm, Supelco, Bellefonte, PA) was conditioned at 240 °C. Then, headspace volatiles were absorbed onto the fibre at 45 °C for 30 minutes using solid phase microextraction (SPME). Next, GC-MS analysis was conducted with a Trace 1310 gas chromatograph and a TSQ 7000 single quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, US). A polar DB-WAX capillary column (60 m length ×0.25mm internal diameter×0.25 µm film thickness, J&W Scientific, Folsom, CA, US) was used. Oven temperature changed at a constant ramp rate of 5 °C/min (hold = 8 min) from 50 °C to 220 °C. Splitless injector mode was set along with an injector temperature of 240 °C. Carrier gas was set at a constant flow of 1.6 mL for helium. Electron impact (EI) ionization mode was used at 70eV for mass spectra detection in full scan mode (range of 33–300 amu) at 0.2 s. Temperatures of the MS transfer line and ion source were set at 220 °C and 240°C, respectively. $NIST_{14}$ (National Institute of Standards and Technology) database aided in probability-predicted conformity of the mass spectra, followed by peak integration across all the samples. Finally, semi-quantification of the identified volatiles was performed via division of areas of target compound with that of the ISTD, according to Elmore (2015). Total soluble solids (TSS) were estimated from °Brix levels using a Brix meter (Atago Co. Ltd., Tokyo, Japan).

### 2.4.3. Sensory evaluation

Sensory evaluation studies were conducted in six steps: one ethical review evaluation, two introductory training sessions and three final analyses sessions. In the first step, a plethora of documents were submitted for approval of the Ethics Committee for Human Sciences, Humanities and Social Sciences Division, University of Turku, Finland; in relation to the ethicality of the proposed research. Documents included in the application were the research plan plus summary, ethics assessment, material management plan, privacy notice, impact assessment, and documents to be delivered to panellists (i.e., consent form, information sheet and recruitment explanation).

In the second step (i.e., training session A), 12 non-allergic and healthy panellists above 18 years of age with prior sensory evaluation experience and alcohol familiarity were recruited post ethical approval. They were introduced to the study aim, familiarized with taste standards' intensities, ortho-nasal odour wheel attribute determination, and asked for a written consent to participate. 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. The third step (i.e., training session B) introduced 10 odour references that were chosen from the Wine Aroma Wheel® originally developed for red or white wines by Ann C. Noble. (University of California Davis; https://www.winearomawheel.com/); and intensity determination of ortho-nasal odour references plus taste standards.

In the latter three steps (i.e., main sensory tests; replicate sessions with triplicate samples but different codes), equal quantities of 5 fermented LB samples and 1 untreated LB juice control (5 mL) poured inside a standard wine glass that bore semblance to a tulip were covered with a petridish, marked with random three-digit codes, and presented in an order based upon the balanced Williams Design model for each panellist where the LB juice was fixed as last sample in all sessions (generated by Compusense). The final tests contained two separate sections: ortho-nasal odour section and taste section. These included intensity line scales, ranging from 0-10 for relevant attributes chosen after the training sessions that are listed in Table 9. The sample arrangement on trays was identical between all panellists on each day to avoid any bias. Water, crackers, and ground coffee were served for palate cleansing conforming with the laboratory requirements set under ISO 8589. The trainings and evaluations were conducted using Compusense20 (Compusense Inc., Guelph, ON, Canada).

*Table 9. A list of the chosen orthonasal odour references and taste standards used in the sensory evaluation sessions.*

| # | **Chosen Attribute** | **Reference/ Standard Materials** |
|---|---|---|
| 1 | Lingonberry Odour | 2 mL of 1:1 water diluted LB juice from Pirkka (Kesko, Helsinki, Finland) |
| 2 | Yeasty Odour | 2 mL of blueberry wine from Chymos (Cloetta Fazer, Sundbyberg, Sweden) |
| 3 | Alcohol Odour | 2 mL of blueberry wine from Chymos (Cloetta Fazer, Sundbyberg, Sweden) |
| 4 | Ester Odour | 1*1 $cm^2$ filter paper with 50 mL of 1:100 ethyl propionate diluted in ethanol |
| 5 | Forest Odour | 1*1 $cm^2$ filter paper with 50 mL of 1:100 linalool, terpen-4-ol, p-cymene, and eucalyptol diluted in ethanol |
| 6 | Sweet Taste | 10 mL of 2% sucrose |
| 7 | Sour Taste | 10 mL of 0.3% citric acid |
| 8 | Bitter Taste | 10 mL of 0.08% caffeine |
| 9 | Astringent Taste | 10 mL of 0.07% aluminium sulphate |
| 10 | Lingonberry Taste | 10 mL of 1:1 water diluted commercial LB juice |

### 2.4.4. Data processing and statistical analyses

For sensory studies, the raw data was imported from Compusense into excel format. Panel performance was evaluated using PanelCheck 1.4.2 (Nofma, Tromsø, Norway) and values were standardized, according to workflow described by Næs, Brockhof, and Tomić in 2010. For volatiles, Chromeleon 7.3.1 CDS (Sunnyvale, California, US) was used to identify the peaks with odour characteristics (verified from Good Scents Company along with external standard runs). For combined sensomics studies, targeted univariate ANOVA test was conducted in SPSS (version 24, IBM Corp., Armonk, NY, US) with replicate as a random factor. Next, non-targeted unsupervised multivariate PCAs were constructed with Unscrambler X (version 10.3, Camo Software, Oslo, Norway).

# 3. Results

## 3.1. Fermentation outcome

### 3.1.1. TSS, ethanol, and cfu/mL check

Progression of the performed fermentations were monitored by measuring a few key parameters. These parameters included a regular check on the decrease in TSS, increase in ethanol content, and maintenance of colony counts. While TSS and ethanol content works on the principle of reduction in $^{o}$Brix value plus increase in ethanol value because of conversion of soluble sugars to ethanol upon action of the strains, cfu/mL estimates viability of strains throughout the fermentations. Both F-RT and F-IB showed a steady decrement in TSS from 14.0 to 5.3 and 5.1, respectively. Next, ethanol increased from 0% to an average of 7.3% (F-RT) and 7.8% (F-IB), which was in accordance with the theoretically predicted value of 9.5%. 1 $^{o}$Brix corresponds to 1% sucrose; therefore, 140 g in 1 L juice constituted the 14 $^{o}$Brix make-up. From Equation 1, it is evident that 1 mole of sucrose gives 4 moles of ethanol. Hence, the given mass of sucrose (i.e., 140 g) was used to estimate ethanol volume; via moles intermediate calculation. The increased duration of F-RT compared to F-IB was presumed to be a repercussion of temperature-light-air flow fluctuations at room temperature and the difference in observed ethanol was conjectured to result from ethanol oxidation (validated in Section 3.1.2). Further, the cfu/mL illustrated that the strains were consistently viable throughout the process and lagged when TSS approached ~5 $^{o}$Brix.

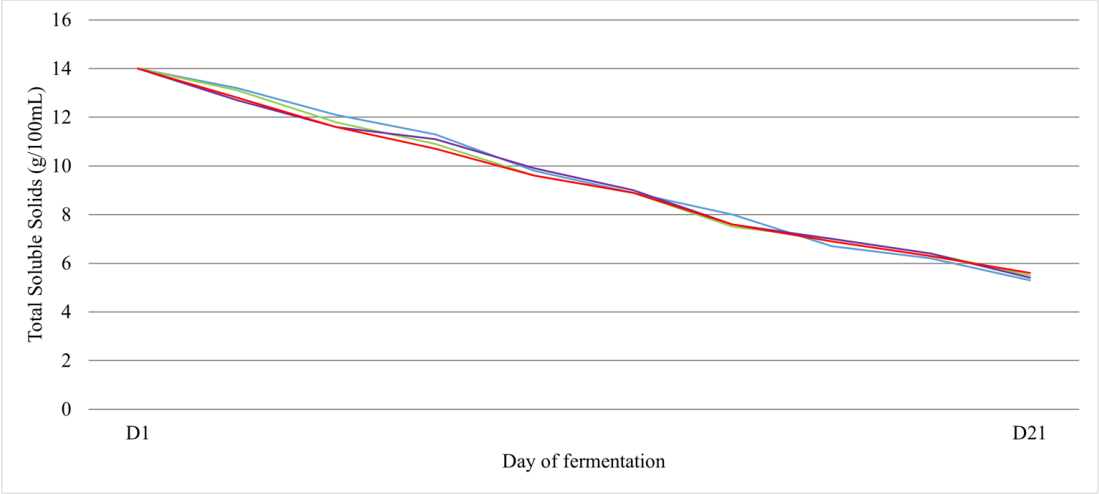
### 3.1.2. Acetic acid and benzoic acid analyses

The quality of the fermented beverages was discerned via acetic acid and benzoic acid estimation. While the former acts as an indicator of oxidative transformation of formulated ethanol, the latter functions as an indirect pointer of strain viability (value < 0.25 g/L) and repugnant sensory perception (sourness and astringency) determination. The observed values remained within satisfactory limits for both the quality checks during fermentations; indicating minimal oxidative damage, antimicrobial action, and unpalatable flavours in the concoctions. Additionally, it was observed that the value of benzoic acid decreased successfully from 0.66g/L and 0.76g/L to 0.04g/L and 0.16g/L for RT and IB juice, respectively after bioprocessing. These values increased during the fermentations. Table 10 depicts the progress and quality of chosen factors throughout the fermentation process for P1, P2. P2 and S; along with separate Cs.

Table 10. Progress of TSS (°Brix), Ethanol, Acetic acid, Benzoic acid and cfu/mL during fermentation of lingonberries at room temperature and in an incubator at 30 °C.

| Samples | Analyses | Fermentation at RT | | | | | | Fermentation in an IB | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Days** | **1** | **5** | **7** | **13** | **17** | **21** | **1** | **3** | **5** | **7** | **9** | **11** |
| P1- *S. cerevisiae* | TSS (°Brix) | 14.0 | 12.1 | 11.3 | 8.0 | 6.7 | 5.3 | 14.0 | 12.1 | 9.6 | 7.0 | 5.8 | 5.1 |
| | Ethanol (%) | 0 | 2.592 | - | 4.458 | - | 7.555 | 0 | 1.496 | 3.761 | 6.093 | 6.574 | 8.066 |
| | Acetic Acid (%) | 0.0007 | 0.0006 | - | - | 0.0009 | 0.0013 | 0.0007 | 0.0005 | 0.0004 | 0.0003 | 0 | 0 |
| | Benzoic Acid (g/L) | 0.02066762 | 0.1037599 | 0.14271876 | 0.16241706 | 0.16712008 | 0.17453578 | 0.08306672 | 0.09230592 | 0.0938479 | 0.09633096 | 0.09629116 | 0.09550216 |
| | Colonies (cfu/mL) | $4.7*10^6$ | $6.7*10^6$ | $7.1*10^6$ | $7.2*10^6$ | $7.2*10^6$ | 0 | $6.5*10^6$ | - | - | $6.8*10^6$ | - | 0 |
| P2- *T. delbrueckii* | TSS (°Brix) | 14.0 | 11.8 | 10.9 | 7.5 | 7.0 | 5.5 | 14.0 | 12.3 | 9.9 | 7.9 | 6.5 | 5.5 |
| | Ethanol (%) | 0 | 2.541 | - | 3.200 | - | 7.557 | 0 | 1.450 | 3.412 | 5.393 | 5.433 | 7.582 |
| | Acetic Acid (%) | 0.0007 | 0.0005 | - | - | 0.0004 | 0.0011 | 0.0007 | 0.0005 | 0.0004 | 0.0003 | 0 | 0 |
| | Benzoic Acid (g/L) | 0.02066762 | 0.09953964 | 0.14133788 | 0.1691104 | 0.18929512 | 0.19609854 | 0.08306672 | 0.10068494 | 0.09493352 | 0.09563178 | 0.09505826 | 0.09679208 |
| | Colonies (cfu/mL) | $5.4*10^6$ | $6.1*10^6$ | $6.9*10^6$ | $5.3*10^6$ | $3.4*10^6$ | 0 | $5.5*10^6$ | - | - | $3.7*10^6$ | - | 0 |
| P3- *M. pulcherrima* | TSS (°Brix) | 14.0 | 11.6 | 11.1 | 7.6 | 7.0 | 5.4 | 14.0 | 12.2 | 9.9 | 7.9 | 6.3 | 5.3 |
| | Ethanol (%) | 0 | 1.411 | - | 3.076 | - | 7.046 | 0 | 1.399 | 3.392 | 5.367 | 6.965 | 7.508 |
| | Acetic Acid (%) | 0.0007 | 0.0003 | - | - | 0.0004 | 0.0003 | 0.0007 | 0.0005 | 0.0004 | 0.0003 | 0 | 0 |
| | Benzoic Acid (g/L) | 0.02066762 | 0.09843906 | 0.1391871 | 0.16452394 | 0.18307684 | 0.19169896 | 0.08306672 | 0.09114226 | 0.09384926 | 0.09503782 | 0.0967061 | 0.09567234 |
| | Colonies (cfu/mL) | $5.1*10^6$ | $8.8*10^6$ | $4.8*10^6$ | $4.2*10^6$ | $3.6*10^6$ | 0 | $2.5*10^7$ | - | - | $4.1*10^6$ | - | 0 |
| S- *S. cerevisiae* + *M. pulcherrima* | TSS (°Brix) | 14.0 | 11.6 | 10.7 | 7.6 | 6.9 | 5.6 | 14.0 | 12.2 | 9.8 | 7.8 | 7.5 | 5.5 |
| | Ethanol (%) | 0 | 1.063 | - | 1.715 | - | 7.197 | 0 | 1.534 | 3.961 | 6.314 | 7.660 | 7.993 |
| | Acetic Acid (%) | 0.0007 | 0.0003 | - | - | 0.0002 | 0.0009 | 0.0007 | 0.0006 | 0.0004 | 0.0003 | 0.0002 | 0 |
| | Benzoic Acid (g/L) | 0.02066762 | 0.08561038 | 0.1220607 | 0.14593514 | 0.1580329 | 0.16436624 | 0.08306672 | 0.09176712 | 0.09328376 | 0.09431172 | 0.09685952 | 0.096267 |
| | Colonies (cfu/mL) | $4.5*10^6$ | $6.5*10^6$ | $7.1*10^6$ | $1.2*10^7$ | $6.4*10^6$ | 0 | $8.5*10^6$ | - | - | $6.1*10^6$ | - | 0 |
| C- Uninoculated non-bioprocessed juice | TSS (°Brix) | 14.0 | 14.4 | 14.5 | 14.5 | 14.4 | 13.6 | 14.0 | 13.1 | 12.5 | 11.4 | 10.8 | 10.3 |
| | Ethanol (%) | 0 | 0 | - | 0 | - | 0.286 | 0 | 0.515 | 1.245 | 2.047 | 2.767 | 3.007 |
| | Acetic Acid (%) | 0.0007 | 0.0008 | - | - | 0.0009 | 0.0009 | 0.0007 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0004 |
| | Benzoic Acid (g/L) | 0.3299238 | 0.2249768 | 0.2346152 | 0.2320106 | 0.2342636 | 0.2402948 | 0.0830667 | 0.0880066 | 0.08907798 | 0.09030032 | 0.09049088 | 0.09445236 |
| | Colonies (cfu/mL) | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |

Line graphs depicting a steady decrease in TSS, generation of ethanol, and progress of acetic acid during RT and IB fermentations are presented in Figures 5, 6, and 7. RT fermentations led to identical decrement in TSS for all inoculations and an increment in final acetic acid concentration except for P3.




*Colours corresponding to different inoculations are as blue for pure S. cerevisiae, green for T. delbrueckii, purple for M. pulcherrima, and red for mixed simultaneous inoculation of S. cerevisiae with M. pulcherrima*

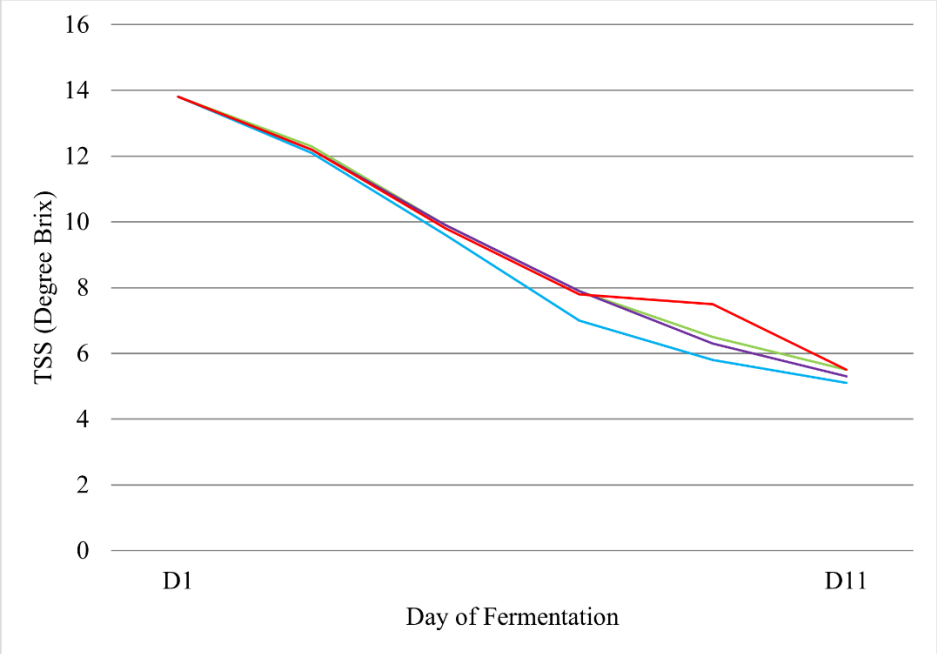
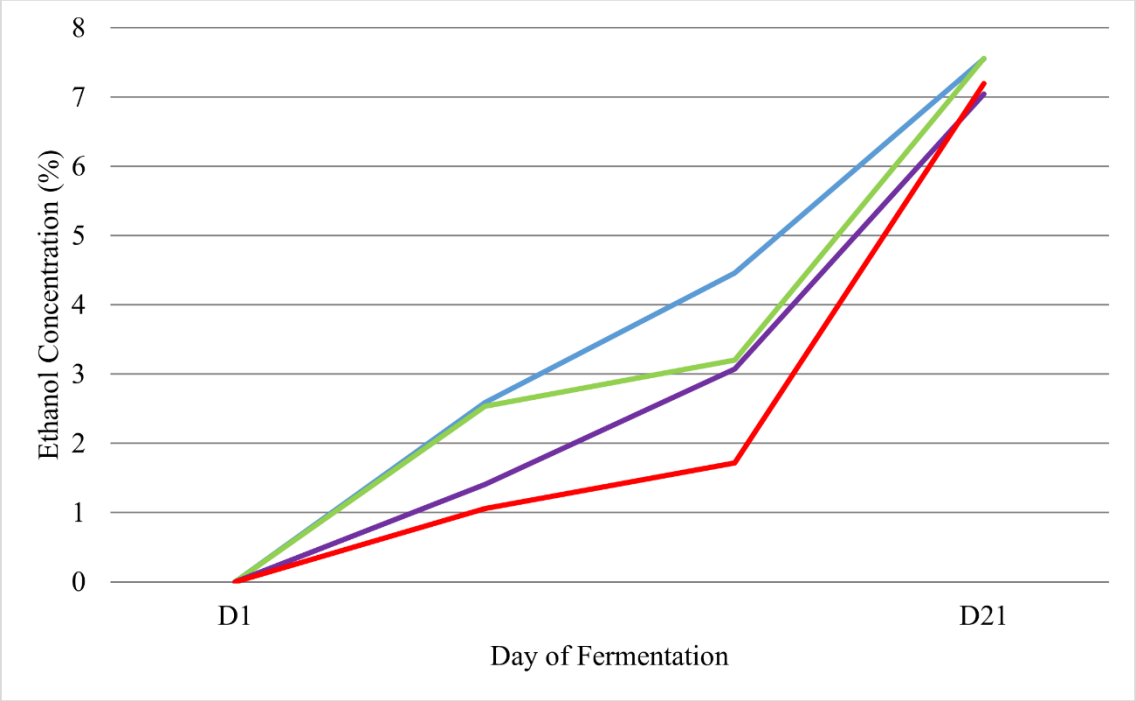



*Figure 5. An average of the total soluble solids change for triplicates over time for RT (top) and IB (bottom) fermentations.*




*Colours corresponding to different inoculations are as blue for pure S. cerevisiae, green for T. delbrueckii, purple for M. pulcherrima, and red for mixed simultaneous inoculation of S. cerevisiae with M. pulcherrima*

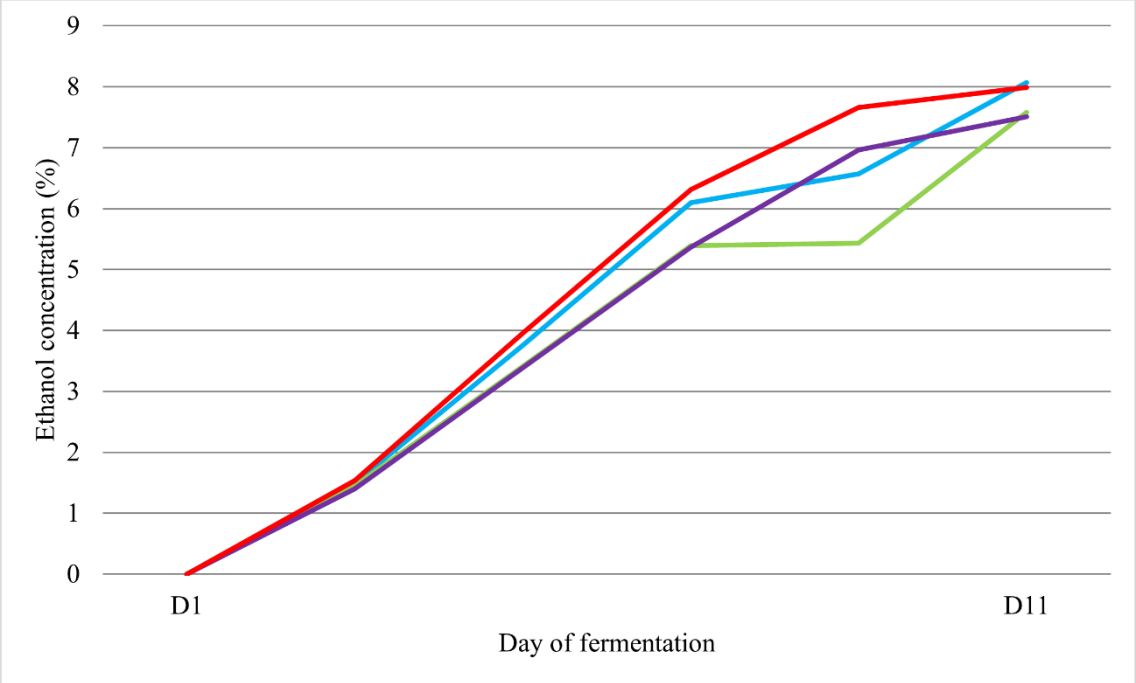
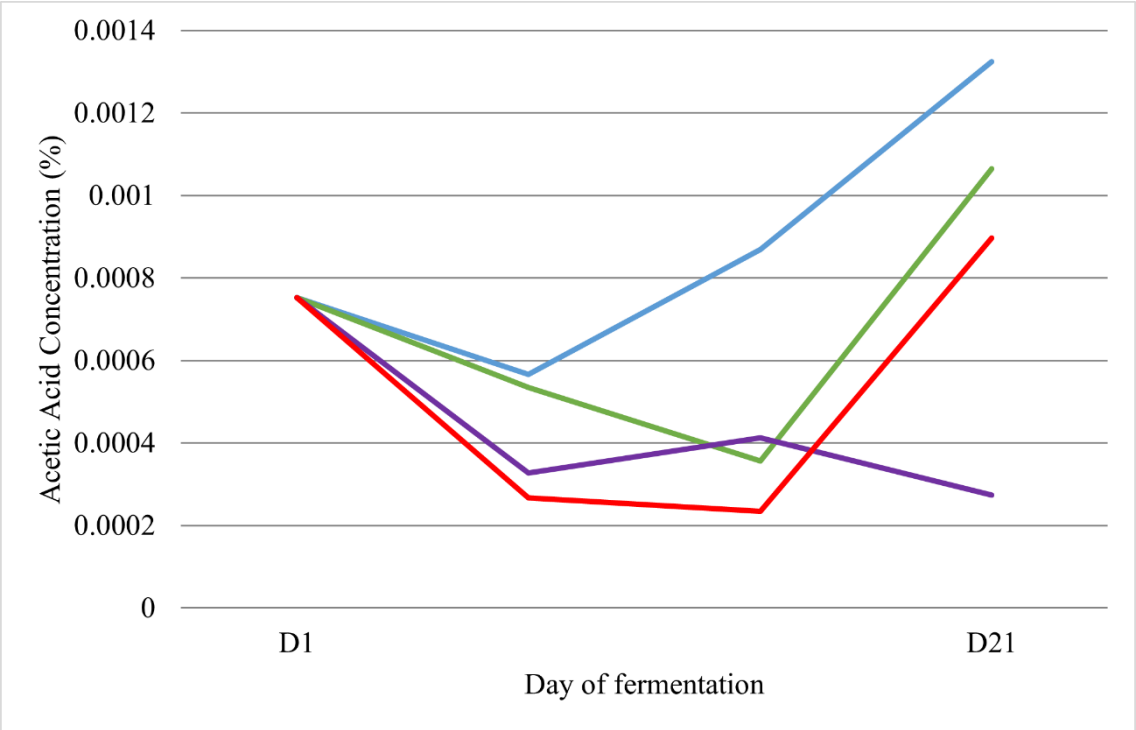



*Figure 6. An average of ethanol generation in triplicates over time for RT (top) and IB (bottom) fermentations.*




*Colours corresponding to different inoculations are as blue for pure S. cerevisiae, green for T. delbrueckii, purple for M. pulcherrima, and red for mixed simultaneous inoculation of S. cerevisiae with M. pulcherrima*

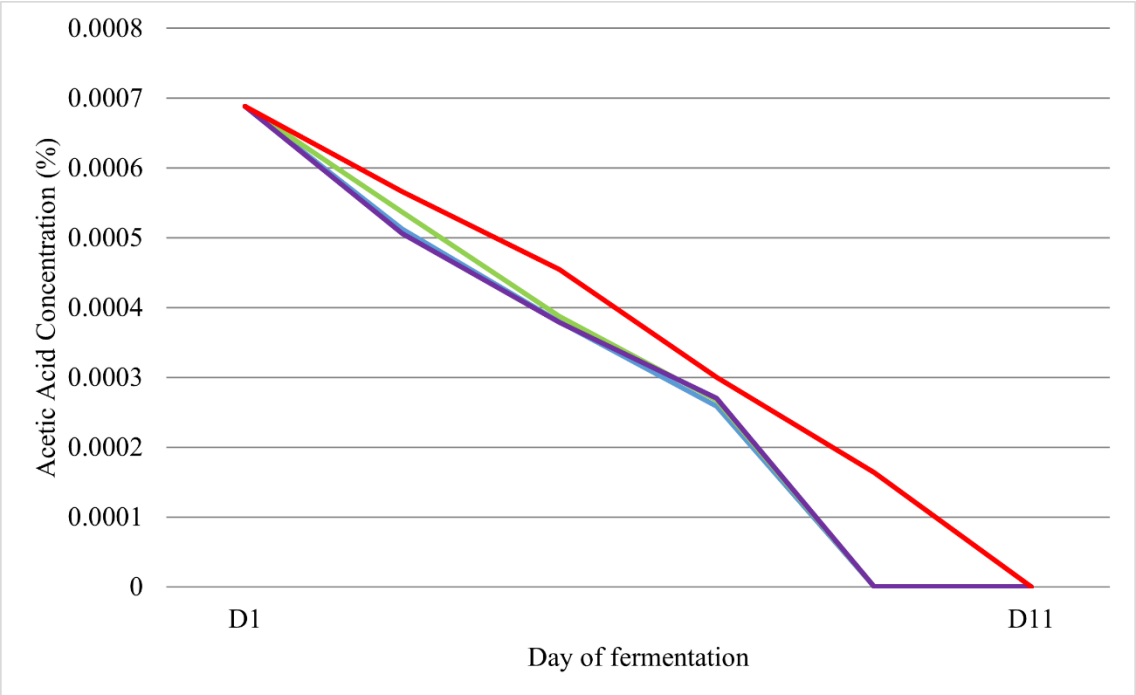



*Figure 7. An average of acetic acid progression in triplicates over time for RT (top) and IB (bottom) fermentations.*

## 3.2. Composition of volatiles

### 3.2.1. Compositional differences

Table 11 provides a summation of the semi-quantified concentrations of volatiles present in the samples. 66 volatile compounds were identified in LB juice and fermented samples. Of these compounds 7 acids, 19 higher alcohols, 6 aldehydes, 3 benzenes, 18 esters, 8 ketones, and 5 terpenes were identified. Generally, higher alcohols (HA) were the most abundant group in P1-RT and S-IB; whilst benzenes (BEN) made up majority of the volatile composition in P2-RT, P3-RT, S-RT, C-RT, and C-IB. Further, the amount of HA in P1-RT was 1250 times that of S-IB; while that of BEN in P2-RT, P3-RT, and S-RT was 20 times and 10000 times than that of C-RT and C-IB, respectively.

In all samples, 2-methyl-propanoic acid was in the highest concentration for all volatile acid (ACI) but nonanoic acid was highest in C-RT. Also, octanoic and nonanoic acid shared the highest concentration with 2-methyl-propanoic acid in S-IB. The second highest was octanoic acid in P1-RT, P3-RT, S-RT; nonanoic acid in P2-RT and C-IB; hexanoic acid in S-IB; and 2-methyl-butanoic acid in C-RT which was absent in rest. Octanoic acid was absent in C-RT. These correspond to acidic, fatty, and waxy odours (Good Scents Company). In all samples, 3-methyl-1-butanol was the most abundant for all higher alcohols (HA) but absent in C-RT. Benzyl alcohol was highest in C-RT. The second highest was phenylethyl alcohol in all fermented wines; 3-hexen-1-ol in C-RT, and benzyl alcohol in C-IB. Additionally, 2-methyl-1-propanol, which was absent in C-RT, shared the second highest concentration in S-IB with phenylethyl alcohol. These correspond to fermented, floral, ethereal, and green odours (Good Scents Company).In all samples, acetaldehyde was in the highest concentration for all aldehydes (ALD) but 2-methyl benzaldehyde in S-RT and hexanal in C-RT were the highest. 2-Methyl benzaldehyde was absent in controls and incubator samples. The second highest was 2-methyl benzaldehyde in all pure RT fermentations; α,4-dimethyl-3-cyclohexene-1-acetaldehyde in S-IB, C-RT, and C-IB; and acetaldehyde in S-RT. C-IB also contained 5-methyl-2-(1-methylethyl)-4-hexenal in second highest concentration which was absent in RT fermentations. These correspond to ethereal, herbal, green, and cherry odours (Good Scents Company).

In all samples, phenylethylene was in highest concentration for benzenes (BEN) concentration except in C-RT which contained methylbenzene. The second highest was methylbenzene in all RT fermented wines and 2,5,8-trimethyl-1,2-dihydro-naphthalene in S-IB, C-RT, and C-IB. Methylbenzene was absent in controls. These correspond to balsamic, sweet, and liquorice odours (Good Scents Company). In all samples, ethyl ethanoate was in highest concentration for esters (EST). S-IB also shared the highest concentration with ethyl benzoate exclusively. The second highest was ethyl benzoate in all, except 4-methyl-2-pentyl acetate in C-IB and ethyl octanoate in S-IB. Ethyl benzoate

and ethyl octanoate were absent in controls. 4-methyl-2-pentyl acetate was absent in RT samples. These correspond to ethereal, fruity, waxy, and minty odours (Good Scents Company).

In all pure RT fermentations, 3-hydroxy-2-butanone was in highest concentration for all ketones (KET). Sequential RT samples had 1-phenylethanone while IB samples had 2-methyl-4-pentanone in highest concentrations. 3-hydroxy-2-butanone was absent in controls and IB samples and 2-methyl-4-pentanone was absent in all RT fermentations. The second highest was 1-phenylethanone in all fermented samples except in S-RT which had 3-hydroxy-2-butanone as second highest. 2,3-butanedione in C-RT and 4-methyl-3-penten-2-one in C-IB were the second highest. These correspond to floral, green, and buttery odours (Good Scents Company). Additionally, 2,3-butanedione (diacetyl) commonly associated with buttery flavour (Viljanen, 2014) was not detected post fermentation; a felicitous finding. In all samples, linalool was in highest concentration for all terpenes (TER). The second highest was α-terpineol in all fermented wines, eucalyptol in C-RT, and terpinen-4-ol in C-IB. S-IB also contained terpinen-4-ol in second highest concentration along with α-terpineol. Eucalyptol and p-cymene were present in all samples except C-RT. These correspond to floral, terpenic, spicy, and herbal odours (Good Scents Company).

*Table 11. Semi-quantitative concentrations of volatile compounds identified from uninoculated non-bioprocessed lingonberry juice controls and lingonberry wine samples fermented at room temperature and in an incubator at 30°C.*

| # | Compound | RI | P1 | P2 | P3 | S | S IB | C RT | C IB | Odour profile |
|---|---|---|---|---|---|---|---|---|---|---|
| **Acids (ACI)** | | | | | | | | | | |
| 1 | 2-Methyl-propanoic acid | 1554 | 20.15 ± 2.4 | 20.19 ± 2.1 | 19.35 ± 1.6 | 20.19 ± 1.3 | 0.01 ± 0 | 0.23 ± 0.3a | 0.02 ± 0 | Acidic |
| 2 | 2-Methyl-butanoic acid | 1664 | ND | ND | ND | ND | ND | 6.37 ± 3.6 | ND | Acidic |
| 3 | Hexanoic acid | 1860 | 4.76 ± 0.7b | 4.06 ± 0.8b | 3.70 ± 0.2b | 4.43 ± 0.8b | 0.004 ± 0 | 0.93 ± 0.3a | 0.002 ± 0 | Fatty |
| 4 | Heptanoic acid | 1975 | ND | ND | ND | ND | 0.001 ± 0 | ND | 0.001 ± 0 | Cheesy |
| 5 | Octanoic acid | 2070 | 16.69 ± 2.5 | 13.89 ± 2.0 | 13.49 ± 1.6 | 17.36 ± 2.5 | 0.01 ± 0 | ND | 0.001 ± 0 | Fatty |
| 6 | Nonanoic acid | 2169 | 11.42 ± 2.1 | 14.21 ± 2.0 | 7.92 ± 3.5 | 8.03 ± 7.5 | 0.01 ± 0 | 6.68 ± 2.5 | 0.004 ± 0 | Waxy |
| 7 | Decanoic acid | 2278 | 8.73 ± 1.2 | 4.80 ± 0.5b | 6.82 ± 1.6 | 8.38 ± 0.1 | 0.002 ± 0 | ND | ND | Fatty |
| **Total ACI** | | | **8.82 ± 1.3** | **8.16 ± 1.1** | **7.33 ± 1.2** | **8.34 ± 1.7** | **0.01 ± 0** | **2.03 ± 1.0** | **0.003 ± 0** | |
| **Higher Alcohols (HA)** | | | | | | | | | | |
| 8 | 4-Penten-2-ol | 1011 | 0.26 ± 0.0b | 0.24 ± 0.0b | 0.26 ± 0.0b | 0.25 ± 0.0b | ND | ND | ND | Fruity |
| 9 | 2-Methyl-1-propanol | 1094 | 17.87 ± 0.5 | 19.78 ± 5.0 | 15.01 ± 1.3 | 17.15 ± 0.4 | 0.01 ± 0 | ND | ND | Ethereal |
| 10 | 3-Methyl-1-butanol | 1211 | 360.89 ± 23.4 | 351.48 ± 45.4 | 328.54 ± 16.1 | 365.22 ± 10.5 | 0.33 ± 0 | ND | 0.01 ± 0 | Fermented |
| 11 | 2-hexanol | 1226 | ND | ND | ND | ND | ND | ND | 0.003 ± 0 | Winey |
| 12 | 1-pentanol | 1255 | ND | ND | ND | ND | ND | 0.33 ± 0.2b | ND | Fermented |
| 13 | 3-Methyl-1-pentanol | 1338 | 2.29 ± 0.3c | 1.57 ± 0.2b | 1.78 ± 0.2bc | 2.29 ± 0.1c | 0.001 ± 0 | ND | ND | Fermented |
| 14 | 2,6-Dimethyl-4-heptanol | 1350 | ND | ND | ND | ND | 0.001 ± 0 | ND | 0.001 ± 0 | Ethereal |
| 15 | 1-Hexanol | 1359 | 1.20 ± 0.1a | 1.20 ± 0.1a | 2.72 ± 2.6a | 1.29 ± 0.1a | 0.0005 ± 0 | 1.14 ± 0.5a | 0.001 ± 0 | Herbal |
| 16 | 3-Hexen-1-ol | 1389 | 0.84 ± 0.1a | 0.78 ± 0.1a | 0.70 ± 0.1a | 0.85 ± 0.0a | 0.0002 ± 0 | 1.34 ± 0.8a | 0.0005 ± 0 | Green |
| 17 | 3,3,5-Trimethyl-cyclohexanol | 1423 | ND | ND | ND | ND | 0.0003 ± 0 | ND | 0.0004 ± 0 | Minty |
| 18 | 1-Octen-3-ol | 1456 | ND | ND | ND | ND | 0.0002 ± 0 | 0.89 ± 0.6b | 0.0002 ± 0 | Earthy |
| 19 | 2-Ethyl-1-hexanol | 1481 | 0.71 ± 0.2a | 0.71 ± 0.0a | 0.57 ± 0.1a | 0.62 ± 0.1a | 0.0004 ± 0 | 1.05 ± 0.0b | 0.0005 ± 0 | Citrus |
| 20 | 2,3-Butanediol | 1523 | 1.53 ± 0.3b | 2.24 ± 0.7b | 1.88 ± 0.3b | 1.93 ± 0.2b | ND | ND | ND | Creamy |
| 21 | 2,4-Hexadien-1-ol | 1570 | ND | ND | ND | ND | ND | 0.06 ± 0.1a | ND | Green |
| 22 | 3-(Methylthio)-1-propanol | 1708 | 1.16 ± 0.1b | 1.22 ± 0.4b | 1.15 ± 0.2b | 1.35 ± 0.2b | 0.001 ± 0 | ND | ND | Meaty |
| 23 | 3,7-Dimethyl-6-octen-1-ol | 1759 | 1.11 ± 0.1b | 1.18 ± 0.2b | 1.01 ± 0.0b | 1.24 ± 0.2b | 0.001 ± 0 | ND | ND | Green |
| 24 | Benzyl alcohol | 1886 | 3.06 ± 0.3a | 3.29 ± 0.3a | 3.23 ± 0.5a | 3.40 ± 0.2a | 0.004 ± 0 | 3.61 ± 2.1a | 0.005 ± 0 | Floral |

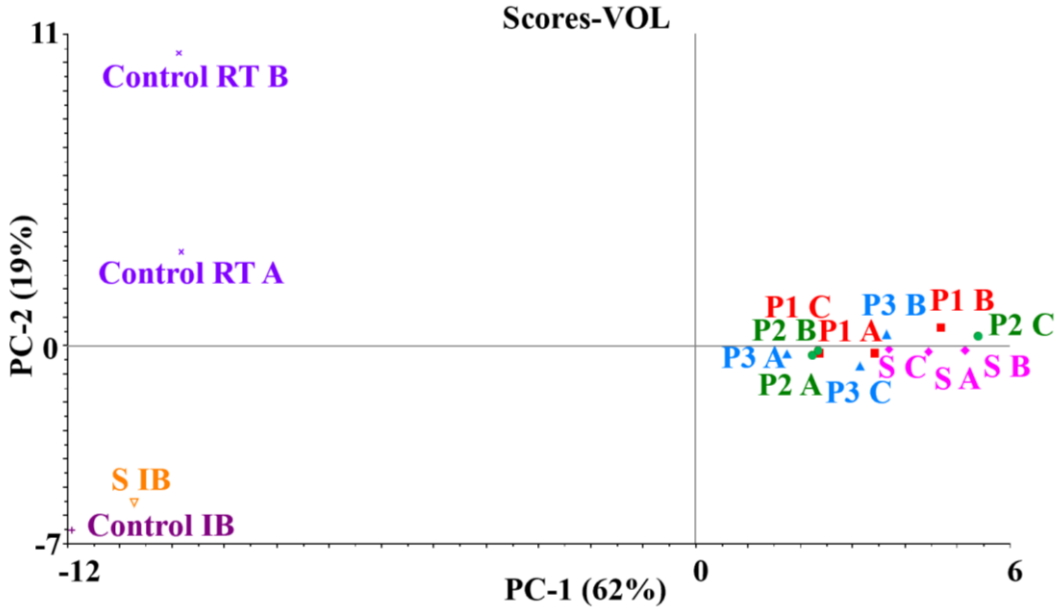
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 25 | Phenylethyl alcohol | 1923 | 91.48 ± 6.2 | 94.54 ± 13.4 | 89.12 ± 2.6 | 99.17 ± 4.6 | 0.01 ± 0 | ND | 0.0006 ± 0 | Floral |
| 26 | 3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol, (E)- | 2042 | 1.59 ± 0.3b | 1.68 ± 0.2b | 1.45 ± 0.2b | 1.71 ± 0.1b | 0.001 ± 0 | ND | 0.0002 ± 0 | Waxy |
| **Total HA** | | | **25.47 ± 1.7** | **25.26 ± 3.5** | **23.55 ± 1.3** | **26.13 ± 0.9** | **0.02 ± 0** | **0.44 ± 0.2** | **0.001 ± 0** | |
| **Aldehydes (ALD)** | | | | | | | | | | |
| 27 | Acetaldehyde | 714 | 3.12 ± 5.4a | 4.97 ± 5.3a | 4.32 ± 3.2a | 0.79 ± 1.4a | 0.004 ± 0 | ND | 0.0005 ± 0 | Ethereal |
| 28 | Hexanal | 1083 | ND | ND | ND | ND | ND | 0.71 ± 0.4b | ND | Green |
| 29 | 2-Hexenal, (E)- | 1219 | ND | ND | ND | ND | ND | 0.31 ± 0.2b | ND | Green |
| 30 | α,4-Dimethyl-3-cyclohexene-1-acetaldehyde | 1620 | ND | ND | ND | ND | 0.0003 ± 0 | 0.35 ± 0.2b | 0.0002 ± 0 | Herbal |
| 31 | 2-Methyl benzaldehyde | 1622 | 1.62 ± 0.1b | 1.70 ± 0.4b | 1.85 ± 0.3b | 1.86 ± 0.2b | ND | ND | ND | Cherry |
| 32 | 5-Methyl-2-(1-methylethyl)-4-hexenal | 1699 | 0.35 ± 0.1b | 0.37 ± 0.0b | 0.41 ± 0.1b | 0.44 ± 0.0b | 0.0002 ± 0 | 0.15 ± 0.1a | 0.0002 ± 0 | Herbal |
| **Total ALD** | | | **0.85 ± 0.9** | **1.17 ± 1.0** | **1.10 ± 0.6** | **0.51 ± 0.3** | **0.001 ± 0** | **0.25 ± 0.1** | **0.0001 ± 0** | |
| **Benzenes (BEN)** | | | | | | | | | | |
| 33 | Toluene/ Methylbenzene | 1041 | 6.61 ± 0.6 | 6.37 ± 1.0 | 5.04 ± 1.0a | 4.66 ± 2.5a | ND | 5.03 ± 0.4a | ND | Sweet |
| 34 | Styrene/ Phenylethylene | 1254 | 55.97 ± 35.4 | 117.25 ± 24.4 | 126.11 ± 68.7 | 125.33 ± 36.1 | 0.01 ± 0 | 0.31 ± 0.2a | 0.01 ± 0 | Balsamic |
| 35 | 2,5,8-Trimethyl-1,2-dihydro-naphthalene | 1999 | 2.00 ± 0.4a | 1.85 ± 0.3a | 2.09 ± 1.0a | 2.20 ± 0.2a | 0.001 ± 0 | 1.20 ± 0.2a | 0.001 ± 0 | Liquorice |
| **Total BEN** | | | **21.53 ± 12.1** | **41.82 ± 8.6** | **44.41 ± 23.6** | **44.06 ± 12.9** | **0.004 ± 0** | **2.18 ± 0.3** | **0.004 ± 0** | |
| **Esters (EST)** | | | | | | | | | | |
| 36 | Methyl ethanoate | 827 | 2.80 ± 2.8a | 2.17 ± 2.6a | 1.22 ± 0.8a | 1.03 ± 1.4a | ND | ND | 0.0004 ± 0 | Ethereal |
| 37 | Ethyl ethanoate | 887 | 125.59 ± 52.9 | 99.79 ± 25.7 | 81.42 ± 20.4 | 105.91 ± 48.8 | 0.04 ± 0 | 0.45 ± 0.3a | 0.01 ± 0 | Ethereal |
| 38 | Ethyl 2-methylpropanoate | 954 | 19.49 ± 3.6 | 19.26 ± 6.2 | 17.06 ± 2.5 | 20.60 ± 0.4 | ND | ND | ND | Fruity |
| 39 | Ethyl butanoate | 1025 | 4.82 ± 0.3b | 4.78 ± 0.9b | 4.50 ± 0.8b | 5.33 ± 0.2 | 0.003 ± 0 | ND | 0.0005 ± 0 | Fruity |
| 40 | Ethyl 2-methylbutanoate | 1042 | 1.19 ± 0.0b | 1.15 ± 0.2b | 1.14 ± 0.3b | 1.40 ± 0.2b | ND | ND | ND | Fruity |
| 41 | Ethyl 3-methylbutanoate | 1060 | 1.72 ± 0.1b | 1.70 ± 0.4b | 1.72 ± 0.3b | 2.07 ± 0.3b | 0.001 ± 0 | ND | 0.0002 ± 0 | Fruity |
| 42 | 4-Methyl-2-pentyl acetate | 1104 | ND | ND | ND | ND | 0.004 ± 0 | ND | 0.005 ± 0 | Fruity |
| 43 | 3-Methylbutyl ethanoate | 1126 | 20.05 ± 9.6 | 14.25 ± 3.9 | 11.79 ± 4.1 | 18.49 ± 10.6 | ND | ND | ND | Fruity |
| 44 | Ethyl hexanoate | 1220 | 8.22 ± 1.4 | 7.32 ± 1.0 | 6.92 ± 0.6 | 9.21 ± 1.3 | 0.01 ± 0 | ND | ND | Fruity |
| 45 | Ethyl 2-hydroxypropionate | 1331 | 0.59 ± 0.1b | 0.66 ± 0.1b | 0.90 ± 0.1c | 0.77 ± 0.1bc | 0.0003 ± 0 | ND | ND | Fruity |
| 46 | Ethyl octanoate | 1421 | 20.45 ± 7.5 | 20.61 ± 7.6 | 20.16 ± 11.2 | 26.83 ± 3.4 | 0.03 ± 0 | ND | ND | Waxy |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 47 | Ethyl decanoate | 1637 | 10.88 ± 0.6 | 9.98 ± 4.3 | 14.93 ± 11.0 | 14.56 ± 3.4 | 0.01 ± 0 | ND | ND | Waxy |
| 48 | Ethyl benzoate | 1666 | 74.82 ± 9.6 | 77.40 ± 12.1 | 65.88 ± 8.1 | 82.17 ± 18.8 | 0.04 ± 0 | ND | ND | Minty |
| 49 | Ethyl 9-decenoate | 1688 | 3.46 ± 0.7a | 3.53 ± 1.8a | 3.67 ± 2.7a | 4.42 ± 0.9a | 0.01 ± 0 | ND | ND | Fruity |
| 50 | 2-Phenethyl acetate | 1826 | 1.36 ± 0.7a | 1.23 ± 0.4a | 0.90 ± 0.3a | 1.57 ± 1.0a | 0.001 ± 0 | ND | ND | Floral |
| 51 | Ethyl dodecanoate | 1829 | 0.73 ± 0.1b | 0.53 ± 0.0b | 0.61 ± 0.2b | 0.75 ± 0.1b | 0.0004 ± 0 | ND | ND | Waxy |
| 52 | Ethyl 3-phenyl-2-propenoate | 2156 | 1.06 ± 0.0b | 1.14 ± 0.2b | 1.06 ± 0.1b | 1.26 ± 0.1b | 0.0003 ± 0 | ND | ND | Floral |
| 53 | Ethyl hexadecanoate | 2255 | 0.73 ± 0.1b | 0.72 ± 0.1b | 0.78 ± 0.2b | 0.83 ± 0.1b | ND | ND | ND | Waxy |
| **Total EST** | | | **16.55 ± 5.0** | **14.79 ± 3.8** | **13.04 ± 3.5** | **16.51 ± 5.1** | **0.01 ± 0** | **0.02 ± 0.0** | **0.001 ± 0** | |
| **Ketones (KET)** | | | | | | | | | | |
| 54 | Acetone | 814 | 0.92 ± 0.1a | 0.35 ± 0.4a | 0.64 ± 0.5a | 1.11 ± 0.8a | 0.0002 ± 0 | 0.34 ± 0.2a | 0.001 ± 0 | Solvent |
| 55 | 2,3-Butanedione | 977 | ND | ND | ND | ND | ND | 0.60 ± 0.4b | 0.0001 ± 0 | Buttery |
| 56 | 2-Methyl-4-pentanone | 1008 | ND | ND | ND | ND | 0.01 ± 0 | 0.44 ± 0.0b | 0.01 ± 0 | Green |
| 57 | 4-Methyl-3-Penten-2-one | 1125 | ND | ND | ND | ND | ND | ND | 0.005 ± 0 | Vegetable |
| 58 | 3-Hydroxy-2-butanone | 1287 | 2.40 ± 0.8ab | 4.45 ± 2.0b | 2.61 ± 1.0ab | 2.52 ± 0.8ab | ND | ND | ND | Buttery |
| 59 | 6-Methyl 5-Hepten-2-one | 1341 | 0.18 ± 0.0a | 0.18 ± 0.0a | 0.17 ± 0.0a | 0.18 ± 0.0a | 0.0001 ± 0 | 0.19 ± 0.1a | 0.0002 ± 0 | Citrus |
| 60 | 1-Phenylethanone | 1670 | 2.38 ± 0.3b | 2.62 ± 0.2b | 2.57 ± 0.5b | 2.63 ± 0.2b | 0.001 ± 0 | 0.76 ± 0.3a | 0.001 ± 0 | Floral |
| 61 | 1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one | 1829 | 0.18 ± 0.0b | 0.19 ± 0.0b | 0.18 ± 0.0b | 0.20 ± 0.0b | 0.0001 ± 0 | 0.08 ± 0.0a | ND | Floral |
| **Total KET** | | | **0.76 ± 0.1** | **0.97 ± 0.3** | **0.77 ± 0.2** | **0.83 ± 0.2** | **0.001 ± 0** | **0.30 ± 0.1** | **0.002 ± 0** | |
| **Terpenes (TER)** | | | | | | | | | | |
| 62 | Eucalyptol | 1212 | ND | ND | ND | ND | ND | 3.59 ± 1.7b | ND | Herbal |
| 63 | p-Cymene | 1298 | ND | ND | ND | ND | ND | 0.24 ± 0.2b | ND | Terpenic |
| 64 | Linalool | 1549 | 9.75 ± 1.1 | 11.19 ± 2.4 | 9.70 ± 0.8 | 10.15 ± 1.2 | 0.01 ± 0 | 3.71 ± 2.5a | 0.003 ± 0 | Floral |
| 65 | Terpinen-4-ol | 1601 | 0.96 ± 0.2a | 0.83 ± 0.1a | 0.88 ± 0.2a | 0.93 ± 0.0a | 0.001 ± 0 | 1.13 ± 0.7a | 0.001 ± 0 | Spicy |
| 66 | α-Terpineol | 1697 | 1.33 ± 0.1b | 1.34 ± 0.1b | 1.29 ± 0.1b | 1.43 ± 0.2b | 0.001 ± 0 | 0.52 ± 0.4a | 0.0004 ± 0 | Terpenic |
| **Total TER** | | | **2.41 ± 0.3** | **2.67 ± 0.5** | **2.37 ± 0.2** | **2.50 ± 0.3** | **0.002 ± 0** | **1.84 ± 1.1** | **0.001 ± 0** | |

*The values depict the means and standard deviations for triplicate. These are multiplied by 100 for easy visualization and ND acts as symbol for not detected. P1, P2, P3, and S are representatives of S. cerevisiae, T. delbrueckii, M. pulcherrima, and mixed simultaneous inoculation of S. cerevisiae with M. pulcherrima. C represents uninoculated non-bioprocessed lingonberry juice controls. a, b, and c represent the grouping of samples based on post hoc Tukey's HSD Test. Retention indices and odour descriptors obtained from NIST and Good Scents Company, respectively.*

### 3.2.2. Non-targeted statistics

A PCA model was constructed using Unscrambler X for the UV-scaled data matrix to analyse the relation between volatile attributes and samples post dimensional reduction. 81% of data variance was explained by the first two principal components. From the scores plot (Figure 8), it was detected that the control and IB samples (C-RT: A/B, C-IB, & S-IB) were negatively correlated to the wine samples' triplicates on PC1. The IB samples (C-IB & S-IB) were negatively correlated to the RT control duplicates on PC2. Further, it was observed that the wine triplicates were scattered quite close to each other with negligible distance. From the loadings plot (Figure 8), a negative correlation between 2-methyl-butanoic acid, heptanoic acid, 2-hexanol, 1-pentanol, 2,6-dimethyl-4-heptanol, 3,3,5-trimethyl-cyclohexanol, 1-octen-3-ol, hexanal, 2-hexenal, α,4-dimethyl-3-cyclohexene-1-acetaldehyde, 4-methyl-2-pentyl acetate, 2,3-butanedione, 2-methyl-4-pentanone, 4-methyl-3-penten-2-one, eucalyptol, and p-cymene (volatiles numbered 2, 4, 11, 12, 14, 17, 18, 28, 29, 30, 42, 55, 56, 57, 62, and 63 from Table 11) versus others (cluster c; RHS volatiles) was detected along P1. The compounds 2-methyl-butanoic acid, 1-pentanol, 1-octen-3-ol, hexanal, 2-hexenal, α,4-dimethyl-3-cyclohexene-1-acetaldehyde, 2,3-butanedione, eucalyptol, and p-cymene (volatiles numbered 2, 12, 18, 28, 29, 30, 55, 62, and 63 from Table 11; cluster a) were negatively correlated with heptanoic acid, 2-hexanol, 2,6-dimethyl-4-heptanol, 3,3,5-trimethyl-cyclohexanol, 4-methyl-2-pentyl acetate, and 4-methyl-3-penten-2-one (volatiles numbered 4, 11, 14, 17, 42 & 57 from Table 11; cluster b) along P2. Here, the former numbers represented C-RT, while the latter constituted incubator samples. Next, upon inspection of the two plots simultaneously, it was concluded that C-RT was positively related to 'cluster a' but negatively related to 'cluster b' plus volatile 56; and vice versa for the IB samples (on P1). The RT fermented wine triplicates were positively related to 'cluster c' (along P2). These were in coherence with the original dimension volatile data.




*Colours corresponding to different inoculations are as red for pure S. cerevisiae, green for T. delbrueckii, blue for M. pulcherrima, and magenta-orange for mixed simultaneous inoculation of S. cerevisiae with M. pulcherrima at room temperature and in an incubator.*

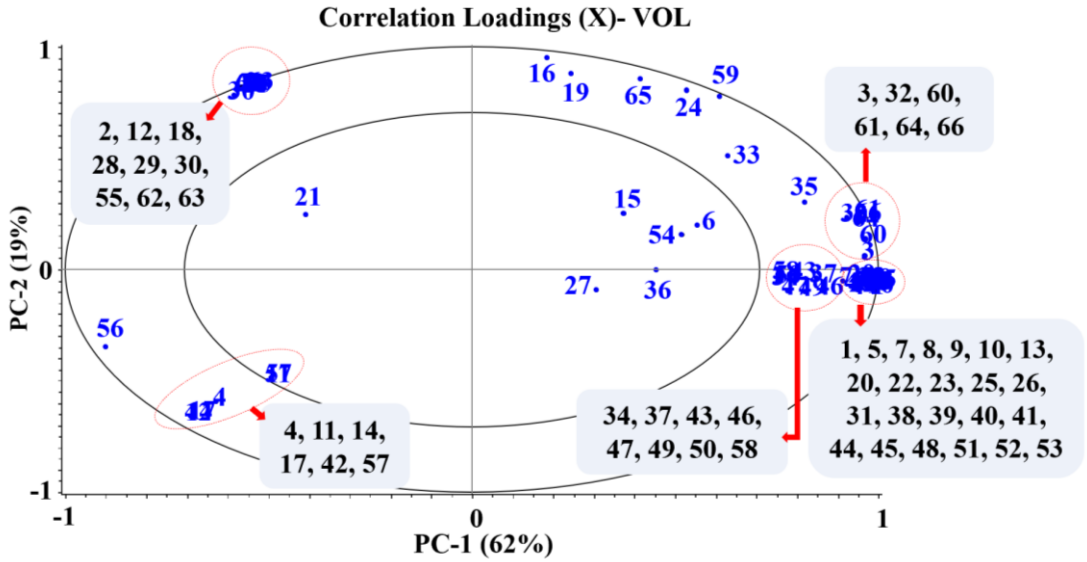



*Figure 8. PCA constructed using Unscrambler depicting the relation between sample triplicates and volatile compounds. The scores plot tells about arrangement of sample triplicates while the loadings plot depicts arrangement of the volatile compounds. Here, the numbers depict various volatile compounds that are listed in Table 11.*

## 3.3. Sensory perceptions

### 3.3.1. Panel performance

Each of the panellist may contribute to individual differences attributed to misunderstandings during training sessions, varied use of attributes, noise perception variation, and different use of scale (Næs et al., 2010). Therefore, it is integral to check performance of the panel for sensitivity, reproducibility and anomalies using PanelCheck. Three main types of plots were used to determine these panel characteristics as follows: 3-way ANOVA plot for identifying significant attributes, Tucker-1 plot for detecting outliers, and p-MSE plot to ascertain sensitivity.

First, the overall 3-way ANOVA overview plot for F values renders systematized colour coded bar graphs where statistical significance decreases from red to orange to yellow corresponding to $p<0.001$ to $p<0.01$ to $p<0.05$ respectively, with grey depicting insignificance. This plot indicated that LB odour, yeasty odour, alcohol odour, ester odour, and sweetness were significant (Figure 9). Alternatively, the data was subject to product- replicate and assessor- replicate/ product interactions. The replicate-related interaction effects were attributed to oxidation in the fermentation sampling bottles (cue acetic acid formulation); while assessor-related interactions were a consequence of variance in scale usage by panellists, as suggested by the large standard deviations amongst each panellists' attributes' mean scores. To account for the latter erroneous effect due to difference in intensity scale usage with respect to means, univariate scaling was conducted. Average of the attribute was divided by standard deviation of attribute calculated against all panellists and replicates.

$Yijkr(new)=yijkr(old)/s(yik)$- Equation 2 for UV scaling

Where i=assessor, k= attribute, s= standard deviation, j= samples, and r= replicate

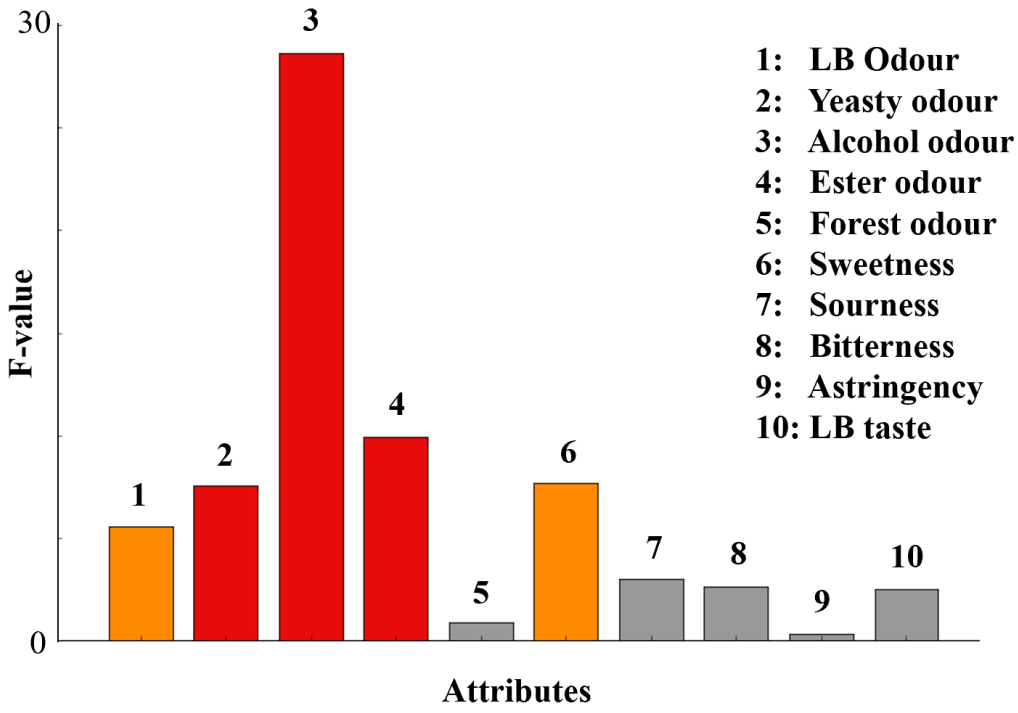
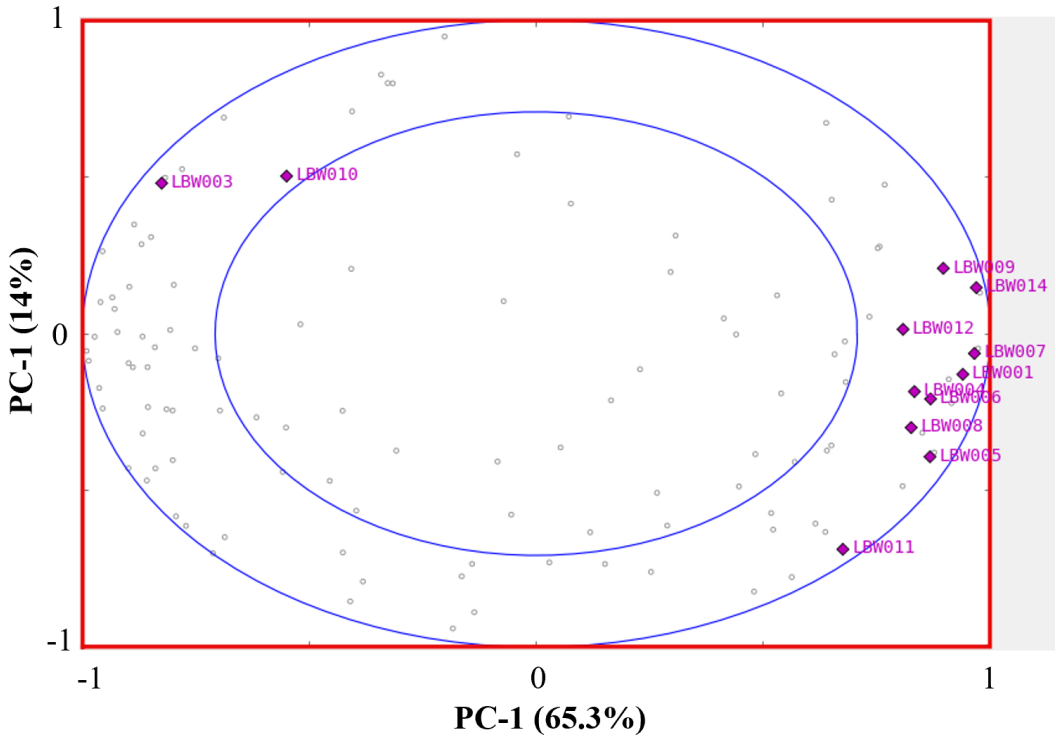


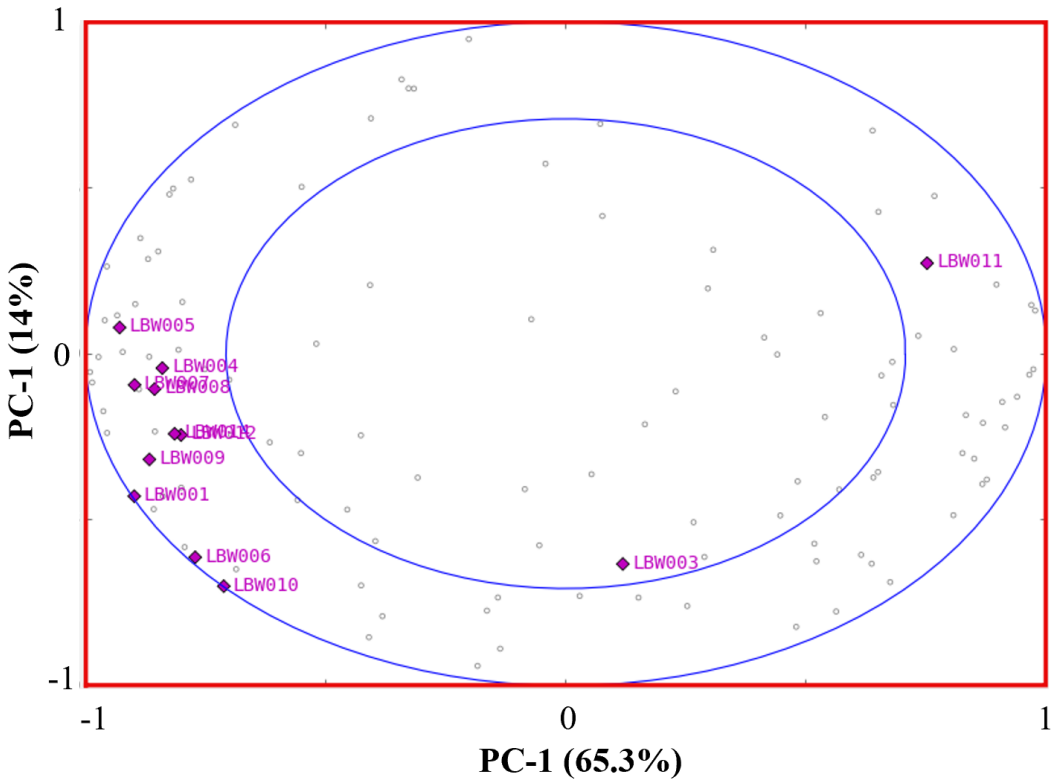

*Figure 9. An overall 3-way ANOVA overview plot for F values under product effect to detect significant attributes. Red and orange colours correspond to $p<0.001$ and $p<0.01$, respectively.*

Second, multivariate Tucker-1 plots aids in panel agreement visualization. Panellists within the concentric ellipses towards the outer periphery contributed significantly, those outside the ellipses were insignificant, while those present outside the clusters but within the ellipses (like panellists 3, 10, 11 & 4) were outliers (Figure 10).

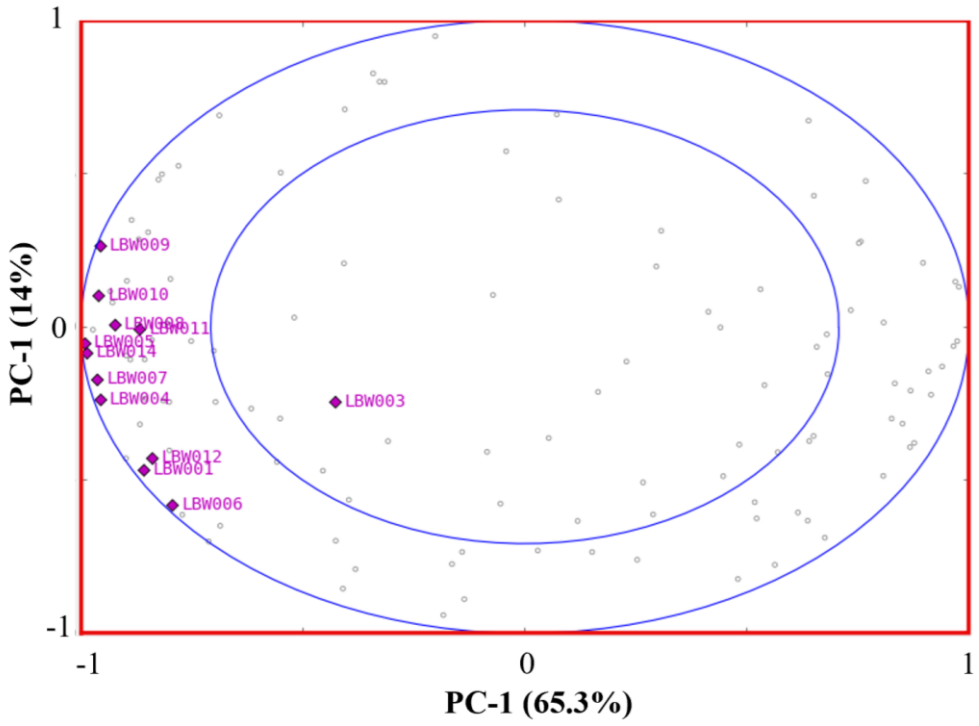
Third, univariate p-MSE plots reveal a panellist's repeatability and discriminability where y axis corresponds to p-value (refers to perceived discriminability amongst samples) and x axis gives Mean Squared Error value (refers to reproducibility of scores for replicates). Some outliers with low sensitivity (like panellists 3, 11, 5 & 6) were observed for significant variables (Figure 11). However, no notable change was observed in results upon exclusion of those panellists and hence, whole panel data was retained.


1
0
-1
PC-1 (14%)
LBW003
LBW010
LBW009
LBW014
LBW012
LBW007
LBW001
LBW004
LBW006
LBW008
LBW005
LBW011
-1
0
1
PC-1 (65.3%)

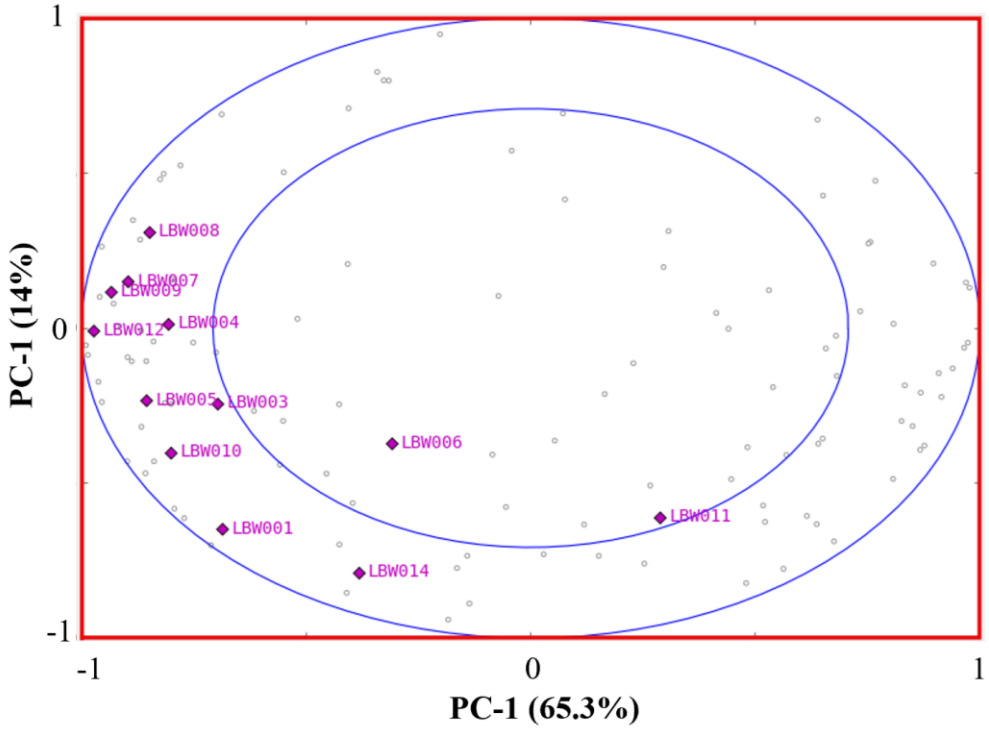
*A*


1
0
-1
PC-1 (14%)
LBW011
LBW005
LBW004
LBW009
LBW001
LBW006
LBW010
LBW003
-1
0
1
PC-1 (65.3%)

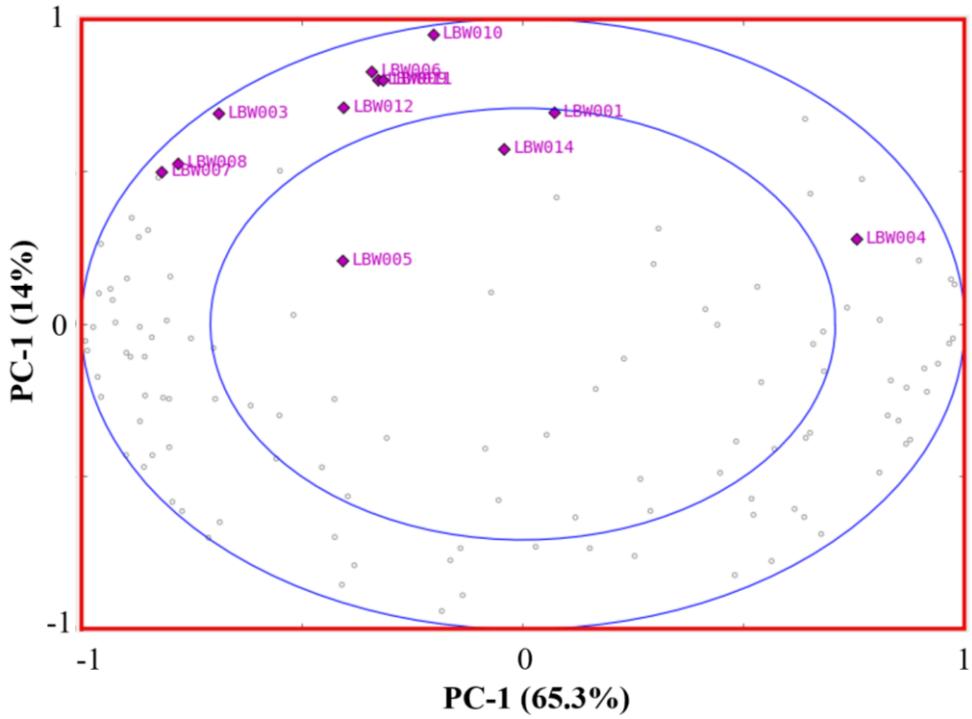
*B*


1
0
-1
PC-1 (14%)
LBW009
LBW010
LBW008
LBW011
LBW005
LBW014
LBW007
LBW004
LBW003
LBW012
LBW001
LBW006
-1
0
1
PC-1 (65.3%)

*C*

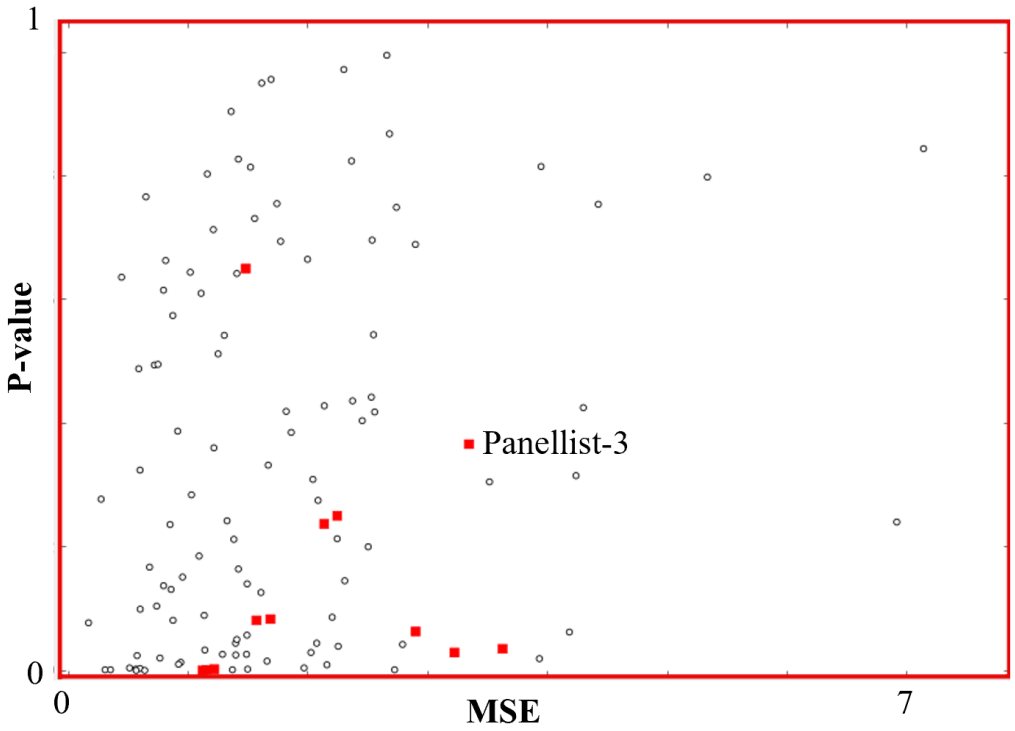

1
0
-1
PC-1 (14%)
LBW008
LBW007
LBW009
LBW012
LBW004
LBW005
LBW003
LBW006
LBW010
LBW011
LBW001
LBW014
-1
0
1
PC-1 (65.3%)

*D*

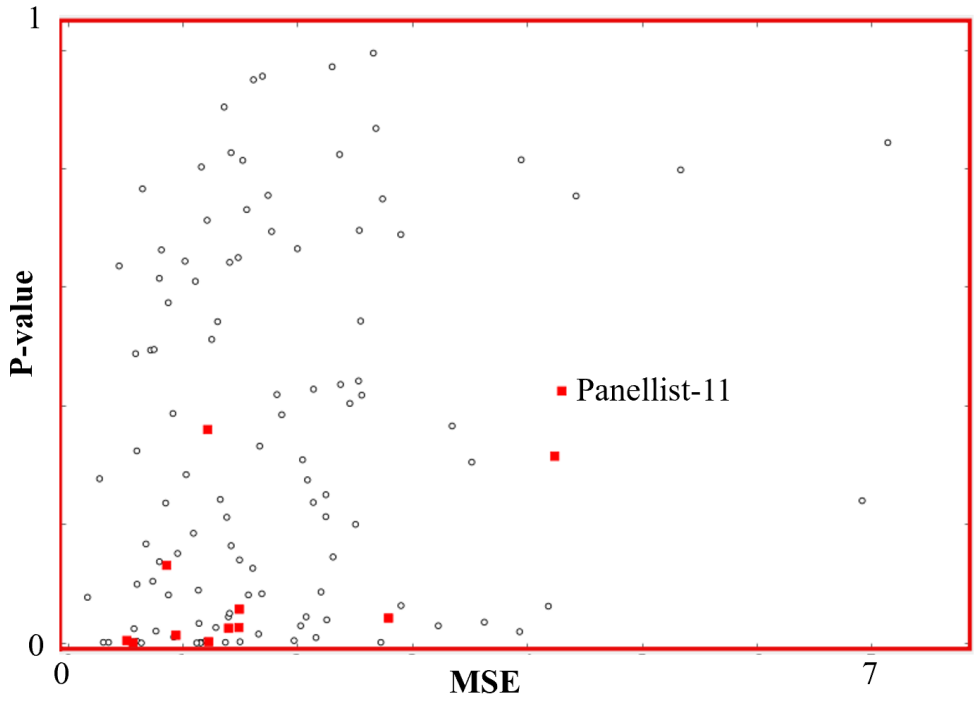



*E*

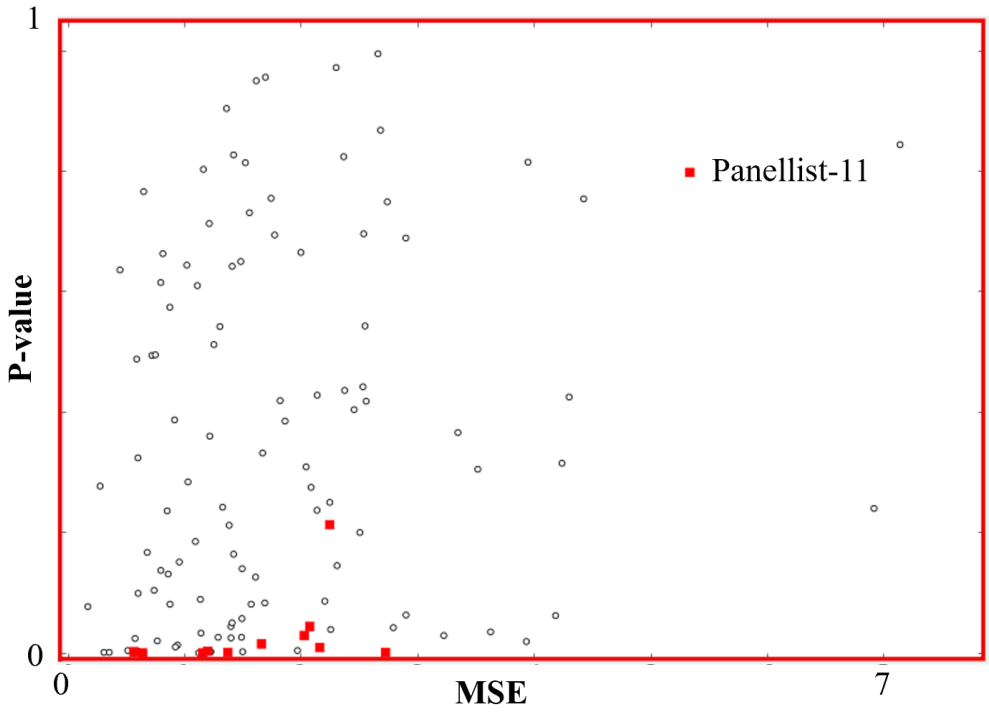
*Figure 10. Multivariate Tucker-1 plots for panel agreement in significant attributes which are A: LB odour, B: Yeasty odour, C: Alcohol odour, D: Ester odour, and E: Sweetness.*



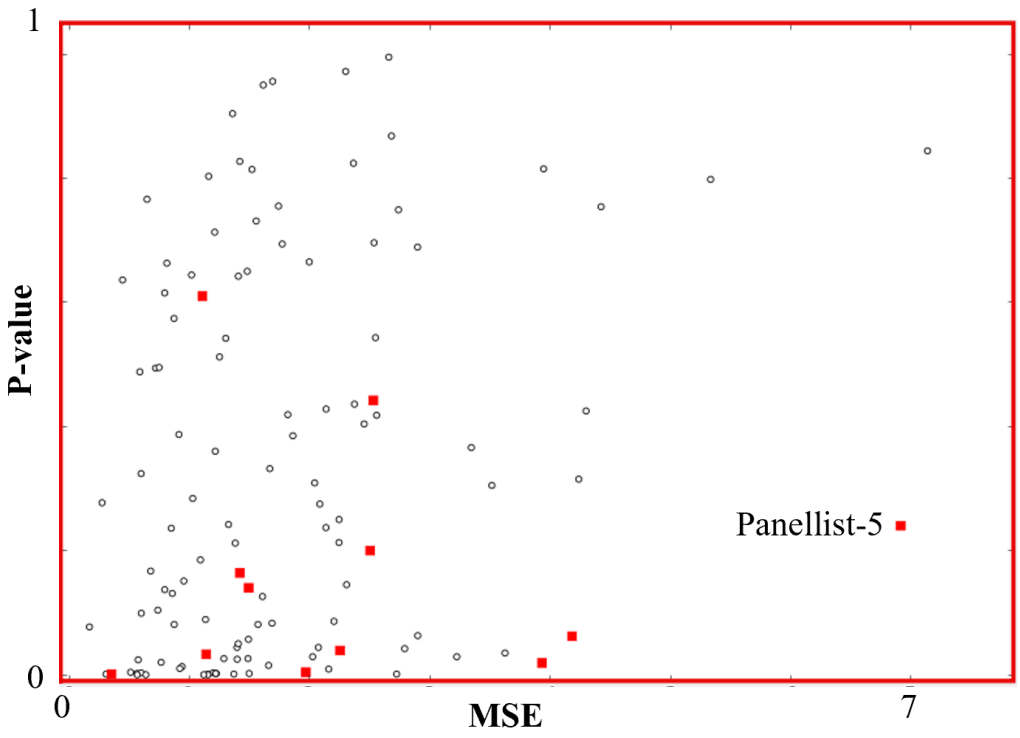

*A*



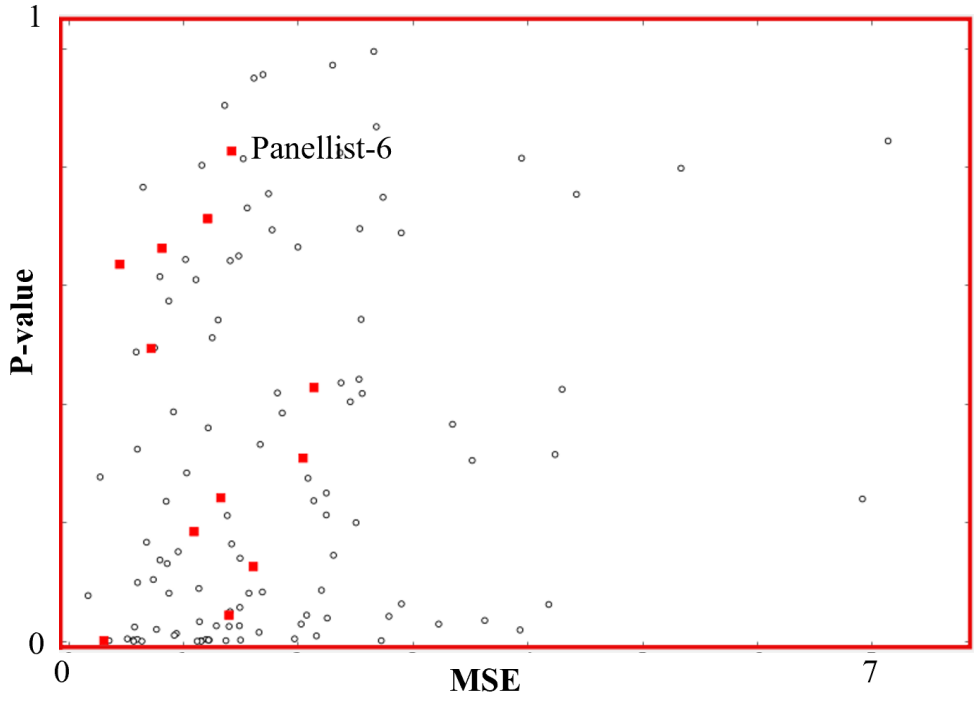

*B*




*C*




*D*




*E*

*Figure 11. Univariate p-MSE plots to determine an assessor's discriminability and repeatability for significant attributes which are A: LB odour, B: Yeasty odour, C: Alcohol odour, D: Ester odour, and E: Sweetness .*

### 3.3.2. Targeted statistics

2-way ANOVA was performed for the UV-scaled significant attributes from PanelCheck (refer to Section 3.3.1.). A significance of $p<0.05$ was detected for LB odour, yeasty odour, alcohol odour, ester odour, and sweetness from SPSS (Table 12). These results corresponded with those from the 3-way ANOVA in PanelCheck and Tukey's Honestly significant difference (HSD) test identified sources of significant difference. The structure was designed such that replicate plus panel effects were kept as random while sample effect was fixed.

*Table 12. A mixed two-way ANOVA depicting main effects for significant attributes along with a post hoc Tukey's HSD Test.*

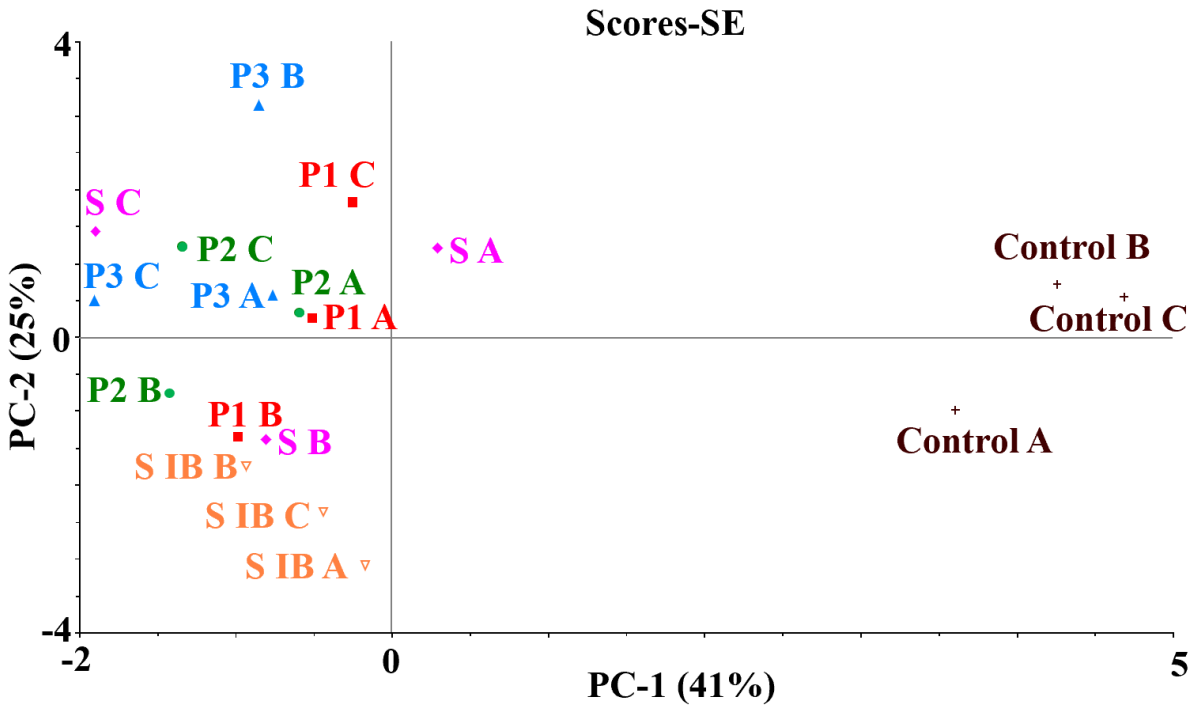
| # | Attribute | Source | F-value | P-value | Tukey's HSD Test | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | P1 | P2 | P3 | S | S-IB | Control |
| 1 | Lingonberry Odour | Sample | 5.589 | 0.002 | 2.15a | 1.95a | 1.97a | 2.12a | 2.25a | 3.26b |
| | | Panellist | 2.584 | 0.014 | | | | | | |
| | | Replicate | 2.307 | 0.145 | | | | | | |
| 2 | Yeasty Odour | Sample | 7.590 | 0 | 1.94bc | 1.80b | 1.85bc | 1.87bc | 2.23c | 0.86a |
| | | Panellist | 6.885 | 0 | | | | | | |
| | | Replicate | 12.699 | 0.001 | | | | | | |
| 3 | Alcohol Odour | Sample | 28.645 | 0 | 2.14b | 2.13b | 2.25b | 2.35b | 2.38b | 0.50a |
| | | Panellist | 2.129 | 0.051 | | | | | | |
| | | Replicate | 0.706 | 0.505 | | | | | | |
| 4 | Ester Odour | Sample | 14.855 | 0 | 2.04b | 2.07b | 1.95b | 1.93b | 2.15b | 0.81a |
| | | Panellist | 1.555 | 0.161 | | | | | | |
| | | Replicate | 0.023 | 0.977 | | | | | | |
| 5 | Sweetness | Sample | 7.692 | 0.005 | 2.39bc | 2.54bc | 2.54bc | 2.63c | 1.79a | 2.11ab |
| | | Panellist | 19.598 | 0 | | | | | | |
| | | Replicate | 3.442 | 0.084 | | | | | | |

*a, b, and c represent the grouping of samples for each specific attribute. P1, P2, P3, and S are representatives of S. cerevisiae, T. delbrueckii, M. pulcherrima, and mixed simultaneous inoculation of S. cerevisiae with M. pulcherrima.*

### 3.3.3. Non-targeted statistics

Another PCA model was designed using Unscrambler X for the UV-scaled data matrix to analyse the relation between sensory attributes and samples post dimensional reduction. 66% of data variance was explained by the first two principal component. From the scores plot (Figure 12), it was detected that the control triplicates (C*A/B/C) were negatively correlated to the wine samples' triplicates on PC1. The IB triplicate cluster (S-IB*A/B/C) was negatively correlated to rest of the wine triplicates on PC2. Further, it observed that the remaining wine triplicates were scattered instead of clustered together based on strains. This was attributed to periodic opening of replicates for progress/quality

checks throughout the fermentation; resulting in oxidation. From the loadings plot (Figure 12), a negative correlation between LB odour-taste and alcohol-ester odour was detected along P1. The attributes of sourness, bitterness and astringency were negatively correlated with sweetness along P2. Next, upon inspection of the two plots simultaneously, it was concluded that the control cluster was positively related to LB odour-taste but negatively related to alcohol-ester odour; and vice versa for the wine samples (on P1). The IB cluster was positively related to bitterness-astringency but negated with sweetness (along P2). Interestingly, P3 RT-B, showed maximal positive relation to sweetness and negation with bitterness-astringency (on P2); backing the oxidation variance speculations amongst replicates.




*Colours corresponding to different inoculations are as red for pure S. cerevisiae, green for T. delbrueckii, blue for M. pulcherrima, and magenta-orange for mixed simultaneous inoculation of S. cerevisiae with M. pulcherrima at room temperature and in an incubator.*

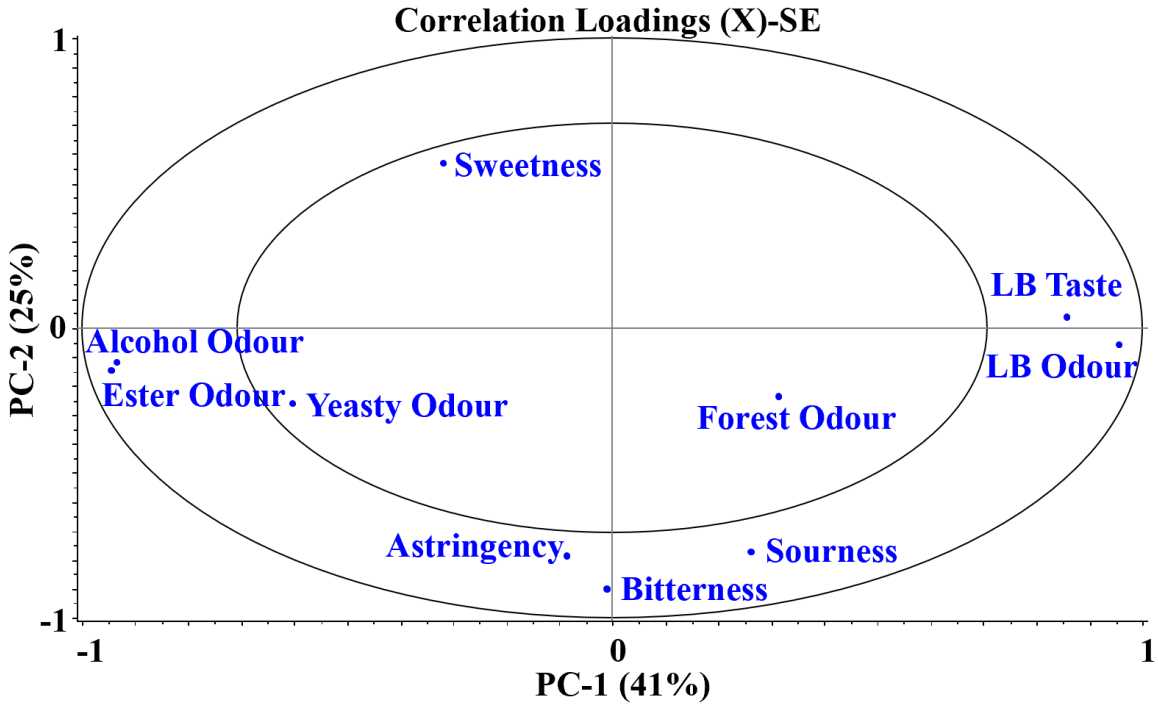



*Figure 12. PCA constructed using Unscrambler depicting the relation between sample triplicates and sensory attributes. The scores plot tells about arrangement of sample triplicates while the loadings plot (B) depicts the arrangement of the sensory attributes listed in Table 12.*

### 3.3.4. Relation between volatile and sensory evaluation data

By comparing the two PCA models for volatile compounds (PCA-1) and results of sensory evaluation (PCA-2), the following conclusions were drawn:

a. The ‘cluster a’ for volatiles corresponding to RT control duplicates in PCA-1, were related to the LB odour-taste and forest odour in PCA-2. These are in accordance with the odour descriptors for the cluster a volatiles with pungent, mushroom, musty, woody, eucalyptus, earthy, green, herbal, buttery, and terpenic odours (Good Scents Company). However, it must be noted that the C for sensory evaluation and volatile analyses were different batches, thereby expected to confer variances in odour perceptions.
b. The ‘cluster b’ for volatiles corresponding to IB-fermented wine (S-IB) in PCA-1, were related to astringency and bitterness in PCA-2. These are in accordance with the odour descriptors for the cluster b volatile with chemical, rancid, mentholic, minty, pungent, and sharp odours (Good Scents Company).
c. The ‘cluster c’ for volatiles corresponding to RT-fermented wines in PCA-1, were related to alcohol and ester odour in PCA-2. These are in accordance with the odour descriptors for the cluster b volatile with fruity, ethereal, winey, fermented, alcoholic, and estery odours (Good Scents Company).

Therefore, a decrease in the perceived intensities of LB odour, LB taste, and forest odour along with an increase in astringency, bitterness, alcohol odour, ester odour, and yeasty odour occurred post fermentation compared to the LB juice. These were in accordance with the increased total esters plus higher alcohols; and decreased terpenes (except linalool and alpha-terpineol) post fermentation. However, a correlation between volatile composition and sensory characteristics was not established due to an unsatisfactory explained variance upon PLS-DA (Partial Least-Squares Discriminant Analysis) modelling (plot not shown).

# 4. Discussion

## 4.1. Volatile literature collation

As stated in section 1.5.5 of introduction, volatile composition of LB juice has been studied in only four research papers till date i.e., Marsol-Vall et al., 2014, Anjou & von Sydow, 1967, Viljanen et al., 2014, and Amundsen et al., 2023. Of these, Viljanen et al., 2014 involved yeast fermentation and related volatile compounds' transformation in lingonberry. Compared to this lingonberry study, 5 volatiles i.e., styrene, ethyl ethanoate, 2,3-butanedione, 6-methyl-5-hepten-2-one, and linalool were detected in all LB juices. For C-RT and C-IB, 15 additional volatiles were identified that were absent from literature; while 87 literature volatiles were absent from the controls. This gap in volatile composition is most likely due to the variance in bioprocessing step and a gap in test years (1967, 2014, 2021, and 2023) between the studies. Columns 6 and 7 of Table 13 indicate the contrast amongst controls from same year, company, and bioprocessing step but different batches (August and October); illustrating occurrence of major volatile variance due to batch differences. Additionally, collating with the Table 6 from section 1.6.3, 4 primary volatile compounds relating majorly to the raw material variety were found as 3 terpenes (eucalyptol, p-cymene, linalool, terpinen-4-ol, and α-terpineol) and 1 thiol (3-(methylthio)-1-propanol). Further, bulk of the composition was made up of volatile compounds categorized as secondary that arise during the fermentation process. These included 5 acids, 13 higher alcohols, 3 aldehydes, 3 benzenes, 17 esters, and 5 ketones that were present in a majority of fermented samples. Tertiary compounds related to ageing were not detected due to a lack of barrel ageing step.

*Table 13. A comparative list of overlapping volatile compounds possessing odour descriptions present in lingonberry between literature references and present study.*

| # | Overlapping Juice Volatiles | Lit-A | Lit-B | Lit-C | Lit-D | C-RT | C-IB |
|---|---|---|---|---|---|---|---|
| 1 | 2-methylbutyric acid | | | o | o | o | |
| 2 | Hexanoic acid | | | | o | o | o |
| 3 | Heptanoic acid | | | | o | | o |
| 4 | Octanoic acid | | | | o | | o |
| 5 | Nonanoic acid | | | | o | o | o |
| 6 | 1-Pentanol | | o | | o | o | |
| 7 | 1-Hexanol | | o | | o | o | o |
| 8 | 3-Methyl-1-butanol | | o | o | o | | o |
| 9 | Benzyl alcohol | | o | o | o | o | o |
| 10 | 2-Phenyl ethanol | | o | | | | o |
| 11 | 3-Hexen-1-ol | | o | o | o | o | o |
| 12 | 1-Octen-3-ol | | o | | o | o | o |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 13 | 2-Ethyl-1-hexanol | | | | o | o | o |
| 14 | Acetaldehyde | o | | | o | | o |
| 15 | Hexanal | o | | o | o | o | |
| 16 | E-2-Hexenal | | | | o | o | |
| 17 | Styrene | o | o | o | o | o | o |
| 18 | Toluene | | o | | | o | |
| 19 | Ethyl ethanoate | o | o | o | o | o | o |
| 20 | Methyl ethanoate | | | | o | | o |
| 21 | Acetone | o | | | | o | o |
| 22 | 2,3-Butanedione | o | o | o | o | o | o |
| 23 | 1-Phenylethanone | | o | o | o | o | o |
| 24 | 6-Methyl-5-hepten-2-one | o | o | o | o | o | o |
| 25 | 4-Terpinenol | | o | | o | o | o |
| 26 | Terpineol | | o | | o | o | o |
| 27 | Cymene | o | o | o | o | o | |
| 28 | Eucalyptol | o | o | o | o | o | |
| 29 | Linalool | o | o | o | o | o | o |

*Lit-A, Lit-B, Lit-C, and Lit-D correspond to studies by Marsol-Vall et al., 2014, Anjou & von Sydow, 1967, Viljanen et al., 2014, and Amundsen et al., 2023. 'o' as symbol for presence.*

Some of the compounds absent in the lingonberry wine literature (but present in the current study) were detected in other *Vaccinium* genus wines that were mentioned in Table 8 of section 1.6.3. These overlapping compounds present in a majority of the developed wines are presented in Table 14. Primary volatile compounds retained in LB wine included linalool, terpinen-4-ol, α-terpineol, and 3-(methylthio)-1-propanol. While a majority of overlapping secondary volatile compounds were detected in other berry wines, linalool was detected exclusively in the LB wine literature. Additionally, terpinen-4-ol and 3-(methylthio)-1-propanol were detected in only bog bilberry and bilberry wine literature, respectively. Further, terpineol was found in all *Vaccinium* berry wines except LB literature. It is important to mention that 10 volatile compounds from the present study were undetected in all of the *Vaccinium* berry wine 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.

*Table 14. A list of overlapping volatile compounds possessing odour descriptions present in present lingonberry wine study and other Vaccinium genus wine literature references.*

| ***Vaccinium* Berry Type** | **Cranberry** | | **Blueberry** | **Bilberry** | | **Bog bilberry** | |
|---|---|---|---|---|---|---|---|
| **Volatile compounds** | **Lit-E** | **Lit-F** | **Lit-G** | **Lit-H** | **Lit-I** | **Lit-J** | **Lit-K** |
| Acetaldehyde | | | | ✓ | ✓ | | |
| 2-Methyl-1-propanol | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ |
| 1-Hexanol | | ✓ | | ✓ | ✓ | ✓ | ✓ |
| 3-Methyl-1-pentanol | | | | ✓ | | ✓ | ✓ |
| 2,3-Butanediol | | | | ✓ | | ✓ | ✓ |
| 3-(Methylthio)-1-propanol | | | | | ✓ | | |
| Hexanoic acid | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| Octanoic acid | ✓ | ✓ | | | ✓ | ✓ | ✓ |
| Decanoic acid | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| Benzoic acid | ✓ | ✓ | | | | | |
| Nonanoic acid | | ✓ | | | | | |
| 2-Methylpropanoic acid | | | | | ✓ | ✓ | ✓ |
| Ethyl butanoate | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ |
| Ethyl 2-methylbutanoate | ✓ | ✓ | ✓ | | | ✓ | ✓ |
| Ethyl hexanoate | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Ethyl octanoate | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 2-Phenyl ethyl acetate | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ |
| Ethyl decanoate | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Ethyl 3-phenylprop-2-enoate | ✓ | ✓ | | | | | ✓ |
| Ethyl 2-methylpropanoate | | ✓ | | | ✓ | | |
| Ethyl 3-methylbutanoate | | ✓ | ✓ | ✓ | ✓ | | ✓ |
| Ethyl dodecanoate | | ✓ | | ✓ | ✓ | ✓ | ✓ |
| Ethyl 2-hydroxypropanoate | | | | ✓ | ✓ | ✓ | ✓ |
| Ethyl 9-decenoate | | | | ✓ | ✓ | ✓ | ✓ |
| Ethyl hexadecanoate | | | | | | ✓ | ✓ |
| Terpineol | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Terpinen-4-ol | | | | | | | ✓ |

*Lit-C, Lit-E/ Lit-F, Lit-G, Lit-H/ Lit-I, and Lit-J/ Lit-K corresponds to lingonberry (Viljanen et al., 2014), cranberry (Zhang et al., 2019/ Zhang et al., 2020), blueberry (Yuan et al., 2018), bilberry (Liu et al., 2019/ Liu et al., 2020), and bog bilberry (Wang et al., 2016/ Lin et al., 2022) wines, respectively. ✓as symbols for presence.*

In case of fermentation volatiles (Table 15), 60% of overlapping volatiles between literature reference (Viljanen et al., 2014) and present study showed the same trend of volatile transformation from LB juice to wine. However, 2-Methylbutyric acid, Benzyl alcohol, Styrene, Ethyl benzoate, 2,3-Butanedione, and 6-Methyl-5-hepten-2-one showed opposite trends between the literature and this study's wines. This contrast could be because of the usage of different yeast strains in the studies along with an additional pH modulation step in literature. Another reason could be the natural variation in composition of LB that is grown unsupervised and foraged from the wild.

*Table 15. A comparative view of overlapping odour possessing volatile compounds' transformation after lingonberry juice fermentation in relation to Viljanen et al., 2014.*

| # | Overlapping Fermentation Volatiles | F-Lit C | F-RT | F-IB |
|---|---|---|---|---|
| 1 | 2-Methylbutyric acid | ↑ | - | ND |
| 2 | 3-Methyl-1-butanol | ↑ | + | ↑ |
| 3 | Benzyl alcohol | ↑ | ↓ | ↓ |
| 4 | Phenylethyl alcohol | + | + | ↑ |
| 5 | 3-Hexen-1-ol | = | ↓ | ↓ |
| 6 | Hexanal | ↓ | - | ND |
| 7 | Styrene | ↓ | ↑ | + |
| 8 | Ethyl ethanoate | ↑ | ↑ | ↑ |
| 9 | Ethyl benzoate | ↓ | + | + |
| 10 | 2,3-Butanedione | ↑ | - | - |
| 11 | 1-Phenylethanone | ↑ | ↑ | + |
| 12 | 3-Hydroxy-2-butanone | ↑ | + | ND |
| 13 | 6-Methyl-5-hepten-2-one | ↑ | ↓ | ↓ |
| 14 | p-Cymene | ↓ | - | ND |
| 15 | Linalool | ↑ | ↑ | ↑ |

*F-Lit C, F-RT, and F-IB are representatives of lingonberry wines from Viljanen et al., 2014 and present study's sample at room temperature and in an incubator at 30$^{O}$C. -, +, ↓, ↑, =, and ND as symbols for removal, addition, decrease, increase, no change, and not detected. Font in red depicts volatile compounds that showed a non-overlapping transformation trend.*

Table 16 gives an overview of changes in specific volatiles affected by wine fermentation using different non-conventional yeast strains for present LB and grape literature studies. Upon comparison with the Tables 2 and 3 from section 1.5.5 of introduction, it was observed that volatile groups followed the same trend despite a difference in raw materials. However, oxidation mediated acetic acid generation via acetaldehyde intermediate formation resulted in opposite trend for those compounds in the RT wines.

*Table 16. A literature comparison for transformation of total volatile functional groups upon non-conventional strain fermentations.*

| # | Volatiles | Literature (grapes) | | | Present Study (lingonberries) | | | |
|---|---|---|---|---|---|---|---|---|
| | | P2 | P3 | S | P2 | P3 | S | S IB |
| 1 | Acetic Acid | ↓ | = | ↓ | ↑ | ↓ | ↑ | ↓ |
| 2 | Higher Alcohols | = | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| 3 | Acetaldehyde | = | ↓ | = | ↑ | ↑ | ↑ | ↑ |
| 4 | Esters | ↑ | = | ↑ | ↑ | ↑ | ↑ | ↑ |
| 5 | Sulphur Compounds | ↑ | = | = | ↑ | ↑ | ↑ | ↑ |

*P2, P3, and S are representatives of T. delbrueckii, M. pulcherrima, and mixed simultaneous inoculation of S. cerevisiae with M. pulcherrima. ↓, ↑, = as symbols for decrease, increase, and no change.*

## 4.2. Sensory evaluation literature collation

Referring to section 1.6.2., the study by Viljanen et al., 2014 on lingonberry wines depicted relation of LB juice controls to fresh and LB flavour while yeast fermentation were related to fermented flavour, sourness, bitterness, and off-taste. This was in accordance with the present study's perceived LB flavour in controls along with alcohol odour, sourness, and bitterness in wines. Since these flavour characteristics are desirable in wines, these should align with the expectations of consumers. Additionally, minimal sensory perception difference was observed between wines fermented using varied yeast strains.

Other Vaccinium berry studies included several methods and parameters that were unexplored in the present study. Lin et al., 2022 studied bog bilberry wines using consumer based CATA and hedonic preference tests. Sourness and sweetness were observed similar to present study. Angeles Varo et al., 2022 compared the impact of partial and complete highbush blueberry wine fermentation on colour plus flavour. Santos et al., 2016 performed ranking tests for calcium carbonate deacidified and glucose syrup chaptalized Rabbiteye wines. Further, the sensory study was related with the phenolic profile composition. Zhao et al., 2023 studied impact of ultrasonic treatment on wine quality. In summation, it would be beneficial to conduct a large scale consumer study employing CATA and hedonic ranking tests to estimate the impact of deacidification, phenolic composition, and ultrasonic treatment on LB wines at different stages of fermentation.

## 4.3. Limitations of study

The findings of this study must be visualized with caution due to a few shortcomings as described next. First, the variance in IB and RT sensomics profile was due to a difference in LB batches and not related to fermentation conditions probably. This is because lingonberries are usually foraged from forests and not cultivated specifically at farmlands. This results in inter-batch composition variability of harvested lingonberries. In

retrospect, the entire study should be conducted using a single LB batch. Second, frequent sampling by bottle opening led to oxidation of RT wine samples. Oxidation lead to the generation of an intermediate (acetaldehyde) and final product (acetic acid) that negatively impact the sensory properties of wines. To counter this, fermentations should be conducted in a bioreactor that acts as a versatile equipment that allows sampling while maintaining the headspace environment of the fermentation vessel. Third, the incubator fermentations were conducted in duplicates instead of triplicates. Apart from this, volatile analytical samples were just analysed as singles. This led to a decrease in precision and complication of targeted ANOVA statistics. Therefore, it is imperative to conduct fermentations and bioanalytical analyses in triplicates to enhance reliability of results and minimize background noise errors.

Fourth, the control used in sensory evaluation was from a different company compared to the controls of the fermentations. This acted as another factor that increased the variance in testing. The same LB batch must be used for all analyses to ensure legit reasonings. Fifth, except a speculative relation, a correlation between volatile analyses and sensory attributes was not determined. This was due to the low explained variance in PLS-DA caused by a lack of cohesion between replicate results due to the factors described before. Sixth, phenolic profile identification, deacidification, ultrasonication, and a large scale consumer sensory test that are performed in other *Vaccinium* berry wines were not performed. In order to completely delve in sensomics, it is important to decode the entire internal composition of a product along with determination of consumer preference. Seventh, the performance of GC-Olfactory analyses was skipped. It is a key interface in sensomics since it enables partial correlation between volatile compound chemistry and perceived odour (Friedrich & Acree, 1998). To sum up, a single batch of lingonberries must be used to prepare wines and analytical samples in triplicates using a bioreactor. Additionally, phenolic profiling, GC-O analyses, and consumer based testing (as described in section 4.2) must be included to minimize the limitations of present study.

# 5. Conclusions

The goal of the research was successfully achieved by development of medium alcohol content (7.7%- IB and 7.3%- RT) non clarified LB wines using pure and simultaneous mixed yeast cultures. This was achieved via preliminary bioprocessing to decrease benzoic acid value below 0.25g/L. After decrement of benzoic acid below critical limit, fermentation was effectively conducted by 1:1 dilution of bioprocessed juice with milli-Q water and TSS adjusted to 14 $^{o}$Brix to ensure proper yeast nutrition.

The volatile composition determined using HS-SPME-GC-MS illustrated an increase in total ester and higher alcohol content, along with a decrease in terpene composition (except linalool and alpha-terpineol) in wines. These coincided with lingonberry wine sensory evaluation data with an increase in astringency, bitterness, alcohol odour, and ester odour along with a decrease in LB flavour perceived intensities post fermentation compared to the LB juice. Two non-targeted volatile related serendipitous findings of inter-batch lingonberry variation and *Vaccinium* genus wines composition overlap with lingonberry wine were comprehended.

As per our knowledge, this is the first promising scientific endeavour at non pH modulated LB wine development at uncontrolled RT that studies conventional and varied non-conventional yeasts' impact on sensomics profile. A prior research by Viljanen et al. (2014) was the previous singular lab-scale attempt at LB wine concoction followed by sensomics profiling using only *H. uvarum* post pH modulation in controlled conditions; with off-taste in SE. This was used as the most prominent literature reference for this study; apart from LB volatile studies by Marsol-Vall et al. (2014) and Anjou & von Sydow (1967). Upon literature comparison, it was concluded that about 60% of the overlapping volatile compounds showed the same transformational trend during fermentation; while the contrasting trend was attributed to variation in bioprocessing and oenology. Additionally, a non-targeted observation showed that volatile composition of LB juice varied extensively based upon company, year, and even between batches. Next, the impact of different non-conventional yeasts of volatile composition was the same as in literature; except oxidation related conflicting trend for acetic acid and acetaldehyde. Finally, the LB wines' sensory attributes were in accordance with the literature, despite a different production methodology but minimal sensory alterations were detected between varied yeast strain wines.

Future studies must focus on optimization of fermentations to eliminate oxidation, replication shortage, and inter-batch variability drawbacks of present study. As a remedy, oxidation elimination through carbon dioxide stoppers or bioreactor usage, analytical triplicates for precision and statistical proficiency, and pooled LB batches for minimized batch mediated contrast, should be used. These could facilitate a reduction in required fermentation times and enable PLS-DA modelling with satisfactorily explained variance.

Similar to literature on Rabbiteye blueberry, analysis of the phenolics must be conducted to estimate the impact of the phenolic composition on colour and flavour of LB wines. Finally, emulating the CATA and hedonic rating tests from bilberry and blueberry wine studies, could ensure a proper representation of the consumer preference.

Further, in order to maintain the health benefits of LB, fermentation could be performed either to generate low levels of ethanol or to forego ethanol in its entirety. The amount of ethanol can be manipulated easily by altering the amount of TSS (reduced sugar addition) in bioprocessed LB juice. Therefore, despite a prolonged processing, fermentation of LB post benzoic acid decrease led to procurement of LB wines sans generation of unpalatable flavours. This could be a constructive method for valorisation of underutilized LB, especially in Nordic countries.

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# 7. Appendix

7.1. Table 17 depicting minimal effect of varied pitching rate and incubation times on Baker's yeast biomass based upon Trial one.

*Table 17. Trial one observations for Baker's yeast biomass increment.*

| Time (hours) | Pitching Rate | | | | |
|---|---|---|---|---|---|
| | 10% | 20% | 30% | 40% | 50% |
| 0 | 304.1 | 311.9 | 305.6 | 316.8 | 334.8 |
| 1 | 303.3 | 310.9 | 304.8 | 315.9 | 333.7 |
| 2 | 303.2 | 310.8 | 304.6 | 315.7 | 333.5 |
| 3 | 303 | 310.6 | 304.5 | 315.5 | 333.4 |
| 4 | 302.9 | 310.5 | 304.4 | 315.4 | 333.3 |
| 5 | 302.8 | 310.4 | 304.3 | 315.3 | 333.2 |

7.2. Table 18 estimating biomass increment at varied pitching rates for 5 hour incubation samples based upon Trial one. It must be noted that a negligible increment in biomass was observed.

*Table 18. Trial one calculations for yeast biomass increment.*

| # | Pitching Rate | | | | |
|---|---|---|---|---|---|
| | 10% | 20% | 30% | 40% | 50% |
| Weight (grams); 5 hours incubated samples | | | | | |
| 1 | 1 | 1 | 1 | 1 | 1 |
| 2 | 0.1 | 0.2 | 0.2 | 0.3 | 0.3 |

7.3. Depiction of impact of baker's yeast mediated benzoic acid decrement at different pitching rates. An observation indicates that higher pitching rate for non-incubated samples led to better benzoic acid decrement, as seen in Table 19.

*Table 19. Trial two results depicting impact of baker's yeast benzoic acid decrease.*

| # | Sample | Benzoic acid concentration (g/L) |
|---|---|---|
| 1 | Juice | 0.61 |
| 2 | 8.33% Incubated (IY) | 0.47 |
| 3 | 12.50% Incubated (IY) | 0.36 |
| 4 | 8.33% Non-incubated (NIY) | 0.29 |
| 5 | 12.50% Non-incubated (NIY) | 0.15 |

7.3. Figure 13 illustrating major steps of lingonberry wine development for this study.

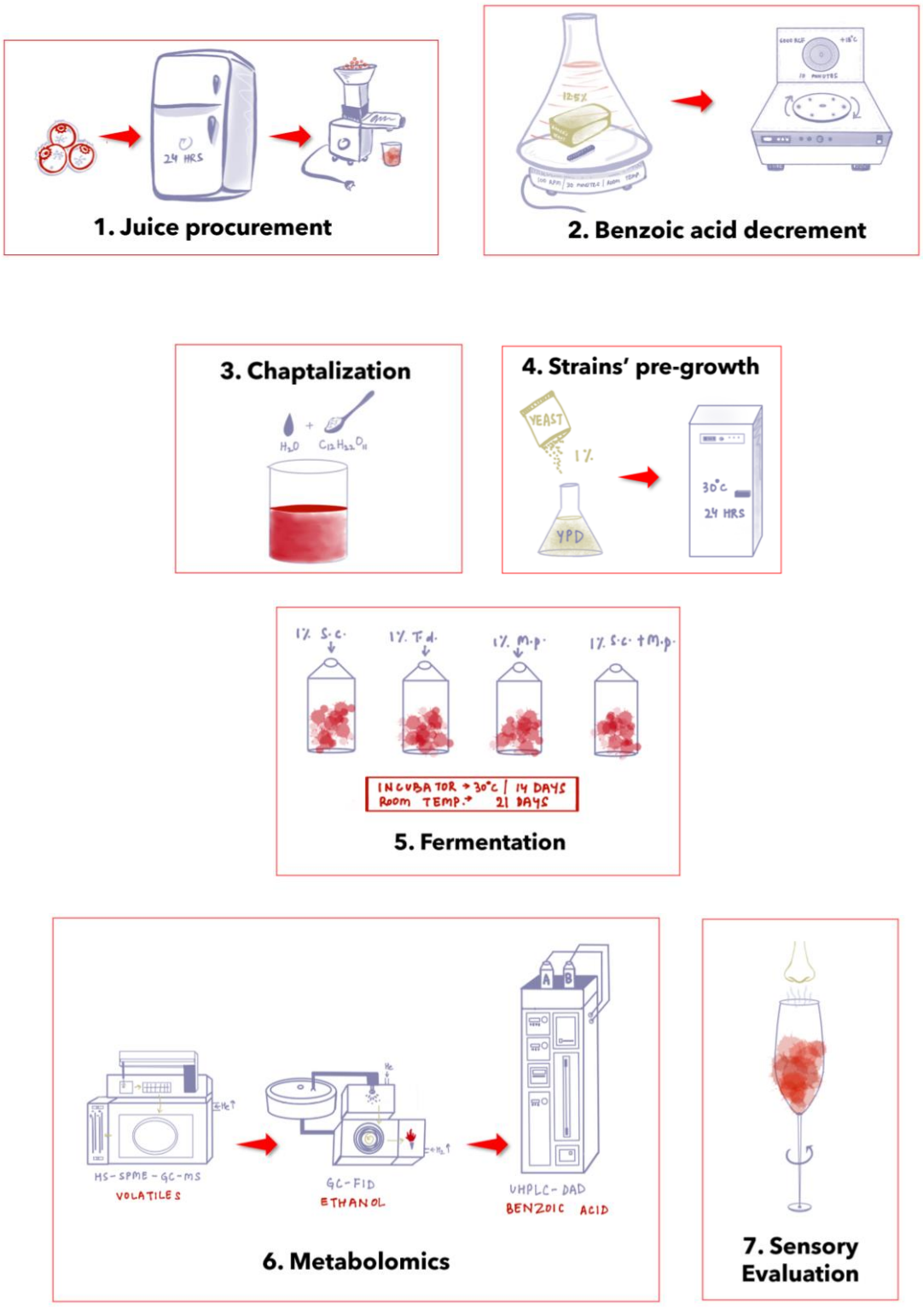


*Figure 13. Lingonberry wine development protocol illustration.*