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Sensory Characteristics of Black Currant Wines

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Fruit wines are fermented alcoholic beverages made from the juice of non-grape fruits, such as black currants, which are one of the largest berry crops in Finland. Fruit wines have a lot of market potential, especially in regions where viticulture does not thrive. In recent years, interest in the so-called non-*Saccharomyces* yeasts has been increasing and they have been reported to have positive effects on the sensory characteristics of wines as well as being potentially useful for producing wines with reduced alcohol content.

The goal of this study was to determine the sensory-chemical differences between alcoholic black currant beverages fermented with *Saccharomyces* and non-*Saccharomyces* yeasts. The beverage samples (n=5) were fermented using different strains of *Saccharomyces*, *Metschnikowia* and *Torulaspora* yeasts. The sensory evaluation was performed by a trained panel (n=11), using a generic descriptive method. Twelve different attributes were analysed, which consisted of five odour attributes, six taste attributes and one texture attribute.

The finished beverages had a strong black currant odour with a very sour and astringent taste. Three attributes had statistically significant differences in the samples: stuffy/musty odour, black currant odour and viscosity. *Saccharomyces bayanus* sample had a stronger stuffy odour compared to the others, as well as the most viscous mouthfeel. *Torulaspora delbrueckii* sample was the least viscous but had the strongest black currant odour. The sourness of black currants was enhanced further by the fermentation process, making it difficult for the panelists to distinguish other taste attributes. Generally, all the non-*Saccharomyces* yeast beverages had stronger black currant odour and weaker stuffy/musty odour, demonstrating their potential for improving the sensory qualities of alcoholic beverages.

Key words: sensory analysis, black currant, fruit wine, non-*Saccharomyces* yeast

Content

Abbreviations

ANOVA	Analysis of Variance
FID	Flame ionization detector
GC	Gas chromatography
GC-O	Gas chromatography-olfactometry
GDPR	General Data Protection Regulation
HS-SPME	Headspace-solid phase microextraction
ISO	International Organization for Standardization
MS	Mass spectrometry
MSE	Mean Squared Error
PCA	Principal Component Analysis
Tukey’s HSD	Tukey’s Honestly Significant Difference

1 Introduction

1.1 Black currant (*Ribes nigrum*)

Black currant is cultivated widely in North America and Europe. It has been the second or third largest berry crop in Finland (Figure 1), with around 1.5 million kilograms produced in 2019 (Official Statistics of Finland 2020). Black currant is known for its bitter and astringent taste, that is partly caused by the high levels of polyphenols in the berry, which are also responsible for most of the potential health benefits that are assigned to black currants (Cortez and Gonzalez de Mejia 2019).

		2014	2015	2016	2017	2018	2019
		Yield (1 000 kg)	Yield (1 000 kg)	Yield (1 000 kg)	Yield (1 000 kg)	Yield (1 000 kg)	Yield (1 000 kg)
BERRIES TOTAL	00	15,579	16,838	14,926	17,081	17,965	21,306
Strawberry	00	12,858	14,389	11,942	13,785	15,333	17,750
Black and green currant	00	1,146	808	950	1,402	990	1,494
Raspberry	00	775	1,009	1,312	1,071	949	1,313
Red currant	00	451	236	337	401	366	376
Highbush blueberry	00	115	145	150	135	128	197
White currant	00	114	69	73	85	65	52
Gooseberry	00	35	51	73	48	27	50
Sea buckthorn	00	34	82	60	111	88	35
Chokeberry (Aronia spp.)	00	34	15	8	10	4	20
Other berries	00	16	34	23	34	15	20

Figure 1. Open-field production of berries in Finland. (Official Statistics of Finland 2020)

1.2 Sensory evaluation

Sensory evaluation is a fairly recent invention, having been born in the late 1940s and then developing quickly alongside the rapidly growing processed food and consumer products industries. Sensory evaluation consists of a collection of techniques that can be used to accurately measure human responses to food, while minimizing the bias and other influencing factors. One widely accepted definition of sensory evaluation is that it is a scientific method used to evoke, measure, analyse and interpret responses to sensory perceptions of products. Essentially, the aim of sensory evaluation is to isolate the sensory characteristics

of a product from everything else and thus provide valuable information to food scientists and product developers. (Lawless and Heymann 2010)

Depending on the aim of the study, different types of tests can be used. There are three commonly used test types that all have their own requirements for participants – discrimination, affective and descriptive. Discrimination tests are the simplest form of testing and just attempt to find out if there are any perceived differences between the products being tested. An example of this can be seen in a study by Laaksonen et al. (2020) where a tetrad test was used to detect differences between black currant juice samples. The results seen in Figure 2 show how many times the panellists were able to distinguish samples from each other and pair them correctly, from which the statistical significance can be calculated. Affective tests can also be very straightforward as the aim is to quantify the degree of like or dislike for a product and the simplest form of it is to find out if there is a preference for one product over another. Descriptive tests, however, are more complex as they attempt to characterize the flavour notes of a product as well as quantify their perceived intensities, making descriptive analysis the most comprehensive and informative sensory evaluation method. (Lawless and Heymann 2010)

Table 3 Discrimination test (tetrad test[a]) for the stored black currant juice samples based on appearance

Storage condition	Timepoints (months)	Enzymatic assistance		No enzymatic assistance	
		Correct answers	*p* value	Correct answers	*p* value
Room temperature	0 vs. 3	24/46	0.005	43/46	<0.001
	0 vs. 12	43/46	<0.001	45/46	<0.001
	3 vs. 12	38/46	<0.001	43/46	<0.001
Fridge, +4 °C	0 vs. 3	20/46	0.096	41/46	<0.001
	0 vs. 12	22/46	0.027	37/46	<0.001
	3 vs. 12	22/46	0.027	22/46	0.027
Room temperature vs. Fridge, +4 °C	3 vs. 3	29/46	<0.001	30/46	<0.001
	12 vs. 12	40/46	<0.001	45/46	<0.001

Figure 2. Example of discrimination test results from a study by Laaksonen et al. (2020) *with 23 panellists and 2 replicates of each sample. The samples were separated in two groups of two by the panellists, based on their similarities and differences.*

1.2.1 Sensory descriptive analysis

Descriptive analysis is one of the most powerful tools in sensory science and as such, its use has steadily increased over the years. In the industry it is used to help create products that match consumer preferences, to check the effects that ingredients and processes have on products and to monitor quality and product

changes over time. In academia it provides valuable information by allowing researchers to make correlations between sensory characteristics and analytical measurements and helping them better understand the mechanisms of sensory perception. (Varela and Ares 2012)

There are several different methods that fit under the umbrella of descriptive analysis which all have their advantages and disadvantages. Murray et al. (2001) described six classical methods and discussed their strengths and weaknesses, while more recently Varela and Ares (2012) reviewed several novel profiling methods developed after the start of the twenty-first century. However, often the best approach is to combine elements from multiple different methods according to the needs of the project, resulting in a method usually referred to as a generic descriptive analysis (Murray et al. 2001).

1.2.2 Statistical analysis in sensory evaluations

While good experiment design is paramount, the importance of statistical methods cannot be understated – they are an extremely valuable tool for the sensory scientist. As is the case for any scientific inquiry, outcomes caused by chance variations must be eliminated in order to draw valid conclusions from the gathered data. Compared to other analytical measurements, however, there is increased variability that is inherent in sensory science, due to the use of human beings as measuring instruments. This especially makes the use of statistical methods a necessity. (Lawless and Heymann 2010)

Statistical analysis helps to identify the characteristics of a product that are most important to consumers. This information can then be used to improve the product and make it more appealing to consumers. Statistical analysis is also needed to compare different products and determine which one is preferred by consumers. This information can be used to make informed decisions about product development and marketing. (Lawless and Heymann 2010)

There are several methods used in statistical analysis for sensory evaluations, with ANOVA (Analysis of Variance) being one of the most common methods. It is a statistical method used to test the differences in means between two or more groups. For example, ANOVA can determine if there are any statistically significant differences in the sensory attributes between different products as can

be seen in the studies by Laaksonen et al. (2012; 2013; 2014; 2020). The method uses the variation within and between groups to determine if the differences in means between groups is significant or just due to random variation. ANOVA can be used to test for differences in a single sensory attribute, or it can be used to test for differences in multiple attributes at the same time. (Lawless and Heymann 2010)

Another method is Principal Component Analysis (PCA) which is a statistical method used to identify the underlying structure of the data in a sensory evaluation. PCA allows, for example, to identify the key sensory attributes that are driving consumer preference, or to find correlations between sensory attributes and specific chemical compounds. An example of this can be seen in Figure 3. The method aims to find the linear combination of the original variables that explain the most variation in the data. The new variables, called Principal Components (PCs) are uncorrelated, and are ranked by the proportion of variance they explain. PCA is useful in reducing the dimensionality of the data, and it allows to visualize the relationships among the variables and the products in a 2D or 3D space. (Lawless and Heymann 2010)

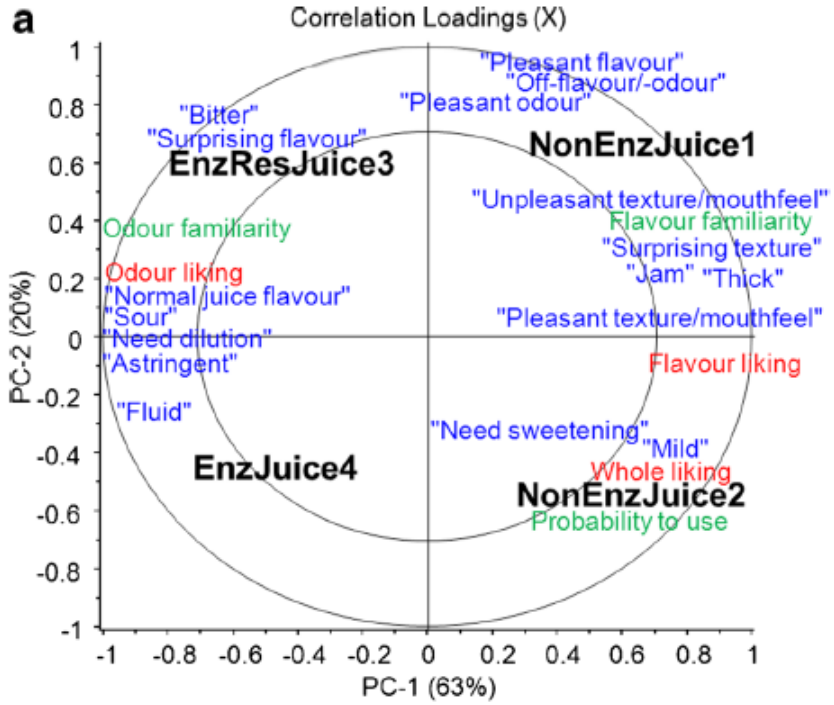

Figure 3. Example PCA plot from study by Laaksonen et al. (2014). *The PCA plot shows correlations between liking, familiarity, and consumer data. Averaged data for four samples with liking (red), familiarity (green), probability to use (green) and consumer comments (blue with quotes).*

1.2.3 Sensory quality of black currant

The sensory characteristics of black currant has been previously studied in several sensory evaluation studies, as can be seen in Table 1. Early studies by Brennan et al. (1997; 2003) and Muir et al. (1998) focused on identifying the various sensory characteristics and the effects of sweetening agents and heat treatment. Sandell et al. (2009) investigated the orosensory properties of different fractions of black currant that were prepared by juice pressing, ethanol extraction and supercritical fluid extraction, while Ng et al. (2012) studied the temporal dominance of sensations of black currant. The effects that different industrial processes, enzymes and storage have on black currant juice have been studied extensively by Laaksonen et al. (2012; 2013; 2014; 2020), Mäkilä et al. (2017) and Marsol-Vall et al. (2019).

Table 1. Examples of sensory evaluation studies of black currant done in the past few decades. Early studies focused on identifying the important sensory characteristics of black currant and later studies focused on the effect of external factors on said characteristics.

Material	Sensory attributes	Method	Panel	Sensory evaluation results	Reference
Juice prepared from 46 different black currant (*Ribes)* genotypes	Appearance (7 terms), aroma (16 attributes), flavour (14 descriptors), aftertaste (6 terms), mouthfeel (4 attributes)	Design, Data Capture and Sensory Profiling Protocol. (Williams et al. 1996)	11 trained panellists	Several attributes were identified as potentially important. Appearance attributes: red hue, purple hue, richness and transparency. Flavour attributes: fruity, sweet, woody, acid/vinegary and black currant. Aftertaste attributes: persistence and natural. Mouthfeel attributes: smoothness and tangy/prickly.	(Brennan et al. 1997)
12 commercial concentrate fruit juice drinks (7 black currant juices, 3 mixed apple and black currant juices, 1 apple juice and 1 strawberry juice) using various sweeteners	Appearance (7 terms), aroma (16 attributes), flavour (14 descriptors), aftertaste (6 terms), mouthfeel (4 attributes)	Design, Data Capture and Sensory Profiling Protocol. (Williams et al. 1996)	15 trained panellists	Sweetening agent used in the juice has an important effect on the perception of flavour, aftertaste and mouthfeel - seemingly even more than the fruit type itself. Especially the "natural" characteristic is heavily influenced by the sweetener type.	(Muir et al. 1998)
Juice prepared from black currant cultivars Ben Lomond and Ben Alder, with and without a heat treatment and with 3 different levels of sweetening (sucrose)	Appearance (7 terms), aroma (16 attributes), flavour (14 descriptors), aftertaste (6 terms), mouthfeel (4 attributes)	Design, Data Capture and Sensory Profiling Protocol. (Williams et al. 1996)	14 trained panellists	Heat treatment and sucrose content did not change the differences in sensory character between the two cultivars, highlighting the importance of the choice of cultivar. Sucrose content had the overall highest effect on sensory characteristics of the juice	(Brennan et al. 2003)

Juice, press residue and ethanol extraction of press residue from black currant variety "Mortti" (MTT Piikkiö, Agrifood Research, Finland)	Total intensity of flavor, roundness, sweetness, fruitiness, sourness, sharpness, bitterness, astringency	Generic descriptive analysis (ISO 8586-1, 8586-2 and 11035)	15 trained panellists (8 female, 7 male) ages 21-57	Ethanol extraction was shown not to affect the sensory profile. Extractions were ranked highest on astringency, despite residue having significantly higher phenolic compound content. Juice was strongest in all other properties except bitterness and astringency.	(Sandell et al. 2009)
Juice, enzymatically pressed juice, heat treated juice and stored juice (0-6 weeks), from black currant variety "Mortti" (MTT Piikkiö, Agrifood Research, Finland)	Fresh odour, fermented odour, total intensity of flavor, fermented flavour, roundness, sweetness, sourness, bitterness, astringency	Generic descriptive analysis (ISO 8586-1)	12 trained panellists	Different enzymes/enzyme combinations tested did not have statistically different sensory properties from each other. Increased astringency correlated with the increased dosage of the enzyme (Macer). Storage time correlated with an increase in sourness in the enzyme-mixture juices.	(Laaksonen et al. 2012)
11 commercial black currant concentrates	Black currant, sweet, tomato ketchup, catty, minty, earthy, acidic, bitter and astringent	Temporal dominance of sensations	11 trained panellists (1 male) ages 30-55	No significant difference was shown in the duration of sweetness and fruitiness between products sweetened by different artificial sweeteners or added sugar products. Samples with complex composition were dominated by sweetness and for longer, while less complex compositions were dominated by fruit flavour.	(Ng et al. 2012)

Juice and enzymatically pressed juice, from four commercial black currant cultivars "Mortti", "Mikael", "Marski", "Ola" and a new breed "Breed15" (MTT Piikkiö, Agrifood Research, Finland)	Sourness, sweetness, bitterness, mouth-drying astringency, puckering astringency, total intensity, berryness, roundness, after taste	Generic descriptive analysis (ISO 8586-1)	Two trained panels (n = 14 and n = 13)	Enzymatic treatment increased bitterness, mouth-drying astringency, puckering astringency, total intensity of flavour and aftertaste, while decreasing the sweetness.	(Laaksonen et al. 2013)
Juice made with different industrial processes from black currant variety "Mortti" (Huittinen, Saarioinen Oy, Finland)	Total intensity of odour, fresh black currant odour, juice-like fermented odour, total intensity of flavour, sourness, sweetness, bitterness, mouth-drying astringency, puckering astringendy, berry flavour, roundness, aftertaste	Generic descriptive analysis, ISO 8586-1	16 trained panellists (9 women, 7 men) ages 23-61	Non-enzymatic juices had more fresh black currant odour, while enzyme aided juices had more juice-like fermented odour. Enzyme aided juices also rated higher on bitterness, sourness and astringency.	(Laaksonen et al. 2014)

Juice made with different industrial processes from black currant variety "Mortti" (Huittinen, Saarioinen Oy, Finland)	Liking, familiarity and preference	Hedonic test (ISO 8589:2007)	117 untrained panellists (82 women, 35 men) ages 19-71	Based on the ratings, the non-enzymatic juices had more pleasant flavour while the enzyme aided juices had more pleasant odour. Enzyme aided juice made from press residue was rated as having most liked and most familiar odour, showing that press residue from non-enzymatic pressing process could be utilized further.	(Laaksonen et al. 2014)
Non-enzymatically pressed juice, enzymatically pressed juice from press residue, enzymatically pressed juice and unpasteurized enzymatically pressed juice, from black currant variety "Mortti" (MTT Piikkiö, Agrifood Research, Finland)	Sourness, sweetness, bitterness, mouth-drying astringency, puckering astringency, total intensity, berryness, roundness, after taste	Generic descriptive analysis (ISO 8586-1)	Two trained panels (n = 14 and n = 11)	Pasteurization seemed to have little effect on the sensory quality of the juices, with only "berryness" attribute rated higher in the pasteurized samples compared to non-pasteurized samples. Storage at room temperature (in the dark) seemed to increased bitterness.	(Mäkilä et al. 2017)
Juice made with different industrial processes from black currant variety "Mortti" (Huittinen, Saarioinen Oy, Finland)	Juice-like matured odour, berry-like odour, total intensity of odour	Generic descriptive analysis	11 trained panellists	Storage in room temperature seemed to decrease the berry-like odour in non-enzymatic juices and increase the juice-like matured odour in the enzymatic juices.	(Marsol-Vall et al. 2019)

Enzymatically pressed juice and non-enzymatically pressed juice from black currant variety "Mortti" (Huittinen, Saarioinen Oy, Finland) stored with different storage conditions	Colour intensity, viscosity, total intensity of flavour, sourness, sweetness, bitterness, astringency, black currant flavour, aftertaste	Generic descriptive analysis	11 trained panellists	Non-enzymatic juices had more intense black currant flavour and higher viscosity, while enzymatic juices had higher total intensity of flavour. Taste properties and astringency was not affected by the storage (12 months) in either storage temperature (room temperature and +4 °C). Black currant flavour diminished in room temperature storage for non-enzymatic juice and total intensity of flavour diminished in room temperature storage for enzymatic juices, indicating the need for cold storage for both juices to preserve initial flavour characteristics.	(Laaksonen et al. 2020)
Enzymatically pressed juice and non-enzymatically pressed juice from black currant variety "Mortti" (Huittinen, Saarioinen Oy, Finland) stored with different storage conditions	Colour, viscosity (visually)	Discrimination test, tetrad	23 untrained panellists	Panellists were able to detect differences between most sample sets comparing storage time of samples stored in room temperature. Panellists appeared to have an easier time differentiating the non-enzymatic juice (even with the samples stored in +4°C), suggesting the storage affected it visually more, compared to the enzymatic juices.	(Laaksonen et al. 2020)

1.3 Anatomy and physiology of taste

Lawless and Heymann (2010) give a thorough description of the physiological aspects of taste in their book “Sensory evaluation of food: principles and practices”. The sense of taste is detected by specialized receptors located in taste buds on the tongue and soft palate. These receptors are modified epithelial cells that have a lifespan of about a week and make contact with sensory nerves. Taste molecules are thought to bind to hair-like cilia at the top of the taste bud, and the taste cells in a taste bud share junctions for common signalling functions. The taste signals are then sent to the brain for processing. Research has shown that two families of receptor proteins, T1Rs and T2Rs, are functional for sweet, bitter, and umami tastes. These receptors are G-protein coupled receptors, which means that when they are stimulated by a taste molecule, they activate other enzyme systems within the cell, causing a cascade of amplified events and leading to the release of neurotransmitters that stimulate the associated nerves.

The taste buds are located in specialized structures on the tongue and the soft palate. These include the papillae, which are small bumps on the tongue that contain the taste buds. There are four different types of papillae: filiform, fungiform, foliate, and circumvallate. The filiform papillae are small cone-shaped structures that serve a tactile function but do not contain taste buds. The fungiform papillae are slightly larger and mushroom-shaped and contain 2-4 taste buds each. The foliate papillae are located along the sides of the tongue and contain several hundred taste buds. The circumvallate papillae are located at the back of the tongue and contain several hundred taste buds. Saliva plays an important role in taste by carrying sapid molecules to the receptors and modulating taste response.

Taste is traditionally divided into four categories: sweet, salty, sour, and bitter. However, other qualities such as metallic, astringent, and umami have been proposed as well. Umami is a taste quality attributed to the taste of monosodium glutamate (MSG) and other substances such as salts of inosine monophosphate (IMP) and guanine monophosphate (GMP). This taste quality is distinguishable from saltiness and is often described as "brothy" or "savory." Umami is an

important taste quality in some ethnic cuisines, particularly in Asian cuisine. Some people can identify it as a distinct taste while others find it hard to distinguish it from the traditional four taste categories. (Lawless and Heymann 2010)

As this study focuses on black currant wine, the most prominent taste attributes are astringency, sourness, and bitterness. Astringency is a common sensation associated with consuming certain foods, particularly those high in phenolic compounds. While it was previously thought that the interaction of phenolic compounds with saliva proteins was the main cause of astringency, it is now understood that this is a complex phenomenon with multiple contributing factors. For example, the interaction of phenolic compounds with oral epithelial cells and the activation of certain receptors in the mouth are thought to play a role. Additionally, different phenolic compounds can have different levels of astringency, suggesting there may be a correlation between a compound's structure and its astringency level. Despite this, there is still a lack of a clear understanding about the mechanisms behind astringency. In recent years, efforts to reduce the negative perception of astringency have been increasing, but more research is needed to fully understand this complex issue. (Huang and Xu 2021)

Sour taste is a complex sensation that is thought to be related to the pH and organic acids in foods, however it is currently not possible to accurately predict and modify the intensity of sour taste in foods. Several studies have attempted to identify the receptors and mechanisms that mediate sour taste, but the physiology of sour taste is still not fully understood. There doesn't appear to be a simple relationship between sour taste intensity and hydrogen ions. Also, the intensity of sour taste of acids cannot be entirely explained by variables such as titratable acidity, molar concentration, buffer capacity, physical and chemical structure. Recent research has proposed that sour taste intensity is directly related to the total molar concentration of all organic acid species with one or more protonated carboxyl group, and the concentration of free hydrogen ions. Despite the recent progress, the psychology of sour taste perception remains controversial, though it seems that at least one specific taste receptor protein has been identified for sour taste. (Neta et al. 2007)

Bitter taste perception is complex process and depends on individual bitterness receptors, the presence of a bitter component, and interactions between flavour components. Bitter receptors are located at the root of the tongue and are

mediated by 25 bitter taste receptors (TAS2R) in humans. Bitter perception involves bitter receptors, signal-coupling proteins, and effector enzymes. Bitter compounds have a diverse range of structures, including peptides, amino acids, lactones, and phenols. The TAS2R receptors, however, have a preference for a specific group of compounds, meaning that a single receptor can respond to different compounds. The perception of bitterness also varies among individuals, and this diversity is thought to be caused by variations in the amino acid sequence of the TAS2R receptors, specifically 25 single nucleotide polymorphisms. Different bitter compounds can activate one or more specific receptors, and variations in bitter receptor genes and mechanisms among individuals can affect, for example, the acceptance of fermented alcoholic beverages. Bitter perception can be defined as a combination of intensity, temporal, and spatial characteristics and is not always unpleasant as different bitter agonists can stimulate specific responses. (Luo et al. 2020)

1.4 Fruit wines

Wines can be sorted into various categories, as seen in Figure 4. Fruit wines are completely, or partially fermented alcoholic beverages made from the juice of non-grape fruits. One the most widely produced fruit wine, is apple wine – commonly referred to as cider. Compared to grapes, many fruits have lower sugar content, and it can be more difficult to extract the existing sugar from the pulp, which is why sugar (or other sweeteners, like honey) may be added before fermentation to increase alcohol content, or post-fermentation, to increase sweetness. Some fruits can have very high acidity, making it necessary to also dilute the beverage to lower its sourness. (Kosseva et al. 2017)

Despite the additional challenges, fruit wines have a lot of market potential, especially in regions where viticulture does not thrive. Fruit wines also have fewer production regulations and unlike grape wine, fruit wines can be produced from concentrates. This eliminates the need for fresh fruit and facilitates year-round production. (Kosseva et al. 2017)

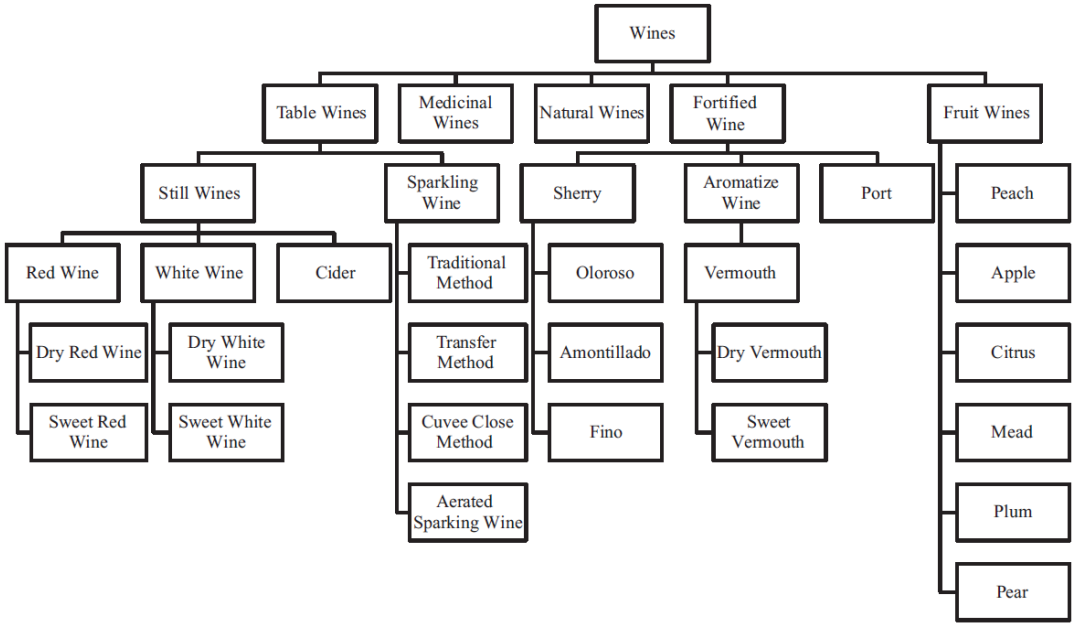

Figure 4. Broad classification of wine types. (Kosseva et al. 2017)

There have been numerous studies done on fruit wines made from various fruits. For example, wine made from passion fruit using different strains of *Saccharomyces cerevisiae* and *Saccharomyces bayanus* were studied by Liu et al. (2022) where they identified passion fruit, mango, green apple, lemon and floral aromas in the sensory evaluation and they were able to determine that passion fruit aroma was significantly affected by the sulphur compounds. In another study, done by Salas-Millán et al. (2022), wine (using *S. cerevisiae*) was made with melons that did not meet the aesthetic standards of supermarket produce and would normally be discarded, despite still being perfectly edible. The sensory evaluation was done using a method from International Organisation of Vine and Wine. Overall, the general impression was that one of the wines was "satisfactory", and the other "very good", hinting at the market potential of using by-products of fresh melon industry to produce novel fruit-based wine.

It can be seen that being able to convert easily perishable fruits into a product with a longer shelf-life and making use of fruits that are not commonly consumed or even "waste products" such as cocoa pod pulp (Kosseva et al. 2017), is one of the biggest advantages of fruit wines.

1.5 Yeasts

Yeasts used for fermentation have a large impact on the aroma and taste of fruit wines. The yeast metabolism results in volatile components from flavour precursors of the fruits being released and new volatiles being synthesized (Lin et al. 2018). There are several hundred of these flavour-active compounds, though not all of them are desirable (Varela 2016). While *S. cerevisiae* is the dominant yeast when it comes to winemaking, the flavour of the resulting wine can be lacking in complexity and mouthfeel compared to a wine produced by spontaneous fermentation, which has been affected by naturally present non-*Saccharomyces* yeasts (Varela 2016; Hu et al. 2018).

Non-*Saccharomyces* yeasts are generally not able to complete alcoholic fermentation by themselves, so they are often used sequentially with *S. cerevisiae* by using *S. cerevisiae* to finish the fermentation (Varela 2016). However, as the ethanol content of wines has increased in the last few decades, this feature of non-*Saccharomyces* yeasts has garnered interest in using them to produce reduced-alcohol wines (Ciani et al. 2016).

The non-*Saccharomyces* yeasts used in this study are *Metschnikowia fructicola, Metschnikowia pulcherrima* and *Torulaspora delbrueckii.* Multiple studies have been done with these yeasts, reporting on their effects on the sensory properties of various fermented beverages. For example, *M. fructicola* has been shown to improve the aroma profile of Treixadura wines by increasing the ester and acetate content, such as 2-phenylethyl acetate, which yields a fruity or floral aroma (Castrillo et al. 2019). In a study with mango wine, both *M. pulcherrima* and *T. delbrueckii* were observed to increase fruity aroma in the wine and were preferred by the sensory panel over the wine fermented with just *S. cerevisiae* (Sadineni et al. 2012). Also in cherry wines, *T. delbrueckii* and *M. pulcherrima* showed increases in compounds associated with fruity aromas, with *M. pulcherrima* causing a significant increase especially in β-Damascone that is associated with sweet, honey-like aroma, while *T. delbrueckii* had an apparent increase in linalool which is perceived as a floral aroma (Sun et al. 2014).

Pichia kluyveri is another non-*Saccharomyces* yeast strain that can influence the aroma profile of wines through its ability to release varietal aromas like thiol-type varietal aromas (4-methyl-4-mercaptopentan-2-one, 3-mercaptohexanol, and 3-mercaptohexyl acetate). In co-inoculation with *S. cerevisiae*, *P. kluyveri* can

enhance the production of these thiol aromas. Some strains of *M. pulcherrima* were reported to have β-xylosidase activity which increases the enzymatic activity during fermentation and release more monoterpenols and 2-phenyl ethanol. (Morata et al. 2020)

Following table (Table 2) shows a summary of some of these metabolites that non-*Saccharomyces* yeasts can contribute.

Table 2. Main metabolites of non-Saccharomyces yeasts, sensory repercussion, and technical impact. Adapted from (Morata et al. 2020)

Non-*Saccharomyces* Species	Metabolite/ Biopolymer	Sensory Repercussion	Technical Impact
Hanseniaspora/Kloeckera	2-Phenylethyl acetate	Floral, rose petals hints	Enhance floral notes
			x2-10 compared to *S. cerevisiae*
	Mannans	Cell wall polysaccharides, mannoproteins	Increased mouthfeel, even perceptible after fermentation
Hanseniaspora vineae	Benzyl acetate	Floral jasmine aroma	Floral
Lachancea thermotolerans	2-Phenylethyl acetate	Floral, rose petals hints	10–50 mg/L
	Ethyl lactate	Strawberry, toffee	>40 mg/L
			High sensory threshold
	Lactic acid	Citric acidity	0.3–16 g/L
			Up to 0.5 pH reductions in oenological conditions
			Slight sugar depletion with some alcohol reduction
Metschnikowia pulcherrima	2-Phenylethanol	Rose-like odour	>30 mg/L
	Monoterpens (e.g., linalool)	Floral	Increase varietal aromas by hydrolysing glucoside terpenes
Pichia kluyveri	Mercaptohexanol (3-MH)	Grapefruit, passion fruit	Fruity smell: > 625 ng/L single fermentation to 3000 ng/L co-inoculation
	Mercaptohexyl acetate (3-MHA)	Grapefruit, passion fruit	Fruity smell: > 500 ng/L single fermentation to 1700 ng/L co-inoculation

Schizosaccharomyces pombe			Enhance the formation of vitisin A derivatives
	Pyruvate	Stable pigments, colour stability	Some strains also vinylphenolic pyranoanthocyanins
	Cell wall polysaccharides, mannoproteins	Better wine structure, softening of the astringency	Increased mouthfeel
Torulaspora delbrueckii	2-Phenylethyl acetate	Flower, honey	1.2-2x compared to *S. cerevisiae* and *Saccharomyces uvarum*
	Ethyl hexanoate	Apple	Fruity smell
	3-Ethoxy-1-propanol	Black currant, solvent	Black fruity smell
Wickerhamomyces anomalus	2-phenylethyl acetate	Flower, honey	
	Isoamyl acetate	Banana	Enhance fruitiness
	Ethyl acetate	Fruity at low concentration	Fruity smell at low concentration
			Enhance complexity

T. delbrueckii is particularly interesting as it is the first non-*Saccharomyces* yeast that has been commercialized and used at the industrial level. According to a review by S. Liu et al. (2022), compared to other non-*Saccharomyces* yeasts, *T. delbrueckii* is characterized by relatively low production compounds that cause off-flavours, such as acetic acid, acetaldehyde, ethyl acetate, acetoin, and hydrogen sulfide. Additionally, *T. delbrueckii* has been found to increase the production of desirable compounds, such as glycerol and fruity esters, that can improve the organoleptic attributes of wine. The maximum ethanol yield of *T. delbrueckii* fermentation is somewhat low at about 9−10%, and for this reason, sequential or simultaneous inoculation of *T. delbrueckii* and *S. cerevisiae* is the common approach to overcome this problem.

Studies have found that fermentation with a coculture of *T. delbrueckii* and *S. cerevisiae* increases ethanol yield significantly while reducing the accumulation of acetaldehyde, fatty acids, and higher alcohols when compared to a pure *S. cerevisiae* fermentation. Additionally, *T. delbrueckii* has been found to alter the profiles of other phenolic compounds, which can affect the mouthfeel and taste attributes of the final wine, such as increasing astringency and bitterness. One sensory evaluation study found that the overall perception, of the wines produced by sequential inoculation, to be better than that of wine produced by monocultures of *S. cerevisiae*. (S. Liu et al. 2022)

1.6 Aim of the study

Based on an earlier study, we know how the chemical composition of black currant wine changes depending on the type of yeast used in fermentation (Kelanne et al. 2020). While some assumptions can be made on how the sensory properties of the wines might have changed based on the chemical composition, it is necessary verify those changes with sensory analysis.

Thus, the aim of this study was to perform a sensory evaluation with a trained panel in order to determine how the sensory qualities of the black currant wine changes, depending on the yeast used for fermentation, and whether those findings correlate with the changes in the chemical composition.

Due to the acidic nature of the black currant berry, it is a challenge to create products with it, without adding a large amount of sugar to combat the sourness. As the resulting acidity and sugar content are among the things influenced by the choice of yeast, this study demonstrated whether these yeasts are suitable for black currant wine production or if the already somewhat unfavourable sensory properties are made worse.

The results of this study were also included in a publication “Comparison of volatile compounds and sensory profiles of alcoholic black currant (*Ribes nigrum*) beverages produced with *Saccharomyces*, *Torulaspora*, and *Metschnikowia* yeasts” by Kelanne et al. (2022) which also investigated the volatile compounds of the wines using GC-FID, HS-SPME-GC-MS and GC-O

2 Materials and Methods

2.1 Materials

2.1.1 Berries

Frozen Finnish black currants (100% Suomalainen Mustaherukka, Pakkasmarja Ltd., Suonenjoki, Finland) were used. The berries were purchased from a local supermarket, and they were from two different batches. Prior to use, they had been stored in -20 °C.

2.1.2 Yeasts

Most of the used yeast strains were manufactured by Lallemand Inc. (Montréal, Quebec, Canada) and were also provided by them: *Saccharomyces cerevisiae* Lalvin W15, *Torulaspora delbrueckii* Level Biodiva, *Metschnikowia fructicola* IOC Gaïa and *Metschnikowia pulcherrima* Level Flavia. *Saccharomyces bayanus* Condessa was from Viinitalo Melkko Ltd. (Lahti, Finland) and was purchased from a local wine equipment store in Turku, Finland.

2.2 Juice preparation

The frozen black currants were thawed in 200 g batches using a microwave twice for 1.5 minutes at 350 W. The thawed berries were then cold pressed into juice with food processor using a horizontal juice press attachment (Kenwood Limited, Havant, United Kingdom). Pasteurization was done by filling 50 mL screw top glass vials with the juice and placing them in boiling water until the juice temperature reached 97 °C. Once the temperature had been maintained for 30 seconds, the vials were placed into an ice bath to cool down to room temperature. Using a scale and a volumetric flask 100 mL of the juice was determined to weigh 105.06 grams. After the juice had cooled down, it was divided into fermentation vessels, each holding 525.3 g of juice.

2.3 Fermentation

Rehydration solution for each yeast was made using 5.4 g of Go-Ferm (Lallemand, Montréal, Quebec, Canada) to 120 mL of 40 °C water resulting in a

4.5% solution. Mixture of 0.75 g of yeast and 20 mL of rehydration solution was made from each of the five yeasts. The rehydration solution temperature was 35 °C for *S. cerevisiae* and *S. bayanus*, for *T. delbrueckii* and *M. pulcherrima* it was 33 °C and for *M. fructicola* it was 30 °C. After 20 minutes, 3.3 mL of the yeast solution was added to the fermentation vessels so that each yeast had 3 fermentation vessels. The fermentation vessels were stored in a covered Styrofoam box in room temperature.

Approximately 23 hours later, the *M. fructicola* and *M. pulcherrima* batches were inoculated with *S. cerevisiae* by adding 3.33 mL of yeast solution into the fermentation vessels. The yeast solution was made with 1.5 g of yeast and 40 mL of 4.5% rehydration solution.

The batches made with just *S. cerevisiae* had very active fermentation that proved to be too much for the smaller fermentation vessels which had minimal airspace, so the three batches were combined and transferred into a larger vessel during the second day of fermentation.

After 8 days of fermentation, 5 mL of 5% (w/v) yeast stopper (potassium sulphate-potassium sorbate mix, 1:1) was added to each fermentation vessel, except the ones with *S. bayanus* which were allowed to ferment additional 4 days. The wines were centrifuged to remove the yeast, their °Brix was measured and then they were divided into smaller bottles and stored in -80°C.

2.4 The panel

The panel (n=11) consisted of nine women and two men within the age range of 20-59 years. Majority of the panel were university students with the rest being university staff. None of the panellists were smokers and only two of them had never participated in a sensory evaluation before. Five members of the panel had participated in sensory evaluations multiple times.

Panellists' information was handled according to GDPR, with their e-mail addresses being used only for correspondence during the evaluation and then immediately deleted afterwards. When analysing the results, the panellists were referred to only by a code number, so they were not identifiable. At the start of the study, the panellists were informed of their right to withdraw at any point.

Due to the ongoing Coronavirus COVID-19 pandemic, panellists were asked to notify the organizers if they had any symptoms of respiratory infection or other illnesses prior to the evaluation, so their participation could be cancelled, or their schedule adjusted as necessary. Panellists were directed to disinfect their hands when they arrived. Training was done in small groups, so that panellists could maintain a safe distance from each other in the room and when using the testing booths, one booth was left empty between each panellist. All surfaces were disinfected after each use.

2.5 Sensory evaluation

Samples were served in wine glasses at room temperature. Taste reference samples were served in small glass beakers and odour references were in foil covered small glass bottles with screw tops. Samples were labelled by random three-digit codes. Each panellist also received a large glass of water, a cracker, and a plastic expectoration cup. They were instructed to drink water or eat some of the cracker between each sample.

The training consisted of four sessions, each lasting up to 60 minutes. The evaluations were written on paper, except for the fourth one where a computer was used instead. During the first training session the panellists were served 2x5 reference samples and asked to identify whether the sample was sweet, sour, bitter, astringent, or just water as listed in Table 3. 1st training basic taste test . Samples were served in randomized order. After each session, there was a group discussion on the suitability of the reference samples and the anchored reference points on the scales.

Table 3. 1st training basic taste test samples.

Descriptor	Content
Astringent	0.07% Caffeine
Bitter	0.1% $AlSO_4$
Blank	Water
Sour	0.07% Citric acid
Sweet	2% Sucrose

Panellists were then asked to describe the odour, the appearance, and the taste of the wine samples (*M. pulcherrima, T. delbrueckii* and *M. fructicola)*, using their own words. And finally, they were asked to evaluate specific attributes in the wine samples, with the help of the reference samples, and place them on a scale from 0 to 10. Each attribute had two reference samples with different concentrations, as seen in Table 4.

Table 4. 1st training basic taste reference samples in the order they were evaluated in.

Descriptor	**Content**	
Sweet	2% Sucrose	4% Sucrose
Sour	0.07% Citric acid	0.14% Citric acid
Bitter	0.07% Caffeine	0.14% Caffeine
Astringent	0.1% $AlSO_4$	0.2% $AlSO_4$

In the next training the panellists were given two wine samples (*T. delbrueckii* and *S. bayanus)*, the same reference samples as in first training (Table 4) and five different aroma references (Table 5). They were asked to evaluate the intensity and suitability of the different aroma reference samples on six different attributes (Table 6), and the intensity of the basic reference samples (sweet, sour, bitter, astringent) by placing them on a scale from 0 to 10. Then they were asked to evaluate the wine samples and add them on the same scales as the reference samples.

Table 5. 2nd training aroma reference samples.

Reference	**Content**
A	Black currant juice
B	Diluted black currant concentrate
C	Black currant berries
D	Commercial black currant wine
E	Black currant leaves

Table 6. 2nd training aroma attributes in the order they were evaluated in.

Descriptor	**Eligible references**
Sweetness	A, B, C

“Black currantness”	A, B, C, E
“Wineness”	D
Sourness	D
Sharpness	Any
Richness	Any

In the third training, the panellists were asked to evaluate the aroma (Table 7), structure (Table 8) and taste (Table 9) of the two wine samples (*M. fructicola* and *S. cerevisiae*) and the 13 reference samples (Table 10) using a 0 to 10 scale.

Table 7. 3rd training aroma attributes in the order they were evaluated in.

Descriptor	**Eligible references**
Total intensity	Wine samples
Black currant aroma	Wine samples, B, C
Sweetness	Wine samples, A, B
Sourness	Wines samples, J, P
Sharpness	Wines samples

Table 8. 3rd training structure attributes in the order they were evaluated in.

Descriptor	**Eligible references**
Visual viscosity	Wine samples, V1, V2
Viscous mouthfeel	Wines samples, V1, V2

Table 9. 3rd training taste attributes in the order they were evaluated in.

Descriptor	**Eligible references**
Total intensity	Wine samples
Black currant taste	Wine samples, MH
Sweetness	Wine samples, M1
Bitterness	Wines samples, K1, K2
Sourness	Wine samples, H2
Astringency	Wines samples, A1, A2

Table 10. 3rd training reference samples.

Reference	Content
A, MH	Black currant juice
B	Undiluted black currant concentrate
C	Black currant berries
J	Yoghurt
P	Buttermilk
V1	Black currant smoothie
V2	Black currant soup (“mehukeitto”)
M1	Sucrose 2%
H2	Citric acid 0.14%
A1	AlSO4 0.10%
A2	AlSO4 0.20%
K1	Caffeine 0.07%
K2	Caffeine 0.14%

The fourth and final training was performed using Compusense Cloud version 20.0 (West Guelph, Ontario, Canada). The training was mostly the same as the third one, except the wine samples were changed (*M. pulcherrima* and *S. bayanus*), reference samples V2, J and P (Table 10) were removed, and some changes were made to the attribute list (Table 11).

Table 11. 4th training attribute list. Except for “Sharp odour”, this was the final selection of attributes that were used in the actual sensory evaluation. During evaluation the attributes were split into two sections: “Aromas” and “Structure and taste”.

Descriptor
Total intensity of odour
Black currant odour
Sweet odour
Sour odour
Sharp odour
Stuffy, musty odour
Viscosity, mouthfeel
Total intensity of taste
Black currant flavour

Sweetness
Bitterness
Sourness
Astringency

For the actual sensory evaluation, all samples were evaluated in triplicate (in three sessions) and the testing was performed with the Compusense software in the sensory evaluation laboratory (ISO 8589, University of Turku, Finland). 10 mL of each sample was served in normal wine glasses (with a glass lid) and the sample order was randomized for each panellist and for each session. One minute break was set in the software between each sample during which the panellists were instructed to cleanse their palate using water or a cracker. With the exception of “Sharp odour”, the list of evaluated attributes was the same as in the fourth training session (Table 11).

3 Results and Discussion

PanelCheck V1.4.2 (Nofima, Tromsø, Norway) was used to evaluate the panel and panellist performance by looking at agreement, discrimination and repeatability and ranking. There were some outliers among the panellists in agreement as seen in Figure 5. One panellist had clear poor replicate performance as seen by their high MSE value in Figure 6. There was also some disagreement in the ranking for few panellists, especially concerning the black currant odour, which can be seen in the profile plots in Figure 7.

While some of the results suggest that a few panellists would have benefitted from additional training, we can see that no panellist had systematically poor performance in all samples. As there was little effect in excluding one or two of the extreme assessors, all panellists were included in the data analysis.

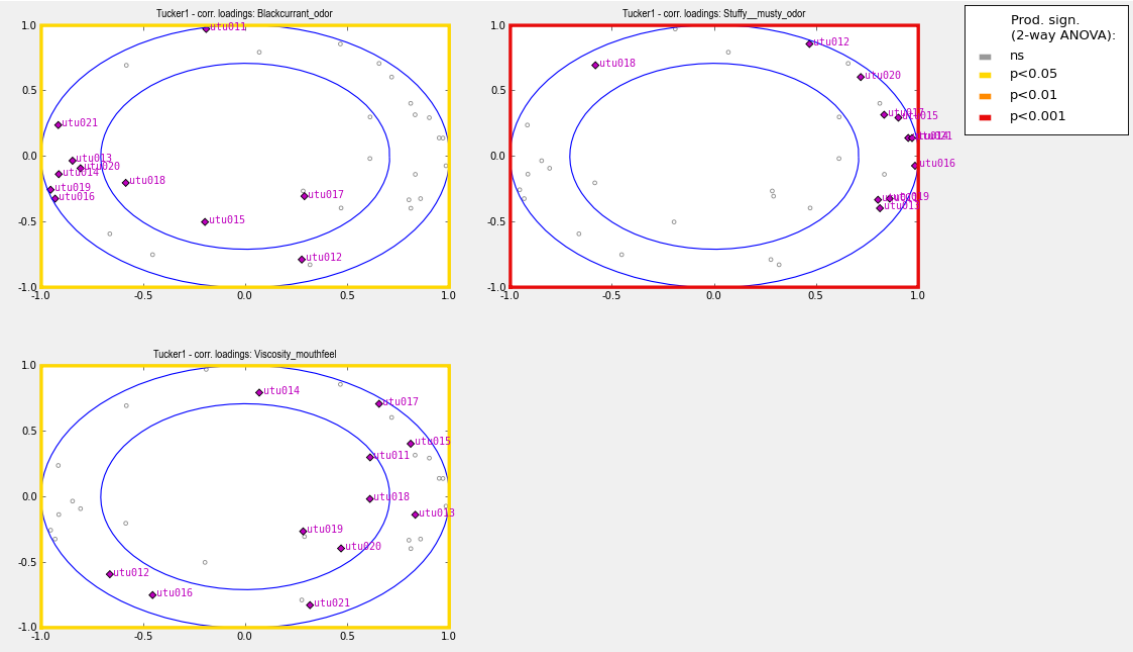

Figure 5. Tucker-1 (Consensus PCA) plots of attributes with statistically significant differences. The two ellipses represent 50% and 100% explained variance. The closer to the outer ellipse, and the more clustered the points are, the better the panel consensus is for that attribute. (Næs et al. 2010)

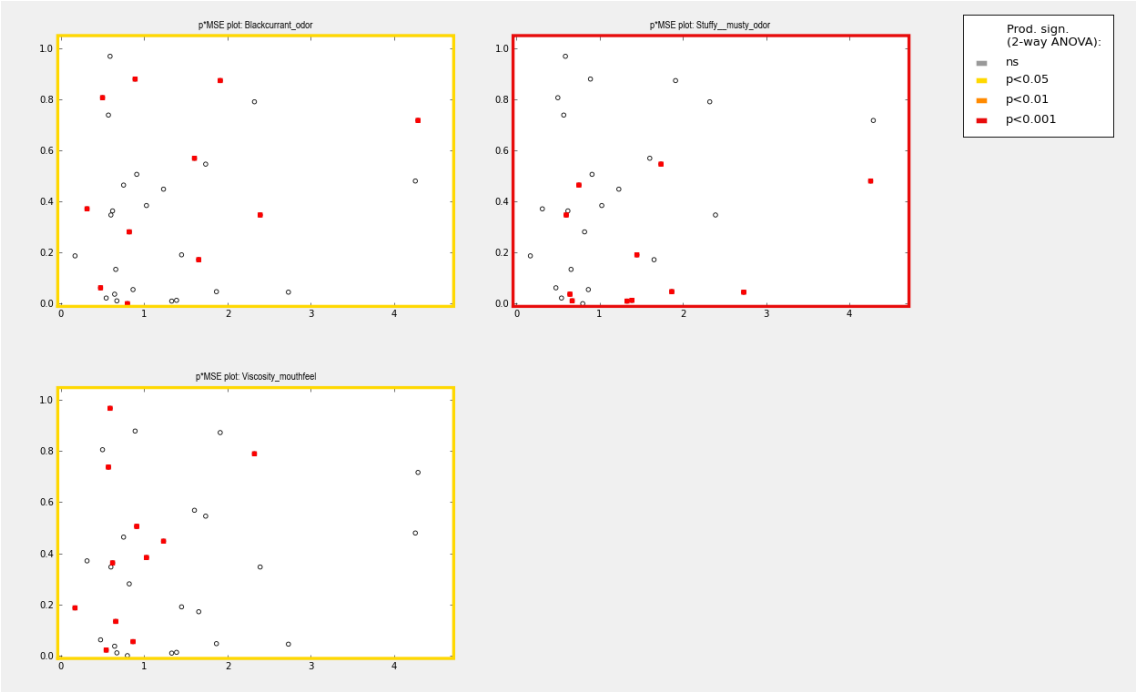

Figure 6. p-MSE plots of attributes with statistically significant differences. MSE-values are on the x-axis and p-values on the y-axis. MSE-values are a measure of repeatability and p-values are a measure of discrimination. The lower both values are, the better the panellist has performed. (Næs et al. 2010)

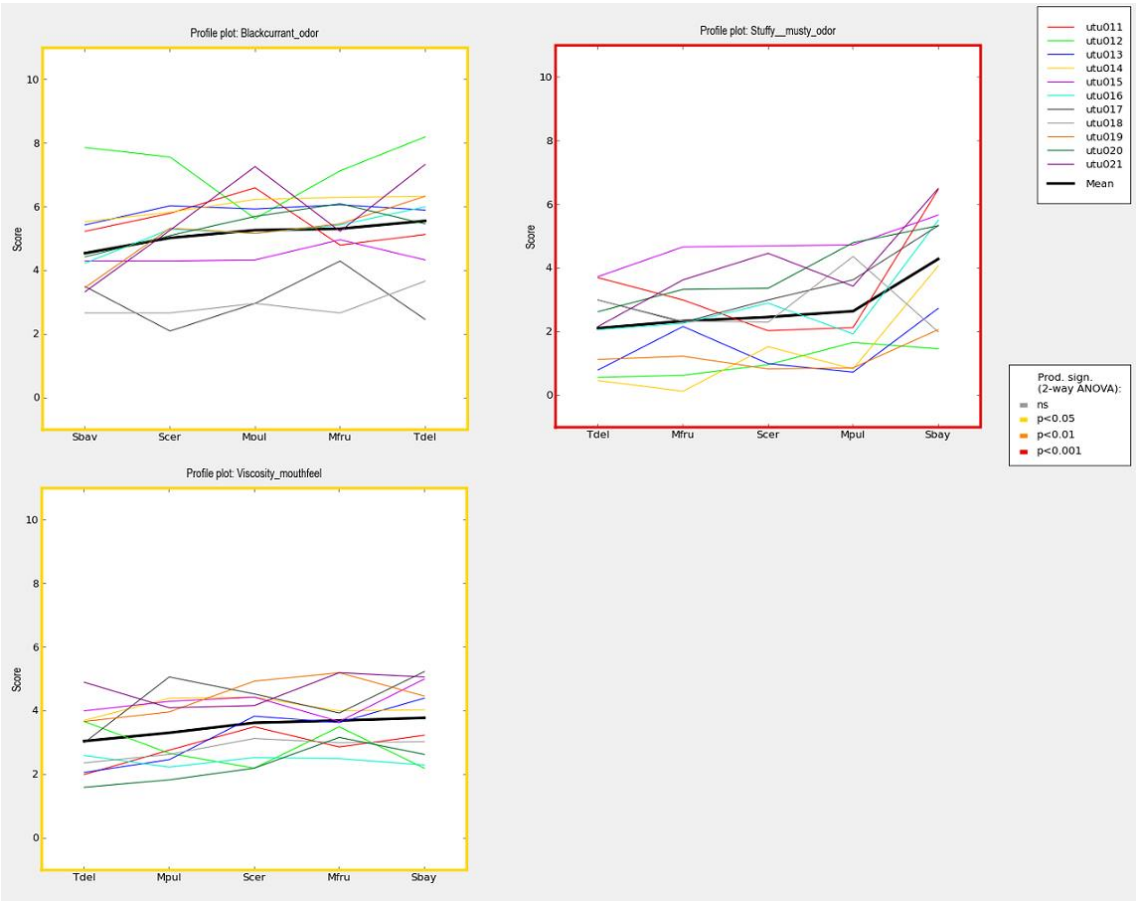

Figure 7. Profile plots of attributes with statistically significant differences. Samples are on the x-axis and scores for each panellist are on the y-axis. The leftmost sample is ranked lowest in attribute intensity by the consensus. (Næs et al. 2010)

2-way ANOVA (Tukey's HSD) calculation done using Compusense software was used to determine the statistical significance of the differences between the sensory properties of the samples.

Of all the sensory attributes being evaluated, only the "black currant odour", "stuffy, musty odour" and "viscosity, mouthfeel" showed statistically significant differences between samples. As seen in Figure 8, *S. bayanus* and *T. delbrueckii* had statistically significant difference in "black currant odour" ($p < 0.05$), while S. bayanus was significantly different from all other samples when it came to "stuffy, musty odour" ($p < 0.01$). For the "viscosity, mouthfeel" attribute, *S. bayanus* and *M. fructicola* showed statistically significant difference compared to other samples ($p < 0.01$).

Multivariate analysis (PCA) results were also obtained from Compusense software, and as seen in Figure 9, they showed clear correlation between more desirable attributes (sweetness, sweet odour, black currant odour, black currant flavour, total intensity of odour) and *T. delbrueckii*, while *S. bayanus* and *S. cerevisiae* correlated more with less desirable attributes (sourness, sour odour, musty odour).

The aromatic characteristics of the *T. delbrueckii* sample was perceived to be the best and this result seems to be in agreement with findings from previous studies (Sadineni et al. 2012; Sun et al. 2014; S. Liu et al. 2022) and what was shown in Table 2. In a recent study it was suggested by Kelanne et al. (2020) that using *Metschnikowia* yeast sequentially with *S. cerevisiae* would be ideal for preserving the phenolic compounds while maximizing the sugar-to-acid ratio. While the sensory characteristics of the *M. fructicola* and *M. pulcherrima* samples did not appear significantly different from the *S. cerevisiae* sample, they were still rated highest after *T. delbrueckii* on the "Black currant odour" and were significantly better in odour attributes, when compared to *S. bayanus.*

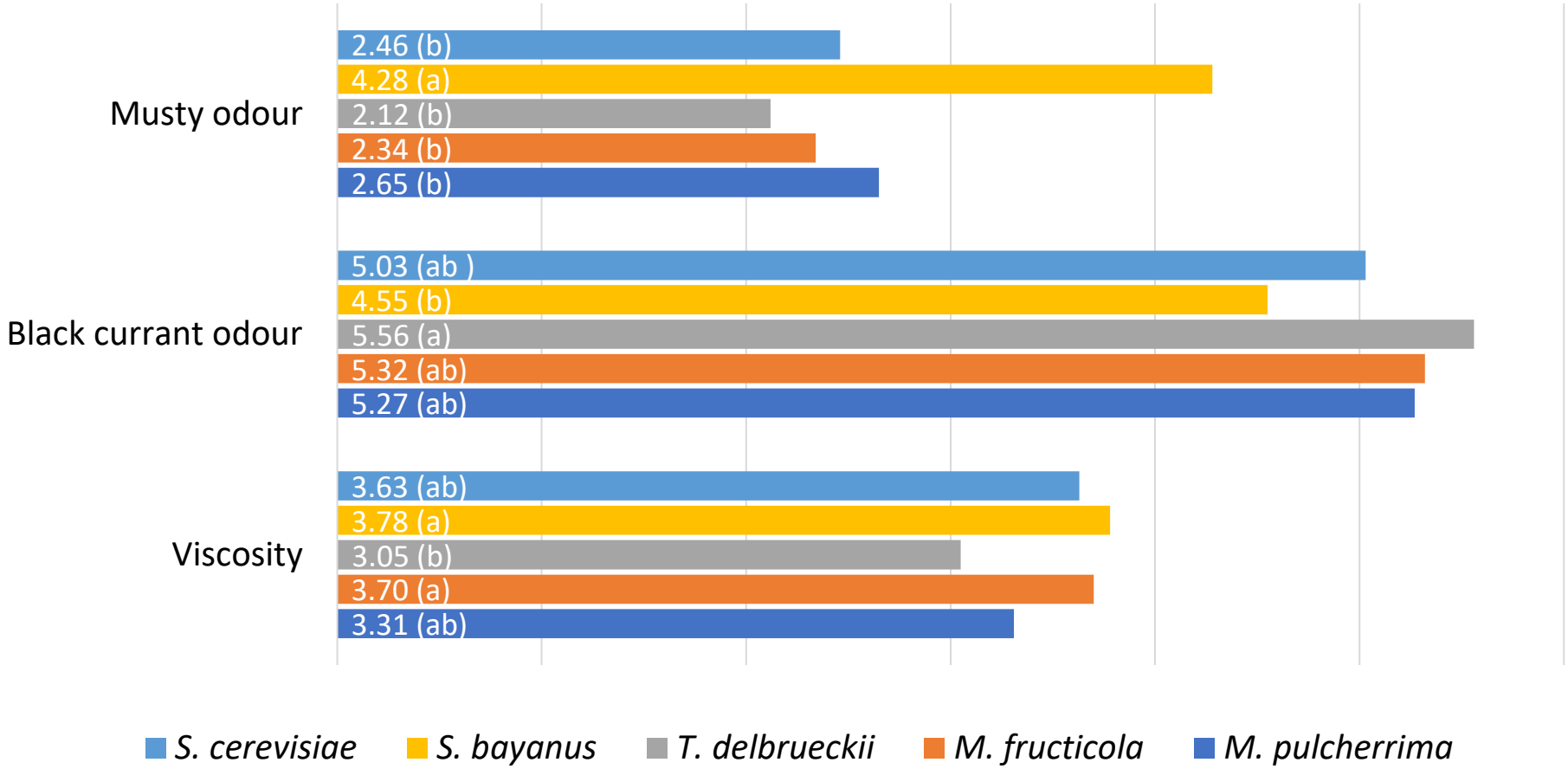

Figure 8. Mean scores of statistically significant attributes calculated by two-way ANOVA (Tukey's HSD). Letters inside the bars signify the groups each sample belongs to. Samples that belong to same groups are not significantly different from each other.

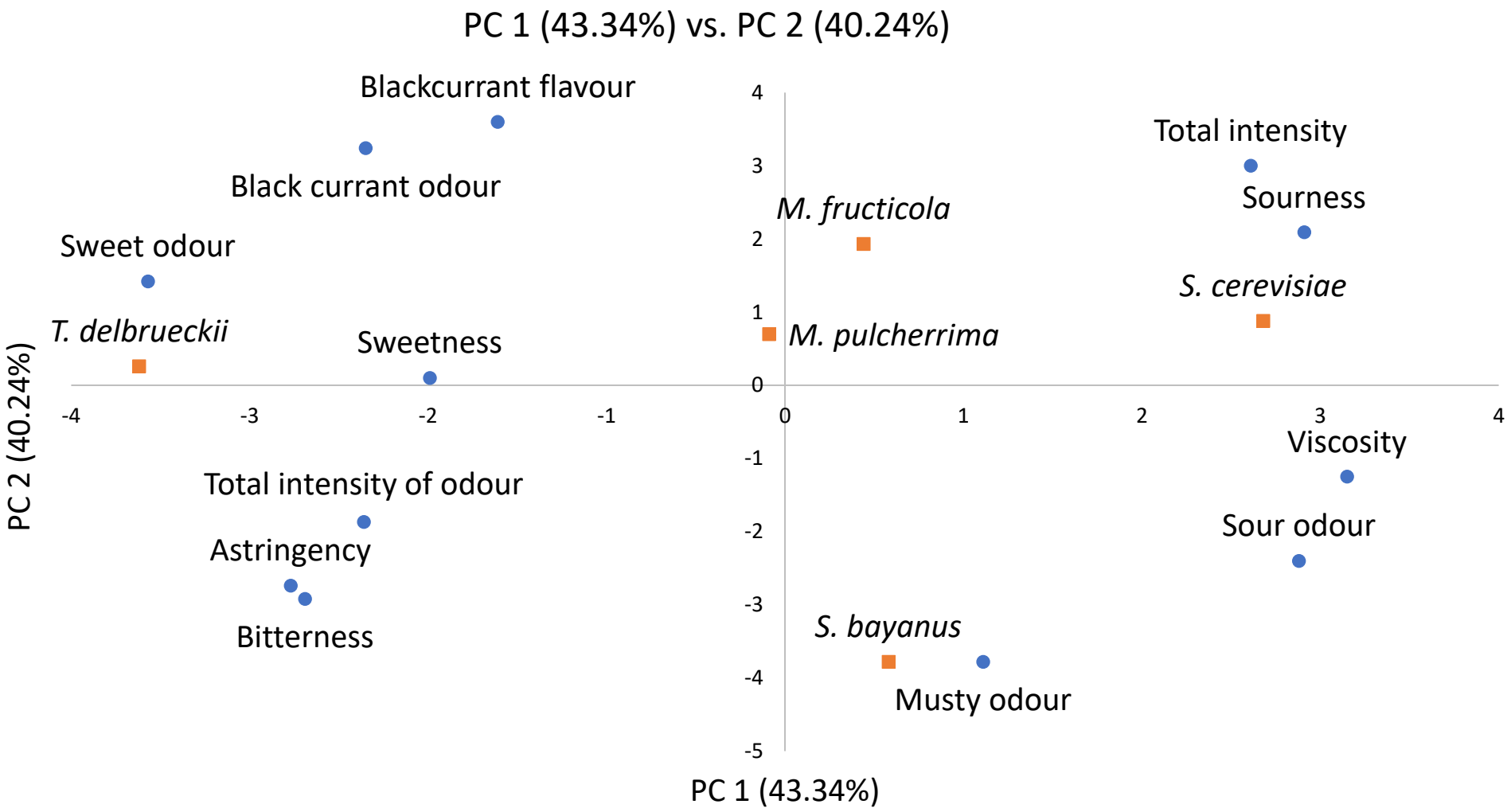

Figure 9. Bi-plot PCA with all measured attributes. Blue circles represent the evaluated attributes (n=12), and orange squares represent the different samples (n=5).

All samples exhibited extreme sourness which made it impossible for the panellists to evaluate taste attributes properly. Black currants are a sour berry to begin with and the sourness was enhanced by the fermentation process. Sweetening the fermentation product afterwards could have alleviated the sourness and allowed the panellists to find differences in the taste attributes as well. One interesting research avenue is looking into using non-*Saccharomyces* yeasts that are known to degrade citric acid during fermentation process, as that could potentially yield less sour product.

However, despite the lack of evidence showing improvement in taste attributes, just the improvements in the odour attributes means that *T. delbrueckii* is a very promising yeast for making black currant wine and that in general, non-*Saccharomyces* yeasts used either alone or sequentially with *Saccharomyces* yeasts have a lot of potential when it comes to improving the sensory qualities of wines.

4 Conclusions

Based on the results of the sensory evaluation study, it appears that there can be significant differences in the sensory characteristics of black currant wines fermented with different yeasts. In particular, the wines fermented either fully or partially with non-*Saccharomyces* yeasts had, in general, stronger black currant odour and weaker musty odour compared fermentation products of *S. bayanus* and *S. cerevisiae.*

These findings suggest that the selection of yeast strain can be an important factor in the production of black currant wine and can potentially be used to develop different flavour profiles and mouthfeels. Further research may be needed to fully understand the mechanisms behind these differences and to explore the use of other non-*Saccharomyces* yeasts in wine production.

As one of the issues with this specific sensory evaluation was the sourness of the black currant, which was further enhanced by the fermentation process, it could be worth looking into using non-*Saccharomyces* yeasts that are known to degrade citric acid during fermentation as they could offer a potential solution to the sourness issue. Additionally, it may be interesting to investigate the potential impacts of different fermentation conditions on the sensory characteristics of the wine.

This study also had a methodological issue concerning the disparity between the samples being evaluated. *Saccharomyces* samples were the “baseline” against which the non-*Saccharomyces* samples were intended to be compared. However, the two *Metschnikowia* samples were fermented in sequence with *S. cerevisiae,* while the *T. delbrueckii* samples was used on its own. Due to this, comparing the *Metschnikowia* samples to the *Torulaspora* sample, or their relative performance in comparison to the *Saccharomyces* samples, was not possible in a meaningful way.

As such, another study where all non-*Saccharomyces* samples are fermented sequentially with *S. cerevisiae* or alternatively, monocultures are used for all samples, could be of interest, to compare the aroma enhancing capabilities of *Torulaspora* and *Metschnikowia* yeasts.

In conclusion, this study demonstrates the importance of yeast strain in the production of black currant wine and highlights the potential for using non-*Saccharomyces* yeasts to develop unique sensory characteristics in the finished product.

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