Fermentation of *Pyrus communis* var. 'Conference' with Native Yeasts and Evaluation of Sensory Properties of Produced Pear Distillates

Bachelor Thesis

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Abstract

For several millennia, yeasts are involved in the production of alcoholic beverages via the fermentation process. Yeast metabolism is responsible for the aromatic compounds produced during fermentation. Different yeasts create different aromatic compounds, or different concentrations of compounds during fermentation because of the metabolic differences between yeasts. In this bachelor thesis, the effect of utilizing native yeasts on the sensory properties of the produced alcoholic beverage is evaluated. A total of 83 isolates were obtained from the surface, juice, and 7-days-rested juice of fresh pears of Conference variety. Among these isolates, 19 of them were identified as yeast samples. For native yeast sample used in sensory analysis, among known species in the sample, *Saccharomyces cerevisiae* has the highest relative abundance, followed by *Saccharomyces eubayanus*. The odor scores were significantly higher for native yeast sample points than the odor scores of LT8 points. The difference between the total sensory analysis scores was not significant. The results showed that the native yeast performed as good as the commercial yeast in terms of sensory properties, with a significantly better odor perception and higher production of ethanol.

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List of Abbreviations

Abbreviation	Full Form
A. arborescens	*Alternaria arborescens*
A. heteromophus	*Aspergillus heteromorphus*
A. namibiae	*Aureobasidium namibiae*
A. pullulans	*Aureobasidium pullulans*
A. subglaciale	*Aureobasidium subglaciale*
acetyl-CoA	Acetyl-coenzyme A
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
et al.	*et alii (and others)*
H. uvarum	*Hanseniaspora uvarum*
K. africana	*Kazachstania africana*
K. marxianus	*Kluyveromyces marxianus*
L. lanzarotensis	*Lachancea lanzarotensis*
L. thermotolerans	*Lachancea thermotolerans*
L. thermotolerans	*Lachancea thermotolerans*
Lachancea thermotolerans	*Lachancea thermotolerans*
M. pulcherrima	*Metschnikowia pulcherrima*
M. larici-populina	*Melampsora larici-populina*
M.osmundae	*Mixia osmundae*
MEA	Malt Extract Agar
P. glucosiphilum	*Pseudomicrostroma glucosiphilum*
P. membranifacencs	*Pichia membranifacens*
P. scopiformis	*Phialocephala scopiformis*
PBS	Phosphate-buffered Saline
R. Graminis	*Rhodotorula graminis*
R. mucilaginosa	*Rhodotorula mucilaginosa*
R. toruloides	*Rhodotorula toruloides*
S. cerevisiae	*Saccharomyces cerevisiae*
S. eubayanus	*Saccharomyces eubayanus*
S. passalidarum	*Spathaspora passalidarum*
S. pasteuri	*Staphylococcus pasteuri*
S. pastorianus	*Saccharomyces pastorianus*
Spathaspora passalidarum	*Spathaspora passalidarum*
T. blattae	*Tetrapisispora blattae*
T. delbrueckii	*Torulaspora delbrueckii*
T. phaffii	*Tetrapisispora phaffii*
TNTC	Too numerous to count
W. anomalus	*Wickerhamomyces anomalus*
Wickerhamomyces anomalus	*Wickerhamomyces anomalus*

1. Introduction

Since ancient times, yeasts are involved in the production of various foods and beverages. Among these, alcoholic beverages are known to have a part in the life of humans for several millennia. One of the types of alcoholic beverages produced is fruit spirits. According to the Regulation (EC) No 110/2008 of the European Parliament and of the Council, fruit spirits can be described as alcoholic beverages produced for human consumption by the alcoholic fermentation and distillation obtained from the raw material that is relevant for the spirit drink produced (European Parliament, 2008). Fermentation and distillation of fruits are the method for obtaining fruit spirits (Einfalt et al., 2020).

1.1. Fermentation

1.1.1. Production of Ethanol

The main process of producing alcoholic beverages is the conversion of fermentable sugars into alcohol, which is known as fermentation (Varela, 2016). Fermentation can be simply described as the process, which belongs to central metabolism, that an organism produces alcohol or an acid by using carbohydrates such as sugars or starch as the substrate (Maicas, 2020).

Biochemically, fermentation can be explained as the process that complex organic compounds anaerobically broken down into similar compounds, together with ATP generation (Berenjian, 2019). Ethanol production during fermentation can be summarized in two overall reactions, the first one being the conversion of fermentable sugars into pyruvate, which can be simplified as follows:

$$2\ \text{Glucose} + 2\text{ADP} + 2\text{Pi} + 2\text{NAD}^+ \rightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\text{NADH}^+ + 2\text{H}^+$$

The first overall reaction of the fermentation can be recognized as the glycolysis (Figure 1).

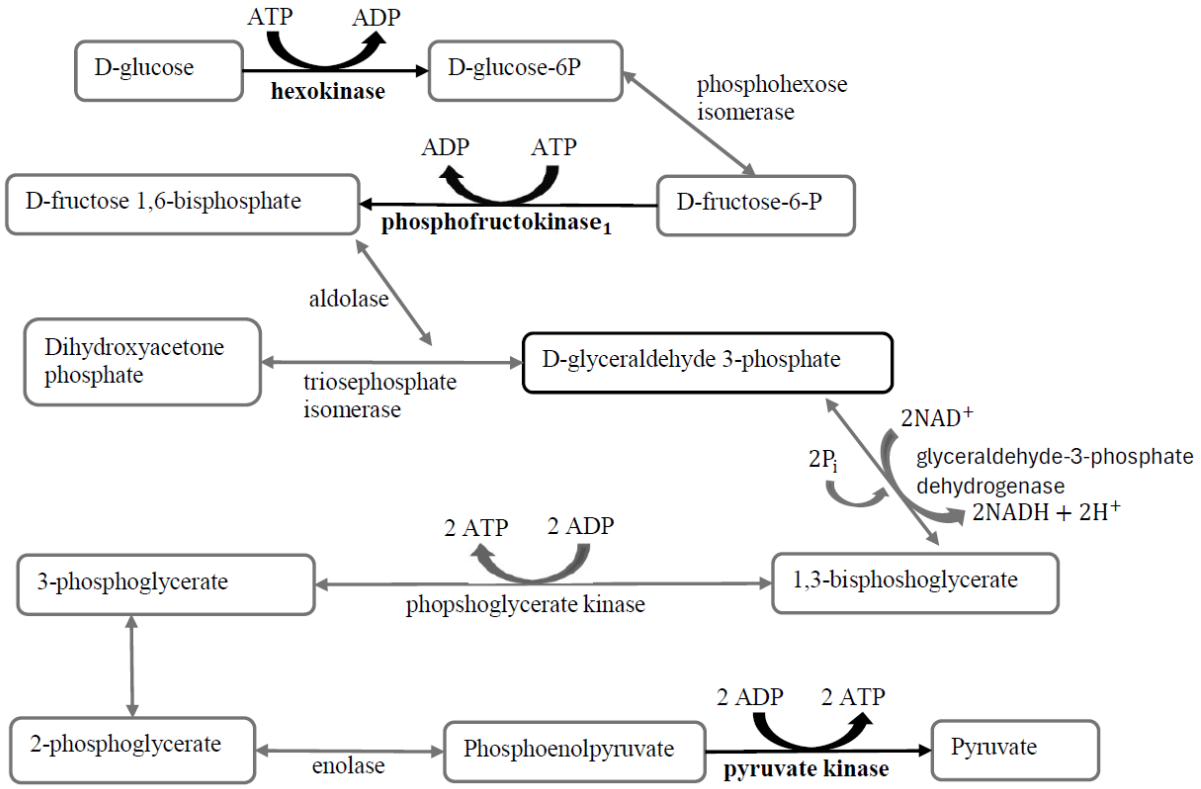


Figure 1 Schematic view of the reactions taking place in Glycolysis. Molecules and involved enzymes are named, thermodynamically irreversible steps are indicated in bold black. Simplified from (Fromm and Hargrove, 2012).

Glycolysis is the pathway that enables ATP generation in anaerobic conditions. For every molecule of glucose, 2 molecules of ATP are produced as the end product of the glycolysis (Fromm and Hargrove, 2012).

In the glycolytic pathway, hexokinase, phosphofructokinase-1 and pyruvate kinase, in which the irreversible steps of the pathway, are also important for the regulation of the pathway with several possible causes to manipulate the activity of said enzymes, such as product inhibition and transcriptional fluctuations changing the concentration of mentioned enzymes (Chandel, 2021). Major reason for the inhibition of hexokinase enzyme is glucose-6-phosphate; the product of the reaction; elevated levels of ATP is considered as an effective inhibitor of phosphofructokinase-1 and pyruvate kinase, along with citrate for phosphofructokinase-1 and L-alanine for pyruvate kinase (Fromm and Hargrove, 2012).

The second reaction being the conversion of pyruvate into ethanol, which can be simplified as follows:

2 Pyruvate + 2NADH +$2H^+$ → $2NAD^+$ + 2 Ethanol + 2 CO_2.

In this overall reaction, acetaldehyde is the intermediate molecule that produced from the pyruvate and the ethanol is produced from the acetyl-CoA. There are alternatives for production of Acetyl-CoA from pyruvate. Acetyl-CoA can be directly produced by utilizing pyruvate dehydrogenase from pyruvate in mitochondria, or alternatively, by utilizing pyruvate decarboxylase, which is responsible for the conversion to acetaldehyde from pyruvate, then by utilizing acetaldehyde dehydrogenase which is responsible for the conversion of acetaldehyde to acetate, and finally acetyl-CoA synthetase which utilized in the conversion of acetate into acetyl-CoA (Rodrigues et al., 2006).

1.1.2. Aromatic Compounds in Spirits

The principle of the alcoholic fermentation process does not change between different alcoholic beverages; but the proportion of the aromatic compounds shows notable variation between alcoholic beverages (Stanzer et al., 2023). Alongside with the production of the ethanol, yeast metabolism is responsible for the formation of hundreds of aromatic compounds (Varela, 2016). Aromatic compounds formed either from the sugar metabolism, derived from pyruvate; or as the nitrogen metabolism by-products (Dupour et al., 2003).

Aromatic compounds are the substances in which the product takes its flavor from, affected by their presence, and their concentration in product. For aromatic compounds to have an impact on the sensory quality of the product, it is not enough for them to be present; depending on the odor threshold, some compounds need to be concentrated sufficiently in the product, and for some, being at a lower concentration could be enough for their detection (Stanzer et al., 2023).

Higher alcohols, volatile esters, organic acids, phenolic and carbonyl groups, and sulfur-containing-molecules are the six main groups of aromatic compounds produced from alcoholic fermentation (Saerens et al., 2010). As the fruit spirits are produced by distilling the mash after the fermentation, they only contain volatile compounds; and the higher alcohols, acids, aldehydes and esters are the compounds that can be found in highest quantities compared to other aromatic compounds, making them the most important substances that give the aromatic characteristics of the spirits (Stanzer et al., 2023).

Esters may be formed by several different ways. Malolactic fermentation, distillation, and maturation of spirits may produce esters, though most of them are produced during fermentation as byproducts (Stanzer et al., 2023).

In fermentations, esters are accountable for a variety of odors. Presence of a large number of esters in fermentations with different yeast species are verified; and generally, low concentrations of esters are present in fermentations (Phaff et al., 2014). As the esters have low odor threshold in alcoholic beverages, concentration changes may have large effects on the overall sensorial quality (Saerens et al., 2010). Generally, esters contribute the aroma characteristics of the products as giving floral and fruity notes (Stanzer et al., 2023)(Table 1).

Acetate esters and ethyl esters are the two main groups of esters that contribute to the aromatic characteristics of alcoholic beverages (Saerens et al., 2008). Ethyl esters are formed during fermentation by esterification, during distillation and maturation, and are the most abundant esters in fermentations (Stanzer et al., 2023).

Table 1 Examples of esters present in pear distillates and their aroma descriptions, (Stanzer et al., 2023 ; Spaho et al., 2021; García-Llobodanin et al., 2007)

Esters	Description
Ethyl acetate	Pineapple, apple-like odor, with astringent, brief taste, glue-like
Isobutyl acetate	Banana, fruity, apple
Ethy butanoate	apple, pineapple, ripe fruit
Ethyl 2-methylbutyrate	Fruity, pineapple, apple
Ethyl isovalerate	Fruity, sweet
Isoamyl acetate	Banana-like
Ethyl octanoate	Fruity, pineapple, pear, flowery
Ethyl lactate	Dairy, green fruity
Ethyl decanoate	Flowery, fruity, fatty
Ethyl dodecanoate	Leaf, fruity, sweet, creamy
Ethyl (E,Z)-2,4-decadienoate	Pear-like, metallic
Ethyl (E,E)-2,4-decadienoate	Pear-like, metallic
Ethyl tetradecanoate	Ether, sweet, flowery
Ethyl hexadecanoate	Waxy, oil

Influence of the higher alcohols on the aromatic characteristics of the spirits are significant, as they are greatly concentrated during distillation (Stanzer et al., 2023). When the higher alcohols are present in the spirits in large quantities, they contribute to the aroma characteristics of the spirits by giving off a strong, fusel, pungent aroma (Stanzer et al., 2023) (Table 2).

During fermentation, higher alcohols are produced directly or indirectly, from sugars or from amino acids respectively (Stanzer et al., 2023). When produced from amino acids, the Ehrlich pathway is employed. With the Ehrlich pathway, fusel alcohols and fusel acids are produced from aromatic amino acids tryptophan, tyrosine and leucine, branched-chain amino acids

valine, leucine and isoleucine, and sulfur-containing amino acid methionine catabolism (Hazelwood et al., 2008)

In the case of higher alcohol production, the process has three steps, being deamination, decarboxylation, and aldehyde reduction; with utilizing amino transferase, pyruvate decarboxylase, alcohol dehydrogenase enzymes in the steps respectively (Gu et al., 2020) (Figure 2).

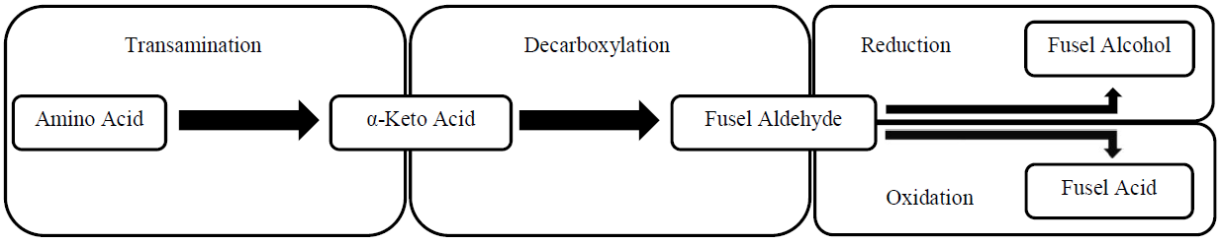


Figure 2 Schematic description of Ehrlich pathway. Simplified from (Hazelwood et al., 2008)

Straight-chain alcohols are present in great quantities compared to other alcohols present in spirits; the most dominant being the amyl alcohols followed by hexanol and butanol as the second group (Stanzer et al., 2023). Methanol is a compound that is intensively produced during fermentation, even though it is not a by-product of the process; it is produced from pectin via demethoxylation (Spaho, 2017).

Table 2 Examples of higher alcohols present in pear distillates and their aroma descriptions, (Stanzer et al., 2023; Spaho et al., 2021; García-Llobodanin et al., 2007)

Alcohol	Description
Methanol	Narcotic ether smell, irritation, burning
3-Methyl-1-butanol	Fusel, solvent, pungent
2-Methyl-1-butanol	Malty, nail polish-like
1-Pentanol	Fruity, sour, pungent
2-Phenylethanol	Flowery, honey-like
1-Hexanol	Grassy, almond-like, green
1-octanol	Fruity

In the production of acids from the Ehrlich pathway, the only difference from the fusel alcohol production is the last step of the pathway; instead of reduction, oxidation takes place by utilizing aldehyde dehydrogenase (Christoph and Bauer-Christoph, 2007).

One of the important groups of aromatic compounds is volatile acids. Produced as a by-product during fermentation, acetic acid is the most commonly observed volatile acid, contamination with acetic bacteria and some errors in distillation causes acetic acid to have high concentrations in the product (Stanzer et al., 2023)(Table 3).

Table 3 Examples of volatile acids present in pear distillates and their aroma descriptions, (Stanzer et al., 2023; Spaho et al., 2021; García-Llobodanin et al., 2007)

Acids	Description
Butanoic acid	Sweaty, cheesy
3-Methylbutanoic acid	Sweaty, dairy
Hexanoic acid	Acidic, cheese, sweaty barbecue
Octanoic acid	Vegetable, fatty sweaty cheese
Decanoic acid	Rancid, fatty, sweaty

Aldehydes are precursors for higher alcohols and acids in Ehrlich pathway. In anabolic and catabolic synthesis of higher alcohols or amino acids, they act as intermediates, thus they can be secreted and act as aromatic compounds in the product, or they can be used to form other compounds during fermentation (Stanzer et al., 2023).

2-methyl-1-propanal, 2-methylbutanal, 3-methylbutanal and acetaldehyde are examples of short-chain aliphatic aldehydes, which are compounds that contribute to the aroma composition by giving fruity flavor (Christoph and Bauer-Christoph, 2007).

Acetaldehyde is a common by-product of fermentation. Giving fruity flavors, acetaldehyde is an important compound in a broad range of fruit flavors by decidedly gives a juicy and fresh aroma; however, at higher concentrations over the odor threshold of acetaldehyde, it is known to give an unpleasant odor to the product (Christoph and Bauer-Christoph, 2007; Stanzer et al., 2023). Compared to other aldehydes, acetaldehyde concentration is considerably higher in spirits (Stanzer et al., 2023).

1.1.3. Yeasts

As the ethanol and aromatic compounds are produced by yeast, the yeast selected for the fermentation has impact on the alcohol yield and the sensory profile of the product (Stanzer et al., 2023). Independent of whether the spores produced externally or internally; yeasts are asexually reproducing fungi that are mainly characterized with single cell growth pattern by utilizing fission or budding primary means for reproduction (Johnson and Echavarri-Erasun, 2011).

In different species of yeasts, the carbon and nitrogen compounds that yeast are able to metabolize and the resulting products are the result of yeasts having considerable differences that can be observed in metabolic potential; even though this differences are not as varied as metabolisms of bacteria (Phaff et al., 2014). In a wide range of disciplines such as food production, medical, and agriculture, yeasts have industrial and essential importance; the

traditional characteristics of yeasts are considered as their role in various food fermentations (Johnson and Echavarri-Erasun, 2011).

Selection of the yeast strains to be used in the fermentation is crucial, not only for increasing the amount of ethanol produced; but also to have a baseline for the sensory quality (Walker and Stewart, 2016b). Being able to grow rapidly, fermenting completely and producing suitable texture and aroma by biochemical activities are the properties in yeasts that are focused, in order to perform desirable fermentations and processes (Fleet, 2003). Being able to remove nutrients effectively during fermentation and effectively tolerating environmental conditions, such as tolerating ethanol are also desired properties of yeast to be used in alcoholic fermentations (Dupour et al., 2003).

The most utilized yeast species in fermentation processes are species from *Saccharomyces* genus, especially *S. cerevisiae*. *Saccharomyces cerevisiae* being the most utilized alternative for ethanol production is a result of its metabolism, the species show characteristics such as a high ethanol productivity and rapid growth (da Silva Fernandes et al., 2022).

Different species in the genus *Saccharomyces* affects the fermented product in terms of composition of aromatics, and fermentation conditions. Utilizing *Schizosaccharomyces pombe* in rum production showed a composition of more esters and less higher alcohols and acids as this yeast performs the fermentation slower (Walker and Stewart, 2016b). *S. pastorianus* is a natural hybrid yeast that is utilized in the lager beer production, as it can perform fermentation at cooler temperatures (Walker and Stewart, 2016b). Similarly, the ethanol sensitivity of *S. eubayanus* increases when the temperature increases; whereas *S. cerevisiae* not being able to grow at such low temperatures is seen as the cause of the higher ethanol sensitivity of *S. cerevisiae* at lower temperatures (Magalhães et al., 2017).

Species in the genus *Saccharomyces* can be observed on pears. In one study, *Saccharomyces cerevisae*, *Schizosaccharomyces pombe*, *Saccharomyces ludwigii* are found to be present on pears and able to naturally perform fermentation (Adedeji and Audu, 2018).

In spontaneous fermentations, in which the fermentations that are not inoculated; non-Saccharomyces yeasts are naturally present and may improve aroma profile of the fermented beverages (Varela, 2016).

Geographic location, climatic and seasonal conditions, type and variety of the fruit and its development and maturity, and usage of agrochemicals can be given as some of the factors that have influence on the microflora of the fruit, which predominantly occur on the surface of the fruit, though a minority is also internally located of the fruit (Fleet, 2003).

Several studies have investigated the fruit microbiota, and isolated native yeasts of the fruits and identified them (Adedeji and Audu, 2018; Lee et al., 2011; Graça et al., 2015; Ruyters et al., 2015). The yeasts on the fruits are belongs to several different genera, and some species have characteristics beneficial for fermentation, and present in several types of fruits. In one study, from apples, apricots, melons, wild strawberries, kiwis and 6 varieties of grapes, a total of 637 isolates of wild yeasts obtained; among these isolates, 120 strains which were classified into 13 species in 7 genera showed at least one of the several properties desired for fermentation, such as better alcohol and glucose tolerance; and *Pichia anomala*, *Wickerhamomyces anomalus, Aureobasidium pullulans, Kodamaea ohmeri* and *Saccharomyces cerevisiae* were the species most found among these species (Lee et al., 2011).

Some species of yeasts can be predominant on fruits. In a study that examined fresh-cut apples, and the found yeast isolates were belonged to 15 species in 10 genera, with the most found isolate species being *Candida sake* and *Pichia fermentans* (Graça et al., 2015). In another study investigating the microflora of the fruits and leaves of 'Williams' pear variety, 18 bacterial isolates and 28 fungal isolates obtained; the yeast species *Metschnikowia pulcherrima* and *Hannaella luteola* were found to be predominant in May and July, respectively (Janakiev et al., 2022).

1.1.4. Fermentation Conditions Related to Fermentation Quality

There are different factors that affect the fermentation quality and aroma profile of the produced alcoholic beverage, such as temperature, pH value, other than the yeast used for the fermentation.

Nitrogen content affects the fermentation in several different ways. It is involved in the rate of fermentation and the production of biomass; and is one of the causes of sluggish or stuck fermentations if the fermentation lacks the nitrogen (Mas et al., 2014). In one study on commercial yeast strains, increasing the initial nitrogen content resulted with a shorter fermentation time (Rollero et al., 2015). Knowing the effect of a specific nitrogen source on the

growth and the fermentation performance of a specific yeast is crucial for optimizing the mentioned parameters (Kemsawasd et al., 2015).

Nitrogen content is an important factor in the production of aromatic compounds. For some aromatic compounds, quality and quantity of the nitrogen source is among the conditions that critical for the formation of the said compounds (Mas et al., 2014). The effects of addition of nitrogen compounds are investigated in different studies. In a study on *S. cerevisiae*, the final liquid concentrations of higher alcohols, all acetates and ethyl esters showed a positive correlation with nitrogen content (Rollero et al., 2015). In another study, on *S. cerevisiae* and *T. delbrueckii*, the complex mixtures of nitrogen sources; and on *L. thermotolerans, H. uvarum,* and *M. pulcherrima*, the single amino acids positively affected all performance parameters (growth rate, viability, glucose consumption, ethanol production), and fermentation performance and growth, respectively (Kemsawasd et al., 2015).

Yeast growth, product formation and fermentation rate are the parameters that pH shows a significant influence (Pramanik, 2003). pH value causes a variation on the ethanol production ability (Pham, 2022).

In one study, between pear fermentations with different initial pH values, the fermentation with an initial pH of 3.0 showed the highest amount of volatile compounds compared to the other pH values that varied between pH 2.5 and pH 4.5 (Pham, 2022).

Temperature is another factor that affects both the alcohol and aromatic compound production in fermentations. Temperature affects fermentative kinetics significantly, ethanol and biomass production are parameters that temperature has an apparent influence (Sablayrolles, 2019; Pramanik, 2003).

Low fermentation temperatures (10-15 °C) enhance the aromatic profile of alcoholic beverages through improving the volatile compound production, nevertheless, sluggish or stuck fermentations may be the outcome of the fermentations that are performed in low temperatures (Sablayrolles, 2019).

In one study, it is found that alcohol production is positively affected with temperatures between 20°C and 30°C during fermentation (Pham, 2022). In the study mentioned, it is found that for pears, ester content was higher in temperatures between 15°C and 20°C and decreased in

temperatures over 20℃; and a proportional increase in the total higher alcohol content is observed when temperature was increased from 10℃ to 25 ℃ (Pham, 2022).

1.2. Distillation

1.2.1. Distillation Systems Used for Fruit Spirit Production

Distillation is the dominating technique in the chemical process industry used for separation, which has been used to concentrate dilute alcoholic beverages for centuries (Patil, 2023). It can be termed as the technique that the liquid mixtures are separated in the basis of boiling points of the components by firstly vaporizing the liquid, and then recondensing in order to collect the components separately (Spaho, 2017).

In the case of a fermented mash, the mixture consists of a multitude of volatile compounds that have a sizeable difference in their boiling points, and water and ethanol, which act as carriers for the volatile compounds; and the characteristics and flavor of the resulting spirit are established according to the volatile compounds profile of the resulting distillate, and the concentrations of volatile compounds present in the finished product (Spaho, 2017). At the end, distillation results in enhanced influence of aromatic compounds by concentrating them in the distillate (Stanzer et al., 2023).

The working principle of the batch distillation systems can be explained as increasing the temperature of the fermented mash until the boiling temperature is reached, continued with passing of the resulting vapor through a rectification system, and finally condensing the vapors in order to obtain distillate (López et al., 2017).

Batch distillation with an alembic copper pot is known as a basic still that is used for producing distillates with an intense flavor (Christoph and Bauer-Christoph, 2007). An alembic still is an enclosed copper pot with a steam pipe that allows the vapor produced with heating the pot to travel through the pipe into a condenser that is cooled down with water, and the condensed vapor collected at the end of the condenser.

For having a high volume of alcohol in the distilled product, the distillations repeated usually twice, with the first distillation performed with the aim of concentrating the alcohol content, and the second distillation performed with the aim of purifying and fractioning the distillate (Spaho, 2017).

Batch distillation columns are equipped with a rectifying column that consists of a number of trays that corresponds to a number of equilibrium stages, and the vapor produced passes through the column (Patil, 2023). From boiler, the vapor mixture goes to the dephlegmator in the beginning of the distillation and gets partially condensed and only a portion of the vapor returns to the trays (Spaho, 2017). Purpose of the dephlegmator is to enable the distillate vapors to pass on to the cooler by acting as a thermal barrier (Heller and Einfalt, 2022). As a result of the reflux mechanism caused by the dephlegmator, the vapor and liquid contacts on the trays of the column, this contact results in with a greater separation of the compounds by making the vapor richer in terms of light components, thus the resulting distillate coming out of the condenser results in an alcohol concentration of 70-87% (v/v) (Spaho, 2017).

1.2.2. Separation of the Distillate

High volumes of ethanol along with high volatile compounds comes through the still at the beginning of the distillation, overtime the volume of low volatile compounds increases, while the volume of water coming through the still increases as the volume of alcohol decreases; and these changes are the basis for cutting the distillate into three fractions namely the heart, the head and the tail (Spaho, 2017).

The head fraction is the first cut of the distillate that comes out of the still and characterized by containing low boiling compounds such as acetaldehyde (Christoph and Bauer-Christoph, 2007). The head fraction is mainly considered as undesirable, as the compounds in this fraction are responsible for giving unpleasant, sharp, and strong flavor to the distillate, and as this part contains some toxic compounds in high concentrations (Spaho, 2017).

As a contrast, the tail fraction is the last fraction that comes out of the still and characterized by high boiling compounds like long-chained fatty acid ethyl esters (Christoph and Bauer-Christoph, 2007). The tail fraction is also considered undesirable, as the longer molecules carried by water and unpleasant fatty and oil compounds present in this fraction giving undesirable flavors to the distillate (Spaho, 2017).

The head fraction is the distillate obtained in between head and tail fractions. This part of the distillate is considered as the consumable product of the distillation for spirit production. The head fraction can be defined as the fraction of the distillate that has high in ethanol content with pleasant aroma compounds (Spaho, 2017). The head fraction is important for the sensory

quality as it has a high volume of aromatic compounds that affects the sensory profile (Christoph and Bauer-Christoph, 2007).

The heart fraction needs to be separated from the head and tail fractions for producing fruit spirits. This separation enables the production of fruit spirits with pleasant sensory profiles as it is the separation of a large group of volatile compounds in the distillate (Spaho, 2017).

The separation of the fractions can be performed with sensory evaluation. As the head fraction has a strong, sharp, and unpleasant flavor because of the composition of the volatile compounds present, the change in the flavor of the distillate can be used as a cut point; and similarly, the change of the flavor can be used as a cut point between the heart and tail fractions, as the flavor that gives a dull, faded character to the distillate starts with the tail fraction (Spaho, 2017).

1.3. Sensory Analysis

Aromatic compounds in the produced alcoholic beverage create a sensory perception on the people consuming the product, and by evaluating this sensory perception, the quality of the alcoholic beverage can be assessed (Stanzer et al., 2023). Smell, taste and overall harmony are determinants for the sensory quality. Orthonasal and retronasal pathways, which can be perceived as odor and aroma respectively, are the pathways used for the most important element for sensory perception, the smell, to reach the brain (Stanzer et al., 2023). However, the overall harmony of the beverage is the important factor for the sensory quality. In order to have a sensory acceptance it is vital that the impression of produced spirit is harmonious and pleasant, which is observed as a result of the aromatic compounds the spirit contains being balanced within each other (Stanzer et al., 2023).

2. Objectives

One of the factors affecting the sensory profile of fruit spirits is the yeasts used during fermentation of the fruits. For enriching the sensory properties of fruit spirits, using native yeasts present on the fruit itself is one of the research areas that is being investigated.

In order to determine the native yeasts present on the fruit itself, it is essential to investigate the microbiota of the fruit that is planned to be used in the production of the fruit spirit. To establish the amount of potential yeast samples to be used in the fermentation, total counts of microorganisms as well as total counts of yeast samples present on the fruit are investigated.

In order to evaluate the ethanol production capabilities of native yeasts obtained, it is necessary to compare the ethanol production of the native yeasts with the commercial yeasts that are currently in use for spirit production. With this aim, laboratory scale fermentations with selected native yeasts and commercial yeast are conducted.

The last part of this study aims to evaluate the sensory perception of the spirit produced with native yeast compared to the spirit produced with the commercial yeast. Based on ethanol production data, fermentation with the commercial yeast is performed along with the native yeast with ethanol production capabilities similar to the commercial yeast, and sensory analysis is conducted to investigate the effects of using native yeast in fruit spirit fermentation on the perception of sensory profile of the produced spirit.

In this bachelor thesis, the effect of utilizing existing yeast on the pears itself in the fermentation of the pears for the sensory properties of the produced alcoholic beverage is evaluated by comparing a strand of commercial *S. cerevisiae*. The hypothesis of fermentation with native yeast of the fruit having a significant effect on the sensory quality of the pear spirits is tested.

3. Materials and Methods

3.1. Isolation and Identification Yeasts from Pear

3.1.1. Isolation of Microorganisms from Pears

For the yeast isolation from pears, *Pyrus communis* var. 'Conference', the samples are collected from the surface of the pears, fresh pear juice, and 7-days-rested pear juice. The isolation procedure repeated for 3 times in order to have 3 batches of isolations.

Surface samples are taken from a $2cm^2$ area, with a cotton swab dipped in phosphate-buffered saline (PBS) solution. The surface samples taken from 2 pears per batch.

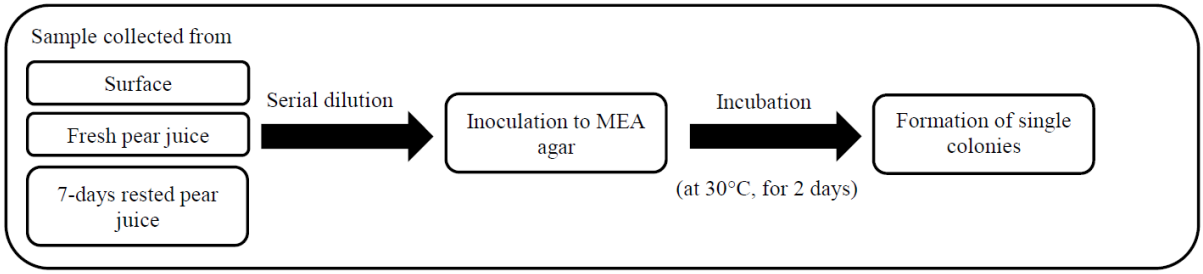


Figure 3 Schematic description of isolation of microorganisms from pears.

The pears are juiced with a juicer, and the fresh pear juice samples are taken right after the juicing process. The remaining pear juice is left in 30°C for 7 days in order to obtain the 7-days-rested pear juice samples.

Surface samples, fresh juice samples and 7-days-rested juice samples are plated on malt extract agar (MEA) plates using spread plate method, after serial dilution with PBS to a minimum of 10^{-5}, and incubated for 2 days at 30°C.

3.1.2. Identifying Yeast Cells

After two days of incubation at 30°C, the single colonies with different characteristics (i.e. color, size, surface type) formed on the agar plates inoculated with the surface, fresh juice, and 7-days-rested samples were isolated by taking the single colonies with a sterile inoculation loop and streaking to new agar plates that were separated into 3 or 4 segments.

After the incubation of the isolated colonies on agar plates in the same incubation conditions, all samples were analyzed under a light microscope at 400x magnification in order to differentiate yeasts and other microorganisms.

All samples deemed as yeasts were inoculated to new MEA agar plates by using sterile inoculation loops in order to obtain separate agar plates for each sample and incubated in the same incubation conditions. The samples were inoculated for 2 more times in order to eliminate contamination of the samples. After all samples are acquired, 4 of them are selected to use in lab scale fermentations.

After the yeast samples are chosen to be used in fermentations, samples from the selected yeasts are sent for DNA analysis in order to get information about the species of the yeasts.

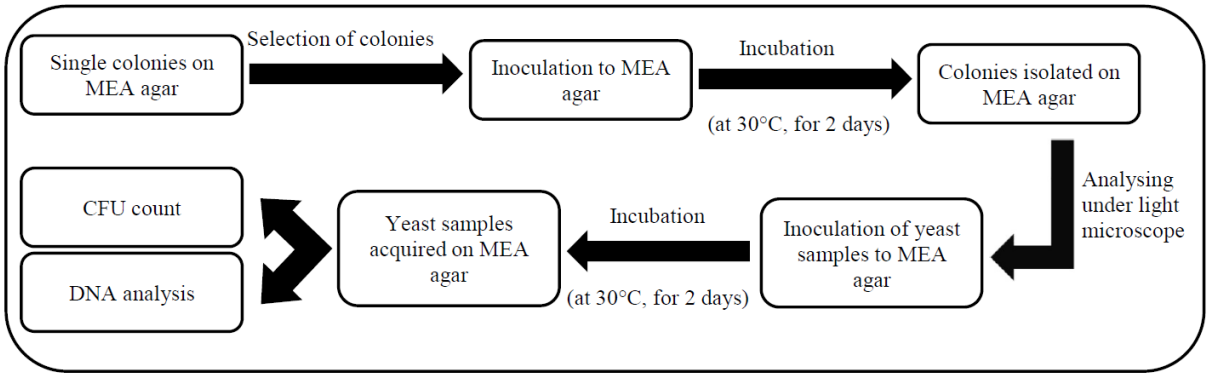


Figure 4 Schematic description of identification of isolates as yeast. Further analysis with CFU count and DNA analysis are also included in the diagram.

The external DNA analysis is performed by CeGaT GmbH (Tübingen, Germany). Illumina bcl2fastq (Version 2.20) was used to perform demultiplexing of the sequencing reads and adapters were trimmed with Skewer (version 0.2.2) (Jiang et al., 2014). Aligning The adapter-trimmed raw forward reads to the filtered RefSeq protein database (version 94) using Diamond in BLASTX mode (Buchfink et al., 2015). Taxonomic placement was performed using the Lowest Common Ancestor (LCA) algorithm implemented in MEGAN6 Ultimate Edition (version 6.18.7) (Huson et al., 2016).

3.1.3. Determination of the Quantity of Inoculum

For determining how much yeast to add to each fermentation, the commercial *Saccharomyces cerevisiae* LT8 (Schliessmann Kellerei-Chemie GmbH & Co (Schwäbisch Hall, Germany)), prepared as instructed on the packaging, with a concentration of 25g/100L. The prepared yeast then serially diluted with PBS to 10^{-5} and plated into MEA agar plates, and incubated for 2 days in 30°C. After incubation, CFU count is performed, and the process was repeated with two more batches of commercial yeast LT8, with one more additional serial dilution step added. The CFU values were calculated using the following formula:

$$\frac{CFU}{mL} = [\frac{(counts\ of\ colonies\ with\ 10-300\ colonies\ present\ in\ plate)}{1 \times (\#\ plates\ with\ lowest\ dilution) + 0{,}1 \times (\#\ plates\ with\ next\ lowest\ d) + \cdots}] \times 10^{(lowest\ dilution\ step)} \times (pipette\ dilution)$$

The mean of the CFU values obtained from three batches was considered as the amount of yeast that needs to be added to the fermentation.

After obtaining the CFU count of the commercial yeast LT8, the same process repeated for the alternative yeasts isolated from the pear samples in order to determine the CFU count of each isolated yeast sample to be used in the fermentations.

3.2. Fermentation

3.2.1. Preparation of the Fermentation

For the fermentations, obtained pears were chopped, stems were removed, then grated in an appropriate device, and the mash was collected into an appropriate container. 0.1mL/L Pectinlyase was diluted into ten times per volume water, then added to the mash and stirred. Pectinlyase is used for reducing the methanol release from pectin in the (Schliessmann Kellerei-Chemie GmbH & Co, 2018).

Then the mash was acidified to pH 3.2-3.3 using approximately 20-30mL/L Acid Combination MS. The acid combination MS is a mixture of malic and lactic acid with a pH value of 1 (at 20°C), which used to eliminate the production of metabolic products of harmful bacteria in fermentation by stopping their reproduction (Schliessmann Kellerei-Chemie GmbH & Co, 2018).

After acidification, the mash was divided into 5 fermentation flasks by volume, each taking 1.9 L $\pm$ 100mL mash, and named alphabetically and as 'LT8' for the commercial yeast. Then each yeast sample were added into different fermentation flasks in equal amounts determined by CFU count calculations. After mixing, the fermentation flasks were closed airtight with stoppers, and placed in a constant temperature of 16℃.

0.3g/L Mash nutrient was dissolved in 200mL/g water and added twice to every fermentation, firstly 1 day after the start of the fermentation, and secondly 2 days after the start of the fermentation. Mash nutrient is used for preventing sluggish or incomplete fermentations due to inefficient amounts of nutrients as it is composed of yeast nutrients and growth substances (Schliessmann Kellerei-Chemie GmbH & Co, 2018).

Two lab scale fermentation batches performed in order to determine the best performing yeast to use in the main fermentation. After the lab scale fermentations, best performing yeast used for the main fermentation, alongside with the control batch with the commercial yeast LT8. Main fermentations were also prepared using the same procedure.

3.2.2. Fermentation Control

After 7 days of fermentation, %Brix data for measuring dissolved sugar content with a digital refractometer, and pH values were monitored with a pH meter in every working day, for each fermentation until the end of the fermentation. For main fermentation, measurements are taken starting from day 10 of fermentation. The fermentations deemed to be completed after 2 days of minimal change in measured sugar content.

3.3. Distillation

For the lab scale distillations, a 2-liter copper distillation kit was used. All lab scale distillations were performed using the same equipment. For the distillation of the main fermentation, a distillation tower is used. Both the natural yeast and the commercial yeast LT8 batches were distilled using the same equipment.

Figure 5 Distillation equipment used for lab scale and main distillations. a) 2-liter copper alembic still. b) 190L distillation column

Lab scale distillations were not separated into head, heart, and tail parts; thus, for each different fermentation sample, all the distillation product was collected into the same flask. For the

products of the main fermentations, the distillates were separated into head, heart, and tail parts via sensory, volumes of each part were measured, and heart part was stored in flasks.

3.4. Sensory Evaluation

For the sensory evaluation, fruit spirits obtained were diluted with distilled water in order to have 40% (v/v) alcohol content. The samples were prepared in approximately 10mL portions and rested in the serving glasses for 15 minutes prior to evaluation.

The sensory analysis carried out with 11 volunteers with 10 of the volunteers having no prior training on sensory testing. The samples are analyzed in terms of color, odor, taste, and by assessing the overall harmony of the sample. Participants evaluated the samples separately, after they have graded both samples, they also asked to compare the samples. Participants also been asked to describe the odor and taste of the pear spirits, and comment on the overall harmony.

3.5. Statistical Data Analysis

Statistical analysis of the color, odor, taste, overall assessment/harmony data, and total scores obtained from sensory analysis was performed using the unpaired t-test method. The difference among the means of the sensory scores of the pear spirits prepared with commercial yeast LT8 and native yeast D was evaluated using the least significant difference test at the significance level of $p<0.05$.

The statistical analysis performed in Microsoft Excel software using the ‘two-sample t-test and z-test’ function of XLSTAT Cloud toolbox (version 5.0.1). The categories in the sensory analysis were evaluated separately, then the total sensory analysis was evaluated by combining the data of all categories together.

4. Results

4.1. Sample Isolation

In Figure 6, the steps of the sample isolation can be observed. The microorganisms obtained from the surface, fresh juice and 7-days-rested juice of the pear samples are serially diluted, isolated according to their visual aspects and finally the yeast samples are further isolated into single agar plates as described in the materials & methods section.

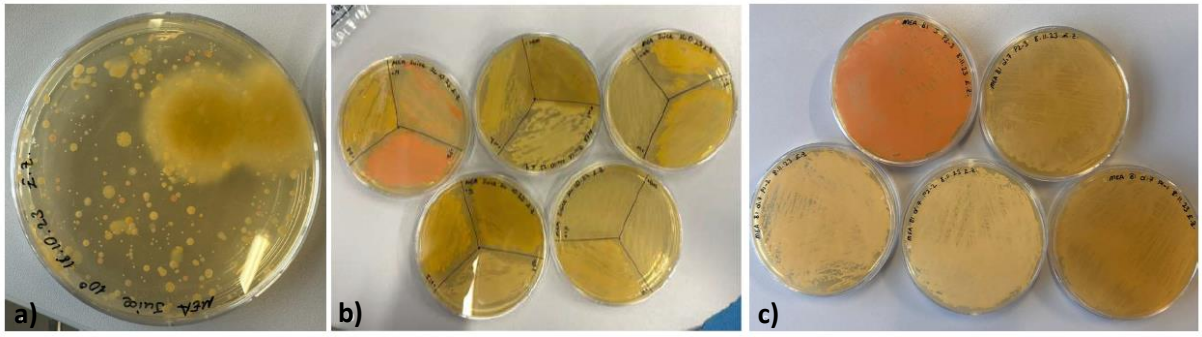


Figure 6 examples of isolation steps. a) Plate inoculated with fresh juice sample, 10^0 dilution. Visible colonies with different colors, sizes, surface characteristics can be observed, b) single colonies inoculated to isolate and identify the microorganisms by dividing one agar plate into 3 parts, c) Isolated yeast samples after microscope evaluation on single agar plates.

From all three batches of isolations, all obtained samples of microorganisms listed in Table 4. A total of 83 isolates obtained.

Table 4 number of samples isolated from surface, fresh juice, and 7 days-rested samples in all 3 batches

Batch #	Surface	Fresh juice	7-days-rested juice
Batch 1	8 samples	20 samples	6 samples
Batch 2	6 samples	15 samples	7 samples
Batch 3	6 samples	9 samples	6 samples

4.2. Identification of Yeast Cells

After isolation of the samples, all samples were analyzed under a light microscope under 400x magnification. Yeast cells were identified according to their morphology, considering cell shape, division, and clustering patterns, and considering their appearance on agar plates.

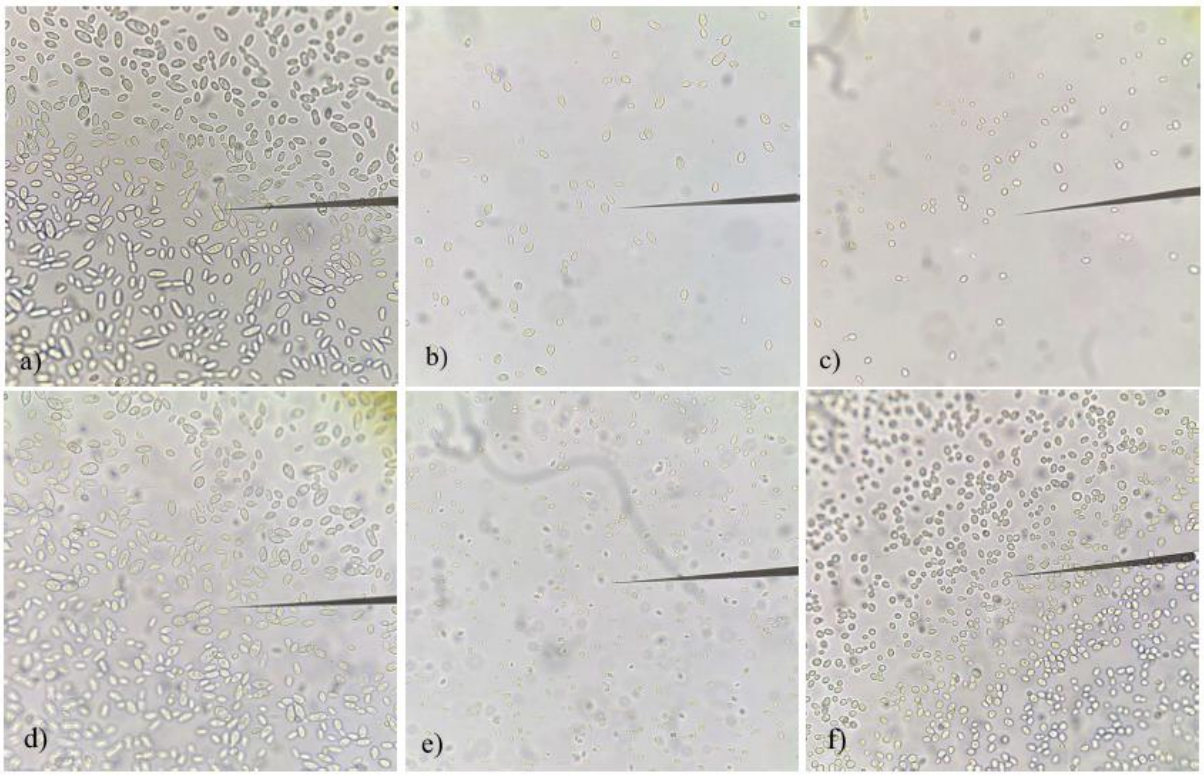


Figure 7 microscope views of some of the samples under 400x magnification: a) surface samples from batch 2, apiculate and elongated budding cells, visible vacuole can be observed in some cells; b) surface samples from batch 2, apiculate singular cells; c) 7-days-rested juice sample from batch 1, circular budding single cells; d) surface sample from batch 2, apiculate and elongated budding cells, visible vacuole can be observed in some cells, this sample is used as 'native yeast C' sample; e) 7-days-rested juice sample from batch 3, apiculate budding single cells; f) 7-days-rested juice sample from batch 2, circular/ovioid budding cells, this sample is used as 'native yeast D' sample. In all samples, smaller microorganisms and visual obstructions on ocular lens can be seen.

Among these 83 samples, after analyzing under a microscope, 19 of the samples are identified as yeasts; in Table 5, the origin of the sample and the corresponding batch can be observed.

Table 5 Numbers of yeast isolates obtained from surface, fresh juice, and 7 days-rested samples in all 3 batches, categories with no yeast sample acquired are marked with '-'.

Batch #	Surface	Fresh juice	7-days-rested juice
Batch 1	-	2 samples	4 samples
Batch 2	4 samples	1 sample	4 samples
Batch 3	1 sample	-	3 samples

Among the 19 identified yeasts, four of them were chosen to be utilized in fermentations. Among these four yeasts, one sample belonged to Batch 1 fresh juice samples, one sample belonged to Batch 3 7-days-rested juice samples, and two samples belonged to Batch 2, one

sample belonging to surface samples and other sample belonging to 7-days-rested juice samples; and named A, B, C, and D; accordingly.

4.3. CFU Count Calculations

For the CFU count of commercial yeast LT8, first serial dilution performed with the highest dilution step being 10^{-5}. The second and third batches were performed with the highest dilution step being 10^{-6} as it was observed that the plates with 10^{-4} dilution in the first batch were too numerous to count (TNTC), and only one of the 10^{-5} plates was usable to calculate CFU.

For both Batch 2 and 3, only the 10^{-4}, 10^{-5}, and 10^{-6} dilution steps were plated and incubated. Instead of 2 plates per dilution step like Batch 1, all dilution steps were plates 3 times in latter batches. After calculating CFU for the batches, the mean CFU value was considered as the CFU count of commercial yeast LT8. In the batch 1, 2 and 3, the CFU values were calculated as 2.13E+08 CFU/mL, 5.93E+08 CFU/mL and 3.03E+08 CFU/mL, respectively. The average CFU value was calculated as 3.70E+08 CFU/mL.

For the CFU counts of the native yeasts, one set of initial serial dilutions were performed with a highest dilution step of 10^{-6}, in order to estimate the densities of the existing plates; with the aim of assessing the required dilutions to perform colony counts accurately, and to concentrate the samples with inadequate colony densities.

In the initial serial dilution, native yeast A was TNTC on all levels of dilution, B and D were TNTC for all dilution steps except 10^{-6}, and only 10^{-3} and 10^{-4} dilution steps were countable for C. After concentrating C, the serial dilutions of each native yeast were performed with the highest dilution step being 10^{-8}. Calculated CFU values for native yeast samples A, B, C, and D were 1.26E+10 CFU/mL, 2.40E+09 CFU/mL, 1.26E+09 CFU/mL, and 2.73E+09 CFU/mL, respectively.

4.4. DNA Analysis

It can be observed that the species that could be specified in Sample A belongs to the genus *Rhodotorula*, with *R. toruloides* having highest sequence count, followed by *R. graminis* (Figure 8). Sample B shows the lowest relative abundance among all the samples, *H. uvarum* showing the highest relative abundance in the sample (Figure 9).

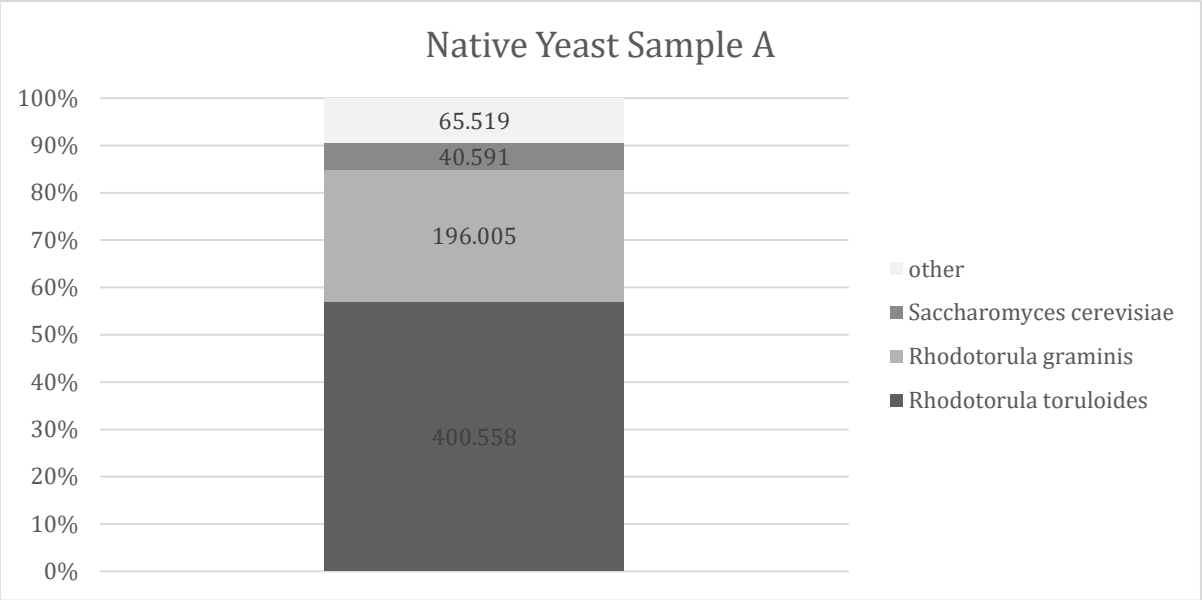


Figure 8 Stacked barplot of the relative sequence counts of the species in the sample A. Most abundant species are specified. Species counts in the samples are given on the plot

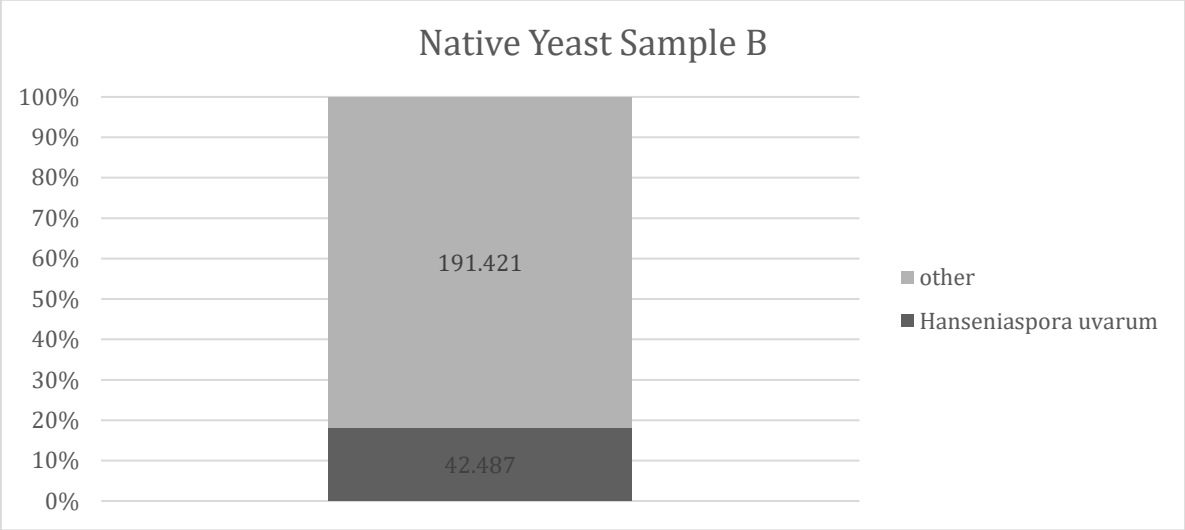


Figure 9 Stacked barplot of the relative sequence counts of the species in the sample B. Most abundant species are specified. Species counts in the samples are given on the plot.

Sample C consists of two known species from genus *Aureobasidium*, when the unspecific and other species present in the sample are overlooked, namely *A. subglaciale* and *A. namibiae* (Figure 10). For sample D, among known species in the sample, *Saccharomyces cerevisiae* has the highest sequence count, followed by *Saccharomyces eubayanus* (Figure 11).

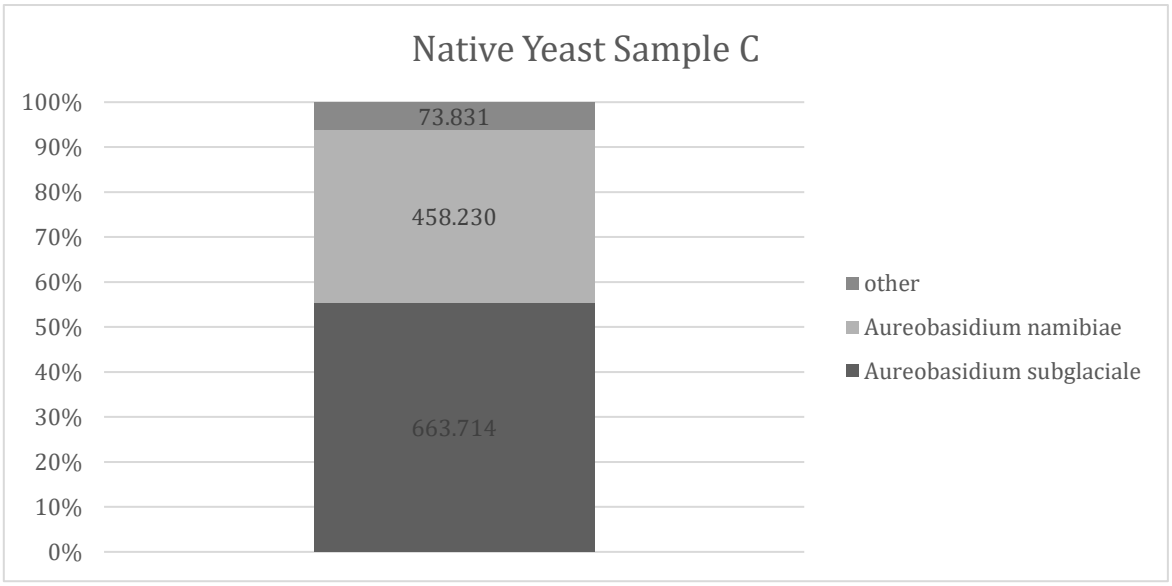


Figure 10 Stacked barplot of the relative sequence counts of the species in the sample C. Most abundant species are specified. Species counts in the samples are given on the plot

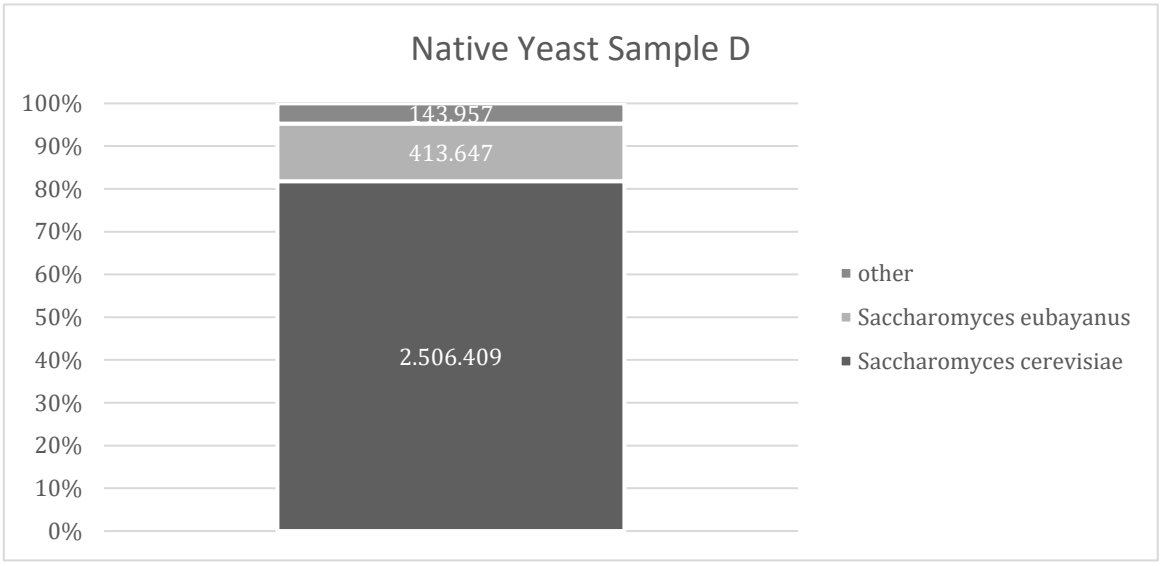


Figure 11 Stacked barplot of the relative sequence counts of the species in the sample D. Most abundant species are specified. Species counts in the samples are given on the plot

According to the sequence counts, the microorganisms in the samples are further investigated.

Species	Sequence count in sample A
Aspergillus heteromorphus	3,012
Melampsora larici-populina	6,777
Mixia osmundae	1,310
Pseudomicrostroma glucosiphilum	1,199
Rhodotorula graminis	196,005
Rhodotorula mucilaginosa	28,015
Rhodotorula toruloides	400,558
Saccharomyces cerevisiae	40,591
Saccharomyces eubayanus	6,875
Spathaspora passalidarum	1,526
Staphylococcus pasteuri	29,145

Table 6 Sequence counts of microorganisms with a sequence count higher than 1000 in native yeast sample A

In native yeast sample A, 11 microorganisms had sequence counts higher than 1000 (Table 6). Microorganisms with the highest sequence counts belong to the genus *Rhodotorula. S. cerevisiae, S. pasteuri,* and *R. mucilaginosa* showed sequence counts between 50000 and 10000 in the sample, followed by *S. eubayanus, M. larici-populina, A. heteromophus, S. passalidarum, M. osmundae,* and *P. glucosiphilum* which are the microorganisms that have the highest sequence counts below 10000 in the sample.

Table 7 Sequence counts of the first 24 microorganisms with the highest sequence counts in native yeast sample B

Species	Sequence count in sample B	Species (cont.)	Sequence count in sample B
Candida glabrata	5,861	*Naumovozyma castellii*	4,806
Agaricus bisporus	3,851	*Naumovozyma dairenensis*	5,005
Aspergillus heteromorphus	4,804	*Pichia membranifaciens*	5,534
Eremothecium gossypii	4,539	*Saccharomyces cerevisiae*	4,503
Eremothecium sinecaudum	4,074	*Saccharomyces eubayanus*	3,945
Hanseniaspora uvarum	42,487	*Spathaspora passalidarum*	4,256
Kazachstania africana	6,478	*Tetrapisispora blattae*	6,468
Kazachstania naganishii	5,107	*Tetrapisispora phaffii*	7,069
Kluyveromyces lactis	5,784	*Torulaspora delbrueckii*	4,807
Kluyveromyces marxianus	6,581	*Vanderwaltozyma polyspora*	5,537
Lachancea lanzarotensis	6,074	*Wickerhamomyces anomalus*	5,089
Lachancea thermotolerans	6,639	*Zygosaccharomyces rouxii*	5,322

In native yeast sample B, the microorganism with the highest sequence count identified as *H.* uvarum (Table 7). There are 48 species in the sample with a sequence count higher than 1000, only the sequence count of *H. uvarum* is higher than 10000. *T. phaffii* shows the highest sequence count after the *H. uvarum* in the sample, followed by *L. thermotolerans, K. marxianus, K. africana, T. blattae,* and *L. lanzarotensis.* The sample also had *S. cerevisiae* and *S. eubayanus* with sequence counts of 4503, and 3945, respectively.

Table 8 Sequence counts of microorganisms with a sequence count higher than 1000 in native yeast sample C

Species	Sequence count in sample C	Species (cont.)	Sequence count in sample C
Agaricus bisporus	1,007	*Diplodia corticola*	1,457
Alternaria arborescens	9,019	*Endocarpon pusillum*	1,093
Aspergillus campestris	1,453	*Hyaloscypha bicolor*	1,284
Aspergillus eucalypticola	1,105	*Marssonina brunnea*	1,009
Aspergillus heteromorphus	1,479	*Mycobacterium kansasii*	3,047
Aspergillus homomorphus	1,222	*Paraphaeosphaeria sporulosa*	1,695
Aspergillus novofumigatus	2,259	*Phialocephala scopiformis*	3,603
Aspergillus sclerotioniger	1,106	*Pseudocercospora fijiensis*	2,117
Aspergillus steynii	2,621	*Pseudocercospora mori*	1,073
Aureobasidium namibiae	458,230	*Ramularia collo-cygni*	1,138
Aureobasidium subglaciale	663,714	*Rasamsonia emersonii*	1,450
Baudoinia panamericana	2,579	*Rhodotorula graminis*	1,407
Cercospora beticola	1,101	*Spathaspora passalidarum*	2,132
Coniosporium apollinis	1,342	*Zymoseptoria tritici*	3,748

28 species of microorganisms with a sequence count higher than 1000 in native yeast sample C (Table 8). The highest sequence counts obtained belongs to the genus *Auerobasidium*. *A. subglaciale* and *A. namibiae* shows the highest sequence counts, followed by *A. arborescens* with a sequence count of 9019. All other species mentioned have a sequence count lower than 4000. The most repeated genus between the microorganisms with a sequence count higher than 1000 in native yeast sample C is the genus *Aspergillus*, with 7 species with sequence counts ranging between 2621 and 1105.

Table 9 Sequence counts of the first 20 microorganisms with the highest sequence counts in native yeast sample D

Species	Sequence count in sample D	Species (cont.)	Sequence count in sample D
Agaricus bisporus	5,764	*Saccharomyces cariocanus*	3,698
Aspergillus heteromorphus	2,836	*Saccharomyces cerevisiae*	2,506,409
Aspergillus novofumigatus	8,178	*Saccharomyces eubayanus*	413,647
Aspergillus steynii	4,483	*Salmonella enterica*	6,728
Bacillus cereus	3,023	*Spathaspora passalidarum*	5,866
Capronia epimyces	2,751	*Stenotrophomonas maltophilia*	5,988
Ogataea polymorpha	7,088	*Streptococcus cristatus*	6,766
Phialocephala scopiformis	2,220	*Thalassotalea mangrovi*	9,743
Pichia membranifaciens	3,827	*Torulaspora delbrueckii*	2,247
Rhodotorula graminis	2,979	*Wickerhamomyces anomalus*	17,508

In sample D, 39 species of microorganisms had a sequence count higher than 1000. The *S. cerevisiae* shows the highest sequence count in the sample, followed by *S. eubayanus*. *W. anomalus* follows the two species mentioned with the highest sequence counts, with a sequence

count of 17,508 (Table 9). All other species in the sample shows a sequence count lower than 10000.

4.5. Fermentation

4.5.1. Ending and Duration of the Fermentation

In the laboratory scale fermentations, the changes in the mash during the fermentation could be observed as the flasks used for the fermentation was made of glass (Figure 12). At the start of the fermentation, it can be observed that the mash was in a homogeneous form. As the fermentation proceeds, the separation of mash can be observed.

Figure 12 Samples of laboratory scale fermentations on different times of fermentation. a) At the start of the fermentation, b) During the fermentation, c) At the end of the fermentation

Laboratory scale fermentations were deemed to be completed in 14 days for batch 1, and in 13 days for batch 2, as the sugar content became stable for at least one of the samples in the batches for 2 consecutive %Brix readings, measured in 2 consecutive days.

In the main fermentations, the fermentation deemed to be completed in 13 days after it is observed that the variation between %Brix readings were around 0.2 %Brix.

As the %Brix readings stabilized, the fermentations moved to be stored in 4°C until distilled.

4.5.2. Change of Sugar Content

All brix measurements are done three times for every sample, and the mean of the measurements are taken as the sugar content value for the Figure 13.

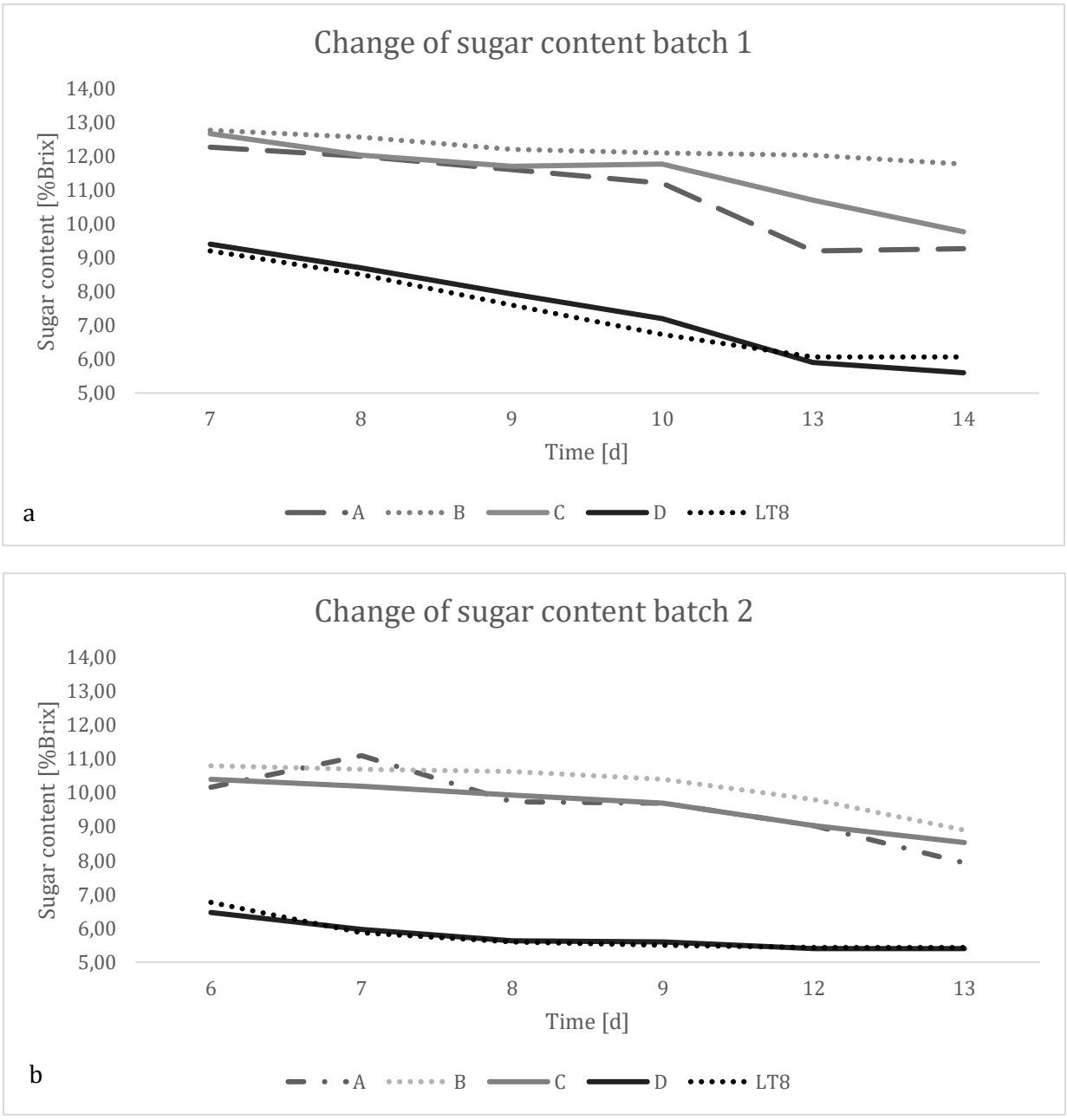


Figure 13 %Brix readings of lab scale fermentations. a) %Brix readings of the first lab scale batch starting from day 7; b) %Brix readings of second batch starting from day 6 of fermentation. 'LT8': commercial yeast LT8, 'A': native yeast sample A, 'B': native yeast sample B, 'C': native yeast sample, and 'D': native yeast sample.

In table both batches, the fermentation with the natural yeast sample D showed a closer performance of metabolizing sugars to the fermentation with commercial yeast LT8 in sugar conversion, when compared with other tested native yeasts.

For both batches, the %Brix values did not reach to 0, the final %Brix value for native yeast D was 5.60, and the final %Brix value for LT8 was 6.07 for laboratory scale batch 1; and for batch 2, the final %Brix values were 5.40 and 5.43 for native yeast D and LT8, respectively.

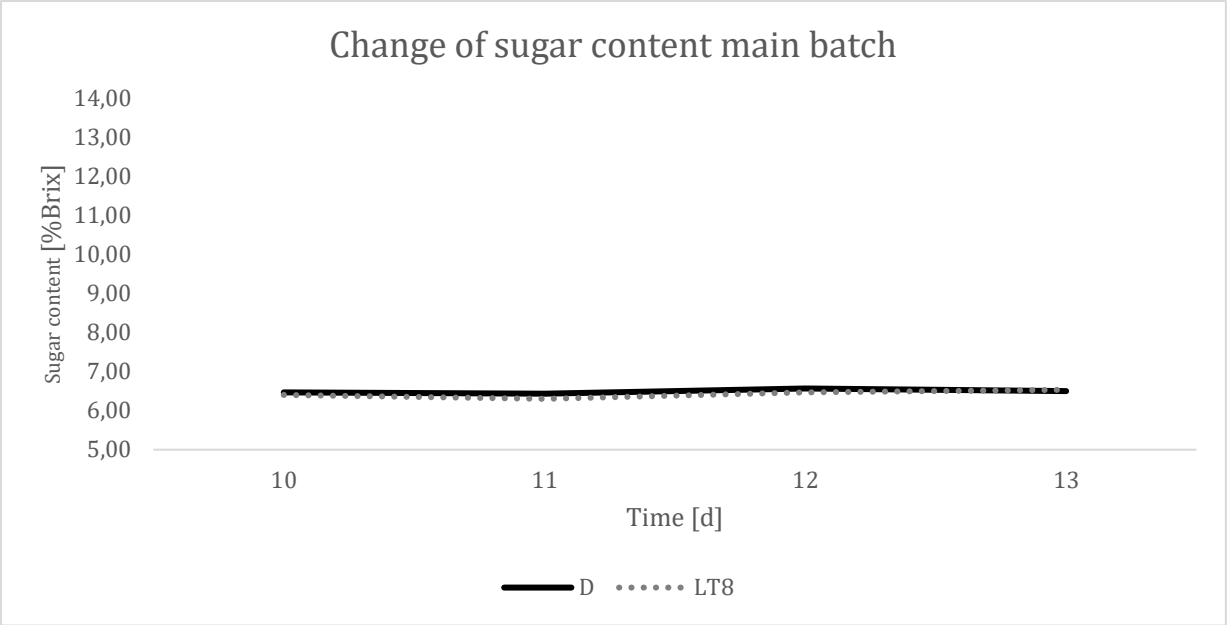


Figure 14 %Brix readings of the main fermentation starting from day 10. 'LT8': commercial yeast LT8, 'D': native yeast sample.

In the main fermentation, the fermentation with the natural yeast sample D showed a closer performance of metabolizing sugars to the fermentation with commercial yeast LT8 in sugar conversion, as it was also observed in the laboratory scale fermentations. For both native yeast D and LT8, the %Brix readings were in the range of 6.30-6.60 for all %Brix readings. The final %Brix values for native yeast D and LT8 were 6.50 and 6.53, respectively.

4.5.3. Change of Acidity

For both fermentations, the pH values stayed between 3,50-3,25 range. The pH fluctuations of both batches can be observed in Figure 15.

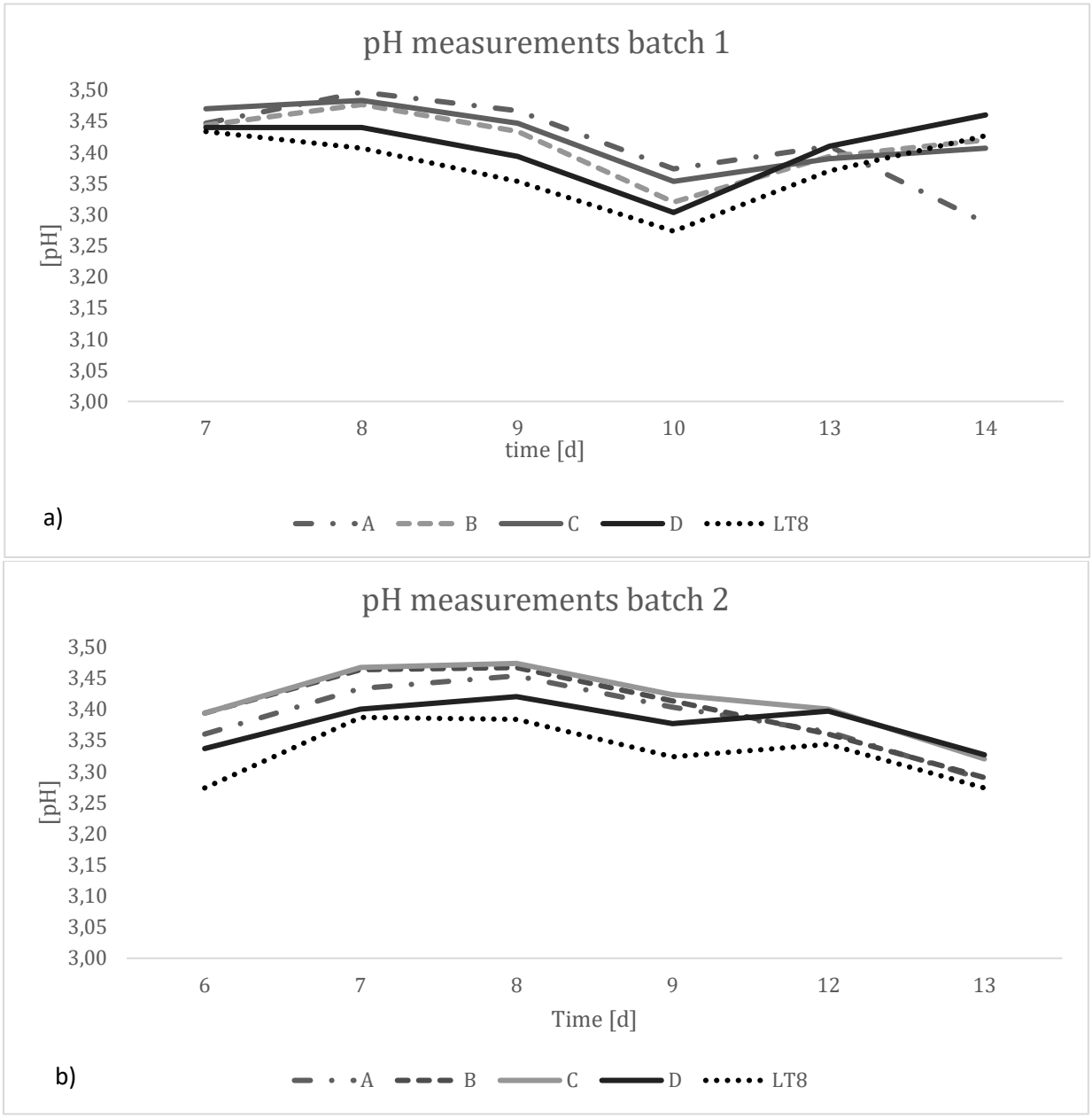


Figure 15 pH readings of lab scale fermentations. a) pH readings of the first lab scale batch starting from day 7; b) pH readings of second batch starting from day 6 of fermentation. 'LT8': commercial yeast LT8, 'A': native yeast sample A, 'B': native yeast sample B, 'C': native yeast sample, and 'D': native yeast sample.

At the end of the fermentation of batch 1, the pH values for A, B, C, D and LT8 were 3.29, 3.42, 3.41, 3.46, and 3.43, respectively. pH values for A, B, C, D and LT8 at the end of the fermentation of batch 2 were 3.29, 3.29, 3.32, 3.33 and 3.27, respectively.

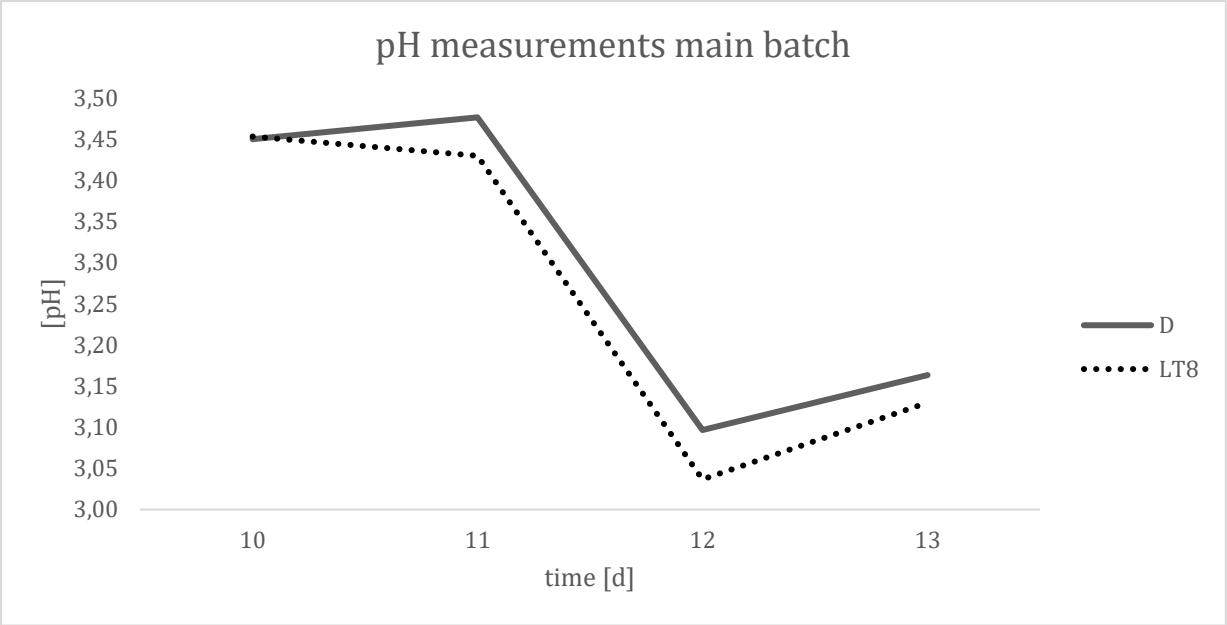


Figure 16 pH readings of main fermentation starting from day 10 of fermentation. 'LT8': commercial yeast LT8, 'D': native yeast sample.

For both fermentations, the pH values stayed between 3,50-3,00 range in the main fermentation. At the end of the fermentation, the final pH values for native yeast D and LT8 were 3.16 and 3.1, respectively.

4.6. Distillation

4.6.1. Laboratory Scale Distillation

For the distillation of the lab scale fermentation batches, since the initial fermentation volumes were small, distillate volumes obtained from the fermentation were between 50-200 mL range, as can be observed in Figure 17.

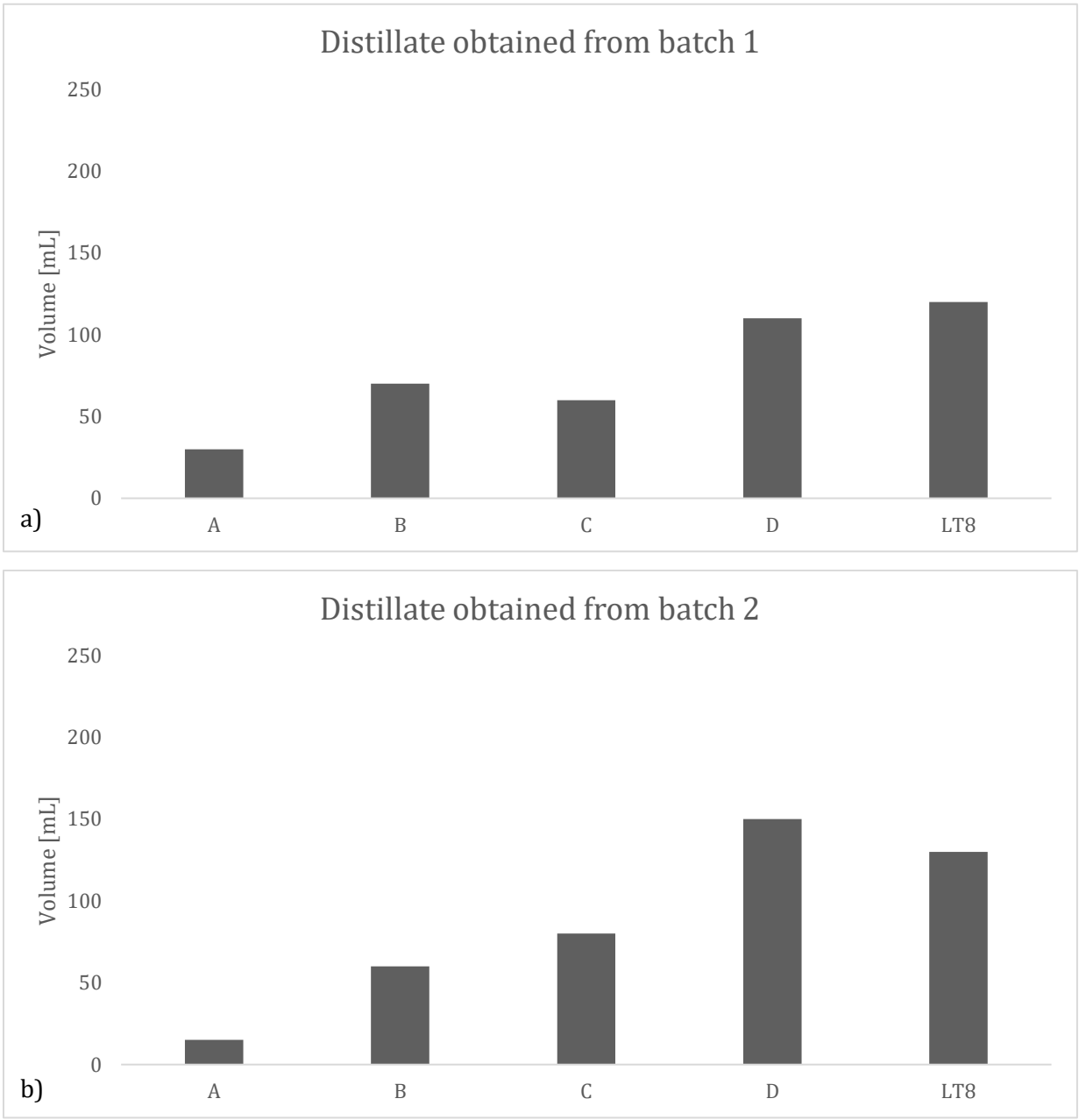


Figure 17 Volumes of distillates obtained from the distillations of lab scale fermentations. a) batch1, b) batch 2. 'LT8': commercial yeast LT8, 'A': native yeast sample A, 'B': native yeast sample B, 'C': native yeast sample, and 'D': native yeast sample.

Both distillations did not been divided to head heart and tail parts because of the small volume of distillate produced. In both batches, the smallest volume of distillate produced from native yeast A. Native yeast D and LT8 produced the highest volumes of distillate among all yeasts tested in fermentations; in the first batch, the highest volume of distillate obtained from the fermentation with LT8, and for the second fermentation, the highest volume of distillate was obtained from native yeast D.

4.6.2. Main Distillation

In the main distillation, 3660 mL of distillate from commercial yeast LT8 fermentation; and 4500mL distillate from native yeast D fermentation were obtained. Pears spirits produced, which correspond to the heart parts of the distillates were 1100mL and 1300mL for commercial yeast LT8 and native yeast D, respectively.

For the main distillation, the volumes of head, heart and tail parts obtained from the fermentation of 106L of mash can be observed in figure 18.

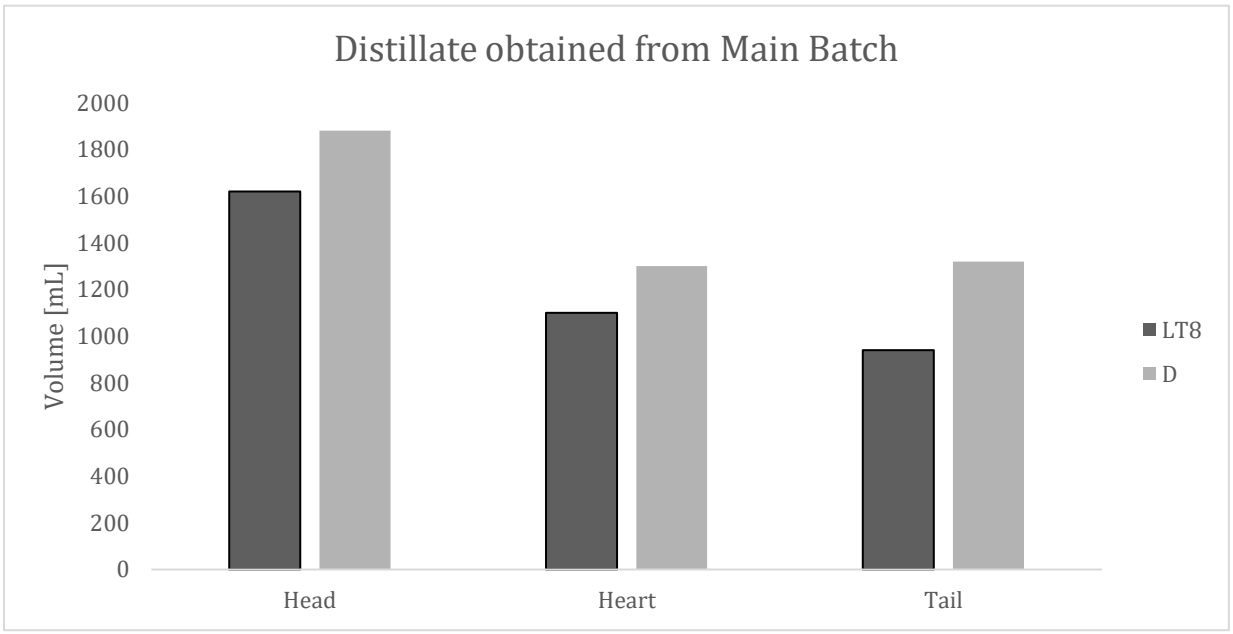


Figure 18 Volumes of head, tail and heart parts obtained from distillation of the fermentation of 106 L mash with commercial yeast LT8 and native yeast D. 'LT8': commercial yeast LT8, 'D': native yeast sample D

For all three parts of the distillate, native yeast D produced more volumes of the corresponding parts compared to that of the commercial yeast LT8.

4.7. Sensory Evaluation

4.7.1. Comparison of Color

The evaluation of the color of the pear spirits showed a significant difference between samples ($p=0.02$), with mean score of LT8 being higher (10.00 ± 0.00 points ($n=11$)) compared to that of the native yeast D (8.73 ± 1.56 points ($n=11$)).

4.7.2. Comparison of Taste

The total taste scores for LT8 and native yeast D were 30.91 ± 4.32 points (n=11), and 31.82 ± 4.51 points (n=11) respectively (Figure 19). The highest score given to the LT8 was lower (36 points) compared to that of the native yeast D (38 points). Similarly, the minimum score given to the LT8 was also lower (22 points) compared to the that of the native yeast D (24 points). The difference between the two samples were not significant (p=0.64).

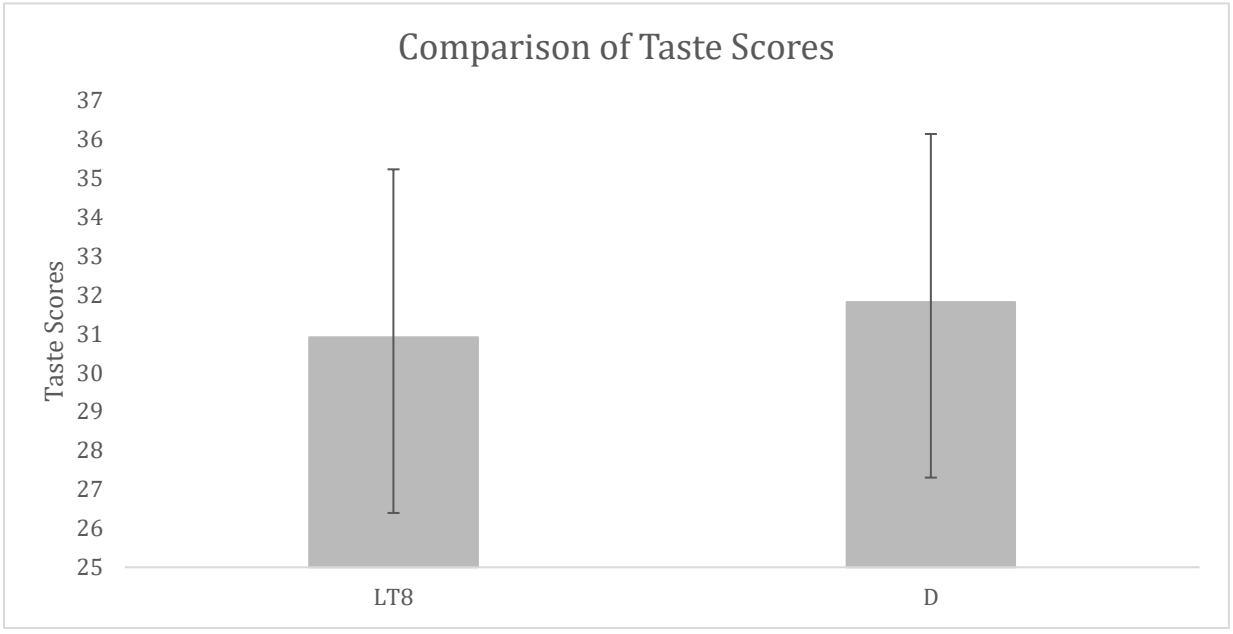


Figure 19 Comparison of sensory analysis scores obtained in taste category. Error bars denote standard deviation for arithmetic mean (n=11). 'LT8': commercial yeast LT8, 'D': native yeast sample D

4.7.3. Comparison of Odor

The highest score given to the total odor evaluation of the native yeast D was the highest possible score with 38 points, and the highest score given to the that of LT8 was 34 points. Similarly, lowest score given to the native yeast D was higher (30 points) compared to that of LT8 (26 points). Standard deviation of native yeast D was also higher (2.72) compared to the standard deviation of LT8 (2.44).

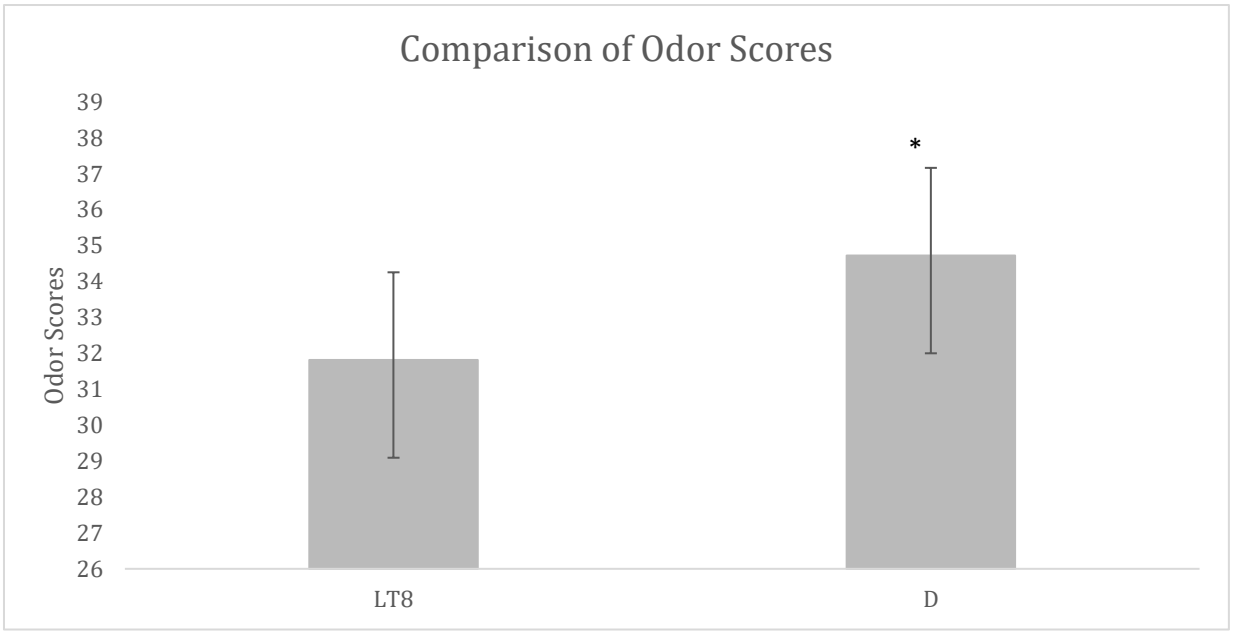


*Figure 20 Comparison of sensory analysis scores obtained in odor category. Error bars denote standard deviation for arithmetic mean (n=11). *≤0.05. 'LT8': commercial yeast LT8, 'D': native yeast sample D*

The odor scores were significantly higher (p=0.02) for native yeast D (34.73 ± 2.72 points (n=11)) than the odor scores of LT8 (31.82 ± 2.44 points (n=11))(Figure 20).

4.7.4. Comparison of Harmony

Scores for the harmony of the pear spirits were 11.09 ± 1.04 points (n=11) for LT8 and 11.27 ±2.05 points (n=11) for native yeast D (Figure 21).

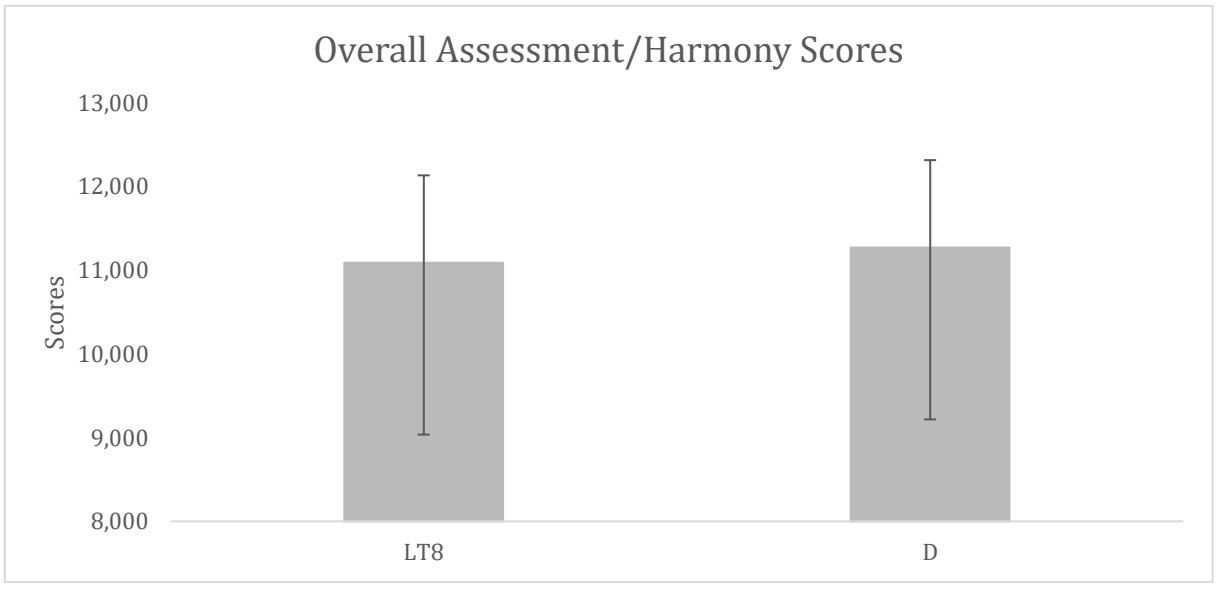


Figure 21 Comparison of sensory analysis scores obtained in overall assessment/harmony category. Error bars denote standard deviation for arithmetic mean (n=11). 'LT8': commercial yeast LT8, 'D': native yeast sample D

The maximum point given for the harmony of LT8 was 12 points, whereas native yeast D was given the highest possible points, 14 points by two participants. However, the lowest point given for the harmony of LT8 was 10 points, the lowest score given to native yeast D was 8 points. Thus, the standard deviation of the scores given to native yeast D was higher (2.05) compared to that of LT8 (1.04). the difference between the harmony scores were not significant (p=0.80).

4.7.5. Comparison of Total Sensory Evaluation

The highest points scored for sensory evaluation for the pear spirits produced with native yeast D and LT8 were 96 and 92, respectively. The lowest point scored for the pear spirit produced with LT8 was 68, and for native yeast D was 73.

Total scores were higher in native yeast D (86.54 ± 7.28 points (n=11), compared to LT8 (83.81 ± 7.29 points (n=11) (Figure 22). The difference between the scores is not significant (p=0.391).

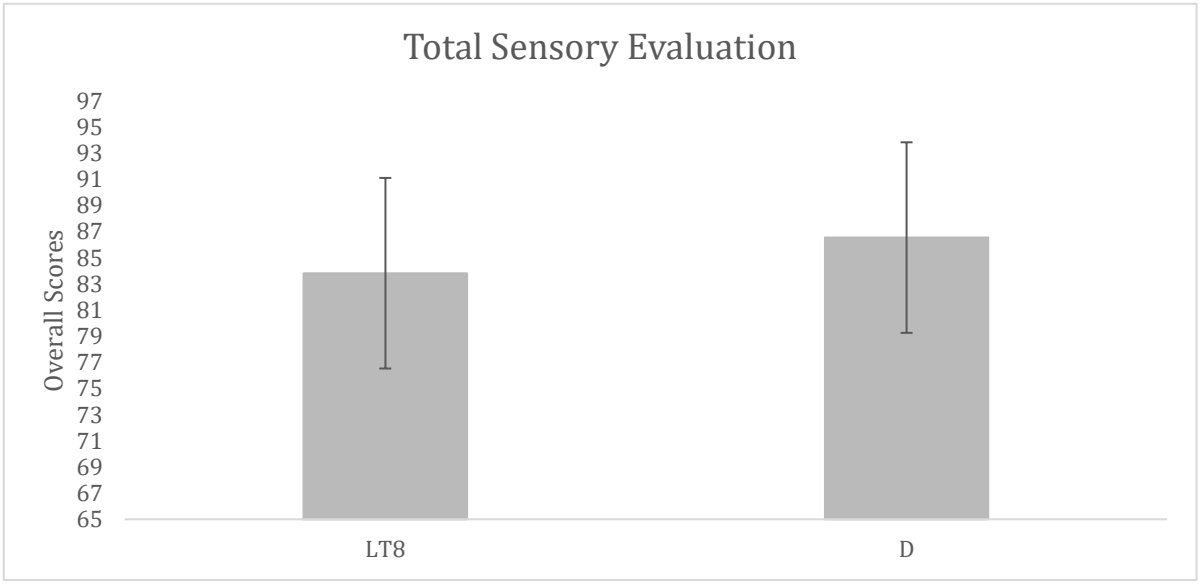
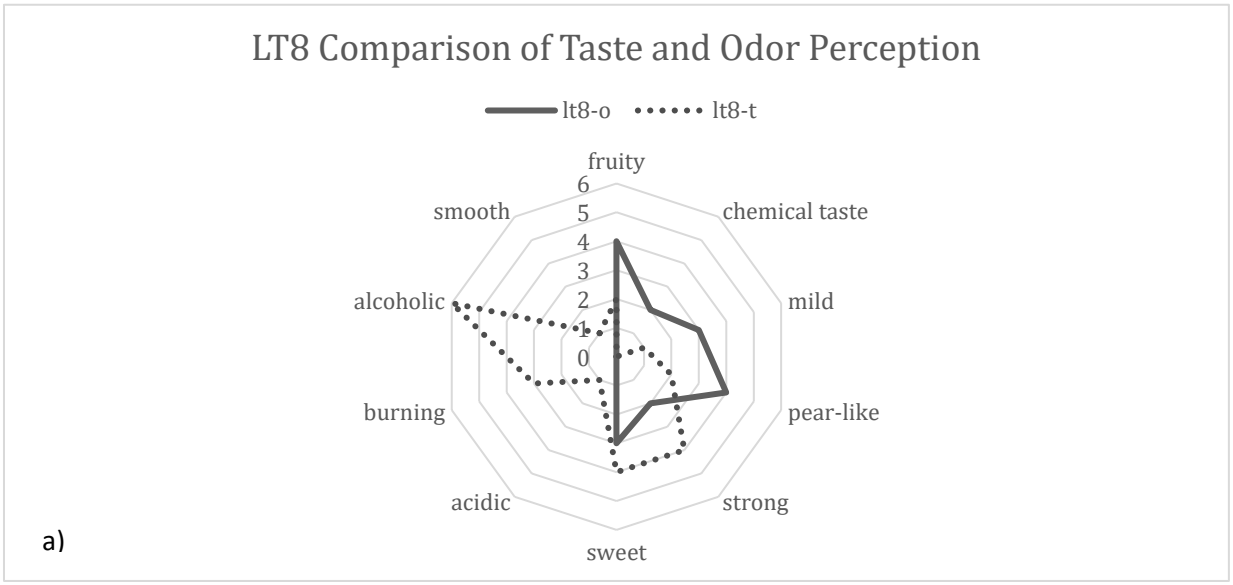


Figure 22 Comparison of all obtained sensory analysis scores combined. Error bars denote standard deviation for arithmetic mean (n=11). 'LT8': commercial yeast LT8, 'D': native yeast sample D

The data is also evaluated with only considering the odor, taste, and harmony scores, thus not taking the color scores into account. The p-value was lower (p=0.22) than the that of total sensory evaluation including the color data (p=0.391). Nevertheless, the difference between the LT8 and the native yeast D was not significant.

4.7.6. Results of Descriptive Evaluation

In the descriptions of the odor of the pear spirit produced with LT8, the most used adjectives were fruity (4 repeats), pear-like (4 repeats), mild (3 repeats), and sweet (3 repeats). For the taste of the pear spirit produced with LT8, the most used adjectives were alcoholic (6 repeats), strong (4 repeats), sweet (4 repeats), and burning (3 repeats). It may be noted that the pear-like adjective used less to describe the taste (2 repeats), compared to its usage frequency for the odor (4 repeats); as well as the pear-like adjective, which the frequency dropped to 2 repeats for taste, compared to 4 repeats for the odor. The 'acidic' adjective was only used once, to describe the taste of the pear spirit produced with LT8 (Figure 23).



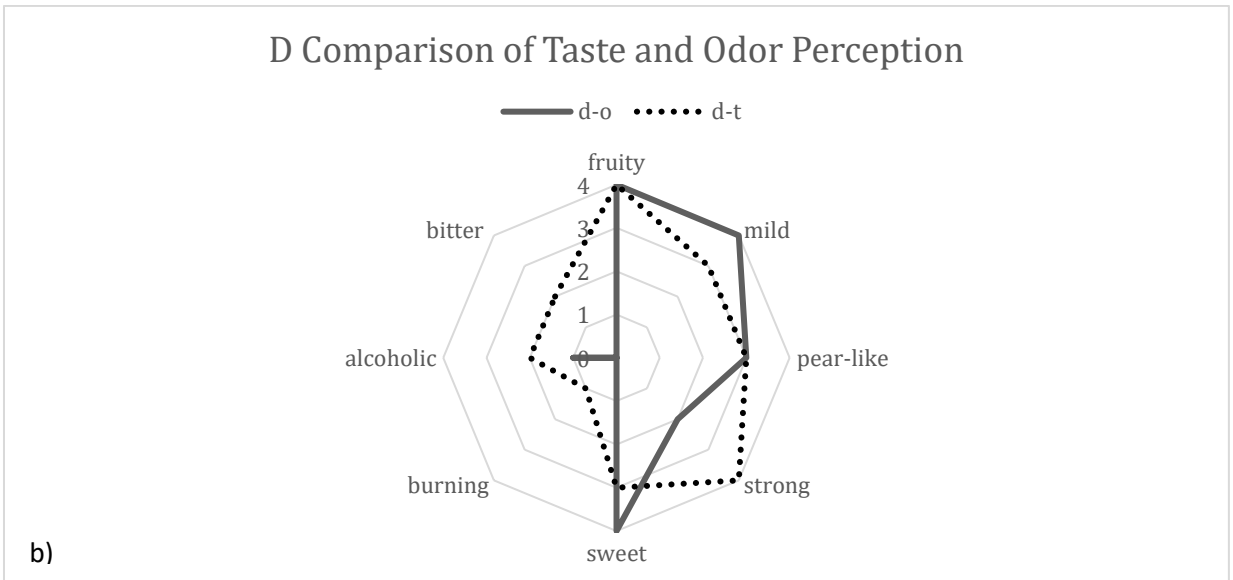


Figure 23 Comparisons of taste and odor perceptions of the produced pear spirits by the participants. a) comparison of the pear spirit produced with using commercial yeast LT8, b) comparison of the pear spirit produced with using native yeast sample D. 'LT8': commercial yeast LT8, 'D': native yeast sample D. 'd-o' represents the native yeast sample D values for odor, 'd-t'

represents the native yeast sample D values for taste ; 'lt8-o' represents the commercial yeast sample LT8 values for odor, 'lt8-t' represents the commercial yeast sample LT8 values for taste.

For the describing the odor of the pear spirit produced with native yeast sample D, fruity (4 repeats), mild (4 repeats), sweet (4 repeats), and pear-like (3 repeats) were the most used adjectives. The most used adjectives were fruity (4 repeats), strong (4 repeats), pear-like (3 repeats), and sweet (3 repeats) for describing the taste of the pear spirit produced with native yeast sample D (Figure 23).

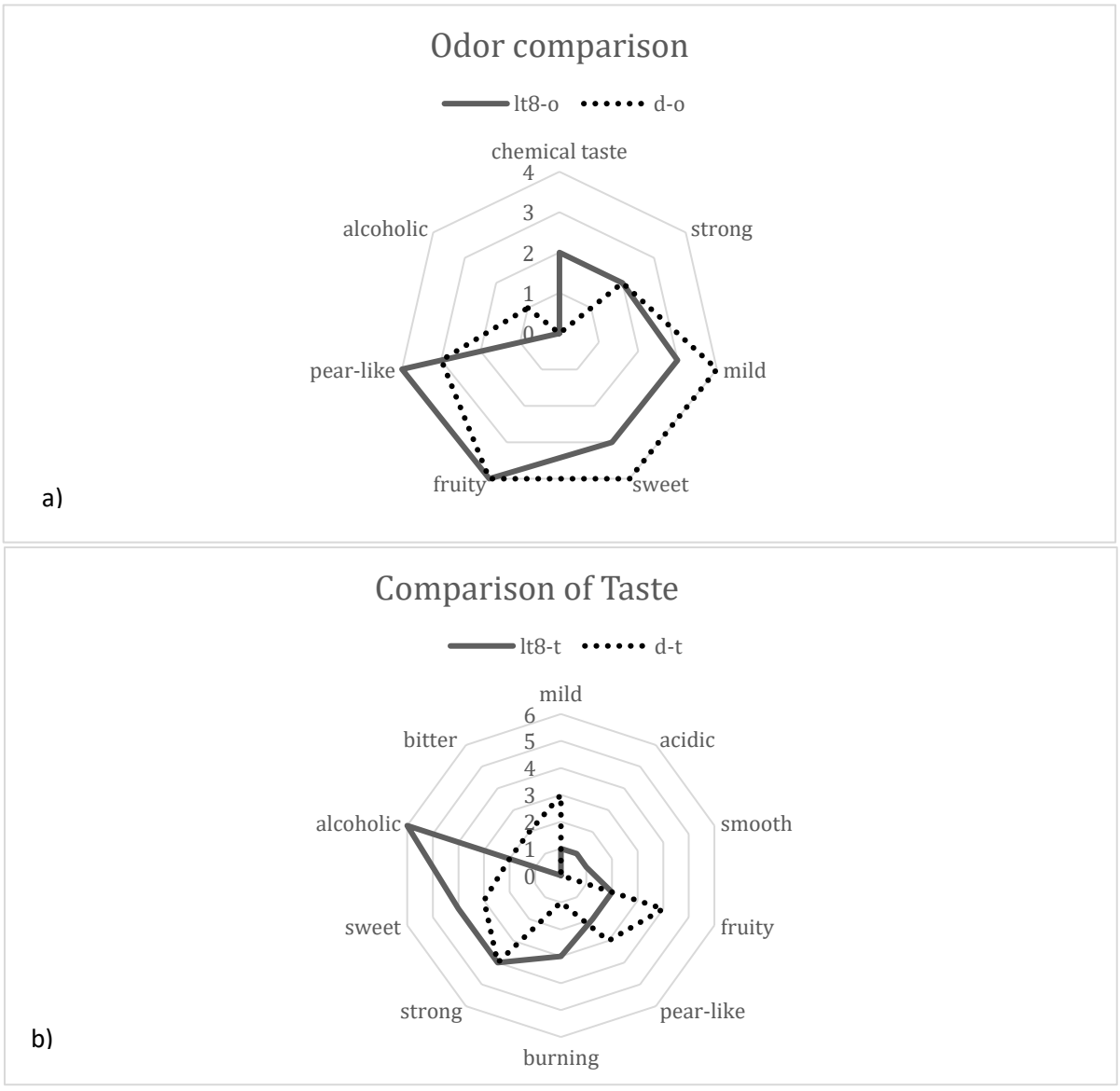


Figure 24 Comparisons of the pear spirits produced in terms of odor and taste. a) Comparison of odor of the pear spirit produced with native yeast sample D and pear spirit produced with commercial yeast LT8. 'LT8': commercial yeast LT8, 'D': native yeast sample D. 'd-o' represents the native yeast sample D values for odor, 'd-t' represents the native yeast sample D values for taste ; 'lt8-o' represents the commercial yeast sample LT8 values for odor, 'lt8-t' represents the commercial yeast sample LT8 values for taste.

The adjective 'alcoholic' was used less frequently for the pear spirit produced with native yeast sample D (2 repeats), compared to that of the pear spirit produced with LT8 (6 repeats). Also,

‘bitter’ was only used to describe the taste of the pear spirit produced with native yeast sample D (2 repeats) and weren’t used to describe either the odor of the samples or the taste of the pear spirit produced with LT8 (Figure 24).

5. Discussion

5.1. Yeast Isolation and DNA Analysis of the Samples

In this study, 83 isolates were obtained from the surface, fresh juice, and 7-days-rested juice of the pear samples (Table 4). Not all isolates were identified with DNA Analysis.

Nevertheless, after the DNA analysis, it has been observed that the chosen native yeast samples were not pure isolates of the yeasts intended to be isolated. Thus, the DNA analysis of the chosen native yeast samples could be used in order to gain an insight about the microbiota of the pear fruits.

As the native yeast samples were isolated several times in order to obtain only the selected yeasts in the sample, their concentrations in the samples were higher. Thus, other microorganisms in the samples show lower sequence counts. As a result, the DNA analysis data was not appropriate for identifying the species abundance on the pears. Similarly, since some species may be eliminated during isolation steps, the DNA analysis results would not be able to represent all species present on the pear samples. The DNA analysis results could only give a perception of the species present on the pear samples.

Along with the yeast species dominating the analyzed native yeast samples, there were several other yeast species present in lower sequence counts. Some of the yeast species that were present in every native yeast sample were *Pichia membranifacens, Lachancea thermotolerans, Wickerhamomyces anomalus,* and *Spathaspora passalidarum*.

One of the yeasts species present in all four native yeast samples is *Pichia membranifacens*. The yeasts belonging to genus *Pichia* are considered as minor species as they have low fermentation capability (Carpena et al., 2021). It is known that *Pichia spp.* are isolated from agave fermentation processes with native yeasts, and also from the wine musts (Walker and Stewart, 2016a). Even though *P. membranifacencs* shows low fermentation capabilities, the species are able to increase ester levels, as well as increasing quality parameters in the wine fermentations (Vicente et al., 2021a).

Lachancea thermotolerans is another yeast that can be observed in the sequence count data of the DNA analysis performed. *L. thermotolerans* is considered popular among the commercialized alternative yeast options (Benito, 2020). The characteristic features of the *L. thermotolerans* include being able to form lactic acid during fermentation, and low production

of acetic acid (Hranilovic et al., 2018). Commercial strains of *L. thermotolerans* are also known as their enhancement of aroma complexity and aroma intensity (Benito, 2020). The species fermentation ability is also considered as one of the highest compared to other non-Saccharomyces yeast (Vicente et al., 2021b).

Wickerhamomyces anomalus is an important non-Saccharomyces yeast in wine industry, one of the important aspect of the species being the metabolic characteristics (Padilla et al., 2018). Results showed that mixed fermentations of *W. anomalus* and *S.cerevisiae* showed enhanced aldehyde and ester contents as well as an increase in the terpene and ester concentration in kiwi wine, compared to pure fermentation of *S. cerevisiae* (Sun et al., 2022*)*. Different studies showed improvements on the aroma in fermentations (Zhang et al., 2023; Yan et al., 2019).

Spathaspora passalidarum is a species of yeast that is more suited to xylose fermenting with a low tolerance to ethanol (Campos et al., 2022). The species is one of the investigated species for biofuel production as fermentation of the xylose is one of the bottlenecks of the utilizing agricultural waste for biofuel production (Selim et al., 2020).

One of the yeast samples that have a higher sequence count in one of the native yeast samples, *Rhodotorula graminis,* was also present in all native yeast samples.

Along with yeast species, other microorganisms were also present in native yeast samples in lower sequence counts. Some of the the species that are present in all four native yeast samples are *Colletotrichum graminicola, Saitoella complicate, Tremella mesentericai, Phtophthora sojae,* and *Phialocephala scopiformis.*

Colletotrichum graminicola is one of the species belonging to genus *Colletotrichum.* Species in the genus *Colletotrichum* are filamentous fungi that cause plant diseases named as anthracnose and recognized as one of the most important plant pathogens (Zimbardi et al., 2013). *Saitoella complicate* is also known as a plant pathogen (Berbee, 2001). *Tremella mesenterica* is known as a mycoparasite (Minter and Soliman, 2022). *Phtophthora sojae* is also known as a plant pathogen. Species in the genus *Phtophthora* are prominent for their damage on both agricultural and ornamental plants (Chen and Wang, 2017).

Phialocephala scopiformis is a foliar fungal endophyte (Frasz et al., 2014). For the plant microbiome, foliar fungal endophytes are considered as a major component as their impact on plant performance is being recognized progressively (Whitaker et al., 2018). It is shown that *P.*

scopiformis can act as an insecticide as the species are able to produce anti-insect compounds are able to reduce the growth rate of spruce budworm species (Frasz et al., 2014).

5.2. Yeasts Used in the Fermentations

5.2.1. Sample A

In the native yeast sample A, the genus that a greater percentage of identified yeasts belongs to genus Rhodotorula (Figure 8). Taxonomically, the genus *Rhodotorula* has 34 basidiomycetous yeast species, and belongs to family Sporidiobolaceae, and the Basidiomycota phylum (Cespedes et al., 2022).

The species *R. toruloides* has the greatest percentage of the identified yeasts in the native yeast sample A. *R. toruloides* has capability to produce carotenoids and lipids naturally, and due to its favorable features, such as stress resistance, seen as a promising microorganism to be used in lipid production (Wen et al., 2020).

The species that has the second greatest percentage of the identified yeasts in the native yeast sample A is *R. graminis. R. graminis* is also a yeast that can produce several compounds that are valuable, such as carotenoids and lipids, and seen as a host organism for bio-refinery processes (Li et al., 2020).

Yeast species in the genus *Rhodotorula* have a quick growth rate, can be grown on several different media and are not demanding in terms of nutrition (Cespedes et al., 2022). Native yeast sample A having higher colony counts and consequently a higher CFU value compared to other native yeast samples can be explained with the native yeast sample A consisting mostly of the yeasts in the genus *Rhodotorula*.

Species in genus *Rhodotorula* are obligate aerobes and cannot perform alcoholic fermentation (Phaff et al., 2014). However, in spontaneous fermentations, it is known that the fermentation may be started by the yeasts such as the yeasts in the genus *Rhodotorula,* and these yeasts provide an increase in the aromatic compounds, thus creating a flavor complexity in the resulting alcoholic beverage (Carpena et al., 2021).

In the laboratory scale fermentation and followed distillations, the volume of distillate obtained from the native yeast A was the lowest compared to the other native yeast samples (Figure 17). This result correlates with the capacity of the yeast species in genus *Rhodotorula* to alcoholic

fermentation. As the native yeast sample A also contains *S. cerevisiae* as yeast species having the third highest percentage in the sample, the produced distillate could be appointed to the presence of *S. cerevisiae* in the sample.

5.2.2. Sample B

For the native yeast sample B, the highest percentage of identified yeast cells in the sample belongs to the *Hanseniaspora uvarum* species (Figure 9). On many fruits, yeasts in the genus *Hanseniaspora* are common isolates (van Wyk et al., 2024).

H. uvarum is one of the most studied non-Saccharomyces species (Vicente et al., 2021b). In one study, in the sequential co-fermentation of *H. uvarum* and S. *cerevisiae,* it is found that at about 12% (v/v) alcohol content three strains of *H. uvarum* showed high concentration levels and stayed viable (Tristezza et al., 2016). However, generally, during fermentation *Saccharomyces* spp. dominate the yeasts belonging to the genus *Hanseniaspora;* and the species belonging to genus *Hanseniaspora* are not considered as good fermenters (van Wyk et al., 2024).

As the genus is considered unfavorable for fermentation, their effect on sensory profile is investigated by inoculating the fermentations with both *Hanseniaspora spp*. and *Saccharomyces spp*. fermentation with addition of yeast belonging to genus *Hanseniaspora* to the fermentations associated with favorable characteristics, biggest beneficence deemed as the flavor properties as the acetate ester content shows a significant increase, even though all compounds that influences aromatic profile have been altered (van Wyk et al., 2024). *H. uvarum* is able to produce higher amounts of higher alcohols, carbonyl groups and esters that enhance the quality *(Januszek and Satora, 2021).*

In this study, the distillate produced from the fermentation with native yeast B sample had a volume higher than that of the native yeast species sample A, but lower than that of the native yeast species D, and commercial yeast LT8 (Figure 17).

As a result, it can be stated that the native yeast sample B, which has the species *H. uvarum* as the microorganism with the highest percentage of known species in the sample, did produce a lower amount of distillate compared to both the commercial *S. cerevisiae* strain, and the native yeast sample which has two species from the genus *Saccharomyces* as the yeasts that have the highest percentage of known species in the sample, which corresponds to the literature.

5.2.3. Sample C

DNA analysis showed that one of the native yeast samples (native yeast C) contain *Aureobasidium subglaciale* and *Aureobasidium namibiae* (Figure 10). Species in the genera *Aureobasidium* are commonly isolated yeast-like fungi (Johnson and Echavarri-Erasun, 2011).

Both *A. subglaciale* and *A. namibiae were* formerly identified as *A. pullulans*, and later identified as separate species (Gostinčar et al., 2014). It is known that the species are known to be irrelevant for fermentation (Barata et al., 2012). After lab scale distillations, it can be observed that the native yeast C sample was able to produce alcohol.

On can assume that the produced alcohol was produced by the yeasts present in lower percentages in the sample.

5.2.4. Sample D

Native yeast sample D showing the most similar results to commercial yeast LT8 can be explained by the sequence counts of the sample D (Figure 11). Genus Saccharomyces accounts for more than 50% relative abundance of the sample, which could mean that this sample has the most similar abilities to LT8 in terms of alcoholic fermentation, as the species of the commercial yeast LT8 is also belongs to genus *Saccharomyces;* precisely, *S. cerevisiae*.

5.3. Co-inoculation with *Sachharomyces cerevisiae*

Aroma, acidity, other quality parameters can be positively altered with non-Saccharomyces species (Benito et al., 2019). In alcoholic fermentation, contributions of non-Saccharomyces species have on the chemical composition considered to be very important (Tristezza et al., 2016).

These yeasts are also appealing as these non-conventional yeasts may be able to produce unusual metabolites (Villarreal et al., 2022). One example for these unusual metabolites could be named as lactic acid, which is known to be produced by *Lachancea thermotolerans;* and carotenoids produced by *Rhodotorula spp.* (Hranilovic et al., 2018; Li et al., 2020).

Species known to benefit fermentations, such as *Rhodotorula spp., Hansenia spp., and Pichia spp.* are also known with low fermentation abilities, without the fermentation ability (van Wyk et al., 2024; Phaff et al., 2014; Carpena et al., 2021). Thus, co-inoculation provides an approach

for producing alcoholic beverages with utilizing the metabolites produced by non-Saccharomyces yeasts in order to enhance the sensory profile.

Several studies confirmed that co-inoculation of *S. cerevisiae* and non-Saccharomyces yeasts have positive impact on the sensory quality of the produced alcoholic beverage (Tristezza et al., 2016; Carpena et al., 2021; Vicente et al., 2021a).

The co-inoculation could have a negative effect on the amount of ethanol produced. In one study researching the co-inoculation of *H. uvarum* and *S. cerevisiae* observed that the fermentation rates were comparable, however, a lower level of ethanol production than that of fermentation using only *S. cerevisiae* were observed *(Tristezza et al., 2016).*

In the light of the information provided above, it can be stated that co-inoculation of non-Saccharomyces species may result in improved sensory profile, however, the amount of ethanol produced may be affected negatively.

Although the mentioned studies are evaluating alcoholic beverages other than fruit spirits, it can be hypothesized that the co-inoculation of non-Saccharomyces yeasts and *S. cerevisiae* for fruit spirit production may result in different sensory characteristics compared to using commercial *S. cerevisiae.* Native yeast samples B and C has showed capability to ferment in lab scale fermentations. Parallel to these information, co-inoculation with *S. cerevisiae* of the native yeast samples B, and similarly native yeast C, may affect the sensory profiles of produced fruit spirits.

5.4. Sugar Content at the End of Fermentation

Both in laboratory batches and the main batches, according to the %Brix values measured, the sugar content in the mashes did not fully metabolized, thus all fermentations performed are considered as stuck fermentations.

One of the parameters that are relevant for stuck fermentations is the yeasts' ability to tolerate high ethanol concentrations (Magalhães et al., 2017). Another parameter that is relevant for stuck fermentations is nitrogen content of the mash, as for developing the highest possible levels of biomass, and for maintaining the fermentative capacity at the present biomass levels, nitrogen is the limiting macronutrient for most of the time (Bisson, 1999). Stuck fermentations could also be a result of low fermentation temperatures (Sablayrolles, 2019).

As for this present study, all fermentations took place in the same fermentation temperature, received the same amount of mash nutrient at the same time, and the fermentation mashes are prepared as a big mash, and divided into the fermentation amounts. Therefore, there were no differences in the fermentation conditions. Further research would be required to determine the reason for incomplete fermentations.

5.5. Ethanol Production of Native and Commercial Yeast

The relative abundance of *S. cerevisiae* is the highest compared to that of other species in the native yeast D sample. In the main fermentation and distillation, it can be clearly seen that native yeast D produced more ethanol compared to LT8 (Figure 18).

Same outcome can be observed in some other studies comparing commercial *S. cerevisiae* and native *S. cerevisiae* found on the fruits. In one study comparing it is found that wild strains of *S.* cerevisiae produces more than 12% (v/v) alcohol (Vigentini et al., 2017). In another study, it is observed that the wild strain isolated had better performance compared to the commonly used *S. cerevisiae* strain in use for industrial bioethanol production (Ruyters et al., 2015). In another study, it is observed that the wild strain of *S. cerevisiae* showed better alcohol fermentation performance compared to commercial wine yeasts, by producing 14% and 18% more alcohol than the compared commercial yeasts (Lee et al., 2011). These findings correlate with the results observed in this study.

S. eubayanus is known for the cold tolerance of the species with a limited sugar utilization ability (Gibson et al., 2013). Even creating hybrids of *S. cerevisiae* and *S.* eubayanus is thought to be beneficial for increasing the fermentation performance; In one study, it is observed that the hybridization brought advantages in aromatic properties (Magalhães et al., 2017).

Considering that the fermentations in this study were performed in a low fermentation temperature, another explanation for the native yeast D producing more product compared to LT8 could be that both *S. eubayanus* and *S. cerevisiae* are present in the native yeast sample D.

5.6. Sensory Perception of Produced Spirits

It can be observed that in this study the evaluation of color showed a significant difference between the pear spirits produced from native yeast D sample and commercial yeast LT8. This difference is caused by floating particles observed in the sample of native yeast D. Probable

explanation for the particles being present could be the excessive foaming observed during the distillation of the native yeast D sample. After observing the excessive foaming, an anti-foaming agent is used while distilling the commercial yeast LT8 sample.

The taste of the pear spirits produced from commercial yeast LT8, and native yeast D sample did not show a significant difference (Figure 19). Both spirits have found strong, fruity, sweet, and pear-like by the sensory analysis participants (Figure 24). The greatest differences in the taste of the pear spirits are that participants perceived a stronger alcoholic taste in the pear spirit produced with commercial yeast LT8, and the pear spirit produced with native yeast D sample perceived bitter by some of the participants. Thus, it can be stated that native yeast has performed as good as the commercial yeast in terms of taste.

The odor of the pear spirit produced with native yeast D sample showed a significantly better result compared to that of the pear spirit produced with commercial yeast LT8 (Figure 20). Both the pear spirits produced with native yeast D sample and commercial yeast LT8 perceived as fruity, mild, sweet and pear-like by the participants (Figure 24). The pear spirit produced with commercial yeast LT8 is found to be having a chemical-like odor by some of the participants. They perceived the spirit produced with LT8 to have a more pear-like aroma, while perceiving the spirit produced with native yeast D sample sweeter. Overall, it is shown that the pear spirit produced with native yeast D sample perceived as significantly better in terms of odor.

The taste and overall assessment/harmony scores of both spirits were similar to each other according to the significance level, by 64% and 80%, respectively. The difference of color and odor scores were significantly different, but these differences could result in an overall significant difference in the total sensory properties of the spirits.

6. Conclusion

In the DNA analysis results, several different species were present with lower sequence counts compared to above mentioned yeast species. According to the DNA analysis of the pears, other yeast species, along with plant pathogens are identified. However, this result could not correspond to definitive research. Thus, investigation is needed for a deeper understanding of the microbiota. Nevertheless, the sequence counts shows that there are commercially important non-Saccharomyces yeast species on the pears, and investigating native strains of the commercially important non-Saccharomyces yeasts offers a possible improvement area for the sensory profile of pear spirits produced with native yeasts.

As the species in other yeast samples are non-Saccharomyces yeasts, their alcohol production was not comparable to sample D. To understand the effect of non-Saccharomyces native yeasts on the sensory properties of pear spirits, it could be suitable to perform co-inoculations.

In the sensory analysis, the pear spirit produced with native yeast showed a significantly better result compared to the commercial yeast in the comparison of odor, together with a higher volume of ethanol production. However, in the total assessment considering color, odor, taste, and overall harmony, the difference between the pear spirit produced with native yeast and the pear spirit produced with commercial yeast was not significant. This result shows that the native yeast strain performed as good as the commercial yeast strain with a higher ethanol production. Utilizing native *S. cerevisiae* strain obtained could be used for producing a higher yield of production without compromising on the sensory quality.

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Declaration

I, Elif Ezgi Zabun, hereby declare that I wrote the present dissertation with the topic: "Fermentation of *Pyrus communis* var. ‘Conference’ with Native Yeasts and Evaluation of Sensory Properties of Produced Pear Distillates" independently and used no other aids than those cited. In each individual case, I have clearly identified the source of the passages that are taken word for word or paraphrased from other works.

I also hereby declare that I have carried out my scientific work according to the principles of good scientific practice.

Kleve, 14.03.2024
City, Date

Signature

Appendix A - List of Materials and Instruments

For the materials and instruments lists, only the items related to fermentation and distillation steps are included. Pipettes, centrifugal tubes, inoculation loops, incubator, vortex stirrer and other materials and instruments are regarded as basic equipment and are excluded.

Material/Instrument Used	Composition/Manufacturer
Saccharomyces cerevisiae (LT8), Art.-No. 5820	Schliessmann Kellerei-Chemie GmbH & Co, (Schwäbisch Hall, Germany)
Pectinlyase, Art.-No. 5015	Schliessmann Kellerei-Chemie GmbH & Co, (Schwäbisch Hall, Germany)
Acid Combination MS, Art.-No. 5852	Schliessmann Kellerei-Chemie GmbH & Co, (Schwäbisch Hall, Germany)
Mash nutrient, Art.-No. 5846	Schliessmann Kellerei-Chemie GmbH & Co, (Schwäbisch Hall, Germany)
Column still 190L	Kothe Destillationstechnik GmbH, (Eislingen, Germany)
CopperGarden® copper still 2L	UNICOBRES GmbH & Co. KG, **(**Eiterfeld, Germany)
pH Meter FiveEasy	METTLER TOLEDO (Ohio, US)
Digital Refractometer HI 96801	Hanna Instruments Deutschland GmbH, (Vöhringen, Germany)

Appendix B - Raw Data

B.1 CFU Counts of LT8 and Native Yeasts

	Batch 1		**Batch 2**			**Batch 3**				
10^(-4)	TNTC		TNTC			TNTC				
10^(-5)	213	332	TNTC			292	349	303		
10^(-6)	-	-	59	56	63	36	31	35		
CFU [CFU/mL]	2.130E+08		5.933E+08			3.030E+08			**Average CFU [CFU/mL]**	3.70E+08

Colony counts and CFU values calculated of commercial yeast LT8. Values did not considered in calculations are marked in the table; and Colony counts too low to include in the calculations are shown with '-' symbol. TNTC: abbr. 'Too numerous to count'

	A		**B**		**C**		**D**	
10^(-6)	TNTC		247	227	105	135	288	249
10^(-7)	115	128	26	27	16	20	33	30
10^(-8)	20	14	-	-	-	-	-	-
CFU [CFU/mL]	1.259E+10		2.395E+09		1.255E+09		2.727E+09	

Colony counts and CFU values calculated of commercial yeast LT8. Colony counts too low to include in the calculations are shown with '-' symbol. TNTC: abbr. 'Too numerous to count'

B.2. Sequence Counts of Native Yeast Samples

B.2.1. Sequence Count of Native Yeast Sample A

Species	Sequence count	Species (cont.)	Sequence count
Rhodotorula toruloides	400,558	*Chondrus crispus*	180
Rhodotorula graminis	196,005	*Trichosporon asahii*	175
Saccharomyces cerevisiae	40,591	*Cutaneotrichosporon oleaginosum*	169
Staphylococcus pasteuri	29,145	*Heterobasidion irregulare*	168
Rhodotorula mucilaginosa	28,015	*Schizophyllum commune*	151
Saccharomyces eubayanus	6,875	*Salmonella enterica*	151
Melampsora larici-populina	6,777	*Ogataea polymorpha*	148
Aspergillus heteromorphus	3,012	*Meira miltonrushii*	147
Spathaspora passalidarum	1,526	*Trametes versicolor*	140
Mixia osmundae	1,310	*Tilletiopsis washingtonensis*	140
Pseudomicrostroma glucosiphilum	1,199	*Streptococcus cristatus*	104
Dichomitus squalens	970	*Pseudogymnoascus destructans*	98
Aspergillus steynii	961	*Stenotrophomonas maltophilia*	89
Agaricus bisporus	913	*Spizellomyces punctatus*	88
Postia placenta	813	*Wallemia ichthyophaga*	86
Sparassis crispa	794	*Aureobasidium subglaciale*	84
Babesia bigemina	730	*Gloeophyllum trabeum*	82
Saitoella complicata	536	*Aureobasidium namibiae*	79
Aspergillus sclerotioniger	515	*Rhizopus microsporus*	72
Aspergillus homomorphus	508	*Lobosporangium transversale*	72
Ustilago maydis	507	*Pseudocercospora fijiensis*	63
Puccinia graminis	489	*Pichia membranifaciens*	62
Alloscardovia sp HMSC034E08	442	*Sugiyamaella lignohabitans*	62
Punctularia strigosozonata	347	*Talaromyces stipitatus*	60
Apiotrichum porosum	330	*Bacillus cereus*	59
Paracoccidioides lutzii	320	*Mitosporidium daphniae*	59
Wickerhamomyces anomalus	306	*Saccharomyces cariocanus*	58
Phycomyces blakesleeanus	288	*Phialocephala scopiformis*	57
Dictyostelium purpureum	273	*Endocarpon pusillum*	55
Tremella mesenterica	269	*Capronia epimyces*	52
Anthracocystis flocculosa	246	*Hyaloscypha bicolor*	51
Acaromyces ingoldii	242	*Torulaspora delbrueckii*	50
Aspergillus campestris	241	*Naumovozyma castellii*	49
Stereum hirsutum	226	*Kalmanozyma brasiliensis*	47
Kockovaella imperatae	217	*Zygosaccharomyces rouxii*	45
Aspergillus novofumigatus	206	*Glarea lozoyensis*	45
Thalassotalea mangrovi	192	*Chaetomium globosum*	44
Coniophora puteana	189	*Amorphotheca resinae*	43
Enterococcus faecium	185	*Tetrapisispora blattae*	42

Sequence count of native yeast sample A (cont.)

Species	Sequence count	Species (cont.)	Sequence count
Rhizophagus irregularis	41	*Vanderwaltozyma polyspora*	23
Enterococcus durans	38	*Yarrowia lipolytica*	22
Botrytis cinerea	37	*Candida glabrata*	22
Burkholderia multivorans	37	*Phytophthora sojae*	22
Colletotrichum graminicola	36	*Rasamsonia emersonii*	22
Arthrobotrys oligospora	35	*Babjeviella inositovora*	21
Parastagonospora nodorum	35	*Penicillium arizonense*	21
Xylona heveae	35	*Bacillus badius*	20
Peribacillus simplex	32	*Meyerozyma guilliermondii*	20
Escherichia coli	31	*Suhomyces tanzawaensis*	20
Coniosporium apollinis	30	*Paraphaeosphaeria sporulosa*	20
Naumovozyma dairenensis	29	*Leptosphaeria maculans*	19
Hanseniaspora uvarum	29	*Brucella melitensis*	19
Batrachochytrium dendrobatidis	29	*Penicillium expansum*	18
Kazachstania africana	28	*Scheffersomyces stipitis*	16
Schizosaccharomyces japonicus	27	*Wickerhamiella sorbophila*	16
Tuber melanosporum	27	*Kluyveromyces marxianus*	14
Lachancea thermotolerans	27	*Ramularia collo-cygni*	14
Phialophora attinorum	27	*Metschnikowia bicuspidata*	13
Aspergillus eucalypticola	26	*Aspergillus terreus*	13
Cyphellophora europaea	25	*Tetrapisispora phaffii*	13
Cavenderia fasciculata	24	*Lachancea lanzarotensis*	13

B.2.2. Sequence Count of Native Yeast Sample B

Species	Sequence count	Species (cont.)	Sequence count
Hanseniaspora uvarum	42,487	*Yamadazyma tenuis*	1,401
Tetrapisispora phaffii	7,069	*Scheffersomyces stipitis*	1,374
Lachancea thermotolerans	6,639	*Peribacillus simplex*	1,311
Kluyveromyces marxianus	6,581	*Aspergillus homomorphus*	1,290
Kazachstania africana	6,478	*Aspergillus steynii*	1,239
Tetrapisispora blattae	6,468	*Suhomyces tanzawaensis*	1,152
Lachancea lanzarotensis	6,074	*Hyphopichia burtonii*	1,149
[Candida] glabrata	5,861	*Meyerozyma guilliermondii*	1,051
Kluyveromyces lactis	5,784	*Phycomyces blakesleeanus*	1,023
Vanderwaltozyma polyspora	5,537	*Aspergillus campestris*	974
Pichia membranifaciens	5,534	*Clavispora lusitaniae*	970
Zygosaccharomyces rouxii	5,322	*Amorphotheca resinae*	964
Kazachstania naganishii	5,107	*Wickerhamiella sorbophila*	960
Wickerhamomyces anomalus	5,089	*Mitosporidium daphniae*	950
Naumovozyma dairenensis	5,005	*Yarrowia lipolytica*	941
Torulaspora delbrueckii	4,807	*Candida orthopsilosis*	936
Naumovozyma castellii	4,806	*Lodderomyces elongisporus*	840
Aspergillus heteromorphus	4,804	*Trametes versicolor*	836
Eremothecium gossypii	4,539	*Bacillus cereus*	794
Saccharomyces cerevisiae	4,503	*Candida viswanathii*	764
Spathaspora passalidarum	4,256	*Sugiyamaella lignohabitans*	721
Eremothecium sinecaudum	4,074	*Saitoella complicata*	718
Saccharomyces eubayanus	3,945	*Candida tropicalis*	680
Agaricus bisporus	3,851	*Aphanomyces invadans*	562
Eremothecium cymbalariae	3,825	*Ogataea parapolymorpha*	513
Cyberlindnera jadinii	3,476	*Cryptosporidium ubiquitum*	499
Wickerhamomyces ciferrii	3,160	*Debaryomyces hansenii*	475
Ogataea polymorpha	2,776	*Saccharomycodes ludwigii*	470
Aspergillus novofumigatus	2,581	*[Candida] auris*	417
Kuraishia capsulata	2,383	*Debaryomyces fabryi*	404
Ascoidea rubescens	2,297	*[Candida] haemuloni*	333
Aspergillus sclerotioniger	2,145	*Gloeophyllum trabeum*	321
Komagataella phaffii	2,011	*Spizellomyces punctatus*	301
Rhodotorula graminis	1,883	*Arthrobotrys oligospora*	272
Babjeviella inositovora	1,871	*Candida dubliniensis*	257
Capronia epimyces	1,851	*Candida albicans*	236
Phialocephala scopiformis	1,676	*Tuber melanosporum*	235
Metschnikowia bicuspidata	1,530	*Rhizopus microsporus*	232
Colletotrichum graminicola	1,403	*Lobosporangium transversale*	231

Sequence count of native yeast sample B (cont.)

Species	Sequence count	Species (cont.)	Sequence count
Schizosaccharomyces pombe	209	*Colletotrichum higginsianum*	61
Wallemia ichthyophaga	186	*Aureobasidium subglaciale*	60
Pseudomicrostroma glucosiphilum	180	*Paraphaeosphaeria sporulosa*	58
Baudoinia panamericana	180	*Puccinia graminis*	54
Schizosaccharomyces japonicus	154	*Cutaneotrichosporon oleaginosum*	54
Trichosporon asahii	149	*Rhodotorula toruloides*	52
Acanthamoeba castellanii	148	*Ramularia collo-cygni*	52
Xylona heveae	143	*Anthracocystis flocculosa*	49
Melampsora larici-populina	141	*Purpureocillium lilacinum*	48
Schizosaccharomyces octosporus	127	*Postia placenta*	46
Monosiga brevicollis	123	*Dictyostelium purpureum*	44
Rhizophagus irregularis	118	*Pseudogymnoascus destructans*	40
Verruconis gallopava	115	*Endocarpon pusillum*	40
Acaromyces ingoldii	114	*Hyaloscypha bicolor*	37
Chaetomium globosum	112	*Rasamsonia emersonii*	37
Meira miltonrushii	107	*Sclerotinia sclerotiorum*	36
Phaeodactylum tricornutum	107	*Sphaerulina musiva*	36
Kalmanozyma brasiliensis	105	*Heterobasidion irregulare*	33
Diplodia corticola	105	*Parastagonospora nodorum*	31
[Candida] duobushaemulonis	105	*Aspergillus eucalypticola*	30
Phialophora attinorum	100	*Aspergillus nidulans*	29
Uncinocarpus reesii	98	*Kockovaella imperatae*	28
Stereum hirsutum	97	*Tremella mesenterica*	25
Perkinsus marinus	97	*Histoplasma capsulatum*	25
Cavenderia fasciculata	95	*Glarea lozoyensis*	24
Dichomitus squalens	94	*Aspergillus terreus*	24
Phytophthora sojae	93	*Alternaria arborescens*	23
Batrachochytrium dendrobatidis	92	*Pseudocercospora fijiensis*	20
Apiotrichum porosum	91	*Punctularia strigosozonata*	19
Cyphellophora europaea	91	*Penicillium arizonense*	17
Galdieria sulphuraria	90	*Ustilago maydis*	16
Byssochlamys spectabilis	90	*Talaromyces stipitatus*	15
Pestalotiopsis fici	89	*Aureobasidium namibiae*	12
Trichoderma atroviride	81	*Leptosphaeria maculans*	12
Mixia osmundae	79	*Marssonina brunnea*	11
Coniosporium apollinis	77	*Tilletiopsis washingtonensis*	10
Sparassis crispa	76	*Paracoccidioides lutzii*	9
Zymoseptoria tritici	72	*Staphylococcus saprophyticus*	8
Schizophyllum commune	61	*Trichoderma virens*	8

B.2.2. Sequence Count of Native Yeast Sample C

Species	Sequence count	Species (cont.)	Sequence count
Aureobasidium subglaciale	663,714	*Penicillium expansum*	569
Aureobasidium namibiae	458,230	*Sphaerulina musiva*	546
Alternaria arborescens	9,019	*Pseudogymnoascus pannorum*	531
Zymoseptoria tritici	3,748	*Nannizzia gypsea*	523
Phialocephala scopiformis	3,603	*Parastagonospora nodorum*	513
Mycobacterium kansasii	3,047	*Exophiala dermatitidis*	493
Aspergillus steynii	2,621	*Trichoderma atroviride*	480
Baudoinia panamericana	2,579	*Penicillium arizonense*	472
Aspergillus novofumigatus	2,259	*Verruconis gallopava*	446
Spathaspora passalidarum	2,132	*Acaromyces ingoldii*	423
Pseudocercospora fijiensis	2,117	*Exophiala spinifera*	417
Paraphaeosphaeria sporulosa	1,695	*Trichoderma virens*	373
Aspergillus heteromorphus	1,479	*Xylona heveae*	372
Diplodia corticola	1,457	*Pyrenophora tritici-repentis*	372
Aspergillus campestris	1,453	*Botrytis cinerea*	371
Rasamsonia emersonii	1,450	*Purpureocillium lilacinum*	365
Rhodotorula graminis	1,407	*Cladophialophora immunda*	332
Coniosporium apollinis	1,342	*Pseudevernia consocians*	328
Hyaloscypha bicolor	1,284	*Amorphotheca resinae*	318
Aspergillus homomorphus	1,222	*Phialophora attinorum*	314
Ramularia collo-cygni	1,138	*Pertusaria propinqua*	310
Aspergillus sclerotioniger	1,106	*Aspergillus terreus*	309
Aspergillus eucalypticola	1,105	*Phialocephala subalpina*	309
Cercospora beticola	1,101	*Sclerotinia sclerotiorum*	300
Endocarpon pusillum	1,093	*Aspergillus mulundensis*	298
Pseudocercospora mori	1,073	*Wickerhamomyces anomalus*	296
Marssonina brunnea	1,009	*Tremella mesenterica*	293
Agaricus bisporus	1,007	*Anthracocystis flocculosa*	271
Talaromyces stipitatus	926	*Colletotrichum higginsianum*	263
Trichoderma reesei	923	*Histoplasma capsulatum*	257
Exserohilum turcicum	919	*Saccharomyces cerevisiae*	248
Alternaria alternata	903	*Uncinocarpus reesii*	228
Colletotrichum graminicola	804	*Mitosporidium daphniae*	164
Aspergillus nidulans	686	*Cyphellophora europaea*	163
Glarea lozoyensis	681	*Arthrobotrys oligospora*	138
Pestalotiopsis fici	648	*Byssochlamys spectabilis*	136
Leptosphaeria maculans	599	*Capronia epimyces*	121
Pseudogymnoascus destructans	584	*Chaetomium globosum*	101

Sequence count of native yeast sample C (cont.)

Species	Sequence count
Paracoccidioides lutzii	79
Saccharomyces eubayanus	51
Tuber melanosporum	51
Lachancea thermotolerans	46
Dichomitus squalens	45
Phytophthora sojae	42
Pichia membranifaciens	40
Gloeophyllum trabeum	39
Dictyostelium purpureum	36
Trichosporon asahii	34
Saitoella complicata	28
Pseudomicrostroma glucosiphilum	28
Yarrowia lipolytica	26
Punctularia strigosozonata	25

B.2.2. Sequence Count of Native Yeast Sample D

Species	Sequence count	Species (cont.)	Sequence count
Saccharomyces cerevisiae	2,506,409	*Staphylococcus saprophyticus*	979
Saccharomyces eubayanus	413,647	*Melampsora larici-populina*	893
Wickerhamomyces anomalus	17,508	*Vanderwaltozyma polyspora*	815
Thalassotalea mangrovi	9,743	*Tetrapisispora blattae*	789
Aspergillus novofumigatus	8,178	*Bacillus badius*	730
Ogataea polymorpha	7,088	*Kazachstania naganishii*	719
Streptococcus cristatus	6,766	*Tetrapisispora phaffii*	694
Salmonella enterica	6,728	*Klebsiella pneumoniae*	688
Stenotrophomonas maltophilia	5,988	*Trichoderma atroviride*	658
Spathaspora passalidarum	5,866	*Lachancea thermotolerans*	635
Agaricus bisporus	5,764	*Lachancea lanzarotensis*	583
Aspergillus steynii	4,483	*Kluyveromyces marxianus*	432
Pichia membranifaciens	3,827	*Saitoella complicata*	337
Saccharomyces cariocanus	3,698	*Ustilago maydis*	319
Bacillus cereus	3,023	*Rhodotorula toruloides*	314
Rhodotorula graminis	2,979	*Hyphopichia burtonii*	306
Aspergillus heteromorphus	2,836	*Zymoseptoria tritici*	271
Capronia epimyces	2,751	*Kluyveromyces lactis*	270
Torulaspora delbrueckii	2,247	*Eremothecium cymbalariae*	224
Phialocephala scopiformis	2,220	*Eremothecium gossypii*	194
Naumovozyma castellii	2,088	*Eremothecium sinecaudum*	178
Peribacillus simplex	1,970	*Meyerozyma guilliermondii*	121
Escherichia coli	1,955	*Metschnikowia bicuspidata*	120
Aspergillus sclerotioniger	1,906	*Babjeviella inositovora*	115
Mixia osmundae	1,880	*Phytophthora sojae*	103
Aspergillus homomorphus	1,834	*Cyberlindnera jadinii*	100
Enterococcus durans	1,778	*Yamadazyma tenuis*	100
Naumovozyma dairenensis	1,768	*Dichomitus squalens*	90
Zygosaccharomyces rouxii	1,731	*Wickerhamomyces ciferrii*	76
Burkholderia multivorans	1,473	*Komagataella phaffii*	73
[Candida] glabrata	1,449	*Sugiyamaella lignohabitans*	69
Aspergillus campestris	1,411	*Kuraishia capsulata*	62
Brucella melitensis	1,369	*Paracoccidioides lutzii*	58
Aspergillus eucalypticola	1,365	*Candida albicans*	57
Enterococcus faecium	1,354		
Kazachstania africana	1,325		
Mitosporidium daphniae	1,229		
Colletotrichum graminicola	1,175		
Tremella mesenterica	1,032		

B.3. %Brix Measurements

B3.1. %Brix Measurements of Batch 1

Batch 1	A			
Measurement #	1	2	3	Mean
day 7	12.3	12.2	12.3	12.27
day 8	12	12	12	12.00
day 9	11.6	11.6	11.6	11.60
day 10	11.2	11.2	11.2	11.20
day 13	9.2	9.2	9.2	9.20
day 14	9.3	9.2	9.3	9.27

Batch 1	B			
Measurement #	1	2	3	Mean
day 7	12.7	12.8	12.8	12.77
day 8	12.6	12.6	12.5	12.57
day 9	12.2	12.2	12.2	12.20
day 10	12.1	12.1	12.1	12.10
day 13	12	12.1	12	12.03
day 14	11.8	11.8	11.7	11.77

Batch 1	C			
Measurement #	1	2	3	Mean
day 7	12.7	12.6	12.7	12.67
day 8	12	12	12.1	12.03
day 9	11.7	11.7	11.7	11.70
day 10	11.8	11.7	11.8	11.77
day 13	10.7	10.7	10.7	10.70
day 14	9.8	9.8	9.7	9.77

Batch 1	D			
Measurement #	1	2	3	Mean
day 7	9.4	9.4	9.4	9.40
day 8	8.7	8.7	8.7	8.70
day 9	7.9	8	7.9	7.93
day 10	7.2	7.2	7.2	7.20
day 13	6	5.9	5.8	5.90
day 14	5.6	5.7	5.5	5.60

Batch 1	LT8			
Measurement #	1	2	3	Mean
day 7	9.2	9.3	9.1	9.20
day 8	8.5	8.5	8.5	8.50
day 9	7.6	7.6	7.6	7.60
day 10	6.7	6.8	6.7	6.73
day 13	6.1	6	6.1	6.07
day 14	6.1	6.1	6	6.07

B.3.2. %Brix Measurements of Batch 2

Batch 2	A			
Measurement #	1	2	3	mean
day 6	10.2	10.1	10.2	10.17
day 7	11.1	11.1	11.1	11.10
day 8	9.8	9.7	9.7	9.73
day 9	9.7	9.7	9.7	9.70
day 10	9.1	9	9	9.03
day 13	8	7.9	7.9	7.93

Batch 2	B			
Measurement #	1	2	3	mean
day 6	10.8	10.8	10.8	10.80
day 7	10.7	10.7	10.7	10.70
day 8	10.6	10.6	10.7	10.63
day 9	10.4	10.4	10.4	10.40
day 10	9.8	9.8	9.8	9.80
day 13	8.9	8.9	8.9	8.90

Batch 2	C			
Measurement #	1	2	3	mean
day 6	10.4	10.4	10.4	10.40
day 7	10.2	10.2	10.2	10.20
day 8	9.9	10	9.9	9.93
day 9	9.7	9.7	9.7	9.70
day 10	9.1	9	9	9.03
day 13	8.5	8.6	8.5	8.53

Batch 2	D			
Measurement #	1	2	3	mean
day 6	6.4	6.5	6.5	6.47
day 7	6	5.9	6	5.97
day 8	5.7	5.6	5.6	5.63
day 9	5.6	5.6	5.6	5.60
day 10	5.4	5.4	5.4	5.40
day 13	5.4	5.4	5.4	5.40

Batch 2	LT8			
Measurement #	1	2	3	mean
day 6	6.8	6.8	6.7	6.77
day 7	5.9	5.8	5.9	5.87
day 8	5.6	5.6	5.6	5.60
day 9	5.5	5.5	5.5	5.50
day 10	5.5	5.4	5.4	5.43
day 13	5.5	5.4	5.4	5.43

B.3.3. %Brix Measurements of Main Batches

Main Batch	D				Main Batch	LT8			
Measurement #	1	2	3	mean	Measurement #	1	2	3	mean
day 10	6.4	6.5	6.5	6.47	day 10	6.5	6.4	6.3	6.40
day 11	6.5	6.4	6.4	6.43	day 11	6.4	6.3	6.2	6.30
day 12	6.5	6.6	6.6	6.57	day 12	6.5	6.5	6.4	6.47
day 13	6.5	6.5	6.5	6.50	day 13	6.6	6.5	6.5	6.53

B.4. pH Measurements

B.4.1. pH Measurements of Batch 1

Batch 1	A			
Measurement #	1	2	3	mean
day 7	3.45	3.44	3.45	3.45
day 8	3.49	3.51	3.49	3.50
day 9	3.48	3.46	3.46	3.47
day 10	3.4	3.36	3.36	3.37
day 13	3.42	3.41	3.4	3.41
day 14	3.3	3.28	3.28	3.29

Batch 1	B			
Measurement #	1	2	3	mean
day 7	3.45	3.44	3.44	3.44
day 8	3.5	3.47	3.46	3.48
day 9	3.44	3.44	3.42	3.43
day 10	3.34	3.33	3.32	3.33
day 13	3.4	3.39	3.39	3.39
day 14	3.43	3.42	3.41	3.42

Batch 1	C			
Measurement #	1	2	3	mean
day 7	3.48	3.47	3.46	3.47
day 8	3.49	3.48	3.48	3.48
day 9	3.46	3.44	3.44	3.45
day 10	3.36	3.35	3.35	3.35
day 13	3.39	3.39	3.39	3.39
day 14	3.42	3.4	3.4	3.41

Batch 1	D			
Measurement #	1	2	3	mean
day 7	3.45	3.44	3.43	3.44
day 8	3.45	3.44	3.43	3.44
day 9	3.41	3.39	3.38	3.39
day 10	3.31	3.3	3.3	3.30
day 13	3.41	3.41	3.41	3.41
day 14	3.46	3.46	3.46	3.46

Batch 1	LT8			
Measurement #	1	2	3	mean
day 7	3.45	3.43	3.42	3.43
day 8	3.42	3.4	3.4	3.41
day 9	3.36	3.35	3.35	3.35
day 10	3.28	3.27	3.27	3.27
day 13	3.37	3.37	3.37	3.37
day 14	3.44	3.42	3.42	3.43

B.4.2. pH Measurements of Batch 2

Batch 2	A			
Measurement #	1	2	3	mean
day 6	3.37	3.36	3.35	3.36
day 7	3.45	3.43	3.42	3.43
day 8	3.46	3.45	3.45	3.45
day 9	3.41	3.4	3.4	3.40
day 10	3.37	3.36	3.36	3.36
day 13	3.3	3.28	3.28	3.29

Batch 2	B			
Measurement #	1	2	3	mean
day 6	3.42	3.38	3.38	3.39
day 7	3.48	3.46	3.45	3.46
day 8	3.47	3.47	3.46	3.47
day 9	3.42	3.41	3.41	3.41
day 10	3.36	3.36	3.36	3.36
day 13	3.3	3.29	3.28	3.29

Batch 2	C			
Measurement #	1	2	3	mean
day 6	3.41	3.39	3.38	3.39
day 7	3.48	3.46	3.46	3.47
day 8	3.48	3.47	3.47	3.47
day 9	3.44	3.42	3.41	3.42
day 10	3.4	3.4	3.4	3.40
day 13	3.32	3.32	3.32	3.32

Batch 2	D			
Measurement #	1	2	3	mean
day 6	3.35	3.33	3.33	3.34
day 7	3.41	3.4	3.39	3.40
day 8	3.42	3.42	3.42	3.42
day 9	3.39	3.37	3.37	3.38
day 10	3.4	3.4	3.39	3.40
day 13	3.34	3.32	3.32	3.33

Batch 2	LT8			
Measurement #	1	2	3	mean
day 6	3.28	3.27	3.27	3.27
day 7	3.41	3.38	3.37	3.39
day 8	3.39	3.38	3.38	3.38
day 9	3.33	3.32	3.32	3.32
day 10	3.35	3.34	3.34	3.34
day 13	3.28	3.27	3.27	3.27

B.4.3. pH Measurements of Main Batch

Main Batch	D			
Measurement #	1	2	3	mean
day 10	3.46	3.45	3.44	3.45
day 11	3.49	3.47	3.47	3.48
day 12	3.11	3.09	3.09	3.10
day 13	3.17	3.16	3.16	3.16

Main Batch	LT8			
Measurement #	1	2	3	mean
day 10	3.48	3.44	3.44	3.45
day 11	3.44	3.43	3.42	3.43
day 12	3.05	3.03	3.03	3.04
day 13	3.13	3.13	3.13	3.13

B.5. Sensory Analysis Questionnaire

Color	Clarity	Excellent (5)			Insufficient (1)	
	Hue	Excellent (5)	Very Good (4)	Good (3)	Sufficient (2)	Insufficient (1)
Odor	Purity	Excellent (10)		Good (6)		Insufficient (2)
	Intensity	Excellent (14)	Very Good (12)	Good (10)	Sufficient (8)	Insufficient (4)
	Quality	Excellent (14)	Very Good (12)	Good (10)	Sufficient (8)	Insufficient (4)
Taste	Purity	Excellent (10)		Good (6)		Insufficient (2)
	Intensity	Excellent (14)	Very Good (12)	Good (10)	Sufficient (8)	Insufficient (4)
	Quality	Excellent (14)	Very Good (12)	Good (10)	Sufficient (8)	Insufficient (4)
Overall Assessment/ Harmony		Excellent (14)	Very Good (12)	Good (10)	Sufficient (8)	Insufficient (4)

B.6. Sensory Analysis

B.6.1. Sensory Analysis of Pear Spirit (LT8)

	Desc.	Point		Point		Desc.		Point		Point		Point		Desc.		Point		Point		Point		Desc.	Point	Total
Color	Colorless, clear	5	Hue	5	Odor	pure smell, not so intense, reflects high quality	Odor(Purity)	10	Odor(Intensity)	10	Odor(Quality)	14	Taste	slight burn at throat, smooth pear aroma, reminds rakija	Taste(Purity)	10	Taste(Intensity)	12	Taste(Quality)	14	OA/H	taste and smell matches, leaves a nice pear taste, doesn't let it feel the amount of alcohol during consumption	12	92
Color	clear	5	Hue	5	Odor	[DE] sehr fruchtig, sour	Odor(Purity)	6	Odor(Intensity)	12	Odor(Quality)	12	Taste	[DE] bissle wie Apfelsaft	Taste(Purity)	6	Taste(Intensity)	10	Taste(Quality)	10	OA/H		10	76
Color	x	5	Hue	5	Odor	pleasant pear-like smell	Odor(Purity)	6	Odor(Intensity)	12	Odor(Quality)	14	Taste	similar to good quality conventional spirits, a bit sweet	Taste(Purity)	10	Taste(Intensity)	12	Taste(Quality)	14	OA/H		12	90
Color	Colorless, clear	5	Hue	5	Odor	sweet, pear like (can smell alcohol), mellow smell	Odor(Purity)	10	Odor(Intensity)	12	Odor(Quality)	10	Taste	slightly sweet, a hint of vanilla, balanced taste	Taste(Purity)	10	Taste(Intensity)	12	Taste(Quality)	12	OA/H		12	88
Color	clear, no floating particles	5	Hue	5	Odor	no errors, pears intensive [DE]schalig, kernig, fruchtig leichte würze(muskat)	Odor(Purity)	10	Odor(Intensity)	12	Odor(Quality)	12	Taste	no errors, pears less intensive than odor [DE] würzig(pfeffer, Muskat) leicht spritig (nach Alkohol)	Taste(Purity)	10	Taste(Intensity)	10	Taste(Quality)	10	OA/H	[DE] in der Nase intensiver als im Mund. Mild, nicht langanhaltend nicht mundausfollend	10	84
Color	clear, no hue	5	Hue	5	Odor	strong smell, fruity, sweet, banana-like smell	Odor(Purity)	10	Odor(Intensity)	12	Odor(Quality)	12	Taste	fruity, slight burn, sweet aftertaste	Taste(Purity)	10	Taste(Intensity)	10	Taste(Quality)	12	OA/H		12	88

Sensory Analysis of Pear Spirit (LT8) (Cont.)

	Desc.	Point		Point		Desc.		Point		Point		Point		Desc.		Point		Point		Point		Desc.	Point	Total
Color	clear	5	Hue	5	Odor	sweet	Odor(Purity)	10	Odor(Intensity)	10	Odor(Quality)	12	Taste	alcohol overtake the taste of pears, strong acidic taste	Taste(Purity)	10	Taste(Intensity)	10	Taste(Quality)	12	OA/H	very good	12	86
Color	Colorless	5	Hue	5	Odor	aroma is not felt much, a bit chemical taste	Odor(Purity)	10	Odor(Intensity)	12	Odor(Quality)	12	Taste	clear pear aroma, strong alcohol taste, sweet aftertaste	Taste(Purity)	10	Taste(Intensity)	12	Taste(Quality)	12	OA/H	alcohol felt strongly but taste like real pear, perfect combination	12	90
Color	Colorless	5	Hue	5	Odor	smells nice, strong, chemical-like	Odor(Purity)	10	Odor(Intensity)	10	Odor(Quality)	10	Taste	purely like a strong alcohol, burned throat	Taste(Purity)	10	Taste(Intensity)	8	Taste(Quality)	10	OA/H	Has a strong taste compared to odor	10	78
Color	similar to commercial spirits	5	Hue	5	Odor	not so intense, fruity	Odor(Purity)	6	Odor(Intensity)	10	Odor(Quality)	10	Taste	mild aroma, alcohol taste is not too strong	Taste(Purity)	6	Taste(Intensity)	8	Taste(Quality)	8	OA/H		10	68
Color	clear, no impurities	5	Hue	5	Odor	slight hints of pear	Odor(Purity)	10	Odor(Intensity)	10	Odor(Quality)	12	Taste	quite sharp but a little bland, tastes like alcohol	Taste(Purity)	10	Taste(Intensity)	10	Taste(Quality)	10	OA/H	very nice with a pleasant aroma	10	82

B.6.2. Sensory Analysis of Pear Spirit (Native Yeast D)

	Desc.	Point		Point		Desc.		Point		Point		Point		Desc.		Point		Point		Point		Desc.	Point	Total
Color	less transparent	5	Hue	4	Odor	more noticable pear smell, not a sharp smell, sweet smell	Odor(Purity)	10	Odor(Intensity)	8	Odor(Quality)	12	Taste	pear sense not as present as smell, intense alcohol taste	Taste(Purity)	6	Taste(Intensity)	8	Taste(Quality)	10	OA/H	inbalanced taste and smell, aroma loses itself in the taste.	10	73
Color	clear with some particles	5	Hue	5	Odor	[DE] sehr fruchtig, sweet	Odor(Purity)	10	Odor(Intensity)	12	Odor(Quality)	14	Taste	[DE] wie Apfelsaft	Taste(Purity)	10	Taste(Intensity)	12	Taste(Quality)	12	OA/H	lighter taste, slight error taste but not very bad	12	92
Color	some visible particles	5	Hue	3	Odor	strong odor	Odor(Purity)	6	Odor(Intensity)	14	Odor(Quality)	14	Taste	very strong taste	Taste(Purity)	10	Taste(Intensity)	10	Taste(Quality)	8	OA/H	x	8	78
Color	clear with some particles	5	Hue	5	Odor	strong smell, pear odor	Odor(Purity)	10	Odor(Intensity)	14	Odor(Quality)	12	Taste	strong, intense taste, slightly bitter, sweet undertones	Taste(Purity)	10	Taste(Intensity)	12	Taste(Quality)	10	OA/H	x	10	88
Color	clear with some particles	5	Hue	5	Odor	no errors, [DE] fruchtig (zwar Birne aber anders) nicht so vielschichtig, einfach)	Odor(Purity)	10	Odor(Intensity)	10	Odor(Quality)	10	Taste	no errors [DE] fruchtig, mild, birne, leichte würze	Taste(Purity)	10	Taste(Intensity)	12	Taste(Quality)	12	OA/H	[DE] langanhaltend, mundausfüllend, nicht harmonisch	12	86
Color	some particles that does not affect clarity	5	Hue	4	Odor	fruity and sweet, not pungent,	Odor(Purity)	10	Odor(Intensity)	12	Odor(Quality)	14	Taste	floral and fruity, mild bitterness	Taste(Purity)	10	Taste(Intensity)	12	Taste(Quality)	12	OA/H	taste and smell elevates each other with fruity and floral	12	91

Sensory Analysis of Pear Spirit (Native Yeast D) (Cont.)

	Desc.	Point		Point		Desc.		Point		Point		Point		Desc.		Point		Point		Point		Desc.	Point	Total
Color	debris can be seen circulating within	1	Hue	5	Odor	has a balance of pear and alcohol smell	Odor(Purity)	10	Odor(Intensity)	14	Odor(Quality)	14	Taste	less acidic and more easy going. Salty-water-like taste	Taste(Purity)	6	Taste(Intensity)	12	Taste(Quality)	12	OA/H	color and odor-wise it is flawless, the alcohol, pear and a milt salted water taste is distinguishable.	8	82
Color	clear but contains some particles	1	Hue	5	Odor	slight yeast odor, not a sharp smell, sweet	Odor(Purity)	10	Odor(Intensity)	14	Odor(Quality)	14	Taste	sweet pear taste, a softer drink	Taste(Purity)	10	Taste(Intensity)	14	Taste(Quality)	14	OA/H	didn't feel strong pear smell or taste but easy to drink	14	96
Color	x	5	Hue	5	Odor	doesn't smell chemical-like	Odor(Purity)	6	Odor(Intensity)	14	Odor(Quality)	14	Taste	strong alcohol taste	Taste(Purity)	6	Taste(Intensity)	12	Taste(Quality)	8	OA/H	taste was strong and odor was really nice	12	82
Color	clear with no hue, some visible particles	5	Hue	5	Odor	fruity, not choking	Odor(Purity)	10	Odor(Intensity)	14	Odor(Quality)	10	Taste	slight burning sensation on tongue, a slight sugar content	Taste(Purity)	6	Taste(Intensity)	14	Taste(Quality)	12	OA/H	good quality, comparable to some industrial products. Alcohol content is easily detectable	12	88
Color	clear, floating particles	5	Hue	3	Odor	pear aroma	Odor(Purity)	10	Odor(Intensity)	14	Odor(Quality)	12	Taste	deep taste, fruity aftertaste	Taste(Purity)	10	Taste(Intensity)	14	Taste(Quality)	14	OA/H	well rounded pear aroma	14	96