# Effect of yeast strains on the chemical composition of apple ciders from Finnish cultivars

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Effect of Saccharomyces and non- Saccharomyces yeast in apple ciders and juices were studied using Gas chromatography fitted with Flame Ionization Detector. Samples were produced from six different Finnish apple cultivars using two different commercial yeasts strains *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*.

Apple juice samples was inoculated with yeast and fermented at 22°C for 30 days. The results showed no notable difference among the ciders obtained. Ethanol content was similar (4.1-8.0 %v/v) SE compared to TD (3.3 -7.6 %v/v). Furthermore, the apple cultivars were significantly different in their sugar (sucrose, fructose, glucose, and sorbitol) contents, Malic, and ascorbic acids compositions. Additionally, a decrease in malic acid among the alcoholic juices was observed with those from TD fermentation slightly higher than SE. Also changes in qunic acid and ascorbic acid content was not detected in both yeast fermentation except in 'Tu1' sample with SE and 'Ra' sample fermented with TD. Both yeasts consumed fructose, glucose, and sucrose completely during fermentation.

Sorbitol an alcoholic sugar was found similar in 'Antonovka' and 'Tunista2' samples and slightly different in 'Alkesanter', 'Musti-Iso Venalainen', 'Tunista2' and 'Rambo' fermented juices samples with *T. delbrueckii* yeast. Citric acid was found present in all apple juice sample but not detected in 'Al' cultivar. Additionally, ascorbic and quinic acids was identified in all apple juice samples however in low quantity. The results illustrate effect was dependent on cultivar and not yeast strains. 'Rambo' cultivar differed from others producing more juice with low malic acid content, highest ethanol (7.8 -8.0% v/v) and total sugar cider and juice.

Therefore, 'Rambo' cultivar was identified as suitable for cider production with *Saccharomyces cerevisiae* or *Torulaspora delbrueckii* however further studies should focus on the phenolic compounds and sensory perception of 'Ra' in comparison to other cultivars. Also, sequential fermentation with both yeast and other non- Saccharomyces yeast like should be considered to produce higher quality alcoholic beverages.

Keywords: fermentation, inoculation, apple cider, yeast, cultivars, malic acid.

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**Content**

**Abbreivations**

| | |
|---|---|
| AFBs | Alcoholic Fermented Beverages |
| SE | Saccharomyces ceravicea |
| TD | Torulaspora delbruckii |
| F-TD | Fermented with Torulaspora delbruckii |
| F-SE | Fermented with Saccharomyces ceravicea |
| GC-FID | Gas Chromatography--Flame Ioniazation Detctor |
| PF | Pure Fermentation |
| RNA | Ribonucleic Acid |
| FDA | Food and Drug Administration |
| FAOSTAT | Food and Agriculture Organization statistics |
| UV-C | Ultraviolet-C |
| TSS | Total soluble solids |
| SSC | Soluble solid content |
| YPD | Yeast Peptone Dextrose |
| MQ | Mili- qui water |
| Rcf | Regenerated Cellulose Syringe Filters |
| PCA | Principal Component Analysis |

## 1. Introduction

Approximately 20% of apples are processed into value-added products, such as apple juice, jam, puree, cider, vinegar, and dehydrated apple products (Shalini and Gupta, 2010). However, the majority of apples (over 60%) are sold and consumed fresh. Cider is an alcoholic beverage made from fermented apples, with an alcohol concentration ranging from 1.2% to 8.5% (v/v) and a varied scent profile (Watson, 2013). Apple cider is one of the fastest-growing drinks in the alcoholic beverage market, with an expected yearly growth rate of 15% by 2021(Jamir et al., 2020, Miles et al., 2020). It has become increasingly popular in Europe, particularly in the UK, France, and Northern European countries. According to the European Cider and Fruit Wine Association, around 7.6% of the overall apple crops in 2018 were used for cider production, and 48.5% were made from cider apples. Cider's taste and aroma can significantly vary from country to country due to the varying preferences of consumers. In France, strong and fruity smells are highly valued and are associated with the tart and sweet apples used as raw materials (Berber 2016). Ciders with higher alcohol content tend to be dry, while those with lower alcohol content are naturally sweet due to residual sugars. Soft ciders have an alcoholic content of 1-4%, while strong ciders have a range of 5-8% (Lea and Peggot 2003; Joish et al., 2017). In recent, aromatic ciders have seen a surge in popularity. Germans tend to favor the traditional golden-yellow, carbonated cider, similar to wine, which is typically served on tap. However, with changing trends, a wider variety of ciders have become available, including those blended with fruit flavors or refreshing additions (Hammel and Arnold 2012). Meanwhile, in Spain, consumers prefer a highly carbonated cider with subtle acetic notes (León-Muñoz et al., 2010). In contrast, British niche producers offer a diverse range of ciders that cater to a wide range of consumer interests. While the typical alcohol content of cider falls between 4-6 percent, in the UK it can reach as high as 8.4% ( Berber 2016).There is a growing interest among cider producers in Northern European countries, especially in Finland, to develop local cultivars such as cider apples. However, there are currently no breeding programs for specialty cider apples in Finland, and no available data concerning the potential of Finnish apples in cider production.

## 1.1 Backgroung of apple cultivation in Finland

In Finland, apple cultivation was not a common practice during the accient times. The southwest region, which experiences a long growing season and warm winter climate, is the sole suitable area for commercial apple production in the country. However, the cultivation of apple trees in gardens in southern Finland dates back centuries, with a rich history tracing back to the 16th and 17th centuries (Aaltonen et al., 2006; Garkava-Gustavssona et al., 2013). In the 1700s, apple trees in Finland were devastated by frost, leading to the widespread cultivation and consumption of apples by the Finnish people. Historical records from the 1820s reveal that over 4000 fruit gardens existed in Finland, mainly concentrated in the southwestern region of the country (Collan, 1934). Despite the harsh winter conditions that often destroyed orchards, planting apple trees from seeds proved to be the most viable method for sustaining apple production in cold climates and ensuring a steady supply of fruit for those residing in the north (Lindén, 2001).

In the past, agronomists in local areas encouraged the cultivation of fruit trees in personal gardens until the mid-twentieth century. To grow this essential source of nutrition and vitamins, Finns utilized various techniques. They would scatter apple fruit seeds from any available fruit, and by chance, some of these seedlings would survive for multiple seasons, producing usable and respectable apples. This method was widespread due to the lack of nearby nurseries in Finland. The preservation of seed-born apples has resulted in the development of a unique and specially adapted native apple germplasm that can be replicated season after season. From the late 1800s to the mid-1900s, apple growers selectively cultivated a broad range of genetically diverse and specialized apples to meet various demands. Maarit and Lidija (2019), note that the first apple saplings were imported from the Baltic states in the 16th century, with subsequent imports from neighboring countries such as Russia, southern Sweden, and countries like Germany, Denmark, and France.

The Finnish breeding program for farmed apples was established in 1958 at the Agricultural Research Center Centre (now known as the Natural Resources Institute Finland) in Piikkiö, southwest Finland (60 250 N, 22 310 E), following extensive pomological studies that encompassed 315 apple varieties were studied from 1935 to 1958). The breeding of apple varieties in Finland aimed to combine the desirable traits of native Finnish seed-born apples, such as early maturation and winter hardiness, with the

fresh fruit quality of imported apple cultivars. Through this process, 17 new apple cultivars were developed, of which 12 had a native Finnish apple variety as a parent. In the 1990s, there was a concerted effort to breed apple cultivars that were resistant to rot, resulting in the creation of three new cultivars, one of which still contains genetic material from local varieties.

In Finland, the collection, preservation, evaluation, and support of plant genetic resources is entrusted to individual nations. These resources are shaped by a variety of factors, including the environment, history, and society. National collections outside of their natural habitat have preserved many valuable types of apple genetic resources. Gene banks for fruits and berries serve as breeding and development programs, while also promoting public awareness and preserving historical genotypes to support fundamental plant research. The Finnish apple cultivar collection was founded in 2001 to preserve genetics and was part of an established breeding program (Aaltonen et al., 2006). Over 50 unique types of indigenous apple cultivars are currently produced in Finland, but the majority have not been accurately classified or traced back to their historical origins.

## 1.2 Background on fermentation and apple cider production

According to historical sources, cider production predates beer and wine, with references in Greek and Roman literature dating back to around 900 BC. Cider is made by partially or completely fermenting fresh or reconstituted juice from apples or pears, with alcoholic content ranging from 1.2% to 8.5% as defined by European Cider and Fruit Wine Association 2018. Some low-alcohol ciders may have less than 1.2% alcohol content and can include added flavoring, water, or sugar. The European Cider and Fruit Wine Association recently published an article on this topic. [Calugar et al., 2021]. For centuries, people have been savoring fermented apple-based beverages. For example, the Jewish community enjoys shekar, a fermented apple drink, while the Greeks produce sikora by fermenting crushed, cooked apples (Sekar, S., and Mariappan, S. 2007).

Additionally, Patagonians brew chicha, a mildly alcoholic fermented apple drink. Moreover, Himalayan locals craft soor from fruits like apples or grains, following their traditional methods (Rena et al., 2004). Various regions utilize different types of apple cultivars to create cider. For instance, in Spain, it is recommended to use Blanquina, Cristalina, Coloradona, Collaos, Marilena, Perezosa, Regona, Prieta, Raxao, Solarina, and Teorica (Merwin et al., 2008). Finland, on the other hand, has distinct apple cultivars with

diverse chemical compositions because of its unique temperature variations and growth conditions. For cider production, the frequently used apple cultivars in Finland are Lobo, Amarosa, and Kenel, which are not conventional types. While cider and perry have a less celebrated history compared to grape wines, they are becoming increasingly popular in Europe, especially in countries such as England, France, Spain, and Northern Europe, due to their high nutritional value and delicious flavor. The beverage industry has a preference for using specialty fruit varieties that are grown in nearby orchards and possess fibrous structures, high tannin levels, and large juice yields. However, Northern European countries face unique environmental challenges when it comes to growing specialist cider apples and/or perry pears. As a result, it is crucial to develop and select specialty cultivars that can serve multiple purposes, such as the production of both juice and cider/perry, in order to meet the demands of modern retail supply chains. Bisson (2004) and Romano et al. (2003) have noted that yeast converts carbohydrates into ethanol, which starts the alcoholic fermentation process. Recent studies (Liu et al., 2016; Dashko et al., 2014) have also explored the production process.

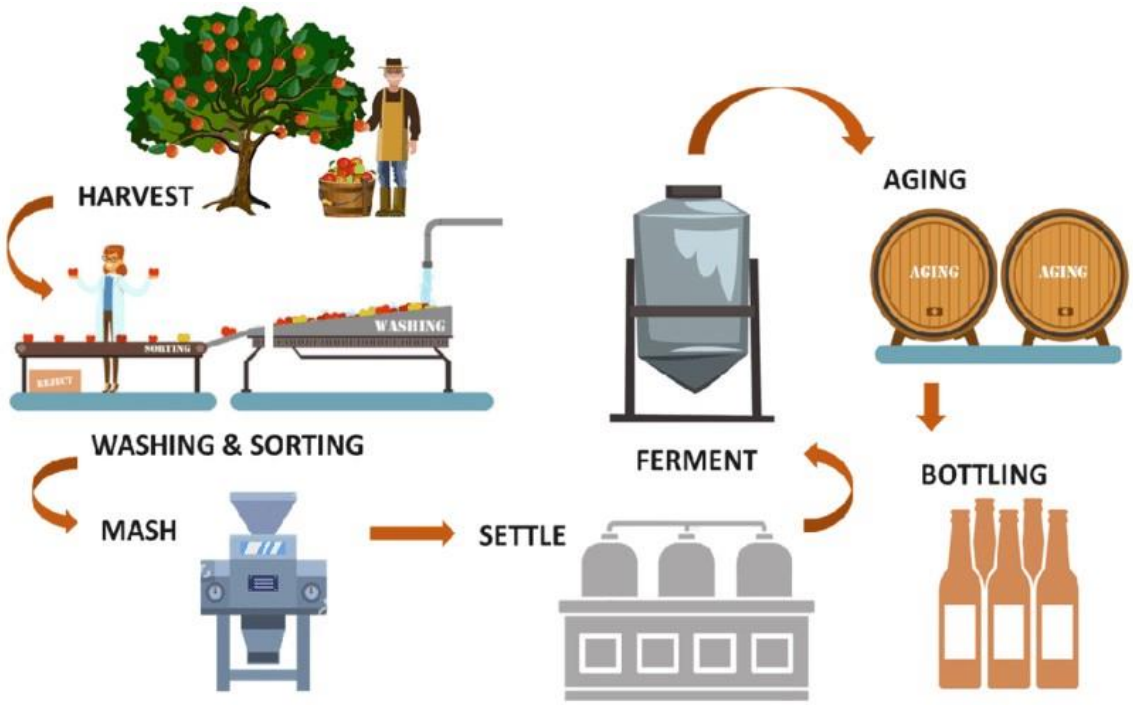



**Figure 1.** An overview of the processing procedures involved in apple cider making industrially. Source: a review on the impact of the physochemical and microbial diversity in apple juice fermentation process by Marina Al Daccache et al., 2020.

Through the process of fermentation, yeast breaks down the sugars found in the substrate. This breakdown leads to the creation of both volatile (such as alcohols, esters, and fatty acids) and non-volatile (such as malic acid) compounds. These compounds have a significant impact on the final aroma and flavor of the cider (Lorenzini et al., 2019, Ramano et al., 2003, and Howell et al., 2006). Research has shown that the development of cider's taste is akin to wine, where flavor-influencing ingredients are derived from grapes, derived from grapes and altered by yeast, and the result of yeast's primary metabolism (Sweiger and peturi 2005). The interplay between the compounds generated by yeast and those naturally present in the fruit juice creates a distinctive sensory experience for the consumer.

Aromatic propertise of cider has a major influence on consumer acceptance and cider product quality these however is determined by the techniques implored in the production of cider (Bingman et al., 2021). The cider industry continues to expand as its product gains growth hence cider producers are exploring various techniques in the craft of cider making. Ciders typically are clear golden fruit juices with colors ranging from pale yellow to amber rose depending on the fruit used with characteristic fruity flavor and sour or tart taste. The act of cider making has evolved throughout the years as industries and individuals gain deeper knowledge on factors that affect the flavors of ciders although it has been since ancient Europe and Asian culture according to archeological evidence.

During fermentation, certain chemicals with a volatile nature, such as higher alcohols, esters, and carbonyl compounds, play a significant role in influencing the organoleptic characteristics of cider. These compounds are primarily produced by the metabolic processes of yeast. It is worth noting that the type of yeast used in cider production has a significant impact on the creation of flavoring compounds (Herrero et., al. 2005). One of the defining traits of yeasts is their ability to convert carbohydrates into essential metabolites including ethanol, carbon dioxide, glycerol, acetate, succinate, pyruvate, higher alcohols, and esters. This quality is vital in the creation of many popular foods and beverages such as bread, beer, and wine, which rely on a process known as alcoholic fermentation. Typically, this process takes place when natural yeasts present in carbon-rich substrates (like grains for bread and beer, and grape musts for wine) spontaneously ferment sugars.

## 1.3 Significance of yeast in commercial food and beverage production

Yeast has been an essential ingredient in human society for millennia. They have proved to be incredibly valuable microorganisms. Yeast has played a vital role in creating a variety of foods, including bread, wine, beer, and kefir. In more recent times, yeasts have been harnessed to produce ethanol for fuel, as well as biochemicals for the pharmaceutical industry, among other uses. Yeasts that cause spontaneous fermentations are naturally present on fruits, vegetables, equipment, homemade starters, and raw biological materials, such as milk. For example, the atmosphere of a cheese factory, including brine and raw milk, can be a crucial source of yeast contamination on the surface of cheese. These yeasts are essential to the maturation process of spread cheeses. According to the research conducted by Fröhlich-Wyder et al., in 2018, *Debaryomyces hansenii* is a vital component in the aging process of surface-ripened cheeses. This yeast aids in raising the pH levels of the cheese through the production of lactic acid, which helps to promote the growth of *Brevibacterium linens*. Moreover, the study highlights the indispensable role of yeasts in the production of a range of fermented products, including blue cheese, white mold cheese, and kefir. Yeast is essential during the cheese ripening process, as they interact with other microorganisms, the lactic acid bacteria produce lactate, which is consumed by yeasts. (Sieuwerts et ai., 2008). They have a mutually beneficial relationship with lactic acid bacteria, benefiting from the bacterium's lactate production. Yeast also deacidifies the cheese and secretes growth hormones that encourage the growth of ripening bacteria such as *Brevibacterium spp., Arthrobacter spp.,* and *Corynebacterium spp*. (Viljoen, 2006; Irlinger and Mounier, 2009) on the cheese's surface. Additionally, yeasts have antagonistic properties that prevent the development of unwanted microbes such as molds, bacteria, and other yeasts for example, *D. hansenii* inhibits *clostridia* (Mufandaedza et al., 2006), and a strain of *K. marxianus* inhibits *Candida albicans* and *L. monocytogenes* (Boldyreva et al., 2016; Ceugniez et al., 2015).

Interactions between yeast and other yeast species, as well as between yeast and fungi, impact the formation of specific sensory qualities in cheese and the dominance and proliferation of specific yeast species. The distinctive odor of blue cheese, which is mainly due to ketone synthesis, is a result of the interaction between *Y. lipolytica* and *Penicillium roqueforti* in blue-veined cheeses (Gkatzionis et al., 2013, 2014). The bitterness of *Penicillium camemberti* in Camembert-type cheeses is reduced by the aminopeptidase activities of *G.candidum* (Boutrou and Gueguen, 2005; Eliskases-

Lechner et al., 2011). A study on the RNA content of gene transcripts found that the development of the characteristic sensory features of Camembert-type cheeses depends on the combined effects of *G. candidum* and *P. camemberti* (Lessard et al., 2014). These interactions, which involve pH shifts, the management of unwanted microbes, and the creation of flavor and aroma in cheese, significantly affect the ripening process and the overall quality of the cheese.

Today, yeasts have expanded their influence on the production of food and beverages beyond Saccharomyces cerevisiae fermentations that produce bread, beer, and wine. Yeast now plays a role in fermenting a vast range of other commodities, working alongside filamentous fungi and bacteria. Additionally, yeasts are utilized to produce various food ingredients and processing aids. Some yeasts also possess potent antifungal activity, making them useful in the biocontrol of food spoilage. While some might be aware that baker's yeast, brewer's yeast, wine yeast, and distiller's yeast are distinct from one another, others may not realize the numerous beneficial roles that various species play in the fermentation of food and beverages. S. cerevisiae*, S. bayanus*, and *S. pastorianus* (Kurtzman 2003) are some of the more recognizable strains, but it is now common knowledge that *Hanseniaspora* (Kloeckera), *Candida, Pichia, Metschnikowia, Kluyveromyces, Schizosaccharomyces*, and *Issatchenkia* can also contribute to the fermentation of wine and cider (Fleet 2003a; Pretorius 2000) while *S. pastorianus* and *S. cerevisiae* are significant in the production of some styles of beer (Dufour et al. 2003). They also play a significant role in the manufacturing of fermented milk products such as koumiss and kefir, as supported by ample research (Frohlich-Wyder, 2003).

Certain yeasts possess a distinctive probiotic property that is increasingly recognized, but they also have a downside. The food and beverage sectors acknowledge that yeast can result in considerable financial losses by spoiling a range of goods. Additionally, there is a mounting apprehension about the possible health hazards linked to yeasts present in food and drinks.

### 1.3.1 Physiological attributes of *saccharomyces cerevisiae* and non-*saccharomyces cerevisiae* yeast and their impact on AFBs

Louis Pasteur introduced the concept of fermentation in the late 1850s. This involves the use of specific microorganisms like yeast to break down sugars during beverage production, altering the taste and increasing shelf life. Even today, the process remains widely used in brewing fruits and malts for both commercial and domestic alcoholic beverages. The breakdown of carbohydrates by yeast is essential in cider production as it results in the production of alcohol (Dashko et al., 2014; Liu et al., 2016). Researchers Bisson (2004) and Romano et al., (2003) noted that yeast converts carbohydrates into ethanol through alcoholic fermentation. According to Wei et al (2020), Saccharomyces cerevisiae is the most used yeast for AFBs (wine and ciders). This yeast is favored by industrial fermentation due to its resilience in harsh conditions such as high sugar content, low pH, and high ethanol levels (Al Daccache et al., 2020). For optimal cider production and favorable consumer reception, it is crucial for both cider producers and yeast manufacturers to acknowledge the impact of yeast strains on critical cider attributes (table 1). This holds even more significance given the vast selection of apple varieties and yeast strains accessible for cider fermentation (Laaksonen et al., 2017).

**Table 1.** Effect of *saccharomyces cerevisiae* and non-*saccharomyces cerevisiae* yeast and their impact in apple cider fermentation**.**

| Apple cultivar | Fermentation type | Yeast strain | General effect of yeast strain on chemical composition | Reference |
|---|---|---|---|---|
| Red fleshed apple variety | PF | *Saccharomyces bayanus and saccharomyces cerevisiae* | Malic acid content was unaffected by both yeast strains. | Lachowicz et al. (2019) |
| Finnish cultivars | PF | *saccharomyces cerevisiae* | Decrease in the content of malic acid was found to be 6-18%, limited capacity for utilizing sorbitol. higher content of ethyl acetate 3-methylbutan-1-ol. higher production of ethyl esters, primarily ethyl pentanoate and ethyl hexanoate, and volatile acids, (2- methylpropanoic), (3-methylbutanoic,pentanoic), and hexanoic acids. | He Wenjia (2022) |
| Finnish cultivars | PF | *Schizosaccharomyces pombe* | A high malic acid consumption was found in the apple beverages fermented (47-89%) and high glycerol level, less ethyl esters, higher alcohols, and volatile acids | He Wenjia (2022) |
| 'Antei', 'Kulikovskoye', 'Melba',and | PF | *Torulaspora delbrueckii with S. cerevisiae* | Low enzyme activities in *Torulaspora delbrueckii as result were similar as with S. cerevisiae alone.* | Laaksonen et al., (2017), Maslov Bandic et al., |

| | | | | |
|---|---|---|---|---|
| 'Orlovskisinap'grown in South Estonia | | | | (2019). |
| Apple cultivar | PF | *Torulaspora delbrueckii* | Increase in total sugar, decrease in higher alcohol, decrease in ethanol, increase acetaldehyde | (Fejzullahu et al, 2021) |
| Apple cultivar | PF | *Torulaspora delbrueckii* | Increase glucose, fructose, sorbitol, and benzyl alcohol, decrease ethanol. | (Lorenzini et al., 2019) |
| Apple cultiva | PF | *Torulaspora quercuum* | Increase ethanol, decrease in citric acid | (Wei et al., 2019) |
| Apple wine | PF& SimF with SE | *Schizosaccharomyces pombe* | Decrease organic acid, increase glycerol, esters, and acetic acid, ethanol, methanol, volatile esters, volatile acids. | (Satora et al., 2018) |

PF: pure fermentation, SimF: sequential

*S. cerevisiae* is known for producing a consistent aroma and flavor, as well as successfully completing the fermentation process despite challenging conditions (Swiegers and Pretorius 2005; Wei et al., 2020). Throughout the process of carbohydrate fermentation to produce ethanol and carbon dioxide, yeast creates various metabolites that can impact the final product's sensory qualities. Some examples of these metabolites include esters, higher alcohols, carbonyl compounds, volatile acids, volatile phenol, and Sulphur compounds (Swiegers and Pretorius, 2005). Studies have shown that *Torulaspora delbrueckii* a non- saccharomyces yeast has the potential to be a promising strain due to its ability to produce lower levels of acetaldehyde, acetoin, and acetic acid compared to other *non-Saccharomyces* strains. This could be beneficial in various applications such as winemaking, where high levels of these compounds can negatively affect the quality and taste of the final product (Benito 2018, Canonico et al., 2016). It's worth noting that many wild yeasts that were previously believed to be spoilage bacteria have recently been found to enhance certain quality factors (Jolly et al. 2014; Varela 2016).

*Torulaspora delbrueckii*, was identified as a spoilage yeast in soft drinks according to Kurtzman, 2011. However, the presence of *T. delbrueckii* can be easily controlled with the addition of preservatives. It's also important to note that *T. delbrueckii* has been known to spoil food items like salads, vegetables, meats, and dairy products (Fröhlich 2003). Researchers in microbiology are exploring the potential benefits of using non-Saccharomyces yeasts to improve the quality of wine and address food safety concerns (Jolly et al. 2014; Padilla et al. 2016; Varela 2016; Petruzzi et al. 2017). One such yeast, *Torulaspora delbrueckii*, has gained significant attention for its potential in commercial and industrial applications. By incorporating *non-Saccharomyces* yeasts during fermentation, quality indicators such as ethanol reduction, glycerol content, aromatic complexity, acidity, anthocyanin content, polysaccharides, and mannoproteins (Domizio et al. 2017) may be improved. Positive results have been reported in studies conducted by Contreras et al. (2014), Benito et al. (2017), and Belda et al. (2017).

Recent studies conducted on the *Torulaspora* genus (Azzolini et al., 2015) have revealed that T. delbrueckii can effectively tackle modern wine-making challenges and enhance the overall quality of wine in comparison to standard S. cerevisiae controls. This is especially significant as *T. delbrueckii* produces minimal levels of acetic acid, a primary quality indicator in wine production (Bely et al., 2008). Additionally, *T. delbrueckii* yields wines with elevated glycerol content and reduced ethanol percentages compared to

conventional fermentations (Contreras et al., 2014; Belda et al., 2015). This attribute is particularly advantageous in combating issues arising from climate change, such as high alcohol content in wines caused by high sugar concentrations in grape must. Winemakers often use *T. delbrueckii* yeast to improve the quality of their wine. This type of yeast can release mannoproteins and polysaccharides, which enhances the mouthfeel and overall taste of the beverage (Belda et al., 2016a). Furthermore*, T. delbrueckii* contributes to the fruity aroma of wines by producing more fruity esters, thiols, and terpenes while also reducing the amount of higher alcohols produced. This helps preserve the original grape character and results in a better-quality wine (Azzolini et al., 2015; Renault et al., 2016; Belda et al., 2017).

Further research in the field has discovered significant differences in important oenological characteristics when examining various *T. delbrueckii* strains at the clonal level. Escribano et al. (2018) observed considerable changes in volatile acidity, with a difference of approximately 0.11 g/L. Additionally, malic acid degradation varied by roughly 19%, and volatile higher alcohols like 1-butanol, or fatty acids like isovaleric acids, varied up to 30%, depending on the clone under investigation. Renault et al. (2009) also found noteworthy differences in ethanol production, ranging from 7 to 10% (v/v), and volatile acidity, which varied between 0.3g/L and around 1g/L in the production of glycerol. When it comes to maximizing fermentation output, it's crucial to select industrial strains with high fermentation power.

However, it's important to consider the parameter of fermentation power and other characteristics of this yeast **(table 2)**. While some cells can persist through alcoholic fermentation, research has shown that the number of viable *T. delbrueckii* cells drops significantly as alcohol levels exceed 8% during sequential fermentation processes (Belda et al. 2015, 2017). This indicates that *T. delbrueckii* has lower tolerance to ethanol compared to S. cerevisiae, making it more challenging to utilize in fermentation-based industries like winemaking. Nevertheless, *T. delbrueckii* is regarded as a rather potent fermenter among non-Saccharomyces yeasts due to only a small number of species, including *Lachancea thermotolerans* or *Schizosaccharomyces pombe*, can ferment sugar to comparable or even higher levels (Benito et al. 2015a). Most non-Saccharomyces yeasts have ethanol resistance that doesn't exceed 4% (v/v). However, because their populations are significantly bigger than those of Saccharomyces during these early stages, non-Saccharomyces yeast species play a very important role during the first stages

of spontaneous alcoholic fermentation when the ethanol concentration is not particularly high (Taillandier et al. 2014).

The prevalence of *Saccharomyces cerevisiae* in alcoholic fermentation processes is attributed to its exceptional fermentative characteristics, remarkable resilience to challenging environmental factors (including high levels of ethanol and organic acids, low pH values, limited access to oxygen, and nutrient depletion), and its ability to outperform other microbial species. Furthermore, *S. cerevisiae* leverages defensive mechanisms such as cell-to-cell communication and the release of antimicrobial peptides to combat other bacteria. The presence of ethanol and high temperature also serves as a natural inhibitor of other yeast species, further solidifying *S. cerevisiae's* dominant position. Although Saccharomyces cerevisiae has long been employed to mitigate harmful substances like biogenic amines or ethyl carbamate in food, non-Saccharomyces strains also possess the potential to contribute to this effort (Benito et al. 2015a). However, incorporating non-Saccharomyces yeasts into industrial processes presents challenges. This is due to their typically limited alcoholic fermentation capabilities, which can hinder proper fermentation in high-alcohol liquids like wine. In large-scale industrial applications, non-Saccharomyces yeasts typically necessitate the use of a more robust fermenter, such as S. cerevisiae.

The most efficient approach to this challenge is sequential fermentation, which allows for the full potential of non-Saccharomyces yeasts to be realized during the initial stages of alcoholic fermentation, without any potential inhibition from the Saccharomyces genus. *T. delbrueckii* is gaining popularity for alcoholic fermentation in industries beyond wine and beer production. This yeast is currently being utilized to enhance the quality of various fermented fruits. For example, in durian fruit fermentations by (Lu et al. 2016), *T. delbrueckii* adds complexity to the aroma of the final product. Additionally, when fermenting lychee fruit, *T. delbrueckii* produces fermented products with higher concentrations of beneficial compounds such as isoamyl alcohol, 2-phenylethyl, ethyl octanoate, ethyl decanoate, and linalool, and lower levels of acetic acid (Chen and Liu 2016). Furthermore, mango fermentations with *T. delbrueckii* result in increased glycerol and reduced volatile and total acidity, which positively impact the fruit's sensory characteristics according to Sadineni et al.,

**Table 2**. Some specific parameters of selection for *T. delbrueckii* strains as proposed by Benito (2018) in review of the impact of *Torulaspora delbrueckii* yeast in winemaking.

| Criteria | Considered characteristics for optimized performance |
|---|---|
| Fermentation | Fermentation power 9.5%(v/v) |
| Acid production and ethanol | Acetic acid ↓ 0.2 g/L<br>Pyruvic acid ↑, glycerol ↑<br>Ethanol ↓<br>Polysaccharides/mannoproteins ↑, succinic acid ↓, ethyl phenol ↓, histidine ↓, malic acid ↑ |
| Volatiles (white wine making) | Aroma complexity ↑, fruity esters↑, terpenes ↑, tiols↑ |
| (red wine making) | Anthocyanin absorption ↓, pyruvic acid ↑, hydrodynamic activities ↑ |

↓: increase ↑: decrease

1.4 Apple fruits microbial flora or microbiome.

According to a growing body of research (Berg et al., 2016), the plant microbiome has a direct or indirect influence on the host's physiology, biochemistry, growth, resistance to disease, stress tolerance, and quality, both before and after harvest. Microorganisms residing in plant roots have been known for a century (Hartmann et al., 2009) crucially for their development and well-being. Recent research has uncovered a mutually beneficial relationship between plants and their associated microorganisms. These plant microbiomes are highly diverse in both form and function, with microbial communities occupying three primary habitats: rhizospheres, phyllospheres, and endospheres. Microbes that coexist with plants engage in vital functional interactions with their hosts, such as stimulating germination and growth, bolstering disease resistance, enhancing plant stress resilience, and affecting overall plant health. Only a select few soil microbes are capable of efficiently colonizing roots due to the strong influence of root exudates on specific microbial populations.

These populations can swiftly respond and migrate towards root exudates through chemotaxis. The carbon molecules present in the exudates offer a readily available source of sustenance and energy for heterotrophic organisms. Furthermore, the exudates contain intricate agents such as carboxylates, phenols, or siderophores that assist in capturing and mobilizing insoluble minerals. Various sections of plants harbor distinct microbial communities, including the phyllosphere (Vorholt, 2012), rhizosphere (Berendsen et al., 2012; Philippot et al., 2013), and endosphere (Hardoim et al., 2015). Among these, the rhizosphere has garnered the most attention from researchers (Berendsen et al., 2012; Hirsch and Mauchline, 2012; Badri et al., 2013; Mendes et al., 2013), thanks to its promising potential for improving plant nutrition and health. With the aid of cutting-edge technologies, a better understanding of the intricate relationships between plants and microbes is now provided, shedding light on how the rhizosphere fosters the growth of microbial species and genotypes, as compared to the surrounding soil and internal tissues as explained by (Bais et al., 2006; Doornbos et al., 2012).

Environmental variables can impact the biosynthesis of leaf metabolites. Additionally, specific plant-microbe interactions can affect the metabolome of the plant by facilitating the production of phytoalexins and other chemical compounds that play a role in plant defense (Badri et al., 2013). It is widely acknowledged that external stressors caused by climate and weather can significantly impact a plant's metabolism. An example of this is *Pisum sativum* leaves producing specific metabolites during drought stress (Charlton et al., 2008). Additionally, the metabolome and proteome of maize plant xylem sap can also be altered in response to drought stress (Alvarez et al., 2008). The metabolome of Arabidopsis also changes in response to heat or cold shock, as demonstrated by the coordinated increase in amino acid pools formed from substances like pyruvate and oxaloacetate (Kaplan et al., 2004).

The composition of plant metabolites can be influenced by soil properties, like nutrient availability. For example, sulfur concentrations can affect the accumulation of glucosinolates (Falk et al., 2007), while salinity stress can increase the quantities of molecules containing nitrile and cyanide (Johnson et al., 2003). However, nitrogen feeding has more profound implications on the metabolome, affecting secondary metabolites, amino acids, and carbohydrates. Other environmental elements, such as soil pH, texture, and humidity, may also cause changes in the plant metabolome. Research on the microbiome of apple trees has shown that various factors such as the tree's genotype,

management techniques, rootstock, and soil characteristics can all impact the composition of the microbiome (Abdelfattah et al., 2016; Liu et al., 2018a; Wassermann et al., 2019a; Abdelfattah et al., 2020; Cui et al., 2020). Several studies have been conducted on the apple microbiome, including a recent comprehensive assessment by Whitehead and colleagues in 2021. However, there have been fewer investigations on the microbiome of pre- and postharvest fruits. Nonetheless, researchers have made strides in managing postharvest infections in apples without relying on synthetic drugs by using different types of microbial antagonists. Furthermore, recent research has revealed that the bacterial and fungal communities found in various parts of an apple - such as the stem-end, calyx-end, mesocarp, and peel - exhibit differences in both their abundance and uniqueness.

There have been multiple studies conducted on this topic, including research by Whitehead et al. (2021), Abdelfattah et al. (2016), Wassermann et al. (2019a), and Abdelfattah et al. (2020), which have elicited similar findings albeit with some variations. However, it is yet to be determined if these trends remain consistent across different regions with diverse farming methods, climates, and apple varieties. Based on research findings according to Abdelfattah et al., 2021, the bacterial and fungal species and their arrangements on apples vary depending on their geographical location. Furthermore, the study highlights that the distribution of these microorganisms varies within the fruit across the world. The diverse microbial makeup of fruits from various regions indicates that geography could potentially contribute to the emergence and prevalence of post-harvest illnesses in different nations. The apple fruit harbors a diverse array of beneficial microbial species, collectively known as the global core microbiome. This research sheds light on the apple fruit microbiome, offering valuable insights that could be leveraged to develop novel methods for preserving the fruit's quality and safety. By mitigating postharvest diseases and laying the foundation for deeper explorations of the intricate microbial dynamics present on the fruit's surface, this study has significant implications for the fruit industry.

### 1.4.1 Effect of low storage temperature on apple microbiome

The duration for which harvested produce can maintain high-quality indicators such as appearance, flavor, texture, safety, and nutritional content before being marketed is known as its storage and shelf-life. To extend the storage and shelf-life of apples, common

techniques such as precooling, washing, and sanitizing, and waxing are used. These simple procedures allow apples to be stored for 6-12 months at low temperatures (1-2°C) before being sent to distributors and eventually retailers. However, fruit washing can affect the natural protective wax layer that covers the surface of a fruit, even though it is meant to remove foreign objects and reduce microbial burden. To preserve the quality of fruits by preventing damage, minimizing water loss, and improving their visual appeal, it's common practice to apply a thin layer of edible wax. This type of wax is made up of higher fatty acid esters combined with monohydric alcohols, hydrocarbons, and free fatty acids, which give the fruit surface a lustrous and polished finish.

The common apple, scientifically referred to as *Malus domestica Borkh*, is a crucial fruit crop that thrives in temperate regions. Farmers rely on both traditional and sustainable methods to cultivate this crop. However, apples, like other fruits, are susceptible to various diseases caused by phytopathogens and microbial colonization. Ongoing research on the apple microbiota concentrates on the microbes that cause economic risks and disease spread. On occasion, biological control agents such as natural enemies can be utilized to combat these pathogens (Graça et al., 2015). Teixidó et ai., In 1999, investigated the microbiota present in "Golden Delicious" apples within an orchard. Their findings indicated that yeasts, fungi, and Cladosporium were the most prevalent microorganisms. Another study on the same type of apples during their production and shelf-life to determine if bacteria from the Enterobacteriaceae family had contaminated them (Abadias et. al., 2006). However, they did not detect any Salmonella or pathogenic Escherichia coli. They did, however, identify small amounts of other enterobacteria, such as *Pantoea, Citrobacter, Enterobacter, Klebsiella, and Escherichia*.

Research has shown that certain harmful microorganisms, such as E. coli*, Listeria monocytogenes*, and *Salmonella spp*., can grow and thrive in fresh fruits like apples and peaches when stored above 10°C or without refrigeration. Even refrigeration at 5°C did not prevent *E. coli* from being found in apple wounds, flesh, peel, and juice (e.g., Abadias et al., 2009, Alegre et al., 2010a, Alegre et al., 2010b). This therefore indicates that fresh-cut fruits can potentially spread food-borne illnesses caused by pathogenic bacteria like *L. monocytogenes, Salmonella spp., and E. coli* (Sivapalasingam et al., 2004). There have been documented cases of foodborne illnesses resulting from E. coli O157:H7, with unpasteurized apple cider (Beuchat, 2002) and strawberries (FDA, 2011) being identified as the sources. Similarly, L. monocytogenes outbreaks have been traced back to

cantaloupe, while fruit salad, cantaloupe, papaya, and watermelon have been linked to cases of salmonellosis (CDC, 2011). It's worth noting that fresh-cut fruits can also be susceptible to fungi, particularly yeasts, which can lead to spoilage. Such spoilage can cause changes in color, gas production, off flavors, and even souring (Loureiro and Querol, 1999; Tournas et al., 2006).

According to a 1999 study by Teixidó et al., intact fruits may harbor yeast communities on their surface. However, when fresh-cut fruits are processed, these populations can pose a contamination risk. In this research Teixidó et al also investigated the effectiveness of using *Candida sake* yeast cells for biocontrol of Golden Delicious apples. The yeast was applied both before and after harvest, and the apples were inoculated with *Penicillium expansum* before being stored in cold conditions for two consecutive seasons. Results showed that postharvest treatment with the yeast significantly suppressed Penicillium rot, regardless of pre- or postharvest wounds. Maximum disease control was achieved with over 80% reduction in lesion diameter and 50% decrease in lesion incidence. In 2010, Ruiz-Cruz et al. and in 2006, Tournas et al. identified various yeast taxa in fresh fruits, including *Candida, Cryptococcus, Debaryomyces, Kloeckera, Kluyveromyces, Pichia, Rhodotorula, Saccharomyces, and Zygosaccharomyces.*

Apples are a beloved fruit across the globe and are especially prevalent in areas with temperate climates. Over the past three decades, apple production has soared, doubling from 41 million tons in 1990 to a staggering 86 million tons in 2018. As of 2020, the total trade value of apples, according to FAOSTAT, sits at an impressive $7.53 billion USD. With proper environmental control, cold storage allows apples to be preserved for several months or even up to a year. To prevent postharvest spoilages and reduce waste throughout the supply chain, a meticulous examination of the microbial communities presents on and within fruits throughout the storage and marketing stages is necessary. By studying these changes, the development of effective strategies to uphold the quality and safety of fruits, ultimately protecting public health and limiting economic losses. The preservation of fresh fruits' quality and safety presents a considerable obstacle due to the growth and development of postharvest pathogens during storage hence, the microbiota of harvested fruits and vegetables plays a critical role in ensuring food safety and preventing the occurrence of foodborne pathogens.

### 1.4.2 Effect of washing on apple microbiome

Maintaining the quality of harvested produce is crucial for its appearance, flavor, texture, safety, nutritional content, and overall consumer acceptance both during storage and marketing. The duration for which the produce can be stored while maintaining these standards is referred to as its storage and shelf-life. To increase the storage and shelf-life of apples, industry-standard practices such as washing, precooling, sanitizing, and waxing are commonly used. These procedures are exceptionally effective in preserving the produce's quality and must be employed to ensure that it remains of the highest quality for as long as possible. Apples can be stored for up to 12 months in cold temperatures (1-2°C) before they reach the distributors and retailers when these treatments are used, although, washing the fruit can remove its natural protective wax covering, leaving it exposed to damage. To protect the fruit from damage, reduce water loss and shrinkage after washing, and add shine and gloss to its surface, a thin layer of edible wax is commonly applied. This wax is typically made of esters of a higher fatty acid with monohydric alcohols, hydrocarbons, and some free fatty acids. For extended storage of apples, it is customary to regulate the amount of oxygen and carbon dioxide present in the storage surroundings.

In general, apples undergo a basic treatment which includes washing, sanitizing, and waxing before being stored. Nonetheless, the treatments may differ based on the preservation approach and fruit type. According to research conducted by Abdelfattah et al. (2020), the act of washing apples has a noteworthy effect on the microbiota of the fruit. The study involved analyzing the microbial communities present in different apple tissues, such as the peel, calyx-end, and stem-end, that had either been unwashed (UW), washed (W), or washed-waxed (WW) using amplicon sequencing. The findings indicate that washing apples has a significant impact on the fruit's microbiota. The study's results showed washing had a notable effect on decreasing microbial diversity on the peel and stem ends of the plant tissues, but not on the calyx ends. Researchers noted that washing caused a significant decrease in microbial loads on the plant surfaces, leading to the reduction in microbial diversity and the rough surface of the stem-end and calyx-end tissues made them more vulnerable to the effects of washing compared to the peel, which has a smooth and waxy surface.

There are varying effects that washing apples can have on different parts of the fruit's microbiome that are important to consider. The calyx-end tissues, which contain remnants of floral tissues, are less impacted by washing due to their protected nature, making it challenging for disinfectants to penetrate. However, washing does significantly alter the microbial community structure and diversity of apple fruit, particularly on the peel's tissues. This can lead to reduced microbial diversity and potentially change the composition of the microbiome. These findings underscore the importance of gaining a better understanding of how postharvest processing techniques affect fruit microbiomes, and the potential implications for food safety and disease control.

### 1.4.3 The effect of growing region on the microbial diversity of apple fruit

It is essential to comprehend the variances in bacterial and fungal communities present on apple fruit, depending on their geographical location. This knowledge can uncover potential correlations between geography and the types and rates of postharvest infections that occur in different countries, ultimately impacting the fruit's quality and safety. Additionally, exploring the regional variations in the apple fruit microbiome can facilitate the development of innovative approaches for monitoring fruit quality and safety. Due to the significant role apples play in global trade, it is essential to maintain a consistent microbiome across different apple growing regions. The microbiome of an apple refers to the diverse community of microorganisms, including bacteria and fungi, that inhabit the apple's surface and internal tissue. A healthy and consistent microbiome in apples is vital because it can impact the apple's taste, texture, and overall quality. Therefore, farmers and producers strive to maintain a uniform microbiome by implementing various practices that ensure the fruit's safety and quality, such as using organic farming methods, reducing the use of harmful chemicals, and carefully monitoring the apple's storage and transportation conditions.

### 1.4.4 Effect of other contaminants on cider quality

Residues of fruit containing fungicides can negatively impact yeast, leading to stalled or halted fermentation. Inadvertently, the residual fungicide can contribute to the formation of hydrogen sulfide, which produces an unpleasant odor. The use of Sulphur-based fungicides can increase the levels of hydrogen sulfide in fermented cider. Other fungicides, such as fludioxonil and fenbuconazole, may also have some impact on fermentation (Boudreau et al., 2016). However, adding methionine supplementation to

cider samples (5 mg/L) was observed to correlate with lower hydrogen sulfide production [100]. Unpasteurized apple juice and cider carry a risk of microbiological contamination. The primary source of contamination with E. coli O157: H7 is animal feces. This contamination occurs when apples are exposed to feces during the growing and harvesting processes (Liu et al., 2007). Cryptosporidium parvum, a pathogen commonly found in the food industry, has been detected in various samples of unpasteurized cider. The presence of microbiological contaminants can be attributed to improper storage conditions and apple contamination with fungi (Simonato et al., 2021). Non-compliant cleaning and sanitation practices can also contribute to contamination. However, pasteurization is the primary method of eliminating these contaminants. Studies have shown that utilizing technology in the cider- making process can effectively decrease the presence of pesticide residue. Notably, pyridaben, an acaricide and pesticide commonly used in apple farming, was detected in finished cider at levels below 0.01 mg/kg, despite its substantial concentration of 2.10 mg/kg in the apples themselves. (Han et al., 2014).

### 1.5 Different Applied Technologies in Apple Juice production

Traditionally, apple juice is obtained by pressing or decanting crushed apples. This method can typically produce between 70-80% juice yield under optimal conditions, but the yield can drop to less than 65% when using dehydrated apples (Cliff et al., 1991). Some manufacturers opt to use higher temperatures during the crushing process to increase juice production and speed up the process. However, this approach can lead to increased energy consumption, changes in color and flavor, and a decrease in vitamin content (Grimi et al., 2011). Alternative techniques, including pulsed electric fields, microwaves, ultrasounds, enzyme treatments, and UV treatments, have been suggested to improve juice yield.

#### 1.5.1 Enzymatic treatment

According to research, enzymatic treatment can improve the amount of juice extracted compared to using hot or cold extraction methods (Sharma et al., 2017). Pectins can be categorized as either soluble or insoluble fibers, based on their chemical makeup. Enzymatically breaking down the pectin in raw juice can decrease its viscosity, resulting in a higher yield. Pectic substances can be further classified into different types, such as arabinans (arabinose polymers), galactans (galactose polymers), rhamnogalacturonan (mixed polymers of rhamnose and galacturonic acid), and arabinogalactans (polymers).

Pectolytic enzymes can break down the pectic components of fruits, leading to a juice with considerably less pectin content (Lee et al., 2006, Satora et al., 2009).

Enzymatic treatments using pectinases have become increasingly popular in the cider industry as they provide a way to clarify juice without affecting the polyphenol concentration (Ma et al., 2018). By optimizing the temperature, duration, and enzyme product concentration, fruit juice extraction can be improved, leading to higher yield, quality, and phenolic content of the final product (Satora et al., 2009). The addition of enzymes to apple juice involves two steps. First, pectinases are applied to crushed apples to facilitate juice extraction. Next, a combination of pectinases and cellulases is used to liquefy the apple pomace and extract all the juice (Will et al., 2000). These processes can be further refined to increase efficiency and quality, ultimately leading to better outcomes for the industry and consumers alike.

### 1.5.2 High pressure processing

Apple juice can be treated effectively to eliminate foodborne germs using a process called high-pressure processing (HPP). It's a great way to maintain the high nutritional content of apple juice during storage. However, the maintenance expenses of HPP equipment can be high for pressures beyond 600 MPa (Petrus et al., 2020). To extend the shelf life of apple juice, reducing the applied pressure and combining HPP with other cost-effective microbial inhibitors can be useful strategies. A combination of dimethyl dicarbonate (100–250 mg/L) and HPP (400–600 MPa) can be applied to inactivate food-borne microorganisms effectively in apple juice. These strategies can help you keep your apple juice fresh and nutritious for longer. The researchers found that a combination of 600 Mpa at 25 °C for 5 minutes proved to be highly effective in almost completely deactivating polyphenol oxidase and peroxidase (Marszalek et al., 2019). They also observed that the stability of different phenolic compounds is significantly influenced by the duration of storage. Catechin was identified as the most stable phenolic compound, retaining its properties for 55 weeks. Furthermore, they found that a lower HPP treatment of 300 MPa could be used to stabilize hazy apple juice by inhibiting enzyme activity (Baron et al.,2006)

HPP technology can be useful in the selective extraction of specific classes of phenolic chemicals. Researchers found that the best combination for increasing the total flavonols (75%), total hydroxycinnamic acids (29%), total flavan-3-ols (58%), total

dihydrochalcones (63%), and total phenolic compounds (54%) was HPP treatment at 600 MPa/35 °C/5 min (Fernández-Jalao 2019). This finding may have significant implications for the food industry as it could represent a more efficient and effective way to extract beneficial compounds from natural sources.

### 1.5.3 High- heat juice extraction

Interestingly traditional methods of extracting apple juice can result in a significant reduction of up to 90% in antioxidant activity and over 58% in phenolic components (Chandrawinata et al., 2013). However, there are some simple ways to improve the quality of your apple juice. One method that has been found to increase the efficiency of juice extraction and improve the extraction of nitrogen which aids yeast fermentation and phenolic components is microwave treatment. Research has shown that heating the apple puree to 60°C yields the best results, with maximum yields in the extraction of juice and phenolic compounds. An important advantage of this method is there are no noticeable differences in the sensory properties of the studied methods, meaning that applying a microwave treatment to apple pulp and peel can lead to a higher-quality juice with a higher content of these valuable compounds (Ma et al., and Massaini et al., 2018). Applying this method to raw apple fruits increases the turbidity, temperature of the puree and soluble solids of the juice. A study has shown that using a microwave treatment can increase the efficiency of juice extraction while enhancing the extraction of flavonoid components at temperatures of 40°C and 60°C (Gerard and Roberts 2008).

### 1.5.4 Radioactivity treatment

Radioactive or ultraviolet technology is a highly effective method for pasteurizing and prolonging the lifetime of beverages. Numerous studies have proven that UV treatment can kill germs without compromising the quality of juice, unlike thermal pasteurization techniques (Islam et al., 2016; Diao et al., 2018 and Yang et al., 2019). However, it is worth noting that the use of UV-C may impact the nutritional and physicochemical properties of juices. The bactericidal action of UV-C radiation takes place when bacteria DNA or RNA absorbs it, resulting in the creation of pyrimidine dimers that render the bacteria unable to replicate. UV-C light falls within the 200 to 280 nm range of the electromagnetic spectrum (Urban et al., 2015). Recent research (Islam et al., 2016) has shown that a UV-C treatment of 40 mJ√cm−2 can safely preserve the polyphenols and antioxidants activity of apple juice. The level of vitamin C in apple juice decreases after

UVC exposure, which is mainly due to the low pigmentation of the apples used in the juice. However, UV treatment can effectively reduce microorganisms without altering the essential characteristics of the juice or cider. When apples are crushed, polyphenol oxidase starts to oxidize the phenolic chemicals, which can be influenced by the variety of apple used. Apple products made from apples with lower levels of polyphenol oxidase activity usually have higher levels of chlorogenic acid. Ultraviolet treatment is an effective way to reduce polyphenol oxidase activity in apple juice, with an irradiance of 13.8 Wm−2 for 33 minutes with UV-C light resulting in a ten-fold decrease in activity.

### 1.6 Sugars in fresh apple juice and yeast metabolism in AFBs

Apples contain a variety of soluble sugars, including fructose, sucrose, and glucose, however, sorbitol is only found in trace amounts in apple products (about 3%). These sugars subsequently undergo glycolytic breakdown to generate pyruvate (Fig.2). Pyruvate is then fermented by yeast into acetaldehyde and eventually ethanol. In 1890, the practice of inoculating pure yeast beverages during fermentation was introduced. Today, yeast companies offer a wide range of dehydrated cultures from various S. cerevisiae strains. The most used yeasts available in the market are derived from lyophilization, a process that involves freeze-drying. These yeasts are carefully chosen from successful natural fermentations, allowing for effective large-scale fermentation and the ability to select specific yeasts for each fermentative substrate or desired final product quality (Morgan et al., 2006).

As soon as the temperature surpasses 10°C, a natural process of fermentation initiates. Interestingly, even if the yeast is not deliberately added, *Rouxii Zygosaccharomyces* can still be found in non-pasteurized apple juice (Wang et. al., 2016). To ensure consistent sensory qualities in large-scale cider production, it's important that the physical and chemical properties of apple juice remain consistent across batches. To achieve this, certain substances can be added to the apple juice before fermentation. For instance, fermentable sugars like glucose syrup can be added up to a certain level to achieve the desired alcoholic concentration (which can be as high as 15% in some cases, if the cider is diluted before packaging) (Lea and Piggot, 2003). During fermentation, yeast strains convert glucose to pyruvate acid, which is essential for producing acetaldehyde (fig 2). The production of alcohol and fermentation rate holds commercial significance. As a result of glycolysis, one molecule of glucose or fructose yields two ethanol and two

carbon dioxide molecules. In theory, 180 g of sugar should yield 92 g of ethanol (51.1%) and 88 g of carbon dioxide (48.9%). Normal fermentation leads to the conversion of approximately 95% of carbohydrates into carbon dioxide and ethanol, 1% into cellular material, and 4% into diverse chemical compounds such as glycerol (Pretorius 2000). In addition to producing ethanol and carbon dioxide, yeasts metabolize carbohydrates and generate hundreds of chemicals throughout the fermentation process that contribute to the final product's flavor.

Yeast can transform carbohydrates into carbon dioxide, ethanol, heat, and energy in an environment without oxygen (anaerobic), however, it retrieves less energy from the substrate's molecules during this process. The percentage of ethanol in apple juice ultimately depends on the starting sugar content and fermentation temperature. At higher temperatures, rapid fermentation can cause some ethanol molecules to be lost (Lea & Piggott 2003). In the first stage of alcoholic fermentation, carbohydrates are transported into the cell using active transport, carrier-facilitated or mediated simple broadcast methods. Glucose and fructose are transported via facilitated diffusion, which is an energy-dependent process. Since yeast cannot directly metabolize sucrose, it secretes an enzyme called invertase to break it down. The breakdown of sucrose involves the hydrolysis of its molecules into glucose and fructose, which are then transported into the cell (Jackson, 2000). Glycolysis, the primary metabolic pathway for glucose and fructose, is involved in both respiratory and fermentative processes and consists of ten distinct enzymatic steps. Throughout this process, the carbohydrate's carbon skeleton is gradually broken down (Jackson, 2000). Figure 2 shows the metabolism of sugars in fruits to release energy in ATP and ethanol.

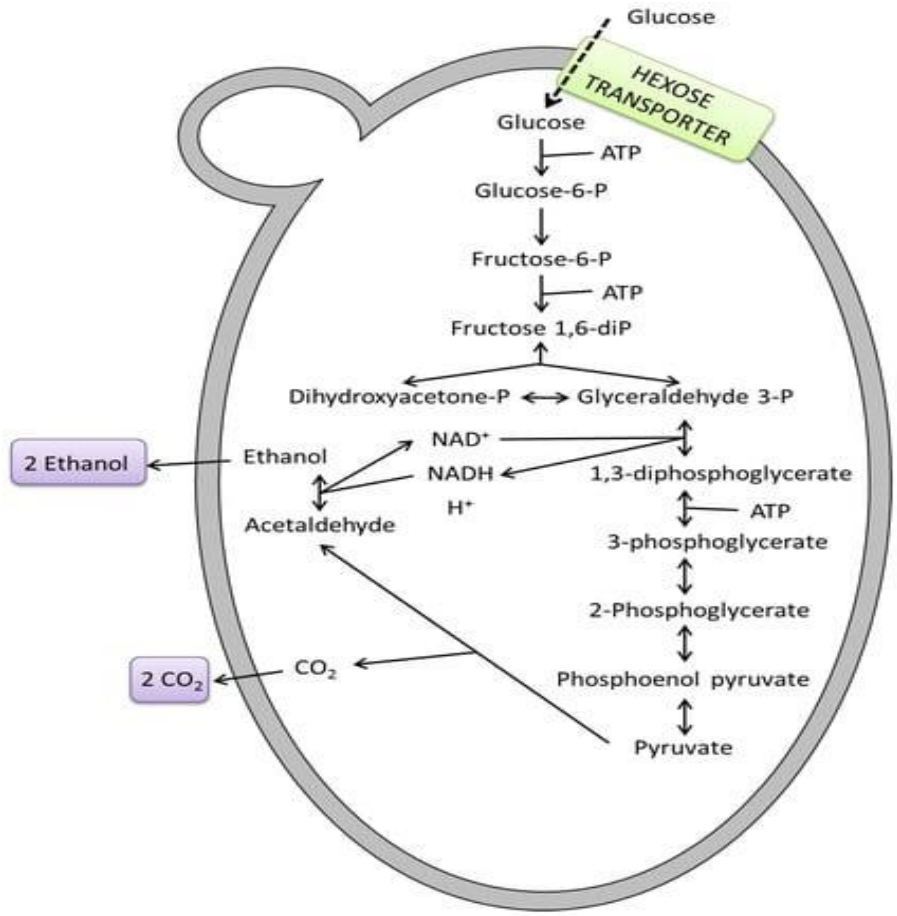



**Figure 2.** Glycolysis and Alcohol fermentation pathway by saccharomyces yeast. Source: the role of yeasts in fermentation processes by Maicas, S. (2020)

In the production of cider, flocculation of yeast is a critical factor. During this process, yeast cells clump together and rapidly settle in the medium or rise to the surface, carried by carbon dioxide. The clarity of the fermentation medium and the recovery of yeast depend heavily on this process (Boulton & Quain, 2006). Energy is received by cells through fermentation, which breaks down nutrients into ATP (adenosine triphosphate). When phosphate groups are removed from ATP to produce ADP (adenosine diphosphate), 7.3 kcal of energy is released per mole of a compound. This energy is then utilized for various cellular functions, including chemical transport, movement, or synthesis. Any excess energy is released as heat (Purves et al., 2001). Fruit wines are categorized based on the amount of unfermented sugars in the AFBs: dry (less than 4 g/L), medium-dry (between 4 and 12 g/L), semi-sweet (between 12 and 45 g/L), and sweet (above 45 g/L). Preferences for sweet or dry wines can differ among age and gender groups and may also vary by region, time, and individual consumers (Dodd, Kolyesnikova, & Wilcox, 2010).

### 1.6.1 Analytical methods for determining sugars and sweetness in fruits.

Various instrumental analytical techniques can be utilized to determine the chemical makeup of fruit and vegetable products and describe their flavor profile. Analytical techniques like gas chromatography (GC), high-pressure liquid chromatography, colorimetric methods, and hydrometers and refractometers (Kader et al., 2003; Saftner et al., 2008; Shanmugavelan et al., 2013) can help us determine a sample's chemical composition and potentially characterize the flavor of different horticultural products. The role of sugar and sweetness is significant in many food items, and several techniques can be used to express the sugar content. Analytical methods for determining sugar content can be classified as either destructive or non-destructive, depending on whether sample preparation is necessary before analysis. In the field of sensory evaluation, various scoring schemes are employed to assess samples.

### 1.6.2. Destructive method

#### 1.6.2.1 Sensory evaluation

Conventionally, the flavour of fruits and vegetables is evaluated through sensory assessment. This procedure involves the use trained and consumer panels to gather accurate and realistic data on human taste and scent perception (Beullens et al., 2006, 2008; Rudnitskaya et al., 2006). However, the sensory evaluation process faces challenges such as training precision, measurement standardization, stability, and repeatability. Furthermore, it is a costly process and panellists may experience taste fatigue. Shewfelt (2009) recommends utilizing at least 24 untrained panellists for reliable test results, but typically, 50-100 panellists are necessary to provide adequate information. Additionally, it is crucial for panellists to represent the demographics of potential product consumers in terms of gender, ethnicity, age group, etc. The hedonic scale is one of the most popular, ranging from 1 (very unpleasant) to 9 (highly enjoyable) (Shewfelt, 2009; Obenland et al., 2009, 2010). Another commonly used scale is the "willingness-to-purchase" scale, which ranges from 1 to 5 and reflects the likelihood of purchase, with 1 meaning "definitely would not buy" and 5 meaning "definitely would purchase". The acceptability scale developed by Dubost et al. (2003) is also utilized, with a range of 1 to 3, where 1 denotes excellent taste, 2 is deemed acceptable, and 3 represents poor taste. The importance of objective analytical procedures in food quality control cannot be overstated. While sensory evaluations are useful in accurately assessing human taste and

scent perception, they are subjective and do not offer quantitative data or reveal the broader relevance of the results. That is why the development of objective analytical procedures that provide this type of information is essential.

#### 1.6.2.2. Hydrometer

According to Nor et al. in 2014, the TSS (Total soluble solids) or Brix levels of fruits and vegetables are typically assessed using hydrometer and gravimetric methods. The density of liquids can be determined by calibrating a hydrometer with a standard liquid. A Brix hydrometer can then be utilized to directly measure sugar content in degrees Brix at a temperature of 20°C, also known as room temperature. While the hydrometer is a popular tool for gauging Brix levels in juices derived from fruits and vegetables, it is susceptible to inaccuracies arising from human error during operation and reading. These inaccuracies, including imprecise meniscus readings, can compromise the reliability of the measurement approach. Furthermore, surface impurities can lead to significant fluctuations in surface tension, exacerbating the potential for imprecision. To address such issues, optical methods have been devised to enable more accurate Brix analysis. Among these alternatives, the refractometer is the most employed and commercially feasible choice.

#### 1.6.2.3. Refractometer

When it comes to measuring SSC or TSS in fruits and vegetables, the refractometer is the most frequently used instrument. By optically gauging the refractive index of juice, this trusty tool can accurately determine the proportion of total soluble solids (TSS) in a pure aqueous sucrose solution, measured in Brix (Pereira et al., 2013). While there are a variety of refractometers on the market, those based on light's critical reflection or refraction (Meeten and North, 1995; Dongare et al., 2014) tend to be the most reliable. In particular, the critical angle-based refractive index refractometer stands out for its precision and versatility. Unlike other refractometers, it can accurately measure Brix in turbid colloidal fluids, like fruit and vegetable juice, without being affected by sample color or suspended solids (Dongare et al., 2014). There are two types of refractometers available: analogue and digital. Digital refractometers offer the convenience of automated temperature correction within a specific temperature range (usually 10-30◦C), eliminating the need for Brix readings to be adjusted for temperature. It's worth noting that readings taken before temperature equilibration or from particles may result in some errors, causing fluctuations

in the refractive index. This sets them apart from hydrometers and analogue refractometers.

#### 1.6.2.4. High performance chromatography

High-performance liquid chromatography (HPLC) is the most efficient and advanced method for analyzing carbohydrates from naturally occurring chemicals as recommend by Ma et al. (2014). However, the conventional approach of HPLC paired with photodiode array detectors cannot determine the quantity and purity of sugars in a sample, as they don't absorb ultraviolet (UV) or visible wavelengths, as pointed out by Peters et al. (2001) and Cools and Terry (2012). Therefore, various techniques have been developed utilizing diverse chromatographic columns and detectors.

ELSD (evaporative light scattering detective) has become increasingly popular due to its ability to remain unaffected by various factors such as sample composition, mobile phase flow rate, and column compartment temperature. This enables the prevention of baseline drift caused by temperature and mobile phase effects (Ma et al., 2014). Unlike RID, ELSD nebulizes the sample and evaporates the mobile phase, rather than relying on the optical properties of the analytes. The principle behind ELSD lies in the remarkable light scattering capabilities of carbohydrate molecules, which is attributed to their size. ELSD has proven effective in detecting a wide range of carbohydrates, including sucrose, fructose, glucose, mannitol, sorbitol, ketose, xylose, and fructans, among others. This technique has been suggested as a viable option for screening carbohydrates in real plant materials (Downes and Terry, 2010). However, it's important to note that the accuracy and performance of ELSD can be affected by various factors, resulting in potential quantification errors. One such factor is the non-linear response curve of the ELSD calibration (Mathews et al., 2004).

The refractive index detector (RID) is a commonly utilized tool for detecting sugars in fruit and vegetable samples. While it may be less sensitive than ELSD (Cools and Terry, 2012), it functions as a differential refractometer by measuring changes in the deflection of a light beam resulting from differences in the eluent's index of refraction caused by the solute. RID operates on the principle that solvents and solutes typically have different refractive indices (Raessler, 2011). However, RID has some limitations. The signal it produces is highly influenced by the density and wavelength of the solute, and gradient elution is not feasible due to baseline drift caused by the eluent's composition. The pulsed

amperometric detector (PAD) is a reliable and widely used technique for identifying sugars and fructans in fruit and vegetable extracts. It is the most accurate and sensitive method for detecting carbohydrates due to its high resolution, selectivity, and sensitivity (LaCourse, 2002). One of the advantages of PAD over other techniques, such as ELSD, is its ability to attract ionized groups of sugars at an alkaline pH with a pellicular quaternary amine stationary phase. Additionally, PAD is not affected by salt concentration, unlike RID and ELSD, allowing for oxide-free detection of aldehyde and alcohol-containing sugar molecules using gold electrodes in alkaline media.

### 1.6.3 Non-destructive methods

The horticultural industry requires reliable and efficient sensing technologies for non-destructive measurement and sorting of food products to meet the high standards of both the industry and consumers. Consequently, there is a significant focus on postharvest technology research, particularly in developing cost-effective analytical methods for non-destructive sweetness analysis. According to Peng and Lu's research in 2005 and 2008, supplying excellent, uniform fruit to the market through non-destructive sweetness evaluation can guarantee consumer satisfaction and approval. Amongst the many non-destructive sensing techniques available, optical methods, particularly visible and near-infrared (vis/NIR) spectroscopy, show great potential for sorting and grading fresh food for internal quality, as per Magwaza et al.'s findings in 2012a and 2013a.

#### 1.6.3.1 Hyperspectral and Multispectral Imaging

To uphold the quality, safety, authenticity, and label compliance of food products, continuous monitoring during key processing stages is imperative. Achieving these goals requires precise and expeditious analytical techniques. While mass spectrometry (MS) and high-performance liquid chromatography (HPLC) have been conventional methods for food monitoring, their drawbacks, including high expenses, time-consuming processes, and sample destruction, have become apparent. The art of capturing images of an object at the same scale, but from various electromagnetic energy spectrum components in the same area is known as multispectral imaging (Magwaza et al., 2012a; Sugiyama and Tsuta, 2010). In contrast, hyperspectral imaging employs a combination of spectroscopy and traditional imaging to extract both the spectral and spatial information of an object. Each pixel in hyperspectral images contains a spectrum for that specific position, comprising hundreds of contiguous wavebands in both the visible range and NIR

regions of the spectrum, for every spatial position of the studied sample. (Mendoza et al., 2011; GómezSanchis et al., 2008; Magwaza et al., 2012a).

The techniques used to measure the sugar content in fruits and vegetables are constantly evolving, as are the tools available for data analysis. This continuous innovation presents new possibilities for estimating sweetness and taste in a more comprehensive, reliable, and sensory-oriented manner. Some of the state-of-the-art techniques that hold great potential for improvement include Nuclear Magnetic Resonance (NMR) spectroscopy, hyperspectral and multispectral imaging, mass spectrometry, capillary electrophoresis (CE) coupled with mass spectrometry (MS), and HPLC. In recent years, several research studies have been undertaken to evaluate the quality of fruits and vegetables. These studies encompass a variety of factors, including the acidity levels of strawberries, dry matter content, and soluble solid content (SSC) of apples. Advanced multispectral and hyperspectral imaging systems are commonly utilized to determine the TSS and SSC of various types of produce. One such example is the work of Lu (2004), who utilized artificial neural networks and hyperspectral imaging in the 500-1000nm wavelength range to estimate the SSC of apple fruits. Hyperspectral imaging has the potential to create multispectral inspection devices, and can be implemented via reflectance, transmission, or fluorescence modes, among others, similarly to other spectroscopic methods.

**Table 3.** Applications of hyperspectral imaging to quantify the TSS and SSC and other parameters of selected fruit vegetable modified from (Magwaza, L. S., and Opara, U. L. 2015).

| Fruits/ Commodity | Quality parameter | Wavelength range (nm) | Accuracy | Reference |
|---|---|---|---|---|
| Apple | SSC | 680–1060 | r = 0.77 | Lu (2004) |
| Apple | SSC | 450–1000 | r = 0.88 | Peng and Lu (2005, 2008) |
| Apple | SSC | 500–1000 | R = 0.68 to 0.88 | Mendoza et al. (2011, 2012) |
| Blueberry | SSC | 500–1000 | r = 0.68 to 0.79 | Leiva-Valenzuela et al. (2013) |
| Grape | SSC | 400–1000 | r2 = 0.93–0.94 | Baiano et al. (2012) |
| Strawberries | TSS | 400–1000 | r = 0.80 | ElMasry et al. (2007) |
| Apples | Bitter pit defect | 900–1700 | R > 0.58 | (Nicolai et al. 2006) |
| Tomatoes | Ripeness level | 396–736 | | Polder et al. (2002) |
| Strawberries | TSS | 400–1000 | R > 0.80 | El Masry et al. (2007) |

R or r, correlation coefficient between vis/NIRS predicted and measured parameter; r2, coefficient of determination.

## 1.7 Aims of the experimental study.

The overall aim of the research was to study the effect of saccharomyces and non-saccharomyces yeast fermentation on the chemical composition of Finnish apple cultivars in alcoholic beverage fermentation process. Ethanol content was analysed with the goal of quantifying the yield of each cultivar with respect to their chemical composition.

Specifically, the study aims at the following:

1) To investigate the effect of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* on the ethanol content of apple ciders made from native Finnish apples.
2) To evaluate the sugars and organic acid composition and concentration of apple juice and fermented apple juice, and to compare the difference among the different apple cultivars used.
3) To compare the chemical characteristics of fermented and unfermented apple juice and investigate the effect of sugars and acid content on ethanol quantity of alcoholic fermented beverages.

## 2. Materials and Methods

### 2.1 Apple cultivars for the study

Finland is the farthest north an apple can be grown, and it is here that it reaches its northern growth limit. The southwest of Finland, which has the nation's longest growing season and the mildest winter climate, it is the only region where apples are currently grown primarily for commercial purposes. The orchard of the Marie Priest's Garden which now has 14 aging apple trees, and one pears tree was established in the early 1900s by Rovast Ivar Markus Tallgrenin. Some of the trees are said to have been planted during the renovation of the garden from fruit trees that were frozen during the harsh winter of 1939–1940. Kalm used dowels and seedling to grow these apple trees. Kalmi was protected by the Turku Academy’s Garden master, Blomberg, who used grafting to create the seedlings.

The seedlings were raised on the experimental farm in Sipsalo and in the garden of the townhouse in Kalmi and were transferred to other gardens. Recently, apple tree varieties from this garden have been identified based on the fruit features for 2020–2021 and the apple trees' DNA fingerprints at the Natural Resources Centre apple trees. Table 6 in this study presents a concise overview of the apple varieties utilized. The apple materials for Studies I and II were Finnish local cultivars that were obtained through seed sowing activities in the 19th and 20th centuries. To guarantee optimal ripeness, the fruits were grown in the Marie Priest's Garden orchard and harvested in the autumn of 2022 by the Natural Resources Institute Finland (Luke) experimental orchard in Piikkiö, Southwest Finland (60°25′N, 22°31′E). They were then refrigerated for one month at +4 °C. The apple resources included six non-commercial cultivars, which comprised of two test cultivars from Russia. For fermentation into alcoholic beverages in Study IV, six native apple cultivars (two unknown test cultivars and four known breeding selections) were selected. Each sample material was meticulously harvested, stored, and chosen to ensure that it was free from any external or internal damage or contamination.

**Table 4.** Apple cultivars used in the present study as provided by Maaria parsonage cider Turku

| **Cultivar name** | **Synonym names in Finland** | **Seasonal category (in Finland)** | **Breed** | **Origin of cultivar** | **Cultivation history in Finland** | **Harvest Time** | **Abbreviations** |
|---|---|---|---|---|---|---|---|
| Antonovka | Antonovka | Winter | Unknown | 19th century from Ukraine | widely cultivated since mid-1800s | 2022.09.16 | An |
| original name (unknown) | Mustialan Iso Venäläinen (Musti- iso) | Autumn | Unknown | early 19th century from Russia | locally popular early 1900s, later rare | 2022.09.28 | Mu |
| Aport | Aleksanteri | Autumn | Unknown | 18th century from Turkey | rather popular during 1840-early 1900s, later rare | 2022.09.28 | Al |
| Rambo | | Winter | Unknown | 17th century North America, | Unknown | 2022.09.16 | Ra |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | pedigree of Swedish origin | | | |
| Unknown, Indet. 3[a] | Tunista 1 | Winter | Unknown | 100yrs Turku area (Kuusisto Manor) | unknown, rare | 2022.09.24 | Ts |
| Unknown, Indet. 9[b] | Tunista 2 | Autumn | Unknown | Unknown | Unknown | 2022.09.29 | Tu |

Cultivar description are available on (https://en.wikipedia.org/wiki/Pehr_Kalm) (1716-1779) and https://www.turunseurakunnat.fi/pehrkalm

The morphological description of these apples data is provided by LUKE.

[a &b] Apple cultivars are yet to be identified and named however, for the purpose of these study both cultivars are assigned Tunista 1&2

2.2 Sample processing methods

The flowchart of sample preparations used in this study are clearly shown in Figure 3. The apples at ripen stage were inspected to remove decay, then washed, and rough diced into smaller pieces to remove the core and seeds with a knife and plastic board. Extraction of the juice was carried out with Kenwood Chef XL juice extractor mixer attachment AT641 (Kenwood Limited, Havant, United Kingdom). The unfiltered juice was collected and stored in 500ml sterile Duran bottles at -22°C temperature in the refrigerator for further chemical analysis and inoculation with the yeast strains. The obtained apple juices were not thermally treated for pasteurization but stored in their fresh natural state without added preservatives, enzymes, and sugar to accelerate fermentation process.

**Table 5.** Apple cultivar quantity used in cider preparation laboratory scale measurement.

| Apple cultivar | Whole measurement (g) | Stalk and seeds(g) | Residue or chaff (g) | Juice quantity(g) | Juice (%) |
|---|---|---|---|---|---|
| Tunnista 2 | 5742.48[a] | 1013.08 | 2478.92 | 2610.2 | 45.5 |
| Antonovka | 5250.83 | 964.79 | 1703.6 | 2521.23 | 48.0 |
| Musti Iso Venalainen | 5188.48 | 836.45 | 1767.86 | 2113.28 | 40.7 |
| Rambo[b] | 3476.66[b] | 584.8 | 1025.07 | 1807.44 | 51.9[c] |
| Aleksanteri | 5121.07 | 1008.11 | 1548.93 | 2156.77 | 42.1 |
| Tunnista 1 | 5291.05 | 1093.27 | 1586.44 | 2407.63 | 45.5 |

[a] highest quantity of whole fruit used, [b] lowest cultivar quantity used, [c] highest percentage in quantity of juice in ratio to raw apple fruit quantity.

Samples were done in batches within the range of 500 grams to 1000 grams, mostly 800g. all cultivars used in the processing of apple cider in this current experiment were in good condition however, some were spotted and damaged internally due to harvest method, chilling, and packaging therefore an increase in the quantity of stalk produced during processing. Subsequently, Tu2 had the highest gram of whole apples used but expressed lower juice quantity compared to Ra which had the least quantity of apples but produced higher juice quantity. This is due to Rambo sizes and juicy nature also Rambo produced least quantity of chaff residue after expression. ‘Antonovka’, ‘Musti- iso’ and ‘Tunista1’ had many damaged spots from internal rot, thick stalk, and less juicy flesh, with ‘Antonovka’ having the highest waste and less juice produced when expressed.

Other measurements used in the processing of the apple cultivars.

Empty plastic jug = 257.70grams

Glass bottle (storage) = 554.91grams

Empty pot used to measure peels, stalk and seeds =345.89grams

Empty pot used for measuring chaff = 343.50grams

Basket used for measuring whole fruits = 223.23grams.

Intial measurements of whole apple fruits in grams before sorting and washing.

- Tunnista (2) (spots) = 8164.5
- Aleksanter (1) = 6642.8
- Rambo = 3560.3
- Mustialan iso Venäläinen = 6282.2
- Tunnista 1 = 5869.8
- Antonovka =10919.1

2.3 Yeast oenology

Lavin PERSY$^{TM}$. 0.9g of nutrient (YPD) was dissolved into 40ml (10 times its weight) MQ warm water at 40°C the resulting mixture was then agitated with a vortex for even mixing. To rehydrate the yeasts, 5ml and 4ml of dissolved nutrient were transferred into two separate 50ml falcon tubes subsequently. 0.5g of active dry *saccharomyces cerevisiae* and 0.4g of *Torulaspora delbrueckii* was added into the falcon tubes respectively and agitated for 1minute. Mixture was allowed to sit for 22 minutes before inoculation with the apple juices. The total rehydration time was about 45mins. 0.35ml of aliquot with *Torulaspora delbrueckii* and 0.45ml of aliquot with *saccharomyces cerevisiae* was then transferred into the fresh thawed apple juice samples at room temperature to ferment. Plate counting on YPD agar (1% yeast extract, 2% peptone, 2% dextrose, and 2% agar) was used to estimate the cell populations of each yeast. Before counting, the plates were incubated at 35 °C for 48–72 hours.

2.4 Fermentation procedure

Fermentations were carried out in sterile 500ml Duran bottles sealed with caps. The glass bottles were filled with 250ml unfiltered and unpasteurized aliquot (fresh apple juice plus yeast) at room temperature and kept in a dark cupboard at 22°C to ferment, fermentation was carried out in triplicate. The cell count for each inoculation was approximately $10^5$ CFU/ml (colony-forming units) and $4.7 \times 10^8$ CFU/mL of *T. delbrueckii*. During

fermentation, the caps of bottles were unscrewed every three days to release $CO_2$ derived from yeast activity. Fermentations was monitored by measuring both °Brix values using °Brix meter (Atago Co. Ltd., Tokyo, Japan) and the change in weight of the bottles every 3 days successively till the completion of fermentation when bottle weights and °Brix values remained constant for four consecutive days during monitoring time points and there was no indication of $CO_2$ gas produced. At the completion of fermentation, 5g of käymisenpysattaja (a mixture of 1:1 potassium sorbate and potassium sulphate) powder was dissolved in water at 60°C, the mixture was stirred until completely dissolved then, 1.5ml of the resulting mixture was added into the fermented samples to kill the yeast and inhibit further production of $CO_2$. The samples were then stored at 4°C fridge to ensure the yeast action has completely stopped before centrifuging. Centrifugation of apple ciders was performed using Beckman coulter avanti JXN-26 centrifuge at 8000g for 10mins before decanting the supernatant to remove yeast pellets and pulp. Supernatant collected was filtered through 450 x 450 Whatman 0858 fitter sheets and 0.22µm rcf filters to remove smaller particles form the juice. Apple cider obtained was stored at -22°C for further chemical analysis. Samples used for chemical analysis were performed in 100 mL Duran bottles with 85 mL unpasteurized fruit juices. **Figure 3** shows the fermentation procedure implemented in this study.

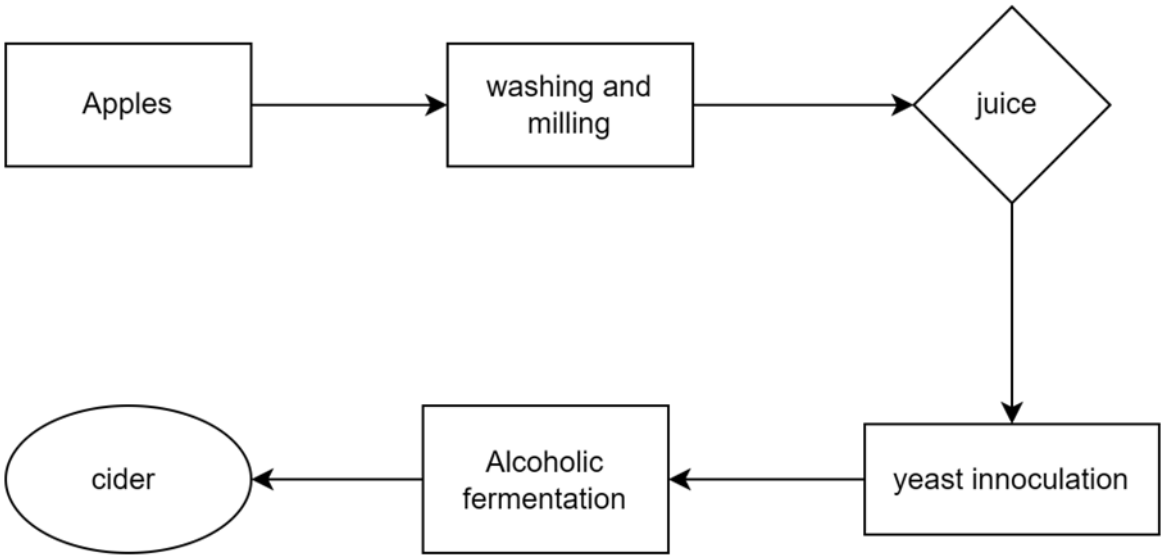



**Figure 3. Laboratory scale fermentation to produce apple ciders in present study.**

2.5 Determination of Ethanol and higher alcohol content of fermented apple juices Quantitative identification of ethanol content of apple ciders produced was carried out with the aid of Gas chromatography coupled with flame ionization detector (GC-FID) Shimadzu corp, Kyoto, Japan. The fermented apple cider was thawed at room temperature before transferring 1ml into autosampler bottles for analysis. A 5- point external standard curves was prepared for quantification of compounds identified in the samples and to prevent carbon hydrogen numbers of compound effecting the intensity of FID signals, a mixture of $H_2O$ and acetone. The dilution points used for standard curve in this study were 10,8,6,4,0 respectively. Analysis was carried out in HP-INNOWax (30 m x 0.25mm x 0.25µm, Hewlett-packard, USA) column with Helium as the carrier gas at a flow rate of 42mL/min at an injection temperature of 220°C and injection volume of 0.2µL. All fermented samples were analysed in both biological and analytical triplicates.

2.6 Determination of sugars and organic acids of apple cider and apple juice with GC-FID

The sugars and acids were quantitatively analysed as trimethyl silyl (TMS) derivatives. Due to polarity, hydrophilic and non- volatile hence derivation in pyridine is essential to volatilize analytes in the temperature of injector and define peak shape. 0.25ml of both fermented, unfermented juice, internal and external were diluted with 4.25mL of MQ water then 0.25mL of tartaric acid and xylitol was added to the mixture and agitated with vortex to ensure thorough mixing. 300µL of aliquot was filter using 0.22µm then transferred into autosampler bottles and evaporated to dryness under nitrogen flow at 50°C for 45 minutes till completely dried. The dried samples were kept overnight in a P2O5 desiccator. TMS derivatives was prepared by adding 500µL of Trisil (Pierce, Rockford, iL, USA) reagent and shaken with vortex (Vortex-Genie, Springfield, MA, USA) for 5 minutes then incubated at 60°C (heat block) for 30mins. After heating the samples were cooled at room temperature for 30mins. Sugars and acid standards were given same heat treatment as the samples. Concentration of internal and external acid and sugar standards used for quantification of compounds was 5g/L of both xylitol (sugars) and tartaric acid (acids). Quinic 5.388g/L, shikimic 2.756g/L, citric 7.268g/L, galacturonic acids 4.952g/L, D-(-)-Glucose 6.916g/L, D-(-)-Frucose 4.904g/L, Sucrose 7.348g/L, Succinic 2.392g/L, Malic acid 5.004g/L. The sugars and organic acids were analysed with gas chromatography (GC, GC-2010Plus, Shimadzu Corp., Kyoto, Japan)

equipped with a flame ionization detector (FID) were analysed to identify and calculate the correction cofactors.

## 2.7 Statistical Analysis

To determine statistical differences among various yeast fermentations, both an independent sample t-test and standard deviation was utilized. The average fermented beverage and the unfermented apple juice were compared using an independent sample t-test. Additionally, Unscrambler X (version 11, CAMO, Inc., Oslo, Norway) was used to execute principal component analysis. PCAs was created using the fermented juice sample, 55 chemical variables, and three biological duplicates of each of the six fermented beverage samples, which were divided into two groups based on the type of yeast and juice. PCA2 was created using three biological replicates of each of the six unfermented beverage samples and 18 chemical variables.

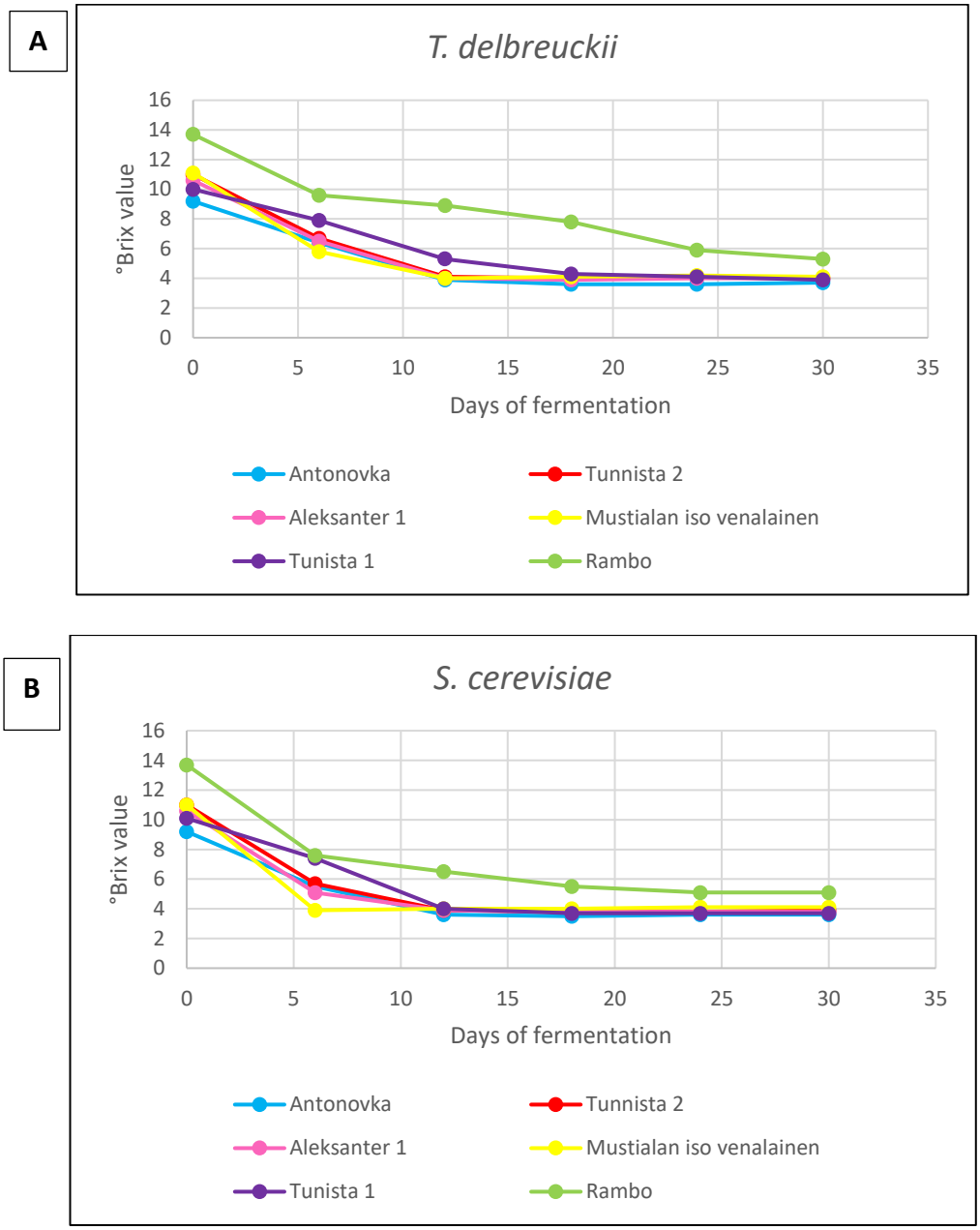
## 3. Results

### 3.1 Fermentation Kinetics of apple juice beverages

There was a noticeable discrepancy in the duration of the fermentation process among the individual yeast strains. The *S. cerevisiae* (Se) strains underwent sequential fermentations that lasted from 10-18 days, while the *T. delbrueckii* (Td) strains required 12-24 days to complete the fermentation process. Figure 4 illustrates the progression of fermentation in terms of variations in the °Brix levels.

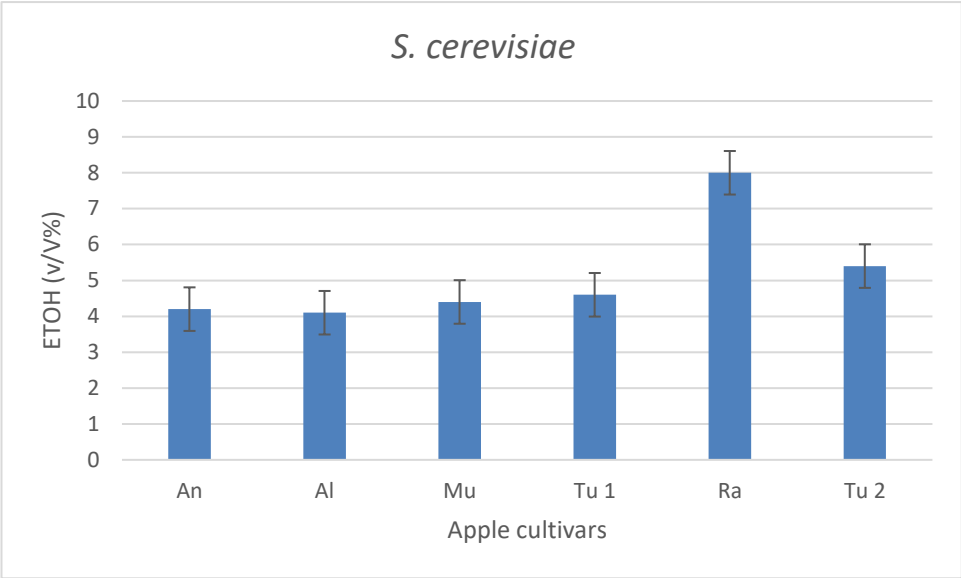
Throughout the fermentation process, we closely monitored several key parameters to track its progress. We carefully tracked the weight difference and regularly checked the TSS drop to gauge how the fermentation was proceeding. The weight check was essential in measuring the decline from the starting weight of the inoculation as the fermentation progressed, while the TSS relied on a reduction in the °Brix value. We observed a consistent decline in TSS for both F-Td and F-Se, from (9.2, 13.7) to (4.1) and (9.3, 13.8), respectively. Additionally, the average weight of the samples decreased, which aligned with the decline in TSS of F-Se and F-Td.

As both fermentations occurred at the same temperature of 22°C, there was no noticeable temperature impact between the two yeast strains. Moreover, the decrease in °Brix values indicated that the strains remained consistently viable throughout the process and lagged only when TSS approached approximately 3.5 and 4.1. The °Brix values of both the Se and Td strains decreased during the fermentation process, based on the initial ºBrix of their respective juices. Notably, the fermentation process utilizing a single strain of *T. delbrueckii* exhibited the longest lag phase, as determined by the initial Brix value which did not correspond to the theoretical predictions and previous study by Kelanne et al 2020.




Line graphs depicting a steady decrease in TSS during fermentations are presented in Figures 4. Both PF fermentations led to identical decrement in TSS for both yeast inoculations (A&B).

**Figure 4.** Fermentation kinetics expressed as decrease in °Brixs values



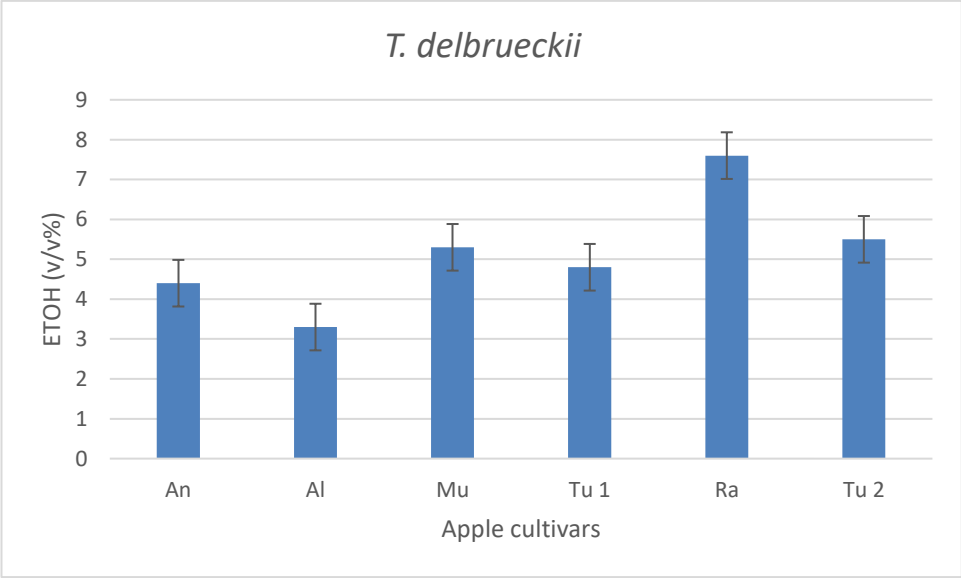
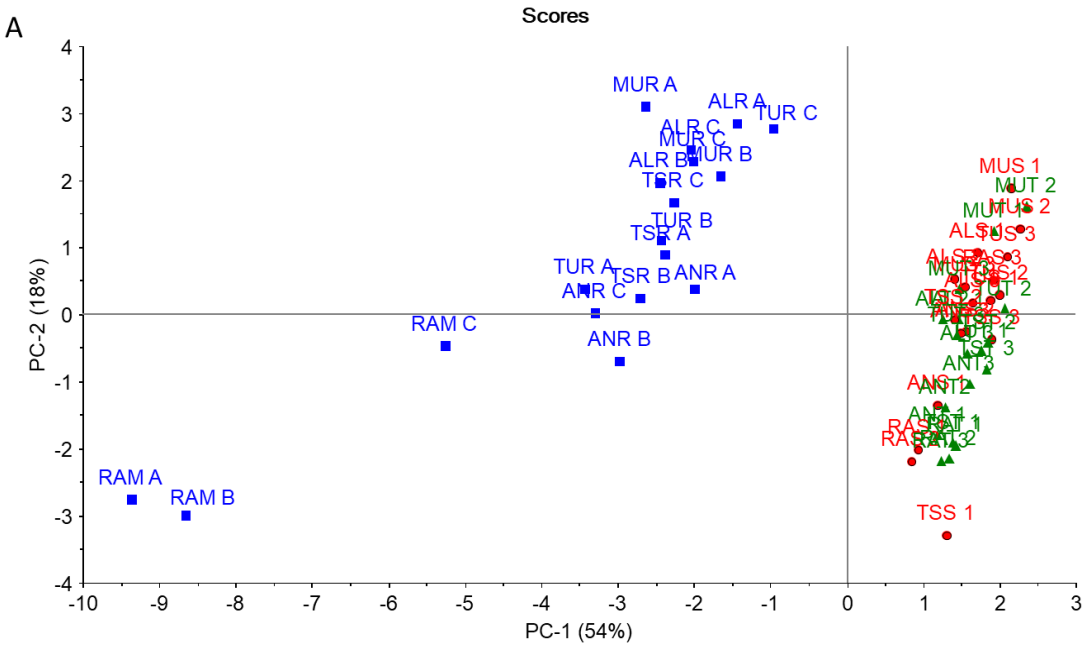




**Figure 5**. Ethanol content of fermented apple juice beverages

Average concentrations of fermented beverages produced by different yeast strains. Names cultivars in abbreviations refer to table 4 above.



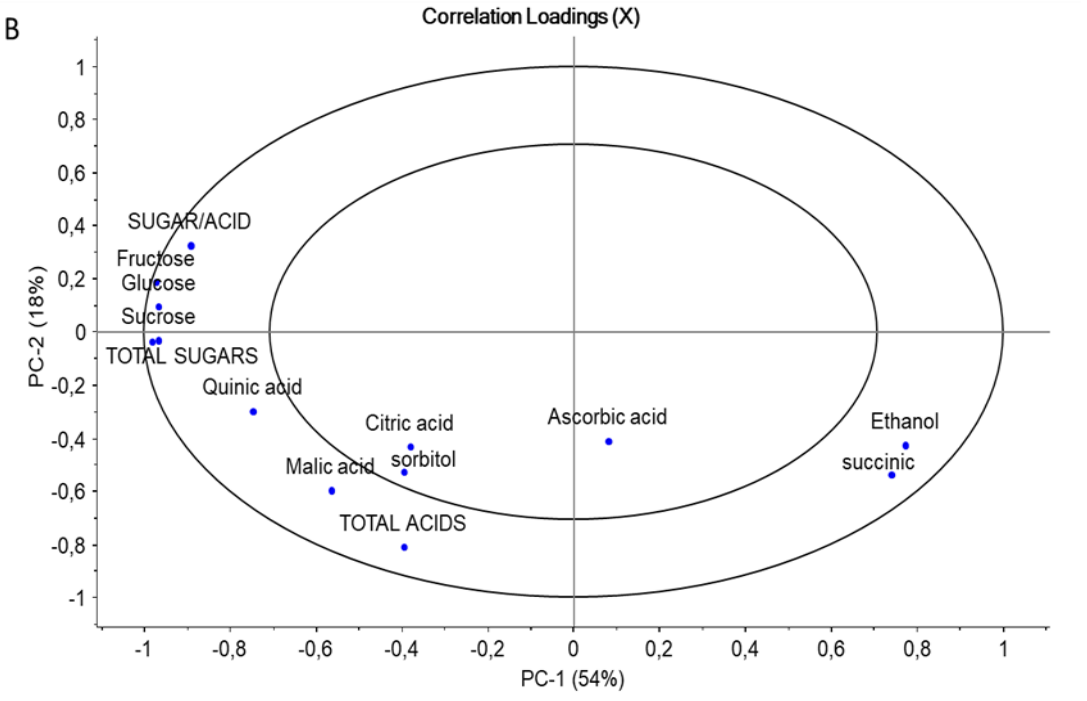




**Figure 6**. PCA model to elucidate the differences between the apple juices and corresponding alcoholic beverages made from S. cerevisiae and T. delbruckii. A. Scores-plot with all samples (juices and ciders, n=54), B. Loadings-plot with all variables (n=13). Labels show juices (blue) and ciders, S. cerevisiae (red) and T. delbrueckii (green)

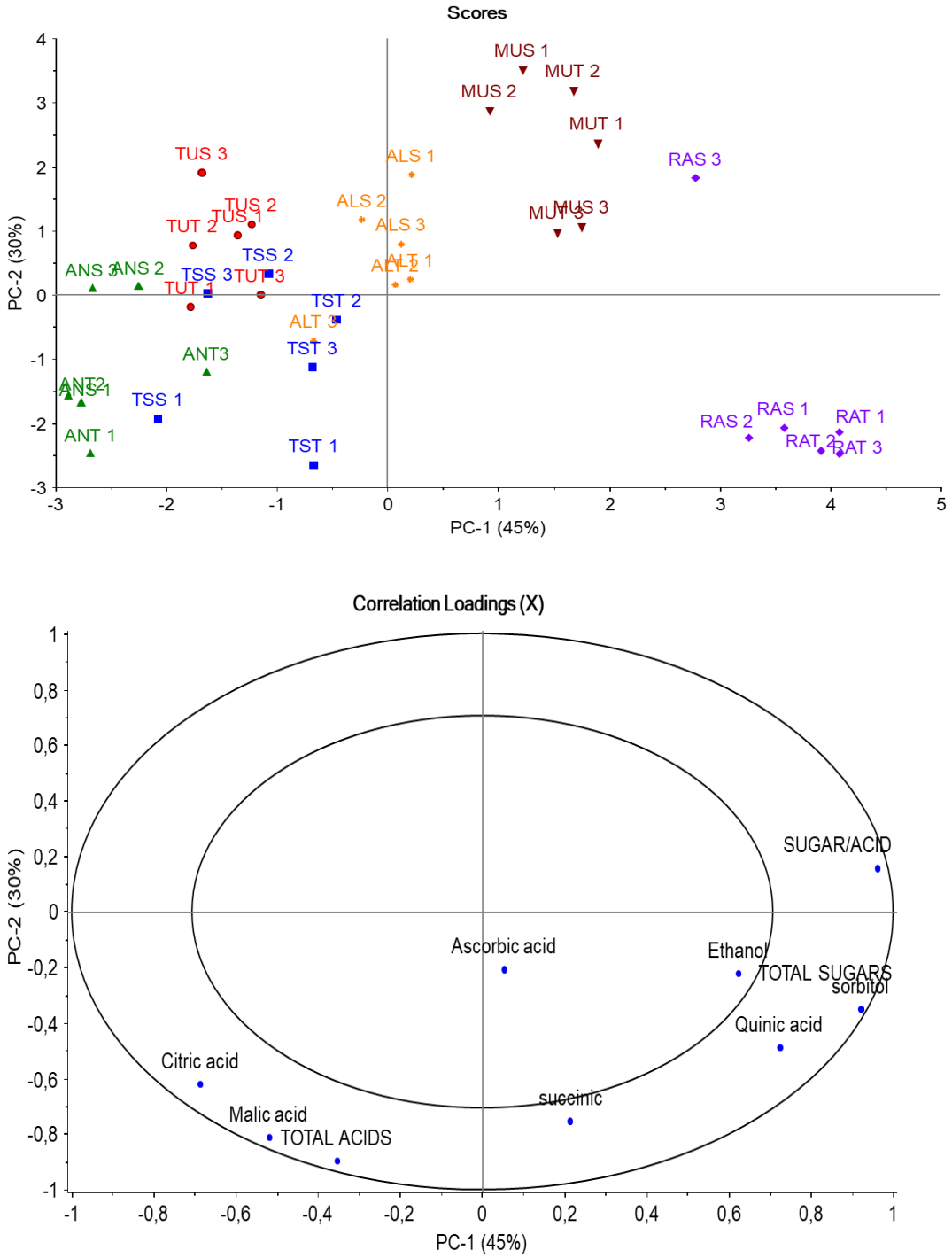



**Figure 7.** PCA model to elucidate the differences between the apple alcoholic beverages made from S. cerevisiae and T. delbruckii. A. Scores-plot with all cider samples based on six cultivars (n=54), B. Loadings-plot with all variables (n=13). Labels show S. cerevisiae (depicted with 'S') and T. delbrueckii (depicted with 'T'). *1, 2 &3 are biological replicate. Abbreviation used are expressed in table 4.

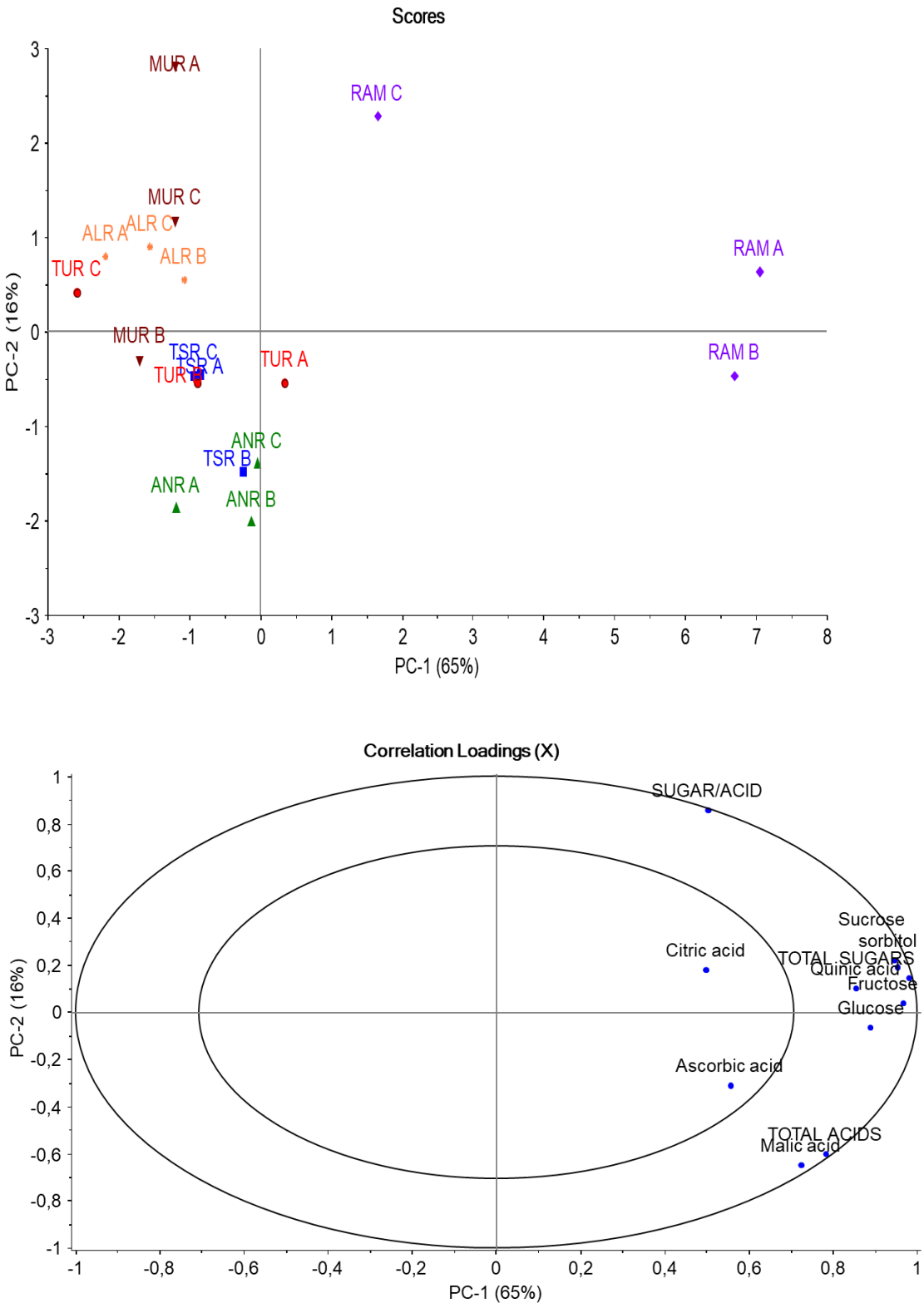



**Figure 8**. PCA model to elucidate the differences between the chemical components of juices A. Scores-plot with all juices based on six cultivars (n=18), B. Loadings-plot with all variables (n=11). Labels show raw apple juice (depicted with 'R' and 'M' for Rambo juice). Abbreviation used are expressed in **table 4** (first two letters). A, B &C biological replicates.

## 4. Discussion: Comparison of Chemical Composition of Beverages Fermented with Different Yeast Strains

### 4.1 Sugars, and organic acids.

The attribute of sweetness is highly sought after in a variety of fruits and vegetables and is typically influenced by the level of sugar content (Terry et al., 2005; Hong et al., 2014). Therefore, accurately measuring, and quantifying sugar and sweetness is essential in numerous fields of plant food research. The sensory attributes of AFBs are significantly influenced by their primary taste and nutritional components, which include sugars, organic acids, and glycerol. The sugar compositions and profiles of fruit cultivar types as shown in figure 8, had the greatest impact on these attributes. All apple cultivar juices contained fructose, glucose, and sucrose, while FAJs showed sorbitol. Juices were primarily composed of sucrose, followed by fructose and sucrose. Fermented apple juices had varying sorbitol contents, with the cultivar 'Antonvka' F-SE having the lowest amount and the cultivar 'Rambo' F-Td having the greatest amount on average. Apple juice fermented with *S. cerevicea* had lower sorbitol content compared to *T. delbruckii*. Malic acid was detected as the major acid component in both fermented and unfermented apple juice, giving the studied beverage products a harsh sour taste. 'Antonovka' had the highest malic acid content (12.8 g/L), followed by 'Tunista 1' (10.4 g/L) and 'Tunista 2' (9.8 g/L). 'Aleksanteri' and 'Rambo' also had relatively high malic acid content, while 'Musti- Iso' had a lower content. Ascorbic and succinic acids were detectable in low amounts in fermented apple juice and unfermented juice but were not detected in 'Aleksanteri' and 'Musti- Iso'. Glycerol was not found in unfermentable fruit juices.

### 4.2 Ethanol and higher alcohols

Based on our experiment, SE proved to be more effective than TD in producing ethanol from the selected apple juice (Fig. 5) Despite the distinct characteristics of the two yeast strains, the ethanol content in the current fermentation matrix was quite similar. F-SE produced ciders 'Antonovka' (4.2 %v/v), 'Aleksanter'(4.1 %v/v), 'Musti-iso' (4.4 %v/v), 'Tunista1' (4.6 %v/v), and 'Tunista2' (5.4 %v/v) while in F-TD 'Antonovka'(4.4 %v/v), 'Aleksanter'(3.3 %v/v), 'Musti-iso' (5.3 %v/v), 'Tunista1'(4.8 %v/v), and 'Tunista2' (5.5 %v/v) respectively. Typically, *T. delbrueckii* is used to reduce ethanol content in grape wines, however our research revealed that F-TD produced a high ethanol content (7.6

%v/v) in ‘Rambo’ apple cultivar juice, which was comparable to F-SE (8.0 %v/v) fermentation of same cultivar. It is worth noting that *T. delbrueckii* has been observed to be highly resistant to harsh conditions such as low pH and temperature, which could explain its comparable fermentation performance in ‘Rambo’ when compared to certain *S. cerevisiae* yeast strains. In an oxygen-deprived environment, carbohydrates can be converted into carbon dioxide, ethanol, heat, and energy through the action of yeast. The concentration of ethanol in apple juice is influenced by various factors such as the initial sugar content and fermentation temperature of the juice While higher temperatures can expedite the fermentation process, some ethanol molecules may be sacrificed in the process. (Piggott and Lea, 2003) this could also explain the amount of ethanol produced as fermentation was carried out at a temperature of 22°C and most of the cultivars had low initial sugar content. Therefore, the quantity of ethanol obtained is proportional to the initial sugar in this present study and not the yeast used. Furthermore, the result from this experiment corresponds to the previous findings of studies conducted by Belda et al. (2015), Renault et al. (2015), and Chen et al. (2018) who found no significant changes in the results. When it comes to wine production, limited fermentation capacity led to significant drops in ethanol levels (more than 1% (v/v) or up to 2% (v/v)), which were attributed to insufficient residual sugar fermentation. Additionally, Bely et al., 2008 also demonstrated Td strains variability in production of alcohol up to 2% (v/v) ranging from (7.4 to 9.38) which explains the result from the ‘Rambo’ cultivars.

### 4.3 Chemical composition of fermented and non-fermented apple juice

An analysis of 18 juice samples and 55 chemical variables of fermented beverages was conducted using PCA to uncover connections between the two categories. The findings depicted in Figure 6, show that PC1 accounts for 54% of the data's variance. PC1 effectively separates the yeast fermentations from the juice samples, highlighting a positive correlation among variables (sugar/acids) and (ethanol) on the loadings. The juices have a negative correlation with fermented beverages. The juices samples grouped together on the same axis on the score plot except for ‘Rambo’ cultivar, exhibit similar fermentation characteristics, high total acid as shown on the loadings (Fig. 6) PC2. Clustered ciders negatively correlated to total acids. PC1 also indicates that variables such as quinic acid, citric acid, and malic acid are positively correlated with their fermented beverage counterpart. Furthermore, PC1 indicates that a higher ethanol yield is correlated with a cultivar with total acid and sugars.

In Figure 6, we can observe the correspondence between the initial juices chemical content and the alcoholic drinks that resulted from the different yeast strains for fermentation. Typically, the apple beverages displayed lower sugar levels following the fermentation process. In the case of the apple juices, the yeast strains SE and TD were able to metabolize fructose, glucose, and sucrose, but sorbitol remained as the primary component in the aftermath of the two yeast fermentations (Fig. 7). This can be attributed to the metabolic properties of the yeast strains, where non-Saccharomyces strains tend to consume less sugar than Saccharomyces strains. The alcoholic apple beverages displayed considerably lower sorbitol concentrations, except for the 'Rambo' cultivar which had high initial sorbitol content in its juice (16.2 g/L), where the yeast strains' ability to utilize sorbitol was limited. Overall, the fermented apple beverages exhibited no difference in residual sorbitol contents except in the case of 'Rambo' cultivar by both yeast strain (8.6g/L) SE and (10.7g/L) TD, which can be attributed to its presence in the initial apple juices (Fig. 8). It is worth noting that sorbitol is known to contribute to the sweetness of the final product. Low-carb dieters often use sorbitol sweetener to add sweetness to their beverages, based on how sweet it tastes (Aprea et al., 2017). Subsequently, during alcoholic apple drinks production, the type of fruit used is as important as the variety. For instance, apple drinks made with 'Antonovka' and 'Aleksanter' had the lowest sugar content compared to other apple types (Fig.8). Fermented apple drinks and juices differed in their quinic and malic acid concentrations (Fig. 7). Malic acid content was higher in apple drinks fermented with TD, while SE fermentation leads to lower levels of malic acid. However, the concentration of these acids did not noticeably increase from initial values. Previous research has shown that *T. delbruckii* yeast can effectively decrease malic acid content in AFBs, resulting in higher pH values that help to reduce the harsh green sourness and astringency in fermented beverage products (Laaksonen, Salminen, Mäkilä, Kallio, & Yang, 2015). Ascorbic acid was detectable (0.3-1.2g/L) in the apple juices used for the apple beverages.

## 4.4 Limitations of study

The study's findings necessitate consideration of certain limitations within the context of this research. It is crucial to address these issues systematically to attain a comprehensive understanding of the study's conclusions. Firstly, the frequent sampling through bottle opening resulted in the oxidation of apple cider samples. This oxidative process gave rise to an acetic acid final product, which has the potential to adversely affect the sensory

attributes of ciders. To mitigate this challenge, it is recommended to conduct fermentations in a bioreactor—a versatile equipment that facilitates sampling while preserving the headspace environment of the fermentation vessel.

Secondly, the prolonged fermentation lag phase poses another limitation. During the fermentation process until dryness, acetic acid was produced in the cider, contributing to undesirable aromas. To address this issue, it is advisable to monitor fermentations closely and promptly cease the process once a consistent brix value is achieved. This proactive approach helps prevent the formation of off-aromas associated with extended fermentation.

## 5. Conclusions

In the present study, the research aimed at examining the effect of *Saccharomyces cerevisiae* and *non-Saccharomyces* yeasts fermentation on the chemical characteristics of Finnish apple cultivars was successfully achieved. The impact of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* strains on the chemical composition of six Finnish apple cultivars was studied using multivariate statistical models. The major difference was observed between apple juice cultivars and ciders produced with yeast fermentation. TD fermentation typically resulted in ciders with high malic acid content in all apple cultivars used, except in the 'Rambo' cultivar. AFBs with high malic acid cause an increase in acidity, characterized by a harsh, astringent, or bitter taste, thereby affecting desirable sensory properties. Therefore, it is crucial to carry out sequential fermentation with *Saccharomyces cerevisiae* to reduce malic acid content in cider. *S. cerevisiae* generally increased the ethanol content and ascorbic acid in 'Rambo' while reducing the malic acid content of the ciders considerably compared to *T. delbrueckii*. Moreover, both yeasts produced a similar impact on all cultivars in terms of quinic acid, succinic acid, ascorbic acid, and citric acid and consumed all the sugars.

On the other hand, the apple cultivar had a very significant role in final cider composition highlighting and verifying the previously known importance of cultivar selection in the cider production. This study included local Finnish apple cultivars with potential to be used in ciders. Apple cultivars used were at optimum ripen stage of the apple which is crucial to optimize the total soluble sugar which impacts on the ethanol composition. Hence apple maturation stage should be focused on during selection for cider production. Juice samples contained malic acids range from 6.1g/L – 13.5g/L with Ra having the highest, however, Rambo cultivar showed great potential in producing cider with high quality amongst other cultivars in this study due to it amount of total sugar and organic acid but, the sensory characteristics, phenolic components and colour intensity must be further investigated.

There have been several studies on the effect of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* strains, this is the first scientific study involving this selection of Finnish native apple cultivar with pure inoculations as per our knowledge. Previous attempt at lab- scale fermentation Impact of apple cultivar, ripening stage, fermentation type and yeast strain on phenolic composition of apple ciders by Laaksonen et al. (2017).

These studies have paved the way for our research in understanding the effect of yeast for this study in terms of yeast effect; in addition to prior studies by Wenjia He et al. (2021) on the effect of processing and raw material on Finnish apples and pears quality of their ciders using *Saccharomyces cerevisiae* and Schizosaccharomyces pombe yeasts without any added carbon sources.

Finally, selection of yeast strains with viable potential to reduce malic acid and utilize total sugars optimally must be focused on for further studies to produce apple ciders with low acidity, also the phenolic composition of the ciders from this study has not been investigated and should be conducted to establish the sensory profile especially of 'Rambo' cultivar.

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**Appendix**

Rambo

Musti-iso-vennala

Tunista 1

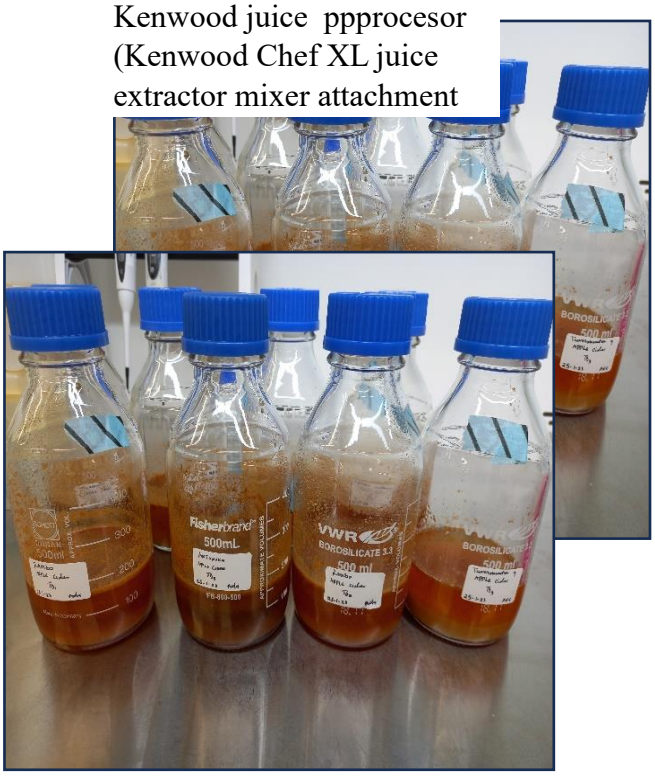
Aleksanter

Tunista 2 Antovaka

**Figure 9.** Gallery of Finnish apple cultivars used in present study.

Kenwood juice ppprocesor (Kenwood Chef XL juice extractor mixer attachment

Apple juice fermented with SE




Apple juice fermented with TD


Apple ciders from SE and TD fermentations

**Figure 10**. Laboratory scale cider production gallery