DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

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Food Development (tech.)

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ABSTRACT

Alcoholic beverages made from non-grape fruits by fermentation, such as apple cider and pear perry, are increasingly popular nowadays, as well as being among the most commonly consumed products in recent years. In general, industrial producers use specific ‘cider apple’ or ‘perry pear’ cultivars mixed with dessert fruit cultivars to obtain alcoholic fruit beverages (AFBs) with a high quality. A stable and low-price supply of local specialty fruit cultivars has been established in countries with a long history of cider making, such as England and France, whereas their availability is limited in the Nordic countries, like Finland. In addition, there are no existing breeding programs in Finland for ‘specialty beverage’ cultivars of apples or pears. Thus, the selection of traditional local cultivars or the development of novel cultivars for AFB production is of high economic importance in the industry. At the same time, alcoholic fermentation is a complex, yeast-driven process. Utilization of non-*Saccharomyces* yeasts in AFBs fermentation to create more versatile flavor profiles can also be considered to be a recent and growing trend in the beverage industry.

The aim of this doctoral research was to investigate the utilization of apple and pear cultivars, as well as *Saccharomyces* and non-*Saccharomyces* strains in the process of producing AFBs. A special focus was placed on the fate of the volatile and non-volatile compounds during the fermentation and their correlations with the sensory properties of the AFBs. The goal was to produce new knowledge about the potential of producing AFBs of high quality and providing technical guidance for the beverage industry. Furthermore, this study promotes the exploitation of Finnish local apple and pear cultivars for commercial utilization in beverage processing.

In **Studies I & II**, the volatile and non-volatile compounds and sensory quality of alcoholic apple beverages were evaluated. The alcoholic beverages were made from selected Finnish traditional and local apple cultivars *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* yeasts without any added carbon sources. The sugars, organic acids, volatile compounds, and phenolic compounds as well as the ethanol and glycerol content were measured using chromatographic and mass spectrometric methods before and after the fermentation. The sensory differences among selected fermented beverages were analyzed using check-that-all-apply tests with untrained panelists. In general, alcoholic fermentation enhanced the contents of the higher alcohols, aldehydes, and acetals but decreased the concentrations of phenolic compounds. Cultivar and yeast selections both significantly influenced the chemical compositions and sensory properties of the obtained beverage products. The utilization of *S. pombe* significantly decreased the malic acid, and these apple beverages were shown to be less sour when compared to beverages fermented with *S. cerevisiae*. Moreover, *p*-coumaric, caffeic, and 5-*O*-caffeoylquinic acids, as well as procyanidin dimers were shown to be potential contributors to the mouth-drying and puckering astringency in the alcoholic apple beverages.

In **Studies III** & **IV**, compositions of phenolic compounds, sugars, organic acids and certain volatile compounds in juices and perries made from unreleased breeding selections and cultivars of the European pear were evaluated. In general, the chemical profiles of the pear juices are primarily dependent on the genetic background, but the effects are complex. Breeding selections sharing the same parental cultivars varied significantly among the cultivars. The 'putative dessert pear' group (determined by the breeders) contained more analyzed volatile compounds, whereas the 'putative perry pear' group was closely associated with the quinic acid derivatives of caffeoyl and coumaric acids and coumaric acid, in addition to (+)-catechin and procyanidin dimers. In **Study IV**, yeast strains of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* were used to produce fermented pear beverages from the selected pear fruits from **Study III**. The sensory characteristics, the chemical profiles of the alcoholic beverage products and the initial pasteurized juices were analyzed using a trained panel. For the alcoholic pear beverages, the fermentation process reduced the content of hydroxybenzoic acids, procyanidins, and flavonols, in addition to increasing the content of flavan-3-ols and hydroxycinnamic acids. The utilization of *T. delbrueckii* led to higher 'cooked pear' and 'floral' odors when compared with *S. cerevisiae* fermentations. Moreover, certain derivatives of hydroxybenzoic and hydroxycinnamic acid, flavan-3-ols, procyanidins, and flavonols were closely correlated with astringency whereas sorbitol associated with sweetness in the fermented pear beverages.

SUOMENKIELINEN ABSTRAKTI

Alkoholipitoiset, käymisteitse valmistetut hedelmäjuomat, kuten omena- ja päärynäsiiderit, kasvattavat jatkuvasti suosiotaan. Siiderin valmistuksessa käytetään tyypillisesti tiettyjä siiderin valmistukseen kehitettyjä lajikkeita yhdessä makeampien, sellaisenaan syötävien lajikkeiden kanssa. Monissa perinteisessä siideriä valmistavissa maissa, kuten Englannissa ja Ranskassa, on vakaat ja kestävät resurssit hyödyntää paikallisia hedelmälajikkeita juomien valmistuksessa, toisin kuin Pohjoismaissa, kuten Suomessa. Suomessa ei tällä hetkellä ole juomien valmistukseen käytettävien omenien tai päärynöiden jalostusohjelmia. Perinteisten paikallisten lajikkeiden valinta tai uusien lajikkeiden kehittäminen on siten taloudellisesti tärkeää teollisuudelle. Alkoholikäyminen on toisaalta monimutkainen, hiivalähtöinen prosessi. *Saccharomyces*-sukuun kuulumattomien hiivojen käytöllä on kasvavaa kiinnostusta juomateollisuudessa viime aikoina alkoholijuomien maun monipuolistamiseksi.

Tämän väitöskirjatutkimuksen tavoitteena oli tarkastella omena- ja päärynälajikkeiden sekä *Saccharomyces*-sukuun kuuluvien ja kuulumattomien hiivojen käyttöä alkoholipitoisten juomien valmistuksessa. Työssä tarkasteltiin erityisesti erilaisten haihtuvien ja haihtumattomien yhdisteiden kohtaloa alkoholikäymisen aikana ja yhdisteiden yhteyttä aistinvaraisiin ominaisuuksiin. Tavoitteena oli tuottaa uutta tietoa juomateollisuudelle korkealaatuisten alkoholipitoisten hedelmäjuomien valmistamisesta ja teknisistä mahdollisuuksista. Tutkimus tukee myös suomalaisten paikallisten omenalajikkeiden ja uusien päärynälajikkeiden kaupallista hyödyntämistä juomien valmistuksessa.

Osatöissä I ja II tarkasteltiin alkoholipitoisten omenajuomien haihtuvia ja haihtumattomia yhdisteitä sekä aistittavaa laatua. Juomat oli valmistettu paikallisista perinteisestä omenalajikkeista käyttäen *Saccharomyces cerevisiae* ja *Schizosaccharomyces pombe* -hiivoja ilman lisättyjä hiililähteitä. Sokerien, orgaanisten happojen, haihtuvien yhdisteiden ja fenolisten yhdisteiden sekä etanolin ja glyserolin määriä mitattiin ennen hiivakäymistä ja sen jälkeen käyttäen kromatografisia ja massaspektrometrisiä menetelmiä. Valittujen näytteiden aistittavaa laatua tarkasteltiin ns. *check-all-that-apply*-menetelmällä kouluttamattoman arvioijaraadin avulla. Alkoholikäyminen lisäsi korkeampien alkoholien, aldehydien ja asetaalien muodostumista, mutta samalla vähensi fenolisten yhdisteiden määriä. Omenalajikkeen ja hiivan valinnat vaikuttivat molemmat merkitsevästi valmistettujen juomien kemialliseen koostumukseen ja aistinvaraiseen laatuun. *S. pombe* -hiivan käyttö vähensi merkitsevästi omenahapon määrää vähentäen samalla juomien happamuutta verrattuna S.

cerevisiae -hiivalla valmistettuihin juomiin. Samalla *p*-kumaari-, kahvi- ja 5-*O*-kaffeoyylikviinihappojen sekä prosyanidiinidimeerien määrät osoittautuivat mahdollisiksi suuta kuivattavaa ja kurtistavaa suutuntumaa aiheuttaviksi yhdisteiksi alkoholipitoisissa juomissa.

Osatöissä III ja IV tarkasteltiin uusista, vielä julkaisemattomista päärynälajikkeista valmistettujen mehujen ja päärynäsiiderien fenolisten yhdisteiden, sokerien, orgaanisten happojen ja tiettyjen haihtuvien yhdisteiden koostumusta. Mehujen kemiallinen koostumus oli pääasiassa riippuvainen päärynöiden perinnöllisestä taustasta, mutta vaikutukset olivat monimutkaisia. Kasvattajien putatiivisesti sellaisenaan syötäväksi määrittelemät päärynät sisälsivät enemmän tutkittuja haihtuvia yhdisteitä, kun taas putatiivisesti siiderin valmistukseen tarkoitetut päärynät sisälsivät enemmän kaffeoyylin ja kumaroyylin kviinihappojohdannaisia ja kumaarihappoa sekä (+)-katekiinia ja prosyanidiinidimeerejä. **Osatyössä IV** käytettiin *Saccharomyces cerevisiae* ja *Torulaspora delbrueckii* -hiivoja siiderien valmistamisessa tietyistä, **osatyössä III** tarkastelluista päärynöistä. Juomista analysoitiin kemiallista koostumusta sekä aistittavan laadun ominaisuuksia käyttäen koulutettua arvioijaraatia. Hiivakäyminen vähensi hydroksibentsoehappojen, prosyanidiinien ja flavonolien määriä samalla lisäten flavan-3-olien ja hydroksikanelihappojen määriä. *T. delbrueckii*-hiivan käyttö lisäsi kypsennetyn päärynän hajua sekä kukkaista hajua verrattuna *S. cerevisiae* -hiivaan. Lisäksi tietyt hydroksibentsoe- ja hydroksikanelihappojen johdannaiset, flavan-3-olit, prosyanidiinit ja flavonolit korreloivat aistitun astringoivuuden kanssa, kun puolestaan sorbitoli yhdistettiin päärynäsiiderien makeuteen.

LIST OF ABBREVIATIONS

AFBs	Alcoholic fruit beverages
HBAs	Hydroxybenzoic acids
HCAs	Hydroxycinnamic acids
PPO	Polyphenol oxidase
DP	Degrees of polymerization
VOCs	Volatile organic compounds
UDP	Uridine 5'-diphosphate
UGTs	UDP-carbohydrate-dependent glycosyltransferases
AAT	Alcohol acyltransferase
ADH	Alcohol dehydrogenases
YAN	Yeast assimilable nitrogen
DAFB	Days after full bloom
UV	Ultra-violet
HDP	High Density Planting
PDNS	Plant-derived nutrient solution
AH	Air humidity
DI	Deficit irrigation
RDI	Regulated deficit irrigation
PGRs	Plant growth regulators
BGs	Bagging treatments
LTC	Low temperature conditioning
RH	Relative humidity
ULO	Ultra-low oxygen
CA	Controlled-atmosphere
1-MCP	1-Methylcyclopropene
RFP	Rack-and-frame press
BAP	Basket press
SP	Screw press
BEP	Belt press
WP	Water press
SFP	Spiral-filter press
OFC	Oxygen-free conditions
TP	Thermal processing
OH	Ohmic heating
NTP	Nanofluid thermal processing
CO_2	Carbon oxidation
PEF	Pulsed electric field
HTST	High temperature short time
US	Ultrasound
HHP	High hydrostatic pressure
HPCD	High pressure CO_2 processing
SC	*Saccharomyces cerevisiae*
S. paradoxus	*Saccharomyces paradoxus*
S. bayanus	*Saccharomyces bayanus*

S. kudriavzevii	*Saccharomyces kudriavzevii*
S. mikatae	*Saccharomyces mikatae*
S. uvarum	*Saccharomyces uvarum*
S. eubayanus	*Saccharomyces eubayanus*
TD	*Torulaspora delbrueckii*
SP	*Schizosaccharomyces pombe*
C	Cider or perry fruit
D	Dessert fruit
GC-FID	Gas chromatography flame ionization detector
HS-SPME	Headspace solid phase microextraction
MS	Mass
DVB/CAR/PDMS	Divinylbenzene/carbozen/polydimethylsiloxane
UHPLC-DAD	Ultra-high-pressure liquid chromatography
Q-TOF	Quadrupole time-of-flight mass spectrometry
CATA	Check-all-that-apply
PCA	Principal component analysis
α-GI	α-glucosidase inhibitory
PLS-DA	Partial least squares discriminant analysis

LIST OF ORIGINAL PUBLICATIONS

I. He, W., Liu, S., Heponiemi, P., Heinonen, M., Marsol-Vall, A., Ma, X., Baoru, Yang., & Laaksonen, O. (**2021**). Effect of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains on chemical composition and sensory quality of ciders made from Finnish apple cultivars. *Food Chemistry*, *345*, 128833.

II. He, W., Laaksonen, O., Tian, Y., Heinonen, M., Bitz, L., & Yang, B. (**2022**). Phenolic compound profiles in Finnish apple (*Malus× domestica Borkh.*) juices and ciders fermented with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains. *Food Chemistry*, *373*, 131437.

III. He, W., Laaksonen, O., Tian, Y., Haikonen, T., & Yang, B. (**2022**). Chemistry composition of juices made from cultivars and breeding selections of European pear (*Pyrus communis* L.). *Journal of Agricultural and Food Chemistry. 70,* 5137-5150.

IV. He, W., Tian, Y., Liu, S., Vaateri, L., Ma, X., Haikonen, T., Yang, B., & Laaksonen, O. Comparison of phenolic composition and sensory quality among pear beverages made from *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* strains. *Submitted*.

1 INTRODUCTION

Apple (*Malus* × *domestica* Bork.) and pear (*Pyrus* spp.), two of the most economically important fruits of the *Rosaceae* family, are widely cultivated and highly consumed throughout the world. Apples are known for their health-promoting effects and good taste, and the proverb ‘an apple a day keeps the doctor away’ is well accepted by consumers. Regarding pears, they are appreciated for their thin peel, rich juice, good taste, pleasant flavor, and high nutritional value (Wei & Wang, 2013). Both of these fruits are rich in bioactive compounds associated with health benefits, such as polyphenolic compounds. However, large quantities of apples and pears are wasted annually due to low fruit quality caused by poor harvesting and transportation practices. Nevertheless, the edible parts of these discarded fruits still contain high levels of nutrients, high dietary fibers, and a low calorie content (Brahem, Renard, et al., 2017; Pasquariello et al., 2013). Thus, it is important to process these fruits into value-added products, such as juices, purees, jams, fermented beverages, and dried fruits.

Alcoholic fermentation technology is well-practiced in processing fruits to increase their preservation capacity and thus to reduce the need for refrigeration (Guiné, Barroca, Coldea, Bartkiene, & Anjos, 2021). Alcoholic fruit beverages (AFBs) made from fruit juices are popular worldwide, mainly ciders, perries, and other fruit wines. In general, the term ‘cider’ refers to a drink made from apples. In European countries, the terms ‘cider’, ‘sidra’ (Spain), and ‘cidre’ (France) are exclusively reserved for apple beverages with alcohol levels of 1.2–8.5% (*v/v*). The term ‘perry’ generally refers to drinks made from pear juices. However, in North America, ‘cider’ refers to cloudy unpasteurized apple juices, whereas ‘hard cider’ refers to the fermented products (Lea & Drilleau, 2003). In Finland, ‘cider’ refers to both the fermented apple and pear products according to Finnish legislation. The studied AFBs in this practical research refer to ‘pure beverages’ (100% juice based alcoholic beverages) without the addition of any sugar or water.

Although the history of cider and perry is not as long and prestigious in comparison to grape wines, these products, due to their high nutritional values and pleasant taste, are gaining increasing popularity in European countries, such as England, France, Spain, and Northern Europe. Generally, the preference of the beverage industry is to use specialty fruit cultivars grown in local orchards, which have fibrous structures, high juice yields, and high tannin levels. However, the distinct climate in Northern European countries limits the growth of specialty cider apples and/or perry pears. Development and selection of speciality cultivars with multi-use potential (juice and cider/perry production) are needed for modern retail supply chains. Until now, breeding programs for specialty cider apples and/or perry pears have not existed in Finland, and compositional data related to Finnish apple and pear cultivars remains scarce.

Saccharomyces cerevisiae is regarded as the most commercially important yeast in wine-making markets, and its genetic background and physiological properties have been well studied. *S. cerevisiae* has a high growth density and compatibility for large-scale fermentation; it also has good resistance and tolerance to phage infection and harsh industrial conditions (Albergaria & Arneborg, 2016). Apart from the *S. cerevisiae* strains, non-*Saccharomyces* yeasts also play important roles in cider and perry production. Among the non-*Saccharomyces* yeasts, *Torulaspora delbrueckii* is the one most commercially used in the industry, whereas *Schizosarrcharomyces pombe* is often used to decrease the malic acid content and to enhance the aroma complexity of fruit wines (Benito, 2018; Mylona et al., 2016). The utilization of these non-*Saccharomyces* yeasts in cider and perry production provides more possibilities for improving the chemical characteristics and sensory properties of specialty cider and perry products to meet customer demands for a pleasant taste, flavor, and odor.

The literature review section comments on the fact that the biosynthesis of volatile and non-volatile compounds is often described, and that this is due to its importance during cider/perry fermentation. In addition, the impact of extraction methods, enzyme treatments, and pasteurized treatments on juice production is well documented in the literature. Cultivars, environmental factors, and storage condition factors are also well covered concerning their effects on the apple/pear/related fruit quality. Furthermore, the utilization of *Saccharomyces* and non-*Saccharomyces* yeasts is comprehensively discussed.

In this thesis, unfermented juices were pressed directly from different traditional apple cultivars and novel pear breeding selections and cultivars in Finland. The cider and perry products were obtained by fermenting pasteurized apple and pear juices with selected yeast strains. Different yeast strains were selected for cider (*S. cerevisiae* and *S. pombe*) and perry (*S. cerevisiae* and *T. delbruekii*) due to their different physiological properties. The aim of this thesis research was to investigate the chemical compositions of the non-alcoholic apple/pear juices and the fermented alcoholic cider/perry products. In addition, the sensory characteristics of selected cider/perry products were studied, and the contribution of the chemical compositions to the sensory properties was investigated. Based on the findings of the thesis research, certain apple/pear cultivars were suggested as potential candidates for the Finnish apple/pear breeding programs for new cider/perry cultivars. This work provides important information which will help the exploitation of Finnish cultivated apple and pear cultivars in commercial application.

2 REVIEW OF THE LITERATURE

2.1 Chemical compounds associated with quality of alcoholic fruit beverages

The nature of fruit wine quality can be described as a multifaceted concept with intrinsic and extrinsic aspects based on seven dimensions of wine quality, including origin, balance, flavor, bouquet, vintage, aging ability, presentation, image, as well as acuteness (mainly associated with the aromatic complexity and the bouquet intensity) (Charters & Pettigrew, 2007). This multifaceted concept can be also applied to describe the quality of alcoholic fruit beverages (AFBs). From the perspective of the consumers, the intrinsic organoleptic aspects (appearance, odor, and taste), which are determined by the chemical compositions of beverages, are the most important dimensions in the determination of product quality.

Quality attributes are important for AFBs, and they are generally evaluated by untrained consumers and/or trained panelists to assess the desirability and typicality of AFBs as well as identifying their faults. The quality assessment usually follows evaluations of the color, aroma, taste, and mouthfeel sensations with a quality score. The quality of pome fruits and AFBs depend on many factors, such as: soil quality, maturity degree of fruits, sunlight exposure, water stress, farming system type, agronomical practices, pre-processing of fruits, pressing methods, fermentation types, yeast species, and other factors. Differences in the fruit, juice, and wine quality can be significant as outcomes of the complex interaction of all these factors.

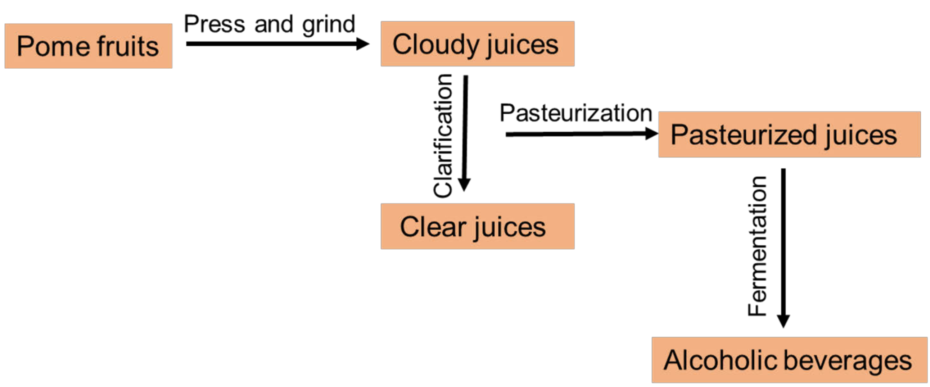


Figure 1. Overview of the processing operations of AFBs

To adequately understand the relationships between volatile and non-volatile components and overall quality of AFBs, it is essential to consider the whole chemical compound metabolism during the fermentation processing. The process flowchart containing the basic steps to producing AFBs is shown in **Figure 1**. The apples and pears are harvested on farms at the technical maturation stage, which is determined by the breeders. Prior to the juice processing, apples and pears need to be cleaned and sanitized by a water bath and sodium hypochlorite solution (50–200 mg/L) to lower the bacterial counts (Granato, de Magalhães Carrapeiro, Fogliano, & Ruth, 2016). In the industry, juices are generally extracted from the whole fruits of apples and pears by using different extraction methods, including hot pressing and cold pressing methods. Alcoholic fruit beverages can be produced from clear juices and/or cloudy juices. Thus, enzymes or further filtration can also be applied in juice processing for juice clarification. Obviously, the juice quality can be influenced by the extraction methods and the temperatures used, as well as the types and concentrations of enzymes utilized in the process. However, excessive clarification leads to sluggish fermentation, whereas small proportions of suspended solids can balance the fermentation properties. Pasteurization and sterilization treatments have also been widely applied to reduce the risk of contamination and to improve safety and shelf-life. During the fermentation steps, different yeast strains can be selected for the alcoholic beverage fermentation under limited oxygen flow. Generally, yeasts are predominant during the modern and large-scale beverage fermentation operations. The targets of the yeast strain selection are highly related to improving the economics of beverage production and its final quality. In addition, some post-fermentation procedures such as maturation and clarification are carried out to stabilize AFBs.

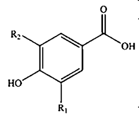
Therefore, it is evident that the fruit cultivars, the juice production process, and the yeast strain selection are the three most related parameters in a successful AFBs industry. This overview highlights the most important examples of these aforementioned parameters that are currently being developed to ensure sustainable, environmentally friendly, and cost-effective fermented products, as well as making improvements to the beverage quality.

2.1.1 Phenolic compounds in apples and pears

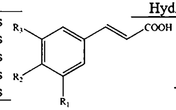
Phenolic compounds are a large group of bioactive compounds derived from the secondary metabolites of plants. They are synthesized through the shikimate and acetate pathways. In general, the polyphenolic components and their derivatives are divided into two major classes, flavonoids and non-flavonoids, based on the different chemical structures. Flavonoid compounds (C6-C3-C6 skeleton) comprise flavan-3-ols, flavanones, flavonols, isoflavones, dihydrochalcones, and anthocyanins, which could be ascribed from the oxidation and heterogeneity level in the C ring. Flavonoids are synthesized through the phenylpropanoid pathway (Zhai et al., 2019). Non-flavonoid compounds include hydroxybenzoates and hydroxycinnaminates.

Phenolic compounds contribute to the sensory properties and qualities of alcoholic beverages made from apples and pears, such as color, bitterness, and astringency perceptions. The phenolic profiles in AFBs mainly depend on the fruit cultivars and vinification conditions. Additionally, these compounds are known to be associated with various health benefits, such as being antioxidant, antidiabetic, antiobesity, anticancer, and anticardiovascular, as well as having anti-inflammation properties (Feng et al., 2021; Sun et al., 2017; Wang et al., 2017). These associations can be related to the inherent structures of phenolic compounds; compounds that have been concluded to involve a structure-activity phenomenon.

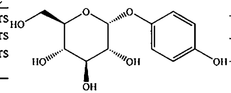
Non-Flavonoid:



Hydroxybenzoic acids	R_1	R_2	Occurrence
Protocatechuic	OH	H	Apples, pears
Gallic	OH	OH	Apples, pears
Vanillic	OCH_3	OH	Apples, pears
Syringic	OCH_3	OCH_3	Apples, pears
p-Hydroxybenzoic	H	OH	Apples, pears

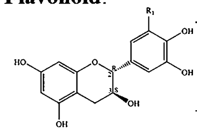


Hydroxycinnamic acids	R_1	R_2	R_3	Occurrence
p-Coumaric	H	OH	H	Apples, pears
Caffeic	OH	OH	H	Apples, pears
Ferulic	OCH_3	OH	H	Apples, pears
Sinapic	OCH_3	OH	OCH_3	Pears

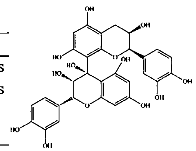


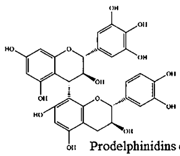
Other phenolic acids	Occurrence
Arbutin	Pears

Flavonoid:



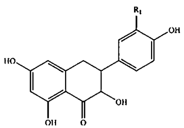
Flavan-3-ols	R_1	C-2	C-3	Occurrence
(+)-catechin	H	*R*	*S*	Apples, pears
(-)-epicatechin	H	*R*	*R*	Apples, pears
(+)-Gallocatechin	OH	*R*	*S*	Pears
(-)-Epigallocatechin	OH	*R*	*R*	Pears



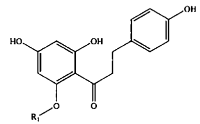


Prodelphinidins dimer B3

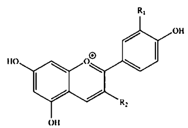
Proanthocyanidins	Extension units	Occurrence
Procyanidin	Epicatechin, Catechin	Apples, pears
Prodelphinidin	Epigallocatechin, Gallocatechin	Pears



Flavonols	R_1	Occurrence
Kaempferol	H	Apples, pears
Quercetin	OH	Apples, pears
Isorhamnetin	OCH_3	Apples, pears



Dihydrochalcones	R_1	Occurrence
Phloretin glucoside	glu	Apples
Phloretin xyloglucoside	xylglu	Apples



Anthocyanidins	R_1	R_2	Occurrence
Cyanidin-3-*O*-glucoside	OH	glu	Apples
Cyanidin-3-*O*-galactoside	OH	gal	Apples, pears
Peonidin-3-*O*-galactoside	OCH_3	gal	Pears

Figure 2. Structures of flavonoid and non-flavonoid compounds existing in apple and pear fruits.

2.1.1.1 Phenolic acids

Phenolic acids are non-flavonoid compounds derived from the secondary metabolites present in apples, pears, and their fermented products. They are derived biosynthetically from phenylalanine and tyrosine through phenylalanine ammonia-lyase via the shikimate pathway (Rosa, Moreno-Escamilla, Rodrigo-García, & Alvarez-Parrilla, 2018). The major phenolic acids present in apples and pears are primarily hydroxybenzoic acids (HBAs) and hydroxycinnamic acids (HCAs), which are shown in **Figure 2**.

In studies, HBAs have been found in low proportions in the total quantitated phenolics, mainly as the derivatives of protocatechuic, gallic, vanillic, syringic, and *p*-hydroxybenzoic acids in pome fruits (Fotirić Akšić et al., 2015; Kalinowska, Bielawska, Lewandowska-Siwkiewicz, Priebe, & Lewandowski, 2014; Vondráková, Malbeck, Trávníčková, Černý, & Cvikrová, 2020). Moreover, gentisic acid derivatives were also commonly found, but in trace amounts (Akšić et al., 2015). Generally, HBAs were present in the form of glucosides in apples and pears (Herrmann, 1989), whereas the HCAs were mainly derived from *p*-coumaric, caffeic, and ferulic acids. Sinapic acid derivatives were found to be rare in apples but have been found in pear cultivars (Feng et al., 2021; Fotirić Akšić et al., 2015). Unlike HBAs, HCAs were found in ester forms with quinic acid or glucose (Mattila, Hellström, & Törrönen, 2006). Chlorogenic acid (5-*O*-caffeoylquinic acid) was found as the most abundant HCA, whereas the other isomers of chlorogenic acid, 3-*O*-caffeoylquinic acid (*neo*-chlorogenic acid) and 4-*O*-caffeoylquinic acid (*crypto*-chlorogenic acid) were also commonly found in the fruit flesh and peel. Surprisingly, in the previous literature there are studies where both 3-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid have been used as chlorogenic acid (Clifford, Johnston, Knight, & Kuhnert, 2003; Naveed et al., 2018).

The oxidation reaction of phenolic acids by fruit polyphenol oxidase (PPO) has been reported to take place during the juice production and fermentation procedures (Yang et al., 2021). The preferential phenolic acids, caffeoylquinic acids, are catalyzed into corresponding *o*-quinones via PPO to form colored pigments, leading to the browning of fruit musts (Bourvellec, Quéré, Sanoner, Drilleau, & Guyot, 2004; Wilska-jeszka, & Pawlak, 1996; Queiroz, Mendes Lopes, Fialho, & Valente-Mesquita, 2008). The oxidation reactions might be enzymatic or nonenzymatic. In addition, the release of free HCAs and monosaccharides through partial hydrolysis of HCAs has been previously reported (Scalzini, Giacosa, Río Segade, Paissoni, & Rolle, 2021). The common free HCAs in AFBs are caffeic acid, coumaric acid, and ferulic acid. Moreover, gallic acid (free HBAs) has also been found in alcoholic beverages produced from apples and pears, and is released from the hydrolysis of hydrolyzable tannins (Xu et al., 2019; Yang et al., 2021). The activities of reductases have also been documented in previous studies (Ripari, Bai, & Gänzle, 2019; Svensson, Sekwati-Monang, Lutz, Schieber, & Gänzle, 2010). For example, strains of *Lactobacillus plantarum* reduced more than 75% of the ferulic acid content and produced dihydroferulic acid (the product of decarboxylation and reduction of ferulic acid) during lactic acid fermentations (Ripari et al., 2019).

2.1.1.2 Flavan-3-ols and proanthocyanins

As a major subclass of the flavonoid family, flavan-3-ols are commonly found in fruits and fruit beverages, mainly as (+)-catechin and (−)-epicatechin (**Figure 2**) (Feng et al., 2021; Kolniak-Ostek & Oszmiański, 2015; Laaksonen, Kuldjärv, Paalme, Virkki, & Yang, 2017). (+)-Catechin and (−)-epicatechin are derived biosynthetically from dihydroquercetin, and they are the main monomeric units of procyanidins (Lancaster, 1992). Moreover, (+)-gallocatechin and (−)-epigallocatechin derived from leucodelphinidin and dihydromyricetin are present in trace amounts and found in apples and pears as previously reported (Lancaster, 1992; Simirgiotis, Quispe, Bórquez, Areche, & Sepúlveda, 2016; Wang, Barrow, Dunshea, & Suleria, 2021).

Procyanidins, derived biosynthetically from the enzymatic reduction of leucocyanidins, are the most abundant phenylpropanoids found in unripe fruits. Both A-type linked via C2-*O*-C7 ether-type bonds and B-type procyanidins linked via C4-C6/C8 carbon-carbon bonds are found in apple and pear fruits. These structures are shown in **Figure 2** (Kolniak-Ostek, 2016b; Schempp, Christof, Mayr, & Treutter, 2016; Simirgiotis et al., 2016). In general, B-type procyanidins are more abundant than A-type procyanidins in pome fruits and their fermented products. In addition, prodelphinidins, consisting of (epi)-gallocatechin units, are also found in pears (mainly as B-type prodelphinidins via C4-C6/C8 interflavan linkage) (Es-Safi, Guyot, & Ducrot, 2006). Generally, the average degree of polymerization (DP) proanthocyanins are low in apples (ranged between 4.2 and 7.5) and pears (ranged between 2.8 and 7.6) (Brahem, Renard, Bureau, Watrelot, & Bourvellec, 2019; Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999). However, the DP of certain cider apples and perry pears can be relatively higher than the dessert fruits; for example, the average DP values of proanthocyanidins in French cider apples ('Guillevic' and 'Averolles') are 40 and 50, respectively, and that of perry pears ('Plant de Blanc' and 'De Cloche') 28 and 20, respectively (Brahem, Eder, Renard, Loonis, & Bourvellec, 2017).

During the fermentation process of AFBs, proanthocyanins are released by the following three types of reactions: 1) formation of low molecular weight proanthocyanins via chemical depolymerization of proanthocyanins, 2) formation of proanthocyanin-anthocyanin adducts, 3) conversion into phlobaphenes resulting in a reduction of both concentration and DP of proanthocyanins (Bourvellec, Watrelot, Ginies, Imberty, & Renard, 2012; Vidal, Cartalade, Souquet, Fulcrand, & Cheynier, 2002). Thus, terminal units are released as free (−)-epicatechin and (+)-catechin. In addition, lower content of proanthocyanins in fruit juice and wine products could be ascribed to its higher retention of cell wall materials and the lower water solubility of these compounds.

Proanthocyanidins are known to interact with the salivary proteins resulting in the tactile sensations of puckering, tightening, and dryness (Guerreiro et al., 2020). The taste (especially astringency and bitterness) of AFBs is influenced by the nature of the proanthocyanidins (condensed tannins), due mainly to their monomer composition and DP (Symoneaux, Chollet, Bauduin, Le Quéré, & Baron, 2014). Generally, higher concentrations and DP of proanthocyanidins leads to a higher intensity of bitterness and astringency in AFBs. The proanthocyanidins are less polymerized in low alcoholic beverages (cider and perry) than in grape wine products. The procyanidins are mainly polymers of epicatechin and catechin in apples and pears whereas those in grapes are in galloyllated forms (Symoneaux et al., 2014).

2.1.1.3 Flavonols

The major flavonols are flavonols in glycosylated forms, mainly kaempferol, quercetin, and isorhamnetin (**Figure 2**). Myricetin has also been detected in some pear fruits as previously reported (Wang et al., 2021). Glucose has been found to be the main sugar residue in the flavonol glycosides. The other sugar moieties like galactose, xylose, rutinose, rhamnose, and arabinose are also common in apple and pear fruits (Lancaster, 1992). Generally, flavonols are glycosylated at their 3-OH and 7-OH position in plant cells, which is achieved by uridine 5'-diphosphate (UDP)-carbohydrate-dependent glycosyltransferases (UGTs) (Yin et al., 2012). Flavonols are mainly concentrated in the fruit peel as the formation of flavonol compounds is highly light-dependent (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007; Lancaster, 1992). Exposure to sunlight generally induces flavonol synthesis and leads to a higher flavonol accumulation during fruit growth when compared with those growing in shade (Takos, Robinson, & Walker, 2006).

Flavonol profiles of AFBs can differed from those of their original fruit types due to the deglycosylation of flavonol glycosides during AFB fermentation. Thus, the flavonol profiles of AFBs comprise a mixture of flavonol glycosides and the corresponding free flavonol aglycones produced by hydrolysis. This results in the precipitation of flavonols in AFBs which can be ascribed to the low solubility and stability properties of an ethanol solution in an acidic environment like AFBs (Makris, Kallithraka, & Kefalas, 2006). Thus, aglycone quercetin was always found to be present in AFBs at low levels. In addition, flavonols may also bind with anthocyanins, a phenomenon known as co-pigmentation, resulting in a haze or precipitation in AFBs.

2.1.1.4 Dihydrochalcones

Dihydrochalcones, which are biosynthesized via the phenylpropanoid pathway, are widely distributed in apples (especially in unripe apples and apple tree leaves) but rarely found in pears, (Stompor, Broda, & Bajek-Bil, 2019). Phloridzin (phloretin glucoside) and phloretin xyloglucoside are the two major dihydrochalcones (**Figure 2**), which exist in apple skin, apple pulp, and seeds. The compositions and profiles of dihydrochalcones are highly dependent on apple cultivars. In addition to apple products, trace levels of phloridzin have also been detected in strawberries (Stalmach et al., 2010). Likewise, free phloretin aglycone is also released via the hydrolysis of phloretin glycosides (Madrera, Lobo, & Valles, 2006).

2.1.1.5 Other phenolic compounds

Anthocyanins are a class of important secondary metabolites formed by linking anthocyanin aglycones with sugars via glycosidic bonds. They are biosynthesized via the phenylalanine metabolic pathway. The main anthocyanin in apples are cyanidin glucosides, whereas both glucosides of cyanidin and peonidin are present in pears (Gao, Jiang, Cui, You, & Li, 2021). For pome fruits, light is necessary for anthocyanin synthesis. Generally, anthocyanins usually exist in the skin of red-skinned pome fruits (Mazza & Velioglu, 1992). In addition, some red-fleshed apple and pear cultivars contain anthocyanidins in both the fruit skin and flesh (Faramarzi et al., 2015; Mazza & Velioglu, 1992). Processing steps involved in the fermentation operations (**Figure 1**) alter the anthocyanin contents of the final AFB. During the fermentation of AFBs, anthocyanin aglycones like malvidin and peonidin can be broken down into syringic and vanillic acids, respectively. Conversion of anthocyanin glucosides into its aglycones and free phenolic acids has also been reported in previous studies. In addition, anthocyanidins may bind with tannins, proteins, and polysaccharides in the fruits, which also results in a haze or precipitation in AFBs (Vergara-Salinas et al., 2013). However, the co-pigments are more stable when compared with the anthocyanins during the wine aging process. Generally, lower concentrations of anthocyanins were found in the apple and pear fermented beverages in comparison with grape and other berry wines.

Arbutin corresponds with neither the phenolic acid groups nor the flavonoid groups; it is a hydroquinone glucoside detected in pear fruits, which has been used as a chemical marker for the authentication of pear products (Spanos & Wrolstad, 1992). Moreover, it has been reported to be a skin lightener used in cosmetics to inhibit the generation of the human pigment melanin (Cui, Nakamura, Ma, Li, & Kayahara, 2005).

2.1.2 Volatile organic compounds in apples and pears

Volatile organic compounds (VOCs) in fruit wine products can generally be divided into the following types: ethyl and acetate esters, higher alcohols, volatile acids, aldehydes, ketones, lactones, phenols, terpenes, and sulfur compounds. Knowledge of the quality-determining aroma and flavor properties in AFBs are essential for winemakers to control and to enhance the overall quality of AFBs. Generally, the perception of wine flavor and aroma is the result of a combination of chemical compounds and sensory receptors. VOCs present in a 'good' wine are usually complex and subtle. Only some of VOCs work as odor-active compounds, mainly in concentrations above their sensory perception threshold. In addition, synergistic or antagonistic interactions may arise from the interaction among different volatile and non-volatile metabolites, which will then determine taste and mouthfeel sensations. The wine type with a single dominant and strong aroma note is generally not highly valued by consumers. Thus, the identification of major VOCs, particularly with the perceived olfactory attributes (orthonasal and/or retronasal) of wines, is one of the long-standing goals of fruit wine research.

Table 1. Main classes of VOCs in AFBs.

Compound classification	***Description***	***Examples***
Primary	Compounds directly originated from fruits 1) persist unchanged 2) altered by fermentation process	Some acetate esters and higher alcohols
Secondary	Compounds derived from the yeast metabolism like sugars and amino acids	Most ethyl esters and fusel alcohols
Tertiary	Compounds formed during AFBs aging and storage conditions 1) bound with other chemicals 2) microbial spoilage or chemical tainting	Lactones, and trichloroanisole

In general, the major VOCs that contribute to the aroma profiles in AFBs can be divided into the following four groups: ethyl and acetate esters, higher alcohols, medium- and long-chain volatile acids, as well as aldehydes. In addition, there are also many minor VOCs like ketones, lactones, phenols, terpenes, and sulfur compounds, contributing to the complex volatile profiles of fruit wines. VOCs can be categorized as primary, secondary, and tertiary VOCs (**Table 1**), depending on whether they naturally originate from the intact fruit issue or are produced from the microorganisms performing fermentation

(Giannetti, Boccacci Mariani, Mannino, & Marini, 2017; Pando Bedriñana, Picinelli Lobo, Rodríguez Madrera, & Suárez Valles, 2020).

Most VOCs are directly derived from the original apple and pear fruits. In ripe apple fruit, the majority VOCs are esters (78–92%) and alcohols (6–16%), primarily in the form of hexyl acetate, butyl acetate, butan-1-ol, and hexan-1-ol (Dixon & Hewett, 2000; Setford, Jeffery, Grbin, & Muhlack, 2017; Yang, Hao, Meng, Li, & Zhao, 2021). In addition to these dominant VOCs, minor VOCs have also been detected, such as aldehydes, ketones, and terpenoids. In ripe pear fruits, the esters detected are the major VOCs, accounting for 60–99% of the total headspace vapors, followed by alcohols (1.5–4% of total headspace vapors), primarily ethyl acetate, butyl acetate, nonan-1-ol, and hepan-1-ol. Moreover, minor VOCs like alkanes, ketones, volatile acids, and aldehydes have also been detected (Chen et al., 2018). Both apple and pear VOCs are comprised of a wide range of esters, and esters provide the fruity and sweet odors of fresh mature fruits. Esters are mainly formed from the esterification of an alcohol (R-OH) and a carboxylic acid (R'-COOH) via enzyme alcohol acyltransferase (AAT) or post-fermentation through acid-catalyzed reaction (non-enzymatic). The AAT activity increases during the fruit ripening period, which resulted in higher production of esters as well as higher VOCs. However, the ester biosynthesis is limited by the accumulation of alcohols.

Linear alcohols are produced from the catabolism of fatty acids, whereas the metabolism of amino acids results in the production of branched-chain alcohols. Aldehydes can also be reversibly converted into alcohol via alcohol dehydrogenases (ADH), whereas its main precursor substances are fatty acids and branched-chain amino acids (Manríquez et al., 2006). In pome fruits, aldehydes predominated in the unripe fruits and then decrease during fruit ripening, in conjunction with the accumulation of esters and alcohols. However, a high concentration of aldehydes can be detected in homogenized juices, primarily hexanals and hexenals. In general, the aromatic amino acids can be the precursors of branched chain alcohols, carboxylic acid, and esters (Espino-Díaz, Sepúlveda, González-Aguilar, & Olivas, 2016). Volatile compounds accumulate quickly as the fruit ripens, and their compositions and profiles are highly dependent on the fruit cultivars. Moreover, there are distinct differences in the volatile profiles of commercial dessert fruit cultivars and wild fruit cultivars (*Section 2.2*).

Yeasts play an important role in producing and releasing large numbers of VOCs (mainly as esters, higher alcohols, and carbonyl compounds) during the fermentation of foods and beverages (Herrero, García, & Díaz, 2006). Higher alcohols tend to be the quantitatively and qualitatively dominant VOCs in AFBs in comparison with the other minor VOCs. Most of the higher alcohols originate from the yeast metabolism and contribute to the winey aroma of AFBs at low concentrations (less than 300 mg/L). The higher alcohols can contribute to a pleasant flavor with a content of less than 300 mg/L of the total higher alcohols, while provoking undesirable sensorial sensations at concentrations over 400 mg/L (Rajko & Janez, 1999). Around 75% of higher alcohols are formed and degraded via amino acid biosynthesis (Calugar et al., 2021). For example, 2-methyl-1-propanol is derived from valine, whereas 3-methyl-1-butanol is produced from leucine (Herrero et al., 2006). In addition, sugars are the main precursors of 25% of the higher alcohols. Thus, the total higher alcohol content in AFBs are closely correlated with the yeast assimilable nitrogen (YAN) contents in fruits. 2-Phenylethanol and 3-methyl-1-butanol were reported as the two primary higher alcohols originated from the yeast metabolism in apple ciders (Ye and Yue, 2014). In addition, some higher alcohols detected in apple juices, such as 6-methyl-5-hepten-2-ol, and (*E*)-2-hexen-1-ol, have been reported to be decreased or diminished during cider fermentation (Ye and Yue, 2014). In pear wines, the concentration of 2-phenylethanol, 3-methyl-1-butanol and hexanol showed relatively high concentrations, accounting for over 85% of total quantified higher alcohols (Yang, Zhao, Yang, Li, & Zhu, 2022).

Some esters originate in the fruit juices, however, the majority of esters are derived from yeast metabolism. In general, esters are the second most dominant group of VOCs in AFBs, and they play an important role in wine aroma by contributing to the sweet and fruity odors (Qin, Petersen, & Bredie, 2018). Ethyl acetate is the predominant ester in apple ciders, contributing to the sweet, pineapple and pungent odors in apple ciders. Other minor esters in apple ciders include ethyl esters (ethyl butanoate and ethyl decanoate) and acetate esters (3-methylbutyl, hexyl and phenethyl acetates). In pear wines, the most dominant esters are ethyl hexanoate, ethyl caprylate, ethyl caparate, and phenylethyl acetate, which together account for over 85% of the total esters (Liu et al., 2022).

2.1.3 Sugars, organic acids, and glycerol

The overall organoleptic character of AFBs produced from pome fruits and their byproducts is largely dependent on the water-soluble chemical compounds, especially sugars and organic acids. They are also indicators of the quality of AFBs during wine fermentation, and their composition and profile are essential for the final quality of AFBs (Ye, Yue, & Yuan, 2014).

The soluble sugars and sugar alcohols in apple and pear fruits are primarily glucose, fructose, sucrose, and sorbitol as well as some other minor sugar components. Fructose and glucose can be directly consumed by yeast strains, whereas sucrose is converted into fructose and glucose via sucrose synthase produced by yeasts, followed by the utilization of these monosaccharides (Amore, Russell, & Stewart, 1989). However, sorbitol is not entirely dydrogenated by only one aldehyde group, thus, it is not metabolized by yeast strains (Amore, Panchal, Russeil, & Stewart, 1988). Generally, the major soluble sugars in apples are fructose, sucrose, and glucose, whereas only around 3% of sorbitol is found in apple products. For example, glucose is transformed into pyruvate acid by yeast strains under fermentation, providing important precursor molecules for the biosynthesis of acetaldehyde (Suárez-Lepe & Morata, 2012). In other words, over 90% of sugars can be consumed by yeasts indicating a powerful fermentation capacity under a suitable fermentation environment.

In contrast to apples, pears have been reported to contain relatively high concentrations of unfermentable polyol sorbitol, resulting in a high concentration of unfermented sugars in the obtained perries (González Flores, Origone, Bajda, Rodríguez, & Lopes, 2021; Tanaka, Murata, Hashimoto, & Kawai, 2020). The concentration of sorbitol in the fruit wines are mainly dependent on the raw fruit materials. For example, the concentration of sorbitol is 4–6 g/L in a final cider, whereas sorbitol contents in pear fruit wines are significantly higher than those in the other fruit wines (Lee et al., 2013). Both apples and pears have a high concentration of sugars, however, in the case of fruits with a low concentration of sugars, such as berries, the addition of sugars or sugar syrup is necessary to keep the balance needed for the yeast to grow. According to the content of unfermented sugars in the AFBs, the fruit wines are categorized into dry wines (less than 4 g/L), medium-dry wines (between 4 and 12 g/L), semi-sweet wines (between 12 and 45 g/L), and sweet wines (over 45 g/L). The preference for dry or sweet wines differs among age and gender groups. Moreover, it may also change over region, time, and individual consumers (Dodd, Kolyesnikova, & Wilcox, 2010).

Glycerol (sugar alcohol) is the third largest byproduct of yeast fermentation after ethanol and CO_2. The concentration of glycerol is highly dependent on the sugar concentration, more glycerol is produced with an increasing content of fermentable sugar during fermentation (Suárez-Lepe & Morata, 2012). In general, the concentrations of glycerol and ethanol are inversely correlated. Glycerol does not relate to the volatile complexity of AFBs but does increase the smoothness and viscosity of AFBs. Previous studies have reported the taste threshold level of glycerol to be 5.2 g/L in wine products; however, the threshold level can be altered by the acidity and ethanol concentrations (Ivit, Longo, & Kemp, 2020).

Here, the term 'organic acid' refers to nonvolatile acids. Organic acids are either synthesized or consumed during yeast fermentation, which influences the overall quality of AFBs. The compositions and profiles of organic acids correlated with total acidity of AFBs. Strong acidity will lead to an excessive sourness and sharp taste; however, too low acidity may result in a flat taste with a less-defined flavor profile. Generally, the major organic acid in apple cider is malic acid, followed by succinic acid and citric acid. Malic acid is present as a residual acid in ciders, whereas succinic acid is the carboxylic acid synthesized during the fermentation process. In pear fermented products, the most abundant organic acids are present as malic and citric acids. Nevertheless, malic acid can be assimilated and degraded by some yeasts during the fermentation process to provide the fermented products with proper acidity and a pleasant flavor (Zhang et al., 2008). Moreover, organic acids can enhance the astringency effect provided by polyphenols (Ye et al., 2014).

2.2 Apple and pear crops

The bioactive compositions and profiles of the final apple ciders and pear perry products are strongly influenced by the apple (*Malus domestica* Borkh.) and pear (*Pyrus spp.*) crops. 'Hillwell', 'Gala', 'Delicious', and 'Fuji', 'Honeycrisp', and 'Granny Smith' are the main dessert apples (*Malus domestica* Borkh.) discussed in this section, whereas *Pyrus communis* L., *Pyrus pyrifolia* Nakai, *Pyrus bretschneideir* Rdhd., *Pyrus ussuriensis* Maxim., and *Pyrus bretschneideri* × *Pyrus communis* are introduced as important examples of pears. Generally, the term 'fruit quality' remains a dynamic concept, which develops constantly with changes in consumer preferences and perceptions. Fruit quality is mainly associated with its appearance (color, shape, size, absence of defects), and external and internal quality (firmness, soluble solid content, titratable acidity, starch, volatiles, and non-volatiles). In this section, the cultivar differences, the maturity levels of the fruit, the environmental factors (pre-harvest), agronomic practices (pre-harvest), and storage conditions (post-harvest) have been comprehensively discussed.

2.2.1 Effect of cultivar types, rootstocks, and fruit development

The fruit quality is directly related to the fruit cultivars, and many quality factors are genetically dependent (Starowicz, Achrem-Achremowicz, Piskuła, & Zieliński, 2020). The chemical compositions and concentrations of pome fruits are highly associated with cultivars and tissues, including the peel, the flesh, and the seeds. As regards phenolic compounds, proanthocyanidins are the dominant phenolic compounds in most of the apple and pear varieties (Heinmaa et al., 2017). However, Carbone, Giannini, Picchi, Scalzo, and Cecchini (2011) found that flavonols represented 51% of the total phenolic contents in the cultivar 'Hillwell' of a new Braeburn clone (*Malus domestic* Borkh.).

The edible parts of apple and pear fruits are the peel and flesh, and higher phenolic components, especially flavonols, anthocyanins, and flavan-3-ols, are located in the fruit peels when compared with the flesh tissues (Galvis-Sánchez, Fonseca, Gil-Izquierdo, Gil, & Malcata, 2006; Wang et al., 2015). However, the arbutin content is higher in the pear flesh than in its peel (Galvis-Sánchez et al., 2006). Phloridzin has been reported as the most abundant individual phenolic compound found in the seeds of apple fruits, accounting for 79–92% of monomeric polyphenols (Fromm, Bayha, Carle, & Kammerer, 2012). Moreover, the seeds of cider apples were found to contain higher amounts of phloridzin and catechin than the seeds of dessert apples (Fromm et al., 2012). In general, cider apples or perry pears were reported to contain higher amounts of polyphenols than dessert apples or pears. In general, the dessert apples and pears are popular, due to their pleasant taste and aroma complexity, whereas the cider apples and perry pears are smaller, bitter and richer in phenolic compounds (Renard, et al., 2017). In addition, pome fruits with red flesh can be used to obtain apple products with appearances that are attractive to consumers and have health benefits. The red-fleshed apples contained high levels of organic acids, mainly malic acid, as well as the phenolic compounds, such as anthocyanins and dihydrochalcones, whereas the white-fleshed apples contained higher concentrations of phenolic acids and flavan-3-ols (Bars-Cortina, Macià, Iglesias, Romero, & Motilva, 2017).

In addition to different cultivar types, fruit cultivars with the same breeding parents may have different phytochemical compounds in the fruits. This could have resulted from the the bulk of genetic variation during fruit development and ripening (Verma et al., 2019). In the case of apples, the majority of cultivated apples are bi-colored with different intensities of the red skin color, and many of these apple productions are based on the limb mutations from 'Gala', 'Delicious', and 'Fuji' (Iglesias, Echeverría, & Lopez, 2012; Iglesias, Echeverría, & Soria, 2008). Some mutations have problems with their appearance, such as their skin color, e.g., decrease in the color of the apple skin, especially in warm climates. This result can be ascribed to the differential methylation of structural gene promoters in apple mutants, which influence the anthocyanin biosynthesis pathway (Jiang et al., 2019). In addition, growth seasons are also important for the apple peel color and anthocyanin content. Moreover, dwarfing rootstocks are usually smaller and can provide better light exposures to the canopy when compared with the semi-vigorous or vigorous stocks (Racsko & Schrader, 2012). A previous study has demonstrated that the rootstock affects apple ripening, quality, size, and mineral composition at harvest and during storage (Autio, 2019). For example, the super dwarfing rootstock P22 was found to result in fruits with a higher content of organic acids (by 45%) and antioxidant activities (up to 44% of free radical scavenging activity) when compared with the P60 dwarfing rootstock (Laužikė, Uselis, & Samuolienė, 2021). The rootstock effects are mainly ascribed to the differences in the sunlight environment during fruit development and ripening.

Pome fruits are harvested at different stages of maturity, for example, unripe fruits are used for cold storage, and ripe fruits are readily sold on the market. In addition, some beverage industries prefer to use the overripe fruits to obtain fermented products with high amounts of VOCs (Harker & Hallett, 2019). The fruit becomes soft and edible during the fruit ripening, and produces intense amounts of VOCs because ethylene production is also associated with the aroma production (Li et al., 2014).

The concentration of carbohydrates changes during the fruit development and ripening period. In general, sorbitol showed a tendency to decrease during the fruit development, whereas fructose and glucose have been reported to increase during the fruit development period after bloom (Zhang, Li, & Cheng, 2010). These results could have been due to the conversion of sorbitol to fructose and glucose by sucrose synthase (Teo et al., 2006; Zhou, Cheng, & Dandekar, 2006). In addition, the accumulation of sucrose before the fruit harvest may result from the sucrose synthase (Berüter, 2004). The concentrations of organic acids showed a trend of increasing during14 days after full bloom (DAFB), and then starting gradually to decrease from 28 DAFB until fruit harvest. The synthesis of phenolic compounds continues until the fruit is fully ripened (Zhang et al., 2010). However, some of the phenolic compounds, such as phenolic acids, flavan-3-ols, and procyanidins in the fruit flesh decrease during the ripening of the fruit before harvest (Hagen et al., 2007). Moreover, most of the flavonols in the fruit peel decrease during the fruit development and ripening (Awad, Jager, Plas, & Krol, 2001). Anthocyanin biosynthesis is developmentally regulated (regulation of skin color in apples); the contents of flavonols and proanthocyanidins are high in juvenile fruit, decrease in concentration during fruit growth, and then increase during fruit ripening (Wojdyło, Nowicka, Turkiewicz, Tkacz, & Hernandez, 2021). In some cultivars, anthocyanins also increase during ripening.

2.2.2 Effects of environmental factors

The fruit quality is highly associated with the environmental conditions found where they developed, especially the levels of sunlight availability, temperature, and water characteristics (Li et al., 2020). For example, pome trees grown in a suitable area produced fruit cultivars which had a better quality and needed less utilization of pesticides.

Table 2. Effects of environmental factors on the chemical compositions and quality of apple and pear crops.

Apple/pear	***Species/Cultivar type***	***Variation of factors***[a]	***Variation of chemical compositions and quality***	***References***
Apple	*Malus domestica* Borkh	Temperature ↑ (25–35 °C)	Anthocyanidins ↑	(Hamadziripi, Theron, Muller, & Steyn, 2014)
Apple	*Malus domestica* Borkh	Temperature ↓ (24–15 ℃)	Fruit size ↓	(Stanley et al., 2000)
Apple	*Malus domestica* Borkh	Sunlight ↑ (1–5 $\mu g \cdot mm^{-1}$)	Color ↑, flavonols ↑, chlorogenic acid ↔	(Jakopic, Stampar, & Veberic, 2009)
Apple	*Malus domestica* Borkh	Water ↑	Chlorogenic acid ↑, hyperoside ↓, protocatechuic acid ↓, caffeic acid ↓	(Huang et al., 2021)
Apple peel and flesh	*Malus domestica* Borkh	Temperature ↓ (7–27 °C)	Phenolic compounds ↑, antioxidant activity ↑	(Yuri, Neira, Quilodran, Motomura, & Palomo, 2009)
Apple and pear leaf	*Malus domestica* Borkh, *Pyrus communis* L.	Temperature ↓ (6–12 °C)	Fruit quality ↔	(Heide et al, 2005)
Pear	*Pyrus bretschneideri* × *Pyrus communis*	Natural light ↑	Anthocyanin ↑, hydroxybenzoic acid ↑, procyanidin ↑, flavonol ↑	(Wei et al., 2020)
Pear	*Pyrus pyrifolia* Nakai	Natural light ↑	Phenolic compounds ↔, anthocyanins ↑, whole fruit quality ↓	(Shi et al., 2021)
Pear	*Pyrus communis* L. var. S. Bartolomeu	Sundrying	Phenolic compounds ↓, procyanidins ↓	(Ferreira et al., 2002)

Compared to normal environments: ↑ increase; ↓ descrease; ↔ similar level.

[a]The variation of temperature/sunlight ranged from the given values.

Pome fruits are grown in many areas of the world, but not all the areas can be described as optimal areas for pome fruit production. Pome trees present a moderate growth in the areas of western Europe with cool days, cool nights, and relatively higher rainfall, and the fruit quality is quite high. In some regions of the US, Italy, and New Zealand, where the climate is characterized as warm days and cool nights together with low rainfall, pome trees grow productively with a balance between the sugars and organic acids. Apple and pear production is relatively high in these areas and in this ideal climate the quality is also good. In the areas of southern Australia, central Chile, and California, the climate is characterized as warm days and moderate nights, and the rainfall remains low; the growth of apple trees is moderate together with a pleasant flower bud formation. However, in those areas with warm nights and days and with a relatively high rainfall, such as China, Japan, and southern France, the productivity of the pome fruit trees is lower than in the other three climates; this can be due to the loss during the warm nights of 40-50% of sugars produced during the day. In addition, growing fruits in greenhouses with elevated carbon emissions and high temperatures from flowering to fruit maturity leads to a reduction in sugar, protein, and mineral contents (DaMatta, Grandis, Arenque, & Buckeridge, 2010).

Temperature has a notable influence on pome fruit production, especially on fruit sizes (**Table 2**). In general, pome fruits grow in two different phases: a cell division phase and a cell expansion phase. Cold weather (low temperature) leads to a reduction in the fruit size during the early exponential cell division period, especially in the first 50 DAFB (Stanley et al., 2000). The expansion rates were temperature dependent during the cell division periods, with high temperatures accelerating fruit development (Mertoğlu, Akkurt, Evrenosoğlu, Çolak, & Esatbeyoglu, 2022). Researchers have found that the phenolic composition and antioxidant properties of berries were significantly higher in winter than those growing in summer (Xu, Zhang, Zhu, Huang, & Lu, 2011). The fruit sizes of berries were 10 times larger when grown at 20 °C rather than 6 °C (Warrington, Fulton, Halligan, & De Silva, 1999). Researchers have found that apples grown in the cooler agro-climatic regions have a high phenolic content and antioxidant capacity (Yuri et al., 2009). In addition, anthocyanin biosynthesis is also light dependent. Agar, Biasi, and Mitcham (1999) found that pears harvested from the warmer growing regions benefit from postharvest cold storage or ethylene treatment to keep the fruits' color. However, Heide and Prestrud (2005) found that low temperature treatments showed no significant effects on apple and pear fruits or their leaves. This can be the result of the inverse correlation between the growing temperature and anthocyanin accumulation. For example, the fruits from the out-layer canopy contain significantly higher concentrations of anthocyanins than those from the inner-layer canopy in apple cultivars. This can be ascribed to the higher average peel temperature of the fruits in the outer canopy (Hamadziripi et al., 2014). These result could also be ascribed to the higher ultra−violet (UV) radiation in the outer-layer canopy of apple trees (Hamadziripi et al., 2014).

Apart from the temperature, sunlight is also highly associated with fruit quality. Skin color is increased by lengthening the exposure of pome fruits to sunlight as the process of anthocyanin biosynthesis is light dependent (Ubi et al., 2006). A major function of phenolic compounds is to prevent the fruits from damage from UV light; plants growing in conditions of high UV radiation contain higher phenylalanine ammonia-lyase (key enzyme for biosynthesis of flavonol), which leads to a high content of flavonols in fruits growing in conditions with considerable sunlight (Schmitz-Hoerner & Weissenböck, 2003). Wei and his co-workers (2020) found that sunlight improved the accumulation of anthocyanins in the fruit peel of 'Xiyanghong' (*Pyrus bretschneideri* × *Pyrus communis*). The content of hydroxybenzoic acids, procyanidins, and flavonols also increased with exposure to natural light (Wei et al., 2020). In a previous study, a higher content of flavonoids and anthocyanins in the coloration was found in the apple cultivar 'Fuji' when grown in sunlight (Jakopic et al., 2009). However, the content of chlorogenic acid showed no difference in this study (Jakopic et al., 2009). Excess UV radiation can lead to crop losses ranging from 5 to 10%, as a result of "sunburn", which has been defined as a physiological disorder resulting from the high UV radiation and has also led to economic problems during the production of pome fruits (Racsko & Schrader, 2012). There are three kinds of sunburn damage: sunburn browning, sunburn necrosis, and photooxidative sunburn. Of these, sunburn browning, which leads to a yellowish and brownish color, is the most common sunburn disease. Ferreira and his coworkers (2002) found that hydroxycinnamic acids and procyanidins in a Portuguese pear cultivar (*Pyrus communis* L. var. S. Bartolomeu) showed a notable decrease in phenolic compounds after sun drying, and procyanidins with a high DP were found to become unextractable. Sunburn necrosis led to cell death when the fruit peel temperature reached 52 °C for 10 min (Pasquariello et al., 2013; Racsko & Schrader, 2012). These diseases can also be caused by relatively low humidity, a high temperature, and a strong wind velocity. To prevent the pome fruits from sunburn damage, new technologies/strategies such as evaporative cooling, shading nets, and fruit bagging treatments have been widely used in orchards growing pome fruits (Racsko & Schrader, 2012).

Pome fruits are commercially important fruits grown in semi-arid and arid regions. Thus, a good water supply is also important for growing pome fruits as pome fruits are very sensitive to water fluctuations. Excessive rainwater can trigger fruit development, e.g., brown coloration in the fruit peel (Shi et al., 2021). Shi and his co-workers (2021) found that a rainfall treatment and a water flushing treatment decreased the final quality of pome fruits from Japanese pear trees (*Pyrus pyrifolia* Nakai). Previous studies have also found that chlorogenic acid in 'Honeycrisp' apple fruits increased with the irrigation levels, whereas the content of hyperoside, protocatechuic acid, and caffeic acid decreased (Huang et al., 2021).

2.2.3 Effect of agronomic practices

Agronomic practices also affect the overall fruit quality, mainly as regards the orchard design, row orientation, pruning, thinning, pollination, and irrigation. Their effects on the quality of apples and pears are clearly shown in **Table 3**. Nowadays, high density planting (HDP) is widely used in orchard design, and is dependent on the following factors: the cultivars and their interaction with the rootstocks, canopy density, orchard layout, tree architecture, as well as the light interception efficiency (Subedi, Atreya, Gurung, Giri, & Gurung, 2020; Wünsche, Palmer, & Greer, 2000). An optimum fruit quality with a good size, ripening uniformity, and a good storage life is the result of a good balance between the canopy and the root, and the pruning currently applied in the commercial orchards. Generally, HDP leads to a decrease in fruit quality and coloration (Lordan, Francescatto, Dominguez, & Robinson, 2018). However, fruit weight was not altered by tree density, spacing geometry, or tree height. The row orientation, however, affects the fruit quality by altering the light interception, especially color of the fruit (Wünsche et al., 2000). An East-West row orientation showed notably fewer light interceptions when compared with North-South rows, and the effects of row orientation on fruit size and quality are higher in the lower latitudes than in the higher latitudes (Li et al., 2020). Moreover, the fruits located on the western side of the row were exposed to increased sunburn damage.

Table 3. Effects of agronomic practices on the chemical compositions and quality of apples and pears

Apple/pear	***Species/Cultivar type***	***Variation of factors***	***Variation of chemical compositions and quality***	***References***
Apple	*Malus domestica* Borkh	HDP	Fruit quality ↓, color ↓, fruit yield ↑, light interception ↑	(Lordan et al., 2018)
Apple	*Malus domestica* Borkh	Root pruning	Tree size ↑, fruit firmness ↑, color ↑	(Khan, McNeil, & Samad, 1998)
Apple	*Malus domestica* Borkh	High AH	Phenolic compounds ↑, color ↑	(Zhang et al., 2016)
Apple	*Malus domestica* Borkh	RDI	Phenolic compounds ↑	(Keivanfar, Fotouhi Ghazvini, Ghasemnezhad, Mousavi, & Khaledian, 2019)
Apple	*Malus domestica* Borkh	BGs	Procyanidin ↓, (−)-epicatechin ↓, flavonol ↓	(Li et al., 2021)
Pear	*Pyrus communis* cv Triunfo of Viena	RDI	No significant difference	(Vélez-Sánchez, Balaguera-López, & Alvarez-Herrera, 2021)
Pear	*Pyrus communis* L.	PGRs	Fruit firmness ↑, total soluble solids ↑, ascorbic acid ↑, total titratable acidity ↑, phenolic acids ↑, flavonols ↑, and antioxidants activity ↑	(Gilani et al., 2021)
Pear	*Pyrus pyrifolia* Nakai	BGs	Anthocyanin ↑, peel color ↑	(Huang et al., 2009)
Pear	*Pyrus bretschneideir* Rdhd.	BGs	Organic acid ↓	(Zhai et al., 2014)

Compared to no agronomic practices: ↑ increase; ↓ descrease; Abbreviations: HDP, high density planting; AH, air humidity; RDI, regulated deficit irrigation; BGs, bagging treatments; PGRs, plant growth regulators.

Pruning can alter the crop load and fruit quality by adjusting the number of flower buds, reducing the competition among the growing fruit, and producing good fruit quality, especially the fruit size (Breen et al., 2014; Lauri, 2009). The size of the apple tree can also be controlled by the root pruning, which helps with fruit firmness and coloration (Khan et al., 1998). There is also the issue of the competition between the growing shoots and the fruit for carbohydrates, and thinning has been proven to have a strong effect on carbohydrate availability; thus, thinning will alter the fruit size and quality, as well as the crop load (the amounts of fruits produced per tree or branch unit). Hand thinning, mechanical thinning, and chemical thinning are widely applied in commercial orchards, and these methods can be used separately or in conjunction with each other (Bound, 2021). Chemical thinning can be one of the most efficient ways to thin fruit trees, and has been categorized into two types: caustic thinners to damage the flower and hormone thinners to induce some form of stress and then alter the physiological events in the pome plants (Dennis, 2000). For example, the utilization of plant-derived nutrient solution (PDNS) can promote the vegetative growth and fruit quality of the 'Whangkeumbae' and 'Hosui' pear cultivars (*Pyrus pyrifolia* Nakai) (Wu et al., 2019). Zhang and his co-workers (2016) found that high air humidity (AH) enhanced the phenolics and coloration in the apple peel of cultivar 'Fuji' when compared with low AH treatment, mainly the chlorogenic acid, ferulic acid, (–)-epicatechin, quercetin-3-*O*-rutinoside, cyanidin-3-*O*-galactoside.

Pollination is important for apple growth as it plays a significant role in the fruit set and fruit quality. The flowers of pome fruits are hermaphroditic, however, the fruit set is relatively low (10%) as most of the pome cultivars have gametophytic self-incompatibility. Insect pollination plays an important role in pome fruit cross-pollination; honey bees are the main pollinators (Wünsche et al., 2000). Inadequate pollination always resulted in a low number of seeds, misshapen fruit as well as low calcium concentrations in the fruits (Barcelos Bisi, Pio, Locatelli, da Hora Farias, & Barbosa Silva Botelho, 2021). In addition, irrigation is also important to the management of soil water during fruit growth. During the fruit growing season, the application of deficit irrigation (DI) technology to control the water supplement helps in the control of the fruit growth and increases the fruit quality by improving the fruit acidity and color (Wünsche et al., 2000). However, deficit irrigation can also lead to a reduction in the crop load and increase sunburn damage (Mpelasoka, Behboudian, & Green, 2001). Vélez-Sánchez, Balaguera-López., and Alvarez-Herrera (2021) found that moderate, regulated deficit irrigation (RDI) did not produce negative effects on the pear fruits (*Pyrus communis* cv Triunfo of Viena). Keivanfar, Ghazvini, Ghasemnezhad, Mousavi, and Khaledian (2019) found that the combination of RDI and a superabsorbent polymer significantly affected the phenolic compounds, soluble solid content, and titratable acidity of apple fruits. Moreover, a reduction of the RDI percentage led to an increase in phenolic compounds in ‘Granny Smith’ apples (Keivanfar et al., 2019). Application of micronutrients and plant growth regulators (PGRs) is regarded to be the best way to enhance nutrient balance and its accumulation in the fruits, and also increases the crop yield and quality. Gilani and his co-workers (2021) found that PGRs significantly enhanced fruit firmness, total soluble solids, pH value, ascorbic acid concentration, total titratable acidity, phenolic acids, flavonols, and antioxidants activity (*Pyrus Communis* L.).

Bagging treatments (BGs) are widely applied in commercial orchards during pome fruit growth. The reduction caused by BGs in the phenolic content of apple fruits, both commercial apples and wild apples, has been well demonstrated in previous study, especially regarding the content of procyanidins, (−)-epicatechin, and flavonols (Li et al., 2021). In addition, BGs have also been reported to influence the anthocyanin content and color visualization of Red Chinese sand pears (*Pyrus pyrifolia* Nakai) (Huang et al., 2009). The re-exposure to the sunlight after BGs slightly altered the concentrations of flavonols and total phenolic compounds, and anthocyanins and reddening occurred extremely rapidly (Huang et al., 2009). However, the effects of BGs were also genotype dependent. The bagging effects on the content of flavonols have also been investigated in the pear cultivar ‘Zaosu’ (*Pyrus bretschneideir* Rdhd.) (Zhai et al., 2019). In addition, the BGs did not affect the sugar content in the fruits but decreased the content of organic acids (Zhai et al., 2019).

2.2.4 Effect of storage conditions

Suitable storage technologies have to be applied to preserve the quality of pome fruits, prevent the loss of nutritional components and maintain a high fruit quality (Francini & Sebastiani, 2013; Neven, Drake, & Shellie, 2001). It is crucial to control the main environmental factors during pome fruit storage, such as storage temperature, relative humidity, light, oxygen, carbon dioxide, and air circulation. A previous study found that phenolic acids and flavonols were stable during storage, except for flavan-3-ols which decreased notably throughout the storage time (Galvis-Sánchez et al., 2006). In addition, the concentration of arbutin was found to increase over time in pear fruits (Galvis-Sánchez et al., 2006).

Storage temperature plays an important role in the storage stability and shelf-life of pome fruits as it alters the metabolic activity of fruit tissues. For example, ‘Granny Smith’ is an apple variety that needs a long ripening period, and low-temperature stress accelerates its maturation (Pérez-Ilzarbe, Hernández, Estrella, & Vendrell, 1997). The total phenolic content behaves differently in the pulp (phenolic composition decreased) and peel (phenolic composition increased) after cold treatment (Pérez-Ilzarbe et al., 1997). Generally, low temperatures decrease metabolic activities and biochemical reactions during storage. Thus, the fruit ripening is slowed down as well as the biological aging process. The low temperature conditions (LTC) decrease the expression levels of lipoxygenase, polyphenol oxidase activities, as well as the content of malondialdehyde in the fruits, thus decreasing the biosynthesis of phenolic compounds (Burda, Oleszek, & Lee, 1990; Li, Cheng, Dong, Shang, & Guan, 2017; Wang et al., 2020). Carbone and his coworkers (Carbone et al., 2011) found that phenolic compounds notably decreased during storage at 0−1 °C for 3 months, with a reduction of 50% in the apple flesh and 20% in the apple peel. However, the content of ascorbic acid in the apple peel did not show significant change (Carbone et al., 2011). Zhou and his co-workers (2015) found that LTC could effectively maintain the esters and fatty acids of ‘Nanguo’ pears (*Pyrus ussuriensis* Maxim.) during storage. Generally, LTC played a crucial role in the stimulation of ethylene biosynthesis during fruit development and the ripening period. In addition, the microorganism activities were also reduced or even inhibited when stored at low temperatures. However, low temperatures can also lead to cold sores (certain physiological disorders) in the fruits, thus, keeping the fruit tissues at moderate temperatures is recommended. Different fruit cultivars show different resistance to low temperatures as has been previously reported (Robiglio, Sosa, Lutz, Lopes, & Sangorrín, 2011). For example, the optimal temperature (storage condition) for apples is from −1 to + 4 °C, whereas that for pears is around 0 °C.

The relative humidity (RH) directly affects the sweating process of the fruit tissues, and storage spaces with low humidity levels may result in an intense sweating process. However, RH significantly increases the growth of microorganisms, leading to fruits with an unpleasant flavor and taste. Generally, moderate RHs (90−95% for apples, 80−90% for pears) in storage environments with a suitable ambient temperature are highly recommended for storing fruit samples (Chen, Yan, Feng, Xiao, & Hu, 2006; Moran, DeEll, & Halteman, 2009). Tu, Nicolaï, and De Baerdemaeker (2000) found that a high RH at 95% could significantly enhance the juice yield and total soluble solids during cold storage at 2 °C of apples when compared with low RH values (30% and 65%). However, high RH was also reported to enhance peel browning and loss of fruit firmness, as well as the incidence of fruit cracking during cold storage (Lee, Mattheis, & Rudell, 2019).

The oxygen and carbon dioxide concentrations greatly affected the storage capacity of pome fruits. Controlling the oxygen concentrations limits the respiratory activity of the fruit tissues, further reducing the oxidation, ethylene production, and physiological disorders of fruits (Bílková et al., 2020). Bílková and his co-workers (2020) found that phenolic compounds benefitted during long-time storage under ultra-low oxygen (ULO) conditions, however, ULO storage conditions did not exhibit significant effects on apples during short-time storage (Bílková et al., 2020). Zlatić and his co-workers (Zlatić et al., 2016) found that ULO helps maintain fruit firmness and suppresses the synthesis of VOCs in ‘Bartlett’ pears, such as esters and aldehydes, when compared with normal atmosphere treatments in cold storage (from −1 to +1 °C). However, the concentration of carbon dioxide increased when the oxygen content decreased (Zlatić et al., 2016). A relatively high concentration of carbon dioxide also reduces respiratory activity, oxidation, and browning, as well as causing a delay in fruit ripening and aging. Undesirable phenomenon occurred in an extensive carbon dioxide environment, such as internal fermentation of the fruit, alcohol accumulation, aldehyde accumulation, caves in the appearance, as well as esters degradation. Air circulation homogenizes the temperature, humidity, oxygen, and carbon dioxide, as well as the VOCs released from the fruit metabolism.

A controlled speed for the air circulation needs to be carried out in the storage system and thus maintaining a stable environment. Controlled-atmosphere (CA) storage is common practice when extending the storage period of pome fruits and maintains the fruit quality and sensory properties. Lara and his co-workers (2003) found that CA suppressed the synthesis of ethyl acetate, hexyl acetate, butyl acetate, acetaldehyde, butan-1-ol, and hexan-1-ol during long-term storage conditions in pear fruits. As an ethylene inhibitor, 1-methylcyclopropene (1-MCP) prevents fruits from the action of both exogenous and self-produced ethylene. Kumar and Thakur (2020) found that ‘Bartlett’ pears treated with 1000 nl/L of 1-MCP together with cold storage significantly increased the shelf-life of the fruit tissue to 120 days, whereas the fruits placed in cold storage lose their sensory acceptability after 90 days. Rizzolo, Cambiaghi, Grassi, and Zerbini (2005) found that both 25 nl/L and 50 nl/L of 1-MCP treatment kept the flesh flavor of ‘Conference’ pears for 14 weeks, such as butanol and ethyl butanoate, whereas the untreated pears became watery or grainy. In addition, CA showed the synergistic effects of 1-MCP treatment on the VOCs of pear fruits.

In addition, light also affects the storage capability of pome fruits, by promoting the redox processes in pome fruits. Thus, it is crucial to keep the fruits in a dark environment. Assumpção and his co-workers found that an increasing UV-B radiation enhanced the concentrations of hydroxycinnamic acids and anthocyanins whereas a reduction in the flavonol content was observed in apple fruits (Assumpção et al., 2018). UV treatment did not influence the emission of volatile compounds (Yang, Balandrán-Quintana, Ruiz, Toledo, & Kays, 2009).

Table 4. Effects of storage conditions on the chemical compositions and quality of apples and pears.

Apple/pear	*Species/Cultivar type*	*Variation of factors*	*Variation of chemical compositions and quality*	*References*
Apple	*Malus domestica* Borkh	UV-B ↑	Hydroxycinnamic acid ↑, anthocyanins ↑, and flavonols ↓	(Assumpção et al., 2018)
Apple	*Malus domestica* Borkh	RH ↑	Juice yield ↑, total soluble solids ↑	(Tu et al., 2000)
Apple	*Malus domestica* Borkh	RH ↑	Peel browning ↑, fruit firmness ↓, fruit cracking ↑	(Lee et al., 2019)
Apple	*Malus domestica* Borkh	ULO	Long-term storage: phenolic compounds ↑, short-term storage ↔	(Bílková et al., 2020)
Apple	*Malus domestica* Borkh	Temperature ↓	Flesh: phenolic compounds ↓, peel: phenolic compounds ↑	(Pérez-Ilzarbe et al., 1997)
Pear	*Pyrus communis* L.	Ethylene ↑	Uniform and ripening ↑	(Agar et al., 1999)
Pear	*Pyrus ussuriensis* Maxim. Cv 'Nanguo'	LTC	Esters ↔, fatty acids ↔	(Zhou et al., 2015)
Pear	*Pyrus communis* L.	ULO	Fruit firmness ↑, esters ↓, aldehydes ↓	(Zlatić et al., 2016)
Pear	*Pyrus communis* L.	CA	Ethyl acetate ↓, hexyl acetate ↓, butyl acetate ↓, acetaldehyde ↓, butan-1-ol ↓, and hexan-1-ol ↓	(Lara et al., 2003)
Pear	*Pyrus communis* L.	CA	Ethyl acetate ↓, hexyl acetate ↓, butyl acetate ↓, acetaldehyde ↓, butan-1-ol ↓, and hexan-1-ol ↓	(Kumar & Thakur, 2020)
Pear	*Pyrus communis* L.	1-MCP	Butan-1-ol ↓, ethyl butanoate ↓	(Rizzolo et al., 2005)

Compared to untreated storage conditions: ↑ increase; ↓ descrease; ↔ similar level. Abbreviations: UV-B, ultra-violet B radiation; RH, relative humidity; LTC, low temperature conditioning; ULO, ultra-low oxygen; CA, controlled-atmosphere; 1-MCP, 1-methylcyclopropene.

2.3 Apple and pear juice processing

The composition of fruit wines also depends on juice production methodologies. Juices used in fermentation are mainly extracted from the whole fruits. To obtain a juice production with a high and reasonable quantity is one of the basic technological aims of the beverage industries (**Figure 1**). In general, juice production includes various steps, such as preparation, extraction, clarification, and stabilization. The final quality of the juice, such as nutritional characteristics, sensory properties, and health benefits, is highly influenced by these aforementioned steps. In addition, utilization and selection of suitable technologies for preventing the deterioration of various volatile and non-volatile compounds during juice production is another challenge for the beverage industry.

2.3.1 Effect of juice extraction methods

The principles and methods of extracting fruit juices from well-prepared fruits differ greatly. Generally, there are three extraction methods that have been widely applied in beverage industries: crushing, milling and fruit-grinding (Fernández-Jalao, Sánchez-Moreno, & De Ancos, 2019). All these extraction steps influence the juice yield, flavor, chemical compositions, and final quality of the obtained products (Álvarez et al., 2012). In addition, the sensory and organoleptic properties of the fruit juices are also highly altered by different extraction methods, as summarized in **Table 5**.

Table 5. Effects of juice extraction methods, enzyme treatments, and pasteurization methods on the chemical and sensory properties of obtained juices.

Processing methods	***Obtained products***	***Effect on the chemical and sensory properties***	***References***
Extraction methods			
RFP, BEP, and WP	Apple juice	RFP: phenolics ↓, sensory properties ↑; BEP: phenolics ↑, sensory properties ↓; WP: total antioxidant activity ↑, sensory properties ↑, VOCs ↑	(Heinmaa et al., 2017)
SP, BAP	Apple juice	SP: juice yield ↑, soluble solids ↑, viscosity ↑, phenolic compounds ↑, antioxidant activity ↑, acidity ↓	(Wilczyński, Kobus, Nadulski, & Szmigielski, 2020)
SFP, BEP	Cloudy apple juice	SFP: juice yield ↑, phenolic compounds ↑	(De Paepe et al., 2015)
SFP	Apple juice	SFP: fresh aroma ↑	(Wibowo et al., 2019)
Grinding under vacuumn condition	Apple juice	Color ↑, antioxidant activity ↑	(Kim et al., 2017)
Enzyme treatments			
Pectinase	Apple juice	Juice yield ↑, phenolic compounds ↑, antioxidant activity ↑, viscosity ↓, turbidity ↓	(Oszmiański, Wojdyło, & Kolniak, 2011)
Pectinase	Apple juice	YAN ↓, amino acids ↑, sulfur off-aromas ↓	(Ma et al., 2018)
Pectinase	Apple mash, juice, and pomace	Methanol ↑, *n*-propanol ↓, iso-butanol ↓, and iso-amylalcohol ↓	(Zhang et al., 2011)
Pectinase	Pear juice	Juice yield ↑, color ↑, titratable acidity ↑, total sugars ↑, pH values↓	(Han, Liu, Li, Wang, & Ni, 2019)
Exopolysaccharide	Cloudy apple juice	Juice stability ↑	(Housseiny, Abo-Elmagd, & Ibrahim, 2013)
Pasteurization methods			
OFC	Apple puree	Phenolic compounds ↑, color ↑, off-flavors ↑	(Kim et al., 2021)
PEF, OH	Apple and carrot juices	Color ↑, antioxidant activity ↑,	(Mannozzi et al., 2019)

Table 5 (Continued)

Processing methods	***Obtained products***	***Effect on the chemical and sensory properties***	***References***
OH (40V/CM, 80 °C)	Apple juice	Ascorbic acid ↑, phenolic compounds ↑, color ↑	(Abedelmaksoud, Mohsen, Duedahl-Olesen, Elnikeety, & Feyissa, 2018)
HPP (600 Mpa, 3 min), PEF (15.5 Kv/CM, 158 KJ/L)	Apple juice	PEF: odor-active VOCs ↑, ((*E*)-2-hexenal ↑ and hexyl acetate ↑), HPP: VOCs ↑	(Kebede et al., 2018)
UV-C light (40 °C, 97.33 mJ/cm^2)	Apple juice	Phenolic compounds ↑, flavonols ↑, antioxidant activity ↑, ascorbic acid ↑, and anthocyanin ↑	(Yıkmış, Barut Gök, Levent, & Kombak, 2021)
UV-C light	Apple and grape juices	Sensory quality ↑	(Barut Gök, 2021)
US	Apple juice	Phenolic compounds ↑, sugar ↑, mineral compounds ↑, and carotenoids ↑	(Abid et al., 2014)
HPCD (65 °C, 20 MPa)	Apple juice	(+)-catechin ↑, (−)-epicatechin ↑	(Murtaza et al., 2020)

↑ increase; ↓ descrease; ↔ similar level. Abbreviations: RFP, rack-and-frame press; BEP, belt press; WP, water press; SP, screw press; BAP, basket press; SFP, spiral-filter press; OFC, oxygen-free conditions; PEF, pulsed electric field; OH, ohmic heating; HPP, high hydrostatic pressure; UV-C, ultra-violet C radiation; US, ultrasound; HPCD, high pressure CO_2 processing.

Pressing is one of the traditional ways of extracting juices from fruit pulps. During pressing, the fruit material is mixed, shredded, crushed, triturated, and then pressed into juices. In general, the fruit proanthocyanidins can be retained in the pomace as they may associate with the cell wall matrix. The phenolic compositions can be changed due to the oxidation process via polyphenol oxidase during the crushing and pressing processes (Weber & Larsen, 2017). Traditionally, a rack-and-frame press (RFP) is used for small-scale fruit juice production. However, it is a slow process with a long exposure of mashed fruits in the air, which leads to a loss of ascorbic acid and phenolic compounds. Heinmaa et al. (2017) found that apple juices produced from RFP contained the lowest amount of most phenolics detected when compared with juices produced with other pressing methods. New pressing technologies have been used in the commercial pressing of fruit juices to speed up the process as well as increase the composition of the obtained juice products. The utilization of a basket press (BAP) leads to a high juice yield, but it is less often used as a commercial pressing method. A screw press (SP) is becoming more popular on small farms, and an SP always results in a higher content of soluble solids and polyphenols and ascorbic acid (Wilczyński, Kobus, & Dziki, 2019).

Moreover, belt presses (BEP) and water presses (WP) are also used by small-scale juice producers (Heinmaa et al., 2017). Thus, it is important to select an appropriate press method during the juice processing procedure. Compared with RFP treated juices, BEP treated juices were reported to contain higher polyphenol contents, but inferior sensory properties. Whereas WP treated juices had a high total anthoxidant capacity as well as high aroma intensity when compared with juices produced from RFP and BEP (Heinmaa et al., 2017). Wilczyński, Kobus, and Dziki (2020) found that the application of an SP for apple juices notably enhanced the extraction juice yield when compared with juice produced using a BAP, and also produced a higher concentration of soluble solids, viscosity, phenolic compounds, and antioxidant activity; although it resulted in lower acidity in the final apple juice (Wilczyński et al., 2020). For certain fruit cultivars, excessive soft fruit pulp leads to more flesh penetration into the fruit juices. For example, apple juices produced from cultivars with high-hardness (‘Granny Smith’ and ‘Modi’) showed higher phenolic compounds and ascorbic acids than those from apples with a medium hardness (Wilczyński et al., 2020). In addition, a spiral-filter press (SFP) has also been applied as a novel extraction technology in commercial juice production to perform the solid-liquid separation of the juices and pomaces in low oxygen environments. The spiral-filter press can be used to produce juices with a higher juice yield and phenolic content when compared to the traditional BEP (De Paepe et al., 2015). Moreover, an SFP helps to maintain the fresh apple juice aroma as previously reported (Wibowo et al., 2019).

Another way to process fruit juices with good quality is the utilization of a grinding technique. It is an effective way to increase the extractability of volatiles and non-volatiles from the fruit pulps. Grinding the fruit pulp increases the expression of phenolic compounds (anthocyanins and proanthocyanidins) in fruit juice products when compared with pressing (White, Howard, & Prior, 2011). However, the contents of flavonols were not significantly altered by the grinding process. Escobedo Avellaneda and his co-workers (2014) have investigated the grinding step during orange juice processing and found that it resulted in a reduction of antioxidant activity, carotenoid contents, hydroxycarotenoid, and carotene, whereas the phenolic concentrations increased during the grinding process. Moreover, grinding in a vacuum environment can improve the color and antioxidant activity of the obtained apple juices (Kim et al., 2017). This result can be ascribed to the limited enzymatic browning that occurs during the low oxygen diffusion in the juice production.

2.3.2 Effect of enzyme treatments

Enzymatic treatments are commonly applied in the industry during recent years to decrease the sugar and soluble dry matter contents, and increase the juice yield and clarification level (**Table 5**). The enzymes help soften the fruit tissues and release the cell contents, leading to a higher yield in the juice produced. During the clarification process, the insoluble materials in the pressed juices are broken down, and a reduction in the viscosity and opacity of the cloudy juices occurs. In general, the enzymatic degradation depends on the enzyme types and concentrations, incubation temperatures and times, agitation, pH values as well as the combinations of different enzymes (Sharma, Patel, & Sugandha, 2017). Moreover, the increasing enzyme dosage and incubation temperature help in the enhancement of juice yield and clarification. The enzymes commonly used in juice production are pectinases, cellulases, hemicellulases, and amylases from food-grade microorganisms.

Pectinases are used in fruit juice production to increase the yield, clarification, liquefaction, and filterability of the obtained juice products, and also to release the flavor compounds, hydrolysis of biomacormolecules, such as proteins, polysaccharides, starch, and agar, and increase the maceration and extraction of fruit tissues (Patidar, Nighojkar, Kumar, & Nighojkar, 2018). There are many types of pectic enzymes used in the extraction and clarification of fruit juices, and the most commonly used for commercial preparations are a mixture of pectin lyases (EC 4.2.2.10), pectin methylesterases (EC 3.1.1.11), and polygalacturonases (EC 3.2.1.15) (Patidar et al., 2018). The action of pectin lyases is to break the glycosidic linkages at C−4 and then eliminate H from C−5, in a non-hydrolytic breakdown of pectates or pectinates. Pectin methylesterase is a carboxylic acid esterase, and it is used for removing the methoxyl groups from pectin. In addition, the polygalacturonases hydrolyze the α-(1-4) linkages between the D-cgalacturonic acid and D-methyl galacturonic acid units. Pectinases affect the chemical profiles, rheology, and sensory characteristics of fruit juices. The yield of apple juices was significantly increased from 82% to 92% with pectinase treatments (Oszmiański et al., 2011). In addition to increasing the juice yields, pectinases can also help in the release of chemical components from cell wall material (Oszmiański et al, 2011). The total polyphenol yields (polymeric procyanidins, dihydrochalcones, and flavonols) and the antioxidant activities were increased by treatment with Pectinex AFP L−4, Pectinex Yield Mash, and Pectinex XXL, reducing the viscosity and turbidity of the juice (Oszmiański et al., 2011). This could be ascribed to the production of a pectin-protein complex, and this product can be removed by centrifugation (Lee, Yusof, Hamid, & Baharin, 2006). However, in some studies, the pectinase treatments lowered the total phenolic compositions in apple juices, blueberry juices, black currant juices, and cherry juices (Landbo, Pinelo, Vikbjerg, Let, & Meyer, 2006; Meyer, Köser, & Adler-Nissen, 2001; Sandri, Lorenzoni, Fontana, & da Silveira, 2013). In addition, pectinase treatments can limit the production of sulfur off-flavors during the cider production (Ma et al., 2018). Pectinase treatments of the apple mash, juice, and pomace have been reported to result in a higher methanol production whereas the concentrations of *n*-propanol, iso-butanol, and iso-amylalcohol were not significantly influenced (Zhang, Woodams, & Hang, 2011). The apple brandy made from pectinase treated apple products were reported to contain higher concentrations of methanol and ethanol (Zhang et al., 2011). Moreover, pectinase treatments can also influence the sensory characteristics of fruit juices. For example, enzymatic treatments with Pectinase 714L significantly increased the total proanthocyanidins and led to higher mouth-drying and puckering astringency in the black currant juices (Simirgiotis et al., 2016). The addition of pectinase produced by *Aspergillus niger* significantly increased the juice yields from 60% to 72% in pear juices, and notably increased the color, titratable acidity, and total sugars of the treated pear juices. In addition, the pH value and relative viscosity of the treated pear juices decreased after enzymatical treatments using *Aspergillus niger* (Han et al., 2019).

Application of cellulases can hydrolysis the cell wall polysaccharides, substituted celluloses and their derivatives, as well as degrade the cellulose into glucose. Cellulases are used for the extraction and clarification of fruit juices as well as the production of oligosaccharides in the juice products. Enzymatic treatments by cellulases produced significant effects on the yield of lingonberry juices increasing the yield from 70% to 81%, and also increasing the concentrations of most phenolic and volatile compounds (Marsol-Vall, Kelanne, Nuutinen, Yang, & Laaksonen, 2021). The content of sugars and organic acids in lingonberry juices showed limited change after enzymatic treatments (Marsol-Vall et al., 2021). Moreover, exopolysaccharides produced from *Eup. Pinetorum* can also be used as a natural flavor stabilizer in cloudy apple juices (Housseiny et al., 2013).

Hemicellulases are used to hydrolyze the hemicelluloses, whereas amylase is used to breakdown the starch into sugars and is also used for juice clarification (Sharma et al., 2017). These enzymes were generally used in two steps: 1) in the partial or complete liquefaction after fruit crushing, which helps to increase the juice yield and shorten the process time; 2) in the clarifying of the fruit juices after juice extraction, which helps to reduce the viscosity of fruit juices, as well as increase the filtration rate and stability of the obtained juices.

2.3.3 Effect of thermal and non-thermal treatments

Different thermal treatments such as the conventional thermal processing, thermovinification, flash release, and infrared radiation, as well as the non-thermal treatments such as pulsed electric fields, high-pressure carbon dioxide, high-pressure processing, sonication, and ultraviolet are used in juice processing (**Figure 1**). They are used to ensure the safety, stability, and shelf-life of the fruit juices by reducing microorganism growth and enzymatic oxidation. In addition, the utilization of these methods can also lead to nutrient loss as well as influence the physical properties of the juice products. Thus, it is important to select the preservation method by assessing the changes in the chemical compounds.

Conventional thermal processing (TP) technologies, including pasteurization and sterilization treatments, have been widely used in commercial juice processing. Conventional thermal processes provide fruit juices with food safety and a longer shelf-life. However, thermal treatments can lead to several undesirable issues in the treated fruit juices, including the degradation of physicochemical compounds and nutrients, as well as the creation of off-flavors (Kim et al., 2021). Thermal treatments also reduce the enzymatic activities such as polyphenol oxidase, thus, minimize the juice spoilage (Kim et al., 2021).

Currently, novel thermal technologies are popular in the beverage industry (**Table 5**). Kim and his co-workers (2021) also found that thermal treatment of apple puree under oxygen-free conditions (OFC) helps maintain the phenolic compounds and juice color. Ohmic heating (OH) generates internal heat in the fruit juices with the application of short burst of high voltage between two electrodes in the fruit juice (Jiménez-Sánchez, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2017). In general, OH helps in destroying the cell membranes of microorganisms with no intended heating (Mannozzi et al., 2019). Mannozzi and his co-workers (2019) found that the application of OH

(80 °C) significantly enhanced the juice color and antioxidant activity. Application of OH (40 V/cm, 80 °C) significantly reduced the PPO activities and increased the content of phenolic compounds, carotenoids, and color values when compared with conventional TP (90 °C, 60 s) (Abedelmaksoud et al., 2018). In addition, OH treated carrot juices obtained a higher level of consumer acceptance than that previously reported (Rodríguez et al., 2021). Nowadays, nanofluid thermal processing (NTP) has been used in several studies for fruit juice processing (Bhattacharjee, Saxena, & Dutta, 2019) as a novel method for thermal pasteurization. In general, NTP provides a notably higher thermal conductivity than the other heating fluids (Jafari, Saremnejad, & Dehnad, 2017). Jafari et al. (2017) concluded that more energy could be saved by the application of alumina nanofluid, resulting in a better retention of lycopene, ascorbic acid, and color when compared with the conventional fluid (water).

Non-thermal processes can help in keeping the original features of fruit juices (**Table 5**). Thus, they can be used as alternative methods to TP. The non-thermal processes used for fruit juice processing include 1) pulsed electric field; 2) ultraviolet irradiation; 3) ultrasound processing; 4) high hydrostatic pressure; 5) high pressure CO_2 processing (Bhattacharjee et al., 2019). A pulsed electric field (PEF) is a promising non-thermal processing technology to inactivate the enzymatic activities and pathogenic microorganisms (Dziadek et al., 2019). The PEF process contains some heat with a maximum temperature at 40 °C during the juice processing which is much less than conventional thermal processing temperatures (Puértolas & Barba, 2016). Kebde et al. (2018) found that apple juices processed with PEF (15.5 KV/cm, 158 KJ/L) showed a high retention of odor-active VOCs, such as (*E*)-2-hexenal and hexyl acetate, whereas the thermal treatments (72 °C, 15 min) led to a reduction in concentrations of VOCs. Microchip-PEF (350 V) treated bilberry juice showed a higher content of ascorbic acid and better sensory qualities when compared with juices treated with high temperature short time sterilization (HTST) (Zhu et al., 2019). Actually, only small changes were found in the juices treated with microchip-PEF regarding odor, taste, and VOCs (Zhu et al., 2019).

UV light processing is a dry cold process which is easy to use in the commercial juice production, and can destroy most of the foodborne pathogens. The UV light varies from 100 to 400 nm and could be categorized as UV-A (320 – 400 nm), UV-B (280 – 320 nm), and UV-C (200 – 280 nm). Among these UV lights, UV-C light is commonly used for the commercial fruit juice processing. In addition, UV-A light treatment showed synergistic interactions with acid treatment, pressure treatment, and mid-thermal treatment in the clarified apple juice that enhanced the bacterial inactivation (Hamadziripi et al., 2014). According to Yıkmış, Barut Gök, Levent, and Kombak (2021), treatment with a moderate temperature UV-C light (40 °C, 97.33 mJ/cm^2) resulted in a higher content of phenolic compounds, flavonols, antioxidant activity, ascorbic acid, and anthocyanin content in apple juices when compared with the other UV-C doses and conventional thermal treatments. Pendyala, Patras, Ravi, Gopisetty, and Sasges (2020) found that UV-C light treatment (40 mJ/cm^2) maintained the aldehydes to a high degree during processing. Moreover, Barut Gök (2021) reported no significant changes in the sensory quality attributes (appearance, color, odor, and taste) of the treated apple and grape juices being caused by UV-C treatment. Ultrasound (US) treatment is also widely applied and it breaks the cell membrane of the juice microorganisms, which leads to the death of microorganisms (Piyasena, Mohareb, & McKellar, 2003). Abid and his co-workers (2014) found that US could enhance the content of several phenolic compounds, sugars, mineral elements, and total carotenoids in apple juices, whereas the total anthocyanins and electrical conductivity showed no significant differences before and after sonication. Moreover, Wang, Liu, Xie, and Sun (2020) found that US (600 W, 10 min) together with UV treatment of mango juice could totally inactivate the pathogen bacteria, PPO, and POD, and the phenolic compounds and carotenoids remained unchanged before and after the treatments.

As a non-thermal cold pasteurization technology, high hydrostatic pressure (HHP) provides juice products with pressure containing fluids such as water. The pressure is maintained between 400 and 700 MPA in the commercial system for a desired holding time. In addition, the pressures used for enzyme inactivation is generally higher than that for microbial inactivation (San Martín, Barbosa-Cánovas, & Swanson, 2002). Wibowo et al. (2019) investigated the retention of VOCs in HPP treated cloudy apple juices and found the best to be among those juices treated with HPP, TP, and PEF. High pressure CO_2 processing (HPCD) helps to damage the microbial growth with a non-toxic gas (CO_2) and it keep the original features of the juice products. Satisfactory results have been obtained with HPCD treatments as it can inactivate the microorganisms and enzymatic activities as well as keep the high amounts of bioactive components present in the original juice (Zhao et al., 2018). Compared with other high pressure treatments, HPCD involves lower pressure (less than 40 MPa) with high commercial benefits (Liu et al., 2010). In addition, the processing temperatures ranging from 20 °C to 60 °C limit the thermal degradation of bioactive compounds. Compared with TP (75 °C), apple juices treated with HPCD (65 °C, 20 MPa) showed higher retention of (+)-catechin and (−)-epticatechin as previously reported (Murtaza et al., 2020). The reason for this could be the high inhibition of PPO and POD enzymes by HPCD.

2.4 Yeast strains used in AFBs

For centuries, fermentation of AFBs was conducted spontaneously with a mixture of native microorganisms with a large amount of non-conventional yeast strains. A well-practiced fermentation technology can be regarded as one of the most crucial factors that can enhance the quality of AFBs. Generally, the concept of controlled fermentations with specific yeast strains has been widely accepted and introduced since the 1970s with the utilization of *Saccharomyces cerevisiae* (*S. cerevisiae*). In the 1980s, researchers then began to investigate the positive effect of several other *Saccharomyces* and non-*Saccharomyces* yeast strains in fruit wine making. In comparison to *Saccharomyces cerevisiae*, other *Saccharomyces* and non-*Saccharomyces* yeasts showed poor fermentability, a slower fermentation rate as well as a lower tolerance to harsh conditions. However, the application of these yeasts in wine making can contribute to the aroma complexity with the proper inoculation and fermentation. In general, yeast selection is a crucial driver of innovative fermentation processes aimed at improving the quality of AFBs, such as the sensory quality, stability, and freshness (Fresno et al., 2022). The traditional role of the yeast is to transform the sugars into ethanol and other metabolites to provide the pleasant organoleptic characteristics of AFBs. The selection of suitable yeast strains is based on the following criteria:

1) the ability to enhance the aroma complexity via the production of VOCs (mainly higher alcohols and esters);
2) the presence of polyalchols (mainly glycerol and 2, 3-butanodiol) as well as the release of mannoproteins and polysaccharides;
3) the absence of *β*-glucosidase activity to prevent the degradation of phenolic compounds;
4) the stabilization of colloidal compounds in AFBs, and to help stabilize the phenolic compounds.

In this section, a summary of yeast classification and identification and their contributions to the fermentation process will be presented. Pure inoculation and co-inoculation of *Saccharomyces* yeasts and non-*Saccharomyces* yeasts and their effects on the quality of AFBs are comprehensively discussed.

2.4.1 *Saccharomyces cerevisiae* in AFBs

S. cerevisae yeast strains are ascosporogenous yeast strains that are widely employed in commercial AFB fermentation, such as apple cider (Cimolai, Gill, & Church, 1987). As conventional commercial yeasts, *S. cerevisiae* strains have a strong fermentative power under harsh environment conditions, for example, low pH values and low oxygen availability (Albergaria & Arneborg, 2016). *S. cerevisiae* yeast is regarded as one of the most fermentative-prone yeast species and exhibits rapid and complete fermentation when compared with the other yeast strains. The optimum pH varies between 4.5 and 6.5 and the temperature between 20 and 30 °C. Therefore, *S. cerevisiae* is widely used as wine starters in AFB processing, such as the fermented beverages of apple, pear, banana, watermelon, red dragon, pineapple, and different berry fruits (Jiang, Lu, & Liu, 2020; Leforestier et al., 2015; Lin et al., 2018; Lorenzini, Simonato, Slaghenaufi, Ugliano, & Zapparoli, 2019; Ogodo, Ugbogu, Ugbogu, & Ezeonu, 2015; Onwuka & Awam, 2001; Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004; Sun, Jiang, & Zhao, 2011;Wang, Xu, Hu, & Zhao, 2004;Yang et al., 2019).

2.4.2 Other *Saccharomyces* yeasts

In addition to *S. cerevisiae*, several species in the genus *Saccharomyces* are also widely applied in AFB fermentation, such as *S. paradoxus*, *S. bayanus*, *S. kudriavzevii*, *S. mikatae*, and *S. uvarum* (**Table 6**) (Bruner & Fox, 2020). They are found to be phylogenetically close to each other showing high levels of sequence similarity (James, Cai, Roberts, & Collins, 1997). These yeast strains represent high growth properties during wine fermentation with strong tolerance to high concentrations of sugars and ethanol. Among these other *Saccharomyces* yeasts, *S. paradoxus* and *S. bayanus* have been studied to investigate their potential use in the production of AFBs (Hutzler, Riedl, Koob, & Jacob, 2012). In comparison to *S. cerevisiae*, other *Saccharomyces* yeast strains present a slower fermentation rate and a lower tolerance to the high alcohols in AFBs (Bruner et al., 2020). However, there are clear benefits supporting the use of these yeast strains in fermentation such as improved sensory complexity (mainly complex VOCs) and a more rounded palate structure in the AFBs.

Table 6. Effects of non-conventional *Saccharomyces* yeast strains on the chemical compositions and sensory properties of AFBs.

Yeast species	*Products*	*Fermenta -tion type*	*Main effects on the chemical compositions and sensory properties*	*Reference*
S. paradoxus	Apple wine	PF	Glycerol ↑, volatile acidity ↓	(Satora et al, 2018)
	Apple wine	SimF SC	Ethanol ↑, methanol ↑, volatile esters ↑, volatile acids ↑	(Satora et al, 2018)
S. bayanus	Apple cider	PF	Free HCAs ↑	(Laaksonen et al., 2017)
	Apple wine	PF	Malic acid cand carbonyl compounds ↑	(Satora et al, 2018)
	Apple wine	SimF SC	Ethanol ↑, methanol ↑, volatile esters ↑, volatile acids ↑, wine quality ↑.	(Satora et al, 2018)
	Apple brandy	PF	Acetate ester↑, ethyl acetate↓, sensory quality ↑	(Januszek et al, 2020)
	Apple brandy	SimF SC	VOCs↑, acetaldehyde↑, methanol↑, fusel alcohols↑, sensory quality↓	(Januszek et al, 2020)
	Pear wine	PF	Hexanoic acid↑, octanoic acid↑.	(Liu et al., 2022)
	Pear wine	SeqF TD	Ester↑, acidc, ethyl esters↑	(Liu et al., 2022)
S. uvarum	Apple cider	PF	2-Phenylethanol ↑	(Lorenzini et al., 2019)

Compared to S. cerevisiae fermentation: ↑ increase; ↓ descrease; PF: pure fermentation, SimF SC: simultaneous fermentation with *S. cerevisiae*, SeqF TD: sequential fermentation with *T. delbrueckii*, HCAs: hydroxycinnamic acids, VOCs: volatile organic compounds.

S. paradoxus was the first wild *Saccharomyces* species found in the early twentieth century, which widely existed in nature. Later, it was demonstrated to be a hybrid of *S. cerevisiae* and *S. eubayanus* based on its genomic sequence data (James et al., 1997). *S. paradoxus* is able to ferment glucose, sucrose, and maltose, but unable to ferment lactose, melibiose, or starch. Generally, *S. paradoxus* is a stable and natural hybrid *Saccharomyces* species widely used in the production of grape wines. However, only limited information exists on the use of this species in the production of AFBs. According to Satora, Cioch, Tarko, and Wołkowicz (2016), the apple wine produced from *S. paradoxus* showed higher residual sugars (mainly fructose), acetone, and acetaldehyde as well as lower volatile esters when compared with products produced with *S. cerevisiae*. The optimal growth temperature of *S. paradoxus* is 15 °C, 7 °C lower than that of *S. cerevisiae* whereas the optimum pH ranges from 4.6 to 4.8. The pure inoculation of *S. paradoxus* has been reported to lead to intense fruit and floral aromas in the final fermented products (Nikulin et al., 2020). In comparison to *S. cerevisiae*, *S. paradoxus* has been reported to produce a significantly higher content of glycerol and lower levels of acetic acid as well as a lower ethanol yield in the final AFBs (Orlić, Arroyo-López, Huić-Babić, Lucilla, Querol, & Barrio, 2010).

S. bayanus (*S. cerevisiae* × *S. eubayanus* × *S. uvarum*) is another hybrid *Saccharomyces* commonly used in AFBs (Ono, Greig, & Boynton, 2020). Generally, *S. bayanus* is represented by two varieties: *S. bayanus* var. *bayanus* and *S. bayanus* var. *uvarum* (Pogorzelski, Kobus, Kowal, Kordialik-bogacka, & Wilkowska, 2007). The optimal growth temperature for *S. bayanus* varies from 10 to 21 °C, and it can be used for the production of low temperature AFBs. It shows both positive and negative fermentation of melibiose, but it is unable to ferment lactose or starch. According to Pogorzelski et al (2007), pure inoculation of *S. bayanus* in pear wines resulted in higher amounts of ethanol (16.5 vol.%), glycerol, succinic acid, and malic acid when compared with *S. cerevisiae*. Moreover, *S. bayanus* significantly increased the malic acid levels and carbonyl compounds in apple wines (Satora, Szczurak, Tarko, & Bułdys, 2018). In addition, fermentation operated by *S. bayanus* has been reported to synthesize more 2-phenylethanol, ethyl lactate, 2-phenylethyl acetate, and other acetate esters as well as producing less isobutanol, isoamyl alcohol, and amyl alcohol (Gamero, Belloch, Ibáñez, & Querol, 2014). Januszek, Satora, Wajda, and Tarko (2020) found that the ulitization of *S. bayanus* in apple brandies produced better acetate esters with the exception of ethyl acetate. A single inoculation of *S. bayanus* in apple brandies enhanced the sensory properties of apple brandies. Moreover, the spontaneous fermentation led to higher acetaldehyde, methanol, and fusel alcohols. This result could be due to the higher β-glucosidase activities of the non-*Saccharomyces* yeasts, which release more VOCs from glycosidic forms. In addition, Liu et al. (2022) found that the utilization of *S. bayanus* in Jinchuan pear wine resulted in higher amounts of VOCs in the final products when compared with products fermented with *S. cerevisiae*, especially esters, acids, and phenols. Co-inoculation of *S. bayanus* with other non-*Saccharomyces* led to a significantly higher content of esters and lower levels of volatile acids when compared with the single inoculation of *S. bayanus*.

S. bayanus var. *uvarum* is fairly well-known in the genus *Saccharomyces* family and often referred as *S. uvarum*. *S. uvarum* has the capacity to ferment glucose, surcose, melibiose, and maltose, but it is unable to ferment lactose (Almeida et al., 2014). In comparison to *Saccharomyces cerevisiae*, the inoculation of *S. uvarum* in grape wines led to a reduction in the acetic acid and an increase of glycerol and succinic acid (Masneuf-Pomarède, Bely, Marullo, Lonvaud-Funel, & Dubourdieu, 2010). Moreover, the utilization of *S. uvarum* in apple ciders resulted in higher amounts of the higher alcohols and acetate esters when compared with *S. cerevisiae* (Lorenzini, Simonato, Slaghenaufi, Ugliano, and Zapparoli, 2019).

2.4.3 Non-*Saccharomyces* yeasts

Nowadays, the utilization of non-*Saccharomyces* yeasts is widely applied in fruit juice fermentation both in pure fermentation or co-inoculation with *S. cerevisiae* or other yeast strains (**Table 7**). In recent studies, different commercial non-*Saccharomyces* have been investigated (Jiang, Lu, & Liu, 2020; Benito, 2019). It has been reported that elected non-*Saccharomyces* could be utilized in AFBs in order to: enhance the content of glycerol, reduce the alcoholic content (*Candida stellata*), decrease the content of malic acid (*Schizosaccharomyces pombe*), decrease the volatile acidity (*Torulaspora delbrueckii*), control the spoilage microbes (*Metschnikowia pulcherrima*), and reduce the production of medium-chain fatty acids (*Lachancea thermotolerans*). In this section, the utilization of *Torulaspora delbrueckii* (*T. delbrueckii*) and *Schizosaccharomyces pobme* (*S. pombe*) are introduced in detail as they were utilized in the apple and pear fermentations in this research work, whereas the other non-*Saccharomyces* yeast strains are only introduced briefly in order to describe their application in the production of AFBs.

Table 7. Effects of non-*Saccharomyces* yeasts on the chemical compositions and sensory properties of AFBs.

Yeast species	***Products***	***Fermentation type***	***Main effects on the chemical compositions and sensory properties***	***Reference***
Torulaspora delbrueckii	Apple cider	PF	Phenolic compositions ↔	(Laaksonen et al., 2017)
	Apple cider	PF	Total sugars ↑, ethanol ↓, acetaldehyde ↑, higher alcohols ↓	(Fejzullahu et al, 2021)
	Apple cider	PF	Gluose ↑, fructose ↑, sorbitol ↑, ethanol ↓, benzyl alcohol ↑	(Lorenzini et al., 2019)
	Apple cider	SimF SC	Total sugars ↓, volatile acidity ↓,ethanol ↓, higher alcohols ↓, esters ↑, fruitness ↑	(Fejzullahu et al, 2021)
	Pear wine	PF	Ethyl hexanoate↑, ethyl decanoate↑, ethyl 9-decenoate↑	(Liu et al., 2022)
	Pear wine	PF	Ethanol ↑, succinic acid ↓	(Wei et al., 2019)
	Pear wine	SeqF SC	VOCs ↑, esters↑, fatty acids ↓, terpenoids ↑	(Yang et al., 2022)
	Apple-blended pear wine	SeqF SC	VOCs ↑, esters↑, fatty acids↓	(Yang et al., 2022)
Torulaspora quercuum	Apple cider	PF	Ethanol ↑, citric acid ↓	(Wei et al., 2019)
Schizosaccharomyces pombe	Apple wine	PF	Organic acid ↓, glycerol ↑, esters ↑, and acetic acid ↑	(Satora et al., 2018)
	Apple wine	SimF SC	Malic acid ↓,ethanol ↑, methanol ↑, volatile esters ↑, volatile acids ↑	(Satora et al., 2018)
Pichia kluyveri	Apple wine	PF	Ethanol ↓,sugar ↑, oxalic acid ↑, malic acid ↑, VOCs ↑, acetate esters ↑	(Wei et al., 2019)
	Apple cider	PF	Hexyl acetate ↑, low ethanol ↓, sugar ↑, ethyl acetate ↑	(Gschaedler, 2017)
	Apple cider	SimF HU	Sugar ↑, ethanol ↓, glycerol ↑, acetic acid ↑, VOCs ↑, acetate esters ↑	(Wei et al., 2020)
Pichia membranaefaciens	Apple cider	PF	Fermentation rate ↓, ethanol ↓, VOCs ↑	(Gschaedler, 2017)
Hanseniaspora uvarum	Apple cider	PF	Hexyl acetate ↑, isoamyl acetate↑.	(Lorenzini et al., 2019)
	Apple wine	PF	Acetic acid ↑, citric acid ↓, succinic acid ↓, VOCs↓	(Wei et al., 2019)

Table 7 (conutinued)

Yeast species	***Products***	***Fermentation type***	***Main effects on the chemical compositions and sensory properties***	***Reference***
Hanseniaspora osmophila	Apple cider	PF	Volatile acids ↓, acetate esters ↓, benzyl alcohol ↑	(Lorenzini et al., 2019)
Candida zemplinina	Pear wine	PF	Glycerol ↑, VOCs ↓	(Wei et al., 2019)
Kluyveromyces marxianus	Apple cider	PF	Ethanol ↓, ethyl acetate ↑	(Gschaedler, 2017)
Zygosaccharomyces rouxii	Apple cider	PF	Ethanol ↓, ethyl acetate ↑	(Gschaedler, 2017)
Zygosaccharomyces bailii	Apple cider	PF	Ethanol ↓, glucose ↑, fructose ↑, volatile acids ↓, acetate esters ↓, benzyl alcohol ↑	(Lorenzini et al., 2019)
Metschnikowia pulcherrima	Pear wine	SeqF SC	VOCs ↑, esters↑, fatty acids↓, higher alcohols ↑	(Yang et al., 2022)
	Apple-blended pear wine	SeqF SC	VOCs ↑, ethyl esters ↑, higher alcohols ↑, terpenoids ↑	(Yang et al., 2022)
Lachancea thermotolerans	Apple cider	PF	Total sugars ↓, ethanol ↓, higher alcohols ↓	(Fejzullahu et al, 2021)
	Apple cider	SimF SC	Higher alcohols ↓, esters ↑, fruitness ↑	(Fejzullahu et al, 2021)
	Apple cider	SimF TD&SC	Total sugars ↑,ethanol ↓, acetaldehyde ↑, higher alcohols ↓	(Fejzullahu et al, 2021)
Starmerella bacillaris	Apple cider	PF	Ethanol ↓, glucose ↑, fructose ↑, volatile acids ↓, acetate esters ↓, benzyl alcohol ↑	(Lorenzini et al., 2019)

Compared to *S. cerevisiae* fermentation: ↑ increase; ↓ descrease; ↔ same level.

PF: pure fermentation, SimF SC: simultaneous fermentation with *S. cerevisiae*, SimF SC: simultaneous fermentation with *S. cerevisiae,* SeqF TD: sequential fermentation with *T. delbrueckii*, SimF TD & SC: simultaneous fermentation with *S. cerevisiae* and *T. delbrueckii,* HCAs: hydroxycinnamic acids, VOCs: volatile organic compounds.

T. delbrueckii is a commercial non-*Saccharomyces* yeast utilized at an industrial level with a good fermentation performance. It is generally used in pure fermentation as well as the simultaneous or sequential fermentation with *S. cerevisiae*. The utilization of *T. delbrueckii* typically produces less acetic acid, and ethanol, and more glycerol. Moreover, it has been found to increase the aroma complexity of AFBs, by decreasing the ethyl esters and increasing lactones and other lesser-known esters. However, it shows a low tolerance to low oxygen environments. In comparison to *Saccharomyces* yeast strains, *T. delbrueckii* produces higher levels of ethyl hexanoate, ethyl decanoate, and ethyl 9-decenoate and lower amounts of hexanoic acid and octanoic acid in Jinchuan pear wines (Liu et al., 2022). Chen and Liu (2016) found that the single culture of *T. delbrueckii* retained better odor-active terpenes and terpenoids from the lychee fruits when compared with *S. cerevisiae*. In the sequential fermentation of *T. delbrueckii* and *S. cerevisiae*, the obtained lycee wine showed lower volatile acids and terpenoids, however, it showed a higher production of ethanol, higher alcohols, and acetate esters. Sadineni, Kondapalli, and Obulam (2012) found that the utilization of *T. delbrueckii* strains alone significantly decreased the ethanol yields in mango wines. However, in a previous study, similar ethanol contents were produced in the co-fermentation of *S. cerevisiae* and *T. delbrueckii* when compared with the monoculture of *S. cerevisiae* in mango wines (Sadineni, Kondapalli, & Obulam, 2012). Moreover, the co-fermentation led to an increase in the glycerol content and a reduction of the volatile acidity in the final mango wine products, thus affecting the sensory properties of the final wine products. Moreover, Yang and his co-workers (2021) found that the single inoculation of *T. delbrueckii* led to higher anthocyanins and enhanced the color and flavor of the low-alcohol strawberry beverages they obtained. In addition to *T. delbrueckii*, a single inoculation of *T. quercuum* has also been applied in apple cider fermentation, leading to an increase in the ethanol content (Wei et al., 2019).

Schizosaccharomyces pombe showed a unique ability to deacidify AFBs by degrading malic acid during AFB fermentation. In general, the degradation of malic acid by the inoculation of *S. pombe* varies from 75% to 100% during fermentation. The reduction of malic acid content helps to reduce the sourness, acidity, and puckering astringency; this makes the AFBs smoother (Benito, Calderón, Palomero, & Benito, 2015; Minnaar et al., 2017). Therefore, *S. pombe* can be used as a potential yeast strain in apple cider fermentation to provide the final apple cider products with higher sensory qualities. Satora and his co-workers (2018) found that the malic acid content was highly reduced by pure fermentation with *S. pombe* and more glycerol, esters, and acetic acid were produced when compared with pure fermentation with *S. cerevisiae*. Minnaar et al. (2017) found that the co-fermentation of *S. pombe* and *S. cerevisiae* significantly decreased the L-malic acid than the mono-fermentation with *S.*

cerevisiae. However, the co-fermentation also led to noticeable off-flavors in the final Kei-apple wines when compared with the monofermentation of *S. cerevisiae*. Moreover, the co-fermentation resulted in higher ferulic and *p*-coumaric acids. Utilization of *S. pombe* in fermentation of kiwi wines resulted in higher VOCs, primarily as phenylacetaldehyde, 2-methylbutan-1-ol, and phenethyl acetate (Li, Bi, Sun, Gao, Chen, and Guo, 2022). In addition, Liu, Laaksonen, Kortesniemi, Kalpio, and Yang (2018) found that fermentation with *S. pombe* almost completely reduced the malic acid and produced more glycerol, acetaldehyde, and pyruvic acid in bilberry wines. In addition, the utilization of *S. pombe* also resulted in a better coloration in the final bilberry wines by increasing the anthocyanins and hydroxycinnamic acid derivatives.

Apart from these two yeasts, several other non-*Saccharomyces* yeasts have also been utilized in alcoholic apple and pear beverage fermentations. For example, the utilization of *Pichia* yeast strains in alcoholic apple beverage production has been reported to result in a significantly lower concentration of ethanol and higher levels of residual sugars and acetate esters (Gschaedler, 2017; Wei et al., 2019). Application of pure fermentation or simultaneous fermentation with *H. uvarum* led to a significant increase in the concentration of acetate esters in apple ciders, especially hexyl acetate and isoamyl acetate (Wei et al., 2020). Similar results were also reported in the pure fermentation of *H. uvarum* in apple ciders (Lorenzini et al., 2019). Moreover, a pure inoculation of *H. uvarum* in apple wines has been reported to result in a reduction of organic acids and ethanol concentrations (Wei et al., 2019; Lorenzini et al., 2019). Moreover, the fermentation with *Kluyveromyces marxianus*, *Zygosaccharomyces rouxii*, *Starmerella bacillaris*, and *Lachancea thermotolerans* also led to a decrease in the ethanol contents whereas that of the total esters increased during AFBs fermentation (Fejzullahu et al., 2021; Gschaedler, 2017). Pear wines, with or without an apple blend, fermented with *Metschnikowia pulcherrima* showed a higher total of VOCs, primarily ethyl esters, higher alcohols, and terpenoids, in comparison to that produced with *S. cerevisiae* (Yang et al., 2022).

2.5 Concluding remarks

The quality of AFBs is highly determined by the fruit cultivars, juice production process, and wine making technology. The composition of the sensory active compounds and the sensory properties are two important factors determining the quality of AFBs.

Pome fruits are rich in sensory active compounds such as phenolic compounds and volatile compounds. The fruit cultivar, rootstock type, and maturity level significantly influence the fruit quality. For example, cider apples or perry pears contain higher levels of phenolic compounds than dessert apples or pears, especially proanthocyanidins with high DP. Apart from the cultivar/rootstock effects, environmental factors also affect the overall fruit quality. The environmental factors are mainly sunlight, temperature, water supplements, and agronomic practices. The agronomic practice are mainly the orchard design, the row orientation, the pruning, thinning, and irrigation. Moreover, the ripeness stage of the pome fruits significantly affects their phenolic compounds, sugars, organic acids, and VOCs. Therefore, the selection of suitable apple or pear cultivars is one of the main concerns of the beverage industry as it is the basis for fruit beverage (with or without an alcoholic content) products with a high quality, e.g. a balanced sweet-tart taste and pleasant flavor.

Juice processing methods also play important roles in AFB production. The basic process includes extraction, clarification, and pasteurization. Enzymes are often used to clarify the juices and increase juice yields by softening fruit tissues and disrupting the cell matrix. The utilization of enzymes may affect the phenolic yields, antioxidant activity, VOCs, and sensory properties of the fruit juices, as they are all dependent on the enzymes and fruit cultivars. For example, the addition of pectinase can increase the total amounts of proanthocyanidins in black currant juices, resulting in higher intensities of mouth-drying and puckering astringency in the black currant juice. In addition, pectinase can also decrease the sulfur off-flavors in further AFB fermentation. The utilization of non-thermal treatments can help to retain the nutritional compounds and VOCs, as well as to maintain the fresh and natural fruit flavor.

The potential of other *Saccharomyces* and non-*Saccharomyces* yeast strains in AFBs have not been sufficiently well investigated. In this study, the utilization of different other *Saccharomyces* and non-*Saccharomyces* yeast in AFBs have been demonstrated based on previous research. For example, apple wines fermented with *Saccharomyces paradoxus* showed higher residual sugars, acetone, and acetaldehyde, whereas lower amounts of volatile esters were found in the obtained apple wines. The pure inoculation with *Saccharomyces bayanus* may increase the ethanol contents, as well as the level of glycerol, succinic acid, malic acid, and carbonyl compounds in AFBs. Spontaneous fermentation with *S. bayanus* and *S. cerevisiae* was found to result in higher amounts of VOCs released in glycosidic forms. The utilization of non-Saccharomyces yeast strains, such as *Torulaspora delbrueckii* and *Schizosaccharomyces pombe*, led to a greater aroma complexity and a lower ethanol content in the final AFBs. These previous findings highlight the potential of other *Saccharomyces* and non-*Saccharomyces* yeasts in AFB production.

Nevertheless, there remains a gap in knowledge about the application of these processing methods in making apple ciders and pear perries. This doctoral thesis research aims to fill this knowledge gap.

3 AIMS OF THE STUDY

The general aim of this research was to investigate the potential utilization of pome fruits as well as the possibility of using *Saccharomyces* strains and non-*Saccharomyces* strains in AFB processes. Volatile and non-volatile compounds were analyzed from fruit juices and AFBs, with the aim of detecting the fate of sensory-active compounds during the fermentation process. The key sensory properties in AFBs were also studied using trained and untrained sensory panelists. Multivariate models were established to pinpoint the correlations between the chemical compositions and sensory properties.

The specific aims of the individual studies were as following:

1) To investigate the effects of *S. cerevisiae* and *S. pombe* strains on the volatile compositions of Finnish apple ciders (**Study I**)

2) To characterize the fate of phenolic compounds during yeast fermentation of apples and pears (**Studies II** and **IV**)

3) To evaluate the phenolic compositions and concentrations of juices made from pear cultivars and breeding selections, and to compare the chemical differences among different pear groups (**Study III**)

4) To investigate the effects of *S. cerevisiae* and non-*Saccharomyces* yeast strains on the sensory properties of AFBs, and to examine the contribution of phenolic compositions to the sensory properties of ciders and wines (**Studies I** and **IV**)

4 MATERIALS AND METHODS

4.1 Plant materials

The apple and pear cultivars used in this research are summarized in **Table 8** and **Table 9**, respectively. The apple materials (**Studies I & II**) were Finnish local cultivars derived from seed sowing activities (19th and 20th century). The fruits were harvested in 2018 and cultivated in the experimental orchard of the Natural Resources Institute Finland (Luke) in Piikkiö, Southwest Finland (60°25′N, 22°31′E). The apples were stored in a refrigerator at +4 °C for 1 month to reach an over-ripeness level.

The pear materials (**Studies III**) include thirteen unreleased selections from the progenies of six controlled crosses of hybrids developed from seven European pear (*Pyrus communis L.*) cultivars, of which two test cultivars originated from Russia, and two were commercial pear cultivars. The unreleased pear selections and the two Russian test pear cultivars were cultivated in the experimental orchard of Piikkiö, Kaarina, Southwest Finland (60°39'N, 22°55'E; 18 m alt.) in 2019, and the pear fruits of the commercial cultivars were purchased from the local supermarket in 2019. Subsequently, eleven pear cultivars (seven unreleased breeding selections, two test pear cultivars, and two commercial pear cultivars) were selected for fermentation into alcoholic pear beverages in **Study IV**. The pears were stored in a fruit storage chamber (from+1 to +3 °C) to obtain the mature fruits. All the sample materials were carefully selected so that they had no external or internal damage.

Table 8. Apple fruits used in the current study. Reprinted from the original publication **I** (He et al., 2021) with permission from Elsevier.

Cultivar[a]	*Seasonal category*	*Breed*	*Origin of cultivar*	*Harvest time*	*Abbreviations*
Alasen Punainen[b]	Summer	Unknown	early 1900s in Joensuu, Finland	2018.09.03	AP
Kersti	Summer	Seedling of Mironchik	early 1900s in Mikkeli, Finland	2018.09.04	Kr
Lepaan Meloni	Summer	Unknown	early 1900s in Lepaa, Finland	2018.09.04	LM
Lohjan Kirkas	Summer	Transparente Blance × Gyllenkroks Astrakan	1920s in Lohja, Finland	2018.09.04	LK
Aino	Autumn	Unknown	1940s in Suomussalmi, Finland	2018.09.03	An
Gustavs Bästa	Autumn	Seedling of Antonovka	1980s in Vaasa, Finland	2018.09.03	GB
Luotsi	Autumn	Bud mutation of Simnoje polosatoje	mid 1900s in Halikko, Finland	2018.09.04	Lt
Pieksämäki	Autumn	Unknown	mid 1800s in Pieksämäki, Finland	2018.09.03	Pk
Turso	Autumn	Unknown	early 1900s in Kangasala, Finland	2018.09.04	Tr
Juuso[c]	Winter	Antonovka × Lobo	1997, breed by Luke, Finland	2018.09.03	Ju
Hyvingiensis[d]	-	Unknown	late 1800s in Hyvinkää, Finland	2018.09.20	Hg

[a] The descriptions of all the cultivars expect for ‘Alasen Punainen’ and ‘Hyvingiensis’ are shown in Finnish Food Authority (https://www.ruokavirasto.fi/).

[b] The morphological description of ‘Alasen Punainen’ is unpublished and the data comes from Luke.

[c] ‘Juuso’ is a commercial apple cultivar derived from the Finnish apple breeding program in Luke.

[d] *Malus* ‘Hyvingiensis’ is a unique seed born cultivar of weeping decorative crap apple in Finland.

Table 9. Pear fruits used in the current study. Reprinted from the original publication **III** (He, Laaksonen, Tian, Haikonen, & Yang, 2022b) with permission from American Chemical Society.

Sample code	*Cultivar or breeding*	*Name of cultivar or cross*[a]	*Harvest time*	*Tentative use by breeders*[b]
Py1	selection	Pepi × Lück	2019.08.30	C
Py2	selection	Pepi × Lück	2019.08.30	D
Py3	selection	Pepi × Lück	2019.08.30	C
Py4	selection	Pepi × Lück	2019.09.12	D
Py5	selection	Pepi × Lück	2019.09.04	C
Py6	selection	Alna × Lück	2019.08.29	D/C
Py7	selection	Alna × Lück	2019.08.29	D/C
Py8	selection	Pepi × Pakurlan Päärynä	2019.09.12	D/C
Py9	selection	Karmla × Pakurlan Päärynä	2019.09.12	D
Py10	selection	Karmla × Pakurlan Päärynä	2019.08.30	C
Py11	selection	Rumnaja Kedrina × Pakurlan Päärynä	2019.08.30	D/C
Py12	selection	Rumnaja Kedrina × Pakurlan Päärynä	2019.08.30	C
Py13	selection	Lukna × Pakurlan Päärynä	2019.08.29	D
Sto	cultivar	Stolishnaja/Stolichnaya	2019.09.10	C
Kru	cultivar	Krupnoplodnaja Susova	2019.09.19	D
Con	cultivar	Conference	-	D
Cla	cultivar	Clara Frijs	-	D

[a] The crosses of the pear selections are expressed by ‘maternal cultivar × pollen cultivar’. The parental cultivars are commercial cultivars originated from different countries.

[b] Tentative use was determined beforehand by the breeders in Luke; the pear selection/cultivars were divided into three sub-groups: ‘putative dessert pears’ (D), ‘putative dessert/perry pears’ (D/C), ‘putative perry pears’ (C).

4.2 Sample processing methods

The overall scheme of the sample preparations and experimental analyses used in this thesis are clearly exhibited in **Figure 3**. The apple and pear fruits were washed, sliced, and pressed into juices, sequentially. The original pear juices were separated and stored in refrigeration conditions at –20 °C until further chemical analysis (**Study III**). The obtained apple juices and pear juices went through a thermal treatment at 95 °C (5 min) for pasteurization.

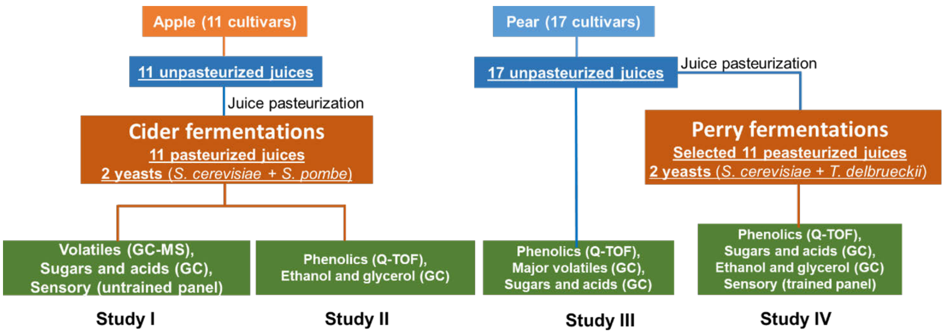


Figure 3. Overall scheme of the sample preparation and experimental analysis.

The cider fermentations (**Studies I & II**) were performed using *S. cerevisiae* 1116 (SC1116) and *S. pombe* 3796 (SP3796), whereas the perry fermentations (**Study IV**) were performed using *S. cerevisiae* 1116 (SC1116) and *T. delbruekii* 291 (TD291). All the yeasts were proliferated in sterilized YPD medium at 25 °C for 48 h. Yeast strains (10^7 CFU/mL) were added into the corresponding juice samples for each fermentation. The fermentation was carried out at 25 °C in duplicate. The CO_2 derived from the yeast fermentation was released by unscrewing the bottle caps every two days. The sign of completion of fermentation process was 1) °Brix values reached constant levels; 2) no CO_2 was produced from yeast growth during four consecutive days. The CO_2 production was monitored every two days by calculating the weight loss of the bottles. The °Brix values were monitored by a °Brix meter (Atago Co. Ltd., Tokyo, Japan). After fermentation, the cider and perry samples were stored in refrigeration conditions at −20 °C for further chemical analysis. The samples used for chemical analysis were conducted in 100 mL Duran bottles with 80 mL pasteurized fruit juices whereas the sensory samples were conducted in 500 mL Duran bottles with 400 mL pasteurized fruit juices. No added sugars and nutrients were applied in the current study.

4.3 Compositional characteristics of juices and fermented products

The sugars and organic acids were analyzed using a Shimadzu GC-FID system (Shimadzu, Japan) together with the trimethylsilyl derivatives of the apple juice and ciders (**Study I**), unpasteurized pear juices (**Study III**), and fermented perries (**study IV**). The 0.25 μm SPB-1 column (30 m × 0.25 mm) was used to separate the sugars and organic acids. The determination was carried out by the comparison of the retention times of analytes with those of the authentic standards. Quantification of the studied compounds was carried out by using *myo*-inositol as an internal standard for sugars and tartaric acid for organic acids.

The major metabolites (glycerol and ethanol) of the apple juices and fermented apple beverages (**Study II**) and pear juices (**Study III**) and fermented pear beverages (**Study IV**) were analysed by the GC-FID (same as the one analyzed for sugars and organic acids) together with a 0.25 μm HP-INNOWAX column (30 m × 0.25 mm). The identification was carried out by a comparison between the retention times of the analytes and the authentic standards. External standard curves were used to quantify the concentrations of each compound.

4.4 Characterization of volatile compounds (Study I)

Volatile compounds of the apple juices and obtained fermented ciders (**Study I**) were determined by using a HS-SPME-GC-MS system as previously described by Liu, Laaksonen, and Yang (2019). The extraction method was conducted from headspace with a divinylbenzene/carbozen/polydimethylsiloxane (DVB/CAR/PDMS) layer fiber (50/30 μm, Supelco, Bellefonte, CA). A Trace 1310 gas chromatograph equipped with a TSQ 8000 EVO mass spectrometer (Thermo Fisher Scientific, Waltham, MA) was used for the analysis of volatile compounds. The volatile separation was conducted with a DB-WAX column (60 m × 0.25 mm × 0.25 μm, J&W Scientific, Folsom, CA) and a SOB-624 column (30 m ×0.25 mm × 0.25 μm, Agilent, Santa Clara, CA). Helium was used as the carrier gas at a flow rate of 1.6 mL/min. The mass spectrometer was operated in electron impact ionization mode, and the ions were scanned in the range from *m/z* 30 to *m/z* 300. Identification of the volatile compounds was performed by the comparison of possibility-based matching between the obtained mass spectra of the analytes and the mass spectra in the databases, including NIST database (https://webbook.nist.gov/chemistry/), VCF database (http://www.vcf-online.nl/VcfCompounds.cfm), and by referring to data published in the literature. A series of C5–C30 alkane mixture was injected under the same condition to determine the RI values of the studied analytes. A comparison of the measured RIs of the studied analytes with the reference samples or the literature databases was used to assist the identification of the volatile compounds. 4-Methyl-2-pentanol was spiked as an internal standard, and semi-quantification was carried out in the current work to quantify the compounds in apple juices and ciders.

4.5 Characterization of phenolic compounds (Studies II, III, and IV)

4.5.1 Extraction of the phenolic compounds

The extraction was carried out with ethyl acetate based on the method of Mäkilä et al. (2016) with slight modifications. In **Study II**, 15 mL ethyl acetate was used to extract 5 g of apple juice or cider samples. In Studies **III** and **IV**, 25 mL of unpasteurized pear juices or perries were extracted with 20 mL of ethyl acetate. The extraction was assisted by 20 min of sonication followed by 15 min of centrifugation at 4,500 × *g*. The extraction process was carried out four times for each sample. The obtained supernatants (four times) were combined and then evaporated (35 °C) until complete dryness. Thereafter, the residue was re-dissolved using methanol (1.5 mL) and filtered (0.2 μm PTFE filter).

4.5.2 Qualitive analysis of phenolic compounds

The identification of the phenolic compounds was conducted by an UHPLC-DAD-ESI-QTOF system (Bruker Daltonik GmbH, Bremen, Germany). The separation was conducted by a Phenomenex Aeris peptide XB-C18 column (150 × 4.60 mm, 3.6 μm, Torrance, CA) at 25 °C. The flow rate for the LC system was 1.0 mL/min, and a splitted flow (0.4 mL/min) into the MS system. A full mass scan (*m/z* 20–2000) was operated in both positive and negative ionization modes. An internal standard (10 mM of sodium formate) was injected into the system to calibrate the Q-TOF. The end plate offset and nebulizer gas pressure were set at 500 V and 2.5 bar, respectively. The flow rate and temperature of the drying gas was set at 11 L/min and 280 °C, respectively. The capillary voltage for positive mode and negative mode was set at 3.5 kV and 3.5 kV, respectively. The ion energy of quadrupole was set at 5.0 eV, whereas the collision energy varied from 5.0 to 12.5 eV. The auto MS/MS program was used for the Q-TOF system in the study. The mobile phase was a combination of water (A) and acetonitrile (B), both containing 0.1% (*v/v*) of formic acid. In **Study II**, the mobile phase was programed by setting the percentage of B as follows: 0–3 min, 0% B; 3–6 min, 0–1% B; 6–15 min, 1–4% B; 15–20 min, 4–5% B; 20–25 min, 5% B; 25–27 min, 5–6% B; 27–32 min, 6–7% B; 32–37 min, 7–9% B; 37–42 min, 9–12% B; 42–47 min, 12–14% B; 47–52 min, 14–18% B; 52–57 min, 18–

20% B; 57–62 min, 20–23% B; 62–67 min, 23–40% B; 67–68 min, 40–70% B; 68–70 min, 70–0% B; 70–72 min, 0% B. In **Studies III** and **IV**, the gradient was changed into the following process: 0–5 min, 2–4% B; 5–10 min, 4–7% B; 10–15 min, 7–8% B; 15–20 min, 8–10% B; 20–30 min, 10–18%; 20–35 min, 18–20% B; 35–40 min, 20–25% B; 40–45 min, 25–35% B; 45–46 min, 35–40% B; 46–49 min, 40–70% B; 49–51 min, 70–2% B; 51–53 min, 2% B. The chromatograms were monitored at 280 nm, 320 nm, and 360 nm. The data was processed by the software of Compass Data analysis.

4.5.3 Quantitative analysis of phenolic compounds

The quantation was conducted using a Shimadzu UHPLC-DAD system (Shimadzu Crop., Kyoto, Japan). External standard curves were constructed for the quantitative analysis of the study. For apple juices and 'cider' products (**Study II**), 5-*O*-caffeoylquinic acid, (+)-catechin, procyanidin B2, quercetin-3-*O*-glucoside, and phloretin-2'-*O*-glucoside were used for the quantification of hydroxycinnamic acids, monomeric flavan-3-ols, procyanidins, flavonols, and dihydrochalcones, respectively. For the unpasteurized pear juices (**Study III**), pasteurized pear juice and 'perry' products (**Study IV**), 5-*O*-caffeoylquinic acid, (+)-catechin, procyanidin B2, quercetin-3-*O*-glucoside, and arbutin were used for the quantification of hydroxycinnamic acids, monomeric flavan-3-ols, procyanidins, flavonols, and other phenolics, respectively. The LC gradient was the same as the one used for the MS system, respectively.

4.6 Sensory evaluation (Studies I and IV)

Sensory evaluations were carried out for the 'cider' (**Study I**) and 'perry' (**Study IV**) products in controlled laboratory conditions (ISO 8589) at the University of Turku. Water and a small piece of unsalted cracker were provided for the panelists between each sample to clean their palates.

In **Study I**, the selected apple 'cider' products were evaluated by 34 untrained panelists (age 20-65, 26 females and 8 males). The 9-point scales were used to examine hedonic scale on the appearance, odor, and flavor. Moreover, the check-all-that-apply (CATA) was applied to examine the 'cider' characteristics by a list of selected sensory attributes (n = 26). The untrained panelists were asked to select all the suitable descriptors of alcoholic apple beverages in the list and/or generate new words during the training sessions. An aliquot of 10 mL of 'cider' samples were placed in a 50 mL transparent plastic cup with glass lid covered.

In **Study IV**, the selected pear 'perry' products were evaluated by 13 trained panelists (age 21-56, 10 females and 3 males). A generic descriptive analysis was used to evaluate the flavor, taste, and appearance of the products. The recruited panelists were trained for three sessions and developed a vocabulary of 'perry' products. Moreover, the given descriptors and reference standards (**Table 10**) should meet the requirements of the panelist group. In the evaluation sessions, 10 mL of samples were served in a 50 mL transparent plastic cups with glass lid covered. Each sample was conducted in triplicates. And a line scale (0 = none, 10 = very strong) was used for the evaluation together with selected reference samples.

A randomized order was created for the samples by a William's design. The panelists were asked to drink the water or eat the cracker to clean their mouth for one minute. Data was collected by Compusense Cloud software.

Table 10. The evaluation attributes, descriptors, and reference samples in the **study IV**.

Attribute	***Description***	***Reference standards***	***Serving size and type***	***Intensity***
Odor				
Pear	Pear odor	Marli pear juice, Eckes-granini, Finland	10 mL pear juice	6
Cooked pear	Cooked pear odor	Piltti pear puree, Nestlé, Finland	2 cm^2 pear puree	5
Pungent	An irritating sharp sensation in the nose	Etax A, Anora, Finland	10 mL 20% ethanol	3.5
Alcoholic	Alcoholic odor	Etax A, Anora, Finland	10 mL 15% ethanol	3
Acidity	Acidic odor	Sicilia lemon juice, Sidag Ag, Switzerland	10 mL lemon juice	6
Floral	Floral odor	Brunneby elderflower concentrate, Eurofoods, Sweden		4
Fermented	Fermented odor	Upcider pear cider (alc. 4.7%), Hartwall AB, Finland	10 mL commercial pear cider	4
Taste				
Sweetness	Sweet taste	Dansukker glucose syrup, Suomen Sokeri, Finland	10 mL 6.00% glucose syrup solution	4.5
Sourness	Sour taste	Alfa Aesar, Germany	10 mL 0.15% citric acid solution	4.5
Bitterness	Bitter taste	Alfa Aesar, Germany	10 mL 0.035% caffeine solution	4
Astringency	The drawing or tightening sensation and the lack of lubrication or moistness	Aluminium ammonium sulfate dodecahydrate, Merck KGaA, Germany	10 mL 0.20% $AlSO_4$ solution	7

4.7 Statistical analysis

The results were expressed as mean ± standard deviation for each study. A one-way ANOVA analysis and a Tukey's test were used to investigate the difference of chemical compositions (**Studies I-IV**) and sensory characteristics (**Study I**). In **Study IV**, a three-way ANOVA analysis, a Fisher least significant difference (LSD), as well as PanelCheck software were also applied in the investigation of the differences between the sensory characteristics.

The statistical univariate analysis (**Studies I-IV**) was constructed with SPSS and multivariate models (**Studies I-IV**) were carried out by Unscrambler X.

5 RESULTS AND DISCUSSION

5.1 Fermentation kinetics and compositional characteristics

5.1.1 Fermentation kinetics of the studied alcoholic apple and pear beverages (Studies II and IV)

The fermentation kinetics, expressed as CO_2 production, during alcoholic apple and pear beverages fermentation (*Saccharomyces* and non-*Saccharomyces*), were illustrated in **Figure 4** (studies **II** and **IV**). The fermentation kinetics varied depending on the different fermentation substrates and yeasts strains. Both of yeast strains and fruit cultivars played important roles in the alcoholic apple and pear beverage fermentations (Belda et al., 2015). As for fermented apple beverages, all the fermentations via *S. cerevisiae* 1116 yeast strains were finished in 10-12 days, and the fermentations with *S. pombe* 3796 completed in 14-18 days.

In the alcoholic pear beverage production, all the fermentation processes with *S. cerevisiae* 1116 were completed within 26 days, whereas the process with *T. delbrueckii* 291 was completed within 30 days. However, the fermentation of ‘Stolishnaja’ and ‘Conference’ was completed within a shorter time period of 10 days and 14 days, respectively. Generally, *S. cerevisiae* showed a faster fermentation performance than the non-*Saccharomyces* yeasts (*S. pombe* 3796 and *T. delbrueckii* 291). Moreover, the fermentation rate of processing alcoholic pear beverages was lower when compared with alcoholic apple beverages. This result can be due to the relatively high concentrations of sorbitol and low glucose/fructose ratio in the pear juices (González Flores et al., 2021).

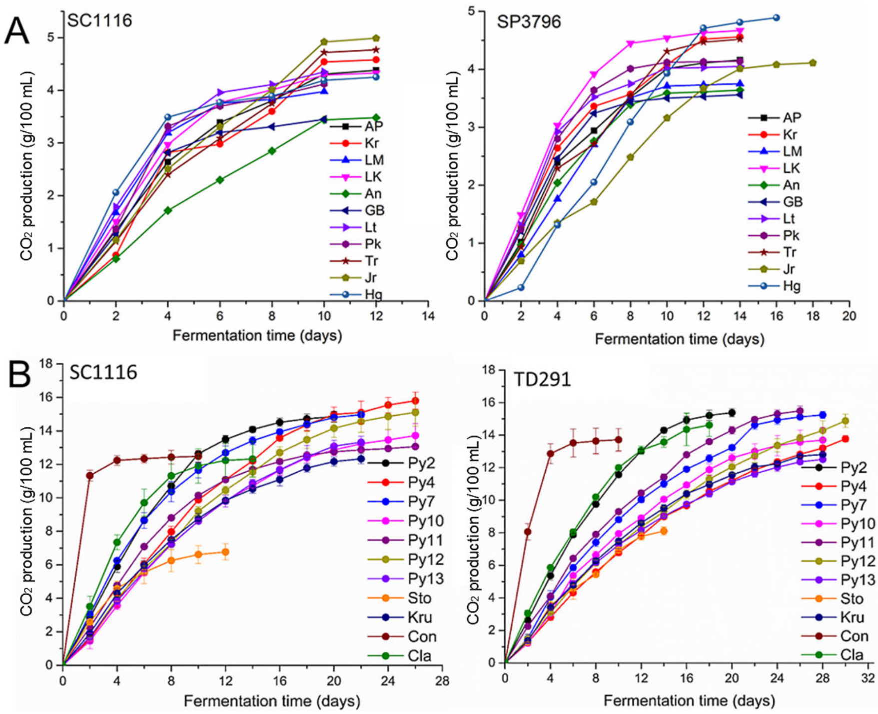


Figure 4. Fermentation kinetics expressed as CO_2 production of apple ciders (**A**) and pear wines (**B**) produced by *Saccharomyces* and non-*Saccharomyces* yeast strains during fermentation. The yeast strains used for apple ciders were *Saccharomyces cerevisiae* 1116 (SC1116) and *Schizosaccharomyces pombe* 3796 (SP3796), whereas those used for pear wines were *Saccharomyces cerevisiae* 1116 (SC1116) and *Torulaspora delbrueckii* 291 (TD291). The abbreviations for apple cultivars and pear cultivars and breeding selections refer to **Table 6** and **Table 7**. **Figure 4A** reprinted from the original publications **II** (He et al., 2022a) with permission from Elsevier.

Moreover, the obtained alcoholic pear beverages contained significantly higher ethanol concentrations (13.4% on average) when compared with the alcoholic apple beverages (6.6% on average), as shown in **Figure 5**. Similarly, the ethanol yield was mainly dependent on the fruit cultivars. For example, the alcoholic apple beverages made from 'Aino' showed quite a low ethanol content (5.4% on average) among the alcoholic apple beverages. Moreover, the ethanol content of alcoholic pear beverages made from pear breeding selection 'Py12' remained at 7.9%. In general, the ethanol yield was highly associated with the fermentable sugar contents of fruit juices. The yeast strains also slightly altered the ethanol yield during the fermentation processes.

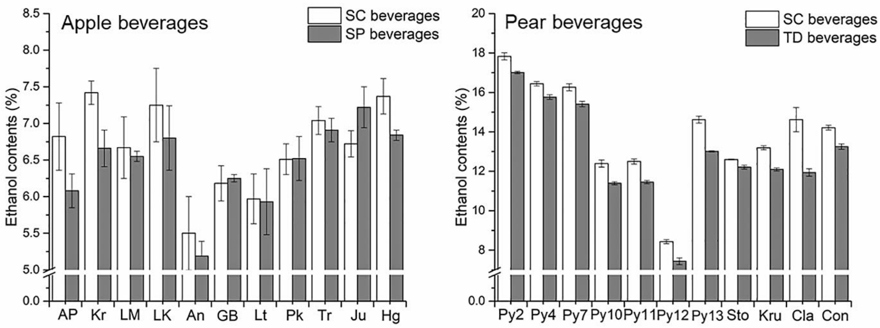


Figure 5. Ethanol contents of apple beverages and pear beverages.

5.1.2 Sugars, organic acids, and glycerol (Studies I, III, and IV)

As the main nutritional and taste compounds, sugars, organic acids, and glycerol played important roles in the sensory properties of the apple and pear beverages. Generally, the sugar compositions and profiles were mainly dependent on the fruit cultivar types. Fructose, glucose, sorbitol, sucrose, and xylose were detected in both apple and pear beverages (**Figure 6**). Among all the detected sugars, fructose was the major sugar presented in both apple (56%) and pear (54%) juices, followed by glucose and sucrose. The sorbitol content in the apple juices was low (5.2 g/L on average), whereas that in the pear juices was detected at the higher level of 15.0 g/L (average content). With regard to organic acids, malic acid was detected as the major acid component in apple and pear juices, which provided harsh sour taste to the studied beverage products. In the apple juices, the highest malic acid content was detected in 'Aino' (18.9 g/L), followed by 'Juuso' (11.3 g/L) and 'Turso' (9.6 g/L). Additionally, the malic acid content was also quite high in 'Stolishnaja' (7.2 g/L). Moreover, ascorbic and succinic acids were not detectable in this current study, whereas they were detected at low amounts in pear juices. Glycerol was not detected in the unfermentable fruit juices.

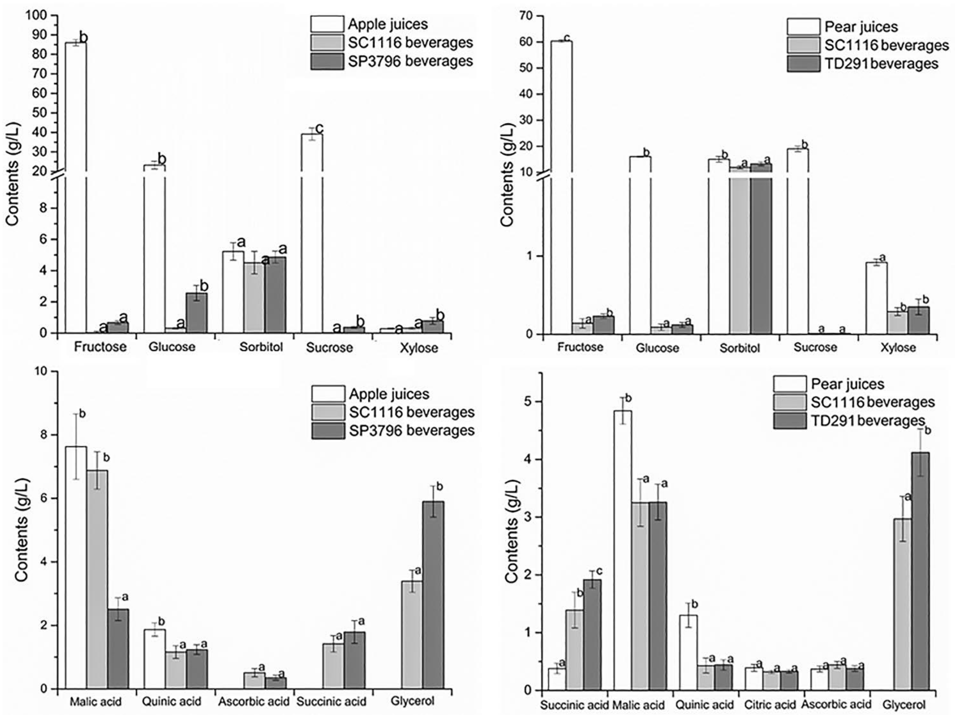


Figure 6. Average concentrations of sugars, organic acids, and glycerol detected in the fruit juices, fermented beverages produced by different yeast strains.

The clear differences between the initial juices and the fermented alcoholic beverages (by different yeast strains) are shown in **Figure 6**. In general, the fermentation process decreased the sugar content in the studied apple and pear beverages. The fructose, glucose, and sucrose in apple juices were almost completely consumed (> 90%) by SC1116, whereas the fermentations with SP3796 (5.2 g/L on average) consumed less sugar in apple juices when compared with SC1116 (9.2 g/L on average). In addition, the alcoholic pear beverages made with TD291 (14.0 g/L on average) showed slightly higher residual sugar contents when compared with SC1116 (12.4 g/L on average), with sorbitol being the dominant residual compound after both fermentations. The differences in the sugar consumption can be ascribed to the different metabolic characteristics of yeast strains. Generally, the sugar consumption capacity of non-*Saccharomyces* strains is lower when compared to *Saccharomyces* strains. Although the alcoholic apple beverages contained significantly lower concentrations of sorbitol, the yeast strains showed a limited capacity for utilising sorbitol. In general, the fermented pear beverages showed significantly higher contents of residual sugars (13.2 g/L on average) when compared with that of apple beverages (7.3 g/L on average). The main residual sugar in the alcoholic pear beverages was detected as being sorbitol, which can be derived from the original pear juices. Sorbitol has been reported to contribute positively to the sweetness perception in the beverages and beverage products, and it is a popular sweetener for low-carbohydrate diets (Aprea et al., 2017). In addition to the fruit types, fruit cultivars also played an important role in the composition of alcoholic apple and pear beverages. For example, the alcoholic apple beverages made from ‘Luotsi’ and ‘Gustavs Bästa’ contained relatively low amounts of residual sugars when compared with other apple cultivars.

As regards organic acids, significant differences between the malic and the quinic acid content were found between the juices and the fermented fruit beverages. A high malic acid consumption was found in the apple beverages fermented with SP3796 (47-89%), whereas the decrease in the content of malic acid was found to be 6-18% in those beverages with SC1116. The potential utilization of *S. pombe* yeasts to reduce the malic acid content in the AFBs has been well demonstrated in previous studies (Benito, 2019). The reduction of malic acid content resulted in an increase of pH values, which helps to mitigate the harsh green sourness and astringency in the fermented beverage products (Laaksonen, Salminen, Mäkilä, Kallio, & Yang, 2015). For the apple beverages, ascorbic and succinic acids were undetectable in the apple juices, whereas their concentrations increased during the alcoholic apple beverage fermentations. In addition, the concentration of succinic acid was relatively high in the alcoholic pear beverages.

Glycerol was another important by-product during the fermentation process of AFBs. The glycerol level was higher in the alcoholic apple beverages fermented with SP3796 (5.9 g/L on average) when compared that with SC1116 (3.4 g/L on average). Additionally, the glycerol level of pear beverages made from TD291 (4.1 g/L on average) was found to be higher than that of pear beverages made from SC1116 (2.9 g/L on average). This could be due to the capacity of non-*Saccharomyces* yeasts to redirect the sugar consumption from ethanol to other alternative metabolites (glycerol and pyruvic acid) during fermentation (Ivit et al., 2020).

5.2 Volatile organic compounds in apple juices and fermented beverages (Study I)

A total of 76 VOCs were identified and quantified in the apple juices and fermented apple beverages (**Table 11**), including 34 esters, 20 higher alcohols, 5 aldehydes, 4 ketones, 3 acetals, 6 volatile acids, 2 benzenes, and other VOCs. The number of VOCs were slightly different between the apple juices and apple beverages made with different yeasts: 44 VOCs were found in the apple juices, 51 VOCs were found in the SC1116 beverages and 54 VOCs were found in the SP3796 beverages. Moreover, twenty-nine VOCs were detected only in the alcoholic apple beverage samples, mainly as ethyl esters and higher alcohols.

5.2.1 VOCs in different pasteurized apple juices

The profiles of VOCs in pasteurized apple juices are summarized in **Table 11**. Among the studied juices, pasteurized apple juices made from the cultivar 'Aino' contained the lowest amounts of total VOCs (140 µg/L), followed by the juices made from 'Hyvingiensis' (168 µg/L). In addition, pasteurized juices made from 'Juuso' contained the highest amounts of total VOCs (829 µg/L). The variations in the VOCs can be explained by the different genetic backgrounds of the apple cultivars and the maturity levels of the fruits (Chen et al., 2018).

Table 11. VOCs identified from the apple juices and their corresponding beverage samples made from SC1116 and SP3796. Reprinted from the original publication **I** (He et al., 2021a), with permission from Elsevier.

No.	*Compound*	*RI*[b]		*BP*[c]	*Formula*	*Identification*[d]	*Odor descriptor*[e]	*Abbrevi-ations*
		DB-WAX	*SPB-624*					
Ethyl esters								
1	Ethyl acetate	894	1203	88	$C_4H_8O_2$	MS, LRI, STD	Pineapple, sweet, pungent[1]	E_1
2	Ethyl propanoate	957		102	$C_5H_{10}O_2$	MS, LRI	Fruity[1]	E_2
3	Ethyl 2-methylpropanoate	965	1342	116	$C_6H_{12}O_2$	MS, LRI	Sweet, tropical fruit, rubber[2]	E_3
4	Ethyl butanoate	1037	1483	101	$C_6H_{12}O_2$	MS, LRI, STD	Fruity[3]	E_4
5	Ethyl 2-methylbutanoate	1053		115	$C_7H_{14}O_2$	MS, LRI	Fruity[1]	E_5
6	Ethyl pentanoate	1134	1643	88	$C_7H_{14}O_2$	MS, LRI	Apple, dry fish, yeast[3]	E_6
7	Ethyl hexanoate	1235	1802	115	$C_8H_{16}O_2$	MS, LRI	Green apple, brandy, fruity[4]	E_7
8	Ethyl octanoate	1440		127	$C_{10}H_{20}O_2$	MS, LRI	Fruity, brandy[3]	E_8
9	Ethyl 3-hydroxybutanoate	1527		117	$C_6H_{12}O_3$	MS, LRI	Grape, roasted nut[5]	E_9
10	Ethyl nonanoate	1545		57	$C_4H_{10}O_2$	MS, LRI	Fruity[5]	E_10
11	Ethyl decanoate	1647		157	$C_{12}H_{24}O_2$	MS, LRI	Brandy, burnt, fruity[4,6]	E_11
12	Ethyl benzoate	1670		105	$C_9H_{10}O_2$	MS, LRI	Fruity, fat, flowery[5]	E_12
13	Ethyl dodecanoate	1850		101	$C_{14}H_{28}O_2$	MS, LRI	Floral, fruity, green apple[7]	E_13
14	Ethyl 3-hydroxydodecanoate	1945		117	$C_{14}H_{28}O_3$	MS, LRI	Apple brandy[4]	E_14
Acetate esters								
15	Methyl acetate	828		74	$C_3H_6O_2$	MS, LRI	Ester, green, sweet[5]	AE_1
16	Propyl acetate	977		73	$C_5H_{10}O_2$	MS, LRI	Celery, floral, pear[4,6]	AE_2
17	2-Methylpropyl acetate	1014		73	$C_6H_{12}O_2$	MS, LRI	Apple, banana, floral[4]	AE_3
18	Butyl acetate	1072	1506	73	$C_6H_{12}O_2$	MS, LRI	Apple, fruit, pungent[6]	AE_4
19	3-Methylbutyl acetate	1121	1607	87	$C_7H_{14}O_2$	MS, LRI	Apple, fruit, sweet[7]	AE_5
20	Pentyl acetate	1175		87	$C_7H_{14}O_2$	MS, LRI	Banana, fruit, sweet[8]	AE_6
21	Hexyl acetate	1274	1825	105	$C_8H_{16}O_2$	MS, LRI	Fruity[4]	AE_7
22	Octyl acetate	1481		83	$C_{10}H_{20}O_2$	MS, LRI	Citrus, fat, wood[5]	AE_8
23	Phenethyl acetate	1831	2123	104	$C_{10}H_{12}O_2$	MS, LRI	Floral, fruit, honey[8]	AE_9
Other esters								
24	Methyl butanoate	986	1362	87	$C_5H_{10}O_2$	MS, LRI, STD	Fruit, ester, floral[7]	OE_1
25	Butyl butanoate	1220	1794	101	$C_8H_{16}O_2$	MS, LRI	Floral[4]	OE_2
26	Hexyl 2-methylpropanoate	1347		101	$C_{10}H_{20}O_2$	MS, LRI	Fruity, apple, beer[6]	OE_3
27	Methyl octanoate	1394		87	$C_9H_{18}O_2$	MS, LRI	Fruity, orange, sweet, wine[6]	OE_4

Table 11 (continued)

No.	***Compound***	***RI***[b]		***BP***[c]	***Formula***	***Identification***[d]	***Odor descriptor***[e]	***Abbrevi-ations***
		DB-WAX	***SPB-624***					
28	Butyl hexanoate	1416		117	$C_{10}H_{20}O_2$	MS, LRI	Fruity, grass, green[7]	OE_5
29	Hexyl butanoate	1420	2014	89	$C_{10}H_{20}O_2$	MS, LRI	Fruity, apple, fresh[4]	OE_6
30	Hexyl 2-methylbutanoate	1432	2054	103	$C_{11}H_{22}O_2$	MS, LRI	Strawberry[6]	OE_7
31	Methyl decanoate	1604		88	$C_{11}H_{22}O_2$	MS, LRI	Fresh, wine[6]	OE_8
32	Hexyl hexanoate	1618		117	$C_{12}H_{24}O_2$	MS, LRI	Apple, fruity[8]	OE_9
33	Butyl 3-hydroxybutanoate	1716			$C_8H_{16}O_3$	MS, LRI	Fruity, brandy[7]	OE_10
34	Diethyl benzene-1,2-dicarboxylate	2041		177	$C_{12}H_{14}O_4$	MS, LRI		OE_11
Aldehydes								
35	Acetaldehyde	705	924	44	C_2H_4O	MS, LRI	Pungent, ripe apple[1,4]	Ad_1
36	Butanal	876	1185	72	C_4H_8O	MS, LRI	Banana, pungent[6]	Ad_2
37	3-Methylbutanal	919	1278	86	$C_5H_{10}O$	MS, LRI	Malt, pungent[7]	Ad_3
38	Hexanal	1088	1510	82	$C_6H_{12}O$	MS, LRI, STD	Grassy, green apple[8]	Ad_4
39	(*E*)-hex-2-enal	1221		98	$C_6H_{10}O$	MS, LRI	Green, pungent[1,5]	Ad_5
Higher alcohols								
40	2-Methylpropan-1-ol	1095	1255	74	$C_4H_{10}O$	MS, LRI	Apple, fusel, malt[6]	HA_1
41	Butan-1-ol	1146	1308	56	$C_4H_{10}O$	MS, LRI	Medicine, fruit[4,5]	HA_2
42	2-Methylbutan-1-ol	1207	1425	70	$C_5H_{12}O$	MS, LRI, STD	Banana, fusel, green, malt[3,4,5]	HA_3
43	3-Methylbutan-1-ol	1211	1421	70	$C_5H_{12}O$	MS, LRI, STD	Alcohol, nail polish[3]	HA_4
44	Pentan-1-ol	1252	1470	88	$C_5H_{12}O$	MS, LRI	Balsamic, fruity[5]	HA_5
45	4-Methylpentan-1-ol	1317		69	$C_6H_{14}O$	MS, LRI	Almond, toasted[3]	HA_6
46	2-Heptanol	1321		83	$C_7H_{16}O$	MS, LRI	Mushroom[4]	HA_7
47	3-Methylpentan-1-ol	1330		84	$C_6H_{14}O$	MS, LRI	Green, pungent, wine[4,6]	HA_8
48	1-Hexanol	1356	1632	69	$C_6H_{14}O$	MS, LRI, STD	Green, herbaceous[5]	HA_9
49	3-Octanol	1400		101	$C_8H_{18}O$	MS, LRI	Citrus, nut, oily[3,5]	HA_10
50	(*E*)-2-Hexen-1-ol	1409		82	$C_6H_{12}O$	MS, LRI	Grass[6]	HA_11
51	1-Octen-3-ol	1452		72	$C_8H_{16}O$	MS, LRI	Thyme[5]	HA_12
52	(+)-(*3R,4R*)-3,4-epoxyhexan-1-ol	1456		85	$C_6H_{12}O_2$	MS, LRI		HA_13
53	Heptan-1-ol	1460		83	$C_7H_{16}O$	MS, LRI	Oily[4,5]	HA_14
54	6-Methyl-5-hepten-2-ol	1467		95	$C_8H_{16}O$	MS, LRI	Rose[5]	HA_15
55	2-Ethylhexan-1-ol	1495		101	$C_8H_{18}O$	MS, LRI	Citrus, green, rose[3,5]	HA_16

Table 11 (continued)

No.	*Compound*	*RI*[b]		*BP*[c]	*Formula*	*Identification*[d]	*Odor descriptor*[e]	*Abbreviations*
		DB-WAX	*SPB-624*					
56	2,4,6-Trimethylheptan-4-ol	1509		140	$C_{10}H_{22}O$	MS, LRI		HA_17
57	Octan-1-ol	1563		84	$C_8H_{18}O$	MS, LRI	Chemical, metal, burnt[4]	HA_18
58	Butane-2,3-diol	1582		75	$C_4H_{10}O_2$	MS, LRI	Fruity[7]	HA_19
59	Pentadecan-8-ol	1559		129	$C_{15}H_{32}O$	MS, LRI		HA_20
Ketones								
60	4-Methylpentan-2-one	1025		100	$C_6H_{12}O$	MS, LRI	Sulfur[6]	K_1
61	3-Hydroxybutan-2-one	1291		88	$C_4H_8O_2$	MS, LRI	Buttery, fatty[6]	K_2
62	6-Methylhept-5-en-2-one	1341		108	$C_8H_{14}O$	MS, LRI, STD	Pungent[6]	K_3
63	2,6,8-Trimethylnonan-4-one	1405		127	$C_{12}H_{24}O$	MS, LRI		K_4
Acetals								
64	1-Ethoxy-1-methoxyethane	845		89	$C_5H_{12}O_2$	MS, LRI	Fruity[3]	Ac_1
65	1,1-Diethoxyethane	897	1352	103	$C_6H_{14}O_2$	MS, LRI	Fruity, cream[6]	Ac_2
66	1-(1-Ethoxyethoxy)pentane	1108	1735	115	$C_9H_{20}O_2$	MS, LRI	Fruity, alcoholic[8]	Ac_3
Benzenes								
67	1-Ethyl-3-methylbenzene	1248		120	C_9H_{12}	MS, LRI		B_1
68	Benzaldehyde	1555		106	C_7H_6O	MS, LRI	Almond, cherry[7]	B_2
Volatile acids								
69	Acetic acid	1466	1267	60	$C_2H_4O_2$	MS, LRI	Sour, vinegar-like[6]	Ai_1
70	2-Methylpropanoic acid	1588		73	$C_4H_8O_2$	MS, LRI		Ai_2
71	Butanoic acid	1652		73	$C_4H_8O_2$	MS, LRI	Rancid[7]	Ai_3
72	3-Methylbutanoic acid	1694		87	$C_5H_{10}O_2$	MS, LRI		Ai_4
73	Pentanoic acid	1703		87	$C_5H_{10}O_2$	MS, LRI		Ai_5
74	Hexanoic acid	1873	1842	83	$C_6H_{12}O_2$	MS, LRI	Sweat[8]	Ai_6
Others								
75	α-Farnesene	1755		93	$C_{15}H_{24}$	MS, LRI		OV_1
76	2,4,5-Trimethyl-1,3-dioxolane	946	1359	101	$C_6H_{12}O_2$	MS, LRI	Fruity, wine[7]	OV_2

[a] Number of volatiles investigated in this study,
[b] Retention indices of volatiles investigated by DB-WAX and SPB-624,
[c] BP: base peak of mass spectrum,
[d] Identification, MS: mass spectrum; LRI: literature retention index; STD: standard. [e] Odor descriptors based on literature. [1]Li et al., 2020, [2]Alberti et al., 2016, [3]Qin et al., 2018, [4]Luan, Zhang, Duan, & Yan, 2018, [5]http://www.vcf-online.nl/VcfCompounds.cfm, [6]Varela, 2016, [7]Tufariello et al., 2019, [8]Niu et al., 2019.

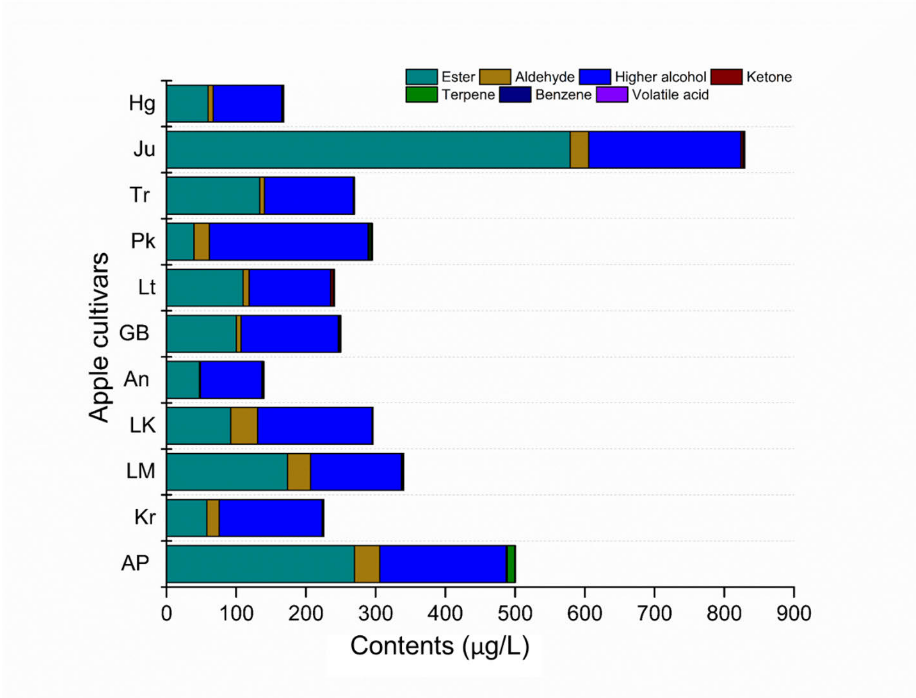


Figure 7. The total content of VOCs in the pasteurized juices made from different apple cultivars.

Among the detected VOCs, esters and higher alcohols are the two major groups of VOCs that were found in the pasteurized apple juices (**Figure 7**), accounting for over 85% of the total VOCs. Generally, esters are a group of VOCs that contribute to the sweet and fruity odors of apple juices. The total content of esters of the studied apple juices ranged from 39 µg/L ('Pieksämäki') to 579 µg/L ('Juuso'), whereas hexyl acetate and butyl acetate were the most abundant esters. Both hexyl acetate and butyl acetate play an important role in the sweet and fruity notes of the apple juices (Varela, 2016). Higher alcohols were regarded as the second largest contributors to the aroma of the apple juices. The total content of higher alcohols in the studied apple juices ranged from 88 µg/L ('Aino') to 227 µg/L ('Pieksämäki'). Among the detected higher alcohols, butan-1-ol and hexan-1-ol were the most dominant higher alcohols that contribute to the positive aroma features of apple juices (Guo, Yue, Yuan, Sun, & Liu, 2020). In addition to the esters and higher alcohols, aldehydes were another group of VOCs in the studied apple juices. No acetals were found in the apple juices in the current study, whereas volatile acids were only found in small amounts.

5.2.2 Comparison of different yeast strains on the VOCs of alcoholic apple beverages

Esters dominated among the detected numbers of VOCs in the alcoholic apple beverages, and included ethyl, acetate, and other esters. Some of the esters were derived from the apple juices, but most of them were produced during the fermentation process by the esterification of alcohols. The major ester detected was ethyl acetate in the alcoholic apple beverages, and contributed to the pineapple, sweet, or pungent odor of the beverage products (Li et al., 2020b). Generally, fermentation with *S. cerevisiae* 1116 led to a higher content of ethyl acetate than that with *S. pombe* 3796. Similar results have also been demonstrated in grape wine production (Peinado et al., 2004). In addition to ethyl acetate, ethyl butanoate, ethyl decanoate, 3-methylbutyl acetate, phenethyl acetate, and hexyl acetate were also found in high concentrations in the alcoholic apple beverages.

Higher alcohols dominated as VOCs in terms of content, and are regarded as important precursors of esters. However, they may produce unpleasant flavors in the final products at excessive levels (Paweł Satora et al., 2018). In general, most of the higher alcohols were produced during fermentation, and the concentrations were 7-25 times higher than that in the initial juices. Fermentation with *S. cerevisiae* produced more higher alcohols than *S. pombe*. Among the detected higher alcohols, 3-methylbutan-1-ol was the most abundant higher alcohol in the apple beverages. In addition, butan-1-ol and pentadecane-8-ol showed relatively high concentrations in the fermented apple beverages when compared with the initial apple juices. Moreover, a significant decrease was found in the concentration of 2-methylbutan-1-ol as a result of the yeast fermentation. In the current study, C6-alcohols, such as 1-hexanol and (*E*)-2-hexen-1-ol, were found to decrease in their concentrations during yeast fermentations.

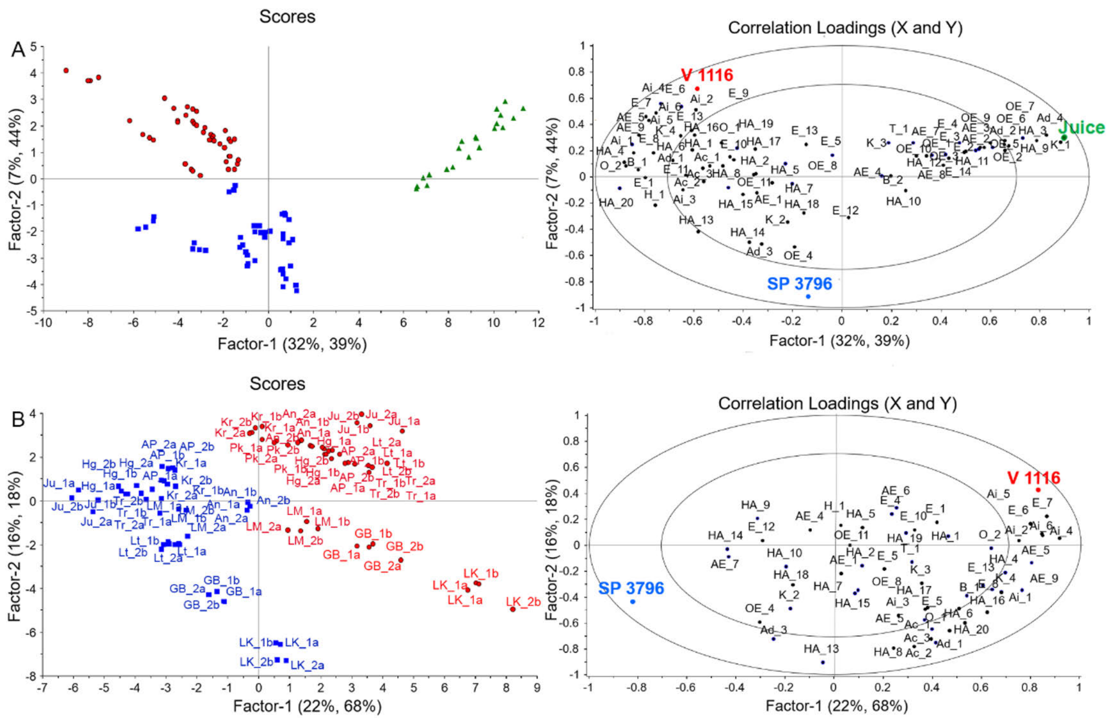


Figure 8. PLS-DA models of volatiles as X-data (n=76) to elucidate the differences between the apple juices and corresponding alcoholic beverages made from *S. cerevisiae* 1116 and *S. pombe* 3796. Juices with green triangles (Juice, n=22), *S. cerevisiae* 1116 alcoholic beverages with red circles (V1116, n=44), *S. pombe* 3796 alcoholic beverages with blue rectangles (SP3796, n=44). Figure reprinted from the original publication **I** (He et al., 2021), with permission from Elsevier.

As regards aldehyde, acetaldehyde was found to be the most abundant in the fermented apple beverages, and was considered to be a contributor to the fruity and nutty odors. However, excessive amounts of acetaldehyde (>110 mg/L) may lead to a pungent and ether aroma in alcoholic fruit beverages (Li et al., 2020b). The accumulation of acetaldehyde in the fermented beverages are known to be cultivar dependent. For example, significantly high levels of acetaldehydes were found in the apple beverages made from 'Lepaan Meloni', 'Lohjan Kirkas', and 'Gustavs Bästa'. Volatile acids have been reported to contribute to the vinegar-like, sweat, and rancid notes of their fermented beverages (Qin et al., 2018). Among the detected volatile acids, acetic acid was found at high levels in all the fermented apple beverages, especially the beverages made from *S. cerevisiae* 1116 yeasts. This result is in line with a study concerning alcoholic apple beverages fermented with *Saccharomyces* and non-*Saccharomyces* yeasts (Madrera et al., 2006).

As shown in the PLS model (**Figure 8A**, R^2=0.9596, validated R^2=0.9132), apple juices have already been separated from the fermented apple beverages along Factor-1. The juices located on the positive side of Factor-1, associate strongly with esters, primarily as hexyl butanoate (OE_6), hexyl 2-methylbutanoate (OE_7), higher alcohols, such as 2-methylbutan-1-ol (HA_3) and 1-hexanol (HA_9), hexanal (Ad_4), and 4-methylpentan-2-one (K_1). After fermentation, the total concentrations of volatile compounds increased significantly. The differences between alcoholic apple beverages made from *S. cerevisiae* 1116 and *S. pombe* 3796 were investigated in an additional PLS model (**Figure 8B**, R^2=0.9721, validated R^2=0.9372). As shown in the PLS model, *S. cerevisiae* 1116 showed a higher production of ethyl esters, primarily ethyl pentanoate (E_6) and ethyl hexanoate (E_7), and volatile acids, primarily 2-methylpropanoic (Ai_2), 3-methylbutanoic (Ai_4), pentanoic (Ai_5), and hexanoic (Ai_6) acids, when compared with *S. pombe* 3796. In general, *S. pombe* 3796 produced less ethyl esters, higher alcohols, and volatile acids when compared with *S. cerevisiae* 1116, which was in accordance with previous results found in bilberry and grape wines (Liu, Marsol-Vall, Laaksonen, Kortesniemi, & Yang, 2020; Peinado et al., 2004).

5.2.3 Comparison of different cultivars on the VOCs of alcoholic apple beverages

Principal component analysis (PCA) models were used to investigate the difference of VOCs among different apple cultivars within juices, *S. cerevisiae* 1116 beverages, and *S. pombe* 3796 beverages. As shown in **Figure 9A** (apple juices), juices made from the cultivar 'Juuso' located on the negative side along Factor-1, whereas the others were located on the positive side along Factor-1. Juices made with 'Juuso' showed higher acetate esters, primarily as propyl acetate (AE_2), butyl acetate (AE_4), hexyl acetate (AE_7), and octyl acetate (AE_8), other esters, primarily as methyl butanoate (OE_1) and diethyl benzene-1, 2-dicarboxylate (OE_11), and higher alcohols, such as 1-octen-3-ol (HA_12), heptan-1-ol (HA_14) and octan-1-ol (HA_18).

As shown in **Figure 9B** (*S. cerevisae* 1116 beverages) and **9C** (*S. pombe* 3796 beverages), the apple beverages fermented with cultivars 'Lepaan Meloni' (LM), 'Lohjan Kirkas' (LK), and 'Gustavs Bästa' (GB) were separated well away from the others along PC-1, which can be due to the difference in acetate esters and higher alcohols. In addition, cultivars 'Lohjan Kirkas' (LK) and 'Gustavs Bästa' (GB) were clearly separated along PC-2 in the model made with *S. cerevisiae* 1116 beverages (**Figure 9B**). The cultivars 'Aino' (An) and 'Luotsi' (Lt) were also clearly separated in the model made with *S. pombe* 3796 beverages (**Figure 9C**), which showed a positive correlation with 2-methylpropan-1-ol (HA_1) and a negative correlation with methyl acetate (AE_1). However, these were not separated in the samples fermented with *S. cerevisiae* 3796 (**Figure 9B**).

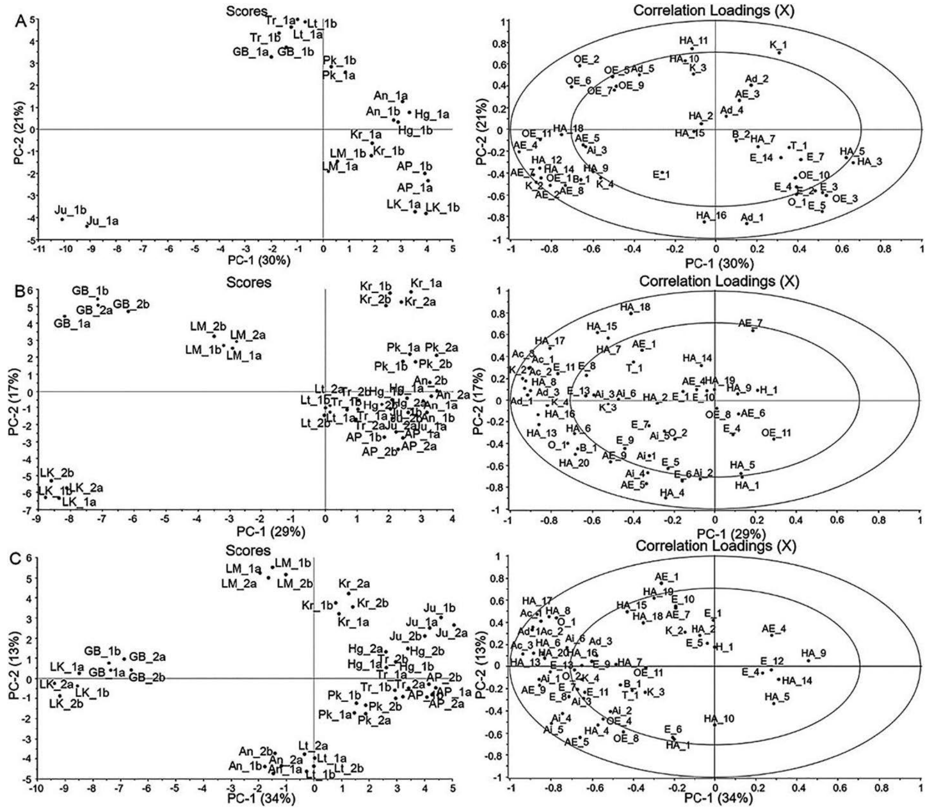


Figure 9. PCA plots of VOCs in apple juices (**A**, 22 samples, 44 volatiles), *S. cerevisiae* 1116 beverages (**B**, 44 samples, 51 volatiles), and *S. pombe* 3796 beverages (**C**, 44 samples, 54 volatiles) made from different apple cultivars. Figure reprinted from the original publication **I** (He et al., 2021), with permission from Elsevier.

In general, the profiles of the VOCs were more dependent on the yeast strains than the differences in cultivars. The PCA models (**Figure 9A-C**) showed similar patterns between the fermented beverages and juices within each cultivar, which could be ascribed to the primary VOCs derived from the initial apple juices. This indicated that the cultivar difference was a potential reason for the variation in the obtained fermented apple beverages. Similar results were also found in previous apple cider studies (Alberti et al., 2016; Rosend, Kuldjärv, Rosenvald, & Paalme, 2019).

5.3 Phenolic compounds (Studies II, III, and IV)

A total of 57 phenolic compounds (3 hydroxybenzoic acids, 25 hydroxycinnamic acids, 2 flavan-3-ols, 6 procyanidins, 16 flavonols, 4 dihydrochalcones, and 1 arbutin) were identified or tentatively identified from the apple beverages (**Study II**) and pear beverages (**Studies III** and **IV**) using a UHPLC-DAD-QTOF-MS system with the aid of external standards and data published in the literature (**Table 12**). The total number of detected phenolic compounds were slightly different between the apple and pear juices: 34 phenolic compounds were identified in apple beverages and 42 phenolic compounds were identified in pear beverages.

5.3.1 Phenolic profiles of juices (Studies II, III and IV)

5.3.1.1 Pasteurized apple juices (**Study II**)

The major phenolic groups and their concentrations in the pasteurized apple juices are shown in **Figure 10**. Among the studied apple cultivars, pasteurized juices made from ‘Aino’ contained the highest total content of phenolic compounds (101 mg/100 mL), whereas ‘Lepaan Meloni’ contained the lowest (18 mg/100 mL). The variations of total phenolic compounds can also be ascribed to the different genetic background of the different apple cultivars (Paweł Satora et al., 2018).

Table 12. Identification of phenolic compounds in apple (**II**) and pear (**III**) juices and their corresponding alcoholic beverages (**II** & **IV**). Adapted from the original publication **II & III** (He et al., 2022a; He et al., 2022b).

Peak	*Tentative identification*	*λ_{max} (nm)*	*Mass (m/z) $[M-H]^-/[M+H]^+$*	*Negative ions in MS^2 (m/z)*	*Positive ions in MS^2 (m/z)*	*Molecular formula*	*Identification method*	*Study*	*Abbreviations*
Hydroxybenzoic acids									
1	Syringic acid hexoside I	275	359 /-	197	-	$C_{15}H_{20}O_{10}$	MS and literature[1]	III & IV	Sya_hex I
2	Syringic acid	277	197/199	-	-	$C_9H_{10}O_5$	MS and literature[2]	III & IV	SYA
3	Syringic acid hexoside II	274	359/-	197	-	$C_{15}H_{20}O_{10}$	MS and literature[1]	III & IV	Sya_hex II
Hydroxycinnamic acids									
4	Caffeoyl N-tryptophan	326	365/-	229	-	-	MS and literature[3,4]	III & IV	Caf_TRY
5	3-*O*-Caffeoylquinic acid	327	353/355	191	-	$C_{16}H_{18}O_9$	MS, standard, and literature[4,5,6]	II & III & IV	3_Caf qA
6	Coumaroylquinic acid isomer I	320	337/339	173	147	$C_{16}H_{18}O_8$	MS and literature[4,5,6,7]	II	Cou_qA I
7	5-*O*-Caffeoylquinic acid	328	353/355	191	163	$C_{16}H_{18}O_9$	MS, standard, and literature[4,5,6,7]	II & III & IV	5_Caf qA
8	Coumaric acid	301	163/165	-	-	$C_9H_8O_3$	MS and literature[3]	IV	Cou_A
9	Caffeic acid	319	179/181	-	-	$C_9H_8O_4$	MS, standard, and literature[4,6]	II & III & IV	Caf_A
10	4-*O*-Caffeoylquinic acid	327	353/355	173	163	$C_{16}H_{18}O_9$	MS and literature[4,5,6]	II & III & IV	4_Caf qA
11	*p*-Coumaric acid	301	163/165	-	-	$C_9H_8O_3$	MS, standard, and literature[4]	II & III & IV	*p* Cou_A
12	Coumaroylquinic acid isomer II	320	337/339	173	147	$C_{16}H_{18}O_8$	MS and literature[4,5,7]	II & III & IV	Cou_qA II
13	Sinapic acid hexoside I	320	385/387	223	235	$C_{17}H_{22}O_{10}$	MS and literature[4]	III & IV	Spa_hex I
14	Caffeoylshikimic acid	326	335/337	179	163	$C_{16}H_{16}O_8$	MS and literature[9]	III & IV	CshA
15	Coumaroylquinic acid isomer III	318	337/339	191	147	$C_{16}H_{18}O_8$	MS and literature[4,5]	II & III & IV	Cou_qA III
16	Di-*O*-caffeoylquinic acid I	327	515/517	353/191	-	$C_{25}H_{24}O_{12}$	MS and literature[3,6]	II & III & IV	Di_Caf_qA I
17	Di-*O*-caffeoylquinic acid II	327	515/517	353/191	-	$C_{25}H_{24}O_{12}$	MS and literature[3,6]	II & III & IV	Di_Caf_qA II
18	Feruloylquinic acid isomer I	327	367/369	193	-	$C_{17}H_{20}O_9$	MS and literature[5]	III & IV	Fa_qA I

Table 12 (continued)

Pe-ak	Tentative identification	λ_{max} (nm)	Mass (m/z) $[M-H]^-/[M+H]^+$	Negative ions in MS^2 (m/z)	Positive ions in MS^2 (m/z)	Molecular formula	Identification method	Study	Abbreviations
19	Feruloylquinic acid isomer II	327	367/369	193, 173	163	$C_{17}H_{20}O_9$	MS and literature[5]	III & IV	Fa_qA II
20	Coumaroylquinic acid isomer IV	318	337/339	173	147	$C_{16}H_{18}O_8$	MS and literature[10]	II	Cou_qA IV
21	Coumaric acid derivative	313	581/583	279, 163	303, 147	-	MS and literature[4]	III & IV	Cou_der
22	Ferulic acid derivative	324	389/391	193	-	-	MS	III & IV	Fa_der
23	Ferulic acid hexoside I	327	355/357	193, 175	195	$C_{16}H_{20}O_9$	MS and literature[11]	II	Fa_hex I
24	Ferulic acid hexoside II	326	355/-	193, 175	-	$C_{16}H_{20}O_9$	MS and literature[11]	II	Fa_hex II
25	Ferulic acid hexoside III	327	355/357	193, 175	195	$C_{16}H_{20}O_9$	MS and literature[11]	II	Fa_hex III
26	Ferulic acid	326	193/195	-	-	$C_{10}H_{10}O_4$	MS and literature[11]	II	FA
27	Sinapic acid hexoside II	320	385/387	223	235	$C_{17}H_{22}O_{10}$	MS and literature[4]	III & IV	Spa_hex I
28	Caffeoylhexose	308	341/343	191, 179	265, 307	$C_{15}H_{18}O_9$	MS and literature[3]	III & IV	CafH
Flavan-3-ols									
29	(+)-Catechin	283	289/291	245, 203	207, 139	$C_{15}H_{14}O_6$	MS, standard, and literature[3]	II & III & IV	Cat
30	(–)-Epicatechin	278	289/291	-	-	$C_{15}H_{14}O_6$	MS, standard, and literature[3]	II & III & IV	E_Cat
Procyanidins									
31	A type procyanidin dimer	279	575/-	449, 289	-	$C_{30}H_{24}O_{12}$	MS and literature[3,7]	III & IV	A Di_I
32	B type procyanidin dimer I	279	577/579	407, 289, 161	427, 409, 291, 247	$C_{30}H_{26}O_{12}$	MS and literature[3,7]	II & III & IV	B Di_I
33	Procyanidin dimer B2	280	577/579	407, 289, 161	427, 409, 291, 247	$C_{30}H_{26}O_{12}$	MS, standard, and literature[3]	II & III & IV	Di_B2
34	B type procyanidin dimer II	280	577/579	407, 289, 161	427, 409, 291, 247	$C_{30}H_{26}O_{12}$	MS and literature[4,7]	II	Di_B II
35	B type procyanidin trimer	280	865/867	-	579, 409, 291,163	$C_{45}H_{38}O_{18}$	MS and literature[4]	III & IV	Tri_B
36	B type procyanidin dimer III	280	577/579	407, 289, 161	427, 409, 291, 271, 247, 163	$C_{30}H_{26}O_{12}$	MS and literature[3,4]	II & III & IV	Di_B III
Flavonols									
37	Quercetin hexoside deoxyhexoside I	353	609/611	301	303, 465	$C_{27}H_{30}O_{16}$	MS and literature[3,4]	III & IV	Que hex deo I

Table 12 (continued)

Peak	***Tentative identification***	***λ_{max} (nm)***	***Mass (m/z) $[M-H]^-/[M+H]^+$***	***Negative ions in MS^2 (m/z)***	***Positive ions in MS^2 (m/z)***	***Molecular formula***	***Identification method***	***Study***	***Abbreviations***
38	Quercetin hexoside deoxyhexoside II	353	609/611	301	465	$C_{27}H_{30}O_{16}$	MS and literature[3,4]	III & IV	Que hex deo II
39	Quercetin hexoside	348	463/465	301	303	$C_{21}H_{20}O_{12}$	MS and literature[4]	II & III & IV	Que_hex
40	Quercetin-3-*O*-glucoside	352	463/465	301	303	$C_{21}H_{20}O_{12}$	MS, standard, and literature[4]	II & III & IV	Que_glu
41	Quercetin pentoside I	350	433/435	301	-	$C_{20}H_{18}O_{11}$	MS and literature[12]	II	Que_pen I
42	Quercetin pentoside II	351	433/435	271, 301	303	$C_{20}H_{18}O_{11}$	MS and literature[12]	II	Que_pen II
43	Quercetin rhamnoside	351	447/449	301	317	$C_{21}H_{20}O_{11}$	MS and literature[12]	II	
44	Isorhamnetin hexoside deoxyhexoside	352	623/625	315	317	$C_{28}H_{32}O_{16}$	MS and literature[4]	III & IV	Iso hex deo
45	Kaempferol hexoside	352	-449		287	$C_{21}H_{20}O_{11}$	MS and literature[4]	II	Kae_hex
46	Kaempferol-3-*O*-glucoside	352	447/449	285	287	$C_{21}H_{20}O_{11}$	MS, standard, and literature[4]	II & III & IV	Kae_glu
47	Isorhamnetin hexoside I	352	477/479	315	317	$C_{22}H_{22}O_{12}$	MS and literature[3,4]	III & IV	Iso_hex I
48	Isorhamnetin hexoside II	352	477/479	315	317	$C_{22}H_{22}O_{12}$	MS and literature[3,4]	III & IV	Iso_hex II
49	Isorhamnetin rhamnoside	358	461/-	315		$C_{22}H_{22}O_{11}$	MS and literature[3,4]	II	Iso_rha
50	Isorhamnetin-acylated-hexoside I	352	519/521	315	-	$C_{24}H_{24}O_{13}$	MS and literature[3,4]	III & IV	Iso acy hex I
51	Isorhamnetin-acylated-hexoside II	354	519/521	315	-	$C_{24}H_{24}O_{13}$	MS and literature[3,4]	III & IV	Iso acy hex II
52	Quercetin	369	301/303	-	-	$C_{15}H_{10}O_7$	MS and literature[12]	II & IV	Que
Dihydrochalcones									
53	Hydroxyphloretin monoglycoside	281	451/453	289, 167	453, 315	$C_{22}H_{28}O_{10}$	MS and literature[12,13]	II	HP_mogly
54	Phloretin-2'-*O*-xyloglucoside	284	567/569	273	275	$C_{26}H_{32}O_{14}$	MS and literature[12]	II	Ph_xyglu
55	Phloretin pentose hexose	283	567/569	273		$C_{26}H_{32}O_{14}$	MS and literature[12]	II	Ph_pthx
56	Phloretin-2'-*O*-glucoside	283	435/437	273		$C_{21}H_{24}O_{10}$	MS, standard, and literature[14]	II	Ph_glu
Other phenolics									
57	Arbutin	282	271/273	109	-	$C_{12}H_{16}O_7$	MS and literature[7]	III & IV	Arb

Identified by [1]Sun, Tao, & Zhang, 2019, [2]Gu, Howell, Dunshea, & Suleria, 2019, [3]Kolniak-Ostek & Oszmiański, 2015, [4]Kolniak-Ostek, 2016a, [5]Clifford, Johnston, Knight, & Kuhnert, 2003, [6]Podio et al., 2015, [7]Wang et al., 2021 , [8]Li et al., 2019, [9]Zhang, Guo, Zheng, & Wang, 2013, [10]Santos, Oliveira, Ibáñez, & Herrero, 2014, [11]Liu, Marsol-Vall, Laaksonen, Kortesniemi, & Yang, 2020, [12]Laaksonen et al., 2017, [13]Kolniak-Ostek, 2016b, [14]Ramirez-Ambrosi et al., 2013.

As shown in **Figure 10**, the major phenolic compounds of the apple juices studied were hydroxycinnamic acids (80%), followed by flavan-3-ols (~10%), flavonols (~5%), and dihydrochalcones (~5%). The juices made from Finnish apple cultivars contained a relatively high content of hydroxycinnamic acids (38 mg/100 mL), which can be ascribed to the lower temperature and better sunlight exposure during the maturity and harvest of the apple cultivars (Fernández-Jalao et al., 2019). In the studied apple juices, 5-*O*-caffeoylquinic acid was detected as the most abundant phenolic acid, with a content ranging from 6 to 44 mg/100 mL. Generally, procyanidins are dominant in apples but 5-*O*-caffeoylquinic acid are more water-soluble when compared with the procyanidins (Fernández-Jalao et al., 2019). Thus, 5-*O*-caffeoylquinic acid represents the most dominant phenolic compound in the apple juices. Phloretin-2-*O*-glucoside and phloretin-2-*O'*-xyloglucoside were detected as the most abundant dihydrochalcones, and their concentrations were also dependent on the genetic background. For example, the detected total dihydrochalcones in 'Aino' was 15 mg/100 mL, which is notably higher than the levels in other apple cultivars.

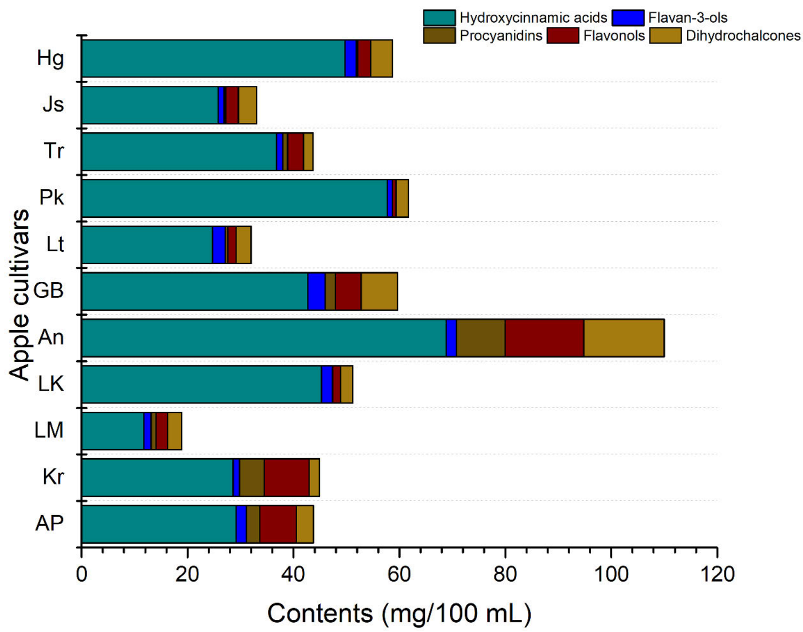


Figure 10. The total contents of major phenolic compounds in the pasteurized juices made from different apple cultivars (Study **II**).

5.3.1.2 Phenolic compounds in pear juices before and after pasteurization (**Studies III and IV**)

The phenolic profiles of the original juices (untreated) made from 17 pear cultivars are summarized in **Figure 11**. The total phenolic concentrations of the studied pear juices ranged from 17 mg/100 mL ('Conference') to 72 mg/100 mL

(‘Py10’ derived from ‘Karmla × Pakurlan Päärynä’). In addition, juices made from the perry pears, such as ‘Stolishnaja’ (65 mg/100 mL) and ‘Py10’ (72 mg/100 mL), contained a higher content of total phenolic compounds, whereas a lower amount of total phenolic content was found in the dessert pear groups, such as ‘Krupnoplodnaja Susova’ (18 mg/100 mL), ‘Conference’ (17 mg/100 mL), and ‘Clara Frijs’ (21 mg/100 mL). In addition, pear breeding selections sharing the same parental cultivars varied in their total phenolic content. For example, the pear selections sharing parental cultivars ‘Pepi × Lück’ ranged from 34 (‘Py4’) to 69 mg/100 mL (‘Py2’). In this case, the genetic effects on the phenolic profiles are complex, which has already been indicated by the different skin bitterness in the pears (Keutgen & Pawelzik, 2008).

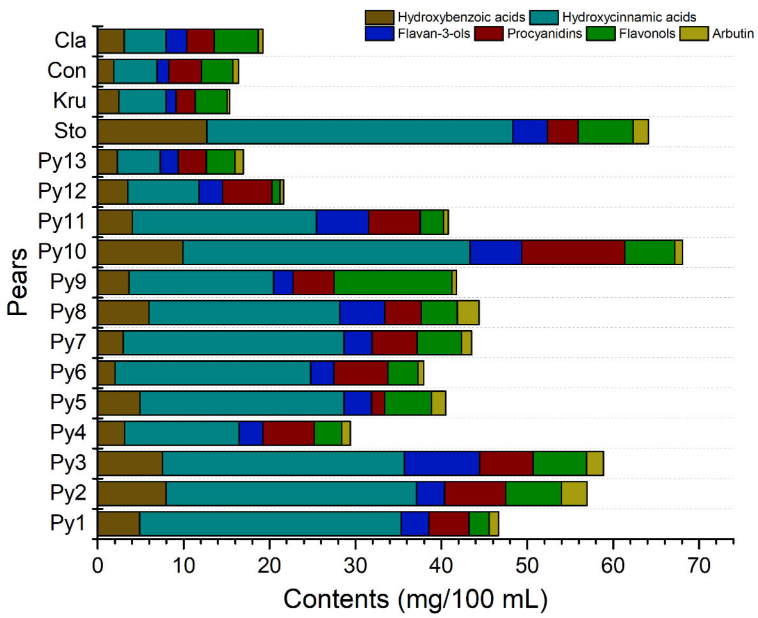


Figure 11. The total content of major phenolic compounds in the original juices made from different pear cultivars and breeding selections (**Study III**).

As shown in **Figure 11**, the pear juice made from ‘Stolishnaja’ was found to contain a higher concentration of total hydroxybenzoic acids at 13 mg/100 mL, whereas that from ‘Conference’ had significantly lower content than the other pears at 2 mg/100 mL. Regarding the hydroxycinnamic acids, the content ranged from 5 mg/100 mL (‘Clara Frijs’) to 36 mg/100 mL (‘Stolishnaja’) in the studied pear juices. In addition, as the primary hydroxycinnamic acid in pear juices, 5-*O*-caffeoylquinic acid accounts for 15–40% of total phenolic content among the studied pear juices. Similar results were also found in the Turkish pear cultivars as previously reported (Smanalieva, Iskakova, Oskonbaeva, Wichern, & Darr, 2020). In comparison with the pear breeding selections and the two test Russian pear cultivars, ‘Conference’ and ‘Clara Frijs’ contained relatively low concentrations of 5-*O*-caffeoylquinic acid. As regards the quantified flavan-3-ols, it ranged from 1 mg/100 mL (‘Krupnoplodnaja Susova’) to 9 mg/100 mL (‘Py3’) in the untreated juices made from the pear breeding selections and cultivars studied. In the studied pear juices, the dominant flavan-3-ols could be either (+)-catechin or (–)-epicatechin. For example, ‘Stolishnaja’ contained high concentrations of (–)-epicatechin, and no (+)-catchin was found. (–)-Epicatechin was also found to be the predominant flavan-3-ols in most European and Tunisian pears as previously reported. The total procyanidins also varied from the pear breeding selections and cultivars, ranging from 2 mg/100 mL (‘Py5’) to 12 mg/100 mL (‘Py10’). The pear breeding selection ‘Py10’ was determined as a ‘putative perry pear’ beforehand by breeders and it showed the highest amounts of total procyanidins up to 12 mg/100 mL in the untreated juices. In general, putative perry pears contain higher concentrations of procyanidins (high DP). The differences in the total flavonols also depended on the pear cultivars, and it varied from 1 mg/100 mL (‘Py12’) to 14 mg/100 mL (‘Py9’). As the characteristic phenolic compound in pear juices, arbutin varied from ‘Py2’ (3 mg/100 mL) to ‘Krupnoplodnaja Susova’ (0.3 mg/100 mL).

The untreated juices underwent a pasteurization process before fermentation. In general, the pasteurization process altered the phenolic compositions and profiles, which was significantly dependent on different pear cultivars. In comparison with the untreated pear juices, the pasteurization process led to a significant decrease (21%) in the total quantified phenolic content. As phenolic compounds are heat-sensitive compounds, they are unstable and easy-degradable under thermal treatments (Rawson et al., 2011). Similar reduction of phenolic compounds has been reported in previous studies on apple juices, orange juices, and mango juices (Guiné et al., 2021; Pala & Toklucu, 2013). However, it showed a slight increase (16%) of total phenolic compounds in ‘Py4’. As previously reported, total phenolic compounds are closely related with α-glucosidase inhibitory (α-GI) activities (Adyanthaya, Kwon, Apostolidis, & Shetty, 2010). In other words, high α-GI activities correlated with high total phenolic compounds in the juices. The α-GI activities might be relatively high in the untreated juices made from pear breeding selections ‘Py4’, but the pasteurization process led to a decrease in the α-GI activities (Alongi et al., 2019). Moreover, this could have also resulted from the thermal inactivation of polyphenoloxidase (PPO), leading to brown reactions in the juices (Alongi, Verardo, Gorassini, & Anese, 2018). Pasteurization led to a decrease in the PPO, preventing the phenolic compounds from producing the brown reactions. Overall, the reactions during the pasteurization process are complex and required more investigations. In addition, the pasteurization effects on different pear cultivars were dependent on the different pear cultivars, which might be due to the differences in the α-GI or PPO activities of different pear cultivars.

5.3.2 Comparison of different yeast strains on the phenolic profiles of fermented products (Studies II and IV)

5.3.2.1 Fermented apple beverages (**Study II**)

In general, the fermented apple beverage products (SC1116: 40 mg/100 mL, SP3796: 37 mg/100 mL) showed significantly fewer phenolic compounds than the corresponding apple juices (49 mg/100 mL). The fermentation process led to the release of free aglycones, such free hydroxycinnamic acids and free flavonols, which could have resulted from the cleavage of the glycosidic bonds during yeast fermentation (Laaksonen et al., 2017). Higher concentrations of free hydroxycinnamic acids and lower total flavonols and dihydrochalcones were found in the apple beverages. A partial least squares discriminant analysis (PLS-DA) was used to visualize the overall effects of yeast strains in the alcoholic apple beverage fermentation and the differences between alcoholic apple beverages and their corresponding juices. As shown in **Figure 12**, 78% of phenolic variables explained 69% of variations in seven factors (R^2=0.8262, validated R^2=0.7401). The apple juices were clearly separated from the alcoholic apple beverages with higher concentrations of flavonols and dihydrochalcones. Generally, the juices were highly associated with isorhamnetin-3-*O*-rhamnoside (Iso_rha), quercetin-3-O-rhamnoside (Qu_rha), quercetin pentoside I (Qu_pen I), phloretin-2’-*O*-glucoside (Ph_glu), phloretin-2’-*O*-xyloglucoside (Ph_xyglu), (-)-epicatechin (E_Cat), PC dimer IV (Di_IV), and 3-*O*-*p*-coumaroylquinic acid (3-ClqA).

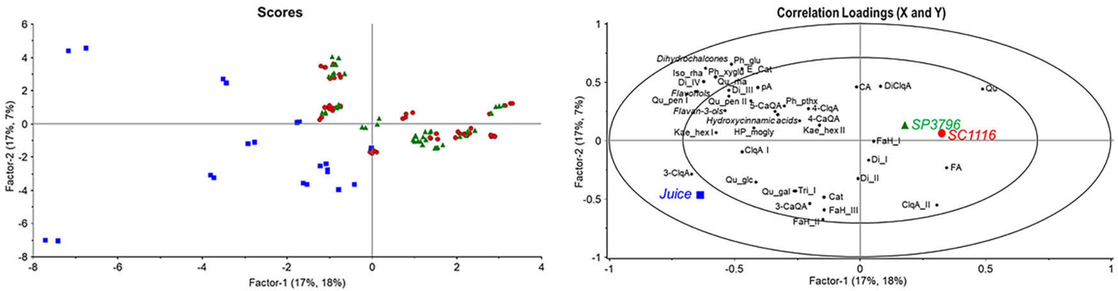


Figure 12. PLS-DA models of phenolic compounds as X-data (n=34) to illustrate the differences between apple juices and fermented apple beverages from *S. cerevisae* 1116 and *S. pombe* 3796. Juices with blue rectangles, SP beverages with green triangles, SC beverages with red circles. For the abbreviations of apple cultivars and phenolic compounds refer to **Table 8** and **Table 12**. Figure reprinted from the original publication **II** (He et al., 2022a), with permission from Elsevier.

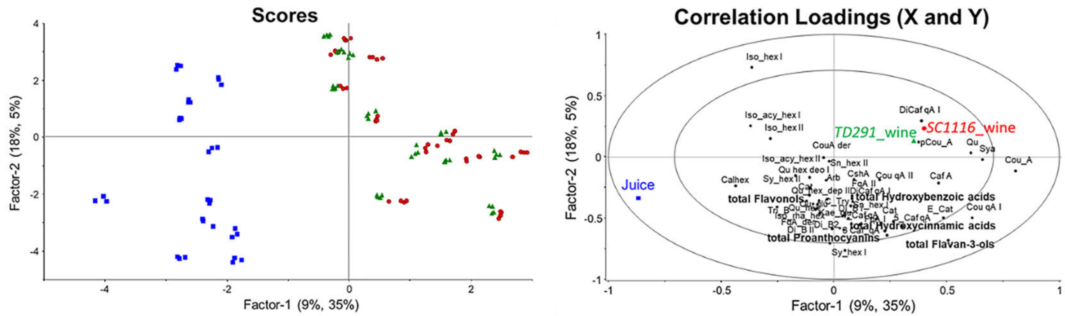


Figure 13. PLS-DA models of phenolic compounds as X-data (n=42) to illustrate the differences between pear juices and fermented pear beverages from *S. cerevisae* 1116 and *T. delbruckii* 291. Juices with blue rectangles, TD beverages with green triangles, SC beverages with red circles. For the abbreviations of pear cultivars and phenolic compounds refer to **Table 9** and **Table 12**.

The phenolic compositions and profiles of the obtained apple beverages were significantly influenced by the apple cultivars, and yeast strains also played an important role in the phenolic profiles. Compared with the procyanidin compositions in the SC1116 beverages (0.9 mg/100 mL on average), beverages made with SP3796 showed slightly higher amounts of procyanidins (1.1 mg/100 mL on average). This could be the result of the higher content of polysaccharides released during the fermentation of *S. pombe* strains (Benito, 2019), and the binding between polysaccharide and procyanidin could help to stabilize the reactive procyanidins. Moreover, SP3796 produced lower concentrations of 5-*O*-caffeoylquinic acid (5-CaQA), 4-*O*-caffeoylquinic acid (4-CaQA), 3-*O*-*p*-coumaroylquinic acid (3-ClqA), and 4-*O*-*p*-coumaroylquinic acid (4-ClqA) when comparing with SC1116. In addition, slightly higher concentrations of (+)-catechin were produced by SP3796 (0.4 mg/100 mL on average) than that with SC1116 (0.2 mg/100 mL).

5.3.2.2 Fermented pear beverages (**Study IV**)

Interestingly, the pear beverages fermented by both SC1116 and TD291 showed a higher number of total phenolic compounds when compared with their corresponding pear juices. For example, more hydroxycinnamic acids and flavan-3-ols, and less hydroxybenzoic acids, procyanidins, and flavonols were found in the alcoholic pear beverages. The increase in the hydroxycinnamic acids can be the result of the release of polysaccharides and mannoproteins by the yeast strains (Kulkarni et al., 2015; Sartor et al., 2019). In addition, the increase in the flavan-3-ols and the reduction in the procyanidins could be the result of the hydrolysis of procyanidins during yeast fermentation (Vidal et al., 2002). Moreover, the decrease of flavonol glucoside and increase of the quercetin aglycone can be ascribed to the hydrolysis of glycosylated flavonols (Makris et al., 2006).

Partial least squares discriminant analysis (PLS-DA) was conducted to visualize the differences between pasteurized pear juices and their fermented alcoholic beverages. In the PLS-DA plots (X-data, n=47) of **Figure 13**, 54% of chemical variables explained 42% of variation in three factors (R^2=0.9386, validated R^2=0.9158). The validated correlation coefficient number is high and this can be due to the fewer factors associated with the separation between pear juices and beverages. As shown in **Figure 13**, the pasteurized juices can be clearly separated from the alcoholic pear beverages along Factor-1. Among the detected phenolic compounds, pear beverages were found to be correlated closely with coumaric acid (Cou A), coumaroylquinic acid isomer I (Cou qA I), and total concentrations of quantified monomeric flavan-3-ols.

5.3.3 Comparison of different fruit cultivars on the phenolic profiles of fermented products (Studies II and IV)

5.3.3.1 Fermented apple beverages (**Study II**)

Principal component analyses (PCA) were established to investigate the relationship between phenolic compounds and apple cultivars. The overall effects of apple cultivars on the phenolic profiles of apple juices and alcoholic beverages are shown in **Figure 14A**, with PC1 and PC2 accounting for 27% and 18%, respectively. As shown in **Figure 14A**, the juices and alcoholic beverages made from 'Aino', 'Gustavs Bästa', and 'Hyvingiensis' were located on the left side along PC-1. Among these cultivars, 'Aino' showed a high relationship with the hydroxycinnamic acids, mainly caffeic acid (CA), *p*-coumaric acid (*p*A), and 4-*O*-*p*-coumaroylquinic acid (4-ClqA), dihydrochalcones, mainly as phloretin-2'-*O*-glucoside (Ph_glu) and phloretin-2'-*O*-xyloglucoside (Ph_xyglu), as well as (-)-epicatechin (E_Cat) and procyanidin dimer IV (DI_IV). In addition, juices and alcoholic beverages made from 'Gustavs Bästa' were closely associated with flavonols, such as quercetin pentoside I (Qu_pen I), quercetin pentoside II (Qu_pen II), quercetin-3-*O*-rhamnoside (Qu_rha), and isorhamnetin-3-*O*-rhamnoside (Iso_rha). In addition to these cultivars, the cultivar 'Pieksämäki' was clearly separate from the other apple cultivars (**Figure 14B**), with PC1 and PC2 accounting for 27% and 15%, respectively. This cultivar 'Pieksämäki' correlated strongly with the hydroxycinnamic acids, such as caffeic acid (CA), 4-*O*-caffeoylquinic acid (4-CaQA), coumaroylquinic acid II (ClqA_II), and 4-*O*-*p*-coumaroylquinic acid (4-ClqA). Similar patterns of cultivar effects were shown in both apple juices and alcoholic apple beverages. Thus, the cultivar difference was the major factor determining the phenolic profiles of the alcoholic apple beverages.

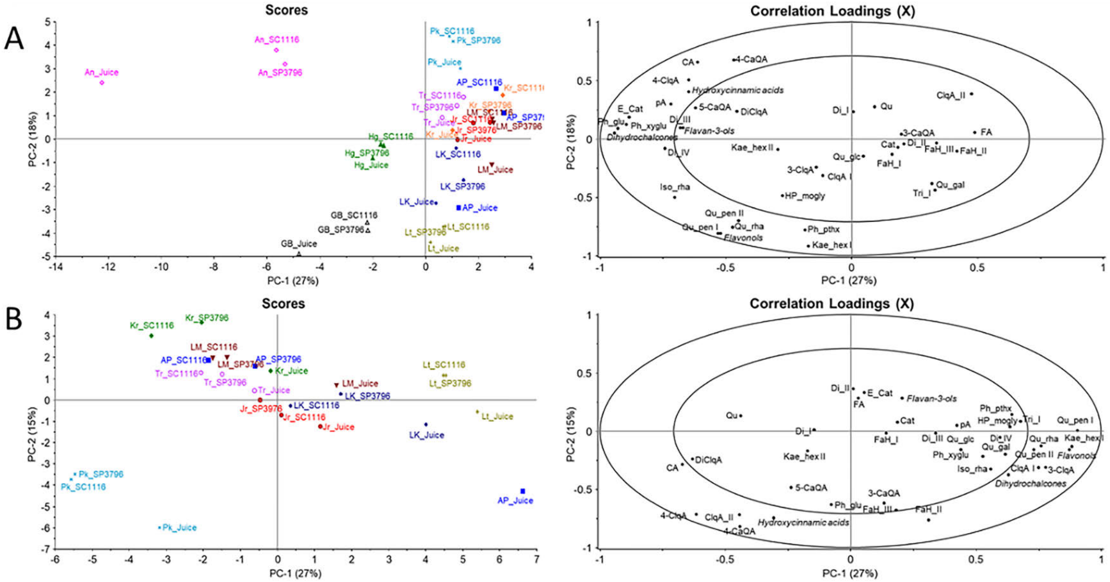


Figure 14. PCA models of phenolic compounds as X-data (n=34) to illustrate the differences between apple juices and their corresponding beverages (*S. cerevisiae* 1116 and *S. pombe* 3796). Apple cultivars are shown in different colors and symbols. **A**: all cultivars; **B**: dominant cultivars ('Aino', 'Gustavs Bästa', and 'Hyvingiensis') in **A** excluded. The abbreviations of the apple cultivars and phenolic compounds can be found in **Table 8** and **Table 12**. Figure reprinted from the original publication **II** (He et al., 2022a), with permission from Elsevier.

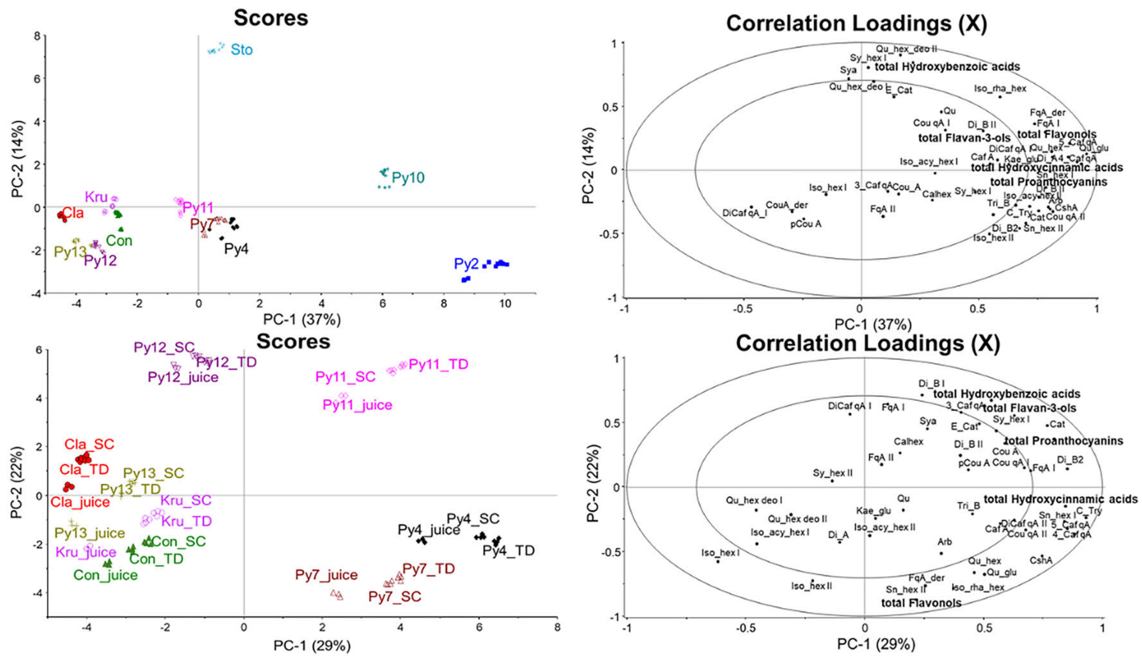


Figure 15. PCA models of phenolic compounds as X-data (n=42) to illustrate the differences between pear juices and their corresponding beverages (*S. cerevisiae* 1116 and *T. delbreuckii* 291). Pear cultivars are shown in different colors and symbols. The abbreviations of pear cultivars and phenolic compounds can be found in **Table 9** and **Table 10**.

5.3.3.2 Fermented pear beverages (**Study IV**)

Similarly, the phenolic compositions and profiles of the alcoholic pear beverages were mainly dependent on the used pear breeding selections and pear cultivars. The total phenolic compounds of alcoholic pear beverages fermented by SC1116 varied from 14 mg/100 mL to 65 mg/100 mL, whereas that of TD291 beverages varied from 14 mg/100 mL to 70 mg/100 mL. Generally, the phenolic compositions and profiles of alcoholic pear beverages were similar to that of pasteurized pear juices.

The overall effects of the phenolic compositions and the studied pear products were investigated using PCA models (**Figure 15**). As shown in the PCA models (Figure 2A), the first two principal components explained 51% of the total variance, with PC1 and PC2 accounting for 37% and 14%, respectively. Pear breeding selections 'Py2' and 'Py10' were clearly separated from the other pear cultivars along PC-1 and located on the right hand side of PC1, with a strong relationship with arbutin (Arb), monomeric flavan-3-ols and procyanidins, primarily as A-type procyanidin dimer (Di_A), B-type procyanidin dimer I (Di_B I), (+)-catechin (Cat), procyanidin dimer B2 (Di_B2), hydroxycinnamic acids, mainly as caffeoyl N-tryptophan (C_Try), 5-*O*-caffeoylquinic acid (5_Caf qA), 4-*O*-caffeoylquinic acid (4_Caf qA), caffeoylshikimic acid (CshA), coumaroylquinic acid isomer II (Cou qA I), feruloylquinic acid isomer I (FqA I), ferulic acid derivative (FqA_der), sinapic acid hexoside II (Sn_hex II), and flavonols, such as quercetin hexoside (Qu_hex), quercetin-3-O-glucoside (Qu_glu), isorhamnetin hexoside II (Iso_hex II), and isorhamnetin-acylated-hexoside II (Iso_acy_hex II). In addition, the pear cultivar 'Sto' was located on the positive side of PC-2, showing a high correlation with hydroxybenzoic acids and flavonols, such as syringic acid (Sya), syringic acid hexoside II (Sy_hex II), quercetin hexoside deoxyhexoside II (Qu hex deo II), and isorhamnetin hexoside deoxyhexoside (Iso hex deo). In general, pasteurized pear juices and fermented pear beverage products made from 'putative perry pears' ('Py10' and 'Sto') were correlated with higher concentrations of phenolic compounds when compared with other pear fruit cultivars except for 'Py12'.

5.4 Sensory properties (Studies I, II, and IV)

5.4.1 Sensory properties of fermented products (Studies I and IV)

5.4.1.1 Fermented apple beverages (**Study I**)

The sensory quality of selected apple beverages, produced from the four apple cultivars, were studied using an untrained panel. The potential differences between the cultivars and yeast strains were characterized using rated sensory attributes and CATA descriptors. As shown in the PCA model (**Figure 16A**), a clear differentiation based on the yeast strains was observed among the apple beverages except for beverages made from the cultivar ‘Gustavs Bästa’ (GB). In general, the *S. cerevisiae* 1116 beverages showed a higher appearance preference together with higher intensities of sourness, puckering and mouth-drying astringency. This could be due to the higher residual malic acid found in the *S. cerevisiae* 1116 beverages. In general, the average ratings were similar and clear differences were only found in the sourness and astringency between the yeast strains. Moreover, the perceived bitterness was similar between the two different yeast strains. In addition, apple beverages made from ‘Gustavs Bästa’ (GB) behaved differently; they were evaluated as less sour and astringent when compared with the other beverages. This could be ascribed to the lower organic acids and higher sugars in this cultivar.

In the CATA evaluation, the studied apple beverages were characterized as fruity, cider-like, fermented, sweet, and floral. As shown in the PCA model with CATA descriptors (**Figure 16**), a clear differentiation based on the yeast strains was observed among the apple beverages along PC-1. *S. cerevisiae* 1116 beverages were located on the positive side of PC-1, and they were described as sharp, dry apple, alcoholic, cider-like, yeasty, earthy, fermented, acidic, and cooked apple. In addition, the *S. pombe* 3796 beverages were characterized as floral, tropical fruity, fruity, honey-like, sweet, and diverse. Previous studies also found that fermentation with non-*Saccharomyces* improved the fruity and mouth-feel of apple ciders and fruit wines (Magalhães, Krogerus, Vidgren, Sandell, & Gibson, 2017; Varela, 2016).

The relationships between the sensory profiles and the phytochemical compounds were further investigated via multivariate models in the current study. As shown in the PCA model (**Figure 17**), the concentrations of hydroxycinnamic acids like caffeic acid (CA), p-coumaric acid (pA), and 5-O-caffeoylquinic acid (5-CaQA), and flavan-3-ols, such as PC dimer III (Di_III), were closely associated with mouth-drying and puckering astringency. No correlation was detected between phenolic composition and bitterness and sourness.

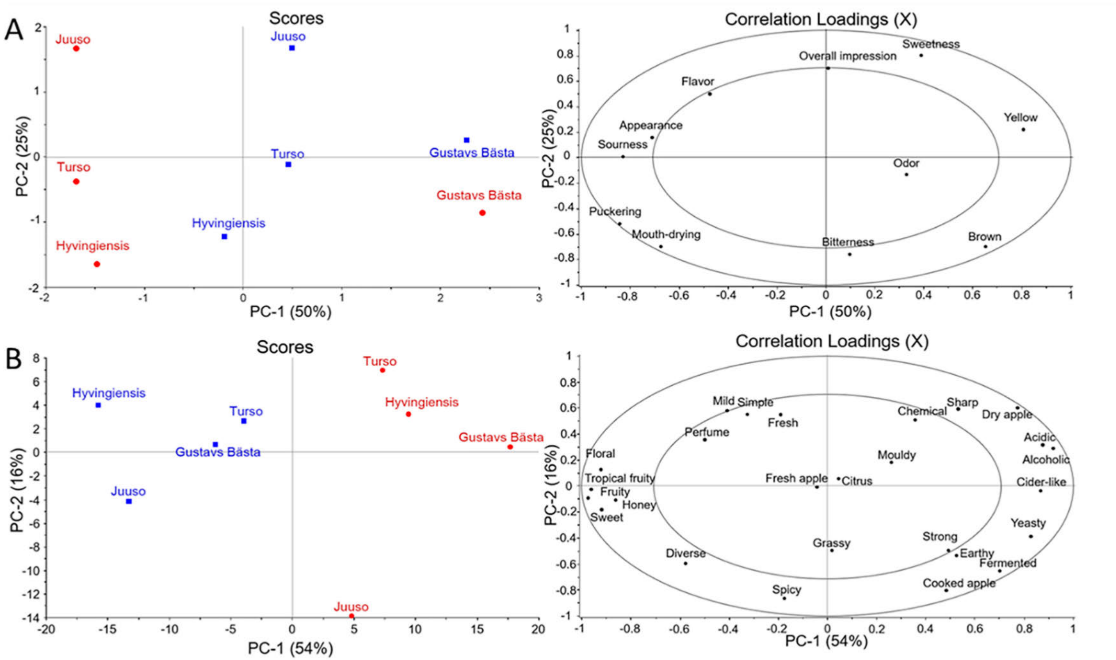


Figure 16. PCA models for sensory attributes of different apple beverages (8). A: rated pleasantness and sensory attributes (n=11); B: CATA attributes (n=26). *S. cerevisiae* 1116 with red circles and *S. pombe* 3796 with blue rectangles. Figure reprinted from the original publication **I** (He et al., 2021), with permission from Elsevier.

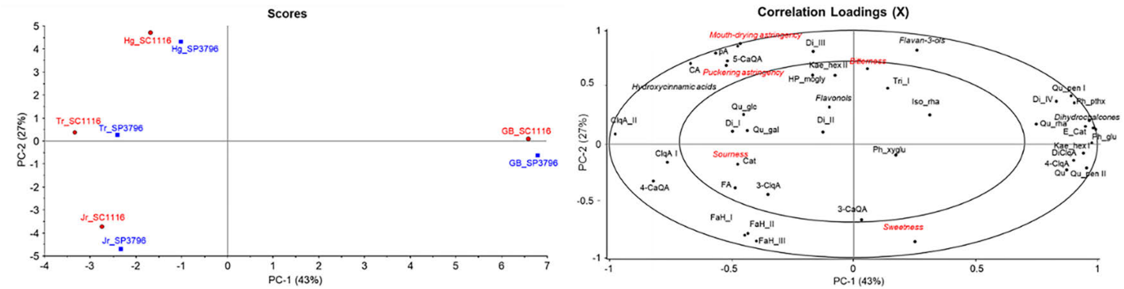


Figure 17. Principal Component Analysis (PCA) models for the chemical and sensory characteristics of alcoholic apple beverages (‘Gustavs Bästa’, ‘Juuso’, ‘Turso’, and ‘Hyvingiensis’, produced from *S. cerevisiae* 1116 and *S. pombe* 3796). SC beverages with red circles, SP beverages with blue rectangles. Abbreviations of apple cultivars can be found in **Table 8**, and abbreviations of phenolic compounds in **Table 12**. Figure reprinted from the original publication **II** (He et al., 2022a), with permission from Elsevier.

5.4.1.2 Fermented pear beverages (**Study IV**)

The sensory quality of the selected pear beverages, produced from six pear cultivars and breeding selections, were studied using a trained panel. In the three-way ANOVA, significant main effects in the pear beverages were observed in 'pear' and 'cooked pear' odors and in the taste attributes (sweet, bitter, sour) and in astringency. In general, the sensory profiles of the studied pear beverages were highly dependent on the pear cultivars and breeding selections whereas the yeast type had notably less effect. In 'cooked pear' and 'floral' odors and sweet taste the samples made with TD291 were evaluated as more intense than the SC1116 samples.

The effect of the pear cultivar can be observed in the sensory consensus plot PCA in **Figure 18** where samples made from 'Sto' (by both yeast strains) were located on the right hand side of PC-1 together with the astringency, sourness, and 'pear' odor variables. The astringency and sourness in the fermented pear beverages made from 'Sto' might be ascribed to the fruit cultivars as it is described as acidic and astringent by breeders. Pear beverages made from 'Py10' (again by both yeasts) were separated and located on the negative side of PC-2, and correlated highly with pear-odor, acidity-odor, and sourness. Moreover, pear beverages made from the commercial cultivar 'Cla' were highly related to the sweetness taste. Generally speaking, the obtained alcoholic pear beverages with higher astringency and sourness were made from sour and astringent pears, whereas the sweet pear beverages were made from the sweet and dessert pear cultivars. In addition, the appearance of the studied pear beverages also showed high correlations with the pear cultivars and breeding selections. The PCA made based on the CATA test frequencies for appearance attributes (**Figure 18B**) showed that the pear beverages produced from 'Sto' and 'Kru' were characterized as clear, brown, and pale, whereas those made from 'Py4', 'Py10', 'Py12', and 'Cla' were characterized as white, yellow, and cloudy.

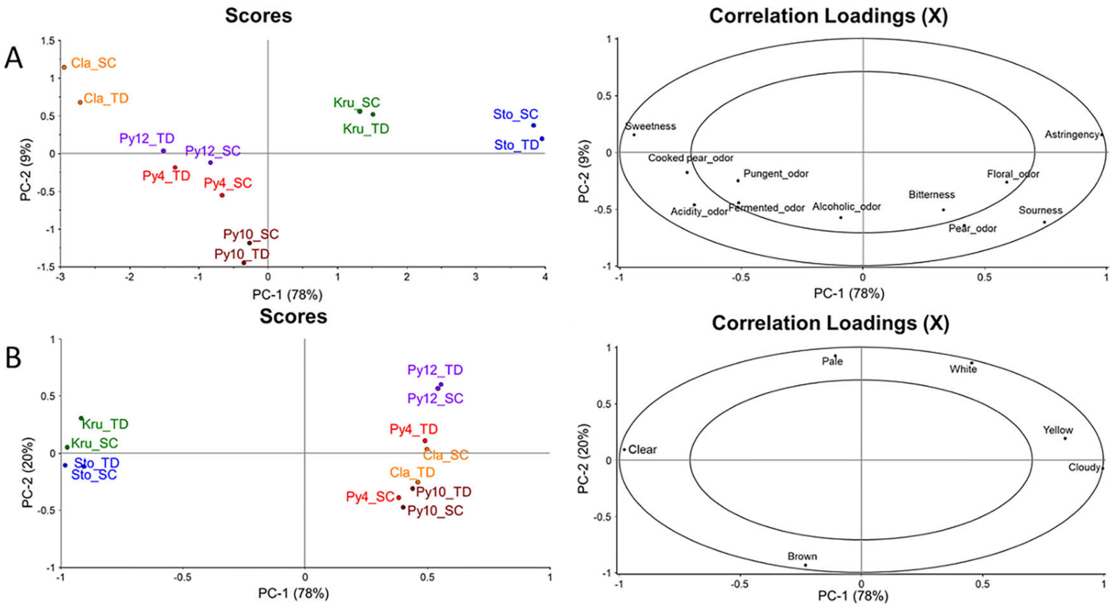


Figure 18. PCA models for sensory attributes of different pear beverages (12). A: rated pleasantness and sensory attributes (n=11); B: CATA attributes for appearance (n=6). Abbreviations of pear cultivars and phenolic compounds can be found in **Table 7** and **Table 10**, respectively.

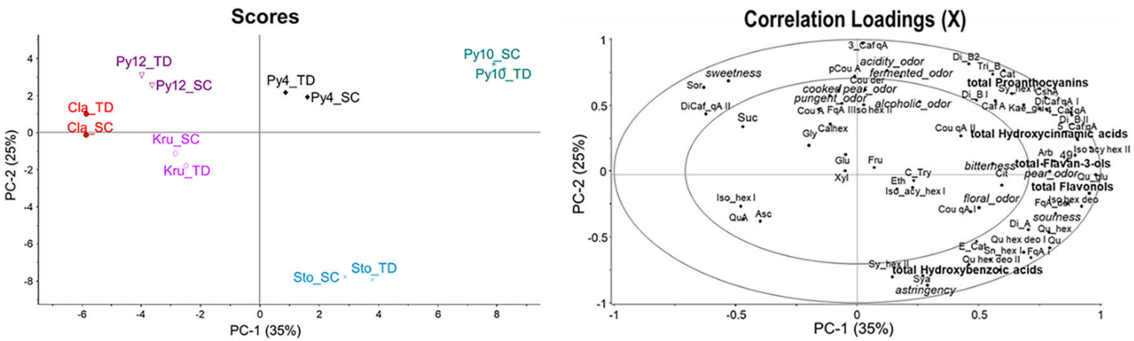


Figure 19. Principal Component Analysis (PCA) models for chemical and sensory characteristics of studied pear beverages ('Py4', 'Py10', 'Py12', 'Sto', 'Kru', and 'Cla' produced from SC1116 and TD291). Pear cultivars are shown in different symbols and colors. Abbreviations of pear cultivars and phenolic compounds can be found in **Table 7** and **Table 10**, respectively.

The relationships between the sensory profiles and the phytochemical compounds were further investigated via multivariate models in the current study. As shown in the PCA model (**Figure 19**), higher concentrations of hydroxybenzoic acids, such as syringic acid (Sya) and syringic acid hexoside II (Sy_hex II), hydroxycinnamic acids, such as coumaroylquinic acid isomer I (Cou qA I), sinapic acid hexoside I (Sn_hex I), and feruloylquinic acid isomer I (FqA I), (+)-epicatechin (E_Cat) and A-type procyanidin dimer (Di_A), as well as the main flavonols, such as quercetin hexoside dexyhexoside I (Qu hex deo I), quercetin hexoside dexyhexoside II (Qu hex deo II), quercetin hexoside (Qu hex), isorhamnetin hexoside deoxyhexoside (Iso hex deo), and quercetin (Qu), they were closely correlated with perceptions of astringency and sourness. Hydroxycinnamic acids and procyanidins were found to contribute to the astringency and sour perceptions, and this has also been demonstrated well in alcoholic apple beverages. In addition, the sweetness perception was shown to be highly correlated with di-*O*-caffeoylquinic acid I (DiCaf qA I) and sorbitol (Sor). The residual sorbitol was found to be the main sweetness contributor in the fermented pear beverage products. No significant relationship was found between the phytochemical compounds and bitterness in the current study, which was in accordance with our alcoholic apple beverage studies.

5.5 General discussion

Apple crops are commercially important in Finland, and they play important roles in the fruit and beverage industry. Currently, domestic apple cultivars are highly appreciated for their characteristics of being natural, fresh, and clean. The selection of proper apple cultivars as the raw material is an important factor determining the quality of alcoholic apple beverages. However, there are no breeding programs for apples for special use to produce alcoholic apple beverages in Finland, and only limited data is available concerning the potential of using Finnish apple cultivars in alcoholic beverage production. In this doctoral thesis research, two different yeast strains (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) were applied to assist the fermentation process of eleven Finnish local apple cultivars. The chemical composition (volatiles and non-volatiles) and sensory properties were comprehensively investigated before and after yeast fermentation. The fate of the sensorial chemical compounds and their contributions to the sensory properties were investigated, mainly for their mouth-drying and puckering astringency. Both cultivars and yeast strains played important roles in the chemical composition and sensory properties. The potential of using *S. pombe* in alcoholic apple beverages and its effect on the reduction of malic acid content and sourness taste, have been demonstrated in this research.

The pear cultivars used in the current study are novel breeding selections developed by Luke, to provide the necessary winter-hardiness and early maturing features for cultivated pears. The phenolic composition of certain unreleased breeding selections and cultivars of European pears were studied. Generally speaking, the chemical composition of pear juices is primarily dependent on the genetic background, but the effects are complex. Even cultivars sharing the same parental cultivars showed significant differences in their chemical compositions. Two yeast strains (*Saccharomyces cerevisiae* and *Torulaspora delbrueckii*) were selected to ferment fruit from eleven pear cultivars to produce alcoholic pear beverages. The phenolic composition of the fermented alcoholic beverages was primarily dependent on the pear cultivars while the yeast strains showed a slight difference in impact on the chemical composition of alcoholic pear beverages. In comparison to *S. cerevisiae*, the application of *T. delbrueckii* resulted in higher 'cooked pear' and 'floral' odors in the fermented pear beverages. Moreover, certain phenolic acids, flavan-3-ols, procyanidins, and flavonols were found to be associated with astringency.

In the current study, the fermented apple and pear beverages were produced from Finnish apple and pear cultivars without the addition of sugar, thus producing a 'natural beverage'. The alcoholic content of the alcoholic apple beverages ranged from 5.5 to 7.4% (*v/v*), whereas that for alcoholic pear beverages it ranged from 7.4 to 17.8% (*v/v*). In comparison to apple fermentation, the fermentation of pear beverages required a longer fermentation time to produce beverages with a higher alcoholic content. This result can mainly be the result of differences in the fruit, e.g., higher concentrations of sorbitol in the pear cultivars. In addition, fruit cultivars were found to be one of the dominant factors in the quality of AFBs. The findings of the current study should help breeders when developing cultivars which are suitable for producing juice or beverages. To select the specialty fruit cultivar for beverage production, the current research focused on the utilization of fruit juices made from a 'single cultivar'. However, the beverage industries prefer to use 'blended juices' made from specific fruit cultivars. Thus, more investigations based on 'blended juices' should be carried out in future studies. In addition, the alcoholic content of the fermented beverages could be studied by controlling the use of several fermentation processes, e.g., shorten the fermentation period or blend the fruit juices with the obtained AFBs.

In the current study, *Saccharomyces cerevisiae* and two non-*Saccharomyces* yeast strains were used in alcoholic beverage fermentation. The beverages made with *S. pombe* significantly reduced the malic acid content, as well as, reducing the sourness taste in alcoholic apple beverages. The potential of *T. delbrueckii*, to produce AFBs such as pear beverages with a low alcoholic content has also been well investigated. Technically, the selection of optimal strains to improve the fermentation performance and the overall quality of AFBs is commercially important for the beverage industry. Thus, it is crucial to understand the potential of using different non-*Saccharomyces* in the fermentation of AFBs to provide a theoretical basis for the beverage industry. Future studies should be conducted on yeast selection with sequential or pure inoculations to produce AFBs with a high quality.

The contribution of phenolic compounds to the sensory properties of fermented beverages has been investigated in the current study, mainly focusing on the association between procyanidins and an astringency taste. More detailed information based on the characterization of procyanidin profiles should be studied in future studies. In addition, more research should be carried out to investigate specific VOCs and their contribution to the sensory properties using gas chromatography-olfactometry (GC-O).

6 SUMMARY AND CONCLUSION

The chemical composition of juices and alcoholic fruit beverages produced from Finnish apple and pear fruits was investigated using gas chromatographic and liquid chromatographic methods together with mass spectrometry. Additionally, the research created new insights into the sensory properties of selected alcoholic beverages and their linkages to the chemical composition. This research provides novel findings on the technological aspects of the fermentation of alcoholic fruit beverages, and promotes the processing and utilization of Finnish local apple and pear fruits.

The chemical composition of untreated pear juices and pasteurized apple and pear juices was thoroughly studied in this research. 5-*O*-caffeyolquinic acid was the predominant compound in both apple and pear juices, followed by flavan-3-ols and procyanidins. Pasteurization processes led to a decrease in the total phenolic content as the phenolic compounds are unstable under thermal treatments. The fermentation process altered the chemical profiles of the studied fruit beverages. Generally, the volatile compositions were highly dependent on the yeast strains, whereas the phenolic profiles were highly dependent on the fruit cultivars.

The alcoholic apple beverages were made from traditional and local apple cultivars, whereas the alcoholic pear beverages were produced from novel pear breeding selections derived from European pear cultivars. The chemical compositions and sensory properties of the obtained fermented beverages were studied. In general, alcoholic fruit beverages made from 'cider' apples or 'perry' pears contained higher concentrations of phenolic compounds together with a strong taste of sourness and astringency, whereas those beverages made from 'dessert' apples or pears were characterized as fruity and floral.

In addition, the lack of suitable fermentation techniques to produce natural alcoholic fruit beverages with an acceptable quality is another challenge for fermentation. The utilization of non-*Saccharomyces* yeasts could provide fermented products with a pleasant taste and aroma complexity. For example, fermentation with *S. pombe* led to a significant reduction of malic acid in the alcoholic apple beverages when compared to *S. cerevisiae*, and this resulted in a reduction of sourness in the final alcoholic apple beverages. In addition, *T. delbrueckii* provided a "cooked pear" and "floral" odor note as well as high intensities of sweetness in the obtained pear beverages.

This research facilitates a better understanding of the potential of the non-conventional yeast strains in alcoholic fruit beverage production and their influence on the sensory quality of the fermented products. This study with apple and pear beverages can provided useful references for studies investigating the variables in fermentation of other fruit beverage.

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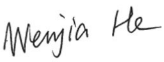

Turku, 2022
Wenjia He

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APPENDIX: ORIGINAL PUBLICATIONS

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IV. Submitted.

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