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Pham Minh Tuan

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PRODUCTION AND QUALITY ANALYSIS OF SOME SPIRITS FROM FRUITS

Pham Minh Tuan

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The Doctoral School

Name: Doctoral School of Food Science

Discipline: Food Science

Head: **Dr. Livia SIMON-SARKADI**
Professor, DSc.
Department of Nutrition Science
Institute of Food Science and Technology
Hungarian University of Agriculture and Life Sciences

Supervisors: **Dr. Quang D. Nguyen**
Professor, PhD
Department of Bioengineering and Alcoholic Drink Technology
Institute of Food Science and Technology
Hungarian University of Agriculture and Life Sciences

Dr. Ágoston Hoschke
Professor, CSc
Department of Bioengineering and Alcoholic Drink Technology
Institute of Food Science and Technology
Hungarian University of Agriculture and Life Sciences

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Approval of the Head of Doctoral School Approval of the Supervisor(s)

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1. INTRODUCTION

Fruit spirits belong to the group known as “Spirit-based” beverages, which are very popular worldwide, especially in Eastern and Central European countries. They are regarded as a traditional alcoholic beverage and a kind of gastronomic heritage (Śliwińska *et al.*, 2015). According to Regulation of European Community EC 110/2008, 'Fruit spirit is a spirit drink produced exclusively by the alcoholic fermentation and distillation of fleshy fruit or must of such fruit, berries or vegetables, with or without stones'. The alcohol content of fruit spirits has been more than 37.5 % v/v and less than 86 % v/v, and they should have an aroma and taste originated from the raw materials. In most cases, the maximum allowed methanol content of fruit spirit is 1000 g/hL absolute alcohol (g/hL a.a.) and cannot be allowed to be flavored artificially (Regulation (EC) No. 110/2008).

Pálinka is a traditional Hungarian spirit drink produced exclusively by the alcoholic fermentation and distillation of any native fruits cultured in Hungary. There are many kinds of pálinka-s with different characteristics based on specific types of fruit used in the fermentation. The most common fruits for production of pálinka are apricot, pear, plum, cherry, grape and apple as well as some exotic fruits such as blueberry, raspberry, black currant, cranberry etc. Fruit spirits as well as pálinka are widely consumed in European countries such as Hungary, France, Spain, Italy, Germany, Austria etc. and some on the world such as the USA, Canada, China, etc. It is protected as a geographical indication by the European Union. Therefore, only fruit spirits fermented, distilled and bottled in Hungary and four regions of Austria can be called “Pálinka”.

Spirits are often made from various sorts of fruits that have the common feature of high sugar content. They can be distributed into three groups, including pome fruits (apples, pears), stone fruits (sour cherries, peaches, plums, and apricots), and small fruits (blackberries and cranberries) (Bajer *et al.*, 2017). Although the principal components of fruit spirits are ethanol and water, their flavor and taste are very varied, mostly coming from the natural aroma of fruits. The variety and characteristics in spirit flavor are caused by the differences in the composition and concentration of a complex matrix containing many volatile compounds. There are a number of publications found on the compositions of volatile compounds in fruit spirits, such as apricot spirits, apple spirits, pear spirits and cherry spirits (Arrieta-Garay *et al.*, 2013, Genovese *et al.*, 2004, Nikićević *et al.*, 2011, Puškaš *et al.*, 2013, Spaho, 2017, Urosevic *et al.*, 2014, Versini *et al.*, 2009, Versini *et al.*, 2012, Willner *et al.*, 2013). Some of them often compare the composition and concentration of volatile compounds in spirits from various fruit types. Besides, studies investigating the authenticity of the products and the identification of their botanical and

geographical origin were carried out (Bajer *et al.*, 2017, Claus and Berglund, 2005, Kovács *et al.*, 2018, Winterova *et al.*, 2008).

The production process of spirits consists of the following stages: fermentation, distillation, and maturation. There are many factors influencing the quality of spirits such as fruit material (the type of fruit, the geographical origin, the method of cultivation, storage, and time of harvest), conditions of the alcoholic fermentation (temperature, pH, yeast strain, nutrient), distillation conditions (equipment type or parameters of distillation), and maturation conditions (time, temperature, the kind of wood) (Spaho *et al.*, 2013, Tomková *et al.*, 2015). To produce high-quality fruit spirits, it is necessary to understand and control these influencing factors. Thus, there are many investigations on the composition of fruit spirits in order to monitor the changes occurring in the production process and to control the content of selected compounds negatively affecting human health as well as compounds influencing the flavor and aroma of spirits. Steger and Lambrechts (2000) screened 107 yeast strains by evaluating higher alcohols, volatile acids, esters and sensory quality for production of premium quality South African brandy products. Their results indicated that the yeast strains had an important role in formation of esters and higher alcohols in the spirits. Peng *et al.* (2015) reported that the changes of fermentation temperature can significantly impact the formation of key aroma compounds and sensory profiles of apple wine. As the investigation of pH adjusting in melon spirits production, the results showed that adjusting the pH of 3.8 significantly decreased the acetaldehyde and methanol contents (Gómez *et al.*, 2008). Initial sugar content increase from 20 g/100 mL to 30 g/100 mL dropped growth rates for some yeasts, and final cell biomass of all yeasts was also decreased (Charoenchai *et al.*, 1998). Besides, studies on optimization of fermentation conditions to obtain more alcohol and volatile compounds are also performed (De León-Rodríguez *et al.*, 2008, Duarte *et al.*, 2011, Jha *et al.*, 2018, Tsegay *et al.*, 2018, Wang *et al.*, 2013). The correct separation of the three distillation fractions (heads, heart and tails) will also be necessary. Much research focuses on investigating the distribution of volatile compounds during spirits distillation to find the appropriate cut-points for separating methanol and others having a negative sensory impact (Awad *et al.*, 2017, Douady *et al.*, 2019, Spaho *et al.*, 2013).

2. OBJECTIVES

Pálinka is regarded as a traditional alcoholic beverage and a kind of gastronomic heritage in Hungary. There are many factors influencing the quality of pálinka, such as fruit material, fermentation conditions, distillation conditions and maturation conditions. To produce this spirit with high quality, it is necessary to understand and control these influencing factors. Therefore, the main goal of PhD research is the production and quality analysis of different pálinka-s. The main tasks are:

- Screening different commercial yeast strains for alcoholic fermentation of fruit spirits. Selection of best one for pálinka production from apple, apricot, cherry and pear
- Investigation of effects of different factors on the alcoholic fermentation process
  - temperature
  - pH
  - initial soluble solid contents
- Optimization of the fermentation process for production of pálinka
- Investigation of effects of the distillation process on distribution of aroma compounds
- Classification of fruit spirits using different chemometric methods such as PCA and LDA.

3. LITERATURE REVIEW

3.1 Pálinka - The Hungarian national fruit spirits

Pálinka is a traditional fruit spirit (or fruit brandy) of Hungary originated in the Middle Ages, produced exclusively by the alcoholic fermentation and distillation of fleshy fruit or must of such fruit, berries, or vegetables, with or without stones. Pálinka is regarded as the commercial name for fruit spirit from Hungary, and the Hungarian Pálinka is protected by the European Union law in 2004 and national law No. LXXIII in 2008. Accordingly, all producers outside of Hungary are not allowed to use the brand "Pálinka" for their products. In other words, fruit spirits produced outside of Hungary are not allowed to trade with the brand "Pálinka", but are freely made and sold under different names. Some similar commercial products occur in the Czech Republic, Poland and Slovakia known as Pálinka as well as in Romania under the name Palincă. In these laws, a Pálinka must be fermented from domestic fruit, distilled, bottled in Hungary with alcohol content at least 37.5 % v/v and not higher than 86 % v/v. Besides, it has a distinctive aroma and taste obtained from the distilled raw materials, not allowing addition of flavors, nor alcohol even alcohol with agricultural origin. Moreover, hydrocyanic acid is not higher than 7 g/hL a.a., and most permitted methanol content in Pálinka is not higher than 1000 g/hL a.a (Regulation (EC) No. 110/2008). Additionally, only apricot spirits produced from four provinces of Austria including Niederösterreich, Burgenland, Steiermark and Wien, can be called as Pálinka (Regulation (EC) No. 110/2008).

With distinctive taste and aroma of the various types of fruit, there are diverse raw materials used for Pálinka production process: types of ripe fruit containing amounts of sugar and pleasant, characteristic flavors. The most popular fruits applied are plums, cherries, apples, pears, apricots and quinces. The fallen ripe fruits are also used to produce the spirits, but the quality is really high, and only used for house-made in the countryside. While Pálinka is traditionally made from a mash of ripe fruit, the European Union law does not mention and control the addition of non-concentrated fruit juice, and notably allows the utility of fruit pulp. Dried fruits are only excluded from the mash and may be applied in the aging process.

In many centuries, some special regions of Hungary have been used for the production of certain fruits because of the climate or soil or special processing methods. Accordingly, Pálinkas produced in these regions have outstanding quality. These areas are protected as separate geographic indications and have their individual well-detailed laws in geographical, farming technical and processing requirements. Take one Pálinka type as an example, a product cannot be labeled as Apricot Pálinka of Kecskemét if not meet the local Protected Designation of Origin

(PDO) requirements even if it is an orthodox Apricot Pálinka from Kecskemét. Some Pálinkas have local PDO on their own such as Plum Pálinka of Szatmár, Apricot Pálinka of Kecskemét, Apple Pálinka of Szabolcs, Plum Pálinka of Békés, Apricot Pálinka of Gönc, Sour Cherry Pálinka of Újfehértó, Pear Pálinka of Göcsej and Pomace Pálinka of Pannonhalma (László *et al.*, 2016).

In the 20th century, the Pálinka distilleries started selling their products not only in the domestic, but also in the international market too. Hungary's Pálinka industry is sharply influenced by the yield of fruit harvested during the year. The amount of fruit produced in a year has a significant influence on the amount of the Pálinka sold the next year. Although the most important market of the Hungarian Pálinka is the domestic market, the foreign markets could also deal with the growing importance. The main export target market is the EU15 countries, especially the German-speaking countries such as Germany and Austria (Török, 2008).

3.2 Raw materials for the production of fruit spirits

3.2.1 Apricot

Apricot (*Prunus armeniaca* L.) is a stone fruit, belongs to the *Rosaceae* family, one of the largest families with about 3,400 species including almonds, peaches, apples, plums, cherries and berries, distributed throughout the northern temperate regions. Apricot is a temperate fruit and is only grown in climates with a seasonal differentiation requires a fairly cold winter and moderate temperatures in spring and early summer such as all Mediterranean countries, South Africa, and South and North America (Ali *et al.*, 2015, López *et al.*, 2017). Apricot is a small tree, 7 – 10 m high, with a trunk up to 40 cm in diameter and a dense spreading canopy (Figure 3.1). The apricot fruit is small with 1.5 – 2.5 cm in diameter, from yellow to orange, and is often red as exposed to the sunlight. Its surface can be smooth or velvety with very short hairs. The pulp is firm and not too unwatery with a taste from sweet to sour. Single seed covered in a hard, commonly referred to as "stone" with grainy texture (Gupta *et al.*, 2018).

In terms of nutritional value, 100g of fresh apricot provides 48 calories including 86 % water, 11 % carbohydrates, 1 % protein and less than 1 % fat. Apricots are an excellent source of fiber, minerals (K, Fe, Mg, P) and vitamins (A, C, E), and they are beneficial in certain cerebrovascular and cardiovascular, Alzheimer's and Parkinson's disease, because flavonoids such as quercetin-3-galactoside, quercetin-3-rutinoside. The quercetin-3-glucoside are the main ingredients in apricot. Besides, it supports other pharmacological effects such as antiemetic,

sedative, antispasmodic, antispasmodic, anti-cough and anti-inflammatory (Ali *et al.*, 2015, Gupta *et al.*, 2018).

Figure 3.1 Apricot fruits from different cultivars measured on a scale bar of 5 cm (Batnini *et al.*, 2016**)**

Apricot has a characteristic aroma and a good taste with a balance of sugar and acidity, and often be served in fresh, juice and dried. A huge apricot amount is produced into fruit spirits (López *et al.*, 2017). When comparing aroma components in distillates from apricot (*Prunus armeniaca*, L. cv. Pellecchiella) and apple (*Malus pumila* L. cv. Annurca), Genovese *et al.* (2004) identified 50 and 45 volatile compounds in the apricot and apple distillates, respectively. The apricot distillate's aroma volatiles were characterized by a high content of higher alcohols and by a variety of specific terpenes including linalool, ocimenol, alpha-terpineol, nerol, geraniol, cis- and translinalool oxide. Gamma-decalactone, gammad-dodecalactone and ethyl cinnamate were also characteristic of the apricot distillate. The olfactometric analysis showed volatile compounds, such as beta-damascenone, ethyl 2-methylbutanoate, linalool, methyl anthranilate, ethyl cinnamate, gamma-decalactone and gammadodecalactone, which may be resulted from the original fruit, had a significant odor activity, while 2-phenylethanol was the key odor effect compound.

3.2.2 Apple

Apple (*Malus domestica*) belongs to a species of genus *Malus*, in the family *Rosaceae*. Apple trees are grown worldwide such as in Asia, Europe and North America, and are the most widely cultivated species in the genus *Malus*. There are more than 7,500 apple cultivars, resulting in a range of desired characteristics. The fruit matures in late summer or autumn with the flesh pale yellowish-white. Matured apple fruits have green, red, pink, yellow color skin depending on the apple variety, covered in a protective layer of epicuticular wax (Figure 3.2). In terms of nutritional value, 100g of fresh apple provides 52 calories including water of 85.6 % sugar of 10.4 %, dietary fiber of 2.4 %, fat of 0.17 %, protein of 0.26 %, fiber, minerals (Na, P, Mn) and vitamins (C, B_6, B_2, K).

Akan Antares Jonagold Cox's Orange

Figure 3.2 Some varieties of apple fruit (Libertyprim, 2021**)**

Apple fruit is often served in fresh, juice, jam, dried or for cooking. A high amount of apple is produced in wine and spirits. The aroma quality of apple brandy is impacted by cider maturation (Madrera *et al.*, 2010). Results indicated that a distillate of superior aroma (with the more sweet and spicy character) is made from the most mature cider. This distillate contains higher levels of ethyl acetate, ethyl lactate and ethyl succinate and volatiles derived from bacterial metabolisms (which is more prevalent in broadly matured cider), such as 2-butanol, 4-ethylguaiacol, eugenol and 2-propen-1-ol.

3.2.3 Cherry

Cherry is also a stone fruit, belongs to the genus *Prunus* with the subgenus *Prunus subg. cerasus*. Cherries were grown in Europe, Western Asia, North America and parts of Northern Africa. They have a short growing season and can grow in most temperate latitudes, blossom in April, and the peak season for the cherry harvest is in the summer.

In terms of nutritional value, 100 g of fresh sweet cherries provides 63 calories including water of 82 % sugar of 12.8 %, dietary fiber of 2.1 %, fat of 0.2 %, protein of 1.1 %, fiber, minerals (Na, P, Mg, Mn, Fe) and vitamins (C, A, variety of B, K) while 100g of sour cherries have 50 calories including water of 86 % sugar of 8.5 %, dietary fiber of 1.6 %, fat of 0.3 %, protein of 1 %, fiber, minerals (Mn, Na, Mg) and vitamins (C, variety of B, K). Sour cherries contain 50 % more vitamin C (12 % DV) and around 20 times more vitamin A (8 % DV), especially β-carotene, compared to sweet cherries.

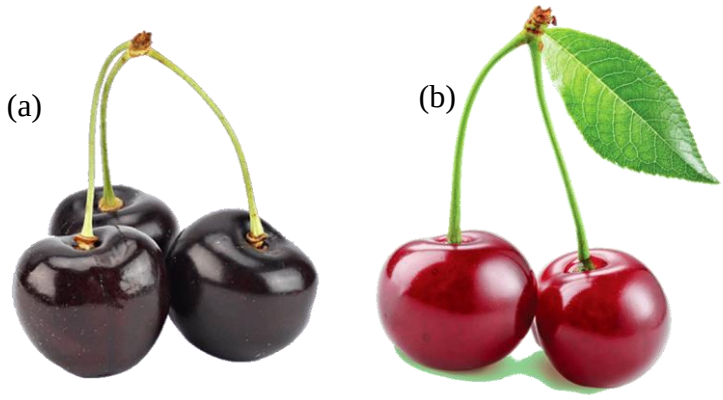


Figure 3.3 Sweet cherries (a) and sour cherries (b) (Michelle, 2021**)**

Most cherry cultivars belong to sweet cherries (*Prunus avium*) serving in fresh, juice, jam or dried while some of them are sour cherries (*Prunus cerasus*), using mainly for cooking (Figure 3.3). Both sweet and sour cherries are suitable for spirits production. Cherry spirits are produced across the world. Nikićević *et al.* (2011) studied the effects of cherry varieties on brandy production assessing both chemical and sensory properties. They identified 32 components such as higher alcohols, esters, benzaldehyde, terpenes and acids, especially the most abundant in ethyl esters. All the tested cultivars made brandies being from very good to excellent quality. The fruit brandies of two cultivars, Celery's 16 and Rexle, were preferred due to the character of high contents of benzaldehyde, linalool, esters and organic acids in harmonious proportions.

3.2.4 Pear

Pear belongs to a species of genus *Pyrus L.*, in the family *Rosaceae*. The tree is medium-sized and native to coastal and mildly temperate Europe, North Africa and Asia. About 3000 known varieties of pears are grown worldwide, which differ in taste, flavor and shape. In the world, the three most grown species are the European pear *Pyrus communis subsp. communis* cultivated

mainly in Europe and North America, the Chinese white pear *Pyrus×bretschneideri* and the Nashi pear *Pyrus pyrifolia*, both cultivated mainly in eastern Asia.

In terms of nutritional value, 100 g of fresh pear provides 57 calories including water of 84 % sugar of 9.75 %, dietary fiber of 3.1 %, fat of 0.14 %, protein of 0.36 %, fiber, minerals (Na, Ca, P, Mg) and vitamins (C, E, K).

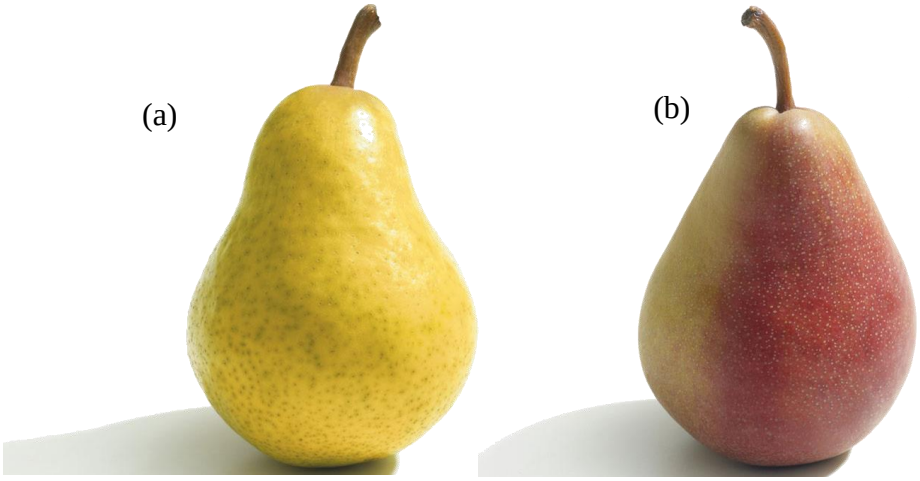


Figure 3.4 Bartlett pear (a) and seckel sugar pear (b) (Scully, 2021**)**

Although pear fruit is often served in fresh, juice, or dried, a significant amount of pear is produced into fruit distillates. Many pear varieties are suitable for spirits production, especially two varieties of seckel sugar pear and Williams or Bartlett pear (T 3.4) providing outstanding distilling qualities (López *et al.*, 2017). Willner *et al.* (2013) reported 26 aroma-active components in the Bartlett pear brandy. The sensorial analysis results revealed that ethyl 2-trans, 4-cis decadienoate and ethyl trans-2-trans-4-decadienoate are key aroma compounds in Bartlett's overall aroma pear spirits. However, these compounds alone cannot mimic Bartlett pear spirits' overall aroma, thus cannot be applied as single quality markers. If Bartlett pear spirit is stored in colorless bottles, the 2-trans-4-cis isomers partially isomerize to the 2-cis-4-trans and 2-trans-4-trans isomers, leading pear spirits to have fewer pear-odors, so the flavor quality of the spirit decreases.

3.3 Production of fruit spirit

Nowadays, fruit spirits have become favorite alcoholic beverages, thus people universally drink them in many parts of the world. They are considered as the national drink that is significantly represented the identity of many countries. For instance, Hungarian people are proud of pálinka while Russians get an impression of vodka. Although hundreds of different compounds

have been detected in fruit spirits with low concentrations, and they play a crucial role in the quality of spirits. The concentration and composition of these congeners are various depending on many factors that consist of the production's raw material, fermentation procedure, yeast strain utilized, storage time of the fermented mash before distilling, distillation technique and spirits aging. Pecić *et al.* (2012) illustrated that the characteristic aromatic complex of fruit brandy is made from components identified by primary aromatic compounds from fruit raw material, aromatic compounds created during alcoholic fermentation, aromatic mixtures produced during the distillation process, and aromas developed in barrels during the maturation process. In general, fruit spirits production consists of four main phases including mashing, fermentation, distillation and aging.

3.3.1 Mashing

The first stage of spirits production is the selection and mashing of fruit. In production, fruits can be used in the classic form of whole fruits, pulp and juice. Some fruits are most often used to make spirits including plums, melons, apples, cherries and pears (Śliwińska *et al.*, 2015). Fruits are collected, sorted and removed from damaged leaves, branches, and fruits. The selected fruits are fresh, free from pests, crushed, or damaged; besides, the fruit must have a suitable maturity for the fermentation process. Then fruits are washed to remove soil, pesticides and some dirt on the skin of fruits. In plants with modern technology, the washing stage is done by specialized machines with conveyor systems and many soaking tubs or high-pressure water spray systems. However, in order to take advantage of the biodiversity of natural yeasts existing on the fruit surface, especially in the case of home-made, this stage is often missed in the traditional way. After that, the fruits are crushed to create advantageous conditions for contacting and consuming sugars in the fruit mash of yeast. In the traditional way, the pit-containing fruits are stoned and crushed by hand while in a modern way, the fruits are ground with stone or metal shafts (László *et al.*, 2016). This way not only is fast but also can be performed in a larger amount to get a higher yield. Particularly for some fruit types with hard stones such as cherry, nectarine and peach, it is necessary to remove their stones before grinding because these seeds contain amygdalin, which can cause the bitter taste of spirits. Moreover, amygdalin can be broken down into bitter aldehydes and highly more toxic hydrogen cyanide. This cyanide hydrogen content of spirits should not exceed 7 g/hL a.a. (Regulation (EC) No. 110/2008). Fruit mashes can be cooled and stored in stainless steel containers for alcohol fermentation. In modern closed systems, the stoning and mashing are carried on in stainless steel pieces of equipment. The fruit is transferred from spout

to masher by a feeder screw. Because the mash's high temperature may raise up to 40 ℃, the mash will be chilled to 18 ℃ by a tube heat exchange system to evade undesired fermentations. The chilled mash is pumped to a stored container to further cooled and mix for alcohol fermentation (László *et al.*, 2016).

3.3.2 Fermentation

The alcoholic fermentation process is the anaerobic conversion of sugars to alcohol and carbon dioxide by yeast presenting in the raw material itself or added.

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2CO_2$$

Hexoses Ethanol Carbon dioxide

During the fermentation process, the yeast begins to metabolize the sugars and other nutrients in fruit juice. Yeasts use all these nutrients to gain growth and develop their population. During the first hours, the yeast population does not increase, known as the lag-phase, which is necessary for the cell to adapt to new environmental conditions. In the case of use of yeast existed on the fruit surface, yeast's initial population is about 10^4 cells/mL, while dry yeasts injected will be controlled to higher $5*10^6$ cells/mL. Once the yeast has adapted to environmental conditions, they begin to grow, called the exponential growth phase. This phase is greatly influenced by temperature, pH and the presence of oxygen. During exponential growth that may last from 3 to 6 hours, the yeast increases the population to $10^7 – 10^8$ cells/mL. The yeast stops raising, and the yeast population remains almost stable because some nutrients are missed, which is called the near-stationary period and can last from 2 to 10 days. After that, the period of decline begins, and the population dwindles until it disappeared almost completely. During this period, yeasts die and fall to the bottom as sediment due to lack of nutrients and because ethanol and other substances produced during alcohol fermentation are toxic. Yeast stops its liveliness whenever all sugars in the mash has been reformed into other compounds or whenever the alcohol content has reached an amount strong enough to block the enzymatic activity of most all yeasts, usually over 15 % v/v. The success of alcohol fermentation depends on maintaining a sufficiently viable yeast population until all types of fermentable sugars have been completely consumed.

It is important to choose yeast in the fermentation process because it greatly determines the quality and taste of the final product. Using directly the yeast existing on raw fruit as well as adding isolated and/or incubated yeast can be applied, which is also considered as a technological secret of processing plants. Wild yeasts can yield high-quality, unique seasoned alcoholic products, but

they are often difficult to control, even contribute to fermented products' spoilage, while isolated yeasts can ferment with predictions. Moreover, yeasts exist on the surface of the fruit, which are a mixture of bacteria. Its biodiversity also depends on several factors such as variety, ripening stage, antifungal treatment and climate conditions. Thus, most notably traditional facilities, wineries often use spontaneous fermentation because they believe it gives the wine more complexity in flavor, but to do this is very difficult, especially in synchronous quality control. Therefore, most wineries prefer isolated yeast supplements to ensure the fermentation process without any deviations. This use usually occurs in a dried or inactive state by reactivating yeasts in warm water or diluted fruit juice before adding a mash.

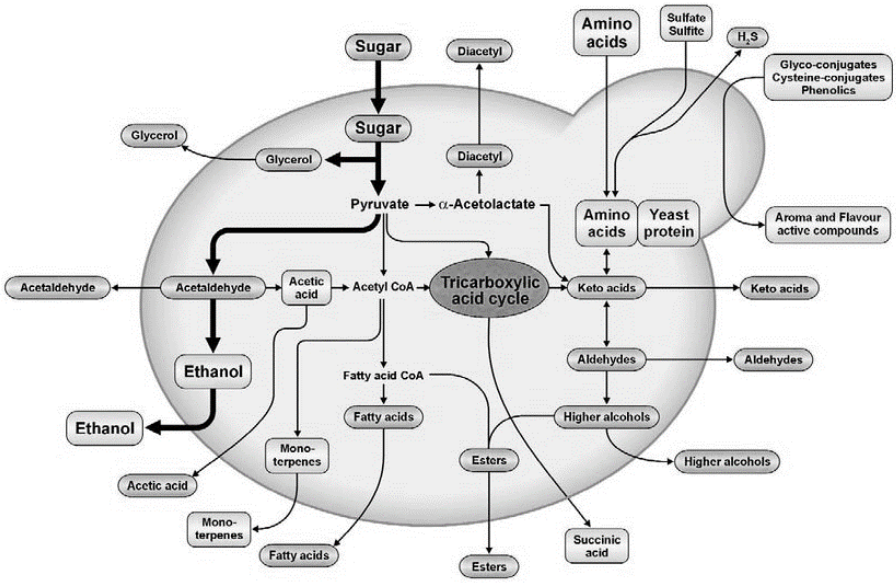


Figure 3.5. The derivation and synthesis pathways of aroma compounds from the metabolism of sugar, amino acids and sulfur under yeast's effect
*(*Lambrechts and Pretorius, 2000*)*

In fact, fermentation is a complicated process because many other chemical, biochemical and physical processes take place simultaneously, which makes fruit juice change into wine. When yeasts on fruit mash start to active, phosphates are added to the sugar, and six-carbon sugar units are broken into three-carbon parts (Figure 3.5). After a series of rearrangement reactions, the carboxylic carbon is liberated in carbon dioxide to form acetaldehyde that will eventually be converted to ethanol by reduction under the condition of absent oxygen anaerobic process. In fermentation, maybe a small amount of acetic acid converted by oxidation can contribute to a fault for spirits, known as volatile acidity, if its excesses. Beside ethanol as the main product, several other compounds that can contribute to the flavor and aroma of spirits are produced and

transformed as well during the fermentation of alcohol, such as higher alcohols (n-propanol, 2-methyl-1-butanol, 1-hexanol, 1-butanol, 1-octanol, fusel alcohols), esters (ethyl butyrate, ethyl hexanoate, phenyl ethyl acetate, isoamyl acetate), aldehydes (furfural, benzaldehyde, heptanal), and organic acids (octanoic acid, hexanoic acid, 2-methylbutanoic acid, succinic acid) through the amino acids metabolism and breakdown of sugars by yeast. Figure 3.5 describes the derivation and synthesis pathways of aroma compounds from the metabolism of sugar, amino acids and sulfur under yeast's effect (Christoph and Bauer-Christoph, 2007).

3.3.2.1 Ethanol and methanol

Ethanol is a major product of alcoholic fermentation. Generally, fruits are relatively rich in glucose, fructose and sucrose. Also, aroma components from fruits often give an attractive fruity and pleasant scent. So, fruits are considered as suitable raw materials for producing fermented beverages. In the alcoholic fermentation process, each glucose unit molds into two ethanol units, two carbon dioxide units and two ATPs. Firstly, the enzyme invertase breaks the glycosidic linkage in sucrose to form glucose and fructose. Then in the glycolysis process, each glucose unit should be split into two pyruvate units. Finally, under catalyzation of pyruvate decarboxylase and alcohol dehydrogenase, pyruvate is converted to ethanol and CO_2, recovery oxidized NAD^+ to provide for the glycolysis process (Figure 3.5).

Ethanol can infinitely dissolve in water. The ethanol and water mixture are also regarded as a suitable solvent for many aroma components. Therefore, in the production of fruit spirits, most of the aroma components presented in the raw fruits as well as formed in the fermentation dissolve well and recover easily through steam-enticing distillation. From about 1.4 % v/v, the human tongue can sense ethanol with bitter and slightly sweet while about 20 % v/v, it contributes to the hotness sensation as capsaicin of chili.

Methanol is not a byproduct of alcohol fermentation. It is a constituent arising from the enzymatic degradation of pectin contained in fruits by de-esterification of methoxy groups in pectin into pectate and methanol (Spaho, 2017). This reaction takes place strongly as fruits ripen with signs of changing from hard to soft. By contrast, some different views indicated that it is a byproduct of fermentation because many yeast strains can produce pectinase to de-esterify in pectin. *S. cerevisiae* strains having a pectin-methyl-esterase activity could produce methanol during fermentation (Ohimain, 2016). Methanol formation is primarily based on pectin content, the raw material component, and pectin-methyl-esterase activity presented during the fermentation process.

The methanol concentration is mainly dependent on the applied technique of fruit treatment and the distillation technical, besides that a little bit from the type and variety of fruit. There are different views on methanol's impact on spirits' flavor, such as contributing to a cooked cabbage odor and being a positive flavor constituent. Still, others reported that it contributed to a mild or bland odor and did not affect spirits' flavor. The molecules of ethanol and methanol cling to each other, so they are notably difficult to divide during distillation despite their different boiling points. However, methanol must be isolated and cut out of distillates because methanol in high concentration is toxic. It will be metabolized to formic acid and formaldehyde that are a dangerous effect on human health. Although the methanol content is considered to be suitable for proving the authenticity of fruit spirits, the methanol level must follow the limits posed by the Council Regulation EC No. 110/2008, not exceed top 12 g/L a.a. (Regulation (EC) No. 110/2008, Spaho, 2017, Winterova *et al.*, 2008).

3.3.2.2 Higher alcohols

Higher alcohols are a byproduct group metabolized from amino acids by yeasts during alcoholic fermentation (Winterova *et al.*, 2008). Higher alcohols include aliphatic and aromatic alcohols. Both of them have critical roles in wine and spirits. Aliphatic alcohols consist of isoamyl alcohol, isobutanol, propanol and active amyl alcohol, whereas aromatic alcohols contain tyrosol and 2-phenyl ethyl alcohol. Higher alcohols can positively and negatively impact wine and spirits' aroma and flavor. In high content, over 3,500 mg/L a.a., they can impart a strong, pungent odor and flavor, while in the optimal amount, they can contribute to the volatile profile of spirits with fruity, pleasant odors and essential characters (Spaho, 2017). Applying different yeast strains offers considerably to alterations in higher alcohol profiles and content in wine and distillates. Amino acid content, the precursors for higher alcohols, also impacts the formation of higher alcohols, where their total production rises as the corresponding amino acid content rises. Moreover, ethanol content, pH, fermentation temperature, the composition of the fruit, fruit variety, etc., influence the composition and content of higher alcohols in final products.

Branched-chain higher alcohols such as isoamyl alcohol, amyl alcohol and isobutanol are synthesized in the yeast via the Ehrlich pathway by the degradation of the branched-chain amino acids such as leucine, isoleucine and valine, respectively (Lambrechts and Pretorius, 2000). The production of higher alcohols is described in Figure 3.5. Firstly, alpha-keto acids are formed through the catabolic or Ehrlich pathway or an anabolic pathway involving the synthesis of branched-chain amino acids via their biosynthetic pathway from glucose. The first step in

branched-chain amino acids' catabolism is transamination to form the respective alpha-keto acids, such as alpha-ketoisocaproic acid from leucine, alpha-ketoisovaleric acid from valine and alpha-keto-beta-methylvaleric acid from isoleucine, under catalyzation of aminotransferases in mitochondrial. Under the impact of pyruvate decarboxylase, alpha-keto acids are converted to corresponding branched-chain aldehydes with lost one carbon atom. Then under the catalyzation of alcohol dehydrogenase, aldehydes formed are transferred to the corresponding higher alcohol. The aldehyde, alternatively, might be oxidized to an acid (Lambrechts and Pretorius, 2000). Researchers are recently interested in increasing yeast branched-chain amino acid transaminase activity to increase higher alcohol production during fermentation, which increases the positive effect flavor of the final product (Lilly *et al.*, 2006).

3.3.2.3 Esters

Ester is one of the largest and most important groups in aroma compounds with mostly pleasant flavor properties such as fruity (iso-phenethyl acetate as pear odor, ethyl hexanoate as apple odor, isobutyl acetate as banana odor) and flowery aromas (2-phenylethyl acetate, ethyl decanoate, ethyl butanoate) (Wiśniewska *et al.*, 2016). Ester often has a shallow threshold of flavor detection. Ethyl acetate in low content impacts some alcoholic beverages' harsh odor, while in high content, it gives a so-called ‘vinegar flavor’ to wine and distillates. In general, the composition and content of esters in wine and spirits depend on the type of raw material, yeast strain applied, pH of mash, etc.

During alcoholic fermentation, esters are produced via the catalyzation of acyltransferases or ester-synthase with energy requirement from the thioester linkage of the acyl-CoA co-substrate, especially acetyl-CoA (Figure 3.5). Acetyl-CoA can be produced either by oxidative decarboxylation of pyruvate or by directly activating acetate with ATP. In general, acetate ester synthesis during fermentation is an energy-requiring process that takes place inside the yeast cell, requires the important metabolite of acetyl-CoA in two stages with contributions of alcohol, fatty acid, CoA, ester-synthesizing enzyme (Lambrechts and Pretorius, 2000):

$$RCOOH + ATP + CoASH \rightarrow R'CO\text{-}SCoA + AMP + Ppi$$

$$RCO\text{-}SCoA + R'OH \rightarrow RCOOR' + CoASH.$$

The role of ester production in yeast metabolism is unclear, but several hypotheses have been proposed. Others suggest that esters might be formed to remove toxic fatty acids from the yeast cell. Another reason for ester formation could be to reduce the acetyl charge, as it is essential

for the yeast cell to maintain a balance between acetyl-CoA and CoA-SH. However, the balance between ester-synthesizing enzymes and esterases is important for ester production. Researchers have recently investigated the effects of these enzymes on ester formation in order to selectively control the biosynthesis of positive-effect esters in alcoholic fermentation, which has important implications in the fermentation industry.

3.3.2.4 Carbonyl compounds

The main carbonyl compounds found in spirits are various volatile aldehydes. In general, volatile aldehydes contribute to the characteristic flavor, especially the contribution of acetaldehyde to green leaves, fruity odor and alcohol oxidation (Spaho, 2017). During fermentation, the most acetaldehyde formation is recorded as the maximum carbon dissimilation rate. Its concentration drops to a low level at the end of fermentation after that slowly rises over time. Generally, increases in acetaldehydes occur over time because of ethanol's oxidation, yeast activity and aeration. Furthermore, acetaldehyde production is considerably influenced by fruit juice composition, fermentation conditions, aeration rate etc. Some results recorded that *S. cerevisiae* strains with sulfate-resistant form much more acetaldehyde than ones without sulfate-resistant (Lambrechts and Pretorius, 2000). Besides, the high sulfur dioxide in fruit mash or increasing fermentation temperature also increases in formation of acetaldehyde (Lambrechts and Pretorius, 2000).

In the yeast fermentation, acetaldehyde is known as one of the major metabolic intermediates before ethanol is formed. Pyruvate is converted to acetaldehyde through pyruvate decarboxylase enzyme. Then acetaldehyde is converted to ethanol by alcohol dehydrogenase enzyme and deoxidizes NADH to NAD^+, providing for glycolysis. The acetaldehyde formation is described in Figure 3.5. In addition, ethanol can also be converted to acetaldehyde when wine and spirits are prolonged storage in a barrel at high temperature, resulting in a lack of freshness and a musty taste in the final product as alcohol oxidation.

3.3.2.5 Volatile acids

The volatile acid is an organic acids group with a short carbon chain. This is a result of fatty acid metabolism under yeast and bacteria's effects. The content usually ranges from 500 and 1000 mg/L, which constitutes 10 % –15 % of the total acid, in which acetic acid contains about 90

% of total volatile acids. 10 % of the rest is almost hexanoic and propionic acids. Acetic acid is the main compound of the volatile acidity of wine or spirits.

In *S. cerevisiae,* acetate is produced as an intermediate of the pyruvate dehydrogenase bypass. Firstly, pyruvate will be decarboxylated to acetaldehyde by pyruvate decarboxylase. Then the acetaldehyde dehydrogenase oxidizes acetaldehyde to acetate that can be transformed into acetyl-CoA in the cytosol or excreted in the culture medium (Figure 3.5). Although *Saccharomyces* can form acetic acid, the high content in wine is mostly the result of ethanol's metabolism by aerobic acetic acid bacteria.

S. cerevisiae strains tend to produce much higher acetic acid in sweet wines than in dry wines, while *S. bayanus* and *S. uvarum* produce lower acetic acid than *S. cerevisiae*. The optimal acetic acid content ranges from 0.2– 0.7 g/L, but that in high content, over 1.1 g/L, will contribute to a vinegar odor for wine and spirits (Lambrechts and Pretorius, 2000).

In short, fermentation is the most important process in fruit spirits production technology. In fact, this process is evaluated not only by the alcohol yield but also by the composition and concentration of the aroma compounds obtained in the final spirits. Therefore, understanding and controlling the volatile compound formation in the fermentation process is a very concerning today issue.

3.3.3 Distillation

A blend of two or more compounds isolated by boiling it to a certain temperature and condensed the occurring vapors is called the distillation process. Thus, distillation is a physical separation process of a mixture comprised of two or more compounds based on differences in boiling points. When a liquid blend of volatile components is heated, the vapor over a boiling mixture becomes richer in more volatile components, making the original mixture have more of the less volatile compounds. The vapor that comes off is cooled, which will be a tendency of a less volatile material to condense with a greater proportion than a more volatile material (López *et al.*, 2017, Spaho, 2017).

Spirits primarily contain ethanol and water in pretty equal portions, but water has a higher boiling point than alcohol (100 ℃ compared to 78.37 ℃ for alcohol). Hence, depending on the ratio of alcohol to water, the boiling temperatures of the mixture will be between 78.5 ℃ and 100 ℃. Because of differences in boiling points, the vapor above the liquid will be richer in alcohol than water at any moment of evaporation. Distillations will make mixtures near the azeotropic ratio of 95.6/4.4 % of ethanol/water, which means the alcohol in the vapor phase is no longer more

concentrated than in the liquid phase. Then fractional distillation no longer happens at this concentration (Spaho, 2017).

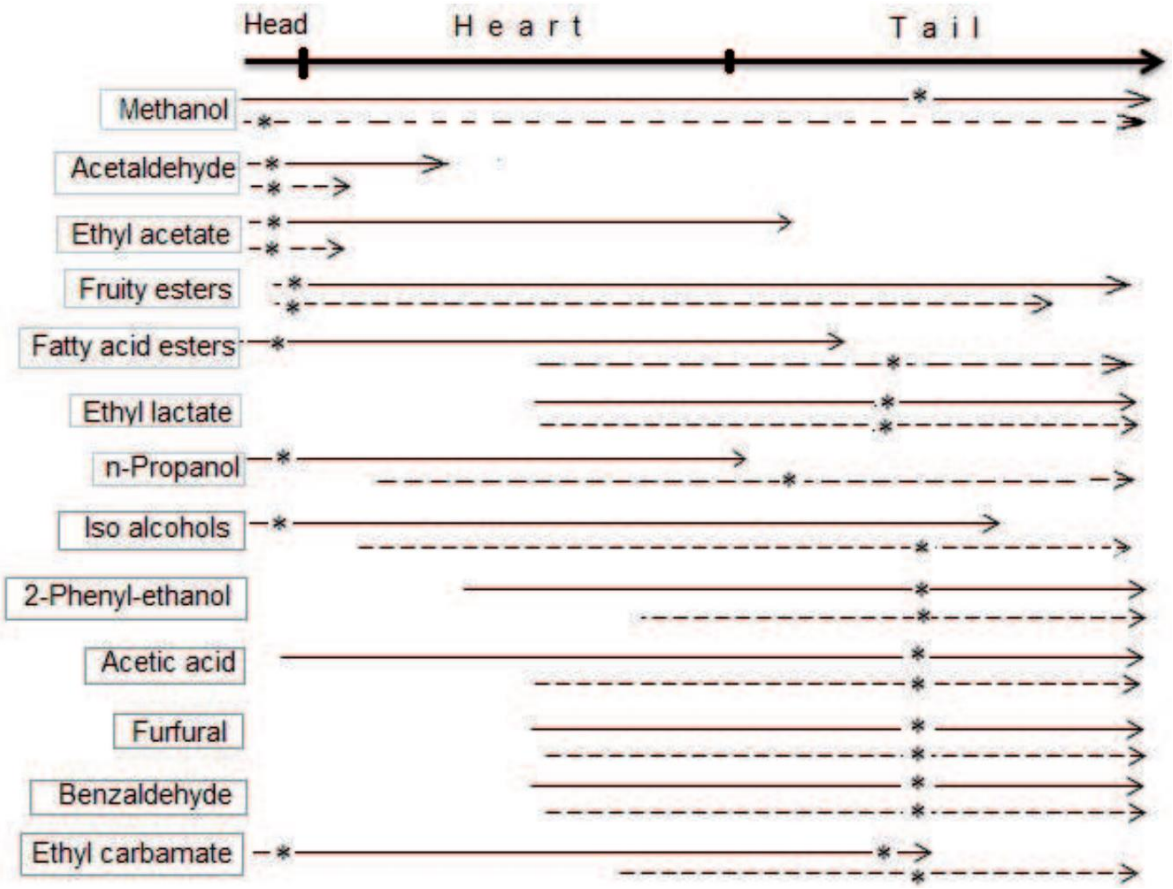


Figure 3.6 Distribution of volatile components under different distillation systems *(*Spaho, 2017*)* Note: A full line for alembic distillation, dashed line for column distillation, and "*" for the cut point

It can be seen clearly that this process is complicated due to the fact there are various kinds of alcohol and other chemical compounds presenting besides ethanol. Although these chemicals usually make spirits character and flavor, some of them are desirable in small quantities and others should be completely removed during distillation. During distillation, the ethanol and water are the two major compounds which obviously carry all other volatiles being aroma-and-flavor compounds in spirits. In the beginning, the high volume of ethanol comes out of the still together with high volatile compounds, then by the time volume of alcohol reduces followed by water, and low volatile compounds rise. The distillate will be divided into three parts including the head, heart and tail fraction. The head one gives a higher concentration of low boiling point components and mainly contains undesirable compounds that usually give an unpleasant, strong and sharp flavor, and maybe have a higher concentration of some toxic compounds, so it should be removed. The

heart is the best part, rich in ethanol carrying pleasant and fruity aromas and has an immaculate taste lacking the head's sharp bite. When the alcohols with lower boiling points evaporated, which leaves the water, proteins, carbohydrates, and less volatile compounds with higher boiling points, better known as the tail fraction. The tail contains unpleasant fatty and oil compounds with the distinctive smell of wet-dog. Thus, the issue of concern is to predict the right moment to separate the stills outflow from the head to the heart and from the heart to the tail. The smaller the heart's ratio is so, the greater the heart's purity, which means to remove more valuable alcohol, so some tail fractions are collected and diluted or redistilled into the head because of their relatively high alcohol concentrations and good aroma compounds. Sometimes, some parts of the tail and the heads will be added to the next distillation to recycle some trapped alcohols in order to fully utilize and enrich these fragrant alcohols (Spaho, 2017).

Based on the compounds' boiling temperature in the distillation mixture, they will evaporate and condense in a certain order: the substance with a lower boiling point will evaporate and condense go out first for others with boiling points higher. This allows manipulating to separate desired substances and to clean unwanted substances. There are three ways applied to determine the cut-point in the distillation process: (i) the capacity of sensory evaluation from the distiller, (ii) the alcohol content and (iii) the distillation temperature (Spaho, 2017). In the first way, taste and smell are the most reliable indicators of determining when to make a cut for the head and the tail fractions. Thus, distillates' aroma profile significantly depends on the distiller's skill; an experienced distiller does this very well by smell. The head fraction has a sharp, strong and unpleasant odor (methanol, acetaldehyde, ethyl acetate) because there is an existing and lack of volatile congeners (2-phenyl ethanol). While the tail fraction has a faded flavor and dull character (fatty acid ester, acetic acid, ethyl carbamate), it should not be hard for sensory evaluation and division (Figure 3.6). In a second way, the spirits' alcohol content can be used as an indicator of cutting points, especially for the heart's division from the tail fraction. However, this value may be changed involving distillation equipment, fruit variety and fermented mash quality. The last way, the vapor's temperature before it entered the condenser, can be used as a sign of the cut-points. The first cutting point to separate the head from the heart is when the vapor's temperature in the copper pipe reaches around 74 ℃–76 ℃. The second cut-point of the heart/tail is when the vapor's temperature in the copper pipe comes to approximately 87 ℃–88 ℃. Finally, the stop-point to be over the distillation is when the temperature gets to near 92 ℃–93 ℃. After all, these methods of determining the cut-points have their own advantages and disadvantages,

depending on facilities and people's conditions to choose the best way to use. Nevertheless, it is better to apply all of them as a guide for the separation during the distillation of spirits.

In the distillation flask, the heat applied can make chemical reactions between the existing compounds and form other compounds, raising the final distillate's complexity through the distillation. The final product could be differently influenced by these minority compounds being known as congeners. Some compounds are not pleasant, even some of them could have toxicity. Parallelly, there are also compounds giving a positive character. However, it can remove negative aromas and boost positive ones and minimize and by determining key congeners in distillation (López *et al.*, 2017).

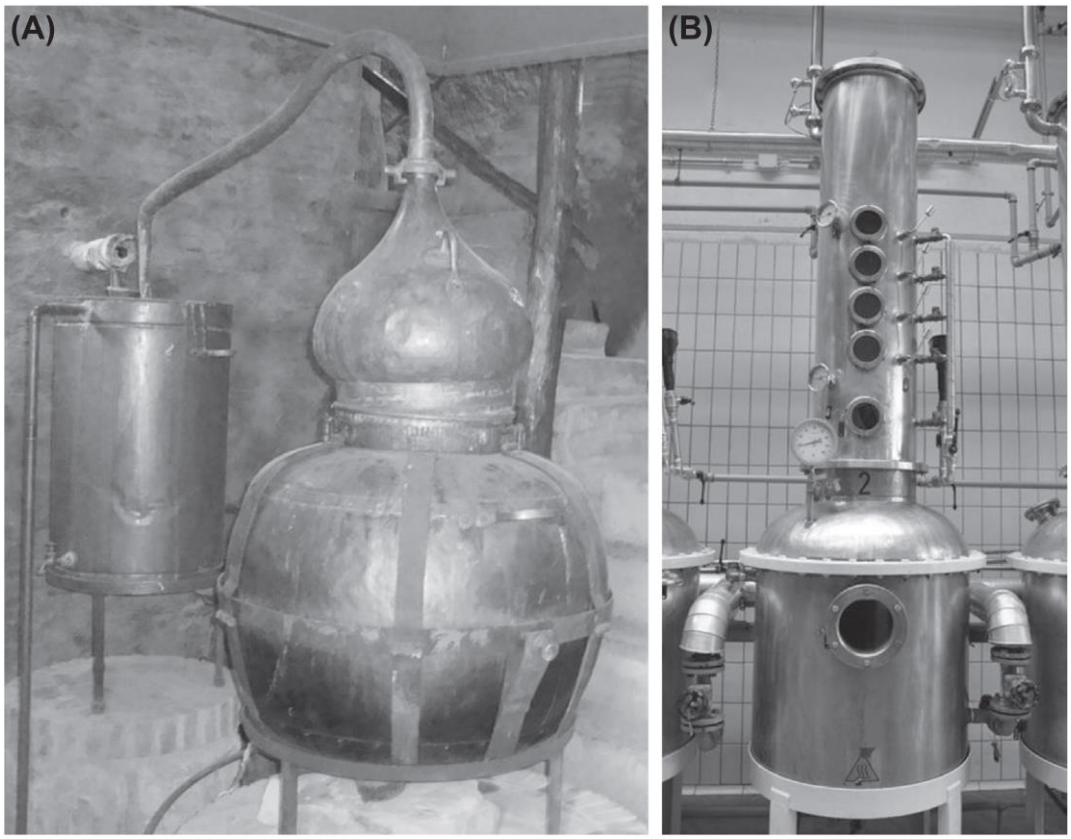


Figure 3.7 Two typical distillation systems for production of fruit spirits: (A) copper Charentais alembic (French-style); (B) batch distillation column (German-style)
*(*López *et al.*, 2017*)*

The distillation process, basically, may be carried out by both types of techniques including batch-wise or continuously, but batch distillation is preferred to produce fruit spirits. In batch distillation, two types of distillation systems with the same operating principle are applied frequently for the production of spirits as copper Charentais alembic follow French-style, and batch distillation columns follow German-style (Figure 3.7) (López *et al.*, 2017). Although both

distillation methods are based on the same theoretical principles, they vary in operational principles. Pot still distillation presents a constant reflux rate while its' batch column distillation changes over a wide range. In batch column distillation, the cooling rate in the partial condenser or the returned condensate stream's flow rate was established to control the reflux rate when the column contained both total and partial condensers or only a total condenser, respectively (García-Llobodanin *et al.*, 2011).

Besides ethanol and water, the distillate contains abundant volatile compounds, which are valuable components of fruit spirits. The composition and concentration of these compounds determine the characteristics of flavor and taste in the spirits' product. Therefore, understanding the distribution of these aroma compounds during the distillation can control the fruit spirits' quality.

3.3.4 Ageing

After the distillation process, the beverage's quality is impacted by many factors, and the priciest one is aging. Fruit spirits might be aged for several months in stainless steel casks to improve the positive characteristics by polymerizing the phenols with oxygen, such as adding flavor and softening the alcohol. In addition, the fruit can be added to the cask or bottle the aging process with a maximum amount of 10 % (w/v) dried fruit for 3 months to upgrade the fruity odor and sweetness for the spirits products. The spirits are then diluted by distilled water to an alcohol content range of 37.5 % v/v to 60 % v/v (László *et al.*, 2016).

During aging, many changes occur involving substances presenting in both the fresh spirits and the wood, which will modify the odor, taste and color of spirits. These changes are associated with the volatile's evaporation via the barrel, reactions by compounds originated from the wood with raw spirits component, substances sorption onto the wood, reactions by the substances in the fresh spirits, and incorporation of substances extracted or derived from the wood (Madrera *et al.*, 2013). Besides metal casks, other wooden barrels having various volumes are used in the aging process to allow the flavors to mature, such as oak or mulberry barrels. Moreover, some aroma compounds can be introduced into spirits from the wood material during aging. Some researchers showed that the material of barrels might influence spirit quality (Bortoletto *et al.*, 2016, Caldeira *et al.*, 2006, Canas, 2017, Granja-Soares *et al.*, 2020). Correctly, Portuguese brandies matured from various wooden barrels have significant differences in flavor and odor profile. Besides, the overall brandy quality grows over aging time, especially some positive sensory attributes such as toasted, vanilla, retronasal aroma, woody, flavor persistence and smoke (Caldeira *et al.*, 2006).

Chestnut wood with appropriate porosity facilitates the spirit's micro-oxygenation and the volatile phenols' abundant release into the distillates during aging (Canas *et al.*, 2016).

In addition to boosting the sensorial properties, adding flavor and softening the spirits, other alternatives methods may be applied to reduce cost, barrel stock management's complexities, and the maturation time shortening. For example, using sticks or wood staves instead of barrels minimizes cost and applying the micro-oxygenation technical in aging systems reduces the maturation time (Canas *et al.*, 2019, Coldea *et al.*, 2020, Granja-Soares *et al.*, 2020).

To sum up, fermentation is the most important process in fruit spirits production technology. This process is evaluated not only by the alcohol yield but also by the composition and content of the aroma compounds obtained in the final spirits. The characteristic properties of the spirits' flavor are primarily based on the variety of fruits used for fermentation. Besides sugars and other nutrients, fruit juices contain volatile compounds and aroma precursors, which precursors will be used by yeast to convert into aroma compounds, positively contributing to the final flavor and taste of the final product. The distillation process will recover, enrich these volatiles as well as remove unexpected aroma compounds formed during the fermentation process, leading to spirits' taste more attractive. The aging process would improve some positive sensorial attributes, add flavor and soften the spirits.

3.4 Some effects on the production of fruit spirits

Fruit spirits not only are a mixture of water and ethanol but also are composed of many aroma components present in original fruit materials, the volatile compounds produced during fermentation by yeast, and interactions between these aroma compounds. To sum up, there are numerous factors influencing alcohol fermentation, mainly relate to fermentation conditions such as temperature and time of fermentation, initial pH and total soluble solids of the fruit juices, yeast strains used, the nutrients involved in the yeast activity.

3.4.1 The influence of fermentation conditions

3.4.1.1 Yeast strains

Different yeast strains influence the volatiles components of wine and fruit distillates. Steger and Lambrechts (2000) screened 107 yeast strains by evaluating higher alcohols, volatile acids, esters and sensory quality to produce premium quality South African brandy products. Results indicated that the yeast strains had an important role in forming esters and higher alcohols in the spirits. Ethyl acetate and iso-amyl acetate at high levels were undesirable, while all other

high content esters contributed a positive effect on the overall potential quality of the brandy product. When examining the impact of five yeast strains of *S. cerevisiae* and *S. bayanus* and two nutrients on the chemical, volatile and sensory characteristics of apricot brandies, Urosevic *et al.* (2014) found that the use of selected yeast and nutrients supported better results than production without selected yeast and nutrients. Apricot brandies from the *S. bayanus* yeast strain and diammonium phosphate as a nutrient received the best total sensory scores. However, as studying the effect of yeast strain and different fruit cultivars on the wine organoleptic properties, Mateos *et al.* (2006) proved that wines produced from different grape varieties and under different fermentation conditions got more homogeneous properties compared to the same yeast strain. The volatile compounds' production depended mostly on the fruit mash's composition and the fermentation condition compared to the yeast strains used. The aroma precursors present in the raw fruits had a greater impact on the character flavor and taste of wine and spirits than yeast strains.

The use of different yeast strains in the fermentation process impacted the quality of wine and spirits. Thus, to obtain excellent quality, producers should first select suitable fruit varieties of good quality. After that, the utility of suitable yeast strains under good fermentation conditions would support in formulating positive aroma compounds and enhancing the expression of characteristic volatile compounds existing in fruit materials.

3.4.1.2 The fermentation temperature

Many pieces of research demonstrated that temperature is a variable directly affecting the growth rate of yeasts on alcohol fermentation, in particular, the alcohol yield and the volatiles content. When investigation of influence of the fermentation temperature on the aroma compound formation during the fermentation at 15 ℃ and 28 ℃, Molina *et al.* (2007) illustrated that although the maximal biomass content was similar at both temperatures, the fermentation rate at 28 ℃, as well as consumption rates of glucose and fructose, was around twice faster than at 15 ℃. The total concentration of volatile products was higher in the fermentation at the lower temperature; in particular, the total ethyl ester and total acid content were higher at 15 ℃ than at 28 ℃. The ethanol content at 28 ℃ was lower, but glycerol content was higher than at 15 ℃. Higher contents of fresh and fruity aroma compounds were recorded at 15 ℃, while higher contents of flowery aroma compounds were recorded at 28 ℃. In investigation of the effect of fermentation temperature on the alcohol yield and volatile profile of plum brandy, spirits obtained from mashes fermented at 18 ℃ contained higher concentrations of aldehydes and esters than that at 30 ℃ (Pielech-

Przybylska *et al.*, 2016). Peng *et al.* (2015) reported that the fermentation temperature changes can significantly impact the formation of key aroma compounds and sensory profiles of apple wine. The content of key aroma compounds in apple wine, including 3-methylthio-1-propanol, isobutylalcohol, isopentylalcohol, benzeneethanol, ethyl caprylate, ethyl acetate, isobutyl acetate, isopentylacetate and ethyl 4-hydroxybutanoate, considerably rose when the fermentation temperature grew from 17 ℃ to 20 ℃. Then they dropped with the temperature increases of 20 ℃ to 26 ℃, except for ethyl 4-hydroxybutanoate. The apple wine fermented at 20 ℃ received the highest sensory score. Fermentation temperature of 20 ℃ was considered the most suitable condition of *S. cerevisiae* AP05 for apple winemaking.

In short, the temperature contributes vital effects on the production of alcohol and volatiles formation related to the final wine and fruit spirits' flavor and taste. A temperature around 20 ℃ is considered to be suitable for supporting the formation of alcohol and positive aroma compounds and inhibiting the formation of negative aroma components in wine and distillates.

3.4.1.3 pH

The pH has a significant effect on the yeast activities, resulting in the formation of the products as well as byproducts during fermentation. The mash pH, ranging from 2.75 to 4.25, is known as a relevant factor for yeasts' survival and growth. Liu *et al.* (2015) indicated that initial pH vital impact on the formation of alcohol, acetic acid and glycerol by *S. cerevisiae* strains. Low initial pH prolonged yeast lag phase, inhibited yeast growth, reduced fermentation rate, increased final content of acetic acid and glycerol, decreased final content of ethanol. Ethanol content reached the maximum at pH 4.50, 3.00, while glycerol and acetic acid content get to the highest at pH 2.5. When examining the initial pH effect on alcohol production of *S. cerevisiae*, Reddy and Reddy (2011) figured out that the alcohol content reached the maximum at pH 5.0. It rose in a pH range of 3.5-5, then dropped in a pH range of 5-6. After studying the impact of the fermentation pH on Blanquilla pear spirits production, García-Llobodanin *et al.* (2010) suggested that the higher alcohols' formation is greatly based on pH through the acidification of the fermentation medium. The contents of 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol in the spirits at the adjusted pH of 3.20 were higher while ethyl acetate contents found were lower than that at the native pH of 4.25. As the investigation of pH adjusting in melon spirits production, the results showed that adjusting the pH of 3.8 significantly decreased the acetaldehyde and methanol contents (Gómez *et al.*, 2008).

Generally, the suitable pH for the yeast to grow well and produce more alcohol ranges from 3.0 to 4.0. Low pH level supports the formation of higher alcohol and glycerol, acetic acid as well. However, it significantly reduces acetaldehyde and methanol formation.

3.4.1.4 Brix

Results of the sugar content effects on fermentation by twenty-two strains of wine yeasts indicated initial sugar content increase from 20 to 30 g/100 mL dropped growth rates for some yeasts and final cell biomass of all yeasts were also decreased (Charoenchai *et al.*, 1998). *S. cerevisiae* utilized glucose and fructose at similar rates as fermented separately; however, as the fermentation media containing an equal content of glucose and fructose, glucose tended to be utilized at approximately twice the rate fructose (D'Amore *et al.*, 1989).

High initial sugar content is more preferred in alcohol fermentation, which may raise the content of ethanol and other products in the fermented mash. Nevertheless, yeast cells in the media with high sugar content may be exposed to high osmotic stress, led to affect the fermentation performance. Thus, many studies have been carried out to determine the high initial sugar content suitable for the fermentation process. They are based on assessing the simultaneous effects of many factors including temperature, initial sugar content, pH, time and culture rate. Duarte *et al.* (2011) pointed out that temperature 20 ℃ and 22 °Brix were optimized fermentation conditions for the *Jabuticaba* spirit production. The *mezcal* production from *Agave salmiana* should be carried out at an optimized temperature of 28 ℃ and an initial sugar concentration of 10.5 g/100 mL (De León-Rodríguez *et al.*, 2008). The best fermentation conditions for ethanol production from palmyra jaggery were 26.2 ℃, pH of 8.4, fermentation time of 4.2 days, substrate of 398.5 g/L, urea of 3.1 g/L and EDTA of 0.51 g/L (Ratnam *et al.*, 2005).

Sugar content influences the fermentation process, so it is essential to find a suitable initial sugar content.

3.4.2 The influence of distillation

During distillation, the composition of fruit spirits was impacted by using different distillation methods or different distillation conditions (such as cut-points, fruits, yeasts etc.) (García-Llobodanin *et al.*, 2011). Therefore, many studies have focused on investigating the effects of distillation equipment operation on the composition of fractions, mainly focusing on changing the cut-points, numbers of distillation, boiler heating and partial condenser cooling rates.

After investigating the effect of double or single distillation and different alcohol content in heart fractions on the aroma volatiles distribution and undesirable compounds during the distillation of plum brandies, Balcerek *et al.* (2017) pointed out that the head fractions contained a relatively high content of aldehydes, acetals, esters and higher alcohols while the tail fraction essentially consisted of aliphatic ethyl carbamate, 2-phenyl ethanol, 1-hexanol, benzyl alcohol and furfural as irrespective of the distillation method used. The alcohol content rise in the heart fractions from 70 % v/v to 90 % v/v would lead to gradual content decreases of all volatile compounds. The heart fractions of double distillation had lower contents of acetaldehyde and benzaldehyde, but higher contents of furfural and esters compared with single distillation. Compared to the single process, the amount increases of methanol and ethyl carbamate from double distillation were found. Similar results were found as studying impacts of cut-point on the distributions of higher alcohols and esters in plum brandy distillation (Spaho *et al.*, 2013). The alcohol and volatile compound content in cutting fractions varied as the cut-point changed. However, ethyl acetate, ethyl propionate and isobutyl acetate occurred in the head fractions with the highest concentration, whereas isopentyl acetate and ethyl lactate were relatively dominated in the tail fractions. García-Llobodanin *et al.* (2011) investigated the influences of operation variability of distilling equipment on the Pear spirits composition. The significant difference in esters including ethyl octanoate, ethyl decanoate and ethyl palmitate, was recorded in the composition of column distilled spirits. Simultaneously, there was a difference in alembic spirit compositions found, consisting of acetaldehyde and acetal. The rectification column distillation unit with only one distilling could produce pear spirits with higher alcoholic content spirits than the alembic distillation unit with two consecutive distilling. Besides, column distillation produced hearts with a lower concentration of toxic compounds (such as acetaldehyde and methanol) but more positive-effect higher alcohols and esters. When Claus and Berglund (2005) studied the relationship between the operating modes and the aroma compound in three different fractions during brandy distillation, they showed that the methanol content obtained had a unique distribution compared to other aroma compounds. Ethanol content raised as all the trays used while the contents of other compounds dropped such as methanol, 1-propanol, isoamyl alcohol, acetaldehyde and ethyl acetate. Four operating modes were applied, including all three trays with the catalytic converter, two trays with the catalytic converter, three trays without the catalytic converter and two trays without the catalytic converter.

3.4.3 The influence of aging

The aging process of wine and fruit spirits is of great research interest. de Aquino *et al.* (2006) reported that low molecular weight phenolic compounds, acids, aldehydes and tannins were considered as aging indicators of sugar cane spirits. The effects of traditional and alternative aging systems on the volatile composition of cider brandy were employed (Madrera *et al.*, 2013). Alternative aging systems with micro-oxygenation supported the spirits maturing compared to the traditional treatment, resulting in a higher oxidation degree, more favouring of benzoic derivatives and total acetaldehyde, a higher content of oak lactones and gallic acid and less 3-methyl-1-butyl acetate and 2-phenylethyl acetate. Xu *et al.* (2017) investigated the aging process of Chinese liquor on aroma components by year. Thirteen of a total twenty-one major aroma components dropped significantly in the first year, but they kept the same levels for the next three years, then reduced again in the fifth year. Conversely, increases of propionic acid, furfural and phenyl ethanol were recorded whereas ethyl lactate was observed to be the most stable component during the aging process.

Generally, aging time, wood composition and oxidation levels are important factors in the variation of aroma components occurring during the aging process. Control factors could shorten the aging time, reduce product costs and increase product quality.

All in all, fruit spirits production depends on many factors, from raw material selection, alcohol fermentation, to distillation, aging and packaging. In particular, the raw fruit varieties dramatically influence the quality of the product of the spirits. Besides, fermentation conditions (yeast strains, temperature, pH, time etc.) and distillation (cut-points, distillation equipment, operation parameters, etc.) equally play important roles. Hence, meticulous control of these factors is required to produce excellent quality fruit spirits products.

3.5 Chemometric statistics

Established at the beginning of the 1970s by Svante Wold *et al.*, chemometrics is the field applying mathematical, statistical and other ways to design, evaluate and provide related chemical information by analyzing chemical data. Chemometrics involves multivariate statistics, mathematical modeling, computer science and analytical chemistry with some important application areas including (a) calibrating, validating and significance testing, (b) optimizing chemical determinations and experimental procedures, (c) extracting the maximum of chemical information from analytical data (Gemperline, 2006).

By applying the "formal" models to prediction and classification, the chemometric approach often detect formal relationships having the causality elements while the classical approach determines new relationships and discovers new natural laws. So, the advantage of the classical approach is to be successful, accepted and well based on the constants in the models having definite physical significance, but the factors are correlated and their effects cannot be separated. The advantage of the chemometric is correlations among variables can be used but sometimes the constants in models do not necessarily have physical relevance. Therefore, the two approaches are complementary, the modern approach cannot be substituted by the classical one and vice versa (Héberger, 2008).

In recent years, issues related to food science and authentication have been of large concern to researchers, buyers and administering objects. The necessity to ensure quality foodstuff has motivated researchers to study for more powerful tools in order to examine and deal with food chemistry problems. As a result, numerous studies have been published regarding applications of the combination of instrumental analysis and different chemometric methods to describe the similarities and dissimilarities between samples based on multivariate data with the primary purposes of traceability, assess the quality and also verify the authenticity of one or a group of products (Bauer-Christoph *et al.*, 1997, Cantarelli *et al.*, 2015, Fernandez-Lozano *et al.*, 2019, Forina *et al.*, 2009, Kovács *et al.*, 2018, Peng *et al.*, 2017, Pérez-Caballero *et al.*, 2017, Sádecká *et al.*, 2016, Sádecká *et al.*, 2009, Schiavone *et al.*, 2020, Zhang *et al.*, 2018). Fifteen commercial whiskies with different years ageing were recognized by using linear discriminant analysis (LDA) and partial least square discriminant analysis (PLS-DA) on molecular absorption spectroscopy data (Cantarelli *et al.*, 2015). By comparing the use of UV absorption, excitation-emission matrix (EEM) fluorescence and synchronous fluorescence spectroscopy and HPLC with fluorescence detection combined with principal component analysis (PCA), parallel factor analysis (PARAFAC) and LDA on the commercial Juniper-flavoured spirit drinks, the method accuracy was enhanced significantly to 97 % in the case of applying LDA on data of eight principal HPLC peak areas (Sádecká *et al.*, 2015). Besides, some studies were conducted multivariate chemometric classification procedures on GC-FID dataset for identifying wines according to their origin and variety (Falqué *et al.*, 2002, Márquez *et al.*, 2008, Rebolo *et al.*, 2000).

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Reagents, chemicals and standards

All chemicals and standards were analytical grades and purchased either from Sigma–Aldrich (USA), Lachner (Czech Republic), VWR Chemicals (USA) or Fluka (Hungary). Basic chemicals were sodium hydroxide, phosphoric acid, anhydrous sodium carbonate, sodium bicarbonate, potassium sodium tartrate, anhydrous sodium sulphate, copper sulphate, sulphuric acid, ammonium molybdate, disodium hydrogen arsenate. Meanwhile standards for HPLC included citric acid, oxalic acid, malic acid, succinic acid, acetic acid, glucose, fructose and sucrose, whereas standards for GC comprised methanol, ethyl acetate, acetaldehyde, ethanol, ethyl formate, 2-propanol, 1-propanol, 2-butanol, 2-methyl-1-propanol, 1-butanol, propyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol were used. Both external and internal techniques were applied for identification and quantification of origin compounds.

4.1.2 Yeast strains

Nine different yeast strains were provided by the Kokoferm Limited Company (Gyöngyös, Hungary; Table 4.1). They are classical strains for red, white and sparkling winemaking.

Table 4.1. Yeast strains used

No.	Trademark	Label	Strain	Provenance
1	Uvaferm SLO	SLO	*S. cerevisiae, var. bayanus*	Lallemand, Canada
2	Uvaferm PM	PM	*S. cerevisiae, var. bayanus*	Lallemand, Canada
3	Uvaferm Danstil A	A	*S. cerevisiae,* N0.342	Lallemand, Canada
4	Fermiblanc Arom	Aro	*S. cerevisiae,* N0.SM102	Oenobrands, France
5	Viniflora Melody	M	*S. cerevisiae, K. thermotolerans, T. delbrueckii*	Chr. Hansen, CA
6	Vin-O-Ferm Roses	R	*S. cerevisiae spp.*	OenoBioTech, France
7	Fermicru AR2	AR	*S. cerevisiae,* N0. 10122	OenoBioTech, France
8	Oenoferm x-treme F3	O1	*S. cerevisiae, var. bayanus*	Erbslöh, Germany
9	Oenoferm x-thiol F3	O2	*S. cerevisiae, var. bayanus*	Erbslöh, Germany

These yeast strains were activated before fermentation by mixing 1 g dry yeast, 1 g yeast nutrients* (Uvavital^{TM}, Lallemand, France) and 100 mL warm water (28 °C), then the mixture was aerated by gentle agitation for 2 hours to grew. The composition of yeast nutrients consisted of

vitamins (thiamine, biotin, folic acid, etc.), amino acids, peptides and polypeptides, proteins, ionic nitrogen, microelements, sterols, unsaturated fatty acids, oxygen-binding compounds, yeast extract.

4.1.3 Fruit juice

Concentrate juices, including sour cherry of 68 °Brix, apple of 70 °Brix, apricot of 65 °Brix and pear of 70 °Brix, were provided by the INNIGHT Company (Budapest, Hungary). Concentrate juices were diluted to the desired strength with tap water, then adjusted to the desired pH by 3N phosphoric acid or 3N sodium hydroxide solution. Tap water was used to provide minerals for enhancing the yeast enzymatic activity in the fermentation process.

4.2 Experiment design

4.2.1 Selection of yeast strain for fermentation of fruit spirits

Fruit juice fermentations with each yeast strain were carried out separately in 500 mL Erlenmeyer conical flasks. Each flask contained 300 mL juice of 18.0 °Brix and 2 % v/v pre-culture of the activated yeast strain, then was mounted by twin bubble airlock to close the air and provide facultative anaerobic conditions. The fermentation was conducted at 20 ℃ statically. Sampling was carried out daily, and pH, Brix, reducing sugar, alcohol and organic acid content were determined excepting volatile compounds analyzed on the last day of the fermentation. Three replicates of the fermentation were performed for each strain to select a suitable yeast strain among fruit juice based on evaluating alcohol production capacity and volatile profile.

4.2.2 Optimization of fermentation process

Based on preliminary results, fermentation temperature, pH and soluble solids content were selected as variables in the ethanol production and formation of volatiles of fruit brandy by *S. cerevisiae*. Many prior studies have also confirmed the crucial impact of these factors (Bandaru *et al.*, 2006, Chen, 1981, Duarte *et al.*, 2011, Hajar *et al.*, 2012, Jha *et al.*, 2018, Sudheer Kumar *et al.*, 2009). Therefore, the fermentation process of fruit juice concentrate and *S. cerevisiae* by using Response Surface Methodology (RSM) coupled with the central composite rotatable design was adapted and used to optimize fermentation conditions through three variables, namely temperature (X_1), pH (X_2) and initial soluble solids content (X_3) on production yield of alcohol (Y_1) and volatile compounds (Y_2). However, to find the appropriate input range of these variables for the RSM

method, three investigations about their independent effects on the production yield of ethanol and volatile compounds were performed.

4.2.2.1 Effect of temperature

Each conical flask 500 mL contained 300 mL fruit juice of 18.0 °Brix, pH 3.0 and 2 % v/v pre-culture of the activated Uvaferm Danstil A strain. The flasks were mounted by twin bubble airlocks to close the air and provide facultative anaerobic conditions. The fermentation was statically conducted at 10 ℃, 15 ℃, 20 ℃, 25 ℃, 30 ℃ and 35 ℃. After eight days, fermented fruit juices were sampled and analyzed by Brix, reducing sugar, alcohol content, total higher alcohol and total ester. Three replicates of the fermentation were performed for each fruit type based on evaluating alcohol and production capacity of aroma compounds.

4.2.2.2 Effect of pH

Each conical flask 500 mL contained 300 mL fruit juice of 18.0 °Brix, adjusted to the desired pH of pH 2.5, pH 2.75, pH 3.0, pH 3.5, pH 4.0 and pH 4.5 by 3n phosphoric acid or 3n sodium hydroxide. The alcoholic fermentation was initiated by adding pre-culture of the activated Uvaferm Danstil A strain in the ratio of 2 % v/v. The flasks were then mounted by twin bubble airlocks and kept statically at 20 ℃. After eight days, fermented fruit juice was sampled and analyzed.

4.2.2.3 Effect of total soluble solids content

Pre-culture of the activated Uvaferm Danstil A strain was added to each 500 mL conical flask containing 300 mL fruit juice with pH 3.0 in the ratio of 2 % v/v, thus the initial total soluble solids content changed of 12 °Brix, 18 °Brix, 24 °Brix, 30 °Brix and 36 °Brix. The alcoholic fermentation was also conducted under similar conditions. Samples were taken and analyzed after eight fermentation days.

4.2.2.4 Optimization of fermentation conditions for alcohol production

Response Surface Methodology (RSM) proposed by Montgomery (1997) was applied to optimize the fermentation conditions. Box-Behnken design with 2^3 points in the corners of the cube representing the experimental domain (Table 4.2), 2x3 axial points in the center of each face

of the cube and 3 points are the replicates in the center of the cube leading to a total number of 17 experiments (Table 4.3) was used.

Table 4.2. Independent variables in the experimental plan

Variables	Coded levels		
	- 1	0	+ 1
Temperature, X_1 (°C)	15	20	25
pH, X_2	2.75	3.25	3.75
Soluble solids content, X_3 (°Brix)	18	24	30

Table 4.3. Box-Behnken experimental design with three independent variables for optimization of fermentation conditions

Exp. No.	X_1	X_2	X_3
1	- 1	- 1	- 1
2	+ 1	- 1	- 1
3	- 1	+ 1	- 1
4	+ 1	+ 1	- 1
5	- 1	- 1	+ 1
6	+ 1	- 1	+ 1
7	- 1	+ 1	+ 1
8	+ 1	+ 1	+ 1
9	- 1	0	0
10	+ 1	0	0
11	0	- 1	0
12	0	+ 1	0
13	0	0	- 1
14	0	0	+ 1
15	0	0	0
16	0	0	0
17	0	0	0

The second-order polynomial function was used to evaluate results obtained.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3$$

where, Y is a predicted response, X_1, X_2, X_3 are independent variables; b_0 is a offset term; b_1, b_2 and b_3 are linear effects; b_{11}, b_{22} and b_{33} are squared effects and b_{12}, b_{23} and b_{13} are interaction terms.

Experiment sets with the conditions in Table 4.2 were carried out. Alcohol, total higher alcohol and total ester contents were determined and analyzed.

4.2.3 Effects of distillation process on aromatic profile

4.2.3.1 Effects of distillation process on distribution of aroma compounds

The effects of the distillation process were investigated with the fermentation of 5.5 L of each fruit juice at the optimum conditions. After the alcoholic fermentation was completed, the mashes were immediately transferred into the glass distillation system with a capacity of 3 L (Figure 4.1). The cool water of around 15 ℃ – 18 ℃ was circulated through the entire system before distillation began. The cool water flow rate was adjusted for the alcohol product in the outflow not exceeding 9 mL/min. The temperature of the heater was set at 102 ℃.

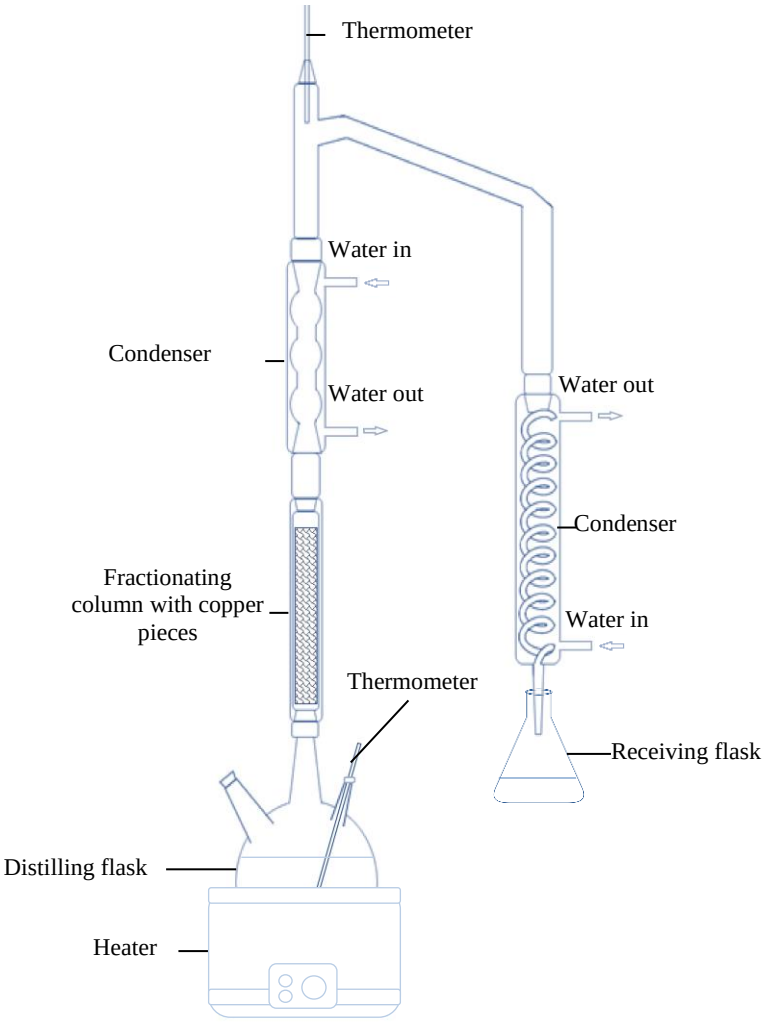


Figure 4.1. The glass distillation system

Distillation was carried out slowly and continuously. It was stopped when the alcohol degree in the outflow was lower than 5 % v/v. In the first distillation, the total volume of distillate in the first distillation reached around 1.8 L with an alcohol content ranging from 23 % v/v to 33

% v/v depending on the fruit mash applied. For describing the distribution of volatile compounds during the second distillation, the first cut volume was 1.5 % of the distillate. Other fractions were collected by volumes of each 100 mL cut until the outlet's alcohol content was below 5 % v/v. Besides, sensory evaluation was also performed adjunctively to find the appropriate cut-off point for the distillation process. Sensory samples for the cut-point of the head to heart fraction were 5 mL at 1%, 1.5% and 2% of the first distillate. Sensory samples for the cut-point of the heart to tail fraction were 20 mL of the distillate of around 50 % v/v, 45 % v/v, 40 % v/v and 35 % v/v. Three replicates of the distillation were performed with each fruit type. Samples of various kinds of fruit spirits were analyzed based on the alcohol content and the aroma compounds. In order to avoid the loss of aroma, all the fractions collected were kept at 4 ℃ until analysis.

4.2.3.2 Profile of spirit products from apple, cherry, pear and apricot

Serries experiments were conducted with the cut-points obtained. After the second distillation, fruit spirits (heart fractions) were stored at room temperature for two weeks for stabilizing their flavor and state. Then, they were diluted to 40% v/v and analyzed for volatile compounds to find the profile of these spirits.

4.2.4 Characterization and classification of pálinkas and fruits

A total of 48 pálinka samples (12 apple pálinkas, 12 apricot pálinkas, 12 pear pálinkas and 12 cherry pálinkas) were covered in this study. All samples were purchased with some well-known producers in Hungary whose labels displayed the corresponding quality seals. They were analyzed alcohol content and aroma compounds. Chemometric statistics methods were applied to confirm the key aroma compounds and to classify fruit spirits and fruits used.

4.3 Analytical methods

4.3.1 Measurement of Brix and pH

The total soluble solids (°Brix) and pH were measured by use of refractometer (Atago, Japan) and pH meter (Mettler Toledo, Switzerland), respectively.

4.3.2 Alcohol content

Two different methods were used to determine the alcohol content of the samples. In case of metabolism analysis, the ethanol concentration was measured by HPLC with RI detector. In all other cases, alcohol content of the fermented mash was determined by distilling and measuring the density of the distillate. Briefly, 100 mL of fermented mash was taken out from each fermentation flask, then one drop of silicone oil (Antifoam B Emulsion, USA) was added to prevent the foaming during the distillation process. The mash was distilled by using steam injection distillation unit (Büchi K-350, Switzerland) in 3 minutes. The distillate was collected in 100 mL volumetric flask and diluted to the mark with distilled water. Alcohol content was measured by a digital density meter (Anton Paar DMA 35N, Austria).

4.3.3 Analysis of reducing sugars and organic acids

Samples were centrifuged at speed of 9,168 g and room temperature for 10 minutes before the analysis process. Sugars and organic acids were detected by HPLC system (Surveyor, Thermo Scientific, San Jose, USA) with Aligent Hi-Plex H column 7.7 x 300mm (Agilent, Santa Clara, USA). Parameters: the mobile phase was 5 mM H_2SO_4 solution, the flow rate for elution was 0.6 mL/min at 45 ℃, injected volume was 10 µL, the temperature of the column was maintained at 45 ℃, the measurement time was 25 min at a constant flow rate. The carbohydrates and organic acids were detected by RI and PDA detectors, respectively. All chemicals of the standards were HPLC grade of purity and used without further purification.

4.3.4 Analysis of volatile compounds by GC-FID

The analyses of the volatile compounds were done with a GC-FID system (Perichrom, ALPHA MOS, France). The compounds were separated on a CHROMPACK CP-WAX 57CB Wcot (Agilent, Santa Clara, USA) fused silica column (polyethylene glycol stationary phase, 50 m*0.25 mm i.d. with 0.25µm film thickness). The temperature program of the oven was as following: initial 60 ℃ (isotherm for 6 min), ramp rate (6 ℃/min to 83 ℃ and afterward to 220 ℃ at a rate of 10 ℃/min), temperatures of injector and detector were 210 ℃ and 220 ℃, respectively. The carrier gas was helium at 3 mL/min.

The commercial pálinka samples were analyzed directly without any preparation process. All other samples were distilled with the distillation unit (Büchi K-350, Switzerland) and stored at -20 ℃ until analysis.

4.4 Statistical analysis

4.4.1 Classical statistics

The unpaired and paired Student's t-test were applied to compare the experimental results. In addition, the one-way analysis of variance (ANOVA), LSD test and Tukey-HSD test were applied to check the regression analysis. Before the statistical procedure, the data were checked for normality. All of the tests were done using R-studio and STATGRAPHICS Centurion XV with a significant level 5 % (α=0.05).

4.4.2 Response surface methodology (RSM)

Response surface methodology was employed to optimization of fermentation conditions. Both the experimental design and data processing were carried on commercial software Modde 5.0.

4.4.3 Multivariate analyses

The data matrix (dimension 48x17) was constructed with 48 rows representing observed analyzed samples and 17 columns corresponding to aroma components of the fruit spirits samples tested. The dataset was auto-scaled and normalized before statistical treatment. The principal component analysis (PCA) and linear discriminant analysis (LDA) were used for classifying and identifying spirits follow to fruit type. All multivariate analyses were done using the R-studio and R version 4.0.0 with some packages, including MASS, ggplot2, scales, gridExtra, FactoMineR, factoextra, corrplot, DiscriMiner, ropls and mdatools.

5. RESULTS AND DISCUSSIONS

5.1 Selection of yeast strains for fruit spirit fermentation

The alcoholic fermentation process has significantly affected the final flavour and quality of fruit spirits. In fermentation, various volatile components were formed through both the release of aroma compounds from the precursors presenting in fruit materials and the synthesis of other volatile compounds under the yeast actions (Molina *et al.*, 2009). However, the composition and concentration of these aromas vary depending on the raw fruit material and yeast strain applied. One aim of this study was to evaluate the capacity of alcohol production among different yeast strains on various fruits, simultaneously to identify the relationship between volatile components and aroma profiles of fruit distillates obtained. Four fruit juices, including apple, pear, apricot and cherry, were fermented individually with nine commercial yeast strains consisting of Uvaferm SLO (SLO), Uvaferm PM (PM), Uvaferm Danstil A (A), Fermiblanc Arom (Aro), Viniflora Melody (M), Vin-O-Ferm Roses (R), Fermicru AR2 (AR), Oenoferm x-treme F3 (O1) and Oenoferm x-thiol F3 (O2). The results are summarized in Table 5.1, 5.2, and Figure 5.1.

Table 5.1 Some physicochemical parameters of apple, apricot, cherry and pear juice before (day 0) and after fermentation (day 8) with different yeast strains

Fruit	Parameter	Juice (day 0)	Fermented juice (day 8)									Sig.
			SLO	PM	A	Aro	M	R	AR	O1	O2	
Apple	pH	3.01±0.00	3.25±0.03	3.25±0.01	3.24±0.03	3.27±0.01	3.27±0.01	3.25±0.02	3.25±0.01	3.21±0.04	3.24±0.02	*
	Brix	17.20±0.00	6.00±0.00	6.07±0.06	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	***
	Organic acids (g/100 mL)											
	Acid malic	0.85±0.06	0.64±0.04	0.66±0.03	0.54±0.11	0.62±0.02	0.58±0.08	0.53±0.30	0.60±0.10	0.64±0.01	0.59±0.08	*
	Acid citric	1.60±0.01	1.32±0.06	1.35±0.19	1.17±0.06	1.30±0.08	1.24±0.22	1.26±0.03	1.32±0.03	1.21±0.17	1.22±0.18	*
	Reducing sugars (g/100 mL)											
	Sucrose	0.94±0.18	0.05±0.00	0.05±0.00	0.04±0.02	0.05±0.00	0.04±0.01	0.05±0.01	0.05±0.01	0.05±0.00	0.06±0.01	***
	Glucose	3.74±0.44	0.24±0.07	0.24±0.06	0.32±0.11	0.23±0.06	0.23±0.07	0.23±0.07	0.27±0.01	0.22±0.06	0.25±0.05	***
	Fructose	8.87±1.15	0.41±0.04	0.42±0.04	0.44±0.04	0.42±0.03	0.40±0.04	0.41±0.04	0.45±0.04	0.41±0.01	0.46±0.02	***
	Alcohol content (% v/v) and yield (% v/v alcohol/ % total reducing sugar)											
	Alcohol	0.00±0.00	9.33±0.12	9.30±0.10	9.43±0.06	9.43±0.15	9.37±0.06	9.20±0.26	9.33±0.12	9.17±0.06	9.37±0.06	***
	Yield	0.00±0.00	0.69±0.01	0.69±0.01	0.70±0.00	0.70±0.01	0.69±0.00	0.68±0.02	0.69±0.01	0.68±0.00	0.69±0.00	***
Apricot	pH	3.02±0.03	3.13±0.01	3.11±0.02	3.15±0.05	3.15±0.04	3.15±0.04	3.15±0.04	3.16±0.03	3.13±0.04	3.11±0.05	*
	Brix	17.55±0.45	9.25±0.90	9.35±0.90	9.20±0.92	9.30±0.98	9.30±0.87	9.25±0.95	9.20±0.61	9.35±0.87	10.00±0.61	***
	Organic acids (g/100 mL)											
	Acid malic	1.58±0.07	1.30±0.02	1.27±0.07	1.33±0.08	1.21±0.08	1.06±0.12	1.10±0.08	0.96±0.12	1.01±0.04	1.11±0.07	***
	Acid citric	0.86±0.08	0.65±0.09	0.73±0.03	0.70±0.02	0.60±0.06	0.60±0.03	0.68±0.08	0.61±0.06	0.61±0.06	0.68±0.08	*
	Reducing sugars (g/100 mL)											
	Sucrose	1.71±0.15	0.58±0.12	0.63±0.18	0.60±0.14	0.63±0.17	0.54±0.09	0.67±0.19	0.58±0.10	0.66±0.21	0.63±0.15	***
	Glucose	3.61±0.10	0.61±0.17	0.71±0.25	0.63±0.17	0.61±0.18	0.51±0.24	0.67±0.48	0.52±0.25	0.61±0.37	0.62±0.16	***
	Fructose	5.25±0.45	0.43±0.21	0.42±0.22	0.39±0.24	0.42±0.18	0.40±0.22	0.44±0.21	0.42±0.24	0.44±0.22	0.64±0.35	***
	Alcohol content (% v/v) and yield (% v/v alcohol/ % total reducing sugar)											
	Alcohol	0.00±0.00	7.00±0.11	6.87±0.12	6.97±0.22	6.83±0.30	7.10±0.33	6.97±0.18	6.87±0.30	6.73±0.24	6.60±0.11	***
	Yield	0.00±0.00	0.66±0.03	0.65±0.03	0.66±0.05	0.61±0.10	0.68±0.03	0.66±0.05	0.65±0.00	0.63±0.01	0.59±0.08	***

Fruit	Parameter	Juice (day 0)	Fermented juice (day 8)									Sig.
			SLO	PM	A	Aro	M	R	AR	O1	O2	
Cherry	pH	3.02±0.00	3.18±0.01	3.16±0.00	3.23±0.00	3.24±0.01	3.21±0.01	3.23±0.02	3.23±0.02	3.18±0.01	3.19±0.04	***
	Brix	17.17±0.06	9.30±0.10	9.33±0.06	9.27±0.15	9.20±0.10	9.27±0.12	9.33±0.12	9.27±0.15	9.33±0.06	9.37±0.06	***
	Organic acids (g/100 mL)											
	Acid malic	1.66±0.04	1.40±0.06	1.43±0.03	1.41±0.03	1.38±0.08	1.39±0.06	1.36±0.04	1.34±0.04	1.39±0.02	1.31±0.08	***
	Acid citric	0.53±0.08	0.31±0.06	0.37±0.02	0.33±0.05	0.29±0.08	0.30±0.04	0.29±0.04	0.29±0.04	0.30±0.03	0.28±0.05	***
	Reducing sugars (g/100 mL)											
	Sucrose	0.34±0.08	0.24±0.12	0.26±0.08	0.25±0.04	0.25±0.04	0.25±0.08	0.24±0.12	0.25±0.08	0.26±0.012	0.25±0.12	ns
	Glucose	5.45±0.12	0.57±0.23	0.71±0.34	0.69±0.16	0.71±0.37	0.68±0.15	0.64±0.23	0.68±0.27	0.69±0.18	0.68±0.21	***
	Fructose	5.20±0.35	0.73±0.12	0.78±0.30	0.74±0.18	0.81±0.24	0.77±0.21	0.73±0.18	0.74±0.11	0.81±0.34	0.77±0.37	***
	Alcohol content (% v/v) and yield (% v/v alcohol/ % total reducing sugar)											
	Alcohol	0.00±0.00	6.13±0.15	6.07±0.06	6.13±0.06	6.2±0.10	6.2±0.10	6.2±0.10	6.2±0.00	5.93±0.06	6.13±0.06	***
	Yield	0.00±0.00	0.56±0.05	0.56±0.03	0.56±0.03	0.57±0.04	0.57±0.03	0.57±0.04	0.57±0.03	0.55±0.04	0.56±0.04	***
Pear	pH	3.01±0.02	3.15±0.11	3.11±0.10	3.14±0.11	3.13±0.11	3.12±0.11	3.12±0.12	3.12±0.09	3.11±0.12	3.09±0.09	ns
	Brix	17.87±0.21	9.17±0.15	9.27±0.15	9.20±0.10	9.23±0.15	9.10±0.20	9.17±0.12	9.20±0.10	9.27±0.05	9.27±0.15	***
	Organic acids (g/100 mL)											
	Acid malic	0.53±0.04	0.16±0.03	0.08±0.03	0.19±0.04	0.14±0.07	0.31±0.12	0.27±0.10	0.32±0.05	0.29±0.08	0.26±0.16	**
	Acid citric	0.36±0.01	0.23±0.02	0.23±0.01	0.21±0.03	0.22±0.02	0.21±0.06	0.18±0.04	0.21±0.06	0.21±0.05	0.21±0.01	**
	Reducing sugars (g/100 mL)											
	Sucrose	0.57±0.07	0.21±0.06	0.2±0.05	0.2±0.05	0.19±0.05	0.19±0.07	0.18±0.05	0.17±0.07	0.16±0.06	0.22±0.07	***
	Glucose	3.06±0.13	0.40±0.03	0.38±0.05	0.37±0.04	0.39±0.04	0.34±0.05	0.32±0.04	0.41±0.06	0.40±0.06	0.39±0.05	***
	Fructose	6.91±0.12	1.86±0.50	1.79±0.54	1.54±0.42	1.54±0.33	1.31±0.73	1.16±0.62	1.28±0.73	1.83±0.66	1.49±0.49	***
	Alcohol content (% v/v) and yield (% v/v alcohol/ % total reducing sugar)											
	Alcohol	0.00±0.00	7.43±0.15	7.27±0.21	7.40±0.10	7.30±0.10	7.27±0.25	7.13±0.06	7.13±0.21	7.20±0.10	7.17±0.06	***
	Yield	0.00±0.00	0.70±0.01	0.69±0.02	0.70±0.00	0.69±0.00	0.69±0.02	0.67±0.01	0.67±0.03	0.68±0.00	0.68±0.01	***

Sig.: significance (*, **, ***) - display the significance at 0.05, 0.01 and 0.001 by least significant difference.
"ns": not significant.

5.1.1 Changes of pH and organic acids

Table 5.1 showed that there was a difference in pH value before and after fermentation, except for the case of pear. After the fermentation process, pH value slightly increased, in detail, from pH 3.01 to pH 3.25, pH 3.25, pH 3.24, pH 3.27, pH 3.27, pH 3.25, pH 3.25, pH 3.21 and pH 3.24, from pH 3.01 to pH 3.13, pH 3.11, pH 3.15, pH 3.15, pH 3.15, pH 3.15, pH 3.16, pH 3.13 and pH 3.11, from pH 3.02 to pH 3.18, pH 3.16, pH 3.23, pH 3.24, pH 3.21, pH 3.23, pH 3.23, pH 3.18 and pH 3.19 for apple, apricot and cherry juices over in the case of strain Uvaferm SLO (SLO), Uvaferm PM (PM), Uvaferm Danstil A (A), Fermiblanc Arom (Aro), Viniflora Melody (M), Vin-O-Ferm Roses (R), Fermicru AR2 (AR), Oenoferm x-treme F3 (O1) and Oenoferm x-thiol F3 (O2), respectively. The increase in pH values related to the biosynthesis of organic acids making the concentrations of some acids in raw materials changed and some other acids formed during fermentation. Fruit juice contained varieties of organic acid compounds, however malic and citric were the primary organic acids in apricot (Fan *et al.*, 2017); apple (Wu *et al.*, 2007), pear (Chen *et al.*, 2007) and sour cherry (Serradilla *et al.*, 2016). A light decrease in citric acid concentration before and after juice fermentation occurred at all yeast strains of SLO, PM, A, Aro, M, R, AR, O1 and O2, respectively, such as apple (from 1.60 g/100 mL to 1.32 g/100 mL, 1.35 g/100 mL, 1.17 g/100 mL, 1.30 g/100 mL, 1.24 g/100 mL, 1.26 g/100 mL, 1.32 g/100 mL, 1.21 g/100 mL and 1.22 g/100 mL), apricot (from 0.86 g/100 mL to 0.65 g/100 mL, 0.73 g/100 mL, 0.70 g/100 mL, 0.60 g/100 mL, 0.60 g/100 mL, 0.68 g/100 mL, 0.61 g/100 mL, 0.61 g/100 mL and 0.68 g/100 mL), cherry (from 0.53 g/100 mL to 0.31 g/100 mL, 0.37 g/100 mL, 0.33 g/100 mL, 0.29 g/100 mL, 0.30 g/100 mL, 0.29 g/100 mL, 0.29 g/100 mL, 0.30 g/100 mL and 0.28 g/100 mL) and pear (from 0.36 g/100 mL to 0.23 g/100 mL, 0.23 g/100 mL, 0.21 g/100 mL, 0.22 g/100 mL, 0.21 g/100 mL, 0.18 g/100 mL, 0.21 g/100 mL, 0.21 g/100 mL and 0.21 g/100 mL). However, in most cases, no significant difference in the citric acid content among yeast strains in the same fruit was found, except comparing with respective initial juices. In general, the difference in malic acid value before and after fermentation was also observed. The reduction might not be attributed to malolactic fermentation due to no production of lactic acid (Redzepovic *et al.*, 2003). Following Coloretti *et al.* (2002), malic acid molecules could enter into yeast cells by passive diffusion, which might be the reason for this reduction.

5.1.2 Changes of total soluble solids, reducing sugars and alcohol

Glucose, fructose and sucrose were major reducing sugars and represented nearly 90 % of total reducing sugars in the apple, apricot, cherry and pear (Chen *et al.*, 2007, Fan *et al.*, 2017,

Serradilla *et al.*, 2016, Wu *et al.*, 2007). They were also the main sugars found in raw materials studied, in which fructose was dominant, followed by glucose and sucrose. *S. cerevisiae* consumes sugar for the growth and development of new cells and fermenting to produce ethanol, carbon dioxide, and other products (Lambrechts and Pretorius, 2000). In Table 5.1, results of the total soluble solid (Brix value) and the residual reducing sugar content before and after fermentation showed that all yeast strains tested fermented the juices to dryness. During eight fermentation days, Brix value reduced rapidly from 17.20 °Brix to around 6.01 °Brix for apple, from 17.55 °Brix to around 9.36 °Brix for apricot, from 17.17 °Brix to around 9.30 °Brix for cherry, and from 17.87 °Brix to around 9.21 °Brix for pear. The average of residual Brix values (6.01 °Brix, 9.36 °Brix, 9.30 °Brix and 9.21 °Brix) were from nine yeast strains over apple, apricot, cherry and pear. Correspondingly, the total reducing sugar content decreased from 13.55 mg/100 mL to around 0.72 mg/100 mL, from 10.57 mg/100 mL to round 1.67 mg/100 mL, from 10.99 mg/100 mL to around 1.69 mg/100 mL, and from 10.54 mg/100 mL to around 2.10 mg/100 mL for apple, apricot, cherry and pear juices, respectively (Figure 5.1).

Figure 5.1 illustrated that the fermentation was solely intense in the first four days, then gradually dropped over the next two days and stabilized until the 8th day. In the most of cases, the healthiest metabolism occurred in the first four days at almost all strains, except for strain O1 in the early five days. In most cases of strain A, alcohol content reached the maximum on the 3rd day, which was best demonstrated by apricot and cherry fermentation. The substrates used were gradually depleting, leading to the fermentation slowly proceeded until the 8th day. At the end of fermentation, alcoholic content reached from 9.17 % v/v to 9.43 % v/v for apple, from 6.60 % v/v to 7.10 % v/v for apricot, from 5.93 % v/v to 6.20 % v/v for cherry, from 7.13 % v/v to 7.43 % v/v for pear. Alcohol yield ranged from 68 % – 70 %, 59 % – 68 %, 55 % – 57 % and 67 % – 70 % in the cases of apple, apricot, cherry and pear, respectively. These alcohol yields in the study were higher than that in some researches of Rita *et al.* (2011) (from 50% – 52 % for apple), Ganatsios *et al.* (2019) (from 44 % – 46 % for sour cherry) and García-Llobodanin *et al.* (2007) (50 % for pear). However, for each fermented fruit juice, no significant difference in the alcohol production capacity among these yeast strains was found (p-value > 0.05). Similar results were reported by various authors (Bordiga *et al.*, 2017, Estévez *et al.*, 2004, Torrens *et al.*, 2008). For instance, when Vararu *et al.* (2016) conducted the fermentation of grape by eight commercial *Saccharomyces* strains, alcohol yields ranged from 54 % to 60 %, and no statistical difference was reflected among ethanol content and pH in wine obtained.

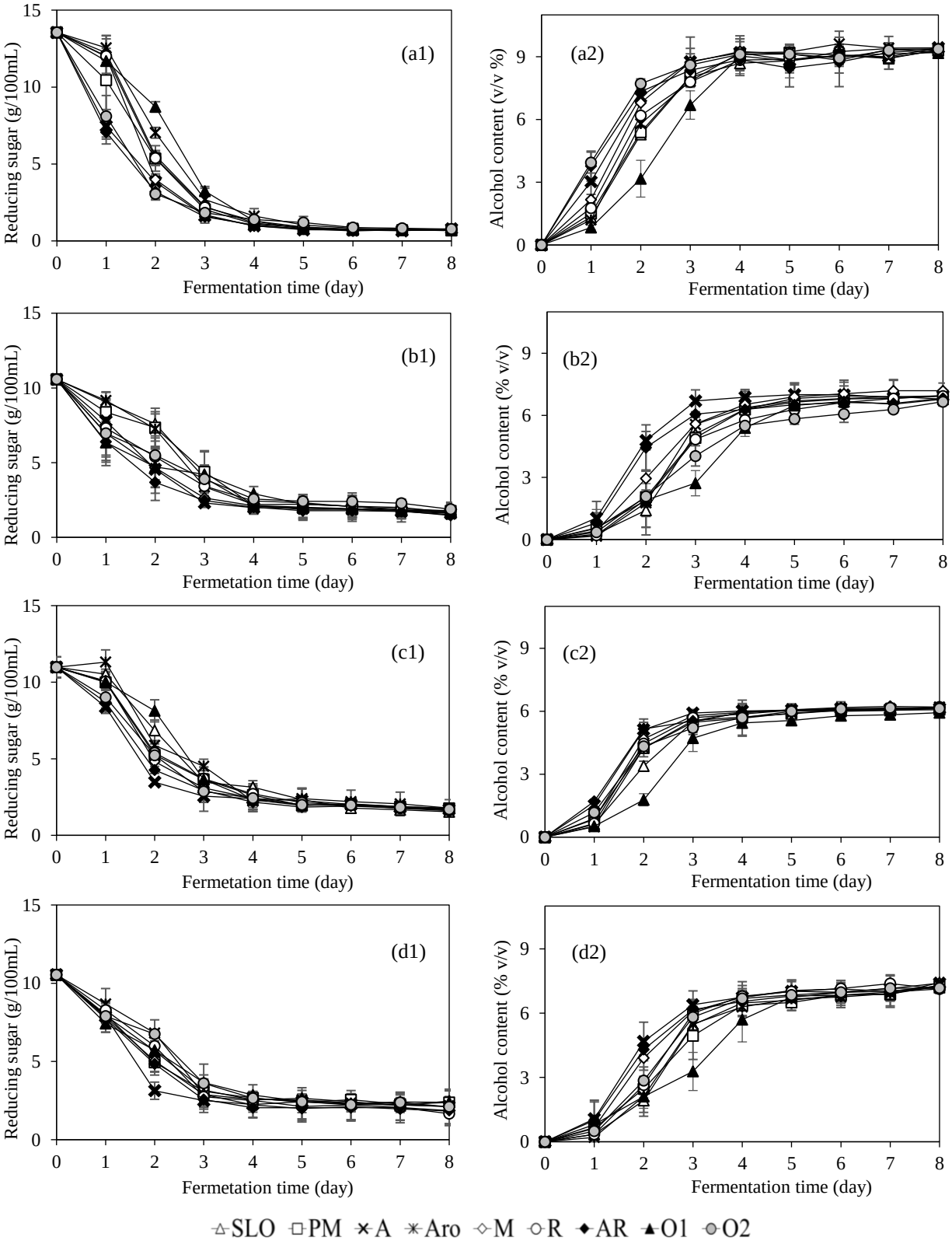


Figure 5.1. Changes of reducing sugar and alcohol content during alcoholic fermentation in apple, apricot, cherry and pear

Note: a1, b1, c1 and d1: changes of reducing sugar during alcoholic fermentation in apple, apricot, cherry and pear, respectively. a2, b2, c2 and d2: changes of alcohol content during alcoholic fermentation in apple, apricot, cherry and pear, respectively.

Although there was no significant difference among the alcohol production capacity of the yeast strains tested, the experimental results indicated that all these commercial yeast strains were strongly suitable for spirit production with a high yield of alcohol production, a short fermentation time and a stable pH during an alcohol fermentation process, especially strain Uvaferm Danstil A.

5.1.3 Volatile compounds in fermented fruit juice by different yeast strains

During the fermentation process, yeasts convert sugars of fruits to ethanol, carbon dioxide, and other metabolites. These by-products might have a wide lower content than ethanol but they dramatically contribute to the quality and flavor of spirit products, especially aroma components. Numerous studies have shown that there is a difference in the content of the volatile compounds when applying various yeast strains in the fermentation process (Carrau *et al.*, 2008, Lorenzini *et al.*, 2019, Urosevic *et al.*, 2014). Differences in the composition and concentration of aromatic substances between fermented mashed depending on the fruit type and yeast strains are shown in Table 5.3.

Fermented mashes from apple (198.99 mg/L – 284.17 mg/L) and cherry (173.35 mg/L – 268.68 mg/L) have total volatile compounds higher than apricot (154.09 mg/L – 249.90 mg/L) and pear (139.73 mg/L – 198.71 mg/L) (p-value < 0.05). In addition, there was a significant difference in volatile compounds content according to various yeast strains, which came from the variance in the formation of volatile compounds, mainly 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and ethyl acetate.

Table 5.2 Volatile compounds in fermented fruit juice by different yeast strains

Fruit type	Volatile compound (mg/L)	SLO	PM	A	Aro	M	R	AR	O1	O2	Sig.
Apricot	**Higher alcohols**										
	1-propanol	23.64±4.26	46.47±3.41	30.65±7.88	26.52±3.63	34.10±4.15	30.39±4.13	33.58±1.30	63.34±4.45	37.24±5.20	***
	2-propanol	4.92±1.16	22.97±4.73	20.38±13.98	11.67±0.89	1.55±0.65	14.17±2.96	5.94±1.41	17.56±4.71	3.49±2.13	***
	1-butanol	0.03±0.03	0.44±0.28	0.24±0.16	0.11±0.09	0.14±0.06	0.36±0.02	0.25±0.14	0.50±0.02	0.13±0.02	**
	2-butanol	0.03±0.04	2.42±0.81	0.05±0.01	0.00±0.01	0.00±0.00	0.54±0.23	0.03±0.01	0.15±0.03	0.02±0.00	***
	2-methyl-1-propanol	14.57±1.91	15.34±6.55	28.00±0.31	25.71±4.09	27.93±7.64	28.76±6.89	29.93±2.94	14.03±5.46	15.54±2.85	***
	2-methyl-1-butanol	12.23±3.14	5.98±1.39	13.14±1.70	7.10±2.75	11.04±7.54	8.00±1.35	11.99±3.43	5.54±2.31	5.78±3.24	ns
	3-methyl-1-butanol	60.17±8.38	72.35±4.08	92.67±11.51	98.14±6.72	87.94±30.46	118.56±13.21	118.91±6.64	74.67±2.24	60.86±11.66	***
	2-phenylethanol	0.15±0.03	0.05±0.01	0.20±0.04	0.02±0.01	0.08±0.05	0.02±0.00	0.08±0.05	0.29±0.10	0.03±0.01	***
	Σ Higher alcohol	115.74±15.04	166.02±8.90	185.34±11.69	169.28±3.25	162.78±21.31	200.79±25.55	200.70±9.07	176.08±8.33	123.10±23.90	***
	Esters										
	ethyl acetate	31.14±5.16	27.41±4.90	25.24±5.40	18.42±3.92	18.21±1.27	38.07±5.37	28.12±11.48	23.24±2.11	24.56±3.87	**
	ethyl formate	0.20±0.13	0.00±0.00	0.11±0.07	0.00±0.00	3.27±1.22	0.00±0.00	0.00±0.00	0.32±0.08	0.00±0.00	***
	ethyl lactate	3.05±0.55	1.31±0.47	6.42±0.90	4.77±2.55	3.73±0.36	9.53±2.16	9.57±0.55	5.10±1.47	4.22±2.19	***
	ethyl hexanoate	1.14±0.40	0.47±0.70	0.95±0.77	0.08±0.13	0.08±0.13	0.65±0.77	0.35±0.46	0.97±1.02	0.43±0.51	ns
	butyl acetate	0.83±0.11	0.08±0.01	0.11±0.08	0.10±0.05	0.09±0.01	0.47±0.08	0.26±0.10	0.15±0.03	0.13±0.10	***
	propyl acetate	0.62±0.34	0.06±0.01	0.00±0.00	0.03±0.01	0.33±0.11	0.05±0.02	0.05±0.03	0.00±0.00	0.00±0.00	***
	isoamyl acetate	1.38±0.17	0.18±0.06	1.86±0.27	0.20±0.01	1.37±0.59	0.33±0.07	0.48±0.32	1.53±1.00	1.88±1.32	ns
	Σ Ester	38.35±4.46	29.52±5.77	34.69±5.33	23.60±6.66	27.08±2.40	49.11±8.15	38.82±11.68	31.32±2.60	31.21±5.52	**
	Total volatile compound	154.09±14.42	195.53±12.64	220.03±12.82	192.89±9.51	189.86±21	16249.90±19.34	239.52±14.55	207.41±5.94	154.31±25.81	***
Apple	**Higher alcohols**										
	1-propanol	15.72±0.85	25.35±0.69	13.50±2.56	17.26±2.52	16.19±2.40	16.55±2.31	26.63±4.74	29.31±4.51	20.15±6.04	***
	2-propanol	1.92±0.74	15.68±1.84	7.10±2.49	30.41±4.66	11.28±0.46	7.63±2.07	32.35±2.61	9.58±1.61	8.90±2.45	****
	1-butanol	0.11±0.08	0.02±0.03	0.12±0.16	0.12±0.07	0.15±0.03	0.16±0.21	0.26±0.17	0.09±0.16	0.01±0.02	ns
	2-butanol	0.00±0.00	0.32±0.08	0.33±0.44	0.18±0.29	0.47±0.82	0.26±0.17	0.82±1.04	0.48±0.51	0.35±0.57	ns
	2-methyl-1-propanol	32.88±3.40	24.14±2.59	38.06±2.74	39.28±4.15	31.17±1.80	29.58±3.27	34.78±0.65	23.75±1.68	26.05±6.35	***
	2-methyl-1-butanol	26.11±2.32	20.52±2.29	38.40±2.30	28.09±2.29	24.63±2.95	29.03±2.14	27.35±4.58	25.41±4.33	22.71±4.78	***
	3-methyl-1-butanol	128.88±9.85	93.45±9.38	141.89±6.52	134.04±2.66	123.66±2.26	137.91±1.82	124.41±10.13	103.84±6.07	91.92±9.94	***
	2-phenylethanol	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.01±0.02	0.00±0.00	0.00±0.00	ns
	Σ Higher alcohol	205.62±12.79	179.48±11.81	239.41±1.23	249.37±15.63	207.56±1.13	221.14±3.07	246.61±6.30	192.46±8.52	170.10±25.30	***

Fruit type	Volatile compound (mg/L)	SLO	PM	A	Aro	M	R	AR	O1	O2	Sig.
	Esters										
	ethyl acetate	20.40±2.12	30.99±2.51	30.95±3.72	33.27±3.81	32.66±2.98	31.29±2.70	30.89±2.54	28.05±4.53	28.08±2.17	*
	ethyl formate	0.00±0.00	1.74±0.27	1.24±0.48	0.00±0.00	0.22±0.15	0.22±0.15	0.00±0.00	0.46±0.10	0.00±0.00	***
	ethyl lactate	0.01±0.01	0.01±0.01	0.02±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	ns
	ethyl hexanoate	0.35±0.11	0.55±0.16	0.43±0.14	0.74±1.27	0.96±1.66	0.30±0.12	0.02±0.04	0.29±0.30	0.63±0.84	ns
	butyl acetate	0.00±0.00	0.00±0.00	0.00±0.00	0.11±0.01	0.01±0.02	0.02±0.03	0.00±0.00	0.03±0.06	0.00±0.00	***
	propyl acetate	0.00±0.00	0.00±0.00	0.03±0.05	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	ns
	isoamyl acetate	0.41±0.16	0.29±0.17	1.05±1.09	0.68±0.69	1.16±0.11	0.85±0.76	0.43±0.25	0.46±0.29	0.18±0.16	ns
	Σ Ester	21.17±2.04	33.58±2.31	33.72±3.59	34.80±1.88	35.01±1.82	32.70±1.65	31.35±2.70	29.30±4.09	28.89±2.06	***
	Total volatile compound	226.79±10.86	213.05±13.77	273.13±3.88	284.17±14.01	242.58±1.88	253.83±4.69	277.96±7.67	221.76±12.60	198.99±26.70	***
Cherry	**Higher alcohols**										
	1-propanol	36.39±4.51	58.35±5.02	34.80±12.32	42.84±4.72	43.28±5.06	38.84±1.70	41.81±4.85	78.12±0.89	54.91±7.77	***
	2-propanol	3.67±1.02	23.48±2.64	2.25±1.40	3.11±0.63	2.79±0.82	19.30±2.74	14.32±1.00	18.47±1.31	15.55±2.33	***
	1-butanol	0.25±0.07	0.47±0.15	0.49±0.26	0.15±0.07	0.41±0.11	0.24±0.03	0.20±0.13	0.31±0.02	0.58±0.05	**
	2-butanol	0.00±0.01	0.28±0.12	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.01	0.01±0.01	0.17±0.05	0.01±0.00	***
	2-methyl-1-propanol	37.29±3.20	23.71±5.77	47.45±9.09	51.65±5.82	44.02±1.04	51.26±9.47	42.87±6.43	24.13±9.07	17.68±1.00	***
	2-methyl-1-butanol	18.56±1.83	16.05±2.14	28.12±6.21	24.98±5.21	23.96±2.06	26.35±3.43	23.61±1.97	12.78±3.91	9.07±3.06	***
	3-methyl-1-butanol	86.45±7.33	80.24±3.62	111.67±8.24	130.63±9.68	107.72±17.90	132.66±11.69	121.39±8.07	85.64±5.75	75.55±4.91	***
	2-phenylethanol	0.01±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	***
	Σ Higher alcohol	182.63±13.50	202.57±13.65	224.78±12.15	253.36±22.65	222.18±14.92	268.68±21.45	244.21±18.83	219.62±7.40	173.35±17.79	***
	Esters										
	ethyl acetate	20.43±3.32	18.32±0.58	21.10±1.93	20.38±6.28	24.34±4.06	21.29±2.42	29.24±1.47	26.48±3.00	27.17±2.20	***
	ethyl formate	0.29±0.16	2.25±0.81	1.17±0.32	0.24±0.07	3.57±0.86	4.88±0.55	0.00±0.00	0.02±0.00	2.29±0.19	***
	ethyl lactate	0.17±0.02	0.02±0.01	0.04±0.04	0.01±0.01	0.01±0.00	0.09±0.02	0.03±0.00	0.01±0.01	0.04±0.04	***
	ethyl hexanoate	0.02±0.01	0.03±0.00	1.48±0.38	0.08±0.02	0.09±0.05	0.29±0.08	0.20±0.07	0.01±0.00	0.05±0.03	***
	butyl acetate	0.02±0.00	0.00±0.00	0.09±0.08	0.02±0.03	0.00±0.00	0.02±0.01	0.01±0.00	0.01±0.00	0.00±0.00	*
	propyl acetate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.02±0.01	0.06±0.02	***
	isoamyl acetate	3.06±0.42	0.09±0.04	3.68±1.05	3.20±0.48	1.54±0.47	5.48±0.30	5.18±0.74	1.33±0.49	2.86±0.74	***
	Σ Ester	23.99±3.01	20.72±1.02	27.56±1.96	23.95±6.71	29.56±2.83	32.07±2.76	34.66±2.26	27.89±3.44	32.46±2.73	**
	Total volatile compound	206.62±11.91	223.29±14.43	252.34±11.08	277.30±29.00	251.75±17.03	300.75±21.43	278.87±16.65	247.51±4.43	205.81±17.65	***

Fruit type	Volatile compound (mg/L)	SLO	PM	A	Aro	M	R	AR	O1	O2	Sig.
Pear	**Higher alcohols**										
	1-propanol	20.30±3.66	36.64±7.11	23.98±0.68	22.85±8.16	26.60±5.04	23.66±9.49	19.71±7.69	45.70±8.84	26.06±3.99	**
	2-propanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	ns
	1-butanol	0.51±0.30	0.38±0.16	2.48±0.68	0.03±0.03	0.29±0.08	0.33±0.12	0.37±0.12	0.60±0.35	0.57±0.19	***
	2-butanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	ns
	2-methyl-1-propanol	15.32±1.64	15.20±4.83	19.09±3.07	21.33±3.30	17.69±3.57	18.30±4.07	17.83±4.39	12.86±2.62	9.14±0.53	*
	2-methyl-1-butanol	6.69±1.30	6.86±1.23	18.09±7.50	7.81±1.17	10.17±3.24	7.81±0.77	7.31±1.98	5.95±1.42	5.36±1.68	**
	3-methyl-1-butanol	66.15±6.58	69.89±7.93	100.99±8.89	100.47±2.99	68.83±10.28	115.01±5.44	117.28±8.13	73.88±5.48	53.36±6.27	***
	2-phenylethanol	0.25±0.24	0.00±0.00	0.00±0.00	0.04±0.04	0.03±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	*
	Σ Higher alcohol	109.23±5.77	128.97±6.25	164.62±3.12	152.54±9.37	123.61±10.56	165.11±12.27	162.50±7.36	138.99±5.36	94.49±4.39	***
	Esters										
	ethyl acetate	26.75±4.34	28.67±3.34	24.97±3.12	24.05±4.73	28.78±1.94	26.86±2.98	28.56±4.31	33.93±2.63	31.31±3.76	ns
	ethyl formate	7.84±2.16	6.47±1.09	5.56±2.37	3.58±1.12	0.00±0.00	1.14±0.13	1.00±0.13	2.04±0.72	12.75±2.00	***
	ethyl lactate	1.33±0.52	1.99±0.26	0.26±0.24	6.80±1.61	1.51±0.20	4.68±2.41	2.13±0.55	1.80±0.34	1.14±0.47	***
	ethyl hexanoate	0.10±0.10	0.00±0.00	0.00±0.00	0.19±0.11	0.12±0.04	0.00±0.00	0.00±0.00	0.18±0.18	0.00±0.00	***
	butyl acetate	0.42±0.43	0.12±0.11	0.12±0.09	0.07±0.07	0.05±0.04	0.00±0.00	0.00±0.00	0.04±0.04	0.00±0.00	ns
	propyl acetate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	ns
	isoamyl acetate	0.35±0.10	0.40±0.05	3.18±0.55	0.04±0.04	0.36±0.39	0.62±0.07	0.00±0.00	0.08±0.05	0.03±0.03	***
	Σ Ester	36.79±6.27	37.66±3.63	34.08±5.07	34.72±7.04	30.82±2.29	33.30±5.04	31.70±4.87	38.08±3.37	5.24±6.17	ns
	Total volatile compound	146.02±11.42	166.63±5.42	198.71±7.11	187.26±3.61	154.42±8.54	198.41±7.76	194.20±5.64	177.07±4.02	139.73±10.08	***

Sig.: significance (*, **, ***) - display the significance at 0.05, 0.01 and 0.001 by least significant difference.
"ns": not significant.

Higher alcohols play an important role in the aroma profile of fruit spirit with a pleasant odor. However, a high amount may have negative effects as being a strong, pungent flavor and taste (Spaho, 2017). Higher alcohols, including 3-methyl-1-butanol (isoamyl alcohol), 2-methyl-1-butanol (active amyl alcohol) and 2-methyl-1-propanol (isobutyl alcohol), contribute to the alcoholic, aromatic and fruity odor of fruit spirits (Miller, 2019). In all fermented mashes from apricot, apple, cherry and pear juices, 3-methyl-1-butanol accounted for the highest concentration, range of 60.17 mg/L – 118.91 mg/L, 91.92 mg/L – 141.89 mg/L, 75.55 mg/L – 132.66 mg/L and 53.36 mg/L – 117.28 mg/L, respectively. In additionally, 2-methyl-1-butanol content run from 8.00 mg/L – 13.14 mg/L, 20.52 mg/L – 38.40 mg/L, 9.07 mg/L – 28.12 mg/L and 5.36 mg/L – 18.09 mg/L; and 2-methyl-1-propanol varied from 14.57 mg/L – 29.93 mg/L, 23.75 mg/L – 39.28 mg/L, 17.68 mg/L – 51.65 mg/L and 9.14 mg/L – 21.33 mg/L. In general, as fermenting apricot, apple and cherry juices, Uvaferm Danstil A (A), Fermiblanc Arom (Aro), Viniflora Melody (M), Vin-O-Ferm Roses (R) and Fermicru AR2 (AR) exhibited an ability to synthesize 3-methyl-1-butanol higher than other strains. Unlike other higher alcohols, 1-propanol was formed by the condensation of pyruvic acid and acetyl CoA (Carrau *et al.*, 2008) and provided pungent and alcoholic odor (Miller, 2019). The content of 1-propanol ranges from 23.64 mg/L – 63.34 mg/L, 13.50 mg/L – 29.31 mg/L, 36.39 mg/L – 78.12 mg/L and 19.71 mg/L – 45.70 mg/L in fermented mashes from apricot, apple, cherry and pear, respectively. The concentration of 1-propanol was observed the highest in all four fermented fruit juices under the influence of strain Oenoferm x-treme F3 (O1), which indicated that these strains had the dominant advantage in synthesizing 1-propanol.

Esters are formed chiefly by the esterification of alcohols with fatty acids during fermentation. Ethyl esters qualitatively represented the largest group in the number and content of aroma components found (Lambrechts and Pretorius, 2000). In tested fermented mashes from apricot, apple, cherry and pear, ethyl acetate was considered as the dominant compound, accounting for 18.21 mg/L – 38.07 mg/L, 20.40 mg/L – 33.27 mg/L, 18.32 mg/L – 29.24 mg/L and 24.97 mg/L – 33.93 mg/L, respectively. In small quantities, ethyl acetate contributed to fruity and sweetish odor, but in large amounts, it gave a sharp and glue smell for fruit spirits (Miller, 2019, Spaho, 2017).

In fermented mashes from apple and pear, the content of total volatile compounds was the highest level in the case of strains Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses and Fermicru AR2, whereas in fermented cherry mashes the total aroma compound peaked in the case of these strains and Viniflora Melody. In addition, in fermented mashes from apricot, it reached

the maximum value in the case of strains Uvaferm Danstil A, Vin-O-Ferm Roses, Fermicru AR2 and Oenoferm x-treme F3. It suggests that Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses and Fermicru AR2 are potential yeast strains in distilled alcohol production with high volatile compound content.

Based on these analysis results, it can be seen clearly that all these commercial yeast strains are suitable for use in the production of distilled alcohol in general and pálinkas in particular, especially Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses and Fermicru AR2. Although there was no difference in alcohol production capacity among the commercial yeast strains applied in this study, strain Uvaferm Danstil A exhibited vigorous fermentation via the rate of sugar to alcohol conversion, short fermentation time, and pH being stable during alcohol fermentation. Besides, strain Uvaferm Danstil A is regarded as one of the strains with the high production capacity of volatile compounds. Therefore, the strain Uvaferm Danstil A was selected to conduct further studies.

5.2 Optimizing alcohol fermentation for fruit spirit production

The production capacity of alcohols and aroma compounds depends strongly on fermentation conditions such as temperature, pH and substrate concentration. Fermentation temperature had an essential effect on ethanol production and volatile compounds levels (Lu *et al.*, 2017, Molina *et al.*, 2007, Peng *et al.*, 2015, Reddy and Reddy, 2011). Molina *et al.* (2007) reported that higher concentrations of compounds related to fresh and fruity aromas were found at 15 ℃, whereas higher concentrations of flowery-related aroma compounds were found at 28 ℃. The effect of pH on ethanol production was found (Liu *et al.*, 2015, Reddy and Reddy, 2011). Lu *et al.* (2017), Narendranath and Power (2005) indicated that the initial pH values had a more significant impact on alcohol production (i.e., isobutyl alcohol and isoamyl alcohol) than temperature. By contrast, the ester production (i.e., ethyl esters and acetate esters) was more influenced by temperature than pH. Besides, the initial substrate concentration has been applied in the optimization methods of the alcoholic fermentation process (De León-Rodríguez *et al.*, 2008, Duarte *et al.*, 2011, Ratnam *et al.*, 2005). Hence, response surface methodology (RSM) was employed for optimizing fermentation conditions of fruit spirits, including temperature, pH and initial substrate concentration on the production capacity of alcoholic and aroma compounds. However, to find suitable input ranges for the RSM algorithm, preliminary experiments on the individual influence of these variables on alcoholic production capacity were carried out.

5.2.1 Effect of temperature

Temperature is known as one of the main relevant environmental variables influencing the growth and metabolic of yeast. The optimal growth temperature of *S. cerevisiae* was around 32 ℃ (López-Malo *et al.*, 2013, Salvadó *et al.*, 2011, Yalcin and Ozbas, 2008). Low temperatures are often used in alcohol fermentation to enhance production and retain more flavor volatiles and greater aromatic complexity. However, low-temperature fermentation had some disadvantages, such as increased lag and reduced growth rates, producing stuck and sluggish fermentations (López-Malo *et al.*, 2013). Thus, the problem was how to balance these weaknesses and to retain more flavors. In my work, the fermentation of cherry, apricot, pear and apple juices at different temperature levels (10 ℃, 15 ℃, 20 ℃, 25 ℃, 30℃ and 35 ℃) were conducted. After 8 days of fermentation with strain Uvaferm Danstil A, alcohol content and residual sugar were recorded. The results were presented in Figures 5.2 and 5.3.

Several authors have suggested that within the specified temperature range, alcoholic production rose proportionally with temperature, and the alcohol yield would drop when the temperature increases excessively (Reddy and Reddy, 2011, Salvadó *et al.*, 2011, Wang *et al.*, 2013). In my study, the temperature range of 20 ℃ – 30 ℃ had a positive effect on alcohol production, except for cherry, a temperature range was 20 ℃ – 25 ℃ and for apple, a temperature range was 20 ℃ – 35 ℃. After 8 days of fermentation, the highest alcohol contents were observed as 7.23 ± 0.06 % v/v, 9.70 ± 0.00 % v/v, 6.67 ± 0.06 % v/v and 7.70 ± 0. 10 % v/v in mashes of apricot, apple, cherry and pear, respectively. In most cases, a reduction trend was found when the fermentation temperature was above 30 ℃ and below 20 ℃, except in the cases of fermented cherry and apple mash. For the cherry case, a reduction trend was found if the temperature was above 25 ℃ and below 20 ℃. For the apple case, the high alcohol content (9.5 ± 0.00 % v/v) was still detected at 36 ℃. Reddy and Reddy (2011), as well as Torija *et al.* (2003b), showed that high-temperature fermentation started faster, but the cell death rate might occur higher, which caused the reduction in the capacity of use of substrates leading to a decrease in the alcohol content. Additionally, the high temperature may cause the acceleration of alcohol evaporation in the mash. Besides, the reduction in alcohol content has probably affected the increase in secondary products of other metabolic pathways. Conversely, fermentation at a too low temperature may inhibit yeast activities resulting in low use of substrates. This evidenced that why alcohol yields in fruit juices were too low in the cases of fermentation at 10 ℃. The alcohol yield in the case of fermentation at 15 ℃ was lower than ones at 20 ℃, 25 ℃ and 30 ℃ (p-value < 0.05), except for cherry, alcohol

yield at 15 ℃ was similar its' at 30 ℃ (p-value > 0.05). It is also can be explained by the effect of temperature on the fermentation process.

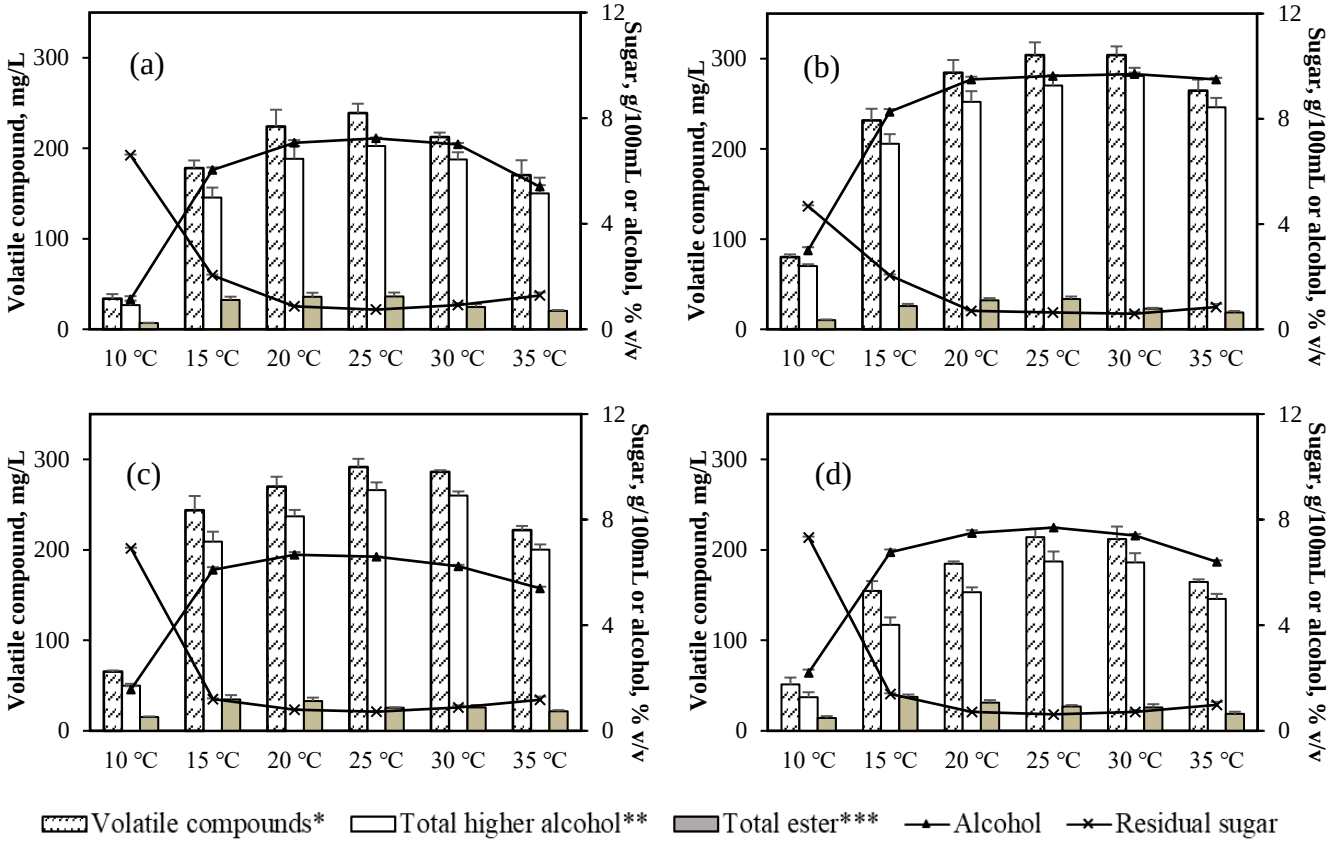


Figure 5.2 The effect of temperature on alcohol content, residual sugar, total higher alcohol, and total ester during fermentation of (a) apricot, (b) apple, (c) cherry, (d) pear

*: Major compounds in volatile compounds were ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate, isoamyl acetate, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol. **: Major compounds in total higher alcohol were 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol. ***: Major compounds in total ester were ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate and isoamyl acetate.

Figure 5.3 indicated the effect of temperature on the content of higher alcohols and esters such as ethyl acetate, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol. In most cases, the total higher alcohol reached the highest values at the temperature range of 25 ℃ – 30 ℃, whereas in the case of apricot, the highest content was observed at the temperature range of 20 ℃ – 30 ℃. The total higher alcohol content increased proportionally with the temperature from 10 ℃ to 25 ℃, except for apricot from 10 ℃ to 20 ℃, and then this trend turned to decrease. Our results were in agreement with the ones reported by Prusina and Herjavec (2008), Ramsay and Berry (1984), Reddy and Reddy (2011). The amount of 3-methyl-1-butanol

in fermented mashes from apricot, apple, cherry and pear increased from 13.50 mg/L to 103.05 mg/L, from 38.70 mg/L to 161.93 mg/L, from 22.08 mg/L to 117.87 mg/L and from 17.81 mg/L to 107.76 mg/L, respectively, as the temperature increased from 10 ℃ to 25 ℃. In contrast, as the fermentation temperature at 35 ℃, their 3-methyl-1-butanol content dropped to 83.81 mg/L, 145.78 mg/L, 100.28 mg/L and 85.80 mg/L, respectively. Likewise, in fermented mashes from apricot, apple, cherry and pear, 1-propanol rose from 4.67 mg/L, 4.51 mg/L, 5.35 mg/L and 7.56 mg/L to 25.18 mg/L, 30.71 mg/L, 32.08 mg/L and 35.56 mg/L, then reduced to 17.88 mg/L, 30.73 mg/L, 20.36 mg/L and 21.96 mg/L, respectively, corresponding to 10 ℃, 25 ℃ and 35 ℃.

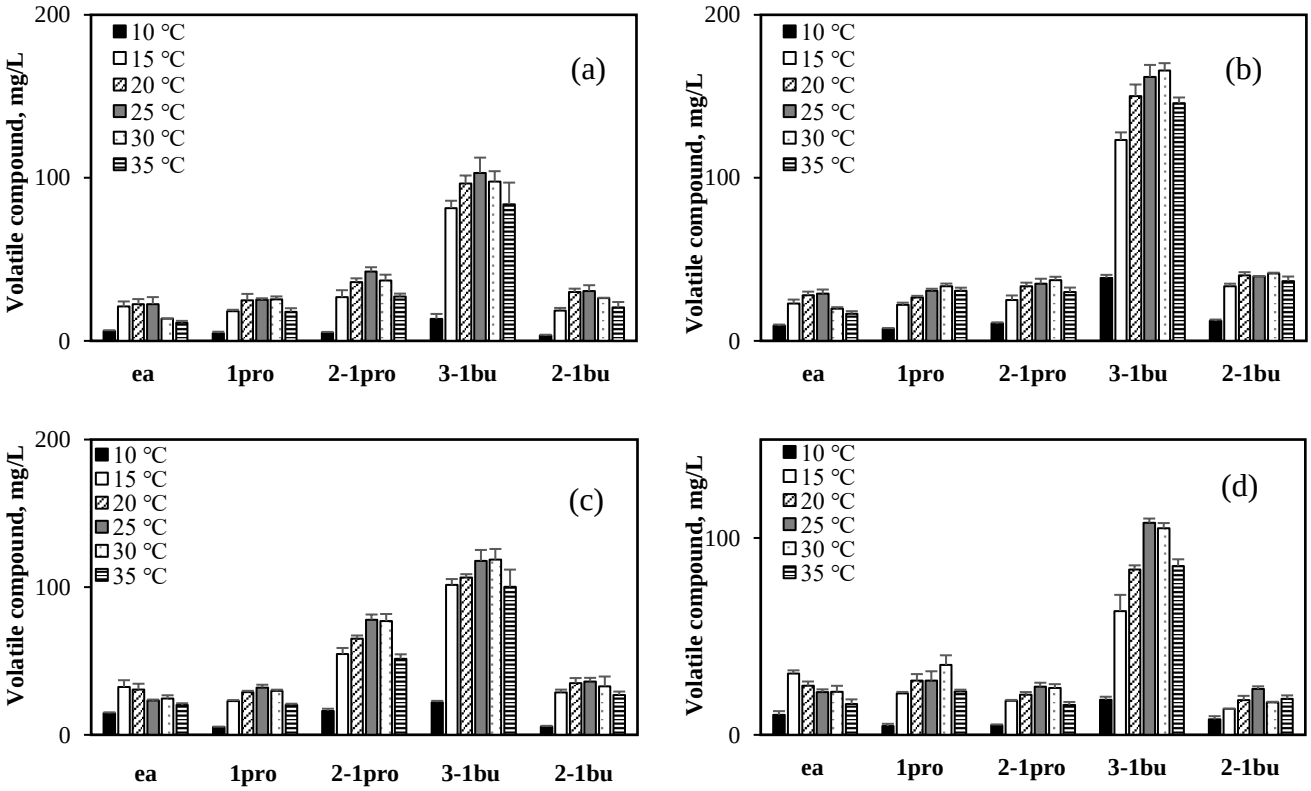


Figure 5.3 The effect of temperature on formation of main volatile compounds during spirits fermentation from (a) apricot, (b) apple, (c) cherry, (d) pear

ea: ethyl acetate, 1pro: 1-propanol, 2-1pro: 2-methyl-1-propanol, 3-1bu: 3-methyl-1-butanol, 2-1bu: 2-methyl-1-butanol

Figures 5.2 showed that the total ester content sharply grew with the increase of fermentation temperature from 10 ℃ to 15 ℃, except for apple case, it raised strongly as the temperature rose from 10 ℃ to 20 ℃ (p-value < 0.05). Total ester content was the highest value at the temperature level of the range 15 ℃ – 20 ℃ for cherry and pear, the range 20 ℃ – 25 ℃ for apple and the range 15 ℃ – 25 ℃ for apricot. The decrease in total ester was observed when the fermentation temperature was over 20 ℃ for cherry and pear and as it was over 25 ℃ for

apricot and apple (p-value < 0.05). In the cases of temperatures of 10 ℃, 15 ℃ and 35 ℃, the ethyl acetate in fermented mashes from apricot, apple, cherry and pear raised from 6.11 mg/L, 9.37 mg/L, 14.55 mg/L and 10.18 mg/L to 21.08 mg/L, 22.93 mg/L, 32.40 mg/L and 31.17 mg/L then declined to 11.12 mg/L, 16.67 mg/L, 20.29 mg/L and 15.72 mg/L, respectively. Many studies indicated that within certain limits, the increase in temperature resulted in lower total ester content (Erten, 2002, Kourkoutas *et al.*, 2003, Molina *et al.*, 2007, Reddy and Reddy, 2011). Mallouchos *et al.* (2003) studied the formation of volatiles in wine fermentation at different temperatures (10 ℃, 15 ℃ and 20 ℃), and they showed the increase in total esters content of both immobilized and free cells in connection with the fermentation temperature. Ramsay and Berry (1984) also reported a similar trend when studying the effect of temperature and pH on the formation of higher alcohols, fatty acids and esters in the malt whisky fermentation. Higher ethyl acetate content was reported at 25 ℃ comparing to at 10 ℃ when fermentation of fruit juice with *S. cerevisiae* (Bilbao *et al.*, 1997). Torija *et al.* (2003a) reported that total ester production at 25 ℃ was higher than at 13 ℃ in the case of *S. cerevisiae* A strain. However, most results presented that the temperature around 20 ℃ was proper for alcohol fermentation with a high aroma compound level and an excellent sensory score (Moreno *et al.*, 1988, Peng *et al.*, 2015, Prusina and Herjavec, 2008, Reddy and Reddy, 2011).

In general, the alcohol content at 20 ℃ and 25 ℃ was higher than that at 15 ℃. In addition, the total volatile compounds in fermented mashes from these fruits were highest in the range of 20 ℃ – 30 ℃, except for pear in the range of 25 ℃ – 30 ℃. Therefore, the fermentation temperature range of 15 ℃ – 25 ℃ was suitable for input on the RSM algorithm of the optimization process.

5.2.2 Effect of pH

Almost *S. cerevisiae* strains were acidophilic organisms and grew well under pH conditions between pH 2.50 and pH 8.50, but the optimal pH range could change depending on temperature, oxygen, substrate concentration (Liu *et al.*, 2015). In my work, initial pHs of pH 2.5, pH 2.75, pH 3.0, pH 3.5 and pH 4.0 were used to find suitable pH and range input for optimization of fruit spirit fermentation from cherry, apricot, pear and apple juices, respectively, alcohol content and residual sugar were determined after fermentation with yeast strain Uvaferm Danstil A for 8 days (Figure 5.4 and 5.5).

The ability of ethanol production varied slightly with the pH change of fruit juices tested. As shown in Figure 5.4, in most cases, the ethanol amount of all fermented mashes at pH 2.75, pH 3.0, pH 3.5 and pH 4.0 was almost the same (p-value > 0.05), except for apricot at pH 2.75 and

pear at pH 4.5, but vitally different from that at pH 2.5 level (p-value < 0.05). The alcohol amount of fermented juice from apricot, apple, cherry and pear at pH 3.0 level accounted for 7.60 % v/v, 9.50 % v/v, 6.70 % v/v and 7.50 % v/v.

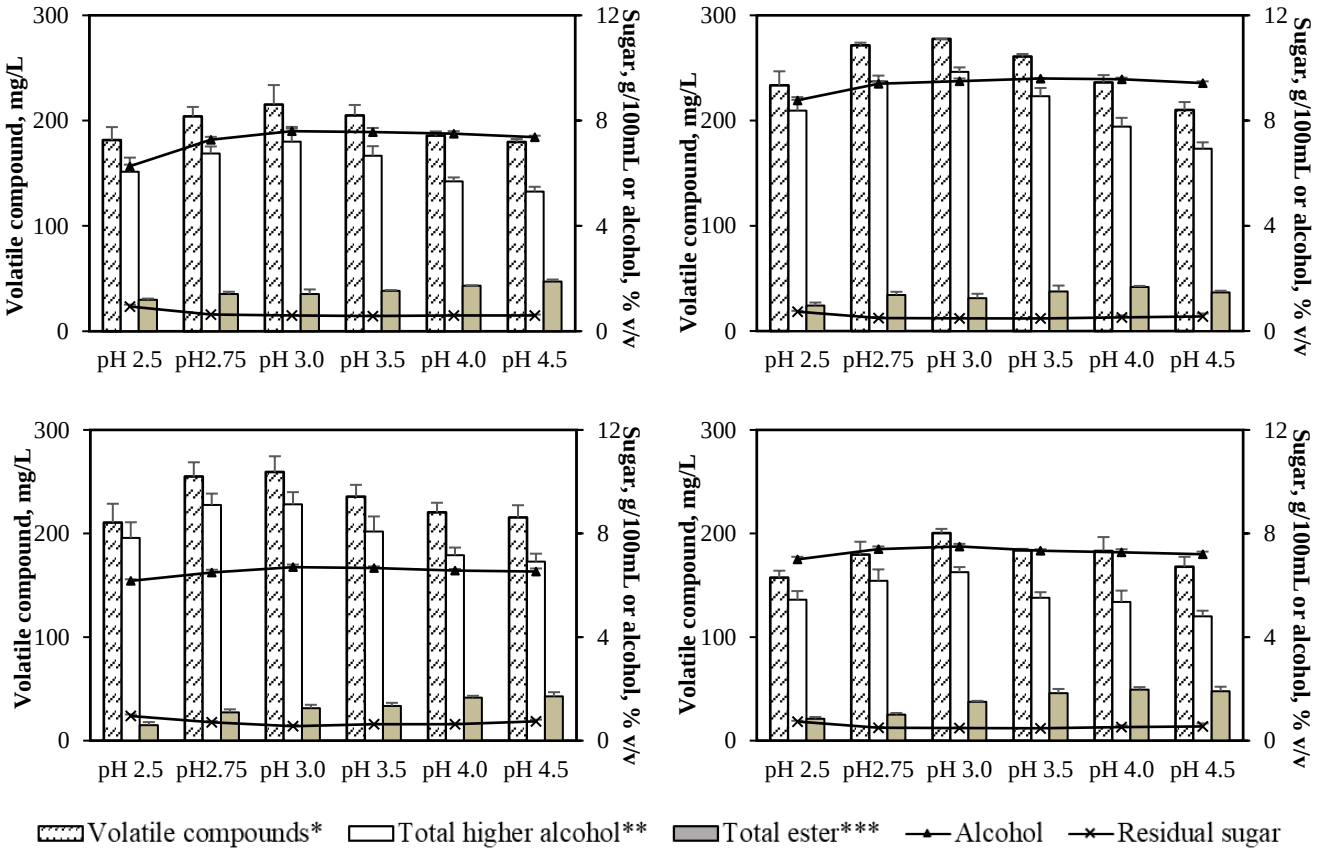


Figure 5.4 The effect of pH on alcohol content, residual sugar, total higher alcohol, and total ester during spirits fermentation from (a) apricot, (b) apple, (c) cherry, (d) pear

*: Major compounds in volatile compounds were ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate, isoamyl acetate, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol. **: Major compounds in total higher alcohol were 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol. ***: Major compounds in total ester were ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate and isoamyl acetate.

In the malt whisky fermentation, altering the initial pH of the wort from pH 4.0 to pH 7.0 made total higher alcohols increasing slightly, while total esters peaked as initial pH range of pH 5.0 – 6.0 (Ramsay and Berry, 1984). In our results, the change of pH from pH 2.5 to pH 2.75 resulted in the increase in total higher alcohol and total ester contents (p-value < 0.05), except for the ester case of pear, pH from pH 2.5 to pH 3.0. Total higher alcohol tended to decrease at pH of over pH 3.5, except for apple and pear at pH of over pH 3.0 (p-value < 0.05). In contrast, in the case of apricot and cherry, total ester contents still raised as pH was over 3.5, and it rose at pH of

over pH 3.0 in the case of pear. At this pH, the total esters content is relatively lower than the total higher alcohols. A similar trend was found in fermenting durian wine (Lu *et al.*, 2017). While more higher alcohols were produced at pH 3.1 than at pH 3.9, whereas much more esters were formatted at pH 3.9 than at 3.1. In the study of (García-Llobodanin *et al.*, 2010), the amount of total higher alcohols in the heart fractions in pH adjusted case (pH 3.27) was higher than the amount in pH native case (pH 4.1), but the total ester was lower. The content of total volatile compounds in fermented apricot, apple, cherry and pear mashes at pH 3.0 reached 251.44 mg/L, 277.46 mg/L, 259.25 mg/L and 200.31 mg/L, respectively.

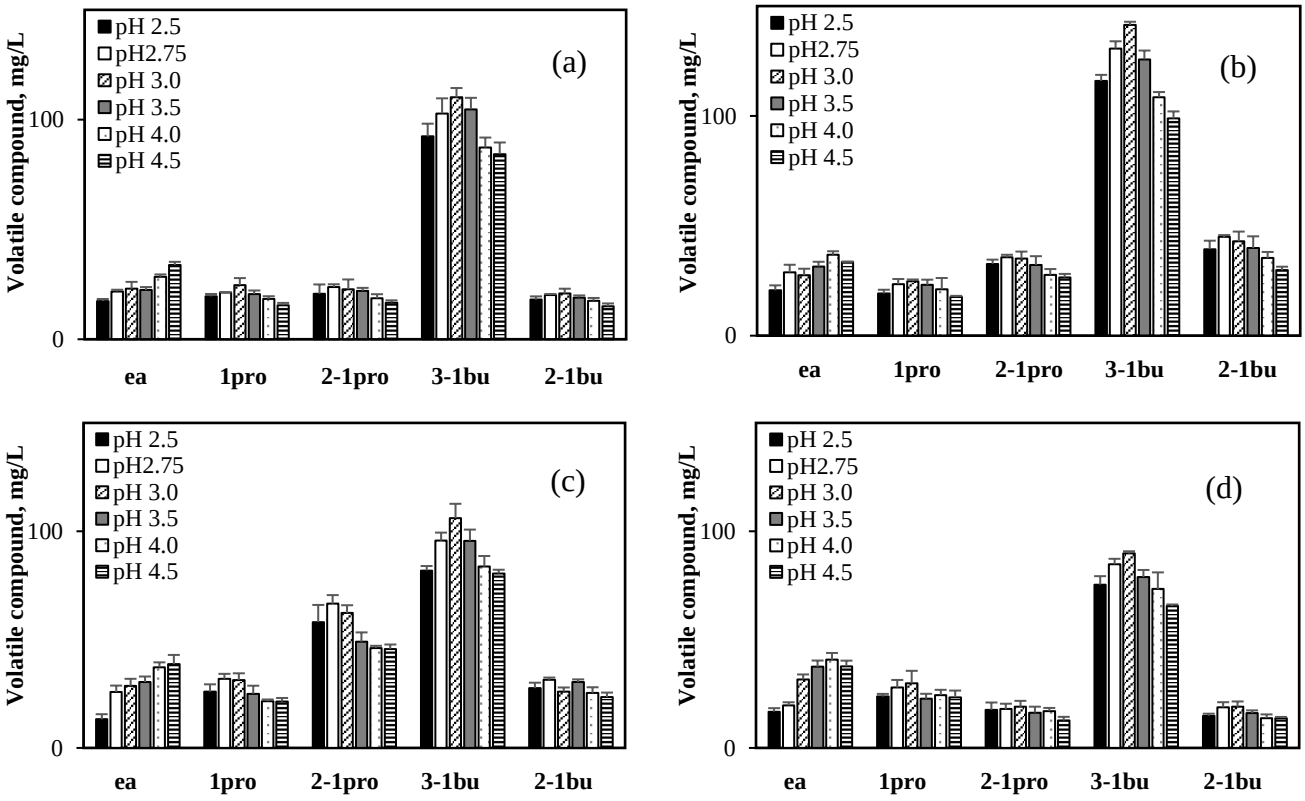


Figure 5.5 The effect of pH on formation of main volatile compounds during spirits fermentation from (a) apricot, (b) apple, (c) cherry, (d) pear

ea: ethyl acetate, 1pro: 1-propanol, 2-1pro: 2-methyl-1-propanol, 3-1bu: 3-methyl-1-butanol, 2-1bu: 2-methyl-1-butanol

Meanwhile, the changes of total higher alcohol content are mainly due to changes in the content of 3-methyl-1-butanol, 2-methyl-1-butanol, 1-propanol, 2-methyl-1-propanol, whereas the total ester variation was from changes in ethyl acetate content (Figure 5.5). Generally, ethyl acetate peaked at pH 3.5 – 4.5, except for apricot, the highest value at only pH 4.5. The ethyl acetate content in fermented apricot, apple, cherry and pear mash at pH 4.5 was 38.80 mg/L, 33.61 mg/L, 38.67 mg/L and 37.71 mg/L, respectively. Additionally, in most cases, the highest 3-methyl-1-

butanol concentration was observed in pH of 2.75 – 3.5, except for pear in the range of pH 2.75 – 3.0 and apple in only pH 3.0. The 3-methyl-1-butanol value in fermented apricot, apple, cherry and pear mash at pH 3.0 was 110.16 mg/L, 141.43 mg/L, 106.00 mg/L and 89.76 mg/L, respectively.

Environmental pH affects the yeast cell wall structure, especially transporting substances throughout the membrane by proteins in the plasma membrane, affecting the yeast growth rate and fermentation products (Narendranath and Power, 2005, Reddy and Reddy, 2011). In all tested fermented fruit juices at pH 2.5, the amount of residual sugar was higher than other pHs; and the low content of both alcohol and volatile compounds is recorded (p-value < 0.05). The low initial pH level caused a fall in yeast internal pH and inhibition of enzymes. It might be the probable reason for low ethanol and volatile compounds at low pH of 2.5. Thus, when the pH increases from pH 2.5 to pH 3.0, the pH gradually changes towards beneficial to the yeast activity, resulting in both the alcohol content and the volatile compounds increasing.

Finally, the pH range of pH 2.75 – pH 3.75 was suitable for input on the RSM algorithm of the optimization process.

5.2.3 Effect of initial soluble solid contents

To find a suitable range of initial sugar content input for optimization of spirit fermentation of cherry, apricot, pear and apple juice, the experiments were designed with various initial soluble solid contents changed at 12 °Brix, 18 °Brix, 24 °Brix, 30 °Brix and 36 °Brix, respectively.

Changes in the concentration of alcoholic and volatile compounds as well as of respective soluble solids content were obtained (Figure 5.6). The initial soluble solid contents affected ethanol and aroma compound production, which was found in all fruit juices tested. An increase in initial soluble solid content from 12 °Brix to 30°Brix had a positive effect on alcohol and volatile compound production. However, they reduced considerably if total soluble solid content in the medium increasing up to 36 °Brix. In most cases, the maximum ethanol was found at 30 °Brix levels; inhere, the ethanol content from fermented apple mashes peaked at both 24 °Brix and 30 °Brix levels. The highest alcohol contents in fermented fruit from apricot, apple, cherry and pear were recorded 10.47 % v/v, 14.17 % v/v, 11.0 % v/v and 12.10 % v/v, respectively. Total volatile compounds reached the highest amount when fermentation of apple and cherry mashes at 30 °Brix level, while it was found in the cases of apricot and pear mashes at both 24 °Brix and 30 °Brix levels. The concentration of the total volatile compounds in apricot, apple, cherry and pear at 30 °Brix were 295.86 mg/L, 379.34 mg/L, 375.48 mg/L and 275.43 mg/L, respectively.

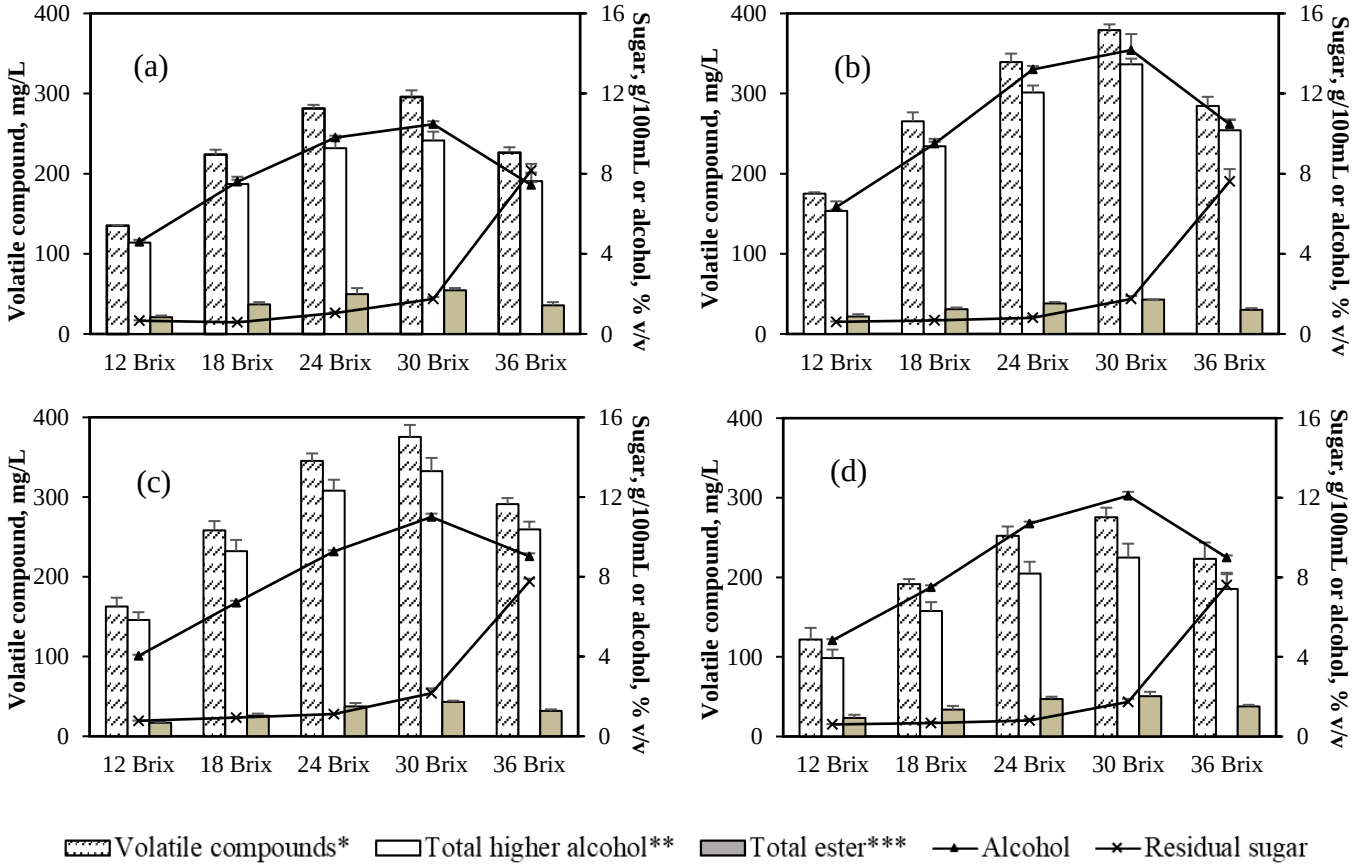


Figure 5.6 The effect of initial soluble solids content on formation of alcohol, residual sugar, total higher alcohol and total ester during fermentation from (a) apricot, (b) apple, (c) cherry, (d) pear

*: Major compounds in volatile compounds were ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate, isoamyl acetate, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol. **: Major compounds in total higher alcohol were 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2 phenylethanol. ***: Major compounds in total ester were ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate and isoamyl acetate.

Generally, the changes of concentration of individual aroma compounds tended similarly to the case of alcohol in different Brix, including ethyl acetate, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol. Ethyl acetate, 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol peaked 25.72 mg/L, 29.18 mg/L, 37.64 mg/L, 132.50 mg/L and 35.28 mg/L in fermented apricot juice, respectively; 36.81 mg/L, 25.06 mg/L, 39.43 mg/L, 198.43 mg/L and 70.96 mg/L in fermented apple juice, respectively, 24.52 mg/L, 32.08 mg/L, 77.97 mg/L, 118.64 mg/L and 36.17 mg/L in fermented cherry juice, respectively, and 37.66 mg/L, 41.30 mg/L, 21.81 mg/L, 116.47 mg/L and 34.32 mg/L, in fermented pear juice, respectively.

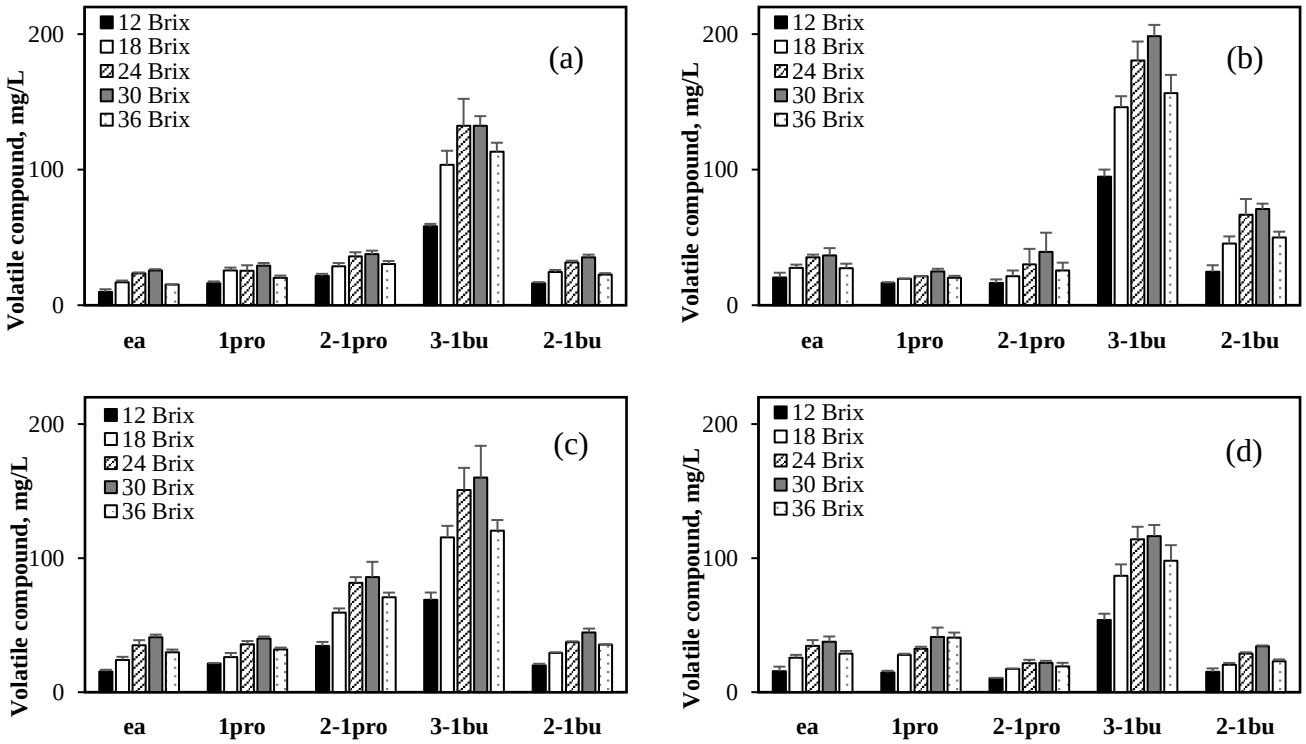


Figure 5.7 The effect of initial soluble solids content on formation of main volatile compounds during fermentation from (a) apricot, (b) apple, (c) cherry, (d) pear

ea: ethyl acetate, 1pro: 1-propanol, 2-1pro: 2-methyl-1-propanol, 3-1bu: 3-methyl-1-butanol, 2-1bu: 2-methyl-1-butanol

In most cases, the residual sugar content did not significantly different as the soluble solid content ranges from 12 °Brix and 24 °Brix, except for apricot of 12 °Brix – 18 °Brix (p-value > 0.05), but initial solid content of over 24 °Brix caused higher the residual sugar concentration after fermentation (p-value < 0.05). The results suggested that the high soluble solid content might inhibit the yeast activity. The higher sugar concentration in the fermentation medium might exert severe osmotic stress on the yeast cells, which could negatively compromise essential cell functions (Narendranath and Power, 2005). Accordingly, the initial soluble solid content of lower than 30 °Brix was appropriate for alcohol fermentation, thus the range of 18 °Brix – 30 °Brix was selected for the RSM algorithm of the optimization process.

5.2.4 Optimization of some fermentation factors

Based on preliminary experiments, the RSM with three variables: temperature (15 ℃ – 25 ℃), pH (2.75 – 3.75) and total soluble solid content (18 °Brix – 30 °Brix) was applied. The polynomial quadratic equations giving the alcohol production yield ($Y_{P/S}$ is a percentage of the amount of alcohol produced by sugar used) and the volatile compound production yield ($Y_{VC/S}$ is

a percentage of the amount of total volatile compounds produced by sugar used) by the types of fruit are presented in Table 5.3. For each equation, variants of fermentation temperature (X_1, ℃), pH (X_2, pH) and total soluble solids content (X_3, °Brix) was included in the function.

In my work, multiple regressions of the experimental data were applied to find the second-order polynomial equations representing the ethanol and main aromas production. Full predictive equations for optimization of fruit alcoholic fermentation were given below (Eq. 1-8).

Apricot juice:

$$Y_{1\ P/S} = 69.09 + 4.47*X_1 + 2.53*X_2 - 5.40*X_3 - 4.91*X_1^2 - 2.37*X_2^2 - 3.44*X_3^2 + 1.46*X_1*X_2 - 0.77*X_1*X_3 - 0.72*X_2*X_3 \quad (Eq.1)$$

$$Y_{2\ CV/S} = 1997.1 + 169.38*X_1 + 34.66*X_2 - 42.57*X_3 - 127.24*X_1^2 - 82.01*X_2^2 - 190.51*X_3^2 - 21.39*X_1*X_2 - 49.65*X_1*X_3 - 2.01*X_2*X_3 \quad (Eq.2)$$

Apple juice:

$$Y_{3\ P/S} = 69.68 + 5.34*X_1 + 1.96*X_2 - 3.10*X_3 - 2.69*X_1^2 - 3.12*X_2^2 - 4.83*X_3^2 + 0.48*X_1*X_2 + 1.42*X_1*X_3 + 1.03*X_2*X_3 \quad (Eq.3)$$

$$Y_{4\ VC/S} = 1797.83 + 171.98*X_1 - 83.58*X_2 - 127.91*X_3 - 86.94*X_1^2 - 140.33*X_2^2 - 22.82*X_3^2 + 9.56*X_1*X_2 - 28.22*X_1*X_3 + 3.2*X_2*X_3 \quad (Eq.4)$$

Cherry juice:

$$Y_{5\ P/S} = 57.91 + 4.11*X_1 + 0.97*X_2 - 2.61*X_3 - 2.87*X_1^2 - 1.63*X_2^2 - 1.98*X_3^2 + 1.01*X_1*X_2 + 0.31*X_1*X_3 + 0.1*X_2*X_3 \quad (Eq.5)$$

$$Y_{6\ VC/S} = 2153.09 + 103.64*X_1 - 76.26*X_2 - 24.30*X_3 - 20.44*X_1^2 - 116.49*X_2^2 - 198.54*X_3^2 + 6.88*X_1*X_2 - 26.22*X_1*X_2 + 8.54*X_2*X_3 \quad (Eq.6)$$

Pear juice:

$$Y_{7\ P/S} = 78.37 + 5.3*X_1 + 2.67*X_2 - 3.95*X_3 - 6.39*X_1^2 - 4.97*X_2^2 - 5.93*X_3^2 + 0.68*X_1*X_2 + 1.89*X_1*X_3 + 1.34*X_2*X_3 \quad (Eq.7)$$

$$Y_{8\ VC/S} = 1881.02 + 156.70*X_1 + 58.39*X_2 - 53.19*X_3 - 0.38*X_1^2 - 106.56*X_2^2 - 126.56*X_3^2 - 4.74*X_1*X_2 - 45.37*X_1*X_3 - 9.09*X_2*X_3 \quad (Eq.8)$$

ANOVA analysis results in Table 5.4 showed these second-order regressions were statistically significant (all $p_{ANOVA} < 0.05$). An insignificant difference in p-values of the lack of fit ($p_{LOF} > 0.05$) indicated these models are sufficiently accurate for predicting the responses in the production yield of alcohol and volatile compound. Besides, all coefficient of determination values (R^2) for all response variables were higher than 0.85, which the value was considered sufficiently

good (Duarte *et al.*, 2011). Adjusted R^2 values of the three dependent variables in the production yield of alcohol and volatile compound were 0.95 and 0.94, 0.97 and 0.94, 0.96 and 0.99, 0.95 and 0.99 in the case of apricot, apple, cherry and pear, respectively. The adjusted determination coefficient values were high and closed to the determination coefficient (Table 5.4), which indicated the high significance of the models. Also, the predictive powers Q^2 of all models from the dependent variables being higher than 0.8 (except for alcohol production yield in the case of apricot was 0.73) revealed a perfect model with good predictive power.

The regression coefficients, including three linear, three quadratic, three interaction terms, and one block term, were listed in Table 5.4. The effects of temperature, pH and total soluble solids content of the fermentation process on the production of ethanol and aroma compounds were found to be significant (at least p-values of linear coefficient and quadratic coefficient or both of them was lower than 0.05). They were shown through the model terms, X_1, X_2, X_3, X_1^2, X_2^2 and X_3^2 with among their respective probability of 95 % (Table 5.4). Moreover, the quadratic response 3D surface plots in Figure 5.8 – 5.11 illustrate clearly the optimization model of fermentation temperature, pH and initial soluble solid content for the production yield of alcohol and volatile compounds from these fruit juices.

Many studies have pointed out that higher alcohols and esters make an essential and a positive contribution to the quality of alcohol fermentation products (Douady *et al.*, 2019, Duarte *et al.*, 2011, Moreno *et al.*, 1988, Spaho, 2017). Therefore, in the optimization process, the production yield of alcohol and volatile compounds were aimed to be maximized in order to increase flavors for the product of the final spirit. After optimizing with the full regression models (Eq.1-8), for fermentation of apricot, apple, cherry and pear juices, the optimal conditions of temperature, pH and soluble solid content were determined to be 23.02 ℃, pH 3.50 and 20.94 °Brix; 24.66 ℃, pH 3.25 and 21.28 °Brix; 24.71 ℃, pH 3.25 and 22.49 °Brix; 24.33 ℃, pH 3.42 and 21.95 °Brix, respectively. Additionally, predicted values of the responses were calculated. Predicted values of alcohol and volatile compounds' production yield were 73.38 (8.98 % v/v) and 2031.64 (248.66 mg/L) for apricot, 72.20 (12.10 % v/v) and 1947.76 (326.39 mg/L) in the case of apple, 59.68 (9.02 % v/v) and 2231.68 (337.37 mg/L) in the case of cherry, 78.63 (10.12 % v/v) and 2039.77 (262.60 mg/L) in the case of pear (Table 5.5).

Table 5.3 Central composite design matrix and results obtained

	Temperature (℃)	pH	Total soluble solid (°Brix)	Apricot		Apple		Cherry		Pear	
				Alcohol yield	Volatile compound yield	Alcohol yield	Volatile compound yield	Alcohol yield	Volatile compound yield	Alcohol yield	Volatile compound yield
Run	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y5	Y_6	Y_7	Y_8
1	15	2.75	18	56.27	1373.99	58.08	1547.58	50.7	1801.08	61.88	1426.74
2	25	2.75	18	65.30	1799.18	64.89	1962.20	56.48	2035.88	66.93	1833.04
3	15	3.75	18	61.12	1465.35	59.25	1373.33	50.7	1605.58	63.46	1564.97
4	25	3.75	18	72.14	1890.54	66.78	1816.96	59.79	1895.42	69.77	1971.27
5	15	2.75	30	50.76	1366.33	47.40	1354.70	44.47	1789.65	47.17	1427.02
6	25	2.75	30	52.85	1678.47	58.69	1647.24	50.75	1947.09	58.34	1670.80
7	15	3.75	30	48.86	1535.20	51.49	1184.02	44.14	1655.82	52.66	1547.84
8	25	3.75	30	60.64	1676.25	65.88	1524.00	55.22	1813.25	68.00	1753.73
9	15	3.25	24	58.31	1671.47	59.78	1582.33	50.22	2032.79	64.64	1719.37
10	25	3.25	24	69.08	2061.65	74.06	1811.36	59.1	2229.64	79.79	2024.09
11	20	2.75	24	62.38	1913.17	63.84	1754.65	54.76	2114.66	70.09	1713.71
12	20	3.75	24	70.08	1910.40	69.13	1532.26	57.04	1955.67	77.19	1817.38
13	20	3.25	18	71.29	1879.75	67.48	1905.45	57.04	2008.55	74.51	1812.85
14	20	3.25	30	59.03	1726.81	62.07	1616.48	54.06	1897.68	70.84	1677.62
15	20	3.25	24	69.79	1927.98	69.83	1854.96	58.48	2149.30	78.14	1915.79
16	20	3.25	24	70.36	2002.03	70.18	1845.71	58.9	2140.80	78.61	1894.48
17	20	3.25	24	69.08	2074.53	69.30	1749.00	57.86	2174.91	77.43	1868.43

- Alcohol yield (%)= alcohol content/total sugar*100
- Volatile compound yield (%) = total volatile compound/total sugar*100
- Major compounds in total volatile compounds were ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate, isoamyl acetate, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol.

Table 5.4 Regression analysis based on ethanol and volatile compounds yield

	Apricot				Apple				Cherry				Pear			
	Alcohol		Volatile compound		Alcohol		Volatile compound		Alcohol		Volatile compound		Alcohol		Volatile compound	
	Coeff. SC	P	Coeff. SC	P	Coeff. SC	P	Coeff. SC	P	Coeff. SC	P	Coeff. SC	P	Coeff. SC	P	Coeff. SC	P
Constant	69.09	***	1997.10	***	69.68	***	1797.83	***	57.91	***	2153.09	***	78.37	***	1881.02	***
X_1	4.47	***	169.38	***	5.43	***	171.98	***	4.11	***	103.64	***	5.30	***	156.70	***
X_2	2.53	**	34.66	ns	1.96	**	-83.58	**	0.97	*	-76.26	***	2.67	**	58.39	***
X_3	-5.40	***	-42.57	*	-3.10	***	-127.91	***	-2.61	***	-24.30	*	-3.95	***	-53.19	***
X_1^2	-4.91	**	-127.24	**	-2.69	**	-86.94	*	-2.87	**	-20.44	ns	-6.39	**	-0.38	ns
X_2^2	-2.37	ns	-82.01	*	-3.12	**	-140.33	**	-1.63	*	-116.49	***	-4.97	**	-106.56	***
X_3^2	-3.44	*	-190.51	***	-4.83	***	-22.82	ns	-1.98	*	-198.54	***	-5.93	**	-126.87	***
X_1*X_2	1.46	*	-21.39	ns	0.48	ns	9.56	ns	1.01	*	6.88	ns	0.68	ns	-4.74	ns
X_1*X_3	-0.77	ns	-49.65	*	1.42	*	-28.22	ns	0.31	ns	-26.22	*	1.89	*	-45.37	***
X_2*X_3	-0.72	ns	-2.01	ns	1.03	*	3.20	ns	0.10	ns	8.54	ns	1.34	ns	-9.09	ns
N = 17	Q^2	0.73	Q^2	0.80	Q^2	0.89	Q^2	0.85	Q^2	0.889	Q^2	0.942	Q^2	0.83	Q^2	0.97
DF = 7	R^2	0.98	R^2	0.97	R^2	0.99	R^2	0.97	R^2	0.984	R^2	0.993	R^2	0.98	R^2	0.99
	$R^2_{Adj.}$	0.95	$R^2_{Adj.}$	0.94	$R^2_{Adj.}$	0.97	$R^2_{Adj.}$	0.94	$R^2_{Adj.}$	0.964	$R^2_{Adj.}$	0.985	$R^2_{Adj.}$	0.95	$R^2_{Adj.}$	0.99
	RSD	1.64	RSD	56.44	RSD	1.20	RSD	53.61	RSD	0.926	RSD	22.52	RSD	2.02	RSD	20.42
	p_{ANOVA}	<0.05	p_{ANOVA}	<0.05	p_{ANOVA}	<0.05	p_{ANOVA}	<0.05	p_{ANOVA}	<0.05	p_{ANOVA}	<0.05	p_{ANOVA}	<0.05	p_{ANOVA}	<0.05
	p_{LOF}	>0.05	p_{LOF}	>0.05	p_{LOF}	>0.05	p_{LOF}	>0.05	p_{LOF}	>0.05	p_{LOF}	>0.05	p_{LOF}	>0.05	p_{LOF}	>0.05

Sig.: significance (*, **, ***) - display the significance at 0.05, 0.01 and 0.001 by least significant difference.
"ns": not significant.

Table 5.5 Experimental and predicted values for dependent variables

	Variable	Apricot	Apple	Cherry	Pear
Optimum values	X_1, temperature (°C)	23.02	24.66	24.71	24.33
	X_2, pH	3.50	3.25	3.25	3.42
	X_3, total soluble solid (°Brix)	20.94	21.28	22.49	21.95
Predicted values	Alcohol production yield (%)	73.38	72.20	59.68	78.63
	Alcohol content, (% v/v)	8.98	12.10	9.02	10.12
	Volatile compound production yield (mg/L)	2031.64	1947.76	2231.68	2039.77
	Total volatile compound, (mg/L)	248.66	326.39	337.37	262.60
Experimental values	Alcohol production yield (%)	75.17	75.79	60.86	80.55
	Alcohol content, (% v/v)	9.20	12.70	9.20	10.37
	Volatile compound production yield(mg/L)	2097.34	2075.47	2339.75	2135.62
	Total volatile compound (mg/L)	257.70	347.79	353.71	274.94

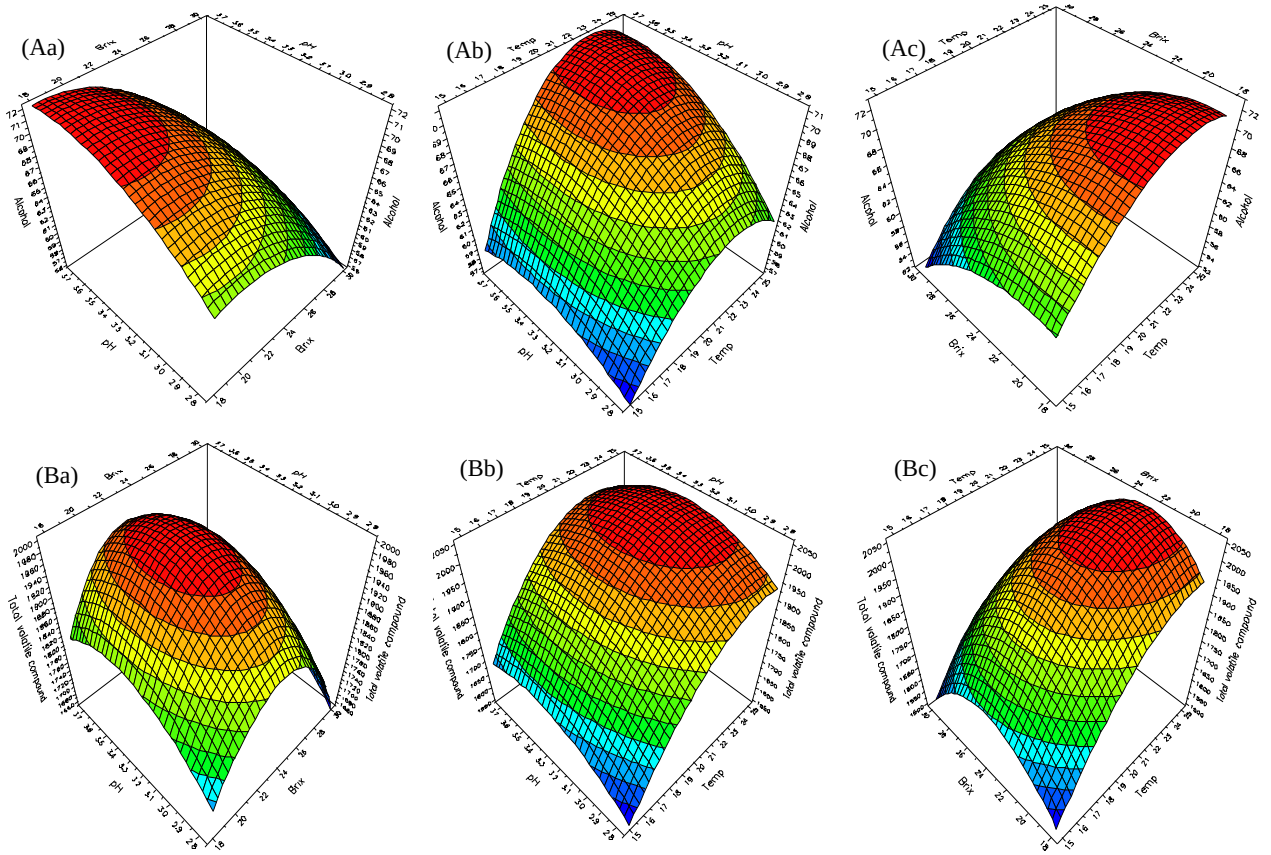


Figure 5.8 Response surface (a) pH vs. Brix, (b) pH vs. temperature and (c) Brix vs. temperature on alcohol (A) and volatile compounds (B) yield in the case of apricot juice

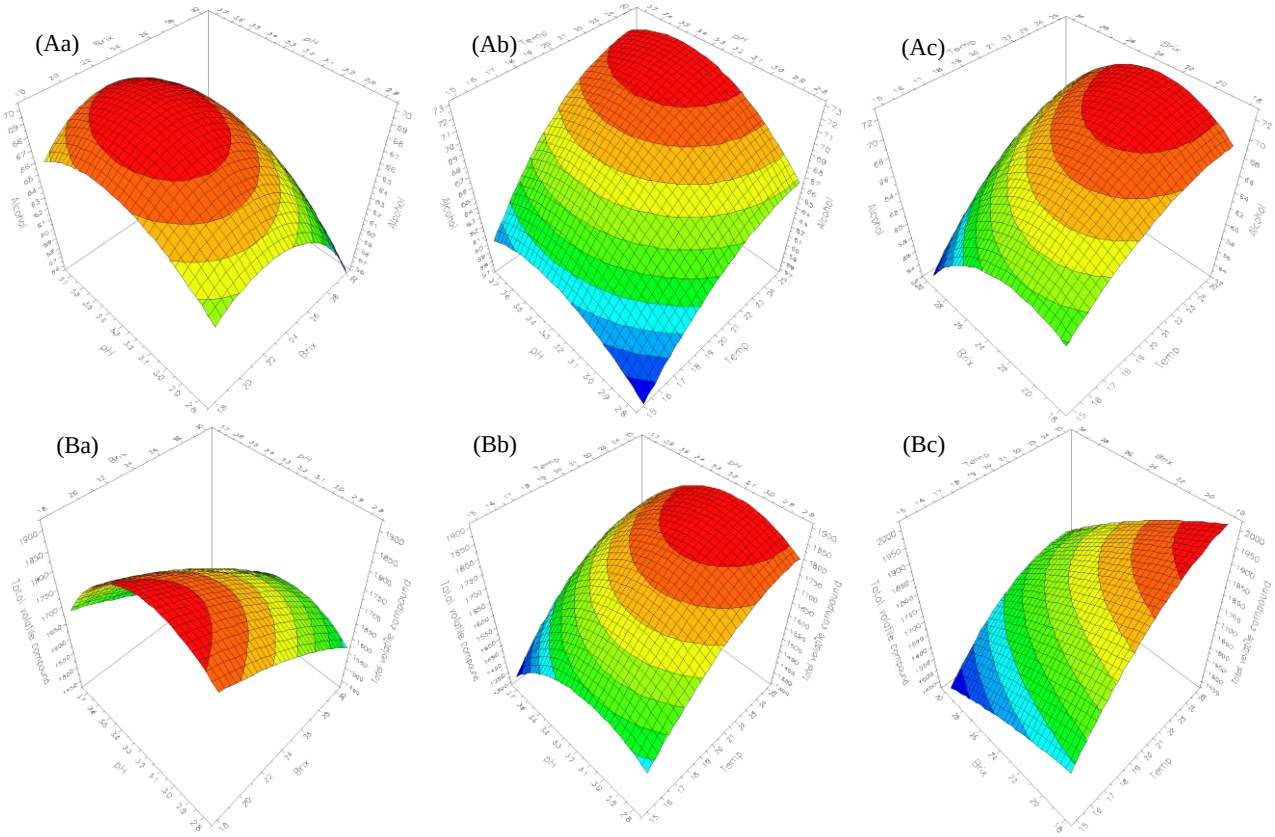


Figure 5.9 Response surface (a) pH vs. Brix, (b) pH vs. temperature and (c) Brix vs. temperature on alcohol (A) and volatile compounds (B) yield in the case of apple juice

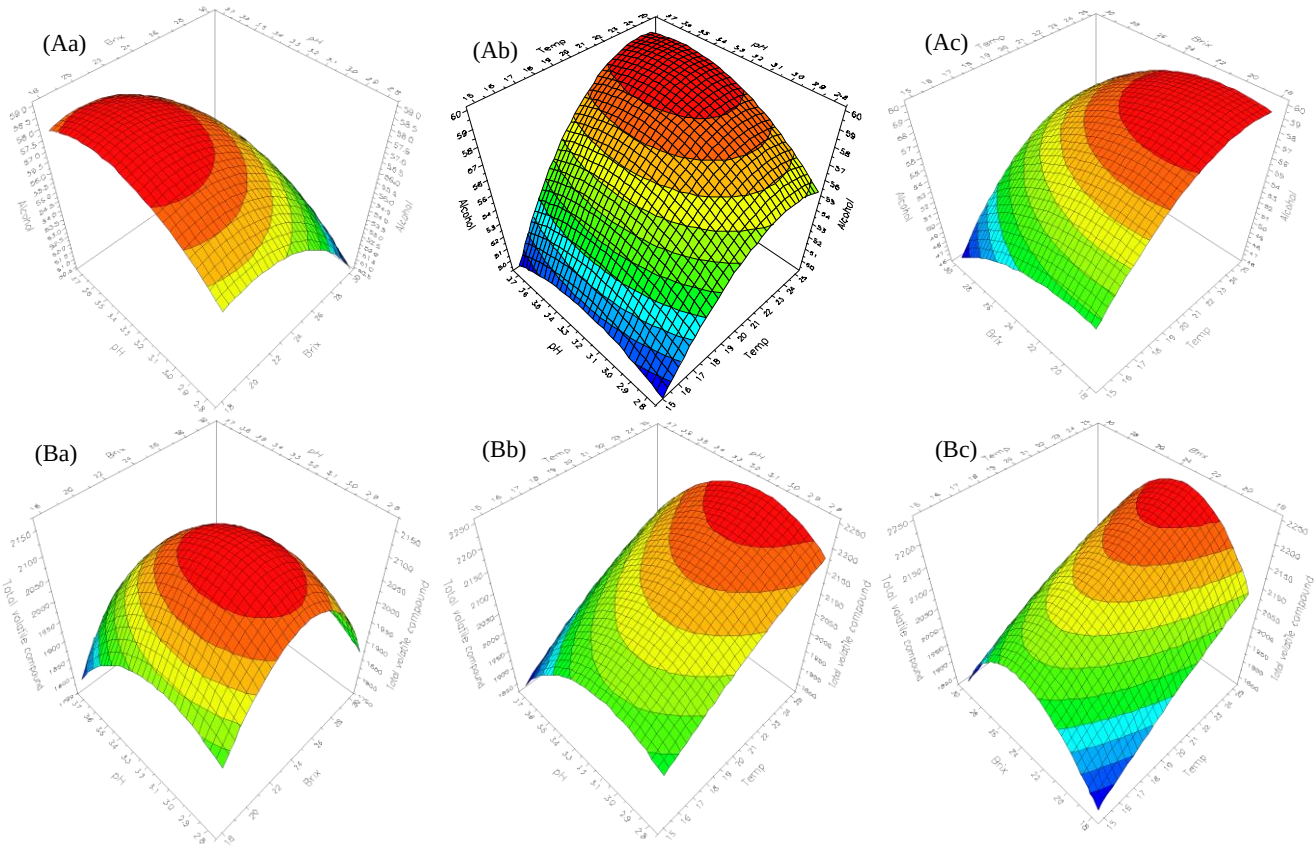


Figure 5.10 Response surface (a) pH vs. Brix, (b) pH vs. temperature and (c) Brix vs. temperature on alcohol (A) and volatile compounds (B) yield in the case of cherry juice

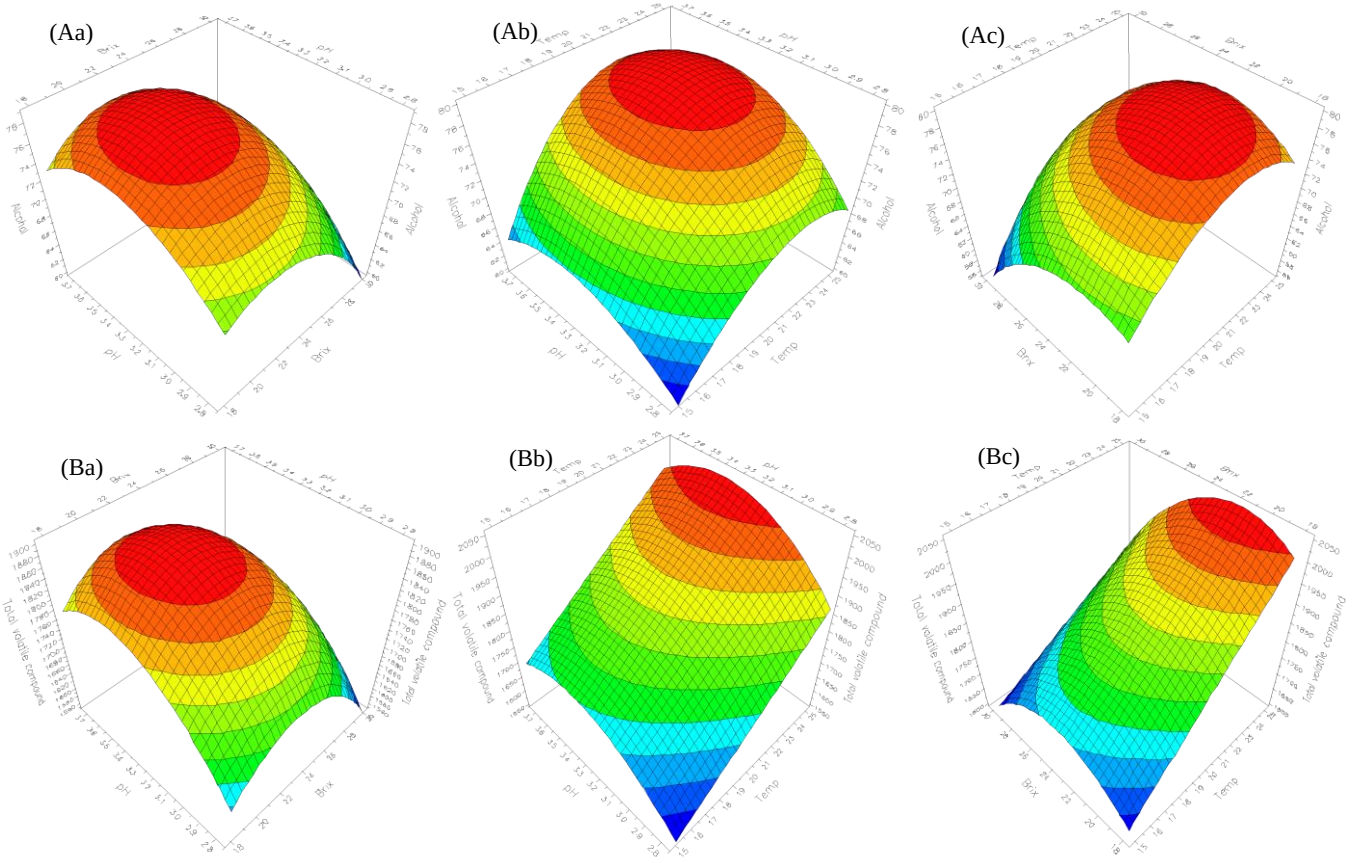


Figure 5.11 Response surface (a) pH vs. Brix, (b) pH vs. temperature and (c) Brix vs. temperature on alcohol (A) and volatile compounds (B) yield in the case of pear juice

To evaluate the estimated optimum values obtained, a confirmatory experiment was carried out by fermentation of the apricot juice under these optimum values. The experimental results illustrated in the table 5.5 were closed to the predicted values. It suggests that the optimization values have been reliable and should be applied in the fermentation of apricot, apple, cherry and pear juices.

5.3 Effects of distillation process on aromatic profile

5.3.1 Effects of distillation process on distribution of aroma compounds

The production process of fruit spirits goes through three crucial stages, namely fermentation, distillation and aging/maturation, which affect the formation and concentration of many aromatic compounds thus, finally, the quality of the beverage. Indeed, improper fermentation conditions cause severe sensory flavor defects for certain products. In addition, the quality of spirits also strongly depends on the distillation process; hence it controlled the separation of negative-effect aroma components from different parts of the distillate. It is important to understand the distribution of the essential compounds in order to find suitable cuts for the head, heart and tail fractions. It helps to eliminate or at least to reduce the harmful compounds. The distribution of the primary aromas during the distillation process is summarized in Figure 5.12.

Acetaldehyde has a lower boiling point than methanol (20.2 ℃ < 64.7 ℃), leading it to appear before methanol in the distillation process. Generally, in the distillates, acetaldehyde is mainly in high concentrations in the head fraction; thus, it might be applied as an indicator for the cut of the head/heart (Spaho, 2017, Zhao *et al.*, 2014). As a very light component, acetaldehyde can be completely soluble in both water and ethanol. It is too hard to separate acetaldehyde from the spirits, so small amounts of acetaldehyde might proceed to the heart fraction of the distillate and steadily decreases during the distillation process. The distribution of acetaldehyde in the second distillation is described in Figure 5.12. The presence of acetaldehyde concentration from apricot, apple, cherry and pear changed significantly in the distillation process and in the first fraction, its' content was up to 2072.13 mg/L, 2980.19 mg/L, 3580.34 mg/L and 1797.30 mg/L, respectively. Then, in the second fraction, it rapidly decreased to 218.48 mg/L, 464.54 mg/L, 500.84 mg/L and 234.98 mg/L, respectively (p-value < 0.05). Finally, in the sixth fraction, it went steadily down 0.87 mg/L, 5.4 mg/L, 2.93 mg/L and 3.41 mg/L, respectively. From the seventh fraction, it was not detected in distillates. The distribution trend of acetaldehyde is similar to the results of Claus and Berglund (2005). In comparison with the classification of volatile compounds

mentioned by Douady *et al.* (2019), the distribution of acetaldehyde in all these cases similarly belonged to type 1.

Although, ethyl acetate is a compound with a boiling point of 77.1 ℃. It is similar in distribution to acetaldehyde (Claus and Berglund, 2005), which means it also belonged to type 1 (Douady *et al.*, 2019). The distribution of ethyl acetate during distillation is depicted in Figure 5.12. Likewise, during second distillation in the case of apricot, apple, cherry and pear distillates, ethyl acetate concentration reached the maximum value in the first fraction with 2549.61 mg/L, 3789.38 mg/L, 2799.48 mg/L and 2760.97 mg/L, respectively. Then, in the second fraction of distillate, it fell significantly to 314.52 mg/L, 316.67 mg/L, 380.03 mg/L and 339.95 mg/L, respectively (p-value < 0.05). Eventually, from the 8th fraction, except for in the case of apricot and apple from the 9th fraction, it reduced steadily to null.

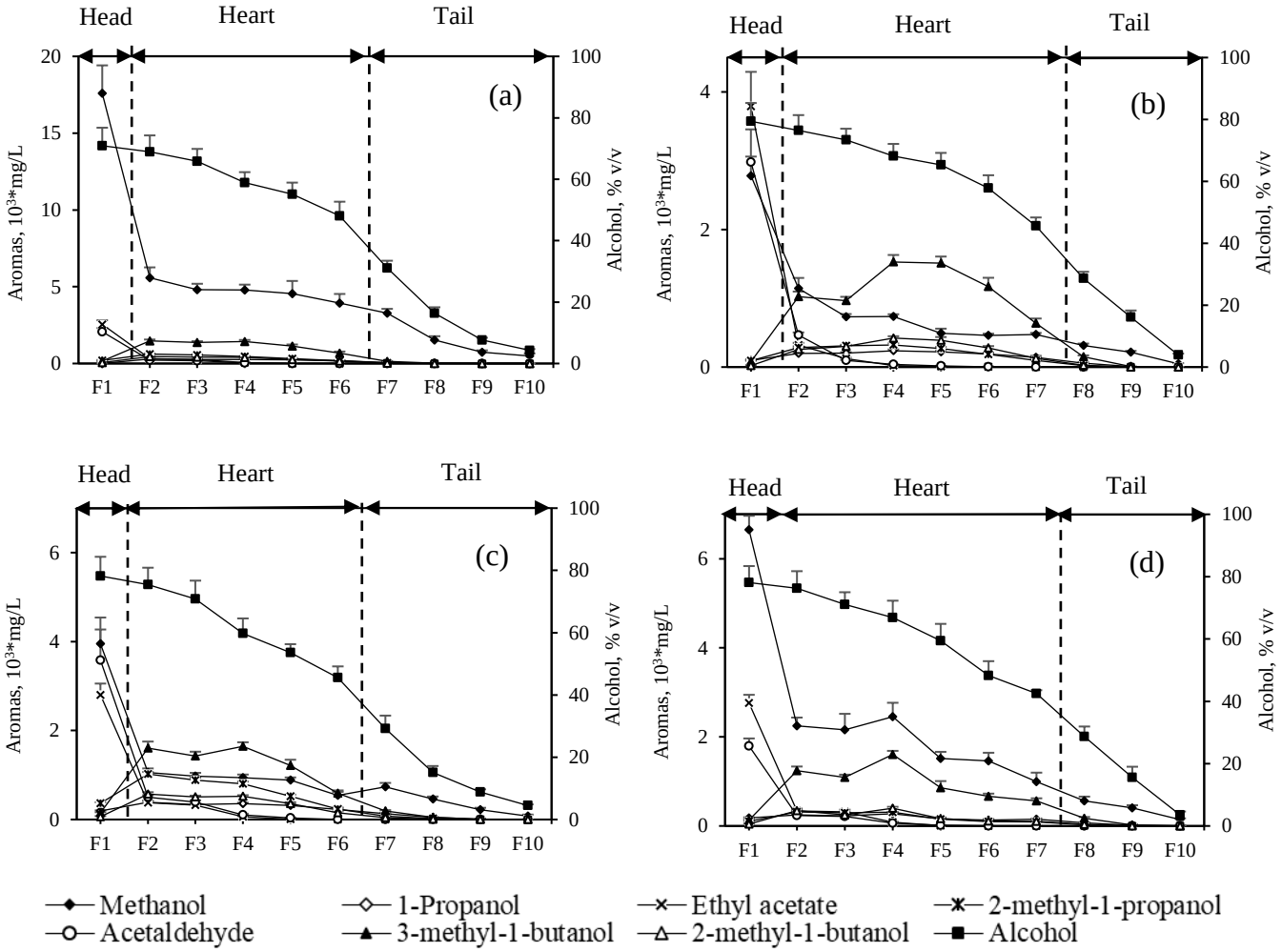


Figure 5.12 The distribution of primary aroma compounds during the distillation process from fermented (a) apricot, (b) apple, (c) cherry and (d) pear juices

* F1 – F10: 1st fraction to 10th fraction

Methanol is generally considered as a positive-flavor compound in distilled spirits even though it might be toxic to customers in high concentrations. However, it is difficult to separate the methanol from the ethanol-water mixture further without losing many other aromas (Claus and Berglund, 2005, Scanavini *et al.*, 2010). Methanol has high volatility and low boiling point, so it is mainly distilled at the first fraction, then its content is firmly reduced during the distillation process. During the distillation process of the mashes of apricot, apple, cherry and pear, methanol content ranged from 17591.82 mg/L to 489.31 mg/L, from 2781.57 mg/L to 47.26 mg/L, from 3948.22 mg/L to 76.69 mg/L and from 6651.74 mg/L to 141.60 mg/L, respectively (Figure 5.12). In the cases of apple and cherry mashes, the methanol distribution was compatible with type 3 of the classification of volatile compounds during batch distillation by Cantagrel (1989). Similar results were reported by Douady *et al.* (2019), Scanavini *et al.* (2012). However, there was a difference between the methanol distribution in apricot and pear distillates compared to in apple and cherry distillates. In the cases of apricot and pear, methanol content relatively remained high from the 2^{nd} to the 7^{th} fraction, resulting in high concentration in heart fractions from 3238.37 mg/L of alcohol 40 % v/v and 1320.53 mg/L of alcohol 40 % v/.v, respectively. Scanavini *et al.* (2010) suggested that other components such as residual sugars, some carbohydrates and acids might change the methanol volatility, which affects the methanol distribution of the distillates.

Higher alcohols (such as 1-propanol, 2-metyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol) contribute greatly to the necessary characteristics of spirits. These alcohols have partial or high solubility in water and moderate regular boiling points, so they tend to emerge mainly in the head and heart fraction. Follow the classification of volatile compounds by Cantagrel (1989), the distribution of these alcohols in the distillates was arranged in type 4; meanwhile, they might be sorted in type 7 of the category of volatile compounds by Douady *et al.* (2019). In our case, their distribution seemed to conform to type 7 under the classification of Douady *et al.* (2019), with the first distribution is not excessively high. The concentrations of these higher alcohols, including 1-propanol, 2-metyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were relatively low in the head fraction, gradually increased at 2^{nd} fractions. In addition, most cases from 4^{th} fraction or 5^{th} fraction, they gradually decreased during the distillation. At the end of the distillation process, all their contents were close to zero. The 2-methyl-1-butanol and 3-methyl-1-butanol concentration reached the highest at 4^{th} fraction in the case of pear, in a range of 2^{nd} fraction – 4^{th} fraction in the case of cherry and apricot, in a range 4^{th} fraction – 5^{th} fraction in the case of apple. Besides, 1-propanol reached the highest in range of 2^{nd} fraction – 4^{th} fraction in

case of pear and cherry, 2nd fraction – 3rd fraction in the case of apricot and 2nd fraction – 5th fraction in the case of apple.

In distillation, distillates' taste and smell could be used as indicators to determine the cut-point for the head and tail fractions (Spaho, 2017). Acetaldehyde, methanol and ethyl acetate accounted for the highest concentration in the head fraction. These compounds often withdrew from the final product because in high concentration, they were undesirable for fruit spirit quality, sharp, strong, unpleasant smell, especially methanol and acetaldehyde. In contrast, the tail fraction contained fatty acid ester, acetic acid, ethyl carbamate, which gave a faded, dull flavor. Several studies suggested that the distillate volume and alcohol content might be regarded as signals for the determination of the cut-points. The head volume should range from 1 % to 2 % of the base wine volume, which is equivalent to 10 % of the absolute alcohol volume of fermented fruit mash (Arrieta-Garay *et al.*, 2014, Balcerek *et al.*, 2017, Spaho, 2017, Spaho *et al.*, 2013, Xiang *et al.*, 2020). The heart/tail cut-point might be applied at the alcohol content dropped from 55 % v/v to 40 % v/v (Claus and Berglund, 2005, Darıcı *et al.*, 2019, Spaho, 2017, Spaho *et al.*, 2013, Xiang *et al.*, 2020). Finally, the tail cut-point could be at the alcohol content dropped to lower than 5 % v/v (Spaho, 2017, Spaho *et al.*, 2013). In this work, the combination of sensory and distillate alcohol content was known as the basic indicator applied to find the cut-point for distillation. In general, the suitable cut-point for a head faction at around 1.5 % of the base wine volume with the sensory signal was the disappearance of the sharp, strong smell in the distillate outflow. The suitable cut-point of heart/tail appeared as the alcohol content dropped to 40 % v/v. Then, the floral and fruity smell of distillates sharply decreased, followed by a slight sour smell of organic acids and a faded, dull flavor of fatty acid ester occurred. These results were completely in consistent with the arguments of other authors (Claus and Berglund, 2005, Darıcı *et al.*, 2019, Spaho, 2017, Spaho *et al.*, 2013, Xiang *et al.*, 2020). In addition, Figure 5.12 illustrated concentrations of higher alcohols significantly reduced when the alcohol content in the outflow dropped below 40 % v/v (p-value < 0.05).

In conclusion, the study provided the distribution profiles of the main components during the distillation process. That would have application potential in supporting a selection of the composition and flavor of expected distillate products. In the distillation of the spirit from apricot, apple, cherry and pear juice, the suitable cut-point of the head faction was at around 1.5 % of the wine volume while the alcohol content in the outflow of heart fraction dropped to 40 % v/v, which would contribute to enhancing the quality of spirits.

5.3.2 Profile of spirit products from apple, cherry, pear and apricot

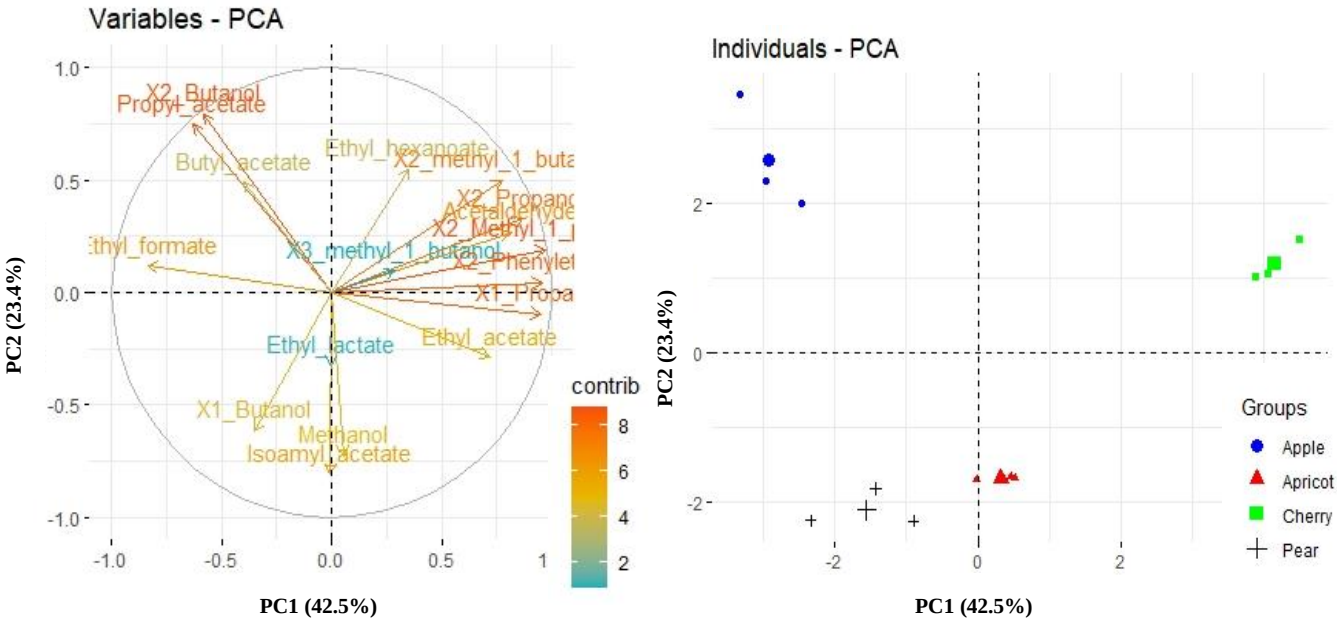
The quality of spirits primarily depends on fruit type, climate, geographical origin, harvest method of fruit used for their production and alcohol processing techniques (Śliwińska *et al.*, 2015). In my work, 17 aroma components mainly including methanol, higher alcohols (1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 2-phenylethanol), esters (ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate and isoamyl acetate) and acetaldehyde in the apple, apricot, cherry and pear spirits were identified (Table 5.6) by GC–FID techniques. By GC-FID, Puškaš *et al.* (2013) determined 28 aroma compounds in the apricot brandy. In comparison with the aroma compounds in apricot and apple raw distillates by HR-GC, 50 volatile compounds were identified in the apricot distillate while 45 compounds were in the apple distillate (Genovese *et al.*, 2004).

The fruit spirits consisted of a large variety of compounds, including methanol, higher alcohols, esters, fatty acids, carboxylic compounds (such as aldehydes, ketones and acetals) and others (Wiśniewska *et al.*, 2016). Besides, the number of volatile substances should be at least equal or exceeding to 2000 mg/L a.a. (equivalent to 800 mg/L alcohol 40 % v/v), while the maximum limit approved for the methanol concentration is from 10,000 to 13,500 mg/L a.a. (equivalent to 4000 mg/L alcohol 40 % v/v – 5400 mg/L alcohol 40 % v/v), depending on the varieties of the fruits (Regulation (EC) No. 110/2008). In my work, the methanol contents in the spirits samples varied from apricot, apple, cherry and pear accounted for 3238.37 mg/L alcohol 40 % v/v, 386.61 mg/L alcohol 40 % v/v, 650.12 mg/L alcohol 40 % v/v and 1320.53 mg/L alcohol 40 % v/v, respectively. According to the Council Regulation EEC No. 110/2008 about the maximum limit approved for the methanol concentration in many authentic fruit spirits, there was no limit to exceed in all fruit spirits obtained. The methanol content of fruit spirit from apple and cherry was smaller than one from Winterova *et al.* (2008). Fruit spirits have been featured a high methanol level. However, there was a large difference in the methanol contents of fruit spirits, which were highly dependent on the pectin content of the raw materials. In my case, apple and cherry concentrate used had lower pectin content than the others.

Table 5.6 Aroma profiles of spirits from apple, apricot, cherry and pear juices

Aroma compound (mg/L alcohol 40 % v/v)	Descriptive	Threshold (mg/L)	Apricot	Apple	Cherry	Pear
methanol	Alcohol, solvent	10000	3238.37±43.28	386.61±34.45	650.12±57.68	1320.53±151.42
Higher alcohol						
1-propanol	Alcoholic, ripe fruit	720	184.55±1.83	130.68±9.24	207.29±16.74	152.00±12.42
2-propanol	Ethanol-odor	1500	0.16±0.11	0.03±0.01	14.73±2.89	0.12±0.04
1-butanol	Alcoholic, pleasant odor	5	1.15±1.29	1.15±1.24	0.21±0.05	21.81±1.71
2-butanol	Alcoholic, pleasant odor	10	0.13±0.02	3.54±1.16	0.29±0.34	0.12±0.04
2-methyl-1-propanol	Malty, ethanol-odor	200	287.95±2.62	157.56±12.87	468.08±38.86	152.89±11.58
2-methyl-1-butanol	Banana, malty, ethanol-odor	32	223.45±2.41	207.57±17.90	268.20±15.53	179.27±12.74
3-methyl-1-butanol	Sweetish, malty, banana	70	821.64±14.92	780.79±64.38	847.01±110.78	823.84±68.44
2-phenylethanol	Roses, sweetish, perfumed	7.5	10.91±0.90	0.24±0.17	18.00±0.87	0.67±0.56
Total higher alcohol			1529.93±24.11	1281.56±106.96	1823.83±186.06	1330.72±107.54
Ester						
ethyl acetate	Ethereal, fruity, sweetish	17	80.51±8.26	56.55±16.88	116.17±13.14	101.89±19.84
ethyl formate	Rum-like, fruity	150	1.72±0.11	33.48±1.36	1.29±0.13	34.17±2.35
ethyl lactate	Artificial strawberry, perfumed	5.8	19.43±0.23	3.98±0.69	1.41±1.31	0.29±0.08
ethyl hexanoate	Apple, fruity, sweetish	1	0.21±0.06	0.64±0.20	0.92±0.40	0.47±0.38
butyl acetate	Banana, fruity	1.83	0.13±0.02	0.37±0.42	0.12±0.04	0.15±0.02
propyl acetate	Sweetish, perfumed	30	0.13±0.02	12.19±0.46	0.08±0.00	0.12±0.04
isoamyl acetate	Banana, apple solvent	15	7.43±0.23	0.26±0.16	6.72±0.76	18.73±2.35
Total ester			109.56±8.93	107.46±20.17	126.72±15.78	155.83±25.08
acetaldehyde	Green leaves, fruity, sharp	10	55.73±8.52	59.30±11.61	136.13±17.64	74.44±3.96

The 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol and 2-methyl-1-butanol were considered as higher alcohols accounting for high levels in these fruit spirits. Total higher alcohol content in apricot, apple, cherry and pear spirits reached 1529.93 mg/L alcohol 40 % v/v, 1281.56 mg/L alcohol 40 % v/v, 1823.83 mg/L alcohol 40 % v/v and 1330.72 mg/L alcohol 40 % v/v, respectively. They mainly made fruity, alcoholic, pleasant odor for spirits. Total higher alcohol of cherry spirit was the highest, followed by apricot spirit and then apple and pear spirits. The ester group in these fruit spirits included ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate and isoamyl acetate, with ethyl acetate being the highest. These esters impacted the floral and fruity aromas' texture of fruit spirits. The total esters in apricot, apple, cherry and pear spirits accounted for 109.56 mg/L alcohol 40 % v/v, 107.46 mg/L alcohol 40 % v/v, 126.72 mg/L alcohol 40 % v/v and 155.83 mg/L alcohol 40 % v/v, respectively. It can be seen clearly that sum of these higher alcohols and esters in the spirits was higher than the minimum limit approved of Council Regulation EEC No. 100/2008.



(a) (b)

Figure 5.13 Principal component analysis: loadings plot (a) and score plot (a) of PC1 and PC2, from all volatile compound in the apricot, apple, cherry and pear spirits

Acetaldehyde contributed a green leaf aroma and a bit pungent odor, which was the main compound of the aldehyde group presenting in these spirits. High acetaldehyde content was recorded in cherry spirits (136.13 mg/L alcohol 40 % v/v compared to pear, apple and apricot

spirits with 74.44 mg/L alcohol 40 % v/v, 59.30 mg/L alcohol 40 % v/v and 55.73 mg/L alcohol 40 % v/v, respectively).

Figure 5.13 presented the distribution of the scores and the loading plot obtained from the principal component analysis (PCA) of aroma compounds of fruit spirits made from apricot, apple, cherry and pear. Therein, the PCA explained 65.9 % of the variability of the primary compounds in two components: PC1 (42.5 %) and PC2 (23.4 %). Aroma compounds extremely contributed to PC1-2 including propyl acetate, 2-methyl-1-propanol, 2-butanol, 1-propanol, 2-phenylethanol, 2-propanol, 2-methyl-1-butanol, acetaldehyde. It is said that fruit spirits are popular alcoholic beverages due to their unique flavor and aroma is no exception. Hence, aroma compounds were known as the key representing and distinguishing between the fruit spirits. It can be observed that the spirits made from these four types of fruit are identified according to their volatile composition. Cherry, apricot, pear and apple spirits were clearly located at the quarters of 1^{st} to 4^{th} in turn. The key aroma compounds were mainly located corresponding to the spirits from cherry and apple. Furthermore, they were both separate in the first and fourth quarters, making it very easy to distinguish between cherry and apple spirits. The 2-methyl-1-propanol, 1-propanol and 2-phenylethanol represented cherry spirits well, whereas propyl acetate and 2-butanol represented apple spirits. Although the pear and apricot spirits were located separately in the second and third quarters, they were relatively close together. It indicated the composition and content of their volatile compound had similarities, and it might be difficult to distinguish these two spirits based on their compounds. Isoamyl acetate and methanol were clearly expressed in apricot spirits, while 1-butanol was explicitly expressed in pear spirits.

Generally, the production of fruit spirits from apricot, apple, cherry and pear has given Pálinka a high content of volatile compounds. Fruit spirit made from cherry reached the highest content of higher alcohols, while pear spirits had the highest esters content. Besides, these spirits obtained from the four fruits could be classified by principal component analysis based on data of their volatile compounds. This presented the potential for application in distinguishing the origin of spirits by chemometric analysis via volatile compound data.

5.4 Classification of fruit spirits by PCA and LDA

After analyzing 48 samples using GC–FID techniques, 17 volatile components in these apple, apricot, cherry and pear pálinkas were identified. Tables 5.7 summarized the results of the volatile compound contents in the commercial samples of individual sorts of fruit spirits. These volatile compounds were important to characterize pálinkas.

Following the Council Regulation EEC No. 110/2008 about the maximum limit approved for the methanol concentration, and minimum limit approved for volatile substances in many authentic fruit spirits, no limit was exceeded in all tested samples.

In mean value, apricot pálinkas samples exhibited the highest contents of ethyl formate, ethyl lactate and propyl acetate while there was a significant peak consisting of 1-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol in apple pálinkas samples. Similarly, volatile compounds accounted for the highest level in pear pálinkas samples, including 1-propanol, 2-propanol, 2-butanol, 2-methyl-1-propanol, ethyl acetate and butyl acetate. Nevertheless, amounts of ethyl hexanoate and 1-propanol were the highest in cherry pálinka samples. The total alcohol reached the highest level in apple and pear spirit samples with 4713.3 mg/L a.a. and 4795.6 mg/L a.a., respectively. Total ester was high in apricot and pear spirit samples with 641.9 mg/L a.a. and 715.5 mg/L a.a., respectively.

According to the results of one-way ANOVA, the mean concentrations of 2-butanol, 2-methyl-1-propanol, 2-phenylethanol, ethyl formate, ethyl hexanoate, butyl acetate and acetaldehyde were not significantly different among sorts of fruit spirit samples (apple, apricot, cherry and pear pálinkas) (p-value > 0.05). In contrast, there was a significant difference in the other average contents of fruit spirits' types (p-value < 0.05), containing methanol, 1-propanol, 2-propanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl acetate, propyl acetate and isoamyl acetate.

Although some other aroma components, as well as other parameters, were not analyzed in this study, the differences in aroma compounds found by origin can still exist. These signs of differences provided reliable and potential parameters for the classification of fruit spirits.

Table 5.7 The volatile compound contents in the commercial pálinka-s determined by GC-FID

Volatile compound (mg/L a.a.)	Apricot (n=12)		Apple (n=12)		Cherry (n=12)		Pear (n=12)		Sig.
	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	
methanol	5666.2±2461.4	767.8-8605.8	3187.2±1565.0	1098.5-6368.3	3191.2±1802.3	1427.2-7740.9	6128.2±2003.3	951.9-8409.4	***
Higher alcohol									
1-propanol	249.0±183.3	97.9-672.8	97.2±42.2	57.8-215.0	610.0±506.3	285.2-1693.2	617.9±752.4	79.0-2404.0	*
2-propanol	8.7±13.9	0.6-47.7	0.2±0.3	0-0.9	2.6±5.8	0.1-20.7	26.8±26.4	0.3-77.1	***
1-butanol	113.8±85.3	25.0-310.2	203.4±87.5	88.7-357.8	39.4±93.6	0.1-334.5	144.0±44.0	73.9-220.3	***
2-butanol	9.1±17.2	0-45.9	11.0±30.9	0-108.4	7.0±20.1	0-70.6	23.2±52.8	0-171.6	ns
2-methyl-1-propanol	727.7±345.0	268.6-1707.1	831.1±286.3	440.1-1344.9	765.2±246.5	480.2-1395.6	884.6±256.4	507.0-1431.1	ns
2-methyl-1-butanol	549.1±154.4	341.5-830.6	773.4±131.1	557.3-976.3	316.6±143.1	122.2-604.8	639.9±155.1	405.5-944.3	***
3-methyl-1-butanol	1994.6±591.8	1265.9-3302.5	2784.9±867.2	1732.9-4357.2	2150.0±696.2	1017.7-3142.9	2454.2±612.4	1277.3-3441.3	*
2-phenylethanol	3.2±5.2	0-15.0	12.2±16.1	0-47.9	3.7±6.8	0-24.2	5.0±0.0	0-27.1	ns
Σ Higher alcohol	3655.1±995.3		4713.3±1083		3894.6±714.6		4795.6±828.3		*
Ester									
ethyl acetate	446.2±410.8	96.2-1495.9	237.1±90.5	130.7-457.1	316.8±199.0	78.5-816.7	665.0±330.8	262.4-1439.6	*
ethyl formate	175.2±198.4	8.7-683.1	47.0±60.4	2.0-175.0	178.9±311.4	1.1-1124.1	94.1±103.4	0.6-309.4	ns
ethyl lactate	40.9±43.1	0-130.3	34.2±28.7	0-84.1	10.8±15.9	0.1-55.3	0.5±0.2	0-0.9	**
ethyl hexanoate	3.7±2.8	0.1-7.5	11.0±23.7	0.3-85.0	18.0±26.2	0.1-74.8	2.0±1.7	0.2-5.8	ns
butyl acetate	4.3±7.2	0-24.5	2.2±4.0	0-14.1	1.2±3.3	0-11.5	8.4±13.4	0-43.2	ns
propyl acetate	4.4±6.5	0-17.9	112.1±134.1	0-400.7	22.5±43.4	0-144.2	32.8±56.2	0-178.7	**
isoamyl acetate	4.2±6.7	0-17.4	0.8±1.4	0-3.9	0.5±0.1	0.4-0.6	11.1±14.4	0-42.8	**
Σ Total ester	678.86±621.4		444.51±217.8		548.54±497.5		813.88±484.6		*
acetaldehyde	423.3±310.9	85.4-763.3	426.4±379.6	6.5-1424.4	466.5±419.2	2.5-1271.8	403.9±129.0	174.4-632.3	ns

Sig.: significance (*, **, ***) - display the significance at 0.05, 0.01 and 0.001 by least significant difference.
"ns": not significant.

5.4.1 Classification of pálinka spirits according to fruit types by principal component analysis

The principal component analysis (PCA) was used to describe the differences in volatile compounds from the tested spirits and to express the important information from the dataset via visual graphics. Seventeen aroma components were subjected to PCA, and the results were indicated in Figure 5.14.

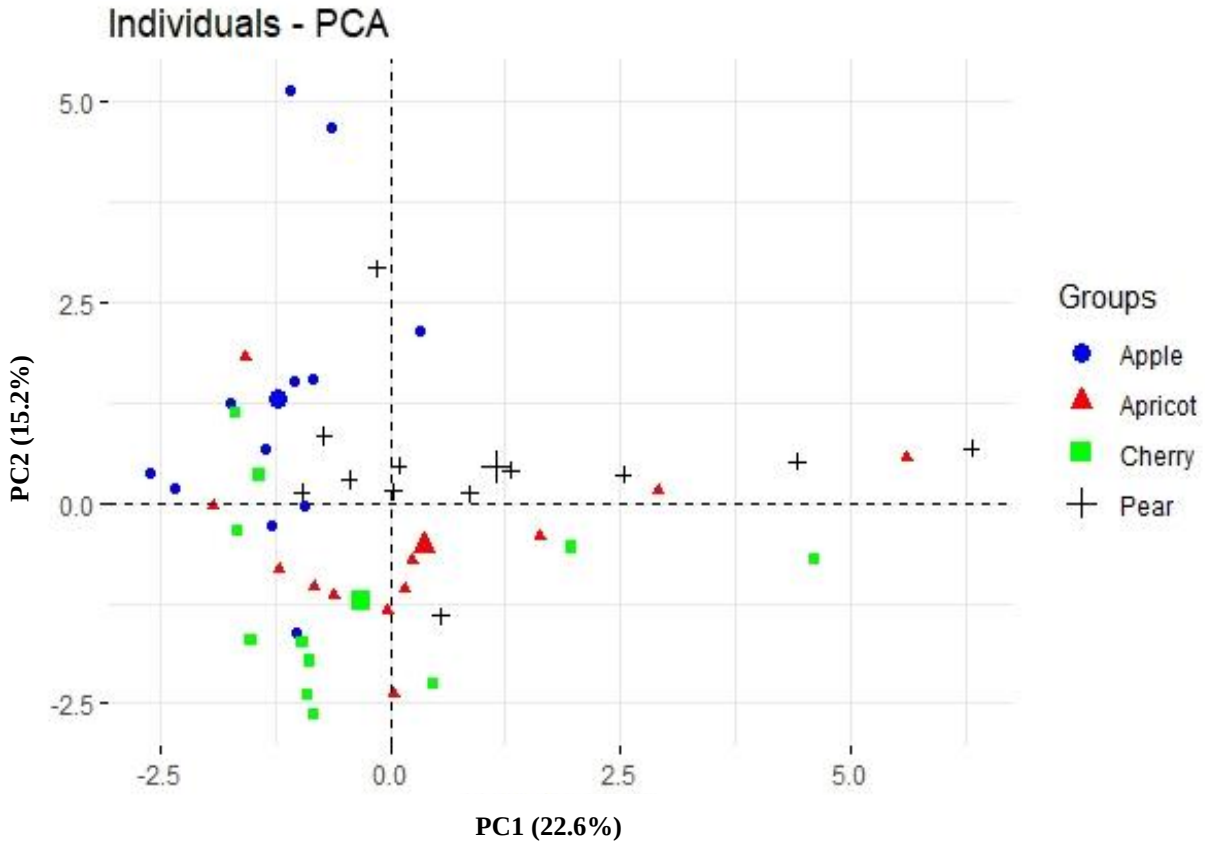


Figure 5.14 Score scatter plots of the first two principal components PC1-PC2

The results (Figure 5.14) showed that the tendencies for the cluster of tested samples did not fulfil expectations because there were both partial overlaps and scatters existed among the four pálinka groups. The reason could be all samples are commercial spirits from different sources; thus, there is a large variation in the content of volatile compounds. It was clearly shown through the low variance contribution rate. The scores of PC1 – PC2 could explain only 37.8 % of the total variances (PC1 contributed 22.6 % and PC2 contributed 15.2 %). Some similar results were found when applying the PCA method in the traceability of foodstuffs, such as the studies of Chung *et al.* (2015) in determining the authenticity of the geographical origin of rice using PCA with the proportion of variation explained of 43.5 % from PC1 – PC2, reports of Ni *et al.* (2018) in discriminant the geographical origin of tea with the contribution of the variances from PC1 – PC2

being 49 %. Thus, other statistical analysis methods (Linear Discriminant Analysis, LDA) should be applied to gain better classification results.

5.4.2 Classification of pálinka spirits by linear discriminant analysis

The spirits discrimination between four fruit types of different origins was verified by a scatter plot of the two functions' scores, using the linear discriminant analysis model, based on their aroma compounds. In the scatter plot of Figure 5.15, two functions explained 97 % of the total variance, with 55 % from LD1 and 42 % from LD2. The results illustrated that there were significant differences between these four groups of tested samples. Although these fruit spirits groups were unequally distributed and concentrated. Depending on the fruit type, the distance between groups was still close. The distribution of the apricot spirit was central and surrounded by apples, pears and cherry spirits. It suggested that apricot was potentially difficult to distinguish because they might easily be mixed with other spirits. Besides, apricot spirits were located near pear spirits, so there was a relatively high confusion in classifying them with others. The cherry spirits were easily distinguished by LD1, while LD2 supported recognizing the apple spirits well.

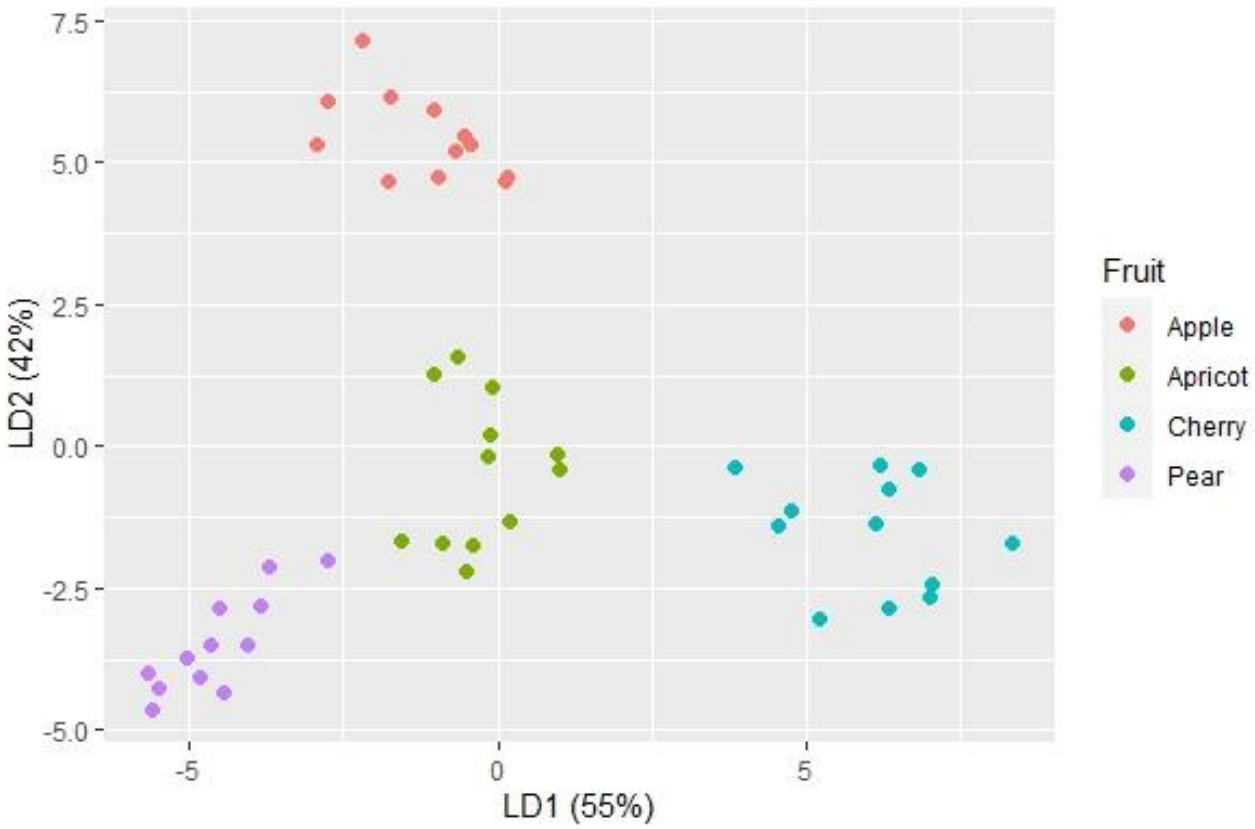


Figure 5.15 Score scatter plots of the constructed standardized discriminant functions LD1-LD2

Conducting Fisher's classification function coefficients for multiple classes via the LDA, pálinka discrimination among the different fruits related to 2-propanol, 2-butanol, butyl acetate, isoamyl acetate, ethyl hexanoate, 2-phenylethanol. The combination of 2-propanol, isoamyl acetate, ethyl hexanoate, and 2-phenylethanol might contribute positively to the pear pálinka grading. In contrast, 2-propanol, butyl acetate, isoamyl acetate, and 2-phenylethanol had high weight in cherry pálinka classification. Likewise, 2-propanol, 2-butanol, isoamyl acetate, ethyl hexanoate and 2-phenylethanol could be applied to clustering apricot spirits. Whereas 2-butanol, butyl acetate, ethyl hexanoate and 2-phenylethanol could be used to classify apple pálinka.

Table 5.8. Classification results as using LDA on pálinka samples among fruits

Actual Group	Validation	Verification Samples	Predicted group				Correctly Classified (%)
			Apple pálinka	Apricot pálinka	Cherry pálinka	Pear pálinka	
Apple pálinka	Without Cross-Validation	12	12	0	0	0	100
Apricot pálinka		12	0	12	0	0	100
Cherry pálinka		12	0	0	12	0	100
Pear pálinka		12	0	0	0	12	100
Total		48	12	12	12	12	100
Apple pálinka	With Cross-Validation	12	12	0	0	0	100
Apricot pálinka		12	0	11	0	1	100
Cherry pálinka		12	0	1	11	0	91.66
Pear pálinka		12	0	2	0	10	83.33
Total		48	12	14	11	11	91.66

The correct classified capacity of the LDA model was 100 % (Table 5.8). A frequent measure of the predictive validity of a regression model is the cross-validated correlation. Accordingly, to validate the predictive ability of the model, the leave-one-out cross-validation method was utilized to generate the appropriate model. As a result, this model's predictive ability (% of the objects belonging to the testing set correctly classified using the developed model) was 91.66 %, which revealed that the LDA model showed relatively satisfactory results for the classification of pálinkas from different fruit types.

Overall, these results indicated that the tested spirits from different fruit types were well distinguished from one another. Thereby, based on the aroma fingerprints, the LDA method can be suitable to verify the origins of pálinka-s.

6. CONCLUSIONS AND RECOMMENDATIONS

In the study, nine commercial yeast strains were screened for spirit fermentation. The results showed that all tested yeast strains were suitable for spirit production. The most promising strains were the Uvaferm Danstil A, the Fermiblanc Arom, the Vin-O-Ferm Roses and the Fermicru AR2. The concentration of total volatile compounds in fermented mashes in the cases of these four strains were higher than that in the cases of the rest ones. The strain Uvaferm Danstil A exhibited strong fermentation ability through the conversion rate of sugar to alcohol and short fermentation time. In my research, fermentation temperature, pH, and initial soluble solid content have been demonstrated to affect the formation of volatile compounds during alcoholic fermentation significantly. Through the Response Surface Methodology method, these influencing factors have been optimized to increase the maximum production yield of alcohol and total volatile compound. Besides, confirmatory experiments for evaluating the optimal values were also conducted, which indicated that the optimal values were reliable and should be applied to fruit spirit production. The distribution of the main aroma components during distillation was also described. That helped easier find suitable cut-points for collecting the heart fraction during distillation. Furthermore, obtained optimum parameters and cut points were applied for pálinka production over apricot, apple, cherry and pear fruit juices. Profiles of pálinka from four types of fruit were also given. Most volatile compounds all had a positive contribution to the flavor and taste of fruit spirits. The process of classifying pálinka based on the database of volatile compounds was also conducted. The results showed that the linear discriminant analysis would be an appropriate method with a model's predictive ability of 91.66% to classify fruit spirits based on fruit origins.

Overall, this study introduced encouraging results in production of pálinka with high volatile compounds and classified them by fruit origins. Some directions for further research can be proposed as follows:

- Collection and normalization of the database of volatile compounds to classify fruit spirits by origin, not only by fruit type, but also by geographical origin, as well as to detect whether it is real or artificial. Those would be considered for further studies.
- Application of optimal parameters for production of fruit wine from tropical fruits such as dragon fruit, banana and mango, as well as finding the aroma profile of these fruit spirits.
- In optimization, sorting the individual volatile compounds into some groups of observed variables that positively affect the flavor and taste of spirits products is recommended. Besides, incorporating sensory evaluation into the optimization process should also be considered.

7. NOVEL CONTRIBUTION

1. Nine commercial yeast strains were screened for alcoholic fermentation of fruit juices. The production capacity of volatile compounds reached the highest level in the cases of strains Uvaferm Danstil A, Fermiblanc Arom, Vin-O-Ferm Roses, Fermicru AR2, and the lowest level in the cases of strain Oenoferm X-thiol F3. The strain Uvaferm Danstil A exhibited strong fermentation ability through the conversion rate of sugar to alcohol in the cases of apple, apricot, cherry and pear and short fermentation time. Thus, it was selected for production of pálinkas.

2. The optimal conditions for alcoholic fermentation of fruit juices for production of pálinkas were determined and optimised. The temperature, pH and soluble solid content for fermentation of apricot, apple, cherry and pear juices were 23.02 ℃, pH 3.50 and 20.94 °Brix; 24.66 ℃, pH 3.25 and 21.28 °Brix; 24.71 ℃, pH 3.25 and 22.49 °Brix; 24.33 ℃, pH 3.42 and 21.95 °Brix, respectively.

3. The effects of the distillation process over apple, apricot, cherry and pear spirits on aroma compounds distribution were described. And the suitable cut-point for the distillation process scientifically was determined experimentally. In the distillation of spirits from apricot, apple, cherry and pear juice, the suitable cut-point of the head faction was at around 1.5% of the wine volume, while the cut-point of the heart fraction was appropriate when the alcohol content in the outflow dropped to 40 % v/v.

4. Chemometric statistics were conducted to classify both obtained and commercial fruit spirits. The linear discriminant analysis method was suitable to verify the origins of spirits from apricot, apple, cherry and pear with a model's predictive ability of 91.66 %. In addition, the spirits discrimination among these different fruits related to 2-propanol, 2-butanol, butyl acetate, isoamyl acetate, ethyl hexanoate, and 2-phenyethanol.

8. SUMMARY

The alcoholic fermentation process has significantly affected the final flavour and quality of fruit spirits. In fermentation, the composition and concentration of these aromas vary depending on the raw material and yeast strain applied. Nine commercial yeast strains, Uvaferm SLO, Uvaferm PM, Uvaferm Danstil A, Fermiblanc Arom, Viniflora Melody, Vin-O-Ferm Roses, Fermicru AR2, Oenoferm x-treme F3 and Oenoferm x-thiol F3, were used for fermenting apricot, apple, cherry and pear juice. Meanwhile, there was no significant difference among the alcohol production capacity of the yeast strains tested, whereas the formation of volatile compounds is greatly affected by tested yeast strains. Strain Uvaferm Danstil A displayed strong fermentation ability via the conversion rate of sugar to alcohol [0.70, 0.66, 0.56 and 0.70 (% v/v alcohol/ % total reducing sugar) in the case of apple, apricot, cherry and pear] and short fermentation time (sugar contents reduced drastically while alcohol contents reached the maximum on the 3rd day). Additionally, strain Uvaferm Danstil A was selected for alcoholic fermentation of fruit juices based on the high production capacity of volatile compounds.

Single-factor experiments were carried out to investigate the effect of fermentation conditions of fruit spirits on the production capacity of alcoholic and aroma compounds. The temperature ranging from 15 ℃ to 25 ℃, pH ranging from 2.75 to 3.75 and initial soluble solids content ranging from 18 °Brix to 30 °Brix were appropriate for the production of fruit spirits with high alcohol content and high level of aroma compounds. They were also suitable input ranges to optimize fermentation conditions by RSM. Full predictive equations for optimization of fruit alcoholic fermentation were described by equations 1-8. After optimizing with the full regression models for fermenting apricot, apple, cherry and pear juices, the optimal conditions obtained, including temperature, pH and soluble solids content, were 23.02 ℃, 3.50 and 20.94 °Brix; 24.66 ℃, 3.25 and 21.28 °Brix; 24.71 ℃, 3.25 and 22.49 °Brix; 24.33 ℃, 3.42 and 21.95 °Brix, respectively. Additionally, predicted values of the response for fermentation were also calculated. Predicted values of alcohol and volatile compounds' production yield were 73.38 (8.98 % v/v) and 2031.64 (248.66 mg/L) for fermentation from apricot juices, 72.20 (12.10 % v/v) and 1947.76 (326.39 mg/L) in the case of apple, 59.68 (9.02 % v/v) and 2231.68 (337.37 mg/L) in the case of cherry, 78.63 (10.12 % v/v) and 2039.77 (262.60 mg/L) in the case of pear.

Improper fermentation conditions cause severe sensory flavour defects for spirits production. Therefore, to improve the quality of fruit spirits, the distillation should be carefully controlled. It is essential to understand the distribution of the vital compounds in order to find suitable cuts for the head, heart and tail fraction, which helps eliminate or at least reduce the

harmful compounds to obtain spirits of better quality. The effects of the distillation process on aroma compounds distribution have been described. In the distillation of the spirits from apricot, apple, cherry and pear juice, the suitable cut-point of the head fraction was at around 1.5 % of the wine volume, while the alcohol content in the outflow of heart fraction dropped to 40 % v/v.

By using GC–FID techniques, 17 aroma components were identified in these apple, apricot, cherry and pear spirits, mainly including methanol, higher alcohols (1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol), esters (ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate and isoamyl acetate) and acetaldehyde. The total higher alcohol content in apricot, apple, cherry and pear spirits reached 1529.93 mg/L alcohol 40 % v/v, 1281.56 mg/L alcohol 40 % v/v, 1823.83 mg/L alcohol 40 % v/v and 1330.72 mg/L alcohol 40 % v/v, respectively. In addition, the ester group included ethyl acetate, ethyl formate, ethyl lactate, ethyl hexanoate, butyl acetate, propyl acetate and isoamyl acetate, with ethyl acetate being the highest. The total ester in apricot, apple, cherry and pear spirits accounted for 109.56 mg/L alcohol 40 % v/v, 107.46 mg/L alcohol 40 % v/v, 126.72 mg/L alcohol 40 % v/v and 155.83 mg/L alcohol 40 % v/v, respectively. The results of the principal component analysis indicated that while 2-methyl-1-propanol, 1-propanol and 2-phenylethanol represented characters of cherry spirits, whereas propyl acetate and 2-butanol were attributed to apple spirits. Isoamyl acetate and methanol were found in apricot spirits, and 1-butanol was explicitly expressed for pear spirits.

A total of 48 commercial pálinka samples (12 apple pálinkas, 12 apricot pálinkas, 12 pear pálinkas and 12 cherry pálinkas) were also analyzed by GC-FID technique. Chemometric statistics methods (such as principal component analysis and linear discriminant analysis) were applied to confirm the key aroma compounds and to classify fruit spirits. Pálinka discrimination among the different fruits was related to 2-propanol, 2-butanol, butyl acetate, isoamyl acetate, ethyl hexanoate and 2-phenylethanol. The combination of 2-propanol, isoamyl acetate, ethyl hexanoate and 2-phenylethanol might contribute positively to the pear pálinka grading. In contrast, 2-propanol, butyl acetate, isoamyl acetate and 2-phenylethanol had high weight in cherry pálinka classification. Likewise, 2-propanol, 2-butanol, isoamyl acetate, ethyl hexanoate and 2-phenylethanol could be applied to clustering apricot pálinka. Whereas 2-butanol, butyl acetate, ethyl hexanoate and 2-phenylethanol could be used to classify apple pálinka.

The alcoholic fermentation and distillation process has obviously influenced the final quality of spirits products. These results provided important information in serving the basic to develop standard pálinka production from apricot, apple, cherry and pear, as well as the application potential of chemometric statistics to classify fruit spirits origin.

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❖ Journal articles

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2. CSILLA FARKAS, JUDIT M. REZESSY-SZABÓ, VIJAI KUMAR GUPTA, ERIKA BUJNA, **TUAN M. PHAM**, KLÁRA PÁSZTOR-HUSZÁR, LÁSZLÓ FRIEDRICH, RAJEEV BHAT, VIJAY KUMAR THAKUR, QUANG D. NGUYEN. *Batch and fed-batch ethanol fermentation of cheese-whey powder with mixed cultures of different yeasts*. Energies, 12, 4495, 2019. (IF: 3.004)

❖ Poster presentations

- ***International conference***

1. **TUAN M. PHAM**, RÉKA VARJÚ, ERIKA BUJNA, ÁGOSTON HOSCHKE, QUANG D. NGUYEN. *Chemical and volatile composition of fresh spirit from apple fermented with different Saccharomyces Serevisiae yeast strains*. EuroFoodChem XIX Conference. 2017, Budapest – Hungary.
2. **TUAN M. PHAM**, RÉKA VARJÚ, AGÓCS GERGELY, ERIKA BUJNA, ÁGOSTON HOSCHKE, QUANG D. NGUYEN. *Effect of different commercial yeast strains on physic-chemical characterizations and volatiles production in fermented apricot juice*. 3rd FoodConf. 2018, Budapest-Hungary.
3. **TUAN M. PHAM**, WEIZHE SUN, ERIKA BUJNA, ÁGOSTON HOSCHKE, QUANG D. NGUYEN. *Study on response surface methodology (RSM) of alcohol fermentation from apple juice by Saccharomyces cerevisiae*. 18th International congress of the Hungarian society for microbiology. 2019, Budapest-Hungary.
4. **TUAN M. PHAM**, WEIZHE SUN, ERIKA BUJNA, ÁGOSTON HOSCHKE, LÁSZLÓ FRIEDRICH, QUANG D. NGUYEN. *Application of response surface methodology for fermentation optimization of cherry by Saccharomyces cerevisiae*. 4th International Conference on Biosystems and Food Engineering BIOSYSFOODENG 2021, Budapest-Hungary.

- ***National conference***

1. **TUAN M. PHAM**, MOHAN MALKANI ANBALAGAN, FANNI HEGEDŰS, RÉKA VARJÚ, QUANG D. NGUYEN. *Fermentation profile of different yeast strains on sour cherry. Chemical Engineering Conference*. 2017, Veszprem – Hungary.

2. **TUAN M. PHAM**, RÉKA VARJÚ, AGÓCS GERGELY, ERIKA BUJNA, ÁGOSTON HOSCHKE, QUANG D. NGUYEN. *Effects of commercial Saccharomyces Cerevisiae strains on fermentation of pear juices and production of volatile compounds*. FIBOK2018 Conference. 2018, Budapest-Hungary.
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