

Apple juice fermentation process

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This work emphasized the apple fermentation process and showed how the fermentation can be affected by the first material composition and the used microorganisms.

Keywords: apples ; cider ; process ; fermentation ; physicochemical composition ; microorganisms

Fermented apple beverages are produced all over the world with diverse characteristics associated with each country. Despite the diversifications, cider producers are confronted with similar issues and risks. The nature of the raw material, also known as the fermentation medium, plays a key role in fermentation. A well-defined composition of apples is, therefore, required to produce cider with good quality. In addition, ferment and its metabolism are important factors in the fermentation process. The producers of cider and other alcoholic beverages are looking in general for novel yeast strains or for the use of native strains to produce “authentic” and diversified beverages that are distinct from each other, and that attract more and more consumers. Research articles on cider production are infrequent compared to wine production, especially on the impact of the chemical composition and microbial diversity of apples on fermentation. Even though the processing of fermented beverages is close in terms of microbial interactions and production, the study of the specific properties of apples and the production challenges of cider production is advantageous and meaningful for cider producers.

1. Introduction

Apples represent a very particular fruit known for their unique symbolic richness over time. Later, the different studies proved the importance of that fruit due to its chemical composition and specifically its antioxidant characteristics. The fruit belongs to the “*Maloideae*” subfamily and to the “*Rosacea*” family. It represents one of the most important deciduous tree fruits that are generally grown in temperate and tropical regions ^[1]. Apple is one of the most produced and consumed fruits in the world. It is ranked as the third-most fruit produced worldwide after bananas and watermelon with a production that reached 75 million tons in 2018–2019 ^[2]. China stands as the largest producer with increasing production of almost 33 million tons per year, followed by the European Union (EU) producing 15 million tons per year (Table 1). The United States comes in the third rank, producing 5.6 million tons of apples in 2019. The main apple producers in Europe are Poland, France, and Italy. Turkey and Iran produce around 3 million tons per year each while the production of Chile, Russia, Ukraine, and Brazil is around 1.2 million tons per year (Table 1).

Table 1. Global production and consumption of fresh apples per year in 2019 ^[2].

Country	Apple Production (kt)	Fresh Domestic Consumption (kt)
China	33,000	38,050
European Union	15,442	7400.6
United States	5564	2589.4
Turkey	3306	2630.5
Iran	3085	1813.9
Russia	1656	1884.4

Chile	1393	229.6
Ukraine	1211	1066.2
Brazil	1156	1325.9

Furthermore, apple juice is the main raw material for several beverages' production. Vinegar, cider, calvados, and apple wine are obtained from apple juice fermentation, depending on the conditions applied. This review focuses on the alcoholic fermentation of apple juice to produce cider. Over the past years, different definitions were accorded to the word "fermentation". The term was first applied to describe the production of wine and specifically the bubbling caused by the production of carbon dioxide. Nowadays, alcoholic fermentation is known as a biological complex process where yeasts convert sugars like glucose, fructose, and sucrose into cellular energy, ethanol, carbon dioxide, and other metabolic byproducts. Different parameters may affect the fermented product such as the composition of the raw materials, the microorganism used during the fermentation, and the process parameters and conditions.

2. Cider-Making Process

Different types of ciders exist in the market since every country has its specialty to produce traditional ciders. French cider is usually produced following a natural process without additives or other modern treatments, compared to the English cider. Due to the different production methods, French cider tends to be fruity while the English one is richer in alcohol. Even if the processes seem to be different, many key steps are common to all of these processes (Figure 1).

Figure 1. Main steps of the cider-making process.

Apples are first transported from the silo to be machine-washed in water. They are sorted by appearance to remove rotten fruits. The remaining apples are transferred for milling where they are crushed into small pieces. In the French cider process, the apple pulp is oxidized from 30 min to up to 5 h. The pulp is then pressed and left to settle. The fermentation step, which in France relies on natural flora, begins with an oxidative phase. Oxygen flow is highly beneficial for this flora at the beginning of fermentation, leading to limited growth of *Saccharomyces* during this step. This stage is considered very important because this is when fruity aromas are generated. The fermentation is conducted later by *Saccharomyces* for 1 to 3 months at a moderate agitation speed. As for wine production, malolactic fermentation can occur due to bacterial growth in cider. Maturation is the next step after fermentation when other yeasts, such as *Brettanomyces anomalus* can grow, which can have a negative influence on the aromatic quality of the cider. Later, a post-fermentation clarification step takes place, leading to a clear product without turbidity and deposits, and which stabilizes the cider and eliminates haziness caused by the action of proteins or tannins. This step can also eliminate microorganisms and ensure better bacterial stability in the final product. Clarification is done either by settling, centrifugation, or filtration. Finally, after blending and final filtration, the cider is bottled with either carbonation or additional yeast to trigger a second fermentation in the bottle.

Some research works have been conducted to investigate the impact of power ultrasound and pulsed electric fields (PEF) on apple juice fermentation for cider production. Ultrasound- and PEF-assisted fermentations [3][4] showed that the treatment of the yeast strain *Hanseniaspora* sp., isolated from a spontaneous fermented Lebanese "Ace Spur" apple juice [5], may contribute to shortening the fermentation time and to reducing the ethanol content in the fermented product, depending on the parameters applied. Further investigations are, nonetheless, required to study the impact of these emerging technologies on the sensory properties on cider.

3. Impact of Apple Juice Composition and Microbial Diversity on Alcoholic Fermentation in the Cider Production Process

Fermentation is a complex metabolic process when sugars are transformed into ethanol, secondary metabolites, acids, alcohols, esters, and carbon dioxide. This transformation can be affected by several parameters related to the fermentation medium. Thus, the choice of apple varieties, as well as the yeast species carrying out the fermentation process, is important (Figure 2).

Figure 2. Impact of apple varieties and yeast type on cider production.

3.1. Impact of Apple Juice Composition on Fermentation

Sugar, acids, and polyphenols represent the three major compounds that affect apple juice fermentation [6]. Accordingly, apple selection is an important step, having a direct impact on the quality of the final product. In countries with ancient cider traditions, special varieties of apples known as “apple cider” are grown for their high levels of acids and phenolic compounds. However, nowadays, dessert apples are more and more frequently used, especially in Germany, Switzerland, and America. Consequently, in order to help cider producers to obtain an optimal mixture, acidity ratios, polyphenols, and alcohols derived from sugars or residual sugars in their products, a quantitative classification system was developed by Long Ashton Cider Research station in the UK [6]. Phenolic compounds have an important effect on the sensory properties of cider such as color, bitterness, and astringency balance, which provide the mouthfeel of cider [6][7]. The phenolic profile may differ from one apple variety to another, but it may also depend on the year of harvest, variety, climate, maturity, storage, and processing [8][9][10][11]. Procyanidins, composed of high molecular compounds, play a major role in astringency, while molecules of lower weights are responsible for bitter taste. In addition, polyphenols can influence the sweetness and acidity, thus affecting overall aroma development during fermentation [12][13]. Not only nonvolatile phenolic compounds play a major role during fermentation, but also the volatile phenolic compounds formed by enzymatic reactions during fermentation contribute to the formation of the aromas of the final product. Another factor to consider is the composition and the concentration of the initial sugars. The nature of the sugar can also affect the fermentation process. Monosaccharides can produce carbon dioxide faster than disaccharides. Furthermore, many other factors can play a role in the progression of fermentation. The glucose and fructose concentrations may influence the yeast growth, i.e., a high sugar concentration will reduce the growth rate of certain yeast strains. For sugar concentrations between 200 and 300 g/L, a decrease in the growth rate of *S. cerevisiae* was observed [14][15]. Furthermore, high sugar levels increase the yeast demand for assimilable nitrogen, which can similarly inhibit the fermentation [16]. For low glucose concentrations, yeasts use sugars either by respiration or fermentation. Aeration induces an increase in the biomass formed (total and per unit of degraded sugar), and at the same time, a decrease in alcohol production and sugar consumption; Pasteur then retained that respiration inhibits fermentation. For high concentrations of glucose, *S. cerevisiae* metabolizes sugars only by fermentation. Even in the presence of oxygen, respiration is impossible. Al Daccache et al. [17] reported different fermentative behaviors of the yeast *Hanseniaspora* sp. during the fermentation of Lebanese “Ace spur” and French “Kermerrien” apple juices. The apples used had different chemical compositions, where the “Ace spur” apple juice had almost the double concentrations of sugars, compared to “Kermerrien” one. Different biomass and ethanol kinetics were obtained. In the presence of an excess of sugar, the yeast cells followed the fermentative pathway from the first hour of fermentation. For the fermentation of “Kermerrien” apple juice, the cells were in a respiratory mode generating biomass in the early hours of fermentation [17]. Some variables, such as temperature and pH, can influence yeast growth rates and the ecology and adaptation of yeast strains [14][15]. Rosend et al. [18] studied the impact of four apple varieties grown in Estonia, Antei, Melba, Kulikovskoye, and Orlovski Sinap, on cider fermentation. Alcoholic fermentation was carried out using the must from the apples at various stages of ripening (i.e., unripe, ripe, overripe) and commercially available yeast strains. The differences in volatile composition between the samples were assessed. The results showed that apple variety stands as the principal attribute influencing the quality and aroma properties of apple cider. The maturity of the fruit was variety-specific, the volatile profiles of Melba variety ciders were the least affected by the ripening stage of apples [18]. Organic acids are indicators of quality during cider fermentation. The dominant flavor of organic acids is sourness, but they also contribute to bitterness and astringency of cider [19]. Some yeasts can assimilate malic acid resulting in its reduction, fluctuating from 5 to 40% [20]. When a second bacterial fermentation occurs, its level is reduced mainly by lactic acid bacteria. During this fermentation, citric acid is transformed into acetic acid, whereas shikimic and quinic acids are metabolized to single phenols, like catechol and ethylcatechol, and other compounds. Organic acids may affect the yeast metabolism. The yeast enzymatic activity and the chemical alterations are also influenced by the juice acidity [21].

3.2. Impact of Yeast on Fermentation

Yeast plays an essential role in the production of all alcoholic beverages, and the selection of an appropriate yeast strain is crucial to control the alcohol yield and to preserve the beverage's sensory quality. Fermentative yeasts can use sugars anaerobically as electron donors, electron acceptors, and carbon sources. However, the yeast action during fermentation is not only limited to the transformation of sugars into alcohol. Yeast metabolism produces different other metabolites and by-products that may have an essential impact on the organoleptic quality of the fermented product [22]. Thus, the criteria to select yeast strains for their use in fermented beverages comprise their capability to dominate the media and to improve desired sensorial characteristics and their inability to produce undesired compounds such as biogenic amines or off odors [23]. During spontaneous fermentation, several yeast species may be present and could play a significant, complex, and unpredictable role [24]. Some yeast species may be present only during the first stage of fermentation, while others, more resistant to ethanol, are dominant during the later stages. This type of yeast is nowadays known as belonging to the *Saccharomyces* strains [25]. *S. cerevisiae* is largely used to produce alcoholic beverages due to its controlled and repetitive behavior as well as for the release of its aroma precursors [26][27][28][29]. Nevertheless, fermentation is the collaboration of different species of yeast and bacteria initially present in the product or found on the

surface of the presses and fermenters. Mixed fermentations are suggested as a feasible way to improve the complexity and enhancing the particular and specific characteristics of the product [30]. The growth of each yeast species is characterized by a definite metabolic activity, which determines the concentrations of flavor compounds in the final product. Therefore, the role of non-*Saccharomyces* yeasts appears important during the fermentation process. The main yeasts present in the early stages of fermentation belong to the genera *Hanseniaspora* and *Candida*. These species are characterized by a low fermentation capacity and are sensitive to an alcohol concentration close to 5 or 6%. In addition, some changes in fermentation parameters may result in the presence of yeasts such as *Brettanomyces*, *Kluyveromyces*, *Schizosaccharomyces*, *Torulaspora*, *Zygosaccharomyces*, and *Saccharomycodes* [31][32][33]. From the above-cited yeasts, some of them may have a positive impact on fermentation by releasing favorable aromas, but others may release undesirable aromas known as off-flavors. Yeasts can affect primary aroma determined by the initial composition of the product and the secondary aromas that are created during the fermentation, as well as the tertiary aromas generated during the maturation of the finished product [34]. *Hanseniaspora*, *Zygosaccharomyces*, and *Schizosaccharomyces pombe* species produce high amounts of volatile fatty acids, such as acetic acid [35][36][37][38][39][40], and low concentrations of higher alcohols [41][42][43][44]. Esters and sulfur compounds are mainly produced by *Candida*, *Hanseniaspora*, *Torulaspora delbrueckii*, and *Kazachstania gamospora* [42][45][46][47]. Lorenzini *et al.* [48] investigated the capacity of *Torulaspora delbrueckii*, *Hanseniaspora osmophila*, *Hanseniaspora uvarum*, *Starmerella bacillaris*, and *Zygosaccharomyces bailii* to ferment apple juice and found that *Hanseniaspora uvarum* was the greatest producer of hexyl and isoamyl acetate. The complex volatile profile of cider suggests the possible strain-specific effects on the aroma formation. Wei *et al.* [49] tried to enhance the flavor complexity of cider by different non-*Saccharomyces* species. The chemical composition and sensory properties of five different fermentations of mixed cultures of *Pichia kluyveri*, *Hanseniaspora vineae*, *Hanseniaspora uvarum*, and *Torulaspora quercuum* were studied for apple juice fermentation. The results indicated that the growth of *P. kluyveri* and *H. vineae* were interreacted and affected by *H. uvarum* and *T. quercuum*. Furthermore, *H. vineae* was able to consume more sugar than *P. kluyveri*. In general, the fermentations involving *H. uvarum* displayed high pH values, whereas those involving *P. kluyveri* and the mixed *P. kluyveri* and *H. uvarum* resulted in high levels of residual sugar, sugar/acid ratio, and glucose-fructose consumption ratio. The pair *P. kluyveri* and *H. uvarum* produced the highest concentration of glycerol. Noticeable variations in organic acids and polyphenols were observed between the different fermentations. The analysis showed that acetate esters contributed the most positively to the roasted and cooked aroma note in all ciders. This was the first study evaluating the simultaneous fermentation of two non-*Saccharomyces* yeasts to produce cider. A recent study described the antagonistic and fermentative properties of *Starmerella bacillaris*. The yeast proved to positively modulate cider volatile profile in the microfermentation trials [50]. *Brettanomyces*, *Kluyveromyces*, *Schizosaccharomyces*, *Torulaspora*, *Zygosaccharomyces*, and *Saccharomycodes* have a negative influence on the product [51]. *Brettanomyces* may produce 2-ethyltetrahydropyridine, 2-acetyltetrahydropyridine, and 2-acetylpyrroline, causing taste defects and unpleasant smell in beverages.

Non-*Saccharomyces* yeasts have high enzyme activity such as β -glucosidase, esterase, and β -lyase. This enzyme activity contributes to a higher concentration of terpenes and thiols that may add a positive fruity aroma and fragrance to the fermented product [52][53][54][55]. De Arruda Moura Pietrowski *et al.* [56] and Wosiacki *et al.* [57] noted that the strains of *Hanseniaspora* sp. have a positive impact on the aromatic profile of cider, thereby accentuating the beneficial role of these yeasts. Nowadays, modern oenology is searching for novel strategies to reduce the final ethanol content in fermented beverages. This trend is due to consumer demand for products with lower ethanol content. The use of non-*Saccharomyces* species reduces the initial ethanol content by approximately 1–2% (v/v), depending on the yeast species and fermentation conditions [58][59][60]. In addition, these yeasts can be used to regulate the acidity of drinks [61][62] as *Saccharomyces* yeasts have no significant influence on acidity [63][64], and conventional chemical methods consist of the addition of expensive and qualified products being of food quality.

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