# **Apple Cider Sensory**

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Keywords: apple cider ; fermentation ; volatile compounds ; sensory profile

### 1. Introduction

Apple cider and pear cider are defined as alcoholic beverages with an alcohol content between 1.2% and 8.5% (low-alcohol cider may have less than 1.2%) obtained by partial or complete fermentation of juice (fresh or reconstituted), with or without the addition of sugar, water or flavouring <sup>[1]</sup>.

According to historical sources, cider began to be obtained at the same time as beer and wine. In Greek and Roman literature (about 900 BC) there is a wide reference in terms of obtaining fermented beverages from apples, and other fruits <sup>[2]</sup>. Many fermented drinks known since antiquity have been obtained from apples and pears. Shekar, a fermented drink derived from apples, is consumed by Jews; Sikora, an alcoholic beverage-specific to Greece, is made from boiled apples, crushed and then fermented <sup>[3]</sup>; Chicha, in its apple-based version specific to Patagonia, is a low alcoholic fermented beverage <sup>[4]</sup>, and Soor is an alcoholic beverage prepared by Himalayan traditional people from either fruit, such as apples, or cereals <sup>[5][6]</sup>. Roman sources mention that when England was conquered by the Roman Empire, the natives of those lands consumed fermented beverages from apples <sup>[7]</sup>.

Global cider production is constantly growing. The world's most important cider consumption areas are Western Europe (55.7%), Africa and North America (12% each), Australia (8%) and Eastern Europe (6.4%) <sup>[8]</sup>.

The UK is by far the world's cider consumption leader. Considering Eastern European countries, the Czech Republic, Romania and Slovenia recently reached the highest increase in cider consumption of 121.13%, 117.6% and 53.68%, respectively. According to 2018 statistics, more than 1 million tons of apples were processed worldwide only in the cider industry. Half of them were specific varieties, sweet and bitter, especially intended for cider production, grown mostly in countries such as: Great Britain, France, Ireland and Belgium <sup>[9]</sup>.

The cider assortments vary from dry to sweet, from low alcohol content to a concentration of 8–9% ABV (alcohol by volume), and include aromatic ciders with the addition of fruit juice or flavours or even 'ice ciders', obtained by fermentation of juice or frozen apples <sup>[10]</sup>.

Depending on consumers preferences, the sensory profile of cider tends to be extremely different from one country to another. In France, a robust and fruity aroma is appreciated, reflecting the strong characteristics of the sweet and sour apples used as raw material <sup>[11]</sup>. Cider with higher alcohol content is usually dry, whereas the one with a lower content is naturally sweet, due to the presence of residual sugars (soft cider: 1-5% ABV; strong cider is above 5-8% ABV) <sup>[12]</sup>. Aromatic ciders gained increasing popularity, lately. The Germans prefer the classic, wine-like, golden-yellow, slightly carbonated cider. It is usually sold as draught cider, and new trends have led to a diversification of the existing range of cider on the market; flavoured or mixed with fruits with a mild and refreshing taste <sup>[13]</sup>. In Spain, the traditional cider with light acetic nuances similar to wine, strongly carbonated remains the favourite by consumers <sup>[14]</sup>. The British, however, have a very diversified range, with niche producers covering all consumer preferences. If the average alcohol content of cider is 4–6%, in the UK it can reach up to 8.4% <sup>[11]</sup>.

## 2. Apple Varieties for Cider-Processing

According to European Cider and Fruit Wine Association, apples for cider are classified into four broad categories: sour, bitter sour, bittersweet and sweet. The main criteria for the classification of apples are their acidity, which gives the astringent flavour <sup>[15]</sup>, phenolic compounds, which impart the bitter taste <sup>[16]</sup> and sugar content, which determines the alcoholic concentration of cider <sup>[17]</sup>. <u>Table 1</u> shows a classification of some apple varieties according to these criteria, with exemplary values of sugar content, titratable acidity and total phenolic content.

**Table 1.** Classification of cider apples in terms of sugar content (°Brix), titratable acidity (TA) and total phenolic content (TPC), with typical examples.

Class	Chemical Composi	tion Based on Cide					
	Variety	Sugar Content (°Brix)	TA (%w/v)	TPC (%w/v)	— TA Range (%w/v)	TPC Range (%w/v)	References
Sour	Golden Russet	17	0.55	0.04	>0.45 <0.2		[2]
	Baldwin	11.4	0.74	0.06		<0.20	[2]
	Roxbury Russet	15.2	0.71	0.06			[2]
	Cox's Orange Pippin	13	0.6	0.07			[18]
	Bramley's Seedling	12.2	0.85	0.08			[19]
	Raxao	12.5	0.6	0.1			[20]
	Judor	-	-	0.11			[21]
Bitter sour	Kingston Black	12.6	0.58	0.19	>0.45	>0.20	[22]
	Foxwhelp	12.6	1.91	0.22			[22]
	Meana	-	0.5	0.3			[2]
	Kermerrien	13.6	-	0.38			[23]
Bittersweet	Coloradona	-	0.1	0.2			[2]
	Michelin	12.6	0.25	0.23			[24]
	Binet Rouge	10.9	0.15	0.24			[25]
	Somerset Redstreak	-	0.19	0.28	<0.45	>0.20	[21]
	Tremletts Bitter	12.4	0.27	0.38			[2]
	Dabinett	14.9	0.18	0.43			[2]
	Yarlington Mill	13.5	0.22	0.46			[22]
Sweet	Duron Arrores	-	0.3	0.1			[2]
	Sweet Alford	15	0.22	0.15	<0.45	<0.20	[22]
	Bedan	14.4	-	0.34			[24]

Several apple varieties were tested for cider processing, such as Guillevic <sup>[26]</sup>, Durona de Tresali, Limón Montés, Perico, Verdialona, de la Riega, Raxao and Regona <sup>[10]</sup>, McIntosh, Gala, Golden Delicious, Red Delicious, Red Rome, Fuji and Granny Smith <sup>[27]</sup>, Marie-Ménard and Petit Jaune <sup>[28]</sup>. Among the dessert apple varieties, the most studied were Pink Lady, Red Delicious and Royal Gala <sup>[29]</sup>, Red Delicious, Pink Lady, Bulmer's Norman and Sturmer <sup>[30]</sup>, Cox, Egremont Russet and Ashemead's Kernel <sup>[31]</sup>.

The chemical composition, namely sugar content, of apple juice, prove its authenticity and its sensory and nutritional properties <sup>[32]</sup>. It is an important factor when deciding the coupage of apple juice varieties to obtain specific cider assortments. Organic acids are important constituents of apple cider as they greatly influence their sensory profile <sup>[33]</sup>. The mineral content of apple juice, with potassium as the most abundant mineral (<u>Table 2</u>), is influenced by variety, ripening stage and the use of some fertilisers <sup>[34]</sup>.

#### **Table 2.** The average composition of cider apple juice.

ttribute	Units	Values	References
Sugars	(g/L)	≈125	[35]
Glucose	(g/L)	14–22	[35]
Fructose	(g/L)	24–65	[35]
Sucrose	(g/L)	14–32	[32]
Sorbitol	(g/100 mL)	0.2–1.0	[36]
Starch	(g/L)	7.5–8.5—unripe apples 2–2.5—ripe apples not detected—stored apples	[37]
Organic acids			
Malic	(g/L)	2.5-4.9	[33][38]
Ascorbic	(mg/L)	800-1100	[38]
Succinic	(mg/L)	420–600	[ <u>33][38]</u>
Oxalic	(mg/L)	150-240	[ <u>38]</u>
Tartaric	(mg/L)	5–7	[38]
Fumaric	(mg/L)	3.5–5	[ <u>38]</u>
Folic	(µg/L)	60–75	[39]
Quinic	(mg/L)	1202	[33]
Pyruvic	(mg/L)	31	[33]
Citric	(mg/L)	343	[33]
Amino acids			
Aspartic acid	(mg/L)	1.2-5.6	[40]
Glutamic acid	(mg/L)	1–3.3	[40]
Serine	(mg/L)	0.1–0.89	[40]
Histidine	(mg/L)	0.31-0.77	[40]
Glycine	(mg/L)	0.03-0.12	[40]
Arginine	(mg/L)	0.26–1.0	[40]
Alanine	(mg/L)	0.22–1.7	[40]
Tyrosine	(mg/L)	0.66–1.4	[40]
Methionine	(mg/L)	0.83–1.4	[40]
Valine	(mg/L)	0.59–1.8	[40]
Phenylalanine	(mg/L)	2.7-13	[40]
Isoleucine	(mg/L)	1.3-2.1	[40]
Leucine	(mg/L)	1.1-1.8	[40]
Lysine	(mg/L)	0.33–0.6	[40]
Minerals			
Potassium	(mg/L)	374–1568	[34]
Phosphorus	(mg/L)	11-76	[34]
Calcium	(mg/L)	69–194	[34]
Magnesium	(mg/L)	27–56	[34]

Attribute	Units	Values	References
Copper	(mg/L)	4.58–1.1	[ <u>34</u> ]
Iron	(mg/L)	0.9–11	[34]
рН		3.3–3.8	[33][40]
Pectin	(g/100 mL)	0.1–1.0	[40]
YAN	(mg/L)	9–249	[41][42][43]

The amine nitrogen content of apple must impact the fermentation rate as it is an important factor for yeast multiplication. The higher the total nitrogen content is, the higher the yeasts population will be <sup>[44]</sup>. Most of the performed studies show that for an efficient and complete fermentation in winemaking, there must be a minimum concentration of 140 mg/L YAN (yeast assimilable nitrogen) and, as a recommendation, the concentration must be between 200 and 300 mg/L YAN. <sup>[45]</sup> <sup>[46]</sup>. In terms of cider production, studies have shown that apple juice is deficient in YAN (usually under 100 mg/L) <sup>[41]</sup> compared to wine production standards <sup>[45][47]</sup>. The composition of apple juice for cider processing is presented in <u>Table 2</u>.

Cider makers should focus on the following apple juice characteristics to optimise cider quality and flavour: lower pH, higher titrable acidity and polyphenols content, moderate to higher YAN <sup>[48]</sup>.

The use of concentrated apple juice may be considered efficient for cider-processing, but some nutrients addition might be needed to assure yeast vitality during fermentation <sup>[49][50]</sup>. Overall, the use of the concentrate could be considered efficient for cider fermentation, although some nutritional supplementation might be required to support the vitality of yeast.

Apple varieties have different chemical characteristics (Table 1 and Table 2), which influence the sensory profile of the finished product. Blending can take place in many phases of the cider production process. This process consists of mixing several varieties of apples or juices and aims to adjust the acidity, bitterness, astringency, sweetness, alcohol concentration, colour and flavours. Apple juices with a pH higher than 3.8 should be brought below this value, and this can be done by blending with other juices with low pH. Blending is the main factor in maintaining the consistency and quality of cider used by large producers on an industrial scale <sup>[2]</sup>. The specific varieties used for cider production differ from one region to another. For example, in Spain, among the varieties recommended in cider production are Blanguina, Cristalina, Coloradona, Collaos, Marilena, Perezosa, Regona, Prieta, Raxao, Solarina, Teorica [2][51]. In Spain, Asturian and Basque apples are the most popular for obtaining cider. There is an old tradition mentioned since the 8th century <sup>[Z]</sup>. In France, the most popular apple varieties used in cider production are the following: Avrolles, Binet Rouge, Bedan, Bisquet, Cidor, Douce Moen, Douce Coet Ligne [2][52]. This cider is mainly obtained from bittersweet and bitter-sharp apple varieties. As a general appreciation, French cider is considered medium to sweet, with a fruity aroma, and the influence of malolactic fermentation is subtler than English cider [53]. Certain varieties of apples are used in the production of French traditional cider, which has a different taste compared to that of dessert apples. The latter is slightly acidic, and the concentration in phenolic compounds is low unlike the apples most commonly used for cider production, in which phenolic compounds are found in concentrations even ten times higher [26].

In the production of cider, of course, two or more varieties of apples can be used. This blending contributes to obtaining ciders with specific flavours. The UK, with a rich history of cider production, also has apple varieties with a long tradition: Broxwood Foxwhelp, Blumers Foxwhelp, Bramley's Seedling, Brown's Apple, Backwell Red, Court Royal, Dymock Red, Cox Orange Pippin, Crimson King, Morgan Sweet, Sweet Alford <sup>[7][54]</sup>. This cider is generally dry, having a complex aroma profile, notable for its high tannin content <sup>[53]</sup>. With globalization, cider has become increasingly popular in the United States. Among the common US apple varieties used in cider production are Northern Spy, Golden Russet, Baldwin and Roxbury Russet <sup>[7]</sup>.

There are two categories of apple cider: standard and special cider. Standard cider refers to cider obtained from apple juice, without the addition of flavours or other fruits. The only ingredient allowed to be added is sugar, but only within certain subcategories, with the role of regulating the level of carbohydrates needed for fermentation or raising the sweetness in the fermented cider <sup>[53]</sup>.

Speciality cider consists of the drink obtained by adding other fruits (the combination of apple and pear juice, berries) or herbs (ginger, cinnamon, nutmeg, lemongrass), by adding sugar, sweeteners or honey (if the character cider remains dominant). Fermented and aged cider in barrels, which have acquired aromas specific to wood, are also part of this category. Ice cider consists of obtaining cider by concentrating the juice for fermentation by freezing apples or freshly squeezed juice, to eliminate water. No additives are allowed to be added to obtain this cider speciality <sup>[53]</sup>.

Consistent with the residual sugar present in cider, five classes have been established: dry, semi-dry, medium, semi-sweet and sweet. The last two classes of cider must contain a significant amount of residual sugar. In this case, the fermentation process must be stopped at a certain time, well determined, or cider can be sweetened afterwards (if the law allows this procedure) with apple juice, paying special attention to the re-fermentation process, which should not occur <sup>[54]</sup>.

Future apple orchards for cider must be sustainable and resilient, and pesticide dependence must be reduced. Pesticides and fungicides can occur in the pulp and juice of fruits if they are not degraded naturally. Moreover, the concentration of residues increases during technological processes and is higher in juice than in fruit <sup>[55]</sup>. Yeast activity can also be affected by pesticides. In addition to the risk they have on the health of the consumer, the presence of residues also harms the quality of the fermented beverages <sup>[56]</sup>.

These characteristics are intended to be obtained without adverse effects on fruit production, in terms of their quantity and quality. Also of great importance is the development of new varieties such as Dabinett, Gala, Lis Gala, Fuji Supreme, which are adapted to climate change and produce quality fruit <sup>[34][58]</sup>.

The different varieties of cider apples have different ripening times, therefore they are sometimes harvested separately <sup>[59]</sup>. After harvest, the fruits can be stored for a certain period to ripen. During storage, the additional formation of sugar and flavouring compounds is allowed. At the same time, the fruits become softer, which facilitates the next process to which they are subjected, namely crushing (crushing or grinding) <sup>[2]</sup>.

### **3. Impact of Processing to Cider Microbial Populations**

#### 3.1. Apple's Microbiota and Pre-Fermentative Treatments

Cider can be obtained by spontaneous fermentation of *Saccharomyces* (83% of total yeast population) and non-*Saccharomyces* (13% of total yeast population) yeast species present on the surface of the apples  $\frac{[60]}{1}$ . The common non-*Saccharomyces* yeast species are *Hanseniaspora*, *Brettanomyces* and *Dekkera*  $\frac{[61]}{1}$ . Other predominant species that can reach levels of 3.6–7.1 log CFU/g are *Candida sake* and *Pichia fermentans*  $\frac{[62][63]}{1}$ . Also, the diversity of yeast types present in apple juice is closely related to the geographical area of the orchards, climatic conditions, fruit variety  $\frac{[64]}{1}$ , water used for irrigation, storage period and conditions  $\frac{[65]}{1}$  and the processing equipment  $\frac{[66]}{1}$ .

In the industrial cider-making, selected yeasts are used, and the spontaneous flora is inactivated by the addition of sulphur dioxide (SO<sub>2</sub>) <sup>[66]</sup>, which possesses bacteriostatic, antifungal and antioxidant properties and at SO<sub>2</sub> concentrations above 50 mg/L contributes to slowing down the fermentation process <sup>[67][68]</sup>. Furthermore, yeast strains (of the genus *Saccharomyces*) are resistant to this compound, thus avoiding competition from other microorganisms and helping the fermentation process <sup>[36]</sup>. In Europe, existing legislation allows the addition of sulphite in apple must or in cider up to 180 mg/L <sup>[69]</sup>, which is a quite low level compared to Brazil, where the addition of up to 350 mg/L SO<sub>2</sub> is allowed <sup>[66]</sup>. Given the high temperatures at the time of processing cider (mean temperature of 25 °C) in Brazil, SO<sub>2</sub> addition is essential otherwise cider is exposed to contamination risks in different processing stages. Contrarily, the addition of SO<sub>2</sub> creates health issues especially to sulphite-sensitive people that might be exposed to reactions similar to ones created by food allergies <sup>[69]</sup>. In European countries, such as France and Spain, where natural fermentation is used, the addition of SO<sub>2</sub> is rare.

Besides, when sanitising the fruits with chlorine and washing water, a strong oxidising effect is produced on a wide range of microorganisms  $\frac{[66][70]}{1}$ . This method is effective when using a concentration of 50–200 mg/L of chlorine in water and applied for 5 to 20 min  $\frac{[71]}{1}$ . Among the disadvantages of using this method of sanitization in high concentrations (up to 250 mg/L) is the loss of fruit aroma  $\frac{[66]}{1}$ .

The surface of apples is also a yeast-rich environment and can contain up to 7.1 logs CFU/g [62].

Horticultural practices may also impact fruit microorganisms <sup>[72]</sup>. Patulin is a mycotoxin produced by *Penicillium expansum* <sup>[73]</sup>. Mould is a major problem for apples and apple products, including cider. This mould generally affects damaged or fallen apples but could infect apples also during storage or processing <sup>[74]</sup>. Apples and apple products are the main sources of patulin in the human diet <sup>[75]</sup>. It was shown that patulin was not found in fresh apple juice obtained from fruits harvested directly from the tree, compared to that obtained from apples harvested from the ground, where it was detected up to 375 µg/L. Also, if ground-collected apples are washed, a 10% to 100% decrease in the level of patulin in the juice can be achieved, depending on the initial level of mycotoxin and the type of solutions used for washing <sup>[72]</sup>.

In the case of obtaining a traditional, artisanal cider, spontaneous fermentation is triggered by yeasts of the genus *Saccharomyces*, which are predominant throughout the process. In the first stage of the process, species belonging to other genera are also encountered, such as *Candida*, *Hansenula*, *Hanseniaspora*, *Kloeckera*, *Metschnikowia* and *Pichia* [76].

The microbiological composition of apples is closely related to pH, acidity and Brix degrees. Even the type of press can influence the type of microorganisms present in the apple juice. The use of the pneumatic press (pressing cycle—8 h) has been shown to determine the presence of the genera *Hanseniaspora* and *Metschnikowia*. The traditional pressing method (slow pressing cycle (3 days) with a mechanical press) leads to the presence of *Saccharomyces* and non-*Saccharomyces* yeasts when spontaneous fermentation was tested <sup>[76]</sup>. The slow pressing cycle enabled the development and growth of the fermentative yeasts coming from the pressing equipment. Usually, the non-*Saccharomyces* yeasts are present only at the initial phases of the fermentation. Still, when the fermentation is conducted slowly, with no SO<sub>2</sub> addition and with sugar content lower than 110 g/L, the low yield of alcohol permits also the presence of apiculate yeasts at the final fermentation stages.

Non-Saccharomyces microorganisms influence also volatile compounds, which increase the complexity of the sensory profile. Using *Wickerhamomyces anomalous* and *Wickerhamomyces saturnus* together with *S. cerevisiae* resulted in a cider of greater sensory complexity <sup>[77]</sup>[78]. Also for this purpose, the following yeasts used together with *Saccharomyces* are of particular importance: *Torulaspora delbrueckii*, *Hanseniaspora osmophila*, *H. uvarum*, *Starmerella bacillaris* and *Zygosaccharomyces bailii* <sup>[79]</sup>. The 3 days of apple juice fermentation with *W. anomalus* and *S. cerevisiae* was able to improve the cider quality compared to a single yeast strain fermentation. Among the volatile compounds formed in notable amounts in mixed culture fermentation were: iso-amyl acetate, ethyl hexanoate, ethyl octanoate, ethyl laureate, ethyl decylate, 3-methyl butyl pentadecanoate, isopentyl hexanoate, isoamyl alcohol, isobutanol, 1-hexanol, nerolidol, hexanoic acid, nonanal and eugenol <sup>[78]</sup>.

Given the diversity of microorganisms found in the raw material, the presence of pathogenic bacteria and toxic byproducts such as mycotoxins and biogenic amines is possible <sup>[80]</sup>.

Until the last decades, apple juice and cider were considered safe in terms of pathogenic microorganisms, due to the high acidity (pH 3.0–4.0), and alcohol content. However, over time, cases of disease associated with these products have been identified. Therefore, certain bacteria and viruses can survive acidic conditions and remain infectious <sup>[81]</sup>. Thus, consumption of unpasteurised cider and apple juice has been associated with infection with *Escherichia coli* O157: H7, *Salmonella* spp., *Cryptosporidium* spp., *Trypanosoma cruzi* and hepatitis A <sup>[81]</sup>.

In addition to common procedures for fruit sanitization and pasteurization of apple juice or cider, there are other methods, less common but efficient in terms of reducing or inactivating microorganisms. Biocontrol of the activity of fungi that produce mycotoxins or have pathogenic characteristics is one of these methods <sup>[75]</sup>.

*Starmerella bacillaris* can be successfully used with *S. cerevisiae* in cider production. A cell concentration of  $3.0 \times 10^5$  CFU/g and a similar growth degree in the first 24 h of fermentation contribute to a significant increase in glycerol and residual sugar content. Glycerol plays an important role in cider and wine processing, by assuring the fullness of taste <sup>[75]</sup>.

#### 3.2. Cider Fermentation

Must fermentation begins when the temperature exceeds 10 °C. Even if the selected yeast has not been added to the apple juice, the non-pasteurised apple juice contains a certain amount of yeasts: *Zygosaccharomyces rouxii* <sup>[82]</sup>, *S. cerevisiae*, *Leuconostoc oenos*, *Candida stellata* <sup>[83][84]</sup>. After the pasteurization process, the fresh juice obtained can contain up to  $10^6$  CFU/mL <sup>[36]</sup>. During the whole fermentation process alcoholic and malolactic fermentation (MLF) occur. The predominant species that are active in alcoholic fermentation are *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. These are part of the spontaneous flora or can be selected yeasts, specific to the fermentative substrate and the type of finished product to be obtained <sup>[63]</sup>.

The fermentation process involves not only the metabolism of carbohydrates by yeasts and the production of ethanol and carbon dioxide but also the formation of hundreds of compounds that contribute to the flavour of the finished product <sup>[77]</sup>. MLF of cider, as in the case of wine, has the same main purpose, namely, to improve the sensory characteristics and define the flavours of the finished product. Therefore, MLF contributes to the sensory characteristics of cider, through the formation of volatile compounds: alcohols, carbonyls, esters and fatty acids <sup>[85]</sup>.

Large-scale cider production requires the apple juice to have the same physicochemical characteristics so that cider also has constant sensory characteristics from one batch to another. In this case, before the fermentation process begins, various compounds can be added to the apple juice. For example, fermentable sugar (glucose syrup) can be added up to

a certain level, so that the final alcoholic concentration is the one desired by the manufacturer (in some cases it can be as high as 15% if the cider is diluted before packaging) <sup>[36]</sup>.

The concept of inoculating the fermentation of pure yeast beverages was introduced in 1890. Today, yeast companies market a wide variety of dehydrated cultures from different strains of *S. cerevisiae*. The most popular types of selected yeasts available in the market are obtained by lyophilization and contain yeast strains that have been selected by producers from various successful natural fermentations. Thus, successful large-scale fermentation is allowed, and specific yeasts can be chosen for each fermentative substrate or the desired quality of the finished product <sup>[86]</sup>.

Under anaerobic conditions, yeast can convert sugars into carbon dioxide, ethanol, heat and energy, recovering less of the energy stored in the molecules of the substrate. The final ethanol concentration depends on the initial sugar concentration in the apple juice, as well as on the fermentation temperature. Some ethanol molecules are lost during rapid fermentation at higher temperatures <sup>[36]</sup>.

The main sugars present in apple juice (fructose, glucose and sucrose) are metabolised glycolytically, obtaining pyruvate. In yeast, under fermentation conditions, pyruvate is decarboxylated into acetaldehyde and further reduced to ethanol. The fermentation rate and the amount of alcohol produced from the sugar molecule are of considerable commercial importance. During glycolysis, one molecule of fructose or glucose produces two molecules of ethanol and two of carbon dioxide. However, the theoretical conversion of 180 g of sugar into 92 g of ethanol (51.1%) and 88 g of carbon dioxide (48.9%) is ideal. In the case of fermentation under normal conditions, about 95% of sugars are converted into ethanol and carbon dioxide, 1% into cellular material and 4% into various chemical compounds (e.g., glycerol) <sup>[73]</sup>.

The energy obtained from fermentation from nutrient degradation is transported to cells as ATP (adenosine triphosphate). When phosphate groups are removed from ATP to produce ADP (adenosine diphosphate), 7.3 kcal of energy is released per mole of a compound, and some of this energy is used for cellular activities (transport of substances inside the cell, movement or synthesis). The rest of the unused energy is dissipated in the form of heat <sup>[87]</sup>.

The first step in alcoholic fermentation is the transport of sugars into the cell. This can be done in one of three ways: simple broadcast facilitated or mediated by a carrier, or by active transport. Fructose and glucose are transported by facilitated diffusion, a process that requires energy consumption. Sucrose cannot be metabolised directly by yeast, and this disaccharide is hydrolysed outside the cell by an excreted enzyme, namely invertase. The monosaccharides resulting from the hydrolysis of sucrose (glucose and fructose) are transported to the cell <sup>[88]</sup>.

The most common route of glucose and fructose catabolism is glycolysis. This pathway is active in both fermentative and respiratory metabolism. Glycolysis consists of 10 steps and each step is catalysed by a specific enzyme. The carbon skeleton of the carbohydrate is gradually dismantled during this process <sup>[88]</sup>. Yeast flocculation is a physical process of great importance in the manufacturing of cider. During flocculation, the yeast cells agglomerate and settle rapidly in the medium, or are entrained by carbon dioxide and rise to the surface. This process is essential for yeast recovery and clarification of the fermentation medium <sup>[89]</sup>.

MLF refers to the conversion of malic acid into lactic acid and  $CO_2$ , under the action of lactic acid bacteria. This conversion is a decarboxylation and leads to a decrease in acidity and an improvement in the stability and flavour of the cider <sup>[90]</sup>. It is known that the production of cider is very similar to that of wine because the fermentable substrate has similar components and the processing techniques are similar <sup>[91]</sup>. MLF generally begins immediately after the completion of alcoholic fermentation or in the final stages, depending on the temperature, acidity and amount of nutrients present. Various genera of bacteria (*Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Oenococcus*) are responsible for MLF. These microorganisms can grow in a medium with low pH (<3.5) and alcoholic concentration <sup>[2][90]</sup>.

MLF is considered by cider producers in France and the United Kingdom as part of the maturation process. Cider subjected to MLF makes an important contribution to the complexity of flavours, compared to the cider that has not been subjected to this fermentation <sup>[92]</sup>. As with wine, MLF leads to a large number of chemical compounds in cider, resulting from the metabolism of bacteria. A concrete example of this is demonstrated by Zhao et al. identifying 51 flavour compounds after alcoholic fermentation and MLF, respectively. At the end of the alcoholic fermentation, they were found in the sample in relatively small quantities, but after the completion of the MLF process, they were found in cider about 200% more. The value given above includes the average of the 51 chemical compounds, and it is noteworthy that only 5 of them were in a smaller amount in cider after MLF <sup>[2]</sup>.

Also during MLF, citric acid metabolises into diacetyl, which at concentrations of about 5 mg/L provides a buttery flavour, but above this value, the flavour turns into that similar to rancid butter. The action of malolactic bacteria leads to the formation of acids, alcohols, esters and phenols. They have a notable influence on the aroma of the finished product,

giving it a fruity, spicy character  $[\underline{2}]$ .

#### 3.3. Advanced Methods Applied for Cider Fermentation Monitoring

There are advanced methods that allow the monitoring of the fermentation process. Villar et al. applied the Vis-NIR system (400–1100 nm) to control alcohol concentration, lactic acid content, amount of glucose and fructose and acetic acid. This process involved the correlation of the spectra obtained by the Vis-NIR sensor system with the cider quality parameters and allowed greater control of the fermentation process and the possibility to take corrective measures in real-time. The sensor system is easily adapted for fermentation vessels, providing real-time results and operators do not require advanced training skills <sup>[93]</sup>. Another method successfully used in monitoring the fermentation process was performed using nuclear magnetic resonance (NMR) spectroscopy <sup>[94]</sup>. By this method, the evolution of the compounds was followed throughout the technological process (was examined the fresh apple juice, during the fermentation process, at the end of the fermentation and the cider) <sup>[95]</sup>. A large part of the compounds of interest could be followed during fermentation. Some of them changed into secondary compounds, others increased (organic acids, amino acids, antioxidants) and others disappeared completely (histidine) <sup>[95]</sup>.

The most commonly used analytical techniques for determining the flavour profiles of fruits are chromatographic techniques, especially gas chromatography. Due to the complex composition of the fruits, the analytes must be isolated before being introduced into the chromatographic system. The most used techniques are the following: solvent extraction, steam distillation, supercritical fluid extraction, static headspace/dynamic headspace analysis, liquid-liquid microextraction [96].

Immobilization of cells in alcoholic fermentation involves several technical and economic advantages compared to the conventional free cell system. The following can be listed as advantages: prolonged activity and stability of immobilization cells, because the immobilization support can act as a protective agent against physicochemical changes (pH, temperature, heavy metals, solvents, etc.,); higher-than-usual cell densities, leading to higher productivity and higher substrate absorption and yield; increased tolerance to higher substrate concentrations and inhibitory substances; reduced risk of microbial contamination and increased fermentation activities; fermentation capacity at low temperature/maturation; regeneration and reuse capacity; reduced maturation time in certain circumstances <sup>[97]</sup>.

For the industrial production of wine and cider, it is important to identify adequate support for cell immobilization, resulting in the advantages mentioned above and the general improvement of the sensory characteristics of the finished product. In the case of cider fermentation, *S. cerevisiae* and *L. plantarum* were immobilised on a sponge-like material. Subsequent fermentation with immobilised yeasts and sequential addition had a positive effect on the development of flavours, enhanced the rate of fermentation and accelerated cider production and maturation <sup>[97][98]</sup>.

#### 3.4. Cider Contaminants Affecting Fermentation

Fungicide residues on the fruit can adversely affect the yeast, which can slow down or block the fermentation process. The fungicide residues could advertently contribute to the production of hydrogen sulphide, an undesirable compound due to its unpleasant aroma. The use of sulphur-based chemicals as a fungicide leads to an increase in hydrogen sulphide in fermented cider but also other fungicides, namely fenbuconazole and fludioxonil, may affect the fermentation process to some extent <sup>[99]</sup>.

Hydrogen sulphide production is also influenced by the nitrogen concentration that can be assimilated by yeasts. It has been shown that hydrogen sulphide production is diminished with the addition of amino acids to apple juice. Sensory differences were observed in cider samples with methionine supplementation (5 mg/L), and these were correlated with lower hydrogen sulphide production  $\frac{1200}{1}$ .

In unpasteurised apple juice and cider, there is a risk of the presence of microbiological contaminants. The most common source of contamination with *E. coli* O157: H7 is animal faeces. This contamination is due to the exposure of apples to faeces during the growing and harvesting processes <sup>[101]</sup>. *Cryptosporidium parvum* is a pathogen commonly found in the food industry, it has also been found in various samples of unpasteurised cider. The presence of microbiological contaminants can be attributed to improper storage conditions when apples are contaminated mainly with fungi <sup>[102]</sup>, non-compliant cleaning and sanitation practices, but the main method for their destruction is pasteurization <sup>[65]</sup>.

On the other hand, the pesticide residue can decrease dramatically during the technological processes of obtaining cider. A concrete example is pyridaben, an acaricide and insecticide used in apple culture, which, although it was present in significant quantities in apples (2.10 mg/kg), was found in levels below 0.01 mg/kg in the finished cider <sup>[103]</sup>.

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