

Review

An Overview of the Factors Influencing Apple Cider Sensory and Microbial Quality from Raw Materials to Emerging Processing Technologies

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Abstract: Given apple, an easily adapted culture, and a large number of apple varieties, the production of apple cider is widespread globally. Through the fermentation process, a series of chemical changes take place depending on the apple juice composition, type of microorganism involved and technology applied. Following both fermentations, alcoholic and malo-lactic, and during maturation, the sensory profile of cider changes. This review summarises the current knowledge about the influence of apple variety and microorganisms involved in cider fermentation on the sensory and volatile profiles of cider. Implications of both *Saccharomyces*, non-*Saccharomyces* yeast and lactic acid bacteria, respectively, are discussed. Also are presented the emerging technologies applied to cider processing (pulsed electric field, microwave extraction, enzymatic, ultraviolet and ultrasound treatments, high-pressure and pulsed light processing) and the latest trends for a balanced production in terms of sustainability, authenticity and consumer preferences.

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

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 [1].

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 [2]. 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 [3]; Chicha, in its apple-based version specific to Patagonia, is a low alcoholic fermented beverage [4], and Soor is an alcoholic beverage prepared by Himalayan traditional people from either fruit, such as apples, or cereals [5,6]. Roman sources mention that when England was conquered

by the Roman Empire, the natives of those lands consumed fermented beverages from apples [7].

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%) [8].

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 [9].

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 [10].

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 [11]. 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) [12]. 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 [13]. In Spain, the traditional cider with light acetic nuances similar to wine, strongly carbonated remains the favourite by consumers [14]. 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% [11].

In this context, this review discusses the contribution of microorganisms in the fermentation of apple juice and their impact on volatile and sensory profiles of cider with an overview of the emerging technologies applied in apple cider production.

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 [15], phenolic compounds, which impart the bitter taste [16] and sugar content, which determines the alcoholic concentration of cider [17]. Table 1 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 Composition Based on Cider Apple Variety				TA Range (%w/v)	TPC Range (%w/v)	References
	Variety	Sugar Content (°Brix)	TA (%w/v)	TPC (%w/v)			
Sour	Golden Russet	17	0.55	0.04	>0.45	<0.20	[2]
	Baldwin	11.4	0.74	0.06			[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]

Table 1. Cont.

Class	Chemical Composition Based on Cider Apple Variety				TA Range (%w/v)	TPC Range (%w/v)	References
	Variety	Sugar Content (°Brix)	TA (%w/v)	TPC (%w/v)			
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	<0.45	>0.20	[2]
	Michelin	12.6	0.25	0.23			[24]
	Binet Rouge	10.9	0.15	0.24			[25]
	Somerset Redstreak	-	0.19	0.28			[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	<0.45	<0.20	[2]
	Sweet Alford	15	0.22	0.15			[22]
	Bedan	14.4	-	0.34			[24]

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

The chemical composition, namely sugar content, of apple juice, prove its authenticity and its sensory and nutritional properties [32]. 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 [33]. The mineral content of apple juice, with potassium as the most abundant mineral (Table 2), is influenced by variety, ripening stage and the use of some fertilisers [34].

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 [35]. 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. [36,37]. In terms of cider production, studies have shown that apple juice is deficient in YAN (usually under 100 mg/L) [38] compared to wine production standards [36,39]. The composition of apple juice for cider processing is presented in Table 2.

Table 2. The average composition of cider apple juice.

Attribute	Units	Values	References
Sugars	(g/L)	≈125	[40]
Glucose	(g/L)	14–22	[40]
Fructose	(g/L)	24–65	[40]
Sucrose	(g/L)	14–32	[32]
Sorbitol	(g/100 mL)	0.2–1.0	[41]
Starch	(g/L)	7.5–8.5—unripe apples	[42]
		2–2.5—ripe apples	
		not detected—stored apples	

Table 2. Cont.

Attribute	Units	Values	References
Organic acids			
Malic	(g/L)	2.5–4.9	[33,43]
Ascorbic	(mg/L)	800–1100	[43]
Succinic	(mg/L)	420–600	[33,43]
Oxalic	(mg/L)	150–240	[43]
Tartaric	(mg/L)	5–7	[43]
Fumaric	(mg/L)	3.5–5	[43]
Folic	(µg/L)	60–75	[44]
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	[45]
Glutamic acid	(mg/L)	1–3.3	[45]
Serine	(mg/L)	0.1–0.89	[45]
Histidine	(mg/L)	0.31–0.77	[45]
Glycine	(mg/L)	0.03–0.12	[45]
Arginine	(mg/L)	0.26–1.0	[45]
Alanine	(mg/L)	0.22–1.7	[45]
Tyrosine	(mg/L)	0.66–1.4	[45]
Methionine	(mg/L)	0.83–1.4	[45]
Valine	(mg/L)	0.59–1.8	[45]
Phenylalanine	(mg/L)	2.7–13	[45]
Isoleucine	(mg/L)	1.3–2.1	[45]
Leucine	(mg/L)	1.1–1.8	[45]
Lysine	(mg/L)	0.33–0.6	[45]
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]
Copper	(mg/L)	4.58–1.1	[34]
Iron	(mg/L)	0.9–11	[34]
pH		3.3–3.8	[33,45]
Pectin	(g/100 mL)	0.1–1.0	[45]
YAN	(mg/L)	9–249	[38,46,47]

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 [48].

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 [49,50]. 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 (Tables 1 and 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 [2]. 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 Blanquina, Cristalina, Coloradona, Collaos, Marilena, Perezosa, Regona, Prieta, Raxao, Solarina, Teorica [7,51]. In Spain, Asturian and Basque apples are the most

popular for obtaining cider. There is an old tradition mentioned since the 8th century [7]. 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 [7,52]. 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 [7,54]. This cider is generally dry, having a complex aroma profile, notable for its high tannin content [53]. 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 [7].

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 [53].

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 [53].

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 [54].

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 [55]. 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 [56,57].

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 [34,58].

The different varieties of cider apples have different ripening times, therefore they are sometimes harvested separately [59]. 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) [2].

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 [60]. The common non-*Saccharomyces* yeast species are *Hanseniaspora*, *Brettanomyces* and *Dekkera* [61]. Other predominant species that can reach levels of 3.6–7.1 log CFU/g are *Candida sake* and *Pichia fermentans* [62,63]. Also, the diversity of yeast types present in apple juice is closely related to the geographical area of the orchards, climatic conditions, fruit variety [64], water used for irrigation, storage period and conditions [65] and the processing equipment [66].

In the industrial cider-making, selected yeasts are used, and the spontaneous flora is inactivated by the addition of sulphur dioxide (SO₂) [66], which possesses bacteriostatic, antifungal and antioxidant properties and at SO₂ concentrations above 50 mg/L contributes to slowing down the fermentation process [67,68]. Furthermore, yeast strains (of the genus *Saccharomyces*) are resistant to this compound, thus avoiding competition from other microorganisms and helping the fermentation process [41]. In Europe, existing legislation allows the addition of sulphite in apple must or in cider up to 180 mg/L [69], which is a quite low level compared to Brazil, where the addition of up to 350 mg/L SO₂ is allowed [66]. Given the high temperatures at the time of processing cider (mean temperature of 25 °C) in Brazil, SO₂ addition is essential otherwise cider is exposed to contamination risks in different processing stages. Contrarily, the addition of SO₂ creates health issues especially to sulphite-sensitive people that might be exposed to reactions similar to ones created by food allergies [69]. In European countries, such as France and Spain, where natural fermentation is used, the addition of SO₂ is rare.

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

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 [72]. Patulin is a mycotoxin produced by *Penicillium expansum* [73]. 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 [74]. Apples and apple products are the main sources of patulin in the human diet [75]. 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 [72].

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 [76]. 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 SO₂ 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 [77,78]. 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* [79]. The 3 days of apple juice fermentation with *W. anomalous* 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 [78].

Given the diversity of microorganisms found in the raw material, the presence of pathogenic bacteria and toxic by-products such as mycotoxins and biogenic amines is possible [80].

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 [81]. Thus, consumption of unpasteurised cider and apple juice has been associated with infection with *Escherichia coli* O157: H7, *Salmonella* spp., *Shigella* spp., *Cryptosporidium* spp., *Trypanosoma cruzi* and hepatitis A [81].

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 [75].

Starmerella bacillaris can be successfully used with *S. cerevisiae* in cider production. A cell concentration of 3.0×10^5 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 [75].

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* [82], *S. cerevisiae*, *Leuconostoc oenos*, *Candida stellata* [83,84]. After the pasteurization process, the fresh juice obtained can contain up to 10⁶ CFU/mL [41]. 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 [63].

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 [77]. 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 [85].

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) [41].

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 [86].

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 [41].

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) [73].

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 [87].

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 [88].

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 [88]. 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 [89].

MLF refers to the conversion of malic acid into lactic acid and CO₂, 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 [90]. 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 [91]. 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 [2,90].

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 [92]. 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 [2].

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 [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 [93]. Another method successfully used in monitoring the fermentation process was performed using nuclear magnetic resonance (NMR) spectroscopy [94]. 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) [95]. 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) [95].

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 [97].

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 [97,98].

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 [99].

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 [100].

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 [101]. *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 [102], non-compliant cleaning and sanitation practices, but the main method for their destruction is pasteurization [65].

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 [103].

4. Changes in Sensory, Volatile and Phenolic Profiles during Cider Processing

There are not many conclusive studies on the link between different apple varieties and the volatile composition of cider, but the chemical composition of apples varies by variety, which confirms the influence of apples on the aroma and taste of the finished product. Regarding the non-volatile composition, apart from the fluctuations of the sugar concentration and acidity, the main differences between the fruit varieties, leave their mark on the phenolic content (Table 1). The level of ripeness of the fruit also has a great impact on the aromatic profile of the cider [104].

Smell and aroma are the most important quality aspects of alcoholic beverages and are essential in determining the preferences of potential consumers. The development of these attributes occurs during each stage of the production process: the selection of raw material, fermentation process, cider maturation [105].

The aroma of cider is strongly influenced by the degree of apples ripening. Cider obtained from fully ripe fruit was 24–52% (depending on the variety) more abundant in volatile compounds than that obtained from unripe fruits [34]. The yeast used in the production of fermented beverages contributes to the aromatic profile, mainly by increasing the level of alcohols and esters. These benefits are closely related to the type of yeast strains, which influence not only the diversity of volatile compounds but also the quantities found in the finished product [106].

Other factors that have a significant influence on the sensory properties of cider are the processing conditions of fruits, juice and cider. The volatile compounds of apples are quickly lost during fruit crushing. The most prone to loss are ethyl alcohol, ethyl acetate, ethyl butyrate, butyl alcohol, 2-methyl propyl alcohol [107]. The method of pressing influences the presence of volatile compounds. For example, if the pressing is done at low speed and low temperature, the acetates (butyl acetate, hexyl acetate and

2-phenethyl acetate) can be found in apple juice in higher proportions [108]. Other processes, such as filtration, centrifugation, thermal pasteurization, lead to a decrease in volatile components. Juice clarification and biomass reduction influence the volatile components of cider. Increased attention of producers is paid to the fermentation process (the type of yeast strains, inoculation time, fermentation temperature) because volatile compounds are greatly influenced by this technological step [108,109].

The most abundant volatile constituents of apples are represented by esters (78–92%), alcohols (6–16%), aldehydes and ketones. Worth mentioning that most of the aromatic compounds in apple juice are not authentic constituents of apples, but they are formed during the processing [110,111].

The traceability of volatile compounds in apple juice, either fresh or fermented 8 and 28 days, was evaluated [110]. Most of the volatile compounds, which were not initially present in apple juice, were detected in fermented juice. Few compounds (acetic acid 2-methyl butyl ester, acetic acid butyl ester, 2-hexen-1-ol, hexanal, 2-hexenal) determined in fresh apple juice were no longer present in cider [110]. A series of seven microorganisms were analysed for the fermentation capacity of apple juice (*Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Torulasporea delbrueckii*, *Hanseniaspora osmophila*, *Hanseniaspora uvarum*, *Starmerella bacillaris* and *Zygosaccharomyces bailii*). Significant differences were observed in the production of volatile compounds (alcohols, esters, fatty acids) [79]. *S. uvarum* was the yeast that produced the highest amount of higher alcohols, while *H. uvarum*, produced the least. *T. delbrueckii* was most favourable in the production of ethyl decanoate (277.4 µg/L) and ethyl hexanoate (108.5 µg/L), up to 9 times more compared to other yeasts. In cider obtained by fermentation with *Saccharomyces* yeast, fatty acids were up to 2.5 times more abundant than non-*Saccharomyces* fermented cider. Hexanoic and octanoic acids were produced in approximately equal amounts by all yeasts [79].

Amino acids in apple juice are the main source of nitrogen for yeast (*S. cerevisiae* Bouquet, 10⁶ cells/mL). It is considered that a sufficient amount of nitrogen to complete the fermentation process is 70–150 mg/L [34]. Many amino acids are intermediates or precursors of volatile compounds, especially higher alcohols [112]. Amino acids such as aspartate, asparagine and glutamate have positively influenced the production of esters in cider. The best results were obtained from the combination of aspartate (43.4%) and glutamate (56.6%). In apple juice supplemented with these two amino acids, a four-fold higher ester amount was obtained compared to the cider that was not supplemented with amino acids [113].

Volatile compounds were also analysed for fermentations with several types of yeasts. Co-fermentations of *Saccharomyces cerevisiae* were performed together with other species (*Hanseniaspora valbyensis*, *Hanseniaspora uvarum*, *Williopsis saturnus*). Although at the end of fermentation, the ethanol content was similar in all tested variants, the chromatographic analysis showed significant differences concerning the volatile profiles. The production of volatile compounds is dependent on the strains used in fermentation, so the use of distinct strains favours the formation of the desired compounds. Co-fermentation results in a more complex volatile profile and influences the aromatic characteristics of cider, thus representing a unique way to obtain different flavours [109,114].

Table 3 contains the classes of chemical compounds identified in cider, the characteristic aroma it offers, as well as the microorganisms used in the fermentation process [84].

Table 3. Volatile compounds, the corresponded odour descriptors and the microorganisms involved in the fermentation of apple cider.

Compound	Odour Descriptor	Microorganism Specie	References
Esters			
Ethyl benzoate	Floral chamomile	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109,115]
2-Phenylethyl acetate	Rose, honey	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[107,109]
Ethyl octanoate	Apricot	<i>S. cerevisiae</i>	[108,115]
Isoamyl 2-methyl butanoate	Apple	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]

Table 3. Cont.

Compound	Odour Descriptor	Microorganism Specie	References
2-methylbutyl	Apple	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
2-methylbutanoate			
Isoamyl butanoate	Pear	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
3-Methylbutyl acetate	Pear	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Ethyl 2-methyl butanoate	Berry	<i>S. cerevisiae</i> , <i>O. Oeni</i>	[116]
Ethyl pentanoate	Berry	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i>	[104]
Ethyl decanoate	Grape	<i>S. cerevisiae</i> , <i>O. Oeni</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[108,109,116]
Methyl octanoate	Orange	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Ethyl 3-methyl butanoate	Pineapple	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i>	[104]
Ethyl acetate	Pineapple	<i>S. cerevisiae</i> , <i>O. Oeni</i>	[116]
Ethyl butanoate	Pineapple	<i>S. cerevisiae</i> , <i>O. Oeni</i>	[116]
Isoamyl acetate	Banana	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[104,109]
Ethyl hexanoate	Banana	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i>	[104,108]
Hexyl acetate	Herbal	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Heptyl acetate	Earthy	-	[109]
Ethyl octanoate	Fruity, candy	<i>S. cerevisiae</i>	[105,115]
Ethyl hexadecanoate	Resinous	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[105,109]
3-methyl butyl octanoate	Coconut	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i>	[104]
Ethyl oleate	Waxy	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Ethyl tetradecanoate	Waxy, ether	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[108,109]
Butyl acetate	Sweet	<i>S. cerevisiae</i> , <i>O. oeni</i>	[116]
2-phenylethyl propanoate	Rose	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
2-phenylethyl acetate	Honey	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[104,109]
Diacetyl	Buttery	<i>S. cerevisiae</i> , <i>O. oeni</i>	[105,116]
3-hydroxy-2-butanone	Buttery	<i>S. cerevisiae</i> , <i>O. oeni</i>	[116]
2-phenylethyl acetate	Honey	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[104,109]
Acids			
Octanoic acid	Fatty, sweat	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[108,109]
Propanoic acid	Rancid	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
2-methyl butyric acid	Rancid	-	[105]
Acetic acid	Vinegar	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
9-decenoic acid	Soapy	<i>S. cerevisiae</i> , <i>O. oeni</i>	[115,116]
Nonanoic acid	Fatty	<i>S. cerevisiae</i> , <i>O. oeni</i>	[116]
Hexanoic acid	Cheesy	<i>S. cerevisiae</i> , <i>O. oeni</i>	[116]
Alcohols			
2-Phenylethanol	Rose, honey	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i>	[104,105]
Eugenol	Spicy	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[105,109]
Amyl alcohol	Malt	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
3-methyl-1-butanol	Malt	<i>S. cerevisiae</i> , <i>O. oeni</i>	[108,116]
Isoeugenol	Smoky	<i>S. cerevisiae</i> , <i>O. oeni</i>	[116]
Methionol	Sulphury, vegetables	<i>S. cerevisiae</i> , <i>O. oeni</i>	[105,116]
Octan-1-ol	Oily	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Benzyl alcohol	Sweet	-	[105]
4-ethyl guaiacol	Spicy, clove	<i>S. cerevisiae</i> , <i>O. oeni</i>	[105,116]
2-phenyl ethanol	Rose, honey	<i>S. cerevisiae</i> , <i>O. oeni</i>	[108,116]
1-octen-3-ol	Earthy	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i>	[104]
1-octen-3-ol	Mushroom	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[104,109]
Phenol	Phenol, medicinal	<i>S. cerevisiae</i> , <i>O. oeni</i>	[116]
1-hexanol	Herbaceous	<i>S. cerevisiae</i>	[115]

Table 3. Cont.

Compound	Odour Descriptor	Microorganism Specie	References
Aldehydes and ketones			
Decan 2-one	Orange	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
2,6-dimethyloct-3-enal	Green melon	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Hexanal	Grass	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Beta-cyclocitral	Mint	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Oct-1-en-3-one	Mushroom	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[108,109]
6-methylhepta-3,5-dien-2-one	Cinnamon	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
(E,E)-hepta-2,4-dienal	Nuts	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Benzaldehyde	Almond	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i>	[104]
(E)-hept-2-enal	Almond	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
3-octanone	Herbal	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i>	[104]
Methional	Rancid	-	[105]
Terpenoids and lactones			
Beta-citral isomer	Lemon	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Beta-ocimene	Herbal	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
γ -nonalactone	Coconut	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
γ -decalactone	Peach	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
γ -butyrolactone	Caramel	<i>S. cerevisiae</i> , <i>H. uvarum</i> , <i>H. valbyensis</i>	[109]
Others			
Vanillin	Vanilla	<i>T. delbrueckii</i> , <i>S. bayanus</i> , <i>S. cerevisiae</i>	[104]
Benzothiazole	Smoky	<i>S. cerevisiae</i> , <i>O. oeni</i>	[116]

The selection of appropriate lactic acid bacteria for the MLF, depending on the specific characteristics of the product, is essential, because environmental factors will interact, ultimately selecting only those strains competitive enough to drive the fermentation process. Uncontrolled MLF can in some cases harm the sensory quality of the end product, while controlled fermentation results in flavour profile improvement. This process contributes to the complexity of cider aromas, by replacement of herbaceous notes with fruity or floral ones. The profile of flavours in fermented alcoholic beverages is determined by the combined effects of several hundred different chemical compounds, at concentrations that, in some cases, can be of the order of ppb (parts per billion). The analysis of these compounds requires extremely selective and efficient, effective steps to fractionate the extracts and allow the selective separation of the aromatic compounds [85].

Polyphenols are important secondary metabolites in apples. In the case of apples intended for cider production, these compounds are involved in essential sensory characteristics, such as colour, bitterness, astringency and colloidal stability, respectively. Also, some phenolic compounds are precursors of cider flavours, the so-called volatile phenols such as 4-vinyl guaiacol, chlorogenic acid, caffeic acid, hydrocaffeic acid, 4-vinylcatechol, 4-ethylcatechol [112,117].

During the fermentation process, polyphenols can also influence important technological steps, such as clarification or fermentation. For example, tannins may act as inhibitors of pectic enzymes involved in the clarification process [118].

Polyphenols are present in the whole fruit, the main fraction being identified in the peel. The peel of apples, constitutes on average, between 6 and 8% of its weight. (+)-Catechin, (-)-epicatechin, rutin and phloridzin are found in apple peel in quantities even seven times larger than the whole fruit. In the peel of the Starkinson Delicious variety, the highest amount of polyphenols was identified (82%) [119]. The only exception in this study was chlorogenic acid, which in all apple varieties was more abundant in the pulp than in the peel [119]. When apples are processed into juices, polyphenols interact with other chemical compounds. During pressing, procyanidins can associate with insoluble polysaccharides in cell walls and can be stored for a certain period. Polyphenols also undergo biochemical changes due to enzymatically catalysed oxidation by polyphenol oxidase when apples are crushed or pressed [118].

The phenolic content of cider can be considered a key indicator of quality. Although the apple variety has a major influence on the final phenolic content of cider, a series of technological operations leave its mark on the content of these compounds in the end product. Operations such as maceration, pressing, pre-fermentation influence phenolic extraction and lead to improvements in cider quality. Maceration is the technological process in which the oxidation of the fruit takes place after crushing, before pressing. When the crushed fruits are pressed to extract the juice, the pressure applied determines the pressing fraction and therefore influences the extraction of the phenolic compounds. Higher pressure can cause the bark and seeds to crush, releasing tannins [30]. Tannins contribute to the organoleptic characteristics of cider and give a bitter taste and astringency [120,121]. Some varieties of sweet apples, which are used to make cider, are poor in tannins and do not provide astringency to cider. Therefore, some cider producers add tannin to apple juice. Following the sensory analysis, the cider obtained from dessert apples and enriched with tannins, have better sensory quality and a more complex aroma [122]. A study on U.S. ciders containing 0.04–0.11% tannin showed cider tannin content has a positive effect on consumer willingness-to-pay [123].

Maceration does not contribute to a notable increase in phenolic content when poor-quality and low phenolic content raw materials are used. However, when raw materials rich in polyphenols are macerated, the polyphenols extraction yield is also higher in apple juice and then in cider. Apples, namely apple peels, present antioxidant activity [124] directly related to the abundance of phenolic compounds [125]. The antioxidant activity of the cider obtained by maceration is higher compared to the cider without the maceration stage. Therefore, the inclusion of this operation in the technological process may be important for the concentration of bioactive compounds [126]. Prolonged maceration and excessive oxidation of phenolic compounds can lead to deterioration of the quality of juice and cider [30,127].

Moreover, polyphenols can also be involved in fermentation processes, by acting as sensory quality protectors. Phenolic compounds contribute to the colloidal stability of cider, through interaction with proteins. From a quantitative point of view, there are several classes of polyphenols identified in apple ciders: flavan-3-ols, procyanidins, flavonols (quercetin present in glycolic forms), dihydrochalcones (phloretin glycosides) and hydroxycinnamic acids and derivatives. Among the most common phenolic acids in apples are caffeic acid (present in an esterified form with quinic acid) and *p*-coumaric acid (present in an esterified form with quinic acid) [111,128].

Polyphenols influence the volatility of aromatic compounds in cider [122]. The volatility of the studied compounds (ethyl butyrate, ethyl octanoate, 4-ethylphenol, 4-vinyl phenol, 1-hexanol, 2-phenyl ethanol) is related to the spatial conformation of polyphenols and their concentration. Epicatechin, hydrocaffeic acid and phlorizin induced a decrease in volatility for most of the hydrophobic aromatic compounds studied. In general, with the increase in the concentration of epicatechin, hydrocaffeic acid and phloridzin, the volatility of esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate) was significantly affected [129].

Understanding the effects of polyphenols on aromatic compounds improves the prediction of flavour profiles by chemical analysis, which helps cider producers to increase the quality of flavours [129].

The aroma profile and sensory properties of cider are primarily influenced by the apple variety (besides yeasts strain, ripening stage, geographical area) [104,130,131]. It has been shown that each variety of apples has a unique range of volatile organic compounds, and is an important factor in obtaining good sensory characteristics and, therefore, a good cider quality [130].

A major factor influencing the quality and quantity of volatile compounds is the geographical origin of apple trees which, in turn, is related to local environmental conditions (temperature, precipitations, water and soil composition). Some chemical compounds can be classified as geographical markers. Due to their discriminating ability, these geographical markers are represented by alcohols (1-hexanol, 1-octanol), esters (methyl acetate,

1-ol acetate, ethyl hexanoate, ethyl nonanoate, ethyl octanoate) and terpenic compounds (limonene) [131,132].

The level of ripeness of the fruit can influence the volatile composition of the cider, but it is closely related to the apple varieties. Some varieties, such as Melba, influence the volatile profile by maturity level, while other varieties do not cause significant differences, having similar flavours from both ripened and over-ripened fruits [104].

Some studies have identified and classified several apple varieties according to their volatile composition [133]. The yeasts used in the cider fermentation process contribute to the aromatic profile of the finished product (Table 3).

5. Emerging Technologies Applied in Apple Cider Production

The conventional process for obtaining apple juice consists of pressing or decanting the crushed apples. In optimal conditions, apple juice yield reaches 70–80% but can decrease under 65% when apples were previously stored due to humidity loss [134].

To obtain a higher yield of juice and to reduce the processing time, some producers apply higher temperatures during crushing. These treatments, however, are always accompanied by high energy consumption, loss of juice quality by decreasing the number of vitamins, or changes in colour and flavour [135]. To increase juice yield, non-conventional treatments have been proposed, such as the application of pulsed electric fields, microwave or ultrasound, as well as enzymatic or ultraviolet treatments.

5.1. Pulsed Electric Field

The combination of pressing and treatment with pulsed electric fields (PEF) is an increasingly used technique for extracting apple juices showing positive effects in terms of yield, the release of phenolic compounds [136] and improved flavour profile [137] through an increase in esters content caused by the electroporation effect. PEF causes changes in the structure or rupture of cell membranes. PEF is proposed as an alternative to heat treatments as it is effective against pathogenic and spoiling microbial populations [138].

The effect of the pulsed electric fields in the inactivation of microorganisms in fresh apple juice was investigated in a continuous flow system. The number of microorganisms decreased with the increasing pulse, temperature (45–50 °C) and decreasing juice flow (3–10 L/h) [139].

5.2. Microwave Extraction

Apple juice extraction treatments can have negative influences on the content of flavonoids and phenolic compounds. Studies showed a decrease of phenolic compounds by more than 58% [140] and up to 90% decrease in antioxidant activity during conventional juice processing. Even juice clarification harms the phenolic and nitrogen content of apple juice [141], this procedure does not affect the quality of the finished cider [142].

Juice extraction was more efficient when microwave treatment was applied [143]. Moreover, microwave treatment at 40 °C and 60 °C increased the extraction of phenolic and flavonoid compounds. The soluble solids and the degree of turbidity also increased with the increase of the temperature of the apple puree. No sensory differences were encountered between the tested methods. Therefore, microwave treatment on apple pulp and peel may lead to a higher-quality juice with a high content of the aforementioned compounds. Heating to 60 °C had the best results, with maximum yields in the extraction of juice and phenolic compounds [143].

5.3. Enzymatic Treatment

Enzymatic treatment can increase the extracted juice yield compared to cold and hot extraction procedures [144].

Pectins, in terms of their chemical form, are classified as soluble or insoluble fibres. Degradation of pectin by enzymatic action leads to a decrease in the viscosity of the

raw juice, which increases the extraction yield. Pectic substances can be classified into several classes: galacturonic (polymers of galacturonic acid), rhamnogalacturonan (mixed polymers of rhamnose and galacturonic acid), arabinans (polymers of arabinose), galatians (polymers of galactose) and arabinogalactans (polymers). The pectolytic enzymes can hydrolyse the pectic substances that are present in the fruit; the resulted juice has a much lower amount of pectin [145,146]. Pectinases are widely used in the cider industry for clarification without affecting the concentration in polyphenols [142].

The extraction of fruit juice with the help of enzymatic treatments is a process that must be optimised in terms of temperature, time and enzyme product concentration, to maximise the yield and quality of the end product and increase the phenolic content of the treated juice [146]. The addition of enzymes to apple juice is a two-stage process. First, crushed apples are treated with pectinases to ease the obtaining of juice, then the liquefaction of apple pomace is carried out with the mixture of pectinases and cellulases, for the complete extraction of the juice [147].

Demethylation of pectin carboxyl groups and free pectin acids may increase the titrable acidity. In terms of volatile profile, according to the demethylation process, the release of 500 mg/L methanol during enzymatic treatment could raise the titratable acidity level by 1.5 g/L [146].

5.4. Ultraviolet Treatment

Ultraviolet (UV) technology is an alternative method of pasteurization and extending the shelf life of beverages. Various studies have shown that UV treatment has a germicidal effect, and compared to thermal pasteurization methods, it has minimal effects on juice quality [148–151]. However, the effects of UV-C on the physicochemical characteristics and nutritional composition of juices cannot be overlooked [152]. This bactericidal mechanism is based on the absorption of UV-C light by the microbial DNA or RNA. The main mechanism is the creation of pyrimidine dimers that prevent the replication of microorganisms, which makes them inactive. UV-C light is the electromagnetic spectrum between 200 and 280 nm [153]. An UV-C treatment of 40 mJ·cm⁻² was proved to be protective for apple juice polyphenols and antioxidant activity [148].

Dong et al., highlighted a non-thermal method of reducing microorganisms and patulin in apple juice. Following seven consecutive exposures of the contaminated matter to UV radiation, the patulin level decreased by 43%. Also, no differences were observed on the compounds in the raw material; pH, Brix degree and total acidity remained the same after exposure to UV radiation. The only compound that showed a slight decrease was ascorbic acid. This method, which uses UV radiation, is easy to implement and offers both UV pasteurization of the juice or cider, as well as the reduction or even elimination of the patulin without affecting the quality of the end product [154,155]. UV-C irradiation is suitable for inactivating *Alicyclobacillus acidoterrestris* spores from apple juice. Better results were obtained than the heat treatment at 95 °C after 8 min of irradiation when the number of spores decreased significantly [156].

Depending on the apple varieties from which the juice was obtained, there were decreases in the content of vitamin C when apple juice was treated with UV-C [157]. The losses can be attributed to the lack of pigmentation of the juice; these were mainly between 4–6% [157]. UV treatment is also effective in reducing microorganisms, and the qualities of apple juice and cider are preserved, without any significant changes [158,159].

The oxidation of the phenolic compounds by the polyphenol oxidase starts with the apple crushing [34]. The oxidation activity can also be influenced by the apple variety [160]. Fermented beverages derived from apple varieties with lower polyphenol oxidase activity, usually contain also higher amounts of chlorogenic acid, a common contributor to the phenolic profile of apple products [49,161–163]. Ultraviolet treatment was effective for the reduction of polyphenol oxidase in apple juice. UV-C light with irradiance at 13.8 Wm⁻² for 33 min contributed to a ten-fold decrease in activity [164].

5.5. Ultrasound Treatments

Ultrasound treatment, with potential industrial application, can be used successfully against juice pasteurization [165,166].

Sonication together with low pressure and temperature can be a method of pasteurising the juice. By pasteurization treatment, the colour of the juice is significantly affected, while heat, generates an important loss of volatile compounds [167]. This alternative pasteurization method consists of the application of high-intensity ultrasound (20–100 kHz and 10–1000 W/cm²). It has been established that this method is safe to use in food processing. The examined indices showed that apple juice pasteurised by sonication has a similar quality to fresh juice and better than that pasteurised by the thermal method. Of the analysed volatile compounds, the majority are found in greater amounts in the cider sonicated, heat-treated compared to the raw apple cider (ethyl 2-methyl butanoate, butyl acetate, 1-butanol, 1-hexanol, butanoic acid, 2-methylbutanoic acid, octanoic acid, hexanoic acid). Therefore, manothermosonication (2–5 atm, 60 °C), thermo-sonication and mano-sonication can be promising methods as alternative to pasteurization of apple juice and cider [168].

Sodium hypochlorite (100 ppm), copper ion water (1 ppm) and sonication (22–44 and 44–48 kHz for 3–5 min) are successful in reducing the *E. coli* O157: H7 and *Listeria monocytogenes* from apples and apple juice. These methods were evaluated either together or separately. For example, using only water with copper ions had no significant effect compared to a simple wash. However, a reduction with 5-log CFU/mL was achievable by using water with copper ions in combination with sodium hypochlorite, followed by sonication at 44–48 kHz [169]. Sonication treatment can be used successfully to improve the nutrients in apple juice. This treatment, correlated with time, temperature, amplitude and frequency, led to an increase in the concentration of polyphenols, carotenoids, sugars and some minerals [170].

Ultrasound-assisted fermentation of apple juice was applied for the stimulation of *Hanseniaspora* sp. yeast [23] by applying a cyclic mode with variable periods of US pulses of $\Delta t_p = 0.5, 1$ and 2 s, followed by pauses of $\Delta t_w = 6$ s. The optimization of the treatment was tested by performing the fermentation during the first 6 h (with both the Lag and Log phases), and lag phase (3 h) or log phase (3 h), respectively. Best results in terms of increased yeast concentration and biomass yield were obtained when treatment time of 1662 s was applied during both in the lag and log phases (6 h) for pulse duration of 0.5 s followed by 6 s pause. Of these, the lag phase encountered higher yeast concentration and biomass yield. These findings may be further applied for the industrial production of low alcohol cider, which is a market trend of the last years as consumers increasingly value the lower alcohol drinks [8,171–174].

5.6. High-Pressure Processing

The high-pressure processing (HPP) represents a highly effective tool for the inactivation of foodborne pathogens in apple juice. As the maintenance costs of HPP equipment increase with pressures beyond 600 MPa, the reduction in pressure applied and combining HPP with other cost-efficient microbial inhibitors represent promising tools for prolonging the shelf stability of apple juice [175]. The application of HPP (400–600 MPa) and dimethyl dicarbonate (100–250 mg/L) was highly effective for the inactivation of foodborne pathogens in apple juice.

The HPP is a useful tool for extending the high nutritional quality of apple juice during storage. The combination 600 Mpa—25 °C—5 min caused almost total inactivation of polyphenol oxidase and peroxidase [176]. The storage time significantly affects the stability of individual phenolic compounds. Catechin was the most stable phenolic compound (stable for 55 weeks). Other authors suggested even a lower HPP treatment, of 300 MPa, for the stabilising of cloudy apple juice by the enzymatic activity inhibition [177].

The use of HPP might be also directed to favour the extraction of specific classes of phenolic compounds. The HPP treatment 600 MPa/35 °C/5 min was the optimal

combination to increase total flavonols (75%), total hydroxycinnamic acids (29%), total flavan-3-ols (58%), total dihydrochalcones (63%) and total phenolic compounds (54%) [178].

5.7. Pulsed Light Processing

Build on the application of short-time light pulses with intense broad-spectrum, pulsed light (PL) is a non-thermal innovative technology, an alternative to traditional disinfection and preservation methods. Among the advantages, the following can be summarised: Effective against a great variety of pathogenic and contaminating agents; does not generate residual compounds (use xenon flash lamps, which are nontoxic and mercury-free); low operation cost considered for each treatment; good consumers acceptance; the possibility to operate both continuous and batch modes; represents a fast method for microorganisms inactivation. PL treatment is successfully applied for microbial decontamination of transparent drinks. As per disadvantages, the most important are high initial investment cost, the short lifetime of lamps, changes in pH and colour at high fluence and overheating and thus the negative changes in sensory characteristics, especially when used by itself [179]. Still, there is recent evidence that a PL treatment below a critical fluence of 3.82 J/cm² minimises the photo-degradation and browning of a phenolic-based solution model [180]. The PL in combination with glutathione and ferrous ions was effective for the degradation of patulin in apple juice up to 97%, but more attention is further needed to oxidative stability and improvement of sensory quality of the product [181].

6. Final Remarks

With the increasingly growing market in some parts of the globe, it is imperative to address cider processing with great attention considering the consumer trends and preferences. Despite the technology applied, some of the most important industry challenges are choosing the best apple varieties based on their composition and the microorganisms involved in the fermentation process. Each variety of apples and each type of microorganism used in fermentation, lead to obtaining cider with a unique volatile profile. Studies on apple cider remain a promising field of research, with great potential for new products development and novel technologies transposed to the industrial level. The focus of cider-producing actors will be on increasing the efficiency in a manner able to satisfy the consumer preferences and assuring a sustainable production. Current emerging technologies play an important role in developing a sustainable cider industry.

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