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Alcohol-free and low-alcohol beers: Aroma chemistry and sensory characteristics

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Abstract

Alcohol-free beers have gained popularity in the last few decades because they provide a healthier alternative to alcoholic beers and can be more widely consumed. Consumers are becoming more aware of the benefits of reducing their alcohol consumption, and this has increased the sales of nonalcoholic alternatives. However, there are still many challenges for the brewing industry to produce an alcohol-free beer that resembles the pleasant fruity flavor and overall sensory experience of regular beers. The aim of this review is to give a comprehensive overview of alcohol-free beer focusing on aroma chemistry. The formation of the most important aroma compounds, such as Strecker aldehydes, higher alcohols, and esters, is reviewed, aiming to outline the gaps in current knowledge. The role of ethanol as a direct and indirect flavor-active compound is examined separately. In parallel, the influence of the most common methods to reduce alcohol content, such as physical (dealcoholization) or biological, on the organoleptic characteristics and consumer perception of the final product, is discussed.

KEYWORDS

alcohol-free beer, aroma chemistry, aroma formation, brewing process

1 | INTRODUCTION

Beer is one of the most popular alcoholic beverages across the world. Besides its pleasant fruity flavor and role as a thirst-quencher, a moderate consumption of beer presents benefits for health, such as a decrease in the risk of cardiovascular disease, a source of minerals and antioxidants, and others (Pilarski & Gerogiorgis, 2019; Sohrabvandi

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et al., 2012). However, the population is becoming more conscious of the risks of abusive consumption of alcohol and, in consequence, there is a clear trend to reduce its intake (Shield et al., 2013). Besides, nonalcoholic beers are beverages that can be an option for when the consumption of alcohol is not recommended or allowed, such as during working hours. In a recent consumer study, they were found to be "youthful" and "trendy" (Delarue et al., 2019). Due to these and other grounds (e.g., religious reasons or prohibition of alcoholic beverages in certain countries) and pushed by a clear quality improvement, alcohol-free and low alcohol beers have experienced a remarkable growth in the market. The best example of the success of alcohol-free beer is Spain, leader in production and consumption of alcohol-free beers in Europe. Alcohol-free beer is considered a beverage of choice for 46% of beer drinkers in Spain, where it comprised 17.1% of total beer consumed in the country in 2018 (Ministerio de Agricultura, Pesca y Alimentación, 2018). In other countries such as the Netherlands, the consumption of alcohol-free beers is much more modest, where it only represented 3.3% of the total sales in 2016, but growing every year (Nederlandse Brouwers, 2016).

Nonetheless, there are still challenges for brewers to overcome in order to improve the sensory quality of low alcohol alternatives and hence the levels of sales. A typical comment about alcohol-free beers is that it does not taste as good as an alcoholic beer. In a survey carried out within South Korean beer drinkers, the main reason (56% of the respondents) not to drink an alcohol-free beer was because "it does not taste good" (Statista Research Department, 2019). In an earlier study of the Italian market, a quantitative concept analysis research revealed that, after packaging and price, flavor was in the top three factors affecting consumer choice within the alcohol-free beer category (Porretta & Donaldini, 2008). Indeed, one of the main challenges when brewing an alcohol-free beer is its flavor: the lack of the much-appreciated fruitiness, the presence of off-flavors, but also the lack of body and sometimes bitterness (Porretta & Donaldini, 2008; Sohrabvandi et al., 2010).

The literature available on the topic is often focused on the optimization of the control of ethanol formation or removal. However, the impact of the brewing method on the aroma compounds and sensory properties is secondary in many studies, not to mention the forgotten role of ethanol as a contributor to flavor and perception. The aim of this review is to give an overview of alcohol-free and low alcohol beers addressing the following questions:

- What are the legal definitions of alcohol-free and low alcohol beers, with a quick look at the current consumer trends?
- How are important aroma compounds formed?
- What is the effect of the lack of alcohol?
- How do different production strategies or methodologies of alcohol-free beer affect the sensory properties of the final product?

In order to present a more comprehensive approach, this review covers beers with an alcohol content below 3.5% alcohol by volume (ABV).

2 | WHAT IS AN ALCOHOL-FREE BEER?

The definition of an alcohol-free beer is not as straightforward as it may sound and varies quite significantly between countries. Most commonly, alcohol-free beers are not defined separately from regular beers, but as a subcategory. A clear example is the United States (Code of Federal Regulations, 2022), where beer is defined as a "fermented beverage (…) containing one-half of one percent or more of alcohol by volume, brewed or produced from malt." Therefore, the products containing less than 0.5% ABV are considered *nonalcoholic malt beverages* since they do not fall into the definition of beer. The term *alcohol-free* is used for the beverages containing 0% ABV.

Alcohol-free or low alcohol beers are not defined in EU regulations. According to the Regulation No. 1169/2011, any beverages containing less than 1.2% alcohol do not need to state the alcohol content on the label (Official Journal of the European Union, 2011). In addition to the European legislation, the member countries may apply their own definition of alcohol-free beers which, as a consequence, may imply different denominations for similar products depending on the country (Figure 1). Other countries have more restrictive regulations: in the case of Morocco, the term *low alcohol* is not defined in the legislation and *alcohol-free beer* only applies to those with an alcohol content equal to 0% ABV (Administration des Douanes et Impôts Indirects, 1977).

Further information regarding the denomination of beers and other alcoholic beverages can be found in the International Alliance for Responsible Drinking's website, where they published a table detailing the alcohol beverage labelling requirements for over 100 countries (https://iard.org/science-resources/detail/Beverage-Alcohol-Labeling-Requirements).

3 | BRIEF OVERVIEW OF CONSUMER BEHAVIOR AND MARKET TRENDS

Abstention from alcohol has risen over the last few decades, especially within the youngest group of

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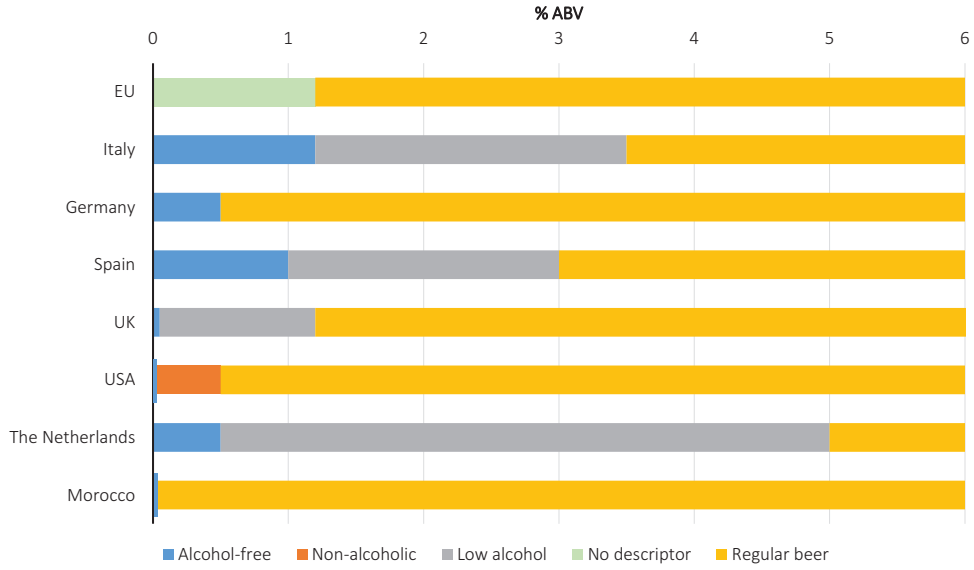

FIGURE 1 Alcohol content ranges for definitions of beers with low content of alcohol in different countries. References: EU (Official Journal of the European Union, 2011); Italy (Decreto del Presidente della Repubblica no. 272, 1998); Germany (Müller et al., 2017); Spain (Boletín Oficial del Estado, 2016); UK (Department of Health & Social Care, 2018); USA (Code of Federal Regulations, 2022); the Netherlands (Nederlandse Brouwers, 2019); Morocco (Administration des Douanes et Impôts Indirects, 1977)

consumers. According to the World Health Organization (2019), the consumption of alcohol by young people from 15 to 24 years old has decreased in most European countries (Figure 2). On the contrary, in 12 other countries, the consumption increased, but in only four of them the increase was greater than 10%. Besides, the consumers in this age group showed a good disposition toward alcohol-free and low alcohol beers. Almost a third of the respondents (aged 18–24 years old) of a survey carried out in Germany agreed that these beers taste "as good" as regular beers (Mintel, 2017).

The increase in sales of alcohol-free beers seems to be a consequence of this changing trend. According to a study of Global Market Insights (2019), the nonalcoholic beer market was estimated to be worth 13.5 billion US dollars in 2016, with a value projection of 25 billion US dollars in 2024. This trend is also observed in other countries like the Netherlands, where in the last decade the consumption of alcohol-free beer has increased more than 400% (Nederlandse Brouwers, 2018). According to a recent study, 47% of the beer consumers in the Netherlands declared that they drink alcohol-free beer (below 0.5% ABV) at least once a month (Nederlandse Brouwers, 2019). This is reflected in the growth of the variety of products worldwide. The world leader in releasing new alcohol-free and low alcohol beers (defined as below 3.5% ABV) is China where 29% of the new beers launched in 2016 belong to this category, whereas the average globally was 8% (Mintel, 2017).

The COVID-19 pandemic has also had an impact on alcohol consumption. In the United States, it has been shown that alcohol consumption decreased for men during the COVID-19 pandemic but was unchanged for women (Tucker et al., 2022), whereas Public Health England (2021) and OECD (2021) found that results were more polarized, with some drinking more and others drinking less. The true picture post-COVID-19 is likely to be complex.

4 | AROMA CHEMISTRY OF BEER

Although beer can be produced from just four ingredients, that is, barley malt, hops, yeast, and water, the result in terms of flavor compounds is a very complex mixture of hundreds of different volatiles and nonvolatiles. Depending on their concentration and how far this is from their perception threshold, their contribution to the overall aroma of beer varies. For this reason, from the hundreds of flavor compounds identified in different beers, the number of character impact compounds is much more limited (Meilgaard, 1975). The most important aroma compounds in beer can be classified into aldehydes, higher alcohols, esters, vicinal diketones, sulfur compounds, and hop-derived compounds. Figure 3 shows a selection of the most important aroma compounds in regular beer, their odor quality and perception threshold in water.

Aldehydes are generally formed by different chemical mechanisms during malt kilning, mashing and wort boiling. Aldehydes have been identified as the main contributors to the characteristic malty and worty aroma of alcohol-free beers, especially those brewed by biological

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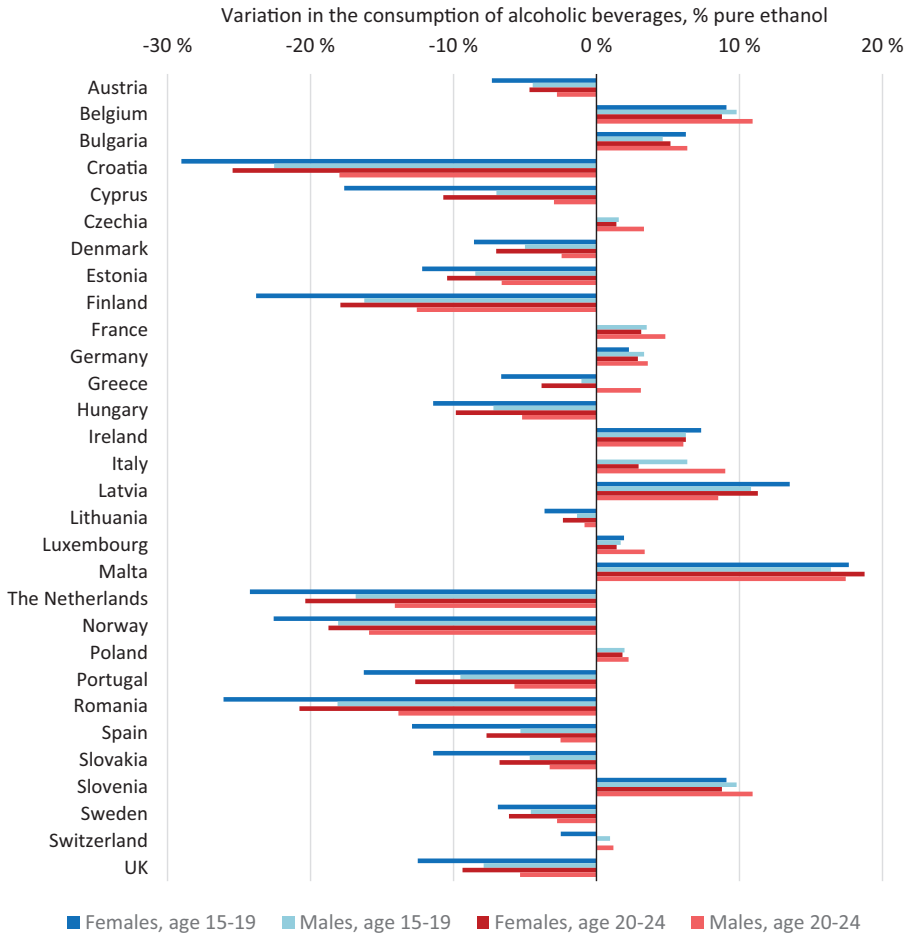

FIGURE 2 Variation of alcohol consumption (in terms of pure ethanol) in young people in Europe between 2010 and 2016. *Source*: Compilation based on data from WHO-Regional Office for Europe (2019)

methods (Perpète & Collin, 1999b; Piornos et al., 2020). This is due to the incomplete conversion of aldehydes into alcohols and esters because the process conditions are inadequate for their formation (see Section 6.2). Nonetheless, while many flavor-active aldehydes have been identified in both alcohol-free and regular beers, such as acetaldehyde, hexanal, (*E*)-2-nonenal, benzaldehyde, furfural, and 5-hydroxymethyl-furfural, the most important ones are 2-methylbutanal, 3-methylbutanal, and methional (Gernat et al., 2020). These three aldehydes, along with methylpropanal and phenylacetaldehyde, have very low perception thresholds, from 23.4 μg/L for 2-methylbutanal to 0.47 μg/L for methional in a model alcohol-free beer (Piornos et al., 2019). Moreover, their concentrations in lager beers (Table 1) are much lower than in low alcohol beers (Table 3) and, consequently, their contribution to the overall aroma of alcohol-free and low alcohol beers is considerably higher.

Higher alcohols (the more commonly used term in brewing for those other than ethanol and methanol) and esters are of key importance in alcohol-free beer flavor, either for their presence or absence. These compounds are responsible for the fruity flavor of regular beers. The most important higher alcohols in beer are 3-methylbutanol, 2-methylbutanol, 2-phenylethanol, and 2-methylpropanol, all of them derived from amino acids and the corresponding Strecker aldehydes (Fritsch & Schieberle, 2005). In Bavarian wheat beers, several esters were found to be key odorants and showed odor activity values (OAV) considerably higher than 1 (i.e., their concentrations were above the threshold) (Bellut & Arendt, 2019). The most

TABLE 1 Concentration of Strecker aldehydes in lager beers from Spain and Czechia

Compound	Concentration, μg/L	
	Spanish beers	Czech beers
Methylpropanal	1.71–28.3–229	4.63–24.0–58.0
2-Methylbutanal	1.80–8.78–60.4	3.16–11.7–34.5
3-Methylbutanal	0.97–7.60–47.2	3.57–13.7–38.2

Note: Data are shown as minimum value–average–maximum value.
Source: Andrés-Iglesias, Nešpor, et al. (2016).

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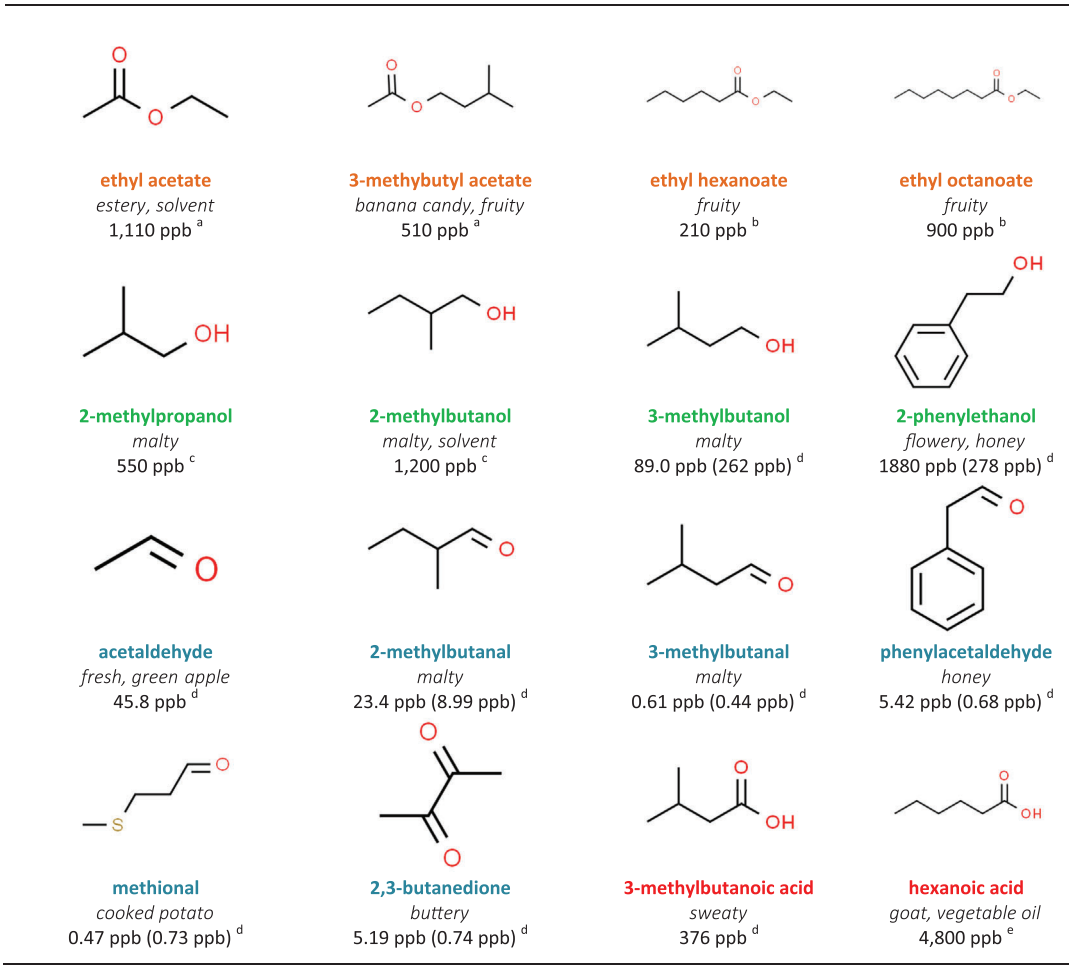

FIGURE 3 Key aroma compounds commonly found in beer and their aroma quality and orthonasal detection threshold (ppb = μg/L; retronasal thresholds in brackets). Esters in orange, alcohols in green, aldehydes and ketones in blue, and acids in red. Molecule structures retrieved from ChemSpider.com. Ref.: [a]Saison et al. (2009), [b]Kobayashi et al. (2008), [c]Czerny et al. (2008), [d]Piornos et al. (2019), [e]Wagner et al. (2016)

important ones were 3-methylbutyl acetate (OAV = 231), ethyl 2-methylpropanoate (225), and ethyl butanoate (115). A common defect of some alcohol-free beers brewed by types of yeasts other than *Saccharomyces cerevisiae* is the presence of one or more of these compounds at a high concentration, this being translated into an aroma that is unbalanced or overpowered by these compounds (Bellut & Arendt, 2019). Nevertheless, for the vast majority of alcohol-free beers, these compounds are present only after the addition of flavors at the end of the production process, either because they were never naturally formed (as it is the case of biological methods) or they were extracted during the process of ethanol removal (physical processes) (Brányik et al., 2012).

Other groups of flavor compounds relevant to the aroma of regular as well as alcohol-free and low alcohol beers are vicinal diketones, sulfur compounds, and hop-derived compounds. Although they are commonly present in these beverages, even at concentrations above threshold, they are usually not key aroma compounds and their role in the overall flavor perception is secondary. 2,3-Butanedione (diacetyl; butter-like aroma) and to a lesser extent 2,3-pentanedione (butter, creamy aroma) are the most relevant vicinal diketones in beer. These are associated with an "unmatured" aroma in fresh beers (Nakatani et al., 1984) or with a defective aroma after long storage (Inoue, 1998). Sulfur compounds are well-known flavor compounds because of their very low perception threshold and characteristic aroma. In beer, the most relevant ones are dimethyl sulfide (DMS; sweetcorn aroma) and 3-methyl-2-butene-1-thiol (MBT; skunky aroma); they are considered as off-flavors above a certain concentration. These compounds are well documented for their very low detection thresholds, namely 0.84 μg/L for DMS and only 0.01 ng/L for MBT (Czerny et al., 2008; San Juan et al., 2012). However, sulfur compounds contribute positively to beer flavor at low levels. Generally, DMS concentration is mainly reduced during wort boiling through evaporation

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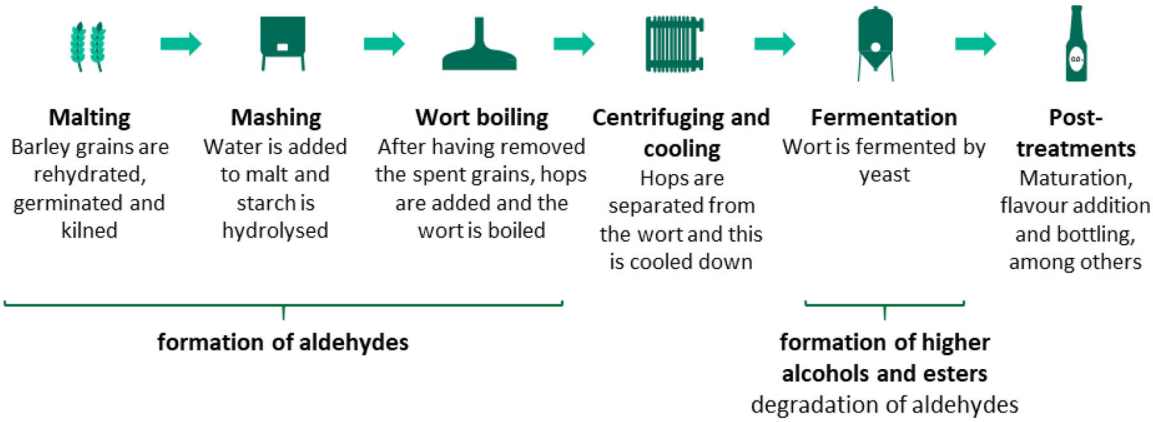

FIGURE 4 Overview of the brewing process and relative impact on flavor compounds. Cereal icon authored by Good Ware (www.flaticon.com)

(Scheuren et al., 2015), whereas MBT is formed from the degradation of the iso-α-acids of hops, brought on by light (De Keukeleire et al., 2008; D. K. Parker, 2012). On the other hand, hops contribute to beer flavor with a huge variety of aroma compounds, mostly terpenes and sesquiterpenes, such as linalool (floral, piney), myrcene (woody, piney aroma), caryophyllene (earthy, spicy), and humulene (hoppy aroma) (Peacock, 1998). Recently, abhexone (5-ethyl-3-hydroxy-4-methyl-2(*5H*)-furanone) and (*E*)-β-damascenone have been shown to contribute to the worty aroma of alcohol-free beer (Piornos et al., 2020) which is a negative attribute in these products and can only be partially masked by the addition of flavorings.

5 | ORIGINS AND FORMATION OF AROMA COMPOUNDS DURING THE BREWING PROCESS OF ALCOHOL-FREE AND LOW ALCOHOL BEERS

The present section aims to cover the principal formation pathways of the key aroma compounds in low and alcohol-free beers that are formed during the brewing process, and those desired aroma compounds present in regular beers. Since the methods for the production of alcohol-free and low alcohol beers are usually modifications of those used for regular beers, the origins and formation pathways of the families of compounds discussed are common to both groups of products. These compounds are mainly derived from amino acids and formed by a variety of mechanisms involving chemical reactions from thermal processes and fermentation. Figure 4 provides a simplified overview of the brewing process and the impact on the aroma compounds.

5.1 | Aldehydes

Methylpropanal, 2- and 3-methylbutanal, methional, and phenylacetaldehyde belong to the Strecker aldehydes. These compounds are responsible for the malty and worty aromas in alcohol-free beers, usually regarded as a major defect since they lack the fruity character very appreciated in alcoholic beers (Perpète & Collin, 1999a; Piornos et al., 2020). They have been detected in the early stages of the brewing process, that is, the malted grains (Beal & Mottram, 1994; De Clippeleer et al., 2010). Aldehydes in malt and beers (both alcohol-free and alcoholic beers) can be formed through the Maillard reaction and Strecker degradation during processes where high temperature is applied, such as kilning of malt, mashing, and wort boiling. Malting is a process in which germinated grains, usually barley, are roasted or kilned under a current of hot air. The temperature of the drying gas (a mixture of combustion gases and air) is controlled, and it influences greatly the characteristics of the finished malt in terms of color and aroma. Temperatures below 100°C are applied to achieve pale malts, whereas roasted or "specialty" malts require temperature in the range of 130–230°C (Yahya et al., 2014). These authors also reported that higher kilning temperature increases the concentration of phenylacetaldehyde in finished malts.

The formation of Strecker aldehydes continues in further stages of the brewing process. Mashing and especially wort boiling are processes where high temperature is applied. Mashing is a process where the optimal temperatures for the enzymatic degradation of starches and proteins are used in the form of a temperature gradient. In this way, the concentration of fermentable sugars and amino acids increases significantly due to the action of

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α- and β-amylases and proteases (Yoshioka & Hashimoto, 1979). During wort boiling, Strecker aldehydes are formed due to the presence of their precursors in the liquid. Under boiling conditions, the flavor volatile compounds are formed through the Maillard reaction and the Strecker degradation, as well as lost by evaporation (Buckee et al., 1982). These compounds are formed at a higher rate at the beginning of the process, and then their concentration stabilizes due to a balance between formation and evaporation (De Schutter et al., 2008a).

5.1.1 | The Maillard reaction

Strecker aldehydes are mainly formed from amino acids via the Maillard reaction which is initiated by the condensation of the carbonyl group in a sugar molecule and an amino group, typically those present in amino acids (Figure 5a). This first reaction is spontaneous but considerably accelerated at high temperature. The carbonyl group in the open form of a reducing sugar is attacked by the amino group, thus forming a Schiff base. This chemical structure can undergo an acid-catalyzed rearrangement to form a series of 1-amino-1-deoxyketoses, which are called the Amadori rearrangement products (ARP), via an eneaminol intermediate. The ARPs are relatively stable intermediate compounds in the Maillard reaction. Those formed from aldohexoses are usually more stable than those from aldopentoses (Parker, 2015). In the case of ketoses, the mechanism is slightly different, and a series of 2-amino-2-deoxyaldoses, called Heyns rearrangement products (isomers of ARPs), are formed. The subsequent stages involve the dehydration and regeneration of the amino compound and the formation of the α-dicarbonyl compounds called deoxyosones. The formation of deoxyosones is followed by series of parallel and consecutive reactions (aldol condensation, retroaldol cleavage, cyclization, dehydration, among others) which results in the formation of both aroma and color compounds (Parker, 2015).

5.1.2 | Strecker degradation

Deoxyosones, such as 3-deoxyhexosulose and 1-deoxyhexulose from glucose, and other α-dicarbonyl compounds which derive mainly from the fragmentation of the deoxyosones (glyoxal, methylglyoxal and even 2,3-butanedione) are unstable Maillard intermediates (Kocadağlı & Gökmen, 2016). The reaction between α-dicarbonyl compounds and amino acids is called Strecker degradation, and it forms volatile aroma compounds with a significant impact on foods submitted to thermal treatments. Figure 5b shows the mechanism of the Strecker degradation from an amino acid and a dicarbonyl compound. A series of aldehydes are formed from their corresponding amino acids, the most important ones in the flavor chemistry of brewing being methional (potato-like aroma), methylpropanal (malty), 2-methylbutanal (malty), 3-methylbutanal (malty), and phenylacetaldehyde (honey) due to their low perception thresholds (Piornos et al., 2019). Their amino acids of origin are methionine, valine, isoleucine, leucine, and phenylalanine, respectively.

5.1.3 | Other lipid-related formation pathways

Other minor pathways for the formation of aldehydes in alcohol-free, low alcohol, and alcoholic beers are by the oxidation of unsaturated fatty acids. This reaction usually leads to the formation of linear aldehydes, such as hexanal, (*E*)-2-nonenal, and (*E*,*E*)-2,4-decadienal (De Schutter et al., 2008b; Gernat et al., 2020). However, some lipid-derived reactive carbonyls can react with amino acids to produce Strecker aldehydes. Hidalgo and Zamora (2016) proposed a formation pathway of Strecker aldehydes from the reaction of an amino acid and a 2-alkenal through an imine intermediate (Figure 6). The fat content in malt barley is lower than 3.4% w/w, of which 58% of the fatty acids correspond to linoleic acid (Anness, 1984). This unsaturated fatty acid forms 2-alkenals and 2,4-alkadienals, such as (*E*)-2-octenal and (*E*,*E*)-2,4-decadienal, under high temperature conditions in the presence of O_2 (Schieberle & Grosch, 1981). The relative contribution of this formation pathway to the total amount of Strecker aldehydes is likely to vary depending on the food matrix and is largely unknown. Nevertheless, the presence of lipids has been proven to contribute positively to the formation of these aldehydes. Gallardo et al. (2008) observed an increase in phenylacetaldehyde in wort when it was supplemented with linoleic acid or its degradation product, (*E*,*E*)-2,4-decadienal (up to 2.77 mmol/L wort for both compounds). The relative increase in the concentration of phenylacetaldehyde was 1.1–2.5 times and 3.6–4.6 times higher than in the control wort, respectively. These results confirmed the role of alkenals in the formation of Strecker aldehydes by reaction with amino acids.

5.2 | Higher alcohols

Fermentation is the main step for the formation of many important aroma compounds in the brewing process, including higher alcohols and esters. In a similar way to the formation of Strecker aldehydes, higher alcohols are formed from amino acids. First, the amino group

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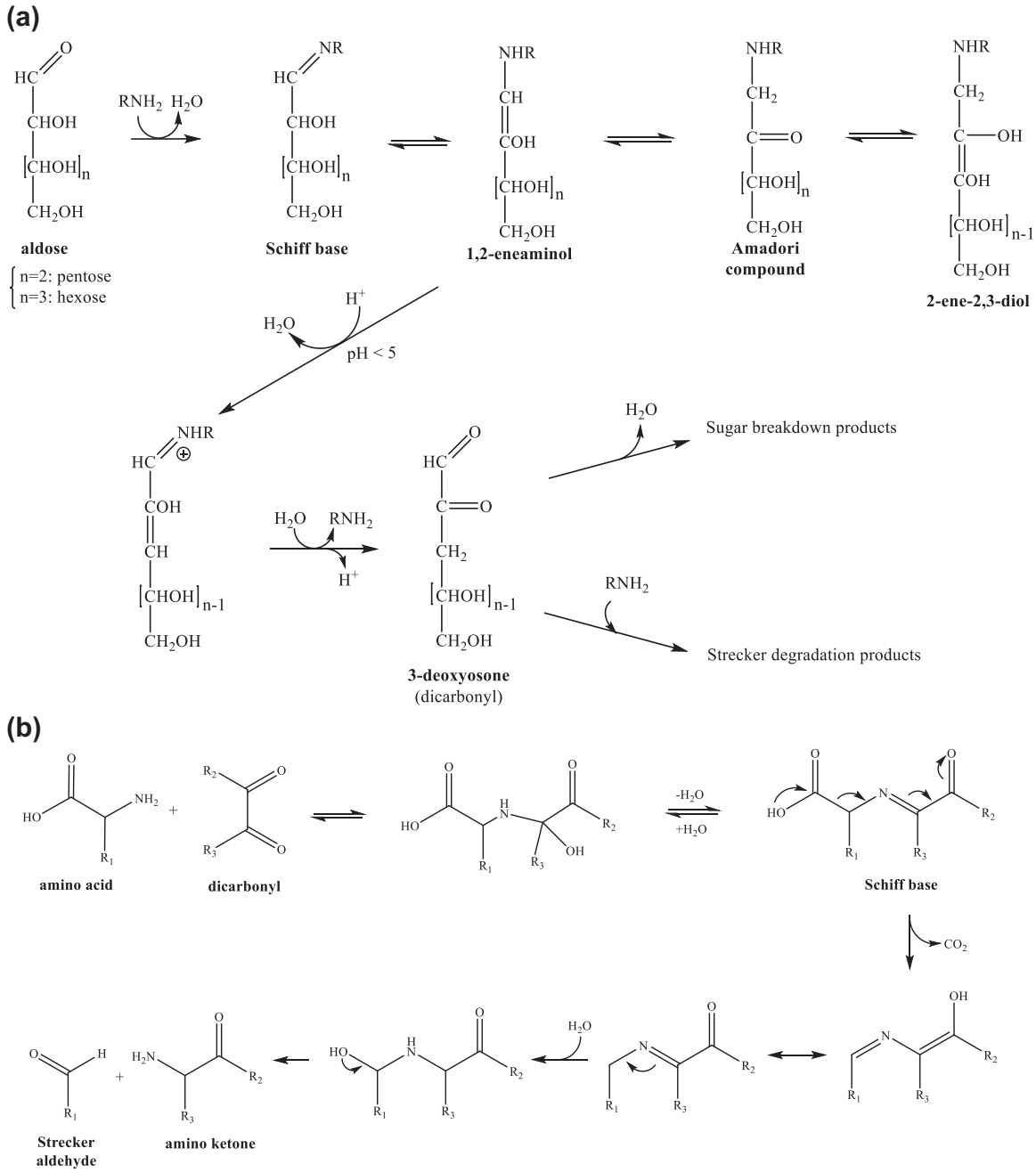

FIGURE 5 Chemical reactions of the Maillard reaction (a) and Strecker degradation (b). Adapted from Gernat et al. (2020)

is substituted by a keto group through a transamination enzymatic reaction, thus forming an α-keto acid (Figure 7). In this reversible reaction, glutamate and α-ketoglutarate act as donor and acceptor of the amino group, respectively (Pires et al., 2014). The next step is the nonreversible decarboxylation of the α-keto acid into a Strecker aldehyde (or fusel aldehyde), with the loss of a molecule of CO_2. The aldehyde, formed either through the Maillard reaction or the Ehrlich pathway, is lastly reduced to the corresponding alcohol by action of alcohol dehydrogenases, which also participate in the production of ethanol by reducing acetaldehyde (Bennetzen & Hall, 1982). Fermentation conditions (short time, low temperature, or both) in production processes for alcohol-free and low alcohol beers such as cold contact fermentation (CCF) and arrested fermentation do not allow enough reduction of the aldehydes (formed during malt kilning, mashing, and wort boiling) and formation of alcohols. As a result, these products miss the pleasant fruity flavor and complexity that would result from the natural presence of these compounds, while other aroma compounds like Strecker aldehydes negatively stand out as off-flavors.

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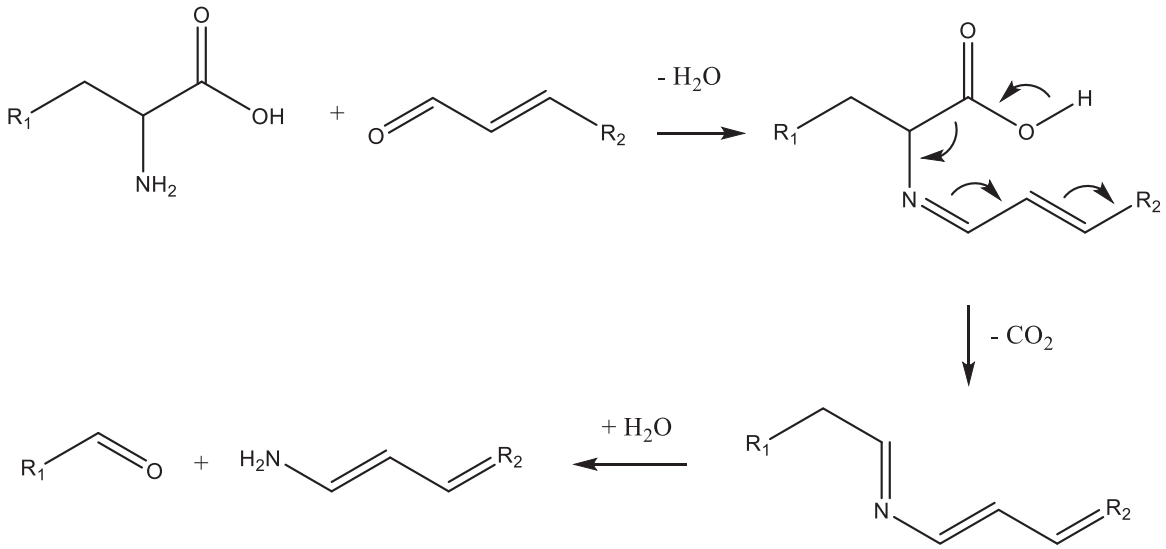

FIGURE 6 Formation pathway of Strecker aldehydes from amino acids and lipid-derived reactive carbonyls. Adapted from Hidalgo and Zamora (2016)

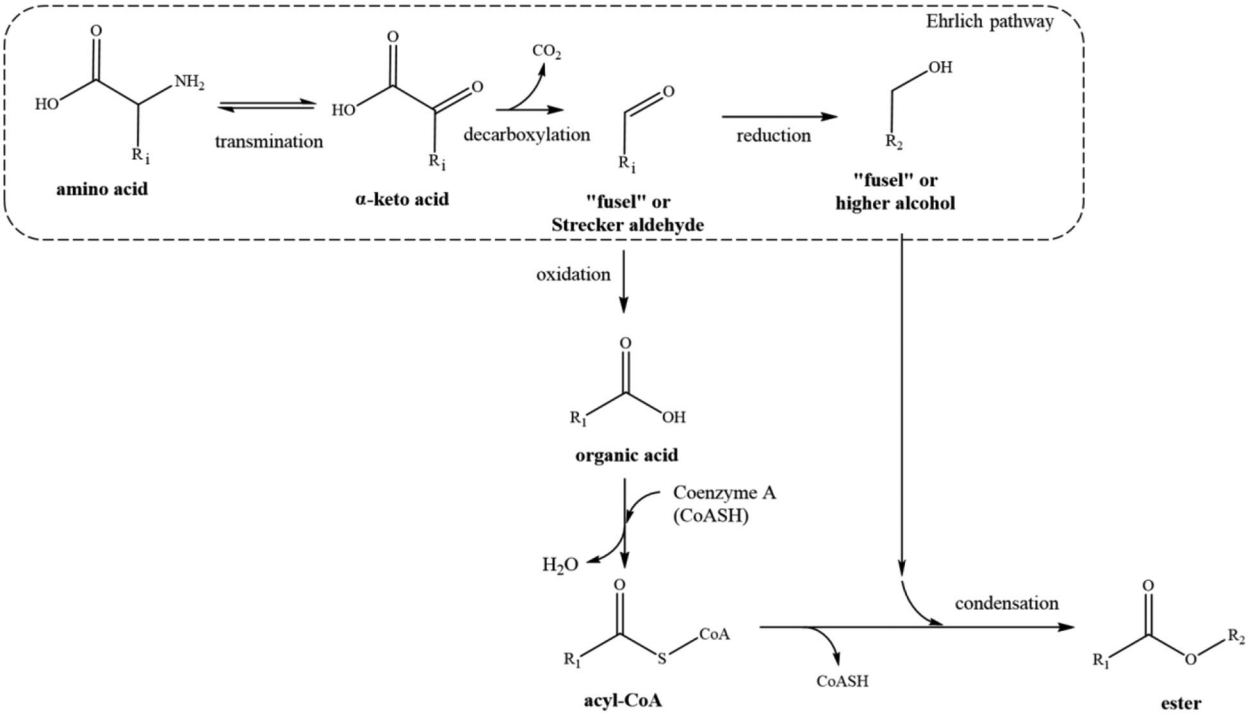

FIGURE 7 Chemical reactions of the Ehrlich pathway and subsequent formation of esters. Adapted from Pires et al. (2014). $R_i = R_1$ or R_2

Another mechanism for the formation of higher alcohols in beer is through the anabolic biosynthetic route from carbon source, mainly sugars. In this route, the α-keto acids are formed from carbohydrate metabolism, and they act as precursors of aldehydes by decarboxylation as shown in the Ehrlich pathway (Chen, 1978). This formation pathway from carbohydrates has usually been related to fermentation media with low or absent nitrogenous nutrients (Van Gheluwe et al., 1975). The contribution of each pathway (catabolic or anabolic) to the final concentration of higher alcohols in the beer is not the same for all these compounds. 1-Propanol is not significantly affected by the lack of one branched-chain amino acid aminotransferases (essential in the Ehrlich pathway) in mutant yeasts, which suggests that this compound might be more readily formed by the anabolic pathway (Eden

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et al., 2001). 2-Methylbutanol and 3-methylbutanol, however, saw their concentration drastically reduced when one of these aminotransferases was absent. The researchers also reported the unexpected formation of higher amounts of these alcohols when two aminotransferases were not coded, which suggested that other formation routes might be involved other than the already known Ehrlich and anabolic pathways.

5.3 | Esters

The formation of esters in beer by yeast fermentation is closely related to that of alcohols, since they are formed by enzymatic condensation of organic acids and alcohols (Pires et al., 2014). Esters can be divided into two groups: acetate esters (those formed from acetic acid and an alcohol) and ethyl esters (those formed from ethanol and an organic acid). First, the formation of organic acids in beer takes place at the end of the Ehrlich pathway, by oxidation of Strecker aldehydes via the action of aldehyde dehydrogenases (Figure 7) (Hazelwood et al., 2008). Although organic acids derived from amino acids, such as 2-methylpropanoic acid, 2- and 3-methylbutanoic acid, or 2-phenylacetic acid, are aroma active, their contribution to beer flavor is not very important, mainly due to their high perception thresholds, usually well above 1000 μg/L (Czerny et al., 2008; Piornos et al., 2019).

The reaction between alcohols and acids to form esters occurs through the formation of an intermediate acyl-CoA molecule from the organic acid. Acetyl-CoA is a special case of acyl-CoA that can enter the Krebs cycle in aerobic conditions, while in the absence of oxygen, it can be esterified with a molecule of ethanol to produce ethyl acetate (solventy aroma), among other esters (Pires et al., 2014). Acetate esters, like ethyl acetate, 3-methylbutyl acetate, 2-phenylethyl acetate, and others, are the most abundant esters in alcoholic beers. The synthases responsible for this condensation reaction have been identified as alcohol acetyl-transferases (Yoshioka & Hashimoto, 1981). The production of ethyl esters by the condensation reaction of acyl-CoA molecules and ethanol is due to the activity of two acyl-CoA:ethanol *O*-acyltransferases (Saerens et al., 2006). For more information regarding the enzymes involved in the production of higher alcohols and esters by yeast, the reader is directed to the review on this topic by Pires et al. (2014).

Several fermentation parameters affect the performance of the yeast in producing fruity alcohols and esters. Amongst them, the most noticeable are the yeast strain and fermentation temperature. The effect of yeast strain is covered in Section 6.2.1. Fermentation temperature has a critical effect on the expression of a gene responsible for encoding a permease that allows the transport of amino acids into the yeast cell (Didion et al., 1996). As one might expect, this has a significant impact on the formation of higher alcohols via the Ehrlich pathway, and thus on the formation of esters. Higher fermentation temperature improves the formation of higher alcohols, esters, and ethanol in beer (Takahashi et al., 1997), and this effect has been observed even at slight increases of temperature such as from 8.5 to 11.5°C in high gravity worts (15.5°P) from Pilsner malt (Kucharczyk & Tuszyński, 2018). For instance, the concentration of 3-methylbutyl acetate increased significantly from 1.46 to 1.82 mg/L when the fermentation temperature increased from 8.5 to 11.5°C, whereas 2- and 3-methylbutanol increased from 67.5 to 74.2 mg/L. Consequently, the fermentation conditions applied in brewing alcohol-free and low alcohol beers is not favorable for the formation of ethanol, the reduction of aldehydes, and the formation of higher alcohols and esters, thus affecting the sensory characteristics of the final product dramatically.

5.4 | (*E*)-β-Damascenone

(*E*)-β-Damascenone is widely known in flavor science because of its extremely low orthonasal detection threshold of 0.00075 μg/L in water (Semmelroch et al., 1995). In Bavarian wheat beers, this compound was reported to have the highest OAV besides ethanol (Langos et al., 2013) and in pale lager and Pilsner, its concentration was 1.6 μg/L (Schieberle, 1991) and 2.3 μg/L (Fritsch & Schieberle, 2005), respectively. Recently, (*E*)-β-damascenone was reported to be one of the compounds with the highest odor impact in alcohol-free beer brewed by cold contact fermentation (Piornos et al., 2020). It had a concentration of 10.4 μg/L which was well above its orthonasal detection threshold 0.23 μg/L (Piornos et al., 2019). This compound contributed to the overall worty flavor of beer with a fruity, jam-like aroma. Its presence was also reported in unhopped wort (De Schutter et al., 2008b).

Although (*E*)-β-damascenone has been found to naturally occur in plants, the evidence shows that its formation in many foods, beverages and other products, such as essential oils, is greatly dependent on the application of heat (Sefton et al., 2011). Chevance et al. (2002) suggested that it originates in the wort, where they reported (*E*)-β-damascenone concentrations of 450 μg/kg. Nonetheless, its concentration decreased remarkably after fermentation (7 days at 20°C). However, (*E*)-β-damascenone has also been found in barley malt (Fickert & Schieberle, 1998; Piornos et al., 2022) explaining its presence in the wort. During mashing and wort boiling, its concentration changes in what is potentially a balance between

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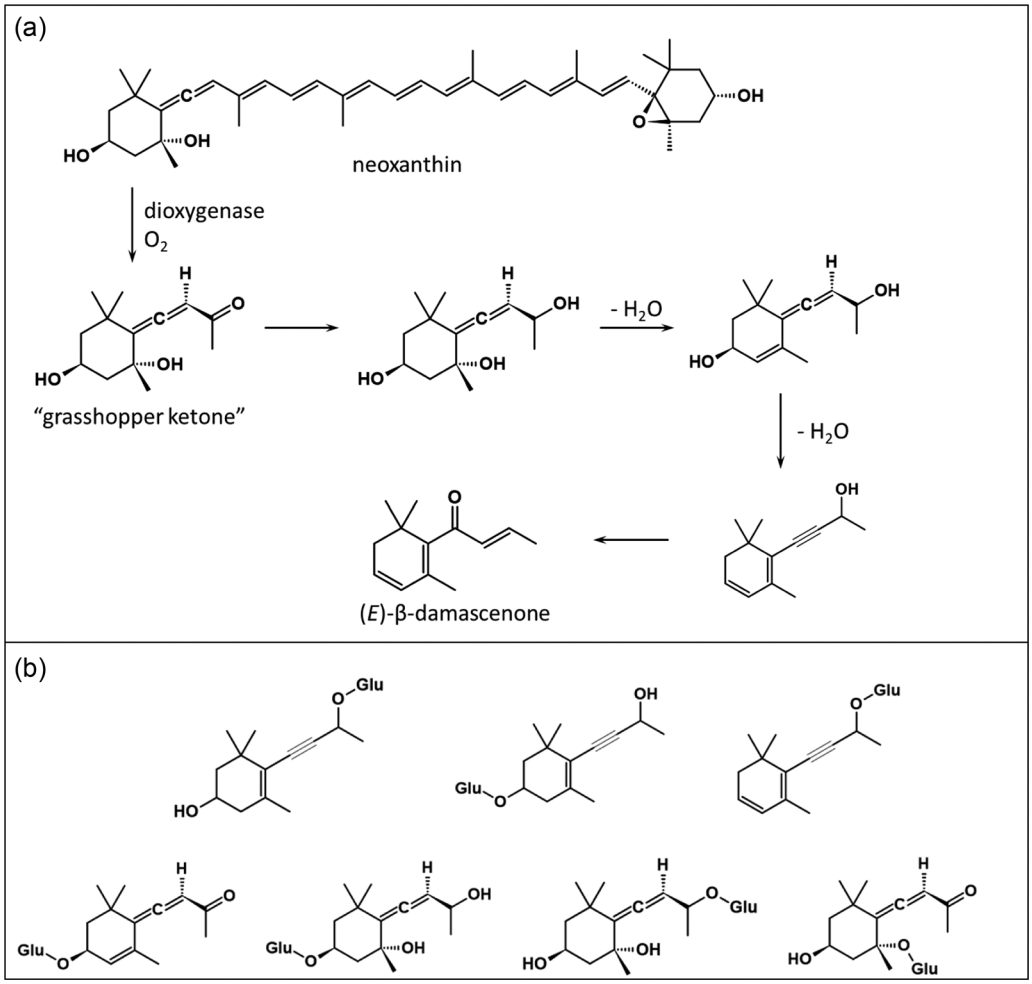

FIGURE 8 Formation of (*E*)-*β*-damascenone from neoxanthin (a) and some glucoside precursors of (*E*)-*β*-damascenone identified in plants (b). Adapted from Ohloff (1978) and Sefton et al. (2011)

formation and loss by evaporation, reaching a maximum at the end of wort boiling (Piornos et al., 2022).

One of the precursors of (*E*)-*β*-damascenone is the carotenoid neoxanthin (Chevance et al., 2002). This carotenoid can be degraded by carotenoid cleavage dioxygenases into the so called "grasshopper ketone" (Figure 8a), which in turn experiences the loss of water and the transposition of oxygen from C_9 to C_7 finally forming (*E*)-*β*-damascenone (Ohloff, 1978; Sefton et al., 2011). These reactions are favored by temperature and acidic pH, as demonstrated in the study of Ohloff et al. (1973). Hops have been described as one possible source of (*E*)-*β*-damascenone, where the presence of its precursor, the *β*-D-glucoside of 3-hydroxy-*β*-damascone, has been previously identified (Kollmannsberger et al., 2006). Other glycoconjugates related to the formation of (*E*)-*β*-damascenone are shown in Figure 8b. For more detailed information about the formation mechanisms of (*E*)-*β*-damascenone, we recommend the comprehensive review by Sefton et al. (2011) on this and other topics related to this compound.

5.5 | Abhexone

Abhexone (5-ethyl-3-hydroxy-4-methyl-2(*5H*)-furanone) is another powerful odorant (curry, spicy aroma) which has been reported in Pilsner beer (Kishimoto et al., 2018), Gueuze beer (Scholtes et al., 2012), and barley malt (Fickert & Schieberle, 1998) and contributes to the "hot honey" character of alcohol-free beer (Piornos et al., 2020). One proposed formation pathway is the aldol condensation of two molecules of *α*-ketobutyric acid originating from the degradation of threonine (Sulser et al., 1967). Figure 9 shows this mechanism in which two molecules of *α*-ketobutyric acid are combined by aldol condensation in an acidic environment to form a dimer. This in turn will undergo dehydration, cyclization, and decarboxylation

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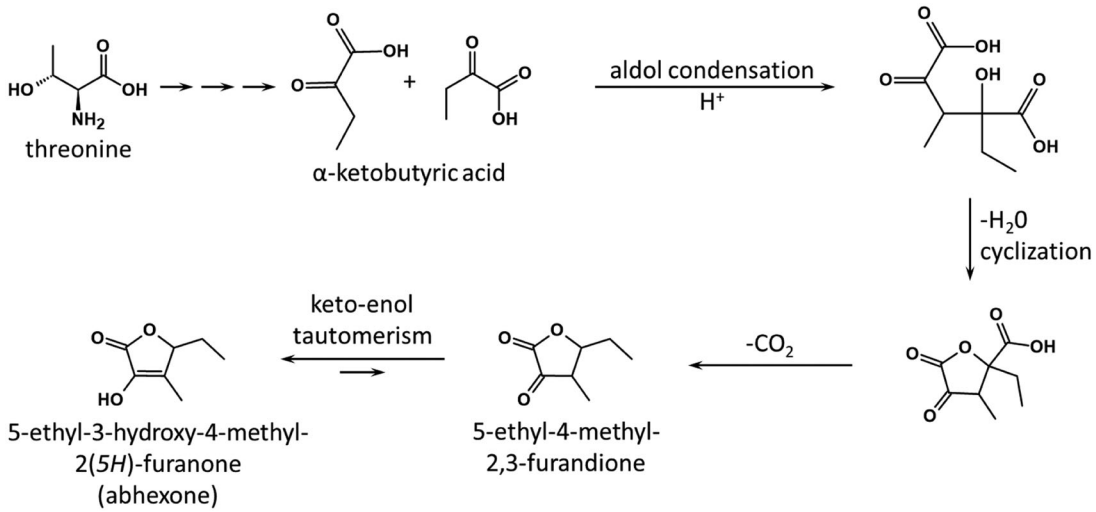

FIGURE 9 Formation of abhexone from the degradation of threonine. Adapted from Sulser et al. (1967)

reactions to further form abhexone and its keto-enol tautomer. The authors indicated that the predominant tautomer was abhexone in its enolic form. Another pathway suggested involves the aldol condensation of α-ketobutyric acid and propanal (Collin et al., 2012). The formation of abhexone was observed after 3 days in wine-like matrices (13% ethanol, pH 1.98) even at room temperature (20°C).

6 | THE ROLE OF ETHANOL IN THE PERCEPTION OF BEER FLAVOR

Ethanol contributes significantly to the sensory and physicochemical characteristics of beer. In terms of the sensory effect, ethanol's role could be divided into two principal functions:

1. Its effect on the volatility of many important aroma compounds (matrix effect),
2. Its own unique flavor and trigeminal sensation.

Related to the first function, indeed, the presence of ethanol has been demonstrated to influence the perception of aroma compounds present in beverages. In order to perceive an aroma compound, this must escape the food matrix, diffuse into the air and reach the olfactory mucosa (Espinosa Díaz, 2004). The release of the compound from the food matrix (a liquid in the case of beer) into the air is governed by the difference of partial pressures of the aroma compound between the liquid matrix and the air, that is, it is dependent on its concentration in both phases (Mackay, 1980). The air-liquid partition coefficient K_{al} is a useful parameter to quantify volatility. It can be expressed in terms of the concentration of the compound (compound "i") in the air and liquid phase, C_i^{air} and C_i^{liq}, respectively (Tsachaki et al., 2008):

$$K_{al} = \frac{C_i^{air}}{C_i^{liq}}. \quad (1)$$

As mentioned above, the composition of the liquid phase affects the partition coefficient and thus the release of volatile compounds into the air. Athès et al. (2004) determined the partition coefficients (at 25°C) of two aroma compounds commonly found in beers, ethyl hexanoate and 3-methylbutanol, in water and ethanol/water mixtures. They found that the values of K_{al} were significantly lower in 10% v/v and 20% v/v ethanol aqueous solutions, around 35%–38% and 58%–66% respectively, when compared to 100% water. This has been associated with the role of ethanol as a "cosolvent" along with water (Tsachaki et al., 2008). These authors related the presence of ethanol with a higher solubility of aroma compounds in comparison to an ethanol-free matrix. Nonetheless, solubility seems to be a consequence of the molecular interaction between the aroma compound (the solute) and the matrix (the solvent), as well as the volatility. The affinity of the aroma molecule to the components of the matrix is closely related to factors such as the size (molecular weight, molecular, or hydrodynamic volume) and nature (functional groups) of both solute and solvent molecules, and resulting physicochemical characteristics (water solubility, hydrophobicity) (Mackay, 1980; Philippe et al., 2003; Tsachaki et al., 2008). Obviously, a higher affinity between an aroma compound and a solvent, which applies to many aromas in ethanol with respect to water, is translated into higher retention of the volatile. This has been clearly demonstrated in several wine studies (Goldner et al., 2009; King et al., 2013; Muñoz-González et al., 2019). Hence, when the concentration of the volatile compound in the

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gas phase is lower, that consequently affects aroma perception. In beer, a concentration as low as 0.5% v/v ethanol in water was found to induce the retention (8%–12%) of 2- and 3-methylbutanal at the liquid phase when compared to 100% water (Perpète & Collin, 2000). These aldehydes were retained up to 32%–39% when the concentration of ethanol reached 5% v/v. Moreover, the detection threshold of methional in a lager-like matrix was higher than in an alcohol-free model solution (0.5 and 0.1 μg/L, respectively) (Perpète & Collin, 2000). This increase in flavor release was confirmed by Ramsey et al. (2020) for nine different aroma compounds. Furthermore, they showed that the presence of ethanol disrupts the protein structure of amylases in saliva, decreasing the hydrophobicity of the protein and thus promoting release of the most hydrophobic aroma compounds (Ramsey et al., 2020). Thus, the ethanol saliva interaction is also important in considering the release of aroma compounds.

In terms of its second function, ethanol stimulates the gustatory system being perceived as sweet and bitter (Blizard, 2007; Scinska et al., 2000), the olfactory system with its characteristic aroma (Czerny et al., 2008), and also the lingual branch of the trigeminal nerve (Clark et al., 2011; Danilova & Hellekant, 2002). Through the trigeminal stimulus, ethanol contributes to the mouthfeel a warming/burning sensation (Clark et al., 2011; Wilson et al., 1973), as well as a perception of body and fullness (Gawel et al., 2007). There is more consensus regarding the bitterness of ethanol, perceived as such even at 0.3% v/v in water (Mattes & DiMeglio, 2001), whereas sweetness does not seem to be affected (Gawel et al., 2007). However, these authors found that even small increases in the ethanol content in Riesling wine, from 11.6 to 13.6% v/v, had a significant positive effect on hotness in the mouth. Additionally, according to Scinska et al. (2000), ethanol is perceived as bitter and sweet, the sweet component being probably associated with the activation of some nerves sensitive to sucrose too. Altogether, the actual sensory character ethanol gives seems to vary depending on the levels present (0%–5%, 5%–10%, 10%–20%, and higher levels), as well as the matrix (aqueous solution, wine, and spirits) (Ickes & Cadwallader, 2018; Nolden & Hayes, 2015; Panovská et al., 2008; Villamor et al., 2013).

Besides, ethanol provokes a sensation of irritation in the nasal cavity, but this is overcome by the trigeminal burning sensation at concentrations above 5% (units not specified) (Rothe & Schrödter, 1996). It has been proven that ethanol stimulates the trigeminal receptors, some of them being also responsive to mechanical and cooling stimuli (Danilova & Hellekant, 2002). These responses were observed by applying aqueous ethanol solutions at concentrations of 0.7 M, that is, 32.2 g/L or approximately 4% v/v. When administered together with a sweetener like sucrose or a bitter compound like quinine, ethanol acts as an enhancer of sweetness or a suppressor of bitterness (Danilova & Hellekant, 2000). In another study, Trevisani et al. (2002) demonstrated that ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1, a heat-gated ion channel that is responsible for the burning sensation produced by capsaicin. This was evidence that ethanol interacts with taste receptors in several ways, besides the trigeminal system.

The content of ethanol has also been related to the perception of "body" in beer. *Body* is a term commonly used in several languages (e.g., *cuerpo* in Spanish, *corpo* in Italian, *σώμα* in Greek, or *Körper* in German) to describe a texture sensory characteristic, but there is no consensus on its definition. The definitions found in literature are in most cases rather vague, ambiguous, or they relate body to other sensory and physical attributes. Clapperton (1974) described body in beer as "a taste of substantial character, a sense of substance as felt by the palate." In his glossary of food texture terms, Jowitt (1974) defined body as "that textural property producing the mouthfeel sensation of substance." Some authors use the terms "body" and "fullness" indistinctively, being described as "the feeling of thickness/fullness as beer is moved around in the mouth" (Ramsey et al., 2018). Others consider body within the category of fullness and related to density and viscosity (Langstaff et al., 1991b), even though they found density and viscosity were poorly correlated with the alcohol content (correlation coefficients 0.41 and 0.50, respectively) (Langstaff et al., 1991a).

The extremely low concentration of ethanol in alcohol-free beers has been also indicated as one of the reasons behind a watery mouthfeel and lack of body. In wine, ethanol has been extensively studied for its effect on the body of the product in which this textural sensation is accentuated at higher concentrations (Demiglio & Pickering, 2008). Ramsey et al. (2018) and Gawel et al. (2007) demonstrated that the addition of ethanol to a commercial alcohol-free beer (0.06% ABV determined analytically) up to 5.25% ABV increased significantly ($p < .05$) the perception of fullness/body. Moreover, the overall liking of these beers was found to be dependent on the alcohol content. Consumers from this study were classified into three different clusters: some consumers preferred the high alcohol beer (22.8% of consumers), other the low or no alcohol beer samples (27.7%), and the third cluster was called "enthusiasts" (49.5%) because of their high liking scorings independent of the alcohol content of the beer.

All in all, the role of ethanol in beer does not have a single straightforward impact because it affects multiple aspects related to perception.

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7 | EFFECT OF PRODUCTION STRATEGY

Alcohol-free beers can be produced following a range of different methods. Brányik et al. (2012) classified the methods for alcohol-free beer production into two main groups: physical and biological processes. Physical methods are based on the removal of ethanol from regular beer by means of thermal processes (vacuum rectification, evaporation) or the application of membrane technology (dialysis, reverse osmosis). On the other hand, biological processes aim to control or avoid ethanol production during fermentation. This can be done by using selected yeast strains ensuring low ethanol production, by reducing the amount of fermentable sugars transferred into wort throughout mashing, or by altering the fermentation conditions in order to reduce yeast activity and thus the production of ethanol.

The composition as well as the nature of the aroma volatiles in alcohol-free beers is quite different depending on the brewing method used. In the case of beers produced by physical methods, their character is driven by the loss of esters and higher alcohols during processing. These compounds are mainly formed by yeasts during fermentation and then lost either by distillation or through the semipermeable membrane. The aroma profile of alcohol-free beers brewed by biological methods, however, is determined from the presence of aldehydes well above their perception threshold, while they are also low in fruity esters and higher alcohols.

7.1 | Physical methods

7.1.1 | Thermal processes

The most widespread physical methods for the removal of alcohol from beer are based on thermal dealcoholization. According to Brányik et al. (2012), in the early stages of the development of these methods, beer was distilled under atmospheric pressure, involving high temperature applied to beer in order to evaporate ethanol. Consequently, the organoleptic properties of the alcohol-free beer were unacceptable due to thermal damage. The principle behind thermal separation resides in the difference in volatility between ethanol and the rest of the components of the beer (mostly water) (Müller et al., 2017). However, compounds with lower or similar volatilities to ethanol will be co-distilled along with the alcohol. Therefore, these techniques lead to the partial or total removal of volatile aroma compounds that normally add a positive character to beer (Liguori et al., 2015), thus affecting the organoleptic characteristics of the final product. Since the boiling point for any chemical compound depends on pressure, researchers turned to the development of dealcoholization processes at milder temperature by reducing the pressure. Among the thermal techniques used for the dealcoholization of beer, vacuum distillation and vacuum evaporation are the most common.

Vacuum distillation. Vacuum distillation consists of the separation of ethanol from the aqueous beer matrix by heat treatment based on the differences in volatility between the two solvents. By reducing the pressure of the system, the boiling temperature of ethanol (and the rest of the volatile compounds present) decreases. In this way, ethanol can be stripped off the beer at lower temperature, hence reducing thermal damage (Mangindaan et al., 2018). The simplest setup for distilling off ethanol by vacuum distillation is a single step process. Andrés-Iglesias, Blanco, et al. (2016) employed a rotavapor for the lab-scale dealcoholization of 16 commercial lager beers from the Spanish market (from 4.6% to 6.5% ABV). Beers distilled at 200 mbar and 67°C showed higher aroma loss than those processed at 102 mbar and 50°C (Table 2). Interestingly, the concentration of 2-phenylethanol increased after distillation, possibly due to the continued formation of this relatively less-volatile compound at those temperatures. However, the authors did not report the final ethanol content of the dealcoholized beers, nor the sensory characteristics.

Vacuum distillation has also been used for the dealcoholization of wines (Gómez-Plaza et al., 1999). Under the conditions applied (67–80 mbar, at 25°C), the aroma compounds behaved similarly to those in the dealcoholization of beers, with substantial or complete losses of the most volatile compounds. The authors reported that the wine produced was not acceptable to the consumers, but the aroma could be potentially improved by adding back the volatiles after trapping them by condensation.

Thin layer evaporators. Another configuration for a distillation apparatus is "thin layer evaporator." In these evaporators, the liquid to be stripped flows as a thin film, which increases the gas–liquid interphase area and thus the mass transfer into the gas phase. In this way, the residence time in the device is reduced, reducing thermal damage. This technology has evolved dramatically since the first beer thin film evaporators. In 1916, Becker and Montgomery patented an evaporator for the dealcoholization of beer in which the beer at 75°C flowed as a thin film on the outer side of a conical steam jacket, and the alcohol (and other vapors) was released to the open atmosphere (Becker & Montgomery, 1916). The authors declared that the dealcoholized beer produced "retained its brilliancy and flavor," which is certainly questionable. An evolution

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TABLE 2 Loss of aroma compounds (mass percentage) after different dealcoholization methods

	Dealcoholization process					
	Distilled at 102 mbar, 50°C[a]	Distilled at 200 mbar, 67°C[a]	Falling film[b]	Dialysis[b]	Reverse osmosis[b]	Osmotic distillation[c]
Higher alcohols	73%	33%	98%	85%	69%	77%
Esters	90%	95%	95%	85%	78%	99%
Aldehydes	n.d.	n.d.	n.d.	n.d.	n.d.	93%
Ketones	n.d.	n.d.	n.d.	n.d.	n.d.	100%
Organic acids (C_5–C_{12})	n.d.	n.d.	45%	70%	71%	n.d.

Abbreviations: n.d., not detected.
[a]Andrés-Iglesias et al. (2016).
[b]Stein (1993).
[c]Liguori et al. (2015).

of these evaporators is the development of the centrifugal thin film evaporator, which has been used widely for the dealcoholization of beer, as well as the concentration of juices, coffee and tea extracts, and others (Shinn, 1971). The contact time between the liquid and the heating surfaces has been reported to be around 1 s, with temperatures as low as 35°C, and a liquid layer of approximately 0.1 mm thick (Flavourtech, 2018). The use of multiple cones increases the evaporation capacity of the apparatus.

Spinning Cone Column. The most used equipment for dealcoholization of beer by vacuum evaporation is Spinning Cone Column (SCC). The beer is transferred into a vertical column containing stationary and rotating inverted cones placed alternately. The forces involved in the movement of the liquid down to the next cone below are a combination of gravitational force, down the stationary inverted cones, and centrifugal force, up to the rotating inverted cones. The beer flows over the upper surface of the rotating cones as a thin liquid film of around 0.1 mm and comes into contact with the gas (usually steam) flowing upward allowing ethanol evaporation (Bae et al., 2020). After optimizing the process, Huerta-Pérez and Pérez-Correa (2018) achieved ethanol recoveries of 94% after stripping an ethanol–water mixture (14.8% ethanol), with a concentration of ethanol in the distillate of 70%. This system requires lower temperature than vacuum distillation (40–45°C) and the residence time of the beer through the equipment is only approximately 20 s to reach ethanol concentrations below 0.05% ABV in a single pass (Alfa Laval, 2019). For the dealcoholization of wines (final ethanol content below 0.04% ABV), temperatures as low as 28°C for one stage process and 24°C for two stages have been reported (Moro González et al., 2012). Because of the mild temperatures used and consequent low damage to flavor compounds, SCC has been also used for the extraction of flavor compounds from foods, like green and black tea (Ma & Wolff, 2007) or citrus fruits (Shinji et al., 2001).

7.1.2 | Membrane processes

Membrane technology is currently used for a wide variety of applications in different fields. The dealcoholization of alcoholic beverages is based on the selectivity of semipermeable membranes which allow certain compounds to pass through. These processes have the advantage of requiring low temperature, mild pressure, and no mobile parts, which lessens maintenance needs. However, membranes are not selective enough to prevent other small compounds from permeating along with ethanol. This usually causes a significant loss of aroma compounds of low molecular weight and the deterioration of sensory characteristics of the dealcoholized beer. Among the membrane processes, the following have been utilized for the dealcoholization of beer: pervaporation, reverse osmosis, osmotic distillation, and dialysis.

Pervaporation. Pervaporation is a two-step separation technique in which permeation is combined with evaporation: a liquid mixture (feed) is put in contact with a membrane, and the permeate is removed by evaporation at low pressure through the membrane (Gorri et al., 2017). The interest on this membrane technique is based on its simple and inexpensive operation and the low amount of chemicals required, if any (Dobrak et al., 2010). This technique is also employed for the recovery of aroma compounds from an alcoholic beer for its use in the aroma reconstitution of a dealcoholized beer. Del Olmo et al. (2014) extracted the aroma compounds from several alcoholic beers ("special" and "reserve" beers) by pervaporation and added them into a low alcohol beer. The latter was submitted to a sensory evaluation in which 90% of the panelists preferred the "enriched" low alcohol beer to the one without the aromas added. Catarino et al. (2009) optimized the dealcoholization of Pilsner beer through a polyoctylmethylsiloxane/polyetherimide (POMS/PEI) composite asymmetric membrane by means of surface response methodology. The researchers found that low temperature (optimal

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temperature 12.4°C) improved the efficiency of the process, as well as the aroma selectivity for higher alcohols and esters and their retention in the beer. The authors also recommended recovering the permeate containing the aroma compounds by condensation at a temperature below −80°C and dealcoholizing it before adding it back to the beer (Magalhães Mendes et al., 2008). The authors did not report any sensory studies in either of these publications.

Pervaporation has also been used to extract the aroma compounds in combination with SCC dealcoholization of beer with 5% ABV (Catarino & Mendes, 2011). The final product was a low alcohol beer (0.45% ABV) with a lower concentration of aroma compounds than the original beer, but similar ratios of aroma compound/ethanol. The addition of the pervaporated aroma to the beer was essential to minimize the almost total depletion of them after SCC dealcoholization; compounds such as propanol, 2-methylpropanol, 3-methylbutyl acetate, ethyl acetate, and 2- and 3-methylbutanol were not detectable in the dealcoholized beer.

The use of pervaporation, however, is not very widespread because of the high initial investment required for the installation and the membranes, and the limitations of the membranes available in the market (Castro-Muñoz, 2019). For these reasons, more research in this promising technology is needed in order to develop more specific membranes and installations for the dealcoholization of beer and other beverages.

Reverse osmosis. Reverse osmosis is based on the selective permeability properties of membranes of defined compounds by terms of their molecular weight and polarity. Commonly used in desalination and purification of water, this technique has also been applied for the removal of ethanol from beer. Beer is pumped and filtered under pressure through the membrane, this way allowing ethanol (permeate) to be separated from beer (retentate). Catarino et al. (2006) succeeded in dealcoholizing beer (initial ethanol content 5.5% ABV approximately) using membrane technology at mild temperatures (assays performed at 4–20°C) and pressures of 2.03 or 4.06 MPa. Although the final concentrations were not low enough to be considered as alcohol-free beer (final alcohol concentration <0.5% v/v), the alcohol rejection rate through the membrane reached up to 63%. The authors mentioned higher retention of aroma compounds at lower temperatures (below 5°C), but further research is needed to optimize the aroma profile and final product quality.

Osmotic distillation. A similar process to reverse osmosis is osmotic distillation or osmotic evaporation, where the solutes to be stripped from the inlet flow travel in gaseous state through a microporous membrane and then are dissolved into the permeate stream (liquid). The use of hydrophobic membranes, for example, made of polypropylene or polytetrafluoroethylene, prevents the liquid water or ethanol passing through, so the process is only driven by the liquid–gas–liquid phase equilibria (Müller et al., 2016). This process requires normal pressure and low temperature, which helps reduce the thermal damage of the beer (Valdés et al., 2009). Liguori et al. (2015) used this technology for the dealcoholization of beer (5% ABV, type unspecified). The final ethanol content achieved was 0.46% ABV. However, the aroma compounds present in the final product were reduced considerably (Table 2) if compared with initial values. No description of the organoleptic characteristics of the dealcoholized beer was reported, but the researchers admitted that the sensorial quality needed to be improved.

Dialysis. Dialysis has been also utilized for the removal of alcohol from beer as a post-fermentation treatment. Beers dealcoholized by this process can achieve ethanol concentrations below 0.5% ABV (Montanari et al., 2008). The principle of the mass transfer is the difference of solute concentrations in two solutions (in this application, beer and dialysate) separated by a semipermeable dialysis membrane (Sohrabvandi et al., 2010). Although it is a technique based on the existence of a concentration gradient, higher pressure in the beer stream also helps maintain the transfer of ethanol from the beer toward the dialysate (Leskošek & Mitrović, 1994). Like other membrane separation processes, dialysis removes key aroma compounds from the beer together with the ethanol. These losses can be as high as 85% for esters, 85% for higher alcohols, and 70% for low chain fatty acids (Table 2) (Stein, 1993). The dialyzing liquid (or "stripping solution") is usually water, which can be recirculated and reused after removal of the ethanol and other components retained (Stein, 1993).

7.2 | Biological methods

These methods do not require any special equipment, as post-fermentation separation techniques (physical methods) do, involving lower investment cost for breweries, and a lower carbon footprint. During the last few decades, several approaches have been used to control the final concentration of ethanol in beer: modification of the microorganisms (use of special yeasts or other microorganisms), modification of the wort to be fermented (low fermentable wort), or modification of the fermentation conditions (arrested fermentation, cold contact fermentation). The presence of off-flavors is a common complaint from the consumer

A general comment about these beers, especially those brewed by modified fermentation conditions and low fermentable wort, is about their sweet taste (Brányik et al., 2012). This is due to the presence of high amounts of unfermented sugars resulting from the unfavorable

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fermentation conditions. They have aobvious sensory impact on the final product affecting mainly the perceived sweetness but also the body and/or fullness of beer. Fermentable carbohydrates in beer come from the activity of α- and β-amylases during mashing in the brewing process (Briggs et al., 2004). In conventional beers, these oligosaccharides are metabolized into ethanol by the yeast during fermentation, whereas in alcohol-free beers produced with biological methods, mainly through cold contact and arrested fermentation, these carbohydrates remain practically intact (Perpète & Collin, 2000). This has a profound impact both in terms of sweetness as well as in the perceived mouthfeel of the final products with the ones made with the latter methods, being perceived mainly as too sweet. Another flaw of beers brewed by biological methods is the presence of off-flavors or an aroma perceived as unbalanced that will be treated more in detail in this section.

7.2.1 | Use of special yeasts or other microorganisms

One strategy to produce alcohol-free beers is based on wort fermentation using different yeast species to the traditionally used *S. cerevisiae*. These alternative microorganisms usually present a low or no capacity to ferment sugars in wort, while desirable esters and higher alcohols are biosynthesized. Beer researchers have tried different types of microorganisms, from special strains of *S. cerevisiae* to yeasts from other genera and even bacteria.

Successful results (type of beers unspecified) have been obtained by fermenting wort with mutant strains of *S. cerevisiae* deficient in the tricarboxylic acid cycle (Navrátil et al., 2002). After an average of 85 h fermentation at 15°C, the authors reported alcohol contents between 0.09 and 0.31% w/w. Furthermore, the concentration of esters (ethyl acetate, 3-methylbutyl acetate, 2-methylpropyl acetate, ethyl hexanoate, and others), higher alcohols (2-methylpropanol, butanol, 2- and 3-methylbutanol, 2-phenylethanol), and 2,3-butanedione were higher than the control (where a regular strain was employed in brewing). The authors explained that the limited production of ethanol by these yeast strains might be due to several factors, such as the higher production of acids. This affects the activities of alcohol dehydrogenase and pyruvate decarboxylase, both with optima at higher pH values. The authors claimed that the organoleptic quality of these beers was comparable to commercial beers, but no formal sensory evaluation was carried out.

Other microorganisms have been used for the fermentation of wort due to their ability to produce flavor-active volatiles considered desirable in beer. Spontaneous mutants of *Saccharomyces pastorianus* have been isolated and used in low alcohol beer (type unspecified) production due to their outstanding capacity to produce 3-methylbutyl acetate and 3-methylbutanol while the ethanol concentration was as low as 13.7 g/L (1.74% ABV) (Strejc et al., 2013). These beers, brewed by arrested fermentation (see section 6.2.3), exhibited a fruity, banana-like aroma and were rated as "good" by a sensory panel. *Saccharomyce pastorianus* has also been used in continuous alcohol-free beer fermentation. Mota et al. (2011) produced alcohol-free beer (type unspecified) fermented with immobilized *S. pastorianus*, and another beer using a strain of *S. cerevisiae* with disruption in the *KGD2* gene. This *S. cerevisiae* strain, deficient in fumarase and α-ketoglutarate dehydrogenase, has been proven to produce alcohol-free beers with an alcohol content below 0.5% ABV (Selecký et al., 2008). However, the concentration of aroma compounds in the final beer was lower than those fermented by *S. cerevisiae ΔKGD2* (Mota et al., 2011). No sensory evaluation was reported in these studies (Mota et al., 2011, Selecký et al., 2008).

The use of microorganisms other than yeasts from the genus *Saccharomyces* has been tried for the fermentation of worts. The most successful yeast used in the industrial production of alcohol-free beer is *Saccharomycodes ludwigii*, the main reason being its inability to use maltose and maltotriose as nutrients (Brányik et al., 2012). Strains of *S. ludwigii* have been used to produce beers (from Pilsner malt) with alcohol contents below 0.75% ABV (Table 3) while they were able to synthesize fruity esters and higher alcohols (De Francesco et al., 2015). The researchers also utilized different strains from *Zygosaccharomyces rouxii*, with higher production of volatiles but also varying amounts of ethanol (0.93%–3.32% ABV, depending on the strain). *Mrakia gelida*, a psychrophilic yeast, has also been used in the production of low alcohol beers, with alcohol contents below 1.40% ABV (De Francesco et al., 2018). The beers were subjected to sensory evaluation with successful results comparable to beers fermented by *S. ludwigii*, used as control in this study, and with higher scores for the attribute "fruity/estery." Bellut et al. (2019) utilized different species of the genus *Cyberlindnera*. All the beers (from Pilsner and acidulated malts) produced contained low levels of ethanol, with a maximum of 0.67% ABV. Moreover, the aroma of most of these beers was described as fruity and banana-like attributed to high concentrations of 3-methylbutyl acetate and 3-methylbutanol. Nonetheless, one of the samples, fermented by *Cyberlindnera misumaiensis*, presented a strong solventy aroma at an unacceptable level, related to a high concentration of ethyl acetate. The authors concluded that, although the concentration of esters was high and well above the

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TABLE 3 Ethanol content and flavor compounds in low alcohol beers fermented by different yeast species

Compounds	*S. ludwigii*[a]	*Z. rouxii*[a]	*M. gelida*[b]	*S. ludwigii*[b]	*C. misumaiensis* 837A[c]	*C. fabianii NT* Cyb[c]	*C. jadinii* L1[c]	*C. subsufficiens* C6.1[c]	*C. mrakii* CBS1707[c]	*C. subsufficiens* CBS5763[c]	*P. kluivery* A[d]	*P. kluivery* B[d]
Ethanol, % ABV	0.51–0.88–1.36	0.93–2.02–3.32	1.16	1.23	0.55	0.63	0.66	0.63	0.54	0.67	0.1	0.2
Esters, mg/L												
Ethyl acetate	1.17–4.33–14.9	2.12–23.7–70.9	0.6	9.3	65.70	22.55	9.27	4.90	8.10	5.17	n.d.	n.d.
3-Methylbutyl acetate	0.008–0.011–0.022	0.008–0.064–0.21	n.d.	0.03	0.90	n.d.	0.15	1.60	1.67	1.03	1.96	4.94
Ethyl hexanoate	0.014–0.016–0.020	0.017–0.024–0.045	0.009	0.011	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03	0.07
Ethyl octanoate	0.010–0.012–0.013	0.011–0.016–0.020	0.006	0.009	n.d.	n.d.	n.d.	–	n.d.	n.d.	0.12	0.13
Higher alcohols, mg/L												
1-Propanol	3.13–4.01–5.57	5.64–14.5–33.6	5.7	2.6	4.03	3.73	4.40	3.27	2.93	3.33	n.d.	n.d.
2-Methylpropanol	5.27–9.90–15.3	14.6–39.6–68.9	9.8	13.0	7.57	7.70	8.27	8.03	5.33	7.20	n.d.	n.d
3-Methylbutanol	14.1–18.2–28.9	16.4–46.1–75.8	6.0	14.3	11.2	16.4	23.2	11.7	11.9	10.5	2.00	2.00
2-Methylbutanol	3.72–5.22–6.98	6.76–13.3–20.4	1.4	5.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.
2-Phenylethanol	13.7–15.4–20.0	9.80–18.8–34.5	2.6	6.8	n.d	n.d.	n.d.	n.d.	n.d	n.d	n.d.	n.d.
Carbonyl compounds, μg/L												
Acetaldehyde	1850–2870–4420	5580–7260–8150	2300	918	9700	8050	2600	3370	3830	2570	n.d.	n.d.
2-Methylbutanal	7.95–24.8–79.1	7.73–29.6–46.8	1.3	1.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-Methylbutanal	31.9–69.9–119	17.1–68.4–143	7.2	6.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hexanal	1.06–1.57–2.13	0.91–1.77–2.34	1.0	0.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Furfural	19.2–24.9–28.6	8.06–10.0–13.4	7.3	10.3	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	–	–
Methional	7.31–11.4–17.2	9.60–17.5–39.9	7.8	5.6	n.d.	n.d.	n.d.		n.d.	n.d.	–	–
Phenylacetaldehyde	22.6–38.7–76.9	13.3–23.5–36.7	9.2	10.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	–
2,3-Butanedione	5.41–8.31–15.8	234–460–851	7.8	8.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	–

Note: Data rounded up to three significant figures.
Abbreviations: ABV, alcohol by volume; n.d., not detected.
[a]De Francesco et al. (2015) (data are shown as minimum value–average–maximum value).
[b]De Francesco et al. (2018).
[c]Bellut et al. (2019).
[d]Saerens & Swiegers (2014).

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threshold, their profile was unbalanced and so was the overall aroma of the beer.

Saerens and Swiegers (2014) used two strains of *Pichia kluyveri* (strains A and B) for the fermentation of alcohol-free beers from Pilsner barley and wheat. The authors claimed that the beers produced by this microorganism had a flavor very close to that of commercial alcoholic beers. These beers contained between 0.1% (strain A) and 0.2% ABV (strain B) and high concentrations of esters and 3-methylbutanol (Table 3). These yeasts also produced less 2,3-butanedione and organic acids, like hexanoic, octanoic and decanoic acid, than *S. ludwigii*. *Pichia kluyveri* was found to be an interesting yeast species for the fermentation of alcohol-free beer because of its ability to consume only glucose and not other sugars. Another yeast of the *Pichia* genus, *P. farinosa*, has also been used for the fermentation of alcohol-free beers. A German patent claimed that a beer with less than 0.5% ABV can be produced by *P. farinosa* with a fruity flavor (Beubler et al., 1983). However, most of the beers reported in this section were well above 0.05% ABV, and could not be labeled as "alcohol-free" in many countries. For this reason, more research is needed in order to improve these promising biological methods.

7.2.2 | Production of low fermentable wort

Mashing is the first stage in the preparation of wort, and it consists of mixing ground malted cereals and water. This mixture is heated following a specified temperature schedule that differs depending on the final beer desired. These temperatures are commonly chosen to be within the range of optimal activity for the major starch-digesting enzymes present in wort: α- and β-amylases. The degradation of starch into maltose is carried out by β-amylase at an optimal temperature range of 60–65°C, while α-amylase converts starch into fermentable and nonfermentable glucose-polysaccharides (glucose, maltose, maltotriose, and higher dextrins) at a temperature optimum of 65–70°C (Montanari et al., 2005). At the end of mashing, temperature is raised to 76–78°C for enzyme deactivation. By modifying the mashing temperature regime, Sieben and Zastrow (1991) reduced the fermentability of the mash by about 60%, reaching final ethanol concentrations of less than 2%. Part of the mash was kept at around 49°C, while a portion of it was transferred into a cooker and boiled for 20 min for the deactivation of the amylolytic enzymes. The authors claimed that, by following this procedure, a low alcohol beer having the same taste, aroma, and mouthfeel as a regular alcoholic beer was obtained, but no formal sensory evaluation was reported. Other authors believe that the strategy of controlling or restricting amylolysis is rarely successful for the production of alcohol-free beers and that it usually requires combining this method with others, such as the control of the fermentation (Briggs et al., 2004).

7.2.3 | Arrested fermentation

Also known as "stopped," "limited," or "restricted" fermentation, arrested fermentation's principle is stopping yeast activity at the right time for a desired concentration of ethanol. The biological process can be stopped by temperature inactivation of yeasts (most used is rapid cooling to 0°C or pasteurization) or by removing cells from the liquid (by employing centrifugation or filtration) (Brányik et al., 2012). Arrested fermentation has been applied to beers fermented either in batch or continuous reactor. Lehnert et al. (2009) compared the characteristics of beers (type unspecified) brewed by both methods. The fermentation in batch was stopped once 0.4% ABV was reached, and in both systems the temperature was kept constant at 8°C. The aroma of the beers brewed in the continuous reactor was described by a sensory panel as worty, estery, or medicinal, depending on the strain of *S. pastorianus* used. On the other hand, the beers brewed in batch reactor presented an aroma described as "normal," with a yeasty off-flavor.

The extract content has been also used as a criterion to stop a fermentation for alcohol-free beer. In brewing, the extract content is defined as the concentration of dissolved solids in a wort or beer. It is measured in degrees Plato (°P), which are equivalent to grams of extract per 100 g of wort. Jiang et al. (2017) combined arrested fermentation with vacuum distillation for brewing an alcohol-free beer from barley and wheat malts with no off-flavors. In fact, excess ethanol and undesired aroma compounds were removed during the distillation process. *Saccharomycodes ludwigii* was used for a batch fermentation maintained at 18°C until the extract content was lower than 1.5°P. This condition was reached after about 15 days. Then, the beer was kept at 0°C for two days aiming to obtain a "young beer" with less than 1% ABV. Next, the beer was submitted to vacuum distillation in a continuous vacuum evaporator (0.05–0.06 MPa, 64–68°C). Following this method, the authors claimed that an alcohol-free beer, with an ethanol content below 0.5% ABV and "rich, smooth and round" flavor could be obtained, albeit no formal sensory evaluation was reported.

7.2.4 | Cold contact fermentation

Cold contact fermentation (CCF) is another one of the common processes for the industrial production of

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alcohol-free beer. The purpose of this kind of processes is to reduce yeast activity by applying a very low temperature during fermentation, thus reducing or even avoiding the production of ethanol (Schur, 1982). This method for the preparation of alcohol-free beers with a yeasty aroma consists of putting the wort in contact with the yeast at a temperature below 3°C, preferably as close as possible to the freezing point, at −0.5 to −0.4°C. After fermentation for 48 h, an alcohol-free beer with an ethanol content below 0.05% ABV was prepared following this method. However, these beers are characterized by an aroma reminiscent of malt or wort, lacking the fruity character of alcoholic beers. The formation of esters and higher alcohols, responsible for the fruity aroma, has been found to be positively dependent on temperature (Landaud et al., 2001). Moreover, researchers have shown that the malty, worty character of beers brewed by CCF is caused by Strecker aldehydes, especially 2-methylbutanal (malty aroma), 3-methylbutanal (malty), and methional (cooked potato) (Perpète & Collin, 1999a). Unlike their correspondent alcohols and acetate esters, the perception thresholds of these Strecker aldehydes are extremely low, below 1 μg/L for 3-methylbutanal and methional (Figure 3). Therefore, even small concentrations of them can impart a strong malty, worty flavor to the beer. A study by Piornos et al. (2020) confirmed the importance of 3-methylbutanal and methional, but not 2-methylbutanal. They used a sensomics approach to identify 27 odor-active compounds in a CCF alcohol-free beer, of which 3-methylbutanal, (*E*)-β-damascenone, abhexone, and phenylacetaldehyde were the most odor active.

8 | CONCLUSIONS AND FUTURE PERSPECTIVE

In this study, the flavor and sensory aspects of alcohol-free beer have been comprehensively reviewed. The literature reveals a clear increase in popularity of these beverages across the world, accompanied by a decrease in the consumption of alcoholic drinks. The increasing interest of consumers toward nonalcoholic drinks has been reflected in a surge of new products in the market worldwide and the improvement of the ones already available. Still, being able to produce an alcohol-free beer, with very similar organoleptic characteristics to its alcoholic equivalent product, remains a complex task and a scientific and technological challenge.

Indeed, among the existing variety of production strategies and methods, none of them fulfills all the requirements for an acceptable, full-bodied, fruity beer, and thus a combination of them, or a post-fermentation treatment such as flavor reconstitution, is required in most of the cases. Physical methods affect the aroma chemistry of these beers in a completely different way from biological ones, and therefore improvement strategies must be addressed in different ways. For physical methods, the focus is on reducing the loss of desirable aroma compounds. The dealcoholization process, both distillation and membrane processes, need to be optimized to not only eliminate ethanol, but also to control the losses of desired aroma compounds. Furthermore, the addition of the extracted aroma compounds to the already dealcoholized beer depends on the capacity to separate ethanol this time from the flavor-rich fraction, and this is another field where more research is required to develop separation methods for the brewing industry. The identification and quantification of the extracted flavor compounds is critical to reconstitute the aroma profile of the beer, where losses cannot be avoided.

In the case of biological methods, the focus is on removing off-notes, particularly Strecker aldehydes, which are ordinarily reduced, removed, or transformed into pleasant, fruity higher alcohols, and esters during a full fermentation process. This issue can be addressed from two different perspectives: the elimination or separation of off-flavor and the mitigation of their formation. Gernat et al. (2020) reviewed the different technologies applied for aldehyde removal from beer, the more promising being the physical adsorption onto hydrophobic resins or activated carbon. On the other hand, in order to mitigate the formation of off-flavors, the optimization of precursor concentration and processing conditions is essential to reduce those aroma compounds not desired in the final product.

Besides, the use of nontraditional or modified microorganisms has shown very optimistic results flavor-wise, although sometimes the flavor of these beers was perceived as unbalanced. However, some of the authors in the literature reviewed claimed that the alcohol-free or low alcohol beers produced presented a fruity, pleasant flavor, confirmed by sensory evaluation. Further research is encouraged in this area in order to find microorganisms able to produce a beer both alcohol-free and with a pleasant, fruity flavor. Additionally, in the biological methods, the mouthfeel and sweetness of the final products is completely different from a conventional alcoholic beer as, in most cases, the initial fermentable sugars of the wort are not metabolized during the process. For this reason, mashing and wort boiling steps should be optimized to adapt the content of sugars in the wort to the fermentation conditions used in alcohol-free beer.

Another pathway to be considered is the use of hops with the double aim of providing another layer of aroma and taste to the alcohol-free beers, and also of masking the undesired aromas already present. A promising approach followed by some researchers is dry-hopping, a process

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consisting of adding the hops after wort boiling when this is already cold, so the loss of volatile aroma compounds by evaporation or thermal damage is minimal (Forster & Gahr, 2013). Dry-hopping has been demonstrated to have a positive effect in both adding new appreciated aroma compounds, such as terpenes and esters, as well as to mask off-notes (Brendel et al., 2020a). Moreover, hops provide a source of organic acids that are able to react with ethanol, even at low concentrations, thus producing fruity ethyl esters of methylpropanoic acid, 2- and 3-methylbutanoic acids (Brendel et al., 2020b).

In the literature reviewed to date, most researchers focus on the optimization of the removal of ethanol but do not report formal sensory evaluations of the beers produced. This reflects a gap in the deeper knowledge of the acceptability of these beers and how the processes affect their flavor and mouthfeel. Thus, we would recommend that researchers put more effort into providing formal sensory descriptions of their beer prototypes. An account on the sensorial consequences from the various processing practices should be included in future studies in order to evaluate the applicability of each technology.

Furthermore, other important information that we have consistently found to be missing in the literature is the type of alcohol-free or low alcohol beer produced or used in the experiments. Since the vast majority of these beers available in the market are lager from barley malts, in most of the cases we assumed that this was indeed the type the researchers were referring to. However, we have demonstrated that both production methods and raw materials (this, of course, linked to flavor precursors) have a key role on the aroma compounds in the final beers. Consequently, we would recommend inclusion of these details in future publications on the topic.

Finally, the contribution of ethanol to the perception of alcohol-free beers has often been neglected or ignored. Even though its role in aroma release and bitterness is well documented, there is lack of knowledge as to how it affects other sensory aspects of the final flavor especially for beer (body, fullness, other sensory aspects). Nevertheless, many have tried to mitigate the effects of the lack of ethanol in alcohol-free beers by the addition of replacers or "ethanol-mimic" components aiming to produce a similar sensory experience in the product. Glycerol has been proposed as an ethanol replacer due to its capacity to retain undesirable worty aldehydes in alcohol-free beers (Perpète & Collin, 2000). A concentration of 4.5% glycerol was capable of reducing the release of 2- and 3-methylbutanal into the headspace by up to 40%. The role of ethanol in beer and other beverages is very important, and finding a replacement that is able to compensate for its multifunctional sensory effects has become a great challenge for brewers, product developers, and scientists.

In conclusion, being able to produce an alcohol-free beer, with very similar organoleptic characteristics to their alcoholic counterparts, remains a complex task and a scientific and technological challenge. This challenge needs to be addressed from a combination of different perspectives: optimization of the brewing process, research on the use of nontraditional microorganisms, removal of off-flavors, and research on ethanol-mimic components.

AUTHOR CONTRIBUTIONS

Conceptualization, data curation and manuscript draft: José Piornos. Conceptualization, funding acquisition, project administration, supervision, and writing—review and editing: Elisabeth Koussissi. Conceptualization, funding acquisition, supervision, and writing—review and editing: Dimitris P. Balagiannis. Conceptualization, funding acquisition, supervision, and writing—review and editing:: Eric Brouwer. Conceptualization, Funding acquisition, Project administration, Supervision, Writing—review & editing: Jane K. Parker.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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