

Chapter

Non-Conventional *Saccharomyces* Yeasts for Beer Production

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Abstract

Beer is a world-famous beverage, second only to tea and coffee, where the yeasts traditionally used are *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* for the production of ale and lager beer, respectively. Their production, especially craft beer production, has grown in recent years, as has the development of new products. For this reason, research has focused on the selection of yeasts with good fermentation kinetics, as well as beers with outstanding aromatic profiles. The final flavor and aroma of beer is a combination of hundreds of active aroma compounds produced mostly during fermentation as a result of yeast metabolism (higher alcohols, esters, aldehydes, and vicinal diketones). Likewise, several studies have demonstrated the potential of wild yeasts of the genus *Saccharomyces*, both in aromatic production and in the production of healthy compounds of interest such as melatonin. This chapter therefore focuses on non-conventional *Saccharomyces* yeasts as they have the capacity to produce outstanding aroma compounds, as well as compounds that can provide health benefits, under moderate consumption.

Keywords: non-conventional yeast, *Saccharomyces*, beer, aromatic compounds, functional beer

1. Introduction

Nowadays, there is a growing global interest in craft beer, as well as in the production of new beers that meet market needs. For this reason, brewers have focused especially on the use of yeasts, especially non-conventional yeast, to innovate in the brewing sector [1–5]. Wild yeast strains can provide different aroma and flavor characteristics with which to obtain varieties and styles of beer that are alternative and new to existing ones [6]. However, the use of these domesticated yeasts may show variable fermentative characteristics and affect the consistency and quality of the beers produced [4, 7]. Unlike the current commercial strains of *Saccharomyces* yeast, their domestication has occurred over millennia, resulting in brewers obtaining consistent beers with established aromas, flavours and fermentation conditions [4, 8]. Nowadays, however, all the steps leading to yeast domestication can be done in a faster way [2, 9], since the metabolism pathways of yeast are better known, as well as the production pathways of the different aromatic compounds (higher alcohols, esters, phenols, acids, and monoterpenes) [2, 10–13]. It is for this reason that brewers

and researchers have focused on the search for new yeasts from different environments using current techniques [14]. Determining the suitability of new yeast strains can be done by evaluation by expert panels; however, this must be coupled with their analysis using physicochemical analytical techniques to show correlation with the aroma compounds produced. Such compounds are produced in smaller proportions than ethanol and carbon dioxide, which are the main products of fermentation, but are nevertheless of great importance [10, 15]. Secondary metabolites can be divided into different categories and include sulfur-containing aroma compounds, undesirable carbonyl compounds, volatile phenols, organic acids, fusel alcohols, esters, and monoterpene alcohols [10, 15–17]. These groups of aromatic compounds play a number of roles in the impact of flavor on beer, as many of them can act synergistically, thus increasing flavors, despite being below their threshold [11, 15, 16].

The present review focuses on the use of non-conventional yeasts of the genus *Saccharomyces* for fermenting beer wort, obtaining an outstanding organoleptic profile, as well as the production of secondary metabolites with healthy characteristics for potential use by brewers.

2. Beer ingredients

Beer is a worldwide-known beverage and the most popular after tea and water. However, people do not focus their attention on how it is brewed, as well as on its ingredients, which are barley as the main cereal, water, hops, and yeast. Other cereals or adjuncts may also be added [18, 19].

Water is the main component of beer, accounting for 85–92% of beer, as well as serving as the basis for brewing the beer wort. Water quality is an important factor to take into account since the presence of any odor, taste, and impurity is unacceptable because of its influence on the sensory properties of the beer. The enzymatic and colloidal reactions that occur during brewing are influenced by the content of dissolved ions found in water such as Ca^{2+} , Mg^{2+} , Na^{+} , K^{+} , SO_4^{-2} , Cl^{-} , HCO_3^{-1} , and CO_3^{-2} [20]. Calcium (Ca^{2+}) is involved in protein coagulation and polyphenol and carbohydrate formation, forms and precipitates calcium oxalate and calcium carbonate, reduces pH during mashing and boiling, and promotes yeast metabolism and flocculation, and its concentration is key to residual hardness and alkalinity. Magnesium (Mg^{2+}) is a vital nutrient for yeast and necessary for the isomerization of humulone to isohumulone in hops. Sodium (Na^{+}) can contribute to the beer body. Chloride (Cl^{-}) in low amounts enhances malt sweetness. Bicarbonate (HCO_3^{-}) functions as a buffering agent, buffering pH variations [21].

The basis of beer production is **malted barley**. The malting process is carried out for the yeast fermentation, as well as proteins that promote and enhance foam stability [22]. Malt can provide the beer with aromas of biscuit, honey, cinnamon, bread, chocolate, cocoa, coffee, and roasted and/or smoked, among others [23].

Hops (*Humulus lupulus* L.) is the ingredient that will mainly provide the bitterness of the beer due to the iso- α -acids (iso-normal-humulone, iso-co-humulone, and iso-ad-humulone) obtained by the isomerization of the α -acids (normal-humulone, co-humulone, and ad-humulone) during the wort boiling and whirlpool process in brewing. These α -acids are contained in the resins of hop flowers [24]. In addition to bitterness, hop also contributes to beer aromas, such as citrus, floral (geraniol, linalool), herbaceous, and fruity (limonene, myrcene, β -pyrene), due to the resins, proteins, and essential oils they possess [25]. Hops are sensitive to oxidation in contact

with air, so it is important that they are stored under vacuum in a cool, dry place; otherwise they can transmit unpleasant aromas to the beer (such as isovaleric acid with rancid cheese aroma).

Yeast is an important ingredient in beer brewing, as it is responsible for fermentation and thus for the conversion of the sugars in the beer wort into ethanol and carbon dioxide (CO₂). However, it will also produce different by-products, the main ones being aromatic compounds that will characterize the aromatic profile of the beers.

In the past, these four ingredients were not well-known, but it was known that yeast was necessary for the production of fermented beverages. It was not until the development of lager beers that the exact composition of yeast was discovered. Although yeasts have been used for centuries to brew beer, they were first identified as responsible for the fermentation of malted barley water in the 19th century. The first principles of yeast function were discovered during the 17th and 18th centuries [26]; however, it was not until the mid-19th century that the French scientist Louis Pasteur was able to demonstrate that yeast is made up of living cells responsible for the fermentation process [27]. In the same vein, sugars were also not known to be another essential ingredient and can be classified according to the type of fermented beverage, whether from sugar cane (sucrose), milk (lactose), fruit or honey (fructose and glucose), or cereals (maltose) [19].

3. Beginnings in the use of yeast for beer production

The beginnings of beer production date back to the Neolithic civilization. Beer is a traditional product and valued for its physicochemical properties that give it quality. Therefore, the history of brewing is not only the history of scientific and technological developments but also the history of people: their government, their culture, and their daily life [28]. Early brewers, winemakers, and bakers realized that by using small portions of finished products that had already been fermented, it was possible to obtain products with faster and more predictable fermentation. Thus, the ability of *Saccharomyces cerevisiae* yeast to convert sugars into ethanol, as well as into other compounds that provide organoleptic characteristics to foods, in addition to alcohol to beverages, began to be exploited [2, 29]. However, the use of pure yeast cultures did not begin until sometime after the pioneering work of Pasteur and Hansen in the 19th century [27]. It should be noted that it has not yet been determined whether the yeast lineages used industrially originated from the loss of contact with their natural niches and limited dispersal or from adaptation to the industrial niche (domestication) [30, 31]. Domestication therefore is about artificially selecting and breeding wild species with improved characteristics adapted to artificial environments. However, this domestication can lead to genome breakdown, polyploidy, chromosomal rearrangements, gene amplifications and deletions, horizontal gene transfer, and loss of genetic diversity due to bottlenecks [32, 33].

The most studied yeast at the industrial level is *Saccharomyces cerevisiae*, where its diversity lies both in genetic drift by bottleneck and small isolated populations as well as in selection and niche adaptation. In yeasts used for winemaking, horizontal gene transfer events [34–36] and copy number variations [37–40] have been described that increase sugar and nitrogen metabolic activity, which favors fermentation of grape must, as well as better tolerance to chemicals used in vineyards and in wine [41]. In contrast, the most significant changes are found in the yeasts used for brewing, as yeasts are generally reused for several fermentation batches. This continuous growth in a very specific industrial niche has resulted in a continuous selection imposed by

the brewing environment. Therefore, the yeasts currently used at the industrial level could be considered as the result of a centuries-long evolutionary experiment in a highly selective niche.

4. Brewing potential of wild *Saccharomyces*

The increase in the consumption of craft beer [42], as well as consumers' interest in trying new beer styles [43], has encouraged the application of new yeasts in brewing [44, 45]. These yeasts include the wild yeast *Saccharomyces eubayanus* (ancestor of today's lager yeast hybrid), as well as various species such as *Torulaspora delbrueckii*, *Lachancea thermotolerans*, and *Mrakia gelida* [46–48]. These non-conventional yeasts have functional advantages over commercial yeasts as they provide novel flavor profiles, production of flavorful beers with low alcohol content, reduced caloric content, and primary acidification, in addition to their use to improve existing beer styles or create new styles. Currently, the use of new yeasts focuses not only on the isolation of wild yeasts but also on mutagenesis and breeding. However, the most attractive option for brewers remains the isolation of yeast strains from different niches [2]. The advantages offered by wild yeast isolation are extensive:

- The natural biodiversity of microorganisms representing the habitat of a geographic region can be tapped.
- These new species can bring added value to beer in terms of organoleptic qualities.
- Some yeasts isolated from nature already have the status of Generally Recognized as Safe/Qualified Presumption of Safety.
- Current regulations favor the use of unmodified genetic stocks, as they add identity and uniqueness to differentiate the production line.

On an industrial level, not only ethanol and glycerol production but also the utilization of available sugars in the wort, hop tolerance and resistance to low temperatures, and the relative production of aromatic compounds such as esters and higher alcohols, as well as low levels of acetic acid and hydrogen sulfide, are important [2, 45, 49]. Not all species are able to ferment the sugars present in the wort (glucose, fructose, maltose, and maltotriose). In the case of *S. eubayanus*, it seems highly efficient at utilizing maltose in addition to presenting an efficient production of biomass at low temperature (ideal for lager brewing), thus balancing with the non-capacity to ferment maltotriose. Wild yeasts are noted for their potential to produce remarkable aroma compounds in brewing [50]. The type strain of *Saccharomyces arboricola* has been found to produce high levels of ethyl esters [50]. However, it should also be noted that numerous *Saccharomyces* wild yeast strains tend to produce significant perceivable levels of unpleasant phenolic aroma (POF), mainly through the production of 4-vinyl guaiacol from ferulic acid [51], imparting a clove-like aroma to beer. This is considered undesirable in most beer styles. In some cases, POF aromas do not represent a problem, but are desired in the final fermented product, such as wheat beer, where clove aroma is part of the normal flavor profile [52]. Therefore, the ability of *Saccharomyces* wild yeasts to ferment at low or high temperatures, to obtain new

aromatic profiles, high or low ethanol levels and a reduction in H_2S production, as well as to supporting stressful wort conditions, represents a potential for new processing strategies. Cubillos et al. [53] collect in their study the application of various strains of wild yeast for application in beer.

Another advantage of wild yeasts is that they can be subjected to different fermentation conditions to observe their behavior. In this case, from a technological point of view, the application of aeration during fermentation is an interesting tool for controlling yeast metabolism during fermentation [54]. First, due to Crabtree-negative yeasts, when the oxygen concentration in the medium is saturated, the yeast metabolism begins to be predominantly oxidative, thus reducing the ethanol content and increasing the yeast biomass. On the other hand, aeration may also affect aromatic compounds by reducing or increasing their concentration in beer (the acetaldehyde content may decrease, and the concentration of higher alcohols increases) [55–57]. Within the non-conventional strains of *Saccharomyces*, there are strains that may present the Kluver effect with aeration during fermentation, as in the study conducted by Postigo et al. [58]. In this study, during fermentation with continuous aeration, it was observed that one of the tested strains (CLI 1057) that under anaerobic conditions only fermented glucose, fructose, and sucrose, when changing to aeration conditions, began to ferment part of the maltose present in the must [59, 60]. This behavior resulted in a variation in the concentration of aromatic compounds where isoamyl acetate, DMS, diacetyl, and 2,3-pentanedione decreased considerably, as observed in other studies such as Mauricio et al. [61]. However, the rest of the compounds increased, resulting in a more phenolic beer than without aeration.

5. Aroma production by non-conventional yeast

The beverage industry has focused on the search for fruity and floral aromas, which is why consumers demand beers with fruitier aromatic profiles. The ingredients in beer that can provide such aromas and flavors are hops, but mostly yeast during the fermentation process, as it will provide a fruitier organoleptic profile to beers. Various aroma compounds can be found in beer, although studies mainly focus on the alcohols and esters produced by yeast as they will provide the main aromas found in beer [10, 62, 63].

Meilgaard elaborated the “beer aroma wheel,” where all important beer aromas were included, including all yeast-derived aromas, as well as all other raw materials used during brewing [64].

Main aromatic compounds relevant in beer:

Sulfur compounds: yeasts can produce sulfur dioxide (SO_2) and hydrogen sulfide (H_2S) during fermentation [65, 66]. SO_2 has several functionalities, including acting as an antimicrobial agent, bleaching agent, oxygen scavenger, reducing agent, and enzyme inhibitor [67]. However, the maximum levels of SO_2 allowed in beer depend on the regulations of each country, always taking into account its perception threshold (20 mg/L), as it can impart unpleasant aromas to beer [68]. Although it should be noted that it is a positive characteristic desired in some bottom-fermented beers [69], this compound is usually found in concentrations of less than 10 mg/L, being a secondary or feedback inhibition product of amino acid anabolism [65, 69]. On the other hand, H_2S (rotten egg aroma) has a lower perception threshold (0.005 mg/L) and can mask desired aromas in beer [70, 71]. Its production occurs mainly during the cell maturation cycle, but it is assimilated later in the budding cycle [66].

Carbonyl compounds: within this group, the most prominent compounds that produce undesirable aromas in beer are vicinal diketones and acetaldehyde, produced by yeasts during the fermentation process. Vicinal diketones include diacetyl and 2,3-pentanedione. Both have low thresholds (0.15 mg/L and 0.9 mg/L, respectively [72]) and a buttery or toffee-like aroma. Diacetyl is a by-product of valine anabolism during glucose metabolism. Later, during the beer maturation phase, yeasts take up diacetyl and reduce it to 2,3-butanediol, which has no unpleasant aroma [73]. 2,3-pentanedione, on the other hand, is produced during the synthesis of the amino acid isoleucine in the mitochondria of yeast cells, but like diacetyl, its content is reduced during maturation [73, 74]. Acetaldehyde is produced during the yeast growth phase as a result of sugar metabolism and is subsequently converted to ethanol [75]. It has an undesirable green apple or grassy taste, with a threshold of 30 mg/L [72, 76, 77].

Phenolic compounds: these compounds are generally referred to as phenolic off-flavors (POFs) for most beers but are accepted in Belgian-style beers or wheat beers. These compounds contribute clove, smoky, spicy, medicinal, and burnt aromas [78, 79]. The compounds responsible for these aromas are generally 4-vinylguaiacol, 4-vinylphenol, 4-ethylguaiacol, 4-ethylphenol, 4-vinylsyringol, styrene, eugenol, guaiacol, and vanillin [80]. Although they depend on the presence of certain precursors in the wort for their production, generally the type of yeast used is largely responsible for their formation. The precursors that can be found in the wort are phenolic acids with a high flavor threshold, such as ferulic, coumaric, and cinnamic acids, which are derived from malt [51, 80]. These compounds usually have low thresholds: 4-ethylphenol, 0.9 mg/L (phenolic aroma, astringent); 4-ethylguaiacol, 0.13 mg/L (phenolic aroma, sweet); 4-vinylguaiacol, 0.3 mg/L (phenolic aroma, bitter, clove); guaiacol, 3.88 µg/L (smoky aroma); and 4-vinylphenol, 0.2 mg/L (phenolic aroma, smoky) [72, 81].

Organic acids: within the organic acids are non-volatile and volatile acids, the latter being the most prominent in beer as they generally contribute along with the rest of the off-flavors [82]. In beer, the main organic acids that can be found are propionic, isobutyric, butyric, isovaleric, valeric, caproic, acetic, caprylic, caprylic, capric, and lauric, the last four being the ones that most influence the flavor of beer [83, 84]. Their concentration plays an important role since in high concentrations, they can contribute acidic, salty, or even cheesy and sweaty flavors [16, 83–85]. Acetic acid, with a vinegar-like aroma, has a high threshold of 175 mg/L, whereas caprylic acid, with a goat-like aroma, has a much lower threshold of 15 mg/L. Capric acid contributes waxy-like aroma with a threshold of 10 mg/L, and lauric acid is described as soapy above the threshold of 6.1 mg/L [16]. On the other hand, the most prominent non-volatile organic acids are oxalic acid, citric acid, malic acid, fumaric acid, succinic acid, lactic acid, and pyruvic acid, with thresholds higher than those of the volatile acids, ranging from 220 to 700 mg/L, most of which provide acidic flavors [16, 83, 84, 86–88]. This group of organic acids is dependent on the yeast strain used and is a by-product of glycolysis, the citric acid cycle, and the metabolism of amino acids and fatty acids [88].

Higher alcohols: higher alcohols are produced by yeasts as a by-product of amino acid metabolism and catabolism. During catabolism, yeasts take up the amino acids and they are transaminated by four transaminase enzymes [89]. The resulting product is an α -keto acid, which through an irreversible reaction forms the higher alcohols, being known as the Ehrlich route [90, 91]. The main aromas provided by the higher alcohols are floral, fruity, or herbaceous, the most important in beer being n-propanol, isobutanol, isoamyl alcohol, and 2-phenylethanol [10, 16, 92]. N-butanol

provides a sweet taste, but its threshold is high (600 mg/L) [92]. Isobutanol and amyl alcohol have solvent-like aromas, with thresholds of 100 and 50–70 mg/L, respectively. Isoamyl alcohol and 2-phenylethanol give fruitier aromas, whereas isoamyl alcohol shows more banana and alcohol flavors with a threshold of 50–65 mg/L. The 2-phenylethanol compound has a gummy bear and rose flavor at a threshold of 40 mg/L [16, 92].

Esters: in addition to higher alcohols, esters are also an important group of aromatic compounds because they have low thresholds and provide fruity aromas to beer [10, 11]. The esters can be divided into two groups, the acetate esters whose concentration is majority in beers and the medium-chain fatty acid ethyl esters [10, 11]. The most important esters that can be found in beer are ethyl acetate (solvent flavor with a threshold of 33 mg/L), isoamyl acetate (banana flavor with a threshold of 1.6 mg/L), isobutyl acetate (fruity and sweet flavor with a threshold of 1.6 mg/L), and phenylethyl acetate (aroma of rose, apple, and honey with a threshold of 3.8 mg/L) [16, 93, 94]. Moreover, medium-chain fatty acid ethyl esters are formed from a medium-chain fatty acid and an ethanol radical. The most prominent are ethyl hexanoate (apple, anise flavor, with a threshold of 0.23 mg/L) and ethyl octanoate (acid apple flavor, with a threshold of 0.9 mg/L) [10, 11, 16].

The use of non-conventional yeasts in winemaking has been extensively studied in both *Saccharomyces* and non-*Saccharomyces*, as they have shown great potential in the production of aromatic esters during vinification, in both mixed and sequential inoculation [95–97]. However, despite the great aromatic potential of non-conventional yeasts, there are fewer studies on beer than on wine [6, 98]. In *Saccharomyces* species, mainly the Ehrlich pathway for fusel alcohols or the enzymes responsible for ester formation have been studied as main pathways for the formation of aroma compounds [99–101]. Gamero et al. [102] also carried out their studies with non-*Saccharomyces* yeasts with a prominent organoleptic profile, so these non-conventional yeasts should also present such metabolic pathways involved in the formation of this type of flavor compounds, such as the Ehrlich pathway, or the specific enzymes responsible for the synthesis of esters (Atf1p, Atf2p in *S. cerevisiae*). However, the regulation of their expression or the functionality of these enzymes may vary from non-*Saccharomyces* yeast to *Saccharomyces* yeast.

The study carried out by Postigo et al. [103] with 114 *Saccharomyces cerevisiae* yeast strains isolated from wine environment resulted in the production of aromatic compounds similar to those produced by commercial strains such as SafAle S-04 (Fermentis, Lesaffre, France). However, the relative proportions between the specific volatiles, or the flavor profiles, were different. This diversity of aromatic profiles was immense, and, interestingly, some of these strains could produce greater amounts of aromatic compounds than the commercial *Saccharomyces* strain. The strains studied were characterized by the production of higher concentrations of alcohols, such as isoamyl alcohol, isobutanol, and β -phenylethanol, as well as esters such as isoamyl acetate and ethyl butyrate compared to the commercial strain. On the other hand, it was also observed that some of these strains showed guaiacol production above the threshold (3.88 ppb) (smoked flavor) [104], giving a phenolic character to beers. A particularity of non-conventional yeasts is the production of POFs, generally unwanted in some beer styles but appreciated in wheat beers [52]. In other studies, such as the one carried out by Rossi et al. [105] with 12 yeast strains isolated from environments such as grape must, bakery, wine, and apple stillage showed behaviors similar to those observed in the previous study. **Table 1** shows the different yeast strains isolated from non-conventional environments and the production

Yeast strain	Species	Source of isolation	Apparent attenuation (%)	Flavor compound	Concentration (mg/L)	Reference
DBVPG 1058	<i>Saccharomyces cerevisiae</i>	Baker's yeast	71.25	Ethyl acetate	32.00	[105]
				Isoamyl acetate	1.90	
				Ethyl hexanoate	0.60	
M4	<i>S. cerevisiae</i>	Craft beer Ale	69.85	Acetaldehyde	164.92	[106]
				Isoamyl alcohol	161.04	
MT-15	<i>S. cerevisiae</i>	Sourdoughs	60.29	Acetaldehyde	43.14	[106]
				Isoamyl alcohol	101.88	
Granvin2	<i>S. cerevisiae</i>	Norwegian kveik	n.a.	Ethyl caproate	0.37	[107]
				Ethyl caprilate	4.56	
				Ethyl decanoate	0.46	
Grancin6	<i>S. cerevisiae</i>	Norwegian kveik	n.a.	Ethyl caproate	0.37	[107]
				Ethyl caprilate	5.01	
				Ethyl decanoate	0.88	
G 520	<i>S. cerevisiae</i>	Organic cellar	72.00	Isoamyl acetate	2.34	[103]
				Guaiacol	0.05	
CLI 1109	<i>S. cerevisiae</i>	Vineyard	71.00	Isoamyl acetate	1.90	[103]
				Ethyl hexanoate	0.60	
				Guaiacol	0.03	

n.a.: not appear.

Table 1.

Aromatic compounds found in different beers fermented with non-conventional Saccharomyces yeasts whose concentrations are above their threshold.

of different aromatic by-products, the most important of which are shown below. Some of the strains studied exhibited fermentative behaviors close to commercial strains (Safbrew-S33 and Nottingham), with concentrations of aromatic compounds higher in some cases than those in commercial ones. Another peculiarity of the non-conventional yeasts of *Saccharomyces* is that there are some strains that will only ferment in the wort glucose, fructose, and sucrose but not maltose [58, 103]. This makes them ideal candidates for brewing low ethanol beers, as they can produce levels of aromatic compounds similar to those of other *Saccharomyces* strains that do ferment maltose, which can cause the characteristic wort flavor of some beers with low ethanol content [108, 109]. This also prevents the use of mechanical methods to remove ethanol content. Furthermore, residual sugars that may remain in beer can influence aspects such as the viscosity that contributes to the sensation in the mouth and body of beer [110, 111]. Likewise, this type of strains can be used in cofermentation with other strains of *Saccharomyces* to improve the aroma of beer [106].

6. Functional beer

Functional beer is defined as a beer that can provide health benefits under moderate consumption. Functional beers include those that have a low ethanol content, as well as those that provide high concentrations of compounds such as fiber, vitamins, minerals, polyphenols, and probiotics [1].

Recent years have seen an increase in consumption and interest in low-alcohol beers. This is mainly due to health and safety reasons, in addition to an increase in strict social regulations [112]. Low-ethanol beers can have health benefits due to the healthy components they contain, besides a lower energy intake and the total absence of negative effects of alcohol consumption.

Several studies have shown that with the use of non-conventional yeast, functional beers can be obtained, as they not only possess the ability to produce remarkable aromatic compounds, as well as other by-products such as melatonin [6, 113].

Functional beers also include beers that are gluten-free, thus covering consumers suffering from coeliac disease, which is a gluten-sensitive, immune-mediated enteropathy.

Probiotic beers are also included in functional beers. Probiotics include those live microorganisms that are added to food and that under certain doses can be potentially beneficial for human health, especially for the intestinal microbial balance [114]. Therefore, a probiotic beer is one obtained by using probiotic microorganisms during the fermentation process. The best known microorganisms used for their probiotic characteristics are lactic acid bacteria (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*, or *Streptococcus*) [115]. However, studies have also been extended to other microorganisms such as yeasts, where experiments have been carried out with *Saccharomyces cerevisiae* var. *boulardii*, since it has been found to have the ability to prevent diarrhea associated with the use of antibiotics, as well as helping with infections caused by *Escherichia coli* or *Clostridium difficile* [116, 117]. In addition, beer trials have also been carried out with *S. cerevisiae* var. *boulardii*, where the results have shown an increase in the antioxidant capacity due to metabolite secretion by the yeasts in the craft beers obtained [118]. However, studies have focused not only on the study of non-conventional yeasts of the *Saccharomyces* genus but also on the use of other non-*Saccharomyces* genera (*Lachancea*, *Kluyveromyces*, *Torulaspora*, *Metschnikowia*, *Kazachstania*, *Brettanomyces*, *Pichia*, *Candida*, *Hanseniaspora*, *Rhodotorula*, *Rodosporidobolus*) since they have been found to have great potential for use in the production of craft beers with probiotic characteristics [119]. Craft beer is generally not unfiltered or unpasteurized; thus, the yeast cells remaining after fermentation can provide a probiotic character to the beer and can be considered beneficial to health. This could be seen in the studies carried out by Mulero et al. [118] comparing unfiltered beers fermented with *S. cerevisiae* var. *boulardii* and a commercial *S. cerevisiae* strain. According to the studies of Hill et al. [120], the minimum recommended dose of live probiotic cell count per product sample is 9 Log colony forming units (CFU). On the other hand, industrial beers, after being subjected to high temperatures during pasteurization, may eliminate such probiotics. For this reason, craft rather than industrial brewing would be more appropriate since viability is crucial for the efficacy of probiotics [106]. However, it should be noted that the presence of yeasts in beer can give beer a unique taste but reduce its shelf life as during storage, cell lysis can negatively affect its quality. This is why yeast strain selection is important in the production of craft beers [121].

6.1 Melatonin production

Melatonin is a mammalian hormone that regulates sleep and has antioxidant properties. It is produced by yeasts during fermentation and can therefore be a source of exogenous melatonin for the body, since as people age, less melatonin is produced in the body. In addition, it provides beer with antioxidant, antiaging, anti-inflammatory, antitumor, and immunomodulatory capacities [122–124].

Much of the food and beverages we consume on a daily basis contain melatonin. Therefore, their intake helps to increase the melatonin level in the body and its antioxidant status in human serum, which is the reason that this molecule is absorbed in the gastrointestinal tract [123, 125, 126] and readily crosses all morphophysiological barriers and tissue and cell membranes [124–129]. Likewise, melatonin interacts with toxic reagents, generating other metabolites that are in turn direct free radical scavengers. The combined actions of melatonin and its derivatives greatly enhance the efficacy of melatonin in protecting against free radical damage and reducing the likelihood of human disease [130].

Melatonin is a by-product of yeast that is produced in the final stages of fermentation and is excreted into the medium during the stationary phase of the yeast fermentation [131]. In the studies carried out by Postigo et al. [103, 132] with different strains of wild *Saccharomyces* and non-*Saccharomyces* yeast, it was generally observed that *Saccharomyces* yeasts produce melatonin in a higher percentage than non-*Saccharomyces* yeasts. However, the concentration of non-*Saccharomyces* yeasts (ranged between 6.69 and 102.98 ng/mL) is in some cases double that of *Saccharomyces* yeasts (between 5.04 and 56.51 ng/mL). Furthermore, in studies carried out with sequential fermentations, where both yeast genera participate, melatonin concentrations were detected in almost all beers (values from 33.63 to 66.57 ng/mL) [133]. On the other hand, in the studies carried out by García-Moreno et al. [122], the melatonin values found in different commercial beers ranged from 58 to 169 pg/mL. As Valera et al. [134] determined in their studies, the presence of *Saccharomyces* seems to promote a higher presence of melatonin in the medium. In the studies carried out by Fernandez-Cruz et al. [135] at the intracellular level with wine yeasts, melatonin was only detected in non-*Saccharomyces* yeasts. Also, Juhnevicá-Radenkova et al. [131] mention in their work that melatonin is produced in the final stages of fermentation and is excreted into the medium during the stationary phase of the yeast fermentation. In comparison with other types of food such as bread, tomato, or yogurt, we can find different melatonin values of 28.9, 138.1, and 126.7 pg/mL, respectively, being therefore lower than what we could find in some beers fermented with non-conventional yeasts [136]. The comparison of the different concentrations can be seen in **Figure 1**.

These levels of melatonin that can be found in various foods, as well as in beer, are concentrations below those studied that have supposed positive effects on health (1–10 mg) [137, 138]; however, if ingested together with other foods, they can contribute to increase its concentration in the human serum. Studies carried out by Maldonado et al. [123] determined that the intake of beer with high melatonin content (169.7 pg/ml) contributed to increase the antioxidant properties of human serum. However, although melatonin can act as a strong antioxidant, it can be degraded in the presence of oxygen, light, or free radicals that are present during the aging process [139]. This fact could be observed in the studies of lambic beer carried out by Postigo et al. [140], where the analysis at different maturation times of the lambic beers brewed determined their degradation over time. It should also be taken into account whether the final product is subjected to final heat treatments to prolong

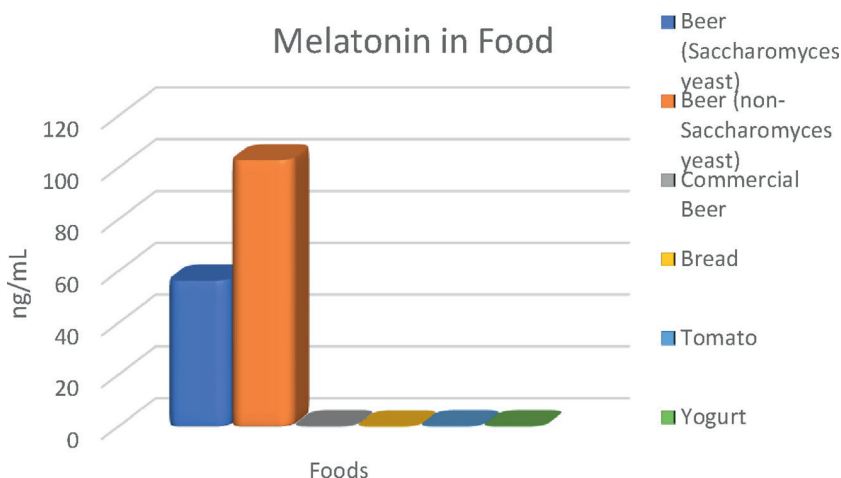


Figure 1. Melatonin concentrations found in different foods. Beer (*Saccharomyces* yeast) [103], beer (non-*Saccharomyces* yeast) [132], commercial beer [122], bread, tomato, and yogurt [136].

the stability of the product (such as pasteurization), since it has been shown that high temperatures can also degrade it and reduce its concentration in food [136].

6.2 Antioxidant capacity

Different compounds such as phenolics can be found in beer [141]. These substances can also be found naturally in fruits, vegetables, nuts, seeds, and beverages [142]. When studying the antioxidant fraction of beer, phenolic compounds are the most studied, which are found in hops (20–30%) as well as in malt (70–80%) [141]. Hops provide the beer with phenolic acids, prenylated chalcones, flavonoids, catechins, and proanthocyanidins [141]. Malt contains an overall phenolic mass of 1.0–1.9 mg g⁻¹ dry matter [143]. Likewise, the yeast used in brewing can also influence the phenolic composition and antioxidant capacity of the final product [106]. Several studies, such as the one carried out by Viana et al. [144], showed that the use of certain yeast strains for the production of Pale Ale beer significantly influences the antioxidant capacity of the beers.

Antioxidant capacity is related to parameters such as total phenolic and flavonoid content; 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity; and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity [145]. In the studies carried out by Postigo et al. [103] with different strains of wild yeast, it was observed that there were no major differences between the yeasts of *Saccharomyces* (9.50 to 13.67 mmol TE/L) and the commercial strain S-04 (11.18 mmol TE/L), as well as in sequential fermentations (9.63 to 13.70 mmol TE/L) (**Table 2**). However, in those strains where maltose was not fermented, or where there was competition between species in sequential culture, the antioxidant capacity was lower [133]. In contrast, in the studies carried out by Granato et al. [113], the values found in beers (ale and lager) were lower, between 424.77 and 10,508.47 μmol TE/L. These antioxidant capacity values may also vary depending on the method used for their analysis, since by the Oxygen Radical Absorption Capacity (ORAC) method in another study, values between 3.70 and 29.11 mmol TE/L were obtained. The ORAC method is based on the absorption capacity of oxygen radicals, whereby the decrease

Beer	Concentration	Method	Reference
Ale (<i>Saccharomyces</i> yeast)	9.50 to 13.67 mmol TE/L	TEAC	[103]
Ale (sequential fermentation)	9.63 to 13.70 mmol TE/L	TEAC	[133]
Commercial Ale (S-04)	11.18 mmol TE/L	TEAC	[103]
Commercial Ale	0.76 to 10.51 mmol TE/L	ORAC	[113]
Commercial Lager	0.44 to 7.72 mmol TE/L	ORAC	[113]
Commercial Ale	3.70 to 29.11 mmol TE/L	ORAC	[113]
Commercial Lager	0.58 to 1.02 mmol TE/L	TEAC	[146]
Commercial Ale	0.73 to 1.19 mmol TE/L	TEAC	[146]
Commercial Lager	3.70 to 14.79 mmol TE/L	ORAC	[146]
Commercial Ale	10.65 to 29.11 mmol TE/L	ORAC	[146]

ORAC (Oxygen Radical Absorption Capacity), TEAC (Trolox equivalent antioxidant capacity).

Table 2.
Antioxidant capacity of different ale and lager beers analyzed by different methods.

in fluorescence emission is measured [146, 147]. Another method used to determine the antioxidant capacity is that using the ferric-reducing antioxidant power, whose principle is the determination of the reduction of a ferric-tripyridyltriazine complex to its ferrous form, colored, in the presence of antioxidant components, where values of 3125 $\mu\text{mol Fe}^{2+}/\text{L}$ can be found in ale beer.

7. Conclusions

The use of non-conventional *Saccharomyces* yeasts provides beer with additional natural flavor variants that differentiate it from other commercial beers. These characteristics can be obtained either in pure culture, in sequential culture, or with aeration during the fermentation, which makes them very versatile. Furthermore, such wild yeasts can provide bio-healthy properties to beer under moderate consumption, as they may present production of compounds such as melatonin, with concentrations higher than those of commercial strains and even those of other foods.

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Conflict of interest

The authors declare no conflict of interest.

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