Chapter

Perspectives and Uses of Non-*Saccharomyces* Yeasts in Fermented Beverages

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Abstract

Fermented beverages such as wine, cider and beer are normally fermented with *Saccharomyces* yeasts due to their well-known fermentative behavior. These yeasts have been extensively investigated and are used in commercial processes. On the other hand, non-*Saccharomyces* yeasts were always considered contaminants in winemaking and brewing. Most researchers in the past argued that these yeasts produce several compounds that may alter the sensory quality of wine and beers. However, recent studies have demonstrated that their fermentative metabolism can be regulated and addressed to the production of compounds of sensory importance. Currently, some non-*Saccharomyces* yeasts belonging to the genera *Kloeckera, Candida, Hanseniaspora* are getting importance due to their high potentiality to be used in the production of fermented beverages such as special wines and craft beers. The emergence of new consumption patterns and market niches demanding products with new sensory characteristics has catapulted the exploitation of these yeasts.

Keywords: non-*Saccharomyces* yeasts, fermented beverages, wine, craft beers

1. Introduction

Fermentation of wines, beers and ciders is traditionally carried out with *Saccharomyces cerevisiae* strains, the most common and commercially available yeast. They are well known for their fermentative behavior and technological characteristics which allow obtaining products of uniform and standard quality. *Saccharomyces cerevisiae* is the most used yeast in fermentative processes. In wine fermentation, strains with specific characteristics are needed, for instance, highly producers of ethanol to reach values of 11–13% v/v, typically found in this beverage. On the other hand, beers and ciders contain less amounts of ethanol with a balanced and distinctive sensory profile characteristic of each one. In recent years, new consuming trends and requirements for new and innovative products have emerged. This situation led to rethink about the existing fermented beverages and to meet the demands of consumers. Yeasts are largely responsible for the complexity and sensory quality of fermented beverages. Based on this, current studies are mainly focused on the search of new type of yeasts with technological application. Non-*Saccharomyces* yeasts have always been considered contaminants in the manufacture of wine and beer. Therefore, procedures for eliminating them are routinely utilized such as must pasteurization, addition of sulfite and sanitization of equipment and processing halls. In recent years, the negative perception about non-*Saccharomyces* yeasts has been changing due to the fact that several studies have shown that during spontaneous fermentations of wine, these yeasts play an important role in the definition of the sensory quality of the final product. Based on this evidence, the fermentative behavior of some non-*Saccharomyces* yeasts is being studied in deep with the purpose of finding the most adequate conditions and the most suitable strain to be utilized in the production of fermented beverages.

2. Yeasts

Yeasts are eukaryotic microorganisms that inhabit a variety of ecological niches such as water, soil, air and the surface of plants and fruits. Commonly, they are present during the decomposition of ripen fruits and participate in the fermentation process. In this natural environment, the yeasts find nutrients and substrates necessary for their metabolism and fermentative activity [1, 2]. Yeasts are not nutritionally demanding compared to other microorganisms such as lactic acid bacteria. For supporting their growth, they need common compounds such as fermentable sugars, amino acids, vitamins, minerals and also oxygen. Morphologically the yeasts are very diverse, being the round, ellipsoidal and oval shapes mostly predominant. During the identification, the microscopic evaluation is the first resource followed by microbiological and biochemical tests; subsequently, assays of sugar fermentation and assimilation of amino acids are necessary [3]. The production and tolerance to ethanol, organic acids and SO_2 are also important tools to differentiate among species. The reproduction of yeasts is mainly by budding, which results in a new and genetically identical cell. Budding is the most common type of asexual reproduction, although cell fission is a characteristic of yeasts belonging to the genus *Schizosaccharomyces* (**Figure 1**). Cultivation conditions leading to the starvation of nutrients such as the lack of amino acids induce sporulation, which is a mechanism used by yeasts to survive under unfavorable conditions. As a consequence of the sporulation, yeast cells undergo genetic variability. In industrial

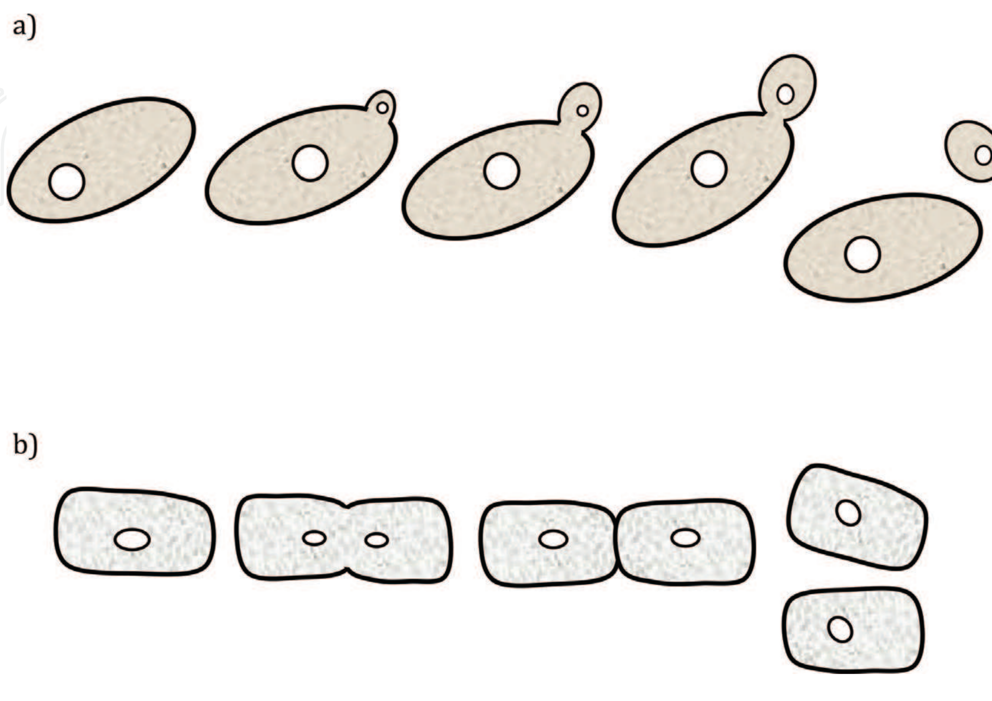

Figure 1.
Asexual reproduction of yeasts. (a) Budding, typically observed in Saccharomyces cerevisiae, Candida, Kloeckera, Brettanomyces *and (b) fission, typically observed in* Schizosaccharomyces pombe.

fermentation processes, asexual reproduction of yeasts is preferable to ensure the conservation of the genotype and to maintain their fermentative behavior over time. Regarding their metabolism, yeasts are usually characterized by fermenting a broad spectrum of sugars, among them, glucose, fructose, sucrose, maltose and maltotriose, which are found in ripen fruits and processed cereals. In addition, yeasts tolerate acidic environments with pH values around 3.5 or even less. According to technological convenience, yeasts are divided into two large groups namely *Saccharomyces* and non-*Saccharomyces*. Morphologically, *Saccharomyces* yeasts can be round or ellipsoidal in shape depending on the growth phase and cultivation conditions. *S. cerevisiae* is the most studied species and the most utilized in the fermentation of wines and beers due to its excellent fermentative capacity, rapid growth and easy adaptation. They tolerate concentrations of SO_2 that normally most non-*Saccharomyces* yeasts do not survive. However, despite these advantages, it is possible to find in the nature representatives of *S. cerevisiae* that do not necessarily present these features.

3. Non-*Saccharomyces* yeasts

Non-*Saccharomyces* yeasts are a group of microorganisms genetically diverse with specific metabolic characteristics and high potential for using in fermentation processes. In the past, many of them have been considered contaminants due to the production of compounds that alters the sensory quality of wines [4, 5]. With the purpose of eliminating them and avoiding their fermentative activity, for instance, in wine processing, disinfection of fermentation tanks and containers with sulfite is commonly performed. However, over time, the importance of non-*Saccharomyces* yeasts in spontaneous fermentation has been demonstrated since they contribute positively to the definition of the sensory quality of wines. These yeasts predominate at the initial stage of the spontaneous fermentation [6–8] until certain concentration of ethanol is reached (usually between 4 and 5% v/v), which are then inhibited due to the effect of the ethanol and the depletion of dissolved oxygen [9, 10]. At the end of the process, *Saccharomyces* yeasts, the most resistant to ethanol, predominate and complete the fermentation. It has been reported that some non-*Saccharomyces* yeasts are able to survive toward the end of the spontaneous fermentation and exert their metabolic activity, thus contributing positively to the sensory quality of wines. Based on this evidence, in recent years, many researchers have focused their studies in understanding the nature and fermentative activity of the non-*Saccharomyces* yeasts [8, 11–21]. The findings demonstrated the enormous potential of these yeasts for use in the fermentation of traditional and nontraditional beverages. Despite the fact that most non-*Saccharomyces* yeasts show some technological disadvantages compared to *Saccharomyces cerevisiae* such as lower fermentative power and production of ethanol, non-*Saccharomyces* yeasts possess characteristics that in *S. cerevisiae* are absent, for instance, production of high levels of aromatic compounds such as esters, higher alcohols and fatty acids [22, 23]. In addition, it has been reported that the fermentative activity of these yeasts is manifested in the presence of small amounts of oxygen which leads to an increase in cell biomass and the decrease in ethanol yield, a strategy that can be used to reduce the ethanol content of wines produced in coculture with *S cerevisiae* [24–26]. With the aim of exploiting the positive characteristics of non-*Saccharomyces* yeasts and reducing their negative impact, fermentations with mixed and sequential cultures with *S. cerevisiae* can be performed to produce fermented beverages with different sensory profiles [27–29]. The most important fact is related to the potential for producing a broad variety of compounds of sensory importance necessary to improve the organoleptic quality of wines and beers. The findings reported so far in literature have led to rethink the role of these yeasts in fermentative processes and to evaluate their use in the development of new products. Among the most studied non-*Saccharomyces* yeasts that reached special importance for researchers include *Candida*, *Kloeckera*, *Hanseniaspora, Brettanomyces, Pichia*, *Lanchacea* and *Kluyveromyces*, among others.

3.1 Fermentative metabolism of sugars

Either non-*Saccharomyces* or *Saccharomyces* yeasts share common pathways for the central metabolism of carbon; thus, both groups metabolize glucose through glycolysis. However, the mechanisms involved in the regulation of respire-fermentative metabolism can differ significantly among them [30]. The glycolysis operates indistinctly under aerobic and anaerobic conditions, and through it, the glucose is metabolized to pyruvate by means of a series of biochemical reactions (**Figure 2**). Under anaerobic or oxygen-limited conditions, pyruvate is converted to acetaldehyde and then to ethanol, and as a result, two net moles of ATP are generated. Under fully aerobic conditions and in the absence of any repression effect, the

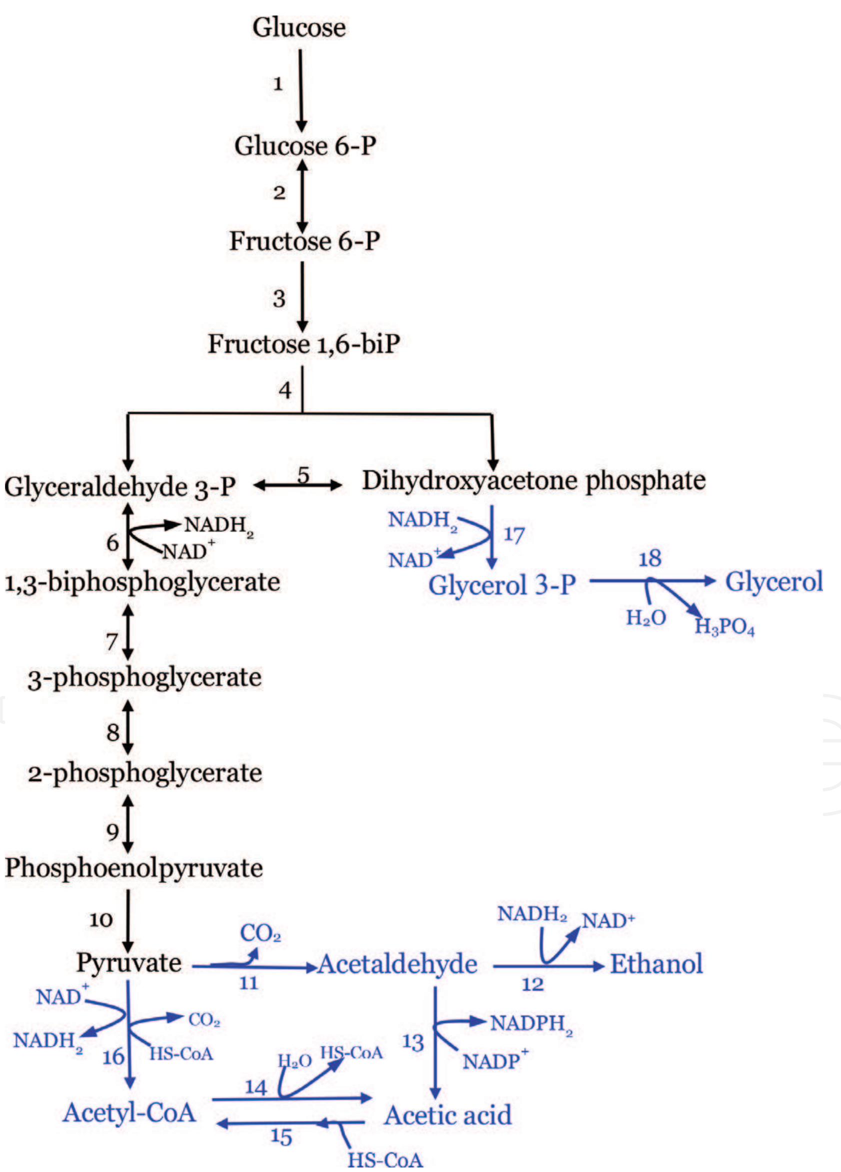

Figure 2.
Fermentative metabolism of glucose by yeasts: Glycolysis (black lines) and ethanol and glycerol production (blue lines). Enzymes: 1, hexokinase; 2, phosphoglucose isomerase; 3, phosphofructokinase; 4, fructose 1,6-bisphosphate aldolase; 5, triosephosphate isomerase; 6, glyceraldehyde 3-phosphate dehydrogenase; 7, phosphoglycerate kinase; 8, phosphoglycerate mutase; 9, enolase; 10, pyruvate kinase; 11, pyruvate decarboxylase; 12, alcohol dehydrogenase; 13, aldehyde dehydrogenase; 14, acetyl-CoA hydrolase; 15, acetyl-CoA synthetase; 16, pyruvate dehydrogenase; 17, glycerol 3-P dehydrogenase; 18, glycerol 3-phosphatase.

generation of energy is greater since glucose undergoes a complete oxidation, and as a result 36 net moles of ATP per mole of glucose are generated. The low-energy yield obtained by yeasts under anaerobic conditions forces the cell to increase the flow of glucose consumption in order to obtain a higher amount of energy in the form of ATP. As consequence, the ethanol accumulates in the fermentation medium and exerts its inhibitory effect, thus stopping the fermentative activity of the yeasts [31]. The low amount of energy generated under anaerobic conditions is used by the yeast cells in requirements for maintenance and growth. Glucose is easily transported and metabolized inside the cell; however, disaccharides such as sucrose, maltose or lactose must be first hydrolyzed to their simple forms (hexoses) which are then catabolized in the glycolysis pathway. Sucrose is hydrolyzed to fructose and glucose, maltose to two glucose units and lactose to glucose and galactose. The disaccharides are preferably hydrolyzed in the periplasmic space before entering the cytosol. Under anaerobic conditions besides ethanol, glycerol is also produced, thus contributing to restore the redox balance inside the cell. The production of glycerol increases in fermentations with musts of high specific gravity as a response to the osmotic stress [32]. It has been found that yeasts unable of metabolizing dihydroxyacetone (**Figure 2**) are not capable of producing glycerol, and as a consequence dihydroxyacetone accumulates and inhibits the fermentation. Moreover, glucose apart of being metabolized via glycolysis, it is also broken by complementary pathways that are not necessarily related to the generation of energy. The hexose monophosphate pathway (HMP) also known as the pentose phosphate cycle usually accompanies the glycolytic pathway [33]. In addition, yeasts during fermentation produce small amounts of acetic acid either from acetaldehyde or acetyl-CoA (**Figure 2**). Acetic acid is the main organic acid produced by yeasts during the fermentation of glucose, and it is responsible for the acidification and the decrease of pH of the medium. Ethanol is the most important fermentation by-product, and from the technological point of view, the production capacity of yeasts is an important parameter that determines their usability in fermentative processes. Gay Lussac defined a stoichiometric theoretical relationship to explain the production of ethanol by *Saccharomyces cerevisiae* yeasts which is:

$$\underset{\text{glucose}}{1\ C_6H_{12}O_6} \rightarrow \underset{\text{ethanol}}{2\ C_2H_5OH} + \underset{\text{carbon dioxide}}{2\ CO_2} \tag{1}$$

According to this relationship, from 180.0 grams of glucose, 92.0 grams of ethanol and 88.0 grams of carbon dioxide are produced, which results in a theoretical yield of 0.511 g ethanol/g glucose. However, in practice, besides ethanol and CO_2, the production of biomass, glycerol and other minority compounds also happens, that is:

$$C_6H_{12}O_6 + \text{nitrogen} \rightarrow C_2H_5OH + CO_2 + \text{glycerol} + \text{biomass} + \text{minority compounds} \tag{2}$$

At industrial scale, a yield of 0.45 g ethanol/g glucose is acceptable [34]. In the case of fermentations with non-*Saccharomyces* yeasts, lower yields are commonly observed. Regarding to glycerol, in the case of *S. cerevisiae,* its production represents approximately 3% of the utilized sugar. Minor compounds are represented by higher alcohols, esters, aldehydes and organic acids, among others.

3.1.1 Importance of oxygen

Oxygen is an important element during the complete oxidation of glucose since it serves as final acceptor of electrons under aerobic conditions. It is also essential for other metabolic processes such as the synthesis of structural components of the cytoplasmic membrane of yeasts. During alcoholic fermentation, as ethanol accumulates, it exerts a detrimental effect on the integrity and stability of the cytoplasmic membrane [31]. Under this condition, the supply of small amounts of oxygen to the medium through aeration promotes the synthesis of unsaturated fatty acids and sterols (mainly ergosterol) which are important components of the yeast cell membrane. Thus, the produced compounds can be used to replace the damaged fraction caused by the effect of ethanol that acts as a solvent [35, 36]. The replacement of unsaturated fatty acids and sterols is important to maintain the cell viability and allow the yeasts to complete successfully the fermentation. From the technological point of view, the supply of small amounts of oxygen is recommended in fermentations with musts of high specific gravity in order to avoid some drawbacks such as sluggish fermentation. It is also necessary for promoting the fermentative metabolism of non-*Saccharomyces* yeasts which are unable to ferment under fully anaerobic conditions [37]. The optimization of the aeration rate is very important to ensure the predominance of the fermentative metabolism and to reach the highest ethanol yield. In Crabtree-negative yeasts, as the concentration of oxygen in the medium increases above a certain value, the metabolism may become predominantly oxidative; thus, the ethanol yield decreases and the production of biomass increases. The highest ethanol yield is possible to achieve, adjusting properly the aeration rate of the fermentation medium. Aeration also affects the production of glycerol by yeasts; thus, as the concentration of oxygen increases, the production of glycerol decreases. From the technological point of view, aeration of the fermentation medium is an interesting tool to control the metabolic activity of non-*Saccharomyces* yeasts during fermentation, for instance, wines and beers [38, 39]. In addition, aeration can be also used in winemaking to improve the quality of wines since it provokes the transformation of phenols, which reduces the astringency.

3.2 Production of higher alcohols

During alcoholic fermentation, either non-*Saccharomyces* or *Saccharomyces* yeasts produce diverse volatile compounds of sensory importance such as higher alcohols, aldehydes, fatty acids and esters in different concentrations depending on the species of yeasts and the fermentation conditions. The harmonic balance of the compounds determines the sensory quality of the fermented beverage. Higher alcohols are a group of compounds that mostly confer unpleasant organoleptic character when present at high concentrations [40, 41]. In adequate concentrations, they contribute positively in defining the organoleptic quality of alcoholic beverage such as wines, beers and ciders. They are produced in the cytosol and then exported outside the yeast cell where it accumulates. Higher alcohols result from the decarboxylation of ketoacids that leads to the formation of the respective aldehydes, which are then reduced to form the corresponding higher alcohols (**Figure 3**). Ketoacids can be originated either from the metabolism of glucose or the catabolism of amino acids [42, 43], which are taken by the yeast cell from the fermentation medium. The synthesis of higher alcohols involves the participation of at least three enzymes: a transaminase, a carboxylase and an alcohol dehydrogenase. Factors that increase the metabolism of sugar and amino acids promote the synthesis of higher alcohols. The factors include temperature of fermentation, amino acid concentration and composition of the fermentation medium.

3.3 Production of esters

Esters are a group of compounds that mostly impart positive sensory characteristics to fermented beverages such as wines, beer and ciders. They are formed by the

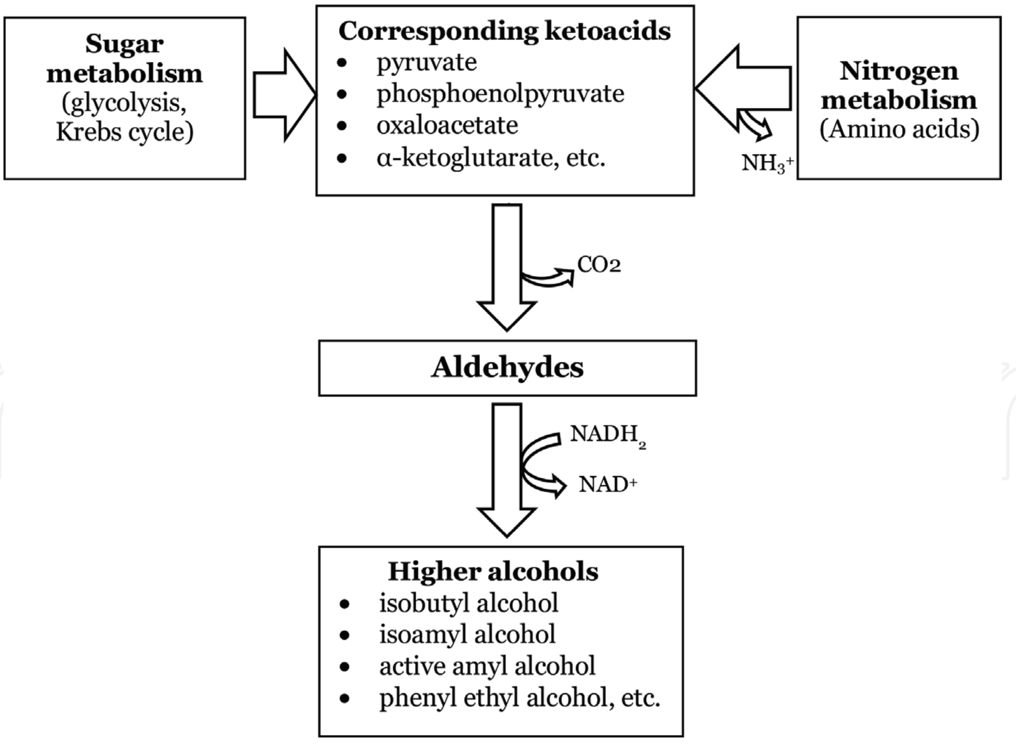

Figure 3.
Production of higher alcohols by yeast. Ehrlich's pathway and glucose catabolism.

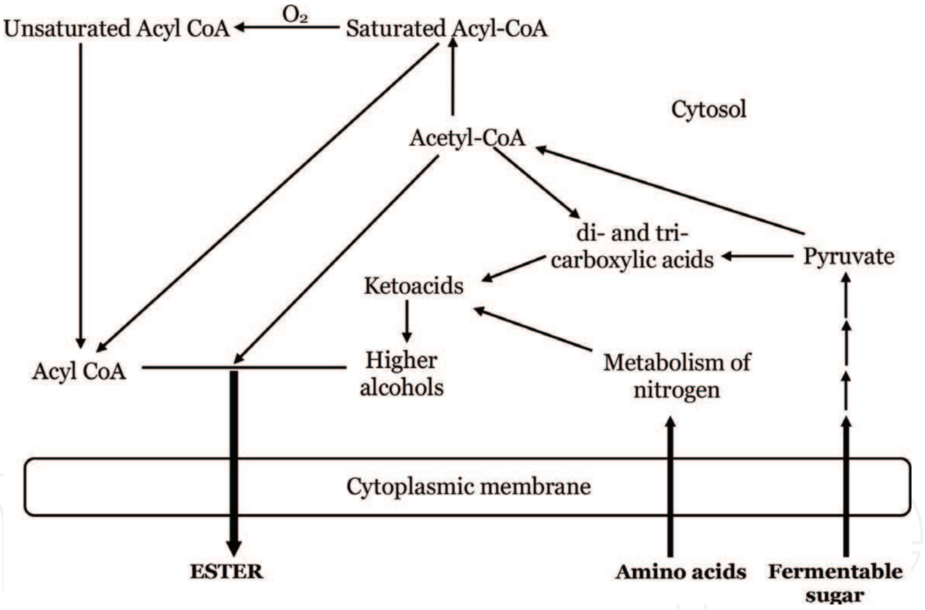

Figure 4.
Mechanisms for the production of esters by yeasts.

action of specific enzymes that catalyze the reaction between an alcohol and a volatile fatty acid (**Figure 4**). The synthesis of esters by yeast initially involves the activation of fatty acids to acyl coenzyme A mediated by energy and the subsequent condensation of the active compound with an alcohol present in the medium to form the corresponding ester [44]. From the sensory point of view, acetate esters are the most important compounds present in fermented beverages, which include ethyl acetate, butyl acetate, propyl acetate, phenyl ethyl acetate and amyl acetate, among others. The esters produced by *S. cerevisiae* involve the activity of at least three acetyltransferases (AAT, EC 2.3.1.84): an alcohol acetyltransferase, an ethanol acetyltransferase and an isoamyl alcohol acetyltransferase [45, 46]. Other enzymes such as ester synthase were also reported to participate in the synthesis of esters.

However, the relevance attributed to the activity of this enzyme is quite limited. Ethyl acetate is the most abundant ester present in wines and largely responsible for the sensory character. Studies carried out with non-*Saccharomyces* yeasts related to the ability of producing esters allowed to select species of *Hanseniaspora* and *Pichia* able to promote esterification of various alcohols such as ethanol, isoamyl alcohol and 2-phenyl ethanol to produce the corresponding esters [47].

4. Most important non-*Saccharomyces* yeasts

4.1 *Candida* yeasts

In the last years, the fermentative behavior of some *Candida* yeasts has been studied with respect to the production of wines and beers. The most studied species include *Candida stellata*, *C. zemplinina* and *C. pulcherrima*, among others [16, 20, 21, 48–50]. Representatives of *Candida* yeasts have been isolated from the early stages of spontaneous fermentation of different types of wines [8, 19, 51, 52]. The isolated species were characterized by being round in shape and smaller than *S. cerevisiae*. These yeasts are able to sediment toward the end of fermentation in a similar manner as *S. cerevisiae* [20]. Currently, the most important characteristics reported include the production of considerable amounts of ethanol and glycerol and a balanced production of volatile compounds of sensory importance, for instance, esters, fatty acids, aldehydes and higher alcohols. The production of ethanol is an important feature to define the use of yeasts in the production of fermented beverages with high ethanol contents such as wines. It has been reported that *C. zemplinina* strains are capable of producing ethanol up to 11.0% v/v [53], amount normally reached during the fermentation of sweet and semidry wines with *S. cerevisiae*. In addition, it has been demonstrated that *Candida* yeasts are capable of producing high amounts (up to 25.0 g/L) of glycerol [53–56], compound that contributes positively to the sensory quality of wines, beers and other beverages. The fermentative behavior of these yeasts was also evaluated as mixed cultures with *S. cerevisiae* [57]. The results were promising and interesting for being scaled-up to pilot fermentations. For instance, fermentation experiments of mixed cultures of *C. stellata* with *S. cerevisiae* produced higher levels of esters and fatty acids than monocultures of *S. cerevisiae* [19, 57]. Fermentations with mixed and even sequential cultures of yeasts are an interesting field of research to evaluate the potential use of non-*Saccharomyces* yeasts to produce sensory differentiated beverages. In addition, individual fermentations with *C. stellata* and *C. zemplinina* strains using immobilized systems have been also performed [53, 58]. The results showed the improvement of some technological properties such as the fermentation rate, ethanol production and the reusability of the strains in successive fermentations. Currently, studies to evaluate the usability of *C. zemplinina* strains in beer fermentation have been carried out using malt wort of 14 and 20°P, typically used in beer fermentation processes [21, 22]. The yeast strains showed a suitable fermentative behavior for the production of lager and ale beers. One interesting feature is that *Candida zemplinina* is unable to ferment maltose, the main fermentable sugar of the malt wort. This characteristic is of special importance since it would enable the production of beers with low ethanol content and particular sensory profiles.

4.2 *Kloeckera* yeasts

Yeasts species belonging to this genus have recently become of interest for the production of fermented beverages. Species such as *Kloeckera apiculata, K. javanica* and *K. corticis* were isolated from a variety of niches including the spontaneous fermentation of grape must and ciders [6, 8, 49, 59]. Most representatives present a lemon shape (apiculate yeasts) and asexual reproduction with bipolar budding. It was reported that these yeasts participate positively in the early stage of the spontaneous fermentation of wine [59, 60], strains of *Kloeckera apiculata* being the most dominant [19, 49, 51, 52]. During spontaneous fermentation, as the ethanol concentration increases, the fermentative activity of these yeasts slows down and stops toward the end of fermentation by the effect of the ethanol [61]. These yeasts are characterized by producing amounts of ethanol around 4–5% v/v, values typically found in commercial beers. It was reported that the control of aeration during fermentation has effect on the production of ethanol and compounds of sensory importance such as esters, higher alcohols and organic acids [14]. Based on the information available in literature, these yeasts are promissory for being used in brewing; however, before defining a strategy of exploitation, it is necessary to carry out more in-depth studies on the effect of temperature, wort composition and inoculation rate in the fermentative activity of these yeasts. In addition, it is also necessary to carry out studies on the behavior of these yeasts in fermentations with mixed and sequential cultures with *Saccharomyces cerevisiae* and the production of compounds of sensory importance. Studies carried out with pure cultures of *Kloeckera corticis* showed that these yeasts are capable of producing acetic acid, acetaldehyde, ethyl acetate and acetoin at high concentrations [62]. In addition, it has been reported that strains of *Kloeckera apiculata* are capable of producing higher concentrations of ethyl and isoamyl acetate than other non-*Saccharomyces* yeasts [14, 63]. From the technological point of view, techniques of cell immobilization can be an additional strategy to improve the fermentative behavior and the production of compounds of sensory importance. The ability of these yeasts to produce a variety of aromatic compounds with positive impact on the sensory quality makes them attractive and potentially exploitable in fermentation processes.

4.3 *Hanseniaspora* yeasts

Few studies have been conducted regarding the potential use of yeasts belonging to the genus *Hanseniaspora* (apiculate yeasts) in the production of fermented beverages. The studied yeasts were isolated from the spontaneous fermentation of grape musts [6, 8, 59] and include species of *Hanseniaspora uvarum*, *H. osmophila* and *H. guilliermondii*, among others. It has been shown that these yeasts play an important role during the early stage of spontaneous fermentation of wine and strains of *Hanseniaspora uvarum* (also called *Kloeckera apiculata*) are dominant [19, 51, 52]. They are characterized by tolerating and producing low amounts of ethanol that do not exceed the values of 5.0% v/v [61]. This limitation explains why these yeasts do not participate actively toward the end of spontaneous fermentation of wines where the ethanol content reaches values even higher than 10%v/v. However, the fermentative capability of these yeasts is enough to produce beers of standard ethanol content similarly to those found in the market (4.5–5%v/v). In addition, they are able to ferment a wide range of sugars including maltose, which is an important feature needed for the production of beers. Regarding the production of compounds of sensory importance, studies have reported that strains of *Hanseniaspora osmophila* are characterized by producing high concentrations of acetic acid, acetaldehyde and ethyl acetate [62]. Additionally, it was also found that strains of *Hanseniaspora uvarum* are able to produce a variety of esters that confer fruitiness to fermented beverages [11, 62, 64]. However, other studies reported that mixed cultures of *H. uvarum* with *S. cerevisiae* produce higher amounts of higher alcohols than monocultures with *S. cerevisiae*

[4, 19]. Regarding fermentation parameters, the control of aeration and temperature exerts an important effect on the dynamics and activity of *Hanseniaspora* yeasts. Both parameters are important to control the production of compounds of sensory importance, which influence the quality of fermented beverages [11, 65]. However, in view of the scarce information on the fermentative behavior of *Hanseniaspora* yeasts, particularly referring to the production of fermented beverages, additional studies are needed to perform in order to find the adequate conditions for their usage, for instance, in the production of beers with new sensory profiles.

4.4 *Brettanomyces* yeasts

Yeasts of this genus do not have a good reputation in fermentation processes such as in winemaking. For instance, representatives of *Brettanomyces bruxellensis* are considered detrimental due to the production of compounds such as 4-ethylguaiacol, 4-ethylphenol and 4-ethylcatechol which impart unpleasant sensory character to wines known as “Bretty” [5, 66]. These compounds result from the activity of a decarboxylase that acts on hydroxycinnamic acids followed by a reduction reaction [67]. The hydroxycinnamic acids are phenolic compounds naturally present in the skin and seeds of grapes. The common representatives of this genus were isolated from the spontaneous fermentation of wine, beer, cider and even kombucha [68–70]. It was also isolated from equipment and utensils utilized in fermentation processes, which are difficult to sanitize. The commonly isolated species include *Brettanomyces bruxellensis*, *B. lambicus*, *B. intermedius* and *B. anomalus*, among others [68, 69]. Particularly, strains of *B. bruxellensis* are able to ferment only in the presence of oxygen (positive Crabtree effect), a broad spectrum of sugars and even maltooligosaccharides which are not fermentable by *S. cerevisiae* [71]. Under anaerobic conditions, these yeasts are unable to ferment and produce ethanol; thus, at low concentration of sugar in the medium, the fermentation of glucose to ethanol is blocked. On the contrary, the fermentation is stimulated in the presence of oxygen, an effect known as Custer or negative Pasteur [72]. Apart from producing ethanol in the presence of oxygen, *Brettanomyces bruxellensis* also produces high concentrations of acetic acid, which acidifies and lowers the pH of the medium. However, yeasts of this genus are not entirely undesirable; some representatives participate, for instance, during the fermentation of certain beers known as “Lambic” and “Gueuze” consumed commonly in Belgium and “Coolship Ales” in North America. The fermentation of “Lambic” beer is a spontaneous process which goes through a complex succession of microorganisms where *Brettanomyces bruxellensis* participates during the final stage acidifying the product [73]. The participation of these yeasts gives the beer its characteristic acidity and dryness and additionally is responsible for the production of compounds such as ethyl phenol, ethyl acetate, ethyl caprylate, ethyl decanoate and ethyl lactate, which synergistically confer their typical aroma character [18, 74]. It has been shown that esters soften the sour taste and add fruity notes to this kind of beers [75]. Based on these findings, it was demonstrated that these yeasts and particularly *B. bruxellensis* contribute positively to defining the floral and fruity character of “Lambic” beers [18]. Beyond the contribution of *Brettanomyces* yeasts in spontaneous fermentation processes, in recent years, their use in controlled fermentations has been investigated, both in pure and in coculture with *S. cerevisiae* [15, 17]. Interesting findings were reported, indicating that the control of aeration during fermentation is a critical point to guide the fermentative metabolism toward the production of important volatile compounds that may contribute to the organoleptic character of fermented beverages.

5. Production of special wines

It is of common agreement that non-*Saccharomyces* yeasts contribute beneficially to the sensory quality of spontaneously fermented wines, an evidence that served as a starting point to pay attention to particular yeast species that could be exploited in fermentations of commercial and noncommercial fermented beverages. Non-*Saccharomyces* species are characterized by producing a greater diversity of compounds of sensory importance than *S. cerevisiae* yeasts. Although these yeasts show a low fermentation power, some species possess important fermentative features, for instance, representatives of *Kloeckera* and *Hanseniaspora* yeasts produce a variety of compounds of sensory impact, particularly esters at concentrations even higher than *S. cerevisiae*. On the other hand, *Candida zemplinina*, a fructofilic yeast, has been shown to produce glycerol in higher concentrations than *S. cerevisiae*. It is also capable of producing ethanol in concentrations high enough to produce different types of wines. In view of the complementary characteristics of both groups of yeasts (*Saccharomyces* and non-*Saccharomyces*), the use of non-*Saccharomyces* yeasts can be proposed in fermentations with mixed or sequential cultures with *S. cerevisiae* as an important strategy to improve sensory complexity and mouthfeel of wines [19, 73]. The fermentative versatility of non-*Saccharomyces* yeasts would enable the production of special wines with different and innovative sensory characteristics. In addition, among the techniques that can be implemented for enabling their practical exploitation include the selection of new strains, the development of fermentation strategies (mixed or sequential cultures with two or more yeast strains), the ratio of both strains in the inoculum (non-*Saccharomyces/Saccharomyces cerevisiae*) and the inoculation rate at the beginning of fermentation [57, 76]. Finally, some technological characteristics of non-*Saccharomyces* yeasts can be also modified by using cultivation techniques in bioreactors with the aim of improving, for instance, the fermentation rate. The possibility of commercializing as starter cultures is an attractive opportunity for the production of different types of wines with special sensory qualities.

6. Production of craft beers

In the last 10 years, the market of craft beers has increased in the USA, Latin America and some countries of Europe [77, 78]. This phenomenon is related to the expectation of consumers for discovering in these beers sensory characteristics different from those routinely found in commercial beers [74]. Current consumers are curious and interested in sensing new flavors and aromas that can satisfy their preferences. As consequence, new market segments have emerged in response to the broad possibility of offering new types of beers produced using different methods and techniques of fermentation. The production of craft beer is generally carried out in small-scale breweries and involves the use of non-technified processing methods. Craft beers are not usually filtered; due to this, their shelf life is relatively short, and therefore, their consumption must be within few days after bottling. There are a variety of innovative alternatives to produce different types of craft beers which include the use of new types of adjuncts either amylaceous (cereal grains) or non-amylaceous (fruit pulps or juices) and selected strains of non-*Saccharomyces* yeasts which have an enormous exploitation potential. Although most non-*Saccharomyces* yeasts produce low concentrations of ethanol, the fermentative capacity of some representatives of *Kloeckera* and *Hanseniaspora* yeasts is adequate to produce beers with an ethanol content typically found in the market

(4.5–5%v/v). Among non-*Saccharomyces* yeasts considered important in beer fermentation, *Brettanomyces lambicus* is the most representative which is involved in the production of "Lambic" and "Gueuze" beers. Currently, some studies with *Candida zemplinina* strains were performed in fermentations with pure malt wort and with different adjuncts (grape or apple juice) at different temperatures and specific gravities. The findings were promissory and showed the capability of these yeasts to ferment at low temperatures (14°C) and in medium with high specific gravity (16°P), which demonstrates the possibility for being exploited in the production of craft beers. In addition, it was also proposed that these yeasts can be used for the production of beers with low ethanol content since they are not able to ferment maltose, the main and most abundant sugar present in the wort [20, 21]. Additionally, other non-*Saccharomyces* yeasts such as *Dekkera anomala, Naumovozyma dairenensis* and *Debaryomyces* spp. have been also reported with a high potential for being used in the fermentation of beers. In view of the different fermentative behavior of non-*Saccharomyces* yeasts and the variety of compounds of sensory importance that they can produce during fermentation, their use in controlled fermentations has aroused the interest of brewers for producing beers with distinctive sensory features [23, 79].

7. Conclusion

Non-*Saccharomyces* yeasts show a great potential to be used in the production of fermented beverages mainly wines and beers. These yeasts show a variety of fermentative patterns, and depending on the fermentation conditions, they produce a wide range of volatile compounds of sensory importance. For their practical application in a particular fermentative process, it is necessary knowing the parameters that directly influence on the fermentative activity and the production of desirable volatile compounds. Among the non-*Saccharomyces* yeasts that have attracted interest of researchers due to their fermentative qualities include strains of *Candida stellata, C. zemplinina, Kloeckera apiculata* and *Hanseniaspora uvarum*. Particularly, strains of *Candida stellata* and *C. zemplinina* have become very attractive for using in fermentations of different types of wines and beers. These yeasts are capable of producing significant concentrations of glycerol, an important compound that imparts a positive impact on the sensory quality of wines and beers. *Candida* yeasts, especially *C. zemplinina*, also produce high concentrations of ethanol, high enough to drive fermentation processes of wines. On the other hand, species of *Kloeckera* and *Hanseniaspora* yeasts are characterized by producing considerable amounts of acetate esters, valuable compounds that contribute positively to the sensory character of beers. Based on this, if a fermentation process that involves the use of non-*Saccharomyces* yeasts is going to be implemented, it is necessary to select the best representatives and then define the appropriate fermentation conditions for the production of fermented beverages with the desired sensory qualities.

Conflict of interest

The author certifies that he has *no* affiliation with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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