Chapter 4

The Role of Yeast and Lactic Acid Bacteria in the Production of Fermented Beverages in South America

Fábio Faria-Oliveira, Raphael H.S. Diniz, Fernanda Godoy-Santos, Fernanda B. Piló, Hygor Mezadri, Ieso M. Castro and Rogelio L. Brandão

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Abstract

Fermentation is one of the oldest forms of food preservation in the world. In South America, most fermented beverages are nondairy products featuring several other food raw materials such as cereals, fruits, and vegetables. Generally, natural fermentations are carried out by yeast and lactic acid bacteria forming a complex microbiota that acts in cooperation. Yeast have a prominent role in the production of beverages, due to the ability to accumulate high levels of ethanol and to produce highly desirable aroma compounds, but lactic acid bacteria are particularly important in fermentation because they produce desirable acids, flavor compounds, and peptides that inhibit the growth of undesirable organisms. Among the South America beverages based on cereals and vegetables, the fermented beverages *chicha*, *caxiri*, *cauim* and *champús*, and *cachaça*, a fermented and distilled beverage, could be cited. Genetic and physiological analyses of *Saccharomyces cerevisiae* strains isolated from *cachaça* have been shown to present interesting traits for beer production, such as flocculation and production of aroma compounds, fundamental to high-quality beer. The study of these traditional beverages allows the identification of new microorganism strains displaying enhanced resistance or new flavor and aroma profiles that could lead to applications in several industries and ultimately new products.

Keywords: yeast, lactic acid bacteria, fermented beverages, South America, *cachaça*

1. Introduction

Alcoholic beverages have been consumed by mankind since ancient times. These products of fermented sugar-rich goods, namely, cereals, roots, and fruits, are present worldwide since the oldest records [1, 2]. In fact, several of mankind's milestones, such as the dawn of agriculture, are closely linked with the production of some type of alcoholic beverages. Similar processes of fermentation emerged independently in many civilizations across the globe. Interestingly, the main players of the whole process are relatively few, mostly yeast from the *Saccharomyces* genus and lactic acid bacteria (LAB) [3, 4]. Nowadays, such microorganisms have a significant role in several industrial relevant processes, including the production of beer, wine, cheese, and bread. Importantly, the popularity of fermented beverages, namely, beer and wine, is such that their worldwide consumption is second only to nonalcoholic drinks as water, tea, and coffee [5].

This chapter aims to contribute to a comprehensible analysis of the role of yeast and LAB on the production of fermented beverages from South America. The microbiological diversity associated with the fermentation of a wide diversity of raw materials, from sugarcane to cassava, as well as new potential biotechnological applications will be addressed.

1.1. Ethanol and lactic acid fermentation

1.1.1. Yeast diversity and metabolism

Yeast are unicellular fungi, being the simplest eukaryotes. Present in a great number of environments, yeast can be found not only in decomposing fruit, trees, and soils but also in commensal relationships with higher eukaryotes, humans included, and even saltwater. The high diversity of species, almost 1500 species have been described [6], is closely related to this wide distribution. Some of these yeast are adapted to extreme environments, such as high salt concentrations [7], low pH [8], or extremely cold temperatures [9, 10]. The genus *Saccharomyces*, particularly *Saccharomyces cerevisiae*, is strongly associated with the production of fermented products for human consumption, namely, bread, wine, and beer [2]. After several millennia of close coexistence, through phenotypic selection, these species evolved to produce goods with organoleptic properties pleasant to humans. However, given the high degree of diversity found in nature, it is expected to find yeast with new and more interesting characteristics for the industry in new and unexplored niches [11, 12].

Yeast, as other heterotrophic organisms, have the anabolism coupled with catabolism. In one hand, the oxidation of organic molecules, as sugars, yields adenosine 5-triphosphate (ATP) that, in turn, is used as an energy resource for the cell. On the other hand, such organic molecules can also be used as building blocks or to generate intermediary compounds for the synthesis of other compounds, some of which with high commercial value.

The high diversity of environments where yeast can be found is closely related to the variety of carbon sources that can be used. Hexoses such as glucose, fructose, galactose, or mannose are the most common substrates, but some species can use pentoses like xylose or arabinose.

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Several industrial relevant species can metabolize disaccharides as maltose, lactose, or sucrose, and some, as *Saccharomyces diastaticus*, can even metabolize dextrins (glucose polymers) [13, 14]. Nevertheless, glucose and fructose, to a lesser extent, are the preferred substrates.

In order to use glucose as carbon source, first and foremost, yeast have to sense the presence of this sugar in the surrounding environment and then express the adequate proteins to transport it across the plasma membrane [15, 16]. Whenever glucose is sensed in the medium, changes in the cell proteome will occur. Several processes contribute to the overall change in enzymes levels, including alteration of mRNA translation rates, mRNA stability, or protein synthesis and/or degradation. However, the major response is the extensive upregulation of a large number of genes required for the metabolism of glucose, such as genes encoding glycolytic pathway enzymes, leading to the adaptation to the fermentative metabolism. Moreover, in genes encoding for proteins involved in the metabolism of alternative substrates, gluconeogenic and respiratory pathways are repressed strongly by glucose (for reviews, see [17, 18]). In *S. cerevisiae*, a glucose concentration as little as 15 mM is enough to induce such changes [19].

S. cerevisiae presents an extensive family of hexose transporters, including more than 20 members: (i) 18 genes encoding transporters (*HXT*1-*HXT*17, *GAL*2) and (ii) at least two genes encoding sensors (*SNF*3, *RGT*2). Some studies suggest that Gpr1p and Hxk2p may sense glucose levels [17, 20]. The transporters can be divided in two classes regarding glucose affinity: (1) low affinity for glucose and high transport capacity, the most important proteins are Hxt1p and Hxt3p, and (2) high affinity and low transport capacity, the key proteins being Hxt2p, Hxt4p, and Hxt7p.

Following uptake by the hexose transporters, glucose enters the glycolytic pathway in order to be metabolized to pyruvate (Figure 1, steps from glucose to pyruvate), whereby the production of energy in the form of ATP is coupled to the generation of intermediates and reducing power in the form of NADH for biosynthetic pathways [21, 22]. The phosphorylation of glucose to glucose-6-phosphate, requiring ATP, is the initial step of glycolysis, by the action of the hexokinases (Hxk1/2p) and the glucokinase (Glk1p), which are linked to high-affinity glucose uptake. The glucose-6-phosphate is then isomerized to fructose-6-phosphate by the phosphoglucose isomerase, encoded by *PGI1* gene. The next step, done by the phosphofructokinase (Pfk1/2p), also requires energy. The fructose-6-phosphate molecule is converted into fructose 1,6-biphosphate through the transfer of inorganic phosphate from ATP. In turn, yeast aldolase (fructose 1,6-bisphosphate aldolase—Fba1p) is responsible for the reversible cleavage of fructose 1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

These two resulting compounds can be interconverted, in a reversible way, by the action of the triosephosphate isomerase (Tpi1p). Glyceraldehyde 3-phosphate is further metabolized to ultimately yield pyruvate, while some of the dihydroxyacetone phosphate follows gluconeogenesis. This step is fundamental for the osmotic and redox homoeostasis, as the dihydroxyacetone can be converted to glycerol yielding NAD^+. Glyceraldehyde 3-phosphate is first oxidized by NAD^+ and then phosphorylated under the catalysis of the 3-phosphate dehydrogenase (Tdh1/2/3p). The resulting 1,3-diphosphoglycerate is, in turn, converted to 3-phosphoglycerate by the action of phosphoglycerate kinase (Pgk1p), yielding 1 molecule of ATP. The

enzyme phosphoglycerate mutase (Pgm1p) promotes the relocation of the phosphate group from C3 to C2, allowing the dehydration by the enolase (Eno1/2 p), resulting in the phosphoenolpyruvate. Then the pyruvate kinase (Pyk1p) converts this highly energetic molecule to pyruvate, yielding a second molecule of ATP.

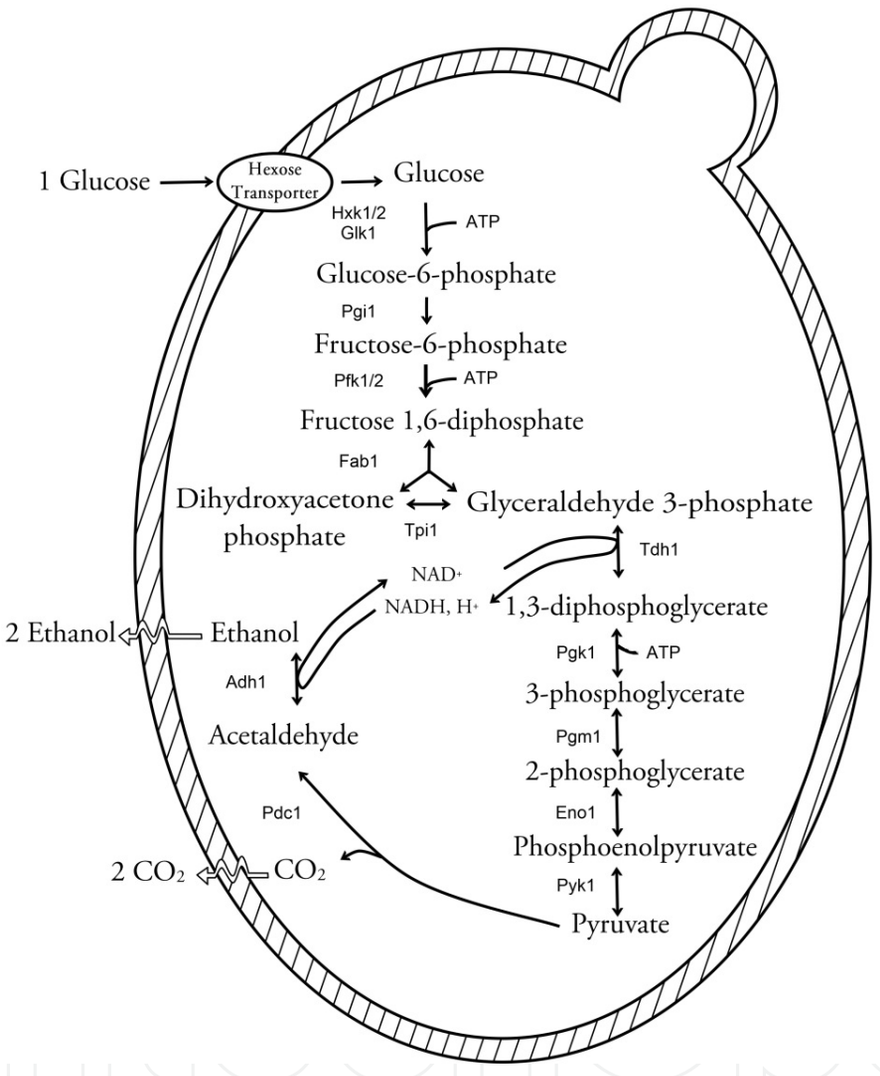

Figure 1. Glycolysis and alcoholic fermentation steps on *S. cerevisiae* (adapted from [23]).

The pyruvate molecule can be further processed through different metabolic alternatives, the respiratory or the fermentative pathways (Figure 2). The selection of one of the route depends greatly on the expression or repression of some genes, which in turn are tightly regulated on the environmental conditions [24]. The genus to which the yeast belongs also plays a role in the prevalence of one route over the other.

The fermentative pathway is particularly relevant to industry, as several important commodities are produced through this process (characteristic of particular organisms). In *S. cerevi-*

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siae, the first step is the decarboxylation of pyruvate to yield acetaldehyde and carbon dioxide (CO_2), through the action of the pyruvate decarboxylase (Pdc1/5/6p). The acetaldehyde can be further reduced to form ethanol by the enzyme alcohol dehydrogenase (Adh1p), allowing the reoxidation of NADH to NAD^+. Besides the direct products of fermentation, ethanol and CO_2, several other by-products are generated during the process, including cell biomass, glycerol, and some organic acids. Overall, the ethanol fermentation is a redox-neutral process since the reduced coenzyme NADH produced during glycolysis, in the oxidation of glyceraldehyde 3-phosphate, is latter reoxidized in the reduction of acetaldehyde to ethanol [25]. Nevertheless, given that biomass is a product of fermentation, and it is in a more oxidized state than glucose, an excess of reducing equivalents may be generated. As mentioned above, glycerol production plays an important role in the redox balance restoration. The glycolytic intermediate dihydroxyacetone is reduced to glycerol 3-phosphate, oxidizing NADH to NAD^+, in a reaction catalyzed by the NAD^+-dependent glycerol 3-phosphate dehydrogenase (Gpd1/2p). Glycerol 3-phosphate is then dephosphorylated to glycerol due to the action of glycerol 3-phosphatase (Gpp1/2p) [5, 26, 27]. The presence of glycerol may contribute to the organoleptic properties in the final product of fermentation, such as wine.

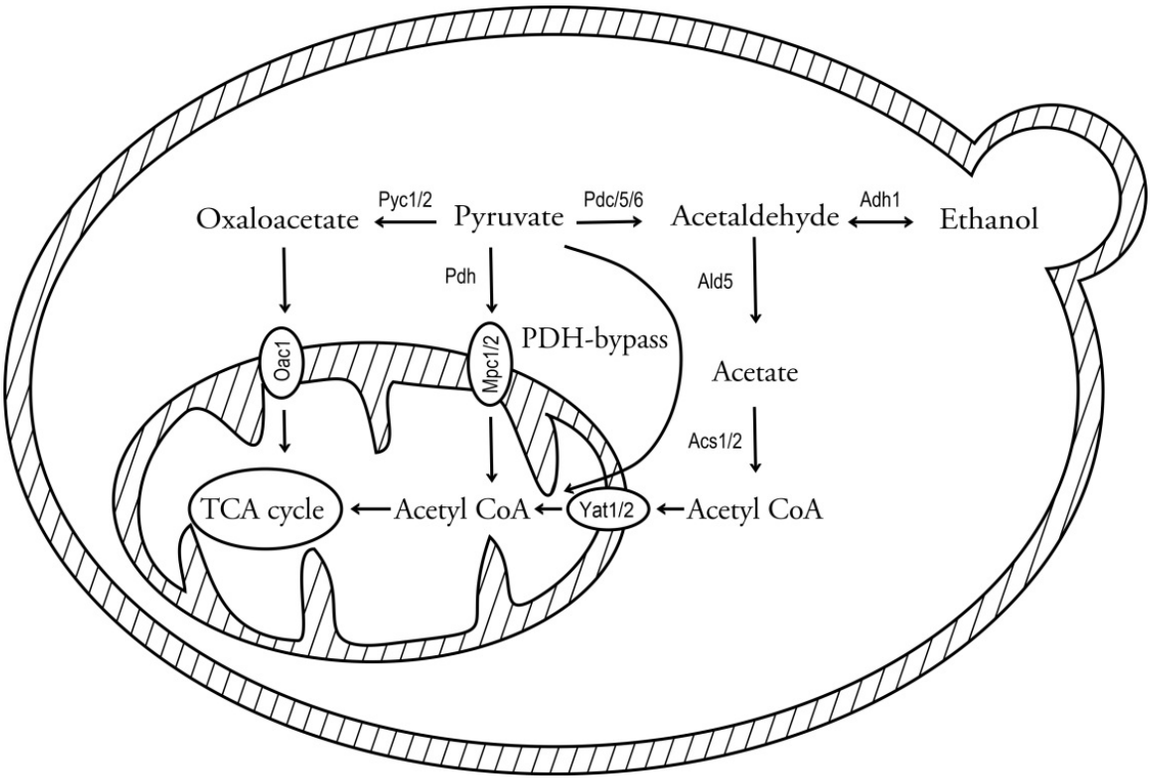

Figure 2. Pyruvate metabolic fates. The pyruvate yielded during glycolysis can be converted into two intermediates of TCA cycle: acetyl-CoA, by the pyruvate dehydrogenase complex (PDH), and/or oxaloacetate, by pyruvate carboxylases (Pyc1/2p). These molecules can be transported into the mitochondria by the pyruvate carriers (Mpc1p or Mpc2p) and the oxaloacetate carrier (Oac1p), respectively. Another alternative is the decarboxylation to acetaldehyde, by the pyruvate decarboxylase (Pdc1/5/6p), which ultimately can yield ethanol. Adh1p—alcohol dehydrogenase; Ald5p—acetaldehyde dehydrogenase; Acs1/2p—acetyl-CoA synthases; Yat1/2p—carnitine acetyltransferases (adapted from [22]).

Although most microorganisms ferment in the absence of oxygen, this is not always the case. Even if oxygen is available, high concentrations of sugars present in the environment will lead yeast to choose fermentation over respiration. This inhibition of aerobic metabolism if glucose is available, both in the presence or absence of oxygen, is denominated the Crabtree effect [28]. *S. cerevisiae* is known as Crabtree positive since it will produce ethanol aerobically if the glucose available is higher than 15 mM [19]. The availability of high sugar concentrations in the surrounding environment stimulates glycolysis, which in turn leads to the production of increasing amounts of ATP, through substrate-level phosphorylation. At the same time, the availability of additional ATP will reduce the respiration and ATP synthesis, through oxidative phosphorylation, leading to a decrease in oxygen consumption. On the other hand, Crabtree-negative yeast do not present a glucose inhibition of aerobic respiration, so these microorganisms resort to this more efficient form of energy metabolism, producing biomass via tricarboxylic acid (TCA) cycle. Nevertheless, these species are able to ferment, but mainly in anaerobic conditions. Importantly, Crabtree is not exclusive to yeast, as it has been detected in many mammalian tumor cells [29–31].

During aerobic respiration (Figure 3), acetyl-CoA is produced by the decarboxylation of the glycolytic pyruvate, by the action of the pyruvate dehydrogenase complex. Then acetyl-CoA will enter the tricarboxylic acid (TCA) cycle, where it will be used to generate reducing equivalents, NADH and $FADH_2$. These molecules will fuel the oxidative phosphorylation, through the highly conserved electron transport chain. Besides the production of reducing coenzymes, the TCA cycle provides intermediates to several other biochemical pathways, including the synthesis of amino acids and nucleotides (for reviews, see [22, 32]).

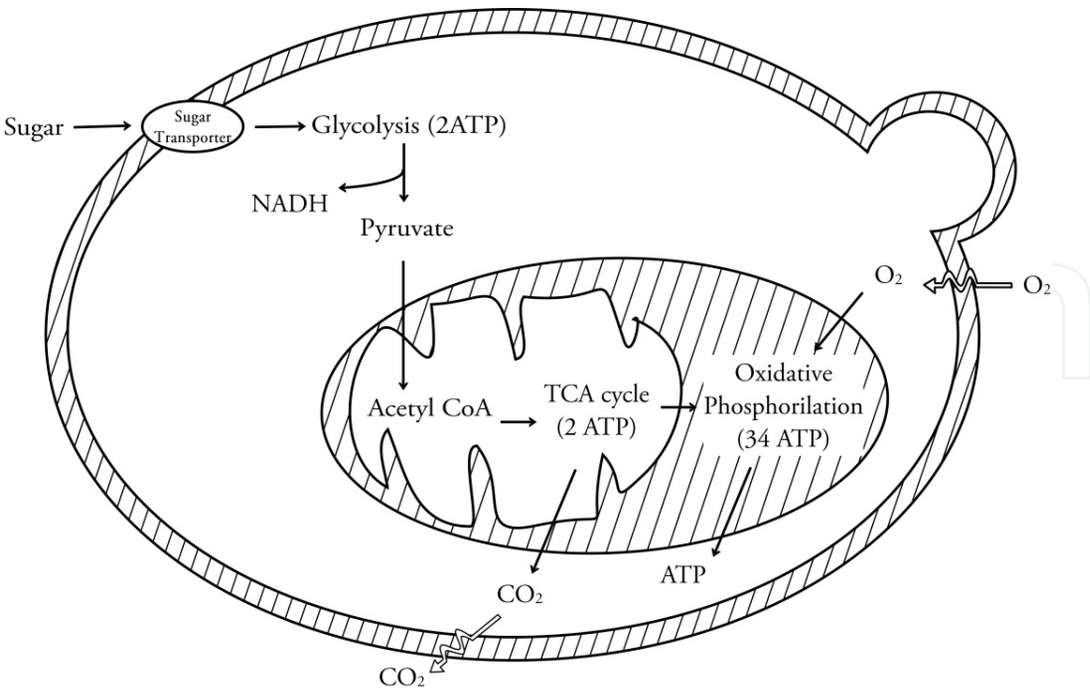

Figure 3. Aerobic respiration in *S. cerevisiae* (adapted from [33]).

1.1.2. Lactic acid bacteria

Lactic acid bacteria (LAB) constitute an ubiquitous and heterogeneous group capable of fermenting carbohydrate with the production of lactic acid as a major end product [34]. LAB are found in diverse nutrient-rich habitats associated with plant and animal's matter, as well as in respiratory, gastrointestinal, and genital tracts of humans [35, 36]. A typical LAB is Gram positive, present a GC content below 55%, generally nonsporulating, usually nonmotile, fastidious, catalase negative (pseudocatalase may occur in some LAB), aerotolerant, and acid tolerant [34]. Taxonomic parameters have distributed LAB members into two phyla, *Firmicutes* and *Actinobacteria*. Within the *Firmicutes* phylum, LAB members belong to the order *Lactobacillales* and comprise the following genera: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Symbiobacterium*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. Regarding LAB members belonging to the *Actinobacteria* phylum, the only species belongs to the *Bifidobacterium* genus [35, 37, 38]. Nevertheless, it is worth mentioning that *Bifidobacterium* is poorly phylogenetically related to typical LAB. These bacteria have been considered as LAB given its physiological similarity and the shared biochemical properties [39, 40].

Usually, LAB members are nonpathogenic organisms with a reputed generally recognized as safe (GRAS) status. The *Lactobacillus* genus includes some of the most important GRAS species involved in food microbiology and human nutrition [41, 42]. The remarkable ability of these bacteria to adapt to different environments resulted in a large number of industrially relevant strains. Among these are *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Bifidobacterium* [35, 43, 44]. Furthermore, given that LAB greatly contribute to the effective acidification of the matrix and consume rapidly fermentable sugars, these bacteria are frequently predominant in the natural fermentation microbiota [44].

1.1.2.1. Pathway of homolactic and heterolactic acid fermentation in LAB

LAB are able to live in the presence of oxygen; however, they obtain their energy by substrate-level phosphorylation. These bacteria do not present a functional respiratory system, as they lack the ability to synthesize cytochromes and porphyrins, key components of respiratory chains [45, 46]. Therefore, an important parameter used in the differentiation of the LAB species is the type of lactate fermentations: homofermentative and heterofermentative [35]. As a general rule, homofermentative lactic acid bacteria use the Embden–Meyerhof–Parnas pathway (EMP pathway or glycolysis) to produce pyruvate, while heterofermentative lactic acid bacteria use the pentose phosphate pathway (PPP). However, a third pathway, the Bifidum pathway, presents distinct reactions (Figure 4) [45, 46].

In the homofermentative lactate fermentation, as the name implies, the major end product generated is lactate. Initially, two ATP molecules are produced per mole of glucose via the oxidation of phosphoglyceraldehyde. In a second stage, NADH molecules resulting from the previous oxidative stage are used to reduce the pyruvate, forming lactate [45, 46]. The overall reaction is as follows:

$$\text{glucose} + 2\,\text{ADP} + 2\,\text{Pi} \rightarrow 2\,\text{lactate} + 2\,\text{ATP}$$

Some representative homolactic LAB genera include *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, and *Pediococcus* species [38].

Conversely, in the heterofermentative lactate fermentation pathway, lactate is not the only end product; significant amounts of CO_2 and ethanol, or acetate, are also produced. In this pathway, lactate is produced by the decarboxylation and isomerization reactions of the PPP. Glucose is oxidized to ribulose-5-phosphate that is isomerized to xylulose-5-phosphate, which in turn is cleaved to form phosphoglyceraldehyde and acetyl phosphate. The phosphoglyceraldehyde molecule is oxidized to pyruvate by reactions of glycolytic pathway, whereas the acetyl phosphate is reduced to ethanol [45, 46]. The overall reaction is as follows:

$$\text{glucose} + \text{ADP} + \text{Pi} \rightarrow \text{ethanol} + \text{lactate} + CO_2 + \text{ATP}$$

Some representative heterolactic LAB genera include *Leuconostoc*, *Oenococcus*, and *Weissella* [38]. It is worth mentioning that heterofermentative lactate fermentation produces only one ATP molecule per glucose, while the homofermentative lactate fermentation produces two ATP molecules per glucose.

1.1.2.2. Bifidum pathway

The Bifidum pathway is a particular metabolic route found in *Bifidobacterium bifidum*, which uses reactions of the PPP and homofermentative pathway, producing primarily acetate and lactate [38, 45]. In this pathway, 2.5 ATP molecules are produced per glucose. As such, ATP yields are greater than for the homofermentative or heterofermentative pathways, due to the presence of key enzymes, fructose-6-phosphate phosphoketolase and xylulose-5-phosphate phosphoketolase. These proteins catalyze two important steps: the cleavage of one molecule of fructose-6-phosphate, yielding one molecule of erythrose-4-phosphate and one of molecule acetyl phosphate, and the cleavage of two xylulose-5-phosphate into two glyceraldehyde 3-phosphate and two acetyl phosphate, respectively [45, 46]. The overall reaction is as follows:

$$2\,\text{glucose} + 5\,\text{ADP} + 5\,\text{Pi} \rightarrow 3\,\text{acetate} + 2\,\text{lactate} + 5\,\text{ATP}$$

1.1.2.3. LAB—beverage industry applications

Over the years, LAB has been explored on a large scale in several food industry segments (processing of meats, vegetables, and beverages) occupying a central role in these niches [43, 48–50]. Withal, there are some reasons that explain their use in the food production industry. Among these are the following: the production of antimicrobial substances, which restricts the growth of harmful microorganisms, and the production of

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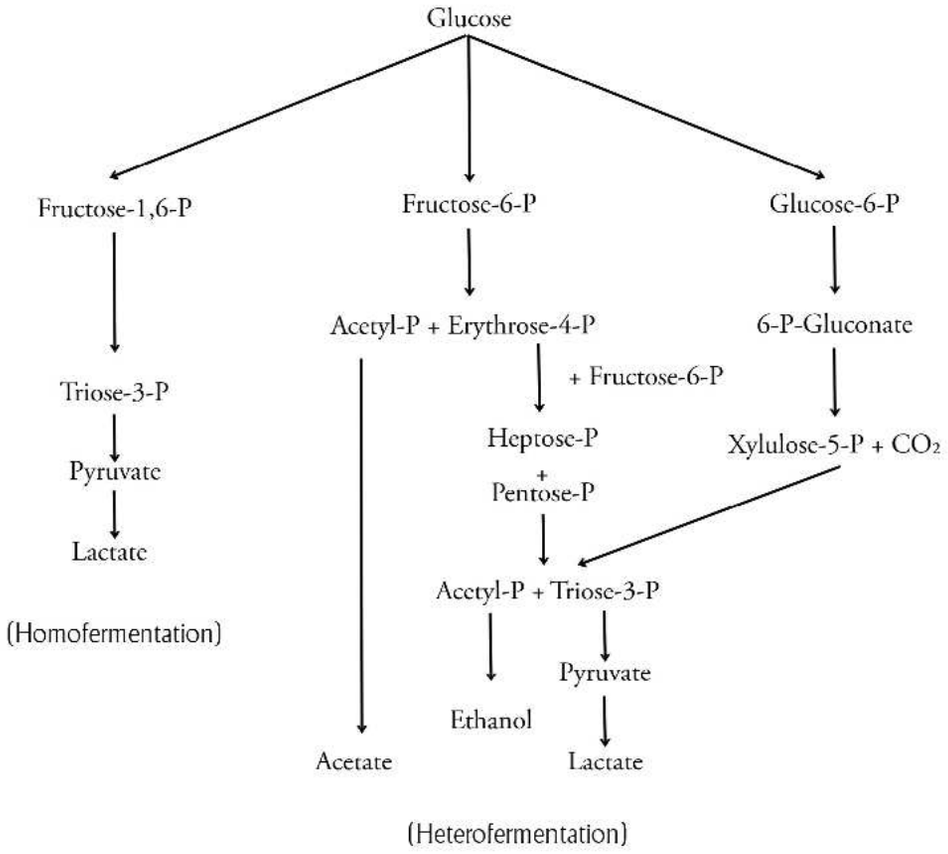

Figure 4. Schematic representation of the metabolism of hexoses by lactic acid bacteria (adapted from [47]).

metabolites, which influences the nutritional, texture, and organoleptic qualities of the end products [36, 51]. Moreover, LAB have also been used as probiotics, which shows several potential health benefit [52]. Thus, in general, LAB enhances the shelf life and microbial safety of end products [43]. However, based on the microorganisms profile present in the raw material, their effects may be either beneficial or disadvantageous to the food processing. For instances, malolactic fermentation (MLF) is a secondary fermentation in wine normally carried out by LAB, especially by *Leuconostoc oenos*, which usually occurs at the end of alcoholic fermentation by yeast [53]. In this metabolism, L-malic acid is decarboxylated to L-lactic acid and CO_2, a reaction catalyzed by the malolactic enzyme without the release of intermediates. As a consequence of this pathway, the acidity is reduced which turn it a crucial process in wine and cider production [53]. However, it is noteworthy that MLF is not only important as a deacidification process in wine, but for the aroma and microbial stability of wine [53–55]. Additionally, this fermentation prevents the malic acid utilization by other undesirable microorganisms. Another industrial application of LAB is the use of starter cultures as inoculants during the malting process, a complex biological process essential to the production of fermented beverages, in order to improve the malt quality and safety. In these conditions, LAB can improve the extraction, fermentability, and nitrogen yield of wort and the foam stability, color, and flavor of beer [50]. Moreover, another important effect of the LAB in the malting process is their ability of antimicrobial

substances production (e.g., bacteriocins) that restricts the growth of harmful bacteria to malting [50, 56–58].

2. Fermented beverages of South America

The traditional foods, mainly those produced by spontaneous fermentation, are present in the daily life of the population and play an important role in the cultural identity of different communities [59]. Indigenous or traditional fermented foods refer to the products that, since the beginning of history, are an integral part of the diet and can be prepared in household or cottage industry, using simple techniques and equipments [60].

In South America, there are various traditional fermented beverages, mainly produced by fermentation of cereals, vegetables, and root tubers. Among these beverages could be cited the traditional beverages *cauim* and *caxiri*, made by Brazilian natives and the traditional beers *chicha* and *champús*, typical of the Andes [59, 61–64]. *Cachaça* is also a typical beverage, from Brazil, where the fermented sugarcane juice is then distilled to produce a spirit. Table 1 shows some characteristics of these traditional beverages.

Beverage	Raw material	Microorganisms	Country
cauim	cassava, rice, peanuts	Lactic acid bacteria (LAB), *Saccharomyces cerevisiae*, other yeast	Brazil
caxiri	cassava	LAB, *Bacillus* spp., *S. cerevisiae* (predominant yeast), other yeast	Brazil
champús	maize	*S.cerevisiae*, other yeast	Colombia
chicha	maize	LAB, *S.cerevisiae* (predominant yeast), *Aspergillus* spp.	Peru
cachaça	sugarcane	*S. cerevisiae*; LAB, other yeast	Brazil

Source: [59, 61-64]

Table 1. Catalogue of traditional fermented beverages of South America.

2.1. *Caxiri*

Caxiri is an alcoholic fermented beverage made from cassava (*Manihot esculenta* Crantz) and produced by Brazilian natives. The *Yudjá-Pakaya* native tribe is located in the Xingu Park in the Mato Grosso state, Brazil [64]. Initially, cassava roots are incubated for 2 days in running water to soft the skin. After this stage, the cassava is peeled, cut into small pieces, and placed in handmade straw press for removing the excess of water. The resulting paste from pressing is grated into flour. Then the flour is roasted for about 2 hours. Subsequently, the flour is mixed

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with water and grated sweet potato. The mixture is placed in special open vessels for fermentation to occur. After 24–48 hours, the beverage is ready for consumption [64]. In the course of the fermentation process, a change of pH occurs, decreasing from pH ~5.8 to ~3.1–3.2. The final beverage has an ethanol concentration of approximately 80 g/L, and 28 g/L of lactic acid.

Ramos et al. (2010) investigated the microbiota involved in *caxiri* by culture-dependent and culture-independent methods of cultivation. These authors found that the bacterial populations varied from 3.05 to 5.33 log CFU/mL, and the populations of yeast from 3.27 to 7.34 log CFU/mL. Yeast dominate fermentation after 48 hours, being *S. cerevisiae* the dominant yeast species. Other yeast species, such as *Rhodotorula mucilaginosa*, *Pichia membranifaciens*, *Pichia guilliermondii* and *Cryptococcus luteolus* were also found. The bacteria belonging to the genus *Bacillus* were the most prevalent in *caxiri*. LAB species were found at the beginning of the fermentation and after 24 hours of fermentation. The microbiota found in *caxiri* may be from different sources, such as raw materials, environmental, or utensils used in the preparation of the beverage [64].

2.2. *Cauim*

Cauim (Kawi) is a nonalcoholic fermented beverage produced from various substrates, such as banana, cassava, cotton seed, maize, pumpkin, peanuts, and rice. This drink is produced in Brazil, in the Mato Grosso state, by *Tapirapé-Tapi'itãwa* native tribe [59, 63]. *Cauim* represents great importance to the tribe since it is consumed by adults and children as part of their daily meal [59, 63]. For the production of *cauim* from cassava, initially this substrate is fermented for 3 to 5 days in running water. Afterward, the cassava is peeled, cut into pieces and placed on sun to dry. The dried pieces are crushed, and the obtained flour is added to water. The mixture is boiled for 2 hours and then cooled at room temperature. An inoculum obtained from the chewed sweet potato (*Ipomoea batatas*) prepared by indigenous women is then added to the porridge to start the fermentation. After 24–48 hours, the beverage is ready for consumption [59].

For the preparation of beverages with other substrates, the procedure is similar to *cauim* made from cassava. The main substrate is cooked for approximately 2 hours and cooled at room temperature. After cooled, the inoculum is added to start the fermentation [63]. During fermentation, a progressive acidification occurs, and the pH of beverage decrease from ~5.5 to ~3.4 [59].

Almeida et al. (2006) found LAB as the dominant microorganisms during *cauim* fermentation. Among the LAB obtained in that work, *Lactobacillus pentosus* and *Lactobacillus plantarum* were the most prevalent species. Gram-positive sporulating bacteria, as the genus *Bacillus* spp., were found in small amounts. In a recent work, Ramos et al. (2010) monitored the community of yeast and LAB in *cauim* prepared from rice and peanuts, by culture-dependent and culture-independent methods. LAB were found in higher counts, ranging from 7.4 to 8.4 log CFU/mL, while yeast were found at 4.0 to 6.6 log CFU/mL. The most prevalent species of yeast found were *Pichia guilliermondii*, *Kluyveromyces lactis*, *Candida* sp., *Rhodosporidium toruloides*, and *S. cerevisiae*. The most prevalent species of LAB belonged to genus *Lactobacillus*: as *L. plantarum, L. fermentum*, *L. paracasei*, and *L. brevis* [63].

2.3. *Champús*

Champús is a cereal-based fermented beverage, sweet and sour, and with low alcohol content. This drink, typically found in Colombia, can also be found in other countries in South America, such as Ecuador and Peru [62]. *Champús* can be prepared from different cereals (maize, rye, and wheat) alone or in a mixture, with other raw materials, such as pineapple, *lulo* (*Solanum quitoense* Lam.), *panela* (brown sugar paste), herbs (orange leaves), and spices (cloves and cinnamon) [62].

In Colombia, the beverage is produced by boiling the kernels of maize, for about 2 hours. Thereafter, the beans are cooled to room temperature, and then fruits, *panela*, and other ingredients are added. The beverage is cooled to 12°C–15°C, and after 24–48 hours of a spontaneous fermentation process, it is ready for consumption. The final beverage has a low alcohol content (2.5%–4.2%), and the pH is between 3.5 and 4.0 but can vary according to the ingredients [62]. The microorganisms responsible for *champús* fermentation (during storage at low temperatures), such as yeast and LAB, come from the fruit since the microbial derived from corn grains are eliminated during the period of boiling [62]. Osorio-Cadavid et al. (2008) found seven genera of yeast when twenty samples of *champús* from Colombia were analyzed. The most prevalent species of yeast founded in this study were *Pichia fermentans*, *S. cerevisiae*, and *Issatchenkia orientalis* (*Candida krusei*).

2.4. *Chicha*

Chicha is an alcoholic beverage, clear, yellowish, and sparkling, which resembles the taste of cider and that has been consumed by Andean indigenous population for hundreds of years. This beverage is produced in regions of the Andes and sometimes in low-lying regions of Ecuador, Brazil, Peru, Bolivia, Colombia, and Argentina [65]. *Chicha* is a generic name that comprises a series of fermented or nonfermented beverages that can be prepared from various raw materials such as cereals and fruits [66].

Chicha can be produced in different ways. Although the recipes pass from generation to generation, all of them use the conversion of starch into sugar, followed by fermentation of sweet wort. As the production process resembles the brewing process, the traditional *chicha*, made from maize, can be named as the Andean indigenous beer [67].

In the Andean region, the most common maize *chicha* is the *chicha de jora*. This *chicha* is prepared from yellow corn grain (*maíz amarillo*) malted (germinated and dried) or chewed. In *chicha de jora* production process, hydrolysis of starch is obtained by the malting process of the kernels of maize or the action of salivary amylase in the case of chewing [65].

The production process of *chicha de jora* is laborious. After malting, it proceeds to the wort boiling (consisting of maize flour plus water), a process that takes many hours. During the wort boiling, sugar and *panela* as well as herbs and spices may be added. Subsequently, the wort is cooled and filtrated. Then the beverage is placed in special vessels for the occurrence of fermentation. The *chicha* is ready for consumption when its sweet taste disappears and the flavor becomes a little stronger. However, if not consumed immediately, the beverage becomes bitter, and after 7 days, it usually is converted into vinegar [65, 68]. During the *chicha de jora*

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production, a wort acidification occurs, with a decreasing pH values of ~5.7–5.3 to ~3.7–3.5. The final beverage presents an ethanol concentration between 9 and 10 g/L [69].

In some Andean countries is produced the *chicha morada*, a beverage prepared with purple maize. Purple corn is a pigmented variety of *Zea mays* L., originating mainly from Peru and Bolivia. This drink is prepared by boiling purple maize with pineapple, quince peel, cinnamon, and cloves [70, 71].

In Ecuador, in addition to *chicha de jora*, other kind of *chichas* like *chicha de morocho*, prepared with white maize, *chicha de Yamor* or seven-grain *chicha*, and *chicha de yuca* (cassava *chicha*) are also produced. The seven-grain *chicha* is produced from seven varieties of maize as *jora*, yellow corn, white corn, black corn, *chulpi* corn, *morocho* corn, and popcorn [68]. The *chicha de yuca*, produced by the indigenous and mestizo population in the Amazon region of Ecuador, is produced in a peculiar way since chewing is used. After the chewing process and fermentation of cassava, a mixture of Ungurahua palm (*Oenocarpus bataua* subsp. *bataua*, Arecaceae) fruit juice with the fermented mass is made, and thus the beverage is ready for consumption. For the preparation of the juice, the fruits are first harvested and then they are soaked in hot water for the removal of mesocarp. The seeds are dropped and then pieces fermented cassava are added to the mixture of mesocarp and water [68, 72].

In *chichas* from Ecuador and Brazil, LAB, yeast, and *Bacillus* species were found as the microorganisms associated with this beverages [73, 74]. Blandino et al. (2003) found yeast, bacteria, and filamentous fungi in *chichas* of Peru. Elizaquível et al. (2015) found the *Lactobacillus* genus as the most prevalent and the one with the highest diversity of species in *chichas* of Argentina, by culture-dependent and culture-independent methods. In another work, the yeast obtained from *chichas de jora* collected in 10 *chicherías* (*chicha* producers) in Peru were identified as belonging to the species *S. cerevisiae* [66]. Vallejo et al. (2013) considered *S. cerevisiae* as the responsible yeast for the fermentation of Peruvian *chichas de jora*.

The *chicha* microbiota may come from different environments. The LAB found in *chichas* may have been introduced from the raw materials, as many species are commonly found in vegetables and plants, and also transferred from humans and animals, natural hosts of these bacteria [73]. The yeast involved in *chichas* production process come from different sources, such as handlers, raw materials, utensils, and equipment used in the preparation of these beverages or can be carried by insects. The clay vessels and wooden spoons, used in the preparation of *chichas*, provide an ideal microhabitat for yeast, infiltrating into tiny cavities of such utensils [75]. In Quito, Ecuador, two isolates of yeast from old vessels obtained from deep tombs of La Florida archaeological site were recovered, which were identified as *Candida theae*, a new species belonging to the clade *Lodderomyces* [76].

2.5. *Cachaça*

In Brazil, *cachaça* (ka.ʃa.sə) was the name given to (i) waste of sugar production (beginning of XVI century), (ii) waste of sugar production when fermented (around XVI–XVII centuries), and finally (iii) product of the distillation of the fermented sugarcane (XVII century to nowadays). The first mention of *cachaça* occurs in 1622 with the name of "augoa ardente" [aqua vitae] or spirit, in Bahia State (Brazil), and the first use of the name *cachaça*

instead of aqua vitae occurs in 1660. Considering the three ethnic groups that formed the Brazilian nation (Native Brazilians, Africans, and Europeans), scarce information is available about the real contribution of each group to the initial production of fermented sugarcane. However, *cachaça*, which is the result of the distillation of fermented sugarcane juice, was certainly "discovered" by Europeans, the most technologically advanced group, who had knowledge and equipment to do so [77]. Nowadays, *cachaça* is the typical and exclusive denomination to Brazilian spirit produced from sugarcane juice with alcohol content ranging 38%–48% (v/v) at 20°C (68°F), which present unique characteristics (Table 2) [78]. Brazil has an estimated installed capacity of *cachaça* production ranging from 1.2 to 1.5 billion litters/year; however, the production is less than 800 million litters/year. According to the Brazilian Institute of Geography and Statistics (IBGE), almost 15,000 establishments are currently producing *cachaça*. In 2014, 10.2 million liters of *cachaça* were exported to 66 countries, generating US$ 18.33 million in revenue [79].

Compounds	Units	Limits
Copper	mg/L	5.0
Ethyl carbamate	μg/L	210.0
Volatile acidity	mg/100mL anhydrous ethanol	150.0
Total esters	mg/100mL anhydrous ethanol	200.0
Aldehydes	mg/100mL anhydrous ethanol	30.0
Total higher alcohols*	mg/100mL anhydrous ethanol	360.0
Furfural+HMF+	mg/100mL anhydrous ethanol	5.0
Methanol	mg/100mL anhydrous ethanol	20.0
Acrolein	mg/100mL anhydrous ethanol	5.0
Particles in suspension	-	Absent
Dry extract	g/L	6.0
Total sugars	g/L	6.0
		38-48

Source: Ministry of Agriculture, Livestock and Supply - Brazil

*Sum of isobutyl (2-methyl-1-propanol), isoamyl (2-methyl-1-butanol and 3-methyl-1-butanol), and n-propyl (1-propanol) alcohols.

+ 5-(Hydroxymethyl)furfural

Table 2. Components present in *cachaça* and its limits in accordance with the Brazilian law.

2.5.1. Production

The main raw material for the production of *cachaça* is the juice of sugarcane (*Saccharum* spp.). The first step to prepare of the fermentation medium is the extraction of sugarcane juice. Small producers extract the juice by crushing the sugarcane using a mill. Yet, the large producers use a more complex system of extraction: (i) crushing system, (ii) cutting machines, and (iii) shredders [80]. The resulting sugarcane juice is an opaque (color ranging from brown to dark green), viscous, and sweet liquid. The color is due to different pigments such as chlorophyll

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and polyphenols, while the viscosity is due mainly to the presence of colloidal proteins. The sugarcane content in fermentable carbohydrates is sucrose (11%–18%), glucose (0.2%–1%), and fructose (0%–0.6%) [81]. A great number of microorganisms is associated with the sugarcane plant, and during the extraction of juice, these microorganisms can be transferred to the fermentation medium. The yeast present in the juice belong to the genus: *Candida*, *Cryptococcus*, *Kluyveromyces*, *Hansenula*, *Rhodotorula*, *Saccharomyces*, and *Torulopsis*. The main bacteria genus are *Leuconostoc*, *Streptotococcus*, *Lactobacillus*, and *Bacillus* [82]. In order to reduce the number of microorganisms and/or optimize the fermentation, several processes to improve the quality of the fermentation medium can be implemented. The most common procedure is the decantation of the sugarcane juice, thus eliminating coarse particles as soil/sand that can damage the *cachaça* manufacturing equipment. A dilution or concentration step (most unusual process) may be performed, particularly if the crop was harvested at a nonoptimal time. The juice supplementation with nutrients can provide a more robust fermentation; however, such practice is not common among the majority of *cachaça* producers. Studies have shown that addition of ammonium sulfate, magnesium sulfate, cobalt sulfate, and vitamins (especially B complex) provide huge productivity gains. Other actions such as filtration, addition of antibiotics, use of disinfectants, temperature control, and must acidity correction can also be used to improve the quality of fermentations [83].

2.5.2. Microorganisms

As important as the preparation of the medium is the preparation of microorganisms that will ferment the sugarcane juice, the so-called foot-of-vat. Traditionally, *S. cerevisiae* is the most used yeast, but some other species are also utilized, namely, *Pichia* sp. [11, 84]. The cell concentration usually found at the beginning is about 10^6–10^7 cells/mL and about 10^8 cells/mL at the end of the fermentation. Traditionally, the majority of the *cachaça* is produced through self-inoculation, using different protocols. Usually, a homemade mixture is prepared: (i) diluted sugarcane juice (carbon source), (ii) rice bran and/or corn bran (nutrient sources), and (iii) lemon/orange juice (to reduce initial pH) [85]. All these ingredients are mixed and kept at rest for 24 hours, during which wild yeast present in sugarcane juice will multiply—microorganism multiplication is verified through foaming. At the end of the first 24 hours, a new volume of diluted sugarcane juice is added, and the process is restarted [85]. The process is repeated until it reaches 20% of the volume of the fermentation vat. At the end of the process, a wild yeast community is obtained, which is adapted to the physical and chemical conditions of the fermentation. Producers, which aim to improved organoleptic characteristics, tend to use selected yeast in fermentative process. There are two basic types of selected yeast: (i) yeast that are used to produce ethanol or other beverages without being specific for *cachaça* production and (ii) yeast selected from their own vats of *cachaça* fermentation. Yeast selected in their own fermentation tanks have advantages over the first because they are more adapted to the *cachaça* fermentation specific conditions and are able to provide unique features to the product [86, 87]. There are numerous advantages to using selected yeast, including (i) rapid and (ii) homogeneity of the fermentation, (iii) higher fermentation yields, (iv) higher quality of the final product, (v) lowest risk of contaminations, and (vi) highest resistance to stress [88]. The improved aroma obtained in the final product is another advantage of using the selected

yeast [86]. Given that *cachaça* fermentation develops in an environment with few controls, these vats represent a unique ecological niche. Interesting phenotypes, such as the production of flavor and aroma compounds, can be assessed by the detailed characterization of isolated strains [89]. The use of yeast strains producing aroma compounds, as esters (ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate) and higher alcohols (propyl, isoamyl, and isobutyl alcohol), can significantly increase the final quality of *cachaça*. Thus, such profile can be a differential characteristic between several *cachaça* producers or even distinct producing regions—if all producers in a particular region start using a selected yeast strain.

2.5.3. Fermentation

The *cachaça* fermentation can occur in simple batch system (most common process) and fed batch or continuous culture (less common). The batch system with cell recycling at the end of each fermentation is the most widespread in the *cachaça* production. In general, the fermentation occurs in 24/36 hours depending on the system efficiency [90]. After fermentation, the product is generally removed by pump systems, or via a valve near the base of the fermenter, where about 80% of fermented must is removed. The remainder medium, approximately 20%, is composed primarily of sedimented yeast [90]. Some producers can perform specific treatments to decrease the contamination load of the inoculum: (i) acid treatment (Melle–Boinot method and its modifications); (ii) by stirring and spray air with diluted sugarcane juice, or (iii) only add new medium for fermentation and restart a new fermentation cycle, without any special treatment [91]. *Cachaça* production occurs mainly on small farms where financial resources are generally scarce. Control of the fermentation conditions is rarely held, including (i) maintaining the fermentation temperature, (ii) standardization of sugarcane juice, or (iii) fermentation in closed vat, in order to prevent contamination. Moreover, hygiene conditions are not always in conformity to national or international standards for the production of beverages [92]. Quite often, the fermentation vats are kept next to animal facilities or made from improvised materials (such as wood, rubber tires, etc.). Thus, the standardization of the final product is not achieved, and the sale of *cachaça* for more demanding/rigorous markets like Europe or the US is greatly impaired. Thereby, government agencies, research laboratories, and private companies together, and some individual initiatives are trying to change the *cachaça* production outlook in order to not only improve the quality of the final product but also regulate unlicensed producers.

2.5.4. Distillation

After fermentation of sugarcane juice, the medium is taken to steel distillation columns (industrial *cachaça*) or special copper-made distillers called "alembics" (majority of the *cachaça* producers). In this last process, the distillation product can be divided into three parts: (i) head or "strong water" (5%–10%), (ii) heart (approximately 80%), and (iii) tail or "weak water" (5%–20%) [19]. The head is the first distilled portion of a fermented must, and it is rich in substances such as aldehydes, methanol, and esters. The head portion has alcohol content of 65/70% v/v. The next distillate volume is called the heart and displays ethanol concentration ranging from

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35/55% v/v. This fraction has lower content of chemical contaminants that affect negatively the sensory characteristics of the product. The last fraction is known as tail, where several acids and furfurals are found, presenting an alcohol content around 14% v/v. The best quality *cachaça* are produced only with the heart fraction of the distillate, and the remainder is discarded or used to produce other products such as liqueurs or bioethanol for fuel [93].

2.5.5. Aging

The *cachaça* aging process includes the storage of the distillation product in wood barrels under specific conditions (temperature, humidity, aeration, etc.) for a period not less than 1 year. Numerous biochemical reactions occur during the aging process, the main being oxidation and esterification reactions [94]:

$$\mathrm{R-COH(alcohol)} + \mathrm{O_2} \rightarrow \mathrm{R-HCO(aldehyde)(oxidation\ reaction)}$$
$$\mathrm{R-HCO(aldehyde)} + \mathrm{O_2} \rightarrow \mathrm{R-COOH(acid)(oxidation\ reaction)}$$
$$\mathrm{R-HCO\,(aldehyde)} + \mathrm{R-COOH(acid)} \rightarrow \mathrm{R-COO-R(ester)\,(esterification\ reaction)}$$

Alcohols are relatively stable to oxidation but can form significant amounts of aldehydes in the presence of phenol and water. Aldehydes are highly reactive and may oxidize to form the corresponding organic acid. Through esterification reactions, acids react with alcohols to form esters, which soften the odor of aldehydes, giving a pleasant odor to the *cachaça*. Beside the aldehydes, some sulfur compounds also decrease during maturation, such as sulfide and disulfide. In addition, alcohol and water, through capillary penetration and osmosis, pass through the interstices of the timber hydrolysing the hemicellulose and lignin [95]. The hydrolysis products are extracted, enriching the distillate and enhancing the quality of the drink [93]. Sensory gains of the beverage varies according to the chemical composition of wood, the aging time, the capacity of the barrel, the porosity, and the thickness of the timber [96]. Some studies have shown that blending aged with fresh *cachaça* is feasible and reasonable since the final product has better organoleptic and commercial characteristics. Many types of woods can be used for the manufacture of high-quality aging barrels, such as amburana (*Amburana cearencis*), jequitibá (*Cariniana estrellensis* and *Cariniana legalis*), ipê (*Tabebuia* genus), balsam wood (*Myrocarpus frondosus avium* and *Prunus cerasus*)—Brazilian trees, and oak (*Quercus* genus) [97].

3. Yeast and LAB new potential applications

South America presents a wide variety of fermented and distilled beverages, which have several unique characteristics, greatly influenced by the fermentative metabolism of microorganisms. Therefore, those microorganisms present a large potential for utilization in the development of new beverages, or even in new biotechnological applications. In this context,

several scientific works have focused in the isolation and characterization of such microorganisms [11, 86, 87].

3.1. Wild yeast

During fermentation, yeast and LAB cells are submitted to several stress factors, such as: high osmotic pressure and hydrostatic pressure, high concentrations of ethanol, anaerobic atmosphere, temperature, and nutrient limitation [98]. Such pressures promote the genetic adaptation of the individuals, leading to the survival of only the fittest cells. The increasing number of such alterations will lead to changes in the fermentation subproducts, some of which contribute to the organoleptic properties of the final products. Consequently, some of those subproducts may contribute to improve the beverages and, in this way, increasing the diversification of this industrial niche. Furthermore, the utilization of microorganisms isolated from traditional products, as *chicha* or *cachaça*, in the fermentation or maturation of new drinks production can lead to development of new promising products.

Recently, wild yeast isolates from *cachaça* fermentation vats in Brazil were innovatively evaluated in the production of beer [99]. First, in that study, 21 isolates belonging to the collection of the Laboratory of Cell and Molecular Biology /UFOP were surveyed for the production of aroma compounds in the beers. For that, compounds known for their influence in aroma and flavor, such as higher alcohols, esters, acetaldehyde, diacetyl, and ethanol, were analyzed by gas chromatography. After a careful analysis of each aromatic profile, two superior isolates were chosen (LBCM78 and LBCM45). In the same study, LBCM78 and LBCM45 were shown to be suitable to the production of ale and lager beers, respectively. The physicochemical composition of the produced beers were analyzed and compared to similar beers produced by commercial brewer yeast strains [99]. As a result, in the production of a wheat beer, the commercial strain WB-06 showed higher values of n-propanol than LBCM78. However, no significant differences were observed in the isobutanol and isoamyl alcohol levels. Similarly, the LBCM45 and the commercial strain W-34/70 were used to produce a lager beer, and the final products were analyzed as before. Between those two beers, the productions of isobutanol or isoamyl alcohol were similar. Moreover, no differences were observed in the ethyl acetate and diacetyl content of all the different beers produced by the four strains. As a final step of that study, beers were submitted to a sensorial evaluation by a group of trained tasters, from the Craft Brewers Association of Minas Gerais. The wild yeast showed a similar production of ethanol to the commercial strains (3.41–4.80% v/v), and the sensory analysis of the beers produced from LBCM45 and LBCM78 strains showed good acceptance in the evaluation panel [99]. These results suggest that yeast strains isolated from *cachaça* vats have a great potential for the production of new beers since a good production of volatile compounds and ethanol were observed.

3.2. Mixed fermentation yeast/LAB

Recently, our research group started a work to approach the utilization of both yeast and LAB in the fermentation of *cachaça* and beer [100]. In that study, it was verified that the presence of LAB in the fermentation of such beverages appears to contribute to the production of organic

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acids, which may contribute to the formation of aromatic compounds, and to the reduction of the pH, inhibiting the growth of spoiling microorganisms. In a recent collaboration between our group and a known Minas Gerais craft brewery, several LAB were isolated from beers with high acidity level. These isolates were characterized molecularly and identified as *Lactobacillus brevis*, which are mandatory hetero-fermentative microorganisms that produce lactate, ethanol, or acetic acid and CO_2 as final metabolites [40]. Moreover, in the next step of that study, those isolates were tested in fermentations at different temperatures in order to assess their applicability in the production of certain beer styles. The selected isolate LBCM718 showed a good growth in temperatures above 18°C, and then it was tested in beer mixed fermentations with two ale yeast strains, LBCM78 and WB-06. The viability of both yeast and LAB as well as the wort final pH were analyzed [100]. The ethanol production was not affected by the presence of *L. brevis*. Such fermentations are frequent in beers from the lambic or fruit beer style, where LAB contribute with acidity and lactate, yielding ethyl lactate—an important aromatic compound. Moreover, a test was conducted to test the LAB resistance to iso-α-acids, in the concentration range generally found in beer—17 to 55 ppm [101]. There was no formation of inhibition zones, which suggest that isolate can be used in the production of beers.

In a study from another research group, a mixed fermentation of *S. cerevisiae* and *Lactococcus lactis* led to the improvement of *cachaça* quality. When the concentrations of higher alcohols, such as propanol, isobutanol, and isoamyl alcohol from three fractions (head, tail, and heart), were compared, the researchers found higher ethanol levels in the *cachaça* from mixed fermentation than in the *cachaça* produced from a pure *S. cerevisiae* inoculum. Finally, the evaluation comparing the two products was performed by 40 trained tasters, men and women aged between 22 and 50 years old. In that evaluation, both *cachaças* were evaluated for flavor, color, and overall acceptability. The *cachaça* produced by mixed culture obtained higher scores in the categories aroma and appearance, while the *cachaça* produced from pure culture yeast showed higher global acceptance. Both beverages showed similar flavor [102].

Both studies show that the use of bacteria and yeast simultaneously in fermentation apparently does affect the growth of both cultures. Similarly, the ethanol production in these mixed fermentations was the same. Furthermore, the use of mixed fermentations appears to improve the aroma of both beer and *cachaça*, a potential alternative to the development of new products.

3.3. New spirits

Brazil is the country with the world's largest fruit production; however, there is a huge postharvest waste of raw material that generates losses to the farmer. Therefore, there is the necessity to develop new processes and products to reduce these losses. In this context, an alternative is the use of these fruits for the production of alcoholic beverages [103].

In a previous study, a research group developed a fermentation process from *cajá* (*Spondias mombin*) pulp for the production of a new beverage. In that study, *cajá* pulp was inoculated with *S. cerevisiae* and then fermented at 22°C during 10 days. Analyses of amount of alcohol and higher alcohols were carried out to determine the compounds present in the beverage. Simultaneously, the final product was subject to sensory analysis. The amount of alcohol found in the *cajá* beverage was in averaged 12°GL, comparable to those found in wines. The total

amounts of higher alcohols found were about 0.7 g/L, while the values for these in wines ranging from 0.1 to 0.3 g/L [104]. Sensory analysis showed a good acceptance by the tasters [103].

In another study, it was evaluated the quality of fruit spirits produced through different treatments [105]. Mango, grape, and passion fruit were used as raw materials, and the fermentation was performed using *S. cerevisiae* cultures. Distillation was performed in copper still with controlled temperature between 85°C and 90°C, and the amount of alcohol in beverage was standardized at 40°GL. After that, oak chips and umburana chips were added to the spirits for 60, 90, and 120 days. At the end of this period, the samples were conducted to sensory analysis using 10 trained panelists, using the quantitative descriptive analysis method. All three products obtained were well accepted by the tasters, being the passion fruit distillate the best evaluated. Additionally, 90 days was the best period of aging for those particular spirits [105].

As noted in these studies, alcoholic beverages obtained from tropical fruits were well accepted in sensory tests, demonstrating the potential application of these substrates in the production of new beverages.

Another study had as objective to obtain and characterize a new spirit from the fermentation of cheese whey. The cheese whey is a by-product of the dairy industry that has a high impact in the environment. The researchers used the yeast *Kluyveromyces fragilis*, due to its ability to grow in medium containing lactose, in high yields, and without the production of toxins. In order to achieve that objective, the whey powder was acidified and deproteinized, and the resulting supernatant was used for fermentation [106]. The fermentation of the whey with a high concentration of lactose (200 g/L), after 92 hours, obtained a final product with an ethanol content of 9.6% (v/v). After distillation of the fermented beverage, the heart fraction was diluted to 40% (v/v) ethanol content. The chemical analysis revealed that the higher alcohols were the most abundant group of volatile compounds present in this fraction, containing isoamyl, isopentyl, isobutyl, and 1-propanol, all present in large quantities. Among the esters, the ethyl acetate was found the highest concentration. This compound has a significant effect on the organoleptic characteristics of wines and spirits. Furthermore, the authors concluded that it was possible to obtain a spirit with pleasant smell and taste from cheese whey, containing high concentration of lactose, this being an alternative to by-product of dairy industry [106].

From these studies, we can see distinct possibilities for the production of new beverages, by changing the yeast strain/species, or using blends of different microorganisms, such as yeast and LAB. Moreover, it is possible to use several different substrates for the production of these beverages, such as fruit and cheese whey.

4. Conclusion

Studies on South American beverages are scarce when compared to other beverages like wine, beer, or even sake. This is mainly due to years of neglect to research in these countries. Until recently, the economic difficulties of the South American countries prevented investments in scientific research. Nowadays, with the economic stability, these countries increased the scientific funding, and a new reality seems to arise. In this context, the understanding of the

microorganisms present in typical South American beverages opens the door to the development of new technologies, contributing to the overall scientific and economic development of such countries. For example, the isolation of yeast in *cachaça* fermentation vats may lead to the discovery of new strains resistant to different stresses, which can be used not only to produce *cachaça,* but also to produce bioethanol. Moreover, lactic acid bacteria can promote the appearance of new products such as beers with unique or regional flavors. Thus, studies of these microorganisms diversity, present in such unique environments as the traditional beverages, help uncovering new potential applications. Furthermore, the knowledge of those microorganisms can promote the revival of traditional beverages, as *cauim* that was the most consumed beverage in South America in the centuries XVI–XVII. Nevertheless, despite the increased investment in research, few laboratories have the know-how necessary or the availability of resources to invest in the "screening" of yeast or lactic acid bacteria in traditional beverages. Research on South American traditional beverages is important to improve the quality of those beverages, but also to develop new products from these microorganisms.

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Author details

Fábio Faria-Oliveira, Raphael H.S. Diniz, Fernanda Godoy-Santos, Fernanda B. Piló, Hygor Mezadri, Ieso M. Castro and Rogelio L. Brandão*

*Address all correspondence to: rlbrand@ufop.br

Laboratório de Biologia Celular e Molecular, Núcleo de Pesquisas em Ciências Biológicas, Universidade Federal de Ouro Preto, Brazil

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