



## Review

## Research opportunities: Traditional fermented beverages in Mexico. Cultural, microbiological, chemical, and functional aspects



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## ABSTRACT

In Mexico, close to 200 fermented products have been described, of which, approximately 20 are beverages. They were obtained through rustic and ancestral fermentation methods by different indigenous Mexican communities; most of them were used in ceremonies, agricultural work, and other occasions. For their elaboration, different substrates obtained from plants are used, where uncontrolled and low-scale spontaneous anaerobic fermentation occurs. In Mexico, some of these products are considered as nutritional sources and functional beverages; the study of those products has revealed the presence of multiple compounds of biological importance. Additionally, elder generations attribute healing properties against diverse illnesses to these beverages. The aim of this review is to highlight the available information on twelve traditional Mexican fermented beverages, their traditional uses, and their fermentation processes along with toxicological, chemical, nutritional, and functional studies as seen from different areas of investigation. In the literature, pulque, cocoa, and pozol were the beverages with the greatest amount of described health properties; sendechó and guarapo were less characterized. Polyphenols, gallic and ferulic acid, anthocyanins and saponins were the most abundant molecules in all beverages. Finally, it is important to continue this research in order to determine the microorganisms that are involved in the fermentation process, as well as the organoleptic and beneficial properties they lend to the traditional Mexican fermented beverages.

## 1. Introduction

Fermentation is an anaerobic process of glycolysis that produces pyruvic acid without oxygen. There are different types of fermentation (alcoholic, acetic, lactic, butyric, butanedioic, propionic, etc.), and these are carried out by distinct microorganisms such as bacteria, yeast, and mycelial fungi, which are naturally present or intentionally added as in the case of enzymes (Tortora, Funke, & Case, 2010; Pérez-Armendáriz & Cardoso-Ugarte, 2020). It is used as a technique for food preservation, increasing the shelf-life of a broad range of foods such as milk, meat, fish, cereals, fruit, vegetables, legumes, roots/tubers, alcoholic beverages, and non-alcoholic beverages (Pérez-Armendáriz & Cardoso-

Ugarte, 2020). This process improves flavor, aroma, texture, and nutritional value, in addition to detoxifying and destroying undesirable substances or microbes present in raw foods (Narzary, Brahma, Brahma, & Das, 2016; Escobar-Zepeda, Montor, Olvera, Sanchez-Flores, & Lopez-Munguia, 2020). It is estimated that there are approximately 3500 fermented foods and beverages globally (Tamang, Holzapfel, Shin, & Felis, 2017).

Mexico offers an impressive variety of food, most of which is considered native, such as agave, cocoa, corn, chili peppers, and prickly pear, which are primarily used for the production of food and/or fermented beverages by the various indigenous Mexican communities (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003; Ramírez-

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Guzmán, Torres-León, de la Martínez-Medina, Rosa, & Aguilar, 2019; Valerino-Perea, Lara-Castor, Armstrong, & Papadaki, 2019). Specifically, in Mexico there are 200 fermented products, of which approximately 20 are beverages. The consumption of these beverages by the indigenous communities has traditionally occurred during religious events, and many of these products can be found for sale to the public in urban and rural zones (Ramírez-Guzmán et al. 2019).

The processes for the production of these beverages is based on empirical knowledge that is passed down from generation to generation (Godoy, Herrera, & Ulloa, 2003). However, little is known about their characteristics, preparation, and possible health effects. In this regard, this review aims to present the available information on twelve traditional Mexican fermented beverages (See Figs. 1 and 2) as seen from different areas of investigation, such as their traditional uses and fermentation processes, and also to systematically report the toxicological, chemical, nutritional, and functional studies conducted to date.

## 2. Significant research opportunities and future directions regarding traditional Mexican fermented beverages

### 2.1. Fermented beverages made from *Agave* spp.

#### 2.1.1. Pulque

**2.1.1.1. Description and traditional uses.** Aguamiel is derived from the extraction of the sap of agave. It can yield from 600 to 1500 L per plant (Puente-Garza, Espinosa-Leal, & García-Lara, 2018). Aguamiel is extracted from different Agave species, principally *Agave salmiana*, *Agave atrovirens*, *Agave mapisaga*, *Agave americana*, *Agave marmorata*, *Agave scapose*, *Agave ferox*, and *Agave seemanniana* (Escalante et al., 2016; Blas-Yáñez, Thomé-Ortiz, Espinoza-Ortega, & Bordi, 2019; Álvarez-Ríos, Figueredo-Urbina, & Casas, 2020; Pérez-Armendáriz & Cardoso-Ugarte, 2020). The spontaneous fermentation of aguamiel is called pulque (see Table 1).

Pulque was reserved for pre-Hispanic ceremonies, agricultural work, births, and funerals (Escalante et al., 2016; Becerra, 1988). This beverage is sold locally in 'pulquerías' (pulque stores), markets, fairs, or small family businesses, and it is usually consumed in its natural form but it can be mixed with oat, peanut, tomato, pineapple, strawberry, mango, celery, among others, which are known as 'curados' (Escalante et al., 2016; Rojas-Rivas & Cuffia, 2020).

**2.1.1.2. The fermentation process and associated microorganisms.** Pulque is a milky, viscous, slightly acidic liquid with an alcohol content of 4–7 mL of alcohol per 100 mL (Gay Lussac), depending on the production process employed. Production begins with a non-stirred portion of freshly collected sap and the fermentation process takes place under non-sterile conditions (Escalante et al., 2016; Matías Luis, Peña Caballero, Reyna González, Domínguez Díaz, & Martínez-Hernández, 2019). The fermentation time varies depending on aguamiel quality, seed maturity, season, type of agave, and region. The fermentation process usually lasts from 3 to 6 h (Escalante et al., 2004). In this regard, there are Official Mexican Standards (NMX-V-022.1972) that regulate the quality and the sensory properties required for the pulque.

During the fermentation process, the microorganisms involved are: acid-producing bacteria (lactic acid bacteria (LAB); acetic acid bacteria (AAB)) and alcohol-producing microorganisms (yeasts) (González-Vázquez et al., 2015; González-Vázquez & Mayorga-Reyes, 2017; Capozzi, Fragasso, & Russo, 2020). The diversity of microorganisms has been associated with the following phyla: Proteobacteria, Firmicutes, Acidobacteria, Actinobacteria, Cyanobacteria, Fusobacteria, and Nitrospira (Escobar-Zepeda et al., 2020). The microorganisms reported have been *Leuconostoc* spp., which are responsible for the viscosity attributed to the production of dextrans and fructans; *Lactobacillus* spp., responsible for increasing the acidity and the presence of aromatic compounds;

*Acetobacter* spp., for acidity; *Zymomonas mobilis* for the production of ethanol; non-*Saccharomyces* and *Saccharomyces* spp., for the production of ethanol and other secondary metabolites (amino acids, vitamins, and volatile compounds) (Yu & Zhang, 2003; Herrera-Solórzano, Lappe-Oliveras, & Wacher-Rodarte, 2008; Cavanagh, Fitzgerald, & McAuliffe, 2015; González-Vázquez et al., 2015; Escalante et al., 2016; Reyes-Nava & Garduno, 2016; Matías Luis et al., 2019; Álvarez-Ríos et al., 2020). On the other hand, *Candida* sp., *Kluyveromyces* spp., *Microbacterium arborescens*, *Flavobacterium johnsoniae*, *Gluconobacter oxydans*, *Hafnia alvei*, *Lactococcus lactis*, *Erwinia rhamontici*, *Enterobacter* spp., and *Acinetobacter radioresistens* have been described as contributors of pulque fermentation, but they have not been specifically described, until recently when they were reported in metagenomic studies (Torres-Rodríguez et al., 2014; González-Vázquez et al., 2015; Álvarez-Ríos et al., 2020; Escobar-Zepeda et al., 2020).

**2.1.1.3. Harmful compounds or pathogenic microorganisms.** During the production of pulque, the process can be contaminated with pathogenic bacteria. Gómez-Aldapa et al. (2012) observed the survival of pathogens such as *Listeria monocytogenes*, *Salmonella typhimurium*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Brucella* spp., *Campylobacter* spp., and *Vibrio cholera* during sap fermentation and in the final product. However, Escobar-Zepeda et al. (2020) showed that the presence of pathogens occurred in very low proportions (<0.005%): *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *Yersinia* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enterica*. In other words, these pathogenic bacteria were inactivated during the pulque fermentation process, by competition with the rest of the microorganisms present, suggesting that they are not a potential health risk (Gómez-Aldapa et al., 2012).

**2.1.1.4. Chemical and nutritional studies.** As fermentation takes place, the chemical compounds in aguamiel change until pulque is obtained. Aguamiel has more content of dissolved saccharose than pulque does, but when the fermentation is carried out in order to produce pulque, the saccharose content decreases and the ethanol concentration increases due to the yeast present during fermentation. Another outcome of fermentation is the decrease in pH values; thus, pulque has a lower pH than aguamiel (Table 2) (Álvarez-Ríos et al., 2020). Hernández-López et al. (2018) reported that moisture content was ~92%, with a total protein content of 2.71%, in contrast to the 6% reported by Escalante et al. (2016). Pulque has been reported to be a good source of vitamins, minerals, and amino acids, while aguamiel has been reported to be high in calcium, phosphorus, magnesium, iron, zinc, copper, boron, and all of the essential amino acids except methionine (Morales de León, Bourges, & Camacho, 2005; Escalante et al., 2016). Additionally, it provides bioactive compounds such as folates, steroid saponins, and phytase (Tovar, Olivos, & Gutierrez, 2008), as well as enzymes with metabolic and catalytic activities associated with soluble fiber or carbohydrate polymer synthesis (thiamine phosphate kinase, riboflavin, cobalamin, and biotin synthase, fructansucrase, and dextranase) (Escobar-Zepeda et al., 2020). Fructans have been described in the Agaves (designated as agavins) and they are considered an alternative source of prebiotic food ingredients, and the agavins have been associated with metabolic disorders and beneficial health effects (Escalante et al., 2016; Alvarado-Jasso et al., 2020).

**2.1.1.5. Evaluated health effects.** Pulque consumption has been shown to have health-promoting benefits. Since pre-Hispanic times, it has been commonly consumed in postpartum to enhance milk production, and to alleviate chest pain. It has also been drunk for the treatment of gastrointestinal (GI) disorders, intestinal infections, fainting, fever, and to induce menstruation, among others remedies (Backstrand, Allen, Black, de Mata, & Pelto, 2002; Becerra, 1988; Villalpando, Flores-Huerta, Fajardo, & Hernandez-Beltran, 1993). Additionally, pulque

**Table 1**

Traditional fermented beverages consumed in Mexico and their associated microorganisms.

Food	Traditional name	Substrate	Fermented material	Microorganism associated		References	
				Bacteria	Yeast		
Agave	Pulque	<i>Agave salmiana</i> <i>Agave atrovirens</i> <i>Agave mapisaga</i> <i>Agave ferox</i> <i>Agave americana</i> <i>Agave marmorata</i> <i>Agave scapose</i> <i>Agave seemanniana</i>	Aguamiel	<b>AAB:</b> <i>Acetobacter pomorum</i> . <b>LAB:</b> <i>Lactobacillus acetotolerans</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus collinoides</i> , <i>Lactobacillus hilgardii</i> , <i>Lactobacillus kefir</i> , <i>Lactobacillus paracollinoides</i> , <i>Lactobacillus plantarum</i> ; <i>Leuconostoc citreum</i> , <i>Leuconostoc dextranicum</i> , <i>Leuconostoc kimchii</i> , <i>Leuconostoc mesenteroides</i> . <b>Others:</b> <i>Acinetobacter radioresistens</i> ; <i>Enterobacter agglomerans</i> ; <i>Erwinia rhamontici</i> ; <i>Flavobacterium johnsoniae</i> ; <i>Gluconobacter oxydans</i> ; <i>Hafnia alvei</i> ; <i>Lactococcus lactis</i> ; <i>Microbacterium arborescens</i> ; <i>Zymomonas mobilis</i> .	<i>Candida lusitaniae</i> ; <i>Non-Saccharomyces</i> ; <i>Kluyveromyces marxianus</i> ; <i>Saccharomyces cerevisiae</i> ; <i>Saccharomyces carbajali</i> .	González-Vázquez et al., 2015; Escalante et al., 2016; Matías Luis et al., 2019	
Beans	Cocoa	<i>Theobroma cacao</i>	Seeds	<b>AAB:</b> <i>Acetobacter ghanensis</i> , <i>Acetobacter lovaniensis</i> , <i>Acetobacter pasteurianus</i> , <i>Acetobacter senegalensis</i> , <i>Acetobacter tropicalis</i> . <b>LAB:</b> <i>Lactobacillus cacaonum</i> , <i>Lactobacillus fabifementans</i> , <i>Lactobacillus plantarum</i> ; <i>Leuconostoc pseudomesenteroides</i> . <b>Others:</b> <i>Fructobacillus pseudoficulneus</i> , <i>Fructobacillus tropeaoli</i> ; <i>Pseudozyma antarctica</i> .	<i>Candida diversa</i> , <i>Candida inconspicua</i> , <i>Candida sylvae</i> , <i>Candida zemplinina</i> ; <i>Hanseniaspora opuntiae</i> ; <i>Pichia membranifaciens</i> , <i>Pichia kudriavzevii</i> ; <i>Rhodosporidium fluviale</i> ; <i>Saccharomyces cerevisiae</i> ; <i>Zygosaccharomyces bailii</i> .	Daniel et al., 2009; Romero-Cortes et al., 2012; de Melo Pereira et al., 2013; Arana-Sánchez et al., 2015; De Vuyst & Weckx, 2016; Sarbu & Csutak, 2019	
Corn	Pozol	<i>Zea mays</i>	Corn kernels	<b>AAB:</b> <i>Acetobacter</i> spp. <b>LAB:</b> <i>Lactobacillus casei</i> , <i>Lactobacillus fermentum</i> , <i>Lactobacillus mesenteroides</i> , <i>Lactobacillus plantarum</i> ; <i>Leuconostoc citreum</i> . <b>Others:</b> <i>Bacillus subtilis</i> ; <i>Bifidobacterium</i> ; <i>Enterobacteriaceae</i> ; <i>Enterococcus sulfureus</i> ; <i>Lactococcus lactis</i> ; <i>Streptococcus bovis</i> , <i>Streptococcus infantarius</i> , <i>Streptococcus macedonicus</i> . <b>LAB:</b> <i>Lactobacillus</i> spp.; <i>Leuconostoc citreum</i> ; <i>Weissella cibaria</i> . <b>Others:</b> <i>Pantoea anthophila</i> ; <i>Streptococcus</i> spp.	<i>Geotrichum</i> and <i>Candida</i> <i>Aspergillus</i> spp.	Fungi	Díaz-Ruiz, Guyot, Ruiz-Teran, Morlon-Guyot, & Wacher, 2003; Cárdenas et al., 2014; Lasso & Trabanino, 2018; Nuraida, Wacher, & Owens, 1995; Omar et al., 2000; Wacher et al., 1993, 2000
	Tejuino and tesgüino		Corn-stem Sprouted corn				Kennedy, 1963; Cruz & Ulloa, 1973; Paredes-López, Guevara Lara, & Bello Pérez, 2006; Nava Arenas, 2009; Cabezas Elizondo, 2016
	Sendechó		Corn kernels	<b>AAB:</b> <i>Acetobacter</i> spp. <b>LAB:</b> <i>Lactobacillus</i> sp.; <i>Leuconostoc</i> spp. <b>Others:</b> <i>Zymomonas mobilis</i> .			Génin, 1924; Torres-Rodríguez et al., 2014; González-Vázquez et al., 2015; Flores-Calderón et al., 2017; Unai, , 2019; Álvarez-Ríos et al., 2020
Balché	Balché	<i>Lonchocarpus violaceus</i> <i>Lonchocarpus longistylus</i> <i>Lonchocarpus punctatus</i>	Bark	ND			Aguirre-Dugua et al., 2013; Menezes, Neto, Contrera, & Venturieri, 2013; Dozier, 2016
Huapilla	Huapilla water	<i>Bromelia alsodes</i> <i>Bromelia hemisphaerica</i> <i>Bromelia karatas</i> <i>Bromelia pinguin</i> <i>Bromelia chrysanthia</i> <i>Bromelia nidus-puellae</i>	Fruit	ND	ND		Bernard Menna & Lozano Cortés, 2003; Pío-León et al., 2009; Espejo-Serna et al., 2010; Menezes et al., 2013; Arzaba-Villalba et al., 2018
Macaw palm	Taberna	<i>Acrocomia aculeata</i>	Sap	<b>AAB:</b> <i>Acetobacter pasteurianus</i> . <b>LAB:</b> <i>Lactobacillus acidophilus</i> , <i>Lactobacillus bulgaricus</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus plantarum</i> ; <i>Fructobacillus durionis</i> .	<i>Candida tropicalis</i> ; <i>Hanseniaspora guilliermondii</i> ; <i>Kazachstania exigua</i> , <i>Kazachstania unispora</i> ; <i>Meyeromyza guilliermondii</i>	Fungi	Balick, 1990; Alcántara-Hernández et al., 2010; Santiago-Urbina et al., 2016

(continued on next page)

**Table 1** (continued)

Food	Traditional name	Substrate	Fermented material	Microorganism associated		References
				Bacteria	Yeast	
Pineapple	Tepache and garapiña	Ananas comosus sativus	Pineapple peels	<b>Others:</b> <i>Citrobacter freundii</i> ; <i>Enterobacter hormaechei</i> ; <i>Klebsiella pneumoniae</i> ; <i>Pantoea agglomerans</i> ; <i>Sphingomonas dokdonensis</i> ; <i>Zymomonas mobilis</i> . <b>AAB:</b> <i>Acetobacter</i> spp.; <i>Gluconobacter</i> spp. <b>LAB:</b> <i>Enterococcus faecium</i> ; <i>Enterobacter aerogenes</i> ; <i>Lactobacillus acidophilus</i> , <i>Lactobacillus curvatus</i> , <i>Lactobacillus mesenteroides</i> , <i>Lactobacillus pentosus</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus salivarius</i> ; <i>Lactococcus lactis</i> ; <i>Weissella confusa</i> . <b>Others:</b> <i>Bacillus graveolens</i> , <i>Bacillus mexicanus</i> , <i>Bacillus subtilis</i> ; <i>Proteus vulgaris</i> .	<i>Pichia kluveri</i> , <i>Pichia kudriavzevii</i> ; <i>Saccharomyces cerevisiae</i> . <i>Candida boidinii</i> , <i>Candida inconspicua</i> , <i>Candida intermedia</i> , <i>Candida quereetana</i> , <i>Candida pseudointermedia</i> , <i>Candida sake</i> , <i>Candida sorbosa</i> ; <i>Hanseniaspora</i> genus; <i>Kloeckera Africana</i> ; <i>Kloeckera apiculata</i> , <i>Kloeckera japonica</i> ; <i>Pichia membranifaciens</i> ; <i>Saccharomyces bayanus</i> , <i>Saccharomyces cerevisiae</i> , <i>Saccharomyces radai</i> .	Herrera & Ulloa, 1982; Moreno-Terrazas, 2005; Rodríguez-González & Muro-Medina, 2011; Mapes & Basurto, 2016
Prickly pear	Colonche	Opuntia streptacantha	Prickly pear fruit pulp	<b>LAB:</b> <i>Enterococcus</i> spp.; <i>Lactobacillus plantarum</i> ; <i>Leuconostoc</i> spp.; <i>Pediococcus</i> spp.; <i>Weissella</i> spp.	<i>Candida</i> spp.; <i>Kloeckera</i> spp.; <i>Hanseniaspora</i> spp.; <i>Pichia fermentans</i> ; <i>Saccharomyces uvarum</i> , <i>Saccharomyces cerevisiae</i> ; <i>Torulopsis taboadae</i> .	Ulloa & Herrera, 1978; Rodríguez-Lerma et al., 2011; Ojeda-Linares, Vallejo, Lappe-Oliveras, & Casas, 2020
Sugar cane	Guarapo	Saccharum officinarum	Sugar cane stalk	<b>LAB:</b> <i>Enterococcus</i> spp.; <i>Leuconostoc</i> spp.; <i>Pediococcus</i> spp.; <i>Lactobacillus casei</i> , <i>Lactobacillus ferintoshensis</i> , <i>Lactobacillus fermentum</i> , <i>Lactobacillus jensenii</i> , <i>Lactobacillus murinus</i> ; <i>Lactococcus lactis</i> ; <i>Weissella</i> spp.	<i>Candida glabrata</i> , <i>Candida guilliermondii</i> , <i>Candida inconspicua</i> , <i>Candida kérif</i> , <i>Candida lipolytica</i> , <i>Candida pelliculosa</i> , <i>Candida rugosa</i> , <i>Candida utilis</i> ; <i>Cryptococcus laurentii</i> ; <i>Rhodotorula mucilaginosa</i> ; <i>Saccharomyces cerevisiae</i> .	Gomes Fatima & Silva, 2010; Chaves-López et al., 2014; Resende Oliveira et al., 2018

consumption is associated with improved iron status in non-pregnant Mexican women (Backstrand et al., 2002). It has also been proposed that low pulque intake (one daily glass) has a beneficial effect during lactation due to its micronutrient content. However, harmful effects of pulque consumption in pregnancy have been described (Backstrand, Allen, Martinez, & Pelto, 2001; Backstrand, Goodman, Allen, & Pelto, 2004). Pulque has been reported as an excellent source of antimicrobials against *E. coli*, *S. aureus*, and *Helicobacter pylori* (Giles-Gómez et al., 2016; Cervantes-Elizarrarás et al., 2019; Álvarez-Ríos et al., 2020) and anti-inflammatory probiotics such as *Lactobacillus casei* J57, *Lactobacillus plantarum* LBH1064, *Lactobacillus sanfranciscensis* LBH1068, and *Lactobacillus composti* LBH1072 (González-Vázquez et al., 2015; Torres-Maravilla, Lenoir, Mayorga-Reyes, Allain, Sokol, & Langella, 2016; Torres-Maravilla et al., 2016; Escobar-Zepeda et al., 2020; Pérez-Armendáriz & Cardoso-Ugarte, 2020). Moreover, agavins have been associated with improved calcium absorption in postmenopausal women, colon cancer prevention, increased iron absorption, and enhanced antioxidant activity (Escalante et al., 2016; Santos-Zea et al., 2016).

## 2.2. Fermented beverages made from *Theobroma cacao*

### 2.2.1. Cocoa

**2.2.1.1. Description and traditional uses.** In Mexico, cocoa is the main component of chocolate and other beverages. It is necessary to ferment the cocoa beans and dry the seeds to develop the aromatic compounds and to obtain its characteristic flavor (Fowler & Coutel, 2017). The word ‘cocoa’ comes from the Olmec word ‘*kakawa*’ (Kaufman & Justeson, 2007). In pre-Hispanic Mesoamerican cultures, cocoa was an important economic element and it was used in political and ritual contexts. The

Mayas and other pre-Hispanic groups used cocoa as a beverage to celebrate war victories, marriage, and ascension to power, among other events (Mathiowetz, 2019).

**2.2.1.2. The fermentation process and associated microorganisms.** Cocoa flavors develop after the fermentation and drying of the seeds. Many microorganisms are associated with this fermentation; they vary according to the growing region and depend on the utensils used for the process (Fowler & Coutel, 2017). The process begins with the harvesting of the pods; at this time, the cocoa is already naturally inoculated with various fungi and bacteria. The pods are broken and the seeds with pulp are placed in wooden boxes and covered with banana leaves. The microorganisms involved in cocoa fermentation are a complex mix comprising anaerobic yeast, LAB, and AAB, among others (Daniel et al., 2009; Jespersen, Nielsen, Hønholt, & Jakobsen, 2005).

During the fermentation process, cocoa bean pulp undergoes three types of fermentation: acetic, ethanolic, and lactic. The AAB, of which *Acetobacter pasteurianus* is the most prevalent species, carry out acetic fermentation. The desirable AAB oxidize ethanol produced by yeasts into acetic acid, and the lactic acid produced by LAB is oxidized into acetic acid and acetoin, after which the acetic acid is re-oxidized in carbon dioxide and water (De Vuyst & Weckx, 2016). Ethanolic fermentation is performed by anaerobic yeasts, which take the glucose originating from the hydrolysis of sucrose and produce ethanol, carbon dioxide, and glycerol; however, the yeast also produces organic acids and volatile compounds such as some higher alcohols, aldehydes, ketones, and fatty acid esters, thus contributing to the flavor of the cocoa. In the first 24 to 72 h, lactic fermentation is carried out by LAB, which ferment fructose into lactic acid, acetic acid, carbon dioxide, and ethanol, or reduce it to mannitol and *Enterobacteriaceae* are facultative anaerobic such as *Tatumella* species occur frequently but transiently (De

**Table 2**

Traditional fermented beverages and their described chemical and nutritional aspects.

Traditional name	Chemical parameters	Nutritional parameters	Reference
<b>Pulque</b>	Aguamiel: pH 5.4, °Brix 9.6 Pulque: pH 4.0, ethanol 4.36%, °Brix 7.8 Inoculum: pH3.78, °Brix 5.4 Bioactive compounds: folates, steroidol saponins, phytase, agavins	Moisture ~ 92%, Protein 2.71–6% Thiamin (10%), Riboflavin (24%) Niacin (23%), Ascorbic acid (48%) Calcium (8%), Iron (51%) Phosphorus, magnesium, zinc, copper and boron Essential amino acids (Ile, Leu, Lys, Phe, Thr, Trp, Val, His) Not-essential amino acids (Tyr and Cys)	Escalante et al., 2016; Giles-Gómez et al., 2016; Morales de León, Bourgues-Rodríguez, & Camacho-Parra, 2016; Torres-Maravilla & Blancas-Nápoles, 2016; Hernández-López et al., 2018; Cervantes-Elizarraras et al., 2019; Álvarez-Ríos et al., 2020; Capozzi et al., 2020; Escobar-Zepeda et al., 2020.
<b>Cocoa</b>	Very complex, will depend on the fermentation, drying and roasting Bioactive compounds: polyphenols, flavonol, caffeine, theophylline, theobromine	Very complex, will depend on the fermentation, drying and roasting	Murphy, Chronopoulos, Singh, Francis, Moriarty, Pike, Turner, Mann, & Sinclair, 2003; Keen, Holt, Oteiza, Fraga, & Schmitz, 2005; Nogueira et al. 2012; Patel et al., 2019; Singh et al., 2020.
<b>Pozol</b>	Iturin A Surfactin Fengycin Bacilysin Chlorotetaine	White Pozol (WP): Energy 1714 KJ Carbohydrates 84 g 100 g <sup>-1</sup> Protein 9.02 g 100 g <sup>-1</sup> Fiber 1.92 g 100 g <sup>-1</sup> Fat 3.81 g 100 g <sup>-1</sup> Pozol mixed with cocoa (PC): Energy 1849 KJ Carbohydrates 7 g 100 g <sup>-1</sup> Protein 10.2 g 100 g <sup>-1</sup> Fiber 3.0 g 100 g <sup>-1</sup> Fat 12.99 g 100 g <sup>-1</sup> Niacin, thiamin, riboflavin Calcium and fiber Low glycemic index	Méndez-Albores, Arámbula-Villa, Preciado-Ortíz, & Moreno-Martínez, 2004; Banjoko et al., 2012; Jenatton, 2017; Olvera et al., 2017; Trabaniño & Meléndez, 2017.
<b>Tejuino and tesúino</b>	pH < 5, ethanol 2–20% Bioactive compounds: Anthocyanins 9.8–9.45 mg Cyanidine 3-gluco-side 100 g <sup>-1</sup> Phenolic compounds: Gallic acid 100–150 mg 100 mL <sup>-1</sup> and ferulic acid 180–300 mg 100 mL <sup>-1</sup>	Protein 1.1 g 100 mL <sup>-1</sup> Fat 0.69 g 100 mL <sup>-1</sup> Niacin 0.36 g 100 mL <sup>-1</sup>	Cruz & Ulloa, 1973; Silva et al., 2017; Cejudo-Valentín et al., 2019.
<b>Sendechó</b>	pH 4.5–4.8, ethanol 1.6%	Not determined	Flores-Calderón et al., 2017;

**Table 2 (continued)**

Traditional name	Chemical parameters	Nutritional parameters	Reference
<b>Balché</b>		Lactic acid 0.18–0.26 g 100 mL <sup>-1</sup> Bioactive compounds: anthocyanins and phenolic compounds (polyphenols)	Gaxiola-Cuevas et al., 2017
<b>Taberna</b>	Ethanol 1–5%	Not determined	De Smet, 1983; Sotelo et al., 1995; Crane, 2001; Sancho et al., 2013; Carod-Artal, 2015; Quezada-Euán, 2018.
<b>Tepache and garapina</b>	pH 3.22–3.66, ethanol < 1% Lactic acid 0.15–0.45%	Protein 7–33 µg mL <sup>-1</sup> Calcium 353–453.8 mg 100 g <sup>-1</sup> Acetic acid 0.02 to 0.4% Bioactive compounds: Flavonoids, polyphenols, isoflavones, carotenoids, saponins, lignans, xanthin and lycopene	Santiago-Urbina et al., 2013; Coutino et al., 2020.
<b>Colonche</b>		Not determined	Moreno-Terrazas, 2005; de la Fuente-Salcido et al., 2015
<b>Guarapo</b>	Bioactive compounds: Hydroxycinnamic and hydroxybenzoic acids. Total phenolics content: (688.1 ± 34.3 mg of gallic acid equivalents mL <sup>-1</sup> ) Betalains 18.7 ± 0.8 mg L <sup>-1</sup> Organic acids (oxalic, tartaric, malic, lactic, acetic, and other unidentified acids)	Total sugars 104–110 mg mL <sup>-1</sup> Glucose 63.6–68.6 mg mL <sup>-1</sup> Protein 0.8–0.10 mg mL <sup>-1</sup> Ascorbic acid 3–3.22 mg mL <sup>-1</sup>	Navarrete-Bolaños et al., 2013; Verón et al., 2019
	Ethanol 3%, ~16 °Brix Organic acids 1–3% Carboxylic acids 1.1–3% Phenolic compounds: gallic acid, catechin, chlorogenic acid, caffeic acid, vanillin, p-coumaric acid, ferulic acid, m-coumaric, and o-coumaric. Volatile compounds (mainly esters) 3-methyl-1-butanol, 2-methyl-1-butanol,	Moisture 93% Ash 1.5–4.5% Sugars 75–92% (70–88, 2–4, and 2–4% of sucrose, glucose and fructose respectively) Protein 0.5–0.6% Amino acids 0.5–2.5%	Olarte, 2007; Armijos Espín, 2016; Resende Oliveira et al., 2018
		Minerals: potassium, phosphorus, calcium, sodium, magnesium,	

(continued on next page)

**Table 2** (continued)

Traditional name	Chemical parameters	Nutritional parameters	Reference
	2-methyl-1-propanol, 1-octanol, Benzene ethanol, 1,1-diethoxyethane	sulfur, iron, aluminum, copper, zinc, calcium, and iron	

Vuyst & Weckx, 2016). The most common LAB present during cocoa fermentation are *Lactobacillus cacaonum*, *Lactobacillus fabifermenans*, *Lactobacillus plantarum*, *Leuconostoc pseudomesenteroides*, *Fructobacillus pseudoficulneus*, and *Fructobacillus tropeaoli* (De Vuyst & Weckx, 2016).

AAB are involved throughout the entire cocoa fermentation processes (Romero-Cortes et al., 2013). Romero-Cortes et al. (2013) found *Acetobacter tropicalis* and *A. pasteurianus* during the fermentation of cocoa in Tabasco, in which *A. tropicalis* was the dominant bacteria. There are differences between countries and between farms regarding which bacteria are involved in fermentation, but the main AAB report worldwide is *A. pasteurianus*. The differences between microorganisms may be due to the different microorganisms present in the environment (Romero-Cortes, Robles-Olvera, Rodríguez-Jimenes, & Ramírez-Lepe, 2012). Some yeast involved in Mexican cocoa fermentation such as *Pseudozyma antarctica*, *Rhodosporidium fluviale*, and *Zygosaccharomyces bailii* have not been reported in cocoa fermentation in other countries (Arana-Sánchez et al., 2015). In contrast, *Candida diversa*, *Candida inconspicua*, *Candida sylvae*, *Candida zemplinina*, *Hanseniaspora opuntiae*, *Pichia membranifaciens*, *Pichia kudriavzevii*, and *Saccharomyces cerevisiae* are some of the yeasts present during cocoa fermentation in Mexico and other countries (Daniel et al., 2009; Nielsen et al., 2007; de Melo Pereira, Teixeira Magalhães, Gonzaga de Almeida, da Silva Coelho, & Freitas Schwam, 2013; Hamdouche et al., 2015; Sarbu & Csutak, 2019).

**2.2.1.3. Harmful compounds or pathogenic microorganisms.** The biogenic amines in cocoa could negatively affect susceptible patients receiving treatment with monoamine oxidase and diamine oxidase inhibitors because the amines can result in blushing, headaches, blood pressure variation, and cardiovascular shock depending on the quantity ingested (Watson, Preedy, & Zibadi, 2013). Several studies have indicated that cocoa products can be contaminated with lead and cadmium, where soil, pesticides, fertilizers, and other emissions are the source of contamination. It is worth mentioning that the amount of cadmium in the final product cannot exceed 2 mg kg<sup>-1</sup> and for lead the specification is <0.1 ppm according to U.S. FDA guidelines (Fowler & Coutel, 2017). The fermentation process decreases cadmium concentration in the final product (Vanderschueren et al., 2020); however, further study is needed regarding the effect of the fermentation process on cadmium concentration and on the quality of the final product, in order to balance both factors. Other potential contamination risks for cocoa are foreign matter, bacteria, allergens, dioxins and polychlorinated biphenyls, mineral oil hydrocarbons, mycotoxins, and pesticide residues (Ching Lik & Flávio, 2019). In the food industry, all types of contaminants are supervised and controlled in order to produce butter, liquor, and powdered cocoa that are safe for human consumption.

**2.2.1.4. Chemical and nutritional studies.** The chemical composition of cocoa is very complex, and it can change in the fermentation, drying, and roasting processes. There are even differences between varieties of cocoa (Mohamadi Alasti, Asefi, Maleki, & Sadegh SeifiedlouHeris, 2019). In cocoa beans, proteins represent about 60%, and the nonprotein nitrogen is composed of amino acids (0.3% amide and 0.02% ammonia), while free amino acids range from 5 to 25 mg gr<sup>-1</sup> fat-free dry matter in fermented cocoa beans. Additionally, total fat content is approximately 50%, 98% of which comprises neutral lipids (75% triglycerides) and 2% are made up of polar lipids (30% and 70% phospholipids and glycolipids, respectively). With respect to carbohydrates, cellulose represents

12% of the fermented/dried cocoa beans and 0.39% to 3.48% of the soluble carbohydrates (glucose, sucrose, raffinose, fructose, stachyose, and verbascose). In fermented beans, phenolic compounds make up 6% (Watson et al., 2013). The amount of polyphenols depends on the nonfat cocoa solids; in dark chocolate, the polyphenol concentrations are higher than in milk chocolate (Watson et al., 2013). Phenol concentration decreases with the roasting step due to high temperatures and long fermentation times. Organic acids in cocoa beans are the compounds that do not have nutritional properties; if the quantity of these is high, the beans may produce adverse health effects. Some of the most common organic acids are citric, oxalic, malic, acetic, and formic (14–33 gr kg<sup>-1</sup> in total) the composition of which depends on the maturation and fermentation of the cocoa beans. The geographical origin of the cocoa is another important factor (Watson et al., 2013). Cocoa beans also contain other bioactive compounds such as caffeine, theophylline, and theobromine (Peláez, Bardón, & Camasca, 2016); furthermore, cocoa contains a discrete amount of biogenic amines such as 2-phenylethylamine, tryptamine, and tyramine, which have vasoactive effects. It has also been reported that cocoa contains serotonin, which has positive neurological effects, and clovamide, which is an antioxidant with an effect similar to that of ascorbic acid (Watson et al., 2013). According to the composition tables for Mexican foods and food products, 100 g of bitter chocolate without sugar contains 519 kcal, 3.20 g water, 11.84 g crude protein, 36.24 g carbohydrates, and 4.36 g fiber; it also contains some minerals and vitamins (calcium, phosphorus, iron, sodium, potassium, magnesium, copper, zinc, manganese, thiamine, riboflavin, niacin, pyridoxine, and folic acid (Morales de León, Bourgues-Rodríguez, & Camacho-Parra, 2016).

**2.2.1.5. Evaluated functional effects.** Cocoa is a food that contains many compounds such as anti-inflammatory agents and antioxidants with bioactive effects for human health. The flavanol in cocoa can produce nitric oxide and improves arterial vasodilatation. In contrast, theobromine is considered an inhibitor of platelet aggregation (Patel, Jayswal, & Maitrey, 2019). Murphy et al. (2003) observed low aggregation and fewer platelet counts in a group that consumed a cocoa flavanol drink compared with a control group; thus, these compounds may have potential as cardioprotective agents. Some studies observed a reduction in systolic and diastolic blood pressure in a group of patients with stage-1 hypertension when they consumed chocolate with 70% cocoa for 4 weeks; this study suggests that cocoa can reduce blood pressure in stage-1 hypertension (Nogueira et al., 2012; Patel et al., 2019). The quantity of antioxidants present in dark chocolate can protect against aging (Keen, Holt, Oteiza, Fraga, & Schmitz, 2005). Other beneficial effects of dark chocolate are antioxidant protection, which is associated with endothelial (Singh, Agarwal, Agarwal, & Rachana, 2020) and vascular function and also shows effects in blood glucose levels (Patel et al., 2019).

### 2.3. Fermented beverages from *Zea mays*

#### 2.3.1. Pozol

**2.3.1.1. Description and traditional uses.** Pozol is a traditional pre-Hispanic beverage consumed by several communities in southern Mexico and in Guatemala. Its origin dates back to the Mayan culture and it is made from the fermentation of corn (*Zea mays L.*) (Ampe, Omar, Moizan, Wacher, & Guyot, 1999; Trabanino & Meléndez, 2017). Since pre-Hispanic times, pozol has been considered a source of nutrients and an essential food for the Mayan culture ingesting it as a refreshing daily drink, or in traditional ceremonies. Presently, it is consumed in Tabasco, Yucatan, Chiapas, Veracruz, Guerrero, Quintana Roo, and Oaxaca (Lasso & Trabanino, 2018). Additionally, its consumption in some regions was considered to have medicinal properties. For instance, the Lacandones used pozol with honey to reduce fever (Cruz & Ulloa, 1973; Wacher,

Cañas, Cook, Barzana, & Owens, 1993) and the Mayans used it to treat GI disorders, diarrhea, and skin infections (Díaz-Ruiz, Guyot, Ruiz-Teran, Morlon-Guyot, & Wacher, 2003; Pérez-Armendáriz & Cardoso-Ugarte, 2020).

**2.3.1.2. The fermentation process and associated microorganisms.** Pozol is a non-alcoholic fermented beverage that is still prepared as it was done in pre-Columbian times. Dry corn kernels are boiled in the presence of calcium oxide or lime. Cooking time varies according to the region, but generally, it varies from 0.5 to 3 h followed by a soaking period that lasts from 3 h to 14 h (Omar, Ampe, Raimbault, Guyot, & Tailliez, 2000; Cooper-Bribiesca et al., 2018). After boiling, the kernels are washed and de-shelled in order to obtain 'nixtamal,' an uniform dough which is homogeneously tied into small balls and stored in banana leaves until fermentation is complete, which may take a couple of hours or up to 30 days. Then, the dough is dissolved, mixed in water and drunk like any other beverage (Cruz & Ulloa, 1973; Méndez-Albores, Arámbula-Villa, Preciado-Ortíz, & Moreno-Martínez, 2004). Pozol can be mixed with batata dough, toasted cocoa, coconut, cinnamon, or vanilla (Jiménez Vera, Gonzalez Cortes, Magaña Contreras, & Corona Cruz, 2010; Velázquez-López, Covatzin-Jirón, Toledo-Meza, & Vela-Gutiérrez, 2018; Ramírez-Guzmán et al. 2019).

The fermentation increases the proliferation of many bacteria, fungi, or yeasts; as soon it occurs, the pH in the dough is reduced drastically from 7.3 to 3–4 to complete the fermentation (Wacher et al., 1993). Several bacteria have been identified in pozol such as *Streptococcus* species, which dominate the entire fermentation process because they make up 25–75% of the total bacteria. Fermentation also increases LAB, especially amylolytic LAB such as *Lactobacillus fermentum*, *L. casei*, *L. plantarum*, *Leuconostoc* sp. *Bifidobacteria*, and *Enterobacteria* (Omar et al., 2000). AAB such as *Acetobacter* spp. and fungi such as *Aspergillus* genus are present (Cárdenas, Barkla, Wacher, Delgado-Olivares, & Rodríguez-Sanjoa, 2014). Other microorganisms have been identified, and these are listed in Table 1 (Díaz-Ruiz, Guyot, Ruiz-Teran, Morlon-Guyot, & Wacher, 2003).

**2.3.1.3. Harmful compounds or pathogenic microorganisms.** There is no evidence concerning pozol toxicity, but the significant risk of maize-based food is the presence of mycotoxins because some fungi such as *Fusarium*, *Penicillium*, or *Aspergillus* frequently infect corn. The toxic metabolites are aflatoxins produced by fungi such as *Aspergillus* spp. A study from pozol in southern Mexico showed aflatoxin presence in 19 of 111 samples. The levels were too low in white pozol samples, and only one sample mixed with cocoa had high levels. Five point four percent of the samples were contaminated with aflatoxin B2 and traces of aflatoxin AB1. Lower values were attributed to the nixtamalization process, which eliminates aflatoxins very efficiently (Méndez-Albores, Arámbula-Villa, Preciado-Ortíz, & Moreno-Martínez, 2004).

**2.3.1.4. Chemical and nutritional studies.** The nutritional content of pozol has yet to be established. Despite the fact that nixtamalization reduces simple carbohydrate content, fermentation increases production of polysaccharides, oligosaccharides, and maltooligosaccharides (Rizo et al., 2020). Also, there is evidence that pozol contains proteins from different sources such as plants, fungi, yeasts, and high-quality bacteria, as well as some antimicrobial molecules, fiber, antioxidants, and minerals; however, the full chemical characterization has not been determined (Cárdenas et al., 2014). Nixtamalization induces the release of molecules such as niacin, it increases soluble fiber and free calcium, and it makes proteins available that were previously indigestible. Several authors have suggested that the carbohydrates present in pozol have a high nutritional value and a low glycemic index, since the sugar is delivered and digested slowly (Bressani, 1990; Milán-Carrillo, Gutiérrez-Dorado, Cuevas-Rodríguez, Sánchez-Magaña, & Reyes-Moreno, 2017).

The nutrient content of pozol may change depending on the maize

variety and the production process, or on added products such as cocoa. Jenatton (2017) determined the nutrient value of white pozol (WP) and pozol mixed with cocoa (PC); values are shown in Table 2.

**2.3.1.5. Evaluated health effects.** The beneficial effects of pozol may be due to the high bacteria concentration or to the presence of fungi; they can produce molecules that induce antibacterial, analgesic, antipyretic, antiemetic, and antioxidant effects (Cruz & Ulloa, 1973). From Mexican pozol, Ray, Sanchez, O'Sullivan, and McKay (2000) isolated the bacterial strain CS93, which belongs to a *Bacillus* genus, observing that CS93 has a wide array of antibacterial, bacteriostatic and bactericidal molecules such as Iturin A. They also found the presence of bacilysin and chlorotetaine which are known as antimicrobial peptides (Yu, Sinclair, Hartman, & Bertagnoli, 2002; Phister, O'Sullivan, & McKay, 2004; Liu, Zhao, Wang, & Wu, 2020). Subsequent studies confirmed that *Bacillus subtilis* isolated from pozol produces other peptides with antibacterial properties such as surfactin and fengycin (Wacher et al., 2000; Moran, Robertson, Paradisi, Rai, & Murphy, 2010; Wacher-Rodarte et al., 2016).

The isolation of bacteria from pozol, for example, *L. citreum*, produced inulin from sucrose metabolism. Inulin can be used directly as soluble fiber or chemically hydrolyzed to produce fructooligosaccharides with possible prebiotic use (Olvera et al., 2017). However, there are no clinical trials with this beverage that support prebiotic or probiotic effects.

In an experiment conducted by Banjoko et al. (2012) in rats to induce hyperlipidemia by feeding them with a high-fat diet, the authors observed that this diet supplemented with fermented maize (by the addition of *Bifidobacterium bifidum* and *L. acidophilus*) prevented dyslipidemia. Animals supplemented with a diet of fermented maize exhibited inhibition of fat absorption, most likely as a consequence of LAB consumption. In another study, Muñoz Cano, Carrillo Aguilar, and Córdova Hernández (2013) included rats in order to analyze the influence of a traditional maize diet on metabolic syndrome. The authors induced metabolic syndrome by the addition of 30% sucrose in the drinking water; the animals were then fed with a tortilla-based diet or a pozol-based diet and followed for 28 weeks. After this period, there were no differences in food or water (fructose) consumption. The maize-tortilla and pozol feeding significantly reduced triglyceride levels after 8 to 24 weeks with respect to the metabolic syndrome rats fed with a regular diet. Glucose levels did not vary among the groups; however, it was observed that tortilla feeding significantly reduces glycosylated hemoglobin, low-density lipoprotein cholesterol, and alanine aminotransferase levels with respect to the metabolic syndrome animals, showing that maize tortillas or traditional pozol foods protect the liver from the high intake of fructose in metabolic syndrome. Finally, a study was conducted in Chiapas, Mexico, in which the objective was to determine the antioxidant capacity of daily foods. It was observed that pozol with cocoa has antioxidant capability (7.7 mmol Trolox g<sup>-1</sup>). Although this value is below that obtained in foods rich in antioxidants such as guava, it still is considered a health adjuvant by local people in southern Mexico, not only due to its antioxidant status but also as an energy source, and even as regular medicine (Gutiérrez Zavala, Ledesma Rivero, García García, & Grajales Castillejos, 2007; Trabanino & Meléndez, 2017).

There is no clinical evidence that pozol has been studied as a functional beverage, but it would be a good candidate, due to the presence of multiple bioactive metabolites or microorganisms that could have an impact on health and a potential use in the food and pharmaceutical industry.

### 2.3.2. Tesgüino or tejuino

**2.3.2.1. Description and traditional uses.** Tesgüino or tejuino is an alcoholic fermented beverage still produced and consumed by the Tarahumara and other groups in northern Mexico (Kennedy, 1963; Novillo-

Verdugo, 2015; Ramírez-Guzmán et al., 2019). However, it is documented that this beverage was consumed by the Pimas, Yaquis, Tepehuano, and Huicholes in pre-Columbian times (Cruz & Ulloa, 1973).

This beverage is known as Mexican beer or maize beer, and its production dates back to centuries ago. Tesgüino is called '*batári*' or '*pacikí*' by the Tarahumaras, who place high socioeconomic and religious importance on the drink. The Tarahumara used to consume it in religious celebrations such as baptisms, weddings, funerals, and other non-Christian events such as beer parties called '*tesgüinadas*', where nearly the entire town participates. Tesgüino is always made by children or women in all communities. It is considered a marker of social status because the man with the highest rank and social importance drinks it first (Kennedy, 1963; Herrera & Ulloa, 1973).

**2.3.2.2. The fermentation process and associated microorganisms.** As mentioned above, tesgüino is prepared by a traditional method, from dry corn kernels (it is possible to use corn stem) that are soaked for 24 to 48 h and left to sprout for five days in a dark place. This step is fundamental in order to avoid the greening of the shoots and obtain the desired flavor. After germination, the kernels or corn stem are ground and boiled for 12 h after being cooled and strained to obtain yellow atole. The atole is placed in an '*olla tesgüinera*' which is a traditional cooking pot; in this step, sugar or '*piloncillo*' (smoky, caramelly, and earthy sugar) is added. Some catalysts are then added for a fermentation of 24 h or even 72 h. The catalysts are commonly endemic plants such as '*ronínawa*' (*Stevia serrata* Cav), '*rojiswi*' (*Chimaphila maculata*), '*ubitukwari*' (*Datura meteloides* Dunal), '*camomila*' (*Hamaemelum nobile*), and '*frijolillo*' (*Cojoba arborea*). In some parts of Chihuahua, tesgüino is mixed with hallucinogenic plants such as 'peyotl' (*Lophophora williamsii*) (Kennedy, 1963; Cruz & Ulloa, 1973; Herrera & Ulloa, 1973). In Colima and Jalisco, it is simply mixed with '*piloncillo*' in order to obtain a beverage with a low alcohol content called tejuno (Nava Arenas, 2009).

LAB, AAB, and yeasts regulate the fermentation process. Some microorganisms have been isolated, such as *Cryptococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Streptococcus* spp., *S. cerevisiae* (Cruz & Ulloa, 1973; Nava Arenas, 2009; Cabezas Elizondo, 2016), *Candida guilliermondii*, and *Saccharomyces kluyveri* (Cruz & Ulloa, 1973; Paredes-Lopez, Guevara Lara, & Bello Pérez, 2006). The isolation of *Pantoea anthropophila* showed its capacity to produce galactooligosaccharides (GOS), which have shown prebiotic activities in clinical trials (Yañez-Ñeco, Rodriguez-Colinas, Amaya-Delgado, Ballesteros, Gschaeffler, Plou, & Arrizon, 2017; Plata, Cruz, Cervantes, & Ramírez, 2019).

**2.3.2.3. Harmful compounds or pathogenic microorganisms.** Except for alcohol content, there is no evidence that tesgüino is a toxic beverage; laboratory production showed the presence of non-toxic compounds (Cejudo-Valentín et al., 2019).

**2.3.2.4. Chemical and nutritional studies.** Tesgüino is a viscous, yellowish beverage with a sour flavor and a pH ranging from 2 to 5. Nutritional content is reported in Table 2 (Cruz & Ulloa, 1973). The alcohol percentage range is 2–20% due to differences in corn variety and the bacteria and yeasts present (Nava Arenas, 2009; Cejudo-Valentín et al., 2019). It is well known that nixtamalization reduces the protein and carbohydrate content in corn, and it has been reported that anthocyanin and phenolic compounds are reduced in corn dough after boiling, due to high temperatures (Bressani, 1990; Gaxiola-Cuevas et al., 2017). Nava Arenas (2009) determined the polyphenols and anthocyanin concentrations in tesgüino, reporting that the polyphenols, expressed as gallic acid units, were reduced by 42–54% during boiling, increasing slightly after fermentation back up to 74%. Ferulic acid has a similar pattern as that of polyphenols during cooking, but after fermentation, the levels increase radically (see Table 2). Finally, the anthocyanins were drastically reduced during boiling, by 80%, in tesgüino production.

**2.3.2.5. Evaluated health effects.** There are no functional effects of tesgüino described in humans; however, *in vitro* studies show that isolated bacteria such as *Weissella cibaria* and *L. citreum* could potentially be used as probiotics and inhibits pathogenic bacteria. It has also been observed that *L. citreum* improves the colonization of beneficial bacteria in the digestive tract (Silva et al., 2017; Pérez-Armendáriz & Cardoso-Ugarte, 2020). The main limitation of using tesgüino as a functional food is the alcohol content, because in excess it will cause alcohol intoxication; however, the presence of bacteria, yeasts, and some biological molecules could lead to its being considered a beverage with nutraceutical importance.

### 2.3.3. Sendechó

**2.3.3.1. Description and traditional uses.** This is a beverage made from malt-pigmented corn and chili peppers. It is fermented with pulque and its color can be white or red, but the flavor is always soft, sweet, and slightly acidic (Unai, 2019). It is known by the name of sendechó or zeyrecha, and it has been considered a beer since the pre-Hispanic era. This beverage was used mainly by the Otomies and the Mazahuas in their religious celebrations (Génin, 1924).

**2.3.3.2. The fermentation process and associated microorganisms.** Corn kernels are placed in a natural basket with leaves of *Buddleja americana* or '*teposan*', exposed to the sun, and continuously soaked for 4–5 days in order to germinate the grains. Subsequently, the germinated grains are dried and ground with chili pepper (*Capsicum* sp.). The result is a flour, which is cooked in a clay pot with water. Once this mixture is cool, the starter inoculum which is called '*ixquini*' (a portion of previously produced sendechó or pulque) is added, producing a sour beverage called '*cité*' (Lorence-Quiñones, Wacher-Rodarte, & Quintero-Ramírez, 1999; Godoy et al., 2003). Therefore many of the microorganisms that have been described in the fermentation process of pulque are most likely present in sendechó such as *Acetobacter* spp., *Candida* spp., *Lactobacillus* sp., *Leuconostoc* spp., *Kluyveromyces* spp., *S. cerevisiae*, and *Z. mobilis* among others (Torres-Rodríguez et al., 2014; González-Vázquez et al., 2015; Álvarez-Ríos et al., 2020). However, no studies have been specifically conducted regarding the microorganisms associated with sendechó. Recently, a new fermentation process for recovery of sendechó was described by a group in Mexico through the addition of hops and brewer's yeast, in combination with traditional ingredients, i.e. guajillo chili (*Capsicum annuum*) and blue corn malt, in order to develop a new type of sendechó, employing the ale fermentation process to produce different types of corn beer (Flores-Calderón, Luna, Escalona-Buendía, & Verde-Calvo, 2017).

**2.3.3.3. Chemical and nutritional studies.** Flores-Calderón et al. (2017) compared eight styles that resulted from the different combinations of three ingredients (a type of malt, hops, and guajillo chili pepper). The authors determined total reducing sugars, alcohol, pH, total acidity, anthocyanins, polyphenols, and antioxidant activity. They observed a significant difference in total reducing sugars for each style. Mean ethanol content produced in green and mature beers was 1.6% alcohol v<sup>-1</sup>; pH values were 4.5–4.8, total acidity in mature beers was found in the range of 0.18–0.26 g 100 mL<sup>-1</sup> of lactic acid, which is associated with the formation of acetic, formic, pyruvic, malic, citric, and lactic acids. Finally, the authors reported a high anthocyanin and phenolic compound content, which is associated with antioxidant activity. In sendechó, as in the leaves of *B. americana*, the presence of flavonoids and glycosides such as aucubin iridoid and nigrone iridoid has been described (Cáceres et al., 1993; Juárez-Vázquez et al., 2013). In *C. annuum*, capsaicinoid (capsaicin, dihydrocapsaicin, nordihydrocapsaicin), carotenoid, thiamine, and ascorbic acid content has been reported (Sanati, Razavi, & Hosseinzadeh, 2018). To date, there are no nutritional studies of sendechó, but there have been many nutritional

studies of the ingredients used in order to make this beverage.

**2.3.3.4. Evaluated health effects.** Until now, sendechó has only been reported to have antioxidant effects, which are mainly attributable to the synergistic effects of antioxidants from blue corn (polyphenols, anthocyanins, along with p-coumaric, vanillic, and protocatechuic acids), caramel malt (anthocyanins and melanoidins), chili peppers (flavonoids), and hops (prenylated chalcones), which showed considerable levels of antioxidant capacity using ABTS<sup>•</sup> (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH<sup>•</sup> assays (Flores-Calderón et al., 2017).

#### 2.4. Fermented beverages made from *Lonchocarpus* spp.

##### 2.4.1. Balché or pitarrilla

**2.4.1.1. Description and traditional uses.** *Lonchocarpus* spp. is mainly used as an ornamental plant, for traditional medicine, and for the preparation of an alcoholic beverage called balché. This is a fermented and sacred drink made from the bark of *Lonchocarpus longistylus*, *Lonchocarpus punctatus*, *Lonchocarpus violaceus* (synonymous), and *Lonchocarpus yucatanensis* soaked in 'virgin' water with honey. The 'virgin' water is taken from a cenote. Balché tastes sweet, has a pale pink color, and has been mainly used by Maya and Lacandon peoples since pre-Hispanic times for agricultural, domestic, and ceremonial uses (Bernard Menna & Lozano Cortés, 2003; Aguirre-Dugua, Pérez-Negrón, & Casas, 2013; Cox-Tamay, Yamasaki, & Heredia-Campos, 2016; CONABIO, 2020).

**2.4.1.2. The fermentation process and associated microorganisms.** A superficial hole is made manually in the tree bark: inside of it, the virgin water and honey are mixed, then the hole and tree bark are sealed with palm or banana leaves and left to ferment spontaneously. The earliest ethnographic records indicate that the solution is normally meant to be fermented by *S. cerevisiae* for two to three days before it is ready for consumption (Menezes, Neto, Contrera, & Venturieri, 2013; Dozier, 2016). Balché has a low alcohol content (1% to 5% ethanol), and occasionally other ingredients are added (sugarcane, pineapple juice, vanilla, chili pods, and flowers) after the fermentation process is complete (Bernard Menna & Lozano Cortés, 2003). Until now, apart from *S. cerevisiae*, the presence of other microorganisms associated with the fermentation process has not been described.

**2.4.1.3. Harmful compounds or pathogenic microorganisms.** Balché consumption has been associated with psychoactive effects (alkaloids) (Lopez-Maldonado, 2005; Carod-Artal, 2015) such as euphoric consciousness, acute perception, and muscle relaxation, and sometimes it can cause narcotic and analgesic effects. Sometimes the beverage may also produce a mildly psychedelic effect, particularly when a large amount of fresh bark has been used (Rätsch, 1985).

**2.4.1.4. Chemical and nutritional studies.** The alcohol concentration in Balché is very low. The presence of alkaloids has been reported (Quezada-Euán, 2018), mainly nicotine, quinine, caffeine, and morphine (Carod-Artal, 2015; Crane, 2001). In addition, the presence of flavonoids in the bark of *L. yucatanensis* has been described (Borges-Argáez et al., 2017). Studies have been conducted in Brazil on the honey used in this drink by enzymatic assays, chromatographic techniques, and capillary electrophoresis procedures associated with the content of aromatic compounds such as *L*-malic, citric, and *D*-gluconic acids (Sancho, Mato, Huidobro, Fernández-Muiño, & Pascual, 2013).

To date, there are no nutritional studies on balché, but the nutritional characteristics of the seeds and pods of *L. longistylus* show high fiber, amino acid (lysine and tryptophan), and mineral (potassium, iron, and calcium) content compared to other wild legumes from the Yucatan Peninsula (Sotelo, Contreras, & Flores, 1995).

**2.4.1.5. Evaluated functional effects.** Several anthropological or ethno-botanical studies indicate that the leaves of *L. longistylus* are used as a treatment for asthma and headaches, and as an antitussive agent. The beverage has also been associated with antiparasitic effects, certain laxative and antibacterial effects, and finally, as a narcotic effects when used in a balché extract (Crane, 2001; Carod-Artal, 2015; Knowlton & Yam, 2019). Some ingredients that are present in the bark or added during production have exhibited some interesting properties. For example, the resin of this plant is used to treat stomach aches (De Smet, 1983; Crane, 2001) and Maya honey (from *Melipona beecheii*) has antioxidant and antimicrobial properties (Cauich Kumul, Ruiz Ruiz, Ortíz Vázquez, & Segura Campos, 2015; Zamora et al., 2017; Quezada-Euán, 2018).

#### 2.5. Fermented beverages made from *Bromelia* spp.

##### 2.5.1. Huapilla water

**2.5.1.1. Description and traditional uses.** In Mexico, there are six species of the genus *Bromelia* (Mondragón Chaparro, Ramírez Morillo, Flores Cruz, & García Franco, 2011). The products derived from *Bromelia* spp. have mainly been used by the Aztecs, Mayas, Incas, Quechuas, Yanomami, and other peoples for manufacturing living fences, fishing nets and ropes, and ornamental plants. These plants have also been used in traditional medicine and food, including fermented and unfermented beverages using the fruit from *Bromelia alsodes*, *Bromelia hemisphaerica*, *Bromelia karatas*, *Bromelia pinguin*, *Bromelia chrysanthia*, and *Bromelia nidus-puellae*, the first four of which are endemic to Mexico (Morales & Berrones-Benítez, 2007; Mondragón Chaparro et al., 2011; CONABIO, 2020), where the fruits are usually employed in the production of huapilla or piñuela, a refreshing fermented beverage made from *B. karatas* and *B. pinguin* that is characteristic of Tamaulipas and northern Veracruz (Arzaba-Villalba, Chazaro, & Torres, 2018; CONABIO, 2020; Espejo-Serna, Lopéz-Ferrari, & Ramírez-Morillo, 2010), and the production of the fruits is mainly carried out from May to November (Pío-León et al., 2009).

**2.5.1.2. The fermentation process and associated microorganisms.** The process of fermentation is carried out by placing fermenting *Bromelia* fruit peels in water with 'piloncillo' in wooden barrels in order to induce spontaneous fermentation for 10–15 days, without a pre-inoculum (Godoy et al., 2003; Romero-Luna, Hernández-Sánchez, & Dávila-Ortiz, 2017). After the fermentation process, the beverage is ready for consumption. Huapilla water is usually sold in open-air markets and stored in wooden barrels (Morales & Berrones-Benítez, 2007). To date, the microorganisms associated with the fermentation process during huapilla water production have not been identified.

The consumption of fresh *B. karatas* and *B. pinguin* fruits has been associated with a burning sensation in the mouth due to the presence of cysteine proteases (similar to bromelain or pinguinain) (Abreu Payrol, Miranda Martínez, Toledo Carrabeo, & Castillo García, 2001; Meza-Espinoza et al., 2017). Harmful compounds or pathogenic microorganisms have not been reported.

**2.5.1.3. Chemical and nutritional studies.** Producers of huapilla water consider it a low-alcohol beverage; however, there are no reports regarding the drink. At the present time, no physicochemical or nutritional properties of the beverage have been described, but it has been reported that the fruits of *B. karatas* and *B. pinguin* have excellent nutritional properties. These fruits are a very good source of vitamin C, calcium, magnesium, phosphorus, manganese, zinc, and fiber, as well as an important qualitative detection of bioactive compounds (tannins, flavonoids, terpenes, saponins, coumarins, and fatty acids) (Pío-León et al., 2009; Meza-Espinoza et al., 2017).

**2.5.1.4. Evaluated functional effects.** To date, there are no scientific studies that describe the health effects of huapilla water. The only reports that have been published are associated with the health properties of the extract, juice, or fresh *Bromelia* fruit (Meza-Espinoza et al., 2017). For example, the boiled fruits or infusions have been used in the treatment of respiratory tract disorders (Argueta Villamar, Cano Asseleih, Rodarte, & Gallardo Vázquez, 1994), scurvy (Chan-Quijano, Pat-Canché, & Saragos-Méndez, 2013), and urinary tract disorders (Manetti, Delaporte, & Laverde, 2009). Díaz Camacho, Delgado Vargas, Parra Inzuna, and Cristerna González (2013), in a patent for a method of preparation of beverages containing *Bromelia* fruit (*B. pinguin*), indicated that this fruit provides functional properties associated with the prevention of diseases such as the flu, whooping cough, parasites, and others. It is also known that the juice of this fruit has been used as an anthelmintic and an antibacterial agent (Abreu Payrol et al., 2001; Pío-León et al., 2009). The cysteine proteases described in bromelia fruits have been reported to have anti-fungal, anti-inflammatory, anti-thrombotic, anti-fibrotic, and anti-asthmatic properties (Camacho-Hernández, Chávez-Velázquez, Uribe-Beltrán, Ríos-Morgan, & Delgado-Vargas, 2002; Meza-Espinoza et al., 2017). These cysteine proteases may have potential utility in the brewing, detergent, food, and pharmaceutical industries (Meza-Espinoza et al., 2017; Meza-Espinoza et al., 2018).

## 2.6. Fermented beverages made from *Acrocomia* spp.

### 2.6.1. Taberna

**2.6.1.1. Description and traditional uses.** The palm wine known as ‘taberna’ is produced by the natural fermentation of the sap obtained from a palm tree ‘coyol’ or ‘cocoyul’ (*Acrocomia aculeata* or *Acrocomia vinifera*). These are plants of the *Arecaceae* family, native to some tropical regions of the Americas. Taberna is fresh, sweet, and effervescent, with a thick consistency (Coutiño et al., 2020). It is an alcoholic beverage produced in small villages in southern Mexico and other places in Central America (Balick, 1990). It was used by the Mayans as a part of their rituals (Doemel, 2013), but there is no information regarding the origins of taberna production (Gómez Toledo, 2014).

For the production of taberna, palm trees 10–15 years old are needed. They are cut and the working area is cleaned. The workers carefully cut the layers of the sheath base until the portion of the palmito is evident; they never cut the palmito, which is the heart of the palm. A rectangular cut is made, about 15 cm long × 10 cm wide × 10 cm deep. Then the cut is covered with plastic or a piece of wood to prevent the entry of contaminants or insects; the next day the harvest begins: The sap is collected twice per day, usually in the early morning and the late afternoon (Balick, 1990).

**2.6.1.2. The fermentation process and associated microorganisms.** Production of taberna is done under non-sterile conditions; thus, the microorganisms that participate involved in fermentation are present in the environment. There are many microorganisms involved, but little is known about them. Some of the bacteria reported to be involved in taberna production are *A. pasteurianus*, *Citrobacter freundii*, *Enterobacter hormaechei*, *Fructobacillus durionis*, *Klebsiella pneumoniae*, *Pantoea agglomerans*, *Sphingomonas dokdonensis*, *Z. mobilis*, and some *Lactobacillus* spp. Alcántara-Hernández et al. (2010) suggested that bacterial diversity decreases during the fermentation process. Santiago-Urbina, Peña-Montes, Nolasco-Cancino, and Ruiz-Terán (2016), using the PCR-DGGE technique, found a predominance of *Candida tropicalis*, *Hanseniaspora guilliermondii*, *Kazachstania exigua*, *P. kudriavzevii*, and *S. cerevisiae* in different batches of taberna. Other yeasts described in the taberna production process are *Meyerozyma guilliermondii*, *Pichia kluyveri*, and *Kazachstania unispora* (Balick, 1990). However, the microorganism population may change depending on the environment, the temperature, and even the region of production (Table 1).

**2.6.1.3. Harmful compounds or pathogenic microorganisms.** Currently there are no toxicological studies, and no harmful compounds or pathogenic microorganisms have been reported for taberna.

**2.6.1.4. Chemical and nutritional studies.** Ramos, Ramos Filho, Hiane, Braga Neto, and Siqueira (2008) analyzed the composition of *A. aculeata* and found that β-carotene (49 mg g<sup>-1</sup>) makes up 82% of the total carotenoids and that it is more bioavailable than pure β-carotene; the authors also found γ-carotene, β-cryptoxanthin, *cis*-lycopene, and *cis*-flavoxanthin. The composition of sucrose in *A. aculeata* at the beginning of the process is 11.36%, but after 15 days of tapping it is 0.22%, due to the growth of the microorganism. The initial pH of the palm sap is neutral and it decreases due to the production of organic acids during fermentation; two main organic acids are produced: lactic acid (0.26% to 0.48%) which decreases pH levels to 3, and acetic acid (0.02% to 0.4%) (Santiago-Urbina, Verdugo-Valdez, & Ruiz-Terán, 2013). There are different studies on the amount of ethanol present in taberna. Santiago-Urbina et al. (2013) reported an average of 4.25% in the different samples studied while Amoa-Awua, Sampson, and Tano-Debrah (2007) reported 6% ethanol and Coutiño et al. (2020) reported 7.06% and 10.31%. The difference in ethanol concentration is most likely a result of several factors, depending on the microorganisms present in the beverage, time of collection, and temperature. The protein content in taberna is around 7–33 µg mL<sup>-1</sup> and it contains many minerals in addition to calcium, potassium, sodium, magnesium, zinc, and iron (Coutiño et al., 2020).

**2.6.1.5. Evaluated functional effects.** The bioactive or phytochemical substances, minerals, and *Lactobacillus* spp. present in taberna seem to produce beneficial effects on human health. Traditionally, people associate taberna drinking with a healthy GI tract (Coutiño et al., 2020). One of the bioactive peptides produced by *Lactobacillus bulgaricus*, *L. acidophilus*, *L. casei*, and *L. plantarum* is melatonin, which acts on sleep and reproductive behavior and controls immunity, inflammation, and carcinogenesis in mammalian cells (Tan et al., 2014).

In taberna we can find bioactive substances such as flavonoids, polyphenols, isoflavones, carotenoids, saponins, and lignans; these compounds exert a protective effect on the cardiocirculatory system, regulate blood glucose, and stimulate the immune system (Coutiño et al., 2020). However, there are few studies regarding the benefits of taberna on human health. To this end, further study is needed regarding the bioactive compounds it contains and their respective quantities.

## 2.7. Fermented beverages made from *Ananas comosus*

### 2.7.1. Tepache and Garapiña

**2.7.1.1. Description and traditional uses.** *Ananas comosus* belongs to the *Bromeliaceae* family, which is widely distributed in the Neotropics, Mexico, Brazil, and the Amazon, from Guayanas to northern Argentina, as well as in Africa and Asia. This fruit is consumed fresh and canned, but also in the form of juice, yogurt, ice cream, and jam. Vinegar and refreshing beverages such as garapiña and tepache are produced from its juice (CONABIO, 2020).

Tepache and garapiña are alcoholic beverages. Garapiña is made from pineapple pulp and peel while tepache is usually made from pineapple peel only, but it can also be made from other fruits such as apple, orange, guava, and tamarind (Herrera & Ulloa, 1973, 1982). Both beverages have been consumed since pre-Hispanic times and the etymology of the name is unclear. Some authors report that the name derives from the indigenous Nahuatl language: ‘tépiatl’ or ‘tepiatzin’ means water or beverage from maize, a variety named ‘tépitl’ (Herrera & Ulloa, 1982). Tepache and garapiña have a low alcohol concentration and are consumed principally in Mexico City; however, variants can be found and consumed in the Mexican states of Hidalgo, Puebla, Morelos, San

Luis Potosí, Oaxaca, Jalisco, and Nayarit (Ramírez-Guzmán et al., 2019).

**2.7.1.2. The fermentation process and associated microorganisms.** The process of fermentation varies according to the place of preparation, but currently the beverage is produced by placing pineapple peels and brown sugar in water to induce fermentation, which takes place without a pre-inoculum. The ingredients are placed in wooden barrels called 'tepacheras' (Godoy et al., 2003) where the pineapple rind and pulp are spontaneously fermented for 3 days (Romero-Luna et al., 2017). After that, a sweet, refreshing, and pleasant-tasting beverage is obtained. If the fermentation is prolonged, the drink acquires an unpleasant taste due to the acetic acid generated in the process (Herrera & Ulloa, 1982; Moreno-Terrazas, 2005; Corona-González et al., 2013). It has been reported that some of the microorganisms involved in fermentation are yeasts; Moreno-Terrazas (2005) described *Candida intermedia*, *Candida pseudointermedia*, *Candida sake*, *Candida sorbosa*, *Kloeckera africana*, *Kloeckera apiculata*, *Kloeckera japonica*, and *Saccharomyces bayanus* for the first time in different samples of tepache. Other yeasts isolated from tepache are *Candida queretana*, *C. inconspicua*, *Candida boidinii*, *S. cerevisiae*, and *Pichia membranafaciens* (Mapes & Basurto, 2016). With regard to the yeast used in industrial fermentation, the entire yeast population belongs to the *Saccharomyces* genus; however, findings are different in homemade tepache, where 95% of the yeasts belong to the *Hanseniaspora* genus (Rodríguez-González & Muro-Medina, 2011). Other microorganisms present during the fermentation process are LAB such as *Enterococcus faecium*, *Lactobacillus curvatus*, *L. plantarum*, *L. acidophilus*, *L. salivarius*, *L. mesenteroides*, *Lactococcus lactis*, and *Weissella confusa* (Moreno-Terrazas, 2005). There are some LAB present, such as *Lactobacillus pentosus*, only in standardized conditions versus traditional fermentation processes (Escobar-Zepeda et al., 2020). The AAB identified during tepache production are *Gluconobacter* spp. and *Acetobacter* spp. (Moreno-Terrazas, 2005).

In traditional tepache production, there is another method involving 'tibicos' which are macro-colonies of bacteria and yeast in symbiotic association (Moinas, Horisberger, & Bauer, 1980). Tibicos have been used in Mexico to produce refreshing beverages with a low alcohol content. In Europe, tibicos are used in order to produce sweet kefir or ginger beer (Hesseltine, 1965; Pidoux, 1989). Some of the microorganisms described in tibicos are *Saccharomyces radaiisi*, *Bacillus mexicanus*, *B. subtilis*, *Bacillus graveolens*, *E. coli*, *Proteus vulgaris*, *P. membranifaciens*, and *S. cerevisiae* (López Rojo, 2016). The composition of tibicos can vary depending on each product (kefir, tepache, ginger beer, etc.) and it is important to mention the isolation of probiotic microorganisms present in these macrocolonies (Zanirati et al., 2015; Romero-Luna, Peredo-Lovillo, Hernández-Mendoza, Hernández-Sánchez, Cauich-Sánchez, Ribas-Aparicio, & Dávila-Ortiz, 2020).

**2.7.1.3. Harmful compounds or pathogenic microorganisms.** Moreno-Terrazas (2005) reported the presence of *Enterobacter aerogenes*, *E. coli*, and *Klebsiella* spp. in different batches of tepache. It should be noted that *E. coli* is an indicator of poor sanitary quality in tepache production, but this bacteria was present only in one sample.

**2.7.1.4. Chemical and nutritional studies.** Currently there is little chemical information available regarding tepache. Ethanol content is less than 1%, pH values are around 3.22–3.66, lactic acid 0.15–0.45%, and acetic acid 0.021–0.043% (Moreno-Terrazas, 2005). Further study is needed with respect to the nutritional properties of tepache, since there are no published studies of this kind.

**2.7.1.5. Evaluated functional effects.** There is also little information available on the beneficial effects of this beverage. Some studies report the existence of probiotic microorganisms that promote human health due to the production of functional oligosaccharides, which help to prevent GI diseases and colon cancer (Martínez-Cervantes, Wong-Paz,

Aguilar-Zárate, & Muñiz-Márquez, 2019). *L. mesenteroides* has been isolated in tepache and it is reported to have prebiotic properties. Moreover, *L. lactis* and *E. faecium*, which are present in tepache, produce bacteriocins (nisin and enterocin) that may act as natural antimicrobial agents (de la Fuente-Salcido et al., 2015). More research is needed in order to determine whether or not tepache has health-promoting effects.

## 2.8. Fermented beverages made from *Opuntia streptacantha*

### 2.8.1. Colonche

**2.8.1.1. Description and traditional uses.** Plants of the genus *Opuntia* are the most abundant group of the *Cactaceae* family, currently spreading throughout the Americas, Europe, Asia, Africa, and Australia (Ojeda-Linares, Vallejo, Lappe-Oliveras, & Casas, 2020). Prickly pear is usually consumed fresh. To increase its shelf life and commercial value, the artisanal processes have been used, leading to unique products such as prickly pear cheese, candies (jelly and *melcocha*), and prickly pear wine (*colonche*). Colonche is obtained by the natural fermentation of red prickly pear (*O. streptacantha*) juice, and it is valued for its color, flavor, and taste (Navarrete-Bolaños et al., 2013; Díaz-Lima & Vélez-Ruiz, 2017). The name 'colonche' comes from the indigenous Nahuatl language: 'nochtli', from the words 'nochtli' = cactus fruits, and 'octli' = fermented agave sap. In particular, colonche was produced and consumed in pre-Columbian times by the Chichimecas, native indigenous peoples from the northern region of Mexico. Nowadays it is consumed in the arid areas of northwest Mexico by ethnic groups such as the Tarahumaras from Chihuahua and the Yaquis from Sonora. It is also routinely prepared and consumed in San Luis Potosí and Zacatecas. Nevertheless, consumption of this traditional fermented beverage has decreased due to scarce production information (Ulloa & Herrera, 1978; Ojeda-Linares et al., 2020).

**2.8.1.2. The fermentation process and associated microorganisms.** The modern procedure for colonche production has remained nearly unchanged and it seems to be a custom that has survived for centuries (Ulloa & Herrera, 1978). Red prickly pear fruits (*O. streptacantha*) are collected with a sickle and peeled with a knife *in situ*. Ripe prickly pear fruits are selected for colonche production, and when red fruits are scarce, yellow and white fruits can be added, resulting in changes in flavor, color, and texture (Ojeda-Linares et al., 2020). The peeled fruits are lightly crushed by hand and placed inside clay pots to maintain a constant temperature, and for the spontaneous fermentation process that occurs overnight. Occasionally, in order to speed up fermentation, catalysts such as a small amount of old fermented colonche, or prickly pear hulls, which contain native microorganisms (bacteria and yeasts) are added. Several studies have identified different yeast strains such as *Torulopsis taboadae* (Ulloa & Herrera, 1978). Rodríguez-Lerma et al. (2011) observed the existence of two phases of fermentation: an initial phase predominated by species with low fermentative activity such as *Candida* spp., *Hanseniaspora* spp., *Kloeckera* spp., and *Pichia* spp., followed by a second phase where the most ethanol-tolerant yeasts, mainly *S. cerevisiae* and in some cases *S. uvarum*, prevailed. In another study, the main species isolated were *Enterococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Pediococcus* spp., and *Weissella* spp., where *L. plantarum* was one of the most frequently isolated species (Verón, Di Risio, Isla, & Torres, 2017). The colonche obtained after a few hours of fermentation is a slightly butyric acid sweet carbonated drink with a pleasant flavor, from which the seeds are removed with a sieve. It is served immediately for fresh consumption (Ulloa & Herrera, 1978; Ojeda-Linares et al., 2020).

In 2011, a study of prickly pear wine (*O. streptacantha*) described a *Pichia fermentans* strain with a high capacity for production of volatile compounds, which increase the aromatic properties of the wine, and a low capacity for production of ethanol, along with a *S. cerevisiae* strain

that primarily synthesized ethanol (Rodríguez-Lerma et al., 2011).

**2.8.1.3. Harmful compounds or pathogenic microorganisms.** The maximum concentration of methanol in wines is regulated in order to avoid health risks for consumers. Currently in Mexico there is no standard for prickly pear wine, but international standards (United States, Canada, Italy, and Switzerland) can be used as a reference. These regulations establish limits ranging from 15 to 30 mg 100 mL<sup>-1</sup>. Prickly pear wine has 10.84 mg 100 mL<sup>-1</sup> methanol, which compares favorably with these international regulations (Navarrete-Bolaños et al. 2013; Verón, Gauffin Cano, Fabersani, Sanz, Isla, Espinar, Gil Ponce, & Torres, 2019).

**2.8.1.4. Chemical and nutritional studies.** There are no studies on the chemical and/or nutritional characteristics of colonche made with the spontaneously fermented process. However, Verón et al. (2019) conducted a study that evaluated the effect of fermentation using autochthonous *L. plantarum* S-811 on the nutritional composition of fermented prickly pear juice (FPPJ) versus non-fermented prickly pear juice from *Opuntia ficus-indica*. The authors observed a high content of vitamin C in FPPJ and an increased betalain content, by 38%, due to the effect of fermentation with *L. plantarum* S-811 (see Table 2). Navarrete-Bolaños et al. (2013) analyzed the volatile compounds present in prickly pear wine (*O. streptacantha*) fermented by *P. fermentans* and *S. cerevisiae* by gas chromatography (GC). The GC analysis showed the following profile: 96.28% ethanol, 0.14% methanol, 0.12% 1-propanol, 0.7% 2-methyl-1-propanol, 2.5% 3-methyl-1-butanol, and 0.26% phenyl ethyl alcohol, along with the presence of organic acids (see Table 2).

**2.8.1.5. Evaluated functional effects.** Undoubtedly, most of the nutritional and health benefits of fermented products are conferred by the microorganisms involved in fermentation. In this sense, humans have been selecting good microorganisms and removing pathogenic microorganisms from these fermented beverages using different strategies since ancient times (Ojeda-Linares et al., 2020). One study showed that FPPJ with *L. plantarum* S-811, a native lactic acid bacterium, has antioxidant activity associated with the phenolic compounds it contains, which act as reducing agents, free-radical scavengers, and singlet oxygen quenchers. However, the antioxidant mechanisms of hydroxycinnamic and hydroxybenzoic acids, the phenolic compounds present in this FPPJ, are also related to the compounds' ability to modify cellular signaling processes, which increases the levels of antioxidant enzymes, thereby decreasing oxidative stress.

The aforementioned study also shows that the fermentation of prickly pear juice by *L. plantarum* S-811 most likely enables the use of complex polysaccharides and their catabolization to short-chain fatty acids (such as acetic acid or propionic acid) that are involved in improving metabolic functions during obesity. They do this by ameliorating glucose homeostasis, which decreases the inflammatory status and increases the secretion of glucagon, which improves β-cell function, increases insulin sensitivity in muscle, and suppresses insulin signaling in adipocytes, inhibiting fat accumulation in adipose tissue (Verón et al., 2019). The functional effects of FPPJ with *L. plantarum* S-811 described above notwithstanding, no studies have been reported evaluating the functional effect of colonche produced by spontaneous fermentation.

## 2.9. Fermented beverages made from *Saccharum officinarum*

### 2.9.1. Guarapo

**2.9.1.1. Description and traditional uses.** Sugarcane is a plant that is native to Asia. It belongs to the Poaceae family and the *Saccharum* genus (Chen, Zheng, & Lin, 2013; Silva Ribeiro, Ferreira Duarte, Ribeiro Dias, & Freitas Schwan, 2015). Sugarcane as a raw material has given rise to primary products that originate during its harvesting and processing,

such as sugar, bagasse, honey, and cane juice with or without fermentation. The last product listed is known as 'guarapo' (Gómez-Merino et al., 2017).

Guarapo is an indigenous fermented beverage obtained from the first product of cane grinding and it is widely consumed in countries such as India, Brazil, Colombia, Ecuador, and Mexico. It can be extracted in an artisanal mill with an efficiency of 55% per unit, or through industrial grinding when passing through four or five mills with an efficiency of 97% per unit. The juice obtained has 16 to 20% dry matter and it is mainly made up of sucrose and reducing sugars such as glucose and fructose, which are excellent sources of carbon for microbial growth and can thus be directly used as a fermentation medium (Armijos Espín, 2016; Resende Oliveira, Caliari, Soares, Ribeiro Oliveira, & Marques Duarte, 2018).

Once the juice is extracted, it is left to ferment (spontaneously) in order to produce an alcoholic beverage. The production of alcohol is favored by a diverse community of yeasts and bacteria that proliferate in the initial stages of the process, resulting in alcohol-resistant organisms that produce organic compounds characteristic of the aromas and flavors of the beverage (Olarte, 2007; Armijos Espín, 2016; Cobos Dávila, 2016). In many countries, guarapo is consumed with acidic fruit juices added such as lemon, jambolan, and pineapple in order to improve the flavor and make it a more refreshing beverage (Silva Ribeiro et al., 2015; Resende Oliveira et al., 2018).

**2.9.1.2. The fermentation process and associated microorganisms.** In guarapo production, the raw material (sugarcane stem) is selected and washed to prevent the proliferation of pathogenic microorganisms. After going through a mill, the juice is obtained and the bagasse is discarded. The juice is filtered through a sieve and poured into a clay bowl, in which the sugarcane juice is left to ferment for approximately 4 days (Chaves-López et al., 2014; Armijos Espín, 2016). The sugarcane juice fermentation process involves the decomposition of carbohydrate sources to produce alcohol and carbon dioxide using activated yeasts, and the alcohol content increases gradually with the consumption of total sugars (Cuervo Mulet, Angel Ledesma, Duran Vanegas, & Argote Vega, 2010; Chen et al., 2013). The most common yeasts isolated from guarapo are *Candida utilis*, *Candida guilliermondii*, *Candida rugosa*, *Candida lypolytica*, *Candida glabrata*, *Candida pelliculosa*, *Candida kefir*, *Candida inconspicua*, *Cryptococcus laurentii*, *Rhodotorula mucilaginosa*, and *S. cerevisiae* (Vanegas Córdoba & del Yepes Pérez, 2004; Gomes Fatima & Silva, 2010; Chaves-López et al., 2014).

The LAB composition during fermentation allows the guarapo to acquire sensory characteristics (aroma, flavor, and color) that are desirable for the consumer. The most frequent LAB genera in guarapo are *Leuconostoc* spp., *Lactobacillus* spp., and *Pediococcus* spp., which influence the beverage's flavor by means of the secondary compounds that they produce. In Brazilian fermented sugarcane, Gomes Fatima and Silva (2010) reported that *L. plantarum* and *L. casei* were the prevalent LAB. Other species such as *Lactobacillus ferintoshensis*, *L. fermentum*, *Lactobacillus jensenii*, *Lactobacillus murinus*, *Lactococcus lactis*, *Enterococcus* spp., and *Weissella* spp. were present in lesser quantities (Gomes Fatima & Silva, 2010; Chaves-López et al., 2014). However, more studies are needed in this regard, to evaluate all microorganisms involved in the fermentation process of guarapo.

**2.9.1.3. Harmful compounds or pathogenic microorganisms.** In one study, which reports that the fermentation time of guarapo is approximately 4 days, a statistical analysis between coliforms and the different stages of guarapo fermentation was performed. It was found that the total coliforms showed high counts in the initial stage (day 0, 5.69 CFU/mL), and that in the fermentation stage (Days 1 and 2) the counts decreased by more than half (1.99 CFU/mL). In the final stage (Day 3) of fermentation, a total inhibition of coliforms was observed. The authors associated this coliform inhibition with a pH decrease derived from the

presence of LAB and their production of bacteriocins, which prevent the growth of pathogenic microorganisms (Feoli Bonilla, Escobar Guarín, & Marín Mahecha, 1995; Jiménez Vera et al., 2010; Pazmiño, Escudero, & Grijalva, 2014; Armijos Espín, 2016). In relation to the toxic effects of guarapo, there is a concern related to excessive and prolonged consumption, since this beverage has been associated with the development of liver cirrhosis (Olarte, 2007; Chaves-López et al., 2014). In a study conducted in Colombia that included 232 subjects who consumed this beverage daily, 40% of the patients exhibited hepatic cirrhosis, and consumption of guarapo was the main risk factor, with exposure times of more than ten years in 80.6% of the patients (Olarte, 2007). Toxicological studies of this beverage in Mexico and other countries have not yet been carried out. Therefore, it would be interesting to evaluate guarapo consumption in Mexico and its association with the development of liver disease.

**2.9.1.4. Chemical and nutritional studies.** Nutritional studies in Colombia and Ecuador have reported that guarapo has a pH of 3.8, 15.98° Brix and that it contains 3% alcohol and 93% water (see Table 2) (Olarte, 2007; Armijos Espín, 2016). Protein content was present in small quantities and was possibly hydrolyzed during the fermentation process. In relation to mineral content, potassium is present in high concentrations, followed by phosphorus, calcium, sodium, magnesium, sulfur, iron, aluminum, copper, and zinc (Chen et al., 2013; Chaves-López et al., 2014; Armijos Espín, 2016). In a study conducted in Brazil in 2017 by Resende Oliveira et al., eleven volatile compounds were identified in guarapo beverages, which were mainly esters and nine phenolic compounds (see Table 2).

**2.9.1.5. Evaluated functional effects.** There are no studies on the functional effects of guarapo. However, some of the phenolic compounds described have been associated with several health-promoting effects such as antimicrobial, anti-inflammatory, antiproliferative, antidiabetic, anticancer, hypolipidemic, as well as gastro-, cardio-, neuro- and cytoprotective activity (Ramírez-Rodríguez et al., 2020). Different extracts of sugarcane are reported to have hypocholesterolemic, neuroprotective, and antiplatelet effects (Duarte-Almeida, Negri, Salatino, de Carvalho, & Lajolo, 2007; Pallavi & Elakkiya, 2012; Caderby et al., 2013; Khan, Tahir, Lone, Munir, & Latif, 2015).

### 3. Network analysis and associations

We include recent scientific evidence demonstrating these beverages' have multiple functions associated with the fermentation process such as microbiota along with adverse, chemical, nutritional, and functional studies. We completed a network analysis with available microorganisms present in these beverages. Fig. 3 shows that the most frequent species are yeasts (*Saccharomyces cerevisiae*, *Candida inconspicua*), AAB (*Zymomonas mobilis*), and LAB (*Lactobacillus plantarum*, *Lactococcus lactis*, *Lactobacillus acidophilus*, and *Lactobacillus casei*). It is also possible to observe that the beverages from agave, corn, and pineapple have a greater number of common microorganisms compared to sugar cane, balche, palm, and prickly pear. It is worth mentioning that these results may be due to a lack of characterization. Finally, cocoa seems to exhibit particular species of microorganisms.

Fig. 4A shows a heatmap representing the presence of bioactive compounds in all beverages described in the literature; in yellow, we can observe the presence of biomolecules such as polyphenols, phenolic compounds, gallic and ferulic acids, anthocyanins, caffeine, and saponins, which have all been associated with functional effects as well with health benefits. The results show that the guarapo has the most bioactive compounds described, followed by taberna and subsequently aguamiel, pulque, cocoa, pozol, tescuino, tepache, balché, and sendechó. Fig. 4B shows a heatmap describing some therapeutic effects associated with those beverages; red color indicates the presence of known beneficial

health effects in each beverage. It can be observed that antioxidant activity is the most common effect; as well as the presence of several bacteria strains with potential probiotic use, while anti-inflammatory and antibacterial activity were present only in three of the beverages. As we can observe pulque, cocoa, and pozol have better characterization of biomolecules; however, the absence in others such as sendechó and guarapo is due to a lack of deep study. We used Pearson's product moment correlation, a common metric for estimating association based on presence/absence data, to determine the association between the bioactive compounds reported in the literature for each beverage, and the possible health properties attributed to the beverages. Fig. 4C shows some correlations that make with biological sense, for example, alkaloids are strongly associated with narcotic and laxative activity, betalains were positively correlated with leptin and short chain fatty acids production, flavonoids, theobromine and theophylline were positively correlated with aging, hypotensive cardioprotective activity as well as improvement of cognitive function, additionally to inhibition of platelet aggregation, steroidal and folates were related with same effects as, anti inflammatory, alleviate chest and abdominal pain, antipyretic energy source, prevention of colon cancer. However, Pearson's product moment correlation is very sensitive to co-absent values. A high co-absent value tends to overestimate the correlation coefficient. Thus, when a compound and a possible effect are both rare (they have only been described for one beverage), the result is a high association between compound and effect, which is not necessarily true, and may be due to a lack of deep study of the beverages for example surfactin, bacilysin, fengycin, iturin A, and chlorotetaine were strongly correlated with hepatoprotective and hypolipidemic activity.

### 4. Conclusions and future directions

More than 200 fermented products have been documented in Mexico, of which approximately 20 are beverages. This review highlights the fact that Mexican fermented beverages exhibit nutraceutical and health benefits, in addition to industrial interest, through twelve traditional Mexican beverages: pulque, cocoa, pozol, tejuino, sendechó, balché, huapilla water, taberna, tepache, garapiña, colonche, and guarapo, which are produced from agave, cocoa, corn, sugar cane, balche, palm, pineapple, and prickly pear (see Figs. 1 and 2). We describe their importance with regard to their traditional use, production, and empirical therapeutic effects as transmitted by traditional peoples in our country.

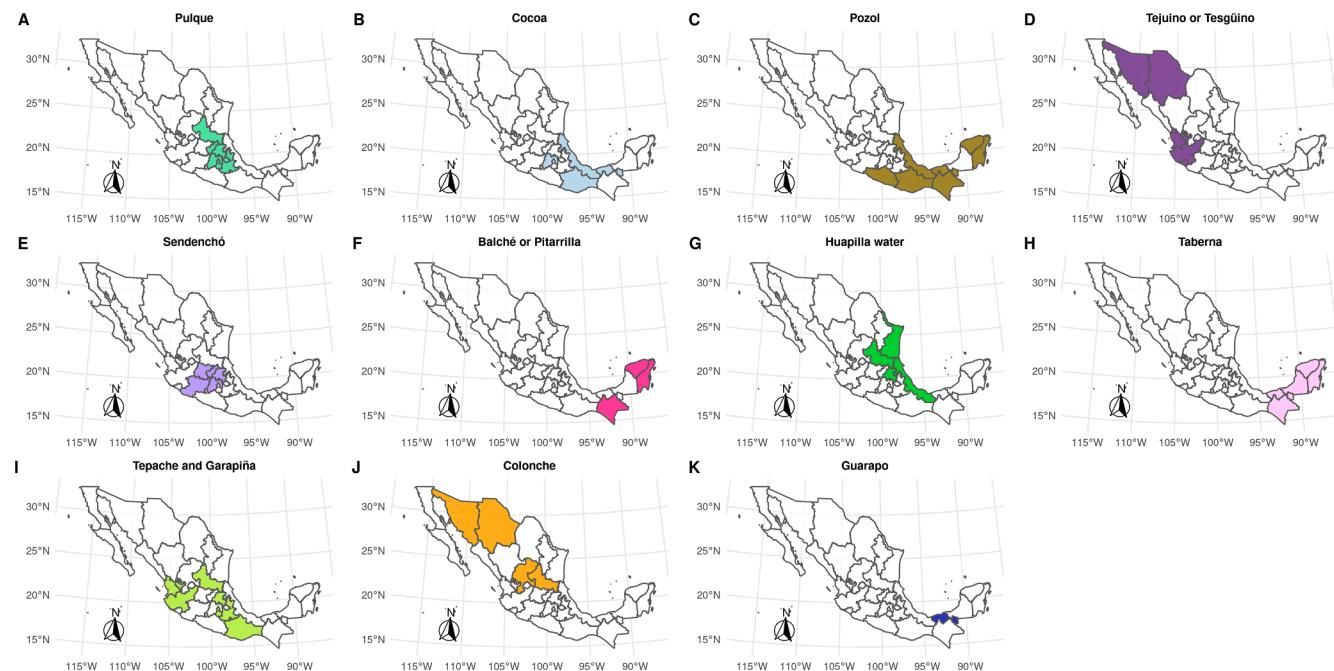
The evidence described here clearly shows that fermented beverages have multiple benefits on human health, contributing directly to better nutrition and production of bioactive chemical compounds such as functional carbohydrates, minerals, vitamins, fructooligosaccharides, antioxidants, polyphenols, carotenoids, and other bioactive compounds. Some of these beverages contain different chemical compounds lacking in nutritional importance such as ethanol. The presence of ethanol can be associated with abusive consumption and negative health consequences, such as alcoholism or hepatic cirrhosis. The presence of alkaloids in balché may have detrimental effects on human health, for instance visual and auditory hallucinations. This beverage is consumed in traditional ceremonies and not as a daily beverage.

Another important point out is the presence of multiple bacteria strains. These have been considered hot topics because they may have a potential use as probiotics. Some of them produce chemical compounds such as GOS, which have been used as prebiotics in several basic and clinical trials. Production of chemical molecules showing antibacterial, antioxidant, antinflammatory and other types of activity.

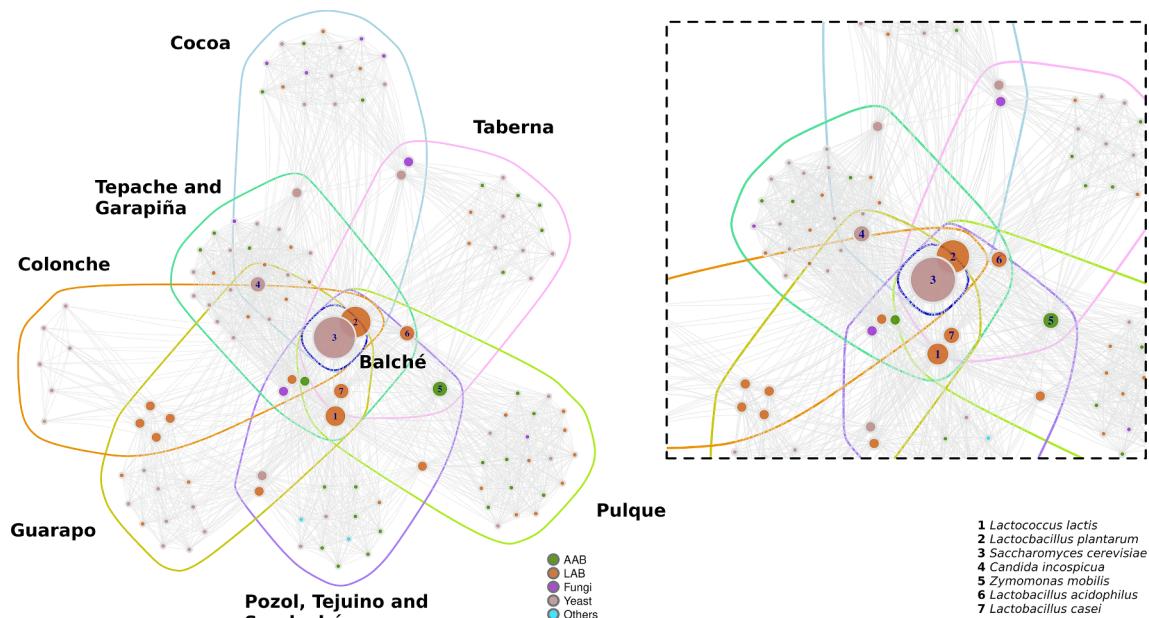
Traditional fermentation is not an industrialized process and the safety of the beverages is not controlled, which permits the presence of pathogenic bacteria. Currently, some of them have been poorly described, but as the authors mentioned, they are in low quantities and transiently disappearing at the end of it. It should be noted that sufficient research has yet to be performed. One example is the characterization of



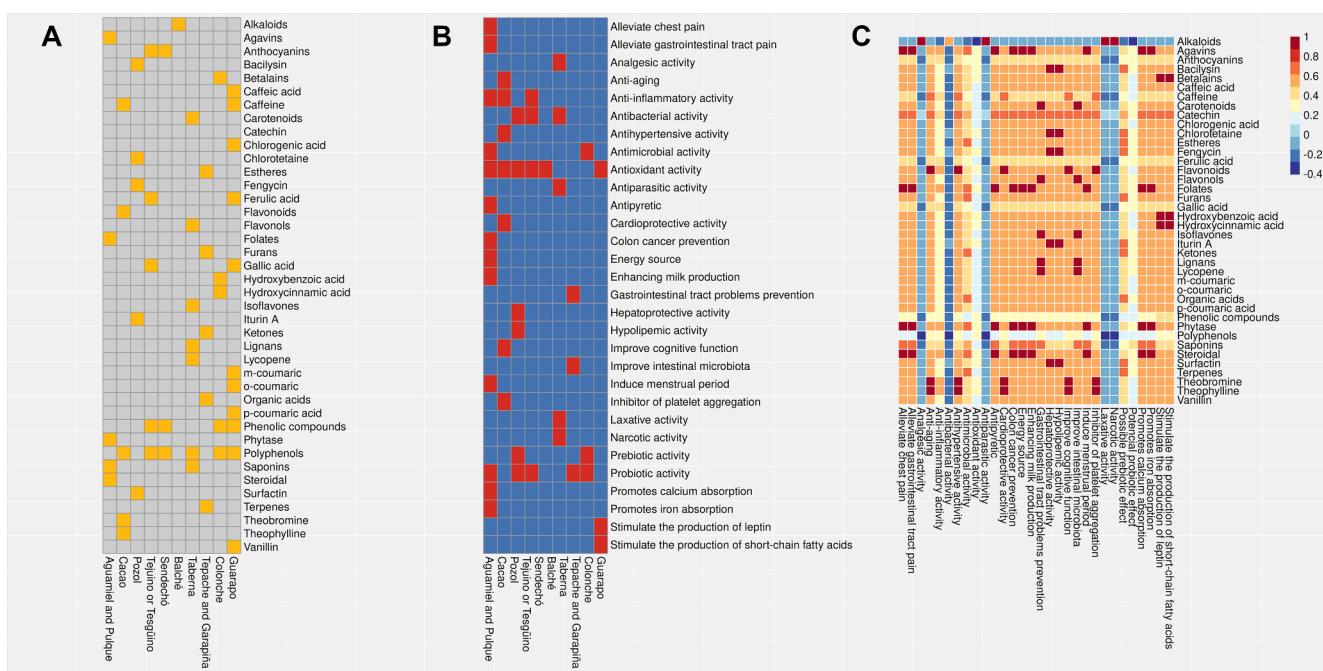
**Fig. 1.** Traditional Fermented Beverages in Mexico. A) *Agave* spp. (a) Pulque and (b) Aguamiel; B) *Theobroma cacao*. (a) Cocoa; C) *Zea mays*. (a) Pozol, (b) Tejuino or tesgüíno (c) Sendechó; D) *Lonchocarpus violaceus*. (a) Balché or pitarrilla; E) *Hechtia glomerata*. (a) Huapilla water; F) *Acrocomia aculeata*. (a) Taberna; G) *Ananas comosus*. (a) Tepache, (b) Garapiña; H) *Opuntia streptacantha*. (a) Colonche; I) *Saccharum officinarum*. (a) Guarapo.



**Fig. 2.** States of Mexico where fermented beverages are produced. A) Pulque: Estado de México, Hidalgo, Querétaro, Mexico City, Morelos, Puebla, Tlaxcala, and San Luis Potosí. B) Cocoa: Oaxaca, Tabasco, and Veracruz; C) Pozol: Guerrero, Oaxaca, Quintana Roo, Tabasco, Veracruz, Yucatán, and Chiapas; D) Tejuino or tesgüíno: Chihuahua, Colima, Jalisco, Nayarit, and Sonora; E) Sendechó: Estado de México, Hidalgo, Querétaro, Guanajuato, Michoacán, Tlaxcala, Puebla, and Veracruz. F) Balché or Pitarrilla: Chiapas, Quintana Roo, and Yucatán. G) Huapilla water: Hidalgo, San Luis Potosí, Tamaulipas, and Veracruz. H) Taberna: Chiapas. I) Tepache and garapiña: Hidalgo, Jalisco, Mexico city, Morelos, Nayarit, Oaxaca, Puebla, and San Luis Potosí. J) Colonche: Chihuahua, Sonora, San Luis Potosí, and Zacatecas. K) Guarapo: Tabasco.



**Fig. 3.** Microbial interaction networks for traditional fermented beverages. Interaction networks were built using the Igraph package of R. Node colors represent a type of microorganism. Green nodes represent acetic acid bacteria (AAB), orange nodes represent lactic acid bacteria (LAB), cyan nodes represent bacteria with different metabolic traits from those of AAB and LAB, white nodes represent yeasts, and purple nodes represent fungi. The size of the nodes represents the number of communities to which each microorganism belongs. Communities are delimited by the beverage where each microorganism was isolated. Pulque, cocoa, pozol, tejuino, sendechó, balché, taberna, colonche, and guarapo. The enlarged zone shows details of what microorganisms are most abundant and more frequently found in all beverages. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** Bioactive compounds and prospective functional properties in Mexican fermented beverages. A) Heatmap of bioactive compounds in fermented beverages. Yellow color represents an association between bioactive compounds and the beverage. Gray color represents the absence of association. B) Heatmap of possible health properties in fermented beverages. Red color represents the potential human health properties in the fermented beverage. Blue color represents a lack of possible health properties in the beverage. These human health properties are associated with the presence of bioactive compounds in raw material and those generated during the fermentation process. C) Pearson's correlation heatmap of bioactive compounds and possible health properties in beverages based on presence/absence data. Pearson's correlation coefficients are shown with continuous gradient colors. Red represents a positive correlation while blue represents a negative correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the genomic data from the microorganisms associated with the production of Mexican fermented beverages and the development of a high-quality product, or bioproducts with biotechnological applications in

the food or the pharmaceutical industries, which will have an economic impact on all producer communities, and on the transmission of knowledge in our culture.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Further reading

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