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# Saccharomyces cerevisiae populations and other yeasts associated with indigenous beers (chicha) of Ecuador



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## ABSTRACT

Chicha, a type of beer made mainly with maize or cassava, is a traditional fermented beverage of the Andean region. There have only been a few studies on yeasts associated with chicha fermentation, and the species diversity occurring during the production of this beverage is not known. The objective of this study was to determine the biodiversity of yeasts in chicha, and to characterize the *Saccharomyces cerevisiae* populations associated with the production of chicha de jora, seven-grain chicha, chicha de yuca, and chicha de morocho in Ecuador. The molecular diversity of *S. cerevisiae* populations was determined by restriction polymorphism mitochondrial profiles. The beverages were characterized based on their physicochemical parameters. Twenty-six species were identified, and the most prevalent species were *S. cerevisiae* and *Torulaspota delbrueckii*. Other yeast species were isolated at low frequencies. Among 121 isolates of *S. cerevisiae*, 68 different mtDNA molecular profiles were identified. These results showed that chichas are fermented by a high number of different strains of *S. cerevisiae*. Some other species provided a minor contribution to the fermentation process. The chicha presented generally similar physicochemical parameters to those observed for other traditional fermented beverages, and can be considered as an acid fermented beverage.

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## Introduction

*Chicha* or maize beer could be regarded as the oldest beverage in Latin America. The name *chicha* possibly originates from the word *chichab*, from the original language spoken in the current territory of Panama, which means maize. Other theories suggest that the name is derived from the word *Chibcha*, a civilization that populated Colombia and Panama, or relate the word *chicha* to *Chichas*, an ethnicity present in southern Bolivia before the establishment of the Incas.<sup>1</sup>

*Chicha* is a clear, yellow, and frothy beverage present in the Andean region and in low-lying regions of Ecuador, Peru, Bolivia, Colombia, Brazil, and Argentina.<sup>2</sup> This traditional beverage is prepared mainly from maize, but currently, the name is considered generic and refers to a variety of beverages, fermented or not, prepared from various other materials, such as cassava, beans (such as rice, oats, and quinoa) and fruits (such as bananas). In Ecuador, the first reports of *chicha* production date back to 200 B.C., before the establishment of the Incas in the region.<sup>1</sup> This beverage was of great importance in traditional indigenous cultures, especially in the Incan culture, wherein it was also linked to festive ceremonies.<sup>3</sup>

In Ecuador, as in the rest of the Andean region, the most common maize *chicha* is *chicha de jora* (Fig. 1). This *chicha* is prepared from the yellow maize grain (*maíz amarillo*), which is malted (germinated and dried). For the preparation of malt, the grains are left in water for a day. This step is necessary to achieve the optimum grain moisture for germination. Subsequently, the water is drained and the maize is placed in baskets of straw to germinate over a period of 13 days. Once germinated, the maize is put into straw mats or plastic tarps under the sun for 2 days to dry completely, which stops the enzymatic activity within the grain. After drying, the beans are ground and the flour obtained is used for the preparation of *chicha*. For this, the *jora* flour is added to cold water and then this mixture is transferred to vessels with hot water, and boiled for approximately 20 min. After boiling, the mixture is strained and then placed in a container to ferment. The clay vessels, formerly used for boiling and fermentation, have been replaced by aluminium pots and plastic containers, respectively. The spent grain obtained after filtration is termed *afrecho* and serves as food for animals. The fermentation vessels are usually open. Usually after two days of spontaneous fermentation by indigenous microorganisms, the beverage is ready for consumption. Some producers typically boil *jora* flour with other ingredients, including *panela* (brown sugar in solid pieces). Others make a mixture of *panela* and herbs and then add this mixture to the *jora* flour and water. There are still those that add pieces of fruit and *panela* to the beverage, after filtering.

Other *chicha* beverages produced in Ecuador include *chicha de morocho*, made with white maize, and *chicha* produced with seven varieties of maize including *jora*, *maíz amarillo* (yellow maize), *maíz blanco* (white maize), *maíz negro* (black maize), *chulpi* (*chulpi* maize), *morocho* (*morocho* maize) and *cangil* (popcorn maize). Seven-grain *chicha* is produced in the town of Otavalo, in northern Ecuador, and is a very famous drink and appreciated throughout the country. The *yuca* (*cassava*, *Manihot esculenta*) is also an important raw material for the

production of *chicha*.<sup>4</sup> This *chicha* is produced by the indigenous and mestizo population in the Amazon region of Ecuador.

Few studies have been performed to identify the yeast species in *chichas*. Vallejo et al.<sup>5</sup> isolated *Saccharomyces cerevisiae* as the single yeast species at the end of fermentation from 10 samples of *chicha de jora* collected from 10 different familiar traditional “*chicherías*” in the Cusco region of Peru. These authors suggested that this species was mainly responsible for alcoholic fermentation in these *chicha* samples. Rodríguez et al.<sup>6</sup> suggest that *Saccharomyces uvarum* is responsible for the traditional fermentation of apple *chicha* elaborated by aboriginal communities of Andean Patagonia (Argentina and Chile). Mendonza et al.<sup>7</sup> showed by high-throughput sequencing and culture-dependent approaches that *S. cerevisiae* was the dominant species in an Argentinian maize-based *chicha*. Other works on *chicha* fermentation linked bacterial populations to this process.<sup>4,8,9</sup> Despite the work of Vallejo et al.<sup>5</sup> and Mendonza et al.,<sup>7</sup> the yeast biodiversity associated with maize and cassava *chicha* production is almost unknown. In this work, *chichas* sold in bulk (Fig. 1), produced with different substrates and different fermentation times, were collected from markets, bars, restaurants, and in villages of Ecuador. The objective was to determine yeast species richness and to characterize the *S. cerevisiae* populations associated with the production of this beverage by restriction polymorphism mitochondrial DNA (mtDNA) analyses. In addition, the physicochemical parameters of the beverages were determined.

## Materials and methods

### Sampling

Forty-two *chicha* samples were collected from August to October of 2010 and April to September of 2012 in two regions of Ecuador: the Amazon region, within the Yasuní National Park (Orellana Province) and the Andean region, in the provinces of Pichincha, Imbabura and Chimborazo. The samples included two *chichas de yuca*, 34 *chichas de jora*, three seven-grain *chichas*, and two *chichas de morocho*. In these samples, the fermentation was considered finished by the producers, and the beverage was ready to drink. One sample of *chicha de jora* was sampled during successive fermentation times (0–5 days). The *chichas* were collected in sterile bottles of 100 mL, transported to the laboratory on ice, and processed the same day.

### Yeast isolation and identification

Aliquots of 25 mL of each *chicha* were added to 225 mL of sterile 0.1% peptone water. For yeast isolation, 0.1 mL of appropriate decimal dilutions, in triplicate, was spread on yeast extract-malt extract (YMA: 1% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, 2% agar, and 0.02% chloramphenicol) and lysine (1.17% YCB, 0.056% lysine, 2% agar, and 0.02% chloramphenicol) agars. The YMA was utilized for the isolation of *Saccharomyces* and non-*Saccharomyces* yeasts while the lysine agar was utilized for the isolation of non-*Saccharomyces* yeasts. The plates were incubated at 25 °C for 5 days and the density of each different yeast morphotype



**Fig. 1** – Chicha de jora (A); Morocho (white maize) (B); Chicha de morocho ready to drink (C); seven-grain chicha (D); cassava for chicha de yuca production (E); Chicha de yuca ready to drink after addition of seeds of the Ungurahua palm (F).

was expressed as the means of each morphotype in each sample in colony-forming units (cfu/mL). Representative colonies of each different yeast morphotype from both culture media were purified on YMA plates and preserved at  $-80^{\circ}\text{C}$  or in liquid nitrogen for subsequent identification.

The yeast isolates were morphologically and physiologically characterized according to Kurtzman et al.<sup>10</sup> Isolates with identical morphological and physiological characteristics were grouped together and subjected to PCR using the core sequences of the primer (GTG)<sub>5</sub> as described by Gomes et al.<sup>11</sup> Isolates with identical DNA banding patterns were grouped and tentatively considered the same species. At least 50% of the yeast isolates of each molecular group were identified by sequencing. Species identification was performed by sequence analysis of the ITS-5.8S region and the D1/D2 variable domains of the large subunit of rRNA gene as described previously.<sup>12–16</sup> Sequencing was performed directly from purified PCR products using an ABI3130 (Life Technologies, USA) automated sequencing system. For yeast species identification, the sequences obtained were compared to those included in the GenBank database using the Basic Local Alignment Search Tool (BLAST at <http://www.ncbi.nlm.nih.gov>).

#### Restriction polymorphism of mitochondrial DNA

Extraction of mtDNA from 121 isolates of *S. cerevisiae* was performed according to the methodology described by Querol and Barrio<sup>17</sup> and modified by Foschino et al.<sup>18</sup> The mtDNA was resuspended in  $25\ \mu\text{L}$  of TE and stored at  $-20^{\circ}\text{C}$ . For

digestion, a mixture containing  $10\times$  buffer (Invitrogen, USA),  $1\ \mu\text{L}$  of RNase A (Invitrogen, USA),  $1\ \mu\text{L}$  of *Hinf* I (Invitrogen, USA), and  $10\ \mu\text{L}$  of DNA (approximately  $1500\ \text{ng}$ ) was used. The volume was completed with deionized water to  $20\ \mu\text{L}$ . The tubes were then incubated at  $37^{\circ}\text{C}$  for 6 h. The mtDNA restriction fragments were separated by electrophoresis on a 1.5% agarose gel (Pronadisa, Spain) in TBE buffer at 80 V for 2 h. The gels were stained with a solution of GelRed (Biotium, USA) and visualized and photographed under ultraviolet (UV) light.

#### Physicochemical analyses

The parameters of pH, total reducing sugars, and ethanol content were determined. The determination of total reducing sugars was performed by the 3,5-dinitrosalicylic acid methodology.<sup>19</sup> The ethanol, glycerol, and lactic and acetic acid contents were determined by high performance liquid chromatography (HPLC) in an Agilent chromatographer model equipped with a column Rezex ROA ( $300 \times 7.8\ \text{mm}$ ).

## Results

#### Yeast identification

Two hundred and fifty-four yeast isolates obtained from chicha samples were identified as belonging to 26 species. *S. cerevisiae* was the most prevalent species occurring in 33 of 42 chicha samples (41 samples plus one sample collected in different time), followed by *Torulaspora delbrueckii* that was identified in

**Table 1 – Yeast species (cfu/mL and number of positive samples for each species) isolated from chicha samples collected in Ecuador.**

Yeast species	Chicha de jora (n = 35) <sup>a</sup>	Seven-grain chicha (n = 3)	Chicha de morocho (n = 2)	Chicha de yuca (n = 2)
<i>Candida californica</i>	4.0 × 10 <sup>5</sup> (1) <sup>b</sup>	–	–	–
<i>Candida humilis</i>	4.7 × 10 <sup>6</sup> (1)	–	–	–
<i>Candida sake</i>	1.0 × 10 <sup>5</sup> (1)	–	4.0 × 10 <sup>5</sup> (1)	–
<i>Candida solani</i>	3.3 × 10 <sup>5</sup> (1)	–	–	–
<i>Candida sorboxylosa</i>	3.3 × 10 <sup>4</sup> –4.6 × 10 <sup>5</sup> (3)	–	–	–
<i>Candida tropicalis</i>	–	–	–	3.3 × 10 <sup>4</sup> (1)
<i>Candida vinaria</i>	3.3 × 10 <sup>4</sup> (1)	–	–	–
<i>Candida zeylanoides</i>	8.6 × 10 <sup>5</sup> (1)	–	–	–
<i>Dekkera anomala</i>	3.3 × 10 <sup>5</sup> (1)	–	–	–
<i>Dekkera bruxellensis</i>	8.0 × 10 <sup>5</sup> –4.0 × 10 <sup>6</sup> (2)	1.6 × 10 <sup>5</sup> (1)	–	–
<i>Galactomyces candidum</i>	3.3 × 10 <sup>4</sup> –6.6 × 10 <sup>4</sup> (2)	–	–	–
<i>Galactomyces geotrichum</i>	2.0 × 10 <sup>5</sup> –1.0 × 10 <sup>6</sup> (5)	–	–	–
<i>Hanseniaspora opuntiae</i>	–	–	–	3.3 × 10 <sup>4</sup> (1)
<i>Hanseniaspora spp.</i>	3.3 × 10 <sup>4</sup> –5.2 × 10 <sup>7</sup> (9)	–	–	–
<i>Kazachstania exigua</i>	3.3 × 10 <sup>5</sup> –5.6 × 10 <sup>7</sup> (3)	–	–	–
<i>Kodamaea ohmeri</i>	–	–	–	3.3 × 10 <sup>4</sup> (1)
<i>Meyerozyma guilliermondii</i>	3.33 × 10 <sup>4</sup> (1)	–	–	–
<i>Pichia fermentans</i>	1.0 × 10 <sup>5</sup> –1.6 × 10 <sup>6</sup> (5)	–	3.3 × 10 <sup>5</sup> (1)	–
<i>Pichia kluyveri</i>	6.6 × 10 <sup>5</sup> (1)	–	–	–
<i>Pichia kudriavzevii</i>	3.3 × 10 <sup>5</sup> –1.1 × 10 <sup>7</sup> (2)	2.6 × 10 <sup>5</sup> –6.6 × 10 <sup>5</sup> (3)	–	6.7 × 10 <sup>4</sup> (1)
<i>Pichia manshurica</i>	–	6.6 × 10 <sup>4</sup> (1)	–	–
<i>Rhodotorula mucilaginosa</i>	3.3 × 10 <sup>4</sup> (1)	–	–	–
<i>Saccharomyces cerevisiae</i>	3.3 × 10 <sup>4</sup> –3.8 × 10 <sup>7</sup> (28)	3.3 × 10 <sup>4</sup> –5.6 × 10 <sup>7</sup> (3)	–	1.5 × 10 <sup>7</sup> –2.1 × 10 <sup>7</sup> (2)
<i>Saccharomyces ludwigii</i>	2.0 × 10 <sup>5</sup> –1.7 × 10 <sup>6</sup> (2)	–	7.0 × 10 <sup>6</sup> (1)	–
<i>Torulaspora delbrueckii</i>	3.3 × 10 <sup>4</sup> –1.1 × 10 <sup>7</sup> (14)	–	3.3 × 10 <sup>5</sup> –7.0 × 10 <sup>6</sup> (2)	1.6 × 10 <sup>5</sup> –4.3 × 10 <sup>5</sup> (2)
<i>Zygoascus hellenicus</i>	3.3 × 10 <sup>4</sup> (1)	–	–	–

<sup>a</sup> n = number of samples analysed.

<sup>b</sup> Number of samples that the species was isolated. When the yeast species was isolated in more than two samples, the results show in cfu/mL the lowest and the highest yeast counts for each species.

18 samples. These two species as well as *Pichia kudriavzevii*, *Candida sake*, *Dekkera bruxellensis*, *Pichia fermentans*, and *Saccharomyces ludwigii* were isolated from at least two types of chicha (Table 1). The other yeast species were present in only one type of chicha. The highest yeast counts were obtained for chicha de jora and seven-grain chicha. *S. cerevisiae* populations contributed to the highest yeast counts in most samples of chichas de jora, seven-grain chichas, and chichas de yuca. In most samples that this species was detected, the population counts were above 1.0 × 10<sup>7</sup> cfu/mL. However, this species was not isolated from two samples of chicha de morocho, and *T. delbrueckii* and *S. ludwigii* presented the highest counts (7.0 × 10<sup>6</sup> cfu/mL) in this beverage.

One sample of chicha de jora was studied during five days of fermentation (Table 2). At the start of fermentation, *S. cerevisiae* was present at a concentration of 6.6 × 10<sup>5</sup> cfu/mL, and this species remained in the beverage until the fifth day, at which time counts were 4.5 × 10<sup>6</sup> cfu/mL. Other yeast species such as *T. delbrueckii* and *Rhodotorula mucilaginosa* appeared after the third day of fermentation.

The 121 isolates of *S. cerevisiae* studied represented 68 different restriction mtDNA patterns. For chicha de yuca, 11 isolates of *S. cerevisiae* were analysed representing three different mtDNA restriction profiles in two samples studied. For seven-grain chicha, 28 isolates of *S. cerevisiae* were studied, representing 19 different mtDNA restriction profiles in three samples studied. In chicha de jora samples, 82 isolates of *S. cerevisiae* were analysed, and 46 mtDNA restriction profiles were

found (Table 3). Two strains (mtDNA profiles 1 and 2) were found in four different chicha de jora samples, but most chicha samples yielded a set of strains with unique mtDNA restriction profiles that not occurs in another sample.

Four different mtDNA profiles of *S. cerevisiae* were identified in chicha de jora studied during the five days of fermentation (Fig. 2).

Only the *S. cerevisiae* strain with mtDNA profile 3 occurred from the first to the fifth day of fermentation (Table 2). Strains with mtDNA profiles 2 and 3 showed increasing counts over the course of the fermentation. In contrast, the strain with mtDNA profile 1 decreased in number during the fermentation process, and after three days it was not detected.

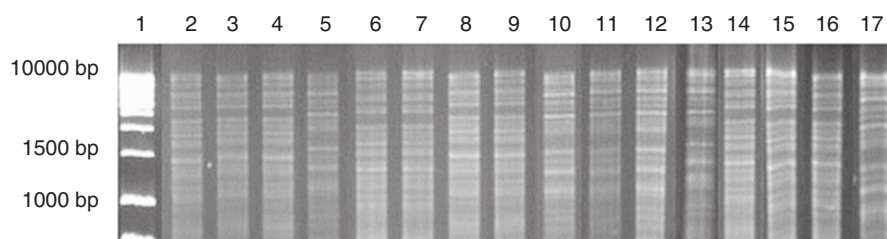
### Physicochemical analyses

The highest pH value was 4.15, in sample 1 after 24h of fermentation, and the lowest value was 2.62, in sample 35 after 8 days of fermentation (Table 3). The lowest value of total reducing sugars was 1.63 g/L and the highest value was 36.24 g/L, both found in chicha de jora samples. The largest amount of glycerol was 3.65 g/L, found in chicha de jora. Lactic acid contents ranged between 1.59 and 6.90 g/L. Ethanol was not detected in 10 chicha samples. The highest value of ethanol was 5.97 (%v/v) in sample 5 of chicha de jora after 24h of fermentation.

**Table 2 – Mitochondrial restriction (mtDNA) profiles of the *Saccharomyces cerevisiae* strains isolated from *chicha de jora* during 5 days of fermentation and identified yeast species at each time point.**

Days of fermentation	Yeast species/counts (cfu/mL)	Population counts (cfu/mL) of each <i>S. cerevisiae</i> mtDNA profile			
		1	2	3	4
0	–	–	–	–	–
1	<i>Saccharomyces cerevisiae</i> ( $6.9 \times 10^5$ )	$3.3 \times 10^5$	$3.3 \times 10^4$	$3.3 \times 10^5$	–
2	<i>S. cerevisiae</i> ( $9.9 \times 10^4$ )	$6.6 \times 10^4$	$3.3 \times 10^4$	–	–
3	<i>S. cerevisiae</i> ( $5.3 \times 10^5$ ), <i>Torulopsis delbrueckii</i> ( $3.3 \times 10^4$ )	–	$9.9 \times 10^4$	$4.3 \times 10^5$	–
5	<i>S. cerevisiae</i> ( $4.5 \times 10^6$ ), <i>T. delbrueckii</i> ( $5.0 \times 10^6$ ), <i>Rhodotorula mucilaginosa</i> ( $3.3 \times 10^4$ )	–	–	$2.2 \times 10^6$	$2.3 \times 10^6$

Analysis of *S. cerevisiae* populations by restriction mtDNA profiles.



**Fig. 2 – Mitochondrial DNA restriction patterns obtained from a *chicha de jora* sample at different times of fermentation. Column: 1: 1 kb DNA ladder; 2–7: profiles found with 1 day of fermentation (2–4: pattern 1; 5: pattern 2; 6–7: pattern 3); 8–10: profiles found with 2 days of fermentation (8–9: pattern 1; 10: pattern 2); 11–15: profiles found with 3 days of fermentation (11–13: pattern 2; 14–15: pattern 3); 16–17: profiles found with 5 days of fermentation (16: pattern 3; 17: pattern 4).**

## Discussion

Our results showed that in most *chicha* samples studied, *S. cerevisiae* was the predominant species, followed by *T. delbrueckii*, *P. kudriavzevii*, *C. sake*, *D. bruxellensis*, *P. fermentans*, and *S. ludwigii*, but generally at much lower counts. All these yeast species may contribute to the sensory quality of the beverage. *S. cerevisiae* was also the dominant species in two productions a maize *chicha* prepared by local producers from Maimará and Tumbaya villages (Quebrada de Humahuaca region) in Northwest of Argentina.<sup>7</sup> However, *Candida parapsilosis* and an undescribed *Pichia* species were the second and third most abundant yeasts in these fermentations in Argentina. Vallero et al.<sup>5</sup> isolated only *S. cerevisiae* from samples of *chicha de jora* in 10 traditional “chicherías” in Cusco region in Peru. These results show that the fermentation process associated with maize-based *chicha* is carried by *S. cerevisiae* and other non-*Saccharomyces* species that occur in minor frequencies. The non-*Saccharomyces* species found in our study can be isolated from different substrates worldwide, including those of fermentation processes for beverage production.<sup>1,5,20–22</sup> The origin of the non-*Saccharomyces* species associated with the *chicha* samples studied might be explained by the different manufacturing processes for these beverages. *Chicha de jora* is prepared in different ways, employing a wide variety of raw materials with additional ingredients such as fruits, herbs,

spices, brown sugar, and sugar. Seven-grain *chicha* is produced with seven varieties of maize flour and *chicha de yuca* with few raw materials including cassava and the seed of the *Ungurahua* palm. *Chicha de morocho*, despite also being manufactured from a wide variety of ingredients, was collected at restaurants in La Mariscal, a district of Quito (data not shown), where the beverage was produced following Good Manufacturing Practice. Some ingredients used in the *chicha* manufacturing process, as fruits, herbs and spices, are added after boiling the *jora* flour. Based on this information, the yeast populations could originate from these ingredients used in beverage preparation, and from other sources such as handlers, utensils, and vessels used during the fermentation process.

A high number of different mtDNA profiles of *S. cerevisiae* were found associated with the *chicha* production process in our study. The occurrence of these different mtDNA profiles of *S. cerevisiae* associated with traditional beverages is well documented.<sup>21,23,24</sup> In addition, the occurrence of different strains during the fermentation process, as observed in the *chicha de jora* studied during the five days of fermentation is very common in spontaneous fermentation during traditional beverage production.<sup>21,25,26</sup> As each mtDNA profile represents a different genetic strain of *S. cerevisiae*, these strains could be contributing to different sensory properties of the beverage. The selection of the best *S. cerevisiae* strains for *chicha* production is interesting, and these studies could be based on strain

**Table 3 – Physicochemical parameters of the *chicha* samples and the occurrence of the different mtDNA profiles of *Saccharomyces cerevisiae* strains in each sample.**

Chicha	Days of fermentation	pH	Total reducing sugars (g/L)	Glycerol (g/L)	Lactic acid (g/L)	Ethanol (%v/v)	mtDNA profiles of the <i>S. cerevisiae</i> strains
<i>yuca</i>							
1	1	4.15 ± 0.01 <sup>a</sup>	34.83 ± 4.51	1.40 ± 0.19	4.72 ± 1.10	2.28	33, 35
2	4	3.94 ± 0.00	7.93 ± 0.03	1.34 ± 0.19	4.58 ± 2.44	3.15	33, 34, 35
<i>jora</i>							
3	1	3.79 ± 0.01	12.42 ± 0.14	1.12 ± 0.30	1.70 ± 0.95	1.07	36, 37, 38
4	1	3.64 ± 0.02	9.11 ± 0.09	1.27 ± 2.49	2.11 ± 3.36	1.87	41
5	1	3.23 ± 0.03	6.81 ± 0.13	3.35 ± 1.71	4.50 ± 2.18	5.97	57, 58
6	1	3.23 ± 0.01	20.6 ± 0.11	1.99 ± 2.47	1.93 ± 1.82	2.75	59
7	1	3.20 ± 0.01	6.97 ± 0.07	–	2.17 ± 0.76	–	11, 12, 13, 16, 31
8	1	3.89 ± 0.01	6.98 ± 0.14	–	–	–	1, 2, 17
9	1	3.56 ± 0.02	5.78 ± 0.23	0.96 ± 0.32	2.17 ± 0.74	0.65	8
10	1	3.89 ± 0.01	7.19 ± 0.09	–	2.46 ± 1.27	0.46	19, 20, 21
11	1	3.87 ± 0.01	7.12 ± 0.03	–	1.90 ± 0.57	–	4, 1, 22
12	1	4.13 ± 0.03	3.40 ± 0.18	–	–	–	–
13	1	3.73 ± 0.02	7.26 ± 0.03	ND <sup>b</sup>	ND	ND	1, 5
14	1	3.62 ± 0.01	5.84 ± 0.09	ND	ND	ND	24, 25, 26
15	2	3.45 ± 0.00	36.24 ± 0.10	2.04 ± 1.65	3.77 ± 2.92	2.76	40
16	2	3.30 ± 0.00	10.08 ± 0.03	0.83 ± 0.21	3.79 ± 0.42	1.21	60
17	2	3.34 ± 0.02	1.63 ± 0.02	–	2.23 ± 1.23	0.20	10
18	2	3.88 ± 0.01	7.17 ± 0.12	–	1.80 ± 1.79	–	1, 17, 22, 23
19	2	3.47 ± 0.01	4.77 ± 0.04	ND	ND	ND	–
20	3	3.55 ± 0.09	34.20 ± 1.87	2.51 ± 2.91	1.94 ± 1.95	2.40	55
21	3	3.24 ± 0.01	6.94 ± 0.06	–	2.27 ± 0.06	–	13, 14, 16, 32
22	3	3.68 ± 0.00	6.92 ± 0.06	–	1.83 ± 2.00	0.37	5, 18
23	3	3.93 ± 0.01	6.64 ± 0.06	ND	ND	ND	2, 4, 6
24	4	2.84 ± 0.01	6.93 ± 0.05	1.53 ± 0.58	3.74 ± 0.95	1.51	–
25	4	3.49 ± 0.01	6.94 ± 0.03	3.65 ± 0.17	2.91 ± 0.43	3.02	8
26	4	3.05 ± 0.01	6.89 ± 0.04	1.80 ± 0.57	1.59 ± 0.80	1.47	9
27	4	3.37 ± 0.01	1.66 ± 0.19	–	3.03 ± 0.62	–	–
28	4	3.37 ± 0.01	6.97 ± 0.05	–	1.95 ± 0.77	–	13
29	4	3.56 ± 0.02	3.31 ± 0.09	–	1.95 ± 1.64	–	–
30	5	4.04 ± 0.01	10.91 ± 0.31	ND	ND	ND	7, 27, 28, 29, 30
31	6	4.14 ± 0.00	6.56 ± 0.06	–	–	–	–
32	7	3.01 ± 0.01	7.91 ± 0.07	–	3.40 ± 0.95	1.71	54, 56, 61
33	7	3.20 ± 0.01	2.65 ± 0.06	ND	ND	ND	–
34	8	2.62 ± 0.00	1.86 ± 0.15	–	3.51 ± 0.28	1.21	15
35	8	3.02 ± 0.01	2.06 ± 0.18	2.66 ± 0.42	5.88 ± 1.01	3.02	2, 17
36	ND	3.39 ± 0.00	16.70 ± 0.59	3.38 ± 1.10	3.79 ± 1.09	4.22	2, 39
<i>seven grain</i>							
37	ND	3.71 ± 0.03	14.71 ± 0.62	2.59 ± 0.85	6.90 ± 2.11	2.99	42, 43, 44, 45, 46, 47
38	ND	3.30 ± 0.01	7.48 ± 0.21	–	–	0.71	62, 63, 64, 65, 66, 67, 68
39	ND	3.31 ± 0.00	2.76 ± 0.07	2.13 ± 1.00	6.66 ± 1.03	1.98	43, 48, 49, 50, 51, 52, 53
<i>morocho</i>							
40	1	3.79 ± 0.01	9.17 ± 0.10	ND	ND	ND	–
41	2	3.84 ± 0.02	7.82 ± 0.23	ND	ND	ND	–

<sup>a</sup> Standard deviation.<sup>b</sup> ND = not determined.

diversity with different mtDNA restriction profiles occurring during the fermentative process.

The low pH values of the *chicha* studied might be associated with acid content, specifically, lactic and acetic acids, produced during fermentation. These organic acids can contribute negatively to the sensory quality of the beverage, as off-flavours can result when these acids are present in high amounts.<sup>23</sup> The total reducing sugars varied widely, possibly

due to sampling at different fermentation times and the diversity of raw materials used in *chicha* preparation. This variation might be related to the efficiency of the microbial community in utilizing these reducing sugars. Glycerol is an alcohol of great importance in alcoholic beverages, as it provides a sweet aroma and contributes to the viscosity of the final product. The largest amount of glycerol was 3.65 g/L, found in *chicha de jora*. It is important to note that glycerol in high

amounts is not desirable because it gives a stale flavour to the beverage.<sup>27</sup>

Altay et al.<sup>28</sup> noted physicochemical parameters very similar to those found in this study when studying *shalgam* juice, a fermented non-alcoholic beverage produced from the lactic fermentation of black carrot. According to the authors, the pH of this beverage was between 3.15 and 4.25 and the major fermentation products obtained were lactic acid (from 5.18 to 8.05 g/L), acetic acid (0.57–0.83 g/L), ethanol (0.79–6.41%) and volatile aromatic compounds. Low concentrations of ethanol present in some samples and the presence of lactic acid make the possibility that fermentation by lactic acid bacteria instead of alcoholic fermentation had occurred in major scale in these samples. With respect to *boza*, a beverage fermented by yeasts and lactic acid bacteria, the pH ranged from 3.16 to 4.63 and the ethanol content ranged from not detectable to 0.39%. Mendoza et al.<sup>7</sup> reported ethanol concentration around 1% in Argentinean maize-based *chichas*, result similar to the *chichas* analysed in our study. Therefore, the *chicha* samples studied presented generally similar physicochemical parameters to those observed for other traditional fermented beverages. Finally, the Ecuadorian *chichas* can be considered acid fermented beverages, and the yeast species associated with its fermentative process should contribute to the singular aroma and unique flavour of this beverage.

### Conflicts of interest

The authors declare no conflicts of interest.

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### REFERENCES

- Gomes FCO, Lacerda ICA, Libkind D, Lopes C, Carvajal EJ, Rosa CA. Traditional foods and beverages from South America: microbial communities and production strategies. In: Krause J, Fleischer O, eds. *Industrial Fermentation: Food Processes, Nutrient Sources and Production Strategies*. New York, USA: Nova Science Publishers Inc; 2009:79–114.
- Steinkraus KH. *Handbook of Indigenous Fermented Foods*. USA: CRC Press; 1995.
- Jennings J. La chichera e el patrón: chicha and the energetics of feasting in the prehistoric Andes. *Archeol Pap Am Anthropol Assoc*. 2005;14:241–259.
- Freire AL, Zapata S, Mosquera J, Mejia ML, Trueba G. Bacteria associated with human saliva are major microbial components of Ecuadorian indigenous beers (chicha). *PeerJ*. 2016;4:e1962.
- Vallejo JA, Miranda P, Flores-Félix JD, et al. Atypical yeasts identified as *Saccharomyces cerevisiae* by MALDI-TOF MS and gene sequencing are the main responsible of fermentation of chicha, a traditional beverage from Peru. *Syst Appl Microbiol*. 2013;36:560–564.
- Rodríguez ME, Pérez-Través L, Sangorrín MP, Barrio E, Querol A, Lopes CA. *Saccharomyces uvarum* is responsible for the traditional fermentation of apple chicha in Patagonia. *FEMS Yeast Res*. 2017;17:1–11.
- Mendoza LM, Neef A, Vignolo G, Belloch C. Yeast diversity during the fermentation of Andean chicha: a comparison of high-throughput sequencing and culture dependent approaches. *Food Microbiol*. 2017;67:1–10.
- Elizaquível P, Pérez-Cataluña A, Yépez A, et al. Pyrosequencing vs. culture-dependent approaches to analyze lactic acid bacteria associated to chicha, a traditional maize-based fermented beverage from Northwestern Argentina. *Int J Food Microbiol*. 2015;198:9–18.
- Puerari C, Magalhães-Guedes KT, Schwan RF. Physicochemical and microbiological characterization of chicha, a rice-based fermented beverage produced by Umutina Brazilian Amerindians. *Food Microbiol*. 2015;46:210–217.
- Kurtzman CP, Fell JW, Boekhout T. *The Yeasts: A Taxonomic Study*. Elsevier; 2011.
- Gomes FC, Safar SV, Marques AR, et al. The diversity and extracellular enzymatic activities of yeasts isolated from water tanks of *Vriesea minarum*, an endangered bromeliad species in Brazil, and the description of *Occultifur brasiliensis* sp. nov. *Antonie van Leeuwenhoek*. 2015;107:597–611.
- White TJ, Bruns T, Lee S, Taylor J. *Amplification and Direct Sequencing of Fungal rRNA Genes for Phylogenetics*. New York, NY: Academic Press; 1990.
- O'Donnell K. *Fusarium* and its near relatives. In: Reynolds DR, Taylor JW, eds. *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics*. Wallingford, UK: CAB International; 1993:225–233.
- Kurtzman CP, Robnett CJ. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek*. 1998;73:331–371.
- Lachance M-A, Bowles JM, Starmer WT, Barker JSF. *Kodamaea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian Hibiscus flowers. *Can J Microbiol*. 1999;45:172–177.
- Rosa LH, Vaz ABM, Caligiorno RB, Campolina S, Rosa CA. Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv. (Poaceae). *Polar Biol*. 2009;32:161–167.
- Querol A, Barrio E. A rapid and simple method for the preparation of yeast mitochondrial DNA. *Nucleic Acids Res*. 1990;18:1657.
- Foschino R, Gallina S, Andrighetto C, Rossetti L, Galli A. Comparison of cultural methods for the identification and molecular investigation of yeasts from sourdoughs for Italian sweet baked products. *FEMS Yeast Res*. 2004;4:609–618.
- Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem*. 1959;31:426–428.
- Sefa-Dedeh S, Sanni AI, Tetteh G, Sakyi-Dawson E. Yeasts in the traditional brewing of pito in Ghana. *World J Microbiol Biotechnol*. 1999;15:593–597.
- Badotti F, Belloch C, Rosa CA, Barrio E, Querol A. Physiological and molecular characterisation of *Saccharomyces cerevisiae* cachaça strains isolated from different geographic regions in Brazil. *World J Microbiol Biotechnol*. 2010;26:579–587.
- Escalante A, Soto DRL, Gutiérrez JEV, Giles-Gómez M, Bolívar F, López-Munguía A. Pulque, a traditional Mexican alcoholic fermented beverage: historical, microbiological, and technical aspects. *Front Microbiol*. 2016;7:1026.
- Querol A, Barrio E, Ramón D. Population dynamics of natural *Saccharomyces* strains during wine fermentation. *Int J Food Microbiol*. 1994;21:315–323.

24. Romano P, Capece A, Jespersen L. Taxonomic and ecological diversity of food and beverage yeasts. In: Querol A, Fleet GH, eds. *Yeasts in Food and Beverages*. Springer; 2006:13–53.
25. Jeyaram K, Tamang JP, Capece A, Romano P. Geographical markers for *Saccharomyces cerevisiae* strains with similar technological origins domesticated for rice-based ethnic fermented beverages production in North East India. *Antonie van Leeuwenhoek*. 2011;100:569–578.
26. Paez-Lerma JB, Arias-Garcia A, Rutiaga-Quinones OM, Barrio E, Soto-Cruz NO. Yeasts isolated from the alcoholic fermentation of *Agave duranguensis* during Mezcal production. *Food Biotechnol*. 2013;27:342–356.
27. Remize F, Roustan JL, Sablayrolles JM, Barre P, Dequin S. Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in by-product formation and to a stimulation of fermentation rate in stationary phase. *Appl Environ Microbiol*. 1999;65:143–149.
28. Altay F, Karbancıoğlu-Güler F, Daskaya-Dikmen C, Heperkan D. A review on traditional Turkish fermented non-alcoholic beverages: microbiota, fermentation process and quality characteristics. *Int J Food Microbiol*. 2013;167: 44–56.