

Microbiological and physicochemical characterization of a traditionally fermented corn product: "Champús"

Caracterización microbiológica y fisicoquímica de un producto de maíz tradicionalmente fermentado "Champús"

Yuli López Cadena¹

Natalia Restrepo Escobar²

Francia Valencia García³ *

¹ Biotransformation Research Group, School of Microbiology, Universidad de Antioquia, Medellín, Colombia, *M.Sc.* Pharmaceutical and Food Sciences.

² Research Group on Management and Application of Science and Technology, Center for Services and Business Management, Servicio Nacional de Aprendizaje (SENA), Medellín, Colombia, *Ph.D.* Biotechnology

³ Biotransformation Research Group, School of Microbiology, Universidad de Antioquia, Medellín, Colombia, *Ph.D.* Pharmaceutical and Food Sciences,

*Correspondence author: Francia.valencia@udea.edu.co

Abstract

Background: Many native fermented foods in Latin America are made by indigenous communities through processes that preserve their traditions. Among these products is champús, a fermented drink or dessert native to Colombia, Ecuador, and Peru. It has a characteristic flavor between sweet and sour with a low alcohol content, is made from cereals such as corn, wheat, or a mixture of these, and can include fruits and spices.

Objectives: This research evaluated the microbiological, physicochemical, and sensory quality characteristics of champús to revalue the consumption of traditional foods.

Methods: For this purpose, the microbiological safety criteria, fermenting microorganisms, and physicochemical and sensory parameters were evaluated for two batches of champús prepared by five producers and taking samples at different stages.

Results: It was found that the average temperature and relative humidity for processing were between 16.5 ± 2.56 °C and 61.6 ± 9.14 %, respectively, and the products exceeded microbiological criteria such as total coliforms and some microbiological criteria for *Escherichia coli* and *Staphylococcus aureus*. There was also a high prevalence of lactic acid bacteria and yeasts throughout the process, with the occurrence of the genera *Weissella*, *Leuconostoc*, and *Lactobacillus*. The presence of organic acids (lactic, acetic, and propionic) and ethanol was evidenced. Champús seems to be a source of calcium, phosphorus, and free amino acids.

Conclusions: This information provides a basis to propose improvement plans for small producers and, at the same time, to conduct more detailed studies on the native microbiota of champús.

Keywords: Artisanal products, fermented food, indicator microorganisms, lactic acid bacteria, safety food.

Resumen

Antecedentes: Muchos de los alimentos nativos fermentados de América Latina son elaborados por las comunidades indígenas mediante procesos que preservan sus tradiciones. Entre estos productos se encuentra el “champús”, una bebida o postre fermentado originario de Colombia, Ecuador y Perú. Presenta un sabor característico entre dulce y ácido con un bajo contenido de alcohol, está elaborado a base de cereales como maíz, trigo o la mezcla de estos, y puede llevar entre sus ingredientes frutas y especias.

Objetivo: Esta investigación evaluó las características de calidad microbiológica, fisicoquímica y sensorial del champús para revalorizar el consumo de los alimentos tradicionales.

Métodos: Se evaluaron criterios de seguridad microbiológica, microorganismos fermentadores, parámetros fisicoquímicos y sensoriales de dos lotes de champús elaborados por 5 productores, tomando muestras en diferentes etapas de proceso.

Resultados: Como resultados, se encontró que la temperatura y la humedad relativa promedio de elaboración estuvieron entre $16,5 \pm 2,56$ °C y $61,6 \pm 9,14$ % y que los productos superaron los criterios microbiológicos como coliformes totales y algunos criterios microbiológicos como *Escherichia coli* y *Staphylococcus aureus*. También hubo una alta prevalencia de bacterias lácticas y levaduras a lo largo del proceso, identificándose los géneros *Weissella*, *Leuconostoc* y *Lactobacillus*. Se evidenció la presencia de ácidos orgánicos (láctico, acético y propiónico) y de etanol. El champús parece ser una fuente de calcio, fósforo y aminoácidos libres.

Conclusión: Esta información sirve de base para proponer planes de mejora para los pequeños productores y al mismo tiempo para realizar estudios más detallados sobre la microbiota nativa del champús.

Palabras clave: Alimento fermentado, seguridad alimentaria, productos artesanales, microorganismos indicadores, bacterias lácticas.

Received: 26/03/2022

Accepted: 03/08/2023

1. Introduction

Worldwide, the growing interest in the consumption of fermented foods has been noted, exploring low-cost traditionally fermented products containing an important source of nutrients, probiotic microorganisms, and help to improve the health and well-being of consumers. For this reason, many countries seek to rescue traditional foods and their consumption by exploring their therapeutic properties through scientific research that supports the importance of their consumption and incorporation into the diet (1-4).

Latin America is home to many indigenous communities that feature a wide range of fermented foods as part of their cultural principles and are symbolically preserved within their natural wealth and legacy. Some authors state that at least 21 indigenous foods are part of the daily preparations and those have been preserved in different cultures by recipes transferred from generation to generation (5). For this reason, some foods are considered human culinary heritage that maintain nutritional contribution to the population in providing essential macro and micronutrients for humans at any stage of growth (5-7).

Thus, traditional fermented foods have been elaborated from different matrices of local agricultural products, such as: sugar cane (*Saccharum officinarum*), cassava (*Manihot esculenta*), rice (*Oryza sativa*), coffee (*Coffea*), cocoa (*Theobroma cacao*) or corn (*Zea mays*) (8). Corn is one of the most widely used cereals to produce different types of indigenous fermented foods given its properties as a good substrate for the growing of different beneficial microorganisms. Some examples of these foods include: chicha (alcoholic fermentation), masa añeja (lactic fermentation) and champús (mixed fermentation), among others (9–11).

Champús is a traditional beverage or dessert mainly consumed Colombia, Ecuador, and Peru (8,12). It has a characteristic sweet to sour taste with a low alcohol content, and its preparation have some variations depending on each population; it is generally made from cereals such as corn, wheat or a mixture of these, however, may contain additional ingredients like pineapple (*Ananas comosus*), lulo (*Solanum quitoense*), panela honey (sugar cane), spices such as cinnamon (*Cinnamomum verum*), cloves (*Syzygium aromaticum*), vanilla (*Vanilla*), and aromatic plants such as orange tree (*Citrus X sinensis*) or arrayán (*Luma apiculata*) (7,9). Champús is made from raw corn kernels that go through a grinding process, water is added, and the corn is left to ferment at room temperature for a period of 24 to 48 h. At the end of the fermentation period, a second grinding process is carried out to obtain fine and homogeneous particles that are again left to ferment from 24 to 48 h. The resulting mixture is subjected to cooking, where ingredients such as spices and aromatic plants are added until the desired viscosity is reached. The name Champús comes from the Quechua "*Chapusca*" that means mixture, or "*Chapuy*" which means to stir or beat. Finally, the mixture is allowed to cool and, for consumption, panela honey and "*mote*" (from the Quechua: mut'i) are added (14).

In general, fermented corn preparations are characterized by low cost, easy making and the availability of raw materials at any time of the year (14). However, these products are produced following poorly controlled processes without appropriate preservation techniques. Fermented corn preparations are produced on a small scale and marketed locally. This is the reason why it is recommended to monitor their hygienic and sanitary quality to prevent the transmission of foodborne infections (12,15).

Therefore, the purpose of this research was to establish a baseline for the microbiological and physicochemical quality of champús traditionally made by indigenous populations of southwestern Colombia and to study the microbial communities occurring in these fermented products aiming to reappraise these traditional foods.

2. Materials and methods

2.1 Participation of producers and collection of samples of champús

The samples were provided from five artisanal producers of champús from the municipality of Córdoba (Nariño, Colombia), located in the extreme southwest of the country in the Andean region (0°51'12"N, 77°31'04"W). The producers agreed to participate voluntarily signing an informed consent form document.

Each producer made two batches of champús keeping their own methodologies during the process. During the elaboration of each batch, the environmental conditions of

temperature and relative humidity (T and % RH) were measured and four samples were taken for microbiological and physicochemical analysis at different stages of the process (EP): EP0 (the mixture of corn in water before starting fermentation), EP1 (the mixture at an intermediate time of fermentation, 48 h), EP2 (the mixture after finishing fermentation, after reaching pH <4) and EPf (final product). The samples were collected aseptically in whirl pack bags and frozen at -18 ± 2 °C and then sent in less than 48 hours to the laboratory of the Universidad de Antioquia (Medellín) for analysis.

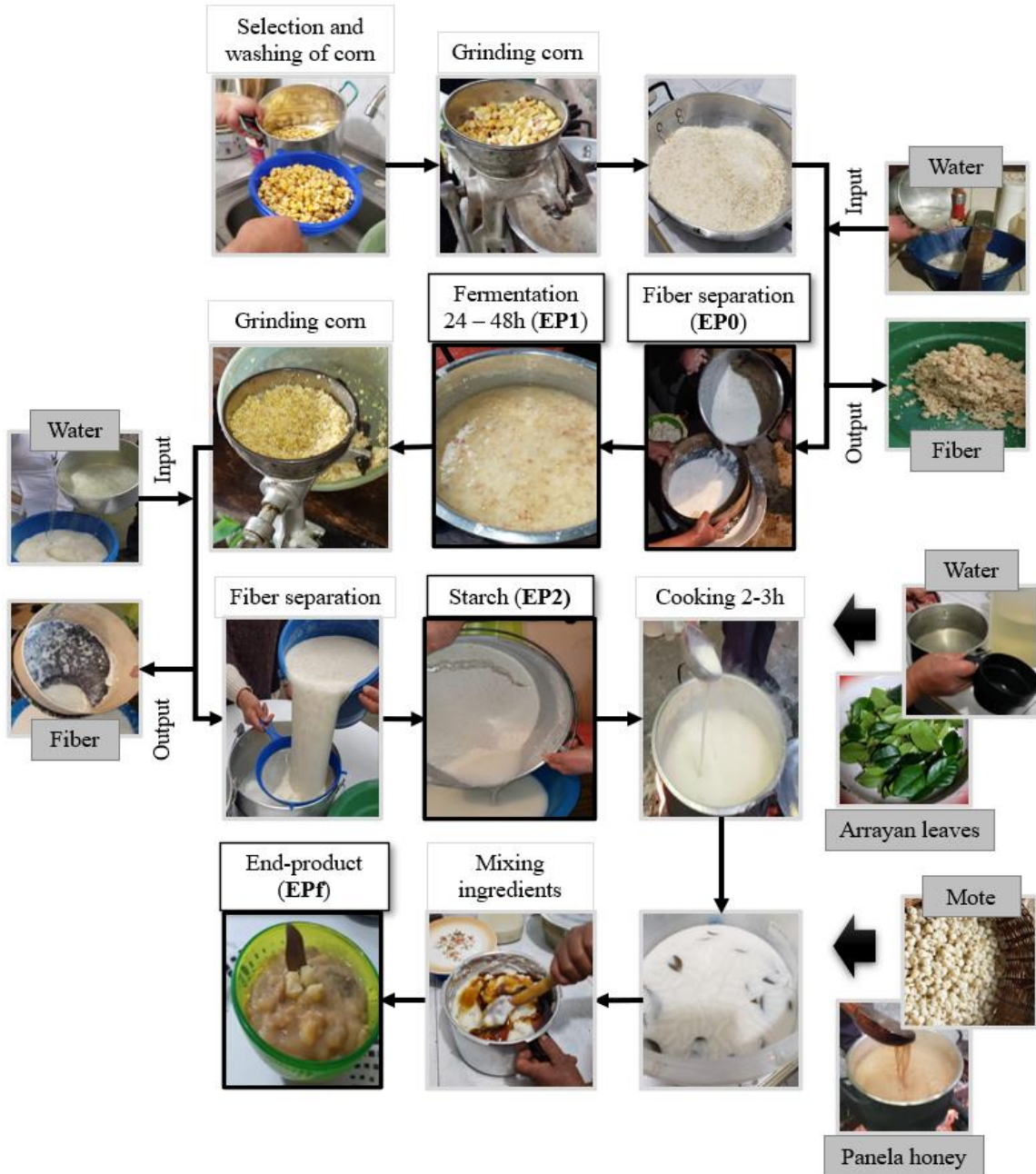


Figure 1: General flow diagram for the traditional production of champús.

2.2 Microbiological evaluation of champús

Each end product of champús (EPf) was evaluated for microbiological criteria: Total mesophilic counts (ISO 4833-2:2013), total coliforms (TC) and *E. coli* (ISO 4832:20068), *Staphylococcus aureus* (ISO 6888-3:2003), *Bacillus cereus* (ISO 7932:2004), molds and yeasts (M&Y) (ISO 6611:2004), as well as the test for the absence or presence of *Salmonella* spp. (ISO 6579-1:2017). In addition to these tests, surface seeding was performed for the count of acetic acid bacteria (AAB) using WL agar (Scharlau - Spain). After the corresponding incubation, the colonies were counted and expressed as Log CFU/g, for subsequent statistical analysis.

2.3 Lactic acid bacteria and yeast isolation

The abundance of lactic acid bacteria (LAB) and yeasts was evaluated during all stages of the process (EP0, EP1, EP2 and EPf). Culture media were used for LAB : Man Rogosa Sharpe (MRS - Oxoid - Spain) and M17 supplemented with 0.5 % lactose (Oxoid - Spain) and yeast extract - glucose - lactose - peptone - meat (YGLPB) prepared according to the instructions of the Spanish Type Culture Collection (CECT, <http://www.cect.org>). All media were supplemented with 100 µg/mL cycloheximide, and the Petri dishes were incubated at 30 ± 2 °C for 48 h in microaerophilic conditions. Yeast abundance was determined according to ISO 6611:2004 methodology. Finally, colony counts were performed and expressed as Log CFU/g.

From the LAB colonies obtained, those with different morphotypes were randomly selected and isolated on the agar from which they were initially obtained; after incubation under the above-mentioned conditions, those isolates that met the basic characterization of negative catalase, negative oxidase and positive Gram staining were considered presumptive LAB and preserved at - 80°C in a suitable broth containing 20 % glycerol. Molecular identification of LAB isolates was performed by amplification and sequencing of the 16S ribosomal gene using the primers 785F (3' GGA TTA GAT CCC TGG TA 5') and 907R (5' CCG TCA ATT CCT TTR AGT TT 3'). The 16S rRNA sequences were compared with references LAB from the NCBI GenBank using BLASTN algorithms (<http://www.ncbi.nlm.nih.-gov/blast>).

2.5 Physicochemical analysis of champú

The pH was measured in real time during the fermentation process, in at least one of the batches from each producer using a pH-meter manufactured by the Interfaculty Instrumentation Center of the Universidad de Antioquia. In addition, the samples collected at different times were assessed for titratable acidity, expressing the result as percentage of lactic acid (% w/v; ISO/TS 11869:2012).

The content of organic acids (lactic, acetic, and propionic), simple carbohydrates (glucose, fructose and maltose) and ethanol were determined by high performance liquid chromatography (HPLC) according to the methodology proposed by Valencia *et al.* (16). Soluble solids (°Brix) were measured by refractometry considering as a reference the Colombian Technical Standards (NTC 440:2015). CIEL*a*b* coordinates for color were evaluated on X-Rite SP-64 portable spectrophotometer with D65 illuminator and 2° observer with attached specular and 4 mm observation window (17). In addition, to determine the percentage of crude protein (Volumetric - Kjeldahl), free amino acids, ash

(ISO 5984:2002), moisture (NTC 2227) and the concentration of calcium, iron, zinc, phosphorus (UV-VIS spectrophotometric) a bromatological analysis was performed in the bromatological analysis laboratory at Universidad Nacional de Colombia (Medellín) (Regulatory document PRE-010 VOO2018) (18).

2.6 Sensory analysis

The samples that met the microbiological criteria were analyzed by quantitative descriptive analysis by five trained judges who evaluated the objective attributes of color, odor, flavor, and appearance, with a hedonic scale where excellent score = 10 and very poor score = 0.

2.7 Statistical analysis of data

The statistical difference between treatments was determined using the 95 % standard deviation limits. An analysis of variance (ANOVA) was performed using IBM SPSS version 25 and Statgraphics version 18 to compare the results of the counts among process stages and producers.

3. RESULTS

3.1 Microbiological evaluation of champús

A total of 40 samples were collected from the producers at different production times. The temperature and relative humidity during sampling were between $16.5 \pm 2.56^{\circ}\text{C}$ and $61.6 \pm 9.14\%$. The total coliforms (TC) were above the permitted limits ($< 1 \text{ Log CFU/g}$), considering as a reference the microbiological the Colombian Technical Standards NTC 805:2005 for dairy products and fermented milks, NTC 3594:2014 for milling products: precooked corn flour for human consumption and the resolution 1407 of 2022 where microbiological criteria are established that must be met by food and beverages destined for human consumption and which include fermented beverages whose alcohol content is less than or equal to 2.5 %. The average count of the Log CFU for *E. coli* in some champús were above the permitted limits ($< 1 \text{ Log CFU/g}$). For *S. aureus* all the champús met the established control limits for this microorganism ($< 2 \text{ Log CFU/g}$). The mold and yeast (M&Y) count averaged $4.43 \pm 2.45 \text{ Log CFU/g}$ and the acetic acid bacteria (AAB) showed an average of $6.18 \pm 2.37 \text{ Log CFU/g}$. None of the samples evaluated showed growth of *B. cereus* and *Salmonella* sp. (Figure 2).

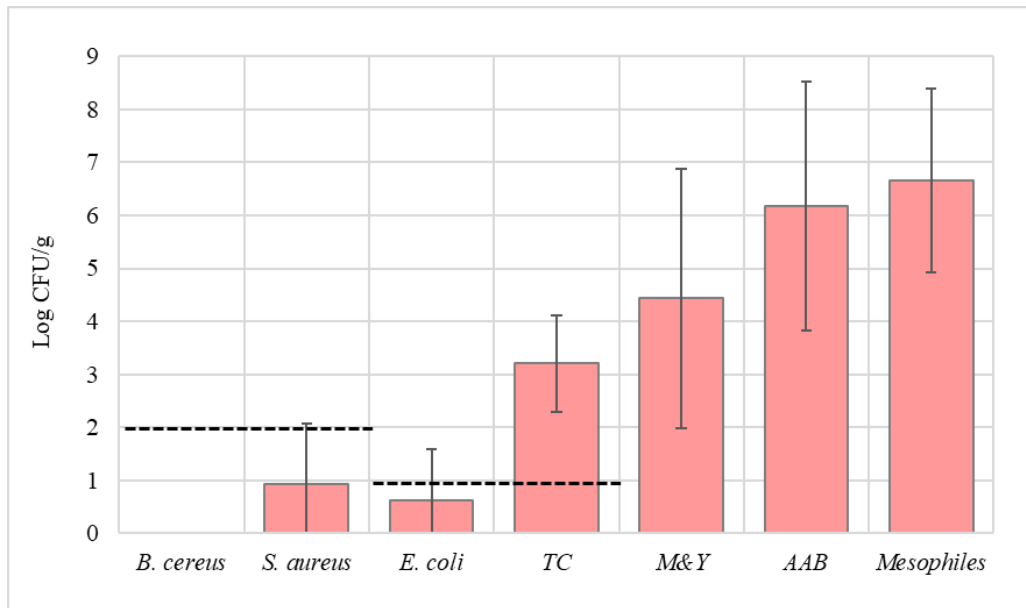


Figure 2. Averages and standard deviation of microorganism counts as indicators of food safety and quality, expressed in logarithms of CFU/g in the final products of champús. The dotted lines indicate the permitted limits by the Colombian Technical Standards NTC 805:2005 and NTC3594:2014.

3.2 Lactic acid bacteria and yeast isolation

The average LAB and yeast log CFU counts reveal the predominance of fermenting microorganisms during the process stages in which the samples were taken; these counts show greater variation for the initial and final time of the process (Figure 3). The average initial concentration of LAB was 5.1 ± 0.99 Log CFU/g and presented a significant increase ($p < 0.05$) in EP1, reaching the maximum cell concentration for the batch (8.9 ± 0.42 Log CFU/g). these LAB from EP1 did not exhibit statistically significant differences with EP2 ($p=0.068$). In EPf, they showed an average of 6.6 ± 0.3 Log CFU/g. On the other hand, the yeast counts show a greater dispersion of data in EP0 (<1 and 4.07 Log CFU/g) and in EPf (4.22 ± 1.42 Log CFU/g), obtaining statistically significant differences between EP0 ($p=0.000$) compared to EP1, EP2 and EPf that show homogeneity among the counts ($p=0.777$).

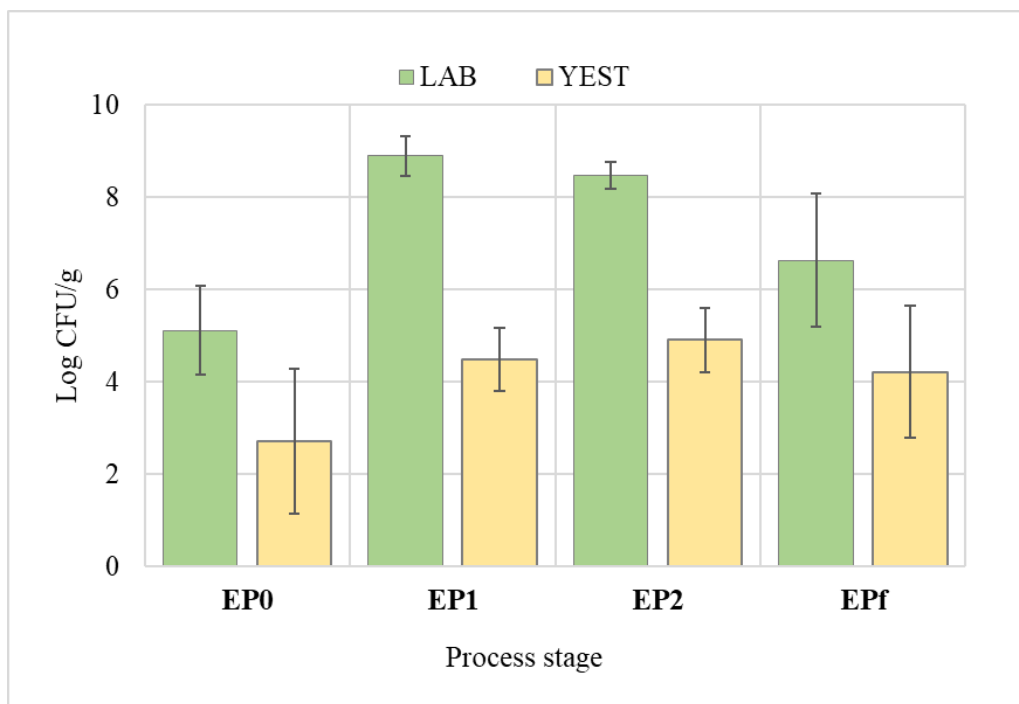


Figure 3. Averages and standard deviation expressed in Log of CFU/g of lactic acid bacteria and yeasts during each stage for fermentation process of champús.

From the samples taken at the different stages of the process, a total of 171 isolates were obtained among the five producers; these isolates met the basic criteria characteristic for LAB presumptive colonies. The percentages of isolates per producer were as follows: P1 23.98 %, P2 21.64 %, P3 15.2 %, P4 16.37 % and P5 with 22.81 %. For their identification, 50 isolates with antagonistic activity against pathogens were selected (Figure 4) and complied with their safety characterization (hemolysis, collagenase, lecithinase, and biogenic amines, these data were not shown). A fragment of ~ 1000 pb of the 16S rDNA gene was obtained and BLASTN results revealed the presence of *Weissella*, *Leuconostoc*, *Lactobacillus*, and *Lactococcus*, with a percentage of identity from 99,2 – 100 % and with a higher prevalence of *Weissella cibaria* (56 %) and *Leuconostoc mesenteroides* (14 %).

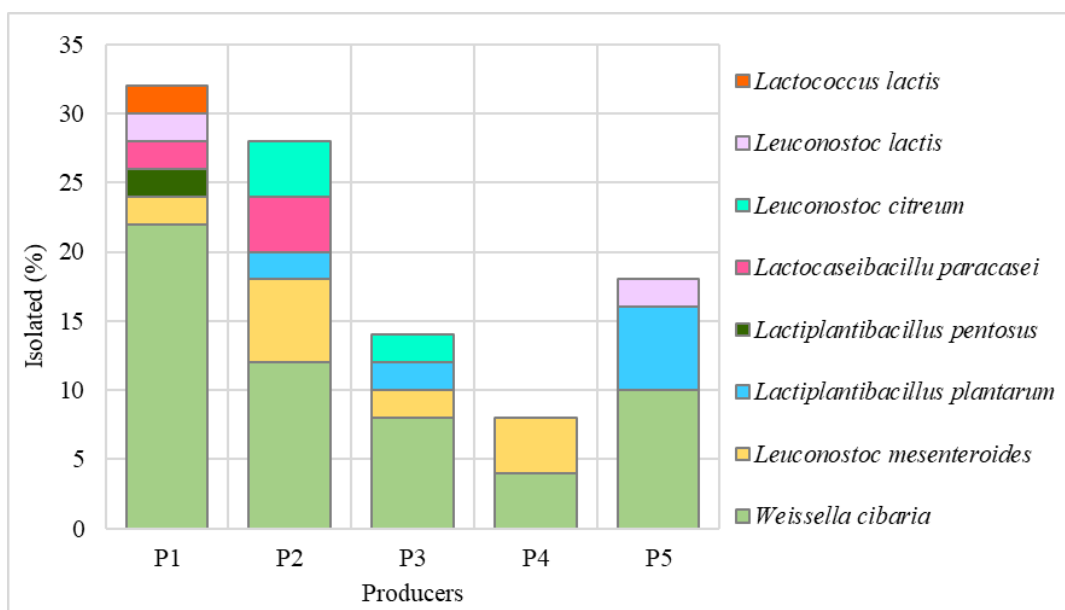


Figure 4. Identification and predominance of lactic acid bacteria isolated from champús by producer expressed as percentage.

3.3 Physicochemical analysis of champús

The values of pH and titratable acidity expressed as percentage of lactic acid (%LA) evaluated in different stages of the process are shown in Figure 5. Three homogeneous groups were observed during fermentation. The first group formed by the EP0 data with an average pH 6.02 ± 0.16 , the second group by the EP1 data and the third homogeneous group was formed by EP2 and EPf, reaching an average pH 3.82 ± 0.25 .

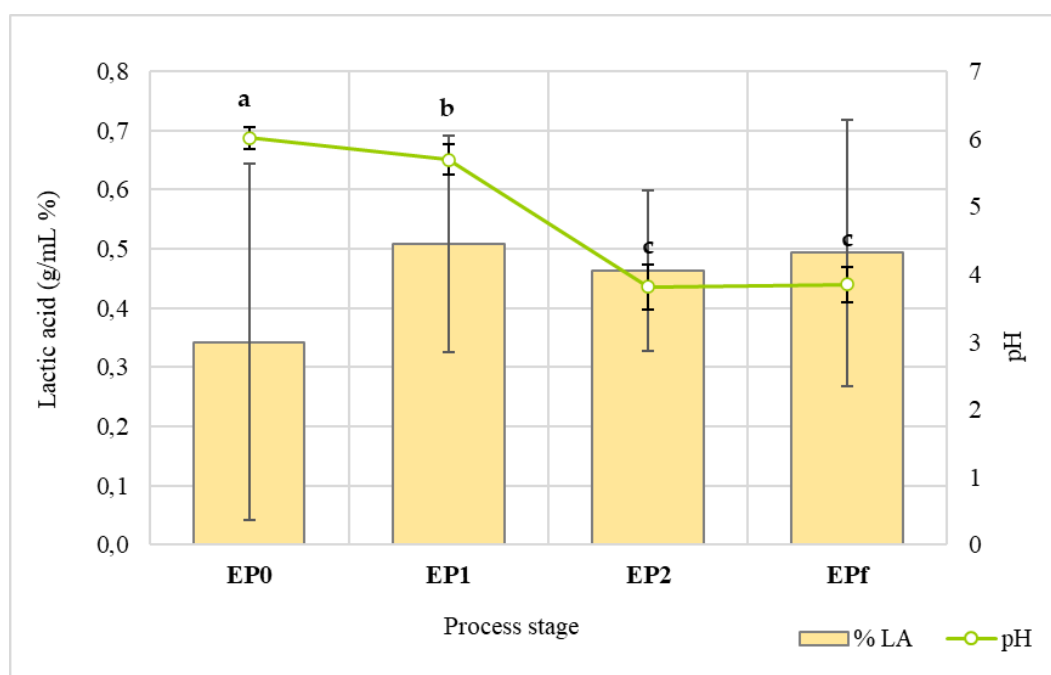


Figure 5. Averages and standard deviation of acidity expressed as percentage of g/mL of lactic acid and averages and standard deviation of pH during the different stages of the champús production process.

Regarding titratable acidity, no statistically significant differences were observed among the stages of the process ($p = 0.203$), although the EP0 data with an average acidity of 0.34 ± 0.3 % LA (g/mL) showed greater variation. In addition, although the pH barely varies between EP2 and EPf, the acidity tends to increase in the finished product, maintaining a high variation among samples.

The results of organic compounds measured on HPLC in EPf (Figure 6) confirmed the presence of fermentation metabolites. Lactic acid and ethanol had the highest concentration in the fermentation metabolite concentration test, followed by acetic acid and propionic acid, which was below the detection limit range in some test samples (< 0.05 g/L). The concentrations of these compounds showed a statistically significant difference between them ($p < 0.05$). In addition, a 2:1 ratio was observed between the means of lactic acid and acetic acid.

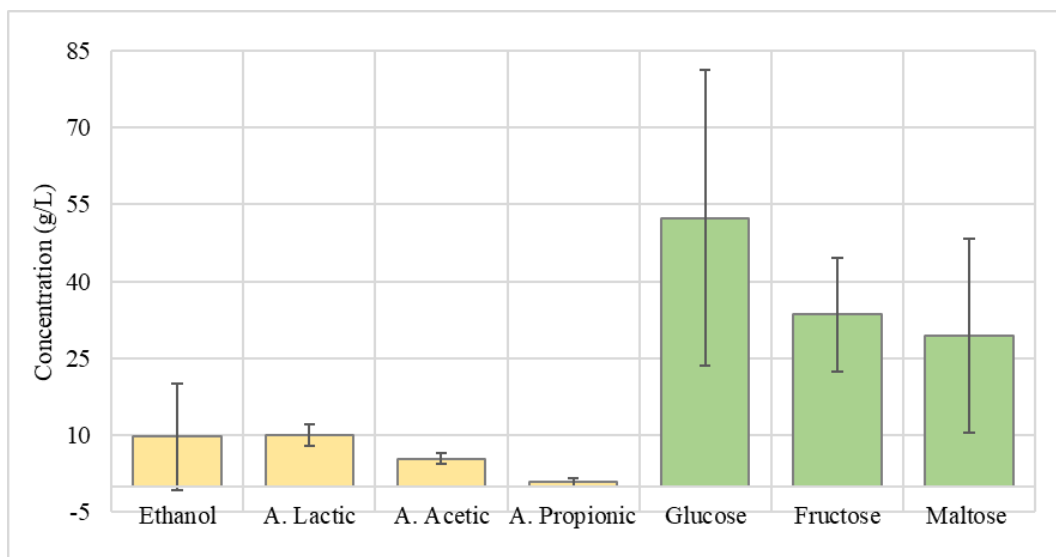


Figure 6. Concentration of metabolites of interest. Mean and standard deviation of ethanol, organic acids, and simple carbohydrates concentration expressed as (g/L).

Likewise, in ethanol production, a high variation was found among the data (from 2.8 to 27.58 g/L). Furthermore, the presence of glucose and maltose as a product of corn starch hydrolysis was also evidenced. The presence of fructose probably resulted from the addition of panela honey to the final product. All these sugars showed great variation in their concentrations.

The results of the bromatological analyses, mineral content, free amino acids, moisture percentage and soluble solids are presented in Table 1. The data obtained show that champús is a rich source of minerals and free amino acids.

Table 1. Physicochemical and bromatological characterization of the champús

Composition	Units	Mean \pm SD
Humidity	g/100g	76.73 \pm 5.10
Soluble solids	° Brix	16.67 \pm 3.90
Free amino acids	mM	0.68 \pm 0.23
Ashes	g/100g	0.27 \pm 0.07
Phosphorus	mg/kg	321.20 \pm 111.52
Calcium	mg/kg	119.20 \pm 73.97
Zinc	mg/kg	< 2.50
Protein	g/100g	< 2.50

Regarding color, the ΔE (1.86 ± 1.56) shows that color differences are not perceptible by the observer (< 2). Brightness (L^*) presented an average of 37 ± 2.70 with a predominance of yellow tones ($b^* = 11.62 \pm 1.51$ and $a^* = 1.43 \pm 0.51$).

Finally, the average of the data obtained in the sensory profile for four producers is shown in Figure 7. It can be observed that the intensity of the descriptors was below 6.15. The panelists highlighted descriptors such as honeycomb, sour, acidic flavors, and panela honey. Another descriptor named was spicy odor. In addition, there is evidence of variation between producers due to changes in the manufacturing processes and the lack of standardization in the amounts of raw materials added.

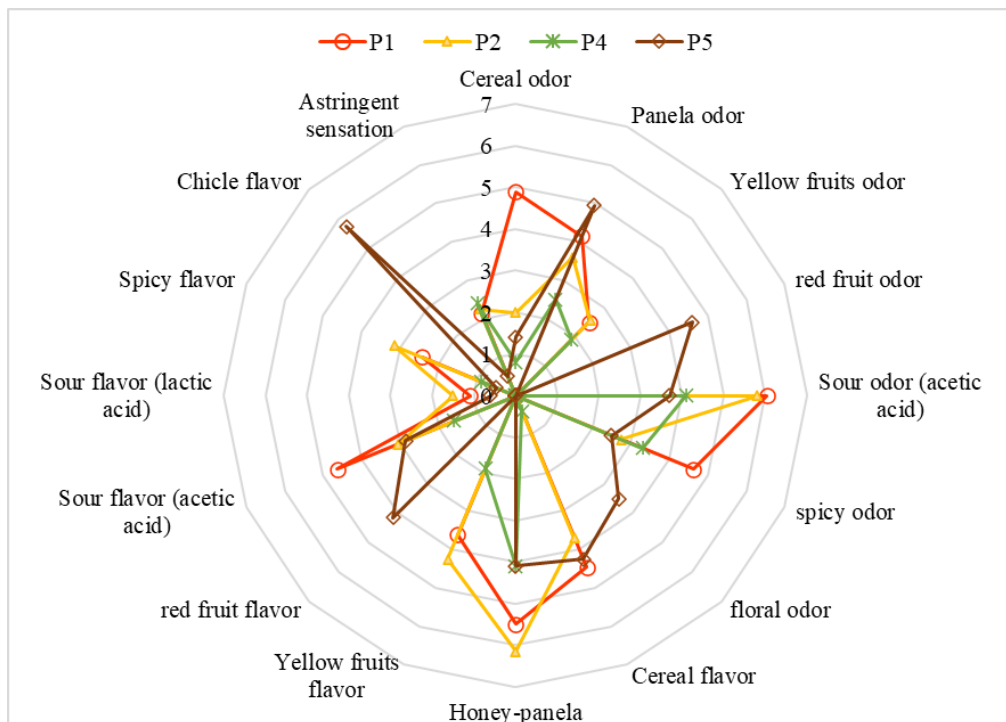


Figure 7. Average of the data obtained in the quantitative descriptive sensory analysis of champús.

4. Discussion

The results obtained in the microbiological criteria featured a high dispersion of the data, probably associated to the lack of standardization and contamination problems after the cooking process. This contamination also may be due to the deficiencies in the facilities (water used, utensils, wooden tables, or presence of insects) and the addition of ingredients that are not heat-treated, such as mote and panela honey. Fermented products with similar contaminations have been discussed by other authors in the artisanal production of champús (11, 14) and fermented rice beverages (16, 19, 20). The presence of microbiological safety criteria such as *S. aureus* and *E. coli* in a ready-to-eat product indicates that this food could be a vehicle for the transmission of foodborne toxoinfections. Consequently the presence of these microbial groups is undesirable and should be controlled (21).

It should be noted that there are no established microbiological criteria for fermented foods such as champús; this regulatory deficiency has been reported by other authors who have evaluated traditional fermented foods (8, 19, 22–24).

Regarding AABs, they are usually found in environments rich in carbohydrates, polyols or ethanol. They produce acetic acid which could explain the evidenced concentrations of this microbial group. Still, the role of these microorganisms is unclear in terms of functionality and microbiological interactions (25), but acid production reduces the presence of acid-sensitive coliforms and enterobacteria. In fact, producers with higher AAB counts are the ones that presented lower pathogen counts.

On the other hand, LAB presented similar counts compared to those found in studies on rice (19) and corn-based (26, 27) fermented foods and beverages. Variations in the results could be equally related to the use of utensils, water sources and others. These microorganisms are part of food systems and are of great importance in natural fermentation processes, since their survival capacity is related to the ease of metabolizing a wide variety of carbohydrates and adapting to stress conditions (e.g., low pH) (28).

In addition, it is observed that yeasts like LAB play an important role in the fermentation of champús since they can be found as autochthonous microbiota in corn (19). Thus, Osorio et al., in their research identified wild yeasts in champús, obtaining 235 isolates (22). This indicates that in fermentations made with cereals such as corn, rice, or wheat, yeasts are also part of the diverse microbiota of these foods, as mentioned by other authors (10, 29–31). The different treatments of the champús process contribute to the prevalence of some microbial species and the interactions favoring the coexistence of these species. Several studies have reported the presence of some microbial genera in fermented corn-based products, among which the more common genera are *Lactobacillus* (*L. plantarum* and *L. fermentum*), *Pediococcus*, *Leuconostoc* (*Lc. lactis*, *Lc. mesenterioides*, *Lc. citreum*), *Enterococcus*, *Lactococcus* and *Weissella* (*W. cibaria* and *W. confusa*) as dominant microorganisms in fermented foods from Latin America and Africa (15, 21, 26, 27). Some of these species were isolated from the final products in this study.

Indeed *W. cibaria* is a microorganism found in a wide variety of habitats, like soil and fresh vegetables and its presence is associated with the milling of corn kernels (20, 28). Its probiotic characteristics together with those of *Lc. citreum* were evaluated in a fermented food from Mexico where the ability of *W. cibaria* to inhibit the growth of pathogens such as *S. aureus*, *Salmonella enterica* biovar *Typhimurium* and *Listeria Monocytogenes* was identified (35). This ability is explained by the production of antimicrobial compounds such as bacteriocins and short-chain organic acids (formic, propionic and butyric acids) (36, 37).

Leuconostoc is part of the secondary (non-fermentative) microbiota in fermented vegetable foods (20). Additionally *Lc. citreum* is remarkable for its high production of short-chain fatty acids such as propionate and butyrate (29, 35, 38).

Other studies highlight that LAB belonging to the genera *Leuconostoc* and *Weissella* are exopolysaccharide producers, although this characteristic is strain dependent. This metabolite favors some physicochemical characteristics of the product such as viscosity and texture. Finally, *L. plantarum*, *L. lactis* and *W. cibaria* strains have been used as starters for beverage fermentation (39–41).

The pH results obtained in this research is similar to those in previous reports (4, 20, 31, 32, 34). Additionally, the acidity presented in the EPO could be related to the physicochemical characteristics of corn, which may contain a titratable acidity between 0.18 to 0.3 % depending on the corn variety. This acidity could be due to the organic acids present, and these are associated with the soil conditions during cultivation (43). Although the other stages of the process varied, they showed tendencies to increase the acidity concentration probably due to the degradation of the substrates and the production of organic acids resulting from the metabolism of the microorganisms present. Furthermore, the variation in these stages could be related to the presence of different types of microorganisms (Figure 3) (19, 30). Moreover, these differences could also be due to the partial dissociation of weak acids that may be in fermented products (44).

Otherwise, the process of making champús is fulfilled in several stages, beginning with the reduction of the particle size of the corn grains which are then hydrated for 24 h. Later fermentation begins with physicochemical changes experienced by champús. When the grains are soft, they undergo a second grinding where water is added. Finally, grinded

corn is boiled for 2.5 ± 0.5 h with a ratio of water and grinded corn of 2:1 until complete starch jellification is reached. These steps lead to physical and chemical changes during fermentation, in which both the washing and the water addition decrease the concentration of the produced acids and cause the longer acidification steps. This prevents the increase in percentage acidity and, at the same time act as selective agents of the microbiota responsible for fermentation and modification of the substrate (21, 34).

The proportions of the produced acid possibly provide to the product different sensory characteristics, e.g., lactic acid is sensed with a "mild acid" taste while acetic acid is closely related to unpleasant sensory characteristics such as sour tastes and odors (28,45). On the other hand, propionic acid production has been described as a final product produced by the metabolism of some microorganisms and it can inhibit growth and microbial activity of molds and some bacteria. Propionic acid is therefore used as a food preservative (37, 46, 47).

The presence of ethanol is closely related to the metabolism of yeasts and the variations in the percentage of ethanol could be related to the elevated counts of yeasts presented in the final products (Figure 2 and 3), since this group of microorganisms hydrolyzes starch into sugars for use in the ethanol production (7, 25, 41). This sugar consumption is associated with the lower glucose concentration found in some of the champús samples. Additionally heterofermentative LAB (*L. plantarum*, *L. pentosus*, *L. paracasei* and *Weissella* among others) are also present and they could metabolize glucose by the phosphoketolase metabolic pathway and produce lactic acid, CO₂ and ethanol (22, 42).

The bromatological results of the champús are related to the composition of corn, which contains minerals and vitamins that can change in concentration rate according to its genetic variety and the type of soil where it was planted. For example, the iron content was only evident in some of the champús samples (6 ± 1.4 mg/kg) made with white corn, providing 60 % of the daily food requirements in children between 4 and 8 years old (50, 51). On the other hand, the iron content of those made with yellow corn was below the detection limit (<5 mg/kg). Corn also stands out for being a good source of carbohydrates and digestible fiber, and although it is not a good source of protein, it provides free amino acids (FAA), with leucine being the most abundant according to studies and the most important in children between 9 and 13 years old (35, 43, 44).

It should be mentioned that within the LAB metabolism, the fermentation process induces the hydrolysis of proteins and peptides due to proteolytic enzymes transforming raw materials, increasing the concentration of FAA in the food through the metabolic pathway of amino acid metabolism (54), producing fatty acids. However, α -carboxylic metabolites involved in LAB metabolism, act as antimicrobial agents and confer specific organoleptic characteristics and biogenic amines that may be beneficial for LAB survival but are toxic for to human consumption (54–56).

Some studies have shown an increase in FAA in fermented foods compared to their controls. Ahmads *et. al.* evaluated fermented milks noting that the FAAs produced provide complexity towards the sensory attributes and could impart the kokumi flavor of the final product. Finally, amino acid metabolism involving glutamine, glutamic acid, and arginine helps LAB adapt to the acidic environment (54, 55, 57).

Additionally, champús has minerals such as calcium providing 10 % of the daily nutritional requirements for an adult (51, 58) and despite being in a low concentration, its consumption is of great importance. Especially, when a challenge has currently been evidenced in food safety by finding deficiencies in the consumption of this mineral, hence champús may promote the intake of calcium in the diet (59–63). Phosphorus provides up to 46 % of the daily nutritional requirements of phosphorus needed by children over 4 years of age and adults (51,64), and champús is a rich source of this mineral.

The chromatic coordinates of champús are also associated with the phytochemical properties of corn. Yellow corn contains a relatively larger number of carotenoids, anthocyanins and phenolic compounds than white corn (17, 65, 66). Besides, panela honey is added to the product at the time of consumption. Panela honey is obtained by the Maillard reaction that takes place when Panela (a sugar cane product) is submitted to high temperatures resulting in a brown and carbohydrate-rich syrup. When added, it contributes to the flavor and color of the champús (67).

In relation to sensory characteristics, these products have strong characteristic aromas and flavors that can sometimes be unpleasant for consumers. Many of these descriptors are related to metabolites formed during fermentation processes and included in raw materials used for their preparation, as well as the amounts used, the type of microorganisms found in greater abundance in the matrices and the processing and fermentation times.

Finally, the elaboration of handmade fermented foods like champús is susceptible to improvement without affecting tradition since some of these products are elaborated with poorly controlled processes without adequate technology.

5. Conclusions

Some of the products do not meet the microbiological quality criteria and there is a high variability in the physicochemical results. All this is related to the lack of standardization of the production processes and opens doors for further analysis where the combination of independent and dependent culture techniques may be used for a detailed and reliable investigation in difficult isolation microbial communities present during each of the fermentation processes of the champús.

Fermented foods despite having quality problems are a good source of beneficial microorganisms and nutrients that could become part of the regular diet of consumers.

These studies provide relevant information that can be used by the competent authorities to monitor and to improve production processes of champús, as well as to establish specific standards that include appropriate criteria for acceptance or rejection, ensuring the safety of this handmade fermented food. Finally, these studies also provide information that points to the isolation of microorganisms as defined starter cultures offering aggregated value to healthcare contribution.

Conflicts of Interest: The authors declare no conflict of interest.

Funding: This work was carried out within the framework of the project "*Plant foods with probiotic functionality for malnourished infant populations PROINFANT*", developed as a strategic project for international cooperation approved by the Ibero-American Program of Science and Technology for Development (CYTED) and funded by the Ministry of Science, Technology and Innovation of Colombia (Minciencias) contract 304 of 2018 and access to genetic resources and derived sub-products contract No 0126 of May 13, 2016 file RGE156-8 of the Ministry of Environment and Development of Colombia (Minambiente).

Author Contributions: Conceptualization, resources, supervision, project administration and funding acquisition F.G.V.; methodology, formal analysis, investigation, writing original draft Y.L.C. and F.G.V.; validation and data curation, Y.L.C.; software, Y.L.C. and N.R.E.; writing review and editing, Y.L.C., N.R.E. and F.G.V.; All authors have read and agreed to the published version of the manuscript.

Acknowledgments: We would also like to thank the Tecnoparque SENA nodo Medellín for the availability of its equipment and facilities.

References

1. Gu Q, Yin Y, Yan X, Liu X, Liu F, McClements DJ. Encapsulation of multiple probiotics, synbiotics, or nutrabiobiotics for improved health effects: A review. *Adv Colloid Interface Sci.* 2022; 309: 102781. DOI: <https://doi.org/10.1016/j.cis.2022.102781>
2. Qiao Y, Zhang K, Zhang Z, Zhang C, Sun Y, Feng Z. Fermented soybean foods: A review of their functional components, mechanism of action and factors influencing their health benefits. *Food Res Int.* 2022; 158: 111575. DOI: <https://doi.org/10.1016/j.foodres.2022.111575>
3. Srinivash M, Krishnamoorthi R, Mahalingam PU, Malaikozhundan B, Keerthivasan M. Probiotic potential of exopolysaccharide producing lactic acid bacteria isolated from homemade fermented food products. *J Agric Food Res.* 2023; 11: 100517. DOI: <https://doi.org/10.1016/j.jafr.2023.100517>
4. Das D, Wann SB, Kalita J, Manna P. Insight into the efficacy profile of fermented soy foods against diabetes. *Food Biosci.* 2023; 53: 102665. DOI: <https://doi.org/10.1016/j.fbio.2023.102665>
5. Meyer R, Duval A, Jensen H. Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New phytol.* 2012; 196: 29-48. DOI: <https://doi.org/10.1111/j.1469-8137.2012.04253.x>
6. Díaz L. Como los alimentos pueden prevenir o curar las enfermedades de la vida moderna. *Inf Tecnol.* 2015; 26(6): 1. DOI: <http://dx.doi.org/10.4067/S0718-07642015000600001>
7. Nazir M, Arif S, Khan RS, Nazir W, Khalid N, Maqsood S. Opportunities and challenges for functional and medicinal beverages: Current and future trends. *Trends Food Sci Technol.* 2019; 88: 513–526. DOI: <https://doi.org/10.1016/j.tifs.2019.04.011>
8. Väkeväinen K, Valderrama A, Espinosa J, Centurión D, Rizo J, Reyes-Duarte D, Díaz-Ruiz G, von Wrigth A, Elizaquível P, Esquivel K, Simontaival AI, Aznar R, Wachter C, Plumed-Ferrer C. Characterization of lactic acid bacteria recovered from atoleo agrio, a traditional Mexican fermented beverage. *LWT - Food Sci Technol.* 2018; 88: 109–118. DOI: <https://doi.org/10.1016/j.lwt.2017.10.004>
9. Chaves-Lopez C, Serio A, Delgado-Ospina J, Rossi C, Grande-Tovar CD, Paparella A. Exploring the bacterial microbiota of colombian fermented maize dough “Masa agria” (Maiz añejo). *Front Microbiol.* 2016; 7: 1168. DOI: <https://doi.org/10.3389/fmicb.2016.01168>
10. López-Arboleda WA, Ramirez-Castrillón M, Mambuscay-Mena LA, Osorio-Cadavid E. Diversidad de levaduras asociadas a chichas tradicionales de Colombia. *Rev Colomb Biotecnol.* 2010; 12(2): 176–186.

11. Osorio-Cadavid E, Chaves-López C, Tofalo R, Paparella A, Suzzi G. Detection and identification of wild yeasts in Champús, a fermented Colombian maize beverage. *Food Microbiol.* 2008;25(6):771–7. DOI: <https://doi.org/10.1016/j.fm.2008.04.014>
12. Alvarez B. Evaluación de procesos tecnológicos para contribuir a la competitividad de los alimentos autóctonos producidos en el bajo Sinú. [M.Sc. Thesis]. [Montería, Colombia]: Univerisidad de Cordoba: 2014. 252 p.
13. Faria-Oliveira F, Diniz RHS, Godoy-Santos F, Piló FB, Mezdari H, Castro IM, Brandão RL. The Role of Yeast and Lactic Acid Bacteria in the Production of Fermented Beverages in South America. In: Ayman E, editor. *Food Production and Industry*. IntechOpen. Egipto; 2015. Chapter 4. Available from: <http://dx.doi.org/10.5772/60877>
14. Ojeda A, Ubillús B. Formulación y obtención de champús deshidratado, a base de maíz (*Zea mays*) y harina de quinua (*Chenopodium quinoa wild*). [Grade work]. [Lambayeque, Perú]: Universidad Nacional Pedro Ruiz Gallo: 2015. 133p.
15. Becerra M. Bebidas fermentadas a partir de maíz y arroz: elaboración, control y conservación. *Aliment hoy.* 2014; 22(1): 96–103.
16. Valencia GF, Palacios AP, López CY. Base line to provide added value to fermented handcrafted rice based products “Masato.” *Ital J food Sci.* 2019; (Special):79–89.
17. Valencia García FE, Cortés Rodríguez M, Román Morales MO. Cinética del color durante el almacenamiento de caramelos blandos de uchuva adicionados de calcio y sin sacarosa. *Rev Lasallista Investig.* 2012; 9(2): 11–25.
18. León Niño JC. Métodos de ensayo utilizados en la determinación de amino nitrógeno libre (NFA) en materiales que cursan o están destinados a procesos de fermentación alcohólica. [Grade work]. [Bogotá, Colombia]: Universidad Nacional Abierta y a Distancia - UNAD; 2021. 157p.
19. 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. DOI: <https://doi.org/10.1016/j.fm.2014.08.009>
20. Ramos CL, de Almeida EG, Freire AL, Freitas Schwan R. Diversity of bacteria and yeast in the naturally fermented cotton seed and rice beverage produced by Brazilian Amerindians. *Food Microbiol.* 2011; 28(7): 1380–1386. DOI: <https://doi.org/10.1016/j.fm.2011.06.012>
21. Chaves-López C, Rossi C, Maggio F, Paparella A, Serio A. Changes Occurring in Spontaneous Maize Fermentation: An Overview. *Fermentation.* 2020; 6(1): 36. DOI: <https://doi.org/10.3390/fermentation6010036>
22. Byakika S, Mukisa IM, Byaruhanga YB, Male D, Muyanja C. Influence of food safety knowledge, attitudes and practices of processors on microbiological quality of commercially produced traditional fermented cereal beverages, a case of Obushera in Kampala. *Food Control.* 2019; 100: 212–219. DOI: <https://doi.org/10.1016/j.foodcont.2019.01.024>
23. Ntuli V, Mekibib SB, Molebatsi N, Makotoko M, Chatanga P, Asita A. Microbial and Physicochemical Characterization of Maize and Wheat Flour from a Milling

- Company, Lesotho. *Internet J Food Saf.* 2013; 15: 11–19.
24. Shankar I, Usha A. Assessment of the microbiological quality of koozh, a fermented millet beverage. *African J Microbiol Res.* 2014; 8(3): 308–312. DOI: <https://doi.org/10.5897/AJMR2013.6482>
 25. De Roos J, De Vuyst L. Acetic acid bacteria in fermented foods and beverages. *Curr Opin Biotechnol.* 2018; 49: 115–119. DOI: <https://doi.org/10.1016/j.copbio.2017.08.007>
 26. Elizaquível P, Pérez-Cataluña A, Yépez A, Aristimuño C, Jiménez E, Cocconcelli PS, Vignolo G, Aznar R. 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. DOI: <http://dx.doi.org/10.1016/j.ijfoodmicro.2014.12.027>
 27. Jiménez E, Yépez A, Pérez-Cataluña A, Ramos Vásquez E, Zúñiga Dávila D, Vignolo G, Aznar R. Exploring diversity and biotechnological potential of lactic acid bacteria from tocosh - traditional Peruvian fermented potatoes - by high throughput sequencing (HTS) and culturing. *LWT - Food Sci Technol.* 2018; 87: 567–74. DOI: <https://doi.org/10.1016/j.lwt.2017.09.033>
 28. Salmerón I, Thomas K, Pandiella SS. Effect of substrate composition and inoculum on the fermentation kinetics and flavour compound profiles of potentially non-dairy probiotic formulations. *LWT - Food Sci Technol.* 2014; 55(1): 240–247. DOI: <https://doi.org/10.1016/j.lwt.2013.07.008>
 29. Chaves-López C, Serio A, Grande-Tovar CD, Cuervo-Mulet R, Delgado-Ospina J, Paparella A. Traditional fermented foods and beverages from a microbiological and nutritional perspective: the Colombian heritage. *Compr Rev Food Sci Food Saf.* 2014; 13(5): 1031–1048. DOI: <https://doi.org/10.1111/1541-4337.12098>
 30. Ramos CL, de Almeida EG, Pereira GV, Cardoso PG, Dias ES, Schwan RF. Determination of dynamic characteristics of microbiota in a fermented beverage produced by Brazilian Amerindians using culture-dependent and culture-independent methods. *Int J Food Microbiol.* 2010; 140(2–3): 225–231. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2010.03.029>
 31. 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. DOI: <https://doi.org/10.1016/j.fm.2017.05.007>
 32. Blandino A, Al-Aseeri ME, Pandiella SS, Cantero D, Webb C. Cereal-based fermented foods and beverages. *Food Res Int.* 2003; 36(6): 527–43. DOI: [https://doi.org/10.1016/S0963-9969\(03\)00009-7](https://doi.org/10.1016/S0963-9969(03)00009-7)
 33. Motato KE, Milani C, Ventura M, Valencia FE, Ruas-Madiedo P, Delgado S. Bacterial diversity of the Colombian fermented milk “Suero Costeño” assessed by culturing and high-throughput sequencing and DGGE analysis of 16S rRNA gene amplicons. *Food Microbiol.* 2017; 68: 129–136. DOI: <https://doi.org/10.1016/j.fm.2017.07.011>
 34. Escobar Gutiérrez P, Turriago Borrero C, Pineda Ruiz DM, Holguín Posada JS,

Ledesma Ríos N. Índice de Desnutrición Crónica 2020. Envigado, Colombia; 2020 [cited 2021 Jun 3].

35. Silva MS, Ramos CL, González-Avila M, Gschaedler A, Arrizon J, Schwan RF, Dias DR. Probiotic properties of *Weissella cibaria* and *Leuconostoc citreum* isolated from tejuino – A typical Mexican beverage. *LWT - Food Sci Technol.* 2017; 86: 227–232. DOI: <https://doi.org/10.1016/j.lwt.2017.08.009>
36. Heredia-Castro PY, Hernandez-Mendoza A, Gonzalez-Córdoba AF, Vallejo-Cordoba B. Bacteriocinas de bacterias ácido lácticas: mecanismos de acción y actividad antimicrobiana contra patógenos en quesos. *Interciencia.* 2017; 42(6): 340–346.
37. Özcelik S, Kuley E, Özogul F. Formation of lactic, acetic, succinic, propionic, formic and butyric acid by lactic acid bacteria. *LWT Food Sci Technol.* 2016; 73: 536–542. DOI: <https://doi.org/10.1016/j.lwt.2016.06.066>
38. Assouhoun-Djeni NMC, Djeni NT, Messaoudi S, Lhomme E, Koussemon-Camara M, Ouassa T, Chobert J-M, Onno B, Dousset X. Biodiversity, dynamics and antimicrobial activity of lactic acid bacteria involved in the fermentation of maize flour for doklu production in Côte d’Ivoire. *Food Control.* 2016; 62: 397–404. DOI: <https://doi.org/10.1016/j.foodcont.2015.09.037>
39. Coda R, Rizzello CG, Trani A, Gobbetti M. Manufacture and characterization of functional emmer beverages fermented by selected lactic acid bacteria. *Food Microbiol.* 2011; 28(3): 526–536. DOI: <https://doi.org/10.1016/j.fm.2010.11.001>
40. Han J, Zhang J, Lin X, Liang H, Li S, Yu C, Zhu Beiwei, Ji Chaofan. Effect of autochthonous lactic acid bacteria on fermented Yucha quality. *LWT-Food Sci Technol.* 2020;123:109060. DOI: <https://doi.org/10.1016/j.lwt.2020.109060>
41. Santos CC, Almeida EG, Melo GV, Schwan RF. Microbiological and physicochemical characterisation of caxiri, an alcoholic beverage produced by the indigenous Juruna people of Brazil. *Int J Food Microbiol.* 2012;156(2):112–121. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2012.03.010>
42. Chaves-López C, Tofalo R, Serio A, Paparella A, Sacchetti G, Suzzi G. Yeasts from Colombian Kumis as source of peptides with Angiotensin I converting enzyme (ACE) inhibitory activity in milk. *Int J Food Microbiol.* 2012;159(1):39–46. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2012.07.028>
43. Salvador-Reyes R, Rebellato AP, Lima Pallone JA, Ferrari RA, Clerici MTPS. Kernel characterization and starch morphology in five varieties of Peruvian Andean maize. *Food Res Int.* 2021; 140: 110044. DOI: <https://doi.org/10.1016/j.foodres.2020.110044>
44. Fennema O, Tannenbaum S. Introducción a la química de los alimentos. In: Damodaran S, Parkin KL, Fennema O, editors, *Química de los alimentos.* third edition. Barcelona: Editorial Acribia. 2010. Chapter 1.
45. Almeida EG, Rachid CC, Schwan RF. Microbial population present in fermented beverage “cauim” produced by Brazilian Amerindians. *Int J Food Microbiol.* 2007; 120(1–2): 146–151. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2007.06.020>
46. Chen L, Yuan XJ, Li JF, Wang SR, Dong ZH, Shao T. Effect of lactic acid bacteria and propionic acid on conservation characteristics, aerobic stability and in vitro gas

- production kinetics and digestibility of whole-crop corn based total mixed ration silage. *J Integr Agric.* 2017; 16(7): 1592–1600. DOI: [https://doi.org/10.1016/S2095-3119\(16\)61482-X](https://doi.org/10.1016/S2095-3119(16)61482-X)
47. Peirotén Á, Gaya P, Álvarez I, Landete JM. Production of O-desmethylangolensin, tetrahydrodaidzein, 6'-hydroxy-O-desmethylangolensin and 2-(4-hydroxyphenyl)-propionic acid in fermented soy beverage by lactic acid bacteria and *Bifidobacterium* strains. *Food Chem.* 2020; 318: 126521. DOI: <https://doi.org/10.1016/j.foodchem.2020.126521>
48. López Miras MM. Identificación y caracterización de comunidades microbianas presentes en pinturas sobre lienzo. Estudio de su capacidad como agentes de biodeterioro. [Doctoral Thesis]. [Granada, España]: Universidad de Granada: 2011. 263p
49. Yépez Latorre A. Potencial biotecnológico de bacterias lácticas aisladas de productos fermentados de Latinoamérica y su aplicación en alimentos funcionales. [Doctoral Thesis]. [Valencia, España]: Universidad de Valencia: 2018. 246p
50. National institutes of health. Iron- Fact Sheet for Health Professionals. <https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/> 2021 (accessed 14 de abril de 2021)
51. Ministerio de salud y protección social. Resolución No. 810de 2021. Available from: https://minsalud.gov.co/Normatividad_Nuevo/Resolución No. 810de 2021.pdf
52. Dongmo H, Tambo ST, Teboukeu GB, Mboukap AN, Fotso BS, Tekam Djuidje MC, Klang JM. Effect of process and variety on physico-chemical and rheological properties of two corn flour varieties (Atp and Kassai). *J Agric Food Res.* 2020; 2: 100075. DOI: <https://doi.org/10.1016/j.jafr.2020.100075>
53. Loy DD, Lundy EL. Nutritional properties and feeding value of corn and its coproducts. In Serna-Saldivar SO, editor. *Corn: Chemistry and Technology*, Third edition. AACC International Press; 2019. Chapter 23. Available from: <https://doi.org/10.1016/B978-0-12-811971-6.00023-1>
54. De Souza EL, de Oliveira KÁR, de Oliveira MEG. Influence of lactic acid bacteria metabolites on physical and chemical food properties. *Curr Opin Food Sci.* 2023; 49: 100981. DOI: <https://doi.org/10.1016/j.cofs.2022.100981>
55. Gao X, Li C, He R, Zhang Y, Wang B, Zhang Z-H, Ho C-T. Research advances on biogenic amines in traditional fermented foods: Emphasis on formation mechanism, detection and control methods. *Food Chem.* 2023; 405: 134911. DOI: <https://doi.org/10.1016/j.foodchem.2022.134911>
56. Yi Y, Li P, Zhao F, Zhang T, Shan Y, Wang X, Lui B, Chen Y, Zhao X, Lü X. Current status and potentiality of class II bacteriocins from lactic acid bacteria: structure, mode of action and applications in the food industry. *Trends Food Sci Technol.* 2022; 120: 387–401. DOI: <https://doi.org/10.1016/j.tifs.2022.01.018>
57. Ahmad R, Dalziel JE, Nguyen HTH, Rounce J, Day L, Maes E. Investigation of free amino acids in lactic acid bacteria fermented milk and their ability to activate the calcium sensing receptor. *Int Dairy J.* 2023; 141: 105568. DOI: <https://doi.org/10.1016/j.idairyj.2022.105568>
58. National institutes of health. Calcium -Fact Sheet for Health Professionals.

<https://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/> 2021 (accessed 14 de abril de 2021)

59. Nguyen B, Murimi MW. Lack of calcium rich foods in the diet, low knowledge on calcium level recommendations and severe food insecurity predicts low calcium intake among Vietnamese women. *Appetite*. 2021; 163: 105242. DOI: <https://doi.org/10.1016/j.appet.2021.105242>
60. Shokunbi OS, Adepoju OT, Ramaite IDI, Shokunbi OS, Mojapelo PEL, Akinyele IO. Potassium, sodium, calcium and magnesium levels of commonly consumed foods and estimates of dietary intakes of selected Nigerian adults. *Heliyon*. 2023; 9(3): e13729. DOI: <https://doi.org/10.1016/j.heliyon.2023.e13729>
61. Muscariello R, Rendina D, Giannettino R, Ippolito S, Romano O, Coretti F, De Vita S, Martino M, Sepe C, Nuzzo V; ASL NA1 GP Network Researchers. Calcium daily intake and the efficacy of a training intervention on optimizing calcium supplementation therapy: A clinical audit. *Nutr Metab Dis*. 2021; 31: 354–360. DOI: <https://doi.org/10.1016/j.numecd.2020.08.005>
62. Vallinoto P, Moreira EG, Maihara VA. Estimation of daily dietary intake of essential minerals and trace elements in commercial complementary foods marketed in Brazil. *Food Chem Adv*. 2022; 1: 100039. DOI: <https://doi.org/10.1016/j.focha.2022.100039>
63. Bacchetta J, Edouard T, Laverny G, Bernardor J, Bertholet-Thomas A, Castanet M, Garnier C, Gennero I, Harambat J, Lapillonne A, Molin A, Naud C, Salles JP, Laborie S, Tounian P, Linglart A. Vitamin D and calcium intakes in general pediatric populations: A French expert consensus paper. *Arch Pédiatrie*. 2022; 29(4): 312–25. DOI: <https://doi.org/10.1016/j.arcped.2022.02.008>