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PHYSICOCHEMICAL, MICROBIOLOGICAL AND ORGANOLEPTIC CHARACTERIZATION OF AGUAMIEL AND PULQUE FROM ALTO MEZQUITAL, HIDALGO

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ABSTRACT

The production of pulque in the Alto Mezquital region, Hidalgo, represents an important source of economic income among the producers of maguey xamini *(Agave salmiana)*. This drink is mainly made by adults in a traditional way. Aguamiel (*Agave sap*) has high protein content and pulque, due to its composition, enables the growth of probiotic bacteria that has both high nutritional and even medicinal value. The objective of this study was to carry out physicochemical, microbiological and organoleptic characterization of aguamiel and pulque from the Alto Mezquital region, and to document the process used for the extraction of aguamiel and the elaboration of pulque. Producers who make pulque in a traditional way were identified, and an interview was applied in order to learn about the processes for making the drink; then samples were taken for laboratory analysis. As part of the results, the process of making pulque in the study region was documented. Physicochemical analyzes allowed pulque to be classified according to the quality standards with reference NMX-V-037-1972. In the case of aguamiel, the reference was NMX-V-022-1972, which meant it was categorized as quality I. The amount of yeasts and lactic acid bacteria in aguamiel, seed (pulque starter), punta (first extraction) and commercial pulque was evaluated. Finally, sensory evaluation made it possible to characterize the organoleptic properties of the pulque from Alto Mezquital. It has thus been concluded that aguamiel and pulque from the study region have high nutritional value, with ideal characteristics for use in the food, biotechnological and medicinal industries.

Keywords: nutritional value, parameters, quality, sensory evaluation, standards.

INTRODUCTION

Pulque is a white Mexican alcoholic beverage, with a strong and putrid smell, produced from the fermentation of the sugary sap of the maguey xamini *(Agave salmiana)*, known as aguamiel, in which microorganisms fluctuate in abundance and diversity for the advancement of this process (Chacón *et al.*, 2020). Archaeological evidence suggests that it was part of the diet of ancient Teotihuacán (150 BC to 650 AD) (Correa *et al.*, 2014); several historians consider that this alcoholic beverage is the oldest in Mexico, and that the Otomi culture was the first to produce it around the year 2000 BC, (Valadez *et al.*, 2012). The first systematic studies carried out on the nutritional benefits of pulque were undertaken among a population of regular consumers, who form part of

the indigenous-Otomí population in the Valley of Mezquital, Hidalgo, where pulque is the second most important food after the tortilla, underlining the contribution it makes in terms of vitamin C, proteins, calories, amino acids, calcium and iron (Anderson *et al.*, 1946). Within this region, pulque is used during lactation and postpartum as a source of nutrients and energy. Currently, pulque is a popular drink; its consumption varies according to age and type of consumer, and currently it has attracted special interest due to its nutritional properties.

In recent years, special interest has evolved to define the nutritional and functional value of aguamiel and pulque, because they contain very varied substances. Several authors have analyzed both drinks in order to assess their nutritional contribution; the main components of pulque include: saccharide, pulp, ashes, pantothenic acid, thiamin, 4-aminobenzoic acid, pyridoxine, biotin, ascorbic acid, riboflabin, protein (Escalante *et al.*, 2008; Tovar *et al.*, 2008), amino acids (isoleucine, leucine, lysine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, methionine and histidine) (Morales de León *et al.*, 2005; Ortiz *et al.*, 2008). Among components of aguamiel are: crude fiber, proteins, highly digestible carbohydrates, minerals (N, Ca, P, Mg, Fe, Zn, Cu and B), inulin, fructans, sugars (saccharide, fructose, glucose and fructooligosaccharides) (Velázquez *et al.*, 2014). Due to the large number of food components in aguamiel and pulque, it attracts great interest, in addition to presenting a low glycemic index and beneficial effects on health (Santos *et al.*, 2016). Similarly, aguamiel and pulque are a source of microorganisms with high biotechnological potential, as their nutritional, pro and prebiotic properties confer benefits on the human digestive system, such as acting as reducers of enzymes involved in gastric cancer as well as being antimicrobials (Castro *et al.*, 2015a; Correa *et al.*, 2014). The pulque production process involves three fermentations: acid, alcoholic and viscous; the microorganisms involved have potential as transporters of sugar, hydrolytic enzymes, exopolysaccharides, lactic acid or ethanol producers, which can be isolated from this beverage (Escalante *et al.*, 2012). The microorganisms involved in fermentation are found naturally in aguamiel and are the same ones that prevail during the collection, transport, inoculation and treatment, among which the most common are; various species of yeasts and bacteria, including homo- and heterofermentative lactic acid bacteria, alcohol-producing bacteria *Zymomonas mobilis*, and the dextran-producing bacteria *Leuconostoc mesenteroides* (Castro *et al.*, 2015a). Recently this has been recognized as a probiotic product, due to the presence of a large population of lactic acid bacteria such as *Lactobacillus acidophilus*, *Leuconostoc mesenteroides, Zymomonas mobilis* (Castro *et al.*, 2015b), due to these characteristics, it is considered as a food with high nutritional value; the combination of macro and micronutrients, together with lactic acid bacteria give it pre and probiotic properties (Correa *et al.*, 2014; Escalante *et al.*, 2012).

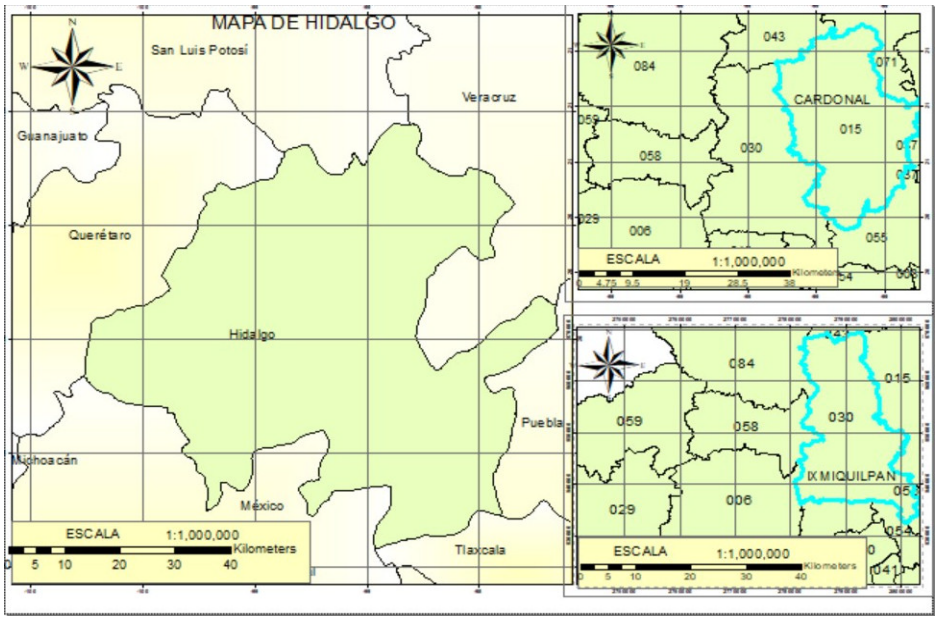
The traditional way of making pulque consists of adding a small amount of mature product or seed (previously produced starter) at the beginning of a new fermentation, which enables the reproduction of these beneficial microbial communities and their metabolic transformation into food and drink (Wolfe and Dunton, 2015). The time for the alcoholic

fermentation process varies from a few to 48 hours, at an average temperature of 24 °C, depending on the quantity and quality of the aguamiel harvested. This process has concluded once foaming ceases and acetic fermentation begins, when a dense layer becomes visible (Escalante *et al*., 2016; Lappe *et a*l., 2008). The quality of viscosity is produced by the synthesis of exopolysaccharides and has been the main criterion to determine degree of fermentation, to produce either fresh or mature pulque, as required; these processes do not include the addition of preservatives (Valadez *et al*., 2012). The process has concluded when the beverage reaches the alcoholic strength, density, refractive index, total solids, sugars, pulp and total acidity –as defined by that established in the NMX-V-037/Secretary of Commerce and Industrial Development (SECOFI) (SECOFI-1972b).

Despite the fact that aguamiel and pulque are beverages with characteristics and properties of interest, there is little documented information regarding the production process typical of different regions, where they still continue to make this beverage in a traditional way, as well as the factors that give rise to the organoleptic characteristics of the drink, such as the plant itself and the agroclimatic conditions where it grows. The Alto Mezquital region and local Otomí culture preserve this knowledge concerning the elaboration of pulque, which is why it is important to rescue it and document these processes. The investigation contemplates aspects of the aguamiel extraction process and the elaboration of pulque, including physicochemical, microbiological and organoleptic analyses. As is well known, the gastronomic heritage that includes maguey, aguamiel and pulque are important as cultural heritage, however, this has been devalued and displaced by other beverages. One of the factors that has had negative impact on the consumption of pulque is lack of dissemination concerning its nutritional properties. That is why the present work aims to describe the process of making pulque made in the Alto Mezquital region and analyze its physicochemical, microbiological and organoleptic characteristics. The hypothesis put forward here, is that aguamiel and pulque produced in the Alto Mezquital have characteristics and properties of interest, due to their high nutritional value and as a nutraceutical food.

MATERIALS AND METHODS

The study region was Alto Mezquital (Ixmiquilpan and Cardonal), Hidalgo (Figure 1). The first stage of the study consisted of identifying the main producers of pulque, who elaborate the process in a traditional way; 20 producers. Due to the fact that the state of Hidalgo does not have a register of producers, a visit was made to the municipal authorities in order to attain information on their pulque producers. Those who had the most experience were selected according to the criteria established during the interview, consisting of a questionnaire of 20 questions, which mainly focused on knowledge about the characteristics and preparation of the plant for the extraction of aguamiel and the elaboration of pulque. The second stage was to collect the pulque samples in triplicate from the 20 producers, in 200 mL screw cap shott bottles, 50 mL was studied for each stage of the process; aguamiel (liquid extracted from the maguey



Source: self elaboration.
Figure 1. Map showing the location of the municipalities of Cardonal and Ixmiquilpan, Hidalgo.

strain), seed (starter, fermented for at least 4 days), punta (result of aguamiel and pulque 1:1 after 24 hours) and commercial pulque (pulque after 48 hours). The samples were taken in compliance with the provisions of the Official Mexican Standard NOM-SSA1-109-1994 (Goods and services) and transported to the laboratory for analysis. The physicochemical, microbiological and organoleptic analyzes were carried out at the Universidad Technológico del Valle de Mezquital (UTVM), complying with the following conditions.

Determination of Brix degrees. This was carried out using a refractometer; a drop of pulque was placed on the refractometer prism (A.S.T. brand) and was observed against the light to determine the results in Brix degrees.

Determination of alcoholic strength. A 250 mL sample of pulque was placed in a test tube and then an alcohol meter (Gay-Lussac brand) was introduced to determine the level of alcohol, according to density.

Determination of acidity (lactic acid). A 9 mL sample was placed in a 125 mL Erlenmeyer flask, 4 drops of phenolphthalein were added, then the acidity test was carried out by titration with 25 mL of 0.1 N NaOH, until it turned slightly pink.

Determination of viscosity. 5 mL of the sample was added through the capillary in an Ostwald viscometer, and a syringe was used to suck the liquid sample up to point A; from there the time was taken until it descended to point B.

Determination of density. This was measured with the help of a pycnometer (25.95 mL volume) and the sample was added using a 5 mL pipette.

Determination of pH. A 25 mL sample of pulque was placed in a beaker and a pH indicator rod (COCIBA brand) was placed within it; this was left for about 2 minutes to obtain the results.

Ethanol extraction. The ethanolic fraction contained in 100 mL of pulque was distilled using a rotary steamer at 60 °C; an alcohol meter was used to calculate the alcohol content, which was measured at different times, in order to evaluate the fermentation process.

Determination of total sugars. This was carried out using the anthrone method, described by Witham *et al*., (1971). Absorbance was read at 600 nm using a spectrophotometer. Sugar concentration was estimated from a standard curve of 20 to 200 µg of glucose mL-1.

Determination of humidity and total ashes. This was carried out using the tray drying method; 5 mL of mixed pulque sample in aluminum trays at constant weight was weighed every two hours until constant weight was obtained. The dry sample was incinerated in a muffle furnace at 800 °C until white ash was obtained.

Determination of proteins. A NOVATECH brand protein digester was used. The Kjeldahl method was applied to analyze organic nitrogen. This technique involves the proteins of liquid or powdered pulque and other organic components being digested with sulfuric acid in the presence of catalysts such as potassium sulfate and copper sulfate. Total organic nitrogen is converted to ammonium sulfate. The digested mixture is neutralized with 30% sodium hydroxide and further distilled into 3% boric acid solution. The resulting borate anions are titrated using standard hydrochloric acid (0.1 N HCl), which is converted into the nitrogen of the sample.

Isolation of microorganisms. Dilutions of 10^{-1} up to 10^{-3} of the pulque were made using peptone water as diluent. The three dilutions were spread-plated using an angled rod in Petri dishes with Yeast Peptone Glucose (YPG), Macconkey, Man, Rugosa and Sharpe (MRS) agar and trypticase soy agar (AST). Boxes were incubated at 30 °C for 42 hours. Pure cultures of each strain were obtained by reseeding. The pure strains were cross-streaked in the different media to observe the change in morphology.

Resistence and increase in acid pH. Tolerance to different pH values was evaluated in three 250 mL flasks with 90 mL of YPG broth, formulated from yeast extract (Bioxon) 1% (p/v), peptone (Bioxon) 2% (p/v) and glucose (Reasol) 2% (p/v) and three flasks of MRS (Difco) with pH 2 with concentrated HCl and controls for each medium were mounted. Each flask was inoculated with a 10^{-3} dilution of pulque diluted with peptone water (Difco), at 10% (Ortiz *et al*., 2008). The samples were incubated at 28 ± 2 °C for 24 hours, finally turbidity indicated microbial growth.

Sensory analysis. This was carried out using a simple methodology, which was applied to 40 panelists, and consisted of rinsing the mouth for 20 seconds, in order to eliminate flavors that were already present; then the pulque was imbibed to determine the visual quality, taste and olfactory attributes. The sensory properties of pulque were analyzed according to what is described in the Mexican Standard NMX-V-1972 (Pulque produced in bulk) (SECOPFI, 1972b).

RESULTS AND DISCUSSION

Pulque is a drink considered as a symbol of Mexican identity; its benefits have been documented in at least 8 codices, since pre-Hispanic times (Goncalves de Lima, 1956). It is white in color and viscous, with a strong flavor and smell of maguey, its alcoholic strength does not exceed 7.5% (v/v) (Guzmán and Contreras, 2018, NOM-199-SCFI-2017). In recent years, greater interest in this drink has developed, by promoting it in cultural exhibitions, causing greater consumption among young people (Trejo *et al.*, 2020), this has been strengthened by recent studies that highlight its nutritional value, as it has high levels of carbohydrates, mineral salts, vitamins and minerals, in addition to containing pre and probiotic microorganisms that help improve intestinal microbiota (Correa, 2014; Escalante *et al.*, 2008). The Otomi are considered to have been the first to make pulque, so it is important to rescue the knowledge surrounding the process of making this drink in a traditional way from ancestral recipes (transmitted from generation to generation), in order to promote its consumption, by highlighting the characteristics and properties of a natural and quality product. The pulque elaboration process was documented from information provided by the producers and was standardized for the Alto Mezquital, as described below.

Traditional pulque making process

The production of pulque in the study region is mainly undertaken by producers who have plantations of no more than half a hectare, where 60 percent use the plant to form borders, 20 percent have their land covered with maguey plantations and the rest buy ripe magueyes to collect the aguamiel.

Cutting. Mature plants (approximately 8 to 10 years old) are selected, cut and the most tender set of leaves in the center of the plant; the embryonic floral peduncle that is surrounded by the flower bud (quiote), are removed. The central leaves of the plant (meloyote) are removed, using a sharp instrument.

Chopping up. This must be undertaken, one to two weeks before the scraping and extraction of the aguamiel is going to begin; a cavity (bowl) is opened at the heart of the plant, which consists of making a hole or hollow in the body of the plant or mezontete, which will serve as a recipient through which the aguamiel flows. This is done with the help of a pry bar.

Repose. Once the maguey has been cut down, the resting stage begins, which lasts from 1 week to 6 months.

Extraction. Using an acocote (gourd with two holes) the tlachiquero (person who extracts the aguamiel from the maguey) introduces this into the mezontete to extract the aguamiel by sucking with his mouth. Once all the aguamiel has been extracted, the cavity is covered with a rock or piece of maguey stalk to avoid contamination with external factors and to protect it from the environment.

Scraping. After extracting the aguamiel, the surface from which the meyolot sprouts, must be scraped with a tool in order to form the mezontete and cause the induction of sap.

Extraction and scraping are carried out twice a day. The production of aguamiel takes from 3 to 6 months depending on the age and size of the plant, and the frequency of the scraping process. The amount of aguamiel obtained each day also varies depending on these factors, but it consists of approximately 3 to 5 liters per day with a production throughout its life of 60 to 90 liters. Scraping is carried out over 1 to 4 weeks, the average scraping stage takes place from 1 to 4 months and the end of the scraping is after 4 to 6 months, later abandoning the maguey. All these stages depend on the time dedicated to the scraping process.

Transport to the tinacal. The tinacal is the place where the pulque fermentation process takes place, which may consist of a closed room of 3 × 3 m or much larger, but it must maintain room temperature conditions in order to better preserve the drink.

Seeding. This process consists of the aguamiel being allowed to ferment for at least 4 days, to produce the starter, where the fermentation process has already occurred. This process takes place spontaneously in a room at room temperature

Sowing. This consists of adding the strong pulque (seed), to the new aguamiel that is extracted in order to accelerate the fermentation process.

Fermentation (maturation). Once the seed and the aguamiel have been mixed, the fermentation process takes approximately between 24 and 48 hours, a process that was once carried out in cattle hides, but is currently carried out in plastic containers and in closed rooms, to which the aguamiel that has been collected is later added. The degree of fermentation depends on the taste of the producer, but is considered adequate when it has the characteristic degree of alcohol (4-6%) and becomes viscous.

Transportation to point of sale. Traditionally, this was transported in skins (made from goat leather), which preserves the proper temperature of the pulque to maintain its quality.

Consumption. There is no need to add any ingredient prior to consumption, which takes place in the tinacales or in specific sale locations, where traditionally, the pulque is drunk from a gourd.

The liquid extracted from the maguey is called aguamiel; its quantity and quality are variable, depending on the level of humidity, as well as soil and climatic conditions, time of year, age of the plant, species or variety of plant. It is important to avoid the addition of any elements which might alter its quality or flavor being added during the process. Various authors (Escalante *et al.*, 2012; Escalante *et al.*, 2016; Lappe *et al.*, 2008), indicate that variations regarding the process of making pulque in different states of Mexico are minimal, however, it is important to document these processes in order to preserve the traditional ways of making pulque.

Fermentation process. The fermentation process is a key factor in the elaboration of pulque, for which variations among producers were identified, due to the recipe that was inherited or due to modifications that they themselves made to improve their product. Table 1 shows the 3 variants identified in the fermentation process among producers in the Alto Mezquital region.

The time dedicated to making the seed is a very important factor when making pulque, as this is what is used to obtain the final product. During the fermentation process,

Table 1. Variants in the fermentation of pulque.

Day	Case 1	Case 2	Case 3
Day 1	The mead is left to rest for its natural fermentation.	The seed is added to the mead and left to ferment.	To pulque 1 (tip) is added mead.
Day 2	More mead is added to continue fermentation.	More mead is added to the previous pulque.	To pulque 2 is added to pulque 1.
Day 3	More mead is added and its condition of maturation is evaluated.	The remaining pulque is eliminated and the process being again.	To pulque 2 is added to pulque 3.
Day 4	It is used as a seed in other pulques.	-	To pulque 3 is added to pulque 4.
Day 5	-	-	To pulque 4 is added to pulque 5.
Day 6	-	-	To pulque 5 is added to pulque 6
Day 7	-	-	To pulque 6 is added to pulque 7 (tail).

Source: authors.

the aguamiel becomes acidic, while lactic acid is formed. Pulque is a white, viscous, liquid, with around 45 g/L of ethanol and a pH of 3.4 (Santos *et al.*, 2016). For the elaboration of this drink, three main fermentations take place during the process: an alcoholic fermentation that is caused by *Zymomonas mobilis* and various types of yeasts such as *Saccharomyces cerevisiae*, a lactic fermentation and a viscous fermentation process during which *Leuconostoc mesenteroides* produces dextran, which gives the drink a viscous texture (Escalante *et al.*, 2004). The fermentation time can last from 0 to 48 hours at a temperature of 25 °C, taking care that the containers do not have any substance that inhibits mesophilic microorganisms (detergents, perfumes, disinfectants, among others). As time passes, important changes occur, for example an increase in the percentage of ethanol and the formation of exopolysaccharides such as β-glucans and dextrans; that generate an increase in viscosity transforming the fluid from Newtonian to non-Newtonian (Escalante *et al.*, 2012). In the pulque samples analyzed, the level of fermentation was evaluated according to the percentage of alcohol present from 0 to 24 hours (Table 2). This makes it possible to establish the basic parameters to indicate their state of fermentation.

Table 2. Changes that pulque undergoes during fermentation.

Time	0-2 hours	2 a 5 hours	6 a 10 hours	10 a 24 hours	After 24 hours
State of fermentation	Alcohol free (0-0.6%)	Slightly fermented (0.6-2%)	Intermediate fermentation (2-3.5%)	Haeavily femented (> de 4%)	Pitched (vinegary) alcohol decreases

Source: authors.

Other changes identified during fermentation include Brix degrees, alcoholic strength, total acidity and viscosity. Values that were identified in the initial stage, 24, 48 and 72 hours (Table 3), where the Brix degrees decrease with respect to a longer fermentation time, whereas alcoholic strength, total acidity and viscosity increase. The importance of identifying the level of acidity in food by pH lies in the fact that it makes it possible to determine the type of microorganisms capable of developing in them, as a result of prebiotic activity relating to the carbohydrates in it (Guzmán and Contreras, 2018).

Aguamiel contains bioactive compounds such as saponins, amino acids and fatty acids that make it qualify as a functional food, in the same way that pulque enables the growth of microorganisms that are used in the production of food products (Guzmán and Contreras, 2018). The results make it possible to generate information to corroborate the nutritional and functional properties of pulque, to promote recognition by the food and biotechnology industry, in addition to raising awareness of its use, as for many years it has been complementary to the diet of marginalized communities.

Physicochemical properties of aguamiel and pulque. Aguamiel is the sap obtained from the maguey; white in color, slightly cloudy, sweet tending to slightly acidic; it is composed of water, sugars, proteins and mineral salts, among its main components (Escalante *et a*l., 2008). According to the Mexican regulation NMX-V-022 (SECOFI, 1972a), two types of aguamiel are identified for the production of pulque. Type I refers to the best quality aguamiel (this is purer and contains a greater amount of sugar), pH of 6.6-7.5 and 0.9-1.03 mg of lactic acid/100 mL, type II is a slightly acidic aguamiel, pH of less than 4.5 and less than 4mg lactic acid/100 mL. The composition and quantity of aguamiel produced during the production period varies according to the agave species, agronomic management of the plant and agroclimatic conditions of the region. According to the characteristics of the aguamiel evaluated in the region (Table 4), this corresponds to quality I, however, it is important to consider that these values are susceptible to change, due to factors such as scraping time, which directly influence the quality and properties of aguamiel, as the characteristics are not the same at the beginning, in the middle and at the end of the scraping of a maguey plant. Analyzes of aguamiel from Tecamachalco, Puebla, also report significant levels of antioxidant capacity of 5.01 ±0.06 trolox (Castro and Guerrero, 2014). Besides this, essential amino acids have been identified for human consumption (lysine, phenylalanine, isoleucine, leucine, valine and methionine) and

Table 3. Perceptible changes in pulque during fermentation.

Characteristic	0 hours	24 hours	48 hours	72 hours
Brix degrees	11	6	4	3.5
Alcohol content	1.23	2.85	5.34	6.30
Total acidity	0.85	1.35	2.5	2.35
Viscosity	1.26	1.30	1.34	1.51

Source: authors.

Table 4. Physicochemical properties of mead and pulque g L^{-1}.

Parameters	Mead	Seed	Tip	Commercial pulque
°Brix (sugar)	10	6	5.4	4.3
% of alcohol	0.1	5.8	6.3	4.5
Density	1.025	1.018	1.20	1.011
% total acidity	1.60	1.12	1.05	1.25
pH	6.8	3.45	4.00	4.01
% Glucose	6.33	4.75	4.65	3.30
% Sucrose	6.1	4.05	3.50	2.34
% Crude protein	1.18	0.95	0.85	0.33
% Ashes	0.24	0.30	0.32	0.36

Source: authors.

aguamiel can be considered as an alternative and economic source of amino acids (Romero *et al.*, 2015).

Regarding pulque, the Mexican regulation NMX-V-037 (SECOFI, 1972b) refers to 2 types of pulque: type I consists of pulque seed and type II refers to commercial pulque. Pulque seed is prepared in order to increase the natural microbiota that enables adequate fermentation of pulque for later sale. Type I aguamiel is inoculated with the seed pulque, in order to achieve biochemical balance between the substrates to be fermented and the microorganisms (Ramírez *et al.*, 2004, Lappe *et al.*, 2008). Table 4 shows the results for the physicochemical composition of the aguamiel, seed, puntas and commercial pulque, the parameters evaluated fall within the acceptable standards for a quality pulque. It is important to consider that these parameters may be affected during the course of the year, due to the presence or absence of rain and particular characteristics of the plant.

The Mexican Standard NMX-V-037, describes 2 types of pulque, Type I (seed and punta) and Type II commercial pulque. Type I physicochemical properties are specified to consist of a pH less than 3.5-4.0, sugar content 0.10-0.80 g/100 mL of reduced sugars, expressed as glucose equivalent and 6-9% alcohol content (v/v). Type II has a final pH of 3.0-4.0, sugar content of 0.20-0.50 g/100 mL of reduced sugars and alcohol content of 4.0-6.0% (v/v) (SECOFI, 1972b). According to the quality specifications established by the NMX-V-037, the seed and puntasfall within Type I and the commercial pulque corresponds to Type II, according to the results obtained. The ash content is low compared to other fermented beverages (Escalante *et al.*, 2016). The protein determination in the samples falls within the range identified by Cervantes and Pedroza, (2007) for other pulque samples. Importantly the small producer determines the quality of the pulque based on his traditional experience.

Microbiological properties. There are more than 30 microorganisms identified in the process and elaboration of pulque; however, these vary according to the region, elaboration process and species of agave (Matías *et al.*, 2019). Those that have been identified in greater quantity and frequency are: *Zymomonas sp., Leuconostoc sp., Saccharomyces sp., Lactobacillus*

sp., Bacillus simplex (Lappe *et al.*, 2008; Escalante *et al.*, 2004; 2008; Castro *et al.*, 2015a). Scientific evidence demonstrates the effects of pulque consumption, where probiotic bacteria are important for the development of functional non-dairy products (Matías *et al.*, 2019). The microorganisms identified in the process of making pulque in the study region were: Lactobacilli and Leuconostoc. Particularly during the fermentation process, *Saccaromices cerevisae, Kluyveromyces, Zymomonas mobilis, acetobacteria,* and *gluconobacter* were identified in the analyzed samples. Several of the microorganisms pertaining to the Leuconostoc and Lactobacillus species, present in aguamiel and pulque, show resistance to the antimicrobial barriers of the gastrointestinal tract, in addition to adherence to the intestinal mucosa (Escalante *et al.*, 2016; Castro *et al.*, 2015b). Therefore, the traditional treatments for infections and gastrointestinal disorders relate to the antimicrobial activity of the lactic acid bacteria present in its fermentation and to moderate consumption of pulque.

Pulque is a drink with great nutritional value; it is considered as a resurgent drink that represents an adequate and healthy food option due to its pre and probiotic properties, which are considered as drinks with organisms and substances that contribute to the intestinal microbial balance, and its daily consumption is considered as a source of energy, vitamins, with a presence of amino acids such as lysine and tryptophan, which are scarce in the Mexican diet. Pulque is also an important source of vitamin C, thiamin, riboflavin, calcium and iron (Matías *et al.*, 2019, Peralta *et al.*, 2020). For this reason, pulque represents a good option. Agave nectar or aguamiel, is rich in vitamins and minerals, and prior to fermentation, can be a source of food for children (Escalante *et al.*, 2012). Likewise, if it is added as a probiotic food, it increases its nutritional and functional value as a contribution to the daily diet, improving people's health condition, because vitamins and minerals have been identified in *A. atroviren,* where for example vitamin C is found in greatest concentration (17.99 mg/ 100 g), followed by vitamin B3 a level of 4.77 mg/ 100 g and to a lesser extent vitamin B6, vitamin B2 and vitamin B1 (Romero *et al.*, 2015).

Organoleptic attributes of pulque. According to the Mexican Standard NMX-V-037-1972, the organoleptic specifications of pulque are based on the color, which must be white, the flavor and smell are unique and must not present different flavors and smells that are inappropriate. The organoleptic attributes of pulque were identified as: visual (color, cleanliness and foam), olfactory (persistence and aroma quality) and taste (quality and taste tests), which were analyzed as an indicator of good and bad quality (Table 5).

Among visual attributes, a white color is considered an ideal characteristic and identified as synonymous with quality for pulque. In terms of purity, ideally it should be without any impurities and clear, with perceptible foam. In terms of olfactory sensation; although its presence must be brief and not prolonged, the primary aromas (tender and fruity maguey) are those most preferred, whereas for the taste parameter, the ideal is bittersweet and sweet, and for the palate tests, a sweet flavor was preferred.

As mentioned previously, the quality of the pulque can be affected by internal and external factors, which are presented in Tables 6 and 7. It was important to document this, as

Table 5. Organoleptic indicators in the evaluation.

Characteristic	Good quality indicators	Defect indicators
Color and appearance	White, clear, sparkling and free of imprities	Yellowish, watery, greasy, slimy (viscous), presence of impurities
Odor	Fruity, almondy, ripe maguey (capon)	Vinegary or sours (sour), brincado (putrid), presence of uncharacteristic puqlue odors
Taste	Fruity (melon: slightly fermented; papaya, well fermented), bittersweet, maguey flavor of origin, alcohol	Guixi (astringent to tender penca), ñanmfi (oldpenca), Sour, bitter, putrid, stinky, straggler, watery
Resabio	Sweet, fruity sensation, with absence of strong and persistent favors	Viscous, scratchy, rancid, persistent putrefaction

Source: authors.

these have an important influence on the extraction process of the aguamiel and in the elaboration of the final product (pulque).

Each of the factors considered in Table 6 influence the quality of the pulque. In particular, the degree of fermentation is a function of the number of microorganisms participating in the process, together with climatic conditions that are favorable for their proliferation. The scraping time will depend on the age of the plant and its morphological characteristics, as well as the origin and form of propagation, as development varies according to type of propagation. The agroclimatic conditions in which the aguamiel extraction and pulque elaboration are carried out is a key factor, because rain, heat, cold and drought, give rise to different taste characteristics and appreciations in pulque, as described in Table 7, giving rise to the different qualities of the drink.

Similarly, the utensils used for the extraction and preparation of pulque, the people who produce the aguamiel and pulque, the service containers, the hygiene that must be observed in the extraction and preparation of pulque, the process for the preparation of the drink, are factors to consider, which affect the quality of pulque, due to the fact that in each particular municipality or region, there are variations concerning the way of preparing the seed, the time periods dedicated to fermentation for the subsequent sale of the final product.

Table 6. Factors influencing pulque organoleptic quality.

Factor	Cause
External	• Degree of fermentation • Scratching time • Climate and season • Plant origin
Internal	• Hygiene in the process • Preparation technique • Environmental conditions in the tinacal

Source: authors.

Table 7. Climate and season.

Climate	Characteristics	Taste appreciation
Rain	Watery and tasteless	Poor quality
Heat	Viscoues, strong alcohol content and putrid	Intermediate quality
Cold	Good balance	Good quality
Drought	High alcoholic content, Good taste	Good quality

Source: authors.

CONCLUSIONS

The Alto Mezquital region is characterized by the production and sale of pulque, because it is a maguey xamini producing area. These activities continue to provide economic income to producers. Here, the extraction of aguamiel and the elaboration of pulque, are processes carried out in a traditional way, which have been inherited by generations. During the process of making pulque from this region, the aguamiel, the seed, the punta and the commercial pulque fall within the quality standards defined by Mexican standards, due to their analyzed physicochemical, microbiological and organoleptic characteristics. Moreover, the bacteria and yeasts identified in the pulque give it pre- and probiotic properties with high nutritional potential. Therefore, there is a need to reassess aguamiel and pulque, in addition to raising awareness of their use for the isolation of microorganisms and the production of metabolites of interest in the food industry, while generally promoting the great number of benefits that they have as traditional beverages with high nutritional value.

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