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Pulque Fermentation

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43.1 Introduction

Pulque is probably the oldest and most traditional Mexican alcoholic beverage, prepared, and consumed since pre-Hispanic times. Due to its great historical, religious, social, medical, and economical importance, it is the most widely studied beverage from the anthropological and scientific points of view (Loyola-Montemayor 1956; Godoy et al. 2003).

It is a milky white, viscous, slightly acidic alcoholic beverage traditionally produced by spontaneous fermentation of *aguamiel*, the sugary sap extracted from several *Agave* species that grow in the central Mexican plateau states (Loyola Montemayor 1956; Steinkraus 1996; García-Mendoza 1995, 1998; Godoy et al. 2003; Blomberg 2000). In pre-Hispanic times, pulque played an important role in the religion, rituals, divination, and curing of the indigenous communities (Bruman 2000). Currently, pulque persists as a typically popular beverage, and it is sold in *pulquerías* as well as in some restaurants. It is also produced at a small industrial scale using a mixed starter culture and controlling the quality and safety of the product, which is canned and exported (Ramírez et al. 2004).

In this chapter, a broad view of pulque is covered: a historical review is presented, from its discovery and relevance during the pre-Hispanic period, its role during colonial times, its consolidation, and production from the 15th to the 20th centuries, until the recent scientific and technological efforts to reincorporate pulque to the Mexican traditional cuisine. The different *Agave* species used in the production of pulque are mentioned as well as their actual distribution and density in the agave plantations. The traditional and industrial pulque production processes are described, including the microbiota involved as well as the biochemical changes that take place both in the spontaneous and the controlled fermentation. To understand the relevance and potential benefits of pulque among consumers, its main chemical and nutritional properties, including its health benefits, are discussed. Finally, the chapter concludes with a description of the actual status of pulque in Mexican society as well as the social, scientific, and technological challenges that have to be faced in order to achieve the rescue of this ancestral beverage.

43.2 History of Pulque

43.2.1 Origins and Pre-Hispanic Production

Pulque represents a Mexican icon and is probably the oldest and most traditional Mexican alcoholic beverage, as attested by archaeological vestiges. The ancient Aztecs called it *metoctli* or agave wine (from the nahuatl language *metl* = agave or maguey + *octli* = wine) as well as *iztacoctlli* or white wine (from *izac* = white + *octli* = wine): they referred to the spoiled beverage with unpleasant odor and flavor as *poliuhquioctli* (from *poliuhqui* = spoiled or rotted + *octli* = wine), so it is probably from this last term that the word *pulque* was adopted by the Spaniards to designate the freshly produced beverage (Robelo 1948).

It is highly probable that ancient Otomíes were the first to prepare pulque toward the year 2000 BC, inheriting the elaboration process to almost all cultures of the central Mexican plateau (Martín del Campo 1938; Blomberg 2000). However, different legends attribute its origin to the Tolteca civilization (990–1021 AD) in the city of Tula. One of these legends considers that maguey and pulque are both a gift of the goddess *Mayahuel* (Gonçalves de Lima 1978; Ramírez Castañeda 1994). There is consensus among historians based on archeological evidences (200 BC–300 AD) and information from pre- and post-Hispanic codices from several important Mesoamerican cultures that pulque had a dominating presence in their daily life and a primordial role in religious and war rituals (Diguet 1928; Martin del Campo 1938; Sánchez-Marroquín 1949; Gonçalves de Lima 1978; Vallejo 1992).

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Aztec civilization developed a full dominion of aguamiel and pulque production techniques incorporating to their diet its nutritional benefits and healing properties. This civilization considered pulque a divine food, a medicine, and a ritual element that allowed a closer relation with their cosmology. As the dominant culture in the Mexican plateau, the Aztecs established the moral guidelines for pulque consumption and religious use: only the children and youths, the elder, pregnant or nursing women to increase milk production, priests and warriors, or anyone before sacrifice were allowed to consume it, but excessive consumption was harshly punished, in some cases including the death penalty (Loyola-Montemayor 1956; Corcuera de Mancera 1991; Godoy et al. 2003). Aztecs' conquered cultures were forced to deliver aguamiel and pulque as tribute (Gonçalves de Lima 1978, 1990; Soberón-Mora 1998).

43.2.2 Pulque Production during the Spaniard Colonial Period

When the Aztec Empire fell, pulque lost its religious importance prevailing as a traditional popular beverage until today. At the beginning of the colonial period, the Spaniards promoted its consumption among the indigenous groups and recent immigrant slaves as a mean to control them (Corcuera de Mancera 1991; Ramirez et al. 2004). The fast growth of the productive and commercial activities introduced by the Spaniards during the 17th century gradually incorporated the production and commercialization of pulque. These activities flourished at the end of the 18th century with the development of the *haciendas pulqueras* (large farms producing pulque) devoted to the cultivation of *Agave* plants, extraction of aguamiel, and production of pulque, which was transported mainly to the cities of Mexico and Puebla. These production organizations prospered in the actual central Mexican plateau states of Hidalgo (mainly in the Apan prairies), Tlaxcala, Puebla, Querétaro, Morelos, and Michoacán.

Since 1779, pulque production and commerce was strongly regulated, and special privileges were granted to Spaniards who controlled these agroindustry (Loyola-Montemayor 1956), becoming one of the most important economic activities in the New Spain. Taxes imposed to pulque became the fifth biggest income of the *Real Hacienda* and the Spanish Crown received from the pulque industry 4.3 million pesos in 1962 and 32 million from 1763 to 1809 (Segura 1901).

43.2.3 Production during the 19th and 20th Centuries

During the independence movement (1810–1821), pulque production suffered a drastic reduction. Once México became independent, government regulations regarding pulque production, quality, commerce, and taxes consolidated again the *haciendas pulqueras*, allowing them to cope with the increasing demand of the beverage by the rapidly growing population (Loyola Montemayor 1956; Sánchez Santiró 2007). An important factor in this process was the introduction in 1867 of the railway system, which in particular connected the Apan prairies with the main consumption centers. In this context, the pulque agroindustry flourished and reached its best times at the beginning of the 20th century with an annual production of 500 million liters obtained from around 140 million agave plants. In 1905, 350,000 L of pulque were consumed only in Mexico City. This agroindustry controlled the agave plantations and the production and retail sale of pulque, activities that originated great fortunes and the emergence of a new social economic class called the "pulque aristocracy" (Ramírez-Rancaño 2000; CECULTAH 2010).

In 1909, the owners of more than 50 haciendas created the *Compañía Expendedora de Pulques*, with the purpose of controlling production, distribution, and sale of pulque in Mexico City and to expand the hacienda activities to the elaboration, distribution and commerce of other agave products, including aguamiel syrups, glues, pulque distillates (mezcal), and medical products, increasing the benefits of the agave industry (Gobierno del Estado de Hidalgo: Museo Nacional de Culturas Populares 1988).

By 1910, the pulque industry was affected again by the revolutionary movement (Soberón-Mora 1998). The social instability together with the introduction of the brewing companies and a strong campaign against pulque had a negative impact in this agroindustry. By 1914, *haciendas pulqueras* were seized and land reform started fractionating the agricultural fields. The elimination of *haciendas pulqueras* became a fact with the promulgation of a new constitution in 1917, which established in the 27th article the agricultural reform, which promoted the return and redistribution of the land to the peasants: this was the formal end of the three centuries of splendor of most of the *haciendas pulqueras* (Loyola Montemayor 1956; Ramírez-Rancaño 2000). Despite the land redistribution, the abundance of agave cultivars allowed the development of a new pulque industry that was able to cope with the decreased beverage demand. Therefore and until the 1920s, fresh pulque produced in several regions of the country was delivered daily by train to Mexico City. During 1934–1940, antialcoholic campaigns were established, particularly against pulque, as it was considered dirty and unhealthy, due to the lack of control in the production process and adulteration during its distribution after certification through the former pulque customs of Pantaco, Cuautepec, and Ticoman, where rigorous norms were applied until the seventies to authorize the entrance of pulque barrels to Mexico City (Gobierno del Estado de Hidalgo: Museo Nacional de Culturas Populares 1988). However, during the following decades the already mentioned problems, as well as the excessive and irrational exploitation of the agave plantations and the introduction of beer as an alternative beverage, considerably deteriorated the pulque industry (Loyola-Montemayor 1956; Ramírez-Rancaño 2000).

It was not until 1960 that considerable efforts were made in order to recover this important national heritage with the creation of the *Patronato del Maguey*, with the aim to improve the culture and exploitation of *Agave* through modifications in both the production and the distribution process. Canning was introduced (350-mL cans and commercialized under the registered trade name Magueyin) as an alternative way of distribution, efforts were made to improve the hygienic conditions of the beverage, and new uses of the *Agave* plants were developed to increase the income of the peasants (Gobierno del Estado de Hidalgo: Museo Nacional de Culturas Populares 1988; Steinkraus 1996). Unfortunately, canned pulque was not accepted by the traditional consumer as the product lacked the distinguishing sensorial properties of fresh pulque and had a higher price. As a consequence, this industrialization attempt failed as well as subsequent efforts to commercialize other former patented pulque brands.

In the 20th century, according to the agronomical census, from 1998 to 2002, the surface devoted to *Agave* pulquero was reduced with the consequent decrease in pulque production. Nowadays, the most important agave pulquero production areas are located in the central states of Hidalgo, Tlaxcala, and Estado de México, and in some regions of Querétaro, Puebla, Morelos, and Michoacán. By the end of the last century, the depressed pulque industry situation was the consequence of the loss of cultivars, the lack of an efficient technology, and the low demand by consumers (Loyola-Montemayor 1956; Gobierno del Estado de Hidalgo: Museo Nacional de Culturas Populares 1988; Ramírez-Rancaño 2000).

43.2.4 Situation in the Early 21st Century

Currently, pulque is considered a traditional popular beverage that practically has disappeared from the urban market, only found in *pulquerías* located in popular districts of Mexico City. In the rural areas, near the production regions, pulque is still the preferred stimulating beverage of the low income class. It is consumed as part of their daily diet and is part of the tradition in all sorts of festivities, from births and baptisms to weddings and funerals. It is also common to find production for self-consumption among agave owners (Bennet et al. 1998). Important efforts are being made to fight against its disappearance: it is being promoted in Mexican food restaurants and gastronomic festivals, while traditional *pulquerías* are recovered. In these traditional places pulque is now exclusively prepared with high quality raw materials and under hygienic conditions (Poblet 1995).

Nevertheless, pulque production remains as a predominantly traditional and small-scale industry that has not been able to massively reach local (other than Mexico City) or international markets (Ramírez et al. 2004). However, *Nectar del Razo*, in Tlaxcala, and *Desarrollo Agropecuario del Altiplano*, in Puebla, export their novel canned pulque product to different countries all around the world. In both companies, the traditional elaboration process has been modified to obtain a stable product with a long, shelf-life, which is sold white, that is to say, plain or added with fruits.

43.3 Agave Species Used for Aguamiel Extraction

Pulque is produced from aguamiel extracted from native *Agave* species, mainly *A. salmiana* var. *salmiana* (green or meek agave), *A. atrovirens* (white agave), and *A. mapisaga* (Mexican or long hand agave), and in lower proportion from *A. lehmannii* and *A. altisima* (Loyola-Montemayor 1956; García-Mendoza 1995, 1998), mostly distributed in the states of the central Mexican plateau where pulque has been mainly produced and consumed. These species grow in a vast region of arid, semiarid, and temperate zones, in sandy, poor, well-drained soils, with scare, and irregular precipitations. Preventing soil erosion, they can be found as single crop or protecting or limiting homes or farm lands (Loyola-Montemayor 1956; Steinkraus 1996; Ramírez et al. 2004; Ortiz-Basurto et al. 2008; Lappe-Oliveras et al. 2008).

Agave is a plant relatively easy to cultivate. Propagation may be carried out by transplanting young vigorous offsprings from adult plants, called *matecuates*. An adult plant can produce above 50 *matecuates* that grow around the mature plant. Young offsprings are harvested in the rainy season and transplanted into a new *magueyera* or agave plantation for aguamiel production where the young plants are arranged on parallel long rows called *melgas* or *metepnatle* (maguey wall), separated by 2–5 m. Alternatively, agave seeds can be grown in seedbeds under controlled conditions (Monterrubio 2007).

Aguamiel is extracted from 7- to 10-year-old mature agaves that are about to produce their inflorescence (*quiote*). Mature plants are castrated or broken by eliminating the floral bud, leaving a cavity (*cajete)* in the center of the agave stem. This operation is usually performed in early spring or late autumn. If the plant inflorescence grows, maguey will never produce aguamiel, and if *castration* is performed before agave maturation, the yield in aguamiel production will be scare. The resulting cavity is covered with agave leaves or with a sized stone to avoid the exposition of the cavity to the environment (Loyola-Montemayor 1956; Gobierno del Estado de Hidalgo: Museo Nacional de Culturas Populares 1988). An aging period follows castration to allow the maturation of the central leaves and the increase of sap sugar content: 7%–14% w/v, depending of the agave species (Lappe-Oliveras et al. 2008). The healed cavity is scraped to open the vessels, promoting the sap flow and its accumulation in the cavity. The sap is collected twice a d (at daybreak and dusk) by oral suction through a dried gourd (*Lagenaria siceraria*) called *acocote*. Production of aguamiel lasts from 3 to 6 months until the plant dies, with an approximate production of 1000 L/plant (Loyola-Montemayor 1956; Gobierno del Estado de Hidalgo: Museo Nacional de Culturas Populares 1988; Steinkraus 1996). The volume of aguamiel produced varies throughout the harvesting period; at the beginning the plant produces around 0.4 L/plant/day, amount that increases to 4 or 6 L/day during the next 3–6 months and then decreases to 0.4 L before the plant dies (Ortiz-Basurto et al. 2008). Once the plant dies, it is removed and substituted by an offspring, as already described (Monterrubio 2007; Lappe-Oliveras et al. 2008).

43.3.1 Aguamiel Composition and Properties

43.3.1.1 Physicochemical Characteristics of Aguamiel

Aguamiel is a milky white, lightly cloudy, thick, sweet, fresh-flavored, and lightly acid agave sap. It contains water, sugars, proteins, gums, and mineral salts as the most important components (Ramírez et al. 2004; Ortiz-Basurto et al. 2008; Escalante et al. 2008). The Mexican regulation NMX-V-022 (Banco de Normas Mexicanas 2010a) defines two types of aguamiel for pulque production. Type I refers to a best quality aguamiel (cleanest and with a highest sugar content, pH <6.6–7.5 and <0.9–1.03 mg lactic acid/100 mL) while type II includes slight acid aguamiel (pH <4.5 and <4 mg lactic acid/100 mL).

According to several sources, the main compounds present in the sap are 0.3 to 0.4 g 100/mL of all essential amino acids with exception of methionine, vitamins (mg 100/mL, 0.02 riboflavin, 0.06 thiamine, 0.45 niacin, 19.60 biotin, 21.64 *p*-aminobenzoic acid, 65.17 panthothenic acid, 22.98 pyridoxine, and 9.0 ascorbic acid), sugars (glucose, sucrose, fructose, fructo-oligosaccharides, and inulin), minerals (mg 100/mL, 25 sodium, 9 magnesium, 1.1 iron, 0.8 zinc, 0.3 copper, and 0.3 manganese). Aguamiel constitutes a water substitute or an alternative food in places where water is scare or of bad quality or where protein is not available (Sánchez-Marroquín and Hope 1953; Ortiz-Basurto et al. 2008; García-Garibay and López-Munguía 1993; Steinkraus 1996; Lappe-Oliveras et al. 2008).

The amount and composition of aguamiel during the production period varies depending on the *Agave* species, the cultivation conditions of the plant, the season of the year, the air relative humidity, and the soil properties (Sánchez-Marroquín 1970). However, in the physicochemical analysis of aguamiel from *A. mapisaga* harvested during 5 months, Ortiz-Basurto et al. (2008) found no significant changes in titrable acidity, pH (above 4.5), and dry matter content, but a slight decrement in fructose content was observed in samples collected after 3.5 months, concluding that the quality of aguamiel remains constant during all the harvesting period.

43.4 Pulque Production

The Mexican regulation NMX-V-037 (Banco de Normas Mexicanas 2010b) recognizes two types of pulque: type I includes *pulque de semilla* or *pulque pie de cuba* (seed or starter) and type II refers to commercial pulque. Pulque starter is prepared to increase the natural microbiota that determines the correct fermentation in the elaboration of the beverage in a tank exclusively devoted for this purpose. Type I sap is inoculated with pulque starter, and is used to establish the optimum biochemical equilibrium between the fermentable substrates and the microorganisms involved in fermentation; it is the base of commercial production (Loyola-Montemayor 1956; Ramírez et al. 2004; Lappe-Oliveras et al. 2008).

43.4.1 Traditional Pulque Production

The traditional process of pulque production has remained relatively unchanged. The process starts with the daily collection of aguamiel and its immediately transference to a container, traditionally a pork or goat skin bag called *cuero* (Figure 43.1 shows several stages and utensils used during aguamiel

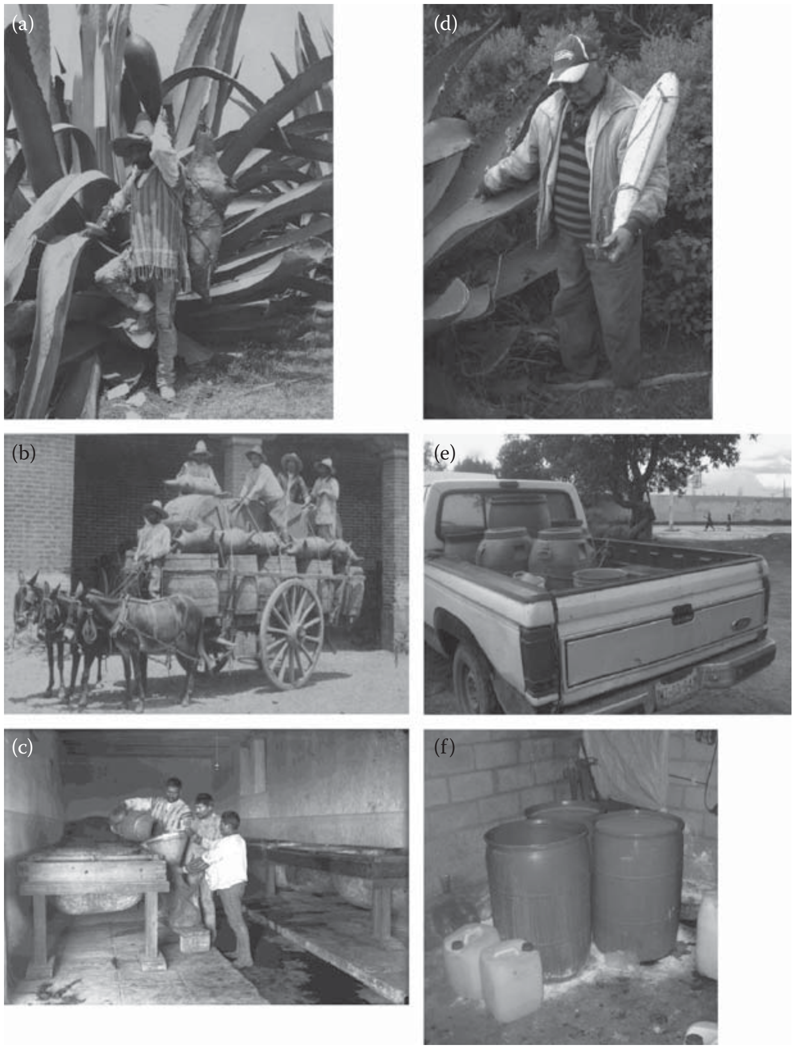

FIGURE 43.1 Aguamiel extraction, transport, and pulque fermentation during the early 20th century (a–c) and at present (d–e, from the aguamiel and pulque production location Las Mesas, Tlaxcala state). (a) Aguamiel extraction by tlachiquero with acococote, cajete scrapper tool (right hand), and skin bag to transport aguamiel to fermentation vats; (b) aguamiel and pulque transportation in skins bags and barrels; (c) former vats made with leather in a large tinacal; (d) modern talchiquero with acocote and scrapper tool; (e) aguamiel and pulque transport to small rural pulquerías; (f) large plastic barrels (200 L) in a small tinacal. (a–c, Reproduced with permission of Sistema Nacional de Fototecas, México. a, ©609476, Conaculta, INAH, Sinafo, FN, México; b, ©611009, Conaculta, INAH, Sinafo, FN, México; c, ©6535, Conaculta, INAH, Sinafo, FN, México.)

extraction and pulque production) or a wood container known as *castaña*; recently, plastic containers have been introduced. After sap collection, the cajete walls are scraped again to maintain the vessels open and a constant aguamiel flow.

Fermentation is carried out for several hours, depending on aguamiel quality, pulque starter maturity, the sap, and seed microbiota, and the season (environmental temperature changes). The degree of fermentation is adequate when a specific alcoholic degree and viscosity are reached (Loyola-Montemayor 1956; Steinkraus 1996).

Pulque fermentation is performed in containers made from different materials (leather, wood, plastic, glass fiber) placed in a closed room (*tinacal*, with small windows to control the temperature), as in the *haciendas pulqueras* of the Spaniard colonial period. In pre-Hispanic times pulque fermentation was carried out in clay pots; during the colonial period these kind of containers were replaced by leather reservoirs that were used until 1900, when wood vats with higher capacities (800–1000 L) were introduced. In most *tinacales*, the fermentation tanks are generally arranged in parallel, while in some others they are placed around a circle (Bellingeri Martini 1980).

To produce the starter, a small amount of aguamiel (10–15 L) preferably with an adequate density and taste is poured into a devoted vat (located in a small room next to the *tinacal*) and spontaneously fermented until alcoholic and acetic tastes are detected or until a white layer called *zurron* is formed on the surface. This process is performed at room temperature and lasts several weeks depending on the season: 1 week in summer, while 3 or 4 weeks in winter (Loyola-Montemayor 1959; Bellingeri Martini 1980; Gobierno del Estado de Hidalgo: Museo Nacional de Culturas Populares 1988; Steinkraus 1996; Lappe-Oliveras et al. 2008).

Small amounts of fresh aguamiel are constantly poured until the vat is filled; this amount of seed should be enough to sustain commercial pulque production. Afterward, the starter is distributed in several vats, process known as *tirar puntas*, and the fermentation is started with the addition of fresh aguamiel to the vats. An empirical rule applied to perform a good fermentation is to mix more starter than aguamiel during the cold season and less starter than aguamiel during the hot season. The fermentation finishes after 6 or 14 hours depending on seed and aguamiel quality, microbial content, and environmental conditions. As all these parameters affect the fermentation process it is not strange to find pulque with a wide variety of properties and compositions (Loyola-Montemayor 1956; Raminez et al. 2004). According to its quality, two types of pulque are recognized: fine or best-quality pulque obtained by fermentation of high-quality *aguamiel* from *A. salmiana* var. *salmiana*, *A. mapisaga*, and *A. atrovirens*, grown in cold weather and dried land, and poor quality pulque or *tlachique* produced by fermentation of poor quality aguamiel (low sugar content) from agaves grown in humid lands and temperate weather (Loyola-Montemayor 1956).

Due to the number and distribution of places where traditional pulque is produced, it is difficult to have a strict regulation and to avoid adulteration of the product, which is sometimes diluted with water to increase the volume of product, with gums to mask a deficient fermentation or with sugars to avoid offending flavors (Monterrubio 2007). Pulque is consumed alone or mixed with single or combined macerated fruits (strawberry, guava, maracuya, figs), vegetables (alfalfa, celery, tomatoes), nuts (pecans, almonds, peanuts, pine nuts, coconut), or condiments (cinnamon, black, or red pepper, coffee), beverages named *curados*.

43.5 Microbiology and Biochemistry of the Fermentation

43.5.1 Microbial Diversity in Pulque: Defining an Essential Microbiota?

Aguamiel constitutes a favorable medium for the proliferation of numerous microorganisms associated to the environment: from the cajete walls, incorporated with the scraping or extraction tools, from dust, or from insects such as *Drosophila* spp. This microbiota complements that of the starter present in the fermentation container to perform a series of distinctive fermentations processes that characterize pulque: acid (lactic and acetic), alcoholic (production of ethanol), and viscous (production of extracellular polysaccharides, EPS) (Sánchez-Marroquín and Hope 1953; Loyola-Montemayor 1956; Gonçalves de Lima 1990; García-Garibay and López Munguía 1993).

Early microbiological studies performed by Sánchez-Marroquín during 1946–1957, resulted in a description of the pulque microbiota and the proposal of *Lactobacillus* spp., *Leuconostoc mesenteroides*, *Zymomonas mobilis*, and the yeast *Saccharomyces cerevisiase* as the essential microorganisms in pulque fermentation. Using purified strains of these organisms, a mixed inoculum was developed and applied in controlled fermentation processes using sterilized aguamiel, demonstrating that the final fermented product was comparable in alcohol content, viscosity, and acidity to the traditional high-quality pulque produced commercially (Sánchez-Marroquín and Hope 1953; Sánchez-Marroquín et al. 1957). Although these results suggest the essential role of the selected microorganisms, recent studies on the microbiology of aguamiel and pulque demonstrate the presence of a complex microbial diversity of bacteria and yeast (Escalante et al. 2004, 2008; Lappe-Oliveras et al. 2008).

Analysis of the bacterial diversity in pulque samples collected from Estado de México, Morelos, and Hidalgo demonstrate the presence of common species in all analyzed samples, but also unique species in pulque from each of the three regions. Lactic acid bacteria (LAB) related to *L. acidophilus* (homofermentative) was the most abundant group present in the samples, while the previously reported *L. mesenteroides* and *Z. mobilis* (considered as essential microorganisms) were detected in a minor proportion only in two of the studied samples (Escalante et al. 2004).

43.5.2 Microbial Dynamics of the Fermentation

Escalante et al. (2008) found in aguamiel an initial total count of 1.3×10^7 CFU/mL mesophilic bacteria, 3.2×10^8 CFU/mL LAB and 3.1×10^4 CFU/mL yeasts; in a sample taken after addition of the fresh aguamiel to the seed (corresponding to the initial fermentation time: T0) the total yeast count increased to 8.8×10^6 CFU/mL, while aerobic mesophilic bacteria and LAB counts were not modified: 1.2×10^7 and 1.5×10^8 CFU/mL, respectively. After 3 hours of fermentation, yeast increased to 1.4×10^7 CFU/mL, maintaining this level until the end of fermentation (6 hours); total bacteria counts remained constant (Escalante et al. 2008).

Bacterial diversity detected in aguamiel was composed mainly by *L. mesenteroides*, *L. citreum*, *L. kimchi*, and the Proteobacteria *Acinetobacter radioresistens*, *Erwinia rhapontici*, and *Enterobacter* spp. At T0, they were detected mainly some species observed in aguamiel and others present in the seed: *L. mesenteroides*, *L. citreum*, *Lactobacillus* spp. (related to *L. acidophilus*), and the Proteobacteria *E. agglomerans* (both in high proportion), *Kluyvera ascorbata*, *Serratia grimensis*, and the acetic acid bacteria (AAB) *Acetobacter malorum*. As fermentation proceed the population of *L. kimchi*, one of the most abundant LAB detected in aguamiel, decreased; those of *L. mesenteroides* and *L. citreum* remained relatively constant, while populations of *Lactococcus lactis* and *Z. mobilis* were detected in low proportion.

After 3 hours of fermentation, important changes in bacterial diversity were observed: *Lactobacillus* spp., *L. mesenteroides*, *E. agglomerans*, and *A. malorum* became the dominant species; *L. acidophilus* and *L. citreum* were detected in low proportion; *L. lactis*, *Z. mobilis*, and *A. radioresistens* remained relatively constant compared to T0, whereas *S. grimensis* and *K. ascorbata* were no longer found. At the end of the fermentation (6 hours), the bacteria diversity was mainly composed by the homofermentative *L. acidophilus* and *Lactobacillus* spp., the heterofermentative *L. mesenteroides*, *Lactococcus lactis* subsp. *lactis*, and *A. malorum*.

These results demonstrate that although no changes occurred in total bacteria counts during a 6-hour fermentation, important changes take place in species distribution.

Lactobacillus species have been previously detected in aguamiel and pulque fermentation, but no detailed information concerning the identity and role of this LAB group is available. Sánchez-Marroquín and Hope (1953) reported that lactobacilli are responsible for the assimilation of 53% of the sugars present in aguamiel. *L. acidophilus*, *L. hilgardii*, and the heterofermentative *L. sanfranciscensis* are capable to produce DL-lactic acid and to ferment glucose and fructose present in aguamiel, but only the former is able to ferment sucrose (Carr et al. 2002).

Lactobacilli are naturally associated to plants, fermented vegetables, fermented doughs, and alcoholic distilled beverages such as malt whisky (van Beek and Priest 2000). The ability of LAB to grow in acid environments and tolerate high ethanol concentrations could explain their high abundance during pulque fermentation (final pH = 4.5 and 4%–6% ethanol, Escalante et al. 2008). The presence of the AAB *A. pomorum*, *A. malorum*, and *Gluconobacter oxydans* in different pulque samples and the high production of acetic acid in comparison to lactic acid after 6 hours of fermentation (Escalante et al. 2004, 2008), suggests an important role of these bacteria in pulque fermentation.

Yeast identified along pulque fermentation belong to *Saccharomyces* (*S. cerevisiae*, *S. bayanus*, *S. paradoxus*) and non-*Saccharomyces* (*Candida* spp., *C. parapsilosis*, *C. lusitaniae*, *Kluyveromyces marxianus*, *K. lactis*, *Hanseniaspora uvarum*, *Pichia* spp., *P. guilliermondii*, *Torulaspora delbrueckii*) are capable to produce ethanol from glucose, fructose, and sucrose and to synthesize nutritive amino acids and vitamins as well as flavor-volatile compounds that influence the quality and the aromatic profile of the beverage. At early fermentation stages, the non-*Saccharomyces* yeasts develops, but as ethanol concentration increases, they are replaced by primary ethanol-producing yeasts such as *S. cerevisiae* and several *K. marxianus* ethanol-tolerant strains. Both yeasts species predominate throughout the fermentation until 6% of ethanol is reached (Lappe-Oliveras et al. 2008).

43.5.3 Physicochemical Changes during Pulque Fermentation

As already described, aguamiel is a sugar-rich sap containing sucrose, fructose, glucose, and fructo-oligosaccharides with a relatively acid pH (7.5–4.5), whereas pulque is an acidic (pH 4.3–3.5, total acidity of 0.75–0.4 g lactic acid/100 mL of pulque), alcoholic (4%–6%), and viscous beverage (Banco de Normas Mexicanas 2010b). Fresh aguamiel used to start fermentation with an initial pH of 6.0, decreases to 4.5 after mixing with the seed, and to 4.1 at the end of the process after 6 hours of fermentation. These environmental conditions favor the appearance of an acid- and ethanol-tolerant microbiota toward the end of the fermentation and probably affect the survival of non–acidic-resistant bacteria and yeast species that apparently disappear during the process as did the possible antimicrobial activity of LAB (production of bacteriocins and H_2O_2) and *Z. mobilis* subsp. *mobilis* (production of acetylmetylcarbinol) (Escalante et al. 2008; Lappe-Oliveras et al. 2008).

Fresh aguamiel has a mild temperature (17°C–20°C), which increases several degrees after inoculation with the seed as a consequence of the microbial activity. In a 6-hour fermentation carried out to assess the overall metabolic activity of the microbial community in aguamiel and pulque samples from the state of Morelos, the concentrations of sucrose, glucose, fructose, and fermentation products were determined during the process. Total sugars consumed after 6 hours of fermentation were 228.4 mM hexose equivalents (equivalent to 1370.5 mM of carbon, which was found as CO_2). It was assumed, in molar terms, that the amount of CO_2 produced is equal to the amount of acetic acid and ethanol. If 176.5 mM of carbon corresponding to 5.3 g/L of carbohydrates in EPS are also included, then 66.2% of total C was recovered in products. Ethanol reached a final value of 142.17 mM carbon equivalents, while 35.73 and 6.32 mM, respectively, of acetic and lactic acid concentrations were reached (Escalante et al. 2008).

Viscosity is one of the distinctive properties of pulque. This characteristic has been associated to EPS (dextran) production by *L. mesenteroides* strains (Sánchez-Marroquín and Hope 1953; Sánchez-Marroquín et al. 1957; Chellapandian et al. 1998; García-Garibay and López-Munguía 1993; Ramírez et al. 2004). However, after 6 hours of fermentation, Escalante et al. (2008) detected also a fructan EPS in pulque. Screening for EPS producing *Leuconostc* species from aguamiel and fermented pulque, allowed the isolation and identification of *L. citreum* as the unique EPS-producing bacteria (Conca 2008), questioning the essential role of *L. mesenteroides* during pulque fermentation.

43.6 Pulque: Nutritional and Traditional Medicine Aspects

Traditionally, pulque has been considered as a healthy beverage due to its nutrient content (described above). Based on a daily consumption, pulque can be considered as an important source of energy, vitamins, and essential amino acids, outstanding content of the essential amino acids lysine and tryptophan, which are deficient in the Mexican diet, which is based on maize. In some rural population in central Mexico where potable water supply is absent, pulque is consumed as water substitute due to its low alcohol content and high availability (Vargas 1999). The modern indigenous Otomíes living in the Mezquital Valley include pulque as part of their basic diet; it represents the second source of energy (after tortillas) and the third source of protein. Moreover, pulque is their main source of vitamin C and second of thiamine, riboflavin, calcium, and bioavailable iron (Vargas et al. 1998). Backstrand et al. (2002) in a study of pulque consumption by several communities of the Estado de México highlands, established that a daily intake of 500 mL supplied 215 kcal, 45% of vitamin C, 10% of niacin, 7% of thiamine, 6% riboflavin, 15% of iron, and microbial protein (with an adequate proportion of lysine and tryptophan).

Considering that pulque is also an alcoholic beverage, it has the same problems associated to excessive consumption of nondistilled alcoholic beverages, but up to now, no toxicological problems other than those related to excessive consumption of any alcoholic beverage has been reported for pulque. Since

TABLE 43.1

Relevant Traditional Medical Uses and Beneficial Effects Associated to the Consumption of Pulque

Proposed Uses	Clinical Trial Evidence/Comments	References
Mixing with ground chili (*Capsicum annuum* L.) and pumpkin seeds (*Cucurbita* spp.) as a tonic	None	Traditional pharmacopoeia
Boiled with chichicpactli (bark of *Garrya laurifolia* Hartw. and *G. ovata* Benth.). Beneficial for chest, stomach, and back pains	None	Traditional pharmacopoeia
Control of: dyspepsia, stomach pain, diarrhea, anemia, anorexia, asthenia, vertigo, severe headache, and some neuralgias, urinal infections, typhus, pityriasis, and syphilis. Diuretic. It was consumed hot to favor diaphoresis, relieve cough, and facilitate expectoration. Sediments of pulque were used in plasters to heal wounds	None Effects probably associated to content of agave saponins (plants sterols with hypocholesterolemic, anti-inflammatory and antibiotic activity). Specific effect of pulque remains unexplored	De la Cruz and Badiano 1964; Peana et al. 1997; Ramírez-Rancaño 2000
Increment of milk production during pregnancy and lactation	Possible galactogogue effect. Few clinical trials related to the content of ethanol in breast milk and effect on the weight and size of infant at moderate consumption	Ortiz de Montellano 1993; Argote-Espinosa et al. 1992; Flores-Huerta et al. 1992; Backstrand et al. 2001, 2004
Agaván (aguamiel syrup) against acute and chronic failure of kidney, bladder, and urethra; also for relief cough and cold and control of anemia	None	Cook et al. 1995; Ramírez-Rancaño 2000
Control of anemia	Avoid ferritin and hemoglobin deficiencies due to a better absorption and iron bioavailability in the presence of ethanol and vitamin C. In particular, the consumption of pulque supplied the Otomí women population with 7% of their daily iron intake	Backstrand et al. 2001
Facilitate cellular respiration and absorption of carbohydrates and proteins	None	Vargas et al. 1998

the middle of the 20th century, several authors have tried to correlate the high incidence of cirrhosis and mortality in rural and indigenous communities in several central states of Mexico with pulque consumption. Nevertheless, it seems that there are other factors associated with the consumption of the beverage, as the presence of endotoxins produced by enterobacteria, the deficient nutrition of the consumers, which increase the rate of the disease or bad sanitary practices during its elaboration (Narro-Robles et al. 1992; Vargas et al. 1998; Kershenovich 1999; Stoopen 1999).

Since the pre-Hispanic period, pulque has been used as traditional medicine to control several diseases (Table 43.1 summarizes principal traditional medical uses and beneficial effects associated to pulque consumption). During the colonial period several medical applications of maguey and its by-products were developed. Nevertheless, until very recently, the medical applications of pulque have been limited to traditional pharmacopoeia. Actually, there is an extensive application of pulque in the treatment of gastrointestinal disorders and intestinal infections that may be explained by pulque's prebiotic and probiotic activities.

43.6.1 Pulque as Probiotic and Prebiotic Agent

Aguamiel and pulque can be considered as probiotic products due to the presence of LAB, as *L. acidophilus* and *L. mesenteroides* (Escalante et al. 2004, 2008; Campos 2010), *Z. mobilis*, which has an antagonist activity particularly against pathogenic bacteria and fungi species (Wuanick 1970; Gonçalves de Lima 1978) and yeast (Steinkraus 1996) and as prebiotics because of its fructan and oligofructan content (Ortiz-Basurto et al. 2008; Ramírez-Higuera 2009).

Several archeological evidences suggest that pulque has been used since pre-Hispanic times as enema. It has recently been proven that this practice may have had beneficial effects on humans' health due to the enrichment of the digestive tract with potential probiotic bacteria (Lemus 2006). In support of the traditional applications of pulque in the treatment of several diseases, Campos (2010) reported the isolation of several *Leuconostoc* species from pulque, with potential probiotic activities such as resistance to acid and bile salts and antimicrobial activity *in vitro* and *in vivo* against pathogenic bacteria. Moreover, in recent experimental reports, Mancilla-Margalli and López (2006) and Ramírez-Higuera (2009) demonstrated the prebiotic activity of aguamiel inulin since it increases the growth of LAB.

43.7 Pulque Industrialization and Major Technological Challenges

43.7.1 Pulque Fermentation Medium Composition

The carbohydrate structure of agave plants and aguamiel is rather complex, combining the fructose polymer structure found in inulins and levans as described a few years ago by Mancilla-Margalli and Lopez (2006). Actually, the term *agavins* has been proposed by these authors due to the clear structural differences with inulin found in agave fructans. Most distilled agave fermented beverages rely on the total hydrolysis of sugars, while pulque fermentation properties are based in the amount of sucrose and monosaccharides found in aguamiel.

A modern production process requires the optimization and reduction in the agave production time. Each *agave pulquero* is capable to generate up to 1000 L of *aguamiel* in a daily extraction basis during 90 to 150 days, with a sugar content of 7% to 14%, depending on the agave species. For instance, in the case of *Agave tequilana* plants, the composition of water-soluble carbohydrates from plants of different ages revealed that 2-year plants exhibited the highest level of free monosaccharide and low-molecular-weight fructans (DP3–DP6) with potential application as prebiotics, while a maximum of fructan polymerization degree was achieved at 4 years with mean DP from 3 to 30 (Arrizon et al. 2009). According to these authors, as the type and amount of sugars accumulated in *A. tequilana* plants varies with age, harvesting time should be optimized in terms of productivity and product quality, a conclusion that could certainly be extrapolated to other agave species. This is in agreement with statements made previously by Sánchez-Marroquín (1970), who found the changes in aguamiel composition with collection time, agave region, and agave varieties an additional inconvenient for the process industrialization.

Surprisingly, Ortiz-Basurto et al. (2008) detected only minor differences in aguamiel composition among samples collected at different time intervals during the 3–6 months *aguamiel* harvest period of an *A. mapisaga* plant. Actually, the analyzed aguamiel contained 11.5 wt% of dry matter, which was composed mainly of sugars (75 wt%). Among these, 10 wt% were fructo-oligosaccharides. The variation of carbohydrate structure among the *agave pulqueros*, and its effect in the process and final product, is a research topic that has not yet been addressed, including the acceptability of industrialized pulque versions. On the other hand, industrialization has to be based in the planned and intensive production (propagation) of selected agave species from the actual wide variety of genetic diversity cultivated in México (García-Martín et al. 2007).

The introduction of hygienic aguamiel collecting and cajete-scraping techniques are also conditio sine quibus non for modernization. Aseptic mechanical extraction systems as well as the proper collecting and easy to handle reservoirs should be introduced. To avoid a preliminary fermentation, the agave juice must be pasteurized by microfiltration or cooled, as fermentation will occur even in the agave basin if not collected daily.

Up to now, no alternative to obtain aguamiel has been reported: however, it is always possible to obtain agave stem extracts by pressing or by extraction in diffusors of the agave stems. This alternative was reviewed previously (Steinkraus 1996), but no mention is made about the yields and sugar composition of the resulting extracts. This possibility may require the hydrolysis of fructans present in the agave stem as in other agave fermentation procedures such as tequila, where the agave stems are either cooked (heat treated) or ground and subjected to water extraction to obtain fermentable sugars, mainly fructose from agavins. The 4–6 months required for aguamiel extraction could then be reduced to a few hours, but the resulting media although feasible for fermentation will no doubt differ from aguamiel and could not be referred as such.

43.7.2 Fermentation

The already described pioneer works of Alfredo Sánchez-Marroquín from 1953 to 1970 (Steinkraus 1996) are important not only in terms of the microbiological characterization of pulque, but also in terms of efforts in the modernization of the production process. Sánchez-Marroquín was the first to introduce a simplification of the enormous diversity of microorganisms isolated from the beverage to a minimum starter formula composed of *S. cerevisiae*, *Z. mobilis*, *Lactobacillus* spp., and *L. mesenteroides* (Sánchez-Marroquín et al. 1957; Sánchez-Marroquín 1970). Although the three types of fermentations (alcoholic, lactic, and viscous) have been generally recognized as an essential element of pulque production, the sensorial properties associated to the products of the three processes (alcohol, lactic acid, and viscosity) can be obtained by alternative microorganisms. For instance, EPS associated to pulque viscosity have always been recognized as dextrans (Sánchez-Marroquín and Hope 1953; Chellapandian et al. 1998) but may also include levans and/or inulins (Goldman et al. 2008; Morales Arrieta et al. 2006; Olvera et al. 2007; Escalante et al. 2008). Isolated from pulque in 1924, *Z. mobilis*, recognized worldwide for its rapid alcohol fermentation, was included once among the essential pulque microbiota, but it is now considered as a "casual microorganism for pulque fermentation" (Escalante et al. 2004).

Besides its ethanol production properties, *Z. mobilis* is also known to produce levansucrase, an enzyme responsible for levan synthesis. Levan is a high-molecular-weight fructose polymer with β2–6 linkages among the fructose molecules, which also induces high viscosity. It is interesting to point out that no author has measured with precision the type and the amount of glycans (glucans and fructans) in pulque. However, and as already mentioned, it has been demonstrated that *Leuconostoc* spp. enzymatically produce dextrans, levans, and/or inulins from the equivalent extracellular or cell-associated enzymes (Chellapandian et al. 1998).

As early as 1967, Sánchez-Marroquín et al. reported that product quality as well as industrial operation would improve if the viscous fermentation is avoided. Actually, to avoid the synthesis of EPS, it is possible to invert all sucrose in aguamiel. Alternatively, glucose or fructose may also be added to support lactic and alcoholic fermentations. Finally, the presence of strains such as *L. mesenteroides* may be avoided. Nevertheless, efforts to preserve the prebiotic properties of fructo-oligosaccharides should be encouraged.

Although Sánchez-Marroquín et al. (1957) did not found differences in product quality following different inoculation strategies, parameters such as inoculation time and density of each of the selected strains remains to be defined in terms of growth rate (LAB grow much faster than yeast), final lactic acid and ethanol content, and eventually, EPS content. Kaffir (also known as Bantu), a traditional African beer is also a complex traditional fermentation beverage that has been successfully produced at large scale. In this case, the lactic acid fermentation of the saccharified sorghum starch takes place during one day, before alcoholic fermentation, which requires an additional 2 to 5 days.

Modern pulque versions will first have to solve the quest that represents the microbial definition of pulque, or alternatively, promote the product resulting from a selected microbial inoculum. Lappe-Oliveras et al. (2008) review the numerous reports dealing with pulque microbial studies in search for the optimal or essential microbial community; however, Escalante et al. (2008) demonstrated, using a polyphasic approach, to study bacterial diversity in aguamiel and pulque (including non–culture-dependent approaches and PCR-based molecular techniques) that the microbial definition of pulque remains as a difficult quest to solve.

43.7.3 Pulque Flavor

As far as flavor is concerned, Sanchez-Marroquin (1970) reports the measurement of an ester profile, but no reference is given regarding the composition and profile of the pulque bouquet. The total amount of esters (as ethylacetate) is reported as 20–30 mg/100 mL, aldehydes (as acetaldehyde) 2.5 mg/100 mL, and higher alcohols (fusel oils) 80–100 mg/L. It has been proposed that the common tradition of combining pulque with fruits or vegetables (*curados*) derives from the intention to correct off flavors or fermentation defects. Offensive flavors derived from bacterial contaminations, frequent in traditional pulque, must absolutely be eliminated in a modern fermentation facility even if some traditional parts of the process are conserved. Some undesirable flavor compounds often result from a combination of metabolic pathways, particularly if a complex microbial diversity is involved. In a recent study, De León-Rodríguez et al. (2008) reported the aroma compounds of agave alcoholic beverages as determined by gas chromatography and headspace solid-phase microextraction–gas chromatography–mass spectrometry as well as a cluster analysis of the major components. There were 11 "major compounds" and around 17 minor compounds that could be used as authenticity markers since they were beverage-specific and provide an interesting fingerprint. It was observed that 3-methyl-thio-1-propanol and nonanoic acids are specific for pulque and may be used as authenticity markers. In this study, pulque is grouped separately from others agave beverages due to its low concentration of ethanol.

43.7.4 Pulque Conservation

The Mexican market in the United States and the ethnic food demands in the globalization era are the major driving forces to develop a stable pulque product produced at a larger scale. One of the main challenges in this direction lies in the difficulties associated to conservation. As pulque is usually consumed fresh after fermentation, mainly in rural areas, little efforts have been devoted to introduce stabilization procedures. Therefore, there are many decisions regarding pulque processing after fermentation that have to be taken into consideration, particularly if pulque production and commercialization continues to rise. These decisions involve the introduction of pasteurization and/or filtration steps as well as the addition of preservatives. If pasteurization had been introduced to pulque production at the same time as in beer we would probably be reading a different story about the evolution of both beverages in the 20th century.

It is almost impossible to bottle fermented pulque without pasteurization, as sugars are usually not exhausted: actually, a residual amount of agavin and fructo-oligosaccharides is desirable in order to increase the prebiotic attributes of the product. On the other hand, filtration or sterilization would increase pulque stability and shelf life but reduce its probiotic content, also recognized as one of pulque nutritional attributes; efforts to preserve or supplement its vitamin (ascorbic acid) and nonheme iron content should also have to be considered. Moreover, additives such as preservation agents, antioxidants, colorants, or texture agents will affect the image of pulque as a natural and, eventually, organic product. In the particular case of the pasteurized bottled brand "Pulque, Cool Passion" (Poliqui SA de CV), the product contains sodium benzoate as preservative, as well as color and sugars. The selection of an adequate packaging requires consideration as biological and photochemical oxidation may also be a problem in storage stability. The paradigm here is that to promote the industrialization of pulque, radical changes in the public perception of pulque have to be made, particularly if new markets are searched. An advantage is that the new pulque producers are probably offering a product that the large majority of the public (particularly outside the country) never tasted before. Actually, it is highly possible that new production processes will require modifications in the Mexican regulation system, as aguamiel as well as pulque are defined specifically in terms of composition and production norms (NMX-V-022 and NMX-V-037) approved in 1972.

According to the Del Razo group, which started canned pulque production in 1980 in the state of Tlaxcala to fulfill the pulque demand in the United States, the market had already grown to more than 100,000 L/month by the start of the 21st century. The Del Razo pulque canning process includes aseptic canning and a controlled process to preserve pulque's sensorial and physicochemical properties. After several unsuccessful efforts, Del Razo group finally developed an effective process and now exports to the United States the brand "Pulque Hacienda 1881" in various flavor presentations as well as natural or plain pulque. According to Del Razo, the modification of their fermentation process has reduced the traditional off-flavor associated with pulque and also its viscosity, which means that neither *Z. mobilis* nor *L. mesenteroides* (nor at least the wild strains) are included in the process. Other trademarks include Jicara, Malinche, Pulquemex, and Pulque Azteca, also competing for the North American market.

After the paradigmatic work carried out by Sánchez-Marroquín, we are now witnessing a renewed interest in this ethnic beverage from both basic and applied research. Pulque is now fully recognized as rich in tradition, microbial diversity, and nutritional value. The recovery of "traditional pulque," as well as the birth of new alternatives of aguamiel processing are promoted by the demand of Mexicans living abroad as well as the curiosity of consumers looking for new gastronomic and nutritional experiences.

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