



REVIEW ARTICLE

## Agave spp.: Sap by-products and health benefits

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### Abstract

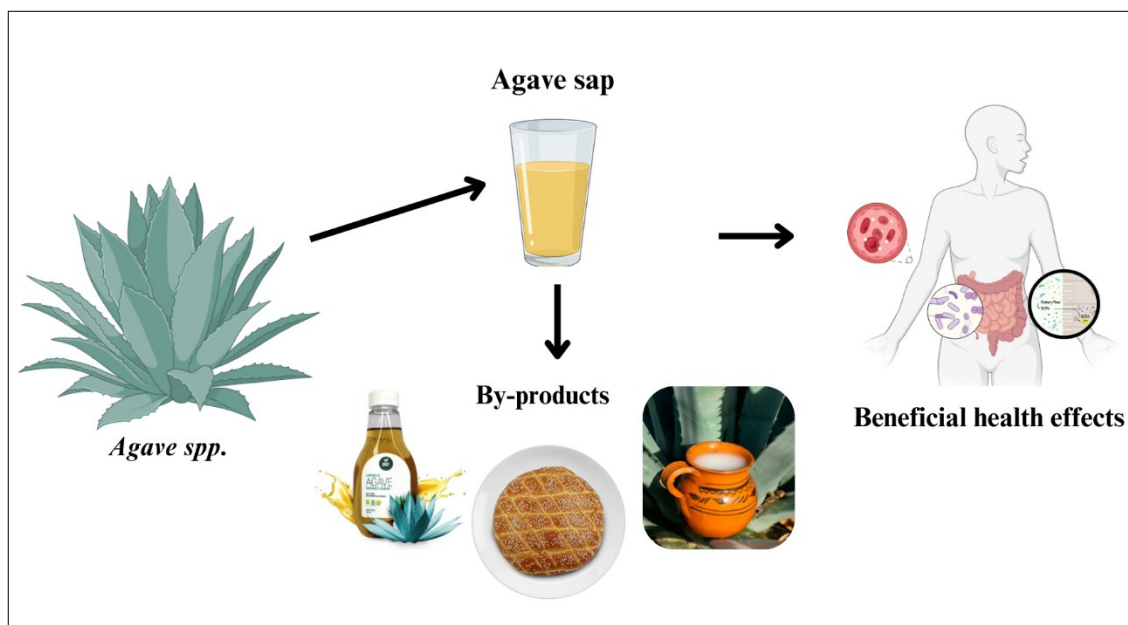
*Agave* spp. is a monocotyledonous plant distributed across the American continent. More than 273 species have been documented, of which 215 are found in Mexico and 151 are endemic to the country. Historically, *Agave* spp. has been used in a variety of applications, including food, the production of alcoholic beverages, textiles and everyday utensils, depending on the culture and species. Commonly referred to as “maguey”, the various species of *Agave* have diverse applications for their residues and particularly the sap. Due to its nutritional composition, which is rich in fructooligosaccharides, vitamins, minerals, saponins and essential and non-essential amino acids, the sap has been associated with several positive health effects. These include antioxidant properties, prebiotic effects, enhanced mineral absorption, inhibition of adenoma and carcinoma precursor lesions and a low glycemic index, classifying it as a functional beverage. Noteworthy species include *Agave salmiana* and *Agave atrovirens*, which are the most significant for sap extraction and the production of pulque, an alcoholic beverage derived from the natural fermentation of *Agave* sap. This review aims to analyze the primary by-products that can be derived from the sap of the commonly used *Agave* spp. species and evaluate their reported health benefits *in vitro* and *in vivo* studies, to promote the increased utilization of *Agave* sap based on scientific evidence.

**Keywords:** *Agave atrovirens*; *Agave salmiana*; antioxidants; by-products; mineral absorption; prebiotics

### Introduction

The *Agave* genus thrives in semi-arid and temperate regions of the American continent. In Mexico, some of the most widely distributed species, such as *Agave salmiana* and *Agave atrovirens*, are traditionally used for the production of “aguamiel”, a sap exuded by the plant upon reaching maturity between 7 and 10 years of age. During this period, the center of the plant is removed before flowering, after which the plant dies. This sap is extracted twice daily during the winter and sometimes up to three times a day in the summer, over approximately three to six months, depending on the species, weather and edaphic conditions of the production area. After this period, production declines and eventually, the plant dies. Following each extraction of sap, the cajete (the hole made in the center of the plant) is scraped so that the sap can re-emerge and be collected (1). Once extracted, the sap is filtered to remove any physical contaminants, such as insects attracted by the sweet taste or soil particles carried by the air. The sap is then stored for “cooking”, during which it is heated for short periods. After cooking, the “aguamiel” is refrigerated until consumed. Traditionally, most of the sap produced is used to make “pulque”, an alcoholic beverage resulting from the natural fermentation of the sap due to its sugar-rich composition and its content of lactic acid and heterolactic bacteria, such as those of the *Leuconostoc* genus, producers of lactic acid, acetic acid and ethanol (2).

Pulque began to be produced and consumed in pre-Hispanic times, becoming a beverage of great cultural importance in Mexico. Initially, it was produced simply and of lower quality, but its production expanded significantly with the establishment of the first *Agave* farms which implemented improved techniques for better quality production (3). However, its consumption drastically declined at the beginning of the 20th century due to various movements and social changes. The introduction of other alcoholic beverages such as beer further decreased its popularity, this led to a reduction in the production of pulque and the cultivation of pulque maguey plantations, primarily *A. atrovirens* (4). Currently, there is renewed interest in reviving the culture and traditions surrounding the cultivation of *Agave* and its by-products (“aguamiel”, *Agave* sap honey, pulque, among others) (5). An important aspect of promoting the consumption of these products has been scientific research, which aims to verify the health benefits attributed to *Agave* sap. If these benefits are confirmed, *Agave* sap could be considered a nutraceutical beverage. Consequently, the potential of sap by-products (syrops, sweeteners, bakery products) has been studied as technological diversification products, offering benefits both to health and to the economy by revaluing maguey cultivation (6). Based on this background, the objective of this paper was to review, discuss and analyze the by-products of *Agave* spp. sap and their potential health benefits. Given the historical and cultural significance of pulque and other *Agave*-derived products (Fig. 1), this research also seeks to explore their role as nutraceutical beverages and functional foods.



**Fig. 1.** Outline of the review.

## Materials and Methods

A systematic review of studies, articles and scientific literature available in databases such as PubMed Central, Google Scholar, Scopus and Web of Science were conducted. The selection criteria focused on documents no older than seven years, using keywords such as “*Agave spp.*”, “*Agave sap*”, “*Agave by-products*”, “*Agave atrovirens*” and “*Agave salmiana*”, “*Agave waste*” selecting only those with relevance in the focus of this review. In this case, 85 documents were selected and analyzed for the synthesis of the most relevant information.

## Results and Discussion

### Maguery

Maguery (*Agave spp.*) is a monocotyledonous plant that can be propagated both by seeds and by scions. It is known for its low nutrient demand and efficient use of water (7), enabling it to survive under extreme conditions of both temperature and water availability. This genus is endemic to the American continent, where it is mostly distributed in arid and semiarid areas of México (8). It has been described as having high agroecological value due to its ability to retain soil, infiltrate moisture and phyto-stabilize and remediate contaminated soils (9, 10). Of the 273 documented *Agave* species, 215 are found in Mexico, where maguery has been used by native peoples since pre-Hispanic times for food, clothing and construction needs (11).

The maguery “pulquero”, unlike other species used for the production of alcoholic beverages, requires low technification in its fermentation processes, thus, it has not undergone major changes since pre-Hispanic times (12). The cultivation and propagation of the different varieties of *Agave pulquero* are primarily intended for the extraction of sap for the production of pulque. However, the plants can also be used for other purposes, as their stalks are as a source of fiber traditionally used for the production of utensils such as brooms. The plant is also commonly used for the preparation of “barbacoa” (a traditional Mexican meat-based dish) and “mixiote” (a Mexican dish made with enchilada beef or chicken), where the meat is wrapped in the skin extracted from the maguery stalk (13). Metzal, the tissue or pulp generated from the scraping of the maguery for sap

extraction, has recently been studied for its nutritional potential. Although, it is mainly used as animal feed or, in times of scarcity, incorporated into foods such as tortillas and tamales, its proximate analysis indicates that it contains significant amounts of sugars (62-79 %), proteins (2-6 %) and fiber (8-21 %). This composition suggests a high potential for developing food products with nutritional benefits (14).

In some species of *Agave*, mainly *A. salmiana* and *A. aplanata*, “chinicuiles” (larvae of the nocturnal moth *Comadia redtenbacheri*), commonly known as red maguery worms, are found and are a highly appreciated as food resource in the cultures of the pulque regions. They are consumed roasted, fried in molcajete sauce, or as an accompaniment to various dishes. The chinicuiles feed on the roots of the maguery and to collect them, the maguery must be tilted and completely extracted from the ground. In the case of the maguery “pulquero”, at the end of harvesting, the base of the plant is cleaned and placed back in the ground to continue growing. However, in the case of *A. aplanata*, a wild maguery, if it is not replanted, it dies. This practice has led to the overexploitation of the resource and reduced populations of both the insect and the magueys (15).

### *Agave atrovirens*

The maguery *A. atrovirens*, commonly known as “maguery cenizo”, is found mainly in semi-desert regions of Mexico (Fig. 2), where it is primarily used for the production of *Agave* sap and pulque. Recently, interest in the integral utilization of maguery plants has led to research aimed at using the residues from *Agave* sap production, such as bagasse. A previous study evaluated the use of bagasse from *A. atrovirens* and *A. salmiana* as a carbon source for lactic acid bacteria, obtaining good results in both cases (16). This suggested the potential of *Agave* bagasse as an ingredient for the development of functional foods. The lignocellulosic material of *A. atrovirens* can be used for bioethanol production. Methods such as cellulase immobilization on magnetic particles coated with chitosan have been proposed for this purpose. This method has proven effective for the hydrolysis of lignocellulosic material, with yields close to those obtained with free enzyme (17). *A. atrovirens* fibers have also been used as support, substrate and inducing sources for the production of cellulolytic complexes by *Trichoderma asperellum*.





**Fig. 2.** *Agave atrovirens* plant in Saltillo Coahuila, Mexico.

Results showed that the fibers of this species are a good source of carbon and support, yielding significant amounts of endoglucanase, exoglucanase and  $\beta$ -glucosidase. The ability to hydrolyze and produce fermentable sugars from these enzymes is important for different industrial processes (18).

Other potential uses of *A. atrovirens* plant residues include serving as microwave absorbers for anechoic chambers, enclosures for electromagnetic compatibility, or antenna measurements. Due to its high carbon content, it is an environmentally friendly alternative that can reduce the generation of polluting carbon dioxide. *Agave* residue has been reported to be the best candidate for the manufacture of low-cost, eco-friendly microwave absorbers compared to other organic wastes such as *Opuntia ficus-indica* cladodes or *Cocos nucifera* L. husk (19).

#### ***Agave salmiana***

*A. salmiana* is distributed in México in both wild and cultivated forms (Fig. 3). Various botanical types and varieties of this species have been described, including *A. salmiana* subsp. *salmiana*, *tehuacanesi*, *Ayoteco* and others. They are known by different names such as Púa Larga, Manso, Chalqueño, Carricillo, Blanco cenizo and Sha'mini, distinguished by their morphological and biochemical

characteristics (20). This species is considered the most suitable for pulque production due to its high acceptance among consumers (1). The plant must reach an age of 8 to 10 years before it can be castrated and begin to produce sap. It has a productive life ranging from 3 to 6 months, during which the plant produces approximately 5 L of sap per day, declining this production towards the end of its productive life. In recent years, the cultivation of this species has significantly declined by 80 % in the central states of Tlaxcala, Hidalgo and Mexico (21). This decline has prompted the search for better substrates and fertilization management to enhance plant growth and sugar yields. It has been found that, in seed reproduction, perlite as a substrate confers the highest concentration of sugars and promotes plant growth, while fertilization has no significant effect. *In vitro* culture via direct organogenesis (medium with hormones that induce the formation of shoots or roots from explants) and indirect organogenesis (medium that promotes the formation of a callus) yielded better results with growth regulators in MS (Murashige and Skoog) culture medium (22). Additionally, the highest CO<sub>2</sub> exchange rate was observed in plants with abundant soil moisture (23).

Several metagenomic studies have explored the microbial diversity of *A. salmiana*. These studies include diazotrophic (nitrogen





**Fig. 3.** *Agave salmiana* cultivation in Saltillo, Coahuila.

-fixing) bacterial communities present in the rhizosphere (soil region surrounding the plant roots), phyllosphere (leaf surface) and endosphere (internal spaces) of the roots and leaves. A previous study identified ten bacterial strains, including two from the Enterobacteriaceae family and one from the *Stenotrophomonas* genus (24). Furthermore, molecular identification of the plant's bacterial endomicrobiota revealed a greater representation of the *Bacillus*, *Enterobacter* and *Leclercia* genera, many of which have potential for nitrogen fixation, production of antimicrobial compounds and growth-promoting compounds (25).

Recently, due to the waste generated from pulque production, several studies have investigated the utilization of *A. salmiana* bagasse. This waste material contains steroid saponins and other phytochemicals that can be recovered using methods such as ultrasound-assisted extraction with water as a solvent. A previous study demonstrated that using water as a solvent provides a higher cavitation intensity, enabling better recovery of bioactives (26). Moreover, *A. salmiana* bagasse has been proposed as an alternative substrate for cultivating edible fungi such as *Pleurotus* spp. This is attributed to its high fiber and nitrogen content. Early research have shown that using *A. salmiana* bagasse as a substrate does not alter the chemical and nutritional properties of the fungi,

making it a viable alternative ecological substrate (27). Additionally, the use of *A. salmiana* leaves as a carbon source (lignocellulosic biomass) for producing cellulases by the fungus *Penicillium* sp. has been reported. A previous study reported a hydrolysis rate of 51.6 % of the parenchyma within 48 hr, indicating the potential of this material for obtaining reducing sugars and enzyme production (28).

### **Agave sap production**

Traditionally, *Agave* sap also known as aguamiel, has been primarily produced for the fabrication of pulque. In Mexico, the *Agave* species commonly used for aguamiel production include *A. atrovirens* Karw, *A. americana*, *A. mapisaga*, *A. hookeri*, *A. inaequidens*, *A. marmorata* Roezl and predominantly *A. salmiana*. Currently, the leading producers of maguey pulquero in Mexico are the states of Hidalgo (comprising 73 % of production), Mexico, Tlaxcala and Puebla (4). Due to historical and cultural factors stemming from the campaign against pulque in the 20th century, its consumption was relegated to economic and social marginalization in its marketing (29). In the last decade, sap production has experienced a significant reduction due to multiple factors. These include challenges in defining political and public procedures to promote the conservation, protection, management and integral use of the plant, along with the loss of biodiversity of these plants and desertification (30).

The plants used for production are selected by individuals with extensive knowledge of the plant, as the process must be carried out just before the development of the flower bud. The central leaves of the selected maguey plants are removed, a task that can be challenging due to the size of the plant and the tools required. A cavity known as a “cajete” is created, with its walls scraped and left to heal before being scraped again to stimulate sap production (31) (Fig. 4). The newly produced sap is slightly amber, with a sweet and herbal flavor. According to the 1972 Mexican standard NMX-V-022-1972, the sap is classified into two types: Type I, defined as high quality with a high sugar content and Type II, defined as lower quality with a lower sugar content. Type II sap is typically selected for pulque production. Quality is measured using parameters established in the standard, such as pH and reducing sugar levels. The volume of production varies depending on the stage in the plant’s productive life and the specific species.

Sap is highly sensitive to spontaneous fermentation processes due to its high sugar content and its great microbial diversity. The sugar content includes glucose, fructose, sucrose and fructooligosaccharides. The content varies depending on the species, climatic and edaphic factors and the method of preservation. Its microbiological diversity includes lactic acid bacteria, exopolysaccharide-producing bacteria and ethanol-producing bacteria. This diversity is also species- and environment-dependent. Notable microorganisms include *Leuconostoc* sp., *Leuconostoc gelidum*, *Lactococcus lactis*, *Enterococcus casseliflavus*, *Pediococcus* sp., *Trichococcus* sp., *Kluyveromyces marxianus*, *Saccharomyces cerevisiae* and recently reported, *Kazachstania zonata* (32). These microorganisms play a crucial role in the flavor and quality of *Agave* sap and after fermentation, in pulque. They confer probiotic properties and the associated health benefits, which will be discussed later.

### Sap by-products

#### Sap concentrate or syrup

This by-product is obtained from sap through the hydrolysis of *Agave fructans*, resulting in the Elimination of a large amount of water, leaving only 20 % to 28 % moisture. The syrup has a high sugar content, primarily fructose (87 %) and glucose (10 %) (33). It has an average shelf life of one and a half years and has gained popularity as a natural sweetener due to its quality, nutraceutical and functional properties. Consequently, it is used as a sugar substitute in food and beverage processing. Characterizations of *A. salmiana* syrup from different Mexican pulque regions (State of Mexico, Hidalgo and Tlaxcala) have been conducted. Parameters such as pH, acidity, degrees Brix, protein content, phenolic content and antioxidant capacity were analyzed. It was found that syrups from Tlaxcala had the highest protein content, while those from the State of Mexico exhibited the highest antioxidant activity and phenolic compound content (34).

*A. salmiana* syrup obtained by low-pressure evaporation of sap could be a superior production method compared to conventional evaporation at atmospheric pressure in an open pot or ladle at boiling temperatures. Low-pressure evaporation may better preserve the nutraceutical and bioactive compounds of sap, as evidenced by the presence of 1-kestose in all syrups obtained by this method (35). A previous study evaluated the expression of recombinant human interleukin-2 in *Escherichia coli* using glucose, fructose and *A. salmiana* syrup as carbon sources (36). The cultures using glucose had the lowest yield, followed by those using fructose,

which saw a 1.9-fold increase in production. The highest yield of interleukin-2 was achieved with *A. salmiana* syrup, resulting in a 3.9-fold increase compared to glucose. Meanwhile, *A. tequilana* Weber syrup, commonly known as blue *Agave* syrup, is available in supermarkets and convenience stores. Unlike *A. salmiana* syrup, it is produced by hydrolyzing the fructans of *Agave* pineapples through enzymatic hydrolysis or acid-thermal hydrolysis, leading to its designation as high fructose syrup (37). The production of syrup from pulque maguey sap could contribute to the sustainable development of pulque-producing communities and promote maguey cultivation. This necessitates further studies on this by-product and the creation of technical and economic information for producers.

#### Pulque

The natural fermentation of sap occurs within 3-6 hr, though this time can be reduced by adding already fermented pulque as an inoculum. This process is facilitated by the carbohydrate content and the presence of microorganisms such as lactic acid bacteria and yeasts. Pulque, a drink traditionally consumed in Mexico, can be enjoyed either naturally or “cured” (mixed with various ingredients to confer different characteristics and flavors, such as fruits). Its importance lies on its historical, economic and social relevance. Pulque has an alcohol content between 3 % and 5 %, depending on the season, the quality of the sap used and the degree of fermentation (38). Its consumption dates back to pre-Hispanic times, with evidence of its use found in various pre-Hispanic cultures such as the Aztec, Toltec, Olmec and Otomi, where it was considered medicinal for its benefits and properties (39). Currently, its production is on a small scale, mainly for self-consumption and local sales, as well as for the production of other pulque by-products such as bread (13).

The lactic acid bacteria in pulque provide different biological activities such as probiotic, anti-inflammatory, immune response modulation, colitis attenuation and antimicrobial (39). Reports on the benefits of pulque date back to 1946, when a study examined the nutritional benefits of consuming 2 L of pulque daily for 7 days in an indigenous group of 100 people from the state of Hidalgo. This study concluded that pulque consumption significantly contributes calories, vitamins (especially vitamin C) and minerals such as calcium and iron (40). Subsequent studies have published its content of vitamins, minerals, saponins and amino acids (41-43).

The shelf life of pulque is three days when refrigerated, after which its taste becomes excessively strong and acidic, rendering it unsuitable for consumption. Consequently, efforts have been directed towards stabilizing and preserving pulque through pasteurization to halt the fermentation process. Currently, various methods and strategies, such as microencapsulation, spray drying and ohmic treatment, have been proposed to enhance pulque processing, aiming to extend its shelf life and consequently boost its production and commercialization (44). Notably, there exist Mexican companies specializing in the production, packaging and global marketing of pulque, including Procesadora de Pulque SA de CV, Pulquemanía SAPI de CV, Poliwhui SA de CV, Pulque Azteca SA de CV and Llanos de Apan (33).

#### Pulque in baking

Pulque bread, an artisanal product originating in the 16th century, is primarily produced in pulque-producing states, notably Tlaxcala and Coahuila, where it holds a significant place in local gastronomy.





**Fig. 4.** *Agave salmiana* sap extraction, Las Mangas ejido. Saltillo, Coahuila.

It represents a cultural fusion, combining Spanish baking techniques with indigenous ingredients such as pulque (45). In the preparation of pulque bread, four main ingredients are utilized: flour, pulque, lard and sugar. Sugar is dissolved in the pulque and combined with lard in the center of the flour. Pulque serves as the fermenting agent for the flour, a process occurring in multiple stages. Initially, during kneading, the ingredients are exposed to oxygen, promoting fermentation by yeasts that metabolize the free sugars in the flour. The second fermentation stage takes place when the dough is left to rest for a period of 3-4 hr. Finally, the last fermentation stage occurs once the dough has been shaped into its final form and divided into pieces. These last two stages occur at room temperature, influenced by climatic conditions. Traditionally, pulque bread is baked in wood-fired ovens, with baking time determined by the oven's incandescence and the baker's judgment (45).

In Saltillo, Coahuila, a city where the tradition of pulque bread production is upheld, historical records indicate that in 1864, pulque bread continues to be produced and enjoyed (46). However,

due to the gradual erosion of traditional practices, pulque consumption has declined in the producing states, with its consumption primarily limited to adults. Therefore, it is imperative to implement initiatives aimed at revitalizing this cultural heritage (45).

Xattle, the sediment residue of pulque containing predominant microorganisms from the *Lactobacillus* and *Saccharomyces* genera, has emerged as a valuable starter culture in dough fermentation for baking. Studies have revealed its potential to lower the glycemic index in bakery products due to the activity of these bacteria and yeasts, offering an opportunity to produce baked goods with reduced glycemic impact (47). Furthermore, *A. salmiana* bagasse (see Fig. 5) has been utilized as a raw material for the production of flour, which is then incorporated into bakery formulations to create muffins with enhanced functional properties. Formulations containing 20 % bagasse flour have demonstrated superior acceptability, suggesting a promising avenue for the development of nutritious bakery products (48).





**Fig. 5.** Bagasse of *A. salmiana*.

### Health benefits of *Agave* sap

#### Antioxidant capacity

Refers to the ability to inhibit the activity of free radicals, known catalysts of degenerative diseases such as cancer (see Table 1). This inhibition is attributed to various classes of compounds capable of interacting with radicals and halting the chain reactions they induce. Among these compounds with antioxidant capacity are vitamins, including vitamins C and E, carotenoids such as carotenes, polyphenols like flavonols, flavones and anthocyanins, as well as tannins, among others (49).

The assessment of antioxidant activity in foods relies on quantifying the scavenging of free radicals by antioxidant compounds. Common methods include ABTS, which measures antioxidant activity equivalent to Trolox (50), DPPH, based on the reduction of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in the presence of antioxidants (51) and ferric reducing antioxidant power (FRAP), which assesses the capacity to reduce iron ions. In this method, the ferric form of iron-tripyridyl-triazine is reduced to the ferrous form, resulting in a blue coloration indicative of antioxidant presence (52).

In *Agave* sap, the antioxidant effect is attributed to its phenolic compounds and vitamin content, particularly vitamin C, which varies depending on the *Agave* species. A previous study report that the antioxidant capacity of *A. salmiana* sap is comparable to other beverages known for their high antioxidant content, such as orange juice (53).

Earlier research advise that the optimal pasteurization time for sap should be less than 30 min, as heat treatment increases the content of reducing sugars and reduces vitamin concentrations, although it positively affects the antioxidant capacity (54). Specifically, in *A. mapisaga* sap, the antioxidant capacity increases by 2 % compared to fresh sap, as determined by the DPPH methodology. In tests conducted on the quantification of antioxidant components and antioxidant activity using DPPH on syrups of *A. salmiana* and *A. mapisaga*, higher antioxidant capacity was observed in comparison to that reported in bee honey, with *A. salmiana* sap exhibiting the highest capacity among the tested sap (55). A previous study evaluated the effects of ozone treatment on the stability and physicochemical and nutraceutical properties of *A. salmiana* sap (56). The study indicated that ozone treatment significantly reduces the antioxidant activity of sap due to the loss of

**Table 1.** *In vivo* and *in vitro* tests on the beneficial health effects of *A. salmiana* and *A. atrovirens* mead and its by-products

Beneficial health effects	<i>A. salmiana</i>		<i>A. atrovirens</i>	
	<i>In vivo</i> assays	<i>In vitro</i> assays	<i>In vivo</i> assays	<i>In vitro</i> assays
Antioxidant effect	X	Syrup, DPPH (55)	X	DPPH and ABTS (57)
Prebiotic effect	Fructans as a dietary supplement for children with malnutrition (63)	Prebiotic effect of fructans on probiotic lactic acid bacteria (63)	X	FOS content (60)
Probiotic effect	X	Identification of microorganisms previously reported to be probiotics (71) and metagenomic study (32)	X	Identification of microorganisms previously reported to be probiotics (71) and metagenomic study (32)
Mineral absorption	X	X	X	X
Glycemic index reduction	Syrup, morphological and metabolic changes in diabetic and non-diabetic rats (80)	Syrup, determination of glycemic index (81)	X	Syrup extract, determination of glucose release inhibition (79)

antioxidant compounds caused by the strong oxidant activity of ozone. Although ozone treatment could improve other qualities of sap, it would not preserve its antioxidant capacity. The superior antioxidant capacity of *Agave* honey confers a characteristic that justifies its classification as a nutraceutical, making it a preferred choice over other commercial honeys. Additionally, ultrasound treatment increased the stability and antioxidant effect (88.14 %, 111.41 and 72.29  $\mu\text{mol TE}$  (Trolox equivalent)/L, respectively) of sap from *A. atrovirens* compared to pasteurized and fresh sap (57).

#### Prebiotic effect

Prebiotics are non-digestible carbohydrates that resist digestion in the small intestine. Upon reaching the colon, they are fermented by the intestinal microbiota and used as a substrate for the selective growth of probiotic bacteria, which maintain balance and promote digestive health. Since ancient times, the laxative effects of certain foods have been noted and later attributed to their “dietary fiber” content. Currently, several collateral health benefits are attributed to the prebiotic effect, such as a reduction in the risk of cardiovascular disease (due to the reduction of low-density lipoproteins), improved glycemic control (as their consumption attenuates the rate of glucose absorption) and a decreased risk of colorectal cancer associated with low-fiber diets (58).

Initially, compounds were called prebiotics because they selectively promoted the growth of beneficial bacteria that help maintain digestive health. However, the definition was later expanded to include their role in selectively inhibiting certain non-beneficial microorganisms. For a fiber to be considered a prebiotic, it must resist gastric acidity and absorption in the upper gastrointestinal tract. Additionally, it must be fermented by the intestinal microbiota and stimulate the selective growth of health-promoting bacteria. Among the compound's considered prebiotics are poly- and oligosaccharides such as inulin, oligofructose and fructooligosaccharides. These have been shown to increase fecal bifidobacteria with an intake of 5-8 g per day (59).

A significant content of fructooligosaccharides (15.51 %) has been reported in *A. atrovirens* sap, primarily consisting of short-chain fructooligosaccharides that are fermented more quickly than those with longer chains (60). Several *Agave* species synthesize and store fructans in their stems, which vary in molecular structure and weight, with degrees of polymerization ranging from 3 to 29. These fructans act as reserve carbohydrates in plant metabolism (61). Additionally, microorganisms such as *Companilactobacillus kimchi* are abundantly found in sap. Together with *L. mesenteroides*, they produce extracellular polysaccharides of great economic importance due to their properties as prebiotics and soluble fiber

(62). Furthermore, the earlier research (63) evaluated the effect of *A. salmiana* fructans on the growth of lactic acid bacteria (*Lactococcus lactis*, *Lactobacillus acidophilus* and *Bifidobacterium longum*), observing that increased concentrations of fructans promoted greater bacterial growth. Supplementing the diet of malnourished children with *A. salmiana* fructans increased lactic acid bacteria, decreased pH and reduced Gram-negative bacteria in fecal samples.

#### Probiotic effect

Probiotics are non-pathogenic microorganisms that, when ingested, can exert a positive influence on health. They help restore the function and composition of the intestinal microbiota, thereby decreasing gastrointestinal disorders (64). Microbiological strains with probiotic potential can be identified through various tests. *In vivo* tests are performed on human and animal models to assess the processing of the microorganism and its interaction with the intestinal microbiota (65). *In vitro* tests evaluate resistance to low pH, bile salts, lysozyme and proteases; antimicrobial activity; antibiotic sensitivity; and gastrointestinal adhesion (65-68).

The probiotic effect of sap is attributed to the presence of lactic acid bacteria from the *Lactobacillus* and *Leuconostoc* genera, as well as yeasts from the *Zymomonas*, *Saccharomyces* and *Kluyveromyces* genera (69). It has been studied *in vitro* by evaluating microbial survival in simulated gastrointestinal tract conditions, where survival rates ranged from 47.8 % to 89.2 %. To be considered probiotic, microorganisms must inhibit the growth of pathogenic microorganisms such as *E. coli*, *S. aureus* and *H. pylori* through the production of sugar catabolites, fat and amino acid metabolites, oxygen and bacteriocins. Sixty percent of the sap isolates showed inhibition of *S. aureus* and *E. coli* and 100 % inhibited *H. pylori* (68).

The production of cellulolytic enzymes by probiotic microorganisms, such as *Enterococcus faecium* and *Lactobacillus lactis*, has been evaluated using sap as a substrate. Enzyme yields of 1460 mg/mL were obtained for *L. lactis* and 1198 mg/mL for *E. faecium*. This result is attributed to the subtly alkaline nature and carbohydrate richness of *Agave* sap, rendering it an excellent substrate for probiotics (70). In a study identifying microorganisms in sap samples from *A. salmiana* and *A. atrovirens* throughout the four seasons of the year, conducted in the previous study (71), bacteria from the *Lactobacillus*, *Leuconostoc* and *Acetobacter* genera were found. These bacteria have been confirmed to exhibit probiotic properties through *in vitro* tests. Additionally, a metagenomic study of the microbial diversity of these two *Agave* species as discussed in the previous study (32) revealed the presence of various species, including *Leuconostoc mesenteroides*, *L. pseudomesenteroides* and *L. gelidum*, all of which are recognized for their probiotic capacity.



Regarding pulque, its probiotic content may vary as lactic acid bacteria increase the acidity of the beverage, while yeasts produce ethanol from sugars (72). It has been reported that the *Kluyveromyces* yeast can survive the harsh gastrointestinal conditions and exhibits antimicrobial activity (Homayouni-Rad). Additionally, *Leuconostoc mesenteroides*, also isolated from pulque, showed high resistance to low pH and bile salts and inhibits the growth of *E. coli*, *L. monocytogenes* and *S. enterica* (67).

#### Increased mineral absorption

Fructooligosaccharides generated by the hydrolysis of *Agave* fructans, besides their prebiotic function, also facilitate mineral absorption in the intestine, potentially offering significant advantages given the prevalence of bone-related conditions such as osteoporosis. This advantage is ascribed to the correlation between fiber intake and mineral assimilation, influenced by alterations in the intestinal microbiota composition, subsequent changes in intestinal pH and immune system regulation (73).

When 10 % *A. tequilana* fructans were incorporated into the diet of mice, an enhancement in calcium absorption, as well as bone mineral retention and an increase of up to 50 % in osteocalcin levels were observed (74). In another study, examining calcium levels following the administration of *A. tequilana* honey to rats with methylprednisolone-induced osteoporosis, it was found that after receiving *Agave* honey three times a week for 3 months, both male and female rats exhibited elevated bone density and calcium levels (75). Conversely, in Macaca mulatta monkeys administered with 8 g of commercial *Agave* inulin from the Metlin® brand daily for 22 months, a positive impact on bone health was noted, potentially due to the favorable calcium homeostasis, which may impede bone resorption processes (76).

Fructans can be derived from pineapple and the leaves of maguey plants (*Agave* spp.), using extraction methods such as pressure extraction, yielding juice with 55 % fructans. Alternately, extraction techniques such as leaching or thermal and acid hydrolysis can be employed, with the latter being commonly utilized in the tequila industry (77). The scarcity of information regarding the enhancement of mineral absorption associated with the consumption of fructooligosaccharides from *Agave* spp. sap may be attributed to the fact that fructans are obtained in smaller quantities.

#### Reduction of the glycemic index

Sap and its by-products are considered to have a low glycemic index (GI) because their primary compounds are fructose, followed by glucose and traces of sucrose (78). The glycemic index measures the rate at which carbohydrate-containing food raises blood glucose levels after ingestion, with glucose (GI = 100) serving as a reference.

As for the hypoglycemic effect of sap, recent studies are limited. The antidiabetic effect of *A. atrovirens* Karw syrup extract has been evaluated through insulin secretion stimulation assays and insulin quantification in the RIN-5F cell line, along with determination of glucose inhibition by *in vitro* enzymatic treatments with invertase and amyloglucosidase. The methanolic extract of the syrup exhibited insulin secretion values similar to those of glibenclamide, a drug prescribed for diabetic patients with hypoglycemic effects. However, the compound responsible for the antidiabetic effect was not identified in the same study, although phytochemical compounds such as glycosylated steroidal saponins

were suggested. Meanwhile, in the enzymatic assays of glucose release, all *Agave* syrup extracts (methanol, acetone) showed inhibitory activity, attributed to the synergistic action of hydroxyflavone molecules (79).

A previous study evaluated the morphological and metabolic changes in diabetic and non-diabetic rats supplemented with *A. salmiana* syrup (80). It was found that the consumption of maguey syrup did not cause alterations in the concentration of metabolic parameters in non-diabetic rats; in the case of diabetic rats, the syrup had a hypoglycemic effect with the three doses evaluated (0.5, 2.0 and 5.0 g/kg) and the concentrations of cholesterol, triglycerides, albumin and creatinine, were not modified with doses lower than 5.0 g/kg body weight. Recently, a determination of the *in vitro* glycemic index of *A. salmiana* sap syrup obtained by rotary evaporation under vacuum resulted in a GI of 36.65, similar to that of commercial syrups from *Agaves pulqueros* and *tequileros* (35.58-52.19), values that remain below 55 established by the American Diabetes Association as low GI (81). Most of the most recent studies are directed to the hypoglycemic effect or the determination of the glycemic index of *Agave* syrups and not to sap, because these are used as a substitute for commercial sweeteners that are recognized as having a high glycemic index.

#### Inhibition of adenoma and carcinoma precursor lesions

It has been suggested that due to the content of *Agave* fructans, sap may have beneficial effects on the inhibition of adenoma precursor lesions and carcinoma (82). However, no *in vivo* or *in vitro* assays have been conducted on sap or any derivatives of *A. salmiana* or *A. atrovirens* to support this claim. A study using *A. sisilana* extract showed that the extract inhibits the survival and migration of osteosarcoma cell lines (a primary tumor that develops in the bone). This effect is associated with the degradation of Yes Associated Protein (YAP) and Tafazzin (TAZ) proteins, which are oncogenic transducers in the Hippo signaling pathway. This pathway plays a crucial role in the development and maintenance of stem cells, regeneration, cancer occurrence and chemoresistance (83).

## Conclusion

Based on the revised literature on *Agave* spp. by-products and their beneficial health effects, it was found that the primary use of sap is for the production of by-products, with the most commonly produced being syrups, pulque and pulque baked goods. Although both *Agave* spp. by-products and sap have showed beneficial health effects in *in vivo* and *in vitro* models, further studies are needed to understand the effects and composition of the most important *Agave* species, such as *A. atrovirens* and *A. salmiana*. Additionally, more information is required on the integral use of the plants of this genus for the development of by-products that allow the reactivation of the economy of the populations linked to its cultivation.

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## Authors' contributions

NCCA is the main author who conducted the research work, with the assistance and guidance of MGS, LPL, LGCM, ACFG, SDNF, SYSB and RRH in the study design, data acquisition and preparation of the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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