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Characterization of Fructan Content from Stem Juices of Different Varieties of Agave Atrovirens, Traditionally Used for the Production of Pulque. Production of a Sweetening Syrup by Chemical and Enzymatic Processing

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Abstract: Agave atrovirens, the hardiest species of Agave in Mexico, thrives in tough, infertile soils and extreme temperatures across a wide range of altitudes, particularly in impoverished rural areas of Central and Northern Mexico. Its potential for agroindustrial use could offer a pathway to social and economic prosperity for many communities. Traditionally, *A. atrovirens* is tapped for its sap, known as aguamiel, which is harvested from the leaves and collected in a cavity, carved into the central stem. This sugar-rich sap serves as the raw material for pulque, an ancient indigenous fermented beverage. However, pulque is unstable with a short shelf life and limited market reach. Despite *A. atrovirens* stems having lower sugar content compared to other species, its high inulin content and large biomass yield, make it a valuable raw material. One potential strategy for *A. atrovirens* is its use in liquid sweeteners. While juices rich in fructans from A. tequilana and A. angustifolia are currently employed in the production of industrial fructans, distilled beverages, and sweeteners, *A. atrovirens* remains relatively underexplored. The total soluble carbohydrate content in Agave juices was, on average, 154.3 g/L. Carbohydrate composition of raw Agave juices (Before Hydrolysis): high molecular fructans 130.0 g/L; sucrose 10.4 g/L, glucose 7.0 g/L and fructose 6.4 g/L). This study aims to characterize stem extracts from three varieties of *A. atrovirens* (Blanco, Cosmimaco, and Xaco), shedding light on its sugar content, juice composition, and the hydrolytic conversion rate of native fructans, by either acid or enzymatic treatment. Experimental results indicate significant differences among the varieties in terms of sugar content and composition Raw stem juices from *A. atrovirens* have a higher concentration of sucrose than glucose or fructose, with the majority being polymerized fructans. Hydrolyzed juices of all three varieties show a higher concentration of fructose (8.65-19.52% w/w); a lower glucose content (4.85-9.68% w/w) and a very low sucrose content (0.13-0.77% w/w). A prototype sweetening syrup derived from enzymatic hydrolysis of mixed fructans from *A. atrovirens* contained (w/v): 48.56% reducing sugars; 29.5% fructose; and 10.8% glucose. As well as 8% w/v fructo-oligosaccharides.

Keywords: Agave Atrovirens, Stem Juices, Carbohydrates, Fructans, Enzyme Hydrolysis

Introduction

Agave atrovirens is undoubtedly the most important species within the agave genus, particularly regarding its ecological benefits. Recognized by the National Commission for Forestry (CONAFOR, Mexico), it plays a crucial role in soil restoration due to its effect on controlling erosion. Additionally, it significantly enhances water infiltration and its extensive root system provides essential structure to soils (FAO-CONAFOR, 2012).

Furthermore, agave atrovirens have received recognition from the National Commission on Biodiversity (CONABIO, Mexico) as one of the key species contributing to national food security and Mexico's fight against poverty (FAO-CONAFOR, 2012).

On the commercial front, fructan-rich juices extracted from Agave tequilana and Agave angustifolia are currently used in the production of distilled beverages such as tequila and mezcal (Pérez-Zavala *et al*., 2020). These juices also serve as raw materials for food specialties like inulin and liquid sweeteners. Due to the high demand for distilled beverages, the cost of Agave stems has steadily increased over the past decades. Notably, A. tequilana, A. angustifolia, and other mezcal Agave species are now cultivated in fertile land, effectively substituting traditional farm products like corn and beans in rural Mexico (Gerritsen *et al*., 2011).

However, Agave atrovirens has primarily been associated with the production of pulque, an ancestral indigenous fermented beverage. The sugar-rich sap extracted from the stems of 6-10-year-old Agave specimens, known as aguamiel, serves as the raw material for pulque (Hernández-Arroyo *et al*., 2018; Villarreal Morales *et al*., 2019).

Despite its historical significance, pulque faces challenges it is poorly standardized, has a short shelf life, and has a limited market compared to other fermented beverages like beer, which are more prevalent in urban areas (Escalante *et al*., 2016). *A. atrovirens* stems have a lower sugar content compared to other Agave species. Its massive stem is much more difficult to cook and convert into fermentable juices when compared to the smaller stems of other Agave species (Narváez Suárez *et al*., 2016). The fact that *A. atrovirens* does not provide interesting raw materials for fermentation and production of distilled beverages may explain the much lower cost of its stems as a raw material for industrial purposes (Personal communication with Dr. Martin Pichardo).

Nevertheless, *A. atrovirens* is a species currently grown in mostly infertile, hardened soils, with virtually no chemical fertilization. It has a remarkable ability to thrive in the highlands of Mexico between 2000 and 4000 meters above sea level, from the slopes of Pico de Orizaba and Cofre de Perote, as well as in the high mountains of the Sierra Madre Oaxaqueña (García, 2002; FAO-CONAFOR, 2012).

Mexico has been recognized as the country with the largest biodiversity of *Agave* species (Díaz Castañeda and Osorio García, 2021). Approximately 200 species have been identified and 75% of them can be found in Mexico (Agricultura and Rural, 2015). A wide variety of these species is used for the production of pulque (Ríos Martínez and López de Juambelz, 2015; Jiménez Muñoz *et al*., 2016). Nowadays, Agave atrovirens is valued for pulque production, although its economic importance is not as significant as that of other species, such as *A. tequilana* (Olvera-Garcia *et al.*, 2015b; Chavez-Parga *et al*., 2016; SAGARPA, 2016).

The traditional use of *A. atrovirens* consists of collecting and extracting phloem fluid or sap derived from photosynthesis from the leaves, into a hollow carved in the central stem. The sugar-rich sap is called aguamiel and it serves as the raw material for pulque production through fermentation (Hernández-Arroyo *et al*., 2018; Villarreal Morales *et al.*, 2019). Pulque production is widely distributed in Mexico, mainly in the states of Hidalgo, Tlaxcala, Mexico, and Puebla, although it is also produced marginally in San Luis Potosí, Michoacán, Querétaro, Morelos, Guanajuato, Veracruz and Oaxaca (García, 2002). One of the main sources of *A. atrovirens* and pulque is the state of Puebla (SAGARPA, 2017). *A. atrovirens* varieties are a bioresource of great importance, economically, socially, and agroecologically. Ethnobotanic studies on *Agave* species reveal a wide variety of uses. Practically the entire plant is utilized for domestic, construction, medicinal, and agricultural purposes (Olvera-Garcia *et al.*, 2015a).

One of the most relevant uses given to a significant number of *Agave* species is their use for the production of mezcal, which is considered a potential axis for the economic development of *Agave*-producing regions (Chavez-Parga *et al.*, 2016). *Agave* species have also been utilized for the diversified production of specialty products related to food, medicine, alcoholic beverage processing, fiber, and fodder (SAGARPA, 2017).

The agricultural landscape in Central Mexico has undergone changes due to the introduction of new cultivation techniques. The loss of traditional crops and the adoption of intensive monocultures (Such as Barley, Oat, and Corn) have led to environmental degradation, resulting in soil erosion and reduced soil fertility (Olvera-Garcia *et al.*, 2015a). A key ecosystem function of *A. atrovirens* is its efficient accumulation of cellulosic biomass. This capacity arises from its adaptation to extreme climatic conditions, allowing it to thrive in dry and cold climates (Narváez Suárez *et al*., 2016). Compared to tequilero and mezcalero *Agave* species, *A. atrovirens* has significantly lower water and fertilization

requirements (Valenzuela-Zapata, 1995). Moreover, it readily grows in the highlands of Central Mexico, even in non-arable land, and can successfully thrive at high cultivation densities (up to 4,000 plants per hectare) (García, 2002). This species is valued as a soil restoration agent, capable of compensating for carbon loss and preventing soil erosion (Ríos Martínez and López de Juambelz, 2015; Servicio Nacional de Inspección y Certificación de Semillas SNICS, 2017). Its production has been encouraged by the Mexican Council for the Knowledge and Use of Biodiversity (CONABIO).

Agave plants have also been described as good sources of biopolymers, including cellulose, lignin, hemicellulose, and fructans (Hernández-Salas *et al*., 2009; Apolinário *et al*., 2017; Álvarez-Duarte *et al*., 2018). *A. atrovirens* plants are rich sources of fructans, specific biomolecules with important health benefits (Olvera-Garcia *et al*., 2015b). The main form of stable photosynthetic products in Agave is fructans, which primarily function as energy storage and provide osmoprotection during drought (Arrizon *et al*., 2010; Olvera *et al*., 2017). *A. atrovirens* specimens used for pulque production are usually 8-10 years old (Hernández-Arroyo *et al*., 2018) and an adult specimen typically contains 40 to 50% total sugars by stem weight (Narváez Suárez *et al*., 2016).

After starch, fructans are the most abundant non-structural polysaccharides in nature. Based on their degree of polymerization, they are classified as inulin, oligofructose, and Fructo Oligo Saccharides (FOS). All of these share linearity and high water solubility (Zimmermann *et al*., 2023). Inulin, an energy reserve carbohydrate, is present in more than 36,000 plant species (Lara *et al*., 2017). Agave fructans are defined as fructose polymers with or without a terminal or internal glucose unit, linked by β-2,1 and β-2,6 bonds. Commercially, they are classified based on their Degree of Polymerization (DP): Fructo Oligo Saccharides (FOS) when DP is below 10 units, or as inulin when DP is higher (Santos-Zea *et al*., 2012). Fructans cannot be hydrolyzed by digestive enzymes until hydrolysis occurs in the colon by the microbiota, providing a reduced caloric value of up to 1-2 Kcal/g (Apolinário *et al*., 2014).

Inulin-type fructans have medium sweetening power, a low glycemic index, and very low caloric content. They serve as safe functional prebiotics due to their resistance to gastric acid and their strong, selective stimulation of beneficial intestinal microbiota (Santos-Zea *et al*., 2012). Agavins, which are the type of inulin found in Agave tequilana, have emerged as prebiotic ingredients of commercial interest for functional foods. They consist of a complex mixture of highly branched fructans bound by β-2,1 and β-2,6 bonds (Muñoz-Gutiérrez *et al*., 2009; Madrigal and Sangronis, 2017; García *et al*., 2019). Agave fructans can stimulate the growth of beneficial microbiota, including Bifidobacteria and Lactobacillus (Gomez *et al*., 2010; García Gamboa *et al*., 2018). Metabolites produced during prebiotic fermentation also exhibit anti-inflammatory and immunomodulatory capabilities, suggesting a potential role in treating various pathological conditions (Guarino *et al*., 2020). Numerous studies have demonstrated that Agave fructans have several health benefits: They decrease blood lipid levels (Roberfroid, 2005), help control blood glucose levels (Roberfroid, 2005; Álvarez- Chávez *et al*., 2021), act as a barrier against pathogens (García Gamboa *et al*., 2018) and improve mineral absorption (Santos-Zea *et al*., 2012). In animal studies, inulin-type fructans have been shown to affect lipid metabolism, primarily by reducing hypertriglyceridemia (Roberfroid, 2005).

The bioprocesses for fructan recovery involve various unit operations of scientific and economic interest, such as drying, grinding, acidic, aqueous, or alcoholic extraction (Salcedo *et al*., 2009). During these processes, differences in biomaterial characteristics, solvent concentration, particle size, and reaction time significantly impact the extractability and yield of inulin (Soto *et al*., 2011). The structure of the biomaterial plays a crucial role in processing, emphasizing the relationship between structure, process, and functionality (Godínez-Hernández *et al*., 2015).

High fructose syrups have been used as the primary liquid alternative sweetener to sucrose in the food industry for at least 40 years (Moeller *et al*., 2009; Parker *et al*., 2010). There is concern that high fructose syrup consumption is associated with an increasing risk of obesity (Ozuna and Franco-Robles, 2022). In Mexico, obesity and overweight are significant public health issues, with nearly 74.2% of adults being overweight (39.1%) or obese (36.1%) (Dávila *et al*., 2014; Barquera *et al*., 2020). Currently, the food industry aims to offer products that contribute to consumer well-being. Agave syrups have gained popularity in the specialty food market due to their natural origin, nutraceutical properties, and low glycemic index (Ozuna and Franco-Robles, 2022). The fructose concentration in Agave syrup products on the market is similar to commercial corn syrup (ranging from 42% to 55%) (Parker *et al*., 2010; Ozuna and Franco-Robles, 2022). Considering that higher fructose content results in a sweeter syrup sustainably produced Agave plants constitute a valuable bioresource for producing syrup with fructose content exceeding 60%, potentially reducing the calorie content in sweetening agents.

Wild and cultivated Agave plants of certain species exhibit significant phenotypic diversity, varying between wild varieties and populations with a long history of cultivation (Romero *et al*., 2015).

The ecological and environmental impact of Agave atrovirens, along with its ability to thrive in low-fertility

soils, positions it as a species traditionally cultivated in impoverished rural areas of Mexico's central highlands. Its potential use for producing specialty products like sweeteners and prebiotics could support sustainable on-site bioprocessing, creating much-needed jobs and contributing to a circular bio-economy model through biorefining.

Agave atrovirens serve as an intriguing source of inulin a sustainable (even organic) raw material with exceptionally high biomass yields per hectare. A viable approach for developing sustainable agro-industrial processes involving *A. atrovirens* is its bioconversion into fructose-rich liquid sweeteners.

However, there remains a significant knowledge gap regarding the characteristics of *A. atrovirens* stem juices and their sugar content, especially when compared to other *Agave* species, particularly those used in tequila and mezcal production.

The study of Agave atroviren's diversity is indeed valuable, especially considering its environmental significance and capacity to store fructans. While there are no published studies specifically comparing the content and characteristics of inulin across different *A. atrovirens* varieties, investigating this aspect could provide insights into its potential as a sustainable bioresource. The study aims to explore how different *A. atrovirens* varieties impact total sugar yield in stem juices, the molecular weight of native inulin, and its suitability for specialty sweetening syrup production.

Materials and Methods

Agave Sources

Pulque is produced in at least 10 states across Mexico: Coahuila, Guanajuato, Querétaro, Aguascalientes, Oaxaca, Morelos, Hidalgo, Tlaxcala, Puebla, Estado de México and Veracruz. However, the most important plantations of *Agave* for pulque production are located in Hidalgo, Tlaxcala, and Puebla (Escalante *et al.*, 2016).

There are at least 10 varieties of "*Agave* pulquero" used in the production of aguamiel and pulque. All of them belong to the genus *Agave*. Most of them fall under the species *A. atrovirens*, *A. salmiana*, *A. mapisaga*, and *A. americana*. Common names for these varieties vary depending on the region. Terms like "chaco" or "xaco," "verde," and "blanco" or "cenizo" are typically applied to different species, again depending on the region (Márquez-Pallares *et al.*, 2024; Delgado-Lemus *et al.*, 2014; Aldrete-Herrera *et al.*, 2019; Olvera-Garcia *et al.*, 2015b).

In our study, we selected a region where three varieties are produced at a commercial scale for pulque production. The plants used in the study were all collected from Zacatlán and Ixtacamaxtitlán, both municipalities in the north of the state of Puebla, Mexico. All specimens were harvested during the same season, late December of 2014, and were physiologically mature for processing (between 6.5 and 7.5 years old). We analyzed three different varieties belonging to Agave atrovirens: Cosmimaco, Xaco, and Blanco.

For the extraction and characterization of fructans, we followed the methods described by Arrizon *et al.* (2010) with a few modifications. Whole *Agave* stems ("piñas") and basal portions of leaves were used for each specimen. *Agave* stem samples were cut horizontally and vertically to reduce particle size, resulting in a homogeneous sample containing 15% of the total mass of the original stem (piña). The *Agave* samples were then milled in a homemade hammer mill and reduced to a particle size of 0.2-0.7 cm to improve extraction yields. All samples were stored at -20°C until further processing to prevent enzymatic depolymerization of fructans and sucrose (Aldrete-Herrera *et al.*, 2019).

In order to determine total solids from *Agave* stem samples, the oven-drying method (A.O.A.C., 2005, method 930.15) was carried out by removing water from the homogenized samples in a Thermo-Fisher brand stove at 100-135°C until a constant weight was recorded. Total Solids (TS) were determined by weight difference.

Recovery of Extracts and Juices from a. Atrovirens Stem Samples

For the extraction of *the Agave* stem, 200 g of the milled stem samples were treated with 96% ethanol (1:1 by weight). Alcoholic extraction allows for the recovery of small molecules and suppresses enzymatic modification of native saccharides and fructans (Aldrete- Herrera *et al.*, 2019). The mixture was thoroughly mixed at room temperature, stirred, and filtered through permeable cloth, resulting in an alcoholic extract and residual bagasse. The residual bagasse from the first extraction was weighed and treated with an equivalent weight of a new batch of 96% ethanol. This procedure was repeated twice. The alcoholic extracts were combined and the total weight and volume were recorded. The dried residual bagasse from the alcoholic extractions was subsequently treated with boiling water (1:1 by weight) twice to quantitatively recover fructans, oligo, and monosaccharides (Olvera-Garcia *et al.*, 2015b). The aqueous extracts were independently weighed and combined with the alcoholic extracts from previous extractions. The residual bagasse was removed by filtration using filter cloth and grade 1 Whatman filter paper. The combined alcoholic and aqueous extracts were concentrated under reduced pressure using a rotary evaporator. The alcohol-free concentrate, an Agave atrovirens Extract (AaE), was stored at -20°C for further analysis.

A simple stem juice was recovered for comparison with the extracts. Using one single water extraction, five portions of boiling water were used with respect to milled stem samples (200 g) from the same *Agave* specimens used for quantitative extraction. After thorough

homogenization, the juices were recovered by filtration using a filter cloth, followed by filtration with grade 1 what man filter paper under vacuum.

Characterization of Aae and Stem Juices

Several analytical methods were used to characterize stem juices from Agave atrovirens samples. Using appropriate dilutions of the concentrated juices, two spectrophotometric methods were employed for quantitating soluble carbohydrates and reducing sugars. The phenol-sulfuric method (Dubois *et al.*, 1951) was used to determine Total Soluble Carbohydrates (TSC), while the dinitrosalicylic acid method (DNS) (Miller, 1959) was used for direct Reducing Sugars (RS) quantification.

Fractionation by Micro and Ultra-Filtration of Aae

Agave fructans were separated into fractions based on their molecular weight using ultrafiltration, which allows for the clean separation of soluble polymeric fructans with different Degrees of Polymerization (DP) (Luiz-Santos *et al*., 2020). To remove insoluble matter, aliquots from diluted AaE were sequentially microfiltered using 70 mm Whatman glass fiber membranes, 47 mm nylon membranes (3.0 µm pore size), and 0.22 µm cellulose acetate filters. The clarified extract obtained was then ultrafiltered using Millipore® lab-scale ultrafiltration equipment with membranes having cut-off values of 5, 3, and 1 kDa. These membranes were used for fractionating high molecular inulin, oligofructans, and fructooligosaccharides, respectively. For each ultrafiltration fraction, TSC and RS were determined.

Acid Thermal Hydrolysis

Hydrolysis of AaE by mineral acids was conducted under conditions similar to those described by Ávila-Fernández *et al*. (2011). Acid hydrolysis of AaE was tested in extracts from all three *Agave* varieties, as well as a pool of concentrated extracts from the three sources (referred to as "Juice A"). Juice A was prepared by combining equivalent weights of AaE from Blanco, Cosmimaco and Xaco samples. Acid hydrolysis was performed as follows: Diluted HC_l or H_2SO_4 was added to AaE at different concentrations (1, 2.5, 5, and 10% v/v). Five different reaction times (15, 30, 60, 120, and 180 min) were evaluated at two different temperatures (60°C and 70°C). The reaction was stopped in an ice/water bath and the solution was adjusted to pH 7 by adding 20% KOH.

For each sample during the acid hydrolysis reactions, TSC, RS, and free monosaccharides (analyzed using HPLC-RID) were quantitatively determined.

Enzymatic Hydrolysis

To enzymatically treat Juice A, we used a commercial inulinase enzyme preparation (Fructolase®, ENMEX, México) at different concentrations: 0.5, 1.0, 2.0, and 4.0 mg/mL. The enzyme solution was microfiltered through membranes with a pore size of 0.22 µm. Juice A was adjusted to pH 4.2, which is optimal for enzyme function according to the manufacturer. Incubation was carried out in Erlenmeyer flasks at 55°C for 6 h with rotary shaking at 150 rpm. After incubation, the reaction was stopped in a boiling water bath for 10 min. Glucose, fructose, and sucrose in the enzyme-treated Juice A were analyzed by HPLC.

Quantification of Monosaccharides and Oligosaccharides by HPLC

Glucose, fructose, and sucrose standards were used for the quantitative determination of saccharides by HPLC. We used Agilent Infinity chromatography equipment (1200 Series) with a refractive index detector. The column employed was a 300×7.8 mm sodium-form ion exchange column (Aminex HPX-87N), typically used for chromatographic separation of saccharides in fruit juices, sugarcane molasses, and other sugar solutions with high salt or conductivity similar to *Agave* juices. The chromatographic method was isocratic and the mobile phase consisted of 4 µM dibasic sodium phosphate. The chromatography was run at a constant temperature adjusted to 45°C.

Results and Discussion

Fructans are widely distributed in nature. They represent the second most common energy storage form of reserve carbohydrates and are present in 15% of flowering plant species (Lara *et al*., 2017; Verma *et al*., 2021). Similar to starches, fructans serve as an essential agro-industrial raw material, valuable for producing inulin and fructose-rich syrups. Plants within the Agavaceae family are a potential source of fructans (Bautista-Justo *et al*., 2001; Iñiguez-Covarrubias *et al*., 2001). Juices rich in Agave fructans have demonstrated various health benefits, including reducing the incidence of digestive disorders, improving intestinal transit, providing gastroprotection, and stimulating the immune system. Additionally, they promote the synthesis of certain vitamins, enhance calcium and iron absorption, reduce cholesterol levels, and lower serum phospholipids and triglycerides (Ávila Fernández, 2014; Romero *et al*., 2015; 2016). Agave atrovirens, particularly in the central-eastern States of Puebla and Tlaxcala, must be considered a significant source of fructans in the Central Highlands of Central Mexico (Álvarez-Duarte *et al*., 2018).

Extraction of Juices from a. Atrovirens Stem Samples

A. atrovirens is a succulent plant that can reach up to 2 meters in height. Its crassulacean acid metabolism allows it to efficiently utilize water from environmental condensation, rainfall, and soil humidity. Agave plants

primarily store fructans as their main carbohydrate reserve (Leopoldo, 2011; Mielenz *et al.*, 2015). This adaptation minimizes water loss by limiting transpiration achieved through stomatal closure during periods of high temperature. The stem juice samples from different *Agave* varieties exhibited varying moisture content: Cosmimaco (80.5%), Xaco (82%), and Blanco (84%). These values depend on the plant's age and climatic conditions (Soto *et al.*, 2011). Additionally, the total solids content was 19.5, 18 and 16% for Cosmimaco, Xaco, and Blanco, respectively. This metabolic strategy enables *Agave*s to thrive under extreme climatic conditions.

The resulting Agave Atrovirens Extract (AaE) obtained from processing stem samples of Cosmimaco, Xaco, and Blanco showed a higher total Soluble Carbohydrates (TSC) content compared to juices obtained by mechanical pressing and filtration from the same specimens (Fig. 1). Notably, alcohol and water extraction proved more efficient in obtaining TSC. Among the varieties, the Blanco extract widely cultivated in the region had the highest extractable percentage of TSC (19.9%) compared to the other two varieties.

Fractionation by Micro and Ultra-Filtration of Aae

The stem extracts (AaEs) obtained from 200 g of fresh stem tissue were subjected to microfiltration using membranes with pore sizes of 70, 47 mm, and 0.22 µm. Subsequently, ultrafiltration was performed to analyze TSC and RS content for each molecular weight fraction. The results are summarized in Table (1) and Fig. (2). Results from Fig. (2) show that the fructan molecular weight distribution, resulting from ultrafiltration analysis, shows important differences depending on the agave variety analyzed. Cosmimaco fructans higher than 5000 Da constitute more than half of all fructans; whereas for Blanco, three fractions (> 5 kDa, 3-5 kDa, and 1-3 kDa) show very similar fructan content.

A. atrovirens presents an economically advantageous option for fructan recovery, particularly from 6-year-old plants, similar to the case of *A. sisalana* (Conceição Apolinário *et al.*, 2020). In the 5 to 7-year age range, *A. atrovirens* specimens reduce vegetative growth, prepare for floral differentiation, and accumulate high molecular weight linear fructans (Arrizon *et al.*, 2010; Leopoldo, 2011). Similar findings were observed in 6-year-old *A. atrovirens* leaves, which exhibited a high concentration of inulin while total carbohydrates decreased, with translocation to the stem during maturity (Leopoldo, 2011). The prevalence of *A. atrovirens* oligosaccharides with Degrees of Polymerization (DP) in the range of 5-13 is comparable to those extracted from other sources such as *A. tequilana* or *A. sisalana* (Apolinário *et al.*, 2017). High molecular weight raw fructans from *A. atrovirens*, including its higher DP fractions, could serve as valuable ingredients in food processing for instance, as fat substitutes in ice creams and other frozen products, as well as prebiotics in food supplements and nutraceuticals (Ávila Fernández, 2014; Madrigal and Sangronis, 2017). Raw fructans from *A. atrovirens*, specifically from the Blanco variety, are likely to have low-calorie content, and strong prebiotic activity and could enhance functional foods and beverages.

Table 1: Distribution of Molecular Weight of Total Soluble Carbohydrates (TSC) based on ultrafiltration fractions in stem juice extracts from different varieties of *A. atrovirens*: Cosmimaco (Co), Xaco (Xa) and Blanco (Bl)

Variety (200 g of stem juices)	TSC content (g)	TSC content (g) per molecular weight interval (percentage TSC in fraction)			
		›5 KDa	3-5 KDa	1-3 KDa	‹1 KDa
Co	2.04	1.09 (53%)	0.3 (15%)	0.38 (19%)	0.27 (13%)
Xa	3.09	1.05 (34%)	0.74 (24%)	1.10 (36%)	0.20 (6%)
Bl	17.1	4.60 (27%)	4.80 (26%)	4.56 (27%)	4.34 (20%)

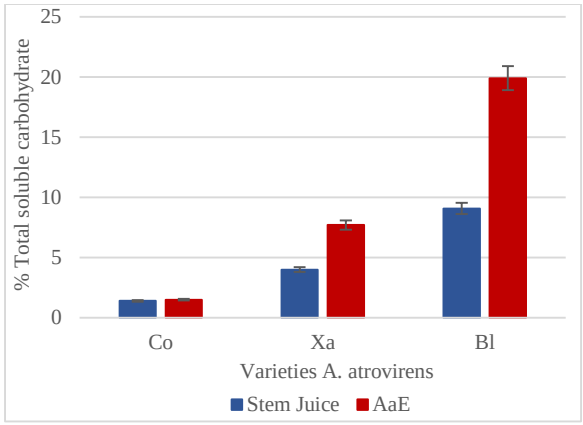

Fig. 1: Effect of *agave* variety on soluble carbohydrate content in both pressed juices (stem juices) and extracts (AaE). The concentration is expressed per weight of fresh stem material. The varieties considered are Cosmimaco (Co), Xaco (Xa), and Blanco (Bl)

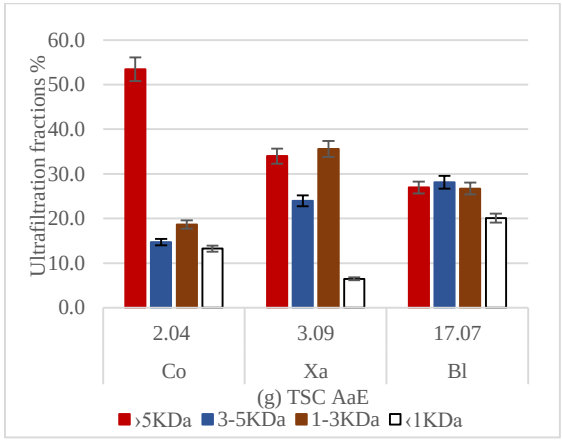

Fig. 2: Total Soluble Carbohydrates content (TSC) and molecular weight distribution in ultrafiltration fractions of AaE from different varieties of *A. atrovirens* Cosmimaco (Co), Xaco (Xa) and Blanco (Bl)

Acid Thermal Hydrolysis of Aae and Juice A

The AaE from three different *Agave* varieties underwent acid treatment at either 60 or 70°C, using four different concentrations of either HCl or H_2SO_4 and four different hydrolysis times. The concentrations of reducing sugars were determined using the DNS method (Table 2).

The concentrations of RS after hydrolysis of AaE varieties treated with HCl at 60°C (as shown in Table 2) were as follows, using the optimal acid concentration (5% w/v): 18.79 - 22.18 for Cosmimaco; 4.05-4.65 for Xaco; and 4.12 - 4.74 for Blanco.

Experimental results reveal significant differences among the three varieties of *A. atrovirens* (Blanco, Cosmimaco, and Xaco) regarding total sugar content, juice composition, and the hydrolytic conversion rate of native fructans.

An *Agave* stem extract from a combined sample (referred to as "Juice A"), comprising equal proportions of stem extracts from Xaco, Cosmimaco, and Blanco, underwent thermal and acid hydrolysis. The effects of different mineral acids and hydrolysis times on reducing sugar and monosaccharide concentration (glucose and fructose) in the hydrolysate are presented in Table (3).

Hydrolyzing Juice, using HCl at 60°C resulted in the highest monosaccharide yield (as indicated in Table 3). Similar results were obtained for the individual varieties of AaE. The optimal acid concentrations ranged from 2.5-5% w/v and the RS content in the hydrolysate was 13.74-18.02% w/v.

The best performance for thermo-acid hydrolysis of fructans was achieved using two different process conditions: HCl at 60°C (resulting in RS concentrations of 125.4-180.2 g/L) and HCl at 70°C (with RS concentrations ranging from 91.3-138.1 g/L).

Fructans serve as the reserve carbohydrates in *A. atrovirens*, primarily composed of fructose. The hydrolysis results indicate that the hydrolysates have a notably high fructose concentration (as shown in Table 4).

Table 2: Effect of acid thermal treatment (5% HCl; 60°C) on the reducing sugars RS Concentration in AaE from Different Varieties of *A. atrovirens*

Variety	Time (min)	RS (g/L)	RS (% w/v)
Co	30	195±0.18	19.54
	60	200±1.64	20.05
	120	188±0.03	18.79
	180	222±0.03	22.18
Xa	30	40±0.08	4.05
	60	44±0.07	4.37
	120	40±0.52	4.02
	180	47±0.20	4.65
Bl	30	41±0.05	4.12
	60	44±0.18	4.43
	120	43±0.27	4.34
	180	47±0.05	4.74

Table 3: Effect of acid, time, and thermal treatment of juice on Reducing Sugars (RS) concentration of the hydrolysate

Concentration	Time (min)	RS HCL (g/L)		RS H_2SO_4 (g/L)	
		60°C	70°C	60°C	70°C
1%	15	151.1±0.4	106.2±0.6	99.9±0.9	99.5±0.2
	30	140.5±1.5	121.2±0.3	111.2 ±1.2	113.2±0.9
	60	135.1±1.1	114.3±0.5	109.7 ±0.9	109.0±0.9
	120	132.3±1.5	123.3±0.2	120.2 ±0.2	94.2±0.7
2.5%	15	180.2±0.8	124.6±0.5	179.5 ±0.8	101.0±0.3
	30	138.4±0.4	135.2±0.3	98.9±0.8	122.1±1.9
	60	137.4±1.9	131.6±0.6	98.3±0.5	102.6±0.3
	120	141.3±0.8	122.1±0.3	124.4 ±1.4	104.1±0.6
5%	15	151.8±1.2	138.1±1.2	88.5±1.5	105.9±0.9
	30	138.5±0.8	130.4±0.7	86.9±0.3	102.8±0.3
	60	142.5±0.8	118.3±1.7	104.8±0.3	68.6±0.4
	120	140.2±0.8	107.5±0.2	62.2±0.3	99.0±0.8
10%	15	143.3±0.9	132.8±1.3	84.0±1.0	98.2±0.8
	30	142.6±0.9	133.9±0.6	100.1±0.5	78.7±0.1
	60	125.4±0.5	104.6±1.3	77.2±0.1	52.2±0.3
	120	136.8±0.9	91.3±1.3	82.4±0.9	83.1±0.3

Table 4: Effect of acid type, concentration, and Temperature on Monosaccharide and Sucrose Concentration in Juice a Hydrolysate. Glucose (G), Sucrose (S), and Fructose (F) were analyzed by HPLC. Juice A without acid and thermal treatment, as control (C)

Juice A	T °C	S (g/L)	G (g/L)	F (g/L)	Total
C		10.4	7.0	6.2	23.7
HCL5%	60	0.59	26.8	80.32	107.7
	70	1.80	29.4	47.65	78.9
$H_2SO_4$5%	60	1.22	22.2	48.83	72.3
	70	0.79	19.9	39.64	60.3

The chemical hydrolysis with low acid concentrations, combined with high temperature, resulted in a syrup with a high reducing sugar content. Temperature plays a critical role: If it is too low, the polysaccharide will not hydrolyze; conversely, if it is too high, thermal destruction and caramelization of sugars occur.

In our present study, the acid-thermal hydrolysis of extracts from the three *Agave* varieties and a pool of concentrated extracts (referred to as "Juice A") showed the best results using HCl at 60°C. Similar approaches have been applied to *A. tequilana* juices for tequila production, where no significant difference was found in extracted sugars when the temperature was reduced to 60°C (Ávila-Fernández *et al.*, 2009).

Studies by Ávila-Fernández *et al.* (2009) and Michel-Cuello *et al.* (2008) suggest that temperature reduction does not adversely affect the free sugar yield. Villegas-Silva *et al.* (2014) found that leaf juices from *A. fourcroydes* Lemaire (henequen) exhibit a stronger dependence on monosaccharide release from acid concentration than from reaction temperature (5% H_2SO_4 (v/v): 74 g/L RS). Our results align with this trend; acid concentrations of 2.5-5% w/v HCl demonstrated optimal saccharification performance. The release rate of reducing sugars during hydrolysis and saccharification depends on the concentration of fructosyl chain ends, which correlates

with the average chain length distribution (Blecker *et al.*, 2002). Interestingly, the hydrolysis rate appears independent of the type of acid used (Ávila-Fernández *et al.*, 2011; Blecker *et al.*, 2002). *Agave*'s native high molecular weight fructans lack reducing power and thus the difference between reducing power and free fructose corresponds to the short-chain oligosaccharides and monosaccharides produced during hydrolysis (Ávila-Fernández *et al.*, 2011). Our results clearly demonstrate that the most successful acid treatment was 2.5-5% HCl at 60°C for 60 min (yielding fructose at 80.3 g/L).

While the cultivation and biorefining of *A. atrovirens* are primarily associated with pulque production (Reynoso *et al.*, 2012), our present study explores the effects of three different *A. atrovirens* varieties on the content and characteristics of stem juices. This investigation aims to define a potential industrial pathway for using *A. atrovirens* as a raw material for fructan and syrup production, as well as for nutraceuticals, prebiotics, and food supplements an approach suggested by other authors (Álvarez- Chávez *et al.*, 2021).

In our results, the non-hydrolyzed Juice A (used as a control) exhibits a higher concentration of sucrose compared to fructose or glucose. Fructose and glucose are the final products of several consecutive and competitive acid-catalyzed reactions (Blecker *et al.*, 2002). Interestingly, our hydrolysis process resulted in only a slight increase in glucose concentration. This phenomenon likely arises because fructans contain one glucose unit per polysaccharide molecule, with the remainder consisting of fructose monomers.

Enzyme Hydrolysis of Agave Stem Extracts

Juice A, an extract obtained from a mixed *Agave* stem sample containing equal proportions of Cosmimaco, Blanco, and Xaco, underwent enzyme hydrolysis using Fructolase®, a locally commercialized inulinase. The results, as determined by both the DNS method and HPLC-RID, are presented in Table (5).

Using 1 g/L of concentrated Fructolase for 6 h at 55°C, the resulting solution exhibited an RS content of 48.65%. Analysis of oligosaccharides revealed a lower sucrose concentration (4.4 g/L) and significantly higher concentrations of glucose (107.8 g/L) and fructose (294.7 g/L). As experimental controls, untreated Juice A (with no enzyme, CNE) and Juice A treated with inactive enzyme (CIE) both showed much higher sucrose concentrations (74.3 and 68.6 g/L, respectively).

The results regarding the effect of enzyme concentration on the production of glucose and fructose through enzymatic hydrolysis and saccharification of juice a are depicted in Fig. (3).

The acid thermal hydrolysis of *Agave* stem extracts may lead to the formation of chemical products toxic to humans. To avoid the production of such toxic chemicals resulting from lignin degradation and sugar oxidation, the use of enzymes is recommended. Enzyme-catalyzed hydrolysis and saccharification not only reduce energy requirements but also improve the efficiency of hydrolysis, simplifying juice processing (Soto *et al.*, 2011). Inulinases, commercially used enzymes for the industrial hydrolysis of fructans, typically contain both exo and endo inulinases (Ávila-Fernández *et al.*, 2009; Muñoz- Gutiérrez *et al.*, 2009; Soto *et al*., 2011; Avila-Gaxiola *et al.*, 2018).

Our findings indicate that the sweetening power and sensory quality of the resulting enzymatic hydrolysis syrup are substantially better than those obtained by acid hydrolysis. Looking ahead, the potential use of fructose-rich syrup could offer a valuable technological alternative to stable liquid sweeteners, which may also contain residual Fructo-Oligosaccharides (FOS) Highly valued prebiotics for advanced nutrition. In an enzymatic hydrolysis study, juices from *A. salmiana* were processed using enzymatic hydrolysis. The authors found that hydrolysis is influenced by temperature, substrate concentration, and type of substrate (Michel-Cuello *et al.*, 2012).

Table 5: Effect of Enzymatic Treatment (Fructolase® ENMEX, 60°C, 30 min) aon Oligosaccharide Concentration and Reducing Sugar Content in the Hydrolysate. Reducing Sugars (RS), Glucose (G), Sucrose (S), and Fructose (F) in Juice A. Controls: Inactivated Enzyme (CIE) and no-enzyme (CNE)

Fructolase (g/L)	S (g/L)	G (g/L)	F (g/L)	Total (g/L)	RS (%) w/v
CNE	74.3	35.3	77.1	187	11.07
CIE	68.6	32.7	117.5	219	28.75
0.5	2.6	99.7	276.5	379	50.97
1.0	4.4	107.8	294.7	407	48.65
2.0	12.8	103.7	271.6	388	48.30
4.0	4.8	84.3	203.2	292	50.81

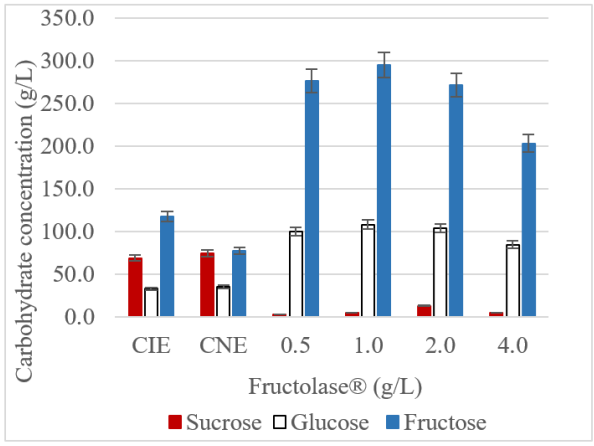

Fig. 3: Effect of Enzyme Concentration on Monosaccharide Release from Juice a Fructolase was applied at 55°C for 6 h. Experimental controls include Juice A treated with Inactivated Enzyme (CIE) and Juice A without enzyme (CNE)

Processing *Agave* juices releases both oligofructans and monosaccharides, derived from either thermo-chemical or enzyme treatment. Oligofructans, or fructo-oligosaccharides (FOS), are increasingly important as functional ingredients in food technology, pharmacy, and advanced nutrition due to their functionality and sensory value (Verma *et al.*, 2021). In our study, we utilized three varieties of *A. atrovirens* (Blanco, Cosmimaco, and Xaco), known for their high aguamiel yield and pulque quality in the states of Tlaxcala and Puebla (personal communication with Dr. Joel Martín Pichardo-Rico). These three varieties were employed in our research. The conservation of *A. atrovirens* diversity holds great importance due to the Mexican ancestral tradition of pulque production the oldest alcoholic beverage in Mexico.

Additionally, Agave atrovirens is valued as a soil restoration agent. However, its production has steadily declined over the past six decades. Challenges faced by its cultivation include low economic profitability, changes in crops, cultivar longevity, decreased pulque consumption, and the destruction of cultivars due to the use of leaf cuticles for gastronomic purposes (Pizarro and Herrera, 2021).

Currently, *A. atrovirens* stems represent the most cost-effective source of fructans among Mexican *Agave* species, while also serving as the most sustainable bioresource for restoring the nutrient-poor soils of the central Mexican highlands.

Efforts are underway to revive this crop both nationally and internationally, recognizing its significance as a biological resource. The National Development Plan 2019-2024 emphasizes the need to protect the *Agave* "pulquero" as an agro alimentary product and a species of great cultural value (Pizarro and Herrera, 2021). Initiatives aim to revitalize pulque production through new packaging presentations and the exploration of novel markets both domestic and international. For instance, the company "Desarrollo Agropecuario de Altiplano" in Puebla commercializes canned pulque and a pulque distilled beverage (Luna, 2007). Therefore, it is crucial to explore innovative production methods and processing technologies for developing Agave atrovirens-based products.

Conclusion

A. atrovirens is a species traditionally used for the production of aguamiel (sweet sap) and pulque, a fermented beverage with ancient roots in indigenous cultures of central Mexico. Stem juices from three different varieties of Agave atrovirens were characterized and processed using either acid or enzymatic methods for saccharification.

Specimens from all three varieties, matured for pulque production, were collected and harvested in the Northern Sierra of Puebla State, specifically in the Zacatlán mountain region, under similar agro-climatic and edaphic conditions. The Blanco variety is widely established in Mexico's Central Highlands.

The Total Soluble Carbohydrates (TSC) content extracted from stems of three *A. atrovirens* varieties showed that Blanco had 19.9 g TSC/kg fresh stem, whereas Xaco had 7.7 g TSC/kg and Cosmimaco had 1.5 g TSC/kg. These yields do not necessarily correlate with the yield and quality of aguamiel (*Agave* Drained Sap), which serves as the raw material for pulque fermentation.

Estimation of the molecular weight of native fructans using analytical ultrafiltration revealed wide differences among *A. atrovirens* varieties. Over 50% of Cosmimaco stem fructans were >5 kDa, whereas only 34% of Xaco fructans and 27% of Blanco fructans were above >5 kDa.

Fructans from the Blanco variety exhibited a more homogeneous MW distribution. Approximately 20% of fructans had an MW under 1 kDa. Fractions corresponding to fractions of 1-3, 3-5, and >5 kDa were 26 and 28.1%, respectively. The results indicate that for the Blanco variety, over 80% of the fructans had an MW of 1 kDa or higher, suggesting that high molecular weight inulin and oligofructose constitute the majority of native fructans recovered. A large proportion of these should provide prebiotic functionality.

The most successful acid treatment (HCl; 60°C, 120 min) of raw, concentrated juices extracted from Blanco *Agave* stems allowed for the production of an *Agave* syrup (13.3% w/v total reducing sugars). This syrup contained only 8.5% (w/v) free monosaccharides, with glucose and fructose in roughly equivalent concentrations. The remaining reducing sugars consisted mostly of oligosaccharides with potential prebiotic effects.

To create a more representative raw material, we combined equivalent parts of stem extracts from specimens of all three varieties (Cosmimaco, Xaco, and Blanco) and analyzed them before and after chemical or enzymatic hydrolysis and saccharification.

The untreated pool of stem extracts (AaE) contained (g/L): 10.4 sucrose, 7.0 glucose, and 6.4 fructose. After acid treatment, the resulting syrup contained (% w/v): 17.8 fructose, 5.9 glucose, and 0.13% sucrose a sweet syrup with a fructose-to-glucose ratio of 3:1.

Enzyme hydrolysis of *Agave* juices for the production of a fructose-rich syrup offers an important technological alternative for stable liquid sweeteners. Such syrups may also contain residual fructo-oligosaccharides (FOS), highly valued prebiotics for advanced nutrition. We enzymatically processed and concentrated AaE using a commercial inulinase (Fructolase, 1%). The resulting syrup contained 48.56% (w/v) reducing sugars, primarily glucose and fructose (% w/v: 29.5 fructose and 10.8 glucose), along with a small proportion of oligofructose (Estimated 8% w/v). With a fructose-to-glucose ratio of

2.7:1, this intensely sweet syrup could potentially reach 85% w/w soluble solids in a stable shelf life. Its sweetening power and sensory quality surpass those obtained by acid treatment. In the foreseeable future, this syrup could serve as a natural, vegan, nutraceutical, and organic sweetener.

Due to its significant benefits in soil restoration, organic production, and remarkable ability to thrive in non-arable soils, *A. atrovirens*, particularly the Blanco variety, should be considered a relevant bioresource for sustainable agroindustry in Mexico.

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Author's Contributions

Karen Gott-Bravo: Conducted experimental work and, wrote the first draft of the manuscript.

Arnulfo Pluma-Pluma: Sample collection, processing, and stabilization.

Victor Eric López-López and Sonia Mayra Pérez-Tapia: Academic and scientific orientation.

Ana Itzel Reyes-Méndez: Performed partial scientific literature review and proofread the manuscript.

Sergio Rúben Trejo-Estrada: Conceived and designed the study and performed data acquisition and analysis. Full coordination of research and data analysis. Drafted, reviewed, and edited the manuscript.

Ethics

This article is original and contains unpublished materials. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

References

Agricultura, S., & Rural, D. (2015). *Pulque, una Delicia Mítica*. Blog Gobierno de México. https://www.gob.mx/agricultura/es/articulos/pulque-una-delicia-mitica

A.O.A.C. (2005). *Official Methods of Analysis 18th Association of Official Analytical Chemists*.

Aldrete-Herrera, P. I., López, M. G., Medina-Torres, L., Ragazzo-Sánchez, J. A., Calderón-Santoyo, M., González-Ávila, M., & Ortiz-Basurto, R. I. (2019). Physicochemical Composition and Apparent Degree of Polymerization of Fructans in Five Wild Agave Varieties: Potential Industrial Use. *Foods*, *8*(9), 404. https://doi.org/10.3390/foods8090404

Álvarez-Chávez, J., Villamiel, M., Santos-Zea, L., & Ramírez-Jiménez, A. K. (2021). Agave By-Products: An Overview of Their Nutraceutical Value, Current Applications and Processing Methods. *Polysaccharides*, *2*(3), 720–743. https://doi.org/10.3390/polysaccharides2030044

Álvarez-Duarte, M. del C., Garcia-Moya, E., Suarez-Espinosa, J., Luna-Cavazos, M., & Rodríguez-Acosta, M. (2018). Conocimiento Tradicional, Cultivo y Aprovechamiento del Maguey Pulquero en Municipios de Puebla Y Tlaxcala Traditional Knowledge, Cultivation and Use of Maguey Pulquero in Municipalities of Puebla and Tlaxcala. *Polibotánica*, *45*, 1405–2768. https://doi.org/https://doi.org/10.18387/polibotanica.45.15

Apolinário, A. C., de Carvalho, E. M., de Lima Damasceno, B. P. G., da Silva, P. C. D., Converti, A., Pessoa, A., & da Silva, J. A. (2017). Extraction, Isolation, and Characterization of Inulin from Agave Sisalana Boles. *Industrial Crops and Products*, *108*, 355–362. https://doi.org/10.1016/j.indcrop.2017.06.045

Apolinário, A. C., de Lima Damasceno, B. P. G., de Macêdo Beltrão, N. E., Pessoa, A., Converti, A., & da Silva, J. A. (2014). Inulin-Type Fructans: A Review on Different Aspects of Biochemical and Pharmaceutical Technology. *Carbohydrate Polymers*, *101*, 368–378. https://doi.org/10.1016/j.carbpol.2013.09.081

Ávila Fernández, Á. (2014). Prebióticos: Alternativas Mexicanas. *HORIZONTE SANITARIO*, *12*(1), 4. https://doi.org/10.19136/hs.v12i1.149

Arrizon, J., Morel, S., Gschaedler, A., & Monsan, P. (2010). Comparison of the Water-Soluble Carbohydrate Composition and Fructan Structures of Agave Tequilana Plants of Different Ages. *Food Chemistry*, *122*(1), 123–130. https://doi.org/10.1016/j.foodchem.2010.02.028

Ávila-Fernández, Á., Galicia-Lagunas, N., Rodríguez-Alegría, M. E., Olvera, C., & López-Munguía, A. (2011). Production of Functional Oligosaccharides Through Limited Acid Hydrolysis of Agave Fructans. *Food Chemistry*, *129*(2), 380–386. https://doi.org/10.1016/j.foodchem.2011.04.088

Ávila-Fernández, Á., Rendón-Poujol, X., Olvera, C., González, F., Capella, S., Peña-Álvarez, A., & López-Munguía, A. (2009). Enzymatic Hydrolysis of Fructans in the Tequila Production Process. *Journal of Agricultural and Food Chemistry*, *57*(12), 5578–5585. https://doi.org/10.1021/jf900691r

Avila-Gaxiola, J., Velarde-Escobar, O. J., Millan-Almaraz, J. R., Ramos-Brito, F., Atondo-Rubio, G., Yee-Rendon, C., & Avila-Gaxiola, E. (2018). Treatments to improve obtention of reducing sugars from agave leaves powder. In *Industrial Crops and Products* (Vol. 112, pp. 577–583). https://doi.org/10.1016/j.indcrop.2017.12.039

Barquera, S., Hernández-Barrera, L., Trejo, B., Shamah, T., Campos-Nonato, I., & Rivera-Dommarco, J. (2020). Obesidad en México, Prevalencia y Tendencias en Adultos. Ensanut 2018-19. In *Salud Pública de México* (Vol. 62, Issues 6, Nov-Dic, pp. 682–692). https://doi.org/10.21149/11630

Bautista-Justo, M., García-Oropeza, L., Salcedo-Hernández, R., & Parra-Negrete, L. A. (2001). Azúcares en Agaves (Agave tequilana Weber) cultivados en el Estado de Guanajuato. In *Acta Universitaria* (Vol. 11, Issue 1, pp. 33–38). https://doi.org/10.15174/au.2001.325

Blecker, C., Fougnies, C., Van Herck, J.-C., Chevalier, J.-P., & Paquot, M. (2002). Kinetic Study of the Acid Hydrolysis of Various Oligofructose Samples. In *Journal of Agricultural and Food Chemistry* (Vol. 50, Issue 6, pp. 1602–1607). https://doi.org/10.1021/jf010905b

Chavez-Parga, M. D. C., Pérez Hernández, E., & González Hernández, J. C. (2016). Revisión Del Agave y El Mezcal. In *Revista Colombiana de Biotecnología* (Vol. 18, Issue 1). https://doi.org/10.15446/rev.colomb.biote.v18n1.49552

Conceição Apolinário, A., Silva Vieira, A. D., Marta Isay Saad, S., Converti, A., Pessoa, A., & da Silva, J. A. (2020). Aqueous extracts of *Agave sisalana* boles have prebiotic potential. *Natural Product Research*, *34*(16), 2367–2371. https://doi.org/10.1080/14786419.2018.1536129

Dávila, T. J., J. González, J., & Barrera, A. (2014). Panorama de la Obesidad en México. *Rev. Med. Inst. Mex Seguro Soc*, *53*(2), 9–240.

Delgado-Lemus, A., Torres, I., Blancas, J., & Casas, A. (2014). Vulnerability and Risk Management of Agave Species in the Tehuacán Valley, México. In *Journal of Ethnobiology and Ethnomedicine* (Vol. 10, Issue 53). https://doi.org/10.1186/1746-4269-10-53

Díaz Castañeda, A., & Osorio García, M. (2021). Los Imaginarios de Los Actores Sociales Del Pueblo Mágico de Ixtapan de la Sal, México. In *Pasos. Revista de Turismo y Patrimonio Cultural* (Vol. 19, Issue 1, pp. 101–115). https://doi.org/10.25145/j.pasos.2021.19.007

DUBOIS, M., GILLES, K., HAMILTON, J. K., REBERS, P. A., & SMITH, F. (1951). A Colorimetric Method for the Determination of Sugars. In *Nature* (Vol. 168, Issue 4265, pp. 167–167). https://doi.org/10.1038/168167a0

Escalante, A., López Soto, D. R., Velázquez Gutiérrez, J. E., Giles-Gómez, M., Bolívar, F., & López-Munguía, A. (2016). Vulnerability and Risk Management of Agave Species in the Tehuacán Valley, México. In *Frontiers in Microbiology* (Vol. 7, p. 1026). https://doi.org/10.3389/fmicb.2016.01026

FAO-CONAFOR. (2012). *Situación de los Recursos Genéticos Forestales en México*. https://doi.org/https://openknowledge.fao.org/server/api/core/bitstreams/747f6e9d-12ec-4e4a-a86b-8224b3e492cb/content

García Gamboa, R., Ortiz Basurto, R. I., Calderón Santoyo, M., Bravo Madrigal, J., Ruiz Álvarez, B. E., & González Avila, M. (2018). *In Vitro Evaluation of Prebiotic Activity, Pathogen Inhibition and Enzymatic Metabolism of Intestinal Bacteria in the Presence of Fructans Extracted from Agave: A Comparison based on Polymerization Degree*. LWT. https://doi.org/10.1016/j.lwt.2018.02.051

García, A. B., Ochoa-Martínez, L. A., Lara-Ceniceros, T. E., Rutiaga-Quiñones, O. M., Rosas-Flores, W., & González-Herrera, S. M. (2019). *Changes in the Microstructural, Textural, Thermal, and sensory Properties of Apple Leathers Containing Added Agavins and Inulin*. Food Chemistry. https://doi.org/10.1016/j.foodchem.2019.03.143

Gerritsen, P. R. W., Rosales Adame, J. J., Moreno Hernández, A., & Martínez Rivera, L. M. (2011). *Agave Azul y el Desarrollo Sustentable en la Cuenca Baja Del río Ayuquila, Costa Sur de Jalisco (1994-2004)*. Región y Sociedad. https://doi.org/10.22198/rys.2011.51.a53

Godínez-Hernández, C. I., Aguirre-Rivera, J. R., Juárez-Flores, B. I., Ortiz-Pérez, M. D., & Becerra-Jiménez, J. (2015). Extracción y caracterización de fructanos de Agave salmiana Otto ex Salm-Dyck. In *Revista Chapingo Serie Ciencias Forestales y del Ambiente* (Vol. 22, Issue 1). https://doi.org/10.5154/r.rchscfa.2015.02.007

Gomez, E., Tuohy, K. M., Gibson, G. R., Klinder, A., & Costabile, A. (2010). In Vitro Evaluation of the Fermentation Properties and Potential Prebiotic Activity of Agave Fructans. In *Journal of Applied Microbiology* (Vol. 108, Issue 6, pp. 2114–2121). https://doi.org/10.1111/j.1365-2672.2009.04617.x

Guarino, M., Altomare, A., Emerenziani, S., Di Rosa, C., Ribolsi, M., Balestrieri, P., Iovino, P., Rocchi, G., & Cicala, M. (2020). Mechanisms of Action of Prebiotics and Their Effects on Gastro-Intestinal Disorders in Adults. In *Nutrients* (Vol. 12, Issue 4, p. 1037). https://doi.org/10.3390/nu12041037

Hernández-Arroyo, M. M., Plascencia-Espinosa, M. Á., Hidalgo-Lara, M. E., Tinajero-Trejo, M., Méndez-Merino, E., & Trejo-Estrada, S. R. (2018). *Novel D-Galactose Isomerases from Lactobacillus Strains Isolated from the Sweet Sap of* Agave atrovirens. American Journal of Biochemistry and Biotechnology. https://doi.org/10.3844/ajbbsp.2018.272.284

Hernández-Salas, J. M., Villa-Ramírez, M. S., Veloz-Rendón, J. S., Rivera-Hernández, K. N., González-César, R. A., Plascencia-Espinosa, M. A., & Trejo-Estrada, S. R. (2009). Comparative Hydrolysis and Fermentation of Sugarcane and Agave Bagasse. *Bioresource Technology*, *100*(3), 1238–1245. https://doi.org/10.1016/j.biortech.2006.09.062

Iñiguez-Covarrubias, G., Díaz-Teres, R., Sanjuan-Dueñas, R., Anzaldo-Hernández, J., & Rowell, R. M. (2001). Utilization of by-Products from the Tequila Industry. Part 2: Potential Value of Agave Tequilana Weber Azul Leaves. *Bioresource Technology*, *77*(2), 101–108. https://doi.org/10.1016/s0960-8524(00)00167-x

Jiménez Muñoz, E., Prieto-García, F., Prieto Méndez, J., Acevedo Sandoval, O. A., & Rodríguez Laguna, R. (2016). Caracterización Fisicoquímica de Cuatro Especies de Agaves con Potencialidad en la Obtención de Pulpa de Celulosa para Elaboración de Papel. *DYNA*, *83*(197), 232. https://doi.org/10.15446/dyna.v83n197.52243

Luiz-Santos, N., Prado-Ramírez, R., Arriola-Guevara, E., Camacho-Ruiz, R.-M., & Moreno-Vilet, L. (2020). Performance Evaluation of Tight Ultrafiltration Membrane Systems at Pilot Scale for Agave Fructans Fractionation and Purification. *Membranes*, *10*(10), 261. https://doi.org/10.3390/membranes10100261

Luna, Z. R. (2007). Economía y Estética de Los Destilados Del Maguey. *En Lo Ancestral Hay Futuro: Del Tequila, Los Mezcales y Otros Agaves*, 175–190. https://doi.org/https://bioteca.biodiversidad.gob.mx/janium/Documentos/15724.pdf

Lara, F., Julián, M., Pérez, A., Benítes, I., & Lara, P. (2017). Avances en la producción de inulina. *Tecnología Química*, *37*(2), 352–366. https://doi.org/http://www.redalyc.org/articulo.oa?id=445551175005

Leopoldo. (2011). Influence of Plant Age on Fructan Content and Fructosyltranserase Activity in Agave Atrovirens Karw Leaves. In *AFRICAN JOURNAL OF BIOTECHNOLOGY* (Vol. 10, Issue 71, p. 15911). https://doi.org/10.5897/ajb11.2254

Madrigal, L., & Sangronis, E. (2017). La inulina y Derivados Como Ingredientes Claves en Alimentos Funcionales. *Archivos Latinoamericanos de Nutrición*, *57*(4), 387-396 2309. https://doi.org/https://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0004-06222007000400012

Márquez-Pallares, L., Aguila-Muñoz, J., Honorato-Salazar, J. A., & Trejo-Estrada, S. R. (2024). MORPHOLOGICAL AND BIOCHEMICAL ANALYSES OF Agave salmiana VARIETIES. *Agrociencia*, *58*(2). https://doi.org/10.47163/agrociencia.v58i2.2841

Michel-Cuello, C., Juárez-Flores, B. I., Aguirre-Rivera, J. R., & Pinos-Rodríguez, J. M. (2008). Quantitative Characterization of Nonstructural Carbohydrates of Mezcal Agave (*Agave salmiana* Otto ex Salm-Dick). *Journal of Agricultural and Food Chemistry*, *56*(14), 5753–5757. https://doi.org/10.1021/jf800158p

Michel-Cuello, C., Ortiz-Cerda, I., Moreno-Vilet, L., Grajales-Lagunes, A., Moscosa-Santillán, M., Bonnin, J., González-Chávez, M. M., & Ruiz-Cabrera, M. (2012). Study of Enzymatic Hydrolysis of Fructans from*Agave salmiana*Characterization and Kinetic Assessment. *The Scientific World Journal*, *2012*, 1–10. https://doi.org/10.1100/2012/863432

Mielenz, J. R., Rodriguez, M., Thompson, O. A., Yang, X., & Yin, H. (2015). Development of Agave as a dedicated biomass source: production of biofuels from whole plants. *Biotechnology for Biofuels*, *8*(1). https://doi.org/10.1186/s13068-015-0261-8

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/10.1021/ac60147a030

Moeller, S. M., Fryhofer, S. A., Osbahr, A. J., & Robinowitz, C. B. (2009). The Effects of High Fructose Syrup. *Journal of the American College of Nutrition*, *28*(6), 619–626. https://doi.org/10.1080/07315724.2009.10719794

Muñoz-Gutiérrez, I., Rodríguez-Alegría, M. E., & López Munguía, A. (2009). Kinetic Behaviour and Specificity of β-Fructosidases in the Hydrolysis of Plant and Microbial Fructans. In *Process Biochemistry* (Vol. 44, Issue 8, pp. 891–898). https://doi.org/10.1016/j.procbio.2009.04.013

Narváez Suárez, A. U., Martínez Saldaña, T., & Jiménez-Velázquez, M. (2016). El cultivo de maguey pulquero: opción para el desarrollo de comunidades rurales del altiplano mexicano. In *Revista de Geografía Agrícola* (Issue 56, pp. 33–44). https://doi.org/10.5154/r.rga.2016.56.005

Olvera, V., Granado, A. B., Jové, M., Cassanyé, A., Cardador, A., Portero, M., & Serrano, J. (2017). Characterization of Antioxidant Properties and Metabolite Profile of Agave Atrovirens Extracts. *BioRxiv 184226*. https://doi.org/https://doi.org/10.1101/184226

Olvera-Garcia, V., Cardador-Martinez, A., & Martin del Campo, S. T. (2015a). Effect of Thermal Process and Filtration on the Antioxidant Activity and Physicochemical Parameters of Agave atrovirens Extracts. In *Journal of Food Research* (Vol. 4, Issue 1, p. 155). https://doi.org/10.5539/jfr.v4n1p155

Olvera-García, V., Martín del Campo, S. T., Gutiérrez-Uribe, J. A., & Cardador-Martínez, A. (2015b). GC–MS and HPLC–MS-TOF Characterization of Agave Atrovirens Extracts. A Preliminary Study. In *Industrial Crops and Products* (Vol. 78, pp. 39–47). https://doi.org/10.1016/j.indcrop.2015.09.068

Ozuna, C., & Franco-Robles, E. (2022). Agave syrup: An Alternative to Conventional Sweeteners? A Review of its Current Technological Applications and Health Effects. In *LWT* (Vol. 162, p. 113434). https://doi.org/10.1016/j.lwt.2022.113434

Parker, K., Salas, M., & Nwosu, V. C. (2010). High Fructose Corn Syrup: Production, Uses and Public Health Concerns. *Biotechnology and Molecular Biology Review*, *5*(5), 71–78. https://doi.org/https://academicjournals.org/article/article1380113250_Parker et al.pdf

Pérez-Zavala, M. de L., Hernández-Arzaba, J. C., Bideshi, D. K., & Barboza-Corona, J. E. (2020). Agave: A Natural Renewable Resource with Multiple Applications. *Journal of the Science of Food and Agriculture*, *100*(15), 5324–5333. https://doi.org/10.1002/jsfa.10586

Pizarro, H. K., & Herrera, T. (2021). Verdes Matas que van de los Valles de Apan a los Estados Unidos. In *México: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO)* (pp. 365–370). https://doi.org/https://bioteca.biodiversidad.gob.mx/janium/Documentos/15852.pdf

Reynoso, S. R., García, A. J., López, W., López, A., Cadena, P., Pérez, M. A., & Domínguez, M. H. (2012). *Identificación Taxonómica de Agaves (Agave spp.) Utilizados Para la Elaboración del Licor Comiteco en Chiapas, México*. Agro Productividad. https://doi.org/https://go.gale.com/ps/i.do?p=IFME&u=googlescholar&id=GALE|A382656329&v=2.1&it=r&sid=bookmark-IFME&asid=af840dea

Ríos Martínez, A., & López de Juambelz, R. (2015). La Etnobotánica y el Paisaje del Maguey Como Recurso Natural Para el Desarrollo del Poblado de San Gabriel Azteca de Zempoala Hidalgo, México. *Estudios Latinoamericanos*, *35*, 45–72. https://doi.org/10.36447/estudios2015.v35.art3

Roberfroid, M. B. (2005). Introducing Inulin-Type Fructans. *British Journal of Nutrition*, *93*(S1), S13–S25. https://doi.org/10.1079/bjn20041350

Romero, M. R., Escobedo, R., Perea, M., & Flores, A. (2016). *Efecto Antiinflamatorio de Aguamiel (*Agave Atrovirens*) en un Modelo Murino de úlcera Gástrica Inducida*. Revista de Ingeniería y Tecnologías Para El Desarrollo Sustentable. https://doi.org/http://reingtec.itsoeh.edu.mx/reingtec/docs/vol1_2016reingtec/ARTICULO_4.pdf

Romero, M. R., Osorio, P., Flores, A., Robledo, N., & Mora, R. (2015). *Composición Química, Capacidad Antioxidante y el Efecto Prebiótico del Aguamiel (*Agave Atrovirens*) Durante su Fermentación in Vitro*. Revista Mexicana de Ingeniería Química. https://doi.org/https://scielo.org.mx/pdf/rmiq/v14n2/v14n2a5.pdf

SAGARPA. (2016). *Agave, Lanta de las Maravillas*. Blog Gobierno de México. https://failover.www.gob.mx/mantenimiento.html

SAGARPA. (2017). *Agave Tequilero y Mezcalero Mexicano*. https://doi.org/https://www.gob.mx/cms/uploads/attachment/file/257066/Potencial-Agave_Tequilero_y_Mezcalero.pdf

Santos-Zea, L., Leal-Diaz, A., Cortes-Ceballos, E., & Gutierrez- Uribe, J. (2012). Agave (Agave spp.) and its Traditional Products as a Source of Bioactive Compounds. *Current Bioactive Compounds*, *8*(3), 218–231. https://doi.org/10.2174/157340712802762410

Servicio Nacional de Inspección y Certificación de Semillas (SNICS. (2017). Generalidades de La Red Agaváceas. https://www.gob.mx/snics/acciones-y-programas/Agave-Agave-spp

García, M. A. (2002). Distribution of Agave (Agavaceae) in Mexico. In *Cactus and Succulent Journal* (Vol. 74, Issue 4, pp. 177–188).

Soto, J. L. M., González, J., Nicanor, A., & Ramírez, EGR. (2011). Enzymatic Production of High Fructose Syrup from Agave Tequilana Fructans and its Physicochemical Characterization. *African Journal of Biotechnology*, *10*(82), 19137–19143. https://doi.org/10.5897/ajb11.2704

Salcedo, J. E., Montes, E. J., & Pájaro, J. L. (2009). Production of Fructose Syrups Through Enzymatic Hydrolysis of Cassava Starch of the Corpoica M Tai-8 and Corpoica Orense Varieties. *Dyna*, *76*(60), 121–130.

Valenzuela-Zapata, A. G. (1995). The Agroindustry of the Tequila Agave, Agave Tequilana Weber. *Botanical Sciences*, *57*, 15–25. https://doi.org/10.17129/botsci.1473

Verma, D. K., Patel, A. R., Thakur, M., Singh, S., Tripathy, S., Srivastav, P. P., Chávez-González, M. L., Gupta, A. K., & Aguilar, C. N. (2021). A Review of the Composition and Toxicology of Fructans and their Applications in Foods and Health. *Journal of Food Composition and Analysis*, *99*, 103884. https://doi.org/10.1016/j.jfca.2021.103884

Villarreal Morales, S. L., Enríquez Salazar, M. I., Michel Michel, M. R., Flores Gallegos, A. C., Montañez-Saens, J., Aguilar, C. N., & Herrera, R. R. (2019). Metagenomic Microbial Diversity in Aguamiel from Two*Agave*Species During 4-Year Seasons. *Food Biotechnology*, *33*(1), 1–16. https://doi.org/10.1080/08905436.2018.1547200

Villegas-Silva, P. A., Toledano-Thompson, T., Canto-Canché, B. B., Larqué-Saavedra, A., & Barahona-Pérez, L. F. (2014). Hydrolysis of Agave Fourcroydes Lemaire (Henequen) Leaf Juice and Fermentation with Kluyveromyces Marxianusfor Ethanol Production. *BMC Biotechnology*, *14*(1). https://doi.org/10.1186/1472-6750-14-14

Zimmermann, A., Visscher, C., & Kaltschmitt, M. (2023). Plant-Based Fructans for Increased Animal Welfare: Provision Processes and Remaining Challenges. *Biomass Conversion and Biorefinery*, *13*(4), 2667–2685. https://doi.org/10.1007/s13399-021-01473-2