Volume 58, January-December 2024, ISSN: 2079-3480

PASTURE SCIENCE AND OTHER CROPS

CUBAN JOURNAL OF AGRICULTURAL SCIENCE

Journal site: https://cjascience.com Cu-ID: https://cu-id.com/1996/v58e26

DETERMINATION OF FRUCTANS IN VEGETATIVE ORGANS OF *AGAVE OFFOYANA* (*ASPARAGACEAE*) AND ITS POTENTIAL USE AS A PREBIOTIC

DETERMINACIÓN DE FRUCTANOS EN ÓRGANOS VEGETATIVOS DE *AGAVE OFFOYANA* (*ASPARAGACEAE*), POTENCIALIDADES DE SU USO COMO PREBIÓTICO

A.R. HERNÁNDEZ MONTESINOS[1]*, D. ALFONSO GONZÁLEZ[2], DAYANNA CORONADO ACOSTA[3], R. REYES FERNÁNDEZ[3], DAYMARA RODRÍGUEZ ALFONSO[3]

[1]*Instituto de Ciencia Animal, C. Central, km 47½, San José de las Lajas, C.P. 32700, Mayabeque, Cuba*
[2]*Jardín Botánico Nacional, Universidad de La Habana, Carretera El Rocío km 3 ½, C.P. 19230. Calabazar, Boyeros, La Habana, Cuba*
[3]*Universidad Agraria de La Habana, Autopista Nacional, km 23 ½, C.P. 32700, San José de las Lajas, Mayabeque, Cuba*

* *Email:andresraulhm@gmail.com*

Fructans are carbohydrates in which the links between fructose residues predominate. They are synthesized in approximately 15 % of angiosperms. These molecules have various applications in food and health, due to the lack of digestibility and their selective use by the beneficial intestinal microbiota in monogastric animals. The objective of this study was to determine, using chromatographic methods, the presence of fructans in vegetative organs of the endemic species *Agave offoyana* (maguey). Plants were collected in three different physiological states during two climatic periods. They were taken from the managed floristic reserve Tres Ceibas de Clavellina, in Matanzas province, Cuba. Samples were analyzed by different chromatographic methods. Thin layer chromatography showed the presence of fructans in stems and leaves, but not in roots. The accumulation was greater in stems. Both organs contain from the trisaccharide to molecules with a high degree of polymerization. The presence of the polysaccharide was greater in the leaves at the base than in the middle and shoot apex. There was an increase in the accumulation of fructans in the organ samples collected during the dry season. High-performance liquid chromatography confirmed the presence of fructooligosaccharides extracted from the stem. Results showed that *Agave offoyana* accumulates fructans in the stem, although they are also present in leaves. This Cuban species can be used to obtain fructans for human and animal feeding and health.

Key words: *chromatography, fructooligosaccharides, maguey*

Los fructanos son carbohidratos en los que predominan los enlaces entre residuos de fructosa. Se sintetizan en aproximadamente 15 % de las angiospermas. Estas moléculas tienen diversas aplicaciones en la alimentación y la salud, debido a la ausencia de digestibilidad y a su utilización selectiva por la microbiota intestinal benéfica en animales monogástricos. El objetivo de este trabajo fue determinar mediante métodos cromatográficos la presencia de fructanos en órganos vegetativos de la especie endémica *Agave offoyana* (maguey). Las plantas se recolectaron en tres estados fisiológicos diferentes durante dos períodos climáticos. Se tomaron de la reserva florística manejada Tres Ceibas de Clavellina, de la provincia Matanzas, Cuba. Las muestras se analizaron por diferentes métodos cromatográficos. La cromatografía de capa fina mostró presencia de fructanos en tallos y hojas, no así en las raíces. La acumulación fue mayor en los tallos. Ambos órganos contienen desde el trisacárido hasta moléculas con alto grado de polimerización. La presencia del polisacárido fue mayor en las hojas de la base con respecto a las del medio y del ápice caulinar. Hubo aumento en la acumulación de fructanos en las muestras de órganos recolectados en el período poco lluvioso. La cromatografía líquida de alta resolución confirmó la presencia de los fructoligosacáridos extraídos del tallo. Los resultados demostraron que *Agave offoyana* acumula fructanos en el tallo, aunque también están presentes en las hojas. Esta especie cubana se puede utilizar en la obtención de fructanos para la alimentación y la salud humana y animal.

Palabras clave: *cromatografía, fructoligosacáridos, maguey*

Received: June 02, 2023
Accepted: September 16, 2023
Conflict of interests: The authors declare that there is no conflict of interest.
CRediT Authorship Contribution Statement: A.R. Hernández Montesinos: **Investigation, Visualization, Writing - original draft.** D. Alfonso González: **Conceptualization, Investigation, Supervision, Writing - original draft.** Dayanna Coronado Acosta: **Investigation, Supervision, Writing - original draft.** R. Reyes Fernández: **Investigation, Writing - original draft.** Daymara Rodríguez Alfonso: **Investigation, Writing - original draft.**

Introduction

Fructans are carbohydrates in which the links among fructose residues predominate. This definition is independent of molecule size, since there are fructans that contain from two to more than 10^6 units (Waterhouse and Chatterton 1993). There are different criteria for the classification of fructans. According to Waterhouse and Chatterton (1993), their grouping is based on three essential criteria: the predominant type of bond among fructose residues, degree of polymerization (DP) of the molecule and its origin. According to this, the most frequent terms that appear in the bibliography are: kestoses, inulin, levan, phlein and graminan, each described with its characteristic structures. Oligomeric fructans, which contain a saccharose linked to one or more fructose residues, are called fructooligosaccharides (FOS) and can be of microbial or plant origin.

Van den Ende (2013) and Franco-Robles and López (2015) established a classification criterion based on the type of glycosidic bond present. These authors grouped fructans into inulins, levans, graminans and neofructans (neoinulins or neoseries inulins and neolevans or neoseries levans). There is a specific type of fructan, which is characterized by a highly branched complex structure, with an external glucose in the graminans and an internal one in the neofructans. These compounds are produced in agaves, which is why they are called agavins (Mancilla-Margalli and López 2006).

In the plant kingdom *Plantae*, approximately 15 % of flowering plants are able to synthesize and store this compound in leaves, stems and roots, mainly in storage organs such as bulbs, tubers and rhizomes (Hendry 1993). These plants are found in a small group of mono and dicotyledonous families: *Amaryllidaceae*, *Poaceae*, *Asteraceae*, *Nolinaceae* and *Asparagaceae* (Franco-Robles and López 2015).

The most studied medicinal property of fructans is their prebiotic action (Ayala *et al.* 2018, Guillot 2018 and Armas *et al.* 2019). In addition, they are involved in the decrease of body mass index, the reduction of total body fat and triglycerides in obese individuals (Padilla-Camberos *et al.* 2018). They also reduce glycemic indicators in individuals with prediabetes and diabetes mellitus, prevent colorectal cancer, osteoporosis and have brain protection properties (Wang *et al.* 2019 and Espinosa-Andrews *et al.* 2021). Fructans are incorporated into foods for their technological properties: emulsifiers, stabilisers, gelling agents and sweeteners (Verma *et al.* 2021). Furthermore, *Agave tequilana* agavins are used in the industrial production of beverages such as tequila and mezcal (Hernández 2018).

Nowadays, the use of fructans as prebiotic additives in animal production is increasing (de Lange *et al.* 2010). García-Curbelo *et al.* (2018) included *Agave fourcroydes* L. agavins in pig diet and obtained modifications in lipid metabolism, related to the decrease of total cholesterol, low-density lipoproteins and total lipids. Alvarado-Loza *et al.* (2017), by supplying 2 % of Agave inulin in rabbit feed, reported its positive influence on digestibility and intestinal microbiota. In addition, Chávez-Mora *et al.* (2019) observed an increase in the percentage of laying and weight of the egg, as well as in its quality indices, in favor of treatments with Agave oligofructose.

Agave genus is considered native to Mexico, where 272 species of the 310 reported are found. Of these, 135 are endemic (Mancilla-Margalli and López 2006).

In Cuba, this genus includes 16 native species (Greuter and Rankin 2017). *Agave offoyana* Jacobi is commonly known as maguey. It is an endemic species (Romero-Jiménez *et al.* 2015) and is distributed on the Northern coast and some inland regions (de Zayas 1980). It grows in evergreen forests, coastal and subcoastal microphylls. It is a local resource, used as a medicinal, honey-producing and ornamental plant (Romero-Jiménez *et al.* 2015).

Several species of *Agave* are used to obtain products for use in health, industry and human and animal food. However, they are not Cuban. Out of the 24 species that grow in Cuba, *A. offoyana* stands out for its size and could store fructans as a carbon source, as occurs in several Mexican species.

The objective of this study was to determine, using chromatographic methods, the presence of fructans in vegetative organs of the endemic species *Agave offoyana* (maguey).

Materials and Methods

Plant material: Specimens of *Agave offoyana* were collected in the Tres Ceibas de Clavellina managed floristic reserve, Matanzas province, Cuba (23º05'48.6" N and 81º39'20.5" W). For plant identification, the characteristics of this species, described in Flora de Cuba, first fascicle (León 1946), were used as a reference.

Samples were taken at two times of the year. The first, in December 2016, during dry period, and the second, in July 2017, rainy season. In December, nine plants were collected, three juveniles, three adults and three in bloom (early stage), close to four, eight and ten years of age, respectively. In July, three adult specimens close to ten years of age were studied.

From each plant collected in December, samples of roots, stems, and a leaf inserted in the base, middle, and apex of the stem, were taken. From those collected in July, only samples of the stem and the leaf inserted in the middle of the stem (fourth leaf from the base of the stem) were taken (figure 1). Samples were transported separately in plastic bags to the plant biotechnology laboratory of the Faculty of Agronomy, of the Agricultural University of Havana.

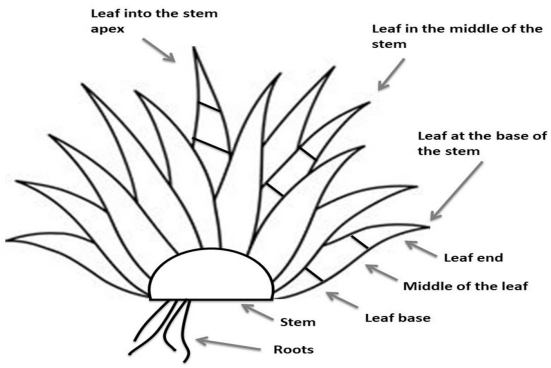


Figure 1. Scheme of sample collecting of vegetative organs in plants of *Agave offoyana* Jacobi

Determination of fructans by thin layer chromatography (TLC): For fructan extraction, the protocol followed by Wack and Blaschek (2006) was modified. All collected samples were processed in the laboratory, where each organ was fractionated, sterilized and crushed separately. Leaves were sectioned at the base, middle and apex (figure 1). The organ segments (stem, leaves and roots) were weighed and sterilized separately, with water, in a 1:2 (w/v) ratio, in an autoclave, at 121 °C and 1 atm of pressure for 40 min. Then, they were crushed separately in a blender until a paste was obtained. They were placed in 1.5 mL tubes and centrifuged at 9000 rpm in a microcentrifuge to obtain the aqueous phase.

The presence of fructans was determined by thin layer chromatography (TLC), according to the method proposed by Trujillo *et al.* (2004). For this purpose, 1.5 µL of the aqueous phase extracted from roots, stems and leaves was applied to a TLC plate (silica gel on aluminum sheets, Fluka, Germany) and introduced three times into a closed chamber containing 10 mL of running solution (1-butanol, 2-propanol and water in a 3:12:0.5 ratio).

The plate was developed by applying a solution (saturated butanol 93 mL, orthophosphoric acid 85 % 7 mL and urea 3 g). Absolute ethanol was also added until the solution became transparent and it was incubated at 80 °C to accelerate the reaction. The combination of these reagents allowed the profiles and the fructose molecule to be visualized. An aqueous extract of onion bulbs (*Allium cepa* L.) was used as a molecular weight standard, as described by Vijn *et al.* (1998).

Determination of fructans by high-performance liquid chromatography (HPLC): To determine the presence of fructans, a stem sample from an adult plant collected in December was used and analyzed by HPLC, as described by Meyer (2010). Prior to performing the HPLC, TLCs were performed on organs of the plants collected in December, which allowed to select the one used as sample.

The run was performed on a Nucleosil NH_2 column (0.8 x 25.0 cm) (Sigma, USA) using 80% acetonitrile in water as the elution solution and a flow rate of 0.4 mL min^{-1} at 37 ºC. The eluted sugars were found with a differential refractometer (Knauer, Germany). For calibration of the equipment, the standards sucrose, 1-kestose and nystose, were used, prepared at 10 mg mL^{-1}.

Results

Product profiles obtained from organ samples from adult plants revealed that *A. offoyana* stores fructans as a carbon source. These carbohydrates were mainly accumulated in the stems and are found in lower concentrations in leaves. They were not found in the roots. Low and high molecular weight polymers were observed in both plant organs (figure 2a).

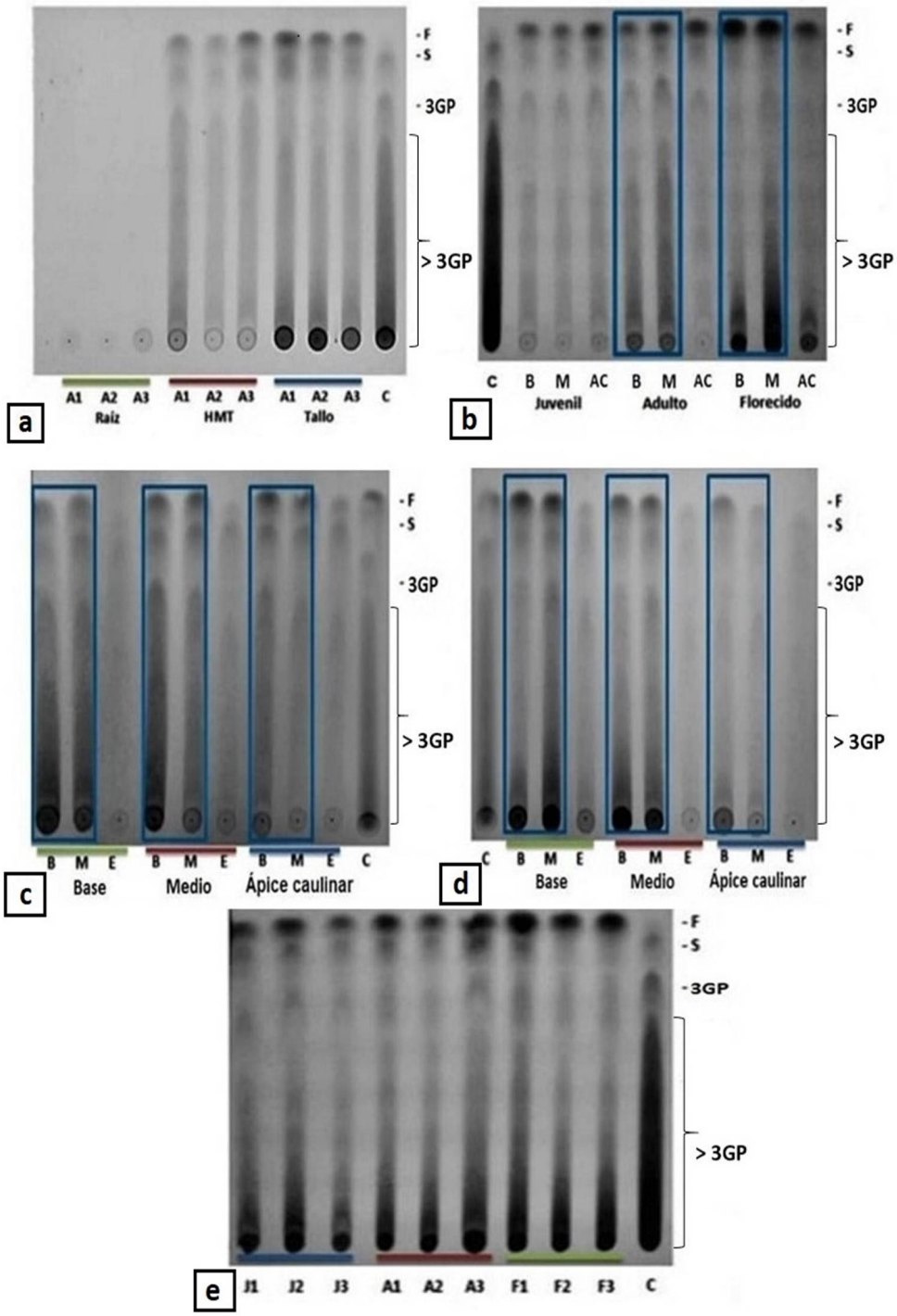
A. offoyana contains fructans in the leaves of juvenile, adult and flowering plants (figure 2b), although in the latter two the concentration is higher than in young plants. In the same figure, TLC also showed that the juvenile plant mainly produces low molecular weight fructans in leaves. However, in the adult and flowering plants, low and high degree of polymerization molecules were observed, which was evidenced by the dark spot present at the application point.

Figures 2c and 2d show that the presence of fructans in leaves inserted at the base and middle of the stem of adult and flowering plants was greater than in the leaves at the stem apex. Furthermore, when these leaves were divided into base, middle and apex, it was found that the concentration of fructans was greater in the areas at the base and middle than at the apex. As demonstrated in the figures, the presence of high molecular weight molecules in the basal area of leaves at the base, middle and stem apex was greater than in the middle. There was little representation of these molecules at the apex of leaves.

Young, adult and flowering plants stored similar amounts of fructans in stem samples taken during dry season (figure 2e). Besides, this figure shows the TLC and the presence of low and high molecular weight polymers during the three phases of plant growth. The concentration of carbohydrates increased slightly with the degree of plant development.

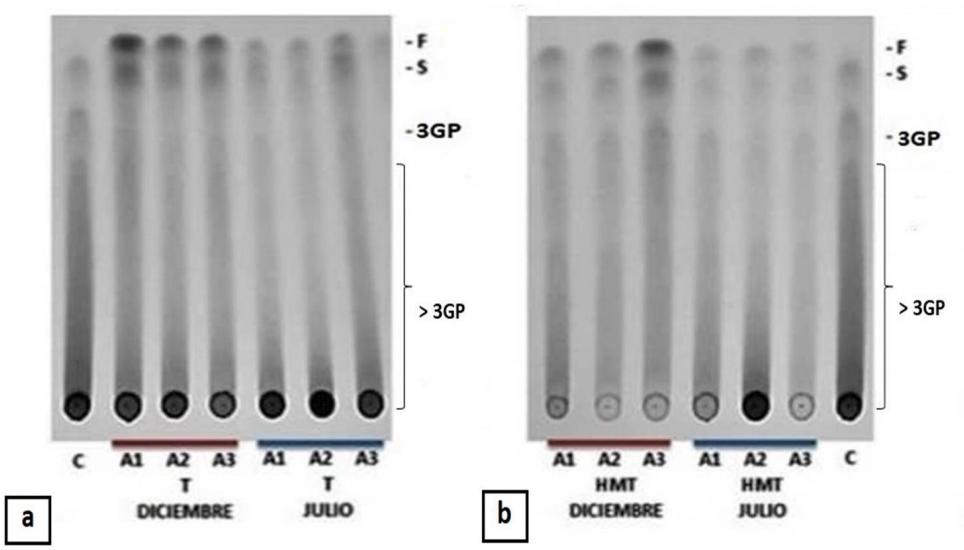
Stem and leaves inserted in the middle of the stem of adult plants stored fructans during dry (December) and rainy (July) periods, although there was a slight increase in the accumulation in these organs during the dry period. The light spots on the dark background at the point of application of some samples indicate the saturation by high molecular weight fructans (figures 3a and 3b).

High-performance liquid chromatography (HPLC) showed the presence of fructans from molecules with DP 3 to DP 6. This technique does not allow the separation of molecules with higher DP, which are seen grouped in a single peak. The polymer with the highest concentration is the one composed of three fructose units (figure 4).



a. Root, leaf* inserted at the base of the stem and stem of three adult plants
b. Leaves* inserted in the base, middle and stem apex in juvenile, adult and flowering plants.
c. Leaves of an adult plant (base, middle and stem apex) sectioned at base, middle and apex.
d. Leaves of a flowering plant (base, middle and stem apex) sectioned at base, middle and apex
e. Stems of juvenile, adult and flowering plants (collected in December). J (juvenile), A (adult), F (flowering), HMT (leaf inserted in the middle of the stem), B (base), M (middle), AC (stem apex), E (apex), C (onion), F (fructose), S (sucrose), 3GP (trisaccharide), GP (degree of polymerization). *Refers to the basal area of the leaf.

Figure 2. Product profiles obtained by TLC from the extracts of the different plant organs of *Agave offoyana* Jacobi



a. Stem of adult plants collected in December and July.

b. Leaves* inserted in the middle of the stem of adult plants collected in December and July. A (adult), T (stem), HMT (leaf inserted in the middle of the stem), C (onion), F (fructose), S (sucrose), 3GP (trisaccharide), DP (degree of polymerization). * Refers to the basal area of the leaf.

Figure 3. Product profiles obtained by TLC of the extracts of different organs of *Agave offoyana* Jacobi plants

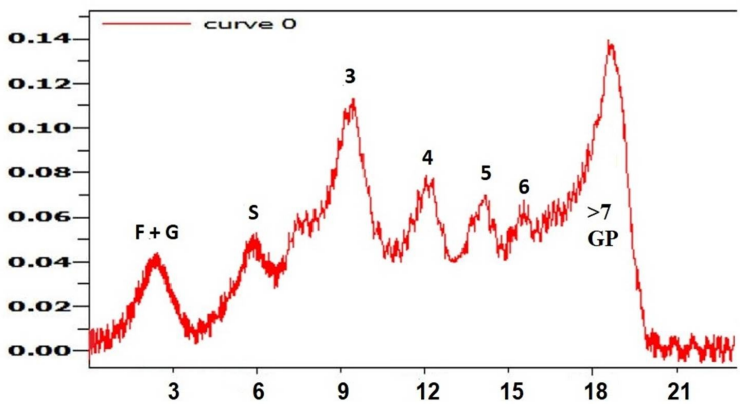


Figure 4. Chromatographic profile obtained by HPLC of extracts of *Agave offoyana* Jacobi stems. G (glucose), F (fructose), S (sucrose), DP (degree of polymerization).

Discussion

Fructans are found in several plant families as reserve carbohydrates. They also function as osmoprotective substances during drought and cold stress (Ritsema and Smeekens 2003). The studied *A. offoyana* plants contain more fructans in stems than in leaves. These polymers are the main reserve carbohydrate present in *Agave* stems (Mellado-Mojica *et al.* 2009). Fructans are also the most abundant carbohydrates in adult plants of *Agave fourcroydes* (García-Curbelo *et al.* 2009). Likewise, *Agave tequilana* has more fructans in stem than in leaves. In this species, the head or cone (stem and leaf bases) stores the largest amount of total non-reducing sugars, where inulin and fructooligosaccharides (FOS) are predominant. This is followed by the base of leaves, where inulin and, to a lesser extent, FOS are also predominant (Montañez-Soto *et al.* 2011 and Ferrer-Serrano *et al.* 2023).

Leaves inserted at the base of the stem of *A. offoyana* are green, without mechanical damage. They grow on previously dead leaves or leaves in the senescence phase.

They represent the most mature leaves of the plant and have the highest amount of fructans. However, in *A. fourcroydes*, intermediate leaves store the highest fructan concentration (García-Curbelo *et al.* 2009). In *A. offoyana* leaves, it occurred at the base of this organ. This coincides with what has been reported in adult plants of *A. tequilana*, where fructan content is equivalent to 68.6 %, with respect of that of the head or cone. The base of leaves constitutes the second fraction with the greatest contribution to the biomass of *A. tequilana* crop. It is mainly composed of inulin, FOS (to a lesser extent) and reducing sugars (Montañez-Soto *et al.* 2011). Also, in the lower leaves of *Agave mapisaga*, 68 % of FOS and 32 % of high molecular weight fructans were found (Plascencia *et al.* 2019).

Fructan accumulation in *A. offoyana* occurs from the juvenile phase until the beginning of flowering. Likewise, carbohydrate content is directly related to the age and physiological stage of *A. tequilana*. The highest concentrations were observed in adult plants, while the lowest concentrations were quantified in juveniles (Mellado-Mojica *et al.* 2009). In a study with plants of *Agave angustifolia* Haw. and *Agave potatorum* Zucc., from one to six years old, it was determined that the concentration of simple carbohydrates was higher in young plants and that fructans with a higher DP predominated in adult plants for both species (Márquez-López *et al.* 2022).

A. offoyana specimens were collected in a natural area. Stems and leaves of juvenile plants (approximately four years old) showed less accumulation of fructans, of low and high DP, compared to adult plants and at the beginning of flowering. In stems and base of the leaves of different *Agave* species, plants from two to four years old exhibited the highest concentrations of free sugars and fructans with low DP. On the contrary, plants aged 10 to 12 years old showed low fructan concentration with higher DP (Aldrete-Herrera *et al.* 2019).

Contrary to the previous result, in *A. fourcroydes* cones, a greater quantity of sugars was obtained in 12-year-old plants, compared to those of seven years (Ferrer-Serrano *et al.* 2023). Similarly, in *A. tequilana*, aged four years, lower concentrations of fructans are shown compared to those of six and eight years. Those corresponding to ten years hydrolyze fructose to supply the energy demand of the flowering stage (Mellado-Mojica *et al.* 2009).

Plants store polysaccharides during the vegetative period to provide the necessary energy during the reproductive stage (Pérez and Martínez-Laborde 1994). However, *A. offoyana* accumulates these carbohydrates even during the beginning of flowering in a similar way to the adult phase, which could be due to the asexual reproduction mechanism that follows the sexual one, and that takes place in the same inflorescence (García-Beltrán *et al.* 2017). Unlike *A. offoyana*, the concentrations of this carbohydrate decreased in *A. tequilana* plants that were in the flowering stage (Mellado-Mojica *et al.* 2009). In most of the studies carried out on species of *Agave* genus, plants were harvested before flowering, because the main function of the storage of fructose polymers is their use during flowering and fruiting (García-Curbelo *et al.* 2009, Arrizon *et al.* 2010, García-Curbelo *et al.* 2015 and Godínez-Hernández *et al.* 2016). As the reproductive stage begins, the development of photosynthetically active leaves is suppressed and the leaves and stems start to age, indicating that carbohydrate reserves were used for the reproductive phase (Delgado *et al.* 2012).

The stem and leaves of adult *A. offoyana* plants stored fructans in both periods of the year, although a slight increase in their accumulation was evident in the dry season (figures 3a and 3b). In Cuba, there are two seasonal periods (rainy and dry) with specific characteristics. More than 80% of the annual precipitation accumulates in rainy season (ONEI 2021). Dry season does not favor the vegetative development of plants due to the scarcity of water. Daily accumulation rate increases when there are high temperatures during the day and low temperatures at night (Taiz and Zeiger 2002). These results coincide with those obtained in *A. tequilana*, in Mexico, where total reducing sugar values (23.68 to 30.80 %) were reported in dry and rainy periods (27.08 to 32.69 %). *Agave* cones have a higher fructan content in dry season, because the juice contains less water, so sugars are concentrated. In humid period, the content decreases due to the increase in the amount of water within the juice (Bautista-Justo *et al.* 2001).

Agave fructans are a complex mixture of FOS and high DP fructans (Márquez-López *et al.* 2022). Thus, high-performance liquid chromatography showed polymers of three and up to six fructose units in *A. offoyana*, although this technique did not allow the separation of molecules with DP greater than seven. Similar results were found in the head or cone of adult plants of *Agave salmiana* and *A. tequilana*, where fructans superior to four DP were identified (Pérez-López *et al.* 2021 and Regalado *et al.* 2021). In a study to search for fructans with different molecular structures, mutant plants of *A. tequilana* were recorded, which stored trisaccharide neokestose in the stem, which has greater nutritional value with respect to 1-kestose (Ángeles-Espino *et al.* 2020).

Other important metabolites are generated in the stem and leaves of Agaves, such as inulin, saponins and flavonoids, which are important for food and pharmaceutical industries (Trujillo-Ramírez *et al.* 2023). The *A. offoyana* species also produces other secondary metabolites, such as saponins, which were found in the inflorescence and leaves (Pérez *et al.* 2013 and Pérez *et al.* 2014).

It is concluded that the Cuban endemic species *A. offoyana* stores fructans as a carbon source. These carbohydrates are present in stem and leaves, but not in the roots of the plant. Adult plants and those at the beginning of flowering accumulate a greater quantity of fructans in the stem. This species stores fructans in the stem and synthesizes them in leaves, mainly in those inserted at the base of the stem and in the area closest to this organ. These carbohydrates accumulate throughout the year, although a slight increase was found in dry season. The polymer with the greatest presence in the stems of adult plants is made up of three fructose units. The plant can be used to obtain fructans with possible uses as prebiotic in human and animal health and feeding.

Acknowledgements

Thanks to all the staff of the plant biotechnology laboratory of the Faculty of Agronomy of the Agricultural University of Havana for their wisdom and professional experience. Gratitude is also expressed to Carlos González, specialist of the Tres Ceibas de Clavellina managed floristic reserve, to Andrés Hernández Rego, for the logistical support for the development of the collections, and to Lázaro Hernández, researcher of the Center for Genetic Engineering and Biotechnology, for his recommendations on the application of chromatographic techniques.

References

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, ISSN: 2304-8158. https://doi.org/10.3390/foods8090404.

Alvarado-Loza, E., Orozco-Hernández, R., Ruíz-García, I., Paredes-Ibarra, F. & Fuentes-Hernández, V. 2017. The 2% of *Agave* inulin level in the rabbit feed affect spositively the digestibility and gutmicrobia. *Abanico Veterinario,* 7(3): 55-62, ISSN: 2448-6132. http://dx.doi.org/10.21929/abavet2017.73.6.

Ángeles-Espino, A., Dimas-Estrada, H.E., Ramírez-Alvarado, D., Cruz-Rubio, J.M., Palmeros-Suárez, P.A. & Gómez-Leyva, J.F. 2020. Molecular characterization of *Agave tequilana* mutants induced with gamma radiation Co^{60} and its effect in the fructooligosaccharides accumulation. *Acta Universitaria,* 30: 1-11, ISSN: 2007-9621. http://doi.org/10.15174/au.2020.2696.

Armas, R.R.A., Martínez, G.D. & Pérez, C.E.R. 2019. Inulin-typefructans: effect on gut microbiota, obesity and satiety. *Gaceta Médica Espirituana,* 21(2): 134-145, ISSN: 1608-8921.

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, ISSN: 2590-1575. http://doi:10.1016/j.foodchem.2010.02.028.

Ayala, M.M.A., Hernández, S.D., Pinto, R.R., González, M.S.S., Bárcena, G.J.R., Hernández, M.O. & Torres, S.N. 2018. Prebiotic effect of two sources of inulin on *in vitro* growth of *Lactobacillus salivarius* and *Enterococcus faecium. Revista Mexicana de Ciencias Pecuarias,* 9(2): 346-361, ISSN: 2448-6698. http://dx.doi.org/10.22319/rmcp.v9i2.4488.

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. *Acta Universitaria,* 11(1): 33-38, ISSN: 2007-9621. https://doi.org/10.15174/au.2001.325.

Chávez-Mora, I., Sánchez-Chiprés, D., Galindo-García, J., Ayala-Valdovinos, M.A., Duifhuis-Rivera, T. & Ly-Carmenatti, J. 2019. Efecto de oligofructosa de agave en dietas de gallinas ponedoras en la producción de huevos. *Revista MVZ Córdoba,* 24(1): 7108-7112, ISSN: 0122-0268. https://doi.org/10.21897/rmvz.1522.

de Lange, C.F.M., Pluske, J., Gong, J. & Nyachoti, C.M. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. *Livestock Science*, 134(1-3): 124-134, ISSN: 1871-1413. https://doi:10.1016/j.livsci.2010.06.117.

de Zayas, A.A. 1980. *Agave cajalbanensis*: una nueva especie de Cuba occidental. *Revista del Jardín Botánico Nacional*, 1(2-3): 33-39, ISSN: 2410-5546.

Delgado, S.S.D.C., Abraham, J.M.J. & Simpson, J. 2012. *Agave tequilana* MADS genes show novel expression patterns in meristems, developing bulbils and floral organs. *Sexual Plant Reproduction,* 25(1): 11-26, ISSN: 0934-0882. https://doi.org/10.1007/s00497-011-0176-x.

Espinosa-Andrews, H., Urias-Silvas, J.E. & Morales-Hernández, N. 2021. The role of agave fructans in health and food applications: A review. *Trends in Food Science & Technology,* 114: 585-598, ISSN: 0924-2244. https://doi.org/10.1016/j.tifs.2021.06.022.

Ferrer-Serrano, C.M., Zumalacárregui-de-Cárdenas, B. & Mazorra-Mestre, M. 2023. Extracción de inulina a partir de piñas de desecho de henequeneras cubanas. *Revista Cubana de Química,* 35(2): 322-337, ISSN: 2224-5421.

Franco-Robles, E. & López, M. 2015. Implication of Fructans in Health: Immunomodulatory and Antioxidant Mechanisms. *The Scientific World Journal,* 2015: 15, ISSN: 2356-6140. http://dx.doi.org/10.1155/2015/289267.

García-Beltrán, J.A., Granado, P.L. & Bécquer, E.R. 2017. Las familias de las angiospermas de la flora de Cuba:

visión diagnóstica desde los sistemas filogenéticos. *Revista del Jardín Botánico Nacional,* 38: 65-117, ISSN: 2410-5546.

García-Curbelo, Y., Ayala, L., Bocourt, R., Albelo, N., Nuñez, O., Rodríguez, Y. & López, M.G. 2018. Agavinas como prebióticos: su influencia en el metabolismo lipídico de cerdos. *Cuban Journal of Agricultural Science,* 52(4): 395-400, ISSN: 2079-3480.

García-Curbelo, Y., López, M.G. & Bocourt, R. 2009. Fructanos en *Agave fourcroydes*, potencialidades para su utilización en la alimentación animal. *Revista Cubana de Ciencia Agrícola*, 43(2): 175-177, ISSN: 0034-7485.

García-Curbelo, Y., López, M. G., Bocourt, R., Collado, E., Albelo, N. & Nuñez, O. 2015. Structural characterization of fructans from *Agave fourcroydes* (Lem.) with potential as prebiotic. *Cuban Journal of Agricultural Science,* 49(1): 75-80, ISSN: 2079-3480.

Godínez-Hernández, C.I., Aguirre-Rivera, J.R., Juárez-Flores, B.I., Ortiz-Pérez, M.D. & Becerra-Jiménez, J. 2016. Extraction and characterization of *Agave salmiana* Otto ex Salm-Dyckfructans. *Revista Chapingo Serie Ciencias Forestales y del Ambiente,* 22(1): 59-72, ISSN: 2007-3828. https://doi:10.5154/r.rchscfa.2015.02.007.

Greuter, W. & Rankin, R. 2017. The Spermatophyta of Cuba. A Preliminary Checklist. Second, updated edition of the The Spermatophyta of Cuba with Pteridophyta added. Botanischer Garten and Botanisches Museum Berlin-Dahlem, Berlín.

Guillot, C.C. 2018. Update in prebiotics. *Revista Cubana de Pediatría,* 90(4): 648, ISSN: 1561-3119.

Hendry, G. 1993. Evolutionary origins and natural functions of fructans a climatological, biogeographic and mechanistic appraisal. *New Phytologist,* 123(1): 3-14, ISSN: 1469-8137. https://doi.org/10.1111/j.1469-8137.1993.tb04525.x.

Hernández, J. de J. 2018. El mezcal como patrimonio social: de indicaciones geográficas genéricas a denominaciones de origen regional. *EmQuestão,* 24(2): 404-433, ISSN: 1808-5245. http://dx.doi.org/10.19132/1808-5245242.404-433.

León, H. 1946. Flora de Cuba, Gimnospermas, Monocotiledóneas. Vol. I Contribuciones ocasionales del Museo de Historia Natural del Colegio La Salle No 8. La Habana. 441 pp.

Mancilla-Margalli, N.A. & López, M.G. 2006. Water-Soluble Carbohydrates and Fructan Structure Patterns from *Agave* and *Dasylirion* Species. *Journal of Agricultural and Food Chemistry,* 54(20): 7832-7839, ISSN: 1520-5118. https://doi.org/10.1021/jf060354v.

Márquez-López, R.E., Santiago-García, P.A. & López, M.G. 2022. Agave Fructans in Oaxaca's Emblematic Specimens: *Agave angustifolia* Haw. and *Agave potatorum* Zucc. *Plants,* 11(14): 1834, ISSN: 2223-7747. https://doi.org/10.3390/plants11141834.

Mellado-Mojica, E., López-Medina, T.L. & López-Pérez, M.G. 2009. Developmental Variation in *Agave tequilana* Weber var. Azul Stem Carbohydrates. *Journal of Agricultural and Food Chemistry*, 3(1): 34-39, ISSN: 1749-0626.

Meyer, V.R. 2010. Practical High-Performance Liquid Chromatography. John Wiley and Sons, Ltd, New York, USA. pp. 5-141. ISBN: 978-0-470-68218-0.

Montañez-Soto, J., Venegas-González, J., Vivar-Vera, M. & Ramos-Ramírez, E. 2011. Extracción, caracterización y cuantificación de los fructanos contenidos en la cabeza y en las hojas del *Agave tequilana* Weber var. Azul. *Bioagro,* 23(3): 199-206, ISSN: 1316-3361.

ONEI. 2021. Anuario estadístico de Cuba 2020. Medio Ambiente. Oficina Nacional de Estadística e Información. Cuba. 60 pp.

Padilla-Camberos, E., Barragán-Álvarez, C.P., Díaz-Martínez, N.E., Rathod, V. & Flores-Fernández, J.M. 2018. Effects of Agave fructans (*Agave tequilana* Weber var. azul) on body fat and serum lipids in obesity. *Plant Foods for Human Nutrition,* 73: 34-39, ISSN: 1573-9104. https://doi.org/10.1007/s11130-018-0654-5.

Pérez, A.J., Calle, J.M., Simonet, A.M., Guerra, J.O., Stochmal, A. & Macías, F.A. 2013. Bioactive steroidal saponins from *Agave offoyana* flowers. *Phytochemistry,* 95: 298-307, ISSN: 0031-9422. https://doi.org/10.1016/j.phytochem.2013.06.020.

Pérez, A.J., Simonet, A.M., Calle, J.M., Pecio, L., Guerra, J.O., Stochmal, A. & Macías, F.A. 2014. Phytotoxic steroidal saponins from *Agave offoyana* leaves. *Phytochemistry,* 105: 92-100, ISSN: 0031-9422. https://doi.org/10.1016/j.phytochem.2014.05.014.

Pérez, F. & Martínez-Laborde, J.B. 1994. Utilización y transporte de los fotoasimilados. In: Pérez García, F. y J. B. Martínez-Laborde (eds.). Introducción a la Fisiología Vegetal. Ediciones Mundi-Prensa. Madrid, España. Pp. 87-94. ISBN: 84-7114-471-9.

Pérez-López, A.V., Simpson, J., Clench, M.R., Gomez-Vargas, A.D. & Ordaz-Ortiz, J.J. 2021. Localization and Composition of Fructans in Stem and Rhizome of *Agave tequilana* Weber var. Azul. *Frontiers in Plant Science,* 11: 1-16, ISSN: 1664-462X. https://doi.org/10.3389/fpls.2020.608850.

Plascencia, A., Gutiérrez-Mora, A., Rodríguez-Domínguez, M., Castañeda-Nava, J., Gallardo-Valdez, J. & Camacho-Ruíz, R.M. 2019. Characterization of fructans extracted from *Agave mapisaga* leaves. In: Gutierrez Mora, A. (ed.). Sustainable and Integrated use of Agave. CONACYT / CIATEJ / Agared, Zapopan, Jalisco, Mexico. Pp. 101-106. ISBN: 978-607-8734-03-0.

Regalado, E., Godínez-Hernández, C.I., Aguirre Rivera, J.R., Camacho, R.M. & Juárez, B.I. 2021. Characterization of fructans from stems of *Agave salmiana* Otto ex Salm-Dyck and *A. tequilana* F.A.C. Weber at full physiological maturity. *Botanical Sciences,* 9(2): 388-397, ISSN: 2007-4476. https://doi.org/10.17129/botsci.2641.

Ritsema, T. & Smeekensm, S. 2003. Engineering fructan metabolism in plants. *Journal of Plant Physiology,* 160: 811-820, ISSN: 1618-1328. https://doi.org/10.1078/0176-1617-01029.

Romero-Jiménez, M., Castañeda-Noa, I. & de las Mercedes, L. 2015. Origen y estado actual de la flora espermatófita en áreas naturales de cayo Las Brujas, Villa Clara. *Revista del Jardín Botánico Nacional*, 36: 31-46, ISSN: 2410-5546.

Taiz, L. & Zeiger, E. 2002. Respiration and Lipid Metabolism. In: Taiz, L. y E. Zeiger (eds.). Plant physiology. Sinauer Associates Inc. Sunderland, Massachusetts, USA. Pp. 245-247. ISBN: 0-87893-823-0.

Trujillo, T.L.E., Gómez, R.R., Banguela, C.A., Soto, R.M., Arrieta, S.J.G. & Hernández, G.L. 2004. Catalytical properties of N-glycosylated *Gluconacetobacterdiazotrophicus* levansucrase produced in yeast. *Electronic Journal of Biotechnology,* 7(2): 115-123, ISSN: 0717-3458. https://doi.org/10.4067/S0717-34582004000200005.

Trujillo-Ramírez, D., Bustos-Vázquez, M.G., Martínez-Velasco, A. & Torres-de los Santos, R. 2023. Integral use of Henequen (*Agave fourcroydes*): applications and trends-a review. *Tropical and Subtropical Agroecosystems,* 26(2): 1-18, ISSN: 1870-0462. http://doi.org/10.56369/tsaes.4619.

Van den Ende, W. 2013. Multifunctional fructans and raffinose family oligosaccharides. *Frontiers in Plant Science*, 4: 1-11, ISSN: 1664-462X. https://doi.org/10.3389/fpls.2013.00247.

Verma, D.K., Patel, A.R., Thakur, M., Singh, S., Tripathy, S., Srivastav, P.P. & 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, ISSN: 1096-0481. https://doi.org/10.1016/j.jfca.2021.103884.

Vijn, I., van Dijken, A., Lüscher, M., Bos, A., Smeets, E., Weisbeek, P., Wiemken, A. & Smeekens, S. 1998. Cloning of sucrose: sucrose 1-fructosyltransferase from onion and synthesis of structurally defined fructan molecules from sucrose. *Plant Physiology,* 117(4): 1507-1513, ISSN: 1532-2548. https://doi.org/10.1104/pp.117.4.1507.

Wack, M. & Blaschek, W. 2006. Determination of the structure and degree of polymerisation of fructans from *Echinacea purpurea* roots. *Carbohydrate Research,* 341(9): 1147-1153, ISSN: 1873-426X. https://doi.org/10.1016/j.carres.2006.03.034.

Wang, L., Yang, H., Huang, H., Zhang, C., Zuo, H.X., Xu, P., Niu, Y.M. & Wu, S.S. 2019. Inulin-type fructans supplementation improves glycemic control for the prediabetes and type 2 diabetes populations: results from a GRADE-assessed systematic review and dose-response meta-analysis of 33 randomized controlled trials. *Journal of Translational Medicine,* 17(410): 1-19, ISSN: 1479-5876. https://doi.org/10.1186/s12967-019-02159-0.

Waterhouse, A.L. & Chatterton, N.J. 1993. Glossary of fructan terms. In: Michio Suzuki, N. y J. Chatterton (eds.). Science and technology of fructans. CRC Press. Boca Raton, USA. Pp. 1-7. ISBN: 0-8493-5111-1.