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**ORIGINAL ARTICLE**

# Fractional Extraction of the Fructans Contained in the *Agave tequilana* Weber blue head based on their average degree of Polymerization

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**ABSTRACT**
*Fructans type inulin and fructooligosaccharides are between the main functional ingredients of foods. In our body, inulin acts as soluble dietary fiber, while fructooligosaccharides play the role of prebiotics agents; ie "promoters of life". Based on the different solubility with respect on their molecular size, the fructans contained in the Agave tequilana Weber blue juicewere separated into four fractions using ethanol concentrations from 20, 40, 60 and 80% w/w. Then the chemical characterization of the four fractions obtained were conducted by determining the content of direct and total reducing sugars, as well as its glucose and fructose content; and from these data, the average degree of polymerization (GPP) and the average molecular weight (PMP) of fructans that integrated each fraction was estimated.In chronological order, the fructans obtained in each one of the fractions was (18.09 + 0.78%, 24.34 + 1.08, 46.05 + 1.30, and 10.77 + 0.44) %w/w, respectively; thus achieving the extraction of the 98.68% of total fructans present in the agavejuice. Each of these fractions were integrated by fructans with different average degree polymerization, which were (27.80 ± 0.83; 18.10 ± 0.7; 10.49 ± 0.4; 6.61 ± 0.30) units and therefore, different average molecular weight (4504 ± 134; 2933 ± 113; 1699 ± 65; 1070 ± 49) g/mol, respectively.*
***Key words:*** *Fructans, agave, inulin, fructooligosaccharides, functional ingredients.*

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## INTRODUCTION

Fructans are fructose polymers, they are the main reserve carbohydrates in some families of the plant kingdom and they arealso produced by microorganisms [1]. They stored in roots, tubers, rhizomes, and inflorescence and immature fruits and are synthesized from sucrose; they containing only one glucose residue at the reducing end of the molecule, have a different degree of polymerization, molecular weight and structure(Ritsema and Smeekens, 2003). According to their structure, fructans are classified into inulins, when the fructose molecules are linked predominantly by $\beta$(2,1) bonds, levans, when in them predominate $\beta$(2.6) bonds; however they can present a variety of complex structures through branches in the point $\beta$(2,6) of the inulin, or in the point $\beta$(2,1) of the levans; these structures are known as "branched fructan". Finally as resulting from the hydrolysis of the above fructans are fructooligosaccharides (FOS), low molecular weight fructans whose degree of polymerization is less than 10 fructose units [3].
Structural differences of the fructans and its average degree of polymerization (ADP), depend on the type and age of the botanical source from which they are obtained and of the conditions of extraction and purification. These differences determines its physical and chemical characteristics and thus their functional properties and with this, its applications in the food industry and pharmaceuticals [4].Betwen

the main functional properties of fructans are its wetting ability, emulsifier, thickener and gelling as well as their ability to lower the freezing point.In addition, its low caloric intake and its neutral color, smell and taste, make of these products the ideal ingredients to replace fats in the development of "light" products [5].On the other hand, since digestive enzymes do not hydrolyze fructans that we ingest through food, these play the role of prebiotic ingredient and soluble dietary fiber, and they promote bioavailability of calcium and magnesium ions [6-9].

Jerusalem artichoke (*Helianthus tuberosus*) tubers and the chicory roots (*Cichorium intybus*) they are between the main potential sources for industrial production of fructans, both with a fructans contain around 80% dry basis.None of these plants are grown intensively in our country, but if have extensively cultivated agave, mainly *Agave tequilana* Weber blue, whose head represents the commercial portion of the plant, because in it carbohydrates are stored that is the raw material for the process of producing "tequila"; mexican alcoholic beverage with higher consumption in the world [10,11].

It is reported that the carbohydrates contained in the *Agave tequilana* Weber blue head are mixtures comprised of inulin-type fructans, neoinulin, graminans and branched neofructans; the latter are highly branched complex molecules in the points β(2-1) and β(2-6), to which has been given the name agavinas[12].It has also been reported that the fructans content in the *Agave tequilana* Weber blue head, are similar to that contained in the tubers of Jerusalem artichoke (*Helianthus tuberosus*) and in chicory roots (*Cichorium intybus*); moreover, these fructans are composed of a mixture of polymers with different degrees of polymerization [13].

The objective of this research is to develop a process for obtaining differential fructans contained in the juice of Weber Blue Agave tequilana, based on their different solubility in ethanol with respect to their molecule size.

## MATERIALS AND METODOS

Three *Agave tequilana* Weber azul heads were harvested of a crop of eight years old, which is located in Atotonilco el Alto, Jalisco. México. The agave heads were transported to the CIIDIR's Laboratory and maintained at 4°C until further preparation and study.

### Raw material conditioning

Before slicing the agave heads their physical characteristics were determined initially, they are their average diameter and height, and their average weight. Later the agave heads were cutting into pieces of 3x3x10 cm approximately, using an electric saw SC-807 model (Surtex, USA). Then the agave pieces were stored at -20°C in a chest freezer Torrey, CHTC-7a model (Torrey, México) until further use.

### Percentage chemical analysis of the agave head

In order to establish the yields in the extraction process of the fructans contained in the agave head, initially the percentage chemical analysis of the agave head was performed. For this the moisture content, ash, fat, protein, crude fiber and total carbohydrate was determined by applying the methodology described in the Official Methods of Analysis [14].

### Getting agave juice

To get the agave juice, the agave head pieces were thawed at room temperature (30 °C) initially and then they were fed in to a juice extractor Robot-Coupe model J80-Ultra (Equipping, Mexico) in order to get the agave juice.Subsequently, the juice was clarified by centrifugation at 5000 rpm for 15 min in a Beckman centrifuge model J2-HS and serial number JA92L23 (Beckman, Germany). The clarified juice was weighed and packaged in PET-containers properly labeled and stored at -20°C in a chest freezer Torrey, CHTC-7a model (Torrey, México), until further analysis.

### Chemical characterization of agave juice

The content of moisture, ash, fat, protein, crude fiber and total carbohydrates in agave juice, were determined according to the Official Methods of Analysis [14]. The soluble solids content expressed as °Brix was determined with a manual refractometer (American Optical Co., USA), while the juice pH was determined with a Criso Digilab digital potentiometer 517 model (Crison, Spain). The water activity was determined with a Thermoconstanter equipment, model RTD33 (Novasina, Switzerland). Finally, the Direct Reducing Sugars (DRS) and Total Reducing Sugars (TRS) were determined [15].

### Differential precipitation of the fructans contained in the agave juice.

The extraction of the fructans present in agave juice was based on the different solubility in water of these polymers, in relation to its molecular weight. In general, the water solubility of the fructans gradually decreases with increasing its molecular weight [16]. Industrial grade ethyl alcohol (95.15% w/w) (Spirits Tamazula, Mexico) was used in the differential precipitation process of the fructans contained in agave juice. Inicially fructans were precipitated at an ethanol concentration of 20% w/w and the obtained precipitate was pelleted for 15 minutes, then decanted, lyophilized, packed and finally labeled for later use. A second, third and fourth precipitation of the fructans were performed by adjusting the supernatant ethanol concentration at 40, 60 and 80% w/w, respectively (Figure 1).

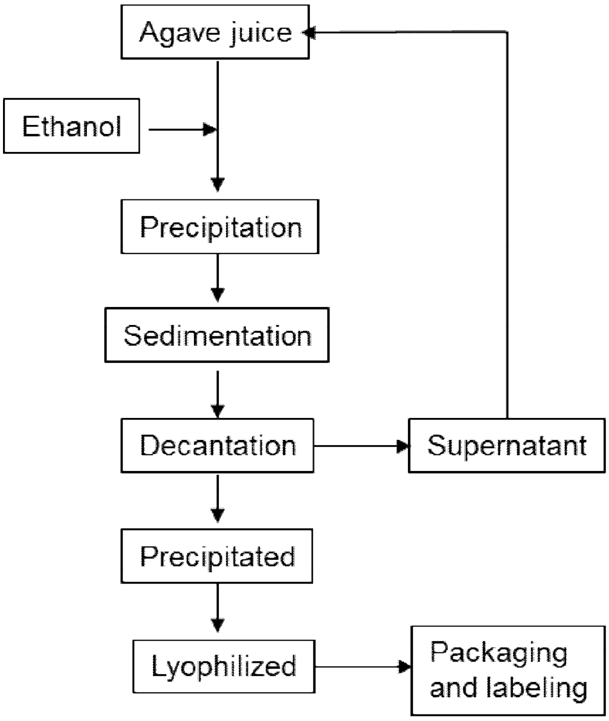


Figure 1. Differential precipitation with ethanol of fructans contained in agave juice

**Fructans chemical characterization**
The moisture and ash content [14], Direct Reducing Sugars (DRS) and Total Reducing Sugars (TRS) [15], Glucose [17] and fructose [18] were determined in each one of the fructan fractions obtained by differential precipitation with ethanol, from the agave juice.

**Average degree of polymerization (ADP)**
The average degree of polymerization of the fructans contained in each of the precipitated fractions was determined according to the proposed method by Greenwood (1976) [19], which determines the average degree of polymerization (ADP) as the relationship between the content of Total Reducing Sugars (TRS) and the Direct Reducing Sugars (DRS) (Eq. 1)

$$ADP = ART / ARD \ldots \text{(Ec. 1)}$$

**The average molecular weight**
Once known the average degree of polymerization (ADP) of the fructans that integrate the different extracts agave juice, and knowing that the molecular weight of each of the glucose residues and fructose that integrate the polymer is 162 g/mol[20], the average molecular weight (AMW) of the fructans is determined by the following expression (Eq. 2).

$$AMW = CPM \times 162 \ldots \text{(Eq. 2)}$$

**Statistical analysis**
All determinations were made in triplicate; the results presented here represent the average of the set ± the series standard deviation.A one-way analysis of variance was conducted and test of Tukey's multiple comparison was done to determine statistical significance ($p>0.05$) between chemical characteristics of fructans obtained at the different concentrations of ethanol used in its precipitation.

## RESULTS AND DISCUSSION

The physical characteristics of the *Agave tequilana* Weber azul heads used in this study were: average diameter of 53 ± 2.5 cm, average height of 62 ± 3 cm, and average weight of 83 ± 5.2 kg. It has been seen that these characteristics depend primarily on the age and condition of agave cultivation [13, 21].Table 1 shows the percentage chemical composition of the *Agave tequilana* Weber azul head. Moisture is the main constituent of the agave head and depends on factors such as plant age and the season crop [22].

 

Table 1: Percentage chemical analysis of the agave head

| Parameter | Content ($\%_{DB}$) |
|---|---|
| Moisture | 70.80 ± 1.20 |
| Ash | 4.11 ± 0.21 |
| Fat | 0.40 ± 0.02 |
| Protein | 2.25 ± 0.10 |
| Crude fiber | 13.65 ± 0.40 |
| Total carbohydrates | 79.59 ± 1.12 |

In dry basis, total carbohydrates are the major constituent of the solids present in the agave head. Its content is similar to that found in Jerusalem artichoke (*Helianthus tuberosus*) and in the chicory roots (*Cichorium intybus* L.), main potential sources of fructans proposals for the industrial production of high fructose syrups and crystalline fructose the first [23], and for the obtention of functional ingredients like fructooligosacharides and inulin the second.

The second most important constituent of the agave head is the crude fiber, which comprises cellulose, hemicellulose and lignin, mainly and, because our body lacks of digestive enzymes to hydrolyze these compounds, the crude fiber is also known as insoluble dietary fiber, since all these compounds are insoluble in water [24].

**Obtention and chemical characterization of the agave juice**

The agave juice yield obtained was 65.7±2.5 $\%_{w/w}$ on a wet basis. This yield was higher than those obtained by others researchers [16], probably due to crop age at harvest. The remaining 34.3 ± 1.2 $\%_{w/w}$ correspond with the residual fiber or agave bagasse. The agave juice obtained presented a soluble solids content of 36.10 ± 0.10 °Brix, pH of 5.24 ± 0.03 units and a water activity of 0.985 ± 0.01, which makes it a highly perishable product, reason why once obtained it was frozen at -20°C until further use. Table 2 shows the agave juice chemical characterization.

The glucose and fructose in "free state" are the principal direct reducing sugars present in the agave juice, which in turn conform the total reducing sugars (TRS) contained therein. On dry basis, the fructans content in the agave juice was 95 ± 0.3%. The difference between total reducing sugars content, less direct reducing sugars content, correspond to the fructans integrated by fructooligosaccharides e inulin [20], so that their content in agave juice, is equal to 30.40$\%_{wb}$ in wet basis (33.25 - 2.85)$\%_{wb}$.

Figure 2 shows the quantities of fructans extracted from the agave juice under the different concentrations of ethanol used in the precipitation process. Most fructans extracted (140 ± 5.4 g) was obtained at an ethanol concentration of 60%, while a smaller fructans quantity (31 ± 1.3 g) was obtained at an ethanol concentration of 80%. At ethanol concentrations of 20% and 40% were obtained 55 ± 2.5g and 74 ± 3.2 g of fructans, respectively. A total of 300 g of fructans were obtained from 1,000 g of agave juice.

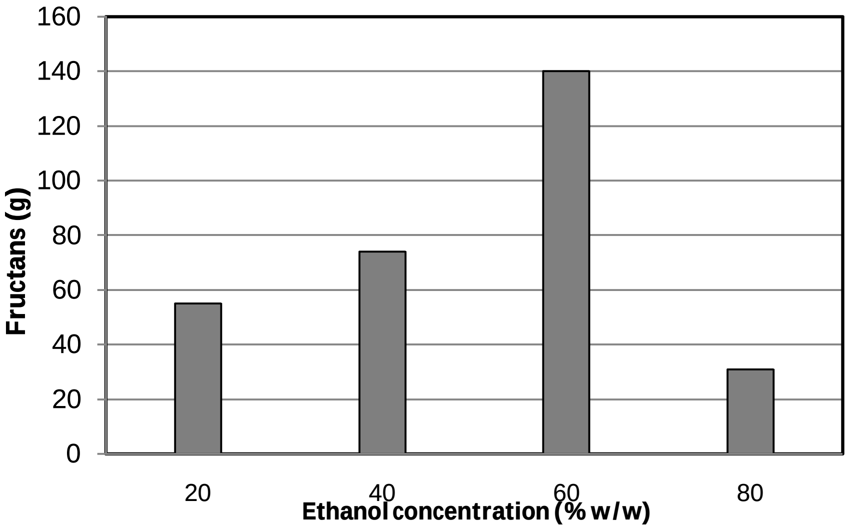


Figure 2. Fructans extraction at different ethanol concentrations

 

Assuming that the fructans total content in the agave juice is of 30.40%, which means that one kilogram of agave juice content 304 g of fructans, so after four precipitation, the total quantity of fructans extracted was of 98.68% total (Table 3). Castellanos et al., (2012) [16], have reported similar results to these.

It is well known that water solubility of the fructans depends on the temperature of the solvent and the size or length of the hydrocarbon chain. Fructans with high degree of polymerization (ADP>20) are practically insoluble in water at low temperatures (T<60 °C) while that fructans with a low degree of polymerization (ADP<10), are highly soluble in water [3].

The precipitation of the fructans present in agave juice is directly proportional to the concentration of ethanol used in the process (Figure 2). Under operating conditions used in the process of differential precipitation of the fructans contained in the agave juice, was possible to obtain four fructans fractions using an ethanol gradient of 20 to 80% w / w, thus achieving the precipitation of 98.68% of all fructans contained in agave juice.

## Fructans chemical characterization

The chemical parameters, which characterize each of the fructan fractions obtained by differential ethanol precipitation from fructan content in the agave juice, are shown in Table 4.The low moisture content and low water activity ensures the preservation over long storage times at room temperature, with no deterioration by microorganisms, from each one of the fructan fractions obtained.

The content of total reducing sugars was nearly 100% and there was not significant statistical difference ($p < 0.05$) among the different fractions obtained fructans. Both the contents of DRS as glucose increased, as increases the ethanol concentration to which the fructans contained in agave juice were precipitated.The opposite occurs with the fructose concentration, which decreases as the increases ethanol concentration to which were precipitated the fructans contained in agave juice.

Since fructans are a mixture of fructose polymers with differing chain length, each of which has a terminal glucose unit at the reducing end of the molecule[25], this means that in a fructan of smaller size, its glucose proportion is higher and less its fructose proportion. Contrary as fructan size increases, the fructose ratio increase while glucose ratio decreases; as demonstrated by the results obtained here. The fructose/glucose ratio on the four fructan fractions obtained at ethanol concentrations of 20, 40, 60 and 80% w/w, were 26.8, 14.2, 9.5 and 5.5, respectively. That is, the higher the concentration of ethanol used for the fructans precipitation, decreases the fructose/glucose ratio on the fructan obtained. Furthermore, the obtained fraction to a lower ethanol concentration is comprised of fructans with a higher degree of polymerization, and as the concentration of ethanol increases, smaller fructans precipitate; that is, of lower average degree of polymerization and thus of lower average molecular weight. The low glucose content and high fructose content in the *Agave tequilana* Weber azul fructans, make this botanical source a promising alternative for the industrial production of high fructose syrups, sweetener widely used in food industry and pharmaceutical current.

Table 2: Agave juice chemical characterization

| Parameter | Content ($\%_{wb}$) |
|---|---|
| Moisture | 65.00 ± 0.07 |
| Ash | 0.63 ± 0.01 |
| ARD | 2.85 ± 0.05 |
| Glucosa | 0.79 ± 0.02 |
| Fructosa | 2.06 ± 0.05 |
| ART | 33.25 ± 0.10 |
| pH | 5.24 ± 0.03 |
| °Brix | 36.10 ± 0.10 |
| $a_w$ | 0.985 ± 0.01 |

Table 3. Yields of the extraction process of the fructans contained in agave juice by differential precipitation with ethanol.

| Etanol concentration (% w/w) | Fructans(g) | Yield based on total | Yield based on content |
|---|---|---|---|
| 20 | 55 ± 2.5[a] | 18.33 | 18.09 |
| 40 | 74 ± 3.2[b] | 24.67 | 24.34 |
| 60 | 140 ± 5.4[c] | 46.67 | 46.05 |
| 80 | 31 ± 1.3[d] | 10.33 | 10.20 |
| Total | 300 ± 12.4 | 100.00 | 98.68 |

 

Table4: Chemical characterization of the fructans obtained at different ethanol concentrations from agave juice

| Parameter | Ethanol concentration ($\%_{w/w}$) | | | |
|---|---|---|---|---|
| | 20 | 40 | 60 | 80 |
| Moisture ($\%_{wb}$) | 2.32 ± 0.03[a] | 2.85 ± 0.02[b] | 2.72 ± 0.02[c] | 3.05 ± 0.05[d] |
| Water activity | 0.22±0.002[a] | 0.27±0.002[b] | 0.24±0.002[c] | 0.30±0.002[d] |
| DRS ($\%_{db}$) | 3.6 ± 0.1[a] | 5.5 ± 0.1[b] | 9.5 ± 0.2[c] | 15.1 ± 0.2[d] |
| TRS ($\%_{db}$) | 99.85 ± 0.2[a] | 99.57 ± 0.2[a] | 99.64 ± 0.2[a] | 99.77 ± 0.2[a] |
| Glucose ($\%_{db}$) | 3.6 ± 0.02[a] | 6.6 ± 0.10[b] | 9.5 ± 0.15[c] | 15.3 ± 0.20[d] |
| Fructose ($\%_{db}$) | 96.4 ± 0.21[a] | 93.4 ± 0.25[b] | 90.5 ± 0.28[c] | 84.7 ± 0.23[d] |
| ADP (residues) | 27.74 ± 0.9[a] | 18.10 ± 0.7[b] | 10.49 ± 0.4[c] | 6.61 ± 0.3[d] |
| AMW (g/mol) | 4493 ± 146[a] | 2933 ± 113[b] | 1699 ± 65[c] | 1070 ± 49[d] |

Different letters in same row indicate statistically significant differences (p>0.05).

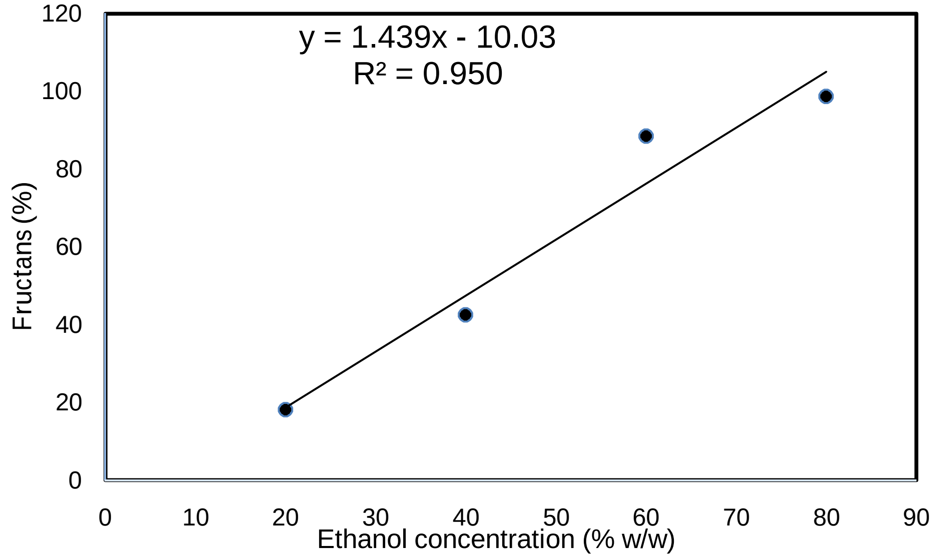


Figure 3. Progress in the precipitation of the agave juice fructans at different ethanol concentrations

## CONCLUSIONS

The high fructans content on the *Agave tequilana* Weber azul head and the diversity in the molecules size, possible to consider this botanical source, as a potential alternative for the industrial production of fructans with different degree of polymerization and thus with different properties physicochemical and functional.

By the technique of differential precipitation with ethanol, it was possible to obtain four fractions of fructans, each of which presented different average degree of polymerization and average molecular weight, and therefore with different physicochemical properties and functional.

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