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Full Length Research Paper

Enzymatic production of high fructose syrup from *Agave tequilana* fructans and its physicochemical characterization

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The conditions for producing high fructose syrup from the fructans contained in the head of the *Agave tequilana* Weber var. blue were determined and their physicochemical properties were compared with those of commercial corn syrup (Frudex-55®). Both syrups behave as Newtonian fluids and showed no significant differences ($p < 0.05$) in density, moisture, water activity, total solids, total reducing sugars and direct reducing sugars. It takes 4.4 kg of fresh agave head to obtain 1 kg of syrup with 70°Brix and 87.92 ± 1.28% fructose. As a result of its highest fructose-glucose ratio, agave syrup has lower viscosity and surface tension than the corn syrup. Due to its high content of fructans, the head of the *A. tequilana* Weber var. blue is a promising raw material for the industrial production of high fructose syrups.

Key words: Syrup, fructose, fructans, *Agave tequilana*, inulin, inulinase.

INTRODUCTION

In the American continent, honey and mead were the unique sweeteners available to the natives to sweeten their food during pre-Hispanic times. After that, sugar-cane was introduced as a crop which marked the beginning of the domain of sucrose in the sweetener market. Today sucrose faces strong pressures that threaten the supremacy that has held for nearly 500 years of leadership in the national and global market for sweeteners (Hernandez et al., 2008). The events that have contributed to the decrease of sucrose consumption are: changes in eating habits, demand of special food for diabetics, the discovery and chemical synthesis of non-caloric sweeteners, commercial production of high intensity natural sweeteners like high fructose syrups (HFS) and, the functionality, availability and price of the available sweeteners (Vuilleumier, 1993).

Today, HFS have become the most demanded sweeteners by the global food and pharmaceutical industry, which is due to its functional and technological advantages over sucrose, including: increased sweetness, increased water and ethanol solubility, increased hygroscopic capacity, less tendency to crystallize, lower cost based on their sweetness, the lower viscosity of solutions, ease of storage, higher osmotic pressure, less cariogenic power, its bioavailability is independent of the hormone insulin, presents the Maillard reactions at lower temperatures, and its sweetness increases with decreasing temperature (Fleming and GrootWassink, 1979).

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Abbreviations: HFS, High fructose syrup; **HFCS,** high fructose corn syrup; **TRS,** total reducing sugars; **DRS,** direct reducing sugars; **G,** glucose; **F,** fructose; **DB,** dry basis; **AW,** water activity.

High fructose syrups are obtained from a variety of starchy raw materials such as maize, potato, rice, tapioca, wheat and cassava, which depends on the sucrose production and the price of agricultural raw materials of each country (Jing et al., 2011). This process uses three different amylolytic enzymes (α-amylase, glucoamylase and pullulanase) to achieve complete conversion of starch to glucose, which is then converted to fructose through glucose isomerase enzyme; syrup corn with 42% fructose is obtained and the residue is mainly glucose and minor quantities of oligosaccharides. That is why to obtain syrups with higher fructose content, it is necessary to perform multiple stages of separation, isomerization and concentration, and this increases the cost of production of these syrups (Palazzi and Converti, 2001; Zhang et al., 2004). Nowadays, research about new plants that produce and accumulate fructans as more viable sources for industrial production of these syrups is in the increment (Bajpai and Bajpai, 1991; Wenling et al., 1999).

Inulin is a major fructans that exist in nature; it is a polymer composed of linear chains of fructose linked by glycosidic β (2.1), contains only one glucose residue at the reducing end of the molecule, its degree of polymerization is variable and can reach up to 60 fructose units, which depends on the botanical source, age of plant and crop agronomic conditions among others (Hendry, 1993). Inulin is among the major reserve carbohydrate in many plants, among them are: the tubers of Jerusalem artichoke (*Helianthus tuberosus*), the roots of Chicory (*Chicory intybus*) and tubers of dahlia (*Dahlia variabilis*) (Kim et al., 1997; Hellwege et al., 1998).

Another potential source of fructans are the plants of the *Agavaceae* family. The *Agave tequilana* Weber var. blue, succulents plants composed of two main parts: the stem and leaves. The leaves represent 45 to 50% of the total weight of the plant and they are the agricultural crop residues, the remaining 50 to 55% consists of the stem and leaf bases that are bind to it; section known too as "head "or" pineapple ", and its weight ranges between 30 to 70 kg. The content of the total reducing sugars (ART) in the head is between 16 to 28%, while in the bases of mature leaves, is between 13.1 to 16% (Iñiguez et al., 2001). The degree of polymerization of fructans contained in the head is between 3 to 29 units and it has been shown that these fructans are composed of links β (2-1) mainly, but they also contains links β(2-6), indicating that these fructans are not of inulin type, therefore they have been called “agavinas” (Mancilla and Lopez, 2006).

The head of the *A. tequilana* Weber var. blue is the raw material in the process of making tequila, alcoholic beverage widely accepted nationally and globally (Lamas et al., 2004; Narvaez and Sanchez, 2009). This plant is grown intensively in the area within the Denomination of Origin of Tequila, which integrates an area of 11,700,000 hectares of which only 120,000 hectares are intended to the cultivation of this plant. Currently, the demand for agave heads for tequila production is over one million tons annually, but due to lack of good planning and control in the system of production, often, agave production is greater than demand, resulting in annual losses over to 200,000 tons of agave heads (CRT, 2011).

Moreover, as in many other countries, in Mexico there is a growing consumption of high fructose syrups. The average national consumption of these sweeteners in the last five years was more than a million tons annually, of which 724 000 tones were produced in our country, the rest (354 thousand tons) were imported to meet domestic demand (INEGI, 2011). Importantly, these syrups are made from corn starch; raw material which is also imported because Mexico is deficient in its production.

The objective of this research was to develop an enzymatic method for the production of high fructose syrups from fructans contained in *A. tequilana* Weber var. blue head seeking to take advantage of the surplus crop of this plant and thus, reduce or eliminate the import volumes of high fructose corn syrups and the maize required for its production.

MATERIALS AND METHODS

Ten *A. tequilana* Weber var. blue plants eight years old were purchased in Atotonilco el Alto, Jalisco, Mexico. Frudex 55®: high fructose corn syrup with 70% solids with 55% fructose and 45% glucose was from Arancia, SA de CV, Mexico; exo-inulinase from *A. niger* (EC 3.2.1.80) powder with specific activity of 1100 units / mg solid, endo-inulinase from *A. niger* (EC 3.2.1.7) powder with 230 units / mg solid of specific activity, exo- and endo-inulinases mixture in 5:1 ratio, respectively, with specific activity of 955 units / mg solid were from Megazyme, Ireland. One unit of enzymatic activity is defined as the amount of enzyme required to release one micromole of fructose per minute under standard assay conditions.

Raw material conditioning

Ten *A. tequilana* Weber azul plants were randomly selected. Its leaves were separated from the head and both fractions were weighed to determine their contribution to the total weight of the plants. The heads were cut into slices of 10 mm thick using an electric saw SC-807 (Surtex, USA) and then were dried in a tray dryer (Mapisa International, S.A. de C.V., Mexico) at 60°C for five days to obtain constant weight. The dried material was weighed again to determine moisture content and then the material was grounded in a micro pulverized mill Micron K-1 (Micropulverizadores, S.A. de C.V. México), to a fine powder passing through a 200 mesh. The flour obtained was packed in an airtight container properly labeled and stored in a cool and dry place.

Proximate chemical composition

The proximate chemical composition of the flour obtained was performed according to the methodology described in AOAC (1997). This analysis included the determination of moisture (925.10), ash (923.03), fat (920.39), protein (920.87), total reducing sugars (Miller, 1959) and crude fiber was calculated by difference.

Extraction of fructans

Initially, it was made a flour-water maceration using a 1/6 (w/w) ratio. The mixture was kept at a temperature of 70°C for 30 min with constant stirring (Montañez et al., unpublished). At the end of time, the mixture was centrifuged at 11,500 gravity units/10 min, and the extract was concentrated to 20%$_{DB}$ of the total reducing sugars (TRS) (Miller, 1959), using a rotary evaporator Flawil SG-CH-9230 model (Büchi, Switzerland) at 60°C.

Determination of hydrolysis time

Hydrolysis of fructans was carried out using each one of the three enzymatic preparations available; exo-inulinase, endo-inulinase and the mixing of both in 5:1 ratio, respectively. Prior to hydrolysis, the pH of the extracts was adjusted to 4.5 units by addition of NaOH 1 M, since at this pH, both enzymes exhibited maximum activity. In three 250 ml Erlenmeyer flasks, 100 ml of the extract were placed with 20%$_{DB}$ of TRS, and the flasks were placed in water bath at 50 ±1°C with constant stirring. In each flask, 2500 units of enzymatic activity were added, of each one of the different enzymatic preparation used. Every 30 min, samples were taken to determine the content of direct reducing sugar (DRS) (Miller, 1959), and thus, the minimum time required to hydrolyze 100% of the fructans present in the extract was determine, which corresponds to the moment that the content of DRS is equal to TRS.

Preparation of high fructose syrups

Once the hydrolysis conditions of the fructans were determined, the extract of 1 kg of flour was obtained, after which it was concentrated to 20% of TRS and its pH was adjusted to 4.5 units. It was then hydrolyzed with the mixing of both inulinases at 50 ± 1°C. Every 30 min, samples were taken and their DRS (Miller, 1959) content was determined to make sure the total hydrolysis of fructans was reached. The hydrolysis was then clarified by passing through a glass column 2.5 cm in diameter and 30 cm high and packed with activated carbon. Then, the syrup was concentrated to 70% of TRS (Miller, 1959) in a rotary evaporator model CH-9230 Flawil / SG (Büchi, Switzerland) at 50°C.

Overall process efficiency

The efficiency of each stage of preparation of the syrup was determined based on the TRS content in the head of agave. With these data, the overall efficiency of the process established under the conditions of operation was estimated and the amount of high fructose syrup with 70°Brix which is obtained from 1 kg of flour from agave head was determined.

Chemical characterization

The high fructose syrup obtained was analyzed to evaluate some of their important chemical and physical properties. At the same time, a commercial high fructose corn syrup was evaluated (Frudex 55®). The chemical parameters evaluated were: moisture and ash contents (AOAC, 1997), DRS and TRS (Miller, 1959), glucose (G) and fructose (F) (Ting, 1956).

Physical characterization

The physical parameters evaluated were: color, soluble solids (°Brix), water activity (aw), surface tension, rheology and viscosity.

The density was determined by a pycnometer Brixco of 25ml and a temperature of 25°C. The soluble solids (°Brix) were determined with an ABBE Refractometer (American Optical Co., USA) to 25°C. The refractometer was adjusted prior to 0°Brix with distilled and deionized water and tempered at 25°C. Subsequently, the syrup was placed in the camera of the refractometer and its soluble solids content (°Brix) was determined.

Water activity (aw) was determined at a temperature of 25°C with a thermoconstanter model RTD33 (Novasina, Switzerland).

Surface tension was determined at a temperature of 25°C with a Surface Tensiometer model 2141 (Analyte, Australia).

Color was determined using a Hunter Lab spectrophotometer with an illuminant D50 and D65 to 10° of the observer. It used the CIE-Lab system which involves the parameters L, a*, and b*. The parameter L refers to the brightness of the sample, that is, the degree to which the sample is able to reflect light. Positive values of the parameter a* indicated colorations toward the brownish tone, while negative values indicated colorations toward the green tone. On the other hand, positive values of parameter b* indicated yellow colorations and negative values of this parameter indicated blue colorations.

The rheological behavior was determined using a Haake RV2 viscometer Rotovisco (Haake, Germany) using the MV1 sensor, and a shear rate of 1000 s-1 at 25°C. In this way, the type of fluid for the syrups was determined and by applying the model of Newton's law also its viscosity was determined.

Statistical analysis

All determinations were made in triplicate; these results presented are the average of these three determinations ± standard deviation. Also, an analysis of variance (ANOVA) was conducted and the Duncan's test was used to find statistically significant differences at a confidence level of 95% in the different treatments using SPSS statistical software 12.0.

RESULTS AND DISCUSSION

The fresh weight of the plant *A. tequilana* Weber var. blue of eight years of age was between 79.2 and 111 kg, with an average weight of 95.1 kg. The head contributes 52.5 ± 3.7% of the total weight of the agave plant, while the remaining 47.5 ± 4.7% was comprised of leaves that are agricultural crop residues and currently not being used (Narvaez and Sanchez, 2009). Iñiguez et al. (2001) reported a ratio of 1.3 between the fresh weight of the head and the fresh weight of leaves of the agave, and this is slightly higher than the ratio found by us, which could be due to the different ages of the selected agave plants.

The moisture content on the agave head ranged 66.9 to 75.3%, which depends on the age of the plant and the cutoff date between others (Iñiguez et al., 2001). In dry basis, total reducing sugars (TRS) represent the main constituent of the agave head (Table 1), its average content was 79.7 ± 2.6% and is similar to that contained in the tubers of Jerusalem artichoke (*Helianthus tuberosus*); main potential source of fructans (Hellwege et al., 1998). After the TRS, the crude fiber was the second more important component of the agave head; in dry basis its average content was 13.8 ± 1.1%. This fraction is made up of indigestible carbohydrates such as cellulose, hemicellulose and lignin, which are also considered components of insoluble dietary fiber

Table 1. Chemical analysis of the head of *Agave tequilana* Weber var. blue.

Parameter	Content (%)	
	Wet basis	Dry basis
Moisture	71.1 ± 1.4	-------
Ash	1.16 ± 0.09	4.00 ± 0.31
Lipids	0.10 ± 0.01	0.4 ± 0.02
Proteins	0.62 ± 0.04	2.2 ± 0.14
Crude fiber	4.00 ± 0.19	13.8 ± 1.1
TRS	23.02 ± 0.74	79.7 ± 2.6

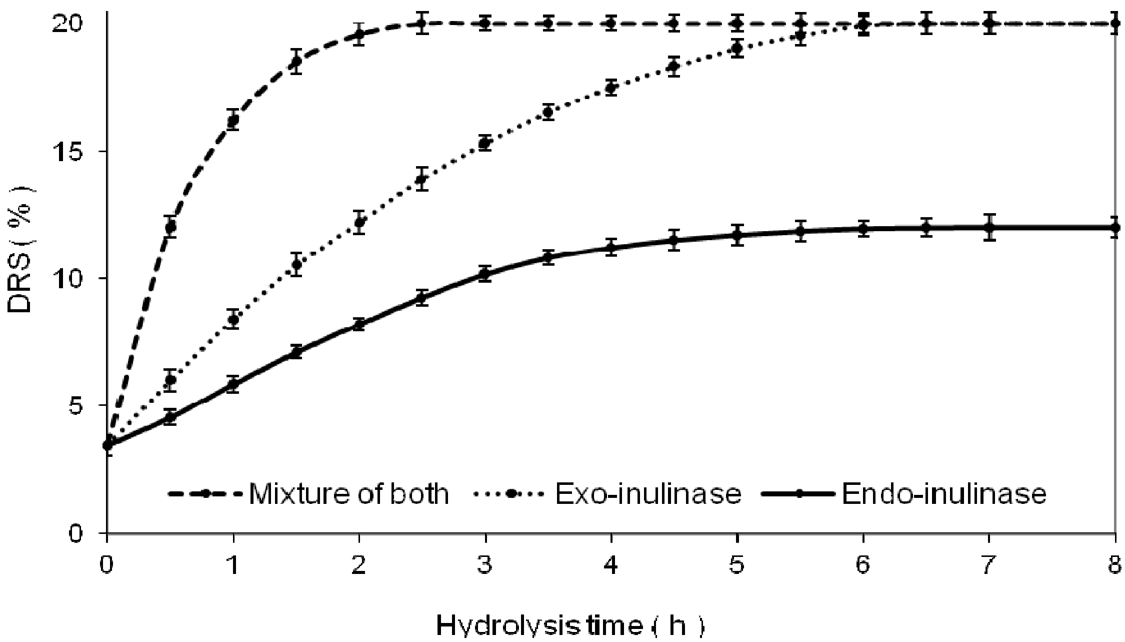

Figure 1. Kinetics of enzymatic hydrolysis of agave extract with different enzyme preparations. Conditions: TRS = 20%, pH = 4.5, T = 50°C and (E) = 25 U/ml.

(Flamm et al., 2001).

Determination of enzymatic hydrolysis time

The kinetics of hydrolysis of fructans is different with each of the enzymatic preparations used for this purpose (Figure 1). When the hydrolysis was carried out only with the endo-inulinase enzyme, the maximum degree of hydrolysis was obtained in a time of 3 h and content of DRS in the extract hydrolyzed was 10.84 ± 0.4% (54.2 ± 2% of TRS). There were no statistically significant differences ($p < 0.05$) in the content of DRS at higher hydrolysis times. On the other hand, when the enzyme exo-inulinase was used only, it was necessary for all the fructans in DRS to be converted at 6 h of hydrolysis time. Hydrolysis of fructans was faster with the mixture of both enzymes that when using each enzyme separately, the minimum time required for complete hydrolysis of the fructans was reduced to 2.5 h with the mixture of both inulinases.

The DRS rate formation at hydrolysis times less than 2.0 h was much higher with the mixture of both inulinases than when each one was used only, and even this was greater than the sum of the reaction rates of individual enzymes. This synergistic effect has been observed by other researchers (Ettaliby and Baratti, 1990) who concluded that the exo-inulinase was more active with decreasing the size of the polymer on which it acts, while the activity endo-inulinase decreased with decreasing size of the polymer.

Overall process efficiency

The critical stages of the obtaining process of high-

Table 2. Overall efficiency of the process of obtaining high fructose syrup from fructans, contained in the head of *Agave tequilana* Weber var. blue.

Parameter	Content
Humidity (%)	71.1 ± 1.4
Total reducing sugars (%$_{DB}$)	79.7 ± 2.6
Extraction of fructans (%)	89.1 ± 2.3
Clarification syrup (%)	85.6 ± 2.1
Concentration syrup (%)	94.7 ± 2.8
Overall efficiency (%)	72.2 ± 2.4
kg of syrup with 70°Brix/kg of flour	0.82 ± 0.03
kg of syrup with 70°Brix/kg of head	0.23 ± 0.01

Tabla 3. Chemical characterization of syrups.

Parameter	Syrup	
	Frudex 55	Agave syrup
Humidity (%)	30.0 ± 0.01^a	30.0 ± 0.01^a
Ash (%$_{DB}$)	0.05 ± 0.001^a	0.27 ± 0.03^b
DRS(%$_{DS}$)	99.80 ± 1.30^a	99.55 ± 1.25^a
TRS (%$_{DB}$)	99.87 ± 1.40^a	99.60 ± 1.10^a
Fructose (%$_{DB}$)	53.12 ± 1.05^a	87.92 ± 1.28^b
Glucose (%$_{DB}$)	46.86 ± 1.18^a	11.64 ± 0.36^b

Different letters between columns of the same line indicate statistically significant differences ($p < 0.05$).

Table 4. Physical characterization of syrups.

Parameter	Syrup	
	Frudex 55	Agave syrup
Soluble solids (°Brix)	70.0 ± 0.1^a	70.0 ± 0.01^a
Density (g/ml)	1.50 ± 0.005^a	1.49 ± 0.003^a
Water activity (aw)	0.702 ± 0.001^a	0.699 ± 0.002^a
Surface tension (dinas/cm)	50.50 ± 1.83^a	45.30 ± 0.95^b
Viscosity (mPa-s)	224 ± 3.9^a	212 ± 3.5^b
Color (a*/b*)	0.04 ± 0.001^a	0.09 ± 0.003^b

Different letters between columns of the same line indicate statistically significant differences ($p < 0.05$).

fructose syrup with 70% (w/w) of TRS from fructans contained in the head of *A. tequilana* Weber var. blue were the fructans extraction and the clarification of the syrup. During fructans extraction, nearly 11% of the TRS contained in the agave head was lost, while in the clarification of the syrup, 14.4% of TRS was lost due to compaction of the column of activated carbon (Table 2). According to the overall process efficiency (72.2 ± 2.4%), the high moisture content (71.1 ± 1.4%) and of TRS (79.7 ± 2.6% DB) in the head, 4.4 kg of fresh head of *A. tequilana* Weber var. blue were needed to obtain 1 kg of syrup with 70% of TRS and a fructose content of 87.92 ± 1.28%.

Chemical characterization of syrups

Since the content of soluble solids in syrups was previously adjusted to 70°Brix, in this way the moisture content, which was 30% was also adjusted (Table 3). The ash content in the agave syrup was higher than the corn syrup, which is indicative of a higher salt content in agave syrup, which must be previously removed by ion exchange resins to avoid altering the sensorial properties of the syrup; this is a common practice in the production of corn syrup (Zhang et al., 2004). There was no significant difference ($p < 0.05$) in the content of TRS and DRS of the produced syrups which indicates that all sugars were present as monosaccharides (fructose and glucose). The fructose content in the agave syrup was much higher than in corn syrup, which was due to the chemical nature of the fructans and the average degree of polymerization of these polymers (Schorr and Guiraud, 1997; Pimienta et al., 2006). The fructose and glucose content in the agave syrup was similar to that obtained from Jerusalem artichoke (*Helianthus tuberosus*), whose fructose and glucose content were 86.4 and and 13.6% (Manzoni and Cavazzoni, 1992) and of 85 and 15%, respectively (Wenling et al., 1999).

Physical characterization of syrups

Table 4 shows the physical parameters of syrups evaluated. Since the moisture content as well as the content and type of solids in the syrup was the same, the density and water activity (aw) were also similar, because their intrinsic properties are directly related to each other (Badui, 1997). On the other hand, the relatively low value of water activity (aw = 0.7) of the syrups, helped maintain physical, chemical and sensorial properties, without resorting to the cooling or the addition of antimicrobial agents for preservation.

Both surface tension and viscosity of the syrup depend on the fructose and glucose content and as a result of the higher content of glucose, both properties were higher in the corn syrup (Frudex-55®), which could be attributed to a stronger association of glucose molecules within the solution, which occurs through hydrogen bonds between them and provides a highly branched polymer effect which increased the strength of the syrup to flow freely (Badui, 1997). These results are consistent with those reported previously by Hernandez et al. (2008), who found that at equal dry matter content and the same temperature, the viscosity of the syrup depends on the type of sugar; this viscosity being lower in fructose syrups, followed in ascending order by the invert sugar syrup (50 glucose and 50% fructose), then by sucrose syrups and finally glucose syrups, whose viscosity was much higher than everyone else. Regarding coloring, agave syrup was slightly darker than the corn syrup (Frudex-55 ®), which is reflected in the greater value of the ratio a*/b* for the agave syrup, indicating colors ranges of yellow clear for the corn syrup, to a yellow shades darker for the agave syrup. The acidity and the high temperatures used during the extraction, hydrolysis and concentration were responsible for the higher pigmentation in the agave syrup; in these stages, the generated compounds were derived from Maillard reactions in addition to strong and persistent color, which impart undesirable odors and flavors, and therefore must be eliminated through a process of clarification and decolorization with activated charcoal, and subsequent filtration (Fleming and GrootWassink, 1979). Among the main compounds from the Maillard reactions that have been identified in extracts of agave heads previously cooked, there are some furans, pyrans, and aldehydes compounds with nitrogen and sulfur among which are 5-hydroxymethyl-furfural, the methyl-2-furoate and 2.3 dihydroxy-3, 5-dihydro-6-methyl-4-pyranone (Mancilla and Lopez, 2002; Kim and Lee, 2009).

Conclusion

The fructans content in the head of *A. tequilana* Weber var. blue is similar to that contained in the tubers of Jerusalem artichoke (*Helianthus tuberosus*), the main fructans potential source proposal for obtaining high fructose syrups.

There is a synergistic effect in the enzymatic activity combined between the exo-and endo-inulinases from *Aspergillus niger* on the agave fructans, that is, the enzymatic activity of the mixture of both enzymes is greater than the sum of the individual enzyme's activities.

The process to make high fructose syrups from fructans of *A. tequilana* Weber var. blue is simpler than the one used from corn starch, moreover, the latter would require four different amylolytic enzymes while in the first, only two enzymes are necessary.

Given the chemical nature of fructans, the syrups obtained from the head of *A. tequilana* Weber var. blue contain a higher proportion of fructose than those obtained from corn starch, a proportion that is directly related to the average degree of polymerization of these polymers.

Regardless of its source, high fructose syrups behave as Newtonian fluids, and therefore its viscosity and surface tension decrease when increasing the fructose content in them.

Due to high crop yields and its high content of fructans, the head of *A. tequilana* Weber var. blue is a promising raw material for obtaining industrial high fructose syrups.

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