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Research Article

Extraction of Inulin from Waste Pineapples of Cuban Henequen Plants

Cándida Magali Ferrer Serrano [1]*, Beatriz Zumalacárregui de Cárdenas [2], Martha Mazorra Mestre[3]

[1,3] Professor Center for the Study of Renewable Energy Technologies.

[2] Full Professor, Faculty of Chemical Engineering, Technological University of Havana.

***Corresponding Author:** Cándida Magali Ferrer Serrano., Professor Center for the Study of Renewable Energy Technologies.

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Abstract

The non-use of henequen pineapples from biodegradable waste as raw material constitutes a problematic to be solved. The presence of inulin in a food is a sufficient condition to be considered a prebiotic food in addition to the variety of applications of inulin in health and in the pharmaceutical industry, so the objective of the work is to extract inulin of henequen pineapples. For the study, henequen pineapples from Mariel and Limonar, qualitative and quantitative analytical techniques were used. An experimental design 23 was carried out through the Stagraphics Centurium statistical package, taking into account the factors that influence the percentage of extraction of this type of polysaccharides. The best results correspond to Limonar henequen pineapples at a temperature of 70 0C time of 60 minutes and 12-year-old plants.

Key words: inulin; obtain; henequen pineapples

Introduction

The advance of science and the social needs of today in correspondence with population growth, together with new diseases, has stimulated the search for products that respond to new conditions, which is why it is required to have sources that are renewable and compatible. with the environment. The Agave Fourcroydes Lem (henequen) is grown in several provinces of the country. This Cuban industry has been fundamentally dedicated to obtaining white fiber with competitive characteristics in the international market. However, the waste generated by the production process has not been used for use in industrial or social processes. From them it is possible to obtain products with a high added value, based on their properties. Several studies indicate that pineapples are rich in carbohydrates with a concentration of 75%. Sugars have been identified such as glucose, dextrin, starch and mainly, inulin, which is contained in 15-20% (1).

Inulin is a fructan-type polysaccharide also known as fructosan, fructo-oligosaccharide (FOS). present in numerous plant species, considered as a prebiotic agent. It has been the subject of numerous studies on a laboratory scale starting from a meticulous analysis of the natural and usable sources of said element, until its transformation into generated by-products. Inulin is identified as an important compound, due to its production possibilities and its variety of applications, as well as the need to develop efficient production technologies. (2,3).

It is a polymer made up of a variable number of fructose units that are joined by a β-glycosidic bond (2→1), normally terminated in a glucose unit through an α-D-glucopyranosyl bond (1→2).

FOS, in recent decades, have been studied intensively for their applications in the food, chemical and pharmaceutical industries. These may be present in the foods themselves, or in turn they may be added to industrialized products (functional foods) (4).

Functional foods cover nutritional aspects, such as: the improvement of gastrointestinal functions, act on the redox and antioxidant system, contribute to the metabolism of macronutrients (mainly carbohydrates, proteins and lipids) and promote the metabolism of micronutrients (vitamins and minerals), among others, which is why there has been a growing interest and consumption of them, mainly those that have a prebiotic character (5).

In particular, research on inulin highlights its positive effects on health and in the food industry, among them: it regulates bacteria in the colon, helping to reduce blood glucose, it allows the bioavailability of calcium and magnesium, and is used for its various beneficial effects in food processing. (4,6,7).

Promising evidence of inulin in the regulation of lipid parameters, reduction of cancer risk, reinforcement of the immune response and protection against intestinal disorders was presented by Escobar (8). Both it and fructans cannot be hydrolyzed by the digestive enzymes found in the upper gastrointestinal tract: stomach and small intestine, but they are hydrolyzed by the bacterial microflora present in the colon. Having this particularity, they are called dietary fiber with a low-calorie content (9-11).

As a prebiotic, they have a great contribution of dietary fiber, low caloric value, hypoglycemic. In a wide variety of food products it is used as: thickener, emulsifying agent, gelling agent, sugar and fat substitute, humectant and freezing point depressant. They are also used in the chemical-pharmaceutical and processing industries as an excipient, additive, technological agent or adjuvant (5,12).

There are scientific studies on the production of inulin, its methodology, variables and methods for quantifying the presence of FOS (6, 13), identifying inulin and oligofructose as the best-known prebiotics. Escobar, (8) and Fiallos et al (2) carried out studies with the purpose of evaluating the organoleptic properties of inulin when it is incorporated into existing products and obtained it through hydrolysis, using 5% oxalic acid and using the roots. and dehydrated leaves of the yacon plant.

Although there are preliminary studies on obtaining fructans from the juice of henequén pineapples, (3) its implementation in Cuba constitutes an interesting problem to solve, since it generates new sources for social and industrial development, in addition to the environmental impact it generates. the use of agricultural or industrial waste. The objective of this research is related to obtaining inulin from Cuban henequen pineapples from the Mariel (Artemisa Province) and Limonar (Matanzas Province) regions, as well as the precision of operational parameters.

Materials and Methods

The research is carried out at the Center for the Study of Renewable Energy Technologies (CETER) and the Faculty of Chemical Engineering of the Technological University of Havana (CUJAE).

Fruits from the Mariel and Limonar regions with ages of 7 and 12 years were used. The precision of some experimental parameters is carried out based on aspects proposed by other authors. (14 -17).

The refrigerated pineapples (1 kg) were brought to room temperature with extraction water, using a Bunsen brand thermostat. To extract the juice, they are washed and cut into small pieces, crushed by an auger machine. The solid-water ratio was set at 1:6 as recommended (3). From the crude extract at room temperature, the pineapple pieces are separated by filtration, dried and weighed. /18/

The residual solid is used as animal feed and in the preparation of compost for the soil. The cloudy juice is centrifuged until clear. The extracted juice was evaporated in an IKA RV Basic type rotary evaporator at a temperature between 60-70ºC, until reaching a volume of 30 mL. The concentrated extract was subsequently subjected to a drying process in an AISET YLD-6000 oven at 90°C for 4 hours. It cools and weighs, (18,19).

The process was carried out according to an experimental design 23 through the Stagraphics Centurium statistical package, taking into account the factors: temperature of the medium, extraction time and age of the plant. The response variable is the % yield of the concentrated extract.

Said extract was subjected to a qualitative identification of inulin by thin layer chromatography, using glass plates with silica gel and standard samples of pure solutions of glucose, fructose, sucrose and inulin. (15)

In addition, it was characterized through the freeze-drying method and an infrared spectrometer (Bruker Tensor 37 FT-IR Spectrophotometer), using KBr pellets. The readings were taken in the range of 450-4000cm-1.

To quantify the inulin obtained in the concentrated extracts, the refractometric technique is used. To do this, a calibration curve is constructed from a standard solution of 5 g of inulin in 50 mL of solution. By extrapolating the curve, the percentages of inulin present can be known.

Figure 1 Inulin standard curve

Humidity, ash and fat percentage were determined by gravimetry.

To determine humidity, the difference in weights between the dried pineapple in the oven at 105°C for 2 hours and the wet pineapple is used, an experiment that is carried out in triplicate and until a constant weight is obtained. The percentage of ash is determined by incinerating the dry sample previously treated with concentrated HCl and carbonized, then placed in a muffle at 800°C for 1 hour. The operation is repeated until a constant weight is obtained. For fats, a Soxhlet extractor is used with ethyl ether as a solvent; the fat is extracted from the sample by reflux for 6 hours. Once the solvent has evaporated, the flask with the fat is dried in the oven at 103°C for 5 minutes. It is cooled in the desiccator and weighed. Acidity and pH by volumetric and potentiometric methods (1,14,16).

The qualitative test for the determination of reducing sugars was carried out based on oxidation-reduction reactions with Fehling's reagent (A and B). and the absorptiometric determination of the sample treated with 3,5 dinitrosalicylic and phenol-sulfuric acid to corroborate the absence of other sugars. (14,16)

Results And Discussion

Characterization of pineapples and their juice. The results are presented in the following table 1.

Property	Mariel (age)		Limonar (age)	
	7	12	7	12
Humidity (%)	82,5	83,2	81,9	83,3
Acidity (g/L)	4,68	6,43	7,20	6,48
Fats (%)	0,46	0,56	0,23	0,36
Ashes (%)	7,06	8,23	7,56	8,34
pH juice	5,50	6,70	5,50	5,70
ºBrix (%) juice	1,7	1,8	4,3	6,6

Table 1: Characterization of pineapples and their juice.

The humidity of the fruits was analyzed for both regions and ages, having a similar behavior between them and previous works. (20, 21) The acidity values found are higher than those reported by Higuera, which indicates the lower ripening of the fruits that coincides with the low Brix value. And the acidic pH determined. (1)

When comparing the percentage of fat in the fruits between the two regions, the values obtained in the Mariel region are somewhat higher than those of Limonar. The ashes, the inorganic matter in the fruit, are higher than those reported by Higueras,(1) (5.04 ± 0.016), possibly due to the use of 9-year-old Agave salmiana in addition to the different geographical area, aspects that influence in natural products.

In the juices from pineapples from both regions, a great difference is observed in total solids (°Brix), due to the level of ripening of the fruit and the edaphoclimatic conditions in general. According to the results obtained from the different parameters, it was decided to continue the study only with the pineapples from the extractions of Limonar pineapples.

Temperature °C)	Extraction time (minutes)	Concentrated extract yield inulin (%)	
		7 years	12 years
70	60	19,76 ± 0,012	29,58 ± 0,011
70	30	10,29 ± 0,016	18,21 ± 0,014
50	60	13,20 ± 0,010	21,08 ± 0,018
50	30	8,23 ± 0,014	14,41 ± 0,015

(Depending on the means of three determinations of extract performance)

Table 2: Pineapple concentrated juice performance in extractions.

Table 3 and Figure 1 show that all the parameters studied and their interrelation significantly influence the response variable, yield of the concentrated extract, given the P-value less than 0.05, with a confidence level of 95%.

From the Pareto diagram and the coefficients of the calculated mathematical model, it is identified that the factors extraction time and age of the plant influence more than the temperature of the medium.

R-squared = 99.43% indicates the fit of the calculated mathematical model.

R-squared (adjusted) = 99.2363%

Statistician's standard error. = 0.6577 Mean absolute error = 0.4289

Below is the equation of the fitted model:

R= 17.0983+ 4.31A+2.865C+ 4.22667 B+1.4 AC+ 0.701667AB+0.708333BC (1)

R= performance (%) C= temperature (ºC) A= time (minutes) B= Age (years)

In this mathematical model, the influence of the factors studied on the performance response variable is corroborated. The Pareto diagram is shown in Figure 1.

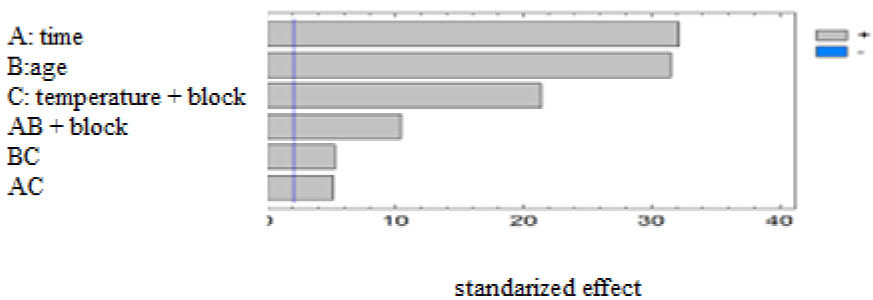

Figure 1: Standardized Pareto diagram for performance.

The highest yields of inulin are obtained in the extractions of 12-year-old pineapples, so it is stated that the age of the plant is an important factor in the concentration and composition of the soluble carbohydrates present in it, as well as the extraction time and temperature.

Characterization of inulin

Qualitative tests

The reducing sugar test was carried out using Fehling's reagent (A and B) and no precipitate of copper oxide I was formed, which shows that there is no presence of them.

When performing the 3,5 dinitrosalicylic acid and phenol-sulfuric acid methods, no positive evidence was obtained, the absorbance was zero in all cases, thus corroborating the absence of other reducing sugars.

Thin layer chromatography

A glass plate with silica gel was used, the standard solutions and the sample of concentrated pineapple extract were added, butanol/isopropanol/distilled water/acetic acid were run in the solvent mixture (5.17). The result showed the presence of inulin in the concentrated extract, with an Rf equal to the standard sample, with no stains corresponding to sugars observed, when comparing with the standard solutions.

Fourier transform infrared spectroscopy

Figure 2 shows the infrared spectrum of the concentrated extract of 12-year-old pineapple. And in table 4 the main bands present are assigned

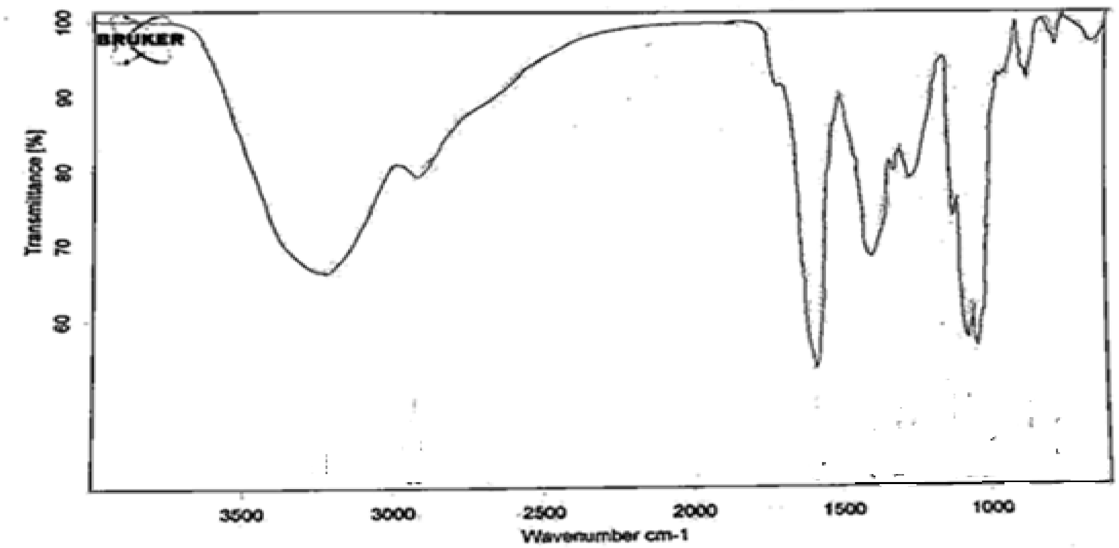

Figure 2: Infrared spectrum of a sample obtained from the concentrated extract of 12-year-old pineapple**.**

Table 4 compares the experimental spectroscopic signals with those obtained by other researchers Vega (19) and Panchev (22) with species from Chile and Bulgaria.

	Experimental	Literature (19)	Literature (22)
γ OH presents In fructose units	3341 cm^{-1}	3422 cm^{-1}	3398 cm^{-1}
γ C-H	2913, 2821cm^{-1}	2934 cm^{-1}	2921, 2848 cm^{-1}
γ C-C rings of 5, 6 members	1400-1382 cm^{-1}	1452 cm^{-1}	1463-1425 cm^{-1}
γ C-O-C of the aglicones	1119-1033 cm^{-1}	1132 cm^{-1}	1150-1050cm^{-1}

Table 4: Comparison of spectroscopic signals.

Quantitative test through the refractometric technique

The concentration of inulin can be determined by the refractometric method (17) so it was decided to determine the concentration of inulin present in each of the samples from the Limonar region, the juices obtained at a temperature of 700C and at extraction times of 60 and 30 minutes. In the following table it can be seen that the percentages of inulin present in each of the samples are similar to those obtained by the gravimetric method.

Temperature (°C)	Time of extraction (minutes)	Índex of refraction	% inulin
70	60	1,3539	29,1
70	60	1,3529	27,9
70	30	1,3447	19,5
70	30	1,3490	24,0

Table 6: Inulin determination in concentrated pineapple extract according to refractometric method.

Conclusions

It was possible to obtain the concentrated extract enriched in inulin, from the extraction carried out on henequen pineapples with a temperature of 70ºC, solid-water ratio 1-6 and 60 minutes, with the best yields being those achieved with 12-year-old plants. old. The method used is feasible, since hydrolysis of the solutions obtained does not occur, which was experimentally corroborated by the absence of sugars. The refractometric method can be used to determine the inulin content of henequen pineapples, being comparable to the gravimetric method.

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