Inulin from tubers of *Dahlia imperialis* Roetz

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Summary

The extraction and isolation of inulin was accomplished, from the tubers of *Dahlia imperialis* Roetz. (Asteraceae), obtaining a yield of 13.7% on dry base. The obtained product was identified by means of physical, chemical, chromatographic and spectroscopic methods; its characterization was made according to the patterns of the United States Pharmacopeia (USP 23). Some assays were made to determine the practical and functional profits of inulin, such as its use as prebiotic food, in the manufacture of fermented milks, with bifidobacteria, specifically *Bifidum infantis*. In these assays, it was observed that inulin stimulates and promotes the growth of these bacteria.

Key words: Inulin – Fructans - *Dahlia imperialis Roetz* – prebiotic - *Bifidum infantis*.

Resumen

Inulina a partir de Dahlia imperialis Roetz

Se llevó a cabo la extracción y aislamiento de inulina, a partir de tubérculos de *Dalia imperialis* Rotez (Asteraceae), obteniéndose un rendimiento de 13.7% en base seca. El producto obtenido fue identificado mediante métodos físicos, químicos, cromatográficos y espectroscópicos; su caracterización fue realizada de acuerdo con los patrones de la Farmacopea de los Estados Unidos (USP 23). Algunos ensayos fueron realizados con el fin determinar la utilidad de la inulina como probiótico en los alimentos, en la elaboración de leches fermentadas con bifidobacteria, especialmente *Bifidum infantis*. En estos ensayos se observó que la inulina estimula y promueve el crecimiento de esta bacteria.

Palabras clave: Inulina – Fructanos - *Dahlia imperialis* Roetz – prebióticos - *Bifidus infantis*.

Introduction

Inulin is formed by units of fructans, which are polymers of D-fructose. Each chain of the polymer contains single unit of glucosyl residue, joint with bonds β 1 $\rightarrow$ 2, such as in the sucrose; this unit is invariably located at the end of the chain, so the chain of fructans forms a series of homologue olygomers and polymers derived from fructosyl-sucrose. From these single fructans three isomers 1-kestose (isokestose), 6-kestose (kestose) and neokestose were isolated. The inulin belongs to the first of them, its structure is shown in Figure 1 (1).

Inulin is a reserve polysaccharide that is stored by some plants, especially from Asteraceae family, although it has been found in families such as Boraginaceae, Indaceae, Gramineae and Asparagus. The name of inulin comes from the plant *Inula helenium*, from which it was firstly isolated (2).

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From the medical perspective, inulin is used in patients with metabolic problems (diabetes and hypoglycemia) because of its poor absorption in the gastrointestinal tract. This is the reason why it is classified as a low calories food, as well as source of dietary fiber from natural origin.

In addition, inulin has many industrial applications because it is a carbohydrate, which produces fructose; it is also used to obtain solvents as: ethanol, buthanol, acetone, 2,3 - buthanoediol. These solvents can be isolated in the different fermentative steps (3).

By the year 2001, the Colombian industry of dairy products imported 90 tons of inulin from Belgium (4), for this reason, this work studied the method to obtain inulin from the tubers of *Dahlia imperialis* Roetz, a native specie in our country.

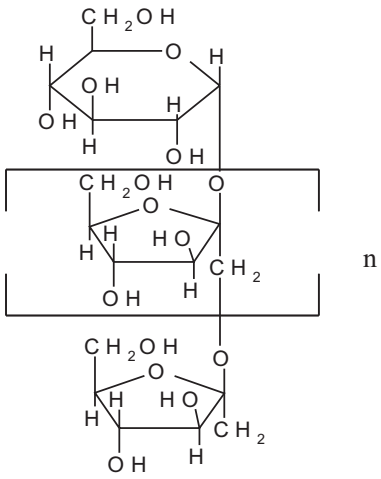

Figure 1. Molecular structure of inulin.

Plant material

D. imperialis Roetz was collected in February 2001, from Colombia, in the Department of Tolima, in the farm San Jose, jurisdiction of Líbano, at a height of 1550 m.o.s.l., at an average temperature of 20°C. A voucher specimen was identified by the botanist Edgar Linares of Instituto de Ciencias Naturales, Universidad Nacional de Colombia.

Extraction

The tubers of *D. imperialis* were selected by sanity and size. Then, they were washed with water and disinfected with sodium hypochlorite, further they were rinsed with water. Then the raw material was size reduced blending it with water in a mechanical juice squeezer. Once blended, a decoction at 65-70 °C during 15-20 minutes was made. Then a single straining for separating the coarse solids was made.

The liquor obtained from the last step was clarified by means of vacuum filtration using filtrating means, as it is done with diatomaceous earth. For crystallizing the inulin, ethanol of 96% was added, slowly, to the clarified liquid. The crystallized inulin was recovered by vacuum filtration and was dried by lyophilization.

Analysis of inulin

The product was characterized according to quality criteria indicated in USP 23, solubility test, specific rotation, iron, reducing sugars and microbial count. In addition, the inulin (1) was assayed for its physico-chemical characterization, by melting point with decomposition, lugol, molisch and resorcinol tests (5-7).

Inulin was identified by means of chromatographic and spectroscopic methods. Liquid chromatography (HPLC) for identification was realized, using a modular chromatograph with pump Waters 510, automated gradient controller Waters, infrared detector Waters 2410, column traded Shodex serie SC-1011 (specific for sugars). The test conditions were: isocratic

isolation, HPLC water as mobile phase, flow 0.6 mL/min, running time 25 minutes, detector temperature of 70 °C, concentration of the sample 10 mg/mL and injection volume 10 μL.

An infrared spectrum was taken, in tablets of KBr at 1% on a spectrophotometer ATI Mattson Genesis, serie FTIR™.

For the characterization and identification, comparative assays with the standard of inulin trademark SIGMA, from the roots of *Chichorium intybus* were made.

Functional properties evaluation

The probiotic cultures can be added to different foods to increase the profits for the consumer. The milky drinks fermented with traditional cultures produce beneficial effects over the human organism. In the case of yogurt, the *Lactobacillus thermophilus, Streptococcus thermophilus* and *bifidobacterium* can exert a hostile action to undesirable microorganisms in the organism.

Some probiotic microorganisms, especially *Bifidobacterium*, require the presence of nutritious ingredients that stimulate their growth, selectively, as inulin does. In this sense, an assay to verify the application of inulin in the manufacture of acid or fermented milks and its incidence in the growth of *Bifidobacterium* was perfomed [8].

Assay with inulin

800 mL of milk at 12 % w/v, were prepared in an erlenmeyer and distributed in four erlenmeyers in fraccions of 200 mL. One of these flask was used a control and to the others with 1, 3 and 5 g of inulin, were added respectively. The erlenmeyers were heated at 90ºC during 20 minutes a water bath, cooled to 37 ºC and then mixed with 0.4 g of *Bifidum infantis* (stock solution) and finally incubated during 5 hours at 37 ºC. The samples and the dilutions of these homogenates were prepared to obtain the number of the neccesary dilutions (10^{-5}) and make the count of ufc (9). The results are expressed as mean values ± SD.

Results and discussion

The procedures used in this investigation for the extraction and purification are easy to carry out. The yield of the inulin obtained was 13.75%.

The inulin obtained by the previous explained methodology and the assays which were subjected to determine the quality control of the product, show clearly that the substance presents a good quality, due to its chemical, physical and microbiological stability and to the completion of the criteria established in the USP, related to good manufacturing practices. The responses inulin to the lugol, molisch and resorcinol tests is that reported in the literature and exhibits a similar behavior respect to the standard inulin.

Inulin (1): white solid, mp 176 – 178 ºC (lit. 177 – 178 ºC [7]); $[\alpha]_D^{20}$: - 43.7 (c = 2, H_2O); IR bands (KBr): 3395, 2940, 1130, 934 cm^{-1}. The spectrum of the inulin obtained from the tubers of *D. imperialis* is similar to the infrared characteristics of the standard inulin.

The chromatographic identification of inulin was made with respect to the retention time of the standard inulin, which corresponds to 8.888 min. The retention time of inulin from *D. imperialis* was 8.854 minutes, regarding that the retention times are similar. The amount of inulin present in the substance obtained was 99.5%, the remaining 0.5% in the composition of the substance corresponding to the presence of fructose. The percentage of inulin present in the sample is high, indicating an efficient

method of extraction. The counting of *Bifidobacterium* obtained is reported in Table 1.

Table 1. Counting of ufc/g in milk reconstituted with inulin

Sample	ufc/g
Control	$1 * 10^5$ (± 0.45)
1 g	$21 * 10^7$ (± 0.5)
3 g	$19 * 10^7$ (± 0.55)
5 g	$17 * 10^7$ (± 0.7)

According to these results, we can verify that inulin stimulates the growth of *Bifidobacterium*, considering the polymer obtained as a prebiotic. Moreover, the inulin did not interfere with the adequate texture of the food. The milk cultured with *Bifidum infantis* presents a minimum content of $1*10^5$ ufc /g of product, showing best results in the cultures with an addition of 1 g of inulin.

Conclusion

The method of inulin obtention is easy and reproducible, and it can be extrapolated to industrial scale. The inulin obtained from the tubers of *D. imperialis* in comparison with the parameters of quality and nutritious functionality presents an excellent behavior.

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