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Identification of fructooligosaccharides and inulin in waste of fique leaves - *Furcraea macrophylla* Baker

Identificación de fructooligosacáridos e inulinas en residuos de hojas de fique -Furc*r*aea *macrophylla* Baker

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Abstract

Colombia is the largest producer of cabuya, natural fiber extracted from fique (*Furcraea macrophylla* Baker*)*. The fique exploitation is characterized by traditional products and processes, low-tech, low value-added and limited plant use, resulting in low profitability. However, residues of this agro-industrial process contain many substances of interest, unexplored yet, including carbohydrates, specifically fructans, such as inulin and fructooligosaccharides which are fructose polymers that are considered dietary fiber and for their characteristics are classified as prebiotics. Through enzymatic and spectrophotometric techniques, in this research is evidenced the presence of FOS and inulin in the residues obtained during the fique leafs defibrating process. Given the wide applications in the food industry, quantification, extraction and characterization is recommended in order to provide added value through the exploitation of these compounds.

Key words: Fique, FOS, fructans.

Resumen

Colombia es un importante productor de cabuya, fibra natural extraída de fique *(Furcraea macrophylla* Baker). La explotación de esta planta se caracteriza por productos y procesos tradicionales, poco tecnificados, de bajo valor agregado y con un aprovechamiento limitado de la planta de fique, lo que se traduce en baja rentabilidad. No obstante, en los residuos de este proceso agroindustrial se encuentran muchas sustancias de interés, aun sin explorar, entre ellas los carbohidratos, específicamente los fructanos, como la inulina y los fructooligosacaridos (FOS) que son polímeros de fructosa considerados fibra dietaría y que por sus características se clasifican como prebióticos. En esta investigación se evidenció la presencia de FOS e inulinas en los residuos obtenidos durante el proceso de desfibrado de las hojas de fique a través de técnicas enzimáticas y espectro-fotométricas. Debido a las amplias aplicaciones en el sector alimentario se recomienda su cuantificación, extracción y caracterización con el fin de proporcionar valor agregado a través de la explotación de estos compuestos.

Palabras clave: Fique, FOS, fructanos.

Introduction

The fique crop in Colombia is for the extraction of a natural fiber known as 'cabuya' which is used in the manufacture of various handicrafts, cordage and biodegradable bags for transporting agricultural products. This agro-industrial production chain is characterized by processes and traditional products with low-tech, low added value and limited exploitation of the plant. The use of the leaf of this plant varies between 4 and 6% (Peinado *et al.*, 2006), which results in low profitability. However, nationwide expectation for the use of its waste is significant; stakeholders recognize the value of the products that come from the fique juice to increase revenue through marketing (Castellanos *et al.*, 2009).

While in Colombia fique is exploited almost exclusively as a source of natural fiber, in countries like Mexico the agave is used - which is a similar plant to fique and belongs to the same family (Agavaceae) to obtain a wide variety of products for different industrial purposes. High fructose syrup, crystalline fructose, soluble dietary fiber and low calorie sweeteners such as fructooligosaccharides (FOS) and inulin, are some of the substances that are exploited for use as food additives and raw materials for producing ethanol citric acid, gluconic acid or sorbitol by fermentation (Chacón et al, 2014. Vargas-Vasquez, 2009).

FOS and inulin are carbohydrates like fructans, polymers of fructose, linked by glycosidic bonds of β type (2-1). Inulins have a degree of polymerization higher than 10 units and FOS, also known as oligofructose, have a lower degree of polymerization with about 3 to 7 units (Olvera *et al.*, 2007). These fructans are considered prebiotics, since they are not digestible by the human digestive tract and have bifidogenic character (stimulate the growth of bifidobacteria). Furthermore, when consumed often favour the absorption of minerals such as calcium, contribute to health and wellness of the colon through the strengthening of its epithelium and prevent diseases such as colorectal cancer (Aguilera-Garcia et al, 2008; Pool-Zobel and Sauer, 2007; Pool-Zobel *et al.*, 2002; Rafter *et al.*, 2007). The aim of this study was to evaluate qualitatively the presence of inulin and FOS in the pulping of fique waste, using an enzymatic and spectrophotometric method.

Materials and methods

The presence of FOS and inulin in agro-industrial pulping waste of fique was determined by enzymatic and spectrophotometric methods, adapted from the technique described by Montañez (Montañez-Soto *et al.*, 2011) and used by Vargas (2009). In this method the acidic hydrolysis of inulin was replace by the inulinase enzyme in order to not overestimate the quantification of carbohydrates. The invertase enzyme EC 3.2.1.26 was used to catalyse the hydrolysis of non-reducing waste of FOS, β-D-fructofuranosidase of fructofuranoside. Thus, components of reducing sugars (AR) were released. To identify inulin the endoinulinase enzyme or β-D- (2-1) -fructane fructohydrolase (EC 3.2.1.7) was used in order to catalyse the hydrolysis of internal bonds of the inulin molecule, resulting in a mixture of fructooligosaccharides (Castillo-Calderon, 2010). Because the endoinulinase do not have invertase activity, the hydrolysis was carried out in two stages. The first with endoinulinase that transforms inulin into FOS. The second with invertase for total hydrolysis of inulin in its reducing sugars, glucose and fructose. To identify the absorbance of present AR the Miller method (Miller, 1959) was used by forming the reduced compound 3-amino-5-nitro-salicylic reddish color. Its absorbance was measured at a wavelength of 540 nm, which is proportional to the amount of AR in the sample. Significant differences between measurement of initial RA, from the FOS and inulin, demonstrates the presence of fructan.

Peinado *et al.* (2006) found that fique juice has free reducing sugars like glucose and fructose, which were determined before and after enzyme action. The first measurement (initial AR) is the baseline to calculate significant differences because an increase of free AR proves the existence of FOS in the pulping waste of the fique leaves.

Sampling of agro-industrial pulping waste of fique was held in the town of Chachagüí, with an important producer of fique in the Department of Nariño (Colombia) (DNP, 2007). During the pulping of fique (*F. macrophylla* Baker), the juice and bagasse were collected from 10 leaves randomly selected from the total harvest for a day's work. These residues were homogenized with an electric processor of food to take a sample of 1 liter, which was cooled and transported in an amber container in order to prevent degradation of fructan by effects of

sunlight and activity of the native microbiota of the plant (Benavides *et al.*, 2012). Samples were analysed in the Chemical Analysis Laboratory of the Cooperative University of Colombia at Pasto. To ensure greater amount of fructan in solution, the sample was held at constant magnetic stirring at 80°C for 1 hour (Montanez-Soto *et al.*, 2011). This procedure also reduces the bioburden of the sample and thus the fermentation of carbohydrates. Afterwards, the sample was vacuum filtered through Whatman paper 90 mm, keeping the filtering in refrigeration.

The retained solids were suspended in 100 ml of distilled water to be heated and filtered again. Both filtrates were mixed to obtain a total volume of 1 litre sample, from which the density and pH were measured. Colour and odour were characterized in order to determine possible changes in the bacterial fermentation of carbohydrates in the sample. Because of the intensity of the colour from the original extract, and based on preliminary testing of this research, it was diluted 1:20 with distilled water.

To recognize by colour the presence of free reducing sugars produced by the plant and present in the agro-industrial waste, 5 ml from the diluted sample were applied to the DNS method (Miller, 1959). A spectrophotometer (Merck Spectroquant Pharo 300) was used in this procedure and the resulting data were the basis of the initial reducing sugars (ARI). All measurements were repeated 10 times. FOS hydrolysis was performed using invertase EC 3.2.1.26. Inulin hydrolysis was conducted with endoinulinase (β-D- (2-1) -fructans fructohydrolase EC 3.2.1.7) in combination with invertase, the two enzymes are thermostable (Vargas Vasquez, 2009). To confirm the presence of FOS, 1 mg of invertase was added to 5 ml of the diluted sample; then the resulting mixture was incubated for 90 min at 55°C. At the end of this procedure the Miller's method was applied (Miller, 1959) and the absorbance values of these samples were recorded.

To determine the inulinase it was used a diluted sample of 5 ml plus 10 ul of endoinulinase (EC 3.2.1.7) that was incubated at 55°C for 1 hour. Based on previous tests, the used time and temperature showed the best yield of reducing sugars. A second consecutive enzymatic stage with 2 mg invertase incubated for 90 min at 55°C was performed. Subsequently, the Miller's method was used to determine the absorbance of the AR. Figure 1 schematically shows the procedure performed to detect the presence of FOS and inulin in the fique residues.

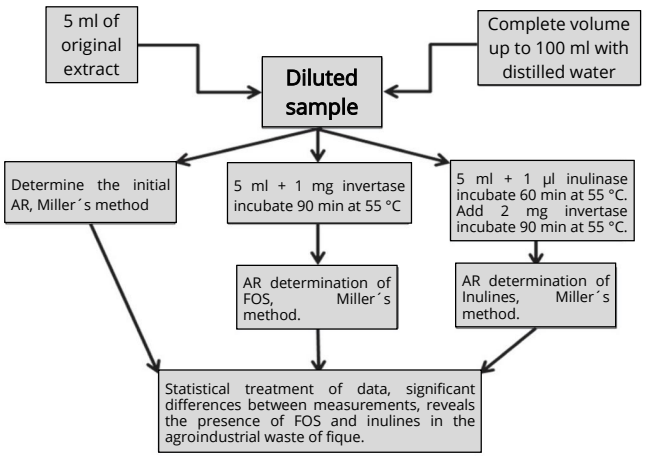

Figure 1. Scheme of the experimental procedure to identify the presence of FOS and inulin in the fique residues.

Results and discussion

Agroindustrial waste during the pulping process of fique leaves are characterized by a heterogeneous mixture composed of liquid juices, vegetable solids from the plant tissue, short fibre and bagasse.

The original extract showed a deep green colour with a strong characteristic odour, pH of 5.48 and density of 1.015 g/ml. These properties were monitored daily and found that after 3 days, the pH began to decrease to a minimum value of 5 after 8 days of storage. All measurements were performed during the following 2 days after the collection of the sample in order to avoid underestimation of the concentration of carbohydrates. Since microorganisms metabolize carbohydrates, the release of acids contribute to the reduction of pH in the solution. Some of the microorganisms that survived the heating performed on the sampling day belong to the native microbiota of the plant, from which are 25 bacterial and 22 yeasts isolates (Benavides *et al.*, 2012), while others are from the environment. Other characteristics such as density, colour and odour remained stable during the period of 8 days of observations.

Montanez *et al.* (2011) suggest the use of acid hydrolysis for quantification of inulin. However, this procedure is not selective to the

type of glycosidic bond for hydrolysis. Therefore, the endoinulinase was used in this research, which is specific for the hydrolysis of the linked β-D- (2-1) -fructose fructose. Since the inulinase do not have invertase activity, all the AR's that come from inulins are the result of the two stages of enzymatic hydrolysis, one by endoinulinase and the other by invertase. To obtain the highest amount of AR from insulins, the resulting conditions from the kinetic enzyme analysis of endoinulinase was made according to Michel-Cuello *et al.* (2012).

Figure 2 shows the results of absorbance measurements of the initial reducing sugars (ARI), reducing sugars from FOS (AR_FOS) and reducing sugars from inulins (AR_INU), calculated by the Miller's method.

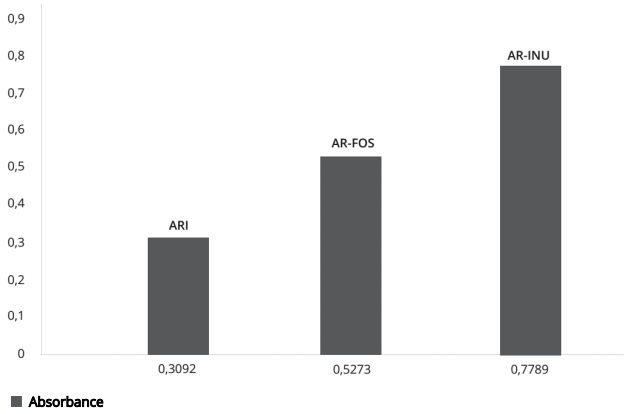

Figure 2. Absorbance of reducing sugars obtained by the method of Miller.

ARI = initial reducing sugars. AR-FOS = reducing sugars from FOS. AR-INU

The absorbance increase is consistent with the hypothesis that FOS and inulin are present in the residues of the pulping from fique. FOS are fructose polymers hydrolyzed by the action of invertase and therefore, release molecules of fructose and glucose. Those molecules are reducing sugars responsible for the increase in absorbance values of ARI. Same process occurs during the treatment with inulinase-invertase which acts upon the inulin to release FOS, and then fructose and glucose.

In applying the test of Fisher to the obtained results with a significance level of 95%, it was found that there are differences ($P < 0.05$) between the samples analysed and each set of measurements is homogeneous. Thus the proposed experimental protocol has good repeatability. In addition, the statistical analysis shows the average absorbance of each group are independent, meaning that the increased amount of AR is due to the proposed enzymatic treatment.

Conclusions

Fique plants cultivated in the municipality of Chachagüí, Nariño (Colombia), store inulin and FOS in their leaves. This feature, as same as with other plants of the Furcraea family and Agave, implies the possibility of finding carbohydrates also in the stem of fique, probably to a greater extent. This result is the basis for initiating studies that quantify and characterize the extraction of FOS and inulin in such plants.

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