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Structural and physicochemical characteristics of starch from sugar cane and sweet sorghum stalks

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ABSTRACT

The starch present in sugar cane and sorghum juice has been considered a problem to the sugar industry. The objective of this work was to study the structural and physicochemical characteristics of the starch present in sugar cane and sweet sorghum. Sugar cane and sweet sorghum starches presented small granules (maximum 5.9 and 7.9 μm), A-type diffraction pattern, high degree of relative crystallinity (44.4 and 42.0%), and low amylose content (17.5 and 16.4%), respectively. Sugar cane starch presented more uniformity in granule shape and size, more homogeneity in amylose chain length, higher number of long lateral chains of amylopectin, and higher susceptibility to enzymatic digestion. Besides being in higher amount in the juice, sweet sorghum starch presented lower values for thermal properties of gelatinization, as well as higher swelling factor, which can cause more problems during processing. Additional studies are needed to evaluate the variety and maturity influence on these properties.

1. Introduction

The sugar (sucrose) and alcohol (ethanol) industry has become an important part of the agro-industrial sector in certain parts of the world. Although sugar cane is widely utilized as raw material for sugar and alcohol production, the use of alternative feedstocks has been tried experimentally. Sweet sorghum can be employed as a complementary option during sugar cane off-season.

Starch is one of the main products of photosynthesis in superior plants, stored in chloroplasts, in the form of granules, as energy storage reserves when photosynthesis is not occurring (Bello-Pérez, Montealvo, & Acevedo, 2006). Starch content in sugar cane juice stalk ranges from 1.6 to 2.6 g/kg (Figueira, Carvalho, & Sato, 2011), depending on season of the year, plant variety, diseases, maturity, processing, method of analysis (Imrie & Tilbury, 1972), climatic conditions, stress, and type of soil (Eggleston, Montes, Monge, & Guidry, 2007b). In recent years higher concentrations of starch in sugar cane have been delivered to factories, mainly because of the processing of unburned sugar cane, environmental conditions, and the use of new varieties with higher amounts of this polymer (Eggleston et al., 2013; Zhou et al., 2008).

The presence of starch in sugar cane poses a great problem, since it affects negatively the quantity and quality of sugar processes and products (Zhou et al., 2008). The increased use of sweet sorghum in agro-industries may aggravate these problems, because this plant species has even higher starch content than sugar cane. In sweet sorghum juice starch content ranges from 76 to 154 g/kg, depending on plant variety, harvest period, and maturity (Zhao, Steinberger, Shi, Han, & Xie, 2012).

Starch is a polysaccharide composed of two macromolecules: amylose, which has a linear chain structure made up of glucose units linked through α-1,4 bonds, and amylopectin, which has a branched chain structure made up of glucose units linked through α(1–4) and α(1–6) bonds. The morphology, chemical composition, and molecular structure of starches are unique for each particular plant species (Bello-Pérez et al., 2006). The adverse effects of starch in sugar cane and sweet sorghum processing are mainly related to the behavior of starch granules during their hydration and heating (Zhou et al., 2008).

Even in small amounts in the juice, the presence of starch can cause problems due to viscosity increase in sugar cane and sorghum mills and refineries. The starch in juice is not soluble, but with the heat applied during clarification and evaporation, starch granules progressively swell, and rupture releasing amylose and amylopectin, which results in an amorphous viscous solution. On cooling, the amylose chains association, a phenomenon known as

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retrogradation, influences the distribution of starch in factory and refinery products (Zhou et al., 2008). According to Eggleston et al. (2013), however, some insoluble starch has been also observed in syrup and raw sugars. This warrants further investigations to determine if it is soluble starch, insoluble granules, or both, which are detrimental to the filtering process.

Starch can raise the cost of production by reducing crystallization and centrifugation rates, increases molasses production in the process of sugar concentration (Kampen, Tan, & Cuddihy, 1998; Zhou et al., 2008), reduces filterability, delays sugar crystal growth and causes its distortion (Roberts, Godshall, Carpenter, & Clarke, 1976), and occludes into the sucrose crystal (Zhou et al., 2008).

Taking into consideration the relevance of the sugar sector in the modern world, studies that characterize in detail the fraction starch both in sugar cane and sweet sorghum are important, since they can provide valuable data for future researches on the control of processes and products. Although some studies characterize sugar cane starch partially (Figueira et al., 2011; Kampen et al., 1998; Park, Martens, & Sato, 1985; Stevenson & Whayman, 1976), to the best of our knowledge, no studies specifically focusing on starch in sweet sorghum stalks have been published so far. Therefore, the objective of the present study was to carry out a comparative assessment of the structural and physicochemical characteristics of starch from sugar cane and sweet sorghum feedstocks.

2. Materials and methods

Sugar cane (RB 867515), third cut in July 2011, was used to extract the juice by pressing the stalks. Sweet sorghum (donated by a private company) were harvested in March 2011, 127 days after planting, and the stalks were pressed to obtain the juice. Both juices were evaluated for starch content according to Copersucar (2001). The starch present in the juice was extracted according to Park et al. (1985) with modifications. The juice was filtered (0.045 mm) and centrifuged at 9000 × *g* for 15 min at 5 °C. The suspension was centrifuged five consecutive times until the supernatant was clear. The method proposed by Stevenson and Whayman (1976) was employed to remove colored compounds. The starch solution was centrifuged with absolute ethanol and distilled water, and the precipitate was collected and dehydrated in an air circulation oven at 30 °C for 12 h. The dry starch was ground in a mortar with a pestle and sieved (0.25 mm).

After isolation, the purity of the starches was evaluated by determining total starch content using the method proposed by Rickard and Behn (1987). The percentage purity of the starches was calculated by dividing the starch content by the weight of the sample. Amylose content was determined using the amylose/amylopectin Megazyme kit (Megazyme International Ireland Limited, Bray, Ireland).

The molecular weight distribution profiles of the starches were determined by gel permeation chromatography (GPC) using a GE XK 26/70 column (2.6 cm diameter × 70 cm height) packed with Sepharose CL-2B gel. The samples were prepared according to the method proposed by Song and Jane (2000). A 4-mL aliquot containing 12 mg starch was injected in the column and eluted in the ascending mode. A solution containing 25 mM NaCl and 1 mM NaOH was used as eluent at a flow rate set to 60 mL/h. Fractions of 125 drops (approximately 4 mL) were collected and total sugar content (CHO) was analyzed using the phenol–sulfuric method (Dubois, Gilles, Hamilt, Rebers, & Smith, 1956) modified to be measured at 490 nm using an absorbance microreader, and the blue value (BV, iodine staining) method at 630 nm (Juliano, 1971).

The appearance of the granules was assessed using a scanning electron microscope (DSM 940 A, Carl Zeiss Inc., Oberkochen, Germany) with an amperage of 80 mA and acceleration voltage of 5 kV. The samples were attached to stubs with double-sided adhesive tape, and the starches were fixed and coated with a thin layer of gold using a sputter coating station (Balzers Med 010, Unaxis Balzers Ltd., Liechtenstein) for 3 min.

The size of starch granules was determined using an optical microscope (Nikon Eclipse E 200, Nikon, Inc., Melville, NY, USA) connected to a digital camera. The samples were diluted with a solution of 50% glycerol in water. The selected images were analyzed using the software Image Tool© (Wilcox, Dove, McDavid, & Greer, 2002). Following the recommendation of Vigneau, Loisel, Devaux, and Cantoni (2000), five slides were mounted and 100 measurements of starch granule size were taken per slide, in a total of 500 measurements. Due to the irregular shape of starch granules, the larger and the smaller diameters were measured. The results were used to construct histograms representing the frequency distribution for the groups of quantitative data.

To obtain the X-ray diffraction profiles, starch humidity was balanced in a desiccator containing saturated $BaCl_2$ solution (25 °C, aw = 0.9) for 36 h. The patterns of X-ray diffraction were determined in an X-ray diffractometer (Miniflex II, Rigaku, Tokyo, Japan) using copper radiation, at a scanning speed of 2° per min, angle 2θ ranging from 4° to 50°, 40 kV, and 40 mA. The relative crystallinity of starch was quantitatively determined following the method proposed by Nara and Komiya (1983) and using the Origin 7.5 software (Microcal Inc., Northampton, MA, USA). The data were smoothed with the Adjacent Averaging tool and plotted in graphs between 2θ angles ranging from 4° to 30°.

The enzymatic digestibility of starch was assessed using the methods described by Zhang and Hamaker (1998) and Benmoussa, Suhendra, Aboubacar, and Hamaker (2006) with some alterations. A 5-mL aliquot of distilled water was added to 200 mg starch. After 20 min in a boiling water bath the material was cooled to 40 °C and 25 mL of porcine pancreatic α-amylase (A-3176, Sigma Chemical Co., St. Louis, MO, USA) in Tris–maleate buffer solution pH 6.9 were added at the proportion of 5 units/mL. The suspension was incubated at 37 °C and at each selected experimental time (10, 20, 40, 60, 90, and 120 min) a 1-mL aliquot was collected. The reaction was interrupted by heating the mixture at 98 °C for 10 min and the content of reducing sugar was determined using the method described by Somogyi (1945).

The thermal properties of both starches were evaluated using a differential scanning calorimeter (Pyris 1 DSC, Perkin-Elmer, Norwalk, CT, USA). An aliquot of 3 mg of starch was placed in high-pressure stainless steel pan (P/N 0219-0062) and deionized water was added using a microsyringe to yield a starch:water ratio of 1:3. The pans were sealed and equilibrated for 24 h at room temperature before the analysis. The equipment was calibrated with indium and an empty pan was used as reference. The scanning was carried out at a heating rate of 5 °C/min and temperature ranging from 30 to 130 °C. Based on the thermograms, the following gelatinization parameters were obtained: onset temperature (T_o), peak temperature (T_p), final temperature (T_f), temperature range ($\Delta T = T_f - T_i$), and enthalpy (ΔH). The swelling factor of starch granules was evaluated according to the direct method proposed by Tester and Morrison (1990), at 50, 70, and 90 °C.

3. Results and discussion

3.1. Starch content in feedstock juices

The starch content in sugar cane juice was 356 ± 20 mg/L, considerably lower than the amount found in sweet sorghum juice, which was 1147 ± 9 mg/L. Andrzejewski, Eggleston, Lingle, and Powell (2013) reported that the stalk starch content was