

A NOVEL APPROACH OF CASSAVA BAGASSE IN DIFFERENT FROM TWO STARCH AND EFFECT OF HYDROCHLORIC ACID

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Abstract - The main objective of the present work was to study the thermal properties and rheological behavior of natural and modified organic cassava starch with standard solutions of hydrochloric acid in concentrations 0.1 and 0.2 mol L⁻¹ and in temperatures 25 and 45°C, respectively, as well as verify the structural changes up to the surface of granules. Cassava is an agricultural crop that occupies an important place in the diet of people of many countries in tropical and sub-tropical regions. Cassava bagasse is a by-product from industrial processing that is generated in great quantities and it can exhibit about 40-50 % of starch in its composition, in dry basis. Two samples, one moist (sample A) and another dry (sample B), were supplied by two cassava starch industries. The samples were analyzed by physicochemical, thermo analytical (TG, DTA, DSC), rheological (RVA) and micro-structural methods (NC-AFM) that demonstrated that the temperature of the industrial drying (around 150 °C) was not very well controlled, reaching higher temperatures, reaching decomposition of organic matter condition causing changes.

Key Words: Modified starch, Thermal Analysis, Cassava, thermal analysis, atomic force microscopy, RVA

1. INTRODUCTION

Starch is a natural polymer, which has many and unique properties. It is a non-toxic, biocompatible, biodegradable and abundant polysaccharide; regenerated from carbon dioxide and water by photosynthesis in plants. Cassava (*Manihot esculenta*, Crantz) is a species of the *Euphorbiaceae* family and one of the most important root crops in tropical regions in terms of food energy production. Tropical root and tuber are rich sources of starches, and vitamins and minerals (traces), and they serve as either subsidiary or subsistence food in parts of the tropical belt. Cassava (*Manihot esculenta*, Crantz) is an important vegetable crop in tropical regions, where on a food energy production basis; it ranks fourth after rice, wheat and corn as a source of complex carbohydrates. These root and tuber crops contain 70-80 % water, 16-24 % starch and small quantities (<4 %) of proteins and lipids and other substances.

Organic foods are produced using methods that do not involve modern synthetic inputs such as synthetic pesticides and chemical fertilizers: they do not contain genetic modifications and are not processed using irradiation, industrial solvents, or chemical food additives. Several countries require producers to obtain a special certification in order to market food as "organic". Organic starch is extracted following the same rules, and for this study. Starches are of great value for the food industry because they contribute greatly to the textural properties of many foods and are used in food and industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent and water retention agent.

Starch, in its native form, does not always have the physical or chemical properties appropriate for certain types of processing. Some of these constraints include, insolubility in cold water, low stability to freeze-thawing and syneresis, which in some cases makes them difficult to use. Starch modifications can be made by chemical, enzymatic and physical methods that promote specific functional properties for industrial purposes.

Cassava (*Manihot esculenta* Crantz) is considered to be an important source of food and dietary calories for a large part of the population in tropical and sub-tropical countries in Latin America, Africa and Asia. It is a rich source of starch and also contains fibres, proteins, lipids, sugars and other substances (Cereda, 2001). Cassava bagasse is a fibrous material resulting as a by-product of the cassava starch processing industry. It is an agro-industrial residue that can offer economic opportunities as an alternative substrate for the fermentation industry. This residue contains starch, about 50 % on a dry weight basis, a considerable quantity of natural fibre and low contents of ash, protein and lipids (Carta, 1999; Matsui *et al.*, 2003; Pandey *et al.*, 2000a; Teixeira *et al.*, 2005).

Starch and cellulose are natural polysaccharides and they are the most abundant renewable resources available to mankind. They are both glucose polymers that are photosynthesized by solar energy. Plants have starch as a reserve and cellulose as the structural basis of the plant cell wall. These polymers also constitute a major energy source

in human and animal diets. They are widely used as raw material in numerous industrial applications such as textiles, food, paper, paint, petroleum, pharmaceutical, bioprocess, and others (Matsui *et al.*, 2003; Pandey *et al.*, 2000a; Pandey *et al.*, 2000b; Richardson *et al.*, 2003; Sivaramakrishnan, *et al.*, 2007; Sun *et al.*, 2013).

The main objective of the present work was to study the thermal, rheological and structural behaviour of natural and modified organic cassava starch with standard solutions of hydrochloric acid (0.1 and 0.2 mol L⁻¹) with controlled temperature (20 and 45 °C). The raw and the hydrolyzed organic cassava starches were submitted to thermal analysis, using the thermo analytical techniques: simultaneous thermo gravimetric and differential thermal analysis (TG-DTA); differential scanning calorimetric (DSC); the structural changes of granules by the non-contact atomic force microscopy (NC-AFM) and pasting properties by the rapid viscoamylographic analysis (RVA).

Starch is the main source of carbohydrates in the human diet due to its abundance in nature. Starch granules are made up of glucose polymers, named amylose and amylopectin, and they are found inside vegetable cells, from where they are extracted for industrial applications by the food, textile, pulp and paper industries among many. Amylose is a mostly linear chain and typically consists up to 3000 anhydroglucose units (AGU) primarily interconnected by α -(1 \rightarrow 4) glycosidic linkages; it is also reported that it contains a few branched chains (Vilaplana *et al.*, 2012). Amylopectin is a highly branched polysaccharide consisting of β -(1 \rightarrow 4) linked glucose with β -(1 \rightarrow 6) linkages at the branch points (Beninca, *et al.*, 2012; Costa *et al.*, 2011; Leivas *et al.*, 2012; Matsuguma *et al.*, 2009; Vilaplana *et al.*, 2012).

Cellulose is a uniform, linear, glucose polymer and is the most abundant of all naturally occurring substances. Polymers constitute approximately one third of all vegetable matter as they are the principal structural cell wall component of all major plants, such as trees, annual plants, mosses, seaweeds and cotton, in which cellulose occurs in varying amounts (Richardson *et al.*, 2003; Abdul-Khalil *et al.*, 2012). According to the literature (Pandey *et al.*, 2000), the industrial processing of cassava is carried out mainly to isolate starch, which generates a great quantity of solid residue that contains 30-50 % of starch in dry.

Whenever a material undergoes a change in physical state, is transformed from one crystalline form to another, or it reacts chemically, heat is either absorbed or liberated as energy (endothermic or exothermic processes, respectively) (Cereda, 2001; Lacerda *et al.*, 2008a). Many of such processes can be initiated simply by raising the temperature of the material. Differential thermal analysis (DTA) and differential scanning calorimetric (DSC) techniques have proven most useful in providing basic information on starch gelatinization and retro gradation (Lacerda *et al.*, 2008b).

Thermogravimetry (TG) is a technique in which the change in sample mass (Δm , mass-loss or gain) is determined as a function of temperature and/or time. DTA is a technique that measures the difference in temperature between the sample and the reference. DSC is a technique whereby the difference in energy input into a substance and a reference material (sample and reference) is measured as a function of temperature, while both materials are subjected to programmed heating or cooling (Wendlandt, 1986). In this present study, cassava bagasse samples were obtained from two starch processing plants with different moisture contents; one industrially dried and another moist. Selected physicochemical, thermo analytical, rheological and microscopic methods were employed for their characterization.

2. MATERIAL AND METHODS

Differential scanning calorimetry (DSC): The DSC curves were obtained using thermal analysis system model DSC-Q200 (TA-Instruments, USA) and were recorded under an air flow of 50 mL min⁻¹, heating rate of 5.0 °C min⁻¹ and samples weighing about 2.0 mg dispersed in 10 μ L of distilled water. The instrument was calibrated using Indium 99.99% purity, m.p. = 156.6 °C, ΔH = 28.56 J g⁻¹. A 4:1 (water:starch, w/w) mixture was prepared and maintained for 60 minutes in order to equilibrate the moisture content. The aluminium crucibles were sealed in order to study the gelatinization process.

Samples of cassava bagasse were obtained directly as a by-product from two starch industries (A) and (B) located in the northwest region of Paraná State and the south of São Paulo State, Brazil. The first sample (A) was obtained with high moisture content (80.5 %) and the sample (B) was obtained with low moisture content (8.2 %); this last sample had brownish coloration due to the industrial drying process that exceeded 150 °C. The samples were milled and sieved at 20, 150 and 270 mesh (840, 105 and 53 μ m, respectively). Physicochemical analysis: The moisture, protein, reducing sugars, ash and pH contents were determined according to protocols of the Association of Official Analytical Chemists (AOAC, 2010) and of Adolfo Lutz Institute, (IAL, 2008).

Simultaneous thermo gravimetric and differential thermal analysis (TG-DTA): The samples were heated from 30 °C to 600 °C using open alumina crucibles with approximately 6.0 mg of sample under a synthetic air flow of 100 mL min⁻¹ at a heating rate of 10 °C min⁻¹. The samples were analyzed in a SDT-2960 thermo balance (TA-Instruments, USA). The instrument was preliminarily calibrated with two weight standard references and with standard calcium oxalate monohydrate. All mass loss percentages were determined using Universal Analysis-2000 data analysis software.

Rapid Viscoamylographic Analysis (RVA): The pasting properties of the samples were determined using RVA-4

(Newport Scientific, Australia). A suspension of 3 g (6 % moisture) cassava bagasse and starch in 25 g of distilled water underwent a controlled heating and cooling cycle under constant shear (160 rpm), where it was held at 50 °C for two min, heated from 50 to 95 °C at 6 °C min⁻¹, and held at 95 °C for 5 min, cooled to 50 °C at 6 °C min⁻¹ and held at 50 °C for 2 min (Sun *et al.*, 2013; Beninca *et al.*, 2012). Non-contact Atomic Force Microscopy (NC-AFM): The micro-images of each sample were observed with high resolution by an Atomic Force Microscope model SPM-9600 (Shimadzu, Japan), using the non-contact method (Beninca *et al.*, 2012; Juszczak *et al.*, 2008). The technique allowed us to observe the surface of the studied cassava bagasse, and it was possible to identify starch granules and natural fibres.

Five portions, each with twenty five grams of cassava starch were separated and identified as (a), (b), (c), (d) and (e). The untreated sample (a) was kept in a desiccators over anhydrous calcium chloride until constant mass. The samples (b) and (d) were treated by one hour with 100 mL of standard HCl solutions 0.1 and 0.2 mol L⁻¹, respectively, with continuous stirring and controlled temperature (45 °C). The samples (c) and (e) were treated by one hour with 100 mL of standard HCl solutions 0.1 and 0.2 mol L⁻¹, respectively, with continuous stirring and controlled temperature (25 °C). After this time, each suspension was filtered, washed with distilled water until complete elimination of chloride ions (test with H⁺/AgNO₃ solution), dried at room temperature and kept in a desiccator over anhydrous calcium chloride until constant mass.

TG and DTA curves were recorded using a simultaneous SDT 2960 System (TA Instruments) under a 100 mL min⁻¹ air flow purge gas, and a heating rate of 10 °C min⁻¹. The initial mass sample was about 7 mg. Alumina crucibles (sample and reference) were used for the TG and DTA experiments [11-13]. DSC curves were recorded using a DSC Q 200 System (TA Instruments) under an air flow of 50 mL min⁻¹, heating rate of 10 °C min⁻¹. A 4:1 (water: starch, w/w) slurry was prepared and maintained for one hour in order to equilibrate the moisture content. The aluminum crucibles were sealed with hermetic lid in order to study the gelatinization process.

The pasting properties were performed in an Rapid Viscoanalyzer RVA-4, (Newport Scientific) was used for studying the starch samples apparent viscosity profile during cooking in the presence of excess water (dispersions of starch at 10 % w/w, dry basis). The Standard analysis profile of the Thermo cline for Windows® software was employed for the analyses and the conditions involved increasing starch dispersion temperature from 50 to 95 °C in a 6 °C min⁻¹ rate, keeping at 95 °C for five minutes and then cooling until 50 °C at the same temperature rate. The apparent viscosity was expressed in centipoises (cP).

3. RESULTS AND DISCUSSION

The non-contact atomic force microscopy technique (NC-AFM) was used to observe the surface of the untreated (Figure 5) and treated organic cassava starch granules, and allowed us to obtain micro-images with high resolution of each sample. The measurements were performed at ambient conditions and a pellet of each sample was fixed directly on an AFM sample holder, which was enough to immobilize the granules and prevent contamination of the starch surface.

In the laboratory, sample A was dried carefully in an oven with forced air circulation at 45 °C for 24 hours, ground and sieved at 20, 150 and 270 mesh, and kept in a desiccators over anhydrous calcium chloride until constant mass. The cassava bagasse is hygroscopic: even in a desiccators it retains moisture content, and the values can be observed from the physicochemical and thermo gravimetric determinations (IAL, 2008; Wendlandt, 1986). All the TG-DTA curves of the untreated and treated samples have shown similarity with mass loss (TG) in three main steps corresponding to endo or exothermic events (DTA).

TG-DTA curves show the first mass loss attributed to dehydration, with corresponding endothermic, followed of stability. The second and third mass losses are due to decomposition of organic matter which begins with a little endothermic samples (a) and (c) attributed to depolymerization of the chains which occurs in an oxidizing atmosphere where are the formation of pyrodextrins [15-16], followed by two exothermic peaks, in an oxidizing atmosphere with ash formation. The little endothermic peaks after stability, not occurs in the thermal decomposition of the samples (b), (d) and (e). The ash content of each sample was: 0.14, 0.12, 0.12, 0.11 and 0.16 %, respectively. Gelatinization process When starch is heated in excess water the amylopectin double helices dissociate, with accompanying loss of crystallinity, and the granules swell (by imbibing water). The starch granules can absorb water and an irreversible swelling takes place in this process. The data for this process are the "onset" or initial temperature (T_o), the "end set" or conclusion temperature (T_c), peak temperature (T_p) and gelatinization enthalpy (ΔH_{gel}). These parameters as well as the viscosity are important for starch industry; they can reflect starch characteristics and play an important role in the application of the starches.

Cooking profile that includes a high viscosity peak followed by granule rupture and consequent cooking instability, ending with a moderate to low retro gradation tendency. In the case of the acid treatments, the viscosity values were inversely related with acid concentrations, as expected, but if one considers the behavior related with the temperature of reaction, with higher temperature (45 °C), the viscosity values were also higher than the reaction promoted at room temperature (25 °C). This was not expected as it is well

known that temperature increase generally is related with higher reaction rate. In an attempt to explain this behavior, it may be speculated that reagent degradation could occur at higher temperatures.

4. CONCLUSION

TG curves of cassava bagasse showed similar behavior; the samples showed three mass losses, the first due to dehydration, followed by stability and subsequently, decomposition (oxidation of starch and cellulose) in two consecutive steps. Each DTA event occurred with one endothermic (dehydration) followed by stability and two exothermic reactions, in agreement with the TG curves. Sample A, which was dried in a laboratory at 50 °C showed gelatinisation process, viscosity and pasting properties in any granulometry. Sample B did not show the gelatinization process or pasting properties due to the industrial drying procedure that caused starch gelatinization as well as a slight darkening of the bagasse.

Simultaneous thermogravimetry and differential thermal analysis (TG-DTA) are techniques that allowed determining the hydration water, the main steps of decomposition and the ash content; all these results were in agreement with endo- or exothermic events and all the cassava starch show similar behavior. Differential scanning calorimetric (DSC) was used in the study of the starch gelatinization process; the major gelatinization enthalpy was found.

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