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Characterization of starch nanoparticles obtained from *Araucaria angustifolia* seeds by acid hydrolysis and ultrasound

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ABSTRACT

Native starch (NS) extracted from the seeds of *pinhão* (*Araucaria angustifolia*) was modified by two methods: acid hydrolysis (AH) and ultrasound (US). The three starch samples were subjected to spray drying and characterized. Chemical composition and rheological characteristics were evaluated and morphology was analyzed by scanning electron microscopy. The starch modified by US and AH achieved nanometric size, with mean particle size of about 453 and 22 nm, respectively. Besides the modified starch reached nanometric size, the three starch preparations were significantly different in four characteristics: starch and amylose content, the percentage of syneresis and colorimetric data. The AH sample differed from the others in terms of solubility (more soluble), hygroscopicity (more hygroscopic) and paste clarity (more translucent). Modified starch nanoparticles obtained by AH and US can be useful for development of novel biocomposites with improved properties to be employed as coating materials or films.

1. Introduction

Starch is widely distributed in plants as a storage carbohydrate and is particularly abundant in cereal grains (40–90 g/100 g dry solids), vegetables (30–50 g/100 g dry solids), tubers (65–85 g/100 g dry solids) and immature fruits (40–70 g/100 g dry solids) (Lajolo & Menezes, 2006). Structurally, starch is a homopolysaccharide composed by amylose and amylopectin chains. Amylose consists of D-glucose units joined by glycosidic α-1,4 bonds, resulting in a linear chain, while amylopectin consists of D-glucose units linked through α-1,4 and α-1,6 bonds, forming a branched structure. The proportions in which these structures appear depend on the botanical source, differences among cultivars of the same species and, even on the degree of maturity of the plant (Eliasson, 2004; Tester, Karkalas, & Qi, 2004). Native starch granules contain between 15 and 45 g/100 g of crystalline material with typical X-ray diffraction patterns. These correspond to two polyforms (A or B) or an intermediate form (C), and their classification is based on variations in the water content and the packaging configuration of double helices (Imberty, Buleón, Tran, & Pérez, 1991).

The market for starches is constantly growing, leading to a continuous search for products with specific features that meet industry requirements. The production of modified starch is an alternative that has been developed in order to overcome one or more limitations of native starches, and thus increase the utility of this polymer in industrial applications (Jimenez, Fabra, Talens, & Chiralt, 2012; Zambrano & Camargo, 2001). The modification by acid hydrolysis has been used to modify the structure of the starch granules and produce "soluble starch" (Murphy, 2000). The differences between the ratio and extent of acid hydrolysis of starches have been attributed to differences in granule size, extent of interactions of starch amorphous and crystalline regions of the granule, extent of phosphorylation, number of α-1,6 bonds, amylose–lipid complexes and extent of distribution of the α-1,6 bonds between the amorphous and crystalline domains (Jayakody & Hoover, 2002).

The ultrasound technique is a very effective method for physical disruption of cellular structures, which enables the extraction of intracellular materials, such as starch contained in the cellular matrix (Suslick et al., 1999). The exposure of polymer solutions to

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high intensity ultrasonic radiation appears to result in the reducing of molar mass as a first effect. The hydrolysis occurs preferably near the middle of the chain, without causing important changes in the chemical structure (Madras & Kumar, 2000; Price & Smith, 1993).

Araucaria angustifolia belongs to the Araucariaciae family and is the only native Brazilian conifer species with economic importance (Zandavalli, Dillemburg, & Souza, 2004). The Araucaria Forest is distributed through Brazil (mainly in the Southern region), Chile, Argentina and Paraguay. The exploitation of this tree is over a hundred years, due to the quality of its wood, which is used by the furniture, building, and ship mast industries. Due to irrational extraction, Araucaria is endangered and currently is under environmental protection (Danner, Zanette, & Ribeiro, 2012). The seed of *A. angustifolia*, namely *pinhão*, is considered a source of starch, dietary fiber and magnesium, and its intake produces a low glycemic index (Cordenunsi et al., 2004). However, reports on nutritional and technological aspects of *pinhão* are scarce in the scientific literature.

The perception for nanotechnology applications in the food industry has become more apparent in recent years (Neethirajan & Jayas, 2011). Starch, being a biodegradable natural polymer, is a good candidate for the formation of nanocrystals or nanoparticles. Recent studies have shown that they could be used as fillers to improve mechanical and barrier properties of biocomposites (Le Corre, Bras, & Dufresne, 2010; Song, Thio, & Deng, 2011). *Pinhão* is primarily composed of starch and can be considered as a new source of this polysaccharide (Thys, Noreña, Marczak, Aires, & Cladera-Olivera, 2010). The aim of this study was to produce nanoparticles from *pinhão* starch, by applying ultrasound and acid hydrolysis, and to characterize the obtained nanoparticles.

2. Materials and methods

2.1. Materials

Pinhão seeds used in this work were obtained in Nova Petrópolis (Southern Brazil), harvested in 2011 and 2012. The seeds were selected by visual inspection, washed with tap water and subsequently stored at −18 °C in polyethylene bags.

2.2. Preparation of starch samples

The seeds were manually peeled and sliced. Water was added to a ratio of 1:2 (mL/mL) and the mixture was grinded. The obtained solution was passed through two sieves (0.250 and 0.074 mm) and collected in a recipient that was maintained at 4–5 °C for 40 min. After the precipitation of starch, the supernatant was discarded and the precipitate was washed with distilled water (the amount of water corresponded to the supernatant). This process was repeated four times. The starch obtained by precipitation was subjected to drying in an spray-drier (1.0 LABMAQ LM, São Carlos, Brazil) using inlet temperature of 90 °C, outlet temperature of 55 °C and a flow rate of 0.61 L/min.

For starch treatment by ultrasound, a solution containing 10 g of starch in 500 mL of distilled water was prepared. This mixture was subjected to a probe-type ultrasound (Unique OF S500, São Carlos, Brazil) using a power of 100 W. The sample was exposed to 30 cycles of sonication for 1 min, followed by 1 min stopped to allow the cooling of the sample in the ice bath. The resulting sample was subjected to spray drying as described above.

Starch treatment by acid hydrolysis was performed with a solution prepared with 1.1 g starch diluted in 500 mL of 2 mL/100 mL HCl, maintained for up to 50 days at 22 °C and stirring daily. After the stipulated time, samples were washed 5 times with distilled water at 4–5 °C. The material obtained was subjected to spray drying, as described above.

2.3. Characterization of native starch and starch nanoparticles

The particle size distribution of native starch was determined using the equipment Cilas 1064 Particle Size Analyzer (Cilas, Madison, USA). The size and polydispersity (PDI) of the nanoparticles was determined by dynamic light scattering (DLS; BI-200M goniometer, Brookhaven Instruments, Holtsville, USA). The XRD patterns were obtained on Siemens D-5000 X-ray diffractometer.

The chemical composition was determined according to the AOAC (1990) methodologies. The moisture content was measured considering the weight loss of samples subjected to heating at 105 °C (protocol 945.15). The measurement of water activity was performed by method number 978.18, using the equipment Aqualab S37E (Decagon Devices, Pullman, USA). The determination of crude fiber was determined based on organic insoluble residue insoluble in the samples, after acid and alkaline digestion, using the protocol 962.09. The methodology used to determine the ash content was based on weight loss of the material subjected to the burning furnace at the temperature of 550 °C (protocol 923.03). The procedure used to determine lipid was based on weight loss of the material subjected to extraction in a Soxhlet extractor with petroleum ether. The amount of protein was determined using the Kjeldal method (protocol 2055), which determines the total nitrogenous in the samples.

The determination of starch was conducted according to the AOAC (1990) methodology, based on the Lane–Eynon method (protocol 923-09). The amylose content of the samples was determined by the colorimetric method of McGrance, Cornell, and Rix (1998). The determination of reducing sugars was performed using the 3,5-dinitrolsalycilic acid method (Chaplin, 1986). The method of Eastman and Moore (1984) modified by Spada, Marczak, Tessaro, and Noreña (2012) was used to measure the percentual of solubility. The hygroscopicity was measured based on the method developed by Cai and Corke (2000), and the method proposed by Singh, Sandhu, and Kaur (2004) was used to determine syneresis and paste clarity. Colorimetric analysis was performed by direct reading in the equipment Chroma Meter CR-400 (Minolta, Osaka, Japan), using the CIELab system; the parameters L^* a^* b^* were used to describe the *Chroma* and *Hue* values (Fante & Noreña, 2012).

The morphological analysis of the native and modified starch samples by scanning electron microscopy followed the method developed by Thys et al. (2008), using the microscope JEOL JSM-6060 (JEOL, Tokyo, Japan) operating at 10 kV.

2.4. Statistical analysis

The analyzes were performed in triplicate and expressed as means ± standard error of measurement (s.e.m.). The statistical evaluations were conducted in SAS 9.3 for comparison of means (Tukey test) and Origin 5.0 software for analysis of crystallinity index.

3. Results and discussion

3.1. Particle size of native and modified starch

The average diameter of native starch was 15.34 μm, and the starch particles were mostly in the range of 12–28 μm. This value was similar to that found by Spada et al. 2012 (15.01 μm) and also in agreement with the range of average diameter of 10–25 μm