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Structural, morphological, and physicochemical properties of acetylated high-, medium-, and low-amylose rice starches

Rosana Colussi[a,*], Vania Zanella Pinto[a,b], Shanise Lisie Mello El Halal[a], Nathan Levien Vanier[a,c], Franciene Almeida Villanova[a], Ricardo Marques e Silva[d], Elessandra da Rosa Zavareze[a], Alvaro Renato Guerra Dias[a]

[a] Departamento de Ciência e Tecnologia Agroindustrial, Universidade Federal de Pelotas, 96010-900 Pelotas, RS, Brazil
[b] Department of Food Science, University of Guelph, Ontario N1G 2W1, Canada
[c] Processed Foods Research Unit, WRRC, ARS, United States Department of Agriculture, 800 Buchanan Street, Albany, CA 94710, United States
[d] Laboratório de Microscopia Eletrônica, Curso de Engenharia de Materiais, Universidade Federal de Pelotas, 96015-560 Pelotas, RS, Brazil

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ABSTRACT

The high-, medium-, and low-amylose rice starches were isolated by the alkaline method and acetylated by using acetic anhydride for 10, 30, and 90 min of reaction. The degree of substitution (DS), the Fourier-transformed infrared spectroscopy (FTIR), the X-ray diffractograms, the thermal, morphological, and pasting properties, and the swelling power and solubility of native and acetylated starches were evaluated. The DS of the low-amylose rice starch was higher than the DS of the medium- and the high-amylose rice starches. The introduction of acetyl groups was confirmed by FTIR spectroscopy. The acetylation treatment reduced the crystallinity, the viscosity, the swelling power, and the solubility of rice starch; however, there was an increase in the thermal stability of rice starch modified by acetylation.

1. Introduction

Starch is composed of amylose and amylopectin molecules and the ratio between both molecules varies according to the botanical origin of starch. Starch is the major constituent of rice grains and is considered an important ingredient that has been used in food preparation (Bao, Kong, Xie, & Xu, 2004; Blazek & Gilbert, 2011). Due to the wide range of amylose levels, rice starch has been used as an ingredient in various food and industrial products, such as desserts, bakery products, and alternatives to fats (Puchongkavarin, Varavinit, & Bergthaller, 2005).

Native starches do not always have the desired properties for certain types of processing. In order to achieve suitable functionalities for various industrial applications, starch has been modified by different methods. Basically, there are four kinds of modifications: chemical, physical, genetic, and enzymatic (Kaur, Ariffin, Bhat, & Karim, 2012). Chemical modifications can promote structural changes and introduce new functional groups that affect the physical and chemical properties of starches (Sandhu, Kaur, Singh, & Lim, 2008).

Acetylation converts the hydroxyl groups of the glucose monomers into acetyl groups (Graaf, Broekroelofs, Janssen, & Beenackers, 1995). The acetylated starches are classified into low, intermediate, or high degrees of substitution (DS). Acetylated starches with a low DS (0.01–0.2) may function as film-forming, binding, adhesion, thickening, stabilizing, and texturing agents, and are widely used in a large variety of foods including baked goods, canned pie fillings, sauces, retorted soups, frozen foods, baby foods, salad dressings, and snack foods. Acetylated starches with intermediate DS (0.2–1.5) and high DS (1.5–3) have high solubility in acetone and chloroform and, thus, have been reported as a thermoplastic material (Luo & Shi, 2012).

Acetylation may be performed to improve the physical, chemical, and functional properties of the starch (Xu, Miladinov, & Hanna, 2004) and has been widely studied by several researchers (Bello-Pérez, Agama-Acevedo, Zamudio-Flores, Mendez-Montealvo, & Rodriguez-Ambriz, 2010; Diop, Li, Xie, & Shi, 2011; Garg & Jana, 2011; Huang, Schols, Jin, Sulmann, & Voragen, 2007; Mbougueng, Tenin, Scher, & Tchiégang, 2012). The changes introduced by acetylation depend on the botanical source, the degree of substitution, the ratio between amylose and amylopectin, and the molecular

* Corresponding author. Tel.: +55 53 32757258; fax: +55 53 32757258.
E-mail address: rosana.colussi@yahoo.com.br (R. Colussi).

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structure of the starch. The number of acetyl groups incorporated into the starch molecule during acetylation and the efficiency of the reaction depend on the type of reagent, reagent concentration, pH of reaction, presence of catalyst, reaction time, botanical origin, and size and structure characteristics of the starch granules (Huang et al., 2007; Huber & BeMiller, 2000).

Several researchers have reported the effects of acetylation on potato, corn, and pea starch properties (Chen, Li, Li, & Guo, 2007; Elomaa, 2004; Graaf et al., 1995; Xu & Hanna, 2005; Huang et al., 2007). A recent studied performed by Luo and Shi (2012) showed effects of acetylation on waxy, normal, and high-amylose maize starch properties. There are few studies about the effects of acetylation of starches with a wide range of amylose contents. Sodhi and Singh (2005) studied the characteristics of acetylated starches from different rice cultivars with an amylose content between 7.83% and 18.86%; however, this study did not consider the effects of acetylation reaction time on starch properties. The aim of this study was to evaluate the effects of acetylation with different DS on FTIR spectroscopy, X-ray diffraction, thermal, morphological, and pasting properties, swelling power and solubility of high-, medium-, and low-amylose rice starches.

2. Materials and methods

2.1. Material

Rice grains of cultivars IRGA 417 (high-amylose), IRGA 416 (medium-amylose), and Motti (low-amylose), with amylose contents of 32%, 20%, and 8%, and purity of 99.4%, 99.5% and 99.1%, respectively, were used. Rice samples were dehulled, polished, and ground in order to obtain rice flour. Rice starch was isolated with 0.1% NaOH as described by Wang and Wang (2004). Rice flour was soaked in 0.18% NaOH at a 1:2 (w/v) ratio for 18 h. Then it was blended, passed through a 63 μm screen, and centrifuged at 1200 × *g* for 5 min. The soft top layer was carefully removed, and the underlying starch layer was re-slurried. The starch layer was then washed twice with 0.18% NaOH and centrifuged. The starch layer was washed with distilled water and centrifuged. The starch was then re-slurried and neutralized with 1.0 M HCl to a pH of 6.5 and centrifuged. The neutralized starch was washed with distilled water three times and dried at 40 °C until 7% moisture content was achieved.

2.2. Starch acetylation

The high-, medium-, and low-amylose rice starches were acetylated according to the method described by Mark and Mehltretter (1972), with some modifications. Starch (200 g) was dispersed in 600 ml acetic anhydride in a closed reactor using 2000 rpm for 5 min (RW 20, IKA, Germany). Afterwards, 20 g of 50% NaOH in water were added to the slurry and the temperature was adjusted to 90 °C for 15 min. The reaction was performed for three different times: 10, 30, and 90 min. When the time of reaction from each treatment was achieved, the temperature was reduced to 25 °C and 300 mL of 92.6° Gl ethanol was added to the slurry in order to precipitate starch. The material was centrifuged at 3000 × *g* for 10 min, suspended in alcohol for four times, and finally dried in an oven at 40 °C for 16 h.

2.3. Determination of acetyl percentage (Ac%) and degree of substitution (DS)

The percentage of acetyl groups (Ac%) and the degree of substitution (DS) of the acetylated starches were determined by the titration method described by Wurzburg (1964). Acetylated starch (1 g) was mixed with 50 ml of 75% ethanol in distilled water. The 250 ml flask containing the slurry was covered with aluminum foil and placed in a water bath at 50 °C for 30 min. The samples were then cooled and 40 ml of 0.5 N KOH were added. The slurry was kept under constant stirring at 200 rpm for 72 h. After this period, the alkali excess was titrated with 0.05 N HCl, using phenolphthalein as indicator. The solution was left to stand for 2 h and then any additional alkali, which may have leached from the sample, was titrated. A blank, using the original unmodified starch, was also used.

$$\text{Ac } \% = \frac{[\text{blank} - \text{sample}] \times \text{molarity of HCl} + 0.043 \times 100}{\text{sample weight}} \quad (1)$$

Blank and sample titration volumes were expressed in mL, sample weight was expressed in g. DS is defined as the average number of sites per glucose unit that possess a (Whistler & Daniel, 1995).

$$\text{DS} = \frac{162 \times \text{acetyl}\,\%}{4300 - [42 \times \text{acetyl}\,\%]} \quad (2)$$

2.4. Fourier transform infrared (FTIR) spectroscopy

The infrared spectra of the native and acetylated starches were obtained using a Fourier transform infrared (FTIR) spectrometer Prestige-21, Shimadzu, in the region of 4000–400 cm^{-1}. Pellets were created by mixing the sample with KBr at a ratio of 1:100 (sample:KBr). Ten readings were collected at a resolution of 4 cm^{-1}.

2.5. X-ray diffraction

X-ray diffractograms of the native and acetylated starches were obtained with an XRD-6000 (Shimadzu, Kyoto, Japan) diffractometer. The scanning region of the diffraction ranged from 5 to 40°, with a target voltage of 30 kV, a current of 30 mA, and a scan speed of 1° min^{-1}. The relative crystallinity (RC) of the starch granules was calculated as described by Rabek (1980) using the equation RC (%) = (Ac/(Ac + Aa))*100, where Ac and Aa are the crystalline and amorphous areas, respectively.

2.6. Thermal analysis

Thermal analysis of the starch samples was performed in a TG–DTA apparatus (DTG model 2010, TA Instruments, New Castle, USA). Change in sample weight against temperature (thermogravimetric analysis, TG) and heat released or absorbed in the sample because of exothermic or endothermic activity in the sample (differential thermal analysis, DTA) were measured. Samples (4–8 mg) were heated from 30 °C to 600 °C at a heating rate of 10 °C/min. Nitrogen was used as purge gas at a flow rate of 50 mL/min.

The gelatinization characteristics of starches were determined using differential scanning calorimetry (DSC) (DSC model 2010, TA Instruments, New Castle, USA). Starch samples (approximately 2.5 mg, dry basis) were weighed directly in an aluminum pan, and distilled water was added to obtain a starch–water ratio of 1:3 (w/w). The pan was hermetically sealed and allowed to equilibrate for one hour before analysis. The sample pans were then heated from 30 to 120 °C at a rate of 10 °C/min. An empty pan was used as a reference. The temperature at the onset of gelatinization (T_o), the temperature at peak (T_p), the temperature at the end of gelatinization (T_c) and the enthalpy (ΔH) of gelatinization were determined.

2.7. Morphology of the starch granules

Starch samples with 7% moisture content were initially suspended in acetone to obtain a 1% (w/v) suspension, and the samples were maintained in an ultrasound for 15 min to eliminate the presence of air bubbles. A small quantity of each sample was spread directly onto the surface of the stub and dried in an oven at 32 °C