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Effect of low and high acetylation degree in the morphological, physicochemical and structural characteristics of barley starch

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ABSTRACT

Barley starch was acetylated at two levels (low degree: LD (0.9), and high degree: HD (2.7)) substitution and the morphological, physicochemical and structural of the resultant acetylated barley starch were determined. The acetylated barley starches presented the signal at 1226 cm^{-1} that corresponds to the C–O stretching of acetyl groups. The morphological study showed fusion of starch granules in the acetylated starch with HD. This effect was evident in the pasting test, because the viscoamylograph profile of HD starch showed the absence in peak viscosity, viscosity breakdown and viscosity setback. The peak gelatinization was similar for native and LD and decrease in the HD acetylated starch. The gelatinization enthalpy value showed difference among the samples, indicating that the loss of the ordered double helices more than the crystallinity loss was higher in the HD acetylated barley starch. In the retrogradation test, acetylation affected both retrogradation and enthalpy value, because acetylated barley starch with HD substitution at three storage days had 3.2 j/g and with LD 4.8 j/g. The molecular weight and z-average radius of gyration values decreased due to the acetylation process, indicating depolymerization of starch components as it was evidenced by the increase in short chains level in the acetylated samples.

1. Introduction

Acetylation is one of the most common methods used to stabilize starch. This type of modification is obtained by esterification of native starch with acetic anhydride, vinyl acetate or acetic acid (Jarowenko, 1986; Rutenberg & Solarek, 1984; Wurzburg, 1964). The acetylated starch is classified depending on its degree substitution (DS) in low and high DS. The acetylated starches with low DS (<0.1) are commonly used in the food industry since they confer consistency, texture and stability. Starches with high DS (>1.0) are used as thermoplastic acetate cellulose substitutes (de Graaf, Broekroelofs, Janssen, & Beenackers, 1995; Xu, Miladinov, & Hanna, 2004). Recently, starch acetates (with low and high DS) were suggested for use of biodegradable materials in packing of foods and diverse pharmaceutical applications (Chen, Li, Li, & Guo, 2007; Elomaa et al., 2004; Heins, Kulicke, Käuper, & Thielking, 1998; Santayanon & Wootthikanokkhan, 2003; Xu & Hanna, 2005).

The properties of acetylated starch depend on the botanical starch source, the DS, the amylose/amylopectin ratio and how the molecular structure of starch is modified. In the acetylation reaction, the number of acetyl groups incorporated in the starch molecule, and the rate and efficiency, depends on the reagent type and concentration, pH, presence of catalyst, reaction time, botanical origin of the starch and on the size and structural characteristics of the native granule (Betancur-Ancona, Chel-Guerrero, & Cañizarez-Hernández, 1997; Huang, Schols, Klaver, Jin, & Voragen, 2007; Huber & BeMiller, 2000; Rutenberg & Solarek, 1984).

The characteristics of acetylated starch of diverse botanical sources have been reported (de Graaf et al., 1995; Heins et al., 1998; Santayanon & Wootthikanokkhan, 2003; Elomaa et al., 2004; Xu & Hanna, 2005; Chen et al., 2007; Huang et al., 2007). Barley grain is mainly used in the brewing and malting industries and for animal feeds. Although the interest in barley as a component of food systems is mainly for the potential health benefits of β-glucans (Song & Jane, 2000), barley still presents an important starch source for food and industrial applications. The principal component of barley grains is starch, which consists of two distinct granules: large, disk-shaped of A-granules and small, spherical of B-granules (Ao & Jane, 2007; You & Izydorczyk, 2007).

Diverse studies have been reported on native barley starch (Stevnebø, Sahlström, & Svihus, 2006; Rolland-Sabaté, Colonna, Méndez-Montealvo, & Planchot, 2007; You & Izydorczyk, 2007;

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Rojas, Wahlund, Bergenstahl, & Nilsson, 2008), but very few were found on chemically modified barley starch. The oxidation of barley starch was carried out, determining that barley starch was not as easily oxidized as potato starch due to their different granular structures (Forssell, Hamunen, Autio, Suortti, & Poutanen, 1995). The encapsulation potential of succinylated barley starch was tested. Succinylated barley starch showed the most promising results for flavor encapsulation (Jeon, Vasanthan, Temelli, & Song, 2003). Barley starch was hydrophobically modified with octenyl succinate anhydride and its emulsification and adsorption properties evaluated (Nilsson & Bergenstahl, 2007). However, studies on acetylation of barley starch are scarce. The objective of this study was to investigate the morphological, physicochemical and structural characteristics of acetylated barley starch at two different levels.

2. Materials and methods

2.1. Materials

Barley grains from variety M-16 (*Hordeum sativum jess*) were provided by the Universidad Autónoma del Estado de Hidalgo. *Pseudomonas amyloderamosa* isoamylase (EC 3.2.1.68) was purchased from Hayashibara Biochemical Laboratories, Inc. (Kayama, Japan). All chemicals were of ACS grade.

2.2. Starch isolation

Barley grains were washed with distilled water to eliminate dust and other adhering substances. Barley starch was isolated using the method of Adkins and Greenwood (1966) with modifications. The grains were soaked in a buffer solution of 0.02 mol/L (pH 6.5) sodium acetate containing 0.01 mol/L mercury chloride. The ratio of soaking solution to grains was 2:1 (2 L of soaking solution and 1 kg of grains). The mixture was kept at room temperature (25 ± 2 °C) and was stirred occasionally for 24 h. The solution was drained off, and the softened grains were washed thoroughly with distilled water. Afterwards the grains were homogenized in a blender (Black & Decker, México, D.F. model BLM2350P) at the maximum speed for 1 min. The solution was successively sifted through sieves no. 40 (425 μm), 100 (150 μm), 200 (75 μm), 270 (53 μm) and 325 (45 μm). In each sieve the residue was washed with distilled water until no more starch was released. The final suspension was kept at room temperature (25 ± 2 °C) for 24 h for starch to settle, and then the water was decanted. The starch was re-suspended in a solution of 0.1 mol/L aqueous NaCl: toluene (7:1) and then mixed at room temperature (25 ± 2 °C) and 50 rpm overnight. The suspension was centrifuged at 9000*g* for 15 min, and the supernatant of the toluene phase containing proteins and fat was discarded. The top grayish layer of the precipitate was carefully removed, and the bottom white layer of starch was repeatedly washed with NaCl–toluene solution. Thereafter, the isolated starch was dried at 40 °C for 24 h and stored at room temperature (25 ± 2 °C) in a sealed container.

2.3. Starch acetylation

The modification by acetylation of the starch was done using the modified method suggested by Mark and Mehltretter (1972). Fifteen gram of starch was weighed (dry basis), and mixed with 120 mL of acetic anhydride (Reasol, Millan, Italia) in a reaction flask with two necks, stirring at 200 rpm with a mixer (Ika-Werke, Cincinnati, Oh.), for 5 min; then, 1.65 g of NaOH solution at 50 g/100 g of water were added. The temperature was increased to 120 °C in about 15 min, using an aluminum container with mineral oil, once reaching this temperature, the reaction was stopped at two different reaction times (0.5 and 6 h) obtaining acetylated starch with low and high degree of substitution, respectively. At the end of the time, the flask was taken out of the container till the reaction media reached 50 °C, then, the starch was precipitated with 100 mL of 96 mL/100 mL ethyl alcohol. At once, the solution was centrifuged (2990*g*, 15 min), washing the residue with ethyl alcohol (100 mL) and then with distilled water (100 mL) till most of the acetic anhydride was eliminated. The resulting paste of these washes was dried at 50 °C for 24 h. Finally, the modified starch was milled and sifted in a 50 (US) mesh to obtain a homogeneous particle size.

2.4. Acetyl percentage and degree of substitution

The percentage of acetylation (% acetyl) and degree of substitution (DS) were determined titrimetrically, following the method of Sodhi and Singh (2005). Acetylated starch (1.0 g) was placed in a 250 ml flask and 50 ml of 75 mL/100 mL ethanol in distilled water were added. The loosely stopper flask was agitated, warmed to 50 °C for 30 min, cooled and 40 ml of 0.5 mol/L KOH were added. The excess alkali was back-titrated with 0.5 mol/L HCl using phenolphthalein as an indicator. A blank, using the original unmodified starch, was also used.

$$\text{Acetyl\%} = \frac{[(\text{Blank(ml)} - \text{Sample(ml)}) \times \text{Molarity of HCl} \times 0.043 \times 100]}{\text{Sample weight(g)}}$$

Degree of substitution is defined as the average number of sites per glucose unit that possess a substituent group.

$$\text{DS} = \frac{(162 \times \text{Acetyl(\%)})}{[4300 - (42 \times \text{Acetyl(\%)})]}$$

2.5. Fourier transform infrared (FTIR) spectroscopy

IR spectra of native and acetylated starch were measured using KBr's method of Pushpamalar, Langford, Ahmad, and Lim (2006). The dry sample was blended with KBr in a ratio starch/KBr 1:4.The blend was pressed to obtain a pellet and introduced in the spectrometer (MIDAC, prospect 269, Costa Mesa, CA, USA). Each spectrum was analyzed in the range of resolution from 400 to 4000 cm^{-1} and 16 scans were collected.

2.6. Scanning electron microscopy (SEM)

The morphology of starch granules was examined using SEM. The samples were fixed to a conductive tape of copper of double glue; which was covered with a layer of coal of 20 nm of thickness. It was deposited at vacuum with an evaporator in a JEOL JSMP 100 (Tokyo, Japan) electron microscope. Later on, samples were covered in the ionizer metals JEOL with a gold layer of a thickness of 50 nm.

2.7. X-ray diffraction

The X-ray patterns of starches were obtained with a copper anode X-ray tube using a Philips Analytical diffractometer (Philips, Almelo, The Netherlands). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 5° to 45° at 0.1° step size with a count time of 2 s. The starch samples were equilibrated in a 100% relative humidity chamber for 24 h at room temperature (25 ± 2 °C). The total area and amorphous area were measured with a planimeter. A straight