



Effect of drying method and hydrothermal treatment of pregelatinized Hylon VII starch on resistant starch content

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ABSTRACT

The effect of pregelatinization and drying method in resistant starch (RS) content of high amylose starch (Hylon VII, 70% amylose content) was studied. Starch was subjected to autoclaving pre-gelatinization (40% starch suspension w/w, 121 °C) for 30 min. Oven-drying (OD) at 40 °C for 24 h and freeze-drying (FD) methods were used. Subsequently, hydrothermal treatment (HT) with 40% water content at 100 °C for 3, 12 and 24 h was applied to dried samples. Pre-gelatinization decreased the RS content by about 25% with respect to the Hylon VII starch. The drying method affected the RS content, with OD increasing and FD decreasing the RS content. HT time affected positively the RS content for OD starch, whilst the opposite effect was obtained for FD starch. XRD analysis revealed that B-type crystallinity of native starch was modified by drying and HT. Weak linear correlation ($R^2 = 0.69$) between RS content and relative crystallinity was found. However, strong exponential correlation ($R^2 = 0.96$) between RS content and short-range ordering quantified by FTIR measurements were found. This suggests that RS is close related to formation of double-helix and amylose-lipid complex (evidenced by the XRD peak at $2\theta = 20^\circ$) during drying and heat treatment of starch.

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1. Introduction

Industrial high-amylose starches ($\geq 50\%$ amylose) are commonly used for fabrication of low-moisture food products containing high fractions of resistant starch (RS). The high amylose content limits the swelling capacity as compared to native starches with lower amylose content (0–30%), positioning modified starches as stabilizers of food matrices. Besides, cooking of high amylose starches leads to low gelatinization degrees while leaving the high RS content as dietary fiber. In practice, high-amylose starches with high RS fraction is produced by heat treatments, where starch with excess water content is subjected to heating by high-pressure autoclaving process at temperature of 121 °C for 20–30 min. This process breaks down the granular starch structure to produce solubilised and non-solubilised fractions of amylose or/and amylopectin structures in the form of gelatinized starch (Chung, Liu, & Hoover, 2009; Miles, Morris, Orford, & Ring, 1985; Thompson, 2000). Afterwards, the cooling down of gelatinized starch promotes the rapid re-association of the leached amylose molecules, whereas

amylopectin molecules require longer times (Arcila & Rose, 2015; Juansang, Puttanlek, Rungsardthong, Pancha-arnon, & Uttapap, 2012; Lim, Chang, & Chung, 2001; Shi & Seib, 1992).

Resistant starch can be formed by different methods. Heating-cooling cycles have shown to increase the RS content of high-amylose starch (Arcila & Rose, 2015; Harder, Khol-Parisini, & Zebeli, 2015; Seib & Woo, 1999; Sievert & Pomeranz, 1989). Starch with high content of amylose (~70%) is commonly recommended since after heating-cooling cycles, re-arrangement of amylose chains is produced faster than in “conventional” starch (25–30%), in a structure that is resistant to enzymatic hydrolysis (Thompson, 2000). The repeating heating of starch can lead to disorganization of amylose and amylopectin and eventually partial breaking of them. The subsequent cooling down of the smaller starch chains can lead to the formation of double-helix aggregates. In turn, this effect favours the formation of denser but smaller crystals, increasing in this way the RS content by limiting the accessibility of digestive enzymes (Guraya, James, & Champagne, 2001; Zeng, Zhu, Chen, Gao, & Yu, 2016). On the other hand, the retrogradation of starch chains leads also to the formation of RS fraction (Zhou, Chung, Kim, & Lim, 2013). The process involves nucleation by intra-molecular initiation of order chain segments helical,

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propagation by growth of crystals from the nuclei by association of chain segments, and maturation by crystal perfection (Kiatpongjar, Tongta, Rolland-Sabaté, & Buléon, 2015; Slade & Levine, 1991). The selection of any of the above-mentioned methods to increase the RS content depends mainly on the specific final purpose of the RS-rich powder (e.g., ingredient in cooked products or ready-to-eat products).

In general, native and modified starches contain a fraction that is slowly digestible, producing sustained glucose supply to the blood flow. This sustained liberation of glucose along the small intestine contributes to different health benefits, mainly for reducing the adverse effects of the so-called metabolic syndrome (Lee, Bello-Pérez, Lin, Kim, & Hamaker, 2013). Hydrothermal treatments are intended to modify the starch digestibility, focusing mainly on obtaining slowly digestible fractions. In such process, native starch is hydrated at specific moisture and stored (typically at 100 °C for 12–24 h) to promote the re-arrangement of starch chains, leading to the generation of fractions that are slowly digestible and/or resistant to hydrolysis by digestive enzymes. The resulting starch structure is slowly digestible and is produced in the nucleation step, whilst the structure resistant to hydrolysis is produced during propagation and maturation steps (Lee, Shin, Kim, Choi, & Moon, 2011; Shin, Kim, Ha, Lee, & Moon, 2005; Son Trinh, Joo Lee, Jun Choi, & Wha Moon, 2012).

The formation of rapidly (RDS) and slowly (SDS) digestible starch fractions, as well as the development of RS components after hydrothermal starch treatments are issues that have not been fully clarified. In general, it is accepted that the relative amounts of these starch fractions depends strongly of the reorganization mechanisms taking place after gelatinization. Recently, Zeng et al. (2016) showed that the drying method has a determinant effect in the digestibility of short chain amylose crystals. However, the analysis of whole starch granules subjected to drying and HT after pre-gelatinization and gelatinization requires some attention. Results in this line should provide valuable insights for the design of proper processes oriented to obtain starch matrices with specified SDS and RS fractions. In this regard, the aim of this study was to evaluate the effect of the drying method of pre-gelatinized high-amylose starch and the change in its digestibility produced by hydrothermal treatment.

2. Materials and methods

2.1. Materials

Hylum VII starch (HVII, 70% amylose content) was supplied by Ingredion S.A. de C.V. (Tlalnepantla, Mexico). Amyloglucosidase (140 AGU/ml, Megazyme International, Ireland) and Pancreatin (Sigma-Aldrich, P-7545, Saint Louis, MO, USA) were used for digestibility tests. All experiments were conducted with deionized water.

2.2. Sample preparation

High amylose maize starch samples were pre-gelatinized by autoclaving (40% starch suspension w/w, at 121 °C for 30 min). Complete gelatinization is not required for hydrothermal treatment. The too low moisture content (about 80%) limits the swelling of starch granules and the leaking out of amylose chains. Instead, pre-gelatinization under limited moisture content is the preferred condition to achieve heat moisture treatment. Two drying methods were considered: oven-dried (OD) at 40 °C for 24 h to give moisture content < 2.0%, and freeze-dried (FD) to obtain moisture content < 1.0%. Oven-drying at 40 °C produces restricted mobility of starch chains partially disorganized during pre-gelatinization,

avoiding reorganization (retrogradation) of starch. In diverse preliminary studies of starch modification, we used drying at 40 °C without important changes in the starch structure. This temperature is below of the range (around 45–50 °C) at which reorganized starch is basically amorphous (Paredes-López, Bello-Pérez, & López, 1994). Hydrothermal treatment (HT) was applied to dried starch samples, which were weighed into a glass container, and the moisture content was adjusted to 40% by adding water. The glass container was sealed and allowed to stand at room temperature for 24 h to reach moisture equilibrium. Then, the sample was stored at 100 °C for 3, 12 and 24 h in an air-drying oven. The prepared samples were dried in an air-drying oven at 40 °C for 5 h to obtain a final moisture content < 1.0%. All samples were ground milled and passed through mesh n° 40.

2.3. Scanning electron microscopy

Starch samples were fixed to conductive tape mounted on a brass disc. Samples were then coated with gold using Polaron E5100 (Polaron Equipment Ltd., Watford, UK). Images of starches were captured using a scanning electronic microscope model JSM-5800LV (JEOL, Tokyo, Japan).

2.4. X-ray diffraction

X-ray diffraction patterns were obtained using a Rigaku diffractometer, model MiniFlex600 (Rigaku, Corporation Japan). The moisture content affects the XRD measurements of the starch samples. To avoid biased results in XRD measurements, the moisture content of the samples was adjusted at 86% by storing the samples in a sealed desiccator for 7 days at room temperature (Song & Jane, 2000). The scanned diffraction angle (2θ) range was 2–60° and the scanning speed was 2°/min.

2.5. FTIR spectroscopy

The Fourier transform infrared (FTIR) spectra of films were obtained by means of a Perkin Elmer spectrophotometer (Spectrum 100, Perkin Elmer, Waltham, MA, USA) equipped with a crystal diamond universal ATR sampling accessory and mirror velocity of 0.4 cm·s⁻¹. During measurements, the sample was in contact with the universal diamond ATR top-plate. A spectrum of the empty cell was used for correcting by background effects. For each sample, the spectrum represented an average of three scans with resolution of 1 cm⁻¹. Also, spectra were baseline-corrected at 1200–900 cm⁻¹ by drawing a straight line below the recorded signal.

2.6. Starch digestibility

In vitro starch digestibility was determined following the methods described by Englyst, Kingman, and Cummings (1992) with modifications. The sample (200 mg d.b.) was mixed with 6 mL of deionized water and 1 mL of 0.5 M sodium acetate (pH = 5.2) in a glass tube, the mixture was adjusted to 37 °C in a water bath. An enzyme solution (2 mL per 200 mg d.b. of starch) containing pancreatin (2.0 mg/mL; 25 USP units of amylase activity per gram) and amyloglucosidase (28.0 U/mL; 20 USP units of amylase activity per gram) were added to tubes in intervals of 1 min at 37 °C in 0.2 M acetate buffer (pH 6.0). After 20 and 120 min, 50 µL aliquots were removed and placed into a tube containing 950 µL deionized water to stop the reaction by boiling. Glucose contents of the hydrolyzed starch were determined using a Glucose Oxidase/Peroxidase (GOPOD) kit (Megazyme, Wicklow, Ireland), and identified as rapidly digestible starch (RDS) the amount digested in 20 min, SDS the amount digested between 20 and

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