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Complex formation between starch and stearic acid: Effect of enzymatic debranching for starch

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ABSTRACT

Effect of debranching for a high-amylose starch (~70% amylose) on its V-complex formation with stearic acid was examined. Gel-permeation chromatograms showed that amylopectin was degraded to smaller molecules as the debranching time increased from 6 to 24 h. Increased formation of debranched starch/stearic acid complexes (recovery yield of stearic acid from 45.17 to 89.31% and starch from 39.92 to 55.43%) was observed with increased debranching time (from 6 to 24 h) and complexation time (from 6 to 24 h). The X-ray diffraction patterns of the debranched starch/stearic acid complexes displayed a mixture of B-type and V-type patterns, with 2θ peaks at 7.6°, 13.1°, 17.2°, 20°, 21.6°, and 23.4°. The melting temperature and enthalpy changes of the debranched starch/stearic acid complexes were gradually enhanced with increasing debranching time. These results suggest that starch can be modified by debranching to produce a significant amount of debranched starch/stearic acid complexes.

1. Introduction

Fatty acids are considerable part of lipids, one of the primary component of biological matter, playing numerous roles in biological system. They are also vital energy substrates covering almost 30% of total energy intake for humans (Tvrzicka, Kremmyda, Stankova, & Ales, 2011). Because of their biological functions, some fatty acids are widely used in foods, pharmaceuticals, and cosmetics (Duric, Sivanesan, & Bakovic, 2012; Zabar, Lesmes, Katz, Shimoni, & Bianco-Peled, 2010). However, most fatty acids are sensitive to heat and light, and easily oxidized during food processing, storage, and consumption, which restricts their application in food and non-food industries (Cheng, Luo, Li, & Fu, 2015; Zabar et al., 2010). To overcome those draw-backs, various encapsulation techniques such as formation of liposomes or complexes have been widely studied and are currently utilized.

Starch is a biodegradable and renewable biopolymer; it is widely used in food and non-food industries as a functional ingredients. It often behaves as a gelling, binding, stabilizing and thickening agent (Reddy, Suriya, Vidya, Vijina, & Haripriya, 2015; Singh, Singh, Kaur, Sodhi, & Gill, 2003). Starch consists of two major glucans: amylose of a primarily linear molecule with limited branches and amylopectin with a highly branched structure (Arijaje & Wang, 2016; Reddy, Kimi, & Haripriya, 2016). Amylose can undergo a conformational change to form a single left-handed helical structure with a hydrophobic cavity that can react with numerous hydrophobic ligands, including alcohols, drugs, fatty acids, iodine, and flavors (Arijaje & Wang, 2016; Chang, He, & Huang, 2013b; Seo, Kim, & Lim, 2015; Wulff, Avgenaki, & Guzmann, 2005). Hence, amylose can be used as an encapsulating agent for various hydrophobic molecules forming inclusion complexes. Meanwhile, the amylose single helices can be arranged to form V-type crystal complexes (Biais, Le Bail, Robert, Pontoire, & Buleon, 2006; Chang, He, & Huang, 2013a). Upon forming an inclusion complex, the guest molecule may be stabilized and protected from oxidative degradation often caused by light and heat (Wulff et al., 2005; Yang, Gu, & Zhang, 2009). Further, an inclusion complex can also serve as a vehicle for controlled release of the guest molecule (Gelders, Goesaert, & Delcour, 2006; Yang et al., 2009).

In recent years, inclusion complexes are used to enhance the functionality of starchy food to develop novel starches (Hasjim et al., 2010; Zhang, Huang, Luo, & Fu, 2012) and used as a delivery system to protect volatile and sensitive ligands, such as poly-unsaturated fatty acids (Chang et al., 2013a; Seo et al., 2015). The characteristics of amylose-fatty acid complexes are commonly influenced by the purity of the starting materials and complexation reaction conditions. However, the stability, complexation, and yield of amylose-fatty acid complexes are influenced by numerous factors, especially the amylose chain length, fatty acid chain length and saturation, reaction time, pH, and incubation temperature (Gelders, Vanderstukken, Goesaert, & Delcour, 2004; Karkalas, Ma, Morrison, & Pethrick, 1995; Yang et al., 2009). Numerous studies on amylose-fatty acid complexes focus majorly on the preparation and characterization of these inclusion complexes. However, limited research has been pursued to improve the formation of V-complexes using debranched high-amylose starch.

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Debranching has been applied to enhance the ability of complex formation using starch. Hasjim et al. (2010) reported a novel resistant starch prepared by debranching high-amylose maize starch with isoamylase and complexing with palmitic acid. Zhang et al. (2012) reported that the prolonged debranching process with high-amylose maize starches using pullulanase (24 h debranching) significantly improved the formation of V-complexes with lauric acid. Cheng et al. (2015) debranched a potato starch using pullulanase, and carried out a complex formation of phosphatidylcholine with the debranched starch. They stated that debranching of starch could significantly improve the formation and stability of V-complexes. Recently, Arijaje and Wang (2016) reported that debranched potato, corn, and high-amylose maize starches using isoamylase alone or combined with a β-amylase formed complexes with stearic acid with an improvement of the formation of starch-fatty acid complexes.

In the present study, defatted high-amylose maize starch was debranched using pullulanase, and then the debranched starch was used to form the V-complex with stearic acid. The effect of debranching level on the formation efficiency and physico-chemical characteristics of the complexes with stearic acid was examined.

2. Materials and methods

2.1. Materials

High-amylose maize starch (~70% amylose) was provided by Ingredion Inc. (Westchester, IL, USA). *Bacillus acidopullulytics* pullulanase (EC 232-983-9P; ≥ 400U/mL) and stearic acid were purchased from Sigma Aldrich Company (St. Louis, Mo, USA). All other chemicals and reagents used in this study were of analytical grade.

2.2. Preparation of debranched starch

Accurately weighed defatted high-amylose starch (400 mg, dry solids) was mixed with an acetate buffer (8 mL, 0.2 M, pH 4.4) to make a 5% dispersion. The starch dispersion was then autoclaved at 121 °C for 30 min for complete gelatinization. The temperature of starch slurry was adjusted to 60 °C, and then debranching was carried out by adding a pullulanase (40 U/g starch), and incubating the starch-enzyme mixture for 6, 12, and 24 h. After the debranching reaction, the pH of dispersion was adjusted to 7 by adding 0.1 M NaOH solution, was adjusted to a final volume of 40 mL by adding deionized water, and then the dispersion was heated for 15 min in a boiling water bath to inactivate the enzyme. After neutralization, the native (without debranching) and debranched-starch dispersion temperatures were adjusted to 60 °C and stored at the same temperature for further use.

2.3. Preparation of starch-stearic acid complexes

To prepare the inclusion complexes between debranched starch and stearic acid, the native and debranched-starch dispersions which had been freshly prepared were mixed with an alcoholic solution of stearic acid (20 mg stearic acid in 2 mL absolute ethanol) by magnetically stirring at 90 °C. The duration for complex formation at 90 °C varied from varied between 6 and 24 h. After the complex formation reaction, the mixtures were cooled to 25 °C and stirred (1000 rpm) for 3 h at 25 °C. The complexes were recovered by centrifuging the reaction mixtures at 25,000 *g* for 30 min, and successively washed with hot deionized water (50 mL, 80 °C) and diethyl ether (50 mL). The complex residues were then freeze dried, milled using a mortar and pestle, and stored at 4 °C for further analysis. The control sample was prepared according to the same procedure without adding the pullulanase.

2.4. Gel permeation chromatography (GPC)

The molecular size distribution of debranched starches was analyzed using a GPC. The mobile phase was 0.02% sodium azide, which had been filtered through a 0.2-μm membrane filter (Millipore Co., USA) and degassed by ultrasonication. The debranched starch samples (50 mg) were wetted with water (0.5 mL) and then dispersed in dimethyl sulfoxide (2.5 mL). The starch dispersions were then heated in a shaking boiling-water bath for 30 min, centrifuged at 5000 *g* for 10 min, and then diluted by adding deionized water (15 mL). The starch dispersion was filtered through a 0.2-μm membrane filter and then injected into the GPC through a 0.1 mL loop (Rheodyne 7072, Cotati, CA). The system consisted of a column (Superdex 75 HR, 10 mm × 300 mm, Amersham Pharmacia, Uppsala, Sweden), a pump (P2000, Spectra System, San Jose, CA), and a refractive index detector (Shodex RI-71, Tokyo, Japan). The flow rate was 0.4 mL/min.

2.5. Analysis of starch and stearic acid in complexes

The starch-stearic acid complex (200 mg, wet basis) was dispersed in 0.5 M methanolic NaOH solution (10 mL) and the dispersion was heated with continuous stirring in a boiling-water bath for 30 min. After incubation, 10 mL of 14% boron trifluoride (BF_3) was added to the solution and continuously heated for 10 min to cause methyl esterification. Extraction of the stearic acid methyl esters was then achieved by adding 5 mL of *n*-hexane and heating for 2 min. After cooling, the mixture was mixed with 30 mL of an aqueous saturated NaCl solution, and the *n*-hexane layer was collected for analysis. The amount of stearic acid in the collected *n*-hexane layer was analyzed using gas chromatography (Hewlett–Packard GCD system HP 5890; Avondale, PA, USA) using a Capillary Omegawax™ 320 column (30 m × 0.32 mm I.D.; Supelco, Bellefonte, PA). The chromatograph temperature was programed initially at 195 °C for 4 min and then ramped to 250 °C at a rate of 4 °C/min. The stearic acid was methylated, and the concentrations of stearic acid were determined from a standard curve prepared using a known concentration of stearic acid.

The amount of starch in the complexes was also measured using a standard phenol-sulfuric acid method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). The complex yields (or percent recovery) of stearic acid and starch were calculated as the percentages of stearic acid and starch in the complexes based on the initial amounts added to the reaction mixtures.

2.6. Thermal transition

The thermal transition of the complexes were analyzed using differential scanning calorimetry (DSC-6100, Seiko Instruments Inc., Chiba, Japan). An empty aluminum DSC pan was used as the reference, and indium was used for calibration. The starch/stearic acid complex (~3.0 mg, dry solids) was placed in the aluminum DSC pan, and 7 μL of deionized water was added. The DSC pans were then hermetically sealed and equilibrated for 2 h at 4 °C. Then, the equilibrated DSC pans containing the samples were directly heated from 30 °C to 160 °C at a rate of 5 °C/min. The calculated values for the gelatinization temperatures (onset, T_0; peak, T_p; conclusion, T_c) and enthalpy (ΔH) were recorded for the crystalline melting of the complexes using the EXSTAR 6000 thermal analysis system (Seiko, Chiba, Japan). All analyses were done in triplicate and the data were reported as the mean values.

2.7. X-ray diffraction analysis

The formation of starch/stearic acid complexes was determined by measuring the X-ray diffraction (XRD) patterns obtained from the freeze-dried samples using a powder X-ray diffractometry (Philips X'PERT MPD, Almelo, Netherlands). The instrument was equipped with Cu-Kα radiation at a target voltage of 40 kV and a target current of 30 mA. Approximately 200 mg of freeze-dried samples were placed in special sample holders, and the samples were scanned over the 2θ angular range from 3 to 30° at a speed of 1.5°/min.