Food Chemistry 217 (2017) 9–17

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of chemical and enzymatic modifications on starch-linoleic acid complex formation

Emily Oluwaseun Arijaje, Ya-Jane Wang *

Department of Food Science, University of Arkansas, 2650 N. Young Avenue, Fayetteville, AR 72704, USA

ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form 11 August 2016
Accepted 22 August 2016
Available online 23 August 2016

Keywords:
Acetylation
Starch inclusion complex
Linoleic acid
β-Amylase
Soluble complex
Insoluble complex

ABSTRACT

This study investigated the complexation yield and physicochemical properties of soluble and insoluble starch complexes with linoleic acid when a β-amylase treatment was applied to acetylated and debranched potato starch. The degree of acetylation was generally higher in the soluble complexes than in the insoluble ones. The insoluble complexes from the acetylated starch displayed the V-type pattern, whereas, the soluble complexes displayed a mixture of either the A-/V-type or the B-/V-type pattern. Acetylation decreased onset and peak melting temperatures for the insoluble complexes, whereas no melting endotherm was observed in the soluble complexes. Acetylation substantially increased the amount of complexed linoleic acid in the insoluble complexes, but had little positive effect on the formation of the soluble complexes. The β-amylase treatment significantly increased the complexed linoleic content in both soluble and insoluble complexes for the low acetylated starch, but not for the high acetylated starch.

1. Introduction

Inclusion complexes using starch as the complexing agent have been extensively studied (Biliaderis, Page, & Maurice, 1986; Cohen, Orlova, Kovalev, Ungar, & Shimoni, 2008; Holm et al., 1983; Mikus, Hixon, & Rundle, 1946; Oguchi, Yamasato, Limmatvapirat, Yonemochi, & Yamamoto, 1998; Putseys, Derde, Lamberts, Goesaert, & Delcour, 2009; Putseys, Lamberts, & Delcour, 2010). Starch is mainly composed of two components, an essentially linear amylose molecule and a highly branched amylopectin molecule. Both amylose and amylopectin are polymers of glucose, and the linear portion of the chains may form a helical structure with a hydrophobic cavity that can include various hydrophobic ligands, such as iodine (Bailey & Whelan, 1961), alcohols (Schoch, 1942), lipids (Biliaderis et al., 1986; Godet, Bizot, & Buleon, 1995; Lay Ma, Floros, & Ziegler, 2011; Mikus et al., 1946), flavors (Wulff, Avgenaki, & Guzmann, 2005), and drugs (Oguchi et al., 1998). When included in the starch helical cavity, the molecules are stabilized and protected from oxidation (Yang, Gu, & Zhang, 2009), enzyme hydrolysis (Lalush, Bar, Zakaria, Eichler, & Shimoni, 2005), and high temperature (Evans, 1986). Nevertheless, starch inclusion complexes are usually crystalline in nature and become insoluble in aqueous solutions (Schoch & Williams, 1944).

The complexation of starch and fatty acids has been reported and is influenced by many factors, such as starch chain length (Godet, Buleon, Trans, & Colonna, 1993; Rutschmann, Heiniger, Pliska, & Solms, 1989), incubation temperature (Biliaderis & Seneviratne, 1990; Evans, 1986; Karkalas, Ma, Morrison, & Pethrick, 1995) and incubation pH (Hahn & Hood, 1987). The thermal stability of starch-fatty acid complexes increases with an increase in fatty acid chain length and decreases with an increase in fatty acid unsaturation (Eliasson & Krog, 1985; Tufvesson, Wahlgren, & Eliasson, 2003a). Additionally, saturated fatty acids have been reported to form more stable complexes with starch compared to unsaturated fatty acids or mono or di-acylglycerols (Galloway, Biliaderis, & Stanley, 1989; Tufvesson, Wahlgren, & Eliasson, 2003b; Tufvesson et al., 2003a). Most studies on starch inclusion complexes focused on the formation of insoluble complexes, and only a few studies have investigated soluble starch complexes (Arijaje & Wang, 2015; Arijaje, Wang, Shinn, Shah, & Proctor, 2014; Wulff, Steinert, & Holler, 1998).

Recently, Arijaje et al. (2014), and Arijaje and Wang (2015) demonstrated that the formation of soluble complexes between starch and stearic (C18:0) and oleic acid (C18:1) could be significantly increased when starch was acetylated and debranched. The acetyl groups hindered starch retrogradation and encouraged its complexing with fatty acids. Low-acetylated debranched starch with a degree of substitution (DS) ~0.04 increased the amount of complexed stearic and oleic acid in both soluble and insoluble

* Corresponding author.
E-mail address: yjwang@uark.edu (Y.-J. Wang).

http://dx.doi.org/10.1016/j.foodchem.2016.08.072
0308-8146/

complexes. High-acetylated starch (DS 0.08–0.09) also increased the amount of complexed stearic and oleic acid in the soluble complexes, but decreased the yield and complexed stearic and oleic acid in the insoluble complexes when compared with low-acetylated starch. Stearic acid was complexed to a greater extent than oleic acid because it is saturated and less soluble in water (Hahn & Hood, 1987). When a β-amylase treatment was incorporated into unacetylated and low-acetylated debranched starch, the amount of complexed stearic and oleic acid generally increased in both soluble and insoluble complexes. However when the β-amylase treatment was combined with high acetylated debranched starch, there was no consistent trend for either stearic or oleic acid (Arijaje & Wang, 2015; Arijaje et al., 2014).

This work continued our two previous studies (Arijaje & Wang, 2015; Arijaje et al., 2014) to investigate the impacts of fatty acid structure on the formation and properties of inclusion complexes with modified starch. Although the kinked structure in linoleic acid (C18:2) has been reported to present steric hindrance in the native starch helix, leading to only partial inclusion (Yamada, Kato, Tamaki, Teranishi, & Hisamatsu, 1998), it is not clear how significant the impact that acetyl groups and degree of polymerization (DP) would have on starch complexation with linoleic acid. Therefore this work was carried out to elucidate their effects.

2. Materials and methods

2.1. Materials

Potato starch (~24% amylose) was obtained from Penford Food Ingredients (Centennial, CO) and used without further treatment. Isoamylase from *Pseudomonas* sp (specific activity 280 units/mg protein), pullulanase from *Klebsiella planticola* (specific activity 34 units/mg protein) and β-amylase from *Bacillus cereus* (specific activity 2660 units/mg protein) were purchased from Megazyme International Ireland Ltd (Wicklow, Ireland). Linoleic acid was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were of ACS grade.

2.2. Acetylation of starch

Starch was acetylated as previously described by Wang and Wang (2002). The acetylation level of starches were determined according to the method of McComb and McCready (1957), and the degree of substitution (DS) was determined according to Wurzburg (1986).

2.3. Enzymatic modification of starch

2.3.1. Debranching

Starch was debranched as previously described by Arijaje et al. (2014). Potato starch (3.75%, w/v, starch db) was gelatinized in a boiling water bath for 1 h with constant stirring. Then the temperature of the solution was equilibrated to 45 °C and the pH adjusted to 5.0 with 0.5 M HCl. To the starch solution, isoamylase and pullulanase (1.33% v/w starch db) each was added, and incubated at 50 °C with constant stirring for 48 h. The starch was recovered with 4-fold volume of pure ethanol, centrifuged at 7000*g* for 10 min, dried at 40 °C for 48 h with a forced air oven (VWR, Cornelius, OR) and ground into powder using a UDY cyclone mill (UDY Corp., Ft. Collins, CO) fitted with a 0.5-mm screen.

2.3.2. β-Amylase treatment

A portion of the debranched starch was subjected to an additional β-amylase hydrolysis to reduce DP. After debranching for 48 h, the starch slurry pH was adjusted to 6.5 with 0.5 M NaOH, and incubated with 0.5% (v/w starch db) β-amylase at 40 °C for 4 h. The enzyme reaction was terminated by boiling for 15 min. The β-amylase-treated starch was recovered as previously described.

2.4. Complexation of starch and linoleic acid

The starch solution (3.75% w/v), debranched or debranched and β-amylase treated, was adjusted to pH 7.0 with 0.5 N HCl, preheated to 80 °C, and mixed with 1 g of linoleic acid that was dissolved in warm 95% ethanol. The mixture was maintained at 80 °C for 30 min with continuous stirring at 400 rpm with the aid of a Barnstead Super-Nuova stirring hot plate (model SP131825; Barnstead/Thermolyne, Dubuque, IA) to allow for efficient complexation, and then the temperature was maintained at 45 °C overnight with continuous stirring. The resulting starch-linoleic acid mixture was centrifuged at 7000*g* for 10 min, from which the precipitate, "*insoluble complex*", was obtained, whereas, the "*soluble complex*" was recovered by precipitating the supernatant with 4-fold volume of pure ethanol. Any uncomplexed linoleic acid was removed from both the *insoluble* and *soluble* complexes by rotating complexes in excess 95% ethanol using a Labquake Rotisserie Shaker (Barnstead/Thermolyne, Dubuque, IA) at room temperature for 2 h, centrifuged at 7000*g* for 10 min, dried at 40 °C for 48 h, milled using a mortar and pestle, sieved through a 250-mm sieve, and stored in the freezer at −8 °C for further analysis.

2.5. Characterization of starch structure

The DPs of debranched unacetylated and acetylated starch without or with β-amylase treatment were determined by recovering starches after complexation with linoleic acid. The recovered starches were characterized using a high-performance size-exclusion chromatography (HPSEC) system (Waters Corp., Milford, MA). Starch (10 mg) was dissolved in 5 mL of 90% DMSO, boiled for 1 h and filtered through a 5.0-μm filter prior to injection into the HPSEC system. The HPSEC system consisted of a guard column (OHpak SB-G, 500 mm × 6.0 mm i.d.), two Shodex columns (OHpak KB-804 and KB-802, both 300 mm × 8 mm i.d.), a 200-μL injector valve (Model 7725i, Rheodyne, Cotati, CA), an inline degasser, a model 515 HPLC pump, and a model 2414 refractive index detector. The mobile phase of 0.1 M sodium nitrate with 0.02% sodium azide was eluted at a flow rate of 0.6 mL/min. The temperature of column was maintained at 60 °C and the detector at 40 °C. Dextran standards of molecular weight of 5200, 11,600, 23,800, 48,600, 273,000 and 410,000 g/mol from Waters Corp. (Milford, MA,) and 1,050,000 g/mol from Sigma-Aldrich (St. Louis, MO) were used to establish the calibration curve.

The amylopectin chain length distributions were characterized by high-performance anion-exchange chromatography equipped with pulsed amperometric detection (HPAEC-PAD) according to the method of Wong and Jane (1995). The chains were divided into DP ranges and classified as follows: A chains (DP 6–12), B1 chains (DP 13–24), B2 chains (DP 25–36), and B3+ chains (DP 37+) (Hanashiro, Abe, & Hizukuri, 1996). The average chain length was calculated as the cumulative sum of the product of DP and percentage relative areas for all the identified peaks.

2.6. Hydrolysis of complexes and linoleic acid analysis

Hydrolysis of complexes was carried out as described by Arijaje et al. (2014). Soluble or insoluble complex (100 mg) was added with 10 mL of 1 M HCl and heated with continuous stirring in a boiling water bath for 1 h. After the complex mixture was cooled to room temperature, 5 mL hexane were added and the solution was rotated on the rotary shaker for 1 h. The hexane layer with