Food Hydrocolloids 79 (2018) 491–497

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Distribution of acetyl groups in acetylated waxy maize starches prepared in aqueous solution with two different alkaline concentrations

Zhi-Gang Luo [a, b, c], Yong-Cheng Shi [b, *]

[a] Carbohydrate Lab, School of Food Science and Engineering, South China University of Technology, Guangzhou, 510640, China
[b] Department of Grain Science and Industry, Kansas State University, Manhattan, KS, 66506, USA
[c] Overseas Expertise Introduction Center for Discipline Innovation of Food Nutrition and Human Health (111 Center), Guangzhou, China

ARTICLE INFO

Article history:
Received 4 November 2017
Received in revised form
11 January 2018
Accepted 15 January 2018

Keywords:
Waxy maize starch
Acetylation
Amylopectin

ABSTRACT

Waxy maize starch acetates with the same degree of substitution (DS) were produced in aqueous medium with 3% or 20% (w/w) sodium hydroxide. The distribution of acetyl groups on amylopectin was studied by enzymatic hydrolysis in combination with chromatographic techniques. Product prepared with 20% NaOH was more resistant to β-amylase but less resistant to α-amylase and isoamylase, indicating more acetyl groups distributed over the external chains or located closer to the non-reducing end of external chains. Settling volume and viscosity of acetylated starch slurry indicated the product prepared with 20% NaOH swelled more than that prepared with 3% NaOH during acetylation. Considering the structure of waxy maize starch, reaction conditions, crystallinity and thermal properties of the acetylated products, it was proposed that high concentration NaOH could improve the reaction process and acetyl groups were more uniformly distributed in amorphous and crystallinity regions of amylopectin in waxy maize starch.

1. Introduction

To extend the use of native starches, functional groups can be introduced into starches by a number of chemical modifications, which can provide starches with improved properties (Wurzburg, 1995). Acetylation of starches is an important substitution method that imparts the thickening needed in food applications. Acetylated starches with a low degree of substitution (DS) may function as film forming, binding, adhesion, thickening, stabilizing and texturing agents (Boutboul, Giampaoli, Feigenbaum, & Ducruet, 2002; Elomaa et al., 2004), 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 (Wurzburg, 1995; Yadav, Mahadevamma, Tharanathan, & Ramteke, 2007).

The properties of acetylated starches depend on the botanical starch source (Chen, Li, Li, & Guo, 2007; Santayanon & Wootthikanokkhan, 2003; Xu & Hanna, 2005), the DS (Bellopérez, Agamaacevedo, Zamudioflores, Mendezmontealvo, & Rodriguezambriz, 2010), the amylose/amylopectin ratio (Liu, Ramsden, & Corke, 1998) and the location and distribution of acetyl groups in starch molecules (Huang, Schols, Jin, Sulmann, & Agj, 2007a). Researchers have studied the distribution of acetyl group in acetylated starches (Huang et al., 2007a; Huang, Schols, Klaver, Jin, & Agj, 2007b; Wang & Wang, 2015). Granule size has been reported to affect the DS values for acetylated potato and sweet potato starches using acetic anhydride as reagent (Chen, Huang, Suurs, Schols, & Agj, 2005; Chen, Schols, & Agj, 2004). The acetyl groups on amylose molecules are more heterogeneously distributed and located more closely to the non-reducing ends for amylose originating from small size granule fractions when compared to amylose from lager sized granules. However, acetyl groups were unevenly distributed over the amylopectin populations. Huang et al. (2007a, 2007b) reported that the distributions of acetyl groups along the amylose and amylopectin chains of cowpea starch were more clustered for modification with vinyl acetate as compared with modification with acetic anhydride. In addition, the method of modification may affect the substitution pattern (Bai & Shi, 2011; Bai, Kaufman, Wilson, & Shi, 2014). Enzymatic hydrolysis in combination with chromatographic

* Corresponding author.
E-mail address: ycshi@ksu.edu (Y.-C. Shi).

https://doi.org/10.1016/j.foodhyd.2018.01.015
0268-005X/

techniques are effective tools to determine the substitution pattern at a molecular level (Bai et al., 2014).

Acetylated starches with a low DS are produced with acetic anhydride or vinyl acetate in aqueous medium in the presence of an alkaline catalyst, such as 3% (w/w) sodium hydroxide (Wang & Wang, 2015). High-DS starch acetates are often prepared by using pyridine as the reaction medium (Wurzburg, 1995). However, reaction in aqueous system avoids harmful solvents and offers advantages in large scale production. Billmers and Tessler (1994) reported a method of preparing starch esters with an intermediate DS using a one-step aqueous process with organic acid anhydride in the presence of 20% (w/w) sodium hydroxide. However, how different NaOH concentrations used in acetylation affect the acetyl group distribution in starch acetates has not been studied.

We hypothesize that the distribution of acetyl group in starch is different when starch acetate is prepared at different alkaline concentrations. In this study, we produced starch acetates with same DS in aqueous medium with different NaOH concentrations as catalysts. The objective of this work was to study the substitution pattern of starch acetates catalyzed by sodium hydroxide with different concentrations. Enzymatic hydrolysis in combination with chromatographic techniques, crystallinity and thermal properties were used to study the substitution pattern at the molecular level.

2. Materials and methods

2.1. Materials

Waxy maize starch was obtained from Ingredion Inc. (Bridgewater, NJ, USA). Alpha-Amylase (EC 3.2.1.1, Type VI-B, from porcine pancreas, 7.7 U/mg), β-amylase (EC 3.2.1.2, TypeII-B, from barley, 55.7 U/mg) and amyloglucosidase (EC 3.2.1.3, from Rhizopus mold, 20.3 U/mg) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). One unit of α-amylase activity released 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C. One unit of β-amylase activity produced 1.0 mg of maltose from starch in 3 min at pH 4.8 at 20 °C. One unit of amyloglucosidase activity generated 1.0 mg of glucose from starch in 3 min at pH 4.5 at 55 °C. Isoamylase (EC 3.2.1.68, from Pseudomonas amyloderamosa, 1410 U/mg) was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). One unit of isoamylase activity causes an increase in A_{610} of 0.1 in 1 h using a homogenized solution of rice starch as a substrate (pH 3.5, 40 °C). Other chemicals and solvents were of analytical grade.

Alpha-amylase or β-amylase was dissolved in sodium acetate buffer (0.01 M, pH 4.8), amyloglucosidase was dissolved in sodium acetate buffer (0.01 M, pH 4.5) and isoamylase was diluted in sodium acetate buffer (0.01 M, pH 4.0), to make solutions containing 0.1, 0.11, 0.1 U/μL and 0.22 U/μL of the enzyme, respectively.

2.2. Acetylation of starch

Native starch (100 g, dry basis) was slurried in distilled water (250 ml) with an overhead mechanical stirring and the pH was adjusted to 8.0 by the dropwise addition of 20% NaOH. The temperature was lowered to 10–15 °C by adding ice to the water bath. Once the proper temperature was reached, the addition of acetic anhydride was started. Acetic anhydride was added dropwise at 10% levels, based on dry weight of starch. Starch suspension was reacted for 40 min. The sample was adjusted to the same concentration (25.0%) with distilled water and pH 6.5 after determining the water content of the samples, and their viscosity was determined by a Brookfield viscometer (Model DV-II+Pro, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a No.S61 spindle to monitor the changes of starches during the reaction. The starch was adjusted to pH 6.5 with 0.5 M HCl, filtered and the residual material was washed twice with distilled water. The final product was oven-dried at 40 °C overnight. Another sample was performed at pH 8.0 by the dropwise addition of 3% NaOH and starch suspension was reacted with acetic anhydride for 120 min.

After each sample was analyzed by the viscometer, it was poured into a 100-ml measuring cylinder; settling volume, which included volume of hydrated starch granules and water between starch granules, was recorded after the sample was held at 25 °C for 24 h.

2.3. Determination of DS

The acetylation percentage (% acetyl) and DS of modified starches were determined following the titrition method of Wurzburg (1964). The starch acetate (ca. 1.0 g) was accurately weighed and added into 50 ml of the aqueous solution of ethanol (75%), then the slurry was kept in the water bath (50 °C) for 30 min. After the slurry was cooled to room temperature, an exact amount of aqueous solution of potassium hydroxide (0.5 M, 30 ml) was added and the solution was stirred for 72 h at room temperature. The excess alkali was back-titrated with 0.5 M HCl using phenolphthalein as an indicator. The reference samples and duplicates were treated in a similar way. The acetyl content (A%) was calculated according to the following equation:

$$A\% = \frac{(V_0 - V_n) \times N \times 43 \times 10^{-3}}{M} \times 100\% \quad (1)$$

where V_0 (ml) was the volume of 0.5 M HCl used to titrate the blank, V_n (ml) was the volume of 0.5 M HCl used to titrate the sample, N was the normality of the HCl used, M (g) was the amount of dry starch acetate sample, factor 43 was the formular weight of acetyl groups. The acetyl content (A %) was used to calculate the degree of substitution, DS, according to the following equation:

$$DS = \frac{162 \times A}{43 \times 100 - (43 - 1) \times A} \quad (2)$$

2.4. Alpha-Amylase, β-amylase and isoamylase hydrolysis

Ten milligrams (dry basis) of native waxy starch (NWS), acetylated waxy starch prepared with 20% (w/w) NaOH as catalyst (AWS-20) or acetylated waxy starch prepared with 3% (w/w) NaOH as catalyst (AWS-3) was mixed with 2 ml of acetic acid buffer (0.01 M, pH 4.8) and heated in a boiling water bath for 1 h, cooled to 25 °C and incubated with 50 μL of α-amylase solution at 20 °C for 12 h. After inactivation by boiling for 25 min, the hydrolysates were vacuum-dried and used for gel permeation chromatography (GPC) analysis.

For β-amylase hydrolysis, 10 mg (dry basis) of NWS, AWS-20 or AWS-3 was mixed with 2 ml of acetate buffer (0.01 M, pH 4.8) and heated in a boiling water bath for 1 h, cooled to room temperature and incubated with 20 μL of β-amylase solution at 25 °C for 8 h and then heated by boiling for 25 min. The hydrolysates were analyzed with high-performance anion-exchange chromatograph (HPAEC). The β-limit value was determined from HPAEC peak with pure maltose as external standard.

NWS, AWS-20 or AWS-3 sample (10 mg, dry basis) was mixed with 9 ml of acetic acid buffer (0.01 M, pH 4.0) in a sealed glass bottle and cooked in a boiling water bath with stirring for 1 h. After the mixture was cooled to 50 °C, 200 μL of isoamylase was added. The mixture was kept at 50 °C with stirring for 24 h and then