Food Chemistry 152 (2014) 113–120

Contents lists available at ScienceDirect

Food Chemistry

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

Branch chain elongation by amylosucrase: Production of waxy corn starch with a slow digestion property

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ARTICLE INFO

Article history:
Received 9 August 2013
Received in revised form 21 November 2013
Accepted 23 November 2013
Available online 3 December 2013

Keywords:
Amylosucrase
Branched chain length distribution
Digestion
Slowly digestible starch
Waxy corn starch

ABSTRACT

Starches with high slowly digestible starch (SDS) contents were prepared by treating completely gelatinized waxy corn starch with amylosucrase. The structural properties of the prepared starches were then investigated. The content of SDS increased by up to 38.7% after amylosucrase modification, and the portion of chains with degree of polymerisation (DP) 25–36 increased, while the portion of chains with DP $\leqslant$ 12 decreased. Amylosucrase-modified starches showed a weak B-type crystalline structure. A slight increase in the degree of relative crystallinity was observed with increased reaction time. The thermal properties, including melting temperature and enthalpy, of the amylosucrase-modified starches were higher than for the control starch. Although the amylosucrase-modified starches showed varying structural properties according to reaction time (1–45 h), their digestibilities did not change much after 6 h. By controlling the reaction time of the amylosucrase treatment, a tailored starchy food containing the desired amount of SDS can be produced.

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1. Introduction

Starch is the major energy source for humans and is related to human health. The release and absorption of glucose generated by the hydrolysis of starch is related to blood glucose level, which is linked directly with health. Starch has been classified into three categories, rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RDS is digested rapidly in the mouth and the small intestine, is found mainly in cooked food, and can be used as an urgent energy source. SDS, which can aid in maintaining energy supply and blood glucose level, is digested completely but slowly in the small intestine. RS resists digestive enzymes, is fermented in the colon, and thus affects intestinal health (Englyst, Vinoy, Englyst, & Lang, 2003). In addition, SDS has many beneficial physiological effects and can be helpful in reducing the severity of many common chronic diseases such as obesity, diabetes, and cardiovascular disease. The glycemic index (GI) is defined as the increasing region in the blood glucose response curve after ingesting a certain amount of carbohydrates in a sample food compared with the same amount of available carbohydrate in a reference food such as glucose or white bread. Foods containing high amounts of SDS and therefore a medium or low GI help to reduce the glycemic load, whereas rapidly digestible food displays a high GI (Ells, Seal, Kettlitz, Bal, & Mathers, 2005; Englyst et al., 2003). Intake of SDS could result in a beneficial metabolic response and protection from diabetes. A meal with a high content of SDS allows for a relatively low postprandial glycemic response to carbohydrates and moderates insulin demand in type 2 diabetes (Ells et al., 2005). Also, since SDS is able to keep a postprandial insulin at a stable and low level, SDS is expected to have a high satiety effect (Lehmann & Robin, 2007).

Research regarding the production, structure, mechanism, and physiological effects of RS has been plentiful, but SDS has yet to be clearly elucidated. Several articles regarding the formation and structure of SDS by enzymatic treatment have been published (Ao et al., 2007; Casarrubias-Castillo, Hamaker, Rodriguez-Ambriz, & Bello-Pérez, 2012; Shin, Choi, Park, & Moon, 2010; Shin et al., 2004). Han et al. (2006) produced a modified maize starch via an α-amylase treatment; the starch maintained its SDS and RS content after cooking and had a low GI. It has also been established that using a high concentration and short reaction time while debranching by pullulanase is an effective strategy for the formation of SDS, and that fast cooling and storage of debranched starches increase the SDS content (Miao, Jiang, & Zhang, 2009). Shin et al. (2004) established optimal conditions for waxy sorghum starch which allow for maximum SDS content; debranching by isoamylase treatment for 8 h followed by storage at 1 °C for 3 days.

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0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.11.145

The researchers reported that SDS consists of mainly imperfect crystalline regions containing small portions of double helices as well as amorphous regions.

Amylosucrase (AS), a glucosyltransferase from *Neiserria polysaccharea*, produces an insoluble α-1,4-linked glucan polymer by consuming sucrose and releasing fructose. This reaction does not require α-D-glucosyl-nucleotide-diphosphate like ADP- or UDP-glucose, but rather uses the energy generated by splitting sucrose in order to synthesise the glucan polymer. Moreover, sucrose as substrate is relatively inexpensive, plentiful, and eco-friendly. Because of these capabilities, many investigators have been interested in the synthesis of amylose by AS. When glycogen is used as the acceptor, elongation of the glucosyl units occurs at the non-reducing ends of the external chains, resulting in the precipitation of modified glycogen (Potocki de Montalk et al., 2000; Rolland-Sabaté, Colonna, Potocki-Véronèse, Monsan, & Planchot, 2004).

A previous study reported that amylose content was significantly correlated with RS content but not with SDS content (Zhang, Ao, & Hamaker, 2008). On the other hand, the fine structure of amylopectin is related to the formation of SDS (Ao et al., 2007; Zhang, Sofyan, & Hamaker, 2008; Zhang, Ao et al., 2008). Although SDS does not have a uniform molecular structure, amylopectin with a high proportion of either short chains or long chains tends to produce a high amount of SDS, showing a parabolic relationship between SDS content and the weight ratio of amylopectin short chains to long chains. SDS with a low ratio of short chains to long chains is a physical entity with long branch chains facilitating association among molecules, whereas SDS with more short chains is a chemical entity inherent in the special structure with high branching density and short chains of amylopectin (Zhang, Ao et al., 2008). Shin et al. (2010) reported very similar findings that the chain elongation of amylopectin and/or amylose by amylosucrase elevated the content of SDS in waxy and non-waxy starches. After the amylosucrase treatment, the proportion of long chains increased, resulting in a decrease in the weight ratio of amylopectin short chains to long chains. It could be a concrete evidence for the production of a relatively high amount of SDS by the amylosucrase treatment on starch. However, the amylosucrase treatment was carried out on all starch samples for 40 h, thus they could not monitor the changes in the branch chain length distribution and the weight ratio of amylopectin short chains to long chains during the amylosucrase reaction. Also, the relationship between the digestion property and the weight ratio of amylopectin short chains to long chains could not be determined during the amylosucrase reaction. Therefore, in this study, we prepared slowly digestible waxy corn starch by the AS treatment and investigated the effect of AS reaction time on its branch chain length distribution. Further, we examined the relationship between the proportion of long chains and their length in AS-treated waxy corn starch and its slow digestion property.

2. Materials and methods

2.1. Materials

Waxy corn starch was obtained from Samyang Genex Corporation (Incheon, Korea). Amylosucrase (AS, 230 U/mL) from *N. polysaccharea* was provided by the Food Microbiology and Bioengineering Laboratory of Kyunghee University. One unit (U) of amylosucrase was defined as the amount of enzyme catalysing the release of 1 μM fructose per min by consumption of sucrose (Potocki de Montalk et al., 2000). All other chemicals were of analytical reagent grade.

2.2. Preparation of AS-modified starches

Waxy corn starch (2%, w/w) was suspended in 100 mM sodium citrate buffer (pH 7.0) and made 100 mM in sucrose added as substrate. The starch suspension was boiled for 30 min and then cooled to 30 °C. Amylosucrase (230 U/mL) was added to the suspension and incubated at 30 °C for 1, 3, 6, 9, 15, and 45 h. Threefold ethanol was added to terminate the reaction, then the AS-modified starch was precipitated by centrifugation at 7000*g* for 10 min. The supernatant was retrieved to measure the content of released fructose during the AS treatment. The precipitate was washed three times with distilled water by centrifugation at 7000*g* for 10 min. The pellet was freeze-dried, pulverized and passed through a 100-mesh sieve. The control was prepared using the same method used for AS-modified starches except without the addition of enzyme. Cooked starch was prepared by boiling starch suspension for 15 min.

2.3. Determination of starch fractions based on digestibility

Starch fractions related to digestibility were determined by the method of Brumovsky and Thompson (2001), although with slight modifications. Pancreatin (2 g, P-7545, activity 8 × USP/g, Sigma–Aldrich, St. Louis, MO, USA) was dissolved in distilled water (24 mL), stirred for 10 min, and centrifuged at 1500*g* for 10 min. The supernatant (20 mL) was mixed with amyloglucosidase (0.4 mL, AMG 300L, activity 300 AGU/mL, Novozymes, Bagsvaerd, Denmark) and distilled water (3.6 mL). This mixture was incubated at 37 °C for 10 min to obtain the enzyme solution, which was newly prepared every time prior to the experiment.

A sample (30 mg) and a glass bead were placed in a 2 mL-microtube, and 100 mM sodium acetate buffer (0.75 mL, pH 5.2) was added. This mixture was then kept in a shaking incubator at 37 °C for 10 min with a stroke speed of 240 rpm. Then, the prepared enzyme solution (0.75 mL) was added to each microtube at regular intervals. The microtubes were then kept in a shaking incubator at 37 °C and tested at 10 and 240 min. To terminate the enzyme reaction, each microtube was boiled for 10 min. The glucose released by the hydrolysis of the starch samples was measured using a GOD-POD kit (BCS, Anyang, Korea) following centrifugation at 5000*g* for 5 min.

Starch fractions were determined according to the degree of hydrolysis. RDS was measured as the amount of glucose released after the reaction was allowed to progress for 10 min. SDS was the fraction digested between 10 and 140 min. The undigested fraction that remained after 240 min was measured as RS.

2.4. Analysis of the soluble fraction after amylosucrase treatment

Following the AS reaction, the remaining sucrose and fructose that were released were retrieved from the supernatant after the first centrifugation (5000*g*, 15 min) to determine the composition of the soluble fraction. Ethanol was evaporated using a SpeedVac Concentrator (Savant AES 1010, GMI, Ramsey, MN, USA) for 4 h. The fraction obtained was redissolved in distilled water. All of the samples were filtered through a 0.45-μm membrane filter and analysed using HPAEC on a CarboPac PA-1 anion exchange column (250 × 4 mm; Dionex, Sunnyvale, CA, USA) with a pulsed amperometric detector (PAD, Dionex). The analysis was performed using 150 mM sodium hydroxide for column equilibration and 600 mM sodium acetate in 150 mM sodium hydroxide for sample elution with a flow rate of 1 mL/min. The linear gradients for the latter were from 0% to 15% for 0 to 10 min and from 15% to 100% for 10 to 15 min.

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