International Journal of Biological Macromolecules 86 (2016) 105–111

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

The optimization of isoamylase processing conditions for the preparation of high-amylose ginkgo starch

Lanlan Hu[a], Yi Zheng[a,b], Yujiao Peng[a], Cheng Yao[a,*], Huanxin Zhang[b,c,*]

[a] College of chemistry and molecular engineering, Nanjing Tech University, Nanjing 211816, China
[b] Jiangsu Agri-animal Husbandry Vocational College, 8 Fenghuang East Road, Taizhou 225300, China
[c] State Key Laboratory of Food Science & Technology, Jiangnan University, Wuxi, Jiangsu 214122, PR China

ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form 4 January 2016
Accepted 12 January 2016
Available online 15 January 2016

Keywords:
Gingko starch
High-amylose
Isoamylase
Morphology
Thermal properties

ABSTRACT

A high-amylose starch was prepared from ginkgo by hydrolysis using isoamylase and its structures (morphology and crystallinity) and physicochemical properties (swelling factor, water solubility and gelatinization) were determined. The experiments used response surface methodology to determine the optimum parameters for enzymatic hydrolysis: pH 5.0 at 52 °C for 170 min, using an enzyme dose greater than 100 IU/ml. The experimentally observed maximum yield of ginkgo amylose under these conditions was 74.74% and the blue value was 0.756. The high-amylose ginkgo starch showed an irregular surface and porous inner structure while the native starch granules were oval with a smooth surface. X-ray showed that the high-amylose starch displayed a V-type structure. Because of its high amylose content and different structural characteristics, high-amylose starch exhibited a higher gelatinization peak temperature (109.25 °C) and water solubility, and a lower crystallinity (19.13%), gelatinization enthalpy (63.83 J/g), and swelling power. The present study has indicated that high-amylose starch prepared using isoamylase has unique functional properties, which lays the foundation for the wider application of ginkgo starch.

1. Introduction

Ginkgo is a non-conventional food resource whose seeds contain about 60%–70% starch, 10%–20% protein, 2%–4% lipid and 0.8%–1.2% pectin [1–3]. It has not been widely studied, but its particular functional properties and potential use have attracted more attention in recent years. Ginkgo starch consists of two main components: linear amylose and highly-branched amylopectin. Amylose has mainly linear molecules with α-1,4 linked D-glucosyl units and a few branches of α-1,6 linkages, whereas amylopectin has large numbers of branch chains which are linked to the linear chains by α-1,6 linkages [4–6]. The ratio of amylose to amylopectin contents in starch varies depending on botanical source. Normal starches consist of 20–30% amylose and 70–80% amylopectin, but waxy starches contain almost 100% amylopectin. As for the ginkgo, the apparent amylose content was reported ranging from 26% to 36% [1–3]. The extent of amylose leaching was 4.0–27.2% in ginkgo starch [1]. It has been reported that amylose leaching was influenced by lipid-complexed amylose, total amylose content and interaction between starch chains [7]. Jane has ever examined the branch chain length distribution of ginkgo. The result showed that the amylopectin had a peak dp number of 13 and chains up to dp number 82. The average chain length for ginkgo is 24.2 [2].

High-amylose starch is of particular interest because its highly retrograded form has been classified as a resistant starch (RS3, the retrograded form) [8]. Amylose retrogradation is a rapid process completed within 48 h, whereas amylopectin retrogradation may continue for several weeks [9]. Because of the structural stability of amylose, some high-amylose cereal crop lines have played an important role in the technology of packaging films, food, medical treatment, textiles, paper making, packaging, petroleum, environmental protection, optical fibers, printed circuit boards, and electronic chips [10–13]. Therefore, great efforts have been made to develop other crops containing high-amylose starch because of its unique functional and nutritional properties. In general, altered amylose is present because of the starch's botanical origins [14–16], gene mutations or gene silencing [17,18] and enzymatic modification [19]. Enzymatic modification, where branch-chains are removed from amylose, give the resultant chains more opportunities to align and aggregate to form perfectly crystalline structures, which in turn leads to forming more high-amylose starch [20].

It has been reported that a debranching procedure for waxy and low-amylose rice starches used isoamylase, but the relevant

* Corresponding authors. Fax: +86 25 5813 9482.
E-mail addresses: yaocheng@njtech.edu.cn (C. Yao), hxinzh@hotmail.com (H. Zhang).

http://dx.doi.org/10.1016/j.ijbiomac.2016.01.045
0141-8130/

reactions were not studied [21]. The objective of the present study is therefore to understand the interactions between efficiency as given by amylose yield and the modification factors (enzyme dosage, pH, temperature and reaction time), and also to locate their optimum levels using response surface methodology. Starches will be isolated from mature ginkgo seeds and modified with isoamylase. Their structures (morphology and crystallinity) and physicochemical properties (swelling factor, water solubility and gelatinization) will be investigated using SEM (Scanning Electron Microscopy), X-ray diffraction and DSC (Differential Scanning Calorimetry).

2. Materials and methods

2.1. Materials

Mature fruits were collected from trees of *Ginkgo biloba* cv. Dafozhi located in Taixing County, Jiangsu Province, China. The granular starch debranching enzyme, isoamylase (1000 IU/ml in 0.02% sodium azide) from *pseudomonas* sp. was obtained from Megazyme International (Bray, Ireland). All other chemicals were reagent grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Starch isolation

Ginkgo starch was isolated in the laboratory using the methods reported by Zheng et al. [22].

2.3. The formation of high-amylose starch

Starch was dispersed in phosphate buffer (at pH values of 4.2, 4.6, 5.0, 5.4 and 5.8) and heated at 100 °C for 30 min to form a starch slurry (1% w/w, dry basis). The temperature of the starch slurry was adjusted to 50 °C and other temperatures (42, 46, 54 and 58 °C). The samples were then debranched using isoamylase enzyme at different concentrations (40, 60, 80, 100 and 120 IU/ml of starch slurry) for different periods (1, 2, 3, 4, 5 and 6 h) in a shaking water bath. After the reaction, two volumes of 95% ethanol were added immediately to facilitate the enzyme reaction. The mixture was centrifuged at 2200 × *g* for 10 min and washed twice with ethanol. The high-amylose samples were dried in an oven at 50 °C for 24 h and then passed through a 100-mesh sieve.

2.4. Starch-iodine absorbance spectra and amylose content

The apparent amylose content was determined by an iodine colorimetric method [23] with a slight modification. 10 mg starch was suspended in 1 ml ethanol and 10 ml NaOH (0.1 M). 1 ml of HCl (1 M) was added after the suspension was heated in boiling water for 30 min to make the solution neutral (pH 7.0). The suspension was made up to 50 ml with distilled water after cooling. A 1-ml aliquot of the solution was added to 0.2 ml 0.5% iodine solution and made up to 10 ml with distilled water. The absorbance spectra and the wavelength of maximum absorption (λ_{max}) were analyzed over a wavelength range of 500–800 nm. A calibration curve was established using a mixture of amylose and amylopectin from potato starch.

2.5. Granule morphology

For the SEM study, the starch granules were mounted on circular aluminum stubs using double-sided adhesive tape and then coated with gold. This was deposited under vacuum with an evaporator in a JSM 6510 electron microscope (JEOL, Tokyo, Japan). All samples were examined using an acceleration voltage of 15 kV, observed in the microscope and recorded photographically using the method of Lu et al. [24].

2.6. Swelling power and water solubility of starch granules

The swelling power and water solubility of the native starch and the modified amylose starch produced by enzymatic hydrolysis using isoamylase were determined according to the method of Karim et al. [25] with a slight modification. Starch (0.5 g) was suspended in 50 ml water and heated in a water bath from 60 to 95 °C in 5 °C steps over 30 min with shaking. The tubes were cooled rapidly to room temperature and then centrifuged at 2200 × *g* for 20 min. The lipid was removed and dried under vacuum for 2 h at 130 °C to obtain the residue and weighed to calculate the starch solubility. The swelling power was determined by calculating the amount of original precipitate and water absorbed by starch after subtraction of the amount of solubilized starch. Three replicate samples were used in this determination.

2.7. Thermal properties

The thermal characteristics of the starches were studied using differential scanning calorimetry (Diamond DSC, PerkinElmer Inc., Waltham, MA, USA) according to the method described by Utrilla-Coello et al. [26] with slight modifications. Each sample (3 mg, dry weight) was placed in an aluminum pan and 6 ml of deionized water was added. The samples were hermetically sealed and allowed to stand for 1 h before heating in the DSC. The analysis was carried out by heating the pan from 30 to 150 °C at a rate of 10 °C/min. The DSC analyzer was calibrated using an empty aluminum pan as a reference. The data of the onset (T_o), peak (T_p), end temperatures (T_e) and the gelatinization enthalpy (ΔH_{gel}) were obtained using the instrument's software.

2.8. X-ray diffraction pattern

The X-ray diffraction patterns of the starches were obtained using the method of Miao et al. [27] with an X-ray powder diffractometer (Rigaku, Tokyo, Japan) operating at 40 kV and 30 mA. The starch samples were scanned at a rate of 2°/min over the diffraction angle (2θ) from 5 to 35° at room temperature. The relative crystallinity was calculated according to the following equation:

$$X_c = A_c/(A_a + A_c)$$

Where X_c is the relative crystallinity, A_c is the crystalline area and A_a is the amorphous area on the X-ray diffractogram.

2.9. Experimental design and statistical analysis

On the basis of single factor studies, response surface methodology (RSM) was used to optimize the conversion yield. Design Expert 8.0 (Stat-Ease Inc., Minneapolis, MN, USA) was used to study the empirical relationship between amylose yield and the four controlled factors: X1: Enzyme dosage; X2: pH; X3: temperature; and X4: time. This design required 29 trials with the independent factors being studied at three different levels. The factors and their levels were: enzyme dosage (70, 100 and 130 IU/ml); pH (4.5, 5.0 and 5.5); temperature (46, 52 and 58 °C); and time (90, 150 and 210 min). All experiments were performed in duplicate with the statistical significance of the terms being examined by ANOVA and a significance test level of 5% ($p < 0.05$).