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Effect of oxidation level on the inclusion capacity and solution stability of oxidized amylose in aqueous solution

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ABSTRACT

The oxidized amyloses with high oxidation level and carboxyl content were successfully prepared through a two-step oxidation method using hydrogen peroxide as the oxidant and copper sulfate as the catalyst. The results showed that oxidation would prevent the oxidized product to crystallize and induce depolymerization of amylose molecules. Accordingly, the helices and inclusion capacity of oxidized amylose molecules were reduced. However, the solubility of oxidized amyloses in water was highly improved due to the introduced carboxyl groups. The solution stability of oxidized amylose-guest inclusion complexes in aqueous solution was efficiently improved to a large extent. The result suggested that the two-step oxidation method was an efficient way to highly broaden the applications of amylose-guest inclusion complexes in water environment.

1. Introduction

Amylose is a resourceful and widely used biomaterial, which is a linear polymer consisting of α-1,4 linked glucopyranose units. Amylose-based products have been widely studied because of their total biodegradability and other unique properties (Fanta, Selling, Felker, & Kenar, 2015; Xie et al., 2015). In aqueous media, amylose can form single, left-handed helices with a hydrophobic cavity which can include guest molecules, such as iodine and esters, via hydrophobic and van der Waals interactions (Kadokawa, Nakaya, Kaneko, & Tagaya, 2003; Nimz et al., 2003; Putseys, Lamberts, & Delcour, 2010). The including of guest molecules can protect the molecules against oxidation, light-induced reactions, volatilization, sublimation, heat-promoted decomposition and limit undesired odors. The release profile of guest molecules can be controlled as well. Moreover, the non-covalent interactions between amylose and guest molecules can provide the inclusion complexes solvent- and temperature-responsive properties. Numerous studies of amylose-guest inclusion complexes have been carried out in recent years (Singh, Byars, & Kenar, 2014; Tian et al., 2013; Wulff, Avgenaki, & Guzmann, 2005).

Two basic methods have been used for amylose-guest inclusion complexes preparation. One method is to insert guest molecules by mixing the raw materials (Putaux et al., 2011; Tomasik & Schilling, 1998). The other method is known as "vine-twinning polymerization" in which amylose is synthesized around the guest molecules via enzymatic polymerization (Kadokawa, Kaneko, Tagaya, & Chiba, 2001). So far, the preparation of amylose-guest inclusion complexes was carried out mostly in aqueous solution. However, disentanglement of the helices of amylose molecules can difficultly happen due to their low water solubility and the strong tendency to retrograde, which brings difficulties to the preparation of amylose-guest inclusion complexes in aqueous solution. Furthermore, the formation of amylose-guest inclusion complexes will induce great reduction of water solubility and solution stability of the products. Hence, the applications of amylose-guest inclusion complexes are limited when they are required to be soluble in water. Therefore, it is of great significance to improve the solubility and solution stability of amylose and amylose-guest inclusion complexes in aqueous solution. The well water-soluble oxidized amylose may potentially be an ideal candidate.

Chemical modification is a common way used to change properties of amylose, among which oxidation is a simple and the most widely used method to improve the performances of amylose (Argüello-García et al., 2014; Chauhan, Chauhan, & Ahn, 2010; Vanier et al., 2012). The introduced carbonyl and carboxyl groups on oxidized amylose molecules will result in a higher solubility and a weaker tendency to retrograde in aqueous solution (Gumul, Krystyjan, Buksa, Ziobro, & Zięba, 2014; Sandhu, Kaur, Singh, & Lim, 2008). However, oxidation can also decrease the helicity of amylose

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to influence the inclusion capacity. As far as we know, the effect of oxidation level on the inclusion capacity and solution stability of amylose in aqueous solution has not been clearly reported yet.

The most frequently used oxidants for starch oxidation are persulfate, sodium or calcium hypochlorite, hydrogen peroxide and periodate (Fiedorowicz & Para, 2006; Zhang, Wang, Zhao, & Wang, 2012). Among them hydrogen peroxide is an environmentally friendly oxidant while the others all produce large quantities of inorganic waste (Tolvanen, Mäki-Arvela, Sorokin, Salmi, & Murzin, 2009). Thus hydrogen peroxide was used as the oxidant in the present study. Considering the poor water solubility of amylose, a new two-step method was used to prepare oxidized amylose, in which amylose was primarily oxidized through dry-oxidation and then transferred into distilled water for further and homogeneous oxidation. The effect of oxidation level on the inclusion capacity and solution stability of oxidized amylose in aqueous solution was investigated.

2. Materials and methods

2.1. Materials

Amylose with 99% purity was purchased from Kangmeida Reagent Inc. (Henan, China). The average degree of polymerization was 1230 according to gel permeation chromatography (GPC) analysis. All other chemicals and reagents were of analytical grade unless otherwise stated.

2.2. Preparation of oxidized amyloses

A modified two-step method was used to prepare oxidized amylose (Lu, Kong, Jing, Hu, & Zhu, 2013). In the first step, amylose was oxidized through dry-oxidation on the surface to destroy the granule structure, thus increasing the solubility of amylose in aqueous solution. 30 g amylose powder was mixed with 30% H_2O_2 in an open beaker. Then 1 mL of 0.05% $CuSO_4$ solution was added. The mixture was transferred into an open flask and then kept at 40 °C in water bath for 15 min to swell the amylose powder. Then the mixture was reacted at 70 °C for 20 min under constant agitation. In the second step, the resultant was taken out and dissolved in 200 mL boiled water in another flask. Further oxidation was continued for 30 min at 100 °C in oil bath and then cooled to room temperature. The solution was centrifuged at 3000 rpm for 20 min and the supernatant was precipitated by absolute ethanol and washed with ethanol/water (80/20, v/v) for several times to remove the copper ion. The precipitate was then freeze-dried. Oxidized amylose with different oxidation level was obtained by controlling the dosage of H_2O_2, which was 10 mL, 15 mL, 20 mL and 30 mL, respectively.

2.3. Carboxyl content determination

The carboxyl content was determined according to the published calcium-acetate method with some modifications (Praskalo et al., 2009). The samples were kept in a vacuum oven at 60 °C for 48 h to remove the absorbed water before the test. 5 g sample was dissolved in 50 mL distilled water and then heated at 100 °C for 10 min. The solution was cooled to room temperature and transferred into a 250 mL volumetric flask. Then 25 mL 0.5 M of calcium-acetate solution and extra water were added to make the final solution 250 mL. After frequent shaking for 30 min, the liquid was vacuum filtered. 50 mL filtered liquid was titrated with 0.05 M sodium hydroxide from colorless to pink, using phenolphthalein as the indicator. A blank determination with unmodified amylose was performed in the same manner. The carboxyl content was calculated as follows:

$$\mathrm{COOH}\,(\%) = \frac{[V_{(\mathrm{NaOH})} - V_b] \times 0.05\,\mathrm{M} \times 45 \times 100}{m} \quad (1)$$

where 0.05 M is the concentration of NaOH; $V_{(NaOH)}$ is the volume (L) of NaOH solution used for sample titration; V_b is the volume (L) of NaOH solution used for blank determination; m is the weight (g) of sample and 45 is molecular weight of carboxyl group. The measurement was done in triplicate.

2.4. Carbonyl content determination

The determination of carbonyl content was following the titrimetric method of Wang and Wang (2003) with a little modification. The samples were kept in a vacuum oven at 60 °C for 48 h to remove the absorbed water before the test. 3 g sample was dissolved in 100 mL distilled water in a 500 mL conical flask and kept in a water bath at 100 °C for 20 min. The solution was then cooled to room temperature and adjusted to pH 3.2 using 0.1 M HCl. Then 40 mL of hydroxylamine reagent was added. The conical flask was stoppered and kept at 40 °C for 4 h under mild agitation. The excess hydroxylamine in the resultant was titrated immediately to pH 3.2 with standardized 0.1 M HCl. A blank determination of original amylose was performed as the same. The hydroxylamine reagent was obtained by dissolving 25 g hydroxylamine hydrochloride in 100 mL 0.5 M NaOH and then adjusting with distilled water to a final volume of 500 mL. The carbonyl content was calculated as follows:

$$\mathrm{CHO}\,(\%) = \frac{[V_b - V_{(\mathrm{HCl})}] \times 0.1\,\mathrm{M} \times 28 \times 100}{m} \quad (2)$$

where 0.1 M is the concentration of HCl; $V_{(HCl)}$ is the volume (L) of HCl solution used for sample titration; V_b is the volume (L) of HCl solution used for blank determination; m is the weight (g) of sample and 28 is the molecular weight of carbonyl group. The measurement was done in triplicate.

2.5. Determination of aqueous solubility

Samples were separately dissolved in distilled water to an over saturated state. After being kept at 20 °C for 24 h under mild agitation, the solution was filtered and the filtrate was freeze-dried and weighed. The aqueous solubility of each sample was calculated as follows:

$$S = \frac{m_1 \times 100}{m} \quad (3)$$

where m_1 is the dissolved mass; m is the mass of distilled water and S represents the solubility in distilled water. The measurement was done in triplicate.

2.6. FTIR spectroscopy analysis

FTIR spectra of amylose and oxidized amyloses were obtained from a Fourier transform infrared spectrometer (Nicolet is10, Thermo Scientific, MA, USA). The samples were kept in a vacuum oven at 60 °C for 48 h to remove the absorbed water before the test. 1 mg sample and 200 mg KBr were mixed, grinded, compressed and then tested. The range of wavenumbers was from 4000 cm^{-1} to 400 cm^{-1} with the resolution of 4 cm^{-1}.

2.7. X-ray diffraction (XRD) analysis

The X-ray patterns of amylose and oxidized amyloses were obtained from an 18 kW rotating X-ray diffractometer (X'Pert