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Effects of carboxyl and aldehyde groups on the antibacterial activity of oxidized amylose

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ABSTRACT

Dialdehyde-amyloses, dicarboxyl-amyloses and dialdehyde-carboxyl-amyloses with different oxidation levels were prepared and used to study the effects of aldehyde and carboxyl groups on the antibacterial activity of oxidized amyloses. The results showed that dicarboxyl-amyloses presented antibacterial activity through acidic pH effect produced by carboxyl groups, which was easily reduced or eliminated by adjusting pH. Dialdehyde-amyloses possessed a broad-spectrum antibacterial activity owing to the reactivity of aldehyde groups rather than acidic pH effect. Aldehyde would irreversibly damage bacterial cell wall and cytoplasmic membrane, resulting in decay and death of bacterial cells. It is interesting that the antibacterial properties of dialdehyde-carboxyl-amyloses were improved to some extent compared to dialdehyde-amyloses. The improvement of antibacterial effect of dialdehyde-carboxyl-amyloses may be due to the increasing dispersibility endowed by carboxyl groups, which could effectively enhance the interaction between dialdehyde-carboxyl-amyloses and bacteria. As a result, carboxyl group could act as a promising synergistic group against bacteria with aldehyde group.

1. Introduction

Control of microbiological food safety is a very important issue in food industry and therefore innovation in antibacterial technology has a high priority (Li et al., 2012). Nowadays, natural ways for food preservation are demanded by increasing consumers, which has led to the development of alternative preservation methods, such as the application of edible biopolymers from industrial by-products and renewable resources (Sanchez-Ortega, Garcia-Almendarez, Santos-Lopez, Raymundo Reyes-Gonzalez, & Regalado, 2016). To improve the antibacterial properties of biopolymers, incorporation of antibacterial compounds by encapsulation has been proposed (Li et al., 2012). Cyclodextrin, starch and liposome are frequently used carriers in encapsulation system to control the odor and improve the physicochemical stability of antibacterial compounds (Lyu et al., 2017; Ye, Zhu et al., 2017; Zhang et al., 2015; Zhou, Li et al., 2016; Zhou, Ye et al., 2016). However, only few of these carriers has a “release-on-demand” functionality in complex food systems (Li et al., 2012). In addition, antibacterial compounds will influence the appearance and flavor of the potentially packaged food. Thus, giving antibacterial properties to biopolymer itself may be a promising option since it can directly suppress bacterial growth rather than import antibacterial compounds with undesirable odor, high price or bad quality.

Natural polysaccharides are representative biopolymers, which have been widely used in food preservation (Kadokawa, Arimura, Takemoto, & Yamamoto, 2012). Chemical modification is a common way used to change properties of polysaccharides, among which oxidation may be the most widely used one (Ashogbon & Akintayo, 2014). In the oxidation process, large amount of hydroxyl groups of original polysaccharide are oxidized into aldehyde or carboxyl groups. The introduced carboxyl and aldehyde groups will improve the performances of oxidized polysaccharides. For example, oxidized polysaccharides present higher solubility and weaker tendency to retrograde in aqueous solution (Gumul, Krystyjan, Buksa, Ziobro, & Zięba, 2014). What is more attractive is that oxidized polysaccharides exhibit good antibacterial effects on various food-related bacteria. Dineen first reported that oxidized regenerated cellulose had antibacterial activity against a broad range of bacteria through pH-lowering effects produced by carboxyl groups (Dineen, 1976; Spangler et al., 2003). Oxidized κ-carrageenan, oxidized β-cyclodextrin and oxidized schizophyllan were reported to possess antibacterial properties against *E. coli*, *S. aureus* and other bacteria (Jayakumar, Kanth, Chandrasekaran, Rao, & Nair, 2010;

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Ye, Ren et al., 2017; Ye, Zhu et al., 2017; Zhu et al., 2017). Dialdehyde starch presented high antibacterial activity owing to the reactivity of its dialdehyde groups (Song et al., 2010). Therefore, oxidation is a simple and promising way to confer polysaccharide itself with antibacterial activity. However, the contributions of aldehyde and carboxyl groups, single or dual, to the antibacterial properties of oxidized polysaccharide have not been systematically studied so far.

Amylose is a linear polymer consisting of α-(1,4) linked glucopyranose units and it is an abundant natural polysaccharide as an energy resource present in starch (Tanaka, Tsutsui, Tanaka, Yamamoto, & Kadokawa, 2017). Amylose-based products have been widely applied in food industry because of their total biodegradability and other unique properties (Fanta, Selling, Felker, & Kenar, 2015; Xie et al., 2015). This work aimed to study the effects of carboxyl and aldehyde groups on the antibacterial activity of oxidized amylose and enlighten the development of more efficient and water-soluble antibacterial agent. The dialdehyde-amyloses (DAAs), dicarboxyl-amyloses (DCAs) and dialdehyde-carboxyl-amyloses (DACAs) with different oxidation levels were prepared through improved preparing methods. The structures of oxidized amyloses were characterized by fourier transform infrared (FTIR). The carboxyl/aldehyde contents and water solubility of oxidized amyloses were studied. Especially, the effects of aldehyde and carboxyl groups on the antibacterial activity of oxidized amyloses were investigated.

2. Material and methods

2.1. Materials

Amylose with 99% purity was purchased from Kang media Reagent Inc (Henan, China). The average degree of polymerization was 1230 according to gel permeation chromatography (GPC). Sodium periodate, sodium chlorite, sodium hypochlorite solution (6%-14% available chlorite) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) were purchased from Aladdin Reagent Database Inc (Shanghai, China). *S. aureus*, *P. aeruginosa*, *E. coli*, and *B. subtilis* were purchased from China Center of Industrial Culture Collection. All other chemicals and reagents were of analytical grade unless otherwise stated.

2.2. Preparation of oxidized amyloses

2.2.1. Synthesis of dialdehyde-amyloses (DAAs)

Dialdehyde-amyloses (DAAs) were prepared by periodate oxidation according to previous method (Yu, Chang, & Ma, 2010). The 4.0 g of amylose were dispersed in 40 mL of deionized water at 40 °C. Then sodium periodate was added into the suspension and the pH value of the suspension was adjusted to 4.0 with sulfuric acid (0.5 M). The mixture was then stirred at 40 °C for 4 h in the dark. After that, the oxidized products were collected by filtration and dialyzed against deionized water to remove superfluous ions. The removing of superfluous ions was confirmed by detecting the conductivity of the dialysate. Then the obtained oxidized amyloses were freeze-dried. The dialdehyde-amyloses were named as DAA1, DAA2 and DAA3 when the molar ratios of sodium periodate to amylose were 0.3, 0.5 and 0.7, respectively.

2.2.2. Synthesis of dicarboxyl-amyloses (DCAs)

Dicarboxyl-amyloses (DCAs) were prepared by further oxidation of DAAs with sodium chlorite through selective conversion of aldehyde groups to carboxyl groups (Dalcanale & Montanari, 1986; Praskalo et al., 2009). The 100 g of DAA1, DAA2 and DAA3 slurries with concentration of 10% were added to a mixture containing $NaClO_2$ (5.4 g, 10.6 g or 15.5 g), 5 M CH_3COOH (11.2 mL, 21.9 mL or 33.6 mL), 30% H_2O_2 (4 mL, 7 mL or 10 mL) and water (70 mL, 287 mL or 393 mL), respectively. Then the oxidation was carried out at room temperature for 48 h under stirring and subsequently neutralized with 1 M HCl. The oxidized products were precipitated with absolute ethanol and collected by filtration. After that, the obtained oxidized products were dialyzed against deionized water to remove superfluous ions. The removing of superfluous ions was confirmed by detecting the conductivity of the dialysate. Then the oxidized amyloses were freeze-dried and named as DCA1, DCA2 and DCA3 when the used DAAs were DAA1, DAA2 and DAA3, respectively.

2.2.3. Synthesis of dialdehyde-carboxyl-amyloses (DACAs)

A modified two-step method was used to prepare dialdehyde-carboxyl-amyloses (DACAs) (Hao, Lu, Xu, Linhardt, & Zhang, 2016; Praskalo et al., 2009; Zhao et al., 1999). In the first step, the 4 g of amylose were dispersed in 250 mL of deionized water at 60 °C under stirring to afford a homogeneous suspension. After cooling to room temperature, 52 mg of TEMPO and 520 mg of NaBr were added to the suspension. Subsequently, 1.3 mL of NaClO solution corresponding to 0.63 mmoL/g amylose were added to the suspension under continuous stirring. The reaction was performed for 2.5 h and then quenched by adding 50 mg of $NaBH_4$. The pH value of the suspension was maintained at about 10 by dropwise addition of NaOH (0.5 M) and the temperature was maintained at 0 °C throughout the reaction. After that, the oxidized products were neutralized with 1 M HCl and precipitated in absolute ethanol. The oxidized products were collected by filtration and dialyzed against deionized water to remove superfluous ions. Then the oxidized product was freeze-dried to obtain carboxyl-amylose (CA) powder. In the second step, CAs were used as raw materials to prepare DACAs by periodate oxidation in accordance with the above steps of DAAs synthesis. DACAs were named as DACA1, DACA2 and DACA3 when the molar ratios of sodium periodate to CA were 0.3, 0.5 and 0.7, respectively.

2.3. Degree of oxidation determination

2.3.1. Aldehyde content determination

The aldehyde content was measured by titrimetric method (Sangseethong, Termvejsayanon, & Sriroth, 2010; Wang & Wang, 2003). Oxidized amyloses were kept in a vacuum oven at 60 °C for 48 h to remove the absorbed water before the test. The 0.5 g of oxidized amylose were dissolved in 100 mL of distilled water and kept in a water bath at 100 °C for 20 min. The solution was then cooled to 40 °C and adjusted to pH 3.2 using 0.1 M HCl. Then 50 mL of hydroxylamine reagent were added under agitation. The hydroxylamine would react with aldehyde group to form oxime. The excess hydroxylamine in the mixed solution after reaction was titrated immediately to pH 3.2 with standardized 0.1 M HCl. A control determination of original amylose was performed as the same procedure. The hydroxylamine reagent was obtained by dissolving 25 g of hydroxylamine hydrochloride in 100 mL of 0.5 M NaOH and then adjusting with distilled water to a final volume of 500 mL. The aldehyde content was calculated as follows:

$$\mathrm{CHO}\ (\%) = \frac{[V_b - V_{(HCl)}] \times 0.1\ \mathrm{M} \times 29 \times 100}{m} \quad (1)$$

where 0.1 M is the concentration of HCl; $V_{(HCl)}$ is the volume (L) of HCl solution used for oxidized amylose titration; V_b is the volume (L) of HCl solution used for control determination; m is the weight (g) of oxidized amylose and 29 is the molecular weight of aldehyde group. The measurement was done in triplicate.

2.3.2. Carboxyl content determination

The carboxylated amylose can react with the salt of weaker acid, such as calcium acetate, forming a salt of carboxylated amylose and releasing an equivalent amount of weaker acid. On the basis, the carboxyl content was determined by the published calcium-acetate method (Praskalo et al., 2009; Zhou, Li et al., 2016). Oxidized amyloses were kept in a vacuum oven at 60 °C for 48 h to remove the absorbed water before the test. The 0.5 g of oxidized amylose, 30 mL of 0.25 M calcium-acetate solution and distilled water were mixed together to a final