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Oxidized amylose with high carboxyl content: A promising solubilizer and carrier of linalool for antimicrobial activity

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ABSTRACT

The oxidized amyloses with different carboxyl content were prepared to include linalool for antimicrobial activity in aqueous environment. The results show that linalool can be effectively reserved from volatilization through encapsulation into amylose and oxidized amyloses. The inclusion ability of oxidized amyloses towards linalool is decreasing with the increase of oxidation level due to the depolymerization of amylose. However, the solubilization effect of oxidized amyloses to linalool is enhanced efficiently owning to the high water solubility of oxidized amyloses. It is interesting that the inclusion complexes have good antimicrobial activity in aqueous environment. Linalool solubilized by oxidized amyloses presents better antimicrobial performance than that solubilized by amylose, mainly resulting from that amylose-linalool inclusion complex would aggregate and retrograde fast in aqueous solution, which is disadvantageous for the release of linalool. The study suggests that oxidized amylose is a promising solubilizer and carrier of linalool for antimicrobial activity in aqueous environment.

1. Introduction

Linalool is one of the most widely used fragrances in light industry and daily use, which exists in essential oils of numerous aromatic plants such as lavender and orange (Bazemore, Rouseff, & Naim, 2003; Hanneguelle, Thibault, Naulet, & Martin, 1992). In recent years, linalool has been reported to possess antimicrobial, insecticidal and pharmacological effects, which provide more possibilities for the application of linalool (Beier et al., 2014; Modjinou et al., 2015; Peana et al., 2002). For instance, linalool can be applied in food systems as an antimicrobial additive (Suppakul, Sonneveld, Bigger, & Miltz, 2011). However, the volatile and hydrophobic nature of linalool would restrict its applications in aqueous environment. Besides, linalool can be easily oxidized under normal temperature and pressure, thus inducing contact allergy when exposed to air (Backtorp et al., 2006; Skold, Borje, Harambasic, & Karlberg, 2004). Hence, measures should be taken to enhance the stability and solubility of linalool.

Microencapsulation is a common way to protect guest molecules against volatilization and undesired reaction and limit undesired odor. The most frequently used carriers in microencapsulation are cyclodextrin, starch and crown ether et al. Starch, chitosan, alginate and β-cyclodextrin have been used as carriers to include linalool to control the volatility and improve the physicochemical stability (Lopez, Maudhuit, Pascual-Villalobos, & Poncelet, 2012; Menezes et al., 2013; Quintans-Junior et al., 2013). The formulations described above could indeed lower the releasing rate of linalool whereas the water solubility of linalool was decreased. The low water solubility is a severe limit to the application of linalool in aqueous environment. Therefore, it is necessary to do further researches on microencapsulation of linalool, including carrier selection and encapsulation method exploration.

Amylose is a linear polymer which has abundant source and broad application in numerous fields. Amylose can form helical structure in aqueous media, which can include guest molecules such as iodine, alcohols and fatty acids through hydrophobic interaction (Katzbeck and Kerr, 1950; Kim & Choi, 1994; Mikus, Hixon, & Rundle, 1946). In the last decades, numerous studies have been conducted to investigate inclusion complexes between amylose and various guest molecules (Putseys, Lamberts, & Delcour, 2010; Ryno, Levine, & Iovine, 2014). However, amylose has low solubility and is unstable in aqueous environment because of the strong trend towards retrogradation (Dintzis, Beckwith, Babcock, & Tobin, 1976; Everett & Foster, 1959). Furthermore, the formation of amylose-guest inclusion complexes will induce great reduction of water solubility and solution stability of the products. This property would bring difficulties to both preparation and application of amylose-guest inclusion complexes in aqueous environment. In

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our previous work, we developed a new two-step oxidation method using H_2O_2 as the oxidizer and $CuSO_4$ as the catalyst to prepare oxidized amylose with high oxidation level. The oxidized amyloses can be used as carriers to include guest to control the releasing and improve the physicochemical stability. Moreover, the solubility and solution stability of the oxidized amylose-guest inclusion complexes in aqueous solutions were enhanced to a large extent, and meantime the helices of oxidized amylose could be partially reserved by controlling the oxidation level (Zhou et al., 2016). The oxidized amylose was an ideal host to prepare inclusion complexes for application in aqueous environment.

In the present work, the oxidized amyloses with different oxidation level were prepared through the two-step oxidation method. Then the oxidized amyloses were used as solubilizer and carrier to encapsulate linalool. The structure, inclusion capacity, releasing behavior and antimicrobial properties of the as-prepared oxidized amylose-linalool inclusion complexes were studied.

2. Material 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. Linalool with 98% purity was purchased from Aladdin Industrial Corporation (Shanghai, China). All other chemicals and reagents were of analytical grade unless otherwise stated.

2.2. Preparation of oxidized amyloses

Oxidized amyloses (OA) were prepared through a two-step method in our previous work (Zhou et al., 2016). In the first step, 30 g of amylose powder was mixed with 30% H_2O_2 in a container. Then 1 mL of 0.05% $CuSO_4$ solution was added. The mixture was transferred into a flask and then kept at 40 °C 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. Further oxidation was continued for 30 min at 100 °C 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) to remove the copper ion. The precipitate was then freeze-dried. Oxidized amylose with different degree of oxidation was obtained by controlling the dosage of H_2O_2.

2.3. Preparation of inclusion complexes

Amylose or oxidized amylose was dissolved in boiled water and kept at 55 °C under magnetic stirring for 30 min. The excess amylose or oxidized amylose was removed to obtain the saturated solutions. Then linalool was added and the mixture was magnetically stirred at room temperature for 30 min. The excess linalool floated on the surface was removed. Then the mixture was centrifuged at 3000 rpm for 20 min. The content of linalool in the supernatant was measured by GC analysis (6890A, Agilent, USA) to evaluate the inclusion ability of amylose and oxidized amyloses towards linalool and the solubility of linalool in saturated amylose and oxidized amyloses solutions. Then the supernatant was freeze-dried to obtain the inclusion complex powder. The inclusion capacity and solubility of linalool were calculated as follows:

$$\text{Inclusion capacity} = \frac{m_1}{m_2} \quad (1)$$

$$\text{Solubility of linalool} = \frac{m_1}{v} \quad (2)$$

where m_1 is the mass of linalool in the supernatant (mg); m_2 is the mass of amylose or oxidized amylose in the supernatant (g) and v is the volume of supernatant (mL).

2.4. Carboxyl content determination

The carboxyl content was determined according to the published calcium-acetate method with some modifications (Wang & Wang, 2003). The samples were kept in a vacuum oven at 60 °C for 48 h to remove the absorbed water before the test. 5 g of sample was dissolved in 50 mL of 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 of 0.5 M calcium-acetate solution and extra water were added to make the final volume of 250 mL. After frequent shaking for 30 min, the solution was vacuum filtered. 50 mL of 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:

$$\text{COOH}(\%) = \frac{\left[V_{(\text{NaOH})}\text{-}V_b\right] \times 0.05\text{M} \times 45 \times 100}{m} \quad (3)$$

where 0.05 M is the concentration of NaOH; $V_{(\text{NaOH})}$ 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.

2.5. Determination of aqueous solubility of oxidized amyloses

Samples were dissolved in distilled water at 100 °C to an over saturated state. After being kept at 20 °C for 24 h under mild agitation, the solution was filtered and undissolved mass was dried and weighed. The aqueous solubility of each sample was calculated as follows:

$$S = \frac{(m_2\text{-}m_1) \times 100}{m} \quad (4)$$

where m_1 is the undissolved mass; m_2 is the total mass added in the solution; m is the mass of distilled water and S represents the solubility in distilled water. The measurements were done at least in triplicates to allow a significant statistical analysis.

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 of sample and 200 mg of 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 diffraction patterns of the samples were obtained from an 18 KW rotating X-ray diffractometer (MXPAHF, Japan) with a fixed CuKα radiation (λ = 1.54 Å). The scanning region of the diffraction angle (2θ) was from 5° to 45° with a rate of 2°/min.

2.8. Morphology observation

The morphology of different samples was observed by a scanning electron microscope (SEM) (Shimadzu, SSX-550). The aqueous