Carbohydrate Polymers 147 (2016) 392–400

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Impact of dual-enzyme treatment on the octenylsuccinic anhydride esterification of soluble starch nanoparticle

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ARTICLE INFO

Article history:
Received 5 December 2015
Received in revised form 3 April 2016
Accepted 5 April 2016
Available online 7 April 2016

Keywords:
Starch nanoparticle
Sugary maize
Dual-enzyme treatment
Esterification
OS groups distribution
Emulsion properties

ABSTRACT

The hypothesis of improving the esterification of sugary maize soluble starch through dual-enzyme pretreatment was investigated. Native starch nanoparticle (NSP) was enzymatically pretreated using β-amylase and transglucosidase (ESP) and then esterified with octenylsuccinic anhydride (OSA). The degree of substitution (DS), reaction efficiency (RE), molecular weight (Mw), molecular density (ρ) and *in vitro* digestibility were determined. Fourier transform infrared spectroscopy and confocal laser scanning microscopy were used to analyze starch particle and its OS derivatives. The emulsification properties of OS-NSP and OS-ESP were also compared. The results showed that dual-enzyme modification increased the DS and RE of OSA modified starch particle compared with the control. Enzymatic modification had a thinning effect at the surface of starch particle, resulting in lower Mw. The extent of reduction in ρ of OS-ESP was greater than that of OS-NSP. At equivalent DS, OSA modification of EPS was more effective than that of NPS in reducing digestibility. Also, there was brighter fluorescence spheres of OS-ESP in comparison to OS-NSP at equivalent DS, suggesting more OS groups were substituted on the chains near the branch points at less density areas. OS-ESP with higher DS (0.0197) had lower zeta-potential and average particle size for superior emulsion stabilization properties with high stability. The results revealed the OS-starch prepared under dual-enzyme pretreatment was a Pickering particle stabilizer for potential application in encapsulation and delivery of bioactive components.

1. Introduction

Starch is the bulk energy storage polymer in cereals, roots and tubers, and is widely available as a renewable resource for industry. Native starch is known to be structurally too weak and functionally too restricted for most industrial applications and is often tailored through physical, chemical or physical modifications to develop desirable functional properties (BeMiller & Whistler, 2009; Miao, Jiang, Cui, Zhang, & Jin, 2015; Tharanathan, 2005). Modification to bring about solubility, pasting properties, dispersion or digestibility can entail glycosidic bond cleavage, the formation of new functional groups, the substitution of free available hydroxyl groups, or the bridging of molecular chains by a cross-linking reagent (Miao, Li, Jiang, Cui, Zhang, & Jin, 2014; Sweedman, Tizzotti, Schäfer, & Gilbert, 2013; Tharanathan, 2005). Among these strategies, esterification reaction using octenylsuccinic anhydride (OSA) has attracted greater attention because of whole molecules with an amphiphilic character (Sweedman et al., 2013). Generally, OS-starch has been prepared in a two-phase system by reacting OSA in slurry with the solid starch, resulting in the hydrophobic groups grafted to hydrophilic glucan chain. Currently, OS-starch is used in a wide range of foods for a variety of purposes; including emulsification, encapsulation, films and coatings, gel production and delivery carrier (Miao, Li, Jiang, Cui, Zhang et al., 2014; Nilsson & Bergenståhl, 2007; Sweedman et al., 2013; Wang, Li, Chen, Xie, Yu, & Li, 2011).

Soluble starch particle (phytoglycogen) is a primary amylopectin analogue with more highly branched structure in the sugary-1 (su-1) mutants of maize, rice, sorghum or barley (Ball et al., 1996; Ball & Morell, 2003; Miao, Li, Jiang, Cui, Lu, & Zhang, 2014; Powell, Sullivan, Sweedman, Stapleton, Hasjim, & Gilbert, 2014). During starch biosynthesis, starch synthase, starch branching enzyme, and starch debranching enzyme work coordinately to produce starch granules (Ball et al., 1996). Su-1 mutant leads to the deficient in debranching enzyme, which is to trim abnormal branches that inhibit the formation of physically organized starch

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http://dx.doi.org/10.1016/j.carbpol.2016.04.012
0144-8617/

crystals and granules (Tetlow, Morell, & Emes, 2004). In the absence of debranching enzyme, highly branched soluble starch particle is formed to replace starch granules. It has been reported that soluble starch particle is a typical nano-scale polymer ranging from 30 to 100 nm and exhibits a spherical shape (Miao, Li, Jiang, Cui, Lu et al., 2014; Putaux, Buléon, Borsali, & Chanzy, 1999; Ye, Miao, Huang, Lu, Jiang, & Zhang, 2014). Soluble starch particle is made up of α-1,4 linked glucose units, forming linear chains with average lengths of 10–12 glucose units that are joined together via α-1,6 linkages as branch points. The branch density and molecular density of soluble starch particle is approximately 7–10% and 1000–2000 g/mol nm^3, respectively, which is higher than that of amylopectin (5–6%, 50–300 g/mol nm^3) (Huang & Yao, 2011; Miao, Li, Jiang, Cui, Lu et al., 2014; Yun & Matheson, 1993). Currently, soluble starch particle as a novel carbohydrate material has received great interest for their biodegradability and functionality. Although several studies on the structural and physicochemical characteristics of modified soluble starch particle have been conducted (Izawa, Nawaji, Kaneko, & Kadokawa, 2009; Miao, Li, Huang, Jiang, & Zhang, 2015; Miao, Li, Huang, Ye, Jiang, & Zhang, 2015; Putaux, Potocki-Véronèse, Remaud-Simeon, & Buleon, 2006; Scheffler, Wang, Huang, Gonzalez, & Yao, 2010), little work has been reported about the effect of dual-enzyme treatment on OSA esterification of soluble starch nanoparticle. In this study, the soluble starch particle from su-1 maize mutant was firstly treated by degradation of β-amylase and transglucosidase and esterification using OSA, and then prepared to determine the changes of molecular structure, distribution of OS groups and emulsion properties of modified starch.

2. Materials and methods

2.1. Materials

Su-1 maize kernels were purchased from the Chinese Academy of Agricultural Sciences (Beijing, China). Transglucosidase from *Aspergillus* (L-500) and β-amylase from barley (OPTIMALT BBA) were donated by DuPont Genencor International Inc. (Wuxi, China). α-Amylase from porcine pancreas (Type VI-B, ≥10 units/mg solid) and 2-octen-1-ylsuccinic anhydride (97%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Amyloglucosidase (3300 U/ml) and the glucose oxidase-peroxidase assay kits (Cat. No. K-GLUC) were obtained from Megazyme International Ireland Ltd. (Wicklow, Ireland). All other chemicals were reagent grade and from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Isolating sugary maize soluble starch particle

The soluble starch particle was isolated from Su-1 maize in our laboratory following the methods of as described in a previous study (Miao, Li, Jiang, Cui, Lu et al., 2014). The sugary-1 maize fresh kernels were ground with five times its weight of deionised water. The mixture was filtered through 100-mesh sieves and then centrifuged at 10,000*g* for 10 min. The supernatant was collected and three volumes of ethanol were added to precipitate the soluble starch. After centrifugation and decanting, the precipitate was placed in a fume hood to remove the residual ethanol. The dried solid with a yield of 29% was ground to form a powder, which was then used for further studies within the next few months.

2.3. Dual-enzyme modification

The soluble starch particles were enzymatically treated with double enzyme according to the method of Miao, Li, Huang, Ye et al. (2015). The soluble starch particles (3 g) were dissolved in 30 ml sodium acetate buffer solution (pH 5.2, 0.02 M). β-Amylase (300 U/g dry weight of starch) and transglucosidase (50 U/g dry weight of starch) were added to the solution. The enzymatic reaction was incubated at 55 °C for 4 h. Three volumes of 90% ethanol (v/v) were added to facilitate reactant precipitation. After centrifugation and decanting, the precipitate was suspended in ethanol and filtered twice. The collected solid (approximately 56% yield) was obtained after removing the residual ethanol and stored in a desiccator for further analysis.

2.4. Octenylsuccinic anhydride modification

The native starch particle (NSP) or enzymatically treated starch particle (ESP) was esterified with OSA according to the method of He, Liu and Zhang (2008). OSA (1% or 3% based on the weight of starch) was added to the starch dispersion (30%, w/w) while the pH was maintained at 8.5. The reaction was conducted at 35 °C and terminated after 8 h by reducing pH to 6.5. To collect substituted starch, three volumes of ethanol were added to the reaction mixture. After centrifugation and decanting, the precipitated was placed in a fume hood to remove the residual ethanol to prepare dry powder of OS-starch. Two type OSA modified samples were named OS-NSP and OS-ESP, respectively.

The degree of substitution (DS) was measured using the titration method as suggested by Kweon, Choi, Kim and Lim (2001) with a slight modification. The DS was calculated using the following formula:

$$DS = \frac{0.162 \times (A \times M)/W}{1 - [0.210(A \times M)/W]},$$

where A was the titration volume of NaOH, M was the molarity of NaOH, W was the dry weight of sample, 162 and 210 were the molecule weight of glucose unit and OS group, respectively.

The reaction efficiency (RE) was calculated as follows:

$$RE = \frac{\text{Actual DS}}{\text{Theoretical DS}} \times 100\%.$$

The theoretical DS was calculated by assuming that all the added OSA reacted with starch particle to form the ester derivative.

2.5. High-performance size-exclusion chromatography (HPSEC) analysis

The weight-average molecular weight (Mw) and z-root mean square radius of gyration (Rz) was analysed by using HPSEC-MALLS-RI method described by Miao, Li, Jiang, Cui, Lu et al. (2014) with a slight modification. The starch samples (10 mg) were completely dissolved with 2 ml deionised water with stirring. Two series tandem columns (300 × 8 mm, Shodex OH-pak SB-806 and 804, Showa Denko K.K., Tokyo, Japan) with an OH-pak SB-G guard column, a DAWN HELEOS II laser photometer fitted with a He-Ne laser (λ = 632.8 nm) with a K-5 flow cell, and an OPTILAB® T-rEX Interferometric Refractometer were used. The flow rate was set at 0.5 ml/min with a mobile phase of distilled-deionised water (pH 6.8) containing 0.02% NaN_3. A dn/dc value of 0.138 was used in molecular weight calculation and data processing was performed using ASTRA software (Version 5.3.4.14, Wyatt Technology). Weight-average molecular weight (Mw) and z-root mean square radius of gyration (Rz) were obtained using the second-order Berry method. The molecular density (ρ) was calculated as Mw/Rz^3.

2.6. In-vitro *digestion*

The digestibility of starch particle was analysed according to the method of Englyst, Kingman and Cummings (1992) with some modifications. The glucose content of *in vitro* enzymatic