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Effects of hydrolysis by xylanase on the emulsifying properties of *Artemisia sphaerocephala* Krasch. polysaccharide

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ABSTRACT

In the present study, *Artemisia sphaerocephala* Krasch. polysaccharide (ASKP) and high-molecular-weight fractions (60P) were treated with xylanase to improve the emulsifying properties. Hydrolyzed ASKP and 60P were structurally characterized using analytical size-exclusion chromatography and ion chromatography system. Results showed that molecular weight and radius of gyration decreased, while the molecular conformation became more extended. After hydrolysis, the monosaccharide composition had no significant changes, indicating that xylanase disrupted the main backbones of 60P and 60P in ASKP without affecting their side chains. Rheological analysis showed that the hydrolyzed 60P and ASKP had lower viscosities than those of native ones. The hydrolysates were also conferred stronger capacities to reduce the interfacial tension at oil/water interface. After treated by xylanase, the hydrolysates demonstrated the better abilities to form smaller droplets in oil/water emulsions and were capable to stabilize the emulsions for 3 days at 60 °C. In conclusion, smaller molecular weight could promote the improvements of emulsifying properties of 60P and ASKP. The improved emulsifying properties allowed the hydrolysates to act as better emulsifiers in food industry. Meanwhile, the hydrolysates with low viscosities were ideal dietary fiber supplements in the fortified foods.

1. Introduction

Artemisia sphaerocephala Krasch. polysaccharide (ASKP) is the crude water-soluble heteropolysaccharide extracted from the outer layer of its seeds using hot water (Guo et al., 2011b). Since the 1980s, ASKP has been widely used as the thickener, stabilizer, water-holding and film-forming in China (Bai, Yong, & Yun, 2000; Liu & Gu, 2006). Using 60% (w/v) ammonium sulfate, ASKP can be precipitated into two main fractions, which were 60P (high Mw: 551 kDa) and 60S (low Mw: 39 kDa) (Guo et al., 2011b). The structures of 60P and 60S were confirmed to be arabino-glucuronoxylan and galacto-glucomannan, respectively (Guo et al., 2011a, 2012).

Dickinson (2003) indicated that an ideal emulsifier is typically with relative low molecular weight. Because of its low molecular weight, Portulaca Oleracea gum shows excellent emulsifying ability to form the oil-in-water emulsion with the droplet size smaller than 2 μm and long-term stability. Conversely, emulsions formed by high-molecular-weight polysaccharides (such as guar and fenugreek galactomannans) are with 5–10 μm droplet sizes and sensitive to external factors, such as dilution and pH (Garti & Leser, 2001). Evaluating emulsifying properties of four citrus pectin samples with different molecular weights (48, 56, 70 and 146 kDa) but same degree of esterification (70%), Akhtar, Dickinson, Mazoyer, and Langendorff (2002) concluded that depolymerized pectin with molecular weight of 70 kDa could form the better emulsion with excellent stability. Similarly, Leroux, Langendorff, Schick, Vaishnav, and Mazoyer (2003) concluded that depolymerized citrus pectins with the molecular weights between 50 and 80 kDa were more efficient to act as the emulsifiers. Lower and higher molecular weights can lead to the partial loss of their emulsifying capacities.

Recent study reported that ASKP showed the ability to reduce the surface tension of oil/water interface which was better than that of gum acacia (Li, Hu, Li, & Ma, 2016). Chen et al. (2014) reported that ASKP possessed a medium molecular weight. Unexpectedly, the emulsifying properties of ASKP were not as good as gum acacia (Li et al., 2016). Against this background, we hypothesize that the poor emulsifying ability of ASKP may partly ascribe to the high molecular weight of 60P in it. Considering the 1,4-linked *xylp* backbone of 60P, hydrolysis by xylanase from *Trichoderma viride* is believed to be an ideal way to reduce the molecular weight

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of ASKP. By hydrolyzing the 1,4-β-D-xyloside linkages in xylan, 60P in ASKP can be disintegrated into low-molecular-weight fractions but with the similar structure (Irfan & Syed, 2012).

In the current study, 60P and 60P in ASKP were digested to the similar molecular weight by xylanase. Our objective of this study is to evaluate the effects of hydrolysis by xylanase on the emulsifying properties of 60P and ASKP. This will interpret the role of molecular weight in acting as an emulsifier for ASKP and 60P.

2. Materials and methods

2.1. Materials

ASK seeds were obtained from a village market in Yulin, Shaanxi province, China. Medium-chain triglyceride (MCT) oil was purchased from Nisshin Oillio Group, Ltd (Tokyo, Japan). Gum acacia (GA) with senegal-quality and xylanase from *Trichoderma viride* were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were reagent grade.

2.2. Extraction and fractionation of ASKP

ASKP was extracted from ASK seeds using hot water and then isolated into 60P and 60S by 60% (w/v) ammonium sulphate precipitation (Guo et al., 2011b).

2.3. Enzyme hydrolysis

In order to reduce the molecular weight of ASKP, xylanase from *Trichoderma viride* was added (0.2U/mL) to 2% (w/v) solution at 30 °C, pH4.5 with constant stirring for 2 h, followed by boiling to stop the reaction, dialysis (molecular weight cut-off 8000Da) for 48 h and freeze-drying. 60P was treated with the same procedure but the period was 6 h. The hydrolyzed samples were designated ASKX and 60PX, respectively.

2.4. Molecular characterizations

Analytical size-exclusion chromatography of samples was performed using HPSEC equipped with a MALLS detector, a RI detector and a UV detector (Wyatt, Santa Barbara, CA, USA) according to our previous report (Li et al., 2016). 0.146 mL/g was used as the dn/dc value for calculation (Guo et al., 2013). Relative peak areas were calculated using Origin 8.5.1 (OriginLab, Northampton, MA, USA).

2.5. Monosaccharide composition and protein content

Analysis of monosaccharide composition of samples was conducted using ICS-3000 Ion Chromatography system (Dionex, Sunnyvale, CA, USA) according to our previous report (Li et al., 2016). After the pretreatment, the solution was subjected to the monosaccharide composition analysis on a CarboPac™ PA20 (3 mm × 150 mm) column (Dionex, Sunnyvale, CA, USA). Protein contents of samples were measured with Bradford method according to Funami et al. (2007). Each sample was measured in triplicate.

2.6. Determination of apparent viscosity

Apparent viscosity by shear rate of polysaccharide solutions (4%, w/v) was measured using an ARES-G2 rheometer (TA instruments, New Castle, USA) according to Guo et al. (2011b). Samples were dispersed in 70 °C water with constant stirring for 2 h, followed by cooling down at room temperature for 1 h. All the measurements were conducted at 25 °C. Parallel-plate (40 mm) with the gap of 1 mm was employed for the test. The shear rate range was from 1 to 1000 s^{-1}.

2.7. Determination of interfacial tension

Interfacial tension measurement was performed on a DCAT 21 surface/interface tensiometer (Dataphysics Instruments GmbH, Filderstadt, Germany) according to our previous report (Li et al., 2016). Polysaccharides were dissolved in water to make 0.75% (w/v) solutions and served as the dispersion mediums. MCT served as the dispersed phase while GA served as the reference. All the measurements were conducted at 20 °C and each sample was measured in triplicate.

2.8. Preparation of emulsion

The emulsion preparation was according to our previous report (Li et al., 2016). Polysaccharides (concentrations varying from 0.4 to 1.6 (w/w)) as the emulsifiers and sodium citrate (0.5%, w/w) as the preservative were added to 25 °C water, followed by constant stirring overnight. MCT (5%, w/w) as the oil source was added to give 100 g and then pre-homogenized using a T18 digital disperser (IKA, Staufen, Germany) at 20,000 rpm for 3min. The final emulsion solutions were obtained with an M-110EH microfluidizer (MFIC, Westwood, MA, USA) at 75 MPa.

2.9. Emulsifying properties

Emulsifying properties were evaluated from droplet size, droplet size distribution and zeta potential by Zetasizer Nano ZS90 (Malvern Instruments Ltd, Worcestershire, UK). Droplet size and zeta potential were measured with the fresh emulsions while the droplet size distribution was after 3 days 60 °C. Before measurements, the emulsions were diluted to 1/20 (v/v) with deionized water (Hou et al., 2012; Nakauma et al., 2008). Each sample was measured three times at 20 °C and the results of droplet size were calculated as the average cumulants mean diameter.

3. Results and discussion

3.1. Molecular characterization

Size exclusion chromatograms of samples are presented in Figs. 1 and 2. Weight average molecular weight (M_w), radius of gyration (R_g), polydispersity index (M_w/M_n), relative peak area and slope of conformation plot are summarized in Table 1. Monosaccharide composition and protein content of samples are listed in Table 2.

Size exclusion chromatography of samples conformed that hydrolysis by xylanase reduced the size of 60P chains and those in ASKP (Figs. 1 and 2). Designate the three main peaks at the retention time of about 11, 16 and 20 min in RI chromatogram as Peak 1, Peak 2 and Peak 3, respectively. Based on the report of Guo et al. (2011b), Peak 1 and Peak 2 were recognized as the two main fractions of ASKP – 60P and 60S while Peak 3 was protein. As shown in Table 1, the relative peak area of Peak 1 and its corresponding M_w/M_n increased for the hydrolysis of xylanase while the M_w and R_g decreased, both in ASKX and 60PX. Chromatogram of RI detector in Fig. 1 showed the increase of small molecular fractions and the decrease of large molecular fractions in Peak 1, indicating the degradation of 60P chains in ASKX. Similarly, the increase of small molecular fractions in Peak 1 of 60PX indicated the breakdown of 60P chains (Fig. 2). In Table 2, the monosaccharide compositions of hydrolyzed samples were similar to those of untreated ones, indicating that xylanase disrupted the main backbone of 60P

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