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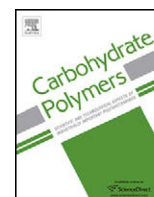

Degradation kinetics and structural characteristics of pectin under simultaneous sonochemical-enzymatic functions

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ABSTRACT

This study investigated the degradation kinetics and structural properties of pectin with combining ultrasound and pectinase treatment. Ultrasound at an intensity of 4.5 W mL^{-1} and a time of 10 min significantly enhanced the enzymatic degradation of pectin weight-average molecular weight (M_w). The degradation kinetics model of pectin followed $1/Mw_t - 1/Mw_0 = kt$, suggesting the randomness of the degradation process. Synergistic effects of ultrasound and pectinase were observed at 20–60 °C and were more effective at lower temperatures. Furthermore, the degree of methoxylation (DM) of sonoenzymolysis pectin significantly decreased whereas the degree of acetylation (DAc) remained unchanged compared to the original and enzymolysis pectin. Simultaneous functions of ultrasound and pectinase caused severe decomposition in pectin homogalacturonan (HG) regions without altering the monosaccharides types, configurations and glycoside linkages of the pectin samples. The complex polymeric structures of pectin transformed into smaller units with simpler branches and shorter chains after sonoenzymolysis reactions.
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1. Introduction

Pectin is an important component of the plants cell walls and is widely applied in the food, pharmacy and cosmetic industries (May, 1990). Characterization of pectin is difficult given its large molecular weight and complex structures. The identified compositions of pectin include homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II) and xylogalacturonan (XG) (Maxwell, Belshaw, Waldron & Morris, 2012). The RG-II and XG regions (which are highly branched structures bound at HG regions) are less common in pectin structures. The HG region is the "smooth region" of pectin and mainly consists of 1, 4-α-D-galacturonic acid (GalUA). Carboxyl groups of GalUA are partially methylesterified or acetylated; degrees of methoxylation (DM) and acetylation (DAc) are known to determine many functional properties (such as gelling, stabilizing and emulsifying abilities) of pectin (Lofgren, Guillotin & Hermansson, 2006). RG-I regions constitute as the "hairy region" and contain the backbone of a repeating disaccharide unit (GalUA and rhamnose (Rha) residues) with neutral sugar side chains binding to the Rha residues. As more and more studies are focused on the structure-function relationships of pectin, the RG-I region has attracted great attention because of its potential anti-cancer activity (Maxwell et al., 2012). A proposed mechanism for this bioactivity is that the specific galactan structures contained in RG-I side chains could restrain galectin-3, a protein whose overexpression is observed in many cancers (Fan et al., 2010; Gunning, Bongaerts & Morris, 2009). Based on this conclusion, pectin structures are modified to design functional products, which are called "modified pectin" (MP).

Generally, production procedures of MP can be divided into two steps: de-polymerization of HG regions and modification of RG-I side chains (Maxwell et al., 2012). As an important process of MP production, degradation of pectin HG regions is commonly conducted by chemical or enzymatic treatments. Despite the high efficiency of these traditional methods, pollution problems and expensive costs restrict their applications. Current studies also utilize ultrasound to degrade pectin (Zhang et al., 2013a; Zhang et al., 2013b; Zhang, Zhang, Liu, Ding & Ye, 2015). A previous study (Zhang et al., 2013a) found that the weight-average molecular weight (M_w) and DM of apple pectin effectively decreased after sonication. Nevertheless, pectin would not be thoroughly degraded by ultrasound

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due to the decrement of energy transmission under high-intensity or long-time ultrasound irradiation (Sun, Ma, Ye, Kakuda & Meng, 2010; Zhang et al., 2013b). In this case, development of a green, efficient and economical method for pectin depolymerization becomes an urgent need.

Recently, there has been growing evidence that the simultaneous function of ultrasound and enzymes is an ideal method for polymer degradation. Effectiveness of this combination method is attributed to the improved enzyme activity under ultrasound irradiation (Prajapat, Subhedar & Gogate, 2016a). Under mild ultrasound conditions, oscillation and rupture of small-amplitude cavitation bubbles lead to the generation of shear forces and free radicals, which could favorably alter enzyme structures and cause the exposure of more active domains (Zhang, Fu & Liang, 2008). Also, ultrasound depolymerizes the polymer substrate, which causes the resultant dispersed structures to be more conducive to enzyme action (Subhedar & Gogate, 2013). Furthermore, microjets produced by ultrasound cavitation accelerate mass transfer in the reaction system (Warmoeskerken, van der Vlist, Moholkar & Nierstrasz, 2002). All these factors contribute to the improvement of enzyme activity under a mild ultrasonic field. However, due to the complexity and susceptibility of enzyme structures, fierce mechanical and chemical effects formed from intense ultrasound conditions will inhibit enzyme activity and retard degradation processes (Islam, Zhang & Adhikari, 2014; Ma et al., 2011; Subhedar & Gogate, 2014). Therefore, variations in enzyme activity strongly depend on operating conditions. Combination of ultrasound and enzymes has been observed to promote degradation of a series of polymers, such as dextran (Bashari et al., 2013), cellulose (Prajapat, Das & Gogate, 2016b; Szabo & Csiszar, 2013), guar gum (Prajapat et al., 2016a), xylan (Sun, Zhang, Xiao & Jin, 2015), pectin (Ma et al., 2016), etc. Our previous study (Ma et al., 2016) applied the combination of ultrasound and pectinase to pectin degradation. Results indicated that reducing sugar productions were increased by 32.59% under an ultrasonic field of 4.5 W mL^{-1} intensity for 10 min, compared with the traditional enzymatic hydrolysis.

Although there have been some studies on sonoenzymolysis treatments of polymers, the research focus is mainly on the yield of products rather than changes in product structures. The present work evaluated the effect of the combination method from a perspective of substrate structures variations. Pectin M_w was designated as an indicator of the degradation extent. Degradation kinetics of sonolysis, enzymolysis and sonoenzymolysis were studied to estimate the synergistic effects of ultrasound and pectinase. Detailed structural information, including the DM, DAc, monosaccharide component, primary structures and nanostructures of the degradation products were investigated in order to elucidate the degradation paths of pectin under simultaneous sonochemical-enzymatic effects and provide a theoretical basis for the practical applications of the combination method in MP preparations.

2. Materials and methods

2.1. Chemicals

Pectinase from *Aspergillus niger* (major component: polygalacturonase), pectin from a citrus peel, standard dextran (M_w: 670, 270, 150, 50, 25 and 12 kDa) and standard monosaccharides (including mannose (Man), Rha, glucuronic acid, GalUA, lactose, glucose (Glu), galactose (Gal), xylose (Xyl), arabinose (Ara) and fucose (Fuc)) were purchased from Sigma–Aldrich (Shanghai, China). Methyl alcohol, acetonitrile, isopropanol and 1-phenyl-3-methyl-5-pyrazolone (PMP) were of HPLC-grade; other chemicals were of analytical grade. All chemicals were used as received from the supplier without further purification.

2.2. Degradation of pectin catalyzed by pectinase

The pectinase and pectin powders were dissolved in 1 mol L^{-1} citric acid-phosphate buffer at a pH of 4.0 with the ultimate concentrations of 2 mg mL^{-1} and 5 mg mL^{-1}, respectively. One milliliter of pectinase samples was added to nineteen milliliters of pectin solution in conical flasks, which were placed in a shaking waterbath. The enzymolysis reactions were carried out at different temperatures ranging from 20 °C to 60 °C for 2 min to 40 min. After incubation, the samples were immediately put in a boiling waterbath at 100 °C for 3 min to inactivate the enzyme.

2.3. Ultrasound treatments

In the sonoenzymolysis reactions, the mixtures were sonicated by a probe ultrasonic processor (JY92-IIDN, Ningbo Scientz Biotechnology Co., Ningbo, China) with a 1 cm diameter horn micro tip. The instrument was operated at 22 kHz and had a maximum power of 900 W. One milliliter of the enzyme sample and nineteen milliliters of pectin solution were mixed in a cylindrical glass reactor (with an inner diameter of about 2.77 cm), and the generator probe was immediately inserted (about 1 cm below the liquid level) to dissipate ultrasonic energy. The reactor was immersed into a low-temperature thermostatic water bath (DC-1006, Safe Corporation, Ningbo, China) in order to keep the solution temperature at a constant. The mixture was processed at different ultrasound intensities ranging from 0.9 to 9 W mL^{-1} for different times (5–40 min) at 30 °C. Sonoenzymolysis reactions were also conducted at 20–60 °C for 2–10 min to investigate the degradation kinetics of pectin molecular weight at each temperature. After the degradation treatments, the mixture was immediately put in a boiling water-bath at 100 °C for 3 min to inactivate the enzyme.

In the sonolysis reaction, 1 mL buffer was added instead of the prepared pectinase sample. The pectin solution was treated with ultrasound as described above.

2.4. Determination of molecular weight and its polydispersity

In this study, the molecular weight was denoted as the weight-average molecular weight (M_w), while its distributions were quantified by calculating the polydispersity index $\left(= M_w/M_N\right)$. The molecular parameters of pectin samples were measured using SEC-HPLC as described by Zhang et al. (2013a) with some modifications. The Waters 1525 HPLC system (Waters, US) was equipped with a TSK-GEL mixed-bed column ($G4000PW_{XL}$, 300 × 7.8 mm, 10 μm; Tosoh Bioscience, Tokyo, Japan). The degradation samples were dialyzed with the dialysis tube MD 34 (M_w: 8000–14000 kDa) in distilled water for 48 h and then filtered through a 0.45 μm membrane before injection. The prepared pectin solution (40 μL) flowed through the system with 0.2 M NaCl as the mobile phase at 0.5 mL/min and 40 °C. After 30 min of elution, the eluent was monitored with a refractive index detector (Waters 2414, US). The molecular weight and its polydispersity of pectin samples were calculated using Breeze 2 GPC.

2.5. Degradation kinetics of pectin M_w and calculations of synergistic coefficients

The degradation kinetics model of the pectin molecular weight was investigated by graphical analysis. The random depolymerization reactions were supposed to abide by the second-order kinetics model according to Roubroeks, Andersson, Mastromauro, Christensen & Aman (2001):

$$1/Mw_t - 1/Mw_0 = kt \quad (1)$$

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