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Degradation of high-methoxyl pectin by dynamic high pressure microfluidization and its mechanism

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High-methoxyl pectin was degraded by dynamic high pressure microfluidization (DHPM). It was found that apparent viscosity, average molecular weight and particle size of pectin decreased, whereas the amount of reducing sugars increased with increasing DHPM pressure. At the same time, the surface topography of pectin was changed from large flake-like structure to smaller porous chips. The mechanism of DHPM-induced degradation of pectin was also investigated. Fourier transform infrared spectra showed DHPM had no effect on the primary structure of pectin. On the other hand, reducing sugars content increased linearly with decreasing average molecular weight, suggesting the degradation may derive from the rupture of glycosidic bond. The breakdown of glycosidic bond may not only result from intensive mechanical forces but also from acid hydrolysis, which was evidenced in the reduction of degradation when the concentration of H^+ was lowered. In addition, neither β-elimination nor demethoxylation occurred with DHPM. Based on these results, a model was proposed to illustrate the degradation of pectin induced by DHPM.

1. Introduction

Pectin is a complex heteropolysaccharide which is widely distributed in the cell walls and middle lamella of most plants. It is safe for human consumption and has been used successfully for many years in food and pharmaceutical industries. In the food industry, pectin is commonly used as gelling agent, thickener, texturizer, emulsifier and stabilizer to modify properties of food systems (Mesbahi, Jamalian, & Farahnaky, 2005). One of the greatest difficulties in their optimal utilization is the tendency to undergo degradation caused by different kinds of physicochemical treatments, such as acid hydrolysis (Krall & McFeeters, 1998), thermal processing (Diaz, Anthon, & Barrett, 2007; Sila, Smout, Elliot, Van Loey, & Hendrickx, 2006), enzymatic treatment (Nikolic & Mojovic, 2007), combination of enzymatic and chemical degradation (Kravtchenko, Penci, Voragen, & Pilnik, 1993), and some mechanical treatments. In the case of mechanical degradation, the results varied according to physical methods used. Seshadri, Weiss, Hulbert, and Mount (2003) found that ultrasonic processing could reduce the average molecular weight and influence rheological and optical properties of high-methoxyl pectin solutions. However, rheological characteristics of pectin were not considerably affected by gamma irradiation (Dogan, Kayacier, & Ic, 2007). High hydrostatic pressure also did not cause degradation of the main chain (covalent bonds) of pectic substance (Kato, Teramoto, & Fuchigami, 1997).

Another physical method, dynamic high pressure microfluidization (DHPM), an emerging dynamic high pressure homogenization technology, has influenced research work on many food constituents, such as macromolecules and colloids, and has received a great deal of attention over the past few years (Tsai, Tseng, & Chen, 2009; Zhong et al., 2011). Recently, this method is tentatively used as a homogenizer in the production of food and medicine that contain pectin, where pectin served as a stabilizer, thickener, texturizer, or emulsifier. However, limited studies have been done to examine the effect of DHPM treatment on the structure and functional properties of pectin.

In the previous studies, DHPM-induced degradation of polymers was attributed to mechanical degradation due to powerful shear, turbulence, high-velocity impaction, high-frequency vibration, instantaneous pressure drop, and cavitation forces generated simultaneously during the treatment (Chen, Huang, Tsai, Tseng, & Hsu, 2011; Liu et al., 2009; Silvestri & Gabrielson, 1991). However, whether other chemical reactions, such as acid hydrolysis, β-elimination and demethoxylation are involved in this physical process remains unknown. In addition, what kind of covalent bond might be broken down during DHPM has not been fully understood.

In this study, the effect of DHPM on high-methoxyl pectin was evaluated, which was characterized by flow behavior, size exclusion

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chromatography, reducing sugars determination, dynamic light scattering and environmental scanning electron microscope. The mechanism of DHPM-induced degradation of pectin was also examined, including what chemical reactions may take place, and what kind of covalent bonds may be broken down.

2. Theoretical considerations

It is well known that monosaccharides of the pectin links with each other through glycosidic bonds. For pectin of N_0 monomers forming L_0 chains, the relationship between the number of reducing ends R_0 and the number of glycosidic bonds G_0 is (Thibault, Renard, Axelos, Roger, & Créeau, 1993):

$$G_0 = N_0 - L_0 = N_0 - R_0 \tag{1}$$

At the time t, the number of glycosidic bonds G_t becomes:

$$G_t = N_0 - L_t = N_0 - R_t \tag{2}$$

with the number of chains $= L_t$ and number of reducing ends $= R_t$.

It was reported that the mechanical degradation of polymer follows a first-order reaction (Harrington, 1965; Tsai et al., 2009). If the degradation is caused by the breakdown of glycosidic bonds, the number of glycosidic bonds will decrease. The relationship between G_0 and G_t is:

$$G_t = G_0 e^{-kt} \tag{3}$$

where k is the rate constant (s^{-1}), t is the time of DHPM (s).

Therefore,

$$N_0 - R_t = (N_0 - R_0)e^{-kt} \tag{4}$$

this can be transformed into:

$$1 - R_t/N_0 = (1 - R_0/N_0)e^{-kt} \tag{5}$$

Supposing that $N_0/R_t \gg 1$, $N_0/R_0 \gg 1$, and $kt \ll 1$, equation (5) becomes:

$$R_t/N_0 - R_0/N_0 = kt \tag{6}$$

Equation (6) is equal to

$$\frac{R_t}{N_A V}\cdot\frac{N_A V}{N_0} - \frac{R_0}{N_A V}\cdot\frac{N_A V}{N_0} = kt, \tag{7}$$

with N_A is Avogadro constant, and V is volume of solution.

Thus,

$$C_t - C_0 = (N_0/N_A V)kt = Kt \tag{8}$$

where C_t and C_0 (mol L^{-1}) is the concentration of reducing sugars at time t and zero, respectively. $K = N_0 k/N_A V$ is the pseudo-rate constant (mol L^{-1} s^{-1}).

In addition, the following relationship has been used in many references to describe the change of molecular weight of polysaccharides induced by DHPM (Kasaai, Charlet, Paquin, & Arul, 2003; Tsai et al., 2009):

$$1/M_t - 1/M_0 = k't \tag{9}$$

where k' is in mol g^{-1} s^{-1}. M_t and M_0 (g mol^{-1}) are average molecular weight of the polysaccharides before and after DHPM, respectively.

Dividing equation (8) by equation (9), a relationship between reducing sugars content and average molecular weight was found to be:

$$C_t - C_0 = (1/M_t - 1/M_0)K', \tag{10}$$

If the equation (10) is established, it is reasonable to infer that the degradation of pectin results from breakdown of glycosidic bonds. Where $K' = K/k'$, is in g L^{-1}.

3. Materials and methods

3.1. Preparation of materials

The apple pectin (P8471, Sigma–Aldrich, Shanghai, China) was purified according to the method of Yapo, Robert, Etienne, Wathelet, and Paquot (2007). Briefly, commercial pectins were dissolved in deionized water, and centrifuged at 30,000*g* for 20 min to remove the water insoluble fraction. The supernatants were filtered through 3 μm Millipore membranes (Millipore Co., Milford, MA, USA) and freeze-dried. The galacturonic acid content of purified pectin was 71.88 ± 1.92% determined by the m-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), and the degree of methoxylation (DM) was 70.76 ± 1.25% determined by a titrimetric method (FCC, 1981, pp. 283–286).

3.2. DHPM treatment

5 mg/mL solution of the purified pectin was dispersed in deionized water and stirred gently at room temperature to achieve complete solubilization. The natural pH of pectin solution was 3.7. DHPM was performed using an M-100EH-30 microfluidizer (Microfluidics Co., Newton, USA) under different experimental conditions: (1) at 40 MPa, 80 MPa, 160 MPa and 200 MPa under pH 3.7 for 5 passes, respectively. (2) Treated 5 passes at 160 MPa under pH 1.0, 2.0, 3.7, 5.0 and 7.0, respectively. The pH was adjusted using 0.5 M HCl or 0.5 M NaOH before the DHPM process, and set back to pH 3.7 immediately after DHPM. Solutions at pH 1.0, 2.0, 3.7, 5.0 and 7.0 were hydrolyzed for 5 min (the time equals to that spent in DHPM) at room temperature as control. Each experiment was done three times with different solutions of pectin in order to verify the repeatability of the DHPM process. Parts of solutions were lyophilized for later Fourier transform infrared (FT-IR) and scanning microscopy analyses.

3.3. Characterization of pectin degradation induced by DHPM

3.3.1. Determination of apparent viscosity

Apparent viscosity of each solution of pectin was measured at 25 °C with a Brookfield DV-IIIUltra Programmable Rheometer (Brookfield, Stoughton, MA, USA), equipped with a CP52 spindle (Spindle Multiplier Constant = 9.83, Shear Rate Constant = 2). Viscosity curves were determined at increasing shear rates from 0 to 500 s^{-1}. Experimental flow curves were compared to power's law model (Barnes, Hutton, & Walters, 1989):

$$\eta = K(\dot{\gamma})^{n-1} \tag{11}$$

where η is the apparent viscosity (Pa s), K is the consistency index (Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-1}), n is the flow behavior index ($n < 1$ for a shear-thinning fluid and $n = 1$ for a Newtonian fluid).

3.3.2. Determination of average molecular weight (M_w)

Change of molecular weight of pectin was determined by the method of Floury, Desrumaux, Axelos, and Legrand (2002) in a high performance size exclusion chromatography (HPSEC) system. The system consists of an Agilent 1200 pump unit, an automatic injector (Agilent Technologies, Waldbroon, Germany) and a refractive index detector (Brookhaven Inc., New York, USA). Columns