The effect of high speed shearing on disaggregation and degradation of pectin from creeping fig seeds


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The effect of high speed shearing (HSS) on disaggregation and degradation of pectin from creeping fig seeds was investigated. It was found that disaggregation and degradation occurred during the whole shearing process. When pectin solution was sheared at 24,000 rpm for less than 8 h, degradation happened but disaggregation was dominant during this period. After 8 h, degradation became obvious, however, a small amount of aggregates remained even after 24 h treatment, indicating that HSS may not eliminate aggregates efficiently. The presence of aggregates is one of the most probable causes for the inaccurate determination of molecular weight of pectin. A new method was proposed for calculating more accurately the molecular weight based on the change of the reducing sugar content and the variation of molecular weight. Determination of unsaturated uronide and FT-IR spectra analysis indicated that neither β-elimination nor demethoxylation occurred during the HSS, and no new functional group was formed during the HSS process.

1. Introduction

Pectin is a polysaccharide extracted from cell walls and middle lamella of plants. It has been extensively used in food and pharmaceutical industries. In the food industry, pectin is commonly used as a gelling agent, thickener, texturizer, emulsifier, and stabiliser to modify properties of food systems. One of the greatest difficulties in the characterisation of pectin is that pectins are highly prone to aggregation in solution, and problems have been identified in several studies on pectin size and conformation (Lopes da Silva & Rao, 2006). In addition, the presence of aggregates may be one of the most probable causes of inaccurate determination of its molecular weight. Considerable success in producing homogeneous solutions of pectin and similar materials has been reported using kinds of “physical” methods. Such techniques include microwave treatment (Ratcliffe, Williams, Viebke, & Meadows, 2005), sonication (Geresh, Adin, Yarmolinsky, & Karpasas, 2002), and the application of heat at an elevated pressure (Wang, Wood, Cui, & Ross-Murphy, 2001). Shearing is also an effective technique that can increase the energy of the component polymer chains and destroy the supramolecular aggregates. However, the effect of shearing for a short period is not enough to change the properties or the change is undetectable. On the other hand little is known about the effect of long time high speed shearing (HSS) on the disaggregation of pectin up to now.

When HSS is evaluated for its efficiency to reduce the aggregates, one inevitable concern is whether this method causes degradation of the polysaccharide, i.e. cleavage of glycosidic bonds in polymer chains. Ultrasonic treatment, homogenisation (including dynamic high pressure microfluidisation), and extrusion are frequently used food processing techniques. All of these processes have been reported to cause the degradation of pectin, and shearing stress was considered as a major mechanical force within these processes (Chen et al., 2012; Corredig & Wicker, 2001; Ralet & Thibault, 1994; Tiwari, Muthukumarappan, O’Donnell, & Cullen, 2010). Very few studies have evaluated the effect of shearing on the degradation of pectin. Mechanical shearing has been shown to be able to generate enough energy to disrupt the polymers. For example, amylpectin molecules were degraded under minimal shear conditions such as gentle agitation (Han & Lim, 2004), whereas according to Silvestri and Gabrielson (1991) a polymer may be mechanically degraded if the number of passes through a conventional capillary viscometer is sufficient. Distinguishing degradation from disaggregation is always an indispensable step when scientists evaluate the stability of polymers.

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Pectin isolated from creeping fig seeds (CFS) is low methoxylated and has high galacturonic acid content, molecular weight, and intrinsic viscosity (Liang et al., 2012). These properties would enable the effect of HSS to be detected easily. Therefore, CFS pectin was chosen in this study for investigating the effect of HSS up to 24 h on the elimination of aggregates and/or degradation of pectin. A new method based on the change of molecule weight and reducing sugar content induced by HSS was also proposed for the more accurate calculation of the molecule weight of those pectins containing a high number of large aggregates.

2. Theoretical considerations

It has been reported that the degradation reaction of a polymer by mechanical shearing follows a first-order reaction (Harrington & Zimm, 1965; Tsai, Tseng, Chang, Hsu, & Chen, 2010). A relationship between the reducing sugar content and the average molecular weight was deduced in our previous paper (Chen et al., 2012):

\[ C_t - C_0 = (1/M_t - 1/M_0)K \]  
\[ (1) \]

where \( C \), and \( C_0 \) (mol L\(^{-1}\)) is the concentration of reducing sugars at time \( t \) and 0, respectively. \( M_t \) and \( M_0 \) (g mol\(^{-1}\)) is the average molecular weight of the polysaccharide at time \( t \) and time 0, respectively. \( K \) is rate constant (g L\(^{-1}\)).

In addition, Eq. (1) also equals to

\[ C_2 - C_1 = (1/M_2 - 1/M_1)K \]  
\[ (2) \]

where \( C_2 \) and \( C_1 \) is the concentration of the reducing sugars at time \( t_2 \) and \( t_1 \), respectively. \( M_2 \) and \( M_1 \) is the average molecular weight of the polysaccharides at the corresponding time. Therefore, if a series or at least one pair of accurate molecular weight and reducing sugars content can be obtained for the degraded samples where no aggregates or aggregates exist at negligible amount, \( K \) can be calculated from the linear relationship of Eq. (2). Then the Mw of other samples containing large aggregates can be more accurately calculated using Eqs. (1) and (2).

3. Materials and methods

3.1. Pectin preparation

The CFS pectin was extracted and purified according to the water extraction procedure used in our previous study (Liang et al., 2012). The galacturonic acid content and degree of methoxylolation (DE) of CFS pectin was 87.65 ± 1.47% and 14.02 ± 1.30%, respectively.

3.2. High speed shearing (HSS) treatment

A 2 mg/mL CFS pectin solution was hydrated and completely dissolved in deionised water for 12 h with continuous mild magnetic stirring (400 rpm). Then, the solution was treated with Ultra-Turrax T25 disperser (IKA-Werke, Staufen, Germany) at 24,000 rpm for 0–24 h. This device consists of a rotor within a stationary stator, works at speed up to 25,000 rpm by using the rotor–stator principle, and permits the continuous operation for more than 24 h. Due to the high circumferential speed, the medium to be processed is drawn axially into the dispersion head and then forced radially through the slots in the rotor–stator arrangement. The high speed and minimal gap between the rotor and stator produce extremely strong shear forces (IKA, 2013). This equipment has been successfully used by Chen, Huang, Tsai, Tseng, and Hsu (2011) to study the degradation kinetics of chitosan induced by shearing treatments.

During shearing treatment, the solution was kept in ice water bath to eliminate the effect of temperature. Then, samples were taken every 4 h and subsequently analysed for intrinsic viscosity, particle size, molecular weight, and reducing sugars content. Parts of the solution were lyophilised for Fourier transform infra-red (FT-IR) and scanning electron microscopy analysis. The solution without shearing treatment was used as a negative control in the study.

3.3. Determination of intrinsic viscosity

The intrinsic viscosity \( ([\eta]) \) of the pectin solutions treated and untreated by HSS was measured at 25.0 ± 0.1 °C, using an Ubbelohde dilution viscometer (diameter = 0.52 mm), which was suspended in a thermostatic water bath under precise temperature control. Four millilitres of pectin solution were applied to this test. The sample was manually diluted with solvent after generating at least three flow time readings at each concentration. The intrinsic viscosity \( ([\eta]) \) was estimated by plotting \( \eta_{sp}/c \) against \( c \) according to Huggins’ equation, and extrapolation of the curves to “zero” concentration.

\[ \frac{\eta_{sp}}{c} = [\eta] + K_1[\eta]^2c \]  
\[ (3) \]

where \( c \) is the concentration, \( K_1 \) is Huggins constant, and \( \eta_{sp} \) is specific viscosity.

3.4. Determination of particle size and its distribution

Dynamic light scattering (DLS) has been reported to be an effective approach to study the aggregation behaviour of macromolecules in dilute solutions (Li, Wang, Cui, Huang, & Kakuda, 2006). The DLS determinations of pectin solutions were performed using a laser particle size analyzer (Nicomp 380 ZLS, PSS Co., Santa Barbara, USA). The solutions were diluted to a concentration of 0.5 mg/mL with deionised water, and all measurements were carried out at 25 °C (Chen et al., 2012).

3.5. Determination of molecular weights

The molecular weight of pectin samples was determined by a high performance size exclusion chromatography (HPSEC) system (Chen et al., 2012). The system consists of an Agilent 1200 pump unit, an automatic injector (Agilent Technologies, Waldbroon, Germany), a refractive index (RI) detector (Brookhaven Inc., New York, USA), and a linear mix column with a guard column. The columns were maintained at 40 °C. Pectin solutions were diluted to 0.5 mg/mL, and then filtered through 0.45 μm filters before injection. A solution of 0.05 M NaNO\(_3\) containing 0.02% NaN\(_3\) was used as mobile phase, while the elution rate was 0.7 mL/min. Dextrans of T-10, T-40, T-70, T-150, T-500, T-1000, and T-2000 were used as standards to construct a standard curve.

3.6. Determination of reducing sugar content

The reducing sugars were measured following a modified 3,5-dinitrosalicylic acid (DNS) assay described by Miller (1959). Briefly, 1.5 mL of the DNS reagent consisting of 3,5-dinitrosalicylic acid (6.5 g), sodium hydroxide (20 g), sodium sulphite (5 g), phenol (5 g), and Rochelle salt (185 g) in 1000 mL of distilled water, was added to 2 mL of the sample. The mixture was heated for 5 min accurately, and adjusted to a final volume of 10 mL. The absorbance of the mixing solution at 540 nm was determined with UV–Vis spectrophotometer (UV-2500, Shimadzu, Kyoto, Japan), using galacturonic acid to create the calibration curve. The analysis was carried out in triplicate.
3.7. Scanning electron microscopy analysis

The microstructure of the HSS treated pectin was observed by environmental scanning electron microscope (ESEM, Quanta200F, FEI Deutschland GmbH, Kassel, Germany) at 30 kV voltage and 3.0 spot size. The freeze-dried samples were attached to a circular specimen stub by sticking the pectin onto double-sided adhesive tape. The ESEM was operated at low vacuum mode (Chen et al., 2012).

3.8. Fourier transform infra-red (FT-IR) spectroscopy

The FT-IR spectra of pectins were recorded on a Nicolet 5700 spectrometer (Thermo Fisher Scientific, USA) using the absorbance mode in a frequency range of 4000–400 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\). The dried samples were mixed with KBr powder (spectroscopic grade) and pressed into KBr pellets prior to FT-IR analysis. The resultant spectra were smoothed to remove the noise, and the data were analysed using the Ominic 7.2 software.

3.9. Determination of the extent of β-elimination

To investigate whether β-elimination occurred during HSS treatment, the absorbance change of pectin solution at 235 nm was measured with a UV–Vis spectrophotometer (UV-2500, Shimadzu, Kyoto, Japan). An extinction coefficient of 5412 L mol\(^{-1}\) cm\(^{-1}\) was used.

3.10. Determination of the extent of demethoxylation

The possibility of demethoxylation of pectin during HSS treatment was monitored by the method of Klavons and Bennett (1986), where an alcohol oxidase was used. Briefly, 30 µL of pectin solution were added to 90 µL of phosphate buffer (pH 7.5, 0.2 N), and then 10 µL of a Pichia pastoris alcohol oxidase (Sigma–Aldrich, Shanghai, China) (1 unit/mL water) were added. The tubes were gently mixed and incubated at 25 °C for 15 min. Subsequently, 2 mL of pentanedione solution (0.02 M 2,4-pentanedione in 2.0 M ammonium acetate and 0.05 M acetic acid) were added and the solution vortex mixed. The tubes were placed in a water bath at 58 °C for 15 min. After cooling with running water to room temperature, the absorbance was measured at 412 nm.

3.11. Statistical analysis

All experiments were conducted in triplicate. Statistical analysis was carried out using SPSS (version 16.0, Chicago, United States). The results were expressed as mean ± standard deviations and compared using the Tukey test at 5% confidence level.

4. Results and discussion

4.1. The effect of high speed shearing on disaggregation and degradation of pectin

The tendency of formation of aggregates for pectin has been extensively reported (Fishman, Chau, Hoagland, & Ayyad, 1999; Sengkhamparn et al., 2010). The CFS pectin used in this study had a large viscosity-average molecular weight (359.2 kDa) so that the chains in the aqueous solution could easily form chain-chain entanglements, while their low degree of methoxylation might facilitate the formation of hydrogen bonds between neighbour molecules, leading to production of aggregates. In addition, the pH of the pectin solutions was about 4.8, which was higher than the pKa of galacturonic acid (3.5). Some residual Ca\(^{2+}\) was also found in the pectin solution at concentrations of 5.34 µg/mL. At pH 4.8, a fraction of the carboxyl groups was ionised, and the calcium binding to the ionised carboxyl groups would further facilitate the formation of aggregates. There was clear evidence that aggregates existed in the original CFS pectin. The refractive index signal for this sample was quite small, as shown in Fig. 1A. This might be because the filters used in Section 3.5 were easily blocked by the aggregates so that only a small amount of pectin with low concentrations could pass. This is a disadvantage of using filtration to eliminate aggregates. The presence of aggregates became more apparent when dynamic light scattering was used to characterise the samples. It was found that the untreated pectin consisted of large particles (Fig. 2A–B), and its average particle size was 1445.6 nm and 9850.5 nm for Population I and II, respectively (Table 1). Such big particles were usually considered to be aggregates of polysaccharides. In addition, after pectin was treated by HSS for 8 h, the bigger particle reduced to 0.1% of the total number of particles (Fig. 2C), however, it accounted for 67.5% of the scattering intensity (Fig. 2D). This is a characteristic feature of aggregates, in which larger particles contribute more to the scattering intensity than the smaller ones. The supramolecular aggregation of pectin in aqueous solution is a critical barrier to the accurate characterisation of the molecular properties of the polymer. Therefore, HSS was used in this study to eliminate the aggregation of pectins. The effect of HSS on disaggregation and degradation of pectin from creeping fig seeds was systematically studied.

The observation of the surface topography of pectin would be one of the most intuitionistic ways to evaluate the effect of HSS on pectin. Although the process of freeze drying had a huge impact

Fig. 1. Effect of high speed shearing on average molecular weight of pectin. (A) High performance size exclusion chromatography (HPSEC); (B) Change of molecular weight (—○— for HPSEC results and —●— for theoretical calculated results) as function of shearing time.
on the surface topography of pectin and the observed microscopy structures by ESEM were clearly not the same particles as seen in solution, the differences in the surface topographies between the samples shown in Fig. 3 could be only due to the process of shearing since all the samples used in microscopy were freeze-dried at same conditions. As shown in Fig. 3A, the morphology for non-shearing-treated pectin was relatively compact with a flake-like lamella structure. A similar topography was found in pectin isolated from other Ficus materials, such as the jelly fig (Ficus Pumila Linn) seed (Jiang et al., 2002). After 4 or 8 h HSS treatment, pectin remained intact but some silk-like structure was observed for the samples (Fig. 3B and C). This filiform texture became more and more obvious with the increase of shearing time (Fig. 3D–G). After 24 h treatment, no flake-like structure was found. Our previous study also found that many porous structures appeared in apple pectin after another mechanical treatment, dynamic high pressure microfluidisation (DHPM). We attributed this to the pectin molecule breaking down into small segments by DHPM so that segments cannot shape into the bigger flake-like structure (Chen et al., 2012). The change of morphology of pectin resulted from disaggregation or degradation of pectin needed to be further investigated through other parameters such as intrinsic viscosity, molecular weight, particle size, and reducing sugar content.

HSS treatment had significant effects on the intrinsic viscosity of CFS pectin. The intrinsic viscosity decreased with shearing time but not linearly. It decreased rapidly in the early 8 h of treatment and gradually tended to a constant value with time. The intrinsic viscosity of the sample at 0, 4, 8, 12, 16, 20, and 24 h was 7.68, 1.86, 1.09, 0.76, 0.61, 0.52, and 0.42 dl/g, respectively (Fig. 4A). Intrinsic viscosity (\(\eta_i\)) is known to relate well with molecular weight according to the Mark–Houwink equation, and the decrease of \(\eta_i\) has been always attributed to the decrease of molecule weight (Laneuville, Turgeon, & Paquin, 2013). Therefore, the Mw and distribution of pectin were determined in our study using high performance size exclusion chromatography (HPSEC). It was found that the decrease of molecular weight as a function of shearing time was quite similar to the decrease of intrinsic viscosity. The molecular weight of CFS pectin decreased quickly in the initial 8 h during HSS, then decreased slowly when the shearing continued (Fig. 1B). For example, the average Mw of untreated pectin was 2566.3 kDa. After treated by HSS at 24,000 rpm for 4, 8, 12, 16, 20, and 24 h, its Mw decreased to 576.1, 425.2, 330.5, 279.1, 265.3, and 212.7 kDa, respectively. Since a large number of big aggregates existed in some pectin solutions and the adopted standards were dextrans, the Mws suggested by HPSEC were only estimations and errors might have occurred. More accurate molecular weight of pectins can be calculated by application of Eqs. (1) and (2) (see the later section for details).

Viscosity and molecular weight loss are often used to reflect the depolymerisation of polysaccharides. For example, Floury,
Desrumaux, Axelos, and Legrand (2002) reported that the significant reduction of the intrinsic viscosity and molecular weight can be explained by the disruption of covalent bonds inside the polymer chain by high pressure homogenisation. However, not only the breakdown of glycosidic bonds could lead to the significant loss in intrinsic viscosity and Mw, but also the disruption of aggregates may give rise to the decrease in intrinsic viscosity and molecular weight as well. For example, Wang et al. (2001) found that the decrease in intrinsic viscosity of detarium xyloglucan in water solution after autoclaving was most likely caused by the disruption of aggregates, rather than the degradation of the polymer chains. Al-Assaf, Sakata, McKenna, Aoki, and Phillips (2009) also reported that the reduction of molecular weight of gum was mainly due to the disruption of hydrophobic bonds, rather than the degradation of
shown in Fig. 4 B. It exhibited a contrary trend to the results of ular aggregates when low mechanical energy was input ( Chen et al., 2012 ). It should be noted that degradation also occurred during this period since the increase of reducing sugar ended (Fig. 4B) and a reduction of particle size for Population I was observed ( Table 1 ).

Beyond 8 h, the degradation of pectin treated by HSS became more apparent as indicated by the rapid increase of reducing sugar content. The reducing sugar content for 24 h treated samples was 1.5-fold more than that of the 8 h treated sample. However, whether the disaggregation also occurred during the period longer than 8 h was another question. Generally, the particles size of both Populations I and II decreased with shearing time ( Table 1 ). It seemed that the aggregates (Population II) decreased to smaller aggregates after 8 h, and aggregates existed in the pectin solution during the whole treatment even if the treatment time was 24 h. There was only 0.1% larger particle left (weighting by number) which accounted for 98.0% of the intensity (weighting by intensity), indicating that aggregates still existed and the disaggregation continued in the period of 8–24 h. The disaggregation was supposed to be easy, since the interactions for aggregates were probably weak non-electrostatic interactions, such as van der waals, hydrophobic interactions, or hydrogen bonding. The energy applied by HSS was strong enough to break down the covalent bond, not to mention the disruption of the above weaker forces. Therefore, the presence of aggregates even after 24 h treatment indicated that the re-association of pectin was very quick. A number of physical and chemical methods have been used to eliminate the aggregates of polysaccharides such as filtration, heating, centrifugation, use of different solvents (e.g. dimethyl sulfoxide, urea, NaOH), and chemical derivatisation ( Anthonsen, Vårum, Hermansson, Smidsrød, & Brant, 1994; Li et al., 2006 ). In our study, it was shown that HSS would not eliminate the aggregates of pectin very efficiently. However, it was noticed that the number of aggregates presented in the samples have been reduced to a negligible amount (0.1% for 24 h treatment and 0.2% for 20 h treatment). The presence of such a small amount of aggregate may not influence the determination of molecular weight by HPSEC because the forced shear flow at an elevated temperature (40 °C) in the HPSEC system may favour the dissociation of aggregates, as noticed by other researchers. For example, Li et al. (2006) reported that wheat β-D-glucan which contained 3.89% aggregates in dilute water solution, which agreed well with those free of aggregates. Therefore, the molecular weight for the samples at 20 and 24 h were considered to be relatively accurate in this study. Based on the change of molecular weight and the reducing sugar content of samples at 20 and 24 h, the K was calculated to be 57.49 g L^{-1} by applying of Eq. (2). Using Eqs. (1) and (2), the molecular weight of the original pectin and pectin treated for 4, 8, 12, and 16 h was calculated to be 670.65, 599.07, 508.21, 388.18, and 297.36 KDa, respectively (Fig. 1B). The calculated molecular weights were smaller than those determined by HPSEC, indicating that the influence of aggregates may be eliminated in these calculations. We noticed that the standard deviations for samples with large molecular weight were somewhat big because only the molecular weight of the samples at 20 and 24 h can be regarded as relatively accurate in the results of HPSEC in this study. The accuracy can be improved when a series of degraded samples with no aggregates are used to calibrate K. Using the change of reducing sugars content and the variation of molecular weight for the more accurate calculation of the molecular weight of polysaccharides that contain a large number of huge aggregates is proposed for the first time, to the best of our knowledge. It may be applied for other polysaccharides when more efficient mechanical degradation methods are used.

4.2. The effect of high speed shearing on structure of pectin

Disruption of aggregates was mostly a physical process, while the degradation was considered as the breakdown of some chem-
rical bonds. Since there was an increase in the reducing sugar contents, it can be deduced that some of the glycosidic bonds were ruptured. However, whether HSS influenced other functional groups such as –COOCH₃, the most characteristic group of pectin, needed to be investigated.

The chemical structures of the original pectin and the pectins treated under different pressure were analysed by FT-IR spectroscopy (Fig. 5). The FT-IR spectra were similar for treated and untreated pectin. No significant difference was observed for the characteristic absorption bands around 3400 cm⁻¹ (O–H stretching), 3000–2800 cm⁻¹ (C–H stretching and bending), 1744 cm⁻¹ (C=O, esterified), 1625 cm⁻¹ (COO⁻ stretching), 1417 cm⁻¹ (COO⁻ stretching), and 1300–1000 cm⁻¹ (C=O, stretching). This showed that HSS treatment had no effect on the primary structure of pectin, and the degradation of pectin may only give rise to a decrease in the degree of polymerisation. Other physical methods such as ultrasonication (Vodeničarová, Dřímalová, Hromádková, Malovíková, & Ebringerová, 2006), dynamic high pressure homogenisation (Hu et al., 2013), and microwave-assisted hydrolysis (Bezáková et al., 2008) were all reported to cause the degradation of polysaccharides but not to influence the primary structure of polymers.

It was reported that the rupture of the polymer chains can be facilitated by a mechanically activated reaction when stress was applied on the polymer chains, even if the applied stress was below a critical value. The mechanical stress prior to the rupture of a polymer chain can result in the deformation of bonds, and the deformed bonds are more reactive because they are in a higher energy state (Casale, 1978). Therefore, the possibility of occurrence of other chemical reactions such as demethoxylation and β-elimination in this physical process were investigated.

The ratio of the peak area of 1750 cm⁻¹ over the sum of the peak areas of 1744 and 1625 cm⁻¹ was used previously to calculate the degree of methoxylation (DE) (Liang et al., 2012). In this study, the DE of the original pectin was determined to be 13.41%. Furthermore, it was observed that the peaks at 1744.4 and 1624.5 cm⁻¹ exhibited no change after HSS, indicating no demethoxylation occurred. On the other hand, the possibility of demethoxylation was also monitored by measuring the formation of methanol using alcohol oxidase. It was found that no methanol was formed for all the treated samples, which was coherent with the results of the FT-IR spectra.

β-Elimination, one of the most common mechanisms of nonenzymic degradation in pectins, was monitored by taking absorbance readings at 235 nm with a spectrophotometer. It has been reported that the β-elimination reaction led to the removal of H atom at C-5 of GalA and the cleavage of the glycosidic linkage at the C-4 position of galacturonic acid residues, resulting in the formation of an unsaturated bond between C-4 and C-5 at the non-reducing end (BeMiller & Kumari, 1972). There was no absorbance at 235 nm for all the pectin solutions in the unsaturated uoride test, indicating that β-elimination did not occur during HSS treatment. Moreover, no new absorption band was detected for the HSS treated pectin and the original pectin, indicating that no new functional group was formed during the process, which further confirmed the absence of β-elimination.

5. Conclusions

High speed shearing treatment may not eliminate the aggregates of pectin very efficiently. Although the disaggregation induced by HSS mainly happened in the initial 8 h treatment and occurred during the whole treatment period (0–24 h), aggregates still existed even after 24 h. The disaggregation of pectin was accompanied by degradation, and significant degradation occurred after 8 h treatment. Based on the relationship between the change of the reducing sugar content and the variation of the molecular weight, a new method was proposed for calculating more accurately the molecular weight of those pectins containing a high number of large aggregates. This method may be further applied for other polysaccharides when more powerful mechanical treatments are used. In addition, HSS treatment did not alter the primary structure of the polysaccharide, and neither β-elimination nor demethoxylation occurred during the process.

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