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Rheological property of extruded and enzyme treated flaxseed mucilage

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ABSTRACT

Rheological properties of flaxseed mucilage under different extrusion conditions with or without enzyme treatment were studied. The steady-shear tests indicated that the apparent viscosity of flaxseed mucilage fitted well to Power law model and all samples showed a shear-thinning behavior. The consistency index decreased and the flow behavior index increased with an increasing temperature and a decreasing screw speed. The addition of initial moisture content improved the degradation of flaxseed mucilage while further increase affected the action negatively. The empirical equations describing relationships between parameters of rheological models and extrusion factors were obtained. The results showed that extrusion can damage the compact fiber structure which improved the effect of enzyme treatment. The apparent viscosity decreased with the increase in hydrolyzing time and enzyme loading amount.

1. Introduction

The world's flaxseed (*Linum usitatissimum* L.) production ranged between 2.0 million and 3.0 million metric tons over the last decade. At present, whole flaxseed is used as an ingredient for many healthy foods. Flaxseed oil is utilized in oil-based industrial products and its meal is used as an ingredient of animal feedstuff or fertilizer (Caragay, 1992). Flaxseed mucilage is a heterogeneous polysaccharide composed of xylose, arabinose, glucose, galactose, rhamnose, and frucose (Erskine & Jones, 1957; Hunt & Jones, 1962). Fibrous flaxseed hulls contain few amounts of protein and oil but abundant polymeric carbohydrates (Wanasundara & Shahidi, 1997). The high water-absorption capacity of flaxseed meal is mainly attributed to the presence of these polysaccharides in the seed hulls (Fedeniuk & Biliaderis, 1994). Flaxseed meal contains 31–45% of protein (Oomah & Mazza, 1993) and mucilage as well when the hulls remain in the defatted flaxseed meal. However, the existence of polysaccharides in flaxseed hulls may hinder the protein separation due to the swelling capability of polysaccharides under aqueous circumstances (Smith, Johnson, & Beckel, 1946; Sosulski & Bakal, 1969).

Recent studies of flaxseed mucilage degradation are focusing on the removal of mucilage in flax fiber based products. Chemical retting (Mooney, Stolle-Smits, Schols, & de Jong, 2001), enzyme retting (Akin, Morrison, Gamble, & Rigsby, 1997), steam explosion (Kessler & Kohler, 1996), etc., are widely applied in flax demucilage and improve the quality of flax fiber. Wanasundara and Shahidi (1997) reported that flaxseeds with less mucilage were prepared by soaking in water or 0.1 M sodium bicarbonate ($NaHCO_3$) solution for 12 h; mucilage in flaxseed could also be removed by enzymatic treatment with carbohydrases (Celluclast® 1.5L, Pectinex™ Ultra SP and Viscozyme® L, Novo Nordisk, Bagsvaerd, Denmark). It was proposed by Foulk, Akin, and Dodd (2001) that enzyme absorption and demucilage of flaxseed were improved while using pressure crimping treatment. Mechanical treatment alters the structure of the flax stalk so that it provides the greatest benefit for increasing enzyme absorption (Foulk et al., 2001). Few investigations on the degradation of flaxseed mucilage were published.

Extruders are widely used in food industry because of their capability of cooking and shaping raw ingredients into intermediate products (Obatolu, Cole, & Maziya-Dixon, 2000; Wu et al., 2008). Although researches which aimed to change properties of flaxseed mucilage using extruders were rarely reported, it has been confirmed that enzyme absorption and demucilage efficiency of flaxseed can be improved through mechanical pretreatment. Some large molecules can be degraded along with the formation of porous structure by high temperature and high shearing speed during extrusion.

The recent studies have been conducted by different drying methods on the rheological behaviors of flaxseed gum (Wang, Wang, Li, Xue, & Mao, 2009). To the best of our knowledge, no study has been published on rheological properties of the extrusion and enzyme treated flaxseed mucilage. The main object of this study was to investigate the influences of both extrusion and enzyme treatment conditions on rheological properties of flaxseed mucilage using steady-shear measurements and analyze the effect of two treatments on degradation of samples.

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2. Materials and methods

2.1. Materials

Flaxseed meal was purchased from a local market in Henan Province of central China. A lipid pulverizer (RT-66S, Beijing Huanya Tianyuan Mechanical Technology Co., Ltd., China) was employed to crush down the flaxseed meal to 40-mesh screen. The moisture content of the mixed sample was adjusted to different values by adding different amount of water in it. All samples were sealed and equilibrated for 24 h before tests.

2.2. Twin-screw extrusion treatment

A co-rotating twin-screw extruder (SLG67–18.5, Beijing Shilian Machine Company, China) with a length-to-diameter ratio of 27.9:1 was used in this study. The diameter of each screw was 47 mm. A circular die with the diameter of 5.2 mm was used. The temperature was controlled by adjusting the electrical resistance. The temperature near the die was measured by a thermocouple.

2.3. Enzyme treatment

Commercially available Pectinex Ultra SP-L (30000 UPTE ml^{-1}) and Pectinex Smash XXL (26000 PG ml^{-1}) were kindly provided by Novozymes Co. Beijing, China. Both of the enzymes were produced from *Aspergillus aculeatus*, and showed brown color and liquid state. The Pectinex Ultra SP-L and Pectinex Smash XXL were selected to hydrolyze the polysaccharides primarily in plant cell walls and middle lamellas by Gouveia, Fiadeiro, and Queiroz (2008). The suitable maceration temperature is between 40 and 45 °C and the optimal pH is between 4.5 and 5.5. Accurate 20.00 g of extrudates was added to 100 ml of acetic acid buffer solution (0.01 M, pH 4.5) at a meal-to-solvent ratio of 1:5 (w/w) and incubated at 40 ± 1 °C for 1, 3 and 6 h. The reaction was terminated by adding 50 ml of 0.10 M NaOH to the mixture (Wanasundara & Shahidi, 1997).

2.4. Analysis of extruded products

2.4.1. Bulk density

The bulk density of dry extrudates was determined by a volumetric displacement method using fine sand (60–140 mesh, or 0.25–0.10 mm). Bulk densities of the extrudates were calculated according to Eq. (1)

$$\rho_b = \frac{W_{ex}}{V_{ex}} = \frac{W_{ex}}{V - V_{fs}} \tag{1}$$

where: ρ_b is bulk density (g cm^{-3}), W_{ex} is mass (g) of the extrudates, V_{ex} is volume (cm^{-3}) of the extrudates, V_{fs} is volume (cm^{-3}) of fine sands displaced and V is the total volume (cm^{-3}) of the fine sands and extrudates (Altan, McCarthy, & Maskan, 2008). Average value of three replicates was represented.

2.4.2. Scanning electron microscopy

The microstructure of the extrudates was observed by scanning electron microscopy (SEM), which was operated at 30 kV (Hitachi S-3400N, Hitachi Instruments Ltd., Japan). Particles of the sample were fixed on the silicon wafer and sputtered with gold powder.

2.5. Extracted mucilage preparation

Flaxseed mucilage was extracted according to Bhatty (1993) with some modifications. Accurate 10.00 g of extruded samples (dry matter) was added into a glass beaker together with 100 ml of boiled water. The extraction lasted for 3 h with continuous magnetic stirring at ambient temperature (22 °C). Over 90% of flaxseed mucilage was extracted following the procedure in the study of Mazza and Biliaderis (1989). The solution was then centrifuged at 10,000*g* for 20 min to obtain a clear supernatant for the rheological measurement.

2.6. Rheological study of treated flaxseed mucilage

Rheological measurements were performed using a rheometer (AR2000ex, TA Instruments Ltd., Crawley, UK), which was fitted with a steel cone geometry (60 mm diameter, 59 μm gap) for steady-shear measurements. The temperature was controlled by a water bath connected to the Peltier system in the bottom plate. A thin layer of silicone oil was applied on the periphery of the samples in order to prevent evaporation. The steady-shear tests were performed at 25 °C over the shear rate range of 0.01–1 s^{-1}.

2.7. Statistical analysis

The experimental rheological data were obtained directly from the TA Rheology Advantage Data Analysis software V 5.4.7 (TA Instruments Ltd., Crawley, UK). The average of the three runs was reported as the measured value with standard deviation. All sample analyses were carried out in triplicate and SAS (Version 6.12, Cary, NC, USA) was used for the statistical analysis. Duncan's multiple range tests were used to estimate significant differences among means at a probability level of 5%.

3. Results and discussion

3.1. Product quality characteristics

3.1.1. Effect of various extrusion parameters on quality of the extrudates

The effects of various extrusion conditions on bulk density and moisture content of the extrudates are shown in Table 1. The bulk density of extrudates decreased from 1.628 to 0.686 g cm^{-3} when the extrusion temperature increased from 80 °C to 160 °C. The decrease in bulk density at high extrusion temperature is probably

Table 1
Effects of various extrusion parameters on the bulk density and moisture content of the extruded samples.

Extrusion conditions	Bulk density (g cm^{-3})	Moisture content of extruded samples (%)
Temperature (°C)		
80	1.628 ± 0.263^{a}	27.57 ± 0.91^{a}
100	1.107 ± 0.081^{b}	24.03 ± 0.51^{b}
120	0.782 ± 0.039^{c}	22.90 ± 0.26^{b}
140	0.686 ± 0.039^{c}	21.07 ± 0.67^{c}
160	0.820 ± 0.034^{c}	17.93 ± 0.91^{d}
Screw speed (rpm)		
60	0.683 ± 0.069^{a}	20.13 ± 0.81^{a}
100	0.618 ± 0.057^{a}	20.54 ± 0.83^{a}
140	0.724 ± 0.078^{a}	20.64 ± 0.37^{a}
180	0.712 ± 0.067^{a}	19.04 ± 1.22^{a}
Moisture content (%)		
18.8	0.629 ± 0.025^{b}	7.55 ± 0.45^{d}
25.1	0.647 ± 0.082^{b}	15.08 ± 0.38^{c}
31.8	$0.743 \pm 0.070^{b,a}$	16.42 ± 0.54^{b}
35.1	0.779 ± 0.035^{a}	22.49 ± 0.59^{a}

Values represent the mean ± standard deviation; $n = 3$.
[a–d] Means in a column with different superscripts were significantly different ($p < 0.05$).

caused by the starch gelatinization and protein denaturalization (Case, Hamann, & Schwartz, 1992; Hagenimana, Ding, & Fang, 2006). The denaturalization of starch gel and protein was enhanced due to the increase in temperature, which enlarged the temperature difference at the end of the die. Meanwhile, moisture loss became more serious with an increasing intensity of flash vaporization. This process was one of the dominating reasons that led to more porous structure, larger volume, as well as decreased bulk density. Decrease in moisture content of extrudates was observed when extrusion temperature increased. This was caused by the faster evaporation of moisture at higher extrusion temperature (Table 1). Moreover, neither the bulk density nor moisture content of extrudates was significantly affected by the screw speed. Similar results were reported by Castells, Marín, Sanchis, and Ramos (2006). The bulk density and moisture content of extrudates were mainly affected by the temperature difference at the end of the die. The rotary speed of the screws was not the significant factor that influenced the temperature or initial moisture content when one of them was fixed. As shown in Table 1, the bulk density of the extrudates increased along with the increasing in initial moisture content while under identical temperature and screw speed. The intensity of water evaporation was not so severe at 120 °C. Incomplete evaporation occurred at relatively high initial moisture content, which might cause the decrease in volume and hinder the formation of a porous structure, contributing to the increase in bulk density. It agrees with Pan, Zhang, and Jane (1998) that the bulk density increased with an increase in initial moisture content at low extrusion temperature.

3.1.2. *Effect of extrusion on the physical structure*

The SEM images of the extrudates at a magnification factor of 200× are shown in Fig. 1. The raw flaxseed meal represented a clear fiber structure (Fig. 1a). Microbes and enzymes can only work on the surface layer of the fiber structure. The extrusion treatment can damage the compact fiber structure so as to form a porous structure inside. The extrusion treatment can also degrade the

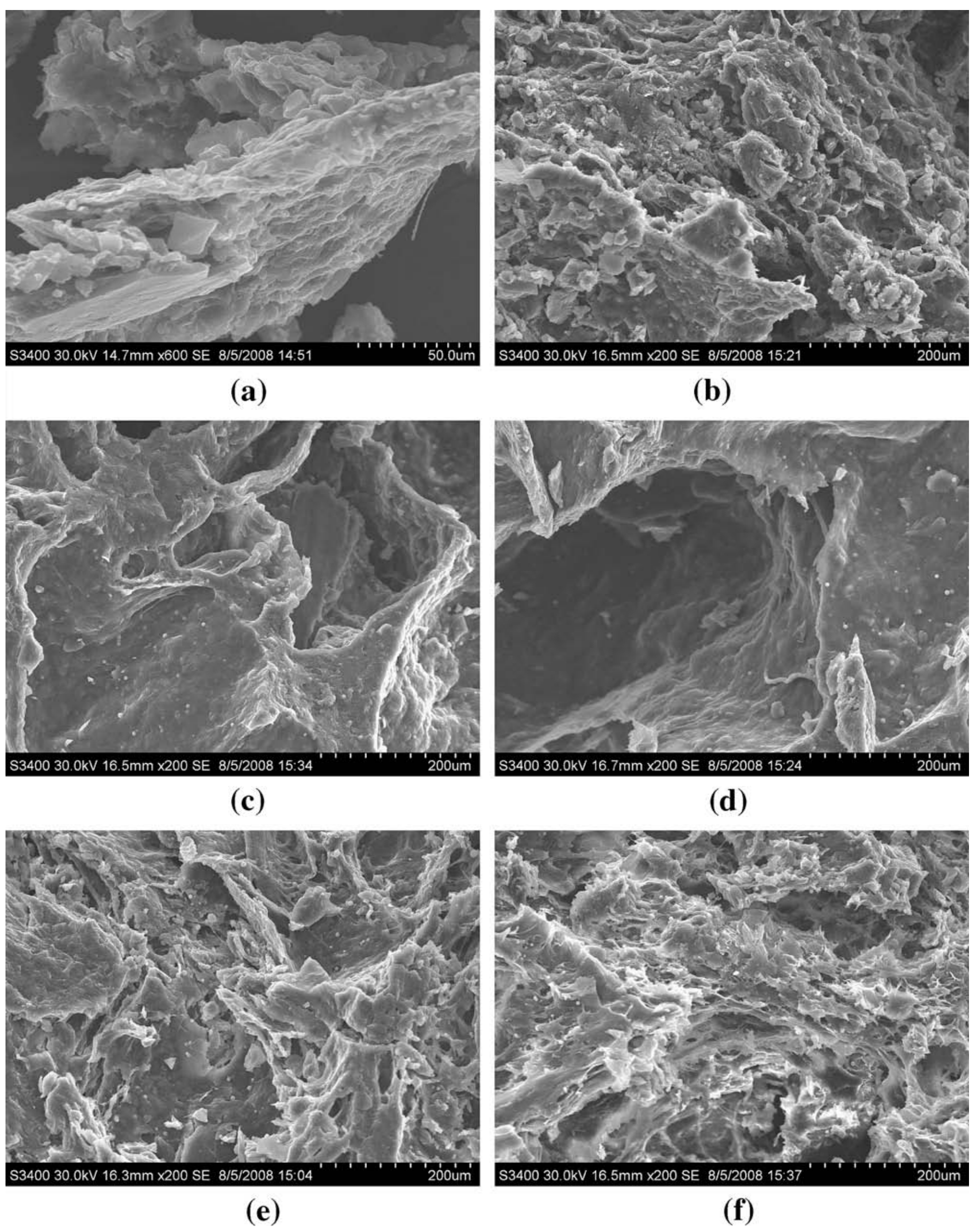

Fig. 1. Scanning electron microscopy images of untreated (a), and treated sample at different extrusion temperatures (80 °C (b), 120 °C (c) and 140 °C (d)) and initial moisture contents (37.7% (d), 25.1% (e) and 18.8% (f)).

flaxseed mucilage to some extent. The microstructure of extrudates was significantly affected by the temperature increasing from 80 to 140 °C (Fig. 1b–d). Larger pores and rougher surface were observed in extrudates obtained at higher initial moisture content and higher temperature (Fig. 1d). Ding, Ainsworth, Tucker, and Marson (2005) reported that an increase in temperature would decrease melt viscosity. The reduced viscosity would favor bubble growth during extrusion. However, excessive high temperature could cause thermal decomposition and degradation of polysaccharides. The initial moisture content also significantly affected the microstructure of extrudates. The water-holding capacity was affected by the porous structure inside the extrudates. The proper initial moisture content can provide a homogenous distribution of pores in the extrudates (Fig. 1e and f). The micrographs of the extrudates showed that extrusion, a form of size reduction, could be implemented for the desired particle size or surface area, which is a decisive factor in chemical reactions (Foulk et al., 2001). The fracture of flaxseed meal allows the enzyme to access to the extrudates' interior from the exterior more easily with an increased surface area.

3.2. Steady-shear measurements of extruded flaxseed mucilage

The flow curves of the extruded flaxseed mucilage are shown in Fig. 2. The apparent viscosities of all samples decreased with the increasing of shear rate, suggesting that the flaxseed mucilage exhibited non-Newtonian behavior. The flow curves were nearly parallel to each other and all the sample represented the shear-thinning behavior, which was widely observed in food gums (Holser, Carriere, Park, & Abbott, 2000). In order to describe the apparent viscosity as a function of shear rate for the extruded flaxseed mucilage, the experimental data were regressed to the power law model seen as Eq. (2), which was used extensively to describe the flow properties of non-Newtonian liquids in theoretical analysis and practical engineering applications.

$$\sigma = K\dot{\gamma}^n \quad (2)$$

where σ is the shear stress (Pa); $\dot{\gamma}$ is the shear rate (s^{-1}); K is consistency index (Pa s^n); and n is the flow behavior index (dimensionless).

The apparent viscosity of extruded flaxseed mucilage decreased with the increasing extrusion temperature. According to Oomah, Kenaschuk, Cui, and Mazza (1995), the decrease in viscosity may be caused by the degradation of polysaccharides with high molecular weight to other polysaccharides with comparatively low molecular weight or monosaccharides during the extrusion process. The decrease in apparent viscosity also might be the result of the increase in temperature, which could accelerate the Maillard reaction. It was proposed that the increase of temperature was one of the reasons that pushing the Maillard reaction between reducing sugars and free amino groups (Hagenimana et al., 2006). As can be seen from Fig. 2b, the apparent viscosity of extruded flaxseed

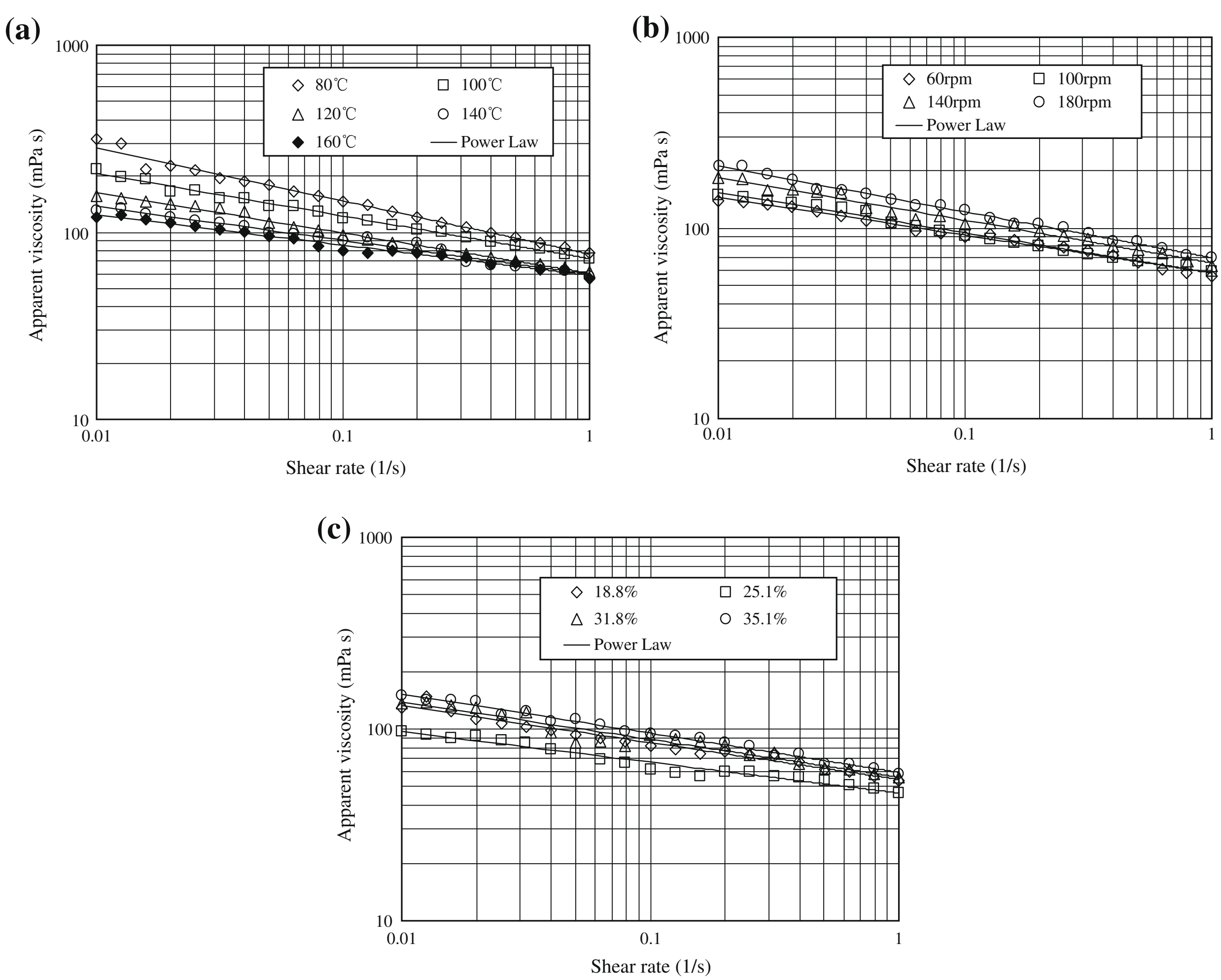

Fig. 2. Effect of various extrusion conditions on the apparent viscosities of mucilage extract from extruded product. Symbols represent the experimental data and the solid lines represent the Power law model (a, temperature; b, screw speed; c, moisture content).

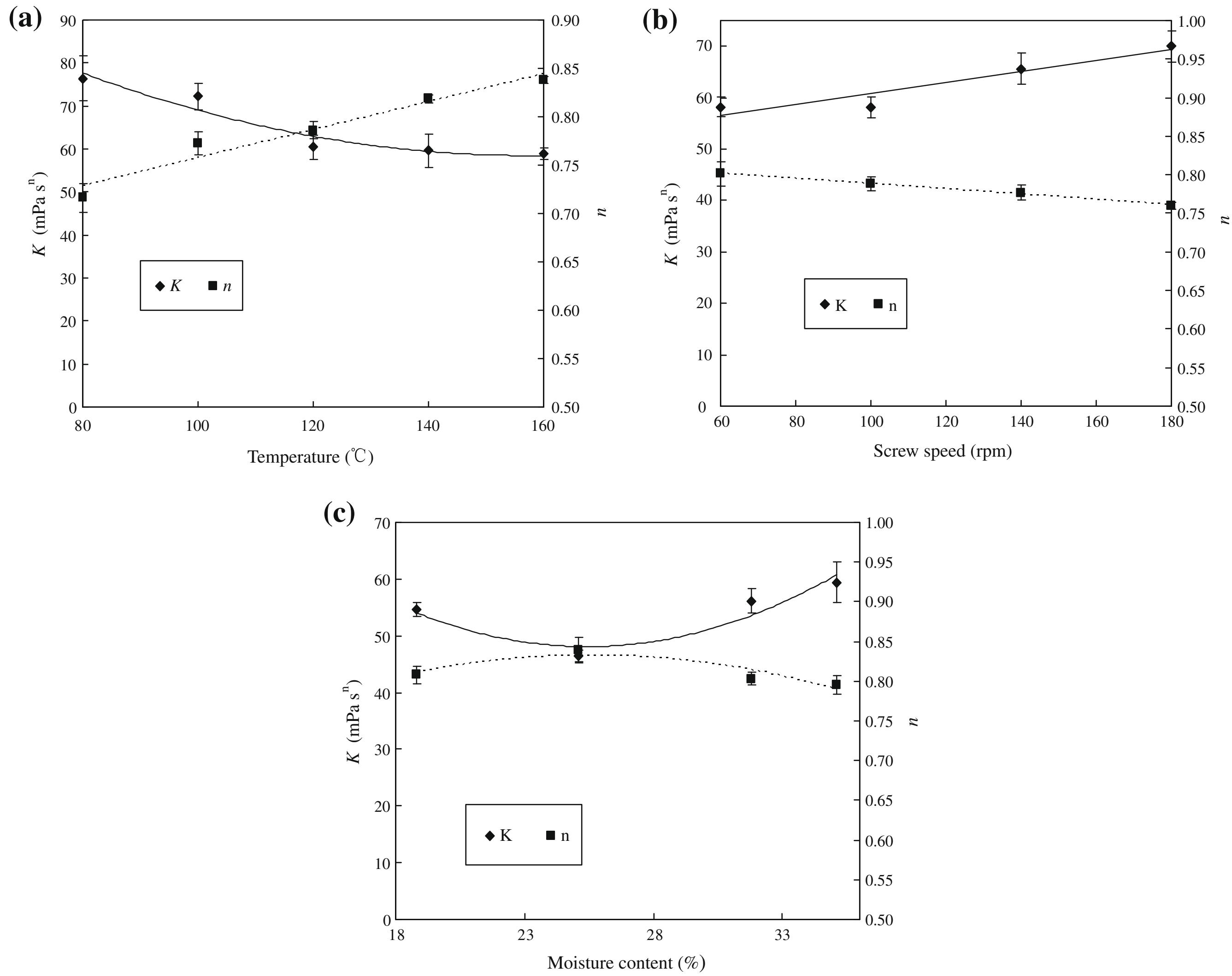

Fig. 3. The K and n of extruded mucilage with different extrusion factors and fitting line (a, temperature T; b, screw speed S; c, moisture content M).

mucilage increased with the increasing of screw speed. This behavior might be attributed to larger shearing force that resulted from the decreased residence time in the extruder's cavity, which led to insufficient degradation of mucilage. Water acts as a plasticizer, which can enhance the molecular motion and reduce the torsion and mechanical loss of polymer chains (Harper, 1989). Certain moisture content can promote the degradation of flaxseed mucilage. As shown in Fig. 2c, when the initial moisture content of meal increased from 18.8% to 25.1%, the viscosity decreased significantly; if the initial moisture content continues to increase, the more moisture content of flaxseed meal would lead to the reduction of viscosity. The increase in initial moisture content resulted in a significant drop in the friction force among flaxseed meal, cylinder and screws, thus bigger pressing force and smaller shearing force from the screws was loaded to flaxseed meal. The degradation of polysaccharides was then weakened by reduced shearing force.

As can be observed from Fig. 3, K decreased with the increasing of temperature (T) and increased with the decrease of screw speed (S), while n changed in the opposite way. In Fig. 3c, K decreased with increase of moisture content (M) from 18.8% to 25.1% and increased with further increase in moisture content from 25.1% to 35.1%. The values of K in Fig. 3 obtained by regressive analysis with power law model were used to investigate the effect of T, S and M on K. It was found that polynomial function was suitable for describing the relationship between K and T, S and M in this study (Table 2). Similarly, the relationship between n and extrusion parameters was also described using polynomial function as shown in Table 2.

3.3. Effect of enzyme treatment on rheological properties of mucilage

Enzyme-assisted degradation of mucilage reduced the mucilage content in the extrudates. It was proposed from Figs. 4 and 5 that the viscosity of mucilage extract in the steady-shear tests

Table 2
Relationships between the parameters of rheological models and extrusion factors (T, S and M) on the flaxseed mucilage.

Parameter[a]	Equation	R^2
K		
Temperature (T, °C)	$y = 0.003T^2 - 1.004T + 137.6$	0.93
Screw speed (S, rpm)	$y = 0.108S + 50.05$	0.90
Moisture content (M, %)	$y = 0.136M^2 - 6.895M + 135.8$	0.88
n		
Temperature (T, °C)	$y = 0.001T + 0.613$	0.95
Screw speed (S, rpm)	$y = -0.0003S + 0.823$	0.99
Moisture content (M, %)	$y = -0.001M^2 + 0.024M + 0.521$	0.82

[a] K and n from the power law model.

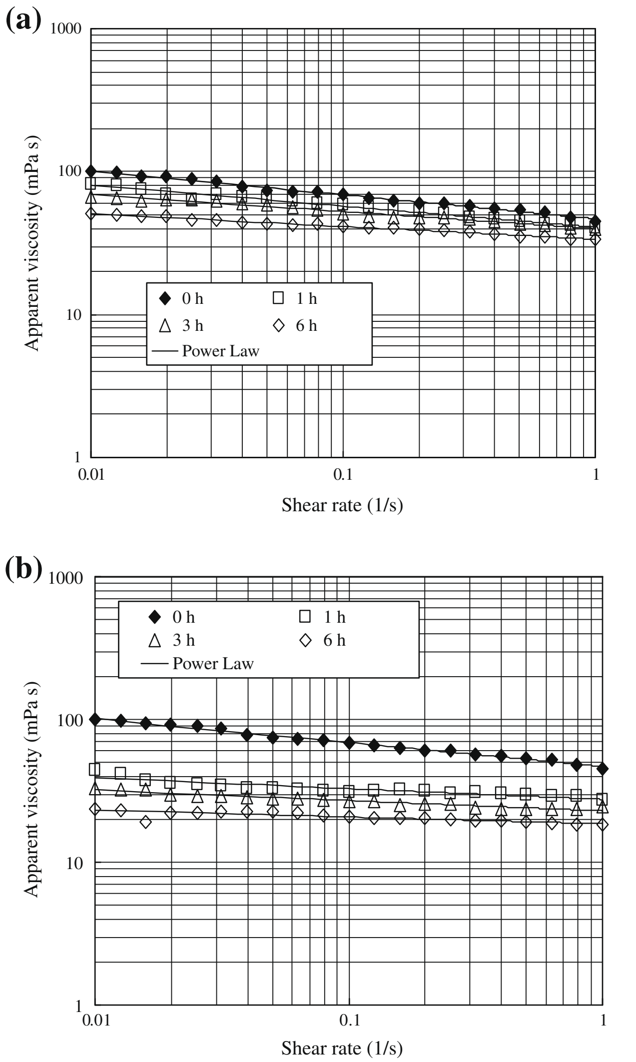

Fig. 4. Effect of hydrolyzing time and loading amount on the apparent viscosities of mucilage extract from enzyme treated product with Pectinex Smash XXL (a, 50 μl kg^{-1}; b, 200 μl kg^{-1}).

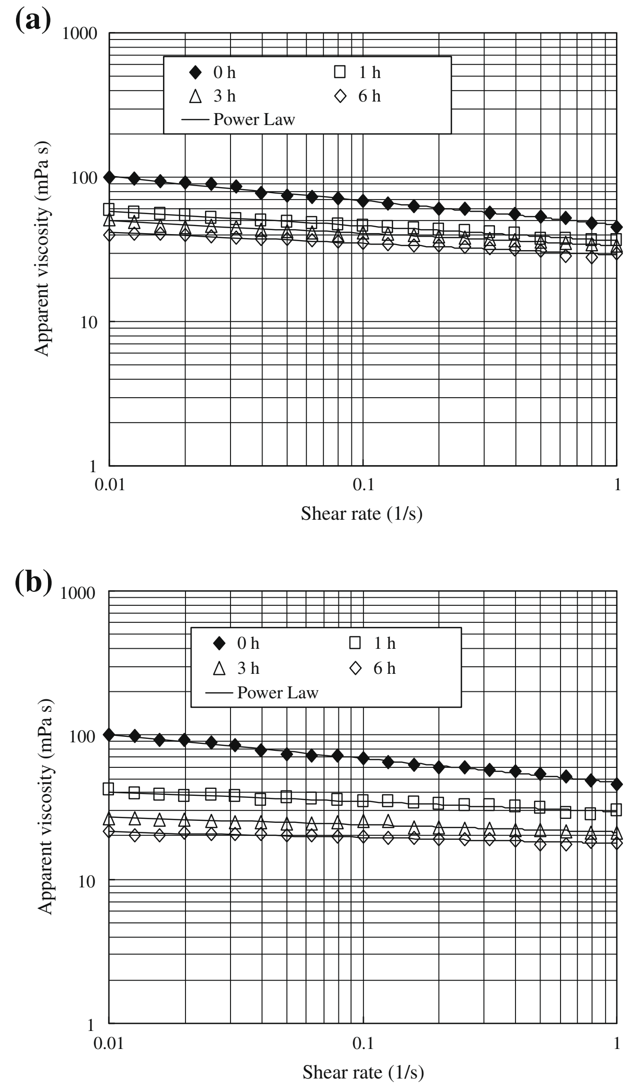

Fig. 5. Effect of hydrolyzing time and loading amount on the apparent viscosities of mucilage extract from enzyme treated product with Pectinex Ultra SP-L (a, 50 μl kg^{-1}; b, 200 μl kg^{-1}).

decreased with the increase both in hydrolyzing time and enzyme loading amount. And the parameters of rheological models (Power law) obtained from regression analysis are summarized in Table 3. As can be seen from Fig. 4, when using SP-L for enzyme treatments, the apparent viscosity of mucilage decreased with increase of hydrolyzing time and the amount of enzyme. As it is seen in Table 3, K decreased from 40.86 to 33.64 mPa s^n and n increased from 0.853 to 0.911 when hydrolyzing time increased from 1 to 6 h. Polysaccharides have strong affinity for water and can easily dissolve in it by continuous hydration. This process changes the intermolecular bindings of the polysaccharides (i.e. polysaccharide–polysaccharide interactions to polysaccharide–water binding). The amorphous regions of polysaccharides which possess little or no intermolecular hydrogen bonds are particularly available for hydration. As a result, the solubility of branched polysaccharides which have no or little intermolecular association is increased (Wanasundara & Shahidi, 1997). The solubility of polysaccharides increased with the increase in soaking time. The enzyme treatments at a loading amount of 200 μl kg^{-1} obtained less apparent viscosity than the loading amount of 50 μl kg^{-1} at same hydrolyzing time (Fig. 4 and Table 3). Moreover, the enzyme treatments at loading amount of 200 μl kg^{-1} for 3 h and 50 μl kg^{-1} for 6 h had a similar effect on the demucilage of samples when Pectinex Smash XXL was used (Fig. 4). As for the treatments of samples with Pectinex Ultra SP-L, treatment with enzyme loading of 200 μl kg^{-1} for 1 h and that of 50 μl kg^{-1} for 3 h had a similar effect in reducing the mucilage content of samples (Fig. 5). It was suggested that the degradation of flaxseed mucilage was the combined effect of amount of enzyme and treatment time. But increasing amount of enzyme is one of the important factors that lead to higher cost of treatment. In addition, as shown Figs. 4 and 5, it was indicated that SP-L showed a better performance of degradation for flaxseed mucilage than that of XXL while under the same amount of enzyme loading and hydrolyzing time.

4. Conclusions

The physical structure and quality characteristic of flaxseed meal has been modified by twin-screw extrusion with the combination of shearing and thermal effects. The effect of extrusion condition (temperature, screw speed and moisture content) and enzyme treatments on the rheological properties of extrusion and enzyme treated flaxseed mucilage were analyzed by steady-shear test. The steady shear data was modeled by Power law equation and correla-

Table 3
Power law parameters of apparent viscosity of enzyme treated flaxseed mucilage prepared by Pectinex Smash XXL and Pectinex Ultra SP-L[b].

Parameters	K (mPa s^n)	n	R^2	K (mPa s^n)	n	R^2
Treatment	Pectinex Smash XXL, 50 µl kg^{-1}			Pectinex Ultra SP-L, 50 µl kg^{-1}		
0 h	46.8 ± 1.24[a]	0.833 ± 0.025[c]	0.9996	46.8 ± 1.24[a]	0.833 ± 0.025[c]	0.9996
1 h	40.9 ± 2.13[b]	0.853 ± 0.012[c]	0.9994	36.2 ± 2.12[b]	0.896 ± 0.015[b]	0.9999
3 h	39.9 ± 1.39[b]	0.881 ± 0.023[b,c]	0.9995	33.6 ± 1.42[c,b]	0.915 ± 0.013[b,a]	0.9998
6 h	33.6 ± 1.70[c]	0.911 ± 0.009[b,a]	0.9998	29.0 ± 1.65[c]	0.921 ± 0.009[b,a]	0.9997
	Pectinex Smash XXL, 200 µl kg^{-1}			Pectinex Ultra SP-L, 200 µl kg^{-1}		
0 h	46.8 ± 1.24[a]	0.833 ± 0.025[c]	0.9996	46.8 ± 1.24[a]	0.833 ± 0.025[c]	0.9996
1 h	27.6 ± 0.72[d]	0.925 ± 0.011[b,a]	0.9986	29.4 ± 0.86[c]	0.930 ± 0.012[b,a]	0.9997
3 h	22.8 ± 0.66[e]	0.927 ± 0.008[b,a]	0.9996	21.2 ± 1.07[d]	0.949 ± 0.019[b,a]	0.9996
6 h	18.6 ± 1.03[e]	0.953 ± 0.008[a]	0.9989	19.1 ± 1.44[d]	0.965 ± 0.005[a]	0.9999

Values represent the mean ± standard deviation; $n = 3$.
[a–e] Means in a column with different superscripts were significantly different ($p < 0.05$).
[b] Means under enzymatic treatment condition: 1:5 (w/v), 0.01 M acetate buffer, pH 4.5 and 40 °C.

tion coefficient (R^2) indicated that power law model could be used for analyzing and modeling of flow properties of mucilage. The results indicated that extrusion could degrade the flaxseed mucilage significantly. This was closely related to the complicated physicochemical changes, including the degradation of polysaccharides, and the Maillard reaction and so on. The physical structure was significantly affected by extrusion temperature and initial moisture content of sample. The best porous and dispersing structure, or the maximum surface area, was obtained at extrusion temperature of 140 °C and initial water content of 18.5–25.1%. The apparent viscosity of mucilage decreased with increasing of extrusion temperature from 80 to 160 °C, and it also decreased with decrease of screw speed. In addition, K decreased with increase of moisture content from 18.8% to 25.1% and increased with further increase in moisture content from 25.1% to 35.1%. Smaller apparent viscosity was observed in the mucilage extracts of enzyme-treated extrudates. The results of enzymatic treatment suggested that the performance of flaxseed demucilage was affected by enzyme loading and degradation time. It was proposed that the loading amount of enzyme could be adjusted to minimize the cost once reaching the desired demucilage performance.

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