Effects of extrusion parameters on rheological properties, chromatism, protein solubility and microstructure of flaxseed-corn mixture

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Abstract: As a kind of significant functional food material, flaxseed can be processed into snacks by extrusion technology. This research mainly studied the effects of different extrusion conditions: temperature, screw speed, material moisture content and proportion of added flaxseed, respectively. Moreover, the rheological properties of steady state were also investigated. Results showed that the steady state shear sweep tendency of extruded disperse system accorded to the power law model of non-Newtonian fluid. By fitting extrusion parameters and the parameters of power law model with polynomial, the maximum of consistency index and minimum of liquidity index can be gained when extruding temperature was 120°C, screw speed was 100 r/min and the proportion of added flaxseed was 20%. Maillard reaction could occur in extruded melt when material moisture content was less than 18.2%, and then formed to be brown by-products; however, the Maillard reaction slowed down and color aberration decreased when the moisture content increased. Protein soluble index observably reduced after extrusion, which indicated that protein denaturation during extruding process and created hydrophobic structure. The study of scanning rheological and electron microscope explained the process of starch, protein and fat in mixture formed steady polymer during extruding process.

Keywords: flaxseed, extrusion, flaxseed-corn blend, steady shear, rheological property, food processing
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1 Introduction

Food extrusion structure reorganization is a technology which uses extrusion technology to change the structure, form and component of raw material. The main components of material, such as starch, protein and fat start chemical and physical are changed during extruding process, and the microstructure of mixture also changed. As an important oil crop, China produces 20% of the world's flaxseed production, only less than that of Canada. Based on years of study, linseed oil[1,2], protein[3,4], lignin[5,6] and physical properties[7] have good functionality and trophism, some of the functionality even better than soybean products.

Plenty of researches detected the extrusion process and products characteristics of crops[8,9] and plant proteins[10,11] in recent years. Starch, as the main component of crops, occurs gelatinization in low-moisture state, different extrusion conditions will directly impact the process and degree of this gelatinization. Furthermore, by action of

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force field and temperature field in machine barrel, protein loses its natural structure and form new ordered-tertiary structure and consecutive sticky dough. This dough changes to laminar flow in channel and the protein molecule orient at drive head along the flow direction, then molecule transform into cross-linked netlike structure and finally created expansile-organized products when extruded from drive head[12]. Starch, protein and fat may recombine their structure and form a new interpenetrating polymer network[13], meanwhile, Maillard reaction takes place during extruding process and product brown pigment composition[14].

Until now, most researches in this field mainly focused on the texture properties of extruded products, and detected how the processing techniques impact the products as a new-style snack; however, using the rheological characteristics of disperse system become more popular in these years. Moreover, some research fields are still lack of results, for example, the physicochemical property and rheological properties of extrusion products with flaxseeds and the new snacks with additional material of flaxseeds.

In this research, a new extrusion instant food with main material of extruding maize flour mixture and additive of flaxseed was studied. The rheological characteristic curve of mixture was detected by steady shear experiment, and the influence of extrusion temperature, screw speed, material moisture content and proportion of added flaxseed on the parameters of rheological model were researched respectively, furthermore, the impact of different extrusion parameters on the color and protein soluble index of products was also studied. The microstructure of products was analyzed by graphics of scanning electron microscope, which could find the influence of extrusion process on the components structure of mixture. This research could provide experimental basis for the multifarious development of flaxseed food.

2 Materials and methods

2.1 Materials

Flaxseed was purchased from a local market in Hebei Province of China (moisture content of 5.6%, protein content of 7.56%). Maize flour was purchased from a local market in Beijing (moisture content of 9.0%, protein content of 25.32%). The moisture and protein (Tecator Kjeltec Auto 2300 Analyzer, protein factor: 6.25, Kjeltec™ 2300, Foss, Sweden), fat (Soxhlet standard extraction mode, B-811, BüchiLabortechnik AG, Switzerland) and ash contents of the flaxseed samples were determined according to approved methods described in AOAC (930.04, 992.23, 996.01 and 930.05, 1990)[15]. The carbohydrate content was calculated by difference.

2.2 Methods

2.2.1 Materials preparation

The mixture of flaxseed with maize flour has eight ratios (use water to adjust the moisture content): 0, 10%, 15%, 20%, 25%, 30%, 40% and 50% (w/w, wet basis), respectively. In order to reach homogeneous water balance, all groups of samples were sealed and equilibrated for 12 h. Finally, the moisture content of mixture was measured again before extrusion.

2.2.2 Extrusion process

All the experiments were only based on single-factor research without interaction influence of different extrusion parameters; therefore, four factors of extruded flaxseed-maize pastes, extrusion temperature (80-160°C), screw speed (60-180 r/min), feed moisture content (11.3%-30.21%, wet basis) and flaxseed content (0-40%), were detected as dynamic rheological properties respectively. All experiments were conducted in duplicate.

A co-rotating twin-screw extruder (SLG67-18.5, Beijing Shilian Machinery Factory, China) with a length-to-diameter ratio of 27.9:1 was used to fulfill the extrusion tests. The diameter of each screw was 47 mm. A circular die with diameter of 5.2 mm was set at the drive head, which could provide the pressure for the mixture when in extrusion, with a thermocouple measuring the temperature near the die.

The extrusion process of flaxseed-maize blend was adopted according to Wu et al.[16] with some modifications. All feeders must be mixed thoroughly before extrusion. The feeding rate was 27.5 kg/h (wet basis) during feeder went through the extrusion process; a screw feeder (Model JD180, China) could control the speed and fed material into machine inlet.

Figure 1 Photograph of extruded flaxseed-corn paste and powder

After machining process, every extruded product was cut into strip of 60 mm in length after completely dried in air drier at 25°C for 24 h, and then samples were separately sealed in plastic bags and stored at 4°C, the moisture contents of each group were kept the same.

2.2.3 Rheological properties

The steady shear statistics was tested by Dynamic rheometer (AR2000ex, TA Instruments Companies of United States) using the aluminum plate clamp (diameter of 40 mm, pitch of 1 mm). The samples were put on the temperature controllable plate and lay down the clamp. The temperature kept at 25°C, while the shearing rate was between 0.01-1 s^{-1} during the steady shear tests. Moreover, a thin layer of silicone oil was used to prevent the edge of sample from drying.

2.2.4 Chromatism (ΔE)

The extrusion products were pulverized with a laboratory grinder and passed through an 80 mesh sieve prior to color analysis. Chromatism test was fulfilled by a Minolta Colorimeter (CR-300, Minolta Co. Ltd., Osaka, Japan).

The color expressed as lightness or brightness (L*), redness (a*), and yellowness (b*) respectively, which L* represents luminance; a* represents red when the number is positive, whereas a* represents green when the number is minus; b* represents yellow when the number is positive, whereas b* represents blue when the number is minus. A calibration standard (L*, 97.10; a*, +0.24; and b*, +1.80) was used to calibrate the equipment prior to the measurements. Total chromatism was ΔE (Equation (1)):

$$\Delta E = \sqrt{(L^* - L_0)^2 + (b^* - b_0)^2 + (a^* - a_0)^2} \qquad (1)$$

where, the subscript "0" means the real test statistics of samples.

2.2.5 Protein soluble index (PSI)

We accurately weighed 5.000 g sample and dissolved in NaCl solution (0.1 moL), and adjusted the pH to 7.0 with magnetic stirring for one hour. Then, the turbid liquid was centrifuged at 600 r/min for 30 min; separated supernatant and transfer to a 50 mL volumetric flask, dilute with buffer solution to volume, and mixed. The protein content of supernatant was measured by Kjeldah method, as Equation (2):

$$PSI(\%) = \frac{\Delta P}{P'} \times 100 \qquad (2)$$

where, ΔP means the protein content that dissolved in NaCl solution; P' means total protein content in samples

2.2.6 Scanning electron microscope

Scanning electron microscope (S-3400N, Hitachi (China) co., LTD) was used to deserve the microstructure of extrusion samples at voltage of 30 V.

2.3 Statistical analysis

The statistics of chromatism, PSI and rheological properties of extrusion productions have been analyzed, and the mathematical relation model for extrusion parameters and rheological model parameters has been established.

Statistical analysis was based on SAS analysis software (Version 6.12, Cary, NC, USA). Duncan variance analysis was used to determine the statistical significance difference of each sample group when the confidence coefficient is 5%. Each experiment had three parallel tests and the average values were used in finally analysis.

3 Results and discussion

3.1 Steady-state rheological properties of extruding dispersed system

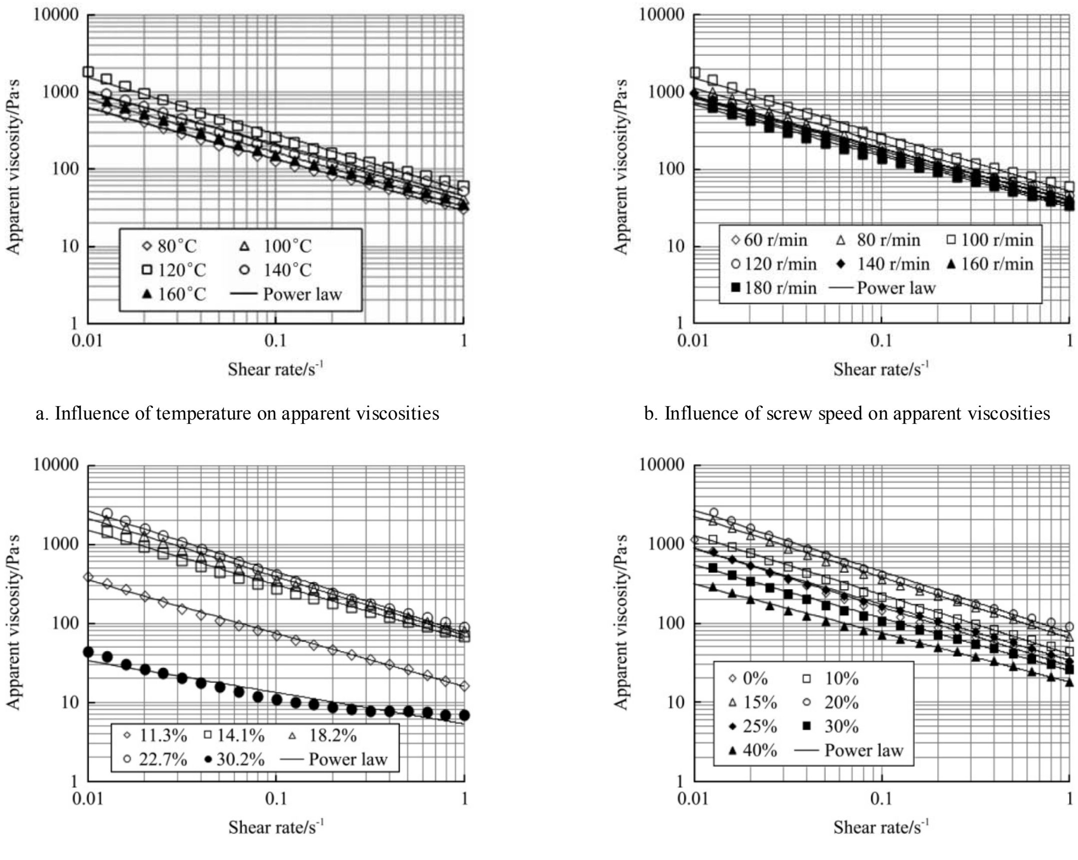
3.1.1 The steady shear scanning of extruding dispersed system

As shown in Figure 2, the apparent viscosity of all samples decreased when the shearing rate increased, and performed the characteristic of non-Newtonian fluid, which fitted the statistics of Power Law model, as Equation (3):

$$\sigma = K\dot{\gamma}^{n} \tag{3}$$

where, σ is shearing stress, Pa; $\dot{\gamma}$ is shearing rate, s^{-1}; K is consistency index, $Pa\cdot s^{n}$); n is liquidity index (dimensionless).

Along with the increase of extruding temperature, the apparent viscosity of extrusion products rose at first and then declined. Since extruding temperature is a determining factor of the chemical reaction in the extrusion process, which plays an important role both in the formation of starch-protein micro network structure and the forming velocity of microstructure[8]. Furthermore, compared with high screw speed (120-180 r/min), low screw speed (60-100 r/min) was beneficial to gain higher apparent viscosity; this may be because high mechanical strength and long residence time resulted in the aggregation of starch, protein and fat to a firmer stable structure. Moisture can acted as plasticizer during extrusion process, which could enhance the molecular motion, reduce torsional and mechanical loss for polymer[17]; thereby, when the material moisture rose from 11.3% to 22.7%, the apparent viscosity of disperse system increased sharply. The apparent viscosity of disperse system reached a high peak, and protein content of the mixture increased to 11.16% when the mass fraction of flaxseed was 20%.



a. Influence of temperature on apparent viscosities

b. Influence of screw speed on apparent viscosities

c. Influence of moisture content on apparent viscosities

d. Influence of flaxseed concentration on apparent viscosities

Figure 2 Influences of extrusion conditions on apparent viscosities of extruded samples

3.1.2 Impacts of extrusion parameters on rheological behavior

As shown in Table 1, when extrusion temperature rose from 80°C to 120°C, K of extrusion products increased whereas n decreased; however, as the temperature rose increasingly, K decreased while n rose up. As the temperature increased, hydrogen bonding structures inside the particles of starch and protein were be disorganized, which led to the swelling. When the temperature reached 160°C, starch and protein were pyrolyzed, especially the disaggregation of starch molecules[8] would significantly impact the stability of creating three-dimensional net structure. Similar conclusion can be verified in recent studies[18,19].

Table 1 Power law model parameters of extruded flaxseed-maize flour blends in steady-shear tests

Test model		$\sigma = K \cdot \gamma^n$		
Parameter	Value	K	n	R^2
T/°C	80	28.72±2.24^c	0.3286±0.0028^a	0.9848
	100	39.19±2.57^b	0.2865±0.0080^c	0.9824
	120	52.48±3.71^a	0.2655±0.0040^d	0.9483
	140	44.51±3.04^b	0.3257±0.0058^a	0.9423
	160	33.33±3.95^c	0.3020±0.0096^b	0.9647
Screw speed /r·min^{-1}	60	35.70±1.72^{cd}	0.3112±0.0107^b	0.9615
	80	43.91±2.98^b	0.2923±0.0088^c	0.9817
	100	52.48±3.71^a	0.2655±0.0040^d	0.9483
	120	45.51±2.28^b	0.3217±0.0041^{ab}	0.9423
	140	39.41±1.65^c	0.3260±0.0091^a	0.9880
	160	34.47±1.92^d	0.3286±0.0090^a	0.9848
	180	32.54±1.78^d	0.3338±0.0118^a	0.9788
Moisture content /%	11.3	15.76±1.92^d	0.3260±0.0136^b	0.9972
	14.1	62.75±4.59^c	0.3112±0.0054^b	0.9919
	18.2	70.23±1.99^b	0.2583±0.0128^c	0.9948
	22.7	75.34±2.51^a	0.2254±0.0038^d	0.9925
	30.2	5.29±0.338^e	0.6015±0.0194^a	0.9272
Flaxseed mass fraction/%	0	28.70±2.15^e	0.2568±0.0129^d	0.9326
	10	40.45±1.92^d	0.2484±0.0225^{de}	0.9553
	15	64.64±3.30^b	0.2310±0.0082^{ef}	0.9600
	20	75.34±2.51^a	0.2254±0.0038^d	0.9925
	25	50.70±2.12^c	0.2927±0.0117^c	0.9819
	30	25.03±1.95^e	0.3350±0.0138^b	0.9798
	40	17.91±2.24^f	0.3791±0.0103^a	0.9845

Note: $^{a-d}$ different superscripts of each tandem unfold that Duncan test different significance level (p<0.05).

Maximum of consistency index (K) and minimum of liquidity index (n) appeared when the screw speed was 100 r/min, and then K declined while n rose when the speed increased from 120 r/min to 180 r/min. Starch based molten mass had shear-thinning behavior, more fat was released to lubricate[20], three dimensional structures formed were relatively weak at this time. Appropriate water content can use as plasticizer to reduce the splitting reaction inside the particles of starch and protein. Furthermore, the increase of water absorbing capacity was easy to form relative stable structure[21]. Moreover, excess water caused the decrease of viscosity of molten masses, which pulled open the crystal structure in starch and protein during swelling process, new molecular groups combined by hydrogen bond were stopped[22]. Similarly, suited flaxseed mass content could reduce the malleability of starch polymer[23], which could promote the forming of complex between lipid and amylose[24]. However, excessive flaxseed led to lipid overflow, which could impede the creation of steady structure[25].

3.1.3 Effects of extrusion parameters on parameters of rheological model

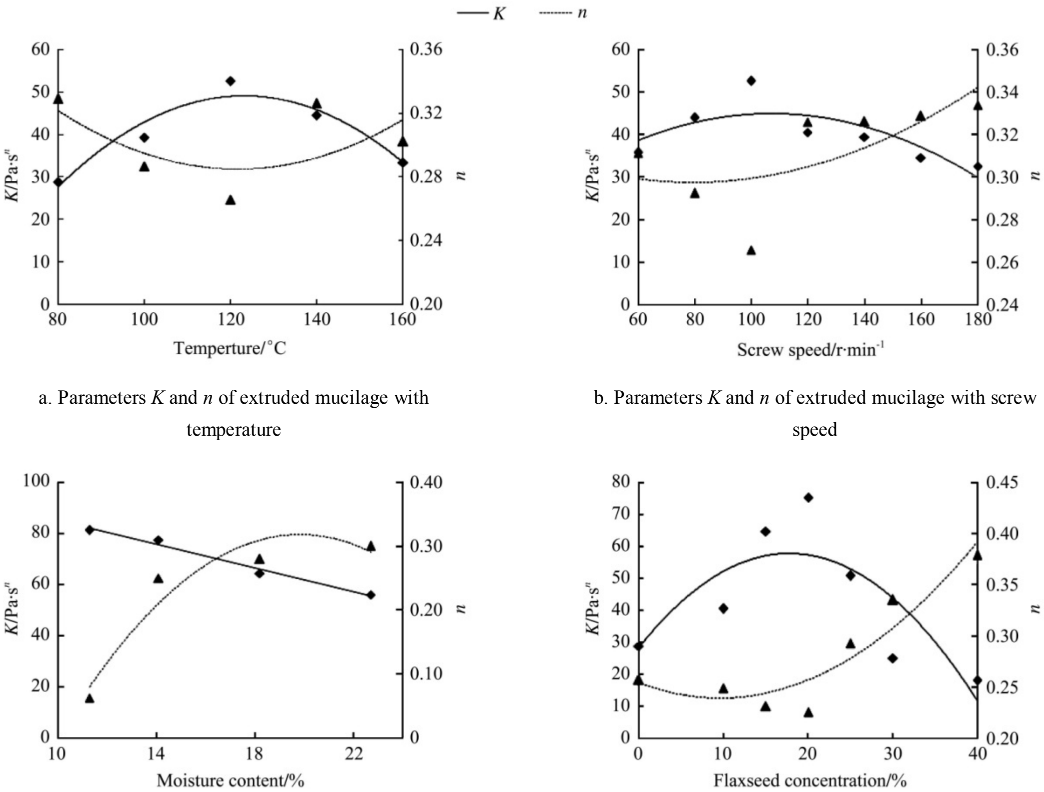
Parameters K and n of model could be calculated using power law model to fitting the statistics getting from rheometer; moreover, these values of K and n could be fitting with extrusion parameter. From the relationship of extrusion parameters and model parameter shown in Figure 3, it can be concluded that with the temperature (T), screw speed (S) and flaxseed content (F) increasing, the value of K increased at first and then dropped, while the value of K unfolded linear decreased when the material moisture content rising.

The Figure 3d showed that when the flaxseed content rose from 0 to 40%, value of K rose increasingly and reached the peak at flaxseed content of 20%, then sharply dropped. The stability of complex in mixture system reduced, and the integral system represented better mobility. According to Figure 3, the fitted polynomial and degree of the relationships of parameters of rheological models and extrusion factors on the flaxseed showed in Table 2. The temperature (T) and moisture content (M) presented a better fit with K by polynomial; moisture content (M) and flaxseed content (F) provided a better fit with n by polynomial and linear equation.

Table 2 Relationships of the parameters of rheological models and extrusion factors on the flaxseed

parameter [a]	regression equation	R^2
	K	
Temperature (T)/°C	$y = -0.0115T^2 + 2.8386T - 125.81$	0.92
Screw speed (S)/r·min^{-1}	$y = -0.0029S^2 + 0.614S + 12.034$	0.62
Moisture content (M)/%	$y = -0.8236M^2 + 32.653M - 243.68$	0.92
Flaxseed content(F)/%	$y = -0.0944F^2 + 3.3553F + 28.04$	0.67
	n	
Temperature (T)/°C	$y = 2\times10^{-5}T^2 - 0.0051T + 0.5966$	0.36
Screw speed (S)/r·min^{-1}	$y = 4\times10^{-6}S^2 - 0.0007S + 0.3251$	0.46
Moisture content (M)/%	$y = -0.0093M + 0.4345$	0.98
Flaxseed content (F)/%	$y = 0.0002F^2 - 0.0032F + 0.2538$	0.88

Note: [a] K and n from power law model.



a. Parameters K and n of extruded mucilage with temperature

b. Parameters K and n of extruded mucilage with screw speed

c. Parameters K and n of extruded mucilage with moisture content

d. Parameters K and n of extruded mucilage with flaxseed concentration

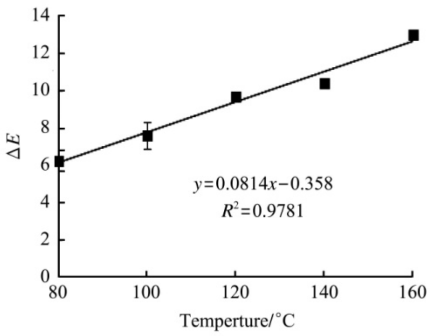
Figure 3 Parameters$K$ and n of extruded mucilage with different extrusion factors and fitting curves

3.2 Effects of extrusion parameters on chromatism ΔE^*

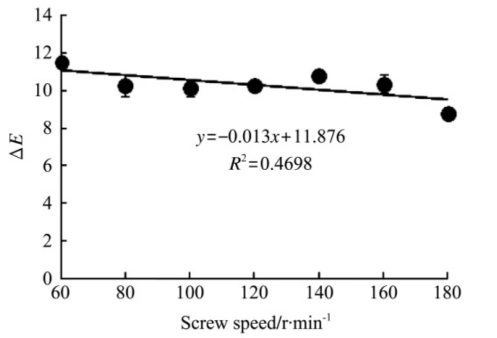
Along with the increasing of extrusion temperature, the total chromatism increased accordingly, because the browning reaction of materials such as Maillard reaction intensifies inside the machine barrel. Maillard reaction also reacted acutely when water content was lower than 18.2%, the viscosity of molten mass respectively high, force of friction and sheared action strengthen that lead to starch particle splitting under this condition, thus, browning reaction happened and brown by-products formed. Maillard reaction slowed down and the total chromatism declined with higher moisture level. Similar conclusion can be found in the study of rice-extrusion by Hagenimana and corn-extrusion by Onyango[26,27]. The total chromatism decreased at first then kept steady and finally decreased again with the the increase of screw speed, tendency of all process was smooth and steady, which may be because the shear action changed during the whole process. The total chromatism showed an exponential rising along with the addition of flaxseed, the more content of protein and fat had, the more micro molecules existed in the mixture, and Maillard reaction could intensify with these molecules. Furthermore, excess fat would cause oxidizing reactions, phospholipid may produce brown productions under high temperature during this period[28]. These results agreed with the report of Rice-glucose-lysine blend extrusion using a co-rotating twin-screw extruder[29].

3.3 Effects of extrusion parameters on PSI

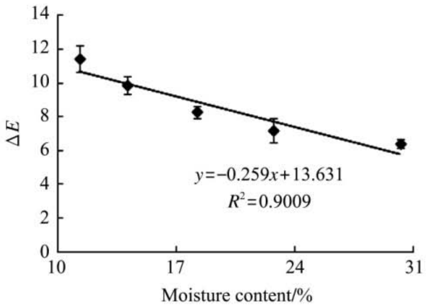
The PSI of puffed food showed a linear decrease tendency along with the extrusion temperature increased, the denaturation of protein intensified under the high temperature and created more insolubility denatured protein; on the other hand, Maillard reaction also enhanced at this condition, which led to more exploded protein reacted carbonyl ammonia reaction with starch, formed more and more by-products or brown products, and reduced the content of soluble protein. PSI increased with screw speed rose. High speed enhanced the shearing act and force of friction of feeders in the machine barrel, so the protein denaturation intensified and then occurred cleavage reaction[30], some of the denatured protein macromolecules were cut into soluble molecule by shearing force. PSI rose from 6.73% to 7.87% when the speed of feeding increased, which let the feeding volume growth, drag increment, and the shearing act and force of friction enhanced the dissimilation of protein. PSI rose accordingly with the increase of material water content, because the moisture reduced the shearing and force of friction inside the barrel, which protected the solubility of some protein molecule.



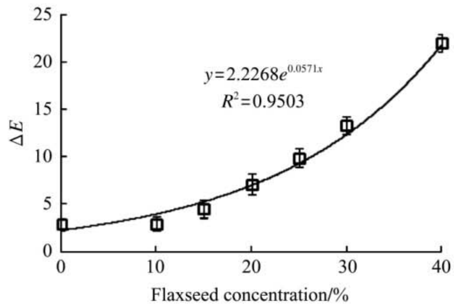
a. Effect of temperature on total color change



b. Effect of screw speed on total color change

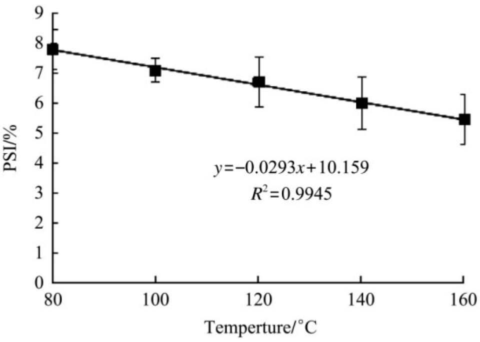


c. Effect of moisture content on total color change

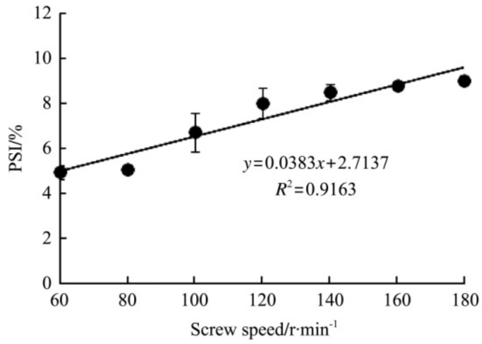


d. Effect of flaxseed concentration on total color change

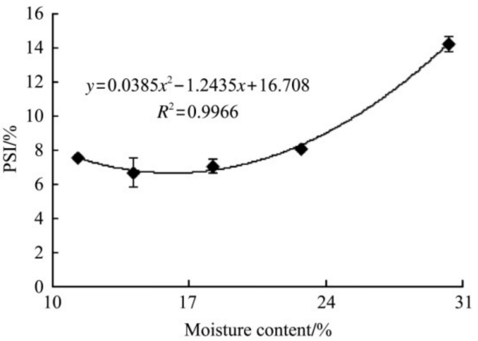
Figure 4 Effects of extrusion conditions on total color change of extruded samples



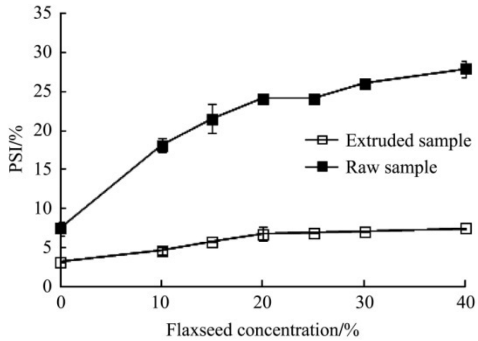
a. Effect of temperature on protein solubility index



b. Effect of screw speed on protein solubility index



c. Effect ofmoisture content on protein solubility index



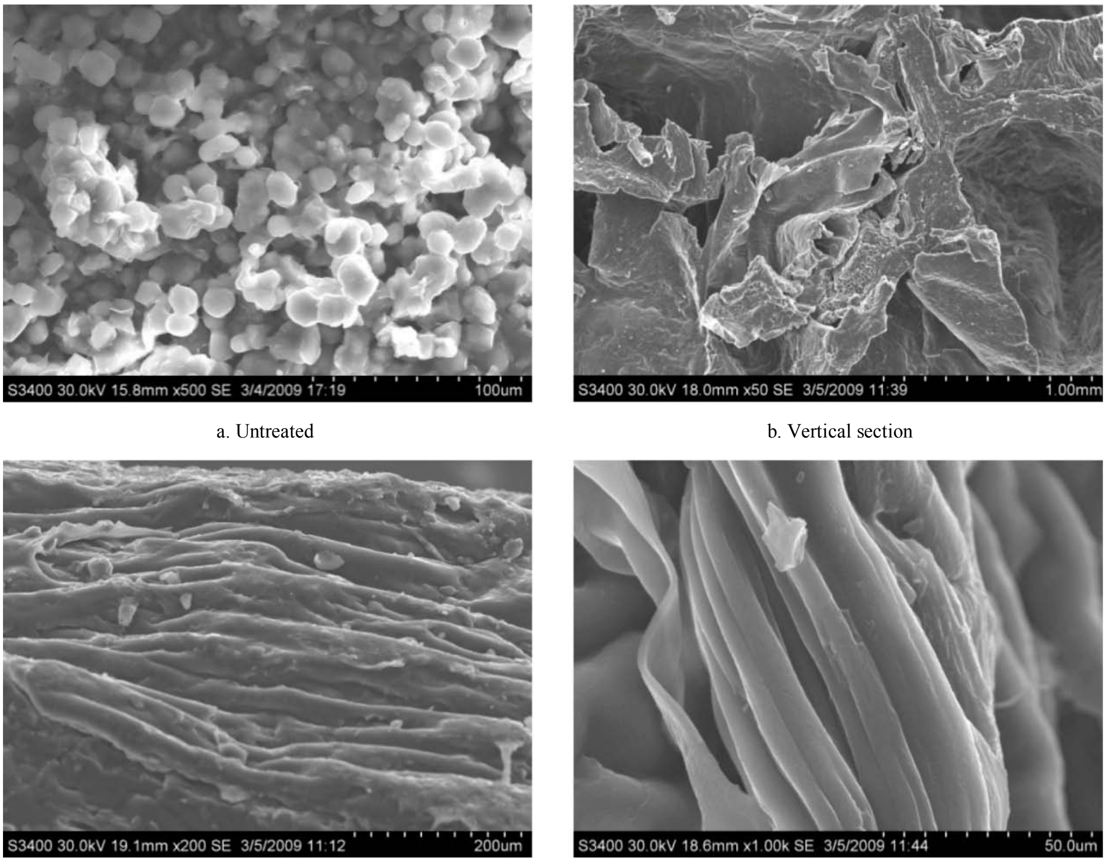
d. Effect offlaxseed concentration onprotein solubility index

Figure 5 Effect of extrusion conditions on protein solubility index of extruded samples

After extrusion, PSI of the disperse system significantly decreased, since the original structure of protein was spread by high temperature and shearing act, hydrogen bond and disulfide bond recombined, while hydrophobic group exposed, therefore formed new chain structure or starch-protein-fat cross-linked networking structure[31]. The flaxseed content has the similar trend of protein content in the mixture, also the same as soluble protein. However, it is still slower compared with the increased trend before extrusion, which means more protein molecules lost their solubility during extruding process.

3.4 SEM analysis of extrusion products

It can be easily determined that extrusions process changed the microstructure of disperse system fundamentally from Figure 6. Figure 6a showed the microstructure of raw materials, many similar size starch particles could be found with some molecular groups which may be original big protein molecules. Figures 6b, 6c are photographs of vertical section and horizontal section of extruded products, respectively. Vertical section showed a porous structure with many dividing thin walls and holes in various sizes, both of them were formed by gel of starch and protein. Horizontal section presented a well-organized orientation laminar flow; conforming to the protein systematism process. In Figure 6d, the polymer constituted by protein and starch showed obviously extrusion screw type organization. After high temperature and high shearing , the biopolymer, which referred to the protein and starch, lost their original molecular structure; Accompanying with protein denaturation and starch congeal, the physical properties of biopolymer groups changed rapidly, which may be because expanding hydrogen bond and disulfide bond formed new molecular polymers[19].



a. Untreated

b. Vertical section

c. Horizontal section

d. Texturized sample

Figure 6 Scanning electron microscopy images of raw samples and treated powder

4 Conclusions

Steady shear experiments proved that the disperse system showed as non-Newtonian fluid, the relationship between apparent viscosity and shearing rate could conform to Power Law model and the matching effect is preferable. Rheological behavior embodied the structural features of flaxseed-corn flour disperse system, in which starch, protein and fat would aggregate and form stronger steady mesh mixture under a suitable extrusion conditions.

Based on the single-factor experiments, the chemical and physical characteristics of extrusion products accordingly changed obviously with the changing of extrusion conditions and flaxseed content, mainly because of the formation of porous structures and Maillard reactions during the extruding process.

The original granular and molecular of starch and protein were spread by higher temperature and shearing act, hydrogen bond and disulfide bond recombined, while hydrophobic group exposed, therefore formed new starch-protein-fat cross-linked networking structure. This structure conclusion can be verified by direct SEM photographs.

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