Journal of Food Engineering 96 (2010) 208–213

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

System parameters and product properties response of soybean protein extruded at wide moisture range

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ARTICLE INFO

Article history:
Received 14 May 2009
Received in revised form 13 July 2009
Accepted 21 July 2009
Available online 25 July 2009

Keywords:
Soybean protein
Extrusion texturization
System parameters
Product properties

ABSTRACT

In order to explore the effect of water during extrusion process, soybean protein isolate (SPI) was extruded using a pilot-scale twin-screw extruder at 28%, 36%, 44%, 52% and 60% moisture content and 140, 150 and 160 °C cooking temperature. The extrusion system parameters like in-line viscosity at die, mean residence time and specific mechanical energy (SME), product textural properties including tensile strength, hardness, chewiness and degree of texturization, and the molecular weight distribution characterized by SDS–PAGE were investigated. And the interrelationship between system parameters and product properties were analyzed. The results showed that moisture content was a more important factor on system parameters and product properties than cooking temperature. Higher moisture content resulted in lower viscosity of dough in the extruder, shorter residence time and lower conversion ratio of extruder mechanical energy into heat energy, finally reducing significantly the tensile strength, hardness, chewiness and the degree of aggregation. The data from extrusion system parameters and product properties correlate well and could be used to explain and control the characteristics of extrudate.

1. Introduction

Extrusion as a continuous efficient cooking, mixing and forming process, has been used increasingly to produce breakfast cereals, baby foods, flat breads, snacks, meat and cheese analogues, modified starches etc. (Harper, 1979; Ding et al., 2006). Despite increased use of extrusion technology, extrusion process is still a complicated multi-input–output system that is yet to be mastered. A simplified system analysis model has been proposed (Meuser and Van Lengerich, 1984), which sorts extrusion parameters into three groups, namely, process parameters (including screw speed, moisture content, barrel temperature, screw configuration, die dimension, raw material characteristics etc.), system parameters (including energy input, residence time etc.), and products properties (including color, nutrition, texture, taste etc.). Among these three kinds of parameters, process parameters have effects on the properties of final products by means of affecting extrusion system parameters. As a result of the "black box" characteristic of extruder and limitation of in-line detection, many researchers focus on the influence of process parameters on product properties, disregarding system parameters (Pham and Del Rosario, 1984; Wang et al., 2001; Ding et al., 2006), and so there are a few literatures related to extrusion system parameters alone, without considering products properties (Akdogan, 1996; Wang, 2005; Kang et al., 2007a,b). In order to better understand the effect of extrusion process on the characteristics of products and to obtain various extrudate with ideal structure and texture, it is imperative to research the correlation between process parameters, system parameters and product parameters comprehensively.

Feed moisture content, as one of the vital process variables, was considered a significant factor affecting the properties of final products (Lin et al., 2000; Wang et al., 2001), and is also an important basis to divide low moisture extrusion and high moisture extrusion (Akdogan, 1999). Due to the limitation of extruder and raw materials, previous studies about the effect of process parameters on system parameters and product properties were implemented at a relative narrow range and numerously at low moisture content level, resulting in incomprehensive even contradictory conclusions. The purposes of this research were to investigate extrusion of soybean protein meat analogue using twin-screw extruder at relative wide moisture range spanning low moisture and high moisture, to study the effect of moisture content and cooking temperature on system parameters and product properties, and to analyze their interrelationship.

2. Materials and methods

2.1. Raw material

Soy protein isolate (SPI) was obtained from Yuwang Group Ltd. (Shandong Province, China). The approximate composition of raw

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doi:10.1016/j.jfoodeng.2009.07.014

material were as follows: water content 8.15%, fat content 0.06% (dry base), ash content 4.81% (dry base), total protein content ($N \times 6.25$) 92.68% (dry base), nitrogen solubility index 63.92%.

2.2. Extrusion

Extrusion was carried out in a pilot-scale, co-rotating, intermeshing, twin-screw food extruder (Brabender GmbH and Co., Germany) with the following dimensions: length/diameter ratio of screw 20:1; at the end of extruder, a slit viscometer die ($2 \times 20 \times 100$ mm) was attached. The barrel is segmented into five temperature-controlled zones which are heated by an electric cartridge heating system and cooled with running water. The extruder response, including motor torque and die pressure, were recorded in-line at a frequency of once per 10 s automatically.

2.3. Experimental design

A 5×3 factorial experimental design was used to investigate the effect of moisture content and cooking temperature. Based on preliminary experiments and the work stability of extruder, the feed moisture contents were selected as 28%, 36%, 44%, 52% and 60% (wet basis), and the cooking temperatures at the middle zone of the extruder barrel were set at 140, 150 and 160 °C. The experimental designs, in total 15 treatments, are shown in Table 1. The feed rate and screw speed were fixed at 20 g/min and 160 rpm, respectively. The temperatures of other four zones except middle zone of the extruder barrel were kept at 80, 110, 135 and 80 °C from feeding zone to die zone, respectively.

2.4. Sample collection and determination of system parameters

When the extruder reached steady state, as indicated by constant values for extruder motor torque and die pressure, samples were collected for system parameters and physical properties determination. Three sets of samples, each about 0.5 kg, were collected and immediately put into airtight plastic bags for future analysis.

The specific mechanical energy (SME) was calculated from the screw speed n (160 rpm), motor torque T (Nm, recorded automatically by computer) and mass flow rate, MFR (g/min, determines the output of extrudate within 3 min) by the formula (Godavarti and Karwe, 1997; Kang et al., 2007a):

$$\mathrm{SME(kJ/kg)} = \frac{2\pi \times n \times T}{\mathrm{MFR}} = 1005.31 \times \frac{T}{\mathrm{MFR}}. \quad (1)$$

Table 1
3×5 factorial experimental design for extrusion operation.

Factors	Levels
Moisture content (%)	28, 36, 44, 52, 60
Cooking temperature at middle zone (°C)	140, 150, 160

The in-line viscosity at die of each treatment was calculated according to the method described by Li et al. (2004) using the formula below, where η is the apparent viscosity (Pa s), τ the shear stress (Pa), γ the shear rate (s^{-1}), ΔP the die pressure drop (Pa), H, L and B the known geometries of viscometer channel (mm), and s the velocity of extrudate coming out from the die (mm/s)

$$\eta(\mathrm{Pa\ s}) = \frac{\tau}{\gamma} = \frac{\Delta PH}{2L} \Big/ \frac{6V}{BH^2} = \frac{\Delta PBH^3}{12LV} = \frac{\Delta PBH^3}{12L(s \times B \times H)} = \frac{\Delta \mathrm{P}H^2}{12Ls} \quad (2)$$

Residence time distribution (RTD) was determined based on the method of Unlu and Faller (2002) and Seker (2005). Active carbon as tracer was rapidly put into feeder in pulse. The L^*–a^*–b^* value of extrudate was determined with constant speed and distance using CR-400 colorimeter (Minolta, Japan), and the period from adding tracer into feeder to no tracer in the outflow extrudate was recorded using a stopwatch. According to the relationship established previously between the L^*–a^*–b^* value and the concentration of tracer in the extrudate, the concentration of tracer in the determination site was calculated, and the $E(t)$ and $F(t)$ function and related parameters could be deduced.

2.5. Analysis of products textural properties

The textural properties of fresh extrudate including tensile strength, hardness, chewiness and shear stress were performed by using a TA.XT2 Texture Analyzer (Stable Micro Systems, UK) immediately after extrusion. A square piece (1.5×1.5 mm) cut from fresh product strip was compressed using a P/35 probe to 50% of its original thickness at a speed of 1 mm/s for 5 s, and the hardness and chewiness data were recorded. A sample shaped like Fig. 1 was pulled using an A/TG probe at a speed of 0.5 mm/s until the strip was broken, and the tensile strength was recorded. A sample chopped into the shape and dimension like Fig. 2, was cut using an A/CKB probe to 75% of its original thickness at a speed of 1 mm/s, along the direction vertical (lengthwise strength, F_L) and parallel (crosswise strength, F_V) to the direction of extrudate outflow from extruder, respectively. The degree of texturization was expressed by the ratio of F_L and F_V. All the determination indices from 12 pieces of each treatment were recorded and averaged.

2.6. SDS–PAGE electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was conducted with 12.5% resolving gel and 5% stacking gel according to the method of Wang and Fan (2000). Protein solutions, extracted by phosphate buffer containing 8 M urea and 0.1 M 2-ME, were diluted with 5× sample diluting solution, and then heated in a boiling water bath for 5 min. After centrifugation at 10,000 rpm for 10 min, a total of 10 μl solution was loaded into each lane and electrically separated. The gels were strained for 30 min with CBB staining solution (0.1% coomassie brilliant blue R, 45% methanol, 10% acetic acid), and destained with 10% methanol and 10% acetic acid. The strained gels were analyzed by using Fluro Chem FC2 Imaging System (Alpha Innotech Cooperation, USA) and

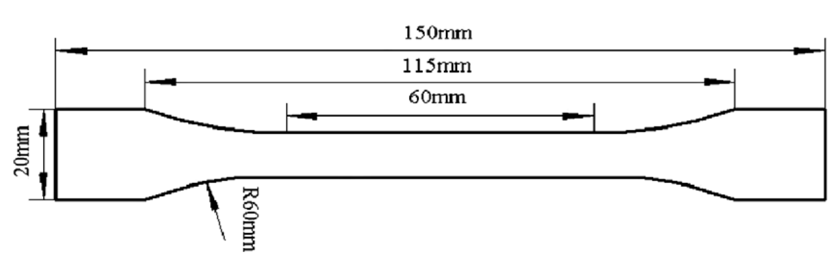

Fig. 1. Sampling sketch for tensile strength test.

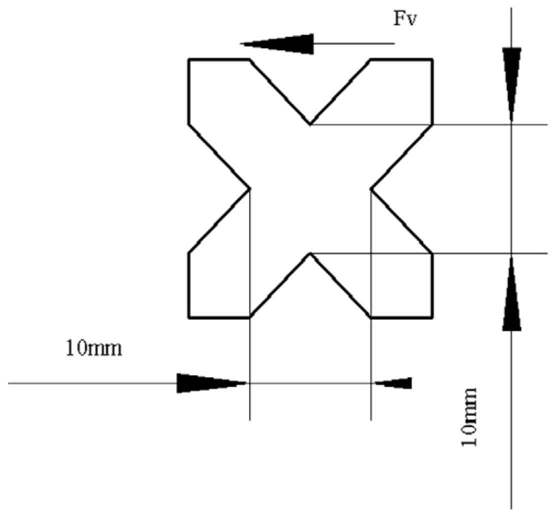

Fig. 2. Sampling sketch for degree of texturization test.

the molecular weight of each subunit was quantified. Low molecular weight markers (Amersham Biosciences, UK) used were rabbit phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), rabbit actin (43.0 kDa), bovine carbonic anhydrase (31.0 kDa), trypsin inhibitor (20.1 kDa) and hen egg white lysozyme (14.4 kDa).

2.7. Statistical analysis

The data of each treatment was analyzed for statistical significance using analysis of variance (ANOVA) function and for statistical interrelation using analysis of correlation (CORR) procedure in Statistical Analysis Software (SAS) V 8.01(SAS Institute, USA). Duncan's multiple range test at 1% and 5% level was used to identify the significant difference of each treatment. *T* test was used to identify the significance of correlation coefficient. The response surface figure was plotted by Statistica V 6.0 (StatSoft, USA).

3. Results and discussion

3.1. Extrusion system parameters

The responses of in-line viscosity, mean residence time and SME as functions of extrusion moisture and cooking temperature are shown in Fig. 3a–c. Analysis of variance indicated that the extrusion moisture content was significant to in-line viscosity at die, mean residence time and SME ($p < 0.01$). As the moisture content increased from 28% to 60% (wet base), the in-line viscosity at die, mean residence time and SME significantly decreased. This is because an increase in moisture content reduced the force required to push wet mass through the die, and resulted in decreasing the friction between raw material and screw shaft and extruder barrel (Lin et al., 2000; Wang et al., 2001; Kang et al., 2007b). Thus the extruder system parameters became lower at higher moisture content.

Increasing cooking temperature from 140 to 150 °C significantly decreased in-line viscosity at die and SME ($p < 0.01$), especially at lower moisture content, and then the changes were little as cooking temperature further increased. However, the mean residence time was hardly affected by cooking temperature. This was probably because temperature and moisture could have synergetic effect on the response of extruder, and the effect of temperature was inferior to that of moisture.

Lo et al. (1998) reported that the viscosity of dough was affected by both temperature and moisture content. When temperature was constant, the viscosity decreased with increasing moisture content. Because of water as a lubricant in the extruder (Hayashi et al., 1992) and the presence of less polymers with higher moisture content, the viscosity of dough in the extruder and at the die was lower (Akdogan, 1996). Our research results (Fig. 3a) were in accordance with these previous reports.

Residence time of feed material in the extruder is supposed to be a degree indicator of raw material experiencing shearing, heating, shaping, mixing and reaction. It was found that increasing moisture content could result in accelerating the flow speed of extrudate coming out from extruder. This was confirmed by the result of effect of moisture content on mean residence time, which is usually considered in two opposite ways (Seker, 2005). On one hand, increasing the moisture content of feed material results in the decrease of viscosity of feed dough in the barrel of an extruder, and lower force is required to pump the melt through the die. On the other hand, temperature in the die due to viscous dissipation is lower, and the lower temperature of feed increases the viscosity at the die, which tends to increase the restriction of flow through the die. The effect of moisture content on the mean residence time is expected to be the result of these two opposite effect of moisture content on rheology of feed material in the barrel and die of the extruder. Fig. 3b shows that the viscosity of dough at the die decreased significantly with moisture content increasing. Therefore, increasing moisture content would result in the mean residence time of dough in the extruder decreasing.

SME is the amount of work input from driver motor into the raw material being extruded, thus provides good characterization of the extrusion process (Godavarti and Karwe, 1997). SME is also an important parameter influencing the final product characteristics such as solubility, density, expansion index, hardness, etc. SME values indicate the extent of molecular breakdown or degradation that the material undergoes during extrusion process. A decrease in SME as the moisture content increased was due to a reduced shear force and mechanical energy input (Fig. 3c). Even though several extrusion system parameters, such as pressure drop at the die, motor torque, SME and product temperature have been studied in previous literatures (Akdogan, 1996; Lin et al., 2000; Wang, 2005), the relationship of cause-and-effect among these system parameters has not been elaborated. In view of previous researches (Jao et al., 1978; Lo et al., 1998) and our research results, some assertions could be made for the relationship among these comprehensible integrated extrusion system parameters. An increase in the moisture content firstly decreases the viscosity of dough in the extruder, shortens the average time of raw material staying in the extruder, and finally reduces the conversion ratio of extruder mechanical energy into heat energy, consequently the SME becomes lower.

3.2. Product properties analysis

Tensile strength, hardness and chewiness, as important quality attributes of un-expansion extruded products, depend on structural characteristics arising from extrusion condition. Degree of texturization is an indicator for fibrous structure formation. The effect of extrusion moisture and cooking temperature on tensile strength, hardness, chewiness and degree of texturization of product are shown in Fig. 4a–d. Analysis of variance indicated that the extrusion moisture content was significant to tensile strength, hardness and chewiness of product ($p < 0.01$). As moisture content increased from 28% to 60%, the tensile strength (Fig. 4a), hardness (Fig. 4b) and chewiness (Fig. 4c) of products decreased sharply. The samples extruded at 60% moisture had the lowest tensile strength, hardness and chewiness, which were likely due to more water contained within the samples (Lin et al., 2000). Moisture content had significant effect on degree of texturization ($p < 0.05$), and the samples extruded at 60% moisture content had the best fibrous

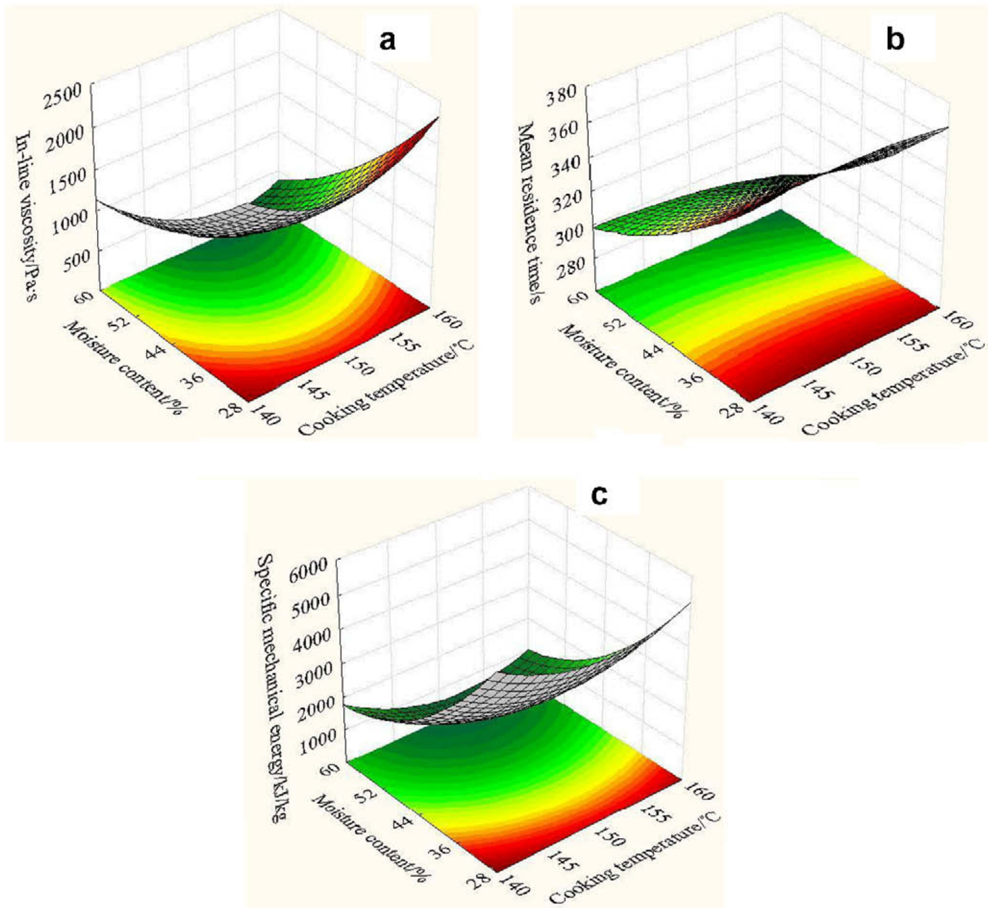

Fig. 3. In-line viscosity (a), mean residence time (b) and SME (c) versus moisture content and cooking temperature with screw speed 160 rpm and feed speed 20 g/min.

structure (Fig. 4d). Kang (2007) reported that increasing moisture content within the range of 35–55% could reduce the hardness and chewiness, and promote the formation of fibrous structure. However, Lin et al. (2000) found that as the extrusion moisture content decreased from 70% to 60%, the structure of the products became more directionally aligned. Liu and Hsieh (2008) found that among the products extruded at the levels of moisture from 60.11% to 72.11%, only the 60.11% having well-defined fiber orientation. These indicate that moisture could help protein molecular unfolding and alignment during extrusion to some extent, but the favorable impact of water on the formation of fibrous structure is not limitless.

The results in Fig. 4a–d show that cooking temperature had a significant effect on tensile strength ($p < 0.01$), but not hardness and chewiness, which could be due to the results of extrusion system parameters affected by cooking temperature. Lin et al. (2000) found that the effect of cooking temperature was significant to the TPA attributes at lower moisture contents, but not at higher moisture contents. Kitabatake et al. (1985) also found that cooking temperature had little effect on the product texture when extruding soy protein isolate at greater than 70% moisture. These indicate that only at lower moisture content is it effective to tailor the texture of meat analogue by controlling cooking temperature.

3.3. SDS–PAGE electrophoresis

The electrophoresis of samples extruded at different moisture content and cooking temperature are shown in Fig. 5. The two major components in soybean protein, viz.11S (glycinin) and 7S (β-conglycinin), had changed greatly after extrusion. When moisture content was increased from 28% to 60%, the bands of 7S (with MW of 79.4, 73.1 and 55.5 kDa, respectively) and 11S (with MW of 39.7 and 18.7 kDa) became darker and wider, and the top of each lane became lighter and narrower, which because the very large molecular weight proteins/protein complex in extrudates were unable to penetrate the pores of the separating gel. This indicated that the raw protein had undergone aggregation and crosslink during extrusion, resulting in the formation of protein–protein and/or protein–non-protein macromolecules polymers with large molecular weight.

On the other hand, increasing cooking temperature from 140 to 160 °C also intensified the polymerization of protein during extrusion. These suggest that the lower moisture content, the higher cooking temperature, the greater protein denaturalization extent. This is corresponding to the effect of moisture content and cooking temperature on SME.

Petruccelli and Anon (1995) found that when temperature was below 80 °C, the proteins of isolate had been denatured, but no aggregation was observed. And increasing soy protein content, namely decreasing moisture content, could enhance thermal aggregation. Li and Lee (1996) reported that the degree of aggregation wheat proteins increased with the increasing extrusion die temperature from 160 to 185 °C. The results reported in our research were consistent with these previous literatures.

3.4. Correlation analysis between system parameters and product properties

The correlation analysis between extrusion system parameters and product properties are shown in Table 2. The product properties

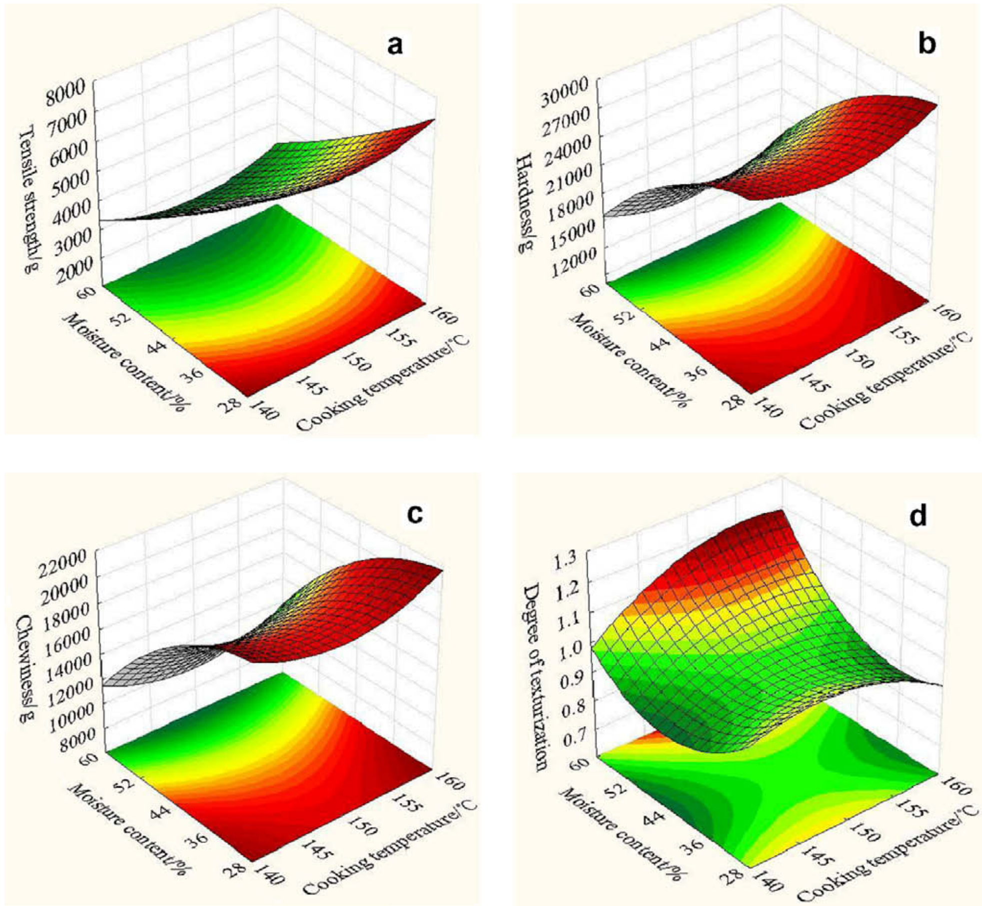

Fig. 4. Tensile strength (a), hardness (b), chewiness (c) and degree of texturization (d) versus moisture content and cooking temperature with screw speed 160 rpm and feed speed 20 g/min.

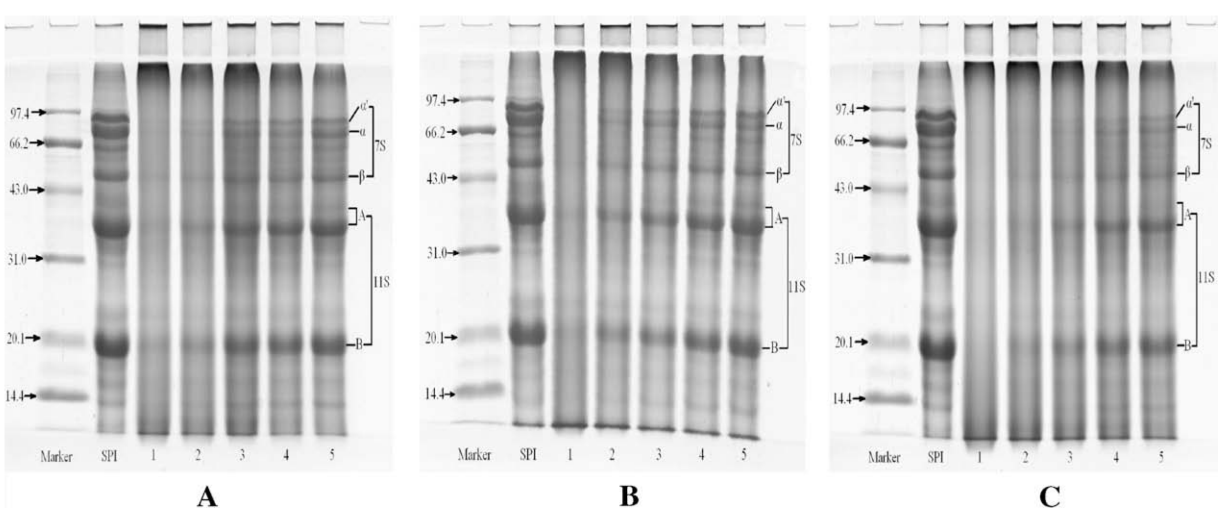

Fig. 5. Vertical SDS–PAGE of samples extruded at (A) 140 °C, (B) 150 °C and (C) 160 °C (1–5 indicates 28%, 36%, 44%, 52% and 60% moisture content, respectively).

except degree of texturization were significantly affected by the extrusion system parameters ($p < 0.01$). This means that the mean residence time and SME have direct impact on the final product quality (Godavarti and Karwe, 1997). The extent of tensile strength was the most affected by the system parameters, followed by hardness and chewiness. Consequently, moisture content and cooking temperature affect the in-line viscosity at die, mean residence time and SME, and finally results in different product textural properties and the extent of polymerization. Therefore, the properties of meat analogue could be regulated by controlling the moisture content and cooking temperature during extrusion (Lin et al., 2000).

Table 2
Correlation analysis between system parameters and product properties.

	In-line viscosity at die	Mean residence time	SME
Tensile strength	0.86**	0.82**	0.96**
Hardness	0.72**	0.76**	0.76**
Chewiness	0.70**	0.75**	0.74**
Degree of texturization	−0.36	−0.45	−0.49

** Significant at $p < 0.01\%$ level.

4. Conclusions

Soybean protein meat analogue extruded at relative wide moisture range was a simple system to study the effect of process parameters, especially moisture content on extrusion system parameters and product properties. The results showed that moisture content was a more important factor on system parameters and product properties than cooking temperature. Increasing the extrusion moisture could reduce dramatically the tensile strength, hardness, chewiness and the degree of aggregation. The fibrous structure was observed in the product extruded at 60% moisture content. There are obvious correlations between system parameters and product properties. Therefore, the properties of extrudate could be controlled and monitored by means of process parameters and system parameters, respectively. In order to explore the effect of water in the extrusion process comprehensively, further studies are needed to investigate the thermal properties, protein solubility and protein structure etc. of the product.

Acknowledgement

The project was part supported by the 11th Five Year Program of China (2006BAD05A08).

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