Effects of extrusion conditions on the physicochemical properties of soy protein/gluten composite

Min Wu[1*], Yang Sun[1], Chonghao Bi[2], Fang Ji[3], Borui Li[1], Junjie Xing[1]

(1. *College of Engineering, China Agricultural University, Beijing 100083, China*;
2. *School of Materials Science and Mechanical Engineering, Beijing Technology and Business University, Beijing 100048, China*;
3. *Chinese Academy of Agricultural Engineering, Beijing 100125, China*)

Abstract: Soybean protein-gluten blend was extruded using a co-rotating twin-screw extruder. Effects of different extrusion conditions on the textural properties of extrudates were analyzed by central composite design through the evaluation of texturization index, water holding capacity, and hardness of extruded protein products. Extrusion process variables including feed moisture content (40%-60%), screw speed (12-20 Hz), extrusion temperature (120°C-180°C), and gluten content (16%-32%) were studied by the nonlinear regression equations analysis. The extrusion process was also optimized using response surface methodology (RSM). The influence of moisture content on the hardness of extrudate was the most significant. The optimized results, including feed moisture content, extrusion temperature, screw speed and gluten content were 49.18%-50.15%, 155.24°C-159.86°C, 16.41-16.73 Hz, 20.00%-20.03%, respectively. The microstructures of TISP products were analyzed by scanning electron microscopy (SEM) and the changes of protein molecules before and after extrusion were also analyzed by SDS-PAGE electrophoresis.

Keywords: soybean protein, gluten, extrusion, response surface methodology (RSM), SDS-PAGE electrophoresis, Microstructural properties

DOI: 10.25165/j.ijabe.20181104.4162

Citation: Wu M, Sun Y, Bi C H, Ji F, Li B R, Xing J J. Effects of extrusion conditions on the physicochemical properties of soy protein/gluten composite. Int J Agric & Biol Eng, 2018; 11(4): 230–237.

1 Introduction

Today, due to increasing consumer demand for healthy diets and concerns about the impact of meat production on the environment, many producers are urgent to increase the appetites for soy products[1]. Soybean protein has many functional benefits in the human diet as plant protein products, which supplies eight essential amino acids and contributes valuable functional properties in the soybean protein processing industry[2]. Both the US Food and Drug Administration (FDA) and Chinese Nutrition Society (CNS) have approved the use of a soy protein health claim on food, and agreed that the digestion of 25 g of soy protein per day as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease[3]. The soybean protein has been used as the replacement of meat protein in food product, the direct consumption of which，although increased by now, is still low. The texturization of soybean protein materials has been a major development in the food industry over the years[4].

Extrusion technique is an important technique widely used in food industry, which is also an efficient continuous processing[5,6]. Soybean protein is an important raw material for processing plant protein extrusion textured products[7]. It could be used, extruded and texturized into food products and has been developed to obtain fibrous meat-like structures[8]. Soybean protein displays an anisotropic structure, with layers oriented in the direction of flow through the die. Shear forces developed in the die would permit denaturation, dissociation and orientation of protein matrix[9,10]. Furthermore, the presence of insoluble gluten proteins could enhance the separation of layers[6,9]. Texturized soy protein has the advantages of blander flavor and major reduction of non-digestible stachyose and raffinose[8]. The high moisture extrusion cooking (HMEC) process is an emerging technology for obtaining fibrous meat-like structures from plant proteins[1]. It has lots of advantages over low or intermediate moisture extrusion[1,11]. Texturization of soy protein has been economically feasible as the meat extenders and replacers and the extrusion textured soybean product is similar to meat's taste and appearance, and rich in nutrition[2,9].

The influence of extrusion parameters such as barrel temperature, screw speed, moisture content, and die diameter on the protein extrudates properties has been investigated[6,11,12]. These extrusion parameters have a great effect on the color[13,14], texturization index, hardness, water holding capacity, and protein solubility of extruded soybean protein[5,15,16]. The evaluation of these physiochemical properties is very complicated process[5]. Therefore, more research is needed to better understand the effect of extrusion process on the characteristics of products, to optimize the processing parameters, and to conduct large-scale production in soybean protein processing industry.

The objectives of this study were to determine the effects of four factors (feed moisture content, screw speed, extrusion temperature and gluten content) on the physicochemical and

Received date: 2018-01-31 **Accepted date:** 2018-06-07

Biographies: **Yang Sun**, Master candidate, research interests: food science and engineering, Email: 815638711@qq.com; **Chonghao Bi**, PhD, Lecturer, research interests: material property characteristics, Email: bichonghao@btbu.edu.cn; **Fang Ji**, PhD, research interests: agricultural engineering, Email: ji_fang1984@163.com; **Borui Li,** Master candidate, research interests: drying technology, Email: boruili@cau.edu.cn; **Junjie Xing,** PhD candidate, research interests: food science and engineering, Email: xingjunjie1202@cau.edu.cn.

***Corresponding author**: **Min Wu**, PhD, Lecturer, research interests: agricultural Engineering, food science and engineering. College of Engineering, China Agricultural University, P. O. Box 50, 17 Qinghua Donglu, Beijing 100083, China. Tel. /Fax: +86 10 62736883; Email: minwu@cau.edu.cn.

functional properties of soy protein/gluten composite[9]. The textural properties of extruded soybean protein were studied from the aspects of texturization index, water holding capacity, hardness, and microstructure. The response surface methodology (RSM) and equations regression analysis were also employed to study the correlation of parameters and properties and to optimize the extrusion processing. This information would be useful for improving soy products used in food industry.

2 Materials and methods

2.1 Materials

Soybean protein isolate (SPI) was obtained from Yuwang Eco Food Group Ltd. (Shandong Province, China). The approximate composition of raw material was as follows: water content 6.48%, fineness 98.9% (dry base), ash content 2.8% (dry base), pH 7.45, gel value 138.8 g, and total protein content (N 6.25) 90.7% (dry base), and the urease activity was negative. The yellowish powder of raw materials were packed in zipper bags and kept in desiccators until used.

The wheat gluten was obtained from Fengqiu Food Eco Group Ltd. (Shandong Province, China). The approximate composition of raw material was as follows: water content ≤ 8%, fat content ≤ 1.0% (dry base), fineness value ≥ 99.5% (dry base, CB30 mesh screen), ash content ≤ 1.0% (dry base), water absorption ≥ 160%, and total protein content (N 6.25) ≥ 75% (dry base).

2.2 Samples preparation

Blends (about 1 kg) were prepared by mixing SPI and wheat gluten materials with different gluten content (16%-32%). The moisture content of these blends samples were adjusted to the high moisture levels of 40%-60% by spraying with a calculated amount of water and mixing completely. These samples were sealed in the zipper bags and conditioned for 12 h to ensure a uniform distribution of water before extrusion.

2.3 Extrusion processing

All extrusion experiments were performed by a laboratory-scale co-rotating twin-screw food DS30-III extruder (Saixin Machinery Co., Ltd, Shandong Province, China). The pump and feeder were calibrated and adjusted beforehand. The moisture level of feeding was 40%-60% (wet basis) and the screw speed was 12-20 Hz. The extruder barrel temperatures were kept constant at 120°C, 135°C, 150°C, 165°C and 180°C, respectively. Extrudates were collected when the operation condition was at a steady state. The texturized isolated soybean protein (TISP) were stored in polyethylene bags at the freezer (–18°C) and used for further analysis.

2.4 Product responses

2.4.1 Texturization index

The texturization index can be used to indicate fibrous structure formation and is expressed as the ratio of lengthwise strength and crosswise strength[5]. The texturization index of extrudate samples were performed using a CT3-4500 Texture Analyzer (Brookfield Engineering Labs. Inc., USA). A square piece (2 cm × 2 cm) cut from TISP sample strip was sheared using a shear geometry TA-SB to 100% of its original thickness along the direction vertical and parallel to the direction of extrudates outflow from the extruder, respectively. The sample was tested at a loading speed of 2 mm/s with trigger point 100 g and distance of 8 mm. The lengthwise strength and crosswise strength data were then recorded. All tests were repeated three times and the data were recorded and averaged.

$$TI = FL/FV \quad (1)$$

where, TI is texturization index, dimensionless; FL is lengthwise strength, MPa; FV is crosswise strength, MPa.

2.4.2 Water holding capacity (WHC)

The WHC is the weight of gel obtained per gram of dry ground sample (g/g). TISP samples were air dried at 60°C for 6 h. The dried samples were ground and then passed through an 80 mesh sieve. Finally, the samples were packed in zipper bags and kept in desiccators until used. The WHC of extrudates was determined according to the AACC method. The TISP extrudate (W_1, about 4 g) was suspended in a centrifuge tube (W_2, g) with deionized water (20 mL) at room temperature, gently stirred during this period. After standing for 10 min, samples were centrifuged for 15 min at 4500 r/min by centrifugal force (Anker GL-20G-Ⅱ, Shanghai, China). The supernatant was decanted and the precipitate was weighed (W_3, g). The WHC was calculated as the weight of sediment obtained after removal of the supernatant per unit weight of original solids as dry basis. WHC were replicated at least three times and calculated as follows:

$$WHC = (W_3 - W_2 - W_1)/W_1 \quad (2)$$

2.4.3 Hardness

The hardness of extrudate samples were carried out using a CT3-4500 Texture Analyzer (Brookfield Engineering Labs. Inc., USA). A square piece (1.5 mm × 1.5 mm) cut from fresh product strip was compressed using a cylinder TA 39 probe to 50% of its original thickness at a speed of 1 mm/s for 5 s. All the other processing parameters were the same with the texturization index measured. The hardness data were recorded in three times and averaged.

2.4.4 SDS-PAGE electrophoresis

The TISP samples were air dried and ground to pass through an 80 mesh sieve. TISP extrudate (1 g) was suspended into a centrifuge tube with 5.0 mL phosphate buffer and treated by ultrasound for 30 min. After centrifugation at 10 000× g for 20 min (Anker GL-20G-Ⅱ, Shanghai, China), the supernatant was collected into a new centrifuge tube and stored in the refrigerator at 4°C until used.

Electrophoresis (SDS-PAGE) experiments were performed on a vertical large gel system (Mini-Protein 3, Bio-Rad, Hercules, CA, USA) using 12 g/100 mL separating gels and 5 g/100 mL stacking gels. 10 μL solutions of the above supernatant were loaded into each sample lane and electrically separated. The test voltage was adjusted to 80 V before loading sample to the gels and increased to 120 V after the indicator has reached to interface of separating gels and stacking gels. When the dye front reached the bottom of the separating gel, electrophoresis was terminated. The gels were stained with Coomassie brilliant blue staining solution, and then de-stained. The stained gels were analyzed using an imaging system and the Mw of each subunit was accurately quantified.

2.5 Microstructural properties

The morphology of TISP sample was investigated using scanning electron microscope (SEM, S-3400N, Hitachi, Japan). Samples were mounted with double-sided carbon adhesive tabs on aluminum stubs, and sputter coated with gold-palladium (60%-40%). The sample patterns were observed at an accelerating voltage of 2 kV or 5 kV.

2.6 Experimental design and data analysis

The central composite design for three independent variables was performed. The independent variables considered were feed moisture content (X_1), extrusion temperature (X_2), screw speed (X_3) and gluten content (X_4). The independent variables and various levels were shown in Table 1. The levels of each variable were established according to literature data and preliminary trials. Dependent variables were feed moisture content, extrusion temperature, screw speed and gluten content as product responses. RSM was applied for experimental data using a commercial statistical package, and Design-Expert version 8.0 (Stat ease Inc., Minneapolis, MN, USA) used for the generation of response surface plots. The same software was used for statistical analysis of experimental data. The coded levels for the independent variables are listed in Table 1.

Table 1 Coded levels for the independent variables

Parameters	Code	Variable level codes				
		–2	–1	0	1	2
Feed moisture content/%	X_1	40	45	50	55	60
Extrusion temperature/°C	X_2	120	135	150	165	180
Screw speed/Hz	X_3	12	14	16	18	20
Gluten content/%	X_4	16	20	24	28	32

All of the above-mentioned tests were carried out in triplicate. The values are averaged and are expressed as mean ± standard deviation. SPSS 17.0 software (SPSS Inc., Chicago, USA) was used for analysis of variance (ANOVA) and significant differences were analyzed by Duncan's multiple range tests. A 0.95 confidence level (p<0.05) was applied to determine if significant difference existed between two mean values.

3 Results and discussion

3.1 Establishment of regression model

The response values of central composite design were listed in Table 2. The results were analyzed by a multiple linear regression method, which describes the effects of variables in first order, a two-factor interaction (2FI) and second order polynomial models. Experimental data were fitted to the selected models and regression coefficients were obtained as follows:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i x_i + \sum_{i=1}^{3}\beta_{ii} x_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} x_i x_j \quad (3)$$

Statistical significance of the terms in the regression equation was examined by analysis of variance (ANOVA) for each response[9].

A regression analysis was carried out to fit mathematical models to the experimental data. The predicted model for texturization index, water holding capacity, and hardness could be described by the equation in terms of coded values as listed in Table 3, which will be analyzed in the next parts in detail.

3.2 Response surface analysis of texturization index

A 4×5 factorial experimental design was used to investigate the effect of extrusion temperature, feed moisture content, screw speed and gluten content characteristic on of extruded sample. The 30 experimental tests were listed in Table 2. The quadratic model for texturization index in terms of coded levels of the variables was listed in Table 3. The full ANOVA to evaluate the results which include the F value, p value and significance were listed in Table 4. The relative coefficients indicated that the interactions between feed moisture content and screw speed, between extrusion temperature and screw speed, and between extrusion temperature and screw speed have a great effect on the textural properties[17].

Table 2 Experimental results of central composite design

Tests	X_1	X_2	X_3	X_4	Texturization index	Water holding capacity	Hardness/g
1	–1	–1	–1	–1	1.51	3.17	7120
2	1	–1	–1	–1	1.66	3.29	4123
3	–1	1	–1	–1	1.31	3.35	7859
4	1	1	–1	–1	1.71	3.75	3736
5	–1	–1	1	–1	1.63	3.29	7259
6	1	–1	1	–1	1.3	3.53	4113
7	–1	1	1	–1	1.69	3.32	7473
8	1	1	1	–1	1.19	4.06	3385
9	–1	–1	–1	1	1.55	3.20	7438
10	1	–1	–1	1	1.34	3.37	2917
11	–1	1	–1	1	1.4	2.96	7460
12	1	1	–1	1	1.35	3.27	3583
13	–1	–1	1	1	1.23	3.21	7934
14	1	–1	1	1	0.96	3.21	2195
15	–1	1	1	1	1.63	3.07	6175
16	1	1	1	1	1.82	3.51	4024
17	–2	0	0	0	1.31	3.38	16511
18	2	0	0	0	1.25	3.77	1891
19	0	–2	0	0	1.32	3.03	5923
20	0	2	0	0	1.64	3.41	5378
21	0	0	–2	0	1.13	3.14	5321
22	0	0	2	0	1.34	3.26	5927
23	0	0	0	–2	1.72	3.43	6755
24	0	0	0	2	1.31	3.06	5159
25	0	0	0	0	1.68	3.41	4186
26	0	0	0	0	1.72	3.53	5210
27	0	0	0	0	1.74	3.41	3516
28	0	0	0	0	1.70	3.49	3946
29	0	0	0	0	1.72	3.51	4054
30	0	0	0	0	1.73	3.47	4172

Table 3 Response values corresponding regression equations

Response value (Y)	Regression equation	R^2
Texturization index	Y_1=1.715+0.065X_2–0.064X_4–0.075X_1X_3+0.094X_2X_3+0.083X_2X_4–0.096X_1^2–0.107X_3^2	0.7366
Water holding capacity	Y_2=3.47+0.133X_1+0.074X_2+0.045X_3–0.112X_4+0.085X_1X_2–0.086X_2X_4+0.032X_1^2–0.056X_2^2–0.061X_3^2–0.050X_4^2	0.9337
Hardness	Y_3=4180.667–2495.083X_1+1057.833X_1^2	0.8612

Note: Regression equation significance level is p<0.05.

Table 4 ANOVN of regression equation for RSM model

Response values	Texturization index			Water holding capacity			Hardness		
Variation	F value	p value	Sig.	F value	p value	Sig.	F value	p value	Sig.
X_1	0.86	0.3684	NS	61.03	0.0001	***	76	0.0001	***
X_2	3.82	0.0695	*	18.88	0.0006	***	5.2E-03	0.9436	NS
X_3	2.5E-03	0.9607	NS	6.95	0.0187	**	4.6E-03	0.9468	NS
X_4	3.72	0.0728	*	43.45	0.0001	***	0.9	0.3566	NS
X_1X_2	1.15	0.2996	NS	16.54	0.0010	***	1.15	0.0705	*
X_1X_3	5.39	0.0424	**	1.58	0.2284	NS	4.9E-03	0.9449	NS
X_1X_4	8.5E-03	0.9278	NS	3.01	0.1034	NS	1.12	0.0735	*
X_2X_3	7.3	0.0361	***	1.58	0.2284	NS	0.07	0.7949	NS
X_2X_4	4.1	0.0489	**	17.03	0.0009	***	0.027	0.8719	NS
X_3X_4	0.34	0.5684	NS	1.73	0.2081	NS	6.8E-03	0.9354	NS
X_1^2	9.49	0.0076	***	4.14	0.0598	*	15.61	0.0013	***
X_2^2	2.17	0.1612	NS	12.41	0.0031	***	0.4	0.5345	NS
X_3^2	11.85	0.0036	***	14.72	0.0016	***	0.37	0.5503	NS
X_4^2	1.42	0.2517	NS	9.81	0.0069	***	0.85	0.3711	NS
Regression model	3	0.0215	**	14	15.1	***	6.65	0.0004	***

Note: * is p<0.1; ** is p<0.05; *** is p<0.01; NS is not significant.

The effect of extrusion process on the texturization index of product was shown in Figure 1a. Under lower screw speed, the texturization index of extrudates increased first and then decreased with increased moisture content from 40% to 60%. While the texturization index decreased with increased moisture content under higher screw speed. Preferable fibrous structure was obtained under the moisture content of 50% and screw speed of 20 Hz.

Furthermore, under lower screw speed, there was no significant correlation between extrusion temperature and texturization index (Figure 1b). However, the texturization index was greatly increased with the increased temperatures under the screw speed of 20 Hz. The texturization index also increased and then decreased later with the increased screw speed under constant temperatures. The results showed that the forming of fibrous structure facilitated under the temperature of 150°C and screw speed of 16 Hz, which was due to that the mass and heat transfer rates were increased by these factors, improving the protein interaction and denaturation[9,18].

Figure 1c showed that the texturization index increased with the increased temperature under the gluten content of 20%. However, the effect of gluten content on the texturization index was more important than the extrusion temperature. Cheftel et al.[9] also found that the addition of gluten could benefit the denaturation, dissociation and orientation of protein matrix.

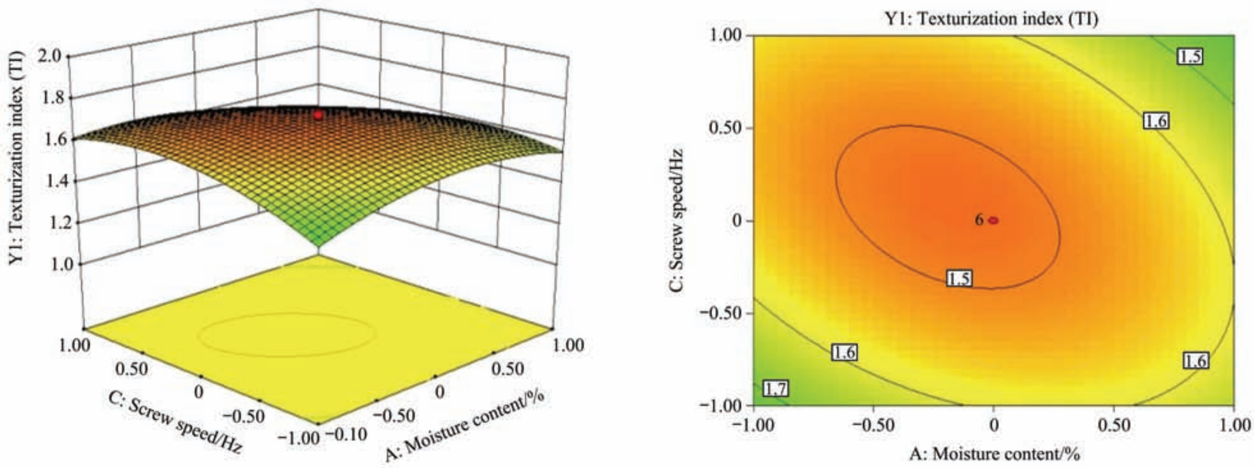
3.3 Response surface analysis of water holding capacity

The quadratic model for water holding capacity in terms of coded levels of the variables was also listed in Table 3. The interactions between extrusion temperature and feed moisture content, and between extrusion temperature and gluten concentration have significant effect on the water holding capacity. Under lower extrusion temperature, there was no significant correlation between moisture content and water holding capacity (Figure 2a). However, the effect of gluten content on the texturization index was gradually increased with increased moisture content under the constant extrusion temperature of 170°C. This was due to that the protein blend became softer with the increase of water content. During the extrusion process, the formation of more air pores inside the melt extruded structure can improve the water holding ability of TISP products. Water played an important role in deciding the denaturation temperature. Protein molecules will aggregate together by hydrophobic interactions under higher moisture content. The intermolecular correlation between disulfide-bonds will take a dominant place with the decrease of moisture content. This finding was similar with other researches[19-21].

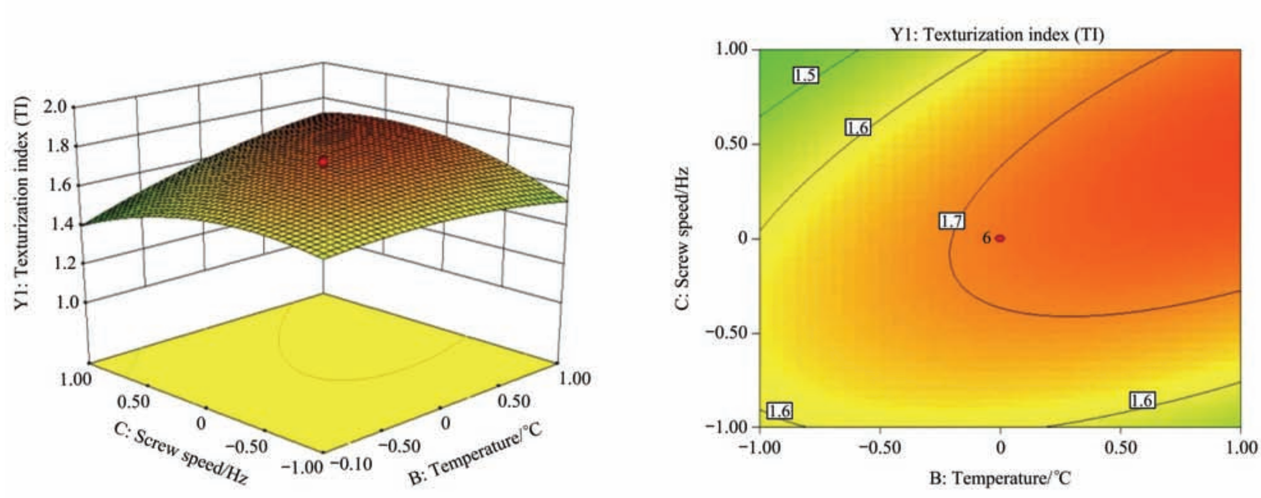
Higher WHC values could be only obtained under higher extrusion temperature with proper gluten content rather than higher temperature with lower gluten content, or lower temperature with lower gluten content. Three-dimensional network structure could be formed after a certain degree of protein denaturation, however, the water holding capacity decreased when protein was fully denatured. Therefore, the degree of denaturation increased with the increased temperature, which could improve the water holding ability. The highest WHC value was obtained under the extrusion temperature of 170°C and gluten content of 20%, which was similar with the research of Altan et al.[15]

3.4 Response surface analysis of hardness

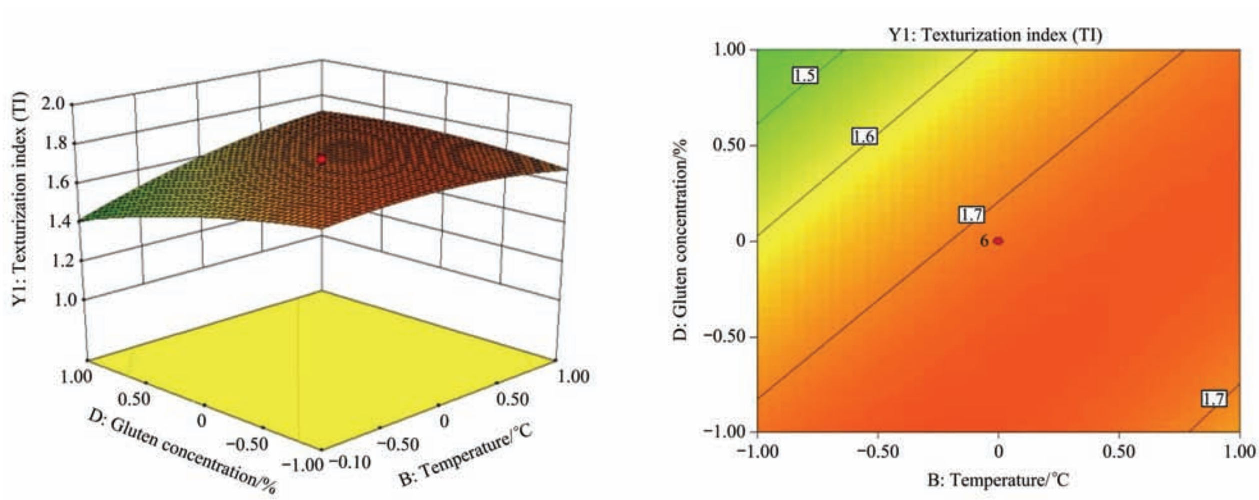
Figure 3 showed that the hardness of the soybean-gluten protein extrudate was significantly affected by linear terms of moisture content rather than temperature, feed speed and gluten content. The quadratic model for hardness was listed in Table 3. The negative coefficients indicated that the hardness decrease with the increase of moisture content, which is the important basis for demarcating extrusion[15]. During extrusion process, water performs several essential functions, such as lubricant, plasticizer and reactant[23]. The hydrophobic interactions, hydrogen bonds, disulfide bonds and their interactions were reported collectively hold the structure of extrudate[11]. Increasing moisture content could increase the interaction between disulfide bonds and hydrogen bonds and between disulfide bonds and hydrophobic interactions.



a. Effects of feed moisture content and screw speed on texturization index of extruded sample

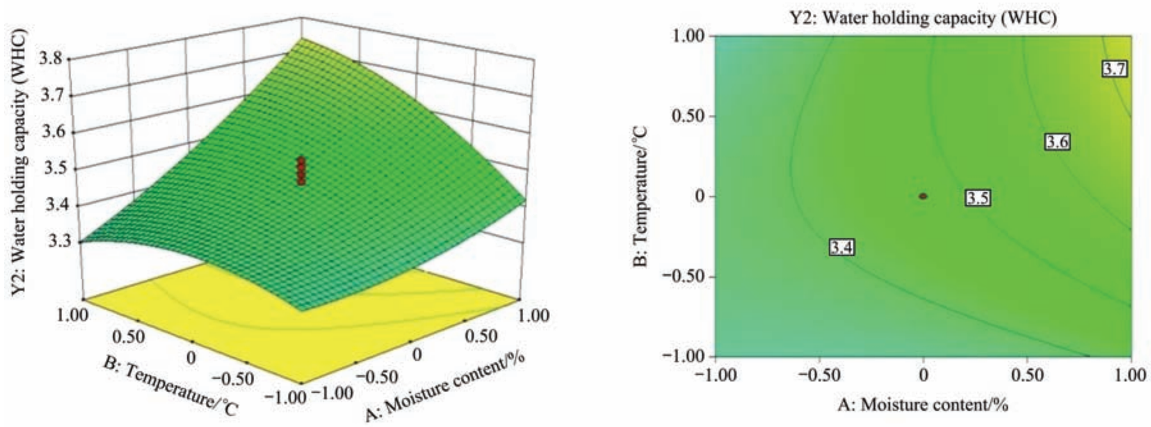


b. Effects of extrusion temperature and screw speed on texturization index of extruded sample

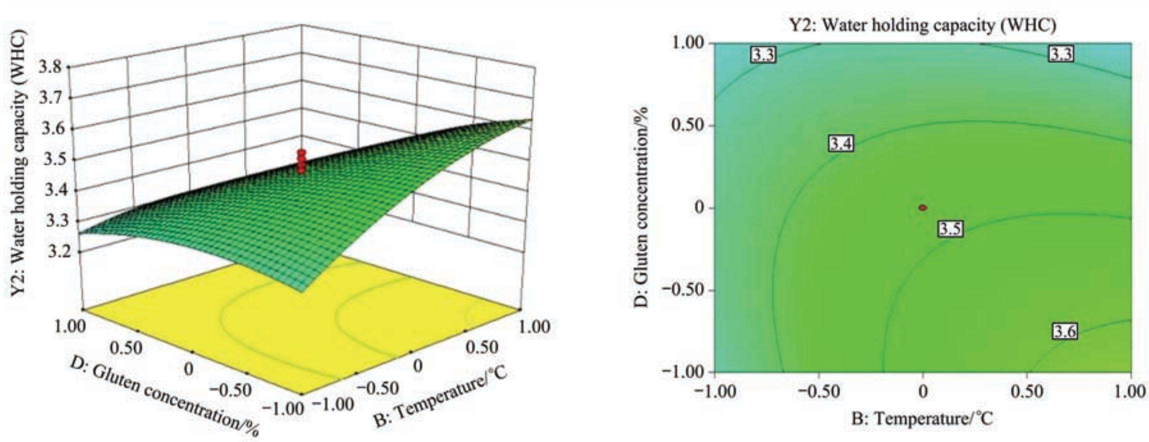


c. Effects of extrusion temperature and gluten content on texturization index of extruded sample

Figure 1 Effects of extrusion temperature, feed moisture content, screw speed and gluten content on the texturization index of extruded sample

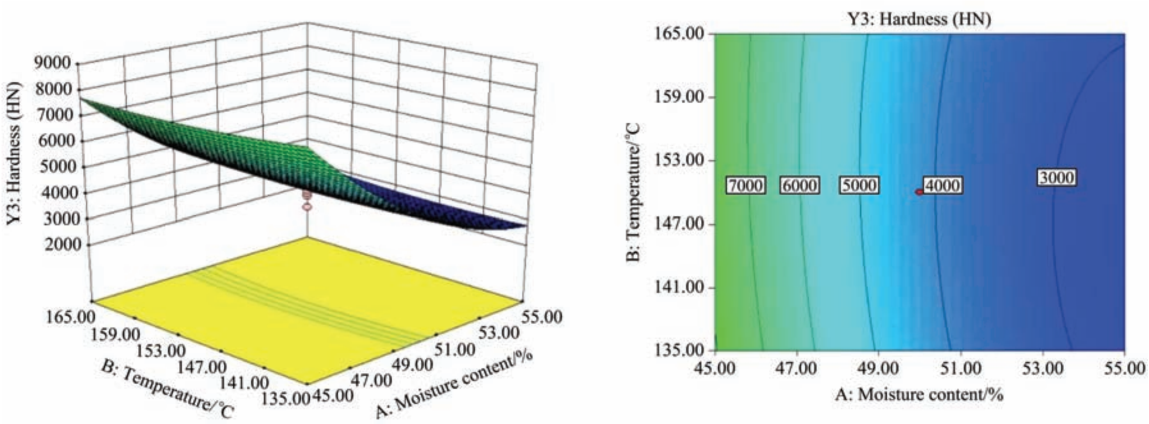


a. Effects of extrusion temperature and feed moisture content on water holding capacity of extruded sample

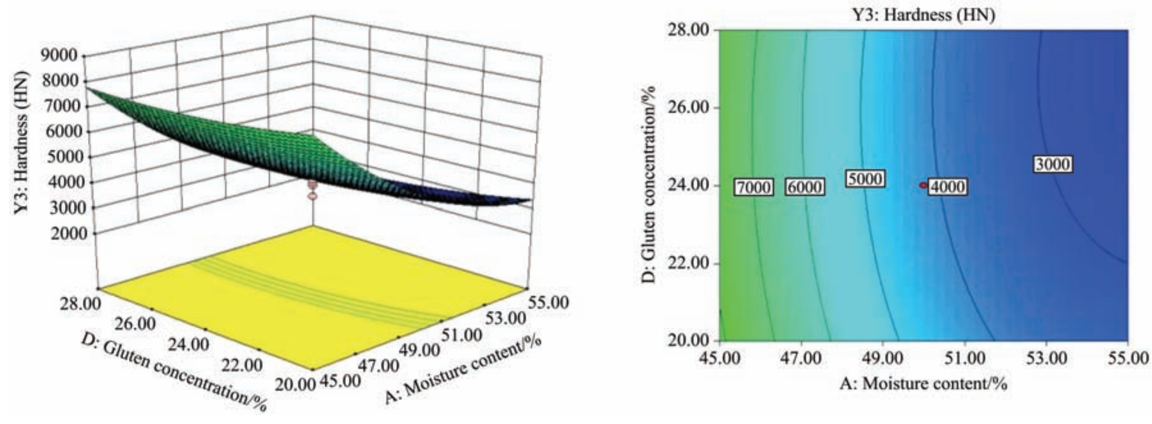


b. Effects of extrusion temperature and gluten concentration on water holding capacity of extruded sample

Figure 2 Effects of extrusion temperature, feed moisture content, screw speed and gluten content on the water holding capacity of extruded sample



a. Effects of feed moisture content and extrusion temperature on hardness of extruded sample



b. Effects of feed moisture content and gluten concentration on hardness of extruded sample

Figure 3 Effects of extrusion temperature, feed moisture content, screw speed and gluten content on the hardness of extruded sample

3.5 Optimization of extrusion parameters

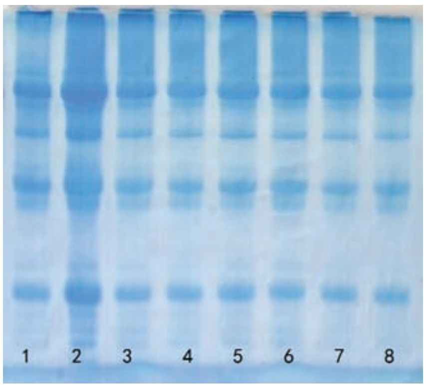
According to the above results, extruded products with higher texturization index and water holding capacity with medium hardness played a key role in the application of products. In this study, the optimum response values of texturization index, water holding capacity, and hardness of extruded protein products were identified as 1.6, 3.5, and 4000-6000 g, respectively. The importance of texturization index, water holding capacity, and hardness were also set into different degrees. The solution of the optimum extrusion conditions were listed in Table 5. The results showed that the processing parameters of six samples could regard as the optimum conditions with feed moisture content (49.18%-50.15%), screw speed (16.41-16.73 Hz), extrusion temperature (155.24°C-159.86°C), and gluten content (20.00%-20.03%).

Table 5 Solution of the optimum extrusion conditions

Number	Moisture content/%	Extrusion temperature/°C	Screw speed/Hz	Gluten content/%	Texturization index	Water holding capacity	Hardness/g
1	49.18	159.84	16.73	20.00	1.71271	3.60048	5125.21
2	49.18	159.73	16.72	20.00	1.71312	3.59984	5124.12
3	49.19	159.86	16.73	20.03	1.71317	3.60025	5117.96
4	49.19	159.27	16.72	20.00	1.71472	3.59731	5118.95
5	49.66	157.00	16.41	20.00	1.72493	3.59561	4860.03
6	50.15	155.24	16.44	20.00	1.73000	3.60318	4626.00
Validation	49.00	160.00	17.00	20.00	1.74000	3.53000	5208.00

3.6 SDS-PAGE

The electrophoresis of extruded samples collected from the extruder under different processing parameters was shown in Figure 4. The extrusion parameters of feed moisture content, extrusion temperature, and gluten content of control group were 50%, 150°C and 20%, respectively. The Mw distribution profiles obtained from SDS-PAGE under reduced conditions for extruded samples with different parameters were in lane 3-8. The soybean protein-gluten blend without extrusion had the band with Mw of 10-95 kD, while after the extrusion process, the molecular weight were from 17-95 kD. This showed that only small part of small molecular weight protein compound in the samples formed to large one. By analyzing the SDS-PAGE distribution of samples in different moisture content (40% and 60%), SDS-PAGE of sample with moisture content of 40% showed clearly band between 10 kD and 17 kD, which became increasing unclear or almost disappeared in sample 60%. Its disappearance might be due to the insolubilisation. This means that increasing moisture content could denature some protein forming a large molecular weight protein that could not be detected on the gels, which is similar with the result of Osen' research[24]. The SDS-PAGE images of the extruded samples showed some changes in the molecular weight distribution compared to the raw protein materials. The protein molecules of extrudates showed lower intensities on the gels. This is due to that protein denaturation occurred and some large molecular weight proteins formed during extrusion process[24,25].

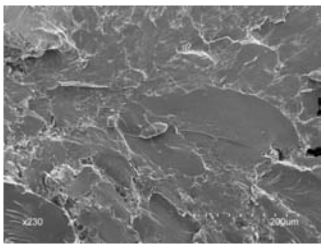


1. Control group 2. Original material 3. Feed moisture content 40% 4. Feed moisture content 60% 5. Extrusion temperature 130°C 6. Extrusion temperature 170°C 7. Gluten content 10% 8. Gluten content 30%

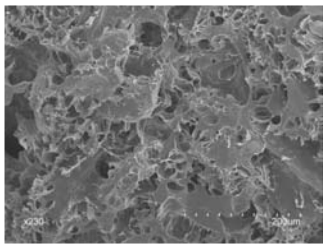
Figure 4 SDS-PAGE images of TISP at different extrusion conditions

3.7 Microstructure analysis

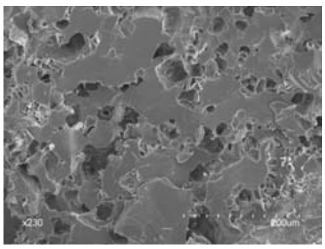
The effects of different extrusion parameters on the structure protein blends could be seen through scanning electron microscopy (SEM) morphology. SEM images of extruded products on SEM (230×) micrographs with different feed moisture content, moisture content, extrusion temperature, extrusion temperature and gluten content were showed in Figure 5. After melted in the die before extrusion process, the TISP products showed uniform texture with compact internal structure, without air cavity and organized structure (Figure 5a). The vaporization happened to TISP structure during the extrusion process with the increase of moisture content, forming honeycomb inner structure. Thus this complicated protein network structure showed a soft and flexible texture with lots of air pores existing in the inner TISP products (Figure 5b).



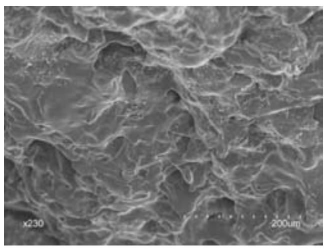
a. Moisture content 40%



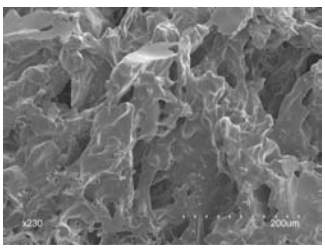
b. Moisture content 60%



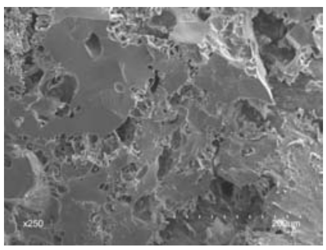
c. Extrusion temperature 130°C



d. Extrusion temperature 170°C



e. gluten content 10%



f. gluten content 30%

Figure 5 Scanning electron microscopy images of TISP at different feed moisture content, extrusion temperature and gluten content

Some small particles were observed in the intersecting surface of TISP product, which means that there were still some untreated proteins that were not fully vaporized (Figure 5c). When the temperature increased up to 170°C, no obvious air cavity structure emerged in the TISP products. These results showed that the higher temperature did not benefit the forming of organized structure[21,22]. Figures 5e and 5f showed the microstructure of TISP extruded under gluten content of 10% and 30%, respectively. The structure of final products showed changes from loosen texture to excellent random network structure with uniform air cavity and compact texture with the increase of gluten content from 10% to 30%. The volume of air cavity was reduced apparently while the numbers of which increased greatly.

4 Conclusions

In this study, the optimization of the extruded products was investigated using the central composite design. The feed moisture content, extrusion temperature, screw speed and gluten content were selected as the influencing factors; the texturized degree, water absorption, and hardness were selected as the response value. The extrusion process was optimized by step-wise nonlinear regression analysis and RSM. The optimized parameters of feed moisture content, extrusion temperature, screw speed and gluten content were established. The microstructures of TISP products were also analyzed by SEM; the changes of protein molecules before and after extrusion were analyzed by electrophoresis (SDS-PAGE).

Acknowledgement

This research project was financially supported by the National Natural Science Foundation of China (31301593), Chinese Universities Scientific Fund (2017GX001) and Research and Development Fund for University's Doctoral Discipline of China (20130008120021).

[References]

[1] Osen R, Toelstede S, Wild F, Eisner P, Schweiggert-Weisz U. High moisture extrusion cooking of pea protein isolates: Raw material characteristics, extruder responses, and texture properties. Journal of Food Engineering, 2014; 127: 67–74.

[2] Asgar M A, Fazilah A, Huda N, Bhat R, Karim A A. Nonmeat protein alternatives as meat extenders and meat analogs. Comprehensive Reviews in Food Science and Food Safety, 2010; 9(5): 513–529.

[3] Shih M C, Hwang T S, Chou H Y. Physicochemical and functional property changes in soy protein isolates stored under high relative humidity and temperature. Journal of Food Science and Technology, 2016; 53(1): 902–908.

[4] Sun J, Zhou W, Yan L, Huang D, Lin L. Extrusion-based food printing for digitalized food design and nutrition control. Journal of Food Engineering, 2018; 220: 1–11.

[5] Chen F L, Wei M, Zhang B, Ojokoh A O. System parameters and product properties response of soybean protein extruded at wide moisture range. Journal of Food Engineering, 2010; 96(2): 208–213.

[6] Guerrero P, Beatty E, Kerry J P, de la Caba K. Extrusion of soy protein with gelatin and sugars at low moisture content. Journal of Food Engineering, 2012;110(1): 53–59.

[7] Martins V B, Netto F M. Physicochemical and functional properties of soy protein isolate as a function of water activity and storage. Food Research International, 2006; 39(2): 145–153.

[8] Riaz M N. Textured soy protein and its uses. Agro Food Industry Hi Tech, 2001; 12(5): 28–31.

[9] Cheftel J C, Kitagawa M, Quéguiner C. New protein texturization processes by extrusion cooking at high moisture levels. Food Reviews International, 1992; 8(2): 235–275.

[10] Guo Q, Joseph M, Setia R, Vikhona H, Sharma K, Alavi S. Extruded corn soy blends: physicochemical and molecular characterization. Journal of Cereal Science, 2018; 79: 486–493.

[11] Chen F L, Wei Y M, Zhang B. Chemical cross-linking and molecular aggregation of soybean protein during extrusion cooking at low and high moisture content. LWT - Food Science and Technology, 2011; 44(4): 957–962.

[12] Wu M, Liu Y, Wang L J, Li D, Mao Z H. Effects of extrusion parameters on physicochemical properties of flaxseed snack and process optimization. Int J Agric Biol Eng, 2015; 8(5): 121–131.

[13] Fang Y, Zhang B, Wei Y. Effects of the specific mechanical energy on the physicochemical properties of texturized soy protein during high-moisture extrusion cooking. Journal of Food Engineering, 2014; 121: 32–38.

[14] Lei H, Ruan R, Fulcher R G, Lengerich B V. Color development in an extrusion-cooked model system. Int J Agric Biol Eng, 2008; 1(2): 55-63.

[15] Altan A, McCarthy K L, Maskan M. Evaluation of snack foods from barley–tomato pomace blends by extrusion processing. Journal of Food Engineering, 2008; 84(2): 231–242.

[16] Gui Y, Gil S K, Ryu G H. Effects of extrusion conditions on the physicochemical properties of extruded red ginseng. Preventive Nutrition and Food Science, 2012; 17(3): 203–209.

[17] Guo X, Sun X, Zhang Y, Wang R, Yan X. Interactions between soy protein hydrolyzates and wheat proteins in noodle making dough. Food Chemistry, 2018; 245: 500–507.

[18] Li S Q, Wu M, Zhao D L, Liu Y, Sun Y, Li D. Rheological properties of flaxseed meal and soybean protein isolate blend by extrusion. Int J Agric Biol Eng, 2017; 10(4): 224–233.

[19] Akdogan H. Pressure, torque, and energy responses of a twin screw extruder at high moisture contents. Food Research International, 1996; 29(56): 423–429.

[20] Sheard P R, Fellows A, Ledward D A, Mitchell J R. Macromolecular changes associated with the heat treatment of soy isolates. Journal of Food Technology, 1986; 21: 55–60.

[21] Wu M, Li D, Wang L J, Özkan N, Mao Z H. Rheological properties of extruded dispersions of flaxseed-maize blend. Journal of Food Engineering, 2010; 98(4): 480–491.

[22] Stojceska V, Ainsworth P, Plunkett A, İbanoğlu Ş. The advantage of using extrusion processing for increasing dietary fiber level in gluten-free products. Food Chemistry, 2010; 121(1): 156–164.

[23] Silva E M M D, Ascheri J L R, Ascheri D P R. Quality assessment of gluten-free pasta prepared with a brown rice and corn meal blend via thermoplastic extrusion. LWT - Food Science and Technology, 2016; 68: 698–706.

[24] Osen R, Toelstede S, Eisner P, Schweiggert-Weisz U. Effect of high moisture extrusion cooking on protein-protein interactions of pea (*Pisum sativum* L.) protein isolates. International Journal of Food Science & Technology, 2015; 50(6): 1390–1396.

[25] Cabrera-Chávez F, Calderón de la Barca A M, Islas-Rubio A R, Marti A, Marengo M, Pagani M A, et al. Molecular rearrangements in extrusion processes for the production of amaranth-enriched, gluten-free rice pasta. LWT - Food Science and Technology, 2012; 47(2): 421–426.