DOI : [http://doi.org/10.22438/jeb/40/3\(SI\)/Sp-01](http://doi.org/10.22438/jeb/40/3(SI)/Sp-01)

Effect of extrusion variables on the extrudate characteristics of *fish muscle-soy* protein blend and optimization of these variables

Paper received: 17. 04. 2018

Revised received: 25. 10.2018

Accepted: 03.01. 2019

Authors Info

G. Zhao^{1,2,4}, W. Bi^{1,2,3}, G. Zhang⁴,
C. Yang⁴, Y. Shi⁴, H. Liu², J. Li²,
C. Zhao⁵ and X. Sun⁶

¹Grain & Corn Engineering Technology Research Center, China State Administration of Grain

²Soybean Research Institute, Heilongjiang Academy of Agricultural Sciences, Harbin-150 086, People's Republic of China

³Heilongjiang Academy of Agricultural Sciences Post-Doctoral Station, Nangang District, Harbin-150 086, People's Republic of China

⁴College of Food Science and Engineering, Harbin University of Commerce, Harbin-150 076, People's Republic of China

⁵Changchun Dacheng Group, Shuangcheng District, Changchun-130 062, People's Republic of China

⁶Quality & Safety Institute of Agricultural Products, Heilongjiang Academy of Agricultural Sciences, Harbin-150 086, People's Republic of China

*Corresponding Author Email : 13603681425@163.com

Edited by

Prof. Muhammad Aqeel Ashraf

Reviewed by

Dr. Y. Wu
Dr. Z. Yang

Abstract

Aim: A single-factor experiment was conducted to study the influence of process variables on the degree of texturization (DTE) by a twin-screw extruder.

Methodology: By using response surface methodology (RSM), the feed rate, feed moisture, screw speed and extrusion temperature were optimized at 35r min⁻¹, 30%, 175r min⁻¹, and 145 °C, respectively. The observations on tensile force and final protein content (after immersion in water) of the extrudates were studied.

Results: Under the optimum conditions, the characteristics of the extrudate including the degree of texturization, tensile force, protein content, fat content, and in vitro digestibility of the extrudate were at 2.12, 3224.6g, 67.9%, 1.56%, and 87.4%, respectively. The texturized extrudate shows a great prospect by providing value-added high protein product.

Interpretation: The data of this study suggest that fish and soybean protein concentrate extruded by twin screw extruder would be of great interest to the researchers.

Key words: Degree of texturization, Extrusion parameter, Fish muscle-soy protein, Twin-screw extruder

Citation: Zhao, G., W. Bi, G. Zhang, C. Yang, Y. Shi, H. Liu, J. Li, C. Zhao and X. Sun: Effect of extrusion variables on the extrudate characteristics of *fish muscle-soy* protein blend and the optimization of these variables. *J. Environ. Biol.*, **40**, 409-417 (2019).

Introduction

Soybean meal with essential amino acids is now widely used as a replacement of fish. Fish are not only excellent source of high nutritional value protein but also good sources of lipid. However, due to its extreme perish ability and non-availability of technology for its proper utilization, a significant portion of fish by products are not utilized as human food (Dun, 2008). Rather, minced boneless fish meat can be utilized from these sources for production of various value-added fishery products (Grantham, 1981; Wahab and Adzmi, 2017; Sahbi *et al.*, 2018). Often minced fish lacks good textural properties which can be improved by processing through food extruders (Choudhury *et al.*, 1995; Halim *et al.*, 2017; Xiao and Liu, 2018). Earlier thermal processing was suggested to improve the texture, palatability and inactivation of heat labile toxic compounds and enzyme inhibitors in several food commodities, including pulses (Sreerama *et al.*, 2008; Basheer *et al.*, 2017; Bumidin *et al.*, 2018). Therefore, manipulation in a cost-effective manner of processing conditions may be required to remove or reduce high calorie components.

Recently, extrusion is becoming popular as a processing technique because it can improve product quality and acceptability. Extrusion is a high-temperature-short-time (HTST) physical treatment during which flour or starch is subjected to high temperature and mechanical stirring at relatively low levels of moisture content (Camire *et al.*, 1990; Khan *et al.*, 2017; Noor *et al.*, 2018). Protein concentration, moisture content, physical and mechanical parameters of an extruder significantly affects the physical and sensory qualities of extrudate (Day *et al.*, 2013; Rahmat *et al.*, 2017). Extrusion promotes cross-linking and polymerization among molecules of proteins and starches to form expanded matrices due to shear, heat pressure and oxygen, thus altering the protein structure, solubility and digestibility (Li *et al.*, 1997; Sa'at and Zama 2017). Functional properties like expansion index, water absorption and solubility indices of the product determines the final texture and sensory properties of products (Oikonomou *et al.*, 2011; Tariq *et al.*, 2017; Hassan, 2018).

These functional properties are affected by extrusion conditions such as feed moisture content, barrel temperature, screw speed, particle size, etc. The particle size of flour and moisture content are important factors influencing the properties of extruded foods (Mian *et al.*, 2007; Ahmad *et al.*, 2017). Effects of extrusion and processing conditions on corn grit have been studied. However, studies on extrusion of soybean protein and fish muscle mixture are still limited (Shevkani *et al.*, 2014; Singh *et al.*, 1998; Atikuzzaman *et al.*, 2018). China is rich in soybean and freshwater fish resources, it is necessary to expand the utilization of these two protein resources through development of both nutritious and readily available products. Based on the above, the objective of this study was to develop a texturized high protein product, utilizing the above two kinds of superior protein rich

resources through screw extrusion technology and also to optimize the extrusion parameters through RSM.

Materials and Methods

Materials: Fresh water fish mirror carp (*Cyprinus carpio* var. specularis) was selected for the study due to its wide distribution. Fresh fish purchased from a local market was prepared by removing the head, fins, tail and viscera. Fish mince was prepared using a meat grinder, pickled in mixture (4.5% perilla leaf powder, 5% vinegar, 1% cooking wine) for 3hrs at 25°C cooked in broth (contain pepper and salt) for 25 min at 125°C fried for 5min at 170°C and then stored in a refrigerator until further use. A food sauce containing vinegar, salt, cooking wine, etc., was purchased from a local supermarket. Soybean protein isolate (SPI, protein 84.32%, ash content 5.88%) was purchased from Harbin high-tech Soy Foods Co., LTD (Harbin, China). The other chemicals (sodium hydroxide, potassium sulfate, acetone, methyl-red dye) were all procured from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China).

Extrusion process: The meshing twin-screw extruder (Brabender DSE-25, Germany Brabender Instrument Company, Germany) was used in this study with 25 mm. outer diameter screw rod (D) The length ratio was 20:1. Length of the extruder was as follows: 4D in feeding area, 6D in I area, 6D in zone II, 4D in zone III, 2D in zone IV, and 6.5D in V area, respectively. The geometrical dimension of the extruded cavity of the square long-distance dies: 200 mm 20 mm 2 mm (L W H, the spacing of the two pressure sensors is 100 mm). The five sections of extruder were equipped with heating, and cooling monitoring system was controlled separately. The pressure monitoring system was installed in area. The screw and feed speed had variable speeds. Controlling the temperature of the fourth region independently had a great influence on the extruded products and it was easy to control variables in the fourth region. The variables were barrel temperature (fourth region), feeding moisture content, feeding rate and screw speed. In a single-factor experiment, in order to study the effect of feeding moisture on DTE, only feeding moisture was set as variable, invariant factors were as follows: barrel temperature 135 °C feeding rate 35 r min⁻¹, screw speed 175 r min⁻¹. In order to study the effects of barrel temperature, feeding rate, and screw speed on DTE, only the above one parameter was set as variable, the other invariant factors were set as follows: barrel temperature 145°C, feeding moisture 30%, feeding rate 35 r min⁻¹, and screw speed 175 r min⁻¹, respectively.

The fish muscle and soybean protein were mixed thoroughly to make a homogenate in 1:2 ratio. The moisture content of the mixture was adjusted to desired levels by adding water and then mixing. Care was taken to avoid lumping. Each lot was packed in sealed polyethylene pouches and allowed to equilibrate overnight in a refrigerator. Before extrusion, the wet feed mixture was brought to ambient temperature and mixed

again. The wet feed mixture was fed manually through hopper. Care was taken to ensure that the manual feeding was controlled to the point of overflow at the feed opening. Under such condition a steady output state was formed, and the only samples obtained under this condition was used for analysis.

Optimization of twin-screw extrusion process by Box-Behnken center combination design: Response surface methodology is a great tool to study the interaction between two or more factors. The factors and levels of response surface design used in this experiment is shown in Table 1.

Table 1 : Response surface experiment factors and level design

Level	X ₁ Feed speed r min ⁻¹	X ₂ Barrel temperature!	X ₃ Screw ¹ speed r min ⁻¹	X ₄ Feed moisture%
-1	30	135	170	28
0	35	145	175	30
1	40	155	180	32

A three-level central composite design was used to analyze the data. The factorial boundaries were set at -1 and +1, the mid-point at 0. Experiments consisting of 29 treatments were conducted to optimize the variables of extrusion process - barrel temperature, feed rate, screw speed and feed moisture to obtain the optimum DTE. The feed rate, barrel temperature, screw speed and feed moisture were delegated by X₁, X₂, X₃, X₄, respectively, Y was delegated by extrusion in DTE.

Determination of DTE of extrudates: Textural profile analysis of extrudate was determined using an Instron Universal Testing Machine (Instron Model 4301, Instron Corp., Canton, MA, USA). The textural parameters were calculated following the method of Mounsey (Mounsey *et al.*, 2001;). Sampling sketch for DTE test is shown in Fig.1. Three samples were analyzed for each batch of extrudates. Determination parameters were set as: pretest speed 2.0 mm s⁻¹, test speed 1.0 mm s⁻¹, post speed 2.0 mm s⁻¹, distance

from the sample 10.0 mm, and application of A/CKB probe. The DTE of the extrudates is expressed in the ratio of transverse and longitudinal shear force. Shear force refers to the size of the force required to cut the sample with a knife. The force size reflects the internal texture orientation of the sample in different directions.

$$DTE = F_L / F_V \text{ (equation)}$$

Where, F_L is the shear force of parallel die extrusion direction; F_V is the shear force of perpendicular to the extrusion direction.

Determination of tensile strength: Textural profile analysis of extrudates as well as textural parameters calculation have been described as above. Sampling sketch for tensile strength test is shown in Fig.2. Three samples were analyzed for each batch of the extrudates. The determination parameters were set as: pretest speed 1.0 mm s⁻¹, test speed 0.5 mm s⁻¹, post speed 1.0 mm s⁻¹, maximum tensile range 15.0 mm and A/TG probe. The tensile strength of the extrudate were expressed as maximum stress.

Determination of *in vitro* digestibility of extrudates: Samples of extrudate were analyzed for protein and fat content (National Standards Authority of China, 2003; Aldaihani *et al.*, 2017). Two grams of each sample after fat-determination were added to the conical plastic tubes containing 150 ml of pre-warmed 0.2% pepsin HCl solution (2g pepsin solubilized in 1l 0.075 mol l⁻¹ HCl) and then incubated with shaking at 100 rpm at 45 °C for 16 hr. After filtration, undigested residue on the filter paper was washed with acetone, the residues together with the filter papers were inserted into the digestive tubes, and National Standard 5009.6-2003 GBT was followed to determine crude protein after digestion. The following formula was applied to calculate the protein digestibility:

$$\text{Protein digestibility} = (A-B) / A \times 100 \%$$

Where, A is the total content of crude protein in the sample; B: crude protein content in the sample after digestion.

Statistical analyses: Three independent assays were carried out for all the experiments. One-way analysis of variance was used to study the difference between means, significant at $P < 0.05$. Data analysis was carried out using SPSS (Windows version 12.0, SPSS Inc., Chicago, IL, USA). All data are presented as mean $\pm$ SE.

Results and Discussion

The influence of feed moisture on DTE of extruded products was analyzed, and the observed variation in DTE of the extruded products obtained at feed moisture of 26%, 28%, 30%, 32%, 34% is shown in Fig. 3. It was also observed that when feed moisture of the products increased from 26% to 28%, the DTE of the extruded products also increased significantly by 8.45%.

Fig. 1 : Sampling sketch for degree of texturization test.

Fig. 2 : Sampling sketch for tensile strength test.

Fig. 3 : Sampling sketch for degree of texturization test.

Fig. 4 : Sampling sketch for degree of texturization test.

When feed moisture of the products were increased from 28% to 30%, the DTE of the extruded products increased significantly by 11.68%. Compared with 30% feed moisture of the products, the DTE of the extruded products made from feed moisture of 32% and 34% decreased by 8.14% and 16.27%, respectively. Therefore, in the response surface test, feed moisture of 28%, 30% and 32% were selected as variables.

The influence of barrel temperature on DTE of the product analyzed the variation in DTE of the extruded products obtained at barrel temperature of 115°C, 125°C, 135°C, 145°C, 155°C is shown in Fig 4. With the increasing temperature of the barrel, the DTE of the products increased, however, there were no significant differences ($P > 0.05$) for the degree of texturization among barrel temperature of 115°C, 125°C, 135°C. No significant differences ($P > 0.05$) were found for the degree of texturization among barrel temperature of 135°C, 145°C, 155°C. However, significant difference ($P < 0.05$) was observed for the degree of texturization between barrel temperature group of 145°C and 155°C, and group of 115°C and 125°C. Significantly higher degree of texturization was obtained from higher barrel temperature of 145°C and 155°C. Consequently, in the response surface test, barrel temperature of 135°C, 145°C, 155°C were

selected as variables. These results are in-line with literature data concerning the extrusion of whey proteins using different process set-up (Queguiner *et al.*, 1992).

The influence of feed rate on DTE of the extruded products was analyzed, and the results are shown in Fig. 5. Variation in DTE of the extruded products obtained at feed rates of 30 rpm, 35 rpm, 40 rpm, 45 rpm and 50 rpm are shown in Fig. 5.

When the feed rate was increased from 30 rpm to 35 rpm, the DTE of the extruded products increased significantly by 13.89%. Compared with the feed rate of 35 rpm, the DTE of the extruded products obtained from the feed rates of 40 rpm, 45 rpm, and 50 rpm decreased by 4.88%, 17.07% and 19.51%, respectively. Therefore, in the response surface test, the feed rates of 30 rpm, 35 rpm and 40 rpm were selected as variables.

The influence of screw speed on DTE of the extruded products was analyzed, and variation in the DTE of the extruded products obtained from screw speed of 170 rpm, 175 rpm, 180 rpm, 185 rpm and 190 rpm are shown in Fig. 6. When the screw speed was increased from 170 rpm to 175 rpm, the DTE of the extruded products increased significantly by 6.6%. When feed moisture of the products increased from 175 rpm to 180 rpm, the

Fig. 5 : Degree of texturization of extrusion products at different feed rate.

Fig. 6 : Degree of texturization of the finished product under different screw speed.

Table 2 : Response surface data of the process parameters of degree of texturization

No.	A Feed rate	B Barrel temperature	C Screw speed	D Feed moisture	Degree of texturization
1	0	-1	-1	0	1.71
2	1	-1	0	0	1.66
3	-1	-1	0	0	1.69
4	1	0	0	-1	1.88
5	0	0	1	1	1.90
6	1	0	0	1	1.81
7	0	0	1	-1	1.91
8	1	1	0	0	1.73
9	0	0	-1	1	1.81
10	0	1	0	-1	1.85
11	0	-1	0	1	1.74
12	0	-1	1	0	1.74
13	0	0	0	0	2.10
14	0	1	1	0	1.78
15	0	1	0	1	1.70
16	0	0	0	0	2.10
17	-1	0	0	1	1.79
18	0	-1	0	-1	1.74
19	-1	0	1	0	1.84
20	0	0	0	0	2.08
21	0	1	-1	0	1.74
22	-1	0	-1	0	1.80
23	0	0	0	0	2.11
24	1	0	-1	0	1.81
25	-1	1	0	0	1.69
26	0	0	-1	-1	1.94
27	1	0	1	0	1.85
28	0	0	0	0	2.10
29	-1	0	0	-1	1.88

DTE of the extruded products increased significantly by 2.38%. Compared with the screw speed of 180 rpm, the DTE of the extruded products obtained from the screw speed of 185 rpm and 190 rpm decreased by 13.95% and 20.93%, respectively. Therefore, in the response surface test, the screw speed of 170

rpm, 175 rpm and 180 rpm were selected as variables. This finding regarding residence time also correspond with the previous studies (Altomare and Ghossi, 1986; Kumar *et al.*, 2010; Ruyck, 1997). The effect of screw speed on the degree of denaturation can possibly be associated with the resulting

Table 3: Regression model analysis of variance

Source of variance	Sum of squares	Freedom	Mean square	F-value	P-value
Model	0.53	14	0.038	889.12	<0.0001
A-Feed rate	2.083E-004	1	2.083E-004	4.89	0.0442
B-Barrel temperature	3.675E-003	1	3.675E-003	86.23	<0.0001
C-Screw speed	3.675E-003	1	3.675E-003	86.23	<0.0001
D-Feed moisture	0.017	1	0.017	395.95	<0.0001
AB	1.225E-003	1	1.225E-003	28.74	0.0001
AC	0.000	1	0.000	0.000	1.0000
AD	1.000E-004	1	1.000E-004	2.53	0.1479
BC	2.500E-005	1	2.500E-005	0.59	0.4565
BD	5.625E-003	1	5.625E-003	131.98	<0.0001
CD	3.600E-003	1	3.600E-003	84.47	<0.0001
A ²	0.17	1	0.17	3969.64	<0.0001
B ²	0.39	1	0.39	9061.19	<0.0001
C ²	0.081	1	0.081	1892.15	<0.0001
D ²	0.060	1	0.060	1417.30	<0.0001
residual	5.967E-004	14	4.262E-005		
Loss of fitting	1.167E-004	10	1.167E-005	0.097	0.9986
Pure error	4.800E-004	4	1.200E-004		
Total error	0.53	28			

[total $r = 0.9989$ r^2 Correction value = 0.9978 r^2 predicted value = 0.9973]

Note: $P < 0.05$ indicates significant difference; $P < 0.01$ indicates very significant difference, $P < 0.0001$ indicates extremely significant difference

Fig. 7 : Effect of interaction between A and B on the degree of texturization (a,b).

product temperature.

Based on the response surface software Design-Expert 7, the design scheme and results of DTE are shown in Table 2. The overall response values, under different conditions, are presented in Table 3. To optimize the process parameters, a response surface model for the overall response values of DTE was developed employing multiple regression techniques and presented as equation 2: $Y = -204.58567 + 0.38728A + 0.74460B +$

$1.46725C + 1.15813D + 3.50000E-004A*B + 0.000000A*C + 5.00000*10^{-4}A*D + 5.00000*10^{-5}B*C - 1.87500*10^{-3}B*D + 3.00000*10^{-3}C*D - 6.46000*10^{-3}A^2 - 2.44000*10^{-3}B^2 - 4.46000*10^{-3}C^2 - 0.024125D^2$ (equation 2). Where, A is the feed rate; B is the barrel temperature; C is the screw speed and D is the feed moisture.

Through ANOVA, it can be seen that the model satisfactorily explained the effect of process parameters on

Fig. 8 : Effect of interaction between B and D on the degree of texturization (a, b).

Fig. 9 : Degree of texturization of extrusion products at different feed rate.

overall response with no significant lacking fit, and R^2 reached 0.9978, indicating that 99.78% of the variability could be explained by this model. Factor contribution rates obtained from F -test were in the following order: feed moisture>barrel temperature = screw speed>feed rate. The interaction terms BD, AB, and CD had a significant impact on DTE, while interaction between AC, AD and BC had no significant effect on DTE. The model of coefficient of variation (CV) was 0.35%, with good repeatability; the multiple correlation coefficient of 0.9989, and small model test error, so the model was appropriate for analyzing and predicting the effect of feed rate, temperature, screw speed and feed moisture content on DTE. The effects of interaction terms BD, AB and CD on DTE were also analyzed in depth. The

impact of interaction between feed rate and temperature on DTE of the products, as seen in Fig. 7, was significant.

With the increasing feed rate and temperature of four areas, initially DTE increased and then decreased, indicating that adequate increase of feed rate and temperature can improve DTE, when DTE reached the maximum, continuous increase of the feed rate and temperature led to a decrease in DTE. On increasing the feed rate, while keeping the screw speed constant, the raw material in the barrel accumulate, resulting in an increase in friction and shear force between the raw material and extruder wall, hence increasing the barrel temperature, the retention time of the raw material in the barrel will prolong, consequently, the

DTE of the extruded products will increase. However, if the feed rate is too high, the raw material inside the barrel will accumulate to a certain degree, resulting in squeezed raw material in the barrel, thereby decreasing DTE of the final product.

As seen in Fig. 8, the effect of interaction between the two factors was significant. With increasing temperature and feed moisture in the four areas, the DTE firstly increased and then decreased. This indicated that adequate increase of feed moisture and temperature could improve DTE. However, when DTE reached maximum, continuous increase of feed moisture and temperature decreased DTE. The raw material viscosity increased with increasing feed moisture, and this also resulted in increased friction between the raw material and the wall of extruding machine. Since retention time of the raw materials in the barrel was extended, the DTE of the extruded products increased.

The effect of interaction on DTE between the two factors was significant. With the increasing screw speed and feed moisture content in the four areas, initially DTE increased and then decreased, indicating that adequate increase of feed moisture content and screw speed may improve DTE. When DTE reached maximum, continuous increasing of feed moisture content and screw speed led decreased in DTE. The viscosity of raw material increased with increasing feed moisture, while this also resulted in increased friction between the raw material and the wall of extruder, the retention time of the raw material in the barrel extended. It was observed that high speed, the retention time of raw material in the barrel reduced the DTE of the extruded products. The experimental and predicted values of the extrudate obtained under these conditions are presented in Table 4. An optimal value of 2.11 was obtained through analysis on regression model using response surface methodology under following conditions: feed moisture 30%, barrel temperature 145°C, feed rate 35 r min⁻¹, screw speed 175 r min⁻¹. Therefore, the above values of the parameter selected as the optimum ones for the extrusion of fish muscle and soybean protein mixture. Other characteristics of the extruded products, including tensile strength, protein content, crude fat content and digestibility were 3224.6 g, 67.9%, 1.56% and 87.4% under optimal process conditions (Aldaihani et al., 2017).

This study concludes that suggested that fish and soybean protein concentrate extruded by twin screw extruder would be of great interest to the researchers. Furthermore, it would be worthwhile studying the structure of products in order to understand their formation and properties

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