

# The Extrusion Conditions Mechanism Effect on the Nutritional Value of Soybean Meal Protein

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## ABSTRACT

**Background:** Soybean tempeh has received great attention due to many advantages such as higher nutritional value, lower production cost. Soy foods have become more familiar to consume worldwide and have become a popular choice of many health conscious valued for their versatility, taste, applications using the soy bean food include: legumes, soy available in boiled soybeans, soy flour, soy oil, soy sauce, soy milk, soy tofu, soy curd, fermented soybeans soy ice cream, soy butter, soy burgers, soy halva and other. **Objective:** The aim of this study was to investigate the mechanism of identifying soybean meal quality by different extrusion conditions, including barrel temperature (45-105 °C), the moisture content of the materials (5-25%), the nozzle diameter (10-28 mm) and the screw speed (60-140 r/min). **Results:** The secondary structure of soybean meal protein was an important factor in the nutritional value. Trypsin inhibitor levels (TIU) was positively correlated to the  $\beta$ -sheet (%) ( $r=0.98$ ) and negatively correlated to the unordered (%) and the  $\alpha$ -helix (%) ( $r=-0.97$ ) at a significant level (barrel temperature 45-105 °C). Nitrogen solubility index (NSI) and protein dispersibility index (PDI) were positively correlated to the turn (%) ( $r=0.89$  and  $0.93$ , respectively) at a significant level. The NSI was negatively correlated to the turn (%) ( $r=-0.95$ ) at (nozzle diameter of 5-20 mm). **Conclusion:** This research may provide practical recommendations for use in determining the quality of soybean protein subjected to extrusion processing.

**KEYWORDS:** Extrusion Soybean meal Nutritional value Fluorescence intensity

## INTRODUCTION

Soybean has received great attention due to many advantages such as higher nutritional value, lower production cost [1]. Soy foods have become more familiar to consume worldwide and have become a popular choice of many health conscious valued for their Versatility, Taste, Applications using the soy bean food include: legumes, soy available in boiled soybeans, soy flour, soy oil, soy sauce, soy milk, soy tofu, soy curd, fermented soybeans soy ice cream, soy butter, soy burgers, soy halva and other. Soy is used for high cholesterol, high blood pressure and preventing diseases of the heart and blood vessels. It is also used for type tow diabetes, asthma, lung Function, all type of cancers (lung cancer, endometrial cancer, prostate cancer and thyroid cancer) as well as preventing weak bone (osteoporosis) slowing the progression of kidney diseases. Other use includes treating constipation and diarrhea, as well as decreasing protein in the urine of people with kidney disease, improving memory and treating muscle soreness caused by exercise. Women use soy for breast pain, menopausal symptoms and premenstrual syndrome [2, 3]. The effects of probiotic soy milk fortified with omega-3 in blood glucose, lipid profile, oxidative stress and the diabetic rats blood diseases has been studied respectively [4, 5]. On the other hand there is study on defects of soybeans as analysis of dental enamel surface submitted to fruit juice plus soymilk by micro x-ray fluorescence: In vitro study [6].

There are some studies about the applications of soy in food as soy souse processes and the fermentation by

bacteria (*Bacillus subtilis* and lactic acid bacteria) and fungi (*Mucor spp*, *Aspergillus spp*, and *Rhizopus spp*) respectively [7, 8]. Change in trypsin inhibitor activity, phytic acid content and total phenolic content in soy, have been studied [9]. There was a study about the improvement of fatty acids profile of bakery products, producing breads with low saturated fatty acid content and with high polyunsaturated fatty acid content, through partial substitution of wheat flour by other ingredients (soy flour, flax flour, and wheat bran) and to analyze the effect of this change on the technological, rheological, and sensorial characteristics of breads [10].

Protein energy malnutrition is a widespread problem throughout the world and has both health and economic consequences. It is the most common deficiency disease in the world, particularly in developing countries. The increasing cost and limited supply of animal proteins have necessitated contemporary research efforts geared towards the study of food properties and the potential of utilising protein from locally available food crops, particularly from under-utilised or relatively neglected high protein oilseeds and legumes [11].

Extrusion technology has been used in the feed industry for almost one century, and extrusion cooking is conducted by using a combination of moisture, pressure, temperature and mechanical shear [12, 13]. Extrusion is a mechanical process exposing material to high temperature, shear force and pressure over a short period of time. Mild extrusion cooking conditions can enhance the digestibility of plant protein; as a result, extrusion normally improves nutrient digestibility, palatability, pellet durability, water stability and pellet storage life [14]. Overheated meals are undesirable because overheating may damage protein and decrease protein digestibility and amino acid availability.

The trypsin inhibitor levels have been decreased rapidly as the duration of autoclaving increased, and protein solubility followed a similar but delayed pattern [15]. The protein dispersibility index (PDI), has been demonstrated to be a simple and effective procedure in assessing the quality of heat-treated soybeans [16]. The Nitrogen Solubility Index is a slight modification of the PDI procedure [17], which is used frequently in the food processing industry [18]. Anti-nutritional factors in soybean meal, including trypsin inhibitors, insoluble carbohydrates, saponins and proteins, which cause an immune response, have been cited as causes for this common effect.

Modifications in the structure of soybean proteins can enhance functional properties compared to natural proteins [19]. As the environment of a protein is gradually altered toward conditions that favour unfolding, the folded structure initially changes little, if at all; suddenly, however, the protein will unfold. These higher-order structures are essential for protein function, whether it is structural, enzymatic, immunologic, neuronal, or hormonal. In general, protein structures are only marginally stable under a limited range of physiological conditions and are easily disrupted by environmental changes, such as shifts in temperature, modifications in pressure or physical disruption that overcome the forces keeping them folded [20]. The remarkable sensitivity of infrared amide bands to changes in the conformation of the peptide backbone of proteins is well established. Because the polypeptide backbone of globular proteins can present diverse structural motifs, registered amide absorption bands result from the possible superimposition of multiple narrow vibration modes that correspond to different types of possible structures. Increasingly, Fourier transform infrared spectroscopy (FTIR spectra) is being used to investigate protein structure and stability. Different conformational types, such as helix, sheet, and turns, among others, give rise to different absorption bands that are usually broad and overlapping. Over the past few decades, the application of fluorescence spectroscopy to the study of the structure and conformation of proteins has proven to be fruitful. In particular, the emission characteristics of tryptophan residues in proteins may provide a convenient mechanism with which to investigate changes in the conformation/structure of proteins that result from a variety of factors, such as substrate/ligand binding, association/dissociation of subunits in oligomeric proteins, and unfolding [21]. Soybean meal protein contains mainly tryptophan (Trp) residues at positions of 295 nm, and the properties of fluorescence can be monitored to investigate changes in its conformation and to study the effect of ligand binding.

Proteins deriving from soybean meal are subjected to a variety of processing conditions to improve their value and functional qualities. During processing, soybeans are cracked to remove the hull and then rolled into full-fat flakes. After the oil has been extracted, the solvent is removed and the flakes are dried, creating defatted soy flakes [22]. Processing destroys the protein structure and impairs the nutritional value of soybean meal. The aim of this study was to investigate the mechanism of identifying soybean meal quality by different extrusion conditions, including barrel temperature (45-105 °C), the moisture content of the materials (5-25%), the nozzle diameter (10-28 mm) and the screw speed (60-140 r/min).

## MATERIALS AND METHODS

### Materials:

Soybean flakes were purchased from (93 Soybean Oil Co., China). The soybean flakes were cleaned, crushed and pressed from the soybeans. The moisture content of the soybean flakes was  $6.41 \pm 0.01\%$ , and other contents were tested on the basis of dry matter weight (Table 1). Soybean oil was extracted using hexane and desolventising in a vacuum at 90 °C.

### Extrusion:

A motorised twin-screw extruder (45 kW of puissance and 380 V of maximal tension) has been used. The

length of the screw was 1.47 mm, and the ratio of the length/diameter was 30 D. Raw granular materials were fed into the extruder at a mass rate of 45.4 kg h<sup>-1</sup>. The temperatures of the six barrel zones were maintained at 45, 60, 75, 90 and 105 °C, from the feeding port to the die section, throughout all experiments. The moisture content of the materials was 5-25% in feed. The screw speeds of the extruder were 60, 80, 100, 120 and 140 r/min. Five nozzles of different diameters (10, 15, 18, 22, and 28 mm) were used to press the materials.

To monitor the temperatures and pressure of extruded products during processing, six thermocouple sensors were inserted along the barrel and in the die plate. A pressure transducer was installed in the die plate. The feed rate, barrel temperatures, dies pressure and screw speed shown on the control panel was recorded. The extruded samples were collected for 5 min while the operating conditions were steady.

#### *Chemical methods:*

The nitrogen solubility index (NSI) and protein dispersibility index (PDI) were determined for the solvent-extracted soybean products. Each feed was analysed using [17], official methods Ba 12–75 and Ba 10–65. NSI is similar to PDI, although it utilised a slower mixing speed of 120 r/min. Trypsin inhibitor (TIU) protein was assayed by the enzymatic method [23]. Benzoyl-DL-arginine-p-nitroaniline (BAPA) was the substrate. Liberation of p-nitroaniline under alkaline conditions (Trizma-buffer pH 8.2) indicated the activity of trypsin. The assay was followed colorimetrically at 410 nm, and serial dilutions of materials were tested for each processing method.

#### *Soy protein isolates (SPI) preparation:*

Soybean protein isolate (SPI) was prepared from soybean meal at room temperature to prevent heat denaturation of the proteins [24]. The soybean meal was suspended in 100 mL of H<sub>2</sub>O, at pH 8.5 and in a ratio of 1:10 (w/v); it was then stirred at room temperature for 1h. Fiber was separated by centrifugation (5000 r/min 5 °C). The supernatant was adjusted to pH 4.5 with 2 M HCl to induce precipitation of soy proteins. After 2h at 4 °C, the dispersion was centrifuged as described above. The precipitate was washed with 10 mM sodium acetate buffer at a pH 4.5 (1:8 ratio (w/v)) and centrifuged as described above; the supernatant from this washing step was discarded. The final precipitate (SPI) was adjusted to pH 7.0 and freeze-dried.

#### *Infrared spectroscopy determination:*

Infrared spectra were measured with a Nicolet 6700 Fourier transform infrared spectrophotometer. For each spectrum, 256 scans were collected with a resolution of 4 cm<sup>-1</sup>, with 40 scans and a 2 cm<sup>-1</sup> interval from the 4000 to 400 cm<sup>-1</sup> region. SPI was added on the sample stage of the ATR accessory and adjusted the pressure of the tower. The relative amounts of the different secondary structure were determined from the infrared second derivative amide I spectra by manually computing the areas under the bands assigned to a particular substructure.

#### *Fluorescence spectroscopy determination:*

Thirty milligram of SPI Freeze-dried samples were homogenised with 20 ml of chloroform–methanol (2:1, v/v) at 90 °C for approximately 1 min. An equal volume of water was added, and after thorough vortex mixing, the samples were centrifuged for 10 min at 3000 g (4 °C). The chloroform-rich layer was taken into a small screw-capped Pyrex tube for subsequent fluorescence measurements. Fluorescence intensities were obtained on a Shimadzu F-4500 fluorescence spectrophotometer with excitation at 295 nm (slit width 5 nm) and the range of the emission wave number at 300-400 nm.

#### *Statistical Analysis:*

All extractions and determinations were conducted in triplicate, and the results were expressed on the basis of dry matter weight. Data are expressed as the means±SD. The means were compared using the one-way and multivariate analysis of variance (ANOVA) followed by Duncan's multiple range tests using SPSS 17.0. The differences between individual means were deemed to be significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

#### *Ingredient composition of the test materials:*

The appropriate inclusion of soybean flake by extrusion (SFE) or a substantial increase in the amount of soybean meal led to an increase in the protein content of the dry matter (Table 1). The protein content of the soybean flake fell between the protein content levels of the soybean and SFE.

#### *The extrusion conditions effect on NSI value of soybean meal protein:*

When the barrel temperature was raised from 45 to 75°C, the moisture content of the materials reached 10%, the nozzle diameter was 18 mm, the speed of the screw was set at 100 r/min, and the NSI value of the soybean meal reached its maximum. However, when the barrel temperature was increased from 75 to 105 °C and the

other extrusion conditions were not changed, the NSI exhibited a slight reduction (Table 2). Table 3 describes the effect of materials' moisture content on their NSI values. The maximum value was reached when the moisture content of materials was 15%, the barrel temperature was 75°C, the nozzle diameter was 18 mm and the speed of the screw was set at 100r/min. The Moisture can protect the structure of the protein in soybean meal from destruction. The nozzle diameter has a significant effect on the NSI level ( $p < 0.05$ ) (Table 4). The value of the NSI increased up to 44.67% with an increase in the nozzle diameter from 10 mm to 22 mm under the extrusion conditions of a barrel temperature of 75°C, a moisture content of 15% and a screw speed of 100 r/min. However, it decreased to 41% when the nozzle diameter was 28 mm. One possible reason for this phenomenon may be that the diameter of the nozzle contributes to an increase in the NSI value. Thus, the larger the nozzle diameter, the lower the level of pressure on the material, which reduces the destructive effect on protein quality. Table 5 describes the effect of screw speed on the NSI under die extrusion conditions in which the barrel temperature was 75°C, the materials' moisture content was 15% and the nozzle diameter was 22 mm. At first, increasing the screw speed of the extruder improved the NSI value. However, when we increased the screw speed of the extruder to 100 r/min, the NSI value decreased. It is reasonable to conclude that breakages in the protein contribute to an increase in the counterpressure on the protein.

*The extrusion conditions effect on the PDI value of soybean meal protein:*

The data on the effects of the extrusion conditions on the PDI value of soybean meal protein were presented in (Tables 2). The PDI value exhibited a similar trend to NSI value, but its value was slightly larger than the NSI value. The PDI values of the complete diets in the current study ranged from 45.19 to 55.61%. However, this is somewhat higher than the 15% to 30% recommended by the National Soybean Processors Association [25]. Also, the PDI has been used as an indicator of quality in the evaluation of different processing methods used with soybean products, results were in agreement. The PDI values of soybeans responded to the length of their exposure to steam and to temperature differently than soybeans of Chinese origin, concluding that soybeans of different origins require different processing conditions to optimise their protein properties [26]. Overheated meals are undesirable because overheating may damage proteins and decrease their digestibility and amino acid availability. Furthermore, the moisture content can decrease the temperature of soybean meal protein to improve its defence against mechanical force damage.

*The extrusion conditions effect on the TIU value of soybean meal protein:*

An increase in the barrel temperature can reduce the TIU value. Overheating soybean meal decreases its TIU, but this is undesirable because overheating can also damage proteins (Table 2). A combination of pressure and temperature was effective in reducing trypsin inhibitor activity, but an increase in pressure alone was not effective [27]. The TIU values were dependent on the moisture content, possibly because moisture can protect the proteins' structures when the materials are exposed to mechanical force (Table 3). In general, the nozzle diameter affected the TIU value of the soybean meal protein (Table 4). A high TIU value was observed in the soybean meal protein when the nozzle diameter was set at 22 mm, and the lowest value was observed when a nozzle diameter of 10 mm was used. Table 5 shows that the increase screw speed increased the protein value, it can reduce the TIU value.

*FTIR spectra studies on the soybean meal protein's secondary structure:*

The conformational sensitive amide I band, which is primarily caused by the carbonyl stretching vibrations of the protein backbone and appears in the range between 1700 and 1600  $\text{cm}^{-1}$ , is frequently used in the assignment of proteins' different secondary structures [28]. There was only one band in the amide I region of  $\alpha$ -helix with a frequency of approximately 1650–1660  $\text{cm}^{-1}$ . A component close to 1645  $\text{cm}^{-1}$ , which had been observed in the infrared spectra of most proteins, was assigned to the unordered structure generally referred to as a 'random coil' and was supported by previous infrared studies [29]. The  $\beta$ -sheet absorption was observed in the frequency regions of 1618–1640  $\text{cm}^{-1}$  and 1670–1690  $\text{cm}^{-1}$ . Little information was available on the turn structure.

Fig. 1 presents the soybean, soybean flake and soybean meal protein measured in the range of 400-4000  $\text{cm}^{-1}$ . Fig. 2 describes the FTIR spectra studies on the soybean, soybean flake and soybean meal proteins' secondary structures from 1600 to 1700  $\text{cm}^{-1}$ . These graphs show that the soybeans' and soybean flakes' proteins exhibited identical secondary structures, but both were different from those of the soybean meal protein. This may be because the crushing mechanism had only a small influence on the proteins, but the extrusion process may have changed the secondary structures of the soybean meal protein.

The  $\beta$ -sheet (%) decreased when the unordered,  $\alpha$ -helix and turn increased as the barrel temperature increased. When the barrel temperature was 75°C, the nozzle diameter was 18 mm, the screw speed was 100 r/min, and the moisture contents of the materials were 5, 10, 15, 20 and 25%. Table 3 demonstrates that the  $\beta$ -sheet (%) was unstable, but it increased when the moisture content of the materials increased from 5 to 25%. However, the unordered,  $\alpha$ -helix and turn all decreased. Table 4 shows the  $\beta$ -sheet (%) increased and then

decreased when the nozzle diameter was 22 mm, while the unordered (%),  $\alpha$ -helix (%) and turn (%) exhibited the opposite trend; under the other extrusion conditions, there was no change when the barrel temperature was 75°C, the moisture content was 15% and the screw speed was 100 r/min. Table 5 shows that the unordered (%) reached its minimum at 1.64% , but the  $\beta$ -sheet (%) and turn (%) reached their maximums of 69.25% and 22.87% when the barrel temperature was 75°C, the moisture content was 15% , the nozzle diameter was 18 mm and the screw speed was 120 r/min. However, the  $\alpha$ -helix (%) was not regular.

#### Fluorescence spectroscopy and spectra analysis:

When the tryptophan fluorescence  $\lambda_{\max}$  is higher than 330 nm, the tryptophan moved from the hydrophobic surrounding to the hydrophilic [30]. However, a  $\lambda_{\max}$  smaller than 330 nm indicates that the tryptophan residue is located within the non-polar environment inside the protein molecule. The emission  $\lambda_{\max}$  and the changes in emission intensity observed under each extrusion condition (Fig. 3). The  $\lambda_{\max}$  measured in this experiment were distributed within the range of 338.4–344.4 nm. It can be concluded that different extrusion conditions, the tryptophan residues are primarily located in the polar environment outside the protein molecules. It was suggested that changes in the extrusion conditions introduced changes in the proteins' conformation.

Fig. 3 (a) presents the fluorescence intensities of the different barrel temperatures. When the temperature was 60°C, the  $\lambda_{\max}$  reached its maximum at 344.4 nm. When the barrel temperature increases, the  $\lambda_{\max}$  of soybean meal first presents a red-shift at 60°C, then decreases (blue-shift) from 60 to 105°C. This may be because the tryptophan residues that were previously located in the non-polar environment inside the structure moved outside, where the polarity of the micro-environment first increased then decreased with an increase in the barrel temperature. Fig. 3 (b) presents the change in the  $\lambda_{\max}$  when the moisture content of the materials changes. It presents a slight red-shift when the moisture content increases from 5 to 10%, a blue-shift from 10-20% and a red-shift from 20-25%. The tryptophan residues are gradually exposed to the surface of the polarity in a micro-environment; they then turn to a non-polar environment, increasing the moisture content of the materials and exposing them once more to the surface of the polarity from 20-25%. Fig. 3 (c, d) describe the effects of the nozzle diameter and screw speed extrusion conditions on  $\lambda_{\max}$ .

#### Relationship of structure to the protein's nutritional value:

Through the determinations of the extrusion conditions effects on the relationships between the functional properties of the soybean meal protein (NSI, PDI and TIU) and the structures of soybean meal protein; results mentioned that as the barrel temperature increases from 45-105°C (moisture content 10%, nozzle diameter 18 mm and screw speed 100 r/min), the TIU value was positively correlated to the  $\beta$ -sheet (%) ( $r=0.98$ ), negatively correlated to the unordered (%) ( $r=-0.89$ ) and  $\alpha$ -helix (%) ( $r=-0.97$ ) with significance ( $p<0.05$ ). The NSI and PDI had no significant relationships with the structure of the soybean meal. As the moisture content of the materials increased from 5-25% (barrel temperature 75°C, nozzle diameter 18 mm and screw speed 100 r/min), the NSI and PDI values were positively correlated to the turn (%) ( $r=0.89$  and  $0.93$ , respectively) and were significant. The TIU value was positively correlated to the  $\beta$ -sheet (%) ( $r=0.91$ ) and negatively correlated to the unordered (%) ( $r=-0.98$ ) and significant. As the nozzle diameter changed from 10 to 28 mm (barrel temperature 75°C, moisture content 15% and screw speed 100 r/min), the NSI value was negatively correlated to the turn (%) ( $r=-0.95$ ) and significant. The nutritional value of soybean meal under different screw speed conditions was not significant to the structure of the soybean meal.

**Table 1:** Chemical compositions of the tested materials

	Protein	Fat	Soluble Fiber	Insoluble Fiber
Soybean	36.18±0.05 <sup>c</sup>	22.56±0.12 <sup>a</sup>	1.16±0.07 <sup>a</sup>	2.51±0.02 <sup>d</sup>
Soybean flake	36.73±0.17 <sup>cb</sup>	20.16±0.02 <sup>b</sup>	1.20±0.01 <sup>a</sup>	2.87±0.09 <sup>c</sup>
Soybean flake extrude	37.35±0.76 <sup>b</sup>	17.16±0.07 <sup>c</sup>	1.04±0.05 <sup>a</sup>	3.34±0.03 <sup>b</sup>
Soybean meal	47.46±0.07 <sup>a</sup>	1.00±0.01 <sup>d</sup>	1.03±0.15 <sup>a</sup>	3.65±0.05 <sup>a</sup>

Each value presented as the mean  $\pm$  standard deviation (n=3). Data with different uppercase superscript letters in the same column of means respectively indicate significant difference ( $P < 0.05$ ) analyzed by Duncan's multiple range test.

**Table 2:** Effect of Barrel temperature

Barrel temp. (°C)	Functional Properties			Secondary Structure				$\lambda_{\max}$ (nm)
	NSI (%)	PDI (%)	TIU/mg	$\beta$ -sheet (%)	Unordered (%)	$\alpha$ -helix (%)	Turn (%)	
45	40.66±0.62 <sup>d</sup>	45.89±0.02 <sup>c</sup>	1057.41±3.33 <sup>a</sup>	35.62±0.01 <sup>a</sup>	25.47±0.01 <sup>c</sup>	22.37±0.03 <sup>c</sup>	16.54±0.05 <sup>c</sup>	341
60	42.79±0.17 <sup>c</sup>	46.66±0.07 <sup>b</sup>	990.89±0.45 <sup>b</sup>	32.16±0.05 <sup>b</sup>	27.10±0.59 <sup>b</sup>	25.08±0.02 <sup>d</sup>	15.99±0.07 <sup>c</sup>	344.4
75	44.58±0.20 <sup>a</sup>	47.89±0.01 <sup>a</sup>	835.50±2.44 <sup>c</sup>	29.18±0.06 <sup>c</sup>	27.19±0.10 <sup>b</sup>	27.27±0.05 <sup>c</sup>	16.36±0.09 <sup>d</sup>	341.4
90	43.62±0.37 <sup>b</sup>	45.74±0.18 <sup>c</sup>	786.72±3.17 <sup>d</sup>	26.05±0.01 <sup>d</sup>	27.84±0.01 <sup>a</sup>	28.89±0.02 <sup>b</sup>	17.23±0.02 <sup>b</sup>	342
105	43.30±0.29 <sup>bc</sup>	45.19±0.22 <sup>d</sup>	621.47±1.53 <sup>e</sup>	23.17±0.05 <sup>e</sup>	28.24±0.01 <sup>a</sup>	30.29±0.01 <sup>a</sup>	18.29±0.06 <sup>a</sup>	340.8

Each value presented as the mean  $\pm$  standard deviation (n=3). Data with different uppercase superscript letters in the same column of means respectively indicate significant difference ( $P < 0.05$ ) analyzed by Duncan's multiple range test.

**Table 3:** Effect of the moisture content of materials

Moisture (%)	Functional Properties			Secondary Structure				$\lambda_{\max}$ (nm)
	NSI (%)	PDI (%)	TIU/mg	$\beta$ -sheet (%)	Unordered (%)	$\alpha$ -helix (%)	Turn (%)	
5	46.83 $\pm$ 0.11 <sup>e</sup>	50.31 $\pm$ 0.02 <sup>d</sup>	757.19 $\pm$ 0.71 <sup>e</sup>	29.50 $\pm$ 0.14 <sup>e</sup>	27.10 $\pm$ 0.02 <sup>a</sup>	32.28 $\pm$ 0.05 <sup>a</sup>	11.12 $\pm$ 0.09 <sup>d</sup>	341.6
10	49.79 $\pm$ 0.16 <sup>c</sup>	52.38 $\pm$ 0.19 <sup>c</sup>	765.90 $\pm$ 1.88 <sup>d</sup>	32.17 $\pm$ 0.07 <sup>d</sup>	26.70 $\pm$ 0.10 <sup>b</sup>	25.09 $\pm$ 0.03 <sup>b</sup>	16.04 $\pm$ 0.05 <sup>b</sup>	344.4
15	53.50 $\pm$ 0.18 <sup>a</sup>	55.80 $\pm$ 0.15 <sup>a</sup>	877.00 $\pm$ 2.38 <sup>c</sup>	31.50 $\pm$ 0.13 <sup>c</sup>	26.16 $\pm$ 0.04 <sup>c</sup>	23.57 $\pm$ 0.15 <sup>c</sup>	18.77 $\pm$ 0.31 <sup>a</sup>	342.0
20	51.65 $\pm$ 0.40 <sup>b</sup>	53.25 $\pm$ 0.09 <sup>b</sup>	900.68 $\pm$ 0.31 <sup>b</sup>	39.19 $\pm$ 0.29 <sup>b</sup>	25.33 $\pm$ 0.03 <sup>d</sup>	21.09 $\pm$ 0.03 <sup>d</sup>	14.39 $\pm$ 0.30 <sup>c</sup>	341.4
25	47.49 $\pm$ 0.39 <sup>d</sup>	50.29 $\pm$ 0.18 <sup>d</sup>	1029.00 $\pm$ 0.67 <sup>a</sup>	46.01 $\pm$ 0.02 <sup>a</sup>	24.08 $\pm$ 0.05 <sup>e</sup>	20.63 $\pm$ 0.55 <sup>d</sup>	9.28 $\pm$ 0.59 <sup>e</sup>	343.6

Each value presented as the mean  $\pm$  standard deviation (n=3). Data with different uppercase superscript letters in the same column of means respectively indicate significant difference ( $P < 0.05$ ) analyzed by Duncan's multiple range test.

**Table 4:** Effect of the nozzle diameter

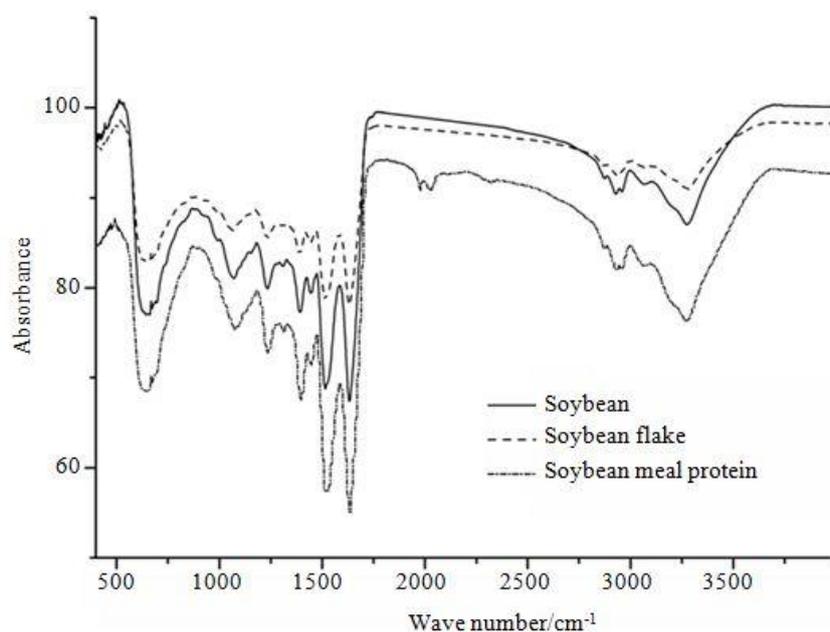
Nozzle diameter (mm)	Functional Properties			Secondary Structure				$\lambda_{\max}$ (nm)
	NSI (%)	PDI (%)	TIU/mg	$\beta$ -sheet (%)	Unordered (%)	$\alpha$ -helix (%)	Turn (%)	
10	40.22 $\pm$ 0.11 <sup>e</sup>	51.25 $\pm$ 0.16 <sup>c</sup>	1051.39 $\pm$ 0.53 <sup>e</sup>	53.57 $\pm$ 0.11 <sup>d</sup>	15.29 $\pm$ 0.01 <sup>c</sup>	15.77 $\pm$ 0.03 <sup>b</sup>	15.37 $\pm$ 0.08 <sup>a</sup>	339.8
15	42.32 $\pm$ 0.18 <sup>c</sup>	52.71 $\pm$ 0.27 <sup>c</sup>	1068.55 $\pm$ 0.69 <sup>d</sup>	57.44 $\pm$ 0.22 <sup>c</sup>	14.35 $\pm$ 0.06 <sup>d</sup>	14.24 $\pm$ 0.21 <sup>c</sup>	13.96 $\pm$ 0.21 <sup>b</sup>	341.0
18	43.44 $\pm$ 0.23 <sup>b</sup>	53.58 $\pm$ 0.32 <sup>b</sup>	1089.16 $\pm$ 0.21 <sup>b</sup>	61.33 $\pm$ 0.06 <sup>a</sup>	13.40 $\pm$ 0.12 <sup>e</sup>	13.82 $\pm$ 0.15 <sup>d</sup>	11.46 $\pm$ 0.15 <sup>d</sup>	339.0
22	44.67 $\pm$ 0.11 <sup>a</sup>	55.53 $\pm$ 0.22 <sup>a</sup>	1090.92 $\pm$ 0.19 <sup>a</sup>	58.33 $\pm$ 0.04 <sup>b</sup>	17.67 $\pm$ 0.20 <sup>b</sup>	15.60 $\pm$ 0.29 <sup>b</sup>	8.40 $\pm$ 0.29 <sup>e</sup>	342.0
28	41.34 $\pm$ 0.31 <sup>d</sup>	52.22 $\pm$ 0.10 <sup>d</sup>	1087.97 $\pm$ 0.31 <sup>c</sup>	46.67 $\pm$ 0.27 <sup>e</sup>	21.94 $\pm$ 0.06 <sup>a</sup>	17.86 $\pm$ 0.14 <sup>a</sup>	13.53 $\pm$ 0.14 <sup>c</sup>	340.4

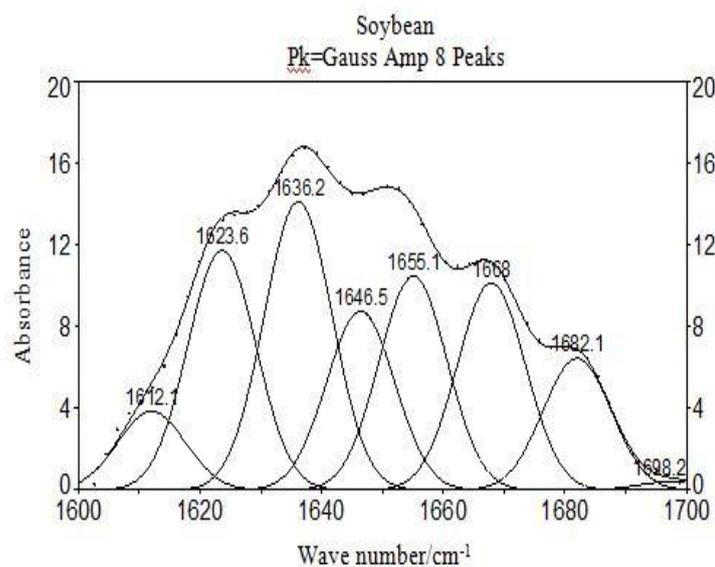
Each value presented as the mean  $\pm$  standard deviation (n=3). Data with different uppercase superscript letters in the same column of means respectively indicate significant difference ( $P < 0.05$ ) analyzed by Duncan's multiple range test.

**Table 5:** Effect of the speed of screw

Screws speed r/min	Functional Properties			Secondary Structure				$\lambda_{\max}$ (nm)
	NSI (%)	PDI (%)	TIU/mg	$\beta$ -sheet (%)	Unordered (%)	$\alpha$ -helix (%)	Turn (%)	
60	49.89 $\pm$ 0.11 <sup>c</sup>	52.57 $\pm$ 0.22 <sup>c</sup>	1031.44 $\pm$ 0.76 <sup>a</sup>	55.62 $\pm$ 0.35 <sup>d</sup>	16.28 $\pm$ 0.04 <sup>b</sup>	14.66 $\pm$ 0.06 <sup>b</sup>	13.44 $\pm$ 0.38 <sup>c</sup>	338.4
80	50.41 $\pm$ 0.19 <sup>b</sup>	53.63 $\pm$ 0.17 <sup>b</sup>	1030.08 $\pm$ 0.21 <sup>b</sup>	57.52 $\pm$ 0.18 <sup>c</sup>	15.35 $\pm$ 0.09 <sup>c</sup>	4.11 $\pm$ 0.01 <sup>c</sup>	13.02 $\pm$ 0.25 <sup>c</sup>	339.4
100	53.42 $\pm$ 0.23 <sup>a</sup>	55.61 $\pm$ 0.16 <sup>a</sup>	993.38 $\pm$ 0.89 <sup>c</sup>	61.58 $\pm$ 0.45 <sup>b</sup>	13.43 $\pm$ 0.17 <sup>d</sup>	13.75 $\pm$ 0.08 <sup>d</sup>	11.25 $\pm$ 0.25 <sup>d</sup>	339.0
120	50.28 $\pm$ 0.10 <sup>b</sup>	52.41 $\pm$ 0.08 <sup>c</sup>	988.41 $\pm$ 0.76 <sup>d</sup>	69.25 $\pm$ 0.14 <sup>a</sup>	1.64 $\pm$ 0.24 <sup>e</sup>	6.24 $\pm$ 0.06 <sup>e</sup>	22.87 $\pm$ 0.16 <sup>a</sup>	340.2
140	48.70 $\pm$ 0.32 <sup>d</sup>	50.48 $\pm$ 0.45 <sup>d</sup>	960.95 $\pm$ 0.31 <sup>e</sup>	50.56 $\pm$ 0.65 <sup>e</sup>	16.64 $\pm$ 0.29 <sup>a</sup>	16.50 $\pm$ 0.20 <sup>a</sup>	16.29 $\pm$ 0.22 <sup>b</sup>	339.8

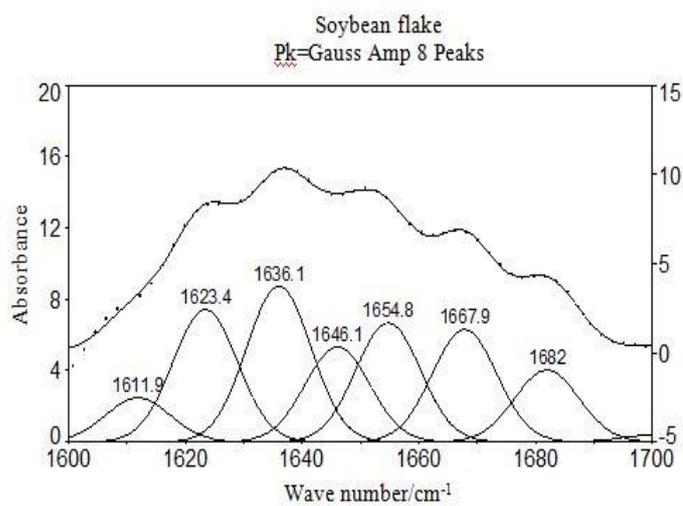
Each value presented as the mean  $\pm$  standard deviation (n=3). Data with different uppercase superscript letters in the same column of means respectively indicate significant difference ( $P < 0.05$ ) analyzed by Duncan's multiple range test.

**Fig. 1:** FTIR spectra of soybean, soybean flake and soybean meal protein

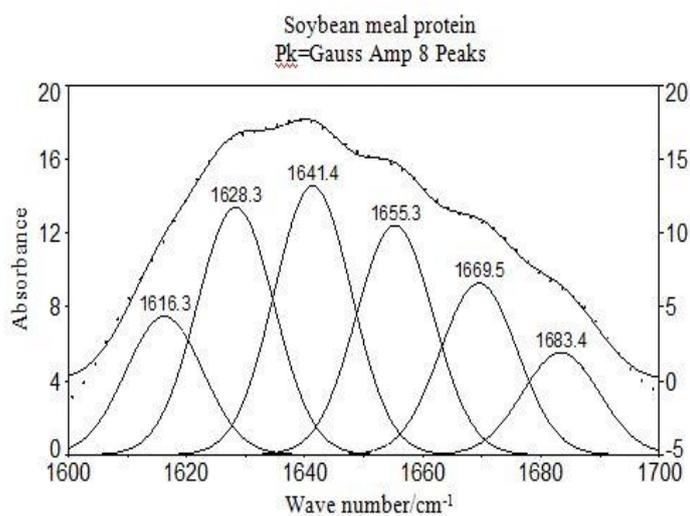


**Fig. 2(a,b,c):** Effect of extruding on FTIR spectra

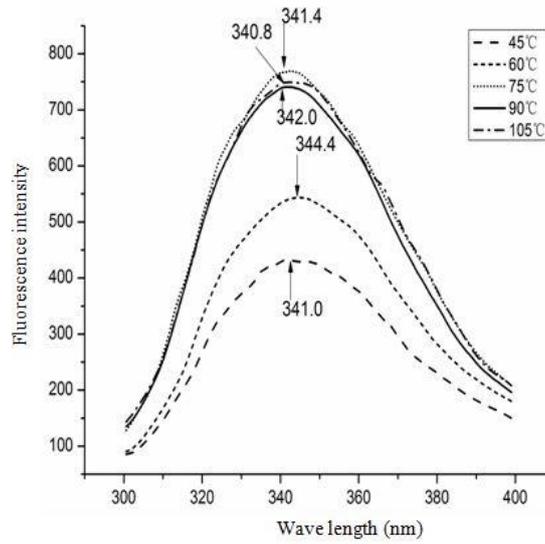
**Fig. 2a:**



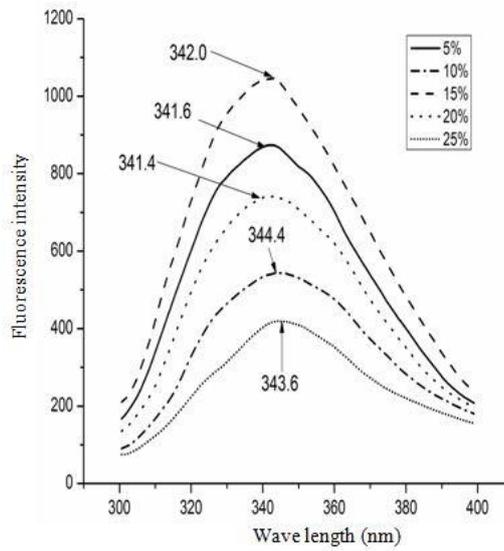
**Fig. 2b:**



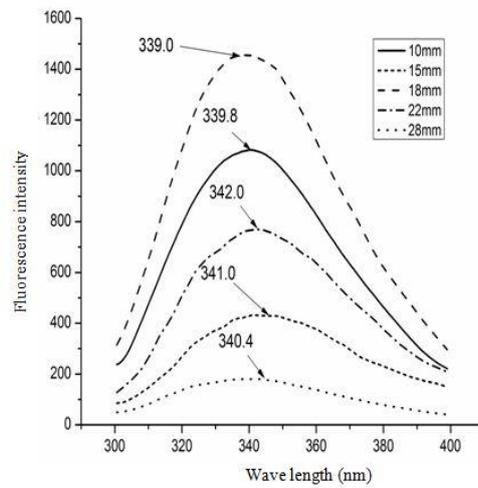
**Fig. 2c:**



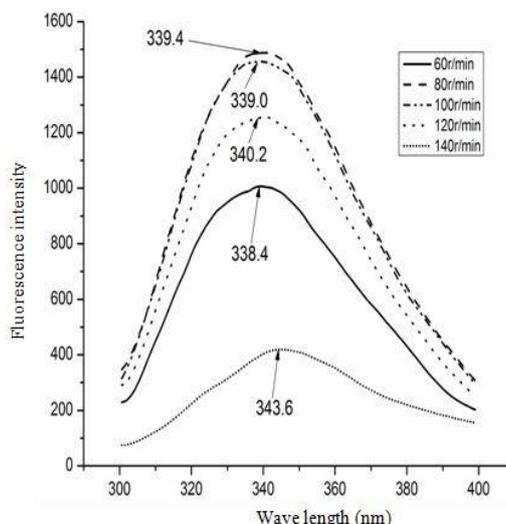
**Fig. 3(a,b,c,d):** Effect of the extruding on florescence intensity  
**Fig. 3a:**



**Fig. 3b:**



**Fig. 3c:**



**Fig. 3d:**

*Conclusion:*

This research demonstrated that extrusion processing can influence the quality and structure of soybean meal. The mechanism of the changes in nutritional value caused by extrusion processing can make the wider use of soybean meal in animal diets possible. In this study, when the barrel temperature increases from 45-105°C, secondary structures play an important role in the TIU level; as the materials' moisture content increases from 5-25%, NSI and PDI were determined by the turn (%), and TIU was changed by the  $\beta$ -sheet (%) and unordered (%); when the nozzle diameter was changed from 10-28 mm, the NSI was affected by the turn (%). There were no significant relationships between the  $\lambda_{max}$  and the quality of the soybean meal. Thus, the results of this research can help to obtain better nutritional value in protein through the diversification rules of the secondary structure of soybean meal protein with appropriate extrusion processing.

*Conflict of Interests:*

The authors declare that there is no conflict of interest is regarding the publication of this paper.

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