

*Research Paper*

## **Effects of soy protein isolate on chemical and physical characteristics of meat analog**

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**Abstract:** Soy protein analog was fabricated using a twin screw extruder. Different soy protein isolate (SPI) contents of 20, 40, 60 and 80% were blended with defatted soy flour (DSF) and then fed into an extruder with the feed rate of 65 g/min. The barrel temperature profile was 60, 90, 140 and 160°C. The screw speed was 250 rpm. Based on protein solubility studies using different buffer solutions containing phosphate, sodium dodecyl sulphate, urea or 2-mercaptoethanol, disulfide bond, hydrophobic interaction and hydrogen bond were major linkages stabilizing meat analog structure. Increasing SPI content from 20 to 80% did not increase these chemical linkages but resulted in a greater expansion ratio ( $p < 0.05$ ). Cutting force, which was monitored by a texture analyzer, decreased with increasing SPI content. Textural characteristics, as judged by tearing, and fibrous characteristics using Quantitative Descriptive Analysis were reduced with increased SPI contents.

**Keywords:** food processing, meat analog, soy protein isolate, chemical linkages, texture, twin screw extruder

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### **Introduction**

Meat analog is a major type of texturized plant protein (TPP) which is extensively used to imitate meat products (Sheard, Ledward and Mitchell, 1984). It is considered to be a health food which contains high protein and fibre, particularly suited to vegetarian diets. The other beneficial characteristics of TPP are year-long shelf stability in dry form and capacity for rehydration without loss in structure, shape and chewy texture (Harper, 1981). Soy protein is a major important component to fabricate the structure of meat analog. The value of soy protein is comparable to that of meat protein (deMan, 1990). Furthermore, the risk of heart disease and cancer of consumers is also reduced (Liu, 1997). Extrusion cooking is the principle processing method used to fabricate meat like texture and

plexilamellar structure for soy protein products (Burgess and Stanley, 1976). It also offers some advantages in terms of cost and the capability for high volume production (Harper, 1981).

The structure of texturized soy protein (TSP) products depends on production conditions and material components (Ha, 1995; Sheard *et al.*, 1984). Quantity and quality of soy protein are the important factors that affect the chemical and physical characteristics of meat analog. Maurice and Stanley (1978) suggested that increasing protein content resulted in a high shearing force on the fine texture of extrudate. Sheard and others (1984) reported that soy carbohydrate also supported the main structure of soy protein extrudate. The fibre and layer structures of meat analog were generated by protein texturization during extrusion. Thermal denaturation set the protein matrix in a fibre-like configuration involving protein cross-linking and interaction between protein and other components during extrusion. Hager (1984) mentioned that chemical bonds, which were disulfide bonds, hydrogen bonds and hydrophobic interaction, were present to support the stabilized extrudate structure and new chemical linkages during extrusion cooking did not appear. However, Hayakawa (1992) reported that the disulfide bond was disrupted during processing, but did not affect the extrudate structure. The objectives of this study were to investigate the chemical linkages and physical characteristics of soy protein meat analog containing different soy protein isolate contents.

## **Materials & Methods**

### ***Materials***

Defatted soy flour (DSF), soy protein isolate (SPI, Profam 970) were supplied by ADM protein specialties, Decatur IL, USA. DSF contained 50% protein and 0.22% fat. The protein content of SPI was 90%. The protein and fat content of materials were determined using the method of AOAC (1995). Urea was obtained from Sigma-Aldrich GmbH, Germany. The other reagents were of analytical grade.

### ***Extrusion processing***

DSF was blended with SPI in order to obtain 20, 40, 60 and 80% SPI. All materials were extruded using an APV Baker MPF 19/25 twin screw extruder (APV Baker, UK) which was equipped with a 3 mm diameter die. The dry feed rate and screw speed were kept constant at 65 g/min and 250 rpm respectively. Water was added to the blends to obtain 30% moisture content. Barrel temperature profile was set at 60, 90, 140 and 160°C from zone 1 to zone 4, respectively. After the product was extruded from the die, it was dried in a hot air oven at 50°C for 3 hrs.

### ***Protein solubility determination***

Protein solubility of both raw materials and extrudates were analyzed using the modified method of Lin *et al.* (2000). Buffer solution used for protein extraction included; (1) 0.035M phosphate buffer (pH 7.6), (2) 1.5% sodium dodecyl sulfate (SDS) in phosphate buffer (P+SDS), (3) 8 M urea in phosphate buffer (P+U), (4) 0.1 M 2-mercaptoethanol (2-ME) in phosphate buffer (P+M), (5) 1.5% SDS and 8 M urea in phosphate buffer

(P+S+U), (6) 1.5% SDS and 0.1 M 2-ME in phosphate buffer (P+S+M), (7) 8 M urea and 0.1 M 2-ME in phosphate buffer (P+U+M).

Approximately 200 g extrudate was finely chopped using a blender for 1 min. Sample (2 g) was extracted with 40 ml of each buffer for 2.5 h. in a 40°C shaking water bath. It was then centrifuged at 20,000 g for 40 min to obtain the supernatant. Protein concentration was determined using Lowry method assay at 750 nm (UV/Visible 916 spectrophotometer, GBC Scientific Equipment Ltd., Australia).

### ***Structural evaluation***

The expansion ratio was defined as the rate of the diameter of extrudate over the diameter of the die. The average for each data was obtained from twenty measurements. The textural appearance of soy protein meat analog was determined using the modified sensory method of Simonsky and Stanley (1982). The meat analog was cut in 5 cm lengths and rehydrated for 5 min at room temperature. The rehydrated samples were then drained on a screen. Quantitative descriptive analysis (QDA) was used as a technique to evaluate the textural appearance of the meat analog using ten trained panelists. Individual strands of meat analog were torn in a longitudinal axis by a judge to observe tearing attributes and fibrous characteristics. A scale of 10 cm was used with two descriptors, tearing and fibrousness. The replication of QDA was conducted in each experiment.

### ***Textural measurement.***

Meat analogs were cut in 3 cm lengths and rehydrated to 80% moisture at room temperature. The texture of soy protein meat analog was measured using TA-XT2 Texture Analyzer (Stable Micro Systems, UK) with Warner–Bratzler shear cell. Cross head speed was 2 mm/s. The maximum force and radius of each sample were recorded. Fifteen measurements were conducted for each sample. The cutting force of extrudate (g/cm) was reported as maximum force per cross-sectional area:

### ***Statistical analysis***

Completely randomized design (CRD) was the method employed and the data were analyzed with the analysis of variance (ANOVA) and the average of each treatment was compared using Duncan's multiple range test (DMRT).

## **Results and Discussion**

### ***Effect of soy protein isolate on chemical linkages***

Protein solubility was introduced to study the intermolecular chemical linkages of protein structure. Urea, SDS and 2-ME were used to break the intramolecular H bond, hydrophobic interaction and disulfide bonds between protein molecules respectively. Disulfide bonds, hydrophobic interaction and hydrogen bonds were formed in the protein structure of meat analog as shown in Table 1. Meat analog containing 20, 40, 60 and 80% SPI showed the highest soluble protein in P+S+M and P+U+M, than that of other buffers ( $p < 0.05$ ). In addition, soluble protein of other buffers was not significantly different

( $p > 0.05$ ). This result indicated that disulfide bonds, hydrophobic interaction and hydrogen bonds were the major linkages in meat analog structure. It is in accordance with Hager (1984) and Predencio-Ferrieira and Areas (1993) who reported the importance of disulfide bonds, hydrophobic interaction and electrostatic interaction in support of soy protein extrudate structure.

**Table 1.** Soluble protein (g/g protein) of soy protein meat analog containing 20 to 80% in 7 buffers.

SPI content							
/Buffer	P <sup>(1)</sup>	P+S <sup>(2)</sup>	P+U <sup>(3)</sup>	P+M <sup>(4)</sup>	P+S+U <sup>(5)</sup>	P+S+M <sup>(6)</sup>	P+U+M <sup>(7)</sup>
20%SPI	0.0816 <sup>y(8)</sup>	0.1687 <sup>y</sup>	0.2949 <sup>y</sup>	0.2462 <sup>y</sup>	0.2085 <sup>y</sup>	0.7994 <sup>x</sup>	0.7198 <sup>x</sup>
40%SPI	0.0817 <sup>y</sup>	0.2107 <sup>y</sup>	0.1723 <sup>y</sup>	0.1749 <sup>y</sup>	0.1705 <sup>y</sup>	0.8367 <sup>x</sup>	0.7952 <sup>x</sup>
60%SPI	0.1172 <sup>y</sup>	0.2816 <sup>y</sup>	0.2406 <sup>y</sup>	0.2512 <sup>y</sup>	0.1352 <sup>y</sup>	1.0184 <sup>x</sup>	1.0122 <sup>x</sup>
80%SPI	0.0540 <sup>y</sup>	0.1369 <sup>y</sup>	0.1668 <sup>y</sup>	0.1886 <sup>y</sup>	0.1051 <sup>y</sup>	0.6307 <sup>x</sup>	0.7117 <sup>x</sup>

<sup>(1)</sup> phosphate buffer

<sup>(2)</sup> phosphate buffer containing sodium dodecyl sulphate

<sup>(3)</sup> phosphate buffer containing urea

<sup>(4)</sup> phosphate buffer containing 2-mercaptoethanol

<sup>(5)</sup> phosphate buffer containing sodium dodecyl sulphate and urea

<sup>(6)</sup> phosphate buffer containing sodium dodecyl sulphate and 2- mercaptoethanol

<sup>(7)</sup> phosphate buffer containing urea and 2- mercaptoethanol

<sup>(8)</sup> Different letters within the same row are significantly different ( $p < 0.05$ ).

The chemical linkages of material feed were not different from the meat analog including disulfide bonds, hydrophobic interaction and hydrogen bonds as observed from the high soluble protein in P+S, P+U, P+M and P+S+U of all materials and their extrudates ( $p < 0.05$ ) (Table 2). Therefore, the extrusion did not alter the type of chemical bond between material and meat analog. The study of SPI content on chemical bond formation in meat analog indicated that increasing SPI content in meat analog did not affect the level of chemical linkage cross-linked in protein molecules. An increase in SPI from 20 to 80% in meat analog did not demonstrate different soluble protein in most of the buffers ( $p > 0.05$ ). Thus, increasing protein content from 58 to 82% did not influence chemical bonds in protein molecules of meat analog.

**Table 2.** Soluble protein (g/g protein) of raw material containing 20 to 80 % in 7 buffers.

SPI content							
/Buffer	P	P+S	P+U	P+M	P+S+U	P+S+M	P+U+M
20%SPI	0.4542 <sup>c(1)</sup>	0.7473 <sup>abc</sup>	0.7168 <sup>abc</sup>	0.5621 <sup>bc</sup>	0.7466 <sup>abc</sup>	0.9888 <sup>a</sup>	0.8932 <sup>ab</sup>
40%SPI	0.4581 <sup>c</sup>	0.8128 <sup>a</sup>	0.7549 <sup>a</sup>	0.6780 <sup>ab</sup>	0.6406 <sup>ab</sup>	0.8776 <sup>a</sup>	0.8982 <sup>a</sup>
60%SPI	0.4472 <sup>b</sup>	0.7695 <sup>ab</sup>	0.8467 <sup>ab</sup>	0.6875 <sup>ab</sup>	0.6276 <sup>ab</sup>	1.0058 <sup>a</sup>	0.9503 <sup>a</sup>
80%SPI	0.3255 <sup>b</sup>	0.6231 <sup>ab</sup>	0.7006 <sup>ab</sup>	0.5900 <sup>ab</sup>	0.4431 <sup>ab</sup>	0.8664 <sup>a</sup>	0.7974 <sup>a</sup>

<sup>(1)</sup>Different letters within the row are significantly different ( $p < 0.05$ ).

Meat analog containing 20, 40, 60 and 80% SPI showed lower soluble protein content than that of feed containing the same SPI content which was extracted by P, P+S, P+U, P+M, P+S+U ( $p < 0.05$ ). This may result from protein denaturation by heat and pressure during the extrusion process and protein reoriented to form the more complex structure. It decreased protein solubility of the extrudate (Jeunink and Cheftel, 1979; Noguchi *et al.*, 1981). Lin *et al.* (2000) reported that the high molecular weights of soy protein molecules were formed by extrusion, resulting in the lower solubility of protein molecules. This is in accord with the result of lower protein solubility of meat analogs as compared with its feed material.

### ***Effect of soy protein on physical characteristics***

Increasing SPI content from 20 to 80% showed that the expansion ratio of meat analog was significantly increased from 2.01 to 2.61 (Table 3). It indicated that material components had an influence on the expansion ratio of meat analog. Yuryev *et al.*, (1995) reported that the damage of dispersed phase from shear stress during flowing in the extruder affected viscosity and elasticity of the system which had an impact on expansion ratio of the product.

Tolstoguzov *et al.*, (1985) and Tolstoguzov (1993) reported that during extrusion molecular weights of biopolymer occurred and incorporated to form an incompatible structure leading to multiple phases being separated. The major component became a continuous phase which was the main structure of the product. The other components became a continuous phase which imparted overall characteristics of the product. A high expansion appeared in high SPI meat analog, indicating that SPI provided elasticity to meat analog dough, but less viscosity was found as indicated by a significant decrease ( $P < 0.05$ ) in torque and die pressure of meat analog containing 80% SPI. The dough greatly expanded after exiting from the die. The expansion of extrudate was a result of the superheated steam flashing when the plastic-like dough exited from the die (Harper, 1981). This result was in accord with Rhee *et al.* (1981) who reported that meat analog

containing higher SPI content had a lower specific density. Bhattacharya and Hanna (1988) also found that high soy protein concentrate added into corn gluten resulted in higher expansion of extrudate. On the contrary, meat analog containing low SPI showed lower expansion ratio because it contained low protein and the dispersed melt carbohydrate embedded on the protein matrix. Thus, the stretchability of protein molecules was decreased resulting in the decreased expansion ratio of meat analog.

**Table 3.** Extrusion parameters and physical characteristics of meat analog containing 20–80% SPI.

Extrusion parameters and characteristics	Soy protein isolate (%)			
	20	40	60	80
Torque (%)	30.00 <sup>ab(1)</sup>	36.00 <sup>a</sup>	35.00 <sup>ab</sup>	28.33 <sup>b</sup>
Die pressure (psi)	183.33 <sup>a</sup>	193.33 <sup>a</sup>	196.67 <sup>a</sup>	116.67 <sup>b</sup>
Expansion ratio	2.01 <sup>d</sup>	2.25 <sup>c</sup>	2.36 <sup>b</sup>	2.62 <sup>a</sup>
Cutting force (g/cm <sup>2</sup> )	2,477 <sup>a</sup>	2,186 <sup>ab</sup>	2,035 <sup>b</sup>	1,446 <sup>c</sup>
Textural appearance score				
- Tearing	6.60 <sup>a</sup>	4.55 <sup>b</sup>	2.03 <sup>c</sup>	1.38 <sup>c</sup>
- Fibrousness	5.36 <sup>a</sup>	3.85 <sup>b</sup>	1.98 <sup>c</sup>	1.30 <sup>c</sup>

<sup>(1)</sup>Different letters within the row are significant different (p<0.05).

The effect of SPI content on textural formation of meat analog was studied by the measurement of textural cutting force. Table 3 shows that the meat analog containing 20% SPI exhibited the maximum cutting force of 2,477 g/cm<sup>2</sup> (P<0.05). Increasing SPI level from 20 to 80% resulted in a reduction of cutting force from 2,477 to 1,446 g/cm<sup>2</sup>. It indicated that the low SPI meat analog (58% protein) had a stronger layered structure than that of higher SPI content (60, 74 and 82% protein). It could be explained that extrudate with high SPI content had high expansion (Table 3) which contained large air cells of loose structure. The low SPI meat analog comprised small air cells because there was low protein to provide elasticity as well as carbohydrate embedded on the protein network: thus, there was limited ability of mass dough to expand. This resulted in a stronger texture of meat analog containing low SPI. This is also relevant to the results of Sheard *et al.*, (1984) in that DSF extrudate showed a higher strength when compared to SPI extrudate.

The structural appearance characteristics of meat analog were evaluated using QDA technique as shown in Table 3. The aim of this study was to develop the fibrous structure of the soy protein meat analog which resemble meat muscle fibres. Therefore, the

characteristics of tearing along the length of extrudate and fibre or layer structure of meat analog were the main parameters of appearance structure examined. Meat analog containing 20% SPI showed the highest tearing and fibrous structure ( $p < 0.05$ ) (Table 3). An increase in SPI level decreased the tearing and fibrousness of extruded product ( $p < 0.05$ ). This was probably due to the higher SPI meat analog showing a higher expansion ratio.

The lowest torque and die pressure was found in extrudate containing 80% SPI. It was not able to build up enough pressure to form the finely fibrous structure during extrusion as supported by Lin *et al.*, (2002). Torque and die pressure of dough were the extrusion parameter variables which were influenced by dough viscosity. An increase in dough viscosity elevated torque on the screw drive and resulted in a high die pressure. Thus, high SPI meat analog dough showed low viscosity, torque and die pressure which resulted in a reduction of fibrous structure of meat analog.

### Conclusion

The protein molecule in the raw material of meat analog was disrupted by extrusion process. The chemical linkages within protein molecules were broken down together with protein arrangement. This led to the textural and structural formation of meat analog. Disulfide bonds, hydrophobic interaction and hydrogen bonds were formed to support the product structure. Increasing SPI content from 20 to 80% did not influence the ratio of these chemical linkages in meat analog but the expansion was greater. The cutting force, tearing and fibrousness of high SPI meat analog were also lower. Meat analog containing low SPI showed strong, continuous, long and finely fibrous structure.

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