PROTEIN-PROTEIN INTERACTION OF SOY PROTEIN ISOLATE
FROM EXTRUSION PROCESSING

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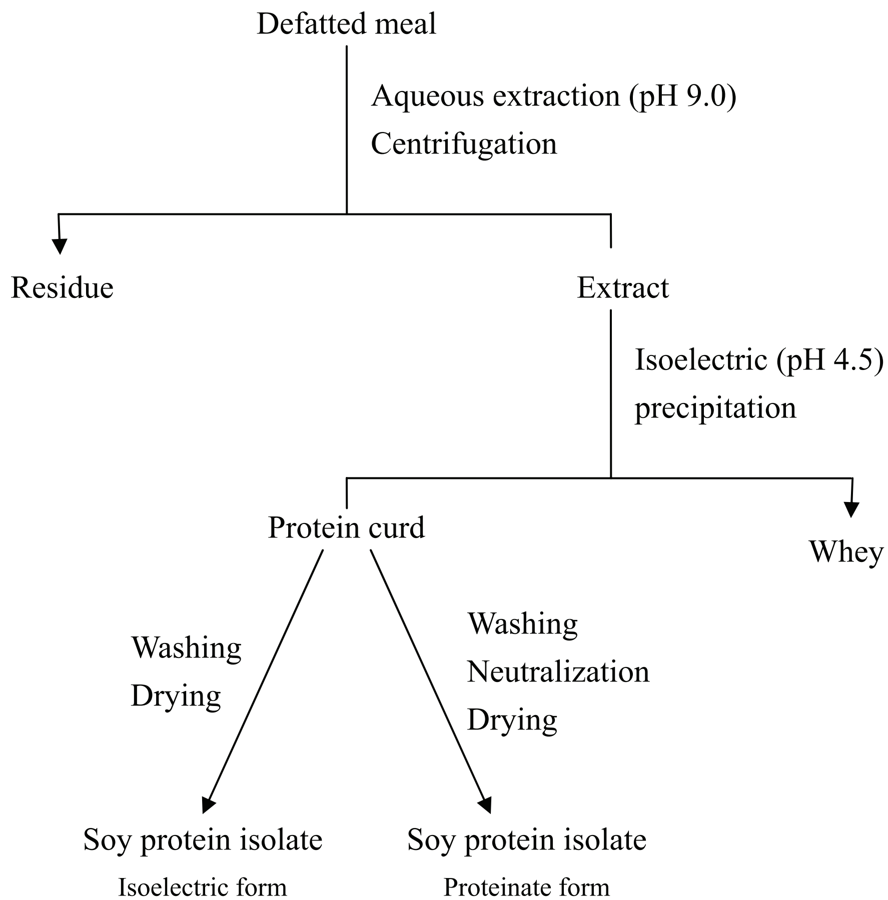

Fig 2.1.4 Outline of commercial processes for soy protein isolates (Hettiarachchy and Kalapathy 1997).

2.2 Food Extrusion

2.2.1 Extrusion Cooking

Food extrusion has been used to produce a variety of food for over 60 years. Extrusion cooking is now widely used in the food industry due to its versatility, high productivity, energy efficiency, and low cost. Extrusion cooking is a continuous thermomechanical process with multi-step or multifunction operation. It is a high-temperature, short-time process and may involve one or more of the following unit operations: mixing, hydration, shear, homogenization, compression, de-aeration, pasteurization or sterilization, stream alignment, shaping, expansion and fiber formation (Harper 1989; Cheftel and others 1992).

The extruder basically consists of a feeder/live bin that feeds the ingredient; screws that rotates inside a cylindrical barrel; and a die that dictates the shape of the extruded products. An example of a single-screw extruder is shown in Fig 2.2.1. The barrel is divided in to six sections from the feeder to before the cool die (zone 1 to 6) according the sensors to record the temperature. The feed is mixed with water and compressed by the screws as they rotate and pushes the feed forward though the heated barrel. Due to the friction and the heat provided inside the barrel, the feed is quickly heated. As the mixture advances along the barrel, pressure and heat build up. This pressurized cooking transforms the mass into a thermoplastic “melt” (Berk 1992). While the proteins undergo extensive heat denaturation, the directional shear force causes alignment of the high molecular components (Berk 1992). At the end of the barrel the melt is forced through the die. The sudden release of pressure leads to instant evaporation of some of the water. This causes puffing of the extrudates, thereby resulting in a porous structure. The extrudate's puffing or porous structure could be partially controlled by manipulating the melt temperature within the die.

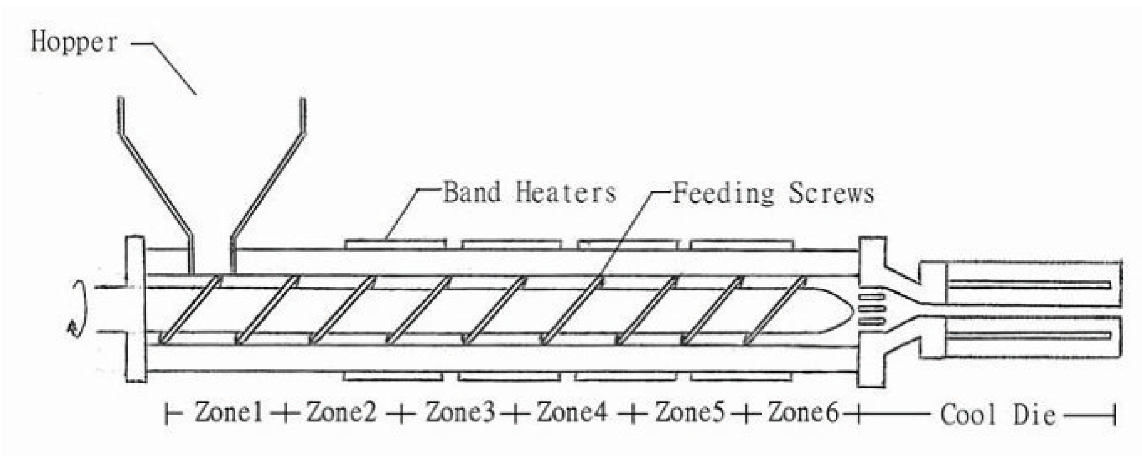

Fig 2.2.1 Example of a single-screw extruder.

2.2.2 Food Extruders

The extruders that were used in food production over 60 years ago are single-screw extruders. Their initial application was to mix and form macaroni and ready-to-eat cereals. As a result of continuous development effort, their versatility expanded and the single-screw extruders were used to produce a variety of foods such as cereals, snacks, croutons, dry pet foods, and precooked infant food in the 1960s. The twin-screw extruders were developed in the 1970s for its expanded operational capabilities and extended range of application (Harper 1989). In addition to manufacturing foods similar to those produced by the single-screw extruders, the twin-screw extruders are used to produce confectionery products.

In comparison to the twin-screw extruders, the single-screw extruders are relatively ineffective in transferring heat from the barrel jackets to the products. This is caused by the poor mixing within the extruder channel (Harper 1989). In single-screw extruders, heat is generated by friction or conversion of mechanical energy to heat or supplied by heated barrels. Twin-screw extruders have considerably more heat exchange capability than single-screw extruders, which expand their application to heating and cooling of viscous pastes, solutions, and slurries. The twin-screw extruders, therefore, are more suitable for processing high moisture materials, due to better heat transfer. In addition, the direction of screw rotation, screw shape, screw configuration and relative position of screw sections minimize pressure and leakage flows (Harper 1989; Noguchi 1989). Twin-screw extruders have less interaction of process variables than single-screw extruders, making them easier to operate and control (Harper 1989). Both types of extruder are widely used in the food industry. Due to their low cost, single-screw extruders remain to be an effective and economical choice to produce pet foods. The twin-screw extruders are mainly applied to products that require better control and operating flexibility. In this study, to have a better control, flexibility and the capability to extrude both dry materials (<35%) and high moisture materials (>50%), a twin-screw extruder is used.

2.2.3 Low Moisture Extrusion

Texturized vegetable protein (TVP), a commercialized meat analog, is produced by thermoplastic extrusion (Atkinson 1970). In this process, defatted soy flour with a mixture of 20-25% moisture is passed through a high pressure extrusion cooker producing a product that is porous and expanded. Although it does not have well defined fiber, it produces particulates that upon hydration have good mouth feel of chewiness and elasticity that symbolizes meat (Berk 1992; Liu 1997). Extrusion of defatted soy flour with moderate water content (up to 35%) have been studied extensively (Kelley and Pressey 1966; Cumming and others 1973; Burgess and Stanley 1976; Jeunink and Cheftel 1979; Hager 1984; Prudencio-Ferreira and Areas 1993).

2.2.4 High Moisture Extrusion

Although low moisture extrusion (up to 35% moisture) produces extrudates that have structured and textured features, it is hard to call the expanded and spongy looking extrudates “meat analogs” based on their appearance. Further disadvantages of low moisture extruded products are the time needed to rehydrate them with water or flavored liquid before use; their lack of meat flavor; and the low level of fat limiting their use as a meat alternative (Noguchi 1989). Therefore, researchers have been investigating the potential of using high moisture extrusion to improve the texturized vegetable protein for the last 20 years.

When soy protein is extruded under high pressure, high temperature and low moisture conditions, the sudden release of pressure upon exiting from the die causes instant water evaporation from the extrudates. This creates expanded and spongy structure of common texturized vegetable protein. To reduce extruder die pressure and extrudate expansion, soy protein needs to be extruded at a higher moisture content (>50%). In addition, a cooling die is essential in high moisture extrusion to increase the viscosity of the hot melt and reduce its fluidity so the necessary pressure and temperature can be maintained (Noguchi 1989). When proper cooling is applied, high moisture protein melt forced through the cooling die is the alignment of proteins due to directional shear force (Noguchi 1989). Unlike low moisture extrusion, the products from high moisture extrusion are dense and fibrous.

2.2.5 Repeated Extrusion

The reuse of material or semi-finished material is essential for the control of the ingredient cost in the food industries. For extrusion operation, it is common to blend ingredients with up to 15% of materials from start-up or shut-down operations or from extruded products that are out of specifications. The experiment of repeated extrusion gives an idea of the physical appearance and chemical characteristic of soy protein extrudates that have been extruded more than once. Isobe and Noguchi (1987) extruded defatted soy flour at 60% moisture with the barrel temperature setting at 130, 140 and 150°C, respectively. The extrudates were cut into small pieces and extruded two more times. The shape of extrudates was similar after repeated extrusion while the soluble protein fractions decreased or disappeared following the first extrusion and gradually decreased after additional extrusions. Extrudates that were extruded at 130°C were not much different from those extruded at 140 or 150°C. These results suggested that multiple extrusions appeared to have little effect on the extrudates.

2.3 Soy Protein Texturization and Effects of Texturization

2.3.1 Texturization of Soy Protein

Soybeans have been a major source of protein source in the eastern countries for centuries. Even though food products incorporated with soy protein are accepted in the western part of the world, the consumption of foods directly converted by soybean protein are still very limited (Berk 1992). Due to differences in culture and preference in texture and flavor, traditional Asian soy foods such as tofu, miso and yaba are not fully accepted in the American household. However, in recent years more and more Americans are becoming aware of the soy-based foods that could provide high quality protein, low fat, no cholesterol, and high fiber. Therefore, numerous efforts have been made to develop soy-based foods that might be acceptable to the western countries.

One notable effort is the texturization of soy protein into meat analogs. Several methods of texturing soy protein have been reported including spinning, thermoplastic extrusion, steam texturization, and enzymatic texturization. Thermoplastic extrusion of soy protein based on several patents, in particular, produces meat-like products that are known as TVP (textured or texturized vegetable protein). These products were first introduced in the 1970s and remain to be an important texturized soy protein food (Atkinson 1970; Liu 1997).

2.3.2 Heat and Shear Effects on Soy Protein

Food extrusion is considered a high-temperature short-time bioreactor that transforms raw feed material into modified intermediate and finished products (Harper 1989). The thermal extrusion exposes the proteinaceous ingredients to high temperature, high pressures and mechanical shear. This converts the soy protein into a continuous plastic “melt”, resulting in protein denaturation, reduce solubility and decrease extrusion effectiveness (Harper 1989). Within the process, water soluble fractions of soy protein (7S and 11S globulins) undergo a complex pattern of association-dissociation reaction (Cheftel and others 1985). Stanley (1989) concluded that the major influence of extrusion is to disassemble the proteins and then reconnect them into a fibrous, oriented structure possessing a characteristic texture.

It is known that thermal treatment of protein results in structural changes such as hydrolysis of peptide bonds, modification of amino acid chains and the formation of new covalent isopeptide cross-links. The effect of heat in the extrusion of soy protein has been studied systematically. Harper (1989) suggested that during extrusion, the protein is denatured and unfolded by shear and high temperature. Berk (1992) stated that while proteins undergo extensive heat denaturation, the directional shear force causes alignment of the high molecular components which lead to the texturization and the fiber formation on the extrudates.

Early works reveal that the obvious consequence of heat treatment to soy protein is the lost of solubility due to the formation of disulfide bonds, hydrogen bonds and hydrophobic bonds (Stanley 1989). Protein solubility is influenced by extrusion temperature, and with the increase of extrusion temperature a more textured product could be produced (Stanley 1989). Hayakawa and Kajiwara (1992) stated that the solubility of soy protein drastically decreased when it was heated to a temperature around 110-120°C. However, the solubility increased at temperatures above 150°C with over 10 min of heating time. Li and Lee (1996) found that when extruding wheat flour extrudates, the increase of die temperature in the extrusion process caused the intensity of higher molecular weight regions (> 25,000) to decrease with a concomitant increase of the intensity of low molecular weight regions (<25,000). They suggested that the fragmentation of high molecular weight proteins might be the reason that the content of soluble proteins in extrudates obtained at a higher temperature was a little higher than at a lower extrusion temperature. The aggregation of proteins during extrusion caused an increase in their molecular weight, which also resulted in a decreased protein solubility.

2.3.3 Pressure Effects on Soy Protein

The pressure produced in the extruder is usually less than 100 MPa (Noguchi 1989). During high moisture extrusion, the pressure is even lower due to low viscosity. Noguchi (1989) summarized the pressure effects on proteins: 1) carboxyl, phenol and amino residues on the protein dissociate and ionize as the pressure increases; 2) formation of hydrogen bonds reduces the volume of the system; 3) hydrophobic bonds increase with the increase of pressure; and 4) covalent bonds are not influenced by pressure.

In the study of the effect of pressure on protein solubility, soy protein isolate was either pressurized at 0, 50, 100 and 250 kg/cm^2 or heated at 110, 140, 170 and 200°C (Hayakawa and Kajiwara 1992). The result shows that protein solubility was strongly dependent on the heating temperature but not dependent on the pressure. Noguchi (1989) also stated that pressure less than 50 MPa do not influence the protein reactions during high temperature treatment.

2.3.4 Ingredient Effects on Extrusion

Major components in the extrusion of texturize soy protein may include soy proteins, water and carbohydrates. Soy proteins are the foundation of texture formation. The increase of protein levels would greatly influence the rheological properties of extrudates (Maurice and Stanley 1978; Rhee and others 1981; Sheard and others 1985). Stanley (1989) concluded that both protein quality and quantity have an important effect on soy protein texturization. Water is also an important factor in extrusion due to the effect on heat transfer during extrusion. Higher moisture content would result in a better heat transfer from the extruder barrel to the feed material and it would also lower viscosity, shear and friction during extrusion.

Another important role of water is in the separation of proteins, which helps the formation of protein fibrous structure (Noguchi 1989). During extrusion, protein undergoes a plastic melt in which protein denatures and aggregates (Harper 1989). While protein is melted into this elastic mass, large amount of water combined with carbohydrate such as starch would lead to a phase separation which enhances protein to protein interaction (Noguchi 1989). Such phase separation with the help of shear force at the die would induce the formation of proteinaceous fibrous structures (Harper 1989; Noguchi 1989). Starch is found to distribute throughout the protein fibrous matrix and never incorporated into the protein fibers (Noguchi 1989). Low moisture extrusion has less fibrous structure due to the limited amount of water that could be absorbed by starch resulting in a weaker phase separation.

2.3.5 Protein Texturization Mechanisms

Many efforts have been made to investigate protein-protein reactions and texturization during extrusion cooking. Intermolecular disulfide bonding was considered the texturization mechanism during extrusion due to its importance in food systems such as wheat dough and spun soy fibers (Kelley and Pressey 1966; Li and Lee 1996). Jeunink and Cheftel (1979) extruded soy protein concentrate at 145°C with 32% moisture. Prudencio-Ferreira and Areas (1993) studied soy protein isolates samples that were extruded at 140,160, and 180°C with 30 and 40% moisture. Lin and others (2000) extruded soy protein isolate that were extruded under 137.8, 148.9 and 160°C with 70, 65 and 60% moisture. All three studies concluded that the major forces responsible for protein insolubilization in the extrudates were due to hydrophobic interaction, hydrogen bonding, and covalent intermolecular disulfide bridges. However, others (Burgess and Stanley 1976; Hager 1984) found that after extrusion of soy protein concentrate there was a major increase in sulfhydryl groups, and the disulfide bond decreased rather than increased (Table 2.3.1). Some researchers also reported that during extrusion of soy protein, there was no significant loss of sulfur amino acids (Jeunink and Cheftel 1979). With these findings Stanley (1989) concluded that because the disulfide bond formation during extrusion was solely based on the indirect evidence from solubility experiments, it cannot be used to infer that intermolecular disulfide bonds contribute significantly to the texturization of soy protein during extrusion. Harper (1989) suggested that during extrusion, cross-linking reactions occur. However, Noguchi (1989) reported that there is little change in the distribution of protein fractions before and after extrusion based on the electrophoresis study. He concluded that the proteins are hardly modified by extrusion.

Table 2.3.1 Disulfide and sulfhydryl concentrations in native and extruded soy concentrate (Hager (1984); Burgess and Stanley (1976)).

Researcher	Extrusion Temp.	(mol/mg)	Native soy concentrate	Extruded soy concentrate
Hager (1984)	140ºC	-S-S- content	22.7×10^{-8}	19.6×10^{-8}
		-SH content	0.5×10^{-8}	4.1×10^{-8}
Burgess and Stanley (1976)	178ºC	-S-S- content	4.5×10^{-8}	0.9×10^{-8}
		-SH content	3.3×10^{-8}	48.9×10^{-8}

2.4 Analysis of Soy Protein

2.4.1 Protein Solubility

The effects of extrusion on proteins are difficult to isolate and determine since the proteins are exposed to several processes simultaneously. There are several ways to analyze extrudate for their chemical properties. One of which is the use of different solvents to analyze protein solubility as a tool to investigate protein-protein interaction and protein texturization of both raw materials and extrudate. As early as 1966, protein solubility was used to investigate the fiber formation of spun soy fibers (Kelley and Pressey 1966). They concluded that hydrogen bond, ionic bond and disulfide bond contribute to the formation of spun soybean fibers. Burgess and Stanley (1976)

CHAPTER 3

EFFECT OF LOW MOISTURE AND HIGH MOISTURE EXTRUSION ON PROTEIN-PROTEIN INTERACTIONS IN SOY PROTEIN ISOLATE

3.1 Introduction

Extrusion cooking is widely used in the food industry due to its versatility, high productivity, energy efficiency, and low cost. It is a continuous thermomechanical process with multi-step or multifunction operation. Texturized vegetable protein (TVP) is one of the patented products produced by thermoplastic extrusion (Atkinson 1970). In this process, defatted soy flour with a mixture of 20-25% moisture is passed through a high pressure extrusion cooker producing a product that is porous and expanded. These low moisture extrusion (up to 35%) soy protein products have been studied extensively (Burgess and Stanley 1976; Jeunink and Cheftel 1979; Hager 1984; Prudencio-Ferreira and Areas 1993). However, the products of low moisture extrusion are expanded and spongy which are hard to call them “meat analogs” based on their appearance. Therefore, researchers have been investigating the potential of using high moisture extrusion to improve the texturized vegetable protein for the last 20 years (Noguchi 1989; Cheftel and others 1992; Akdogan 1999).

When soy protein is extruded under high pressure, high temperature and low moisture conditions, the sudden release of pressure upon exiting from the die causes instant water evaporation from the extrudates. This creates expanded and spongy structure of common texturized vegetable protein. To reduce extruder die pressure and extrudate expansion, soy protein needs to be extruded at a higher moisture content (>50%). In addition, a cooling die is essential in high moisture extrusion to increase the viscosity of the hot melt and reduce its fluidity so the necessary pressure and temperature can be maintained (Noguchi 1989). When proper cooling is applied the result of high moisture protein melt forced through the cooling die is the alignment of proteins due to directional shear force (Noguchi 1989). Unlike low moisture extrusion, the products from high moisture extrusion are dense and fibrous.

Although the development of both low moisture and high moisture soy protein extrusion products have been successful, the mechanism of protein-protein reactions and texturization during extrusion cooking are been disputed. Intermolecular disulfide bonding was considered the texturization mechanism during extrusion due to its importance in food systems such as wheat dough and spun soy fibers (Kelley and Pressey 1966; Li and Lee 1996). Burgess and Stanley (1976) however, investigated the mechanism of thermal texturization of low moisture extruded soybean protein. Their result was that disulfide bonds do not play the most important role in texturization as in the fibers formed during thermal texturization. The intermolecular peptide bond instead contributes mainly to the thermal texturization. Jeunink and Cheftel (1979) extruded soy protein concentrate at 145°C and 32% moisture. Prudencio-Ferreira and Areas (1993) studied soy protein isolates samples that were extruded at 140, 160, and 180°C with 30 and 40% moisture. Lin and others (2000) extruded soy protein isolate at 137.8, 148.9 and 160°C with 60-70% moisture. All three studies concluded that the major forces responsible for texturization of the soy protein extrudates were due to disulfide bonds and noncovalent interaction.

All these studies have been done on either low moisture extrusion products or high moisture extrusion products. Thus, the objective of this study is to investigate: 1) the effect of extrusion temperature on low moisture and high moisture extrusion and 2) comparison between low moisture with high moisture extrusion soy protein isolate products. In addition, 3) changes of protein to protein interaction in high moisture extrusion within the extruder was investigated by the dead stop procedure.

3.2 Materials and Methods

3.2.1 Materials

Soy protein isolate (SPI) (Profam 974) was obtained from Archer Daniels Midland (Decatur, IL) containing a minimum 90% w/w protein. Starch (Midsol 50) was provided *in gratis* by MGP Ingredients, Inc. (Atchison, KS). Their proximate compositions are shown in Table 1. The ingredients were mixed in 9:1 ratio using a Double Action™ food mixer (Model 100DA70, Leland Southwest, Fort Worth, TX) for 10 min to ensure the uniformity of the feeding material.

3.2.2 High Moisture Extrusion and Dead Stop Procedure

An MPF 50/25 co-rotation intermeshing twin-screw extruder (APV Baker, Inc., Grand Rapids, MI) was used. The extruder has a screw length to diameter ratio of 15 to 1 and the diameter is 50 mm. A cooling die with dimensions W × H × L of 30 × 10 × 300 mm was attached at the end of the extruder with 4.4ºC cold water as the cooling media. The screw profile from feed to exit were 100 mm twin lead feed screws, 50 mm 30º forward paddles, 100 mm single lead feed screws, 87.5 mm 30º forward paddles, 175 mm single lead feed screws, 87.5 mm 30º forward paddles, 50 mm 30º reverse paddles, 100 mm single lead feed screws and finally the cooling die. The barrel was divided into six sections and the barrel temperature settings from zone 1 to zone 5 were 22.9, 24, 42.1, 96.3, and 136.1ºC, respectively. Zone 6 barrel temperature and other independent extrusion variables are listed in Table 3.2.1. Dead stop extrusion was also conducted to investigate changes in feed material within the extruder barrel. The temperature at zone 6 was set at 137.8ºC. After steady state was reached, the extruder was abruptly stopped and the barrel was cooled immediately and split opened within 5 min. Samples were collected from zone 2 to 6, cooling die and product. All samples were sealed and stored at -20ºC for further analysis.

3.2.3 Low Moisture Extrusion

The low moisture extrusion was operated with the same extruder as the high moisture extrusion. The screw configuration from feed to exit were 25 mm single lead feed screws, 200 mm twin lead feed screws, 125 mm 30º forward paddles, 50 mm single lead feed screws, 37.5 mm 60º forward paddles, 37.5 mm 60º reverse paddle, 50 mm single lead feed screws, 25 mm 90º paddles, 87.5 mm 30º forward paddles, 37.5 mm 30º reverse paddle, 75 mm single lead feed screws. In order for high moisture extrusion and low moisture extrusion to have similar product temperatures, the final zone temperature of dry extrusion was set according to Table 3.2.2. The temperature settings in the extruder from zone 1 to zone 5 were 19.7, 24.2, 49.8, 89.8ºC, respectively. Zone 6 was set at 118.3, 120.1, 125.2ºC for the product temperatures of 125.3, 134, 140.6ºC, accordingly. All samples were preserved by freezing at -20ºC after extrusion.

Table 3.2.1 Experimental design for high moisture extrusion.

Conditions	Levels
Final barrel temperature setting (zone 6)	137.8, 148.9, 160ºC
Product temperature	124.2, 134, 140.6ºC
Moisture content	60%
Screw speed	200 rpm
Water feed rate	12.2 kg/h (26.8 lb/h)
Dry feed rate	9.1 kg/h (20 lb/h)
No. of Replications	4
Formula	90% SPI, 10% wheat starch

Table 3.2.2 Experimental design for low moisture extrusion.

Conditions	Levels
Final barrel temperature setting (zone 6)	119.6, 130.3, 140.7ºC
Product temperature	121.7, 130.1, 138.6ºC
Moisture content	35%
Screw speed	200 rpm
Water feed rate	4 kg/h (8.8 lb/h)
Dry feed rate	9.1 kg/h (20 lb/h)
No. of Replications	4
Formula	90% SPI, 10% wheat starch

3.2.4 Protein Solubility

Protein solubility was tested on soy protein isolate, extruded products from the high and low moistrue, and samples collected from the dead stop procedure. The following were six solvents used in this study: 1) 0.035 M, pH 7.6 phosphate buffer solution (PBS) (known to extract proteins in their native state); 2) 8 M urea in the phosphate buffer solution (known to dissolve the proteins with hydrogen bonds and hydrophobic interactions); 3) 2% 2-mercaptoethanol (2-ME) in the phosphate buffer solution (known to disrupt the disulfide bonds); 4) 8 M urea + 2% 2-ME in the phosphate buffer solution; 5) 1.5% sodium dodecyl sulphate (SDS) in the phosphate buffer solution (used for their ability to interrupt hydrophobic and ionic interactions); and 6) 8 M urea +

3.4 Conclusion

In both low moisture and high moisture extrusion, increasing product temperature from 125.3 to 140.6°C did not have significant difference on proteins that could be extracted by various solvents. The moisture content did have significant affect on the protein solubility, however no difference in protein subunit distribution was observed. In the dead stop experiment, the results suggest that the network of polypeptide chains interfered and lowered the protein solubility were formed in zone 3 and zone 4. This led to little difference in the protein solubility of after zone 4 of extruder barrel to the final product. The chemical bonds that contributed to the texturization of proteins were the same through zone 4 to the product and proteins were aligned by the directional shear force at the cooling die. Therefore, the major drop in protein solubility after low moisture extrusion, high moisture extrusion and that of zone 3 and 4 in the dead stop procedure, was due to the formation of the three dimensional network of soy protein isolate polypeptide chains. These polypeptide chains aggregated together during extrusion and became less accessible to the solvents used to extract the soluble proteins.

Despite differences in low and high moisture extrusions the solvent combination of PBS+2-ME+Urea extracted the proteins most. This was followed by the solvent combination of PBS+SDS+Urea. In addition, there was no significant change in the molecular weight distribution of protein subunits between the control and extrudates. Based on the results from protein solubility and SDS-PAGE, It appears that the effect of extrusion is to disassemble proteins and then reassemble them together by disulfide bonds, hydrogen bonds and noncovalent interactions forming fibrous structure in extrudates, Despite major difference in appearance, similar protein to protein interactions occurred in both low moisture and high moisture extrusion.

The suggestion by Burgess and Stanley (1976) that intermolecular peptide bonds were the main contributor to protein texturization is questionable since breaking and forming of peptide bonds only occurs at high critical temperatures or very high or low pHs. There was little evidence that peptide bonds could be formed, broken, and formed again during high moisture extrusion cooking at moderate temperatures. If a significant amount of cross-linking exists, the protein subunit of the SDS-PAGE should exhibit a different distribution between the extrudate and the control. Therefore, it appears that the extrusion of soy protein isolates modified very little at the molecular level and that the protein subunits did not have any structural changes.