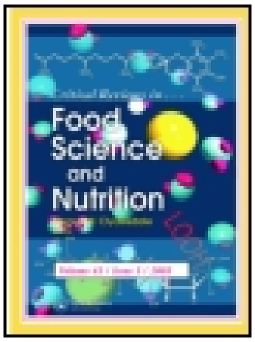

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Changes in conformation and quality of vegetable protein during texturization process by extrusion

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ABSTRACT

Texturized Vegetable Protein (TVP), as the meat analogues, has aroused the attention due to the advantages of health and nutrition. During the extrusion process of TVP, under the comprehensive effects of temperature, shear force, and pressure, complex conformational changes and molecular interactions amongst protein, carbohydrate, lipid, and other components occur, which determine the quality of TVP. Control of the extrusion process is still a big challenge. Therefore, this review summarized the development and current status of food extrusion technology for the production of TVP and gave detailed descriptions about the conformational changes of the main components during the extrusion process, focusing on the effects of barrel temperature, moisture content, feed rate and screw speed on TVP quality. Lastly, we discussed the approaches to characterize the extrusion process and proposed a new system analysis model.

KEYWORDS

Texturized vegetable protein; Extrusion process; Extrusion parameters; Conformational changes; Changes in quality; Characterization approaches

1. Introduction

With an estimated world population of 9 billion by 2050, one of the biggest challenges to global food security is ensuring that protein requirements can be met in a way that is affordable, healthy and environmentally responsible (De, Schösler, and Aiking 2014; Smetana et al. 2017). The environmental limitations of animal protein production combined with increasing advocacy for the health benefits of vegetable protein account for the importance of the research and development of meat analogues (Kumar et al. 2017).

Food extrusion technology has been utilized to produce texturized vegetable protein (TVP) for more than 50 years. The TVP produced by extrusion possesses functional properties such as rich fibrous structure like the muscle fibers in animal meat, high water absorbing capacity (WAC), and high water holding capacity (WHC). It also contains zero cholesterol and has a biological digestion potency that can reach 93%～97% in the human body (Akdogan 1999; Riaz 2001). It has been confirmed that some functional components in vegetable protein such as isoflavones, saponins and some characteristic amino acids such as arginine play an important role for anti-aging, decreasing blood pressure, and promoting mineral absorption (Marcus 2013). Therefore, it may be possible to prevent modern civilization diseases such as obesity, hypertension and cardiovascular disease with regular consumption of TVP as meat additives or meat analogs (Singh, Gamlath, and Wakeling 2007; Conti e Silva, da Cruz, and Areas 2010). Today, TVP is widely used in meat products, frozen food, instant food and snack food. About 500,000 tons of TVP was used in the production of ham sausage, frozen dumplings, instant noodles spices, fish balls and spicy strip in China in 2015 (Zhang, Liu et al. 2017). These years, the world demand for TVP continues to grow which shows that TVP popularity is rising (Jones 2015).

Even though, the market for meat analogues is still quite small. For example, in the Netherlands, the share of meat substitutes is only about 1% of the total market for meat and meat products, probably due to the fact that present meat analogues do not meet consumer preferences with regard to sensory quality. Recently, consumers have started to move towards meat alternatives in order to have a healthy, sustainable and convenient diet and because they want to try new food products. To obtain a larger market share, meat analogues need to compare better to real animal meat (Wild et al. 2014).

The quality of TVP can be affected by the raw materials (e.g. components, granularity, pretreatment conditions and food additives, etc) (Ning and Villota 2007; Day and Swanson 2013), the extruder (e.g. screw configuration, die design and screw length to diameter ratio, etc) (Zhang et al. 2015), and the extrusion parameters (e.g. barrel temperature, moisture content, feed rate and screw speed, etc) (Lin, Huff, and Hsieh 2002). As a biochemical reactor, the extruder can provide temperature, shear force, and pressure by adjusting the extrusion parameters (Gujral, Singh, and Singh 2001). During the extrusion process, under the comprehensive effects of temperature, shear force, and pressure, it induce the simultaneous denaturation, degradation, association, and aggregation of proteins, degradation of carbohydrates, and oxidation of oil (Cheftel, Kitagawa, and Quéguiner 1992; Camire, Camire, and Krumhar 1990).

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Table 1. Overview of the extruders selected for the production of TVP.

Manufacturers	Extruders (length–diameter ratio of the screw)	Research aspects	Research unit	References
Clextral (France)	EV25 (twin screw 24:1)	High moisture extruded-texturized soybean protein	Heilongjiang Academy of Agricultural Sciences of China	(Hong et al. 2016)
	BC45 (twin screw 24:1)	High moisture extruded-texturized compound vegetable proteins	National University of Science and Technology of France	(Thiébaud, Dumay, and Cheftel 1996)
Brabender (Germany)	DSE-25 (twin screw 20:1)	High moisture extruded-texturized soybean protein	Chinese Academy of Agricultural Sciences	(Chen et al. 2010; Zhang et al. 2015)
Coperion (Germany)	ZSK 26 Mc (twin screw 29:1)	High moisture extruded-texturized wheat proteins	Karlsruhe Institute of Technology	(Pietsch, Emin, and Schuchmann 2016)
APV Baker (USA)	MPF 50/25 (twin screw 15:1)	Vegetable protein meat analogues	University of Missouri – Columbia	(Yao, Liu, and Hsieh 2004)
Thermo Fisher Scientific (UK)	Haake16 (25:1)	Pea protein meat analogues	Fraunhofer Institute of Germany	(Osen et al. 2014)
Extrutec (Brazil)	(single screw 20:1)	Low moisture extruded-texturized chickpeas protein	University of Sao Paulo	(Moreira-Araújo, Araújo, and Arêas 2008)
Buhler (Switzerland)	CAMPACtwin^{TM}62 (twin screw 20:1)	Low moisture extruded-texturized wheat protein	Jiangnan University	(Ma 2013)
Saibainuo (China)	SYSLG30-IV (twin screw 20:1)	High moisture extruded-texturized wheat protein	Northeast Agricultural University of China	(Yang 2009)
	SLG-32-II (twin screw 20:1)	High moisture extruded-texturized soybean protein	Zhejiang University of Technology	(Zheng 2009)
	SLG-32-II (twin screw 20:1)	Low moisture extruded-texturized compound vegetable proteins	Tianjin University of Science and Technology	(Chen 2011; Wang 2013)
FUMACH (China)	FMHE-36 (twin screw 24:1)	Low moisture extruded-texturized wheat protein	Hefei University of Technology	(Li et al. 2016)
Beijing JinDi Sanfu (China)	TXLL110 (twin screw)	Low moisture extruded-texturized peanut protein	Lanzhou University of Technology	(Lang, Yan, and Shi 2011)

Additionally, interactions between molecules are accompanied by changing molecular conformation in the extruder, thus forming new conformation and texture (Day and Swanson 2013).

However, control of the extrusion process precisely is still very challenging, which severely hindered the development of extrusion technology. Extrusion is a thermomechanical process, in which the feed components undergo complex physical and chemical changes and exit the extruder as a transformed product, but the incremental changes that occur along the barrel are difficult to understand (Emin and Schuchmann 2016). The complex physical, chemical and biological reactions that occur result in both macroscopic (shape, texture and color) and microscopic changes (molecular conformation and chemical bonds) (Day and Swanson 2013). In order to determine the key control points for quality optimization, it is necessary to discuss the conformational changes of the main components by dividing the extrusion process into several parts.

Therefore, the objective of this review is to summarize the development and current status of food extrusion technology for the production of TVP; to analyze the conformational changes of the main components during extrusion process; to examine the effect of extrusion parameters on the quality forming process of TVP; and to discuss the approaches to characterize the extrusion process.

2. Overview of food extrusion technology for the production of TVP

The development of food extrusion technology can be divided into four stages. The first stage was before the 1940s and mainly involved shaping of products such as enema and pasta by single-screw extruders (Wei, Zhang, and Chen 2011; Alam et al. 2016). Between the 1940s～1980s, food extrusion technology has developed into the second stage with extrusion becoming a high-temperature-short-time (HTST) biological reaction process for producing instant food, snacks and nutritious food for children (Camire, Camire, and Krumhar 1990; Riaz 2000). The 1980s marked the beginning of the third stage, with breakthroughs in the areas of extruder structure, monitoring technology and quality analyzing methods for extrudates. During this period, twin-screw extruders began to replace the single-screw extruder due to their higher processing capacity, lower energy consumption (between 200 and 1200kJ/kg) and wide range of moisture capabilities (from 5% to 95% of the dry feed rate), (Krintiras et al. 2016; Cheftel, Kitagawa, and Quéguiner 1992). Extruders manufactured by some well-known enterprises such as Clextral, Brabender, Coperion, and APV Baker have been widely used for the production of TVP, as shown in Table 1.

Texturization via extrusion technology can make products that imitate the texture and the appearance of meat while providing high nutrient content (Cheftel, Kitagawa, and Quéguiner 1992; Singh, Gamlath, and Wakeling 2007). Based on the moisture content, TVP can be divided into two categories: low moisture TVP (LM-TVP) and high moisture TVP (HM-TVP), which contain about 20～40% and 40～80% moisture, respectively (Riaz 2001; Akdogan 1999). Low moisture extrusion technology was developed earlier than the high moisture extrusion technology (Akdogan 1999). The texture of LM-TVP is similar to that of a sponge, which should be rehydrated and used as a meat additive (Alam et al. 2016). LM-TVP is also the mainstream TVP available in today's market (Wei, Kang, and Zhang 2009; Wild et al. 2014). However, high moisture extrusion technology is a relatively new technology for protein recombination with the potential to create TVP products with higher quality than low moisture process. High moisture extruder systems have a longer cooling die than low moisture, allowing for fibrous structure formation at relatively low temperature (lower than 75°C) (Liu and Hsieh 2008). While for low moisture extrusion technology, the die is shorter and the temperature is usually higher than 120°C at the die for protein texturizing and expanding (Areas 1992; Normelljhoee et al. 2009). HM-TVP possesses a texture similar to animal meat with a rich fibrous structure, dense structure, strong elasticity, and high moisture content (Wild et al. 2014). In addition, some nutrients or bioactive substances can be maximally retained due to the relatively low temperature achieved with high moisture extrusion (Lin, Huff, and Hsieh 2002; Akdogan 1999). HM-TVP can be eaten directly or flavored for added appeal to consumers and product line extensions (Akdogan 1999). At the present, only a few enterprises like the Beyond Meat in America and several European countries are producing HM-TVP. The high moisture extrusion technology is still in the theoretical research stage in China (Zhang et al. 2015). This may be due to the particular requirements of the extruder, poor aroma and taste quality of the products and higher storage costs (Wild et al. 2014; Grabowska et al. 2016). And also, consumers need a certain amount of time to accept a new product (Hoek et al. 2011). For the better application of high moisture extrusion technology in TVP, researchers should focus on the manufacturing of the extruder, improving the quality of the products and revealing the mechanism based on the conformational changes of macromolecules.

Since the 1990s, some new extrusion technologies have emerged after the improvement of the extruder. Supercritical fluid extrusion (SCFX) is a hybrid processing operation that utilizes supercritical carbon dioxide (SC-CO_2) as the blowing agent in lieu of steam (Rizvi and Mulvaney 1992). The low critical temperature of CO_2 (about 31°C) allows for the lowering of process temperature via coolant circulation to below 100°C, better preserving temperature-sensitive vitamins and micronutrients (Sharif, Rizvi, and Paraman 2014). Extrudates produced via SCFX have been shown to possess a more uniform cellular structure and smoother surface, which is attributed to higher nucleation rates, thus limiting the diffusion of gas (Chauvet et al. 2017). Recent research of SCFX has also focused on the incorporation of whey protein in extruded products, which are difficult to extrude under normal extrusion conditions of high temperature and shear due to the high content of protein and carbohydrates that are susceptible to browning (Tremaine and Schoenfuss 2014). Two-stage or multi-stage extrusion, by connecting multiple extruders in series or in parallel, making the components configurational changes easier to control. It has been applied for the production of vegetable protein meat analogues (Wenger, USA) or starch-based materials (Fishman et al. 2006; Tiwari, Patil, and Repka 2016). The combination of extruder and 3D printer has greatly improved the automation of extrusion technology, which has been applied in the pharmaceutical field (Pietrzak, Isreb, and Alhnan 2015). Improving the versatility, visibility and intelligence of the extruder is the developing direction of the new extrusion technology.

3. Main Components and their Conformational Changes during the Extrusion Process of TVP

According to the rheological state of material during the extrusion process, it can be generally divided into four main functional zones: the feeding zone, the mixing zone, the melting zone, and the die. While for the high moisture extrusion, a long cooling zone after the die was necessary, shown in Figure 1 (Cheftel, Kitagawa, and Quéguiner 1992). In the feeding zone, the barrel is always at room temperature. And the screw is just made up of conveying elements, which mainly plays the role of delivery and provides weak shear force (Zhang et al. 2015). So that it could not influence the conformation of protein significantly in this zone. In the mixing zone, the screw is often equipped with some kneading elements. Mixing of the materials with water occurs mainly in this zone. And also, the temperature is always lower than 80°C to ensure that the water can be injected into the extruder normally. The melting zone is the core functional area of the extrusion process with more kneading elements on the screw, in which the significant physical and chemical changes of vegetable protein occur at high temperature (generally higher than 130°C) (Zhang et al. 2017; Thiébaud, Dumay, and Cheftel 1996). The design of the die is often seen as the key for controlling the flowing behavior of the melt and then affecting the TVP quality (Kostic and Reifschneider 2006; Akdogan 1996). When the melt passing through the die, the melt forms a specific shape under the action of shear stress, pressure drop and water evaporation. For high moisture extrusion, the die could promote the macromolecules separate into a continuous and a dispersed phase, which furtherly affect the formation of fibrous structure (Thiébaud, Dumay, and Cheftel 1996). In the cooling zone after the die, the temperature is slightly below 75°C, which ensures the protein molecular rearrangement and promotes to form the fibrous structure (Areas 1992; Yao, Liu, and Hsieh 2004).

3.1. Protein

Protein is the most important component of TVP and includes many types such as soybean protein, peanut protein and wheat gluten (Wei, Kang, and Zhang 2009; Yao, Liu, and Hsieh 2004; Rehrah et al. 2010). Extrusion of TVP generally requires a protein content between 50%~70% in order to form a fibrous structure (Zhang et al. 2007). During the extrusion process, the protein undergoes about four main stages of conformational changes (Areas 1992; Liu and Hsieh 2008), which include unfolding of the molecular chains, association, aggregation, and cross-linking with potential degradation or oxidation, as shown in Figure 2 (Camire 1991; Day and Swanson 2013).

In the mixing zone, a homogenous dough that contains the raw material and water would be formed under the stirring action of the screw (Don et al. 2003; Zhang et al. 2015). The molecular chains of the protein are unfolded along the flowing direction, exposing hydrophobic amino acids that were originally enclosed inside the molecules (Akdogan 1999; Day and Swanson 2013). In the melting zone, the flowing rate of the melt becomes much slower due to the resistance of kneading elements and the high temperature, which would promote the protein-protein interactions and protein-water interactions (Zhang et al. 2017; Manoi 2009; Pietsch, Emin, and Schuchmann 2016). As the results, the association or aggregation of protein was enhanced, showing as the increase of viscosity (Osen et al. 2015; Chen, Wei, and Zhang 2011; Ai et al. 2016). Under the strong shear force, protein molecular chains may also be degraded in this zone. In the die, for low moisture extrusion, the pressure drop

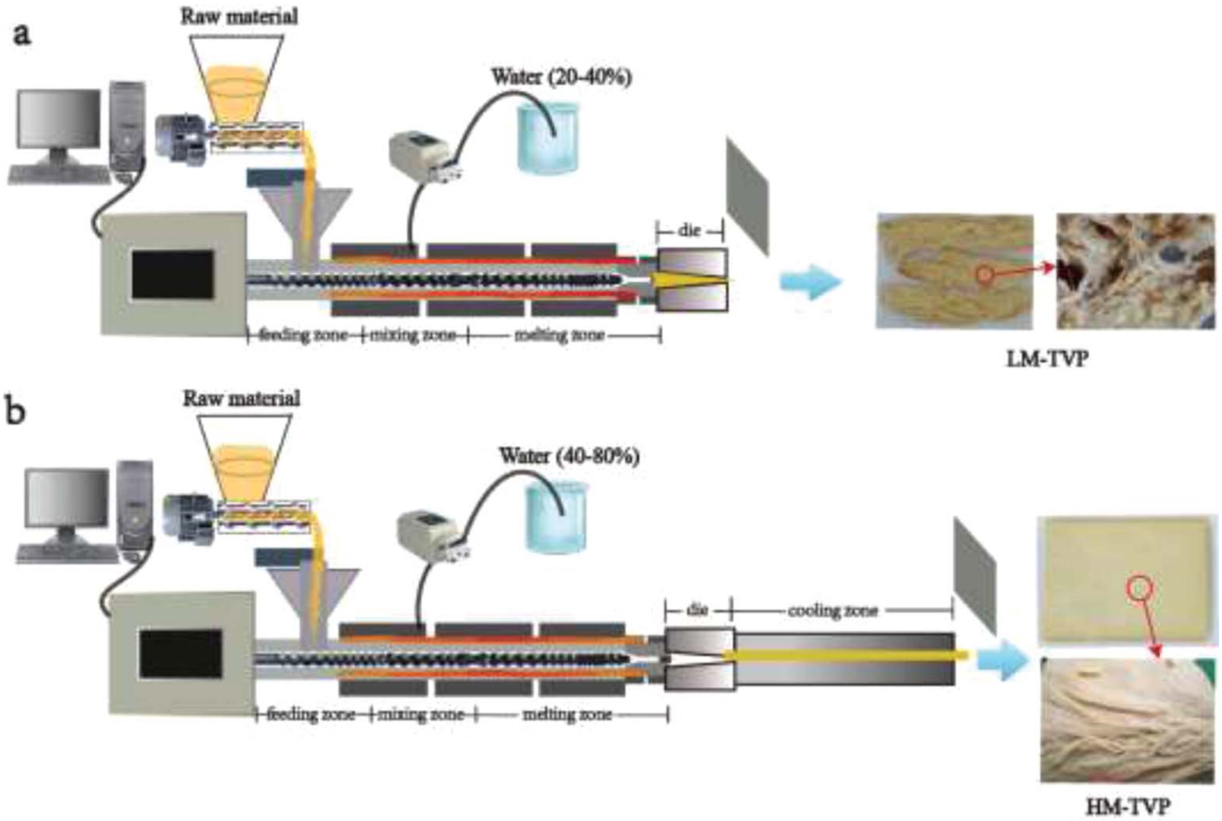

Figure 1. Functional partition of the extrusion process for low moisture extrusion (a) and high moisture extrusion (b).

and water evaporation promote the hot melt to form a puffing structure supported by protein cross-linking. For high moisture extrusion, the die can provide the shear stress perpendicular to the extrusion direction, which lead to the phase separation of macromolecules with protein as the continuous phase (Thiébaud, Dumay, and Cheftel 1996). In the cooling zone, the lowering of the temperature allows for the gradual cooling of the extrudate, which ensures a laminar flow of the melt in this zone (Osen et al. 2014; Maurya and Said 2014). In this zone, rearrangement and cross-linking of protein molecules occur to form a fibrous structure (Areas 1992; Yao, Liu, and Hsieh 2004).

3.1.1. Interactions of protein molecules during extrusion

Interactions of protein molecules determines the change of viscosity, gelation, solubility and other functional properties (Day and Swanson 2013). Studies have shown that during the extrusion process, the interactions which maintain the initial conformation of the protein were changed, but generally the major chemical bonds such as peptide bonds would not be changed (Osen et al. 2015; Shah 2003; Ledward and Tester 1994). Disulfide bonds, hydrophobic interactions and hydrogen bonds have been identified as the main force that determine TVP structure, whether the sample is in the extruder barrel or cooling zone (Liu and Hsieh 2008; Chen, Wei, and Zhang 2011). Under very extreme conditions (high temperature and strong shear force), it is possible to form more covalent bonds (Areas 1992; Cheftel, Kitagawa, and Quéguiner 1992; Zhang et al. 2017). Factors such as protein types, pretreatment approaches and extrusion conditions play an important role in the formation of protein-protein interactions. According to Prudencioferreira and Jag (Prudencioferreira and Jag 1993), the main forces in texturized soybean protein (TSP) were disulfide bonds followed by hydrophobic interactions and electrostatic interactions. These findings were consistent with the research by Ma (Ma 2013) and Hong et al (Hong et al. 2016). But Ning and Villota (Ning and Villota 2007) found that among all molecular interactions, non-covalent interactions such as hydrophobic interactions and hydrogen bonds appeared to be dominant in the fibrous structure of TSP. Disulfide bonds were also found to make an important contribution to the formation of the fibrous structure (Liu and Hsieh 2007). This may be due to the different ratios of 7S (β-conglycinin) and 11S (glycinin) proteins in the material (Nishinari et al. 2014). For texturized peanut protein (TPP), the main forces are non-covalent bonds (hydrophobic interactions and hydrogen bonds) followed by disulfide bonds (Nor Afizah and Rizvi 2014; Wei et al. 2007). Further investigation is needed to clarify these changes in protein conformation during the extrusion process.

Temperature is one of the most important factors that cause conformational changes in protein (Zhang et al. 2017). When the temperature gradually increases during the extrusion process, firstly the hydrogen bonds breakdown, allowing the protein chains to gradually unfold (Shah 2003). In the melting zone, the temperature would increase sharply, which results in the disruption of intramolecular disulfide bonds and the formation of new intermolecular disulfide bonds. If the temperature is higher than 150°C, these newly formed disulfide bonds are subsequently disrupted, thus increasing the content of free thiol (Liu and Hsieh 2008; Hager 1984). In comparison to 135°C, a process temperature of 115°C promoting the formation of intermolecular disulfide bonds for peanut protein (Shah 2003).

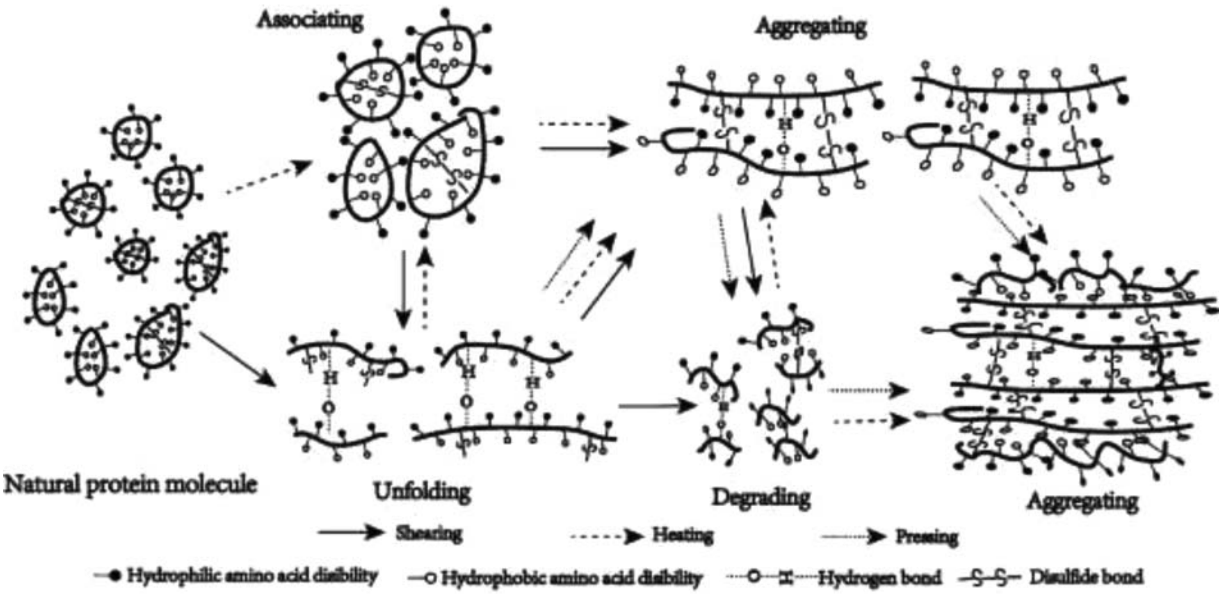

Figure 2. Conformational changes of protein during the extrusion process.

Water is an important medium to induce conformational changes in protein (Li et al. 2014). Protein molecular weight is reduced under low moisture conditions (20∼40%) due to the strong shear force (Vaz and Areas 2010; Smetana et al. 2017). Increasing water content in the range of 20% to 40% would lead to significant increase in the reaction rates of proteins (Emin et al. 2017). Under high moisture conditions (40%∼80%), the large amount of free water reduces the mechanical strength and the degree of polymerization of the protein subunit (Chen, Wei, and Zhang 2011; Liu and Hsieh 2008). In addition, the synergistic effect of disulfide bonds with hydrogen bonds and hydrophobic interactions promote a high degree of fibrous structure formation at a wide moisture range from 20% to 60% (Chen, Wei, and Zhang 2011; Hong et al. 2016).

Shear force would significantly reduce the protein molecular weight which leads to exposure of thiol groups attached to amino acids such as cysteine. With the participation of oxygen, thiol groups are oxidized to form disulfide bonds, thus promoting the formation of large protein aggregates (Vaz and Areas 2010). Shear force in the extruder can be increased by changing the type or angle of the screw element combinations or increasing the screw speed (Zhang et al. 2015). It favoring the aggregation reactions at low shear regions, but the degradation reactions at high shear regions (Emin et al. 2017). Marsman et al. (Marsman et al. 1998) have shown that with a weak shear force combination, the main interactions in TSP were disulfide bonds and non-covalent interactions, however, covalent cross-linking may occur with a strong shear force combination. However, when increasing the screw speed (higher than 140 rpm), some of the disulfide bonds that formed in TPP are disrupted, which is not conducive to the formation of protein network structure (Zhang 2007).

3.1.2. The secondary structural changes of protein during extrusion

Few studies have analyzed the effect of extrusion on the secondary structure of proteins. Temperature is the catalyst for the stable transition of protein conformation (Belitz and Grosch 1999). Kang (Kang 2007) has used the Fourier-transform infrared spectroscopy (FT-IR) method to investigate the secondary structural changes of soybean protein during extrusion. Results showed that during the heating process in the extruder (between 120°C∼160°C), α-helix was the most unstable structure and would gradually transform into a stable corner structure, a transformation that was almost complete at 140°C. However, the β-sheet remained essentially unchanged until the barrel temperature increased above 140°C and the sub-stable β-sheet began to transform into a random coil while the β-turn remained essentially unchanged even at 160°C.

Studies have shown that the plasticization effect of water during extrusion increases as the water content increased from 28% to 60% (Chen et al. 2010). At a moisture content of about 55%, the mobility of the key structure of the protein is enhanced, enabling the peptide chains to be more easily stretched and aligned. High-energy water molecules promote the transition of α-helix to β-turn and β-sheet to random coil and also served to reduce the temperature necessary to form this network (Hager 1984; Kang 2007).

Increasing the screw speed from 60 rpm to 180 rpm causes the amount of β-sheets to gradually decrease while amount of β-turns increases (Kang 2007). Additionally, increasing the feed rate allows more protein molecules to interact in forming the protein network structure. However, as demonstrated in experiments by Kang and Rhee (Kang 2007) on soybean protein, the feed rate has little effect on the secondary structure.

3.2. Carbohydrates

Carbohydrates in the production of TVP can be categorized into small molecular and macromolecular carbohydrate according to the degree of hydrolysis (Zhang et al. 2016; Taranto, Kuo, and Rhee 2006). Small molecular carbohydrates (sugars) such as glucose can participate in the Maillard reaction with free amino acids, which then affect the color and the taste of TVP through browning (Zhang 2007; Wild et al. 2014).

Guerrero et al (Guerrero et al. 2012) found that the formation of the protein-sugar conjugates lead to highly colored and insoluble polymeric compounds, which showed a more ordered structure in extruded soybean protein products. The results also showed that the degree of Maillard reaction was higher for the products with lactose than for the ones with sucrose due to the presence of the free hydroxyl group in the anomeric carbon of the lactose (Sbm et al. 2007). Macromolecular carbohydrates such as starch or crude fibers play a major role in the formation of fibrous structure in TVP, but the adding amount should be not exceed 10% of the feed mixture (Taranto, Kuo, and Rhee 2006; Wang et al. 2002). Starch is the most commonly selected carbohydrate for extrusion formulations, which can affect the structural formation of the extrudate mainly through the reactions of gelatinization and degradation (Zhang et al. 2016).

In the mixing zone, starch is fully mixed with other components in the material and the starch granules begins to gradually expand upon the absorption of water. In the melting zone, starch gelatinization occurs when the hydrogen bonds inter the starch molecules are disrupted. At the same time, the viscosity of the melt can be changed due to starch gelatinization and the protein-starch interactions prevent the unfolding and aggregation of protein molecules, which further affect the formation of fibrous structure in TVP (Verbeek and van den Berg 2010; Zhang et al. 2016). In the die, for low moisture extrusion, the release of the pressure result in water evaporation and puffing of starch granules, which forms the air cells in TVP at low moisture extrusion (Wei, Du, and Zhao 2009). For high moisture extrusion, due to the thermodynamic incompatibility, the starch is embedded in the protein phase as a dispersed phase to prevent the protein from getting close to each other, which stabilizes the hydrophobic interactions of protein molecules at the same time (Zhang et al. 2016; Smith, Mitchell, and Ledward 1982). In the cooling zone, the layered structure will form parallel to the die walls due to phase separation and the layers are close to each other to form a multi-layered structure, which will contribute to formation of the fibres upon tearing (Thiébaud, Dumay, and Cheftel 1996; Shah 2003).

Otherwise, degradation of carbohydrates contributes to color and taste formation in TVP. According to Wei et al (Wei, Du, and Zhao 2009), degradation of starch occurs both in the extruder barrel and in the cooling die. Gomez and Aguilera (Gomez and Aguilera 2006) developed the conformational changes model of starch in extruder barrel, emphasizing that starch degradation and gelatinization occurred simultaneously, especially under the conditions of high temperature and strong shear force. At this severe conditions, starch is degraded into glucose, maltose and other small molecules (Chiang and Johnson 1977; Sokhey and Chinnaswamy 1993). Furthermore, the degradation products of starch also react with amino acids (Maillard reaction) to form the brown color observed on the TVP surface and reduce the content of total free amino acids (Zhu et al. 2010; Harper 1981).

3.3. Lipids

The presence of a small amount of lipids in the material could improve the quality of TVP, and the total content should be strictly controlled within 15% (better within 2%～10%) of the material (Schoenlechner and Berghofe 2000; Vaz and Areas 2010; Gwiazda, Noguchi, and Saio 1987). Studies showed that the lipids play a role as a plasticizer by forming the complexes with starch or protein during the extrusion process, which can distribute on the surface of the protein to prevent its aggregation, and the fibrous structure will be stabilized by protein-lipid interactions (Alzagtat and Alli 2009).

In the mixing zone, the lipids mix with protein or starch to form a protective layer on the surface of the protein molecules, reducing the friction coefficient of the material system which consequently reduces the shear force (Alzagtat and Alli 2009; Areas 1992). In the melting zone, the protein-lipid and starch-lipid complexes are formed on the surface of protein aggregates, thus preventing the unfolding and aggregating of protein (Day and Swanson 2013). In the die, reports for the low moisture extrusion indicated that the volatilization and oxidation of lipids occurs, which promote a smoother surface of the TVP (Arêas and Lawrie 1984).

Extrusion temperature and moisture content are the main drivers of the formation of lipid complexes. At low temperatures (<100℃), the content of these complexes increases with increasing temperature while high temperatures (>100℃) reduced the formation of the lipid complexes (Alzagtat and Alli 2009). Increasing the moisture content, less lipid complexes will be formed in the extrudate (Zhang 2007). These complexes can also reduce the content of free fatty acids and the oxidation rate (Schoenlechner and Berghofe 2000; Vaz and Areas 2010). With the exception of the complexes, studies found that extensive cis-trans isomerism of unsaturated fatty acids occurring during the extrusion process (Wei, Kang, and Zhang 2009). When increasing the temperature from 55℃ to 171℃, the trans fatty acid content increased from 1% to 1.5%. Additionally, Vaz and Areas (Vaz and Areas 2010) found that adding 3.8% of the lipids to bovine rumen protein made more stable the extrusion process without blocking of the barrel and interruptions.

4. Effect of extrusion parameters on quality of TVP

The fibrous structure in TVP can be characterized by degree of texturization, strength of the fibers, hardness and elasticity (Zhang et al. 2015). Otherwise, the color, WHC, and digestibility are also considered as the quality evaluation indicators of TVP. Extrusion parameters including operating parameters (e.g. barrel temperature, screw speed, feed rate, moisture content, and screw configuration) and response parameters (e.g. specific mechanical energy (SME), torque, and pressure) are the key points for controlling the quality of TVP (Wei, Kang, and Zhang 2009). Changing of the operating parameters generates the comprehensive effects of temperature, shear force, and pressure, which will act on the melt in the extruder (Lin, Huff, and Hsieh 2002; Alam et al. 2016).

4.1. Barrel temperature

Generally speaking, the barrel temperature mainly refers to the temperature in the melting zone. It can be heated by an electric cartridge heating system and cooled with running water, which controls the starting and the ending point of the melting state (Tunick and Onwulata 2006). The melting temperature is a critical factor for the conformational changes of protein as previously mentioned, which affects the final quality of TVP (Emin et al. 2016). Hayashi et al (Hayashi, Hayakawa, and Fujio 1991) reported that the barrel temperature was the most important parameter to ensure that the material can be completely melted, and then the protein can be texturized (Osen et al. 2014). In addition, the die temperature can influence the pressure drop, torque, SME and then changing the melt viscosity, which is critical to the formation of the fibrous structure in TVP (Akdogan 1996; Osen et al. 2014). To ensure that the melt can pass through the die successfully and form the fibrous structure, the die temperature should be controlled at least 100°C, especially for low moisture extrusion (Akdogan 1996). For high moisture extrusion, the temperature in the cooling zone might affect the flow velocity profile during the moment of solidification and should be also controlled lower than 75°C (Fang et al. 2013; Cheftel, Kitagawa, and Quéguiner 1992). So that the melt is in a laminar state, in which the melt temperature and flow velocity are higher at the core of the flow channel than close to the cooled zone wall (Osen et al. 2014). Under this condition, the melt starts to display multilayered structures with layers parallel to the zone wall and fine fibers appeared upon tearing (Thiébaud, Dumay, and Cheftel 1996).

For TSP, only when the melting temperature is higher than 130°C, the fibrous structure could be formed (Cheftel, Kitagawa, and Quéguiner 1992). Wei et al (Wei et al. 2006) has found that the texturized structure of TSP was easy to breakdown and the shape was not uniform at a melting temperature below 120°C. As the melting temperature increased from 130°C to 150°C, the degree of texturization increased, which indicated that the material became fully melted gradually enhancing the protein-protein interaction and protein-water interaction (Zhang et al. 2017). When it increased from 150°C to 160°C, the degree of texturization decreased, small pits appeared on the surface, and overall TSP color experienced browning. These results showed that under a relatively higher temperature, the protein would be degraded and the forces that have formed between molecules should be broken (Liu and Hsieh 2008). If the temperature was higher than 160°C, the extruder was unstable and the extrudate was difficult to shape. At the temperature between 140∼160°C, results showed that cooking temperature had a significant effect on tensile strength ($P<0.01$), but not hardness and chewiness (Chen et al. 2010). A melting temperature of about 150°C produced extrudates with the best degree of texturization, a lighter color, and good sensory quality. Compared with 138°C, the TSP with a melting temperature of 149°C or 160°C had higher WAC and an ordered fibrous structure with lower hardness, elasticity and chewiness (Lin, Huff, and Hsieh 2002). The total protein content in the material was the determinant factor for the melting temperature required for texturization (Manoi 2009).

Using low temperature defatted peanut meal (the temperature for desolvenization was 150∼160°C) as the material, Zhang (Zhang 2007) found that at a melting temperature between 100∼120°C, peanut protein denatured, but did not have significant fibrous structure formation. This might be due to the incomplete melt in the extruder as previous mention (Osen et al. 2014) and the poor gel capacity of peanut protein (Wang 2018). When it was higher than 140°C, the structure of the peanut protein became dense and an obvious fibrous structure developed in the extrudates. However, at 155°C, the color became brown and the deformation phenomenon occurred (degradation of protein molecular chain). The best melting temperature for the texturization of peanut protein may be at about 140°C, which is 10°C lower than that of soybean protein (Wei et al. 2006). For high temperature defatted peanut meal (the temperature for desolvenization was 150∼160°C), Lang et al (Lang, Yan, and Shi 2014) found that when the melting temperature increased from 140°C to 150°C, the hardness and elasticity of TPP increased gradually. When it increased higher than 150°C, the hardness and elasticity of TPP began to decrease. Therefore, the best melting temperature for processing TPP with better texturization properties may be at 150°C depending on the conformational state of protein in the material. In addition, Shah (Shah 2003) pointed out that the relatively high melting temperature could promote the unfolding of peanut protein molecule chains, exposing more enzyme sites and subsequent forming the large protein aggregates with loose structure, thus increasing the protein digestibility index (PDI) (Iwe et al. 2004). Wu (Wu 2009) reported the digestibility changes of low temperature defatted peanut meal in the twin-screw extruder. Results showed that the PDI of peanut protein increased from 84.37% to 92.87% as melting temperature increased from 120°C to 140°C. However, at a melting temperature higher than 140°C, the PDI decreased due to the reaction of amino acids with carbonyl compounds.

Wheat gluten has not been used as major matrix material, but as minor ingredient in the production of meat analog products (Liu and Hsieh 2007; Delcour et al. 2012). Both glutenins and gliadins in wheat flour play an important role for the fibrous structure formation of the extrudates (Li and Lee 1996). Pietsch et al (Pietsch, Emin, and Schuchmann 2016) found that during high moisture extrusion of meat analog products by wheat gluten, the texturized structure of texturized gluten protein (TGP) was easy to break at the barrel temperature of 110°C. This is because that when the melting temperature is below 130°C at a high moisture content, wheat gluten cannot be completely denatured to form a fibrous structure (Zheng et al. 2012). At the barrel temperature of 145°C, the inner structure of the sample appeared to be anisotropic which was indicated by a flow oriented fracture behavior (Pietsch, Emin, and Schuchmann 2016). However, when it was higher than 180°C, the color of the extrudate turned black according to Zheng et al (Zheng et al. 2012). These results suggest that the proper barrel temperature for TGP is between 150°C∼170°C under low moisture extrusion.

4.2. Moisture content

Water plays a variety of roles in extrusion processing such as determining the viscosity of the melt, participating in chemical reactions, affecting the temperature and pressure during the extrusion process, and acting as the plasticizer and foaming agent (Chen et al. 2010).

Wei et al (Wei, Zhao, and Kang 2009) found that increasing the moisture content from 35% to 50% led to a gradual increase in the degree of texturization, L^* (an indicator of whiteness) and adhesiveness of TSP while also decreasing chewiness. At a relatively higher moisture content of 45% or 50%, the interactions of protein-protein and protein-water were more severe and more hydrophobic groups exposed, resulting in a lower nitrogen solubility index (NSI) but a higher water holding capacity (WHC), (Wei, Zhao, and Kang 2009; Zhang et al. 2017). Chen et al (Chen et al. 2010) suggested that increasing the moisture content from 28% to 60% led to the reduction of protein aggregation, thus the hardness and chewiness were significantly decreased ($P<0.01$), with fibrous structure formation beginning at a moisture content of 60%. This result was consistent with the finding of Liu and Hsieh (Liu and Hsieh 2008), that only at moisture content of 60.11% the TSP had well-defined fiber orientation. According to Smetana et al (Smetana et al. 2017), a lower extrusion moisture content (40%) caused an increase in shear and friction inside the barrel and die, resulting in a better texture, greater velocity gradient and fiber formation. The inconsistent results between Chen and Smetana may be because of the difference of materials, extruder types and design of the die. Sun showed that when the moisture content increased from 28% to 38%, no significant difference was seen regarding the degree of texturization, hardness, chewiness and WAC. At a moisture content higher than 38%, the degree of texturization and WAC rapidly increased, the color became brighter, and the hardness and chewiness decreased significantly. At high moisture range (60%∼70%), the WAC and solubility of TSP increased with increasing moisture content, which may be also related to the die pressure and protein denaturation (Lin, Huff, and Hsieh 2002).

Zhang (Zhang 2007) has done research on the behavior of TPP at a wide range of moisture content (35%∼60％). Results indicated that at a moisture content of 35%, no fibrous structure was seen and the hardness levels were high. At a moisture content of 40%, fibrous structure began to form, but it was loose and the texture remained dry and hard. When the moisture content increased from 45% to 55％, no significant changes could be seen in hardness and chewiness. At a moisture content of 60%, the hardness and chewiness decreased slightly, which was consistent with the results using high temperature defatted peanut meal as the material (Lang, Yan, and Shi 2014). Wu (Wu 2009) suggested that at a moisture content of 35%, the extrudates produced with low temperature defatted peanut meal had high digestibility at about 92.23%. But if the moisture content increased continuously to 40%, the digestibility decreased gradually.

Pietsch et al (Pietsch, Emin, and Schuchmann 2016) extruded the wheat gluten at the moisture content of 40%. Results showed that wheat gluten polymerization reactions were mainly taking place in the screw section of the extruder and form the anisotropic structure in the die. Zheng (Zheng 2012) found that with the increase of moisture content (12%∼58%), the L^* and b^* of TGP increased, while the a^* and NSI decreased.

4.3. Feed rate

The feed rate mainly affects the filling degree of the material in the extruder, the residence time distribution (RTD) and the die pressure, thereby affecting the mechanical action on the material (Unlu and Faller 2002; Maurya and Said 2014). At low feed rate, the residence time of the material in the extruder is longer, thus resulting in increased protein denaturation and darkening/browning of the extrudate. However, high feed rate does not allow proteins to fully denature due to the decrease of SME (Shah 2003; Unlu and Faller 2002). Compared with HM-TVP, LM-TVP has a higher feed rate requirement, usually 2∼4 times higher than the former, depending on the extruder types (Thymi et al. 2008; Chen, Wei, and Zhang 2011).

Response surface methodology study results indicate that the feed rate has a negative effect on the degree of texturization, hardness, chewiness and color, but positively affect WAC (Kang 2007). When the feed rate increased from 10 g/min to 50 g/min, the degree of texturization decreased from 1.3 to 1.1. At the same time, WHC decreased from 2.2 to 1.6 gH_2O/g, NSI decreased from 9% to 7.5%, surface texture roughened, and chewiness was significantly reduced (Wei, Zhao, and Kang 2009). At a feed rate between 25∼30 g/min, the degree of texturization of TSP is highly acceptable and comparable to the fibrous structure of muscle meat. At low feed rate (10.96∼12.69 g/min), the degree of texturization of TPP made by low temperature defatted peanut meal was also highly acceptable, which was similar to the results of TSP (Zhang 2007). But for high temperature defatted peanut meal, Lang et al (Lang, Yan, and Shi 2014) found that the structure of TPP was loose at a feed rate lower than 350 kg/h using an industrial extruder. Increasing the feed rate to 550 kg/h led to a gradual formation of fibrous structure. However, at a feed rate of 750 kg/h, the structure of TPP became loose again due to the large pressure drop at the exit of the die. When increasing the feed rate from 6 kg/h to 36 kg/h, the digestibility of peanut protein increased firstly and then decreased with the highest digestibility (91.73%) observed at a feed rate of 18 kg/h (Wu 2009). It is worth noting that variation range of feed rate depends largely on the choice of extruder.

4.4. Screw speed

Screw rotation provides shear force and pushed the material passing through the extruder. The viscous dissipation generated by the rotating screws leads to a temperature distribution, and local temperature maxima (Emin et al. 2016). It is possible that a higher screw speed improves the dispersion of the dispersed phase in the continuous phase, resulting in the formation of numerous and thinner fibrous structure (Thiébaud, Dumay, and Cheftel 1996). With an increase in screw speed, the mixing effect is enhanced and the large resistance provided by the screw broadens the residence time distributions, thus increasing the torque and SME (Fang et al. 2013; Iwe, Zuilichem, and Ngoddy 2001). High screw speed consistently causes a decrease of the pressure at the die due to the reduction of viscosity, which is an indicator of less resistance of the melt in the extruder barrel (Shah 2003; Unlu and Faller 2002). Compared with HM-TVP, a higher screw speed is required for LM-TVP, typically higher than 380 rpm depending on the length/diameter ratio of screw (Cheftel, Kitagawa, and Quéguiner 1992; Zhang et al. 2017).

Screw speed should be kept within 80∼100 rpm for an adequate degree of TSP texturization. If the screw speed was higher than 120 rpm, the strength of the fibrous structure was weaken

(Wang, Zhou, and Lin 2001). Results indicated that when increasing the screw speed from 60 to 180 rpm, TSP had a lighter color, a lower WAC and a rougher surface. Response surface methodology studies have shown that high screw speed (180 rpm) was beneficial to the decrease of $\triangle E^*$ (Kang 2007). Wei et al (Wei, Zhao, and Kang 2009) found that with the increase of screw speed (60∼180 rpm), the chewiness and hardness of TSP increased, while the degree of texturization and WHC decreased gradually. For low temperature defatted peanut meal, Zhang (Zhang 2007) found that when the screw speed increased from 60 rpm to 180 rpm, the degree of texturization of TPP significantly decreased. It was suggested that the screw speed should be controlled between 90∼120 rpm for the production TPP. For high temperature defatted peanut meal, when the screw speed increased from 250 to 350 rpm, the degree of texturization of TPP increased gradually. At a screw speed of 450 rpm, the material was subject to a strong shear force, leading to a lower degree of texturization (Lang, Yan, and Shi 2014). With the increase of screw speed from 50 rpm to 130 rpm, the protein digestibility increased from 89.91% to 92.14%. However, it decreased when the screw speed was continuously increased to 210 rpm (Wu 2009). Zhang et al (Zhang et al. 2015) suggested that the shear force could be also changed by adjusting the screw configuration. Results indicated that sufficient shear force provided in the extruder enhances the strength of the fibrous structure.

4.5. Coupling effects between the extrusion parameters

During the extrusion, it is the coupling effects between the extrusion parameters that lead to the conformational changes of the components and then influence the quality of TVP. Kang (Kang 2007) found that a lower feed rate combined with a higher barrel temperature would improve the degree of texturization. Results also showed that under the condition of a higher feed rate and a lower moisture content, a larger springiness would be obtained. In extrusion-like conditions, it showed that the influence of temperature on the rate of the reactions was also a strong function of water content (Emin et al. 2017). High barrel temperatures and low moisture contents promoted Maillard reaction during extrusion (Guerrero et al. 2012). A higher moisture content (at the range of 44%∼60%) combined with a higher barrel temperature (at the range of 140∼160°C) would promote a lower hardness and higher degree of texturization (Chen et al. 2010). At a moisture content between 60% and 70%, Lin et al. (Lin, Huff, and Hsieh 2000) found that a higher moisture content combined with a higher barrel temperature (at the range of 140∼160°C) led to a lower hardness and chewiness, but a lower degree of texturization. These results indicated that only at relatively lower moisture content is it effective to tailor the texture of TVP by controlling barrel temperature. The ratio of feed rate to screw speed is called specific feeding load (SFL), which is an index of degree of barrel fill and has dramatic effect on the residence time of the melt in the extruder (Della, Tayeb, and Melcion 1987). Akdogan (Akdogan 1996) characterized the mass flow rate with SFL, and results showed that an increase of SFL would decrease SME with a lower viscosity of the melt. Unlu and Faller (Unlu and Faller 2002) found that increasing the feed rate and screw speed keeping the ratio constant, a slight increase in barrel fill was observed. Results also showed that the feed rate had more influence on residence time and barrel fill than screw speed.

5. Approaches to Characterize the Extrusion Process

For a long time, food extrusion technology is called "extrusion art" since the control of this process and design of new extruded products are still mostly based on empirical knowledge, leaving the extrusion to be a black box (Emin and Schuchmann 2016). Various advancements in mathematical modeling and spectroscopy have begun to reveal some of the material behavior within the extruder.

Meuser (Meuser F 1984) has used a "system analytical model" to analyze the extrusion process. This model divides the parameters related to the extrusion process into three types, namely operating parameters, response parameters and product quality. It aims at linking these parameters to get information of the structural changes during the extrusion process. To monitor the extrusion process, a measurement slit die with different sensors (Raman, IR spectroscopy, fluorescence spectroscopies, ultrasonic spectroscopy, or dielectric relaxation spectroscopy) was always used to extract specific molecular information or information on chemical composition (Alig, Steinhoff, and Lellinger 2010; Barnes et al. 2007). It has been confirmed that the infrared sensor was able to capture the true temperature variations generated by rotation of screws, which is essential to control the extrusion process and the resulting product characteristics (Emin et al. 2016). Also, the measurement of RTD through particle tracking analysis can be performed to determine the particle trajectories and estimate the flow histories of the material in the extruder (Unlu and Faller 2002). To collect the samples during the extrusion process, dead-stop operation was required (Yao, Liu, and Hsieh 2004). The extrusion operation was intentionally shut down after reaching steady state (dead-stop). Then, the barrel should be cooled and opened immediately. Samples along the extruder are collected for further analysis to characterize fiber formation of TVP (Chen, Wei, and Zhang 2011). Besides the process in a real extruder, the development of computer simulation technology also facilitates the research and control of the extrusion process. Harper (Harper 1981) constructed a digital simulation of the melting zone. He depicted the relationship between the apparent viscosity of the material and operating parameters such as temperature, shear force, and moisture content, from which the apparent viscosity calculation model was proposed. By using computational fluid dynamics (CFD), the flow and rheological characteristics of the material during the extrusion process can be simulated accurately (Emin and Schuchmann 2013). Emin (Emin 2015) classified the modeling approaches of the extrusion process according to spatial model dimensions. Among these approaches, a 3-Dimensional modeling approach offers the most comprehensive analysis of the flow in extruders.

Unfortunately, there is currently no model that can be used to control the conformational change of the components or explain the quality forming process of TVP during the extrusion process. To control the extrusion process, it could be concluded that conformational changes play an important role in the extrusion process and in the determination of final extrudate quality, as summarized in a new 'system analysis model'

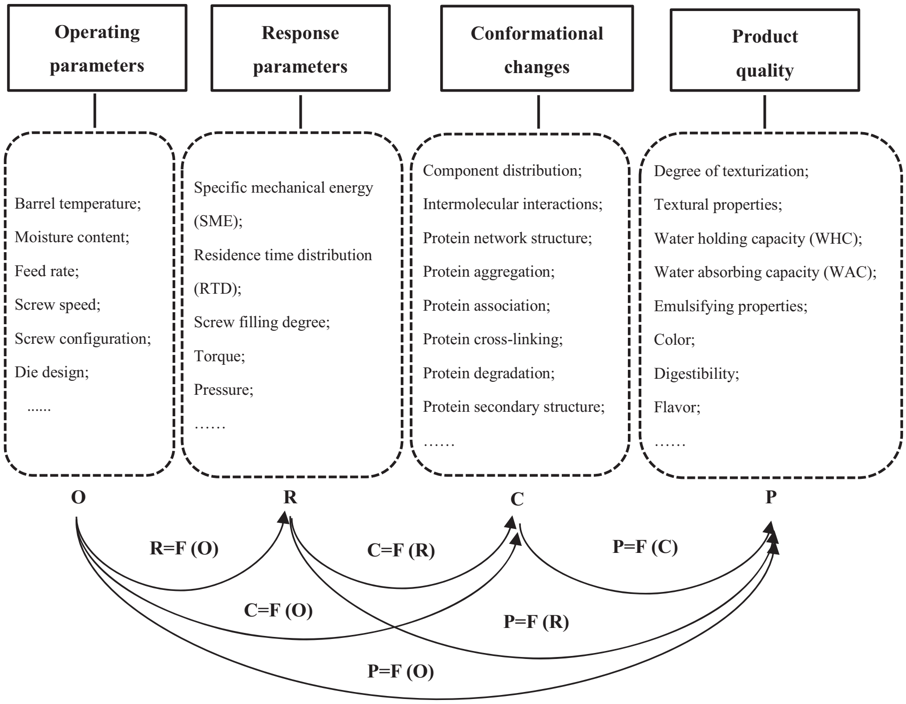

Figure 3. A new system analysis model of the extrusion process. The letters 'O', 'R', 'C', and 'P' represent the operating parameters, response parameters, conformational changes, and product quality, respectively. 'F' means the functional relationship between letters.

(Figure 3). Operating parameters can be controlled directly during the extrusion process. Response parameters and conformational changes are the intermediate links connecting the operating parameters and product quality, with operating parameters controlling the response and conformational changes, which in turn affect the final product quality. There is a definite function relationship between each two types of parameters. Investigating the conformational state of components and analyzing the conformational changes during the extrusion process will be helpful to understand the quality formation mechanism. Furthermore, researchers should try to explain how the operating parameters affect the response parameters and then change the conformation of material components with the forming of product quality. It is also essential for controlling and modeling the extrusion process that establish the relationships among the four types of parameters.

6. Conclusions

Extruder can be seen as a high temperature-short time biochemical reactor that transforms raw ingredients into modified intermediate and finished products. LM-TVP and HM-TVP have different requirements for the extruder and extrusion conditions, thus determining the specific quality of the products. Supercritical fluid extrusion (SCFX), two-stage or multi-stage extrusion and the combination of extruder with 3D printer have attracted great attention, which provides an alternate process for improving the quality of TVP.

Extrusion process disarranges the conformation of the components and promotes to form a new conformation by the rearrangement of the molecules. Due to the thermodynamic incompatibility of different components, phase separation occurs during the extrusion process with protein as the continuous phase, which is critical for forming the fibrous structure in TVP. During the extrusion process, the protein molecules transform from a globular-like structure to a linear-like structure, maintained by the disulfide bonds, hydrophobic interactions and hydrogen bonds. Meanwhile, the α-helixes and β-sheets transform into more stable structures such as β-turn and random coil. As the disperse phase, the macromolecular carbohydrates such as starch or crude fibers are embedded in the protein phase, which can prevent the unfolding and aggregation of protein molecules. Additionally, the present of small molecular carbohydrates (sugars) such as glucose are crucial for the color formation of TVP through Maillard reaction. Moreover, complexes of lipid with starch or protein formed during extrusion can distribute on the surface of the protein aggregates, which prevents the aggregation of protein molecules and stabilizes the fibrous structure. However, these speculations on the conformational changes of the components during extrusion are only a qualitative description since the mechanism of interactions is still unclear due to the purity of raw materials and the complexity of the extrusion process.

Quality forming process of TVP is the result of the main components conformational changes caused by changes in extrusion parameters. The formation of the fibrous structure in TVP requires complete 'melting' of the materials in the extruder, plus laminar flow in the die and the cooling zone. For this purpose, the amount or way of thermal and mechanical energy input should be controlled by changing the extrusion parameters (e.g. barrel temperature, moisture content, feed rate, screw speed). Moreover, the extrusion parameters, the conformational state of raw material components, the distribution degree of components, design of the extruder and the die are also worth to study in the future.

Control of the extrusion process is still very challenging due to comprehensive effects of the thermal and mechanical energy input, coupled with complex physicochemical transformations of the material. To understand the complex biochemical reactions in the extruder, more works should be done on software simulation of the extrusion process, including raw material properties, extrusion parameters, conformational changes of components, and the quality evaluation of the extrudates. A visual platform is the inevitable choice of food extrusion technology to adapt to the industrial 4.0 era.

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