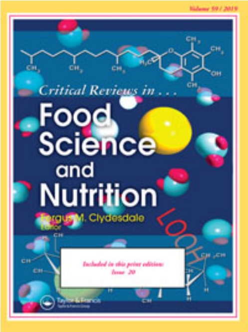

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REVIEW

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

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ABSTRACT
Texturized Vegetable Protein (TVP), as meat analogs, has garnered attention due to the nutritional advantages it offers over conventional animal proteins. 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, all of which influence the quality of TVP. Control of the extrusion process is still one of the largest challenges in its evolution. Therefore, this review aims to summarize the development and current status of food extrusion technology for TVP production and give 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 discuss approaches to characterize the extrusion process and propose a new system analysis model.

KEYWORDS
Texturized vegetable protein; extrusion process; extrusion parameters; conformational changes; extrudate quality; characterization approaches

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. 2018). 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 analogs (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 similar to 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 some of the prevalent diseases in modern civilization 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 2016).

Despite its growing popularity, the market for meat analogs is remains 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, likely due to the fact that presently available meat analogs 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 also due to a curiosity in trying new food products. To obtain a larger market share, meat analogs must better compare to real animal meat in terms of flavor, texture and overall organoleptic acceptability (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, 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,

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and Singh 2001). During the extrusion process, under the comprehensive effects of temperature, shear force, and pressure, the simultaneous denaturation, degradation, association, and aggregation of proteins, degradation of carbohydrates, and oxidation of oil is induced (Cheftel, Kitagawa, and Quéguiner 1992; Camire, Camire, and Krumhar 1990). 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 has 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 TVP production; 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.

Overview of food extrusion technology for TVP production

The development of food extrusion technology can be divided into four stages. The first stage occurred 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). The second stage took place between the 1940s and 1980s, with food extrusion technology developing into a high-temperature-short-time (HTST) biological reaction process for producing instant food, snacks and nutritious food for children (Camire, Camire, and Krumhar 1990). 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 single-screw extruder due to their higher processing capacity, lower energy consumption (between 200 and 1200 kJ/kg) and wider 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.

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, 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 2017)
APV Baker (USA)	MPF 50/25 (twin screw 15:1)	Vegetable protein meat analogs	University of Missouri - Columbia	(Yao, Liu, and Hsieh 2004)
Thermo Fisher Scientific (UK)	Haake16 (25:1)	Pea protein meat analogs	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™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	(Wang 2013; Chen 2011)
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)

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 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 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 the 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 which allows for protein texturization and expansion (Areas 1992; Normelljhoee et al. 2009). HM-TVP possesses a texture similar to animal meat with a rich fibrous and 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 such as Beyond Meat in America and several European countries are producing HM-TVP. High moisture extrusion technology is still in the theoretical research stage in China (Zhang, Wei, 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). Additionally, it takes time for consumers to adopt new food products into their everyday lifestyle (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, new extrusion technologies have emerged that were largely enabled by improvements in extruder design. 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, make the components configurational changes easier to control. It has been applied for the production of vegetable protein meat analogs (Wenger, USA) or starch-based materials (Fishman et al. 2006; Tiwari, Patil, and Repka 2016). The combination of extrusion and 3D printing has also 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 direction of development of the new extrusion technology.

Main components and their conformational changes during the extrusion process of TVP

The rheological state of material during extrusion can be generally divided into four main functional zones: the feeding zone, the mixing zone, the melting zone, and the die. High moisture extrusion also necessitates a long cooling zone after the die, shown in Fig. 1 (Cheftel, Kitagawa, and Quéguiner 1992). In the feeding zone, the barrel is always at room temperature and the screw is composed of conveying elements, which mainly serve to deliver the material into the next zone and to provide weak shear force (Zhang, Zhang, et al. 2015). Thus, the conveying zone does not significantly influence the conformation of protein significantly in this zone. In the mixing zone, the screw is often equipped with kneading elements that mix the feed material with the injected liquid, typically water. The temperature of the injected liquid must be lower than 80 °C to ensure that the water is injected into the extruder uniformly.

The melting zone contains more kneading elements on the screw than the previous two zones and is considered to be the core functional area of the extrusion process in which significant physical and chemical changes of vegetable protein occur at high temperatures (generally higher than 130 °C) (Zhang, Liu, et al. 2017; Thiébaud, Dumay, and Cheftel 1996). The design of the die is often seen as the key for controlling the flow behavior of the melt which in turn affects TVP quality (Akdogan 1996; Kostic and Reifschneider 2006). When the melt passes through the die, it forms a specified shape under the action of shear stress, pressure drop and water evaporation. For high moisture extrusion, the die may promote the separation of macromolecules into a continuous and a dispersed phase, which contribute to the formation of a 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

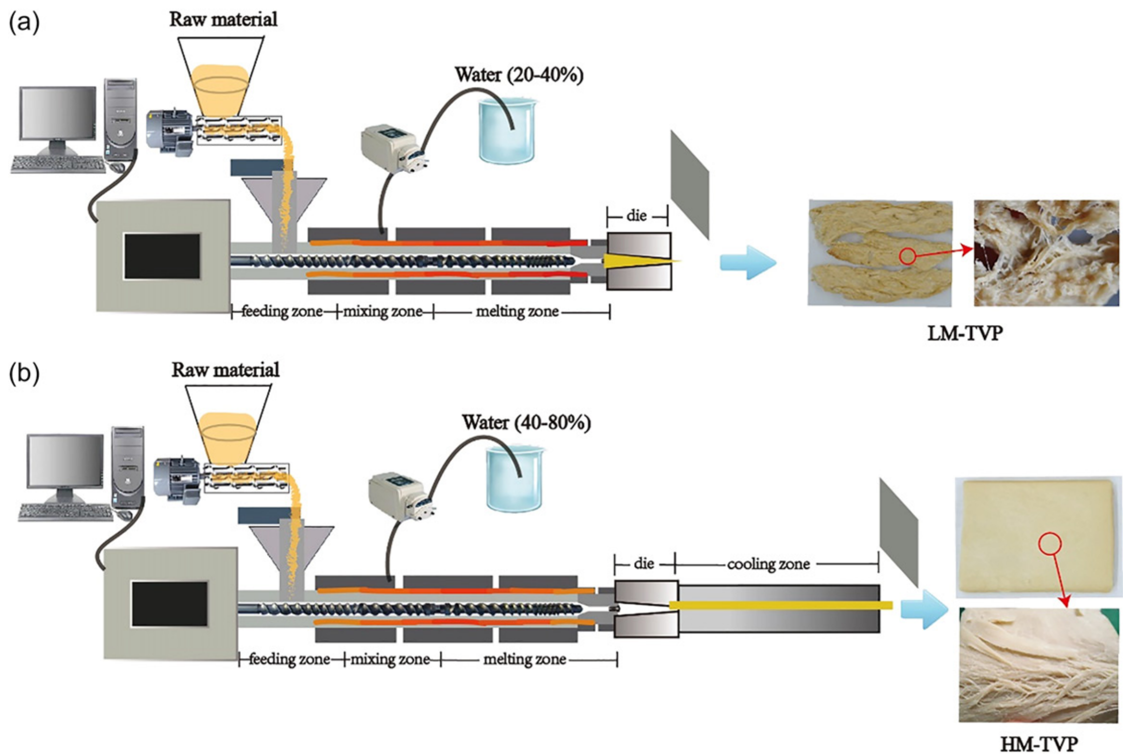

Figure 1. Functional partition of the extrusion process for low moisture extrusion (a) and high moisture extrusion (b).

the fibrous structure (Areas 1992; Yao, Liu, and Hsieh 2004).

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% and 70% in order to form a fibrous structure (Zhang et al. 2007). During extrusion, protein undergoes approximately four main stages of conformational changes (Areas 1992; Liu and Hsieh 2008): unfolding of the molecular chains, association, aggregation, and cross-linking with potential degradation or oxidation, as shown in Fig. 2 (Camire 1991; Day and Swanson 2013).

In the mixing zone, a homogenous dough that contains the raw material and water is formed by the stirring action of the screw (Don et al. 2003; Zhang, Zhang, et al. 2015). The molecular chains of the protein are unfolded along the direction of the flow, exposing hydrophobic amino acids that were originally enclosed inside the molecules (Akdogan 1999; Day and Swanson 2013). In the melting zone, the flow rate of the melt decreases due to the resistance of kneading elements and the high temperature, which promotes protein-protein interactions and protein-water interactions (Zhang, Ying, et al. 2017; Manoi 2009; Pietsch, Emin, and Schuchmann 2017). This promotion of association and/or aggregation of protein has been shown to cause an increase in viscosity (Osen et al. 2015; Chen, Wei, and Zhang 2011; Ai et al. 2016). The strong shear force in this zone may also cause the degradation of protein molecular chains. At the die during low moisture extrusion, the pressure drop and evaporation of water promote the hot melt to form a puffed structure supported by protein cross-linking. During high moisture extrusion, the die can provide shear stress perpendicular to the extrusion direction, which leads 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).

Interactions of protein molecules during extrusion

Interactions of protein molecules determine the change of viscosity, gelation, solubility and other functional properties of extrudates (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 (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, Ying, et al. 2017). Factors such as protein type, pretreatment, and extrusion

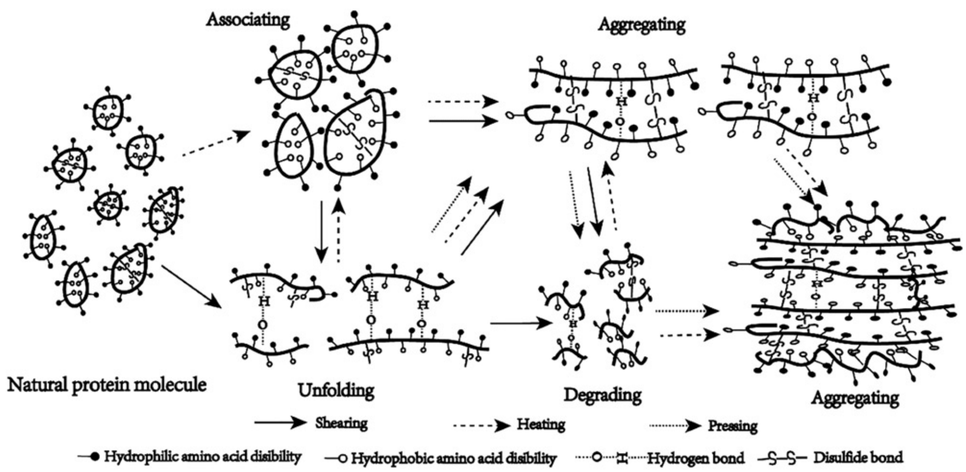

Figure 2. Conformational changes of protein during the extrusion process.

conditions play an important role in the formation and type of protein-protein interactions.

According to Prudencioferreira and Jag (1993), the main forces in texturized soybean protein (TSP) are disulfide bonds followed by hydrophobic and electrostatic interactions. These findings were consistent with research by Ma (2013) and Hong et al. (2016). 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). However, Ning and Villota (2007) found that amongst all molecular interactions, non-covalent interactions such as hydrophobic interactions and hydrogen bonds appeared to be dominant in the fibrous structure of TSP. 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, Ying, et al. 2017). When the temperature gradually increases during the extrusion process, firstly the hydrogen bonds break down, allowing the protein chains to gradually unfold (Shah 2003). In the melting zone, the temperature increases 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 promotes the formation of intermolecular disulfide bonds for peanut protein (Shah 2003).

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. 2018). Increasing water content in the range of 20–40% leads to significant increases 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 significantly reduces protein molecular weight which leads to the exposure of thiol groups attached to amino acids such as cysteine. These thiol groups can be 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, Zhang, et al. 2015). Aggregation reactions are favored at low shear regions and degradation reactions are favored at high shear regions (Emin et al. 2017). Marsman et al. (1998) have shown that with a weak shear force combination, the main interactions between proteins in TSP were disulfide bonds and non-covalent interactions; however, covalent cross-linking may occur when strong shear force is applied. However, when increasing the screw speed (higher than 140 rpm), some of the disulfide bonds that form in TPP are disrupted, which is not conducive to the formation of protein network structure (Zhang 2007).

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 (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 and 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 serve to reduce the temperature necessary to form this network (Hager 1984; Kang 2007).

Increasing the screw speed from 60 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 (2007) on soybean protein, the feed rate has little effect on the secondary structure.

Carbohydrates

Carbohydrates in TVP can be categorized into small molecular and macromolecular carbohydrates based on the degree of hydrolysis (Zhang et al. 2016; Taranto, Kuo, and Rhee 1981). 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. (2012) found that the formation of 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 1981; 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 begin to absorb water and gradually expand. In the melting zone, starch gelatinization occurs when the hydrogen bonds between 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). At the die, for low moisture extrusion, the release of pressure results 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 thermodynamic incompatibility, the starch is embedded in the protein phase as a dispersed phase to prevent the protein from associating, which also serves to stabilize the hydrophobic interactions of protein molecules (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 form a multi-layered structure, which contributes to formation of the fibers upon tearing (Thiébaud, Dumay, and Cheftel 1996; Shah 2003).

Degradation of carbohydrates also contributes to color and taste formation in TVP. According to Wei, Du, and Zhao (2009), degradation of starch occurs both in the extruder barrel and in the cooling die. Gomez and Aguilera (1984) 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 these severe conditions, starch is degraded into glucose, maltose and other small molecules (Sokhey and Chinnaswamy 1993; Chiang and Johnson 1977). 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).

Lipids

The presence of a small amount of lipids has been shown to improve the quality of TVP if kept below 15%, with the best quality at 2–10% of the feed 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 2002).

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 2002; 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). Lipids may also undergo volatilization and oxidation at the die during low moisture extrusion, which promotes a smoother surface on 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 °C), the content of these complexes increases with increasing temperature while high temperatures (>100 °C) reduces the formation of the lipid complexes (Alzagtat and Alli 2002). In terms of moisture content, increasing the moisture content will decrease the formation of lipid complexes (Zhang 2007). These complexes are known to 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 occurs during the extrusion process (Wei, Kang, and Zhang 2009). When increasing the temperature from 55 °C to 171 °C, the trans fatty acid content increased from 1% to 1.5%. Additionally, Vaz and Areas (2010) found that adding 3.8% of the lipids to bovine rumen protein made the extrusion process more stable by preventing blockages in the barrel.

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, Wei, et al. 2015). Otherwise, color, WHC, and digestibility are also considered the main 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 the operating parameters in turn affects 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).

Barrel temperature

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 water or coolant, 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 (Hayashi, Hayakawa, and Fujio 1991) reported that the barrel temperature was the most important parameter to ensure the complete melting of the material and subsequent texturization of the protein. (Osen et al. 2014). In addition, the die temperature can influence the pressure drop, torque, and SME, all of which change 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 and successfully form a 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 solidification and should be kept below 75 °C (Fang et al. 2013; Cheftel, Kitagawa, and Quéguiner 1992). This ensures the melt is in a laminar state, which refers to the 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 these conditions, the melt displays multilayered structures with layers parallel to the zone wall and a structure composed of fine fibers that was visible upon tearing (Thiébaud, Dumay, and Cheftel 1996).

For TSP, the fibrous structure can only be formed when the melting temperature is higher than 130 °C (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, Ying, 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 intermolecular bonds are 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 °C and 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 as the material (the temperature for desolvation was 150–160 °C), Zhang (Zhang 2007) found that at a melting temperature between 100 °C and 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 previously mentioned, (Osen et al. 2014) and the poor gelation capacity of peanut protein (Wang 2018). When the temperature 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 desolvation was 180 °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 (2003) pointed out that the relatively high melting temperature could promote the unfolding of peanut protein molecule chains, exposing more enzyme sites and subsequently forming large protein aggregates with loose structure, thus increasing the protein digestibility index (PDI) (Iwe et al. 2004). Wu (2009) reported the digestibility changes of low temperature defatted peanut meal in a 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, Emin, and Schuchmann (2017) found that during high moisture extrusion of meat analog products by wheat gluten, the 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 a 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 2017). However, according to Zheng et al. (2012), a barrel temperature higher than 180 °C caused the color of the extrudate to turn black. These results suggest that the proper barrel temperature for TGP is between 150 °C and 170 °C under low moisture extrusion.

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, 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 were exposed, resulting in a lower nitrogen solubility index (NSI) but a higher water holding capacity (WHC), (Wei, Zhao, and Kang 2009; Zhang, Ying, et al. 2017). 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 (2008) that only at a moisture content of 60.11% did the TSP display well-defined fiber orientation. According to Smetana et al. (2018), 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 due to the difference of materials, extruder types and design of the die. Sun (2009) 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 (2007) has done research on the behavior of TPP at a wide range of moisture contents (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 (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, Emin, and Schuchmann (2017) extruded 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 (2012) found that with the increase of moisture content (12–58%), the L* and b* of TGP increased, while the a* and NSI decreased.

Feed rate

The feed rate mainly affects the degree of filling of the material in the extruder, the residence time distribution (RTD) and the die pressure, thereby affecting mechanical action on the material (Unlu and Faller 2002; Maurya and Said 2014). At a 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 type (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 and 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.

Screw speed

Screw rotation provides shear force and conveys 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, Liu, 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 weakened (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, 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 (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 and 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, 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.

Coupling effects between the extrusion parameters

During 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 (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 value was 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 less hardness and higher degree of texturization (Chen et al. 2010). At a moisture content between 60% and 70%, Lin, Huff, and Hsieh (2000) found that higher moisture content combined with higher barrel temperature (at the range of 140–160 °C) led to less hardness and chewiness, but a lower degree of texturization. These results indicate 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 (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 (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.

Approaches to characterize the extrusion process

Almost since its inception, food extrusion technology has been called "extrusion art" since the control of this process and design of new extruded products are still mostly based on empirical knowledge, with the actual extrusion process being 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 (1984) used a "system analytical model" to analyze the extrusion process. This model divides the parameters related to the extrusion process into three types: operating parameters, response parameters and product quality. It aims to link these parameters to get information of the structural changes during extrusion. To monitor the extrusion process, a measurement slit die with different sensors (Raman, IR spectroscopy, fluorescence spectroscopies, ultrasonic spectroscopy, or dielectric relaxation spectroscopy) was 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 history 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).

In addition to analysis of the texturization process in a real extruder, the development of computer simulation technology also facilitates the research and control of the extrusion process. 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 (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 predict the conformational change of the components or explain the quality forming process of TVP during

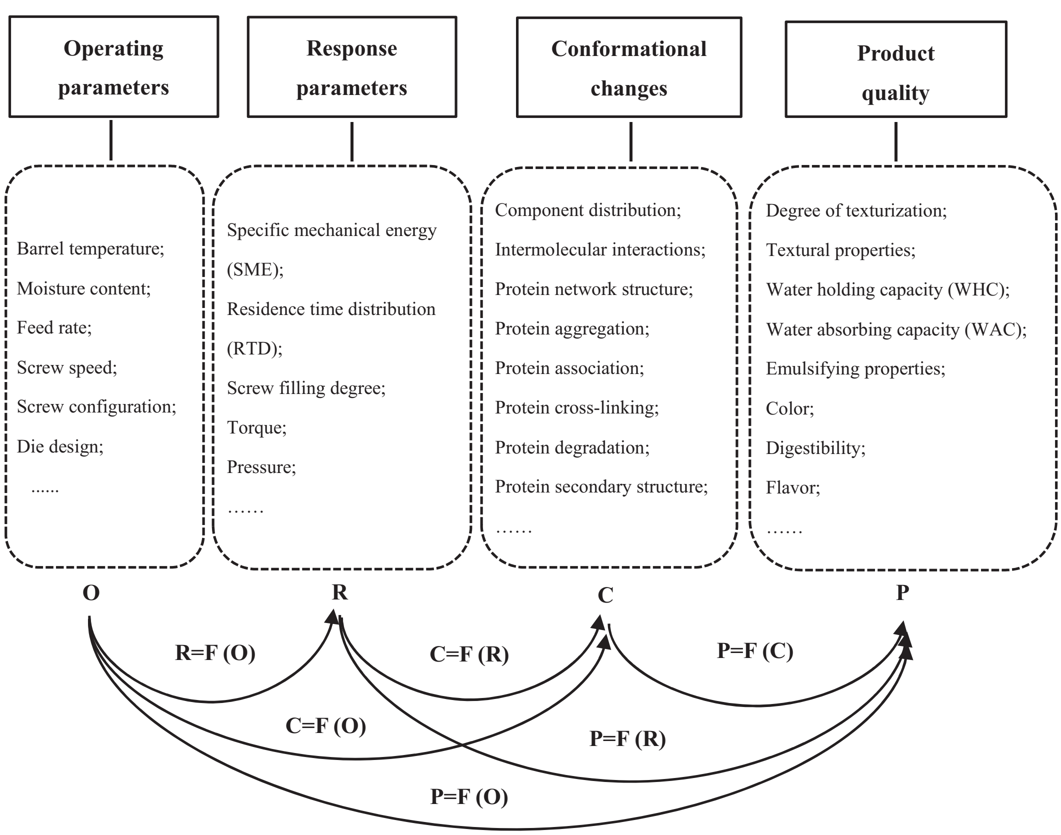

Figure 3. A new system analysis model of the extrusion process. The letters 'O', 'R', 'C', and 'P' represent the operating parameters, response parameters, conformational changes, and product quality, respectively. 'F' means the functional relationship between letters.

extrusion. Conformational changes play an important role in the determination of final extrudate quality, as summarized in a new 'system analysis model' (Fig. 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.

Conclusions

Extruders are high temperature-short time biochemical reactors 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 multistage extrusion and the combination of the extruder with the 3D printer have attracted great attention, which provides an alternate process for improving the quality of TVP.

The extrusion process disarranges the conformation of the components and promotes the formation of 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 disulfide bonds, hydrophobic interactions and hydrogen bonds. Meanwhile, α-helixes and β-sheets transform into more stable structures such as β-turn and random coil. As the dispersed phase, 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 presence 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.

The 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 in addition to laminar flow at the die and the cooling zone. For this purpose, the amount or method 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 and the design of the extruder and the die are also worthy factors for research 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 work should be done on software simulation of the extrusion process, including raw material properties, extrusion parameters, conformational changes of components, and 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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