Effects of particle size distribution and extrusion processing parameters on the techno-functional properties of soybean meal

By

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Abstract

Soybean meal (SBM) is a protein-rich, soybean oil industry by-product which can be extruded for improved techno-functionality and potential utilization as a food ingredient. The effects of SBM particle size distribution (small, medium and large), extrusion feed moisture content (15, 21 and 27 g water/100 g dry SBM) and barrel temperature (110, 130 and 150 °C at the die) on techno-functional properties of SBM extrudates were investigated. A decrease in barrel temperature from 150 °C to 110 °C generally improved nitrogen solubility index, gel forming capacity, oil holding capacity and emulsion capacity of extrudates. In addition, increasing feed moisture content from 15 to 27 g water/100 g dry SBM generally enhanced oil and water holding capacities. Nitrogen solubility index of extrudates made from large particle size distribution (PSD) feed was generally higher than that of medium PSD extrudates. Torque and specific mechanical energy were also affected by SBM's PSD, SBM with large PSD requiring a higher level of mechanical energy input to be processed when compared to that with medium PSD. This work provided a comprehensive understanding of how feed particle size, extrusion moisture and barrel temperature can be manipulated to provide new opportunities in producing novel food ingredients from SBM.

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Dedication

I dedicate this thesis to my mother, Baljinder Kaur and father, Mohinder Singh.

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List of Abbreviations

ANF	Antinutritional factor
BT	Barrel temperature
FMC	Feed moisture content
LGC	Least gel concentration
NSI	Nitrogen solubility index
OHC	Oil holding capacity
PSD	Particle size distribution
SBM	Soybean meal
WHC	Water holding capacity

Chapter 1: Introduction

A shift in dietary consumption patterns from omnivorous diets to vegetarian and flexitarian diets has recently been driving a huge upsurge in the demand for plant-based proteins. Major factors responsible for this shift are environmental, health, ethical and religious issues related to animal meat consumption (Aschemann-Witzel, Gantriis, Fraga, & Perez-Cueto, 2020; Chalvon-Demersay et al., 2017; Kumar et al., 2017). Moreover, increased population pressure requires the need to look for more sustainable food resources, for which plant-based diets have an edge over animal-based diets (Pimentel & Pimentel, 2003). To be used as an animal protein substitute, a plant protein source should ideally have high protein content, good essential amino acid composition and ability to exhibit desirable techno-functional properties, and thus texture (Kinsella, 1979), when used as a food ingredient (Asgar, Fazilah, Huda, Bhat, & Karim, 2010).

Protein-rich fractions of soybeans are one of the most studied and widely consumed plant protein products owing to their high protein content, excellent protein quality, easy availability, low cost and some health-promoting attributes (Kumar et al., 2017; Rizzo & Baroni, 2018). In Western countries, soybeans are primarily processed for their oil (approximately 20 % oil) which is commonly used in the food industry for cooking and frying (Medic, Atkinson, & Hurburgh, 2014). The residue left after oil extraction is termed as defatted soybean meal/flour and is often considered as the best protein source for utilization in animal feed (Stein et al., 2008). Out of the two common oil extraction methods, i.e., solvent extraction and mechanical extraction, a small proportion of solvent extracted soybean meal is further processed to produce high-protein food ingredients such as soy protein concentrates, soy protein isolates and texturized soy proteins (Deak, Johnson, Lusas, & Rhee, 2008). These products are then used in a wide range of foods including infant formulas, bakery products and meat substitutes (Medic et al., 2014). Although few previous research studies

evaluated some techno-functional properties of mechanically extracted soybean meal (Crowe & Johnson, 2001; Heywood, Myers, Bailey, & Johnson, 2002a, 2002b), soybean meal utilization in human foods is still very limited.

The structural characteristics of proteins are the major factors governing their techno-functionality (Kinsella & Melachouris, 1976; Lam & Nickerson, 2013). For instance, the presence of lower molecular weight protein fractions and lower surface hydrophobicity is required for higher protein solubility (Karaca, Low, & Nickerson, 2011); higher surface hydrophobicity is required for higher oil holding capacity (Lin & Zayas, 1987) and better gelation properties (He, He, Chao, Ju, & Aluko, 2014); and high molecular flexibility is required for enhanced emulsion properties (Mozafarpour, Koocheki, Milani, & Varidi, 2019). However, native soy protein's compact globular structure hinders molecular flexibility and thus adversely affects its techno-functionality (Chen, Chen, Ren, & Zhao, 2011; Mozafarpour et al., 2019). In addition, industrial soybean meal production which generally involves thermal processing to inactivate anti-nutritional factors to improve overall nutritional value can deteriorate the techno-functionality of soybean proteins by negatively influencing their solubility (Hettiarachchy & Kalapathy, 1997; Kinsella, 1979). Hence, processing treatments should be carefully designed to produce soybean meal with optimum techno-functionality in order to add value to this oil extraction by-product and to utilize it as a food ingredient. Several processing techniques have been used in the past with an aim to improve the techno-functional properties of soy protein through structural modifications. These processing techniques include extrusion cooking (Ma et al., 2018), high-temperature processing (Cui, Chen, Kong, Zhang, & Hua, 2014), high-pressure processing (Torrezan, Tham, Bell, Frazier, & Cristianini, 2007), enzymatic hydrolysis (L. Chen et al., 2011) and ultrasound processing (Ding et al., 2019).

Extrusion cooking of soybean proteins has been extensively studied in the recent past to produce meat extenders and meat analogs that resemble the fibrous structure of animal proteins (Riaz, 2011; Samard, Maung, Gu, Kim, & Ryu, 2021; Zhang et al., 2019). The high temperatures inside an extruder barrel firstly denature the protein molecules which are then aligned in the direction of flow under high shear and high pressure by the use of specialized dies (e.g., long slit shaped dies with water circulation to cool down the extrudate temperature) where strong protein-protein interactions take place (Riaz, 2011). This process known as texturization results in modified techno-functional properties in the end-product. During texturization, the development of new covalent bonds, as well as non-covalent interactions between protein molecules, can impact protein techno-functionality by influencing their surface hydrophobicity, molecular weight and molecular flexibility (Chen, Wei, & Zhang, 2011; Liu & Hsieh, 2008; Mozafarpour et al., 2019). Although texturization substantially improves techno-functionality of plant-proteins that are to be used in the formulation of meat extenders and meat analogs, extrusion cooking alone can be fine-tuned to enhance extrudate's techno-functional quality attributes, even in the absence of texturization (Alonso, Orúe, Zabalza, Grant, & Marzo, 2000; Téllez-Morales, Herman-Lara, Gómez-Aldapa, & Rodríguez-Miranda, 2020).

Quality of an extrudate is largely dictated by extrusion cooking parameters such as barrel temperature profile, feed moisture content, feed rate, screw speed and raw material characteristics. Hence, these parameters should be carefully controlled to produce extrudates with favorable physical as well as techno-functional properties (Alam, Kaur, Khaira, & Gupta, 2016; Arora, Yoon, Sriram, Singha, & Rizvi, 2020; Day & Swanson, 2013; Zhang et al., 2019). Among these parameters, some, but not all have been investigated. For example, barrel temperature and feed moisture content have previously been reported to affect the protein solubility, emulsion capacity

and emulsion stability of soy protein (Ma et al., 2018; Mozafarpour et al., 2019). Extrusion system parameters, such as specific mechanical energy, torque and die pressure, also play an important role in extrudate quality (Chen, Wei, Zhang, & Ojokoh, 2010; Fang, Zhang, & Wei, 2014). System parameters generally rely on the rheological properties of the raw materials inside the barrel (e.g., melt viscosity), which in turn depend on extrusion cooking parameters (Chen et al., 2010). For example, lowering extrusion barrel temperature and feed moisture content has been previously shown to increase the specific mechanical energy during extrusion cooking by increasing the viscosity of soy protein isolates inside the extruder barrel (Chen et al., 2010). Raw material particle size distribution has also been previously reported to impact the system parameters during extrusion of starch-rich raw materials, as well as the physical properties of extrudates made from them (Al-Rabadi, Torley, Williams, Bryden, & Gidley, 2011; Altan, McCarthy, & Maskan, 2009; Carvalho, Takeiti, Onwulata, & Pordesimo, 2010). However, the effects of raw material particle size distribution on techno-functional properties of protein-rich raw materials, specifically soybean meal, has not been previously studied.

In order to produce novel food ingredients from soybean meal, it is of utmost importance to understand how extrusion cooking and its system parameters impact soybean meal's techno-functionality. Accordingly, the present research work was designed to explore the possibilities of producing novel food ingredients with improved techno-functional quality attributes from soybean meal with the use of extrusion cooking. The specific objectives of this research project were to investigate the effects of particle size distribution and its interaction with extrusion barrel temperature and feed moisture content on nitrogen solubility index, gel forming capacity, oil holding capacity, water holding capacity, emulsion capacity and emulsion stability of soybean meal extrudates.

Bridge to Chapter 2

The following chapter contains a comprehensive review of the current literature on soybean meal, soy proteins, their techno-functional properties and the impacts of extrusion processing parameters on soy protein's structure and techno-functionality. The primary focus is to find research gaps from the current literature to form a basis for the experimental design of this research work.

Chapter 2: Literature Review

2.1. Soybean Meal

SBM is the major by-product of the soybean oil extraction process and is the most widely used ingredient in animal feed amongst oilseed crops (Stein et al., 2008). Although SBM is primarily used in animal feed, several protein rich products made from SBM are widely popular for human consumption as well such as defatted soybean flours or grits, soy protein concentrates, soy protein isolates and texturized vegetable proteins (TVPs) made from them (Hettiarachchy & Kalapathy, 1997; Jones, 2016; Singh, Kumar, Sabapathy, & Bawa, 2008).

The methods for oil extraction from soybeans include mechanical pressing, solvent extraction, or a combination of both (Aydeniz, Güneşer, & Yılmaz, 2014). For the solvent extraction method, the soybean seeds are dried, cleaned, cracked, dehulled, conditioned and flaked before solvent extraction. Conditioning is usually done at around 74 °C before flaking to soften the structure of soybeans, while flaking is done to facilitate and speed up the oil-extraction process (Proctor, 1997). The flakes are then defatted using hexane as a solvent. The resultant meal contains less than 1% oil and about 30-35% residual hexane, hence it needs to be desolventized at high temperatures (e.g., 105-115 °C) to evaporate the residual hexane before it can be used for food or feed consumption (Hettiarachchy & Kalapathy, 1997; Paraíso, Cauneto, Zemp, & Andrade, 2008). In the final step, defatted SBM is ground using hammer mills to produce soy-grits or soy-flour, based on its particle size.

Apart from using solvent extraction for soybean oil processing, mechanical oil extraction through screw presses is utilized to extract cold-pressed soybean oil. Screw presses generate high pressure and thus mechanically squeeze the oil from soybean seeds. The resulting meal from the screw

pressed soybean contains a higher amount of oil, but no hexane, when compared to the solvent extracted meal (Proctor, 1997).

2.1.1. Nutritional Value

SBM is a rich source of proteins, and solvent-extracted SBM contains about 45 – 50 % crude protein content (Lagos & Stein, 2017). The protein content of SBM is not only high in quantity but also in quality, as it has a well-balanced essential amino acid composition with high amounts of lysine - a limited amino acid in cereal protein sources (Stein et al., 2008). Soybean protein is rated among the best protein sources with a Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1 (Hertzler, Lieblein-Boff, Weiler, & Allgeier, 2020; Hoffman & Falvo, 2004). Moreover, soybean proteins are also considered to be good for cardiovascular health. Daily consumption of a minimum of 25 g soy proteins as part of a low-fat diet is recommended for its cholesterol-lowering impacts (Health Canada, 2015).

SBM also contains approximately 32 - 35 % of carbohydrates in the forms of non-starch polysaccharides, starch, sucrose, and oligosaccharides such as stachyose and raffinose. Around half the carbohydrates in SBM are oligosaccharides (Hettiarachchy & Kalapathy, 1997; Singh et al., 2008) which are often considered to be anti-nutritional factors (ANFs) due to their flatulence and abdominal discomfort causing impacts (Kumar Dixit, Kumar, Rani, Manjaya, & Bhatnagar, 2011). However, non-digestible oligosaccharides may also cause several health benefits by modulating the intestinal gut microbiota (Qiang, YongLie, & QianBing, 2009; Slavin, 1999). The total dietary fiber content of SBM is about 21.8 % (Khajali & Slominski, 2012).

The oil content of the solvent-extracted meal lies in the range of 0.5 - 1 %. In terms of micronutrients, SBM contains calcium, phosphorous, potassium, iron, and manganese (Ravindran,

Abdollahi, & Bootwalla, 2014). SBM also contains some water and oil-soluble vitamins in smaller quantities such as vitamin E, thiamin, riboflavin, pantothenic acid, biotin, folic acid, choline, and niacin (Newkirk, 2010).

2.1.2. Anti-nutritional Factors

Anti-nutritional factors (ANFs) are defined as substances produced by the natural defense mechanisms of various plants, and thus are beneficial for plant's survival. However, the presence of ANFs is undesirable if these plants are to be consumed as foods or feeds as they may decrease the availability of nutrients (e.g., proteins and several minerals) to humans and animals (Thangaraj, 2016). The major ANFs present in SBM are trypsin inhibitors, lectins and phytic acid (Gu, Pan, Sun, & Qin, 2010; Yasothai, 2016). The presence of ANFs in soybean protein products are a major hindrance in their utilization in human foods due to their negative physiological effects (Vagadia, Vanga, & Raghavan, 2017).

2.1.2.1. Trypsin Inhibitors

SBM contains approximately 2 % of trypsin inhibitors (Liener, 1994; Vagadia et al., 2017). Trypsin inhibitors decrease the protein digestibility by reducing the activities of trypsin and chymotrypsin (i.e., proteolytic enzymes that aid in protein digestion). The reason behind the decreased activity of trypsin in the presence of trypsin inhibitors is the formation of irreversible trypsin-trypsin inhibitor complexes (Vagadia et al., 2017). In SBM, two types of trypsin inhibitors are present: i.e., soybean Kunitz trypsin inhibitor and Bowman-Birk inhibitor.

2.1.2.2. Lectins

SBM contains approximately 4 % of lectins (Liener, 1994; Vagadia et al., 2017). Lectins are glycoproteins which have antifungal effects (He et al., 2018). Lectins reduce nutrient utilization efficiency by influencing the absorption of amino acids and damage the gut intestinal membrane (Liener, 1994).

Soybean lectins and trypsin inhibitors have been reported to have animal growth depressing effects (de la Barca, Vázquez-Moreno, & Robles-Burgueño, 1991) and SBM should be processed properly before consumption to eliminate these anti-nutrients.

2.1.2.3. Phytic Acid

Phytic acid is present in plants in the form of protein-phytic acid complexes and serves as major phosphorous reserves for plant seeds (Wang & Guo, 2021). The presence of phytic acid is associated with decreased protein digestibility because proteolytic enzymes are unable to digest protein-phytic acid complexes (Joye, 2019; Samtiya, Aluko, & Dhewa, 2020). Phytic acid also makes complexes with several minerals such as calcium, magnesium, phosphorous, iron, zinc, etc., and reduces their availability (Thangaraj, 2016). However, phytic acid has also been reported to provide some health benefits due to its anti-carcinogenic properties (Abdulwaliyu et al., 2019; Kumar, Sinha, Makkar, & Becker, 2010), by reducing cell proliferation through inhibition of various cancer cells (Kumar et al., 2010).

2.2. Important Techno-functional Properties of Soybean Proteins

Techno-functional properties are defined as those properties of proteins that influence their behavior when used in different food systems and produce foods with desirable texture and other

sensory properties (Kinsella, 1979; Mirmoghtadaie, Shojaee Aliabadi, & Hosseini, 2016). Soy protein products have immense potential to be used in various food products such as baked products, comminuted meat products, dessert toppings, etc., provided that the processing operations used during and after oil extraction do not produce negative impacts on their techno-functionality. Some of the important techno-functional properties are emulsification, foaming, gelation, water holding capacity, oil holding capacity, and solubility (Kinsella, 1979). Some of the important techno-functional properties, their mechanism of action, and food uses are given in Table 2.1.

Table 2.1. Important techno-functional properties, their mechanism of action, and the food groups that benefit from these techno-functional properties (Damodaran, 1997).

Techno-functional property	**Mode of action**	**Food**
Solubility	Hydrophilicity	Beverages
Gelation	Network formation through aggregation of protein molecules	Meats, Gels, Cakes, Bakeries, Cheese
Fat holding capacity	Hydrophobic bonding with fats	Low-Fat Bakery Products, Doughnuts
Water holding capacity	Hydrogen Bonding	Meat Sausages, Cakes, Breads
Emulsification	Adsorption at water-oil interface, reducing the interfacial surface tension	Sausages, Bologna, Soup, Cakes, Dressings
Foaming	Film formation at liquid-air interface for air trapping	Whipped Toppings, Ice Cream, Cakes, Desserts

2.2.1. Protein Solubility

Protein solubility is defined as the amount of extractable nitrogen (in water or salt solution) present in a protein product (Sikorski, 2001; Zayas, 1997). The proportion of soluble protein highly depends on the extraction conditions such as pH, ionic strength, extraction time and temperature, etc., as well as the structural characteristics of the protein (Sikorski, 2001). High protein solubility has been shown to be associated with smaller protein aggregates, high surface charge, and low surface hydrophobicity (Karaca et al., 2011). Thermal processing of proteins influences protein solubility by causing changes in protein structure through denaturation and subsequent aggregation. Thermal denaturation generally increases surface hydrophobicity of proteins which leads to protein-protein hydrophobic interactions and thus aggregation of proteins. Accordingly, insoluble protein complexes may occur and thus negatively affect protein solubility (Kinsella, 1979).

Protein solubility is one of the most important and critical techno-functional properties as it significantly impacts other techno-functional properties such as gelation, foaming and emulsification (Khattab & Arntfield, 2009; Wouters, Rombouts, Fierens, Brijs, & Delcour, 2016); high protein solubility often improves emulsification and foaming properties (Hettiarachchy & Kalapathy, 1997; Kinsella, 1979; Wouters et al., 2016). The loss of protein solubility limits the utilization of high protein foods such as SBM by negatively affecting their techno-functionality (Kinsella, 1979; Wouters et al., 2016).

2.2.2. Gelation

Among various techno-functional properties, gelation plays an important role in determining the overall acceptability of food products by imparting desirable sensory and textural properties

(Oakenfull, 1997). In high-protein food products, gelation is a phenomenon of denaturation and subsequent aggregation of protein molecules during which denatured protein molecules aggregate to form a continuous network (Kinsella, Rector, & Phillips, 1994; Totosaus, Montejano, A. Salazar, & Guerrero, 2002). Physical food processing methods which induce protein gelation are high-temperature and high-pressure processing while chemical methods include ion-induced gelation, urea- and acid-induced gelation (Totosaus et al., 2002). Among these methods, one of the commonly used methods to make protein gels is the high-temperature processing that causes unfolding of native proteins, thereby exposing their hydrophobic non-polar residues to their surface, initiating gel formation by aggregation or clustering of protein molecules (Clark, Kavanagh, & Ross-Murphy, 2001; Hettiarachchy, Kannan, Schäfer, & Wagner, 2013). Formation of gels may involve several different interactions such as disulfide bonds, hydrogen bonds, hydrophobic and electrostatic interactions which help in the development and stabilization of protein gels (Banerjee & Bhattacharya, 2012; Foegeding, Bowland, & Hardin, 1995).

2.2.3. Oil and Water Holding Capacities

Oil and water holding capacities are important techno-functional properties of oilseed meals due to their impacts on flavor retention and mouthfeel (Khattab & Arntfield, 2009). The enhancement of these properties improves flavor and mouthfeel by reducing water and fat losses from food products (Khattab & Arntfield, 2009). Thermal denaturation of food proteins causes the non-polar amino acids that generally remain in the interior of native globular protein structure to come to the protein's surface, increasing surface hydrophobicity of the protein. This increased surface hydrophobicity enhances the oil absorption capacity, as oil and the hydrophobic amino acid side chains can more readily interact with each other (Kinsella & Melachouris, 1976; Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014). Along with non-polar amino acids, a significant

proportion of polar amino acids are buried in the interior of the native structure of globular proteins and are therefore unavailable for interacting with water (Damodaran, 1997). The denaturation of globular proteins improves their water-binding capacity by exposing these additional binding sites that were originally buried in the interior of the native structure of globular proteins for water absorption (Khattab & Arntfield, 2009).

2.2.4. Emulsification

Emulsions are mixtures of two or more immiscible liquids (e.g., oil-in-water emulsions). For high quality emulsified food products, emulsifiers that create and stabilize emulsions are needed. Good emulsification properties (i.e., high emulsion capacity and stability) of food help to impart desirable texture and mouthfeel, for example in mayonnaise, coffee whiteners, salad dressings, etc. (Lam & Nickerson, 2013). Food proteins contain both hydrophobic and hydrophilic groups, hence show amphiphilic character when used in food systems. Accordingly, they can act as emulsifiers by reducing the interfacial tension between immiscible liquids like water and oil in foods (Lam & Nickerson, 2013; Phillips, Whitehead, & Kinsella, 1994). Oil-in-water emulsions are formed using high-speed homogenization which breaks a layer of oil into small oil droplets. Emulsifying agents such as soybean protein adsorb to the surface of newly formed oil droplets and prevent their re-coalescence by lowering the interfacial tension between the oil and the water (L. Chen et al., 2011).

Soybean proteins have been extensively studied for their emulsifying capacity (i.e., amount of oil emulsified by a given amount of protein) and stability (i.e., stability of an emulsion over a period of time) as well as how these properties are influenced by several processing methods such as thermal processing (Cui et al., 2014), extrusion processing (Mozafarpour et al., 2019), enzymatic hydrolysis (L. Chen et al., 2011) and high-pressure microfluidization (Chen, Chen, Yu, & Wu,

2016). Native soybean proteins possess low molecular flexibility due to their compact globular structure which hinders their ability to unfold at water-oil interfaces, thus resulting in inferior emulsifying properties (L. Chen et al., 2011; Mozafarpour et al., 2019). Food processing operations, depending on their severity, can cause protein structural changes and thus increase protein's molecular flexibility to enhance emulsifying properties (L. Chen et al., 2011; Mozafarpour et al., 2019). Emulsion properties have also been shown to have a positive correlation with protein solubility (Karaca et al., 2011). Highly soluble proteins migrate more readily to the water-oil interface, hence, favour fast adsorption and improved emulsifying properties (Karaca et al., 2011; Lam & Nickerson, 2013).

2.2.5. Foaming

The unique texture of several food products such as bread, cakes, ice cream and whipped toppings depends on the amount of air incorporated and retained in their structures during processing (Phillips et al., 1994; Shao, Lin, & Kao, 2016). Proteins serve as excellent foaming agents in foods due to their ability to entrap and hold air cells by forming a cohesive film at the surface of these air cells (Hettiarachchy & Kalapathy, 1997; Shao et al., 2016). The foaming properties of proteins greatly depend on their adsorption and subsequent unfolding at the air cell walls (He et al., 2015; Phillips et al., 1994). The improvement of foaming properties can be achieved through the modification of protein structure (Hettiarachchy & Kalapathy, 1997; Kinsella, 1979). To be used as an effective foaming agent, a protein should have the ability to undergo conformational changes for its rapid adsorption at an air-water interface. This rapid adsorption initiates foaming by forming a viscoelastic film around air cells that provide sufficient foam stability (Shao et al., 2016). The foaming properties of soybean proteins show a close correlation with protein solubility (He et al., 2015; Hettiarachchy & Kalapathy, 1997; Ivanova et al., 2018; Kinsella, 1979). High protein

solubility makes it easier for a protein to diffuse to an air-water interface which is an important factor in achieving desired foaming properties (German & Phillips, 1994; Ghumman, Kaur, & Singh, 2016).

2.3. Soy Protein Structure

The major portion of soy protein is composed of seed storage proteins such as glycinin and β-conglycinin. Based on their sedimentation coefficients, the proteins of soybean are classified into four major categories: 2S, 7S, 11S, and 15S (Nishinari, Fang, Guo, & Phillips, 2014; Utsumi, Matasumura, & Mori, 1997). Among these, glycinin (11S) and β-conglycinin (7S) contribute to more than 80% of total soybean proteins. The ratio of β-conglycinin to glycinin is about 0.5-1.3 with a high dependency on soybean varieties (Nishinari et al., 2014; Saio, Kamiya, & Watanabe, 1969). The 2S fraction is composed of some other globulins including trypsin inhibitors and cytochrome (Kinsella, 1979; Luthria, Maria John, Marupaka, & Natarajan, 2018) while 15S is an aggregate of the 11S (i.e. formed by association of small fractions of glycinin) (Luthria et al., 2018; Wolf & Nelsen, 1996).

2.3.1. Glycinin

Glycinin is a hexamer, made up of a combination of five different subunits, and has a molecular weight of 300-380 kDa (Lampart-Szczapa, 2001). Each of the five subunits has an acidic polypeptide A and a basic polypeptide B with molecular weights of 35 kDa and 20 kDa, respectively (Nishinari et al., 2014; Utsumi et al., 1997). The quaternary structure of glycinin is made up of two hexagonal rings that are stabilized by electrostatic interactions and hydrogen bonds, giving it the shape of a hollow cylinder (Badley et al., 1975; Nishinari et al., 2014). Each hexagonal ring contains three acidic and three basic polypeptides that are linked together by

disulfide bonds (Kinsella, 1979; Nishinari et al., 2014). The presence of strong disulfide bonds provides a very compact structure to glycinin molecules (Badley et al., 1975; Nishinari et al., 2014). Furthermore, these subunits of glycinin also show polymorphism and molecular heterogeneity among different soybean varieties (Mori, Utsumi, Inaba, Kitamura, & Harada, 1981). These differences in their structure may give rise to different techno-functional properties of soy proteins from different varieties.

2.3.2. β-conglycinin

The second major protein of soybean is β-conglycinin and it is primarily composed of three subunits: α, α' and β with molecular weights of 67 kDa, 71 kDa and 50 kDa, respectively (Lampart-Szczapa, 2001; Nishinari et al., 2014). In terms of their respective structures, all three subunits exhibit similar primary structure but differ in the composition of their sulfur-containing amino acids, i.e., methionine and cysteine (Utsumi et al., 1997). Unlike glycinin, β-conglycinin has no disulfide bonds, hence making it more prone to denaturation on the application of high temperature or high pressure (Fukushima, 2004).

2.4. Relationship between Protein Structure and Techno-functional Properties

The structural characteristics of proteins such as amino acid profile, size, surface hydrophobicity, and surface charge play vital roles in shaping various techno-functional properties (Akharume, Aluko, & Adedeji, 2021; Kinsella & Melachouris, 1976; Mirmoghtadaie et al., 2016).

In the case of globular proteins, the polar amino acids generally reside on protein surface , hence favoring hydration related properties such as water holding capacity and solubility (Kinsella & Melachouris, 1976). Moreover, the ratio of polar to non-polar amino acids also influences water holding capacity as more polar amino acids generally favor high water holding capacity

(Damodaran, 1997). Similarly, protein solubility also highly depends on the hydrophilicity/hydrophobicity balance. More specifically, the polar amino acids present at the surface of protein structure favor high protein solubility (Damodaran, 1997).

Emulsifying properties, on the other hand, depend primarily on the flexibility of proteins as emulsification involves unfolding of protein molecules at the lipid-water interface (Kinsella, 1979; Mozafarpour et al., 2019). Moreover, the molecular size of proteins also influences the emulsifying properties of proteins, with smaller molecular weight molecules inducing higher emulsion capacities (Lam & Nickerson, 2013). Other factors that influence emulsion properties of the protein molecules are surface hydrophobicity and solubility that regulate protein adsorption to the oil and water phase, respectively (Lam & Nickerson, 2013). The two major protein fractions present in soybean protein, i.e., glycinin and β-conglycinin, show different techno-functional properties that primarily arise as a result of their structural dissimilarities (Nishinari et al., 2014). The presence of disulfide bonds in the structure of glycinin provides it a very compact structure that is responsible for its lower emulsification and foaming properties as compared to β-conglycinin (Nishinari et al., 2014).

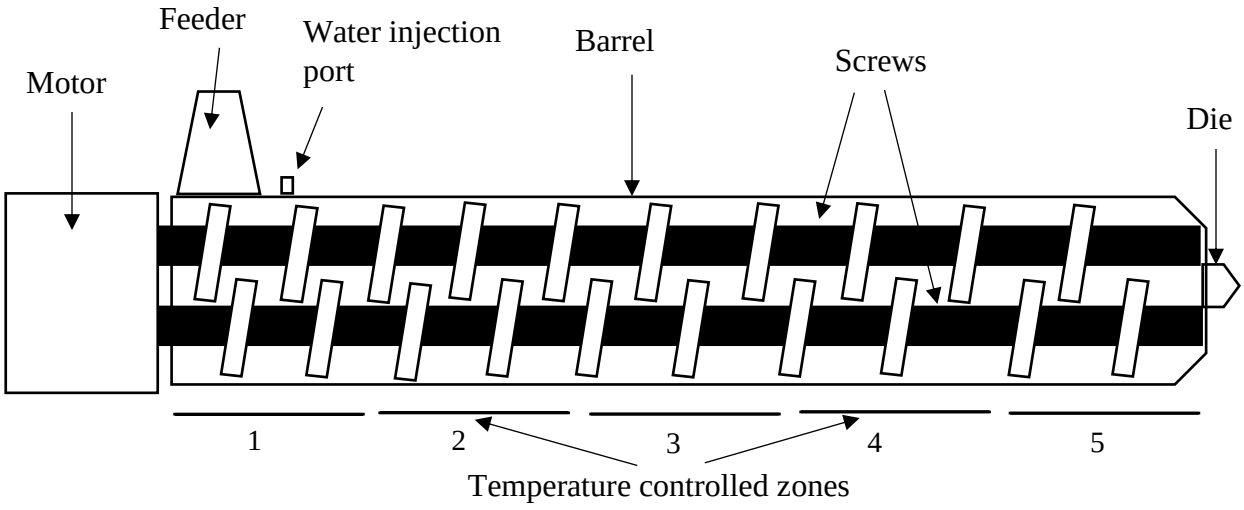
Gelling properties and oil holding capacity of proteins are generally improved with an increase in protein's surface hydrophobicity (Damodaran, 1997). The favorable impacts of increased surface hydrophobicity on gelling properties of proteins may be imparted by an increase in the network formation of protein molecules through extensive hydrophobic bonding, thus requiring lesser protein to form a gel (He et al., 2014). Moreover, the gelling properties are also favored by the presence of larger sized protein molecules through formation of extensive cross-linking structures as compared to smaller sized molecules (Damodaran, 1997; He et al., 2014). In terms of two major soybean globulins, the glycinin molecules require lesser protein concentration to form a self-stable

gel as compared to β-conglycinin (Nakamura, Utsumi, & Mori, 1986a). Moreover, the structure of glycinin gels was found to be stabilized by a combination of disulfide bonds and hydrophobic interactions (Nakamura, Utsumi, & Mori, 1984), whereas, the disulfide bonds were absent in β-conglycinin gels (Nakamura, Utsumi, & Mori, 1986b).

2.5. Extrusion Cooking Process

Extrusion cooking is conventionally used to make puffed snacks and breakfast cereals by forcing a molten mass of raw materials through a small opening (die) with the help of screws, under high temperature and high pressure (Offiah, Kontogiorgos, & Falade, 2019; Singh, Gamlath, & Wakeling, 2007). A schematic diagram of a twin-screw extruder with five temperature-controlled zones is provided in Figure 2.1. The process involves several processing operations like mixing, cooking, shearing, forming and shaping simultaneously which cause structural and techno-functional changes in foods (Alam et al., 2016). According to Zhang et al. (2019), a typical extrusion process involves the processing of raw materials in four functional zones: feeding zone, mixing zone, melting zone, and the die. The raw materials are fed through the feed hopper to the feeding zone and are then conveyed to the mixing zone with the action of rotating screws. In the mixing zone, the raw materials are blended and mixed with incoming water to achieve the desired moisture content (Zhang et al., 2019). Most of the physical and chemical changes in the raw materials take place in the melting zone where strong shearing forces are generated by the screws and high temperatures (generally > 100 °C) used. The ‘melt’ which is formed in the melting zone is then passed through a die to make specific shapes of the ‘extrudate’, i.e., the final product. Leaving the die, the final structure of the extrudate is set due to evaporation of water due to a sudden pressure drop (Zhang et al., 2019).

Figure 2.1. Schematic diagram of a twin-screw extruder



Apart from puffed snacks and breakfast cereals, texturized vegetable proteins (TVPs) (e.g., meat extenders and meat analogs) from plant proteins (e.g., soy, wheat and pea proteins) have become very popular, especially during the last decade (Ismail, Hwang, & Joo, 2020). Extrusion cooking for puffed snacks requires higher starch containing raw materials and lower moisture contents (< 20 %) to facilitate the adequate expansion of extrudates, whereas high protein containing raw materials and higher moisture contents (up to 80 %) are generally used for the production of texturized products (Day & Swanson, 2013). The extrusion cooking of proteinaceous raw materials (such as SBM and other soy protein products) generally produces two distinct types of TVPs i.e., meat extenders and meat analogs. The meat extenders have a porous expanded structure and provide fibrous texture upon rehydration (Riaz, 2011; Samard, Gu, & Ryu, 2019). Hence, proper rehydration after extrusion is required before the final consumption of meat extenders. Meat analogs, on the other hand, have well defined fibrous structure and can be directly consumed

(Samard et al., 2019). Extrusion processing can also be utilized to improve the techno-functional properties of protein-rich raw materials such as SBM to develop new food ingredients.

2.5.1. Impacts of Extrusion Cooking Process on Soybean Protein Structure

Extrusion cooking can induce several changes in protein structure by causing denaturation and subsequent aggregation of protein molecules (F. L. Chen et al., 2011; Liu & Hsieh, 2008; Riaz, 2011). During extrusion processing, high thermal and mechanical energy is provided to the barrel and transferred to the raw materials in the early stages of mixing. This high level of thermal and mechanical energy inputs disrupts or denatures the native protein structure. The screws (to a limited extent) and the die then align the denatured protein molecules in the direction of flow. This may expose the binding sites of proteins and cause aggregation of protein molecules (Riaz, 2011). However, the strong shearing forces and high temperatures in the latter stages of extrusion cooking may also cause some degradation of larger protein aggregates (Mozafarpour et al., 2019; Zhang et al., 2019). The unfolding and aggregation of protein molecules take place simultaneously inside the extruder which makes it very difficult to accurately predict protein-protein interactions at different sections of an extruder (Day & Swanson, 2013). It has also been reported that most of the aggregation of protein molecules takes place at the die, especially during high moisture extrusion where a longer cooling die is generally used (Day & Swanson, 2013). In texturized vegetable proteins, these protein-protein interactions are responsible for the formation of a fibrous structure that can mimic the texture of animal proteins (Riaz, 2011).

It is generally believed that extrusion processing mainly impacts the hydrogen bonds, hydrophobic interactions and disulfide bonds of protein molecules but shows no effects on peptide bonds (F. L. Chen et al., 2011; Liu & Hsieh, 2008; Zhang et al., 2019). However, an earlier research also

reported the formation of new peptide bonds during extrusion texturization of soybean protein (Burgess & Stanley, 1976). To understand the protein-protein interactions that take place during the extrusion texturization of soy protein isolate, F. L. Chen et al. (2011) used a combination of different buffer solutions for protein solubility analysis. Buffer solutions such as phosphate buffer (P), urea (U), sodium dodecyl sulfate (S), and 2-Mercaptoethanol (M) as well as their combinations were used, of which P was used to measure the protein solubility in the native state while the latter three solutions were used to break hydrogen bonds, hydrophobic bonds and disulfide bonds, respectively. The results demonstrated that the protein solubility was highest when a combination of S, M and U was used followed by S and U, M, and P. These results indicated that a combination of non-covalent (hydrophobic and hydrogen) followed by covalent (disulfide) bonds were the most important in forming the characteristic extrudate structure (F. L. Chen et al., 2011). The importance of non-covalent and disulfide bonds in stabilizing the fibrous structure of soybean extrudates was also reported in a separate study, however, the relative importance of disulfide bonds was reported to be higher than that of non-covalent interactions (Liu & Hsieh, 2008).

The formation of new bonding patterns during extrusion cooking of soybean protein concentrates has been reported to cause changes in their secondary structural characteristics (Mozafarpour et al., 2019). Among different secondary structures, α-helix was found to be the most prone to degradation while the amount of β-sheet structures increased with extrusion cooking when compared to the raw soybean protein concentrate (Mozafarpour et al., 2019). Extrusion processing also resulted in the formation of higher molecular weight proteins when compared with raw protein isolates (F. L. Chen et al., 2011; Mozafarpour et al., 2019). The two major soybean proteins, i.e., glycinin and β-conglycinin, showed different stabilities during extrusion processing with glycinin showing higher stability (Ma et al., 2018). All these structural changes can play an important role

in shaping the techno-functional properties of soybean proteins due to their structure-function relationship, thereby effecting their choice as feed ingredients.

2.5.2. Important Extrusion Parameters and their Impacts on Techno-functional Properties

Extrusion cooking transforms raw materials into final products through incorporation of thermal and mechanical energy (Alam et al., 2016; Zhang et al., 2019). This transformation involves a complex interplay between physical and chemical changes in macro- and micro-molecules of raw materials inside the extruder barrel which makes it difficult to accurately predict the outcomes (Zhang et al., 2019). However, the thorough understanding of extrusion parameters and their manipulation can provide effective ways to produce the desired end-products.

The final product characteristics of extrudates mainly depend on two types of extrusion parameters: process parameters and system parameters (Chen et al., 2010). The process parameters include raw material characteristics, barrel temperature, moisture content, feed rate, screw configuration, die shape, etc. (Chen et al., 2010), while the system parameters (specific mechanical energy (SME), residence time distribution, die pressure, etc.) arise as a result of the interactions between various process parameters. Together, they determine the severity of extrusion treatment conditions, thereby, affecting the product characteristics (physical and techno-functional properties) (Alam et al., 2016; Chen et al., 2010). SME is defined as the mechanical energy used by the extruder motor to process a unit mass of raw materials (Godavarti & Karwe, 1997). Among extrusion parameters, only the operating parameters can be directly controlled by an extruder operator, thus making them extremely important in determining the end-product quality.

2.5.2.1. Raw Material Particle Size Distribution

The particle size distribution (PSD) of raw materials substantially affects the properties of extrudates as it dictates how fast particles absorb water and/or how fast they are heated in the barrel (Al-Rabadi et al., 2011; Guy, 2001; Sharifi, Majzoobi, & Farahnaky, 2021). The reason behind the different thermodynamic properties of smaller-sized particles may be their higher contact area (i.e., surface area to volume ratio) when compared with larger particles, allowing other parameters such as barrel temperature and feed moisture to be more effective. As a result, smaller particles get heated up more rapidly (Desrumaux, Bouvier, & Burri, 1998; Onwulata & Konstance, 2006; Sharifi et al., 2021).

Several previous research studies reported the effects of PSD on SME input during extrusion, as well as physical and textural properties of starch-rich extrudates (Alam et al., 2014; Altan et al., 2009; Carvalho et al., 2010; Onwulata & Konstance, 2006). Carvalho et al. (2010) demonstrated that fine fractions caused a higher SME input during extrusion and the resulting extrudates had higher water holding capacity when compared to coarse fractions of corn meal. Similarly, the SME, expansion index and water holding capacity of barley extrudates from fine fractions were higher than those of extrudates from large particles (Al-Rabadi et al., 2011). However, when a pre-conditioning step was used, Altan et al. (2009) reported higher SME in the case of barley grits extrusion as compared to barley flour extrusion. The use of the pre-conditioning step allowed proper hydration of the coarser fractions before entering the extruder which nullified the impacts of water uptake rate of finer and coarser materials inside the extruder barrel (Altan et al., 2009). In terms of properties of extrudates, finer fractions resulted in lower extrudate density (i.e., higher overall expansion) as compared to coarser fractions, but no significant particle size effects were observed on water holding capacity (Altan et al., 2009).

PSD also showed effects on the microstructure of extrudates as measured by scanning electron microscopy. The extrudates obtained from finer fractions of barley and sorghum showed higher disruption of starch granules as compared to extrudates obtained from coarser fractions which showed very little changes in the native starch structure (Al-Rabadi et al., 2011). The lesser disruption of extrudate structure in case of coarser raw materials was related to a lesser water uptake of coarser fractions than the finer fractions that may have reduced the extent of starch gelatinization (Al-Rabadi et al., 2011).

During SBM extrusion for production of meat extenders or meat analogs, PSD can affect the degree of protein texturization, as fiber particles in SBM with larger mean particle size may interfere with the cross-linking of proteins (Riaz, 2004). Grinding of SBM to finer fractions can be used to overcome this problem (Riaz, 2004). Although the impact of raw material PSD on the physical properties of starch-rich extrudates has been studied previously, the PSD effects on the techno-functional properties of protein-rich extrudates have not been reported.

2.5.2.2. Feed Moisture Content

Feed moisture content serves a variety of functions during extrusion processing such as acting as a lubricant, influencing the flow behavior of materials, and determining the overall severity of extrusion processing (Chen et al., 2010; Day & Swanson, 2013; Zhang et al., 2019). Chen et al. (2010) reported for soy protein isolate that an increase in moisture content from 28% to 60% caused a decrease in viscosity of materials inside the extruder barrel through water's plasticizing effect. The decreased viscosity with higher moisture content was responsible for easier flow of materials, resulting in decreased shearing forces accompanied by reduced SME and mean residence time distribution. Moreover, higher moisture extrusion (60 %) resulted in reducing

overall polymerization of soy protein molecules, hence, forming smaller protein molecules as compared to soy protein isolate extrudates obtained at the lower moisture content (28 %) (F. L. Chen et al., 2011). These results may be due to the decreased protein concentration (due to higher water content) and low mean residence time (due to decreased viscosity) of raw materials in the extruder barrel during high moisture extrusion, both of which were previously linked to reduced protein aggregation (Petruccelli & Anon, 1995). Moreover, the decreased shearing forces and residence time distribution during high moisture extrusion may also lead to less denaturation, hence, native protein structure can be retained (F. L. Chen et al., 2011; Mozafarpour et al., 2019). However, lower moisture extrusion may also dissociate the larger protein aggregates during the latter stages of extrusion due to increased shearing forces as compared to high moisture extrusion, as reported for soy protein concentrates by Mozafarpour et al. (2019). Hence, the overall impacts of extrusion moisture on protein-protein interactions are complex and greatly depend on its impacts on the severity of extrusion and protein source.

Among techno-functional properties, extrusion processing generally causes a decrease in protein solubility due to the formation of larger protein aggregates (F. L. Chen et al., 2011; Chen et al., 2010; Ma et al., 2018; Mozafarpour et al., 2019). Moreover, higher extrusion feed moisture content has been reported to further decrease the protein solubility as compared to low feed moisture (Mozafarpour et al., 2019). The lower protein solubility at higher moisture content may be attributed to the lower shearing forces that cause less disintegration of larger protein aggregates. Extrusion processing has also been shown to affect the emulsification properties by inducing changes in solubility and surface hydrophobicity of soy proteins (Mozafarpour et al., 2019). Among different extrusion parameters, an increase in moisture content from 18 % to 25 % resulted

in a decrease in extrudate emulsion capacity but at the same time, emulsion stability increased (Mozafarpour et al., 2019).

2.5.2.3. Barrel Temperature Profile

The major role of temperature during extrusion cooking of protein-rich foods is to induce unfolding of protein molecules which then gives rise to protein-protein interactions to form new protein aggregates (Riaz, 2011). The aggregated protein molecules often have low solubility which results in changes in other techno-functional properties such as gelation, foaming and emulsification (Petruccelli & Anon, 1995). Heating soybean proteins above their denaturation temperature may induce changes in their surface properties by impacting their surface hydrophobicity (Mozafarpour et al., 2019). Petruccelli and Anon (1995) demonstrated that modifications in the soybean protein structure were observed while treating the samples at 90 and 100 °C temperature. However, no modifications were observed below 80 °C heat treatment (Petruccelli & Anon, 1995). Thermal processing (like that during extrusion) generally results in the formation of larger protein aggregates as compared to their native state. However, for soy protein isolates, degradation of these larger protein aggregates has also been reported at very high extrusion temperatures (160 °C) (Liu & Hsieh, 2008). Hence, the temperature profile of the extruder barrel should be carefully selected to induce favorable changes in protein structure (such as formation of lower molecular weight protein molecules for increased protein solubility).

When the impact of the interaction of extrusion temperature profile and moisture content was investigated, changes in temperature profile on protein-protein interactions and product properties were found to be more significant at lower moisture conditions (Chen et al., 2010). For example, during lower moisture extrusion (28 %) of soy protein, the increase in temperature from 140 °C to

150 °C caused a more significant decrease in melt viscosity and SME input as compared to higher moisture extrusion (60 %), both of which have been previously reported to impact protein-protein interactions (Chen et al., 2010). Similarly, Lin, Huff, and Hsieh (2000) reported that an increase in temperature did not produce any changes in protein solubility and texture profile attributes (such as gumminess, hardness, and chewiness) of soy protein extrudates at the feed moisture content of 70 %. In contrast, Mozafarpour et al. (2019) demonstrated that extrusion cooking of soybean protein concentrate at a temperature of 160 °C at 18 % - 25 % moisture content caused dissociation of larger protein aggregates, resulting in increased solubility at higher temperatures. The possible explanation of the difference between the results of the above-mentioned studies is the difference in feed moisture contents as extrusion temperature and feed moisture may produce synergistic effects (Chen et al., 2010).

When techno-functionality is considered, an increase in extrusion temperature at the lower feed moisture content (18 %) has been reported to significantly increase the emulsion capacity of soy protein concentrate extrudates (Mozafarpour et al., 2019). Moreover, the extrudates obtained at higher temperature profile produced emulsions with smaller droplet particle sizes and increased the number of negatively charged droplets at the surface which decreased the occurrence of flocculation of emulsion droplets by increasing the repulsive forces between the droplet particles, thereby resulting in higher stability of emulsions (Mozafarpour et al., 2019).

2.5.2.4. Screw Speed and Shearing Forces

Along with barrel temperature, the shearing forces cause unfolding of protein molecules, and the denatured molecules then aggregate through various covalent and non-covalent interactions (Day & Swanson, 2013). The reassociation of protein molecules mostly take place near the die, hence,

shearing forces in the final stages of the extruder barrel are very important (Day & Swanson, 2013). Screw speed and shearing forces perceived by the melt in the barrel are closely related. The SME during the extrusion process is calculated using the following equation:

$$\text{SME}\ \left(\frac{\text{Wh}}{\text{kg}}\right) = \frac{\frac{actual\ screw\ speed\ (rpm)}{max\ screwspeed\ (rpm)} \times \frac{torque\ (\%)}{100} \times motor\ power\ (W)}{mass\ flow\ rate\ (\frac{kg}{h})}$$

where, the maximum screw speed of an extruder is the extruder rpm when extrusion motor runs on its rated power, and the motor power determines the power of an extruder motor that is required to convert the electrical energy into mechanical energy (Luo & Koksel, 2020). Both maximum screw speed and motor power remain constant based on the brand and model of the extruder.

The actual screw speed impacts the SME in two opposite ways. On one hand, an increase in screw speed decreases the melt viscosity and reduces the severity of extrusion processing, hence, a reduction in torque resulting in lower SME is expected (Meng, Threinen, Hansen, & Driedger, 2010). On the other hand, an increase in screw speed is directly related to SME as shown by the equation above. The impact of screw speed on shearing forces generally outweighs its impact on melt viscosity, resulting in increased SME at higher screw speed (Meng et al., 2010).

The positive correlation between screw speed and SME has been previously reported in corn meal extrusion (Garber, Hsieh, & Huff, 1997; Hsiegh, Peng, & Huff, 1990). SME influences the final product quality by affecting the molecular breakdown of raw material polymers (Chen et al., 2010; Godavarti & Karwe, 1997). Fang et al. (2014) reported that higher SME values were associated with an increase in protein solubility. Moreover, an increase in SME resulted in the formation of new protein aggregates with lower molecular weight (Fang et al., 2014). The possible reason behind increased protein solubility at higher SME was the improvement in mechanical shearing forces that caused the dissociation of larger protein aggregates (Fang et al., 2014).

2.1.3. Inactivation of Soybean Meal Anti-Nutritional Factors

Thermal processing is the most common way to reduce the ANF's of SBM. SBM is conventionally treated at very high temperatures (such as 105 – 115 °C) for longer exposure periods (such as 20-50 min) to inactivate the trypsin inhibitors and lectins (Mustakas, Moulton, Baker, & Kwolek, 1981). Due to these longer processing times, the loss of heat sensitive nutrients from SBM is the major disadvantage of using conventional thermal treatments (Singh et al., 2007). Extrusion processing is highly efficient in destroying ANFs and also capable of reducing the loss of heat-sensitive nutrients due to its short processing time (Alam et al., 2016). Moreover, the inactivation process of ANFs is more efficient when a combination of food processing operations is used (Siddhuraju, Osoniyi, Makkar, & Becker, 2002). Hence, extrusion processing is highly effective in reducing ANFs as it uses high temperature, high pressure and high shearing forces simultaneously (Nikmaram et al., 2017).

Extrusion processing has been shown to significantly reduce the levels of trypsin inhibitors in infant diets formulated from cassava starch, corn protein, and soybean protein concentrate at 170-180°C die temperature and 200-220 rpm screw speed (Omosebi, Osundahunsi, & Fagbemi, 2018). The effectiveness of extrusion processing in decreasing trypsin inhibitor activity in fish meals was also observed at 116-122 °C die temperature (Romarheim, Aslaksen, Storebakken, Krogdahl, & Skrede, 2005). Lectins, the other important ANF of SBM, are highly heat labile compounds. Complete inactivation of lectins has been reported for various pulses and oilseeds with the use of extrusion processing (Batista, Prudêncio, & Fernandes, 2010a, 2010b; Lopes, de Aleluia Batista, Fernandes, & de Andrade Cardoso Santiago, 2012; Morales et al., 2015).

2.6. Research Gap

The structural changes of soybean protein products during the formation of texturized soybean proteins have been studied extensively. Moreover, the impacts of feed moisture and barrel temperature on some of the techno-functional properties (such as emulsification and protein solubility) of extruded soybean proteins have also been reported. However, no research studies are available on the impacts of raw material particle size distribution on the techno-functional quality of soybean protein extrudates. Accordingly, there is a need to systematically evaluate the effects of extrusion cooking on various techno-functional properties of soybean proteins such as emulsification, gelation, water holding capacity, oil holding capacity and protein solubility.

Bridge to Chapter 3

The following chapter contains a manuscript titled “Effects of particle size distribution and processing conditions on the techno-functional properties of extruded soybean meal”. This manuscript was submitted to “LWT - Food Science and Technology” in May 2021 and is currently under review.

Chapter 3: Effects of Particle Size Distribution and Processing Conditions on the Techno-functional Properties of Extruded Soybean Meal

3.1. Introduction

Consumption of plant proteins has been rapidly rising in recent years owing to their potential health, environmental and economical benefits over animal protein sources (Aschemann-Witzel et al., 2020; Chalvon-Demersay et al., 2017). Soybean proteins remain at the forefront of plant protein market in the last few decades due to their high nutritional value, low cost and health benefits, including a health claim for their cholesterol lowering efficacy (Health Canada, 2015; Sun, Ge, He, Gan, & Fang, 2020). Several soybean protein products, predominantly isolates, have been extensively studied in the past for their ability to mimic the characteristic texture of meat in products such as plant-based meat extenders and meat analogs (Lin et al., 2000; Samard et al., 2019; Samard & Ryu, 2019). However, soybean meal, a proteinaceous by-product of soybean oil extraction process, is still underutilized in human foods and currently being primarily used for animal feed. The high protein content of soybean meal makes it a suitable candidate to be used as a food ingredient in the plant-based meat industry through exploration of its techno-functional properties such as protein solubility, emulsification, gelation, water and oil holding capacities.

Extrusion cooking is the most widely accepted and used processing technique to produce puffed snacks, breakfast cereals, and more recently plant-based meat extenders and meat analogs. During extrusion cooking, the complex interplay of various processing operations causes structural and physicochemical transformations of food polymers (e.g., starch gelatinization and protein denaturation) (Alam et al., 2016; Singh et al., 2007). For example, globular proteins, such as soybean proteins, typically undergo firstly denaturation followed by aggregation through development of strong protein-protein interactions (F. L. Chen et al., 2011; Liu & Hsieh, 2008),

leading to changes in their structure (Mozafarpour et al., 2019). It has been previously reported that by fine tuning process conditions, extrusion can be employed as a reactor in which properties of food polymers are modified and novel ingredients with superior techno-functional properties are produced (Alonso et al., 2000; Chen, Chen, Yu, Wu, & Zhao, 2018; Mozafarpour et al., 2019).

Characteristics of extrudates, whether physical or techno-functional, rely on several processing conditions including but not limited to flow rate of raw materials, extruder barrel temperature profile and screw speed (Alam et al., 2016; Zhang et al., 2019). The particle size distribution of raw materials has also been previously shown to impact the quality of starch-rich extruded foods (Al-Rabadi et al., 2011; Onwulata & Konstance, 2006). Particle size distribution of a raw material affects the severity of extrusion cooking by influencing the melt viscosity and the mechanical energy input required to process that melt (Onwulata & Konstance, 2006). Size distribution of particles also affects heat transfer rates during extrusion cooking as smaller-sized particles experience relatively more rapid heating (Guy, 2001). Although several previous research works explored the impact of extrusion conditions on the structural and techno-functional properties of soybean proteins (F. L. Chen et al., 2011; Chen et al., 2010; Fang et al., 2014; Mozafarpour et al., 2019), the impact of particle size distribution on extrudate's techno-functionality remains unknown. Accordingly, this study investigated the impact of soybean meal particle size distribution, extrusion barrel temperature profile and feed moisture content on the techno-functional properties of soybean meal extrudates.

3.2. Materials and Methods

3.2.1. Materials

Mechanically extracted soybean meal (SBM) was received from M & C Commodities (Winnipeg, MB, Canada). The SBM was milled at the Richardson Centre for Functional Foods and Nutraceuticals (Winnipeg, MB, Canada) using a Prater-Sterling M-21 pilot impact mill (Prater Industries, Inc., Bolingbrook, IL, USA). The mill was equipped with three different screen sizes of 0.5 mm (small), 0.75 mm (medium) and 1 mm (large) to obtain SBM with different particle size distributions (PSDs). The PSD analysis of the milled SBM was conducted using a particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., Malvern, UK). Volume mean diameter, D[4,3], and the maximum diameter below which the 10 %, 50 % and 90 % of particles by volume fall, i.e., d(0.1), d(0.5) and d(0.9), respectively, were also measured.

3.2.2. Proximate Analyses

Moisture and crude protein (N × 6.25) contents of raw SBM were determined in triplicates using AACC methods 44-19.01 and 46-13, respectively (AACC, 1999). For the analysis of crude fat and ash contents, the methods described by Min and Ellefson (2010) and Marshall (2010) were used, respectively. Total carbohydrates content was calculated by difference.

3.2.3. Extrusion Cooking

Extrusion cooking was performed on a lab scale, co-rotating, twin screw extruder (MPF19, APV Baker Ltd., Peterborough, UK) with a circular die of 5.5 mm diameter and screw length to diameter ratio of 25:1. The screw speed and feed rate were kept constant at 300 rpm and 2.5 kg/h (dry basis), respectively. The screw configuration was set according to Koksel and Masatcioglu (2018) and

kept constant for all extrusion runs. Using the three different PSDs, i.e., small, medium, large, extrudates were obtained at feed moisture contents of 15, 21 and 27 g water/100 g dry SBM (FMC 15, FMC 21 and FMC 27, respectively) and barrel temperature profiles from the feeder to the die of 55/70/85/100/110, 75/90/105/120/130 and 95/110/125/140/150 °C (BT 110 °C, BT 130 °C and BT 150 °C, respectively). Die pressure and torque values were recorded after steady-state extrusion conditions were reached and specific mechanical energy (SME) was calculated according to Luo and Koksel (2020) using equation (1):

$$\text{SME}\left(\frac{\text{Wh}}{\text{kg}}\right) = \frac{\frac{actual\ screw\ speed}{max\ screw\ speed} \times \frac{torque}{100} \times motor\ power}{mass\ flow\ rate} \quad (1)$$

Extrudates were collected in duplicates and cooled down to room temperature. They were then air dried (Isotemp 180 L Oven Gravity, Thermo Scientific, Langenselbold, Germany) at 50 °C overnight to achieve a moisture content of <10 g water/100 g extrudate and placed at 4 °C in zip lock plastic bags (low density polyethylene) to avoid changes in moisture content. For techno-functionality analyses, raw SBM and the extrudates were ground using a centrifugal mill (Retsch, ZM 200, Haan, Germany) equipped with a 250 μm sieve.

3.2.4. Nitrogen Solubility Index

Nitrogen solubility index (NSI) NSI was determined in triplicates following the AACC method 46-23.01 with minor modifications (AACC, 1999). A mixture was prepared by dissolving 5 g of raw (or extruded) SBM in pre-warmed (30 °C) milli-q water and adding water until the volume of the mixture reached 200 mL. The soluble nitrogen in the mixture was extracted by stirring at 120 rpm in a shaking water bath (SW22, Julabo, Seelbach, Germany) for 2 h at 30 °C. During extraction, the mixture was also manually shaken for 30 sec every 30 min to prevent its particles

from settling. After extraction, 10 mL of the mixture was centrifuged (Sorvall RC6 plus, Thermo Fisher Scientific, Asheville, NC, USA) at 4180 $\times g$ for 10 min and the supernatant was filtered using Whatman no. 2 filter paper. The nitrogen content in 3 mL of supernatant as well as the original raw (or extruded) SBM was determined following the AACC method 46-13 (AACC, 1999). NSI was calculated using equation (2):

$$\text{NSI}\ (\%) = \frac{\%\ \text{water soluble nitrogen}}{\%\ \text{total nitrogen}} \times 100 \qquad (2)$$

3.2.5. Gel Formation Capacity

Gel forming capacity was measured by the least gel concentration (LGC) method reported by Khatkar and Kaur (2018) with some modifications. Different concentrations of protein solutions were prepared in tubes by suspending 1.0 – 1.5 g of raw (or extruded) SBM in 5 mL of distilled water and placed into a water bath at 95 °C for 1 h. The mixture was then immediately cooled to room temperature using cold water and stored at 4 °C for 2 h. After storage, the tubes were inverted and the LGC was defined as the minimum concentration of protein at which the mixture did not fall or slip from the tube when inverted. Measurements were performed in triplicates.

3.2.6. Oil and Water Holding Capacities

Oil and water holding capacities (OHC and WHC) were determined using the method described by Stone, Karalash, Tyler, Warkentin, and Nickerson (2015) with some modifications. In brief, approximately 0.5 g of raw (or extruded) SBM was suspended in 5 mL of oil or water for OHC or WHC measurements, respectively, in a 50 mL centrifugal tube. The suspension was vortexed (G-560, Scientific Industries Inc., Bohemia, NY, USA) for 10 sec every 5 min for a total of 30 min and then centrifuged (Sorvall RC6 plus, Thermo Fisher Scientific, Asheville, NC, USA) at 1100

$\times g$ for 15 min for OHC and 1200 $\times g$ for 18 min for WHC. After centrifugation, the supernatant was decanted, and the remaining solids were weighed. OHC and WHC were then calculated using equations (3) and (4):

$$\text{OHC (g water/100 g dry SBM)} = \frac{W_f}{W_i} \times 100 \quad (3)$$

$$\text{WHC (g oil/100 g dry SBM)} = \frac{W_f}{W_i} \times 100 \quad (4)$$

Where W_f is the final weight of the solid residue after centrifugation and W_i is the initial raw (or extruded) SBM weight. For both OHC and WHC, measurements were performed in triplicates.

3.2.7. Emulsion Properties

Emulsion capacity (EC) and emulsion stability (ES) analyses were carried out according to Stone et al. (2015). For EC, appropriate amount of raw (or extruded) SBM was first dissolved in milli-q water to prepare 1 % (w/w) protein solution. Varying amounts of canola oil were then added to the 1.5 g of 1 % protein solution and homogenized (T 18 basic Ultraturrax, IKA, Germany) at 7200 rpm for 5 min to create a series of emulsions. After homogenization, the electrical conductivity of each emulsion was measured using a benchtop conductivity meter (Orion star A212, Thermo Scientific, Chelmsford, MA, USA) equipped with a 4-electrode conductivity cell. A sudden drop in conductivity was considered as the inversion point of the emulsion, i.e., change of oil-in-water emulsion to water-in-oil emulsion, and the weight of oil that causes this drop was recorded. EC was calculated using equation (5):

$$\text{EC (g oil/g protein)} = \frac{W_b + W_a}{2} \div W_s \quad (5)$$

Where W_b and W_a are the weight of oil added just before and just after the inversion point, respectively, and W_s is the weight of the protein solution used to prepare the emulsion.

For the ES analysis, emulsions were prepared by adding 2 g of oil to 2 g of 1 % protein solution, followed by homogenization at 7200 rpm for 5 min. The emulsions were then transferred to a graduated cylinder and the height of the emulsion layer was recorded immediately after homogenization. After 30 min, the height of the separated aqueous layer was recorded, and ES was calculated using equation (6):

$$\text{ES}\ (\%) = \frac{h_b - h_a}{h_b} \qquad (6)$$

Where h_b is the height of the initial emulsion layer and h_a is the height of the separated aqueous layer after 30 min. Both EC and ES analyses were conducted in triplicates.

3.2.8. Statistical Analysis

Statistical differences among techno-functional properties as a function of extrusion parameters were determined by 3-way ANOVA and Tukey's test ($p < 0.05$) using PROC GLIMMIX procedure (SAS software, version 9.2, SAS Institute Inc., Cary, NC, USA).

3.3. Results and Discussion

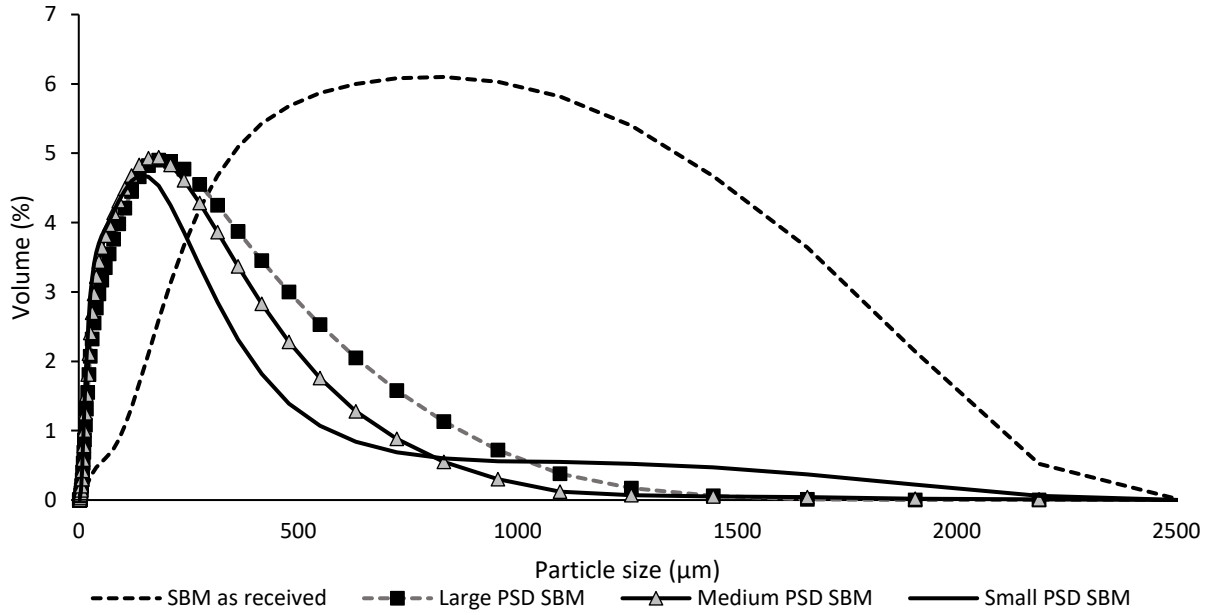
3.3.1. Particle Size and Proximate Composition

The particle size distribution (PSD) characteristics of raw SBM obtained from the three different screen sizes (small, medium, and large) are presented in Figure 3.1. The d(0.1), d(0.5) and d(0.9) values were the lowest for the small PSD SBM (18.2, 87.6, and 343.7 μm, respectively) and subsequently increased for medium (22.4, 106.4, and 366 μm, respectively) and large (24.4, 126.9,

and 448.4 μm, respectively) PSDs. The volume mean diameter, i.e. D[4,3], was the highest for the large PSD SBM (190.3 μm) as expected. Interestingly, D[4,3] remained almost the same for the medium (159.3 μm) and small (159.8 μm) PSD. Small PSD SBM had a slightly higher proportion of larger-sized particles (>1000 μm) as compared to the medium and large PSD SBMs (Figure 3.1) which shifted its D[4,3] to higher values and brought it closer to that of the medium PSD SBM.

The protein content of raw SBM was in the range of 40.8 – 41.4 g protein/100 g SBM (db), and increased significantly ($p < 0.05$) as PSD increased from small to large. Bourré et al. (2019), who studied pulse flour milling, also reported higher protein content for larger particle sizes. When PSD was increased from small to large, fat (9.8 – 10.2 g fat/100 g SBM, db) and total carbohydrates (42.8 – 44.1 g carbohydrates/100 g SBM, db) remained the same, while ash content (5.4 – 5.6 g ash/100 g SBM, db) increased significantly ($p < 0.05$).

Figure 3.1. Particle size distribution (PSD) of raw soybean meal (SBM).



3.3.2. Extrusion System Parameters

Experimental design and the resulting extrusion system parameters are presented in Table 3.1. Regardless of raw material PSD, increases in feed moisture content (FMC) and barrel temperature (BT) caused reductions in die pressure, torque, and specific mechanical energy (SME). A decrease in melt viscosity during extrusion cooking at higher FMC and higher BT (Chen et al., 2010) is possibly responsible for these relatively lower values of system parameters. The decrease in SME at higher BT and higher FMC reflects a decrease in the mechanical energy input and likely a lower level of shearing that SBM experienced in the barrel under these conditions. Die pressure did not change as a function of PSD at the BT and FMC conditions studied. At constant BT and FMC, an increase in PSD from small to medium did not cause a significant change in torque values, except for the treatment of BT 150 °C and FMC 15 where a slight, but significant ($p < 0.05$) decrease was observed. While increasing the PSD further from medium to large generally caused an increase in torque values, a comparison of small with large PSD revealed that torque values remained unchanged, except for the treatment of BT 130 °C and FMC 27. Similar trends for the effect of PSD on torque values at constant BT and FMC conditions were also observed for the effect of PSD on SME values. An inverse relationship between SME and PSD was previously reported during extrusion cooking of corn (Sharifi et al., 2021), barley and sorghum flours (Al-Rabadi et al., 2011). The authors concluded for feed materials with large PSD that water cannot penetrate starch granules as easily, resulting in a lower melt viscosity and lower overall SME at large PSD (Al-Rabadi et al., 2011). In contrast to cereal flours, SBM is rich in protein and low in starch content, making a direct comparison with extrusion of starch-rich materials difficult. Since, system parameters, especially SME, can potentially be used as an indicator of extrudate's physicochemical

quality (Fang et al., 2014), more studies are needed to evaluate the impact of PSD on mechanical energy requirements during extrusion cooking of protein-rich materials.

Table 3.1. Effects of soybean meal's particle size distribution (PSD) and extrusion barrel temperature (BT) and feed moisture content (FMC) on die pressure, torque and specific mechanical energy (SME). Results are reported as mean ± standard deviation (n=2). Values in the same column followed by different letters are significantly different ($p < 0.05$).

PSD	BT (°C)	FMC (g water/100 g dry SBM)	Die pressure (kPa)	Torque (%)	SME (Wh/kg)
Small	110	15	2650 ± 71^{a}	30.5 ± 0.7^{ab}	149.5 ± 3.5^{ab}
		21	1650 ± 71^{bcd}	26.0 ± 0.0^{def}	120.9 ± 0.0^{de}
		27	1250 ± 71^{efg}	24.0 ± 0.0^{ghi}	105.8 ± 0.0^{fgh}
	130	15	1900 ± 141^{bc}	25.5 ± 0.7^{efg}	125.0 ± 3.5^{cd}
		21	1250 ± 71^{efg}	22.0 ± 0.0^{jkl}	102.3 ± 0.0^{ghi}
		27	950 ± 71^{fghij}	19.0 ± 0.0^{no}	83.8 ± 0.0^{mn}
	150	15	1600 ± 141^{bcde}	22.0 ± 0.0^{jkl}	107.8 ± 0.0^{fg}
		21	900 ± 141^{ghij}	19.0 ± 0.0^{no}	88.4 ± 0.0^{klm}
		27	600 ± 141^{j}	17.0 ± 0.7^{op}	77.2 ± 3.1^{no}
Medium	110	15	2550 ± 71^{a}	29.0 ± 0.0^{bc}	143.5 ± 0.0^{b}
		21	1550 ± 71^{cde}	26.0 ± 0.0^{def}	121.4 ± 0.0^{de}
		27	1050 ± 71^{fghi}	24.5 ± 0.7^{fgh}	108.9 ± 3.1^{fg}
	130	15	1800 ± 0^{bc}	25.0 ± 0.0^{efg}	123.7 ± 0.0^{cd}
		21	1050 ± 71^{fghi}	21.0 ± 0.0^{klm}	98.9 ± 0.0^{hij}
		27	800 ± 0^{hij}	19.0 ± 0.0^{no}	84.4 ± 0.0^{lmn}
	150	15	1700 ± 141^{bc}	20.0 ± 0.0^{mn}	98.9 ± 0.0^{hij}
		21	900 ± 141^{ghij}	17.5 ± 0.7^{op}	81.7 ± 3.3^{mno}
		27	600 ± 141^{j}	16.5 ± 0.7^{p}	73.3 ± 3.1^{o}
Large	110	15	2750 ± 71^{a}	31.5 ± 0.7^{a}	155.0 ± 3.5^{a}
		21	1850 ± 71^{bc}	27.5 ± 0.7^{cd}	127.6 ± 3.3^{cd}
		27	1300 ± 71^{def}	25.0 ± 0.0^{efg}	110.2 ± 0.0^{fg}
	130	15	1950 ± 71^{b}	26.5 ± 0.7^{de}	130.4 ± 3.5^{c}
		21	1150 ± 71^{fgh}	22.5 ± 0.7^{ijk}	104.4 ± 3.3^{gh}
		27	950 ± 71^{fghij}	21.0 ± 0.0^{klm}	92.6 ± 0.0^{jkl}
	150	15	1550 ± 71^{cde}	23.0 ± 0.0^{hij}	113.1 ± 0.0^{ef}
		21	1000 ± 0^{fghi}	20.5 ± 0.7^{lmn}	95.1 ± 3.3^{ijk}
		27	700 ± 0^{ij}	18.0 ± 0.0^{op}	79.4 ± 0.0^{no}

3.2.3. Nitrogen Solubility Index

NSI values of raw and extruded SBM are presented in Table 3.2. While raw SBM's PSD did not influence its NSI values, a significant ($p < 0.05$) drop in NSI was observed for all extrudates as compared to their raw SBM counterparts. These findings are in agreement with the previously reported extrusion studies on soy protein concentrates (Mozafarpour et al., 2019; Silva, Arêas, Silva, & Arêas, 2010) and can be explained by the effect of extrusion cooking on proteins. Extrusion causes denaturation of soy proteins, thereby exposing non-polar amino acids to the surface of protein molecules that may lead to strong protein-protein interactions (Riaz, 2011) and formation of high molecular weight protein aggregates with decreased solubility (F. L. Chen et al., 2011; Chen et al., 2010; Mozafarpour et al., 2019). As reported by Fang et al. (2014), an increase in shear forces, as generally reflected by higher SME values, may disintegrate some of these newly formed protein aggregates and cause an increase in protein solubility. Similar trends were observed in the present study as both SME (Table 3.1) and NSI generally increased when temperature decreased from BT 150 °C to BT 110 °C and moisture content decreased from FMC 27 to FMC 15. Decrease in protein solubility with an increase in FMC is in line with the results reported by Mozafarpour et al. (2019). However, these authors also reported an increase in protein solubility with an increase in BT from 110 °C to 160 °C, which they attributed to the disintegration of protein aggregates at higher BT. Considering the inverse relationship that was observed between BT and SME (Table 3.1), it is possible that the disintegration of protein molecules caused by increased SME outweighed any such impact of increasing temperatures in this study. Moreover, decreased protein solubility at higher BT may also be attributed to the more extensive protein denaturation that favors a higher degree of protein-protein interactions and more protein aggregation (Nor Afizah & Rizvi, 2014).

When the PSD was reduced from large to medium, NSI of extrudates significantly ($p < 0.05$) dropped, except for treatments of BT 110 °C and FMC 15, BT 130 °C and FMC 15, and BT 150 °C and FMC 27. Further decrease in PSD reversed this trend for some extrusion treatments (BT 110 °C and FMC 21, BT 150 °C and FMC 21, BT 150 °C and FMC 27) and caused a significant ($p < 0.05$) improvement in NSI. At constant BT and FMC, NSI values of small PSD extrudates and large PSD extrudates were not significantly ($p < 0.05$) different. NSI and SME (Table 3.1) showed similar trends as a function of PSD, in line with previous findings that as SME increases NSI generally increases (Fang et al., 2014). A decrease in PSD generally results in a more rapid heating of particles, mainly due to increases in the particle to particle contact area and friction between smaller sized particles (Desrumaux et al., 1998; Onwulata & Konstance, 2006). Consequently, a more rapid heating may cause earlier initiation of protein denaturation for smaller PSD SBM and allow a relatively longer time for protein aggregation in the barrel when compared to larger PSD, and thus negatively influence protein solubility. Solubility of proteins plays a critical role in their use as food ingredients as higher solubility is generally linked to better overall techno-functionality (Khattab & Arntfield, 2009; Kinsella, 1979). Since, most industrial thermal processing operations for SBM negatively influence its protein solubility (Kinsella, 1979), utilization of SBM with larger PSD as well as lower BT and lower FMC during extrusion cooking may be beneficial to alleviate such losses.

3.2.4. Gel Forming Capacity

LGC of raw and extruded SBM as a function of PSD, BT and FMC is presented in Table 3.2. As LGC is defined as the minimum protein concentration required for gel formation, it is to be noted that a lower LGC means a higher gel forming capacity. LGC was significantly lower for raw SBM as compared to extrudates (i.e., raw SBM had a higher gel forming capacity compared to extruded

SBM), except for treatment BT 110 °C and FMC 27 for medium PSD extrudates, and treatment BT 110 °C and FMC 15 for large PSD extrudates. At constant FMC, gel forming capacity of extrudates showed an increasing trend when temperature was decreased from BT 150 °C to BT 110 °C. However, this improvement was only significant ($p < 0.05$) for small PSD extrudates produced at FMC 21 and FMC 27, and large PSD extrudates produced at FMC 21. Gel forming capacity of proteins is generally favored by high surface hydrophobicity as hydrophobic interactions play a major role in creating and stabilizing three-dimensional gel networks (He et al., 2014). It is possible that more extensive protein aggregation occurred at higher BT and resulted in some of the hydrophobic sites getting buried, rendering them less accessible for gel formation. Increase in FMC has been previously reported to increase the molecular size and surface hydrophobicity of soybean protein extrudates (Mozafarpour et al., 2019), both of which are generally linked with better gel forming capacity (He et al., 2014). In this study, the impact of FMC was only observed for small PSD extrudates produced at BT 110 °C, where an increase in moisture content from FMC 15 to FMC 27 caused a significant ($p < 0.05$) increase in gel forming capacity. Extrudates produced at these conditions would therefore be more desirable to be used in product such as meat extenders (Asgar et al., 2010). At constant BT and FMC treatments, changes in SBM PSD produced variable effects on the LGC of extrudates. Regardless of FMC, at BT 150 °C, medium PSD extrudates had the highest overall gel forming capacity. However, this improvement was only significant ($p < 0.05$) when medium and small PSD extrudates were compared.

Table 2.2. Nitrogen solubility index (NSI) and least gel concentration (LGC) of raw and extruded soybean meal (SBM) as a function of SBM particle size distribution (PSD), barrel temperature (BT), and feed moisture content (FMC). The results are reported as mean ± standard deviation (n=2 for extrusion, n=3 for NSI and LGC). Values in the same column followed by different letters are significantly different ($p < 0.05$).

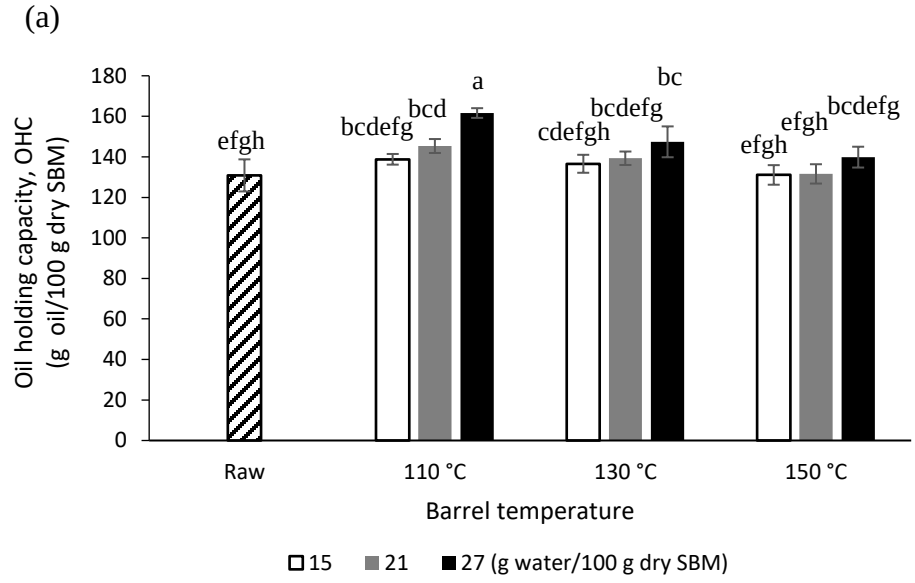
PSD	BT (°C)	FMC (g water/100 g dry SBM)	NSI (%, db)	LGC (%, db)
Small SBM - Raw	-	-	21.12 ± 0.72^{a}	7.73 ± 0.07^{l}
Small SBM - Extruded	110	15	12.17 ± 0.37^{b}	8.41 ± 0.07^{abcde}
		21	10.69 ± 0.46^{defg}	8.26 ± 0.07^{defghi}
		27	9.59 ± 0.32^{ijkl}	8.04 ± 0.28^{ijk}
	130	15	10.94 ± 0.26^{cdef}	8.35 ± 0.06^{cdefg}
		21	9.57 ± 0.27^{jkl}	8.35 ± 0.17^{cdefg}
		27	9.52 ± 0.25^{jkl}	8.34 ± 0.17^{cdefg}
	150	15	9.88 ± 0.30^{ghijk}	8.54 ± 0.06^{abc}
		21	9.50 ± 0.33^{jkl}	8.65 ± 0.17^{ab}
		27	9.82 ± 0.39^{ghijkl}	8.66 ± 0.10^{a}
Medium SBM - Raw	-	-	20.58 ± 0.68^{a}	7.81 ± 0.07^{kl}
Medium SBM - Extruded	110	15	11.84 ± 0.12^{bc}	8.16 ± 0.10^{efghi}
		21	9.73 ± 0.66^{hijkl}	8.27 ± 0.08^{defghi}
		27	9.37 ± 0.61^{jklm}	8.07 ± 0.11^{hijk}
	130	15	10.63 ± 0.09^{defgh}	8.14 ± 0.12^{fghi}
		21	8.88 ± 0.23^{lm}	8.12 ± 0.10^{ghi}
		27	9.01 ± 0.36^{klm}	8.11 ± 0.10^{ghi}
	150	15	9.35 ± 0.35^{jklm}	8.25 ± 0.06^{efghi}
		21	8.51 ± 0.72^{m}	8.29 ± 0.15^{cdefghi}
		27	8.55 ± 0.49^{m}	8.18 ± 0.07^{efghi}
Large SBM - Raw	-	-	20.16 ± 0.46^{a}	7.84 ± 0.07^{jkl}
Large SBM - Extruded	110	15	12.14 ± 0.35^{b}	8.09 ± 0.07^{ghij}
		21	11.33 ± 0.55^{bcde}	8.24 ± 0.07^{efghi}
		27	10.53 ± 0.30^{efghi}	8.31 ± 0.21^{cdefgh}
	130	15	11.50 ± 0.37^{bcd}	8.22 ± 0.11^{efghi}
		21	10.02 ± 0.43^{fghij}	8.38 ± 0.11^{cdef}
		27	10.19 ± 0.34^{fghij}	8.40 ± 0.07^{bcdef}
	150	15	10.55 ± 0.48^{efgh}	8.34 ± 0.05^{cdefg}
		21	10.05 ± 0.42^{fghij}	8.51 ± 0.11^{abcd}
		27	9.28 ± 0.42^{jklm}	8.31 ± 0.12^{cdefgh}

3.2.5. Oil Holding Capacity

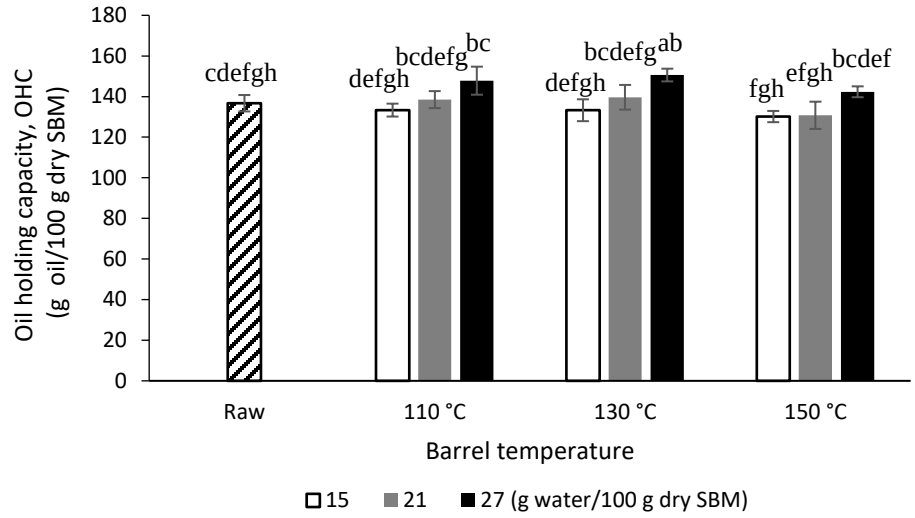
OHC of raw and extruded SBM as a function of PSD, BT and FMC is presented in Figure 3.2. As compared to their raw SBM counterparts, OHC values were significantly ($p < 0.05$) improved for small PSD extrudates produced at BT 110 °C and FMC 21, BT 110 °C and FMC 27, and BT 130 °C and FMC 27, and medium PSD extrudates produced at BT 130 °C and FMC 27. These results indicated that SBM extruded under these conditions has great potential to be utilized as a value-added food ingredient in baked goods and meat products such as burger patties and sausages as high OHC is often required for their improved flavor and mouthfeel. The unfolding of protein molecules that occurs during thermal treatment generally leads to unmasking of surface non-polar amino acids (Khattab & Arntfield, 2009) and can provide additional binding sites for oil particles to interact with protein molecules through hydrophobic interactions (Lin & Zayas, 1987). Accordingly, the increased OHC at above mentioned extrusion process conditions as compared to OHC of raw SBM can possibly be attributed to higher surface hydrophobicity of extrudates. These results are in line with Ma et al. (2018) who reported an increase in surface hydrophobicity for soy protein isolates after extrusion. These authors also found an inverse relationship between surface hydrophobicity and extrusion temperature, possibly explaining the observed improvement in OHC only at lower BT conditions. Overall, an increase in FMC caused an improvement in OHC, however this effect was significant ($p < 0.05$) for only some PSDs and BTs. A higher FMC has been previously reported to increase the surface non-polar sites of extruded soy protein concentrates (Mozafarpour et al., 2019) and mung bean protein isolates (Hossain Brishti et al., 2021). Generally, high surface hydrophobicity favors OHC (Zayas, 1997), while a decrease in surface hydrophobicity has been reported to be associated with increased protein solubility (Karaca et al., 2011). Accordingly, protein solubility and OHC results are expected to exhibit opposite

trends, which was the case for the impact of FMC on NSI and on OHC in the present study. Changes in PSD significantly ($p < 0.05$) affected OHC only at BT 110 °C and FMC 27, where extrudates obtained from small PSD exhibited higher OHC as compared to medium and large PSDs.

Figure 3.2. Oil holding capacity (OHC) of raw and extruded soybean meal (SBM) as a function of feed moisture content (FMC) and barrel temperature (BT) for small (a), medium (b), and large (c) particle size distribution. Errors bars represent ± standard deviation (n=2 for extrusion and n=3 for OHC). Bars assigned with different letters are significantly different ($p < 0.05$).



(b)



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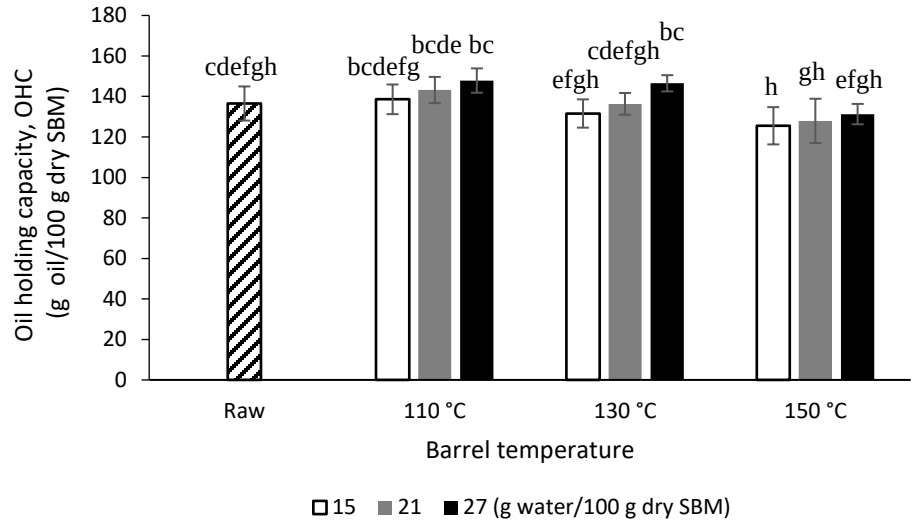
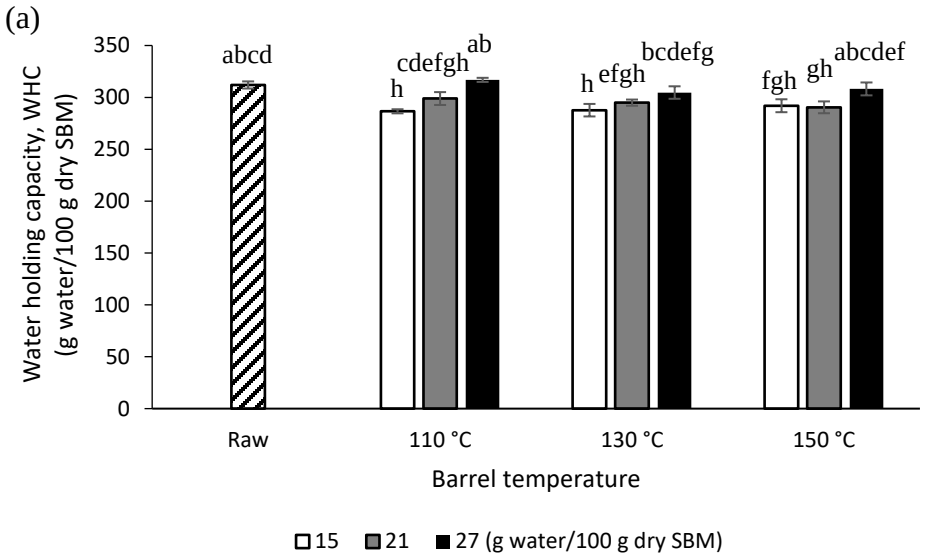


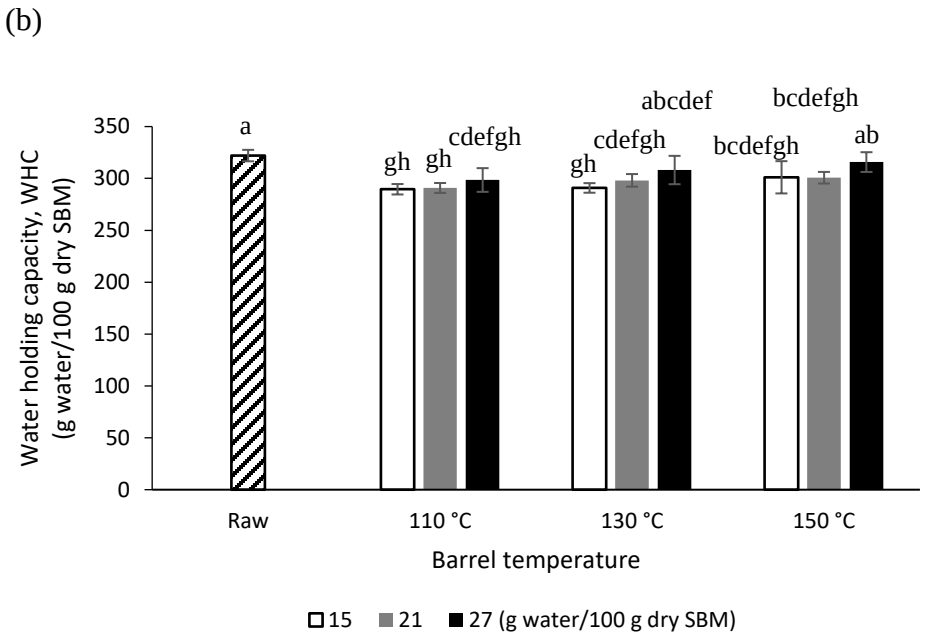
3.2.6. Water Holding Capacity

WHC of raw and extruded SBM as a function of PSD, BT and FMC is presented in Figure 3.3. Regardless of PSD and BT, at the highest FMC, WHC of extrudates were similar to those of their raw SBM counterparts, except for medium PSD extrudates produced at BT 110 °C for which WHC

was lower. For lower FMC, overall, a reduction in WHC of extrudates was observed when compared to raw SBM. In contrast to our findings, Alonso et al. (2000) reported an increase in WHC of extruded pea and kidney bean flours. Nevertheless, the high starch content of pea and kidney bean flours may have played a predominant role in increased WHC results. This is due to the degradation of starch molecules during extrusion cooking as previously reported for yellow pea flour and bread crumb mixtures (Luo & Koksel, 2020) which leads to an increase in starch's ability to interact with water once its granular structure is disrupted. Increasing the moisture content from FMC 15 to FMC 21 did not change WHC of extrudates significantly ($p < 0.05$), however, further increase in moisture content to FMC 27 caused an improvement in WHC values at some PSDs and BTs. High WHC of food proteins has been commonly associated with high molecular weight protein fractions as they exhibit a higher tendency to retain water molecules (Gerliani, Hammami, & Aïder, 2020). Accordingly, the reduction in WHC at lower FMC may be attributed to the dissociation of relatively larger protein fractions possibly due to increased SME at these conditions (Table 3.1). Since, high WHC is an essential feature for food ingredients that are intended to be used in meat extender and baked food formulae, these results demonstrate that extrudates produced at relatively higher feed moisture content might perform better for such applications. The overall impacts of BT and PSD on WHC were not found to be statistically significant ($p < 0.05$).

Figure 3.3. Water holding capacity (WHC) of raw and extruded soybean meal (SBM) as a function of feed moisture content (FMC) and barrel temperature (BT) for small (a), medium (b), and large (c) particle size distribution. Errors bars represent ± standard deviation (n=2 for extrusion and n=3 for WHC). Bars assigned with different letters are significantly different ($p < 0.05$).





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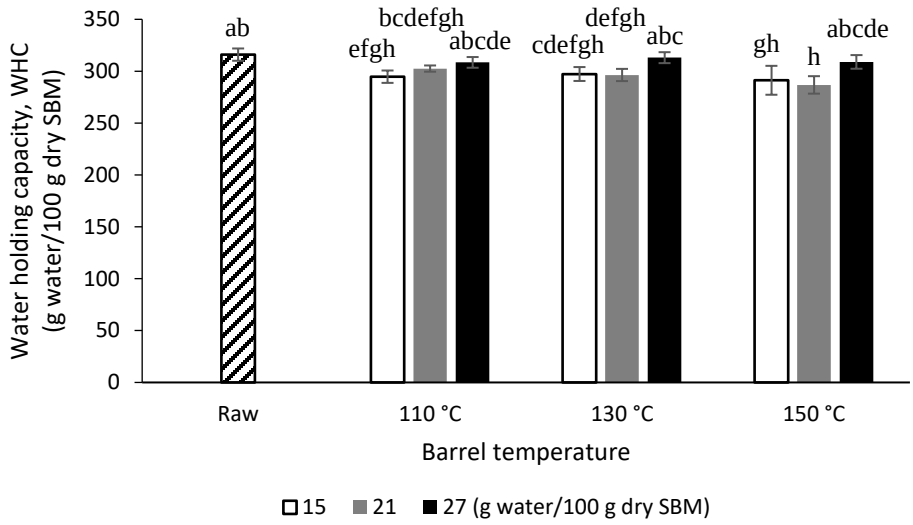

3.2.7. Emulsion Properties

Emulsion capacity (EC) and emulsion stability (ES) of raw and extruded SBM as a function of PSD, BT and FMC are presented in Table 3.3. In general, extrusion cooking negatively impacted the emulsion properties of SBM as EC and of extrudates was significantly ($p < 0.05$) lower when compared to those of raw SBM. The only exceptions to the inferior extrudate emulsion properties were the EC for small PSD extrudates produced at BT 110 °C and FMC 27, and medium PSD produced at BT 110 °C and FMC 15. This decrease in emulsifying capacity after extrusion cooking is in accordance with previously reported research works for extruded whey protein isolates (Téllez-Morales et al., 2020), yellow pea flour (Luo & Koksel, 2020), and wheat flour (Li, Masatcioglu, & Koksel, 2019). High protein solubility is often required for good emulsion properties as increased solubility helps in migration of protein molecules to the water-oil interface, eventually favoring fat adsorption (Karaca et al., 2011). The reduction in protein solubility after extrusion processing (Table 3.2) may be one of the reasons behind the decreased emulsion properties of extruded SBM.

At similar FMC, the effect of BT on EC of extrudates was not significant ($p < 0.05$) for most of the extrudates of small and medium PSD. However, for the large PSD, the EC of extrudates significantly ($p < 0.05$) increased when BT was reduced from 150 °C to 110 °C, in agreement with the results of Ma et al. (2018) for extrusion of soybean protein but at relatively lower temperatures. The higher EC at lower BT was attributed to limited aggregation of protein molecules due to reduced protein-protein interactions at lower temperatures. Although partial denaturation of proteins generally favors emulsion properties by improving fat adsorption through increased solubility, protein aggregation along with reduction in protein solubility has been shown to detrimentally affect the emulsification ability (Nor Afizah & Rizvi, 2014). High emulsion capacity is usually required in several food products such as comminuted meats, mayonnaise and cakes. Hence, manipulation of BT during extrusion cooking, especially the use of lower temperatures, can unlock opportunities in producing ingredients with better emulsifying properties for such applications.

Along with EC, ES also significantly decreased ($p < 0.05$) after extrusion cooking (Table 3.3) which could also be attributed to the drop in protein solubility (Table 3.2) as ES and protein solubility are generally positively associated (Karaca et al., 2011). Protein molecules with high solubility aid in interfacial membrane formation that reduces the coalescence of protein molecules, thereby increasing ES (Nwachukwu & Aluko, 2018). Besides protein solubility, the hydrophilicity/hydrophobicity balance of protein molecules is also important in determining ES (Karaca et al., 2011). More research is warranted to study the structural attributes of SBM extrudates to get more specific answers related to ES. Based on the targeted food product, the need of ES can be of short term, e.g., few hours for cake batter, or of long term, e.g., several months for soft drinks (Nishinari et al., 2014). The lower ES of SBM extrudates as compared to raw SBM

might be a disadvantage if used as an emulsifier in such products. For ES, overall impacts of BT and FMC were not found to be significant ($p < 0.05$) at any PSD studied.

Table 3.3. Emulsion capacity (EC) and emulsion stability (ES) of raw and extruded soybean meal (SBM) as a function of SBM particle size distribution (PSD), barrel temperature (BT), and feed moisture content (FMC). The results are reported as mean ± standard deviation (n=2 for extrusion, n=3 for EC and ES). Values in the same column followed by different letters are significantly different ($p < 0.05$).

PSD	BT (°C)	FMC (g water/100 g dry SBM)	EC (g oil/g sample, db)	ES (%)
Small SBM - Raw	-	-	63.98 ± 2.35^{abc}	78.49 ± 1.09^{a}
Small SBM - Extruded	110	15	57.30 ± 3.22^{defgh}	61.55 ± 1.70^{bc}
		21	57.28 ± 1.69^{defgh}	61.07 ± 1.56^{bc}
		27	59.07 ± 2.84^{bcdef}	62.04 ± 0.81^{bc}
	130	15	$54.96 \pm 2.30^{defghijk}$	61.74 ± 1.32^{bc}
		21	$54.59 \pm 2.64^{efghijkl}$	60.09 ± 1.30^{bc}
		27	$53.88 \pm 2.83^{efghijklm}$	59.76 ± 0.60^{bc}
	150	15	$54.58 \pm 4.79^{efghijkl}$	60.61 ± 1.21^{bc}
		21	50.49 ± 2.42^{ijklm}	59.33 ± 1.95^{c}
		27	$54.53 \pm 3.70^{efghijkl}$	60.87 ± 1.50^{bc}
Medium SBM - Raw	-	-	65.11 ± 4.27^{ab}	78.40 ± 1.21^{a}
Medium SBM - Extruded	110	15	61.14 ± 4.53^{abcd}	62.01 ± 1.80^{bc}
		21	$53.50 \pm 1.23^{fghijklm}$	62.25 ± 0.62^{bc}
		27	56.41 ± 1.32^{defghi}	60.53 ± 2.58^{bc}
	130	15	$53.14 \pm 4.45^{fghijklm}$	62.08 ± 1.02^{bc}
		21	49.24 ± 1.99^{jklm}	61.59 ± 1.93^{bc}
		27	$55.05 \pm 1.88^{defghijk}$	60.01 ± 1.76^{bc}
	150	15	56.07 ± 2.38^{defghi}	61.25 ± 1.13^{bc}
		21	56.41 ± 1.68^{defghi}	60.11 ± 1.36^{bc}
		27	57.27 ± 1.22^{defgh}	60.18 ± 1.69^{bc}
Large SBM - Raw	-	-	67.26 ± 3.36^{a}	79.51 ± 1.16^{a}
Large SBM - Extruded	110	15	57.98 ± 0.81^{cdefg}	62.44 ± 0.79^{b}
		21	55.56 ± 2.14^{defghij}	62.18 ± 0.50^{bc}
		27	59.76 ± 2.76^{bcde}	61.60 ± 0.92^{bc}
	130	15	52.40 ± 1.46^{ghijklm}	61.02 ± 1.01^{bc}
		21	51.33 ± 1.27^{hijklm}	61.55 ± 1.00^{bc}
		27	52.13 ± 1.66^{ghijklm}	59.60 ± 1.16^{c}
	150	15	48.90 ± 4.84^{klm}	62.89 ± 1.18^{bc}
		21	48.09 ± 4.30^{m}	60.54 ± 3.14^{bc}
		27	48.26 ± 3.63^{lm}	60.44 ± 1.68^{bc}

3.3. Conclusion

Techno-functionality attributes of SBM with three different PSDs were investigated as a function of extrusion FMC and BT. The mechanical energy input required to process the SBM during extrusion cooking was influenced by its PSD as extrusion of larger PSD generally had higher SME and torque values than medium PSD. While extrusion cooking negatively impacted SBM's nitrogen solubility as expected for any thermal treatment, the use of relatively larger PSD, as well as lower moisture content (FMC 15) and lower temperature (BT 110 °C) during extrusion cooking produced extrudates with the highest NSI values. Extrusion cooking of SBM at some lower BT and higher FMC treatments enhanced its OHC values, with highest OHC observed for smaller PSD extrudates produced at BT 110 °C and FMC 27. Although LGC, WHC and EC values of SBM generally decreased after extrusion cooking, these techno-functional characteristics were shown to improve for some extrudates upon manipulation of BT and FMC. While decrease in BT was positively associated with OHC, LGC and EC, an increase in FMC positively impacted OHC and WHC values of extrudates. These results indicated that fine tuning of process conditions together with manipulation of feed PSD can open new possibilities for utilization of SBM extrudates as novel food ingredients for use in specific food products such as meat extenders and baked goods which require a combination of several techno-functional properties.

Bridge to Chapter 4

The following chapter contains overall conclusions and some future recommendations that are made on the basis of the results and discussions presented in the previous chapter. Moreover, some additional discussion points are also presented in the following chapter to better summarize the research findings for effective communication of the significance of this research work.

Chapter 4: Overall Conclusions and Future Recommendations

This thesis research work was carried out to evaluate the effects of extrusion cooking on techno-functional properties (nitrogen solubility index, gel forming ability, oil holding capacity, water holding capacity, emulsion capacity and emulsion stability) of mechanically extracted soybean meal (SBM). SBM extrudates were produced from 3 different SBM particle size distributions, PSD (small, medium and large) at 3 different feed moisture contents (15, 21 and 27 g water/100 g dry SBM) and 3 different barrel temperature profiles (110, 130 and 150 °C at the die).

Milling of raw SBM caused slight changes in its proximate composition as raw SBM with large PSD had significantly ($p < 0.05$) more protein and ash content compared to that with small PSD. However, for raw SBM, this difference in protein content as a function of PSD did not affect any of the studied techno-functional properties.

During extrusion cooking, the PSD of raw SBM influenced the mechanical energy output required to process the melt through extruder barrel as SBM with large PSD generally demonstrated higher torque and specific mechanical energy values than that with medium PSD. Since high shearing is generally associated with high protein solubility, it was not surprising to see that large PSD extrudates generally had a higher nitrogen solubility index (NSI) than medium PSD extrudates. In addition, lower feed moisture content was also positively associated with the NSI values (i.e., relatively higher NSI values at lower feed moisture content) of extrudates, in line with the literature on extrusion of soy protein concentrates (Mozafarpour et al., 2019) and canola meal (Zhang, Liu, Ying, Sanguansri, & Augustin, 2017). However, as compared to raw SBM, a significant ($p < 0.05$) reduction in NSI was observed in extrudates which is often an expected outcome after thermal treatments. Considering the importance of high shearing for better protein solubility, future research works are required to look at the interactive effect of PSD and moisture content on

rheological properties (such as melt viscosity) of SBM, so that raw SBM particle size can be optimized to achieve required protein solubility levels for a variety of food products.

In general, lowering the barrel temperature was positively associated with gel forming capacity, oil holding capacity (OHC) and emulsion capacity (EC) of SBM extrudates. In addition, higher feed moisture content was generally associated with better OHC, water holding capacity (WHC) and gel forming capacity of extrudates. Since all these techno-functional properties are highly desirable in food ingredients that are intended to be used as meat extenders, utilization of lower barrel temperatures and higher feed moisture content during SBM extrusion are recommended for future research studies. These process conditions may also be translated to improve the techno-functionality of other emerging plant protein sources, e.g., yellow peas, faba beans, etc.

In the present research, the OHC and WHC of extruded SBM were in the range of 126 – 162 g oil/100 g dry SBM and 287 – 317 g water/100 g dry SBM, respectively. Using similar methods as those used in the present research, Stone, Nosworthy, Chiremba, House, and Nickerson (2019) previously conducted techno-functionality analyses for several Canadian pulse and cereal flours (kabuli chickpeas, desi chickpeas, green peas, yellow peas, full-fat soybean flour, lentils, faba bean, wheat, barley and oats). The reported values of OHC and WHC for these un-processed pulse and cereal flours were in the range of 139 – 193 g oil/ 100 g flour and 101 – 142 g water/100 g flour, respectively (Stone et al., 2019). In a different study, the OHC of extruded yellow pea and lentil flours was reported to be 70 g oil/100 g flour and 69 g oil/100 g flour, respectively, while their WHC was reported in the range of 305 – 360 g water/100 g flour and 318 – 365 g water/100 g flour, respectively (Masatcioglu & Koksel, 2019). These results indicate that extruded SBM had similar or better OHC when compared to the above-mentioned un-processed pulse and cereal flours and extruded yellow pea and lentil flours. When WHC is considered, extruded SBM was

found to lay between un-processed pulse and cereal flours and extruded yellow pea and lentil flours. However, there are still many unanswered questions regarding the techno-functional properties of extruded SBM in comparison to products such as soy and other emerging plant protein isolates or concentrates. Based on current literature, it is very difficult to rank extruded SBM's techno-functionality as a food ingredient in comparison with other protein sources due to the lack of standardized methods for protein techno-functionality analyses as they rely heavily on experimental conditions. For instance, the results of emulsion properties analysis generally depend on protein concentration, pH, homogenizing speed and time, equipment design, oil type and oil addition rate during the test (Pearce & Kinsella, 1978). Hence, similar experimental conditions should be used in the future for fair comparison purposes. This approach will help in ranking extruded SBM as a food ingredient as well as creating a database for food manufacturers about techno-functional properties of novel food ingredients.

One major disadvantage of using raw SBM as a food ingredient is the presence of anti-nutritional factors (ANFs) such as trypsin inhibitors, lectins and phytic acids (Yasothai, 2016). Although the present study did not look at ANFs in extruded SBM, it has previously been reported that extrusion cooking is a valuable processing technique to reduce the amount of ANFs in a variety of pulses and oilseeds including SBM (Batista et al., 2010a, 2010b; Morales et al., 2015; Romarheim et al., 2005), potentially increasing overall nutritional quality of extrudates (Nikmaram et al., 2017; Singh et al., 2007). However, a follow-up study focusing on analyzing extruded SBM's nutritional properties (e.g., protein digestibility and antinutritional factors) can provide more definitive answers regarding its overall nutritional profile.

Overall, the findings of this research provided new insights on how different extrusion cooking parameters, i.e., barrel temperature and feed moisture content, together with the PSD of raw SBM can be optimized for better techno-functionality of SBM extrudates.

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6. Appendices

Appendix 1: Preliminary Experimental Design

Purpose: Preliminary extrusion runs were conducted to shape the actual experimental design by assessing the impacts of extrusion process parameters such as raw material particle size distribution (PSD), moisture content, barrel temperature profile and screw speed on techno-functional properties of soybean meal (SBM). Additionally, where necessary, the methods for analyzing techno-functionality were modified from the existing literature according to the requirements of the raw material used in this MSc project.

Raw material: Cold pressed SBM was purchased from Pristine Gourmet (Waterford, Ontario, Canada) and placed in zip lock bags until further use. The raw SBM contained 47.04 % protein and 8.41 % moisture content. The raw SBM was milled using a laboratory mill (LM 3610, Perten Instruments AB, Hägersten, Sweden) to achieve two different particle size distributions: smaller and larger.

The milled SBM was then sieved using a set of sieves (e.g., 500, 355, 250, 180, 125, and 90 µm) on a sieve shaker (AS 200 basic, Restch, Haan, Germany) for 15 minutes. After 15 minutes, the weight of material on each sieve was measured, recorded and graphed as histograms to characterize the particle size distribution of the milled SBM.

Extrusion cooking process: The extrusion of SBM at each extrusion condition was carried out on a lab scale, co-rotating, twin screw extruder (MPF19, APV Baker Ltd., Peterborough, UK) with a screw length to diameter ratio of 25:1. A circular die of 5.5 mm diameter was used, and the feed rate was kept constant at 2.5 kg/hr.

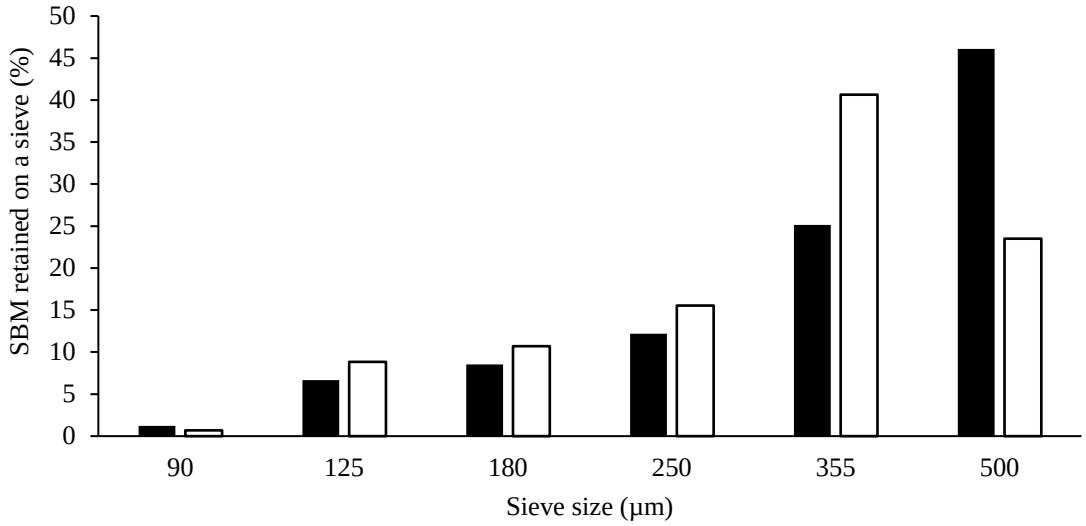
In the first preliminary extrusion run, the SBM with larger particle size was used. Extrudates were obtained at five different moisture contents, i.e., 15, 18, 21, 24, and 27 g water/100 g dry SBM, and three different barrel temperature profiles, i.e., 60/80/100/110/120, 60/80/100/110/130, and 60/80/100/120/140 °C (from the feeder to the die end). The screw speed was kept constant at 200 rpm. Two additional extrusion conditions were studied: (1) at 18 and (2) 27 g water/100 g dry SBM moisture content, both at 400 rpm screw speed and 60/80/100/120/140 °C temperature profile.

In the second preliminary extrusion run, the SBM with smaller particle size was used. Extrudates were collected at two different moisture contents, i.e., 15 and 27 g water/100 g dry SBM, and three different barrel temperature profiles: i.e., 60/75/90/105/120, 80/95/110/125/140, and 100/115/130/145/155 °C (from the feeder to the die end). The screw speed was kept constant at 200 rpm.

Techno-functionality analyses methods: The methods for analyzing techno-functional properties i.e., water holding capacity (WHC), oil holding capacity (OHC), least gel concentration (LGC) and nitrogen solubility index (NSI) of the raw SBM and SBM extrudates in the preliminary experiments were same as described in Chapter 3 of this thesis.

Appendix 2: Raw Material PSD in Preliminary Extrusion runs

Figure A2.1: Differences in the SBM particle size as measured by sieving the raw SBM through sieves of different aperture size. Black and white columns represent larger particle size and smaller particle size SBM, respectively.



Appendix 3: Effects of Raw Material PSD, Extrusion Moisture Content, Barrel Temperature Profile and Screw Speed on WHC of SBM Extrudates

Figure A3.1: WHC of raw SBM, and SBM extrudates as a function of PSD and moisture content. The barrel temperature profile was kept at 60/75/90/105/120 °C. Patterned column represents the raw SBM, while the black and white columns represent extrudates obtained from larger and smaller particle size SBM, respectively. Error bars represent ±1 standard deviation. Columns labelled by different letters are statistically different ($p < 0.05$).

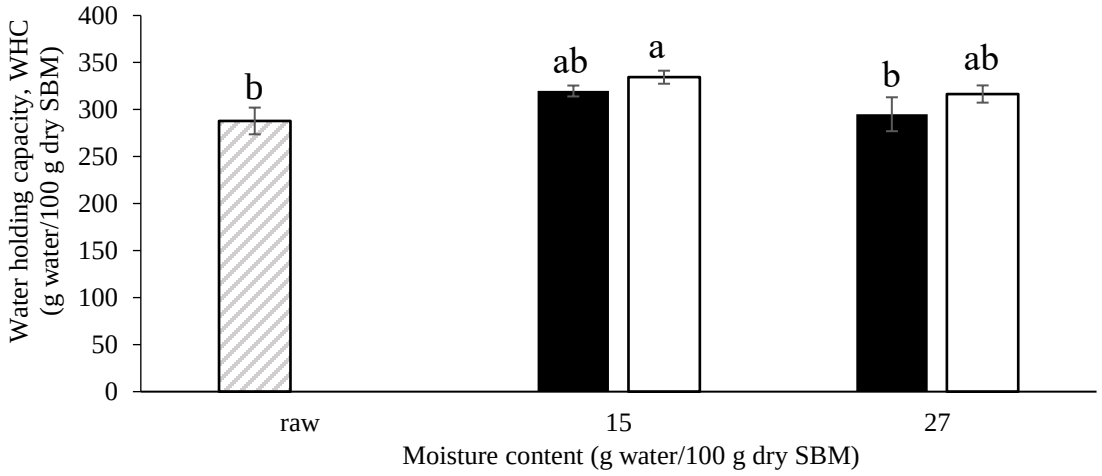
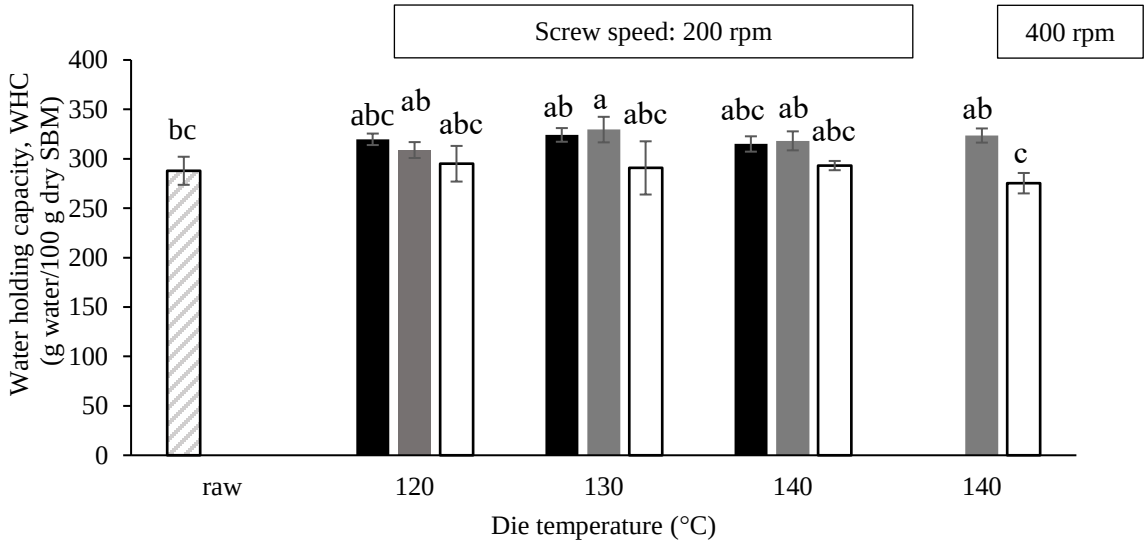


Figure A3.2: WHC of the larger particle size raw and extruded SBM as a function of moisture content, temperature and screw speed. Patterned column represents the raw SBM, while the black, gray and white columns represent extrudates with moisture contents of 15, 18 and 27 g water/100 g dry SBM, respectively. Error bars represent ±1 standard deviation. Columns labelled by different letters are statistically different ($p < 0.05$).



Important data trends for WHC: When the effect of SBM's PSD was considered, compared to the raw SBM, the WHC of extrudates significantly ($p < 0.05$) improved only for the smaller particle size and at a moisture content of 15 g water/100 g dry SBM (Figure A3.1). In general, an increasing trend in extrudate WHC was observed when smaller PSD and lower moisture contents were used. When the effect of extrusion temperature profile was considered, compared to raw SBM, extrusion cooking caused a significant increase in WHC only when the SBM was extruded at 130 °C die temperature and a moisture content of 18 g water/100 g dry SBM (Figure A3.2). Screw speed did not have a significant effect on the WHC at die temperature of 140 °C and at moisture contents of

18 and 27 g water/100 g dry SBM. The positive impact of extrusion cooking relied on the interaction of specific extrusion process parameters such as raw material PSD and moisture content. These preliminary results demonstrated the importance of raw material PSD and moisture content for their impact on WHC of SBM extrudates. Hence, these parameters were selected to be explored in this MSc project.

Appendix 4: Effects of Raw Material PSD, Extrusion Moisture Content, Barrel Temperature Profile and Screw Speed on OHC of SBM Extrudates

Figure A4.1: OHC of raw and extruded SBM as a function of PSD and moisture content. The barrel temperature profile was kept at 60/75/90/105/120 °C. Patterned column represents the raw SBM, while the black and white columns represent extrudates obtained from larger and smaller particle size SBM, respectively. Error bars represent ±1 standard deviation. Columns labelled by different letters are statistically different ($p < 0.05$)

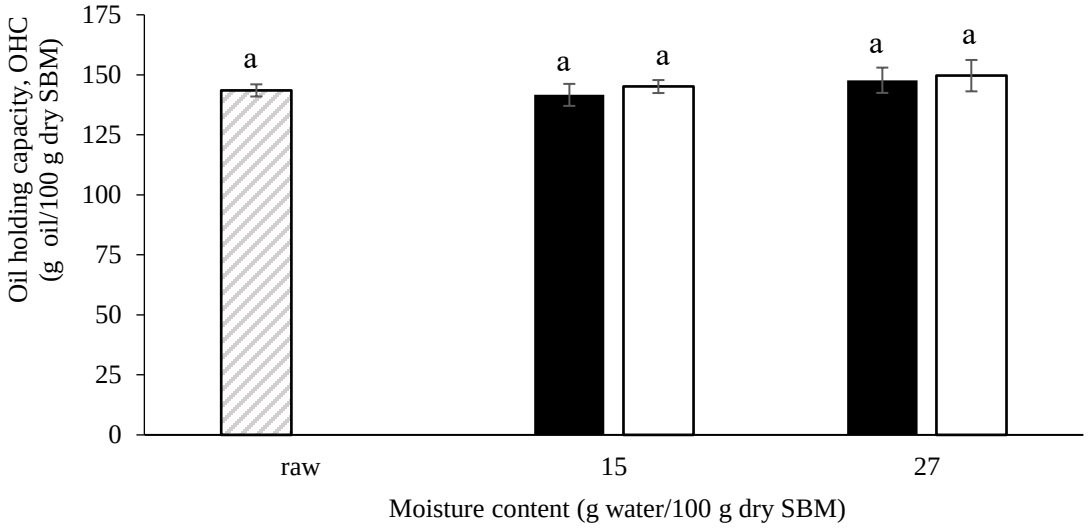
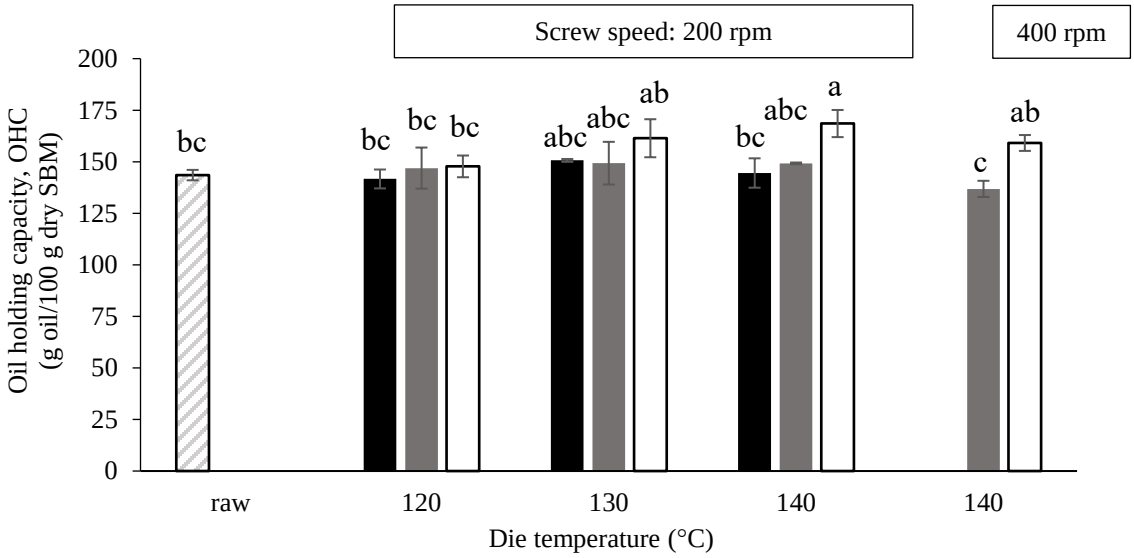


Figure A4.2: OHC of the larger particle size raw and extruded SBM as a function of moisture content, temperature, and screw speed. Patterned column represents the raw SBM, while the black, gray and white columns represent extrudates with moisture contents of 15, 18 and 27 g water/100 g dry SBM, respectively. Error bars represent ±1 standard deviation. Columns labelled by different letters are statistically different ($p < 0.05$).



Important data trends for OHC: OHC was not significantly ($p < 0.05$) affected by SBM's PSD at the extreme moisture contents studied (Figure A4.1). When the effect of moisture content at different extrusion temperature profiles was considered, an increasing trend in OHC was observed with an increase in extrusion moisture content (Figure A4.2). However, this increase was only significant ($p < 0.05$) when moisture content was increased from 15 to 27 g water/100 g SBM and at 140 °C die temperature. When the effect of screw speed was considered, increasing the screw speed from 200 to 400 rpm amplified the effect of moisture content on OHC: At the higher screw speed, an increase in moisture content from 18 to 27 g water/100 g dry SBM resulted in a significant increase in extrudate OHC. The observed trends highlighted the importance of further investigating the interaction of moisture content and barrel temperature profile in this MSc project.

Appendix 5: Effects of Raw Material PSD, Extrusion Moisture Content, Barrel Temperature Profile and Screw Speed on LGC of SBM Extrudates

Figure A5.1: LGC of raw SBM, and SBM extrudates as a function of PSD and moisture content. The barrel temperature profile was kept at 60/75/90/105/120 °C. Patterned column represents the raw SBM, while the black and white columns represent extrudates obtained from larger and smaller particle size SBM, respectively. Error bars represent ±1 standard deviation. Columns labelled by different letters are statistically different ($p < 0.05$).

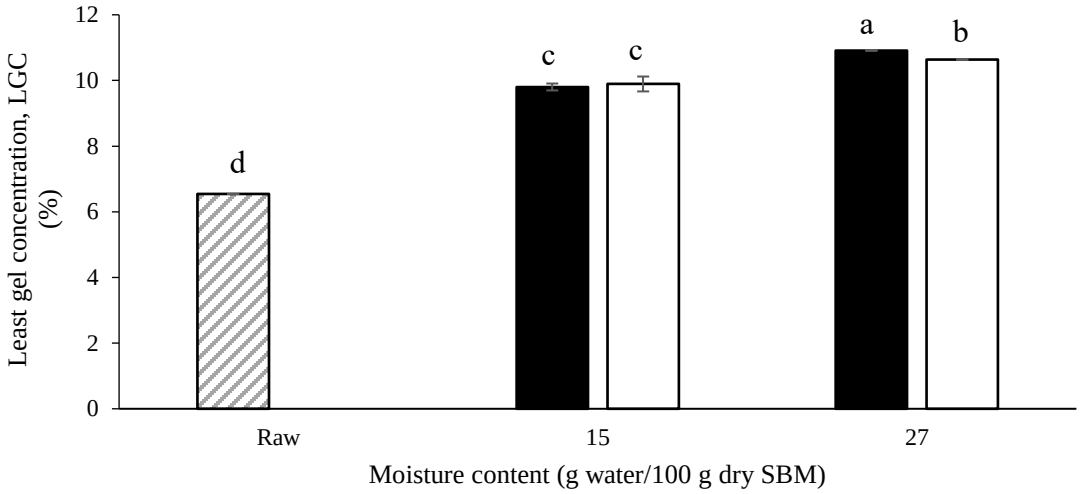
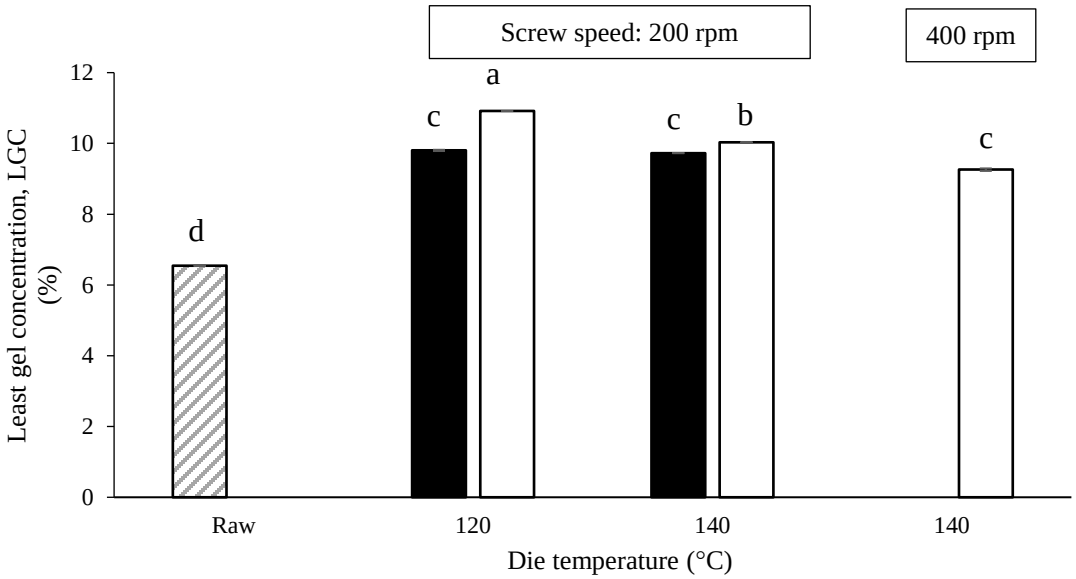


Figure A5.2: LGC of the larger particle size raw and extruded SBM as a function of moisture content, temperature, and screw speed. Patterned column represents the raw SBM, while the black and white columns represent extrudates with moisture contents of 15 and 27 g water/100 g dry SBM, respectively. Error bars represent ±1 standard deviation. Columns labelled by different letters are statistically different ($p < 0.05$).



Important data trends for LGC: LGC was significantly ($p < 0.05$) affected by SBM's PSD at moisture content of 27 g water/100 g dry SBM: LGC significantly decreased when smaller PSD was used as compared to larger PSD (Figure A5.1). Moreover, as compared to raw SBM, LGC increased for extrudates obtained at both PSDs. A significant increase in LGC was observed for SBM extrudates as compared to raw SBM at all extrusion temperature profiles and moisture contents studied (Figure A5.2). While an increase in die temperature at the lowest moisture content did not have a significant effect on LGC, a significant positive impact on LGC (i.e., a reduction in LGC) was observed at the highest moisture content studied (27 g water/100 g SBM). Furthermore, an increase in screw speed at 140 °C and a moisture content of 27 g water/100 g dry SBM also positively affected the LGC of SBM extrudates.

The above results showed interactions between extrusion moisture content and barrel temperature profile. Accordingly, these interactions using a wide range of barrel temperature profiles (such as 120, 140, and 150 °C SBM) and moisture contents (such as 15, 21, and 27 g water/100 g dry SBM) were decided to be investigated during this MSc project.

Appendix 6: Effects of Raw Material PSD, Extrusion Moisture Content, Barrel Temperature Profile, and Screw Speed on NSI of SBM Extrudates

Figure A6.1: NSI of raw and extruded SBM as a function of PSD and moisture content. The barrel temperature profile was kept at 60/75/90/105/120 °C. Patterned column represents the raw SBM, while the black and white columns represent extrudates obtained from larger and smaller particle size SBM, respectively. Error bars represent ±1 standard deviation. Columns labelled by different letters are statistically different ($p < 0.05$).

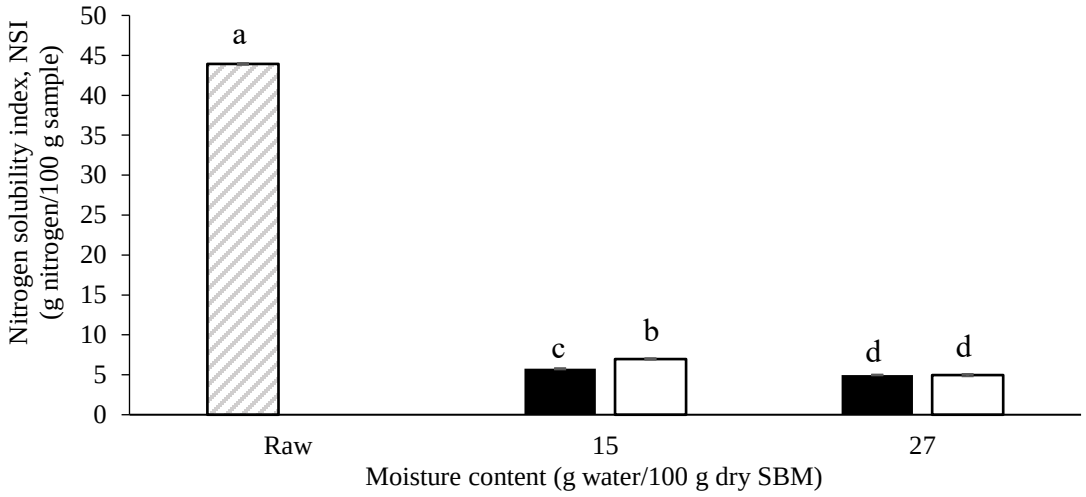
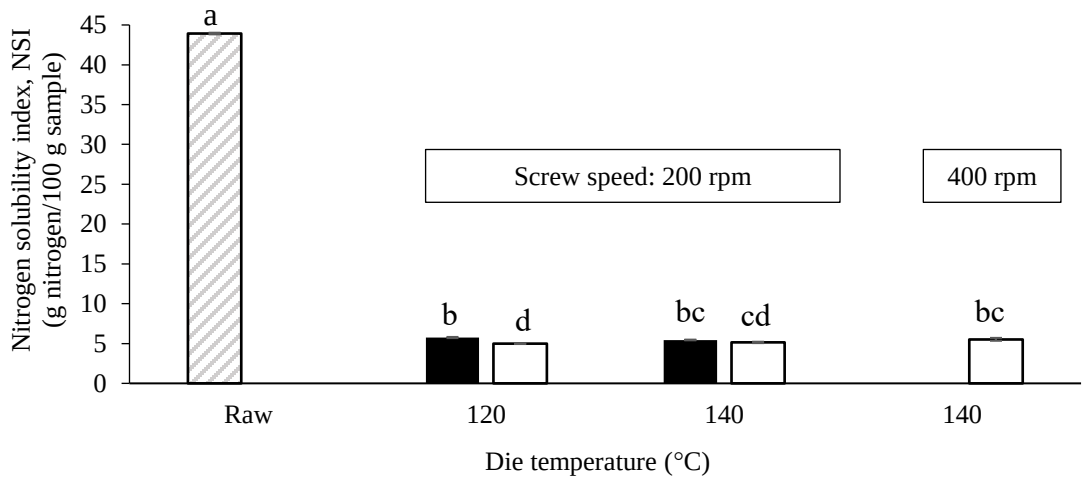


Figure A6.2: NSI of the larger particle size raw SBM, and SBM extrudates as a function of moisture content, temperature, and screw speed. Patterned column represents the raw SBM, while the black and white columns represent extrudates with moisture contents of 15 and 27 g water/100 g dry SBM, respectively. Error bars represent ±1 standard deviation. Columns labelled by different letters are statistically different ($p < 0.05$).



Important data trends for NSI: As compared to raw SBM, extrusion processing caused a significant reduction in NSI of extrudates regardless of their PSD (Figure A6.1). At the lower moisture content, an increase in raw material PSD resulted in a decrease in NSI. When the effect of extrusion temperature profile was considered, at a die temperature of 120 °C, an increase in extrusion moisture content caused a significant ($p < 0.05$) reduction in NSI of extrudates (Figure A6.2). This trend was not observed at the higher die temperature of 140 °C. An increase in screw speed at 140 °C and a moisture content of 27 g water/100 g dry SBM also did not have a significant effect on NSI.

Appendix 7: ANOVA Table

Table A7: Impacts of extrusion process parameters and their interactions on techno-functional properties of extrudates.

Property	NSI	OHC	WHC	LGC	EC	ES
FMC	<0.0001	<0.0001	<0.0001	0.0037	<0.0001	0.0002
PS	<0.0001	0.0003	0.1015	<0.0001	<0.0001	0.3020
BT	<0.0001	<0.0001	0.8199	<0.0001	<0.0001	0.0005
PS*FMC	0.0273	0.2829	0.2050	0.0010	0.3944	0.0829
PS*BT	0.1695	0.0014	<0.0001	<0.0001	<0.0001	0.4005
FMC*BT	<0.0001	0.1486	0.0754	0.0731	0.6503	0.0539
PS*FMC*BT	0.0028	0.2667	0.1415	<0.0001	0.0036	0.6004