Physicochemical properties of pea proteins, texturization using extrusion, and application in plant-based meats

by

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Abstract

With a growing trend for plant-based and clean label products, extrusion texturization of pea protein is becoming more prevalent for plant-based meat applications. However, several challenges have surfaced from this. Large demand for pea protein has made sourcing difficult and expensive. Differences in the physicochemical traits of pea protein compared to traditional textured vegetable proteins, wheat and soy, mean that final product traits are different when switching to pea protein. Additionally, pea protein from different suppliers is sourced and processed differently, and thus require different extrusion processing and lead to varying final product traits. The intent of this research was to better understand the differences between common and upcoming proteins used for texturization, the impact of formulating pea protein with legume flours and fibers, and how various pea proteins differ in raw material physicochemical traits and lead to differences in texturization.

The first study invested how carbohydrate additions up to 20% affected pilot-scale twin screw extrusion and the pea protein extrudate properties. Characteristics of raw material, whole extrudate, and milled extrudate were observed. Adding pulse flour resulted in low water holding capacity (WHC) of the extrudates compared to the control of pea protein isolate (PPI) (241-391% and 496%, respectively). However, fiber increased the WHC from the pea flour treatment (428-442%). An increase in instrumental hardness was observed from PPI to all pulse flour treatments (475 g to 837-2333 g) due to the disruption of protein-networking and protein-based expansion. Both WHC and hardness have a strong relationship with the bulk density of the extrudate. Adding pulse flours to PPI in plant-based meat was shown to increase density and hardness of the extrudate and decrease WHC. However, addition of fiber reduced the negative

impact of starch, and could allow more flexibility in targeting specific qualities while reducing the protein costs and usage for plant-based meats.

In the second study, twin-screw extrusion was used to texturized four pea proteins, as well as soy and wheat proteins for comparison. Pea proteins from commercial sources varied in their functional properties. Proteins that had higher water absorption capacity had the highest peak viscosity. These proteins also required the least specific mechanical energy during processing. Pea protein extrudates displayed similar hardness, chewiness, and springiness, but varied in these traits when formed in a patty. A single trait did not seem to be most impactful in the creation of certain extrudate characteristics, and thus further study should be conducted on understanding the role of multiple factors to create a model which balances the most important factors for desired textured protein outcomes.

This research has proven the importance of understanding raw ingredient sources, composition, and pre-processing for extrusion of plant protein. Textured pea protein shows significantly different texture compared to soy and wheat, and internal structure varies between textured pea protein made with isolates with different functional properties. Even more, starch and fiber can be successfully utilized to reduce necessary protein content for texturized pea protein while simultaneously using a co-product and targeting unique product texture.

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Dedication

For dad. Thanks for knowing my potential before I do.

And for my heavenly father.

Do not work for the food that perishes, but for the food that endures to eternal life, which the Son of Man will give to you. For on him God the Father has set his seal.

John 6:27

Chapter 1 - Introduction

Overview of Textured Vegetable Proteins

Consumers and companies alike are becoming aware of environmental and ethical factors of the animal meat industry which is leading to a rapidly rising interest in plant-based alternatives around the globe. Several alternatives to animal meat, like tofu, tempeh, and seitan, are already established in the market. The main consumer of these are current vegetarians or vegans. Gaining market popularity, though, are products which use textured vegetable protein and target meat-eaters and flexitarians.

Textured vegetable proteins begin as protein isolates from various sources, often soy, wheat, and pea, though research is currently being conducted on a plethora of other sources such as chickpea, faba bean, mung bean, hemp, seaweed, and many others. The protein source is cooked via extrusion which allows for the denaturation and alignment of the protein into a structure that mimics the fibrous nature of meat. The textured vegetable protein is then ready for further processing into the final product application like patties, nuggets, or sausages.

The food market is trending toward plant-based foods, and also toward foods deemed as clean label and allergy friendly. This has caused many companies to research switching from soy and wheat to avoid GMOs or allergens. For this reason, pea protein has been a major focus and significant challenges have been presented in this transition due to differences in plant protein functionality.

This research focused on extrusion texturization of pea protein and its application in plant-based meat products. The intent was to gain insight on the use of pea protein alone and in combination with other raw ingredients to understand formulations and functionality during extrusion and the resulting product qualities. Specific objectives were the following.

Chapter 2 Objectives

Chapter 2 reviews current literature to determine the viability and functionality of pea protein through understanding raw material traits such as protein content and composition, gelation properties, and denaturation temperature. Additionally, the aim was to compare pea protein to traditional meat analog ingredients (soy and wheat) and further explore how other pulse proteins might be used as a major protein ingredient in textured plant protein. A final objective of this literature search was to understand traits of texturized protein product such as protein solubility, water holding capacity, amino acid composition and digestibility, and texture.

Chapter 3 Objectives

The goal of Chapter 3 was to texturize pea protein with multiple varieties of pulse flours and to texturize pea protein with increasing levels of pea fiber. Even more, the aim was to understand raw ingredient functionality (water absorption, flow temperature, denaturation enthalpy, viscosity), the impact of starch sources and pea fiber level on processing (specific mechanical energy), and the final textured pea protein quality (water holding capacity, bulk density, texture properties). An understanding of the role of starch and fiber in the structuring of textured pea protein could lead to the use of co-products from protein isolation, reduced cost, and improved plant-based meat.

Chapter 4 Objectives

The goal of Chapter 4 was to determine raw material characteristics of various commercial pea isolates (water absorption, denaturation qualities, viscosity) and how those qualities may create unique opportunities for textured protein traits (water holding capacity, bulk density, hardness, etc). The intent was also to understand pea protein and its extrudates in comparison to soy and wheat proteins.

Chapter 2 - Chemical and physicochemical features of plant proteins and their extrudates for use in plant-based meat

Abstract

Meat analogs are becoming more widespread as the flexitarian diet becomes popular. Many options on the market are made from soy protein isolate, but other protein sources can be used. Alternate legume protein can be an economically and functionally viable option.

Scope and Approach

This review discusses protein isolate qualities of traditional meat analog proteins (soy and wheat gluten), pea protein, and emerging pulse proteins (chickpea, faba bean, lentil, mung bean). Raw traits of protein are important to extrusion for texturization, and this review focuses specifically on protein sedimentation coefficients, amino acid composition, least gelation concentration (LGC), denaturation temperature, water absorption capacity, and oil absorption capacity, viscosity, and flow temperature. Product qualities (protein solubility, water holding capacity, amino acid composition and digestibility, and texture) of pea-based meat analogs are compared and related to the potential of texturizing emerging pulse proteins.

Raw material characteristics of each isolate should be considered before extrusion, as isolate properties can vary, even when from the same botanical source. The analysis and methods used to change from soy and wheat gluten to pea can be useful to make advancements from pea protein to additional pulse proteins. The quality of pea protein textured vegetable protein (TVP) is heavily dependent on processing parameters such as specific mechanical energy (SME), moisture, and temperature.

1. Introduction

Consumers are becoming more interested in replacing meat proteins with plant proteins due to perceptions of health, environmental impact, and animal welfare. A diet that contains a greater amount of plant foods results in reduced consumption of saturated fats and increased fiber consumption (Forestell, 2018), causing a better balance of some nutrients and potentially increasing cardiovascular protection (Kuchta et al., 2016). Environmentally, more evidence is surfacing which supports the concept that dietary choices can have substantial impact on the environment. Concerns are rising in regard to resource input into the livestock industry as well as greenhouse gas emissions from beef cattle (Heller & Keoleian, 2014; Kumar et al., 2017), and consumers are becoming aware of their personal impact. In addition, animal welfare in the livestock industry continues to be a concern of some consumers.

With health, environment, and animal welfare as the motivators, some consumers are choosing to eliminate meat proteins altogether and some are vying for the "flexitarian" diet where meat consumption is reduced. Flexitarians still enjoy and crave the sensory experience of animal meat, yet simultaneously have desires to increase health and decrease environmental harm, creating a market for plant-based alternatives that closely mimic a meat experience. Meat analogs made from textured plant proteins, commonly called plant-based meats, can deliver on this desire and allow consumers to have an alternative to meat protein without giving up the texture and flavor which they crave. Plant-based meats can potentially have similar texture, mouth feel, flavor, and nutrients compared to animal meats (Wild, 2016).

Often, plant-based meats are made from concentrated proteins from sources such as soy, wheat, and pea (Asgar, Fazilah, Huda, Bhat, & Karim, 2010; Jones, 2016; Schreuders et al., 2019). Two main ways to texturize plant proteins exist: extrusion and centrifugal spinning in a

shear cell (Schreuders et al., 2019). In these processes, a proteinaceous material is mixed with water, heated, and sheared so as to denature the proteins and form thin fibers.

Numerous commercialized products have used soy flour, soy protein concentrates, soy protein isolates, and wheat gluten (Kumar et al., 2017; Riaz, 2004). Soy has been used because of its affordability, availability, and its ability to impart attributes similar to meat (Samard & Ryu, 2019a). Wheat gluten has also been commonly used because it adds greater levels of methionine, a sulfur-containing amino acid, which assists in creating a meat-like product due to disulfide bonding (Roberts, 2013). However, there are additional health and environmental concerns that surface with these protein sources, namely genetic modification of some soy and the allergenicity of both soy and wheat gluten. In order to satisfy the health and environmental concerns of the consumer, there is now movement toward the use of pulse proteins to create plant-based meat.

Using pulse protein isolates can address the previously mentioned concerns as well as improve nutritional quality, texture, and function of texturized vegetable protein (TVP) (Asgar et al., 2010). For example, pea protein generally does not cause allergic reaction or immune response as soy and wheat can (Bajaj, Tang, & Sablani, 2015). While meat proteins naturally contain a complete amino acid profile, pulse proteins from yellow peas and many types of beans may have more satiating effects than meat, keeping consumers fuller longer (Kristensen, Bendsen, Christensen, Astrup, & Raben, 2016). A complete amino acid profile can be obtained by complimenting legume proteins with cereal grains which allows for a complete amino acid profile. Since meat analogs are often paired with a cereal grain, like rice or bread, complete proteins can still be consumed if pea or other legume proteins were to be used for TVP. For these reasons, pea protein utilization in the food industry has taken off in the recent years. The meat substitute market increased the use of pea protein in new product launches by 11% from 2013-

2017 (Innova Market Insights, 2019) and companies are investing more into the production of pea protein, even enough to double the amount of production area (Beyond Meat, 2020; Cargill, 2019). More pulse sources like chickpea, lentils, and faba beans are of rising interest, too, to determine their functionality and use in textured protein.

The goal of this review is to determine the viability and functionality of pea protein, compare it to traditional meat analog ingredients from soy and wheat, and further understand how other pulse proteins can be used as a major protein ingredient in textured plant protein. Important raw material traits are discussed, like protein content and composition, gelation properties, and denaturation temperature. Character traits of the final texturized product such as protein solubility, water holding capacity, amino acid composition and digestibility, and texture are elaborated.

2. Raw Protein Characterization and Sources

Current plant-based meats are made mostly from proteins sourced from soy, wheat, and pea, with minor use of mung bean and rice. As alternative proteins are on the rise, consumers are demanding plant proteins besides soy and wheat, causing a compound annual growth rate of 30% for pea protein from 2004-2019 (Bashi, McCullough, Ong, & Ramirez, 2019). The pea protein market was valued at $19.41 million in 2018 and is predicted to grow to $39.98 million in 2025 (Grand View Research, 2019). Proteins other than the soy, wheat, and pea are also expected to increase in popularity as the alternative protein market continues to grow. Thus, it is important to understand protein sources available and how to transfer current knowledge to new sources.

2.1 Traditional Ingredients

2.1.1 Soy Protein

Defatted soy flour, soy protein concentrates (SPC) and isolates (SPI) have frequently been used for mimicking meat products, with approximate protein contents of 50%, 70%, and 90%, respectfully. Soybeans generally contain 35-40% protein with 20% oil and 30% non-starch polysaccharides (Day, 2013). The protein is separated through dispersing the soy flour in an alkaline solution to dissolve the protein and then precipitating the protein by passing it through its isoelectric point. The major fraction of soy protein are the common legume proteins: 7S vicilin (52% of total protein) and 11S legumin (35% of total protein) (Table 2.1) (Guo & Yang, 2015; Hettiarachchy & Kalapathy, 1998).

Both vicilin and legumin are classified as globulins in the Osborne classification system, meaning they are salt-soluble. Vicilin is a protein composed of three subunits and has a sediment coefficient (rate of sedimentation) of 7S. Legumin is a larger protein than vicilin, composed of six subunits and a sediment coefficient of 11S; the polypeptides are joined by disulfide bridges (Lu, He, Zhang, & Bing, 2019). The ratio of these proteins is very important to the protein functionality in TVP since vicilin contains a lower amount of sulfur-containing residues (cysteine and methionine amino acids) (Lam, Can Karaca, Tyler, & Nickerson, 2018; Lu et al., 2019). Although legumin is a smaller fraction of the protein content in soy, it contains about 20 sulfide groups, allowing for disulfide bonding and a strong ability to texturize. Vicilin has a much lower content of sulfur-containing amino acids. Although soy protein does have antinutritional factors such as phytates, phenols, and trypsin and chymotrypsin inhibitors, heating deactivates the antinutritional factors allowing for better digestion (Abd El-Hady & Habiba, 2003; Fernández-Quintela, Macarulla, Del Barrio, & Martínez, 1997). In summary, legumin is

larger in size, has a greater number of sulfide groups, but is smaller in quantity in soy protein compared to vicilin. Despite its lower concentration, the sulfide groups it contributes make it a vital player in texturizing the protein.

2.1.2 Wheat Gluten

Wheat flour has a protein content of 8-15%, with the remaining content being 75% starch, 1-2% fat, and 5% non-starch polysaccharides (Day, 2013). Vital wheat gluten and isolated wheat gluten have protein contents ranging from 75-80% and 90%, respectively. The major fractions of pulse proteins are globulins, but wheat gluten proteins have extremely unique characteristics that set it apart from any other plant protein due to gliadin and glutenin, which account for 85% of the proteins (Schmiele, Clerici, & Chang, 2013). Gliadin is alcohol-soluble (prolamin), while glutenin is soluble in dilute acid (glutelin) (Urade, Sato, & Sugiyama, 2018). These two proteins complex when mixed with water and create the viscoelastic matrix common in bread dough and also assist in creating disulfide bonds that lead to the fibrous structure in textured plant proteins (Samard, Gu, & Ryu, 2019). Flow of wheat doughs is attributed to gliadin, while elasticity and strength is attributed to glutenin (Urade et al., 2018). The ratio of these two wheat components is important to create the desired viscoelastic properties and resulting product properties (Roberts, 2013).

2.1.3 Pea Protein

The common pea (*Pisum savitum* L.) has a macronutrient composition containing a high amount of protein and fiber relative to cereal grains and some other pulses (Day, 2013; Millar, Gallagher, Burke, McCarthy, & Barry-Ryan, 2019). While the amounts can vary by genotype and environmental factors, the total carbohydrate fraction is the majority of the pea composition at 50-70%. Approximately 20-25% is still protein (Day, 2013; Lam et al., 2018; Lu et al., 2019).

In specific breeding lines of pea, up to 30% protein can be present (Shen, Hou, Ding, Bing, & Lu, 2016). Four Osborne classes of proteins are found in the pea: globulin (55-65%), albumin (18-25%), prolamin (4-5%), and glutelin (3-4%) (Lu et al., 2019). Due to the high concentration of globulin proteins in pea, its functional properties are important. The globulin fraction contains the protein that is also important in soy texturization (Riaz, 2004).

Within the globulin fraction of pea, two specific globulin proteins named vicilin and legumin are present (Lam et al., 2018). Pea protein is roughly 43% vicilin and 28% legumin (Chakraborty, Sosulski, & Bose, 1979), while soy protein has a higher vicilin fraction (52%) and a slightly higher legumin content (35%) (Table 2.1) (Hettiarachchy & Kalapathy, 1998). Overall, pea contains 0.2-0.59 g cysteine residues and 0.3-1.6 g methionine residues per 100 g of raw material (Gorissen et al., 2018; Khattab, Arntfield, & Nyachoti, 2009). Pea protein also contains convicilin, which is absent in other legumes. This storage protein contains more sulfur than vicilin. Due to the importance of disulfide bonds in formation of fibers in TVP and the presence of methionine in the legumin fraction, pea protein may be useful in textured plant protein applications (Lin, Huff, & Hsieh, 2000). While heating rate of the protein does not affect gelling of pea protein, the cooling rate does, and legumin protein has a greater gel strength when cooled at a slower rate (Dong Sun & Arntfield, 2011; O'Kane, Vereijken, Gruppen, & Van Boekel, 2005).

2.2 Other Pulse Ingredients

2.2.1 Chickpea Protein

Chickpeas naturally have protein content ranging between 20-25% (Day, 2013). Chickpea protein has a methionine content of 15-19 mg/g of protein (Paredes-Lópes, Ordorica-

Falomir, & Olivares-Vázques, 1991), and legumin constitutes 32% of chickpea protein, which is very similar to the content of legumin in soy and pea protein (Chakraborty et al., 1979).

2.2.2 Faba Bean Protein

Whole faba beans have a high protein content near 30% db (Gu, Masli, & Ganjyal, 2020). Faba has an 11S fraction of 45%, which is 10% more than soy protein and 17% greater than pea protein. For replacing traditional proteins like soy, the 11S protein content in faba may be important than the content of 7S protein, since the thermal properties of the 11S proteins are more similar than the 7S proteins (Wright & Boulter, 1980). As previously stated, the 11S protein, legumin, contains more sulfur, and is another reason having the same legumin content in faba may be important for textured plant protein (Fernández-Quintela et al., 1997; Zhang, Jiang, & Wang, 2007).

2.2.3 Lentil Protein

Lentils contain between 20-30% protein in their raw form (Jarpa-Parra et al., 2015). Lentils have a low content of sulfur-containing amino acids at 4.9-5.9 mg/g (Aryee & Boye, 2017) and a legumin fraction around 50% of the salt-soluble protein (Jarpa-Parra et al., 2015).

2.2.4 Mung Bean Protein

Mung beans have a protein content around 28% in the flour after removing the hulls (Coffman & Garcia, 1977). Mung bean flour has been extruded at 25% moisture to make textured plant protein (Brishti et al., 2017). When comparing pea protein to other legumes, it contains as much or more methionine and cysteine amino acids—even more than soy (Table 2.1) (Brishti et al., 2017; Tang, Chen, & Ma, 2009), yet a low legumin fraction (Chakraborty et al., 1979).

Table 2.1 Sediment coefficients and amino acids in plant proteins

Pulse Type	7S	11S	Cys	Met	Lys	Reference
Pea Protein	43	28				Chakraborty et al.1979
			0.35-0.59 g/100 g	**0.9-1.6 g/100 g	62.5 mg/g	Khattab et al. 2009
			0.2 g/100 g	0.3 g/ 100 g		Gorissen et al. 2018
			**21 mg/g			Corgneau 2019
Soy Protein	52	35				Hettiarachchy et al. 1998
			3.59 mg/g	117 mg/g	48 mg/g	Brishti et al., 2017
			0.2 g/100 g	0.3 g/ 100 g		Gorissen et al. 2018
			**39 mg/g			Corgneau 2019
Wheat Gluten			0.7 g/100 g	0.7 g/100 g		Gorissen et al. 2018
			56 mg/g			Schmiele et al., 2013
				13.4 mg/g		Mitra et al., 1978
						Woychik et al., 1961
Chickpea Protein	26	32				Chakraborty et al. 1979
			22 mg/g	19 mg/g	6.8 mg/g	Paredes-Lopez et al., 1991
Faba Protein	33	45				Chakraborty et al.1979
			6.2 mg/g	5.4 mg/g		Vogelsang-O'Dwyer et al. 2020
Lentil Protein		50% of globulin fraction				Jarpa-Parra, 2015
			4.9 mg/g	5.9 mg/g	55.7 mg/g	Aryee et al. 2017
Mung Bean Protein	79	6				Chakraborty et al. 1979
	65					Rahma et al. 2000
			43 mg/g	130 mg/g	140 mg/g	Brishti et al. 2017
			2.1 mg/g	12.4 mg/g		Tang et al. 2009

3. Comparisons of raw protein functionality

Proteins from different botanical sources are intrinsically different, thus understanding characteristics of these current and future sources of protein is critical for developing plant-based meat. Several tests are used to determine functional properties of raw protein before their use for extrusion of textured proteins. The tests discussed here are water absorption capacity (WAC), oil absorption capacity (OAC), thermal properties from differential scanning calorimetry (DSC), phase transition analysis (PTA), least gelation concentration (LGC), and rapid visco analysis (RVA). Standards have not been set for these tests, so it is important to note that comparison between protein sources is convoluted and not always straightforward. Even more, isolation methods of proteins, processing history, growing environment, genotypes, and more can affect the properties even within a single pulse source.

3.1 Common Methods

3.1.1 Water Absorption Capacity/ Oil Absorption Capacity

WAC and OAC are determined to understand the polarity of the binding sites available by the amount of water or oil the proteins absorbed. In previous literature, the ability of water to be absorbed by the raw protein has been referred to with several names (water holding capacity, water binding capacity, water absorption capacity). In this review, the term water absorption capacity will refer to the raw protein's ability to retain water after centrifugation and water holding capacity will refer to the amount of water held by a textured protein product after submersion in excess water.

WAC and OAC may be indicative of the extent of denaturation of the protein since proteins unfold and expose more hydrophobic sites for bonding when denatured (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014). WAC and OAC were measured in the

studies reviewed here by mixing 3-10% slurries of protein isolate with water or oil, respectively, then stirred and allowed to sit for 30 minutes. Mixtures were then centrifuged (around 1000-15000 g), and the change in sediment weight or change in free oil or water was measured, resulting in WAC and OAC reported as g or ml oil or water absorbed per g protein (Aydemir & Yemenicioĝlu, 2013; Brishti et al., 2017; Osen et al., 2014).

These functional attributes of protein depend on many factors, however, such as the genotype of the pea, growing conditions, and protein extraction methods (Barac et al., 2010; Osen et al., 2014). With greater heat denaturation, one study has shown that the number of polar sites on the proteins increased, allowing for greater interaction with water (Osen et al., 2014). For three commercial pea protein isolates, the water absorption capacity varied from 2.1 to 5.4 ml/g (Osen et al., 2014). Because these properties are clearly influenced by many factors, it is difficult to directly compare WAC and OAC between plant protein sources.

For comparisons between plant protein sources, WACs and OACs should be considered in light of the many factors that influence these properties. In general, WAC is 1 to 5 g/g and OAC is 1 to 4 g/g (Table 2.2). Based on the lowest WAC if ranges were given, pea, soy, faba, and lentil proteins have similar WACs (1.1-1.7 g/g). Wheat, chickpea, and mung proteins have the highest WAC among the pulse proteins, around 3 g/g. However, pea and lentil have been shown to also reach WACs similar to wheat, chickpea, and mung.

OAC shows that pea, soy, and faba have similar abilities to absorb oil (1.1-1.7 g/g). Lentil and mung bean protein have intermediate OAC around 2-3 g/g. Finally, WG and chickpea have indication of the greatest hydrophobicity with an OAC around 3.5 g/g.

Oil absorption capacity of pea protein isolate were shown to improve after extrusion, however, based on the experiments by Osen et al. (2014) because more hydrophobic sites are

available after the heat treatment while still retaining the hydrophilic sites. So even with oil absorption of the raw material being low, it has potential to increase due to the denaturing during texturization.

3.1.2 Differential Scanning Calorimetry

DSC is a thermal analysis test which determines properties of polymers such as starch gelatinization and protein denaturation temperatures through heat flow changes. Knowing the thermal denaturation characteristics can help determine the strength of bonds due to the amount of energy required to break them for denaturation. A higher protein denaturation temperature shows a greater thermal stability. This can also help determine the extent of denaturation of commercial protein isolates. Results of DSC are highly dependent on rate of heating and the hydration level of the sample. Most of the reviewed literature used a heating rate of 5-10 °C/min, with the exception of Ricci et al. (2018) who used 20 °C/min. The higher heating rate resulted in much higher denaturation temperatures. Samples generally are heated from 20-140 °C, but Brishti et al. (2017) heated to 300 °C. Moistures varied from no water added (Brishti et al., 2017; Leon, Rosell, & De Barber, 2003; Ricci et al., 2018) to 18-30% protein slurries (Cai, McCurdy, & Baik, 2002; Osen et al., 2014; Paredes-Lópes et al., 1991). Studies with no water addition resulted in higher denaturation temperatures.

Overall, each pulse protein had similar denaturation temperatures (Table 2.2). The denaturation temperature of SPI was found to be 157.86 ºC with no water (Brishti et al., 2017), and 86.6-109.2 ºC as a 30% slurry (Cai et al., 2002). Pea protein denatures at 88.5 ºC (Osen et al., 2014). According to Cai et al. (2002), denaturation peaks of 7S and 11S portions of the protein could be differentiated. Lentil, faba bean, and chickpea have similar denaturation temperatures for both the 7S and 11S fractions (roughly 98 ºC and 113 ºC) (Cai et al., 2002).

Wheat gluten has the least thermal stability of proteins reviewed here with a peak denaturation temperature near 55 °C (Leon et al., 2003; Wang et al., 2014).

3.1.3 Least Gelation Concentration

The formation of protein networks for TVP is critical, and gelling is one indicator of the ability of a protein to texturize well (Jones, 2016). LGC is the concentration of protein at which a strong gel is formed with the addition of water and heat, followed by cooling. Extraction processes of proteins can majorly affect the gelation concentration (Aydemir & Yemenicioğlu, 2013), so extracted proteins from the same source can have variable concentrations of gelation. This makes LGC an important test to conduct prior to extrusion, for a lower gelation concentration may extrude a better textured product due to stronger gelling structure. To conduct LGC, protein was dispersed in water in concentrations ranging from 1-14%. Protein solutions were then heated in a water bath at 90-100 °C. After an hour of heating, the protein solutions were cooled immediately via ice bath or running tap water, then chilled at 4 °C for 2 hours. Gelation is observed when the tube is inverted and there is no falling or slipping of the gel (Aydemir & Yemenicioğlu, 2013; Brishti et al., 2017; Wang, Zhao, Yang, Jiang, & Chun, 2007). Lower LGCs are regarded as better since less protein is required to form a strong 3-D network of proteins.

SPI has a reported LGC of 14-16%, (Brishti et al., 2017; Fernández-Quintela et al., 1997). Pea protein isolate requires slightly more protein to gel with an LGC of approximately 18% (Fernández-Quintela et al., 1997). Wheat gluten has a higher least gelation concentration ranging from 16-22% (Wang et al., 2007). Even these ingredients that take the majority of the market share have different ranges of concentration for protein gelling. With the knowledge that soy, pea, and WG can texturize well, and that their LGC vary from 14-18%, this may be a target

LGC for extruding other proteins for plant-based meat, and there is potential that proteins with a higher LGC may texturize well, too.

Chickpea and faba bean protein have LGCs of 14% (Zhang et al., 2007), which is comparable to soy protein and just lower than pea protein. Lentil protein isolate has been shown to have a better LGC than soy and pea protein, ranging from 8 to 14% (Marcela Jarpa-Parra, 2018). Mung bean protein has a LGC of around 10-12% (Brishti et al., 2017; Coffman & Garcia, 1977). Due to these comparable or lower LGC numbers and thus a stronger protein network at a lower concentration, these rising protein sources could give similar or better products as texturized proteins.

Table 2.2 Physicochemical properties of isolated pea, soy, wheat, chickpea, faba, lentil, and mung proteins

Protein Type	LGC	Peak Denaturation Temperature (ºC)	WAC	OAC	Reference
Pea Protein	180 g/L		1.7 g/g	1.2 g/g	Fernandez-Quintela, 1997
		88.5	2.1-5.4 ml/g	0.7-1.7 ml/g	Osen et al, 2014
Soy Protein	160 g/L		1.3 g/g	1.1 g/g	Fernandez-Quintela, 1997
	14% w/v	157.86			Brishti et al., 2017
Wheat Gluten			3 g/g	3.45 g/g	Brishti et al., 2017
	16-22%				Wang et al., 2007
		53.17-56.58			Wang, Xu, et al., 2014
		40.9-64.3			Leon et al., 2003
					Riaz, 2004
Chickpea Protein	140-180 g/L				Zhang et al., 2007
	14-18% w/v				Kaur et al., 2007
		88-94		1.7-2 ml/g	Paredes-Lopez et al., 1991
		204.7			Ricci et al., 2018
		96.8 (7S)			
		115.3 (11S)			Cai et al., 2002
			2.5-3.2 ml/g	1.7-3.96 ml/g	Boye et al., 2010
Faba Protein	140 g/kg				Fernandez-Quintela, 1997
		182.5			Ricci et al., 2018
		99.8 (7S)			
		113.5 (11S)			Cai et al., 2002
			1.8 g/g	1.6 g/g	Fernandez-Quintela, 1997
Lentil Protein	8-14%				Jarpa-Parra, 2018
	13%				Aydemir et al., 2013
		183.4-199.5			Ricci et al., 2018
		97.8 (7S)			
		110.5 (11S)			Cai et al., 2002
			1.1-4.2 ml/g	2.0-2.6 ml/g	Jarpa-Parra, 2018
Mung Bean Protein	12% w/v	157.9	3.33 g/g	3.00 g/g	Brishti et al., 2017
	10% w/v				Coffman et al., 1977
		103.6 (7S)			Cai et al., 2002

3.2 Promising Up-and-Coming Methods

3.2.1 Rapid Visco Analyzer

Viscosity is a crucial parameter to consider in extrusion processes as it changes the flow behavior and the mechanical energy input. While protein gel strength and concentration to achieve such strength is an important consideration, LGC does not quantify viscosity. To have better understanding of viscosity development of proteins in the extruder, recent studies have used the rapid visco analyzer (RVA) (Onwulata, Tunick, & Thomas-Gahring, 2014; Osen et al., 2014; Schut, 2020; Zahari et al., 2020). Using the RVA for proteins is relatively new; therefore, methods are still under development and often are adapted to specific proteins. Heating profiles, stirring speed, protein concentrations are optimized according to protein response. The AACC official RVA method has also been used, meaning that the test is run to 95 ºC and held for a period of time, then the temperature is reduced again to 50 ºC.

Soy protein isolate was found to have a cold swelling peak (Zahari et al., 2020), and to have a lower peak viscosity than animal proteins (egg and whey) (Onwulata et al., 2014). It has become clear that commercial pea protein isolates vary distinctly in viscosity (Osen et al., 2014). Some pea proteins are able to build viscosity upon heating while others have a cold swelling peak and continually decrease in viscosity throughout heating and holding.

RVA is increasing in relevance for protein viscosity properties, especially with the development of the high temperature RVA which allows for temperatures up to 140 ºC and thus greater applicability to the extrusion process (Schut, 2020). Limited information is currently available on each of the pulse proteins discussed here, though it is expected that this analysis will become more prevalent in future research. From these current results, it can be seen that RVA

could be helpful to understand and compare the properties within a singular protein source and between protein sources as well.

3.2.2 Phase Transition Analysis

Recently our research group has used a novel technique, phase transition analysis (PTA), for understanding functionality of proteins in plant-based meat applications (Webb et al., 2020). PTA has been used previously for understanding functionality of starch-based raw materials in extrusion applications (Karkle, Alavi, & Dogan, 2012), but its use for studying transitions related to material flow and viscosity in proteins is unique. For PTA analysis, a small amount of hydrated sample (1.5-2.5 g) is compressed in a test chamber at pressures up to 100 bars and subject to heating at a constant rate from 5-7°C until 120°C or higher until the material starts to flow out of a capillary at the bottom of the chamber. The flow temperature (T_f) is an indirect measure of resistance to flow or viscosity. This technique was effectively used to characterize plant-based meat formulations based on pea protein isolate, containing 0-50% chickpea flour (CPF) (Webb et al., 2020). The PTA data obtained for the raw materials mirrored the specific mechanical energy (SME) trends observed during pilot-scale extrusion based processing of the formulations for texturization into plant-based meat. The PTA flow temperature for pure pea protein isolate (0% CPF) was highest due to the high molecular weight of the protein. T_f progressively decreased as CPF level was increased to 30% due to lower molecular weight of the starch and other components in the CPF. However, as CPF level increased from 30 to 50%, an increase in T_f was observed potentially due to starch forming the continuous phase and building viscosity to a certain extent in the presence of heat. SME during extrusion similarly showed a high at 0% CPF followed by a decline until 20% CPF and then an increasing trend up to 50% CPF. Thus raw material flow temperature using the PTA was an effective predictor of SME,

which is a measure of resistance to flow of melt in the extruder. Moreover, bench-top extrusion of the same formulations followed by comparison of extruded and raw material T_f was used as a tool to determine the texturization or cross-linking potential of raw materials without having to conduct a full-scale extrusion study. The pure pea protein isolate (0% CPF) exhibited a considerable increase in T_f after bench-top extrusion as compared to the corresponding raw material, which indicated high degree of cross-linking, whereas other formulations did not exhibit this effect. The plant-based meat products from the pilot-scale extrusion confirmed this trend. There is opportunity to apply this technique to many other plant protein sources to understand their ability to flow in the extruder.

4. Textured plant protein properties

The initial functions of the proteins determine the processibility and the resulting final properties of the texturized proteins. Chemically, the protein solubility of the final product leads to insight of the type of bonding important for achieving the final structure. Important physical characteristics of the product include water holding capacity (WHC) and textural properties which lead to consumer acceptance. It is also necessary to understand the amino acid composition and digestibility of the product to know the health role the product fulfills.

4.1 Protein Solubility

Comparing protein solubility of raw materials and the extruded product is important for comparing solubility between different solvent types and helps determine which type of bonding is prominent for texturization. Solvents used include urea, sodium dodecyl sulfate (SDS), and redox reagents, which interrupt hydrogen bonding, hydrophobic interactions, and disulfide bonds, respectively. If solubility of the sample increases after being placed in one of these solvents compared to the solubility in the buffer, then the respective bonds are present. Protein

solubility can also be an indicator of the degree of texturization and of processing treatments. With decreased solubility of the protein after extrusion, there is evidence of texturization. SME and moisture content are the most influential parameters for protein solubility, decreasing solubility with higher SME and moisture content (Valle, Quillien, & Gueguen, 1994).

Osen et al. (2015) found that the solubility of pea protein in four different solvents (combinations of phosphate, urea, or dithiothreitol) decreased after high moisture extrusion, owing to the protein denaturation and reassociation, especially of the legumin fraction. Legumin was also found to be insoluble during electrophoresis because of sulfide crosslinking that occurred during the texturization which thereby increased molecular weight. The other major proteins don't have cysteine to make disulfide bonds, and they were soluble. From this finding, it was concluded that disulfide bonding is more important in the fiber structure than noncovalent based on the molecular weight and insolubility of the protein. While there is a higher quantity of non-covalent sites like H-bonds on the proteins, the covalent sulfur cross linkages are thought to be responsible for the fibrous texture, even though lower in quantity. Therefore, with lower protein solubility after extrusion it is understood that greater texturization has occurred, which agrees with the findings in Osen et al. (2014) and Samard and Ryu (2019). With this increase in texturization, the protein properties are altered because of the unfolding and aggregation, as seen with the decrease in protein solubilization.

Similarly, in a soy protein and wheat gluten blend for high moisture extrusion, disulfide bonds were found to be the integral bond in the formation of rigid structure and fiber formation (Liu & Hsieh, 2008), with non-covalent interactions contributing to the structure (Lin et al., 2000). With increasing wheat gluten content, sulfur-containing amino acids increase, creating a greater amount of disulfide bonds upon extrusion and significantly increasing hardness and

texturization of the textured plant protein (Chiang, Loveday, Hardacre, & Parker, 2018). In addition to disulfide bonds, H-bonds and hydrophobic bonds are helpful in the formation of structure, though there is conflict in regard to the extent of its importance (Chiang et al., 2018; Liu & Hsieh, 2008; Osen et al., 2014).

4.2 Amino Acid Composition and Digestibility

For nutritional purposes, it is important to know how extrusion processing affects the total amino acid composition, especially since the health of the product is a large reason why there is interest in pea protein and new legume proteins for TVP. Nutritionally, pea and other legume proteins have been found to have a balanced amino acid profile with a high amount of lysine, an amino acid in which cereal grains are low (Cai et al., 2002; Lu et al., 2019). Wheat gluten, however, has more methionine and cystine, in which legume proteins are low (Cai et al., 2002).

The effect of processing on the total amino acid content of textured pea protein seems to conflict in current literature. Osen et al. (2015) found that the total amino acid content remained the same after extrusion processing while Samard and Ryu (2019b) found that the total amino acid content decreased after pea protein isolate was extruded. The difference in loss of amino acids could be explained by the amount of moisture in each process; with greater intensity of processing, it is expected that there would be greater loss of amino acids. With a processing moisture at 55%, Osen et al (2015) found no decrease in total amino acids, while at 50% moisture content, Samard and Ryu (2019b) did observe a decrease. High moisture, therefore, can be an important factor when considering amino acid composition of pea protein TVP. There is also a decrease in lysine with decreasing moisture content in the process (Wang, Bhirud, & Tyler, 1999). The loss of lysine in processing is attributed to its involvement in the Maillard

reaction, as lysine is very reactive in that set of reactions (Samard & Ryu, 2019a; N. Wang et al., 1999).

Amino acids are better retained with higher moisture, and Wang et al. (1999) found that pea protein digestibility increased with higher moisture as well. Another study also found that digestibility of peas, faba beans, and chickpeas increased after extrusion processing, especially when the seeds were soaked in water first (Abd El-Hady & Habiba, 2003). Digestibility of mung bean protein isolate increases after heat treatment (Tang et al., 2009).

In soy and wheat texturization, it was found that some amino acids increased while other decreased. While many decreased, it is interesting to note that methionine and cysteine were two that actually increased after extrusion (Samard & Ryu, 2019a). The sulfur interactions and crosslinking that occurs during processing also affects the amino acid content. However, all essential amino acids were in lower quantity than found in meats.

Overall, legume seeds are comparable in their amino acid compositions and protein digestibility, making mung, faba, lentil, and chickpea proteins nutritionally viable options for additional legume protein sources for textured proteins. For the best amino acid profile, pairing the legume protein and wheat gluten would round out the deficiencies of lysine, cysteine, or methionine from a single protein source.

4.3 Water Holding Capacity and Oil Binding Capacity

The ability of textured plant protein to hold moisture is important to mimic the juiciness of meat. WHC shows the amount of water that a texturized product can retain due to both its chemical composition and its fibrous structure, and it can be influenced by several factors. Wang et al. (1999) found that increased extruder barrel temperatures or increased screw speed also increased the water holding capacity of textured pea protein. This is similar to the finding of

Osen et al. (2014) in regard to water holding capacity. Ironically, as the moisture content in the barrel increased, the water holding capacity of the final product actually decreased. This is due to the fact that water is a plasticizing ingredient and decreases viscosity, therefore lowering the total amount of energy and reducing heat denaturation and texturization (Osen et al., 2014).

The porosity and air cell size is crucial to the water holding capacity (Samard & Ryu, 2019b; Webb et al., 2020). The WHC of texturized pea protein is less than that of soy, which may indicate less porosity and smaller air cell sizes for textured pea protein. However, potentially due to the presumed smaller cell sizes and a good amount of fiber formation, the integrity index of rehydrated textured pea protein is much greater than that of soy, meaning that pea protein can withstand rehydration, agitation, and retort conditions better (Samard & Ryu, 2019b). The higher integrity index and smaller air cells in pea protein come at the cost of water holding capacity, though. Textured wheat gluten and textured mung bean protein both have lower WHC than textured soy and pea proteins (Samard & Ryu, 2019b), and textured faba bean and lentil protein isolates have been shown to have similar WHC as pea protein isolate (Kim, 2018). The structural properties of extruded faba bean are unique, generating optimistic outlook for further research into its making and application (Gu et al., 2020; Kim, 2018).

At this time, it is unclear how WAC of the native protein may impact the WHC of the textured protein. It is also unknown the extent of importance of the chemical binding of water in the extruded product versus the physical structure holding of water after the texturization of protein.

In the same way that water is crucial to mimic the juiciness of meat, oil is critical in textured plant protein to have the same mouthfeel as experienced with animal proteins (Osen et al., 2014). While WHC gives information on how the physical structure holds water, oil binding

capacity (OBC) tests the chemical ability of the textured protein to bind oil. Overall, pea protein has greater ability to bind with water than with oil, showing that there is a higher quantity of hydrophilic amino acids in pea protein than hydrophobic (Samard & Ryu, 2019b).

Samard and Ryu (2019) found that OBC is greater for textured products made with pea protein or wheat gluten than those made with soy protein or mung bean protein (Samard & Ryu, 2019b). This occurred due to greater content of hydrophobic amino acids in pea protein and wheat gluten, which was confirmed by amino acid assay. Soy and mung bean contained roughly 30-33% hydrophobic amino acids while pea and wheat gluten contained 37-38% (Samard & Ryu, 2019b). The raw materials had similar proportions of hydrophobic amino acids; thus this may be an important characteristic for textured protein manufacturing if targeting a certain OBC.

Textured pea protein also had a greater emulsifying ability and stability than that of textured soy (Samard & Ryu, 2019b). Since textured proteins have added fats in their final formulations, these properties would create an advantage for textured pea protein.

4.4 Texture

In regard to the market, texture is arguably the most important attribute of textured plant protein, as the texture must meet the expectations and desires of flexitarians and meat-eaters. To test the texture of meat analogs, the tensile strength or cutting strength in both the longitudinal and transverse directions of the product can be measured (Osen et al., 2014; Samard & Ryu, 2019b; Schreuders et al., 2019), or Texture Profile Analysis (TPA) can be conducted to determine qualities such as hardness, springiness, and chewiness (Lin et al., 2000; Samard et al., 2019; Samard & Ryu, 2019b; Webb et al., 2020).

An important aspect of texture in meat analogs is the fibrous structure. When fibers form, it takes more force to cut them in the transverse direction than in the longitudinal (parallel)

direction. The difference between strengths in the longitudinal and transverse directions shows the degree and strength of fibers formed during extrusion. To create a fibrous structure with pea protein, it has been found that the processing temperature is critical. Osen et al. (2014) found that the temperature of pea protein must be at least 120°C to allow for full viscosity of the proteins to develop and for denaturation to expose binding sites for crosslinking; the temperature of cooking must be greater than the protein's temperature of denaturation. This was confirmed by Schreuders et al. (2019), who also found that fibers began to form with a pea protein-wheat gluten blend at 120°C. At this temperature, the bonding sites of the macromolecules can be exposed, allowing for more interactions than before and leading to the greater bonding and texturization. At a higher temperature of 140°C, more fibers were formed. According to tensile strength tests, higher temperatures lead to a higher degree of anisotropy, or fibers aligned in the direction of extrusion, and less parabolic pattern (Figure 2.1) (Osen et al., 2014). A smoother surface of the product was also seen with higher temperatures.

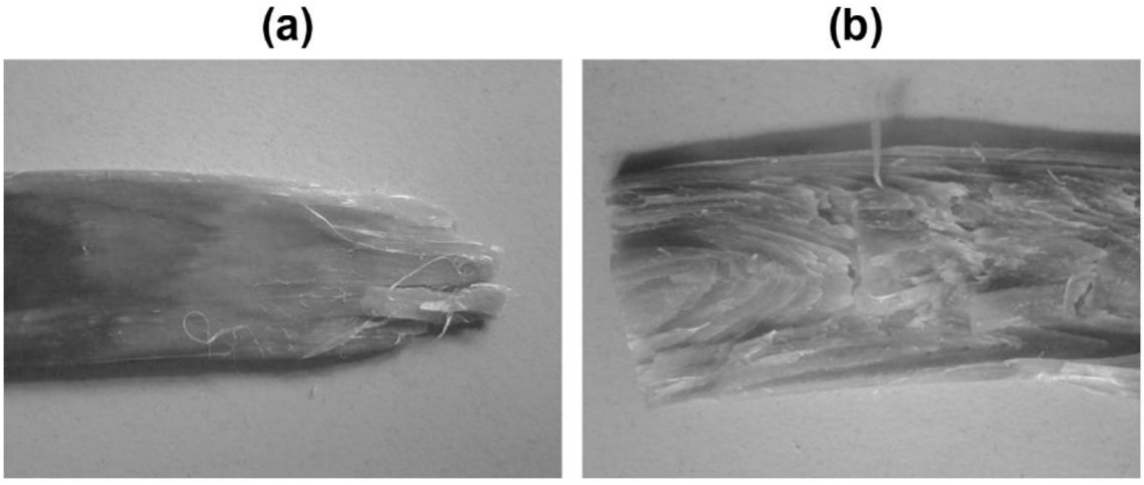

Figure 2.1 Images of extruded pea protein isolate with (a) mostly lengthwise fibrous structures, (b) fibers displaying laminar flow/parabolic patterns, cooked at 130 ºC (Osen et al., 2014)

For soy protein, extrusion at a temperature of 100ºC was found to form fibers, with increasing temperatures also leading to a more fibrous structure (Lin et al., 2000). Formation of fibers occurred at the same temperature for wheat gluten (Pietsch, Schöffel, Rädle, Karbstein, & Emin, 2019).

The importance of the process temperature is thought to be due to two factors. As previously stated, the temperature must be high enough to denature the proteins to expose more bonding sites and to completely denature the mass in the extruder barrel before the die (Kearns, Rokey, & Huber, 1989). Another property of the protein that makes cook temperature crucial is the gelling capability of the protein. At 110°C, the strength of the denatured protein matrix is less than it is at higher temperatures, and only a weak dough is formed at 95°C (Schreuders et al., 2019). A higher temperature also may be needed to help set the structure, since extrusion is a quick heating method and the protein gelation temperature increases when a fast heating rate is used (Dong Sun & Arntfield, 2011; Ricci et al., 2018). Due to protein kinetics, a slower cooling rate may also help the protein to form the greatest gel strength (Dong Sun & Arntfield, 2011), emphasizing the importance of a cooling section for pea protein high moisture meat analogs when product strength is a desirable attribute.

Cutting strength and tensile strength, after processing at 140°C, showed that pea protein is able to form fibers (Samard & Ryu, 2019b). In comparison with other protein sources, the cutting strength of pea protein was significantly less than that of soy, but significantly greater than that of mung bean and peanut protein meat analogs. TPA by Samard and Ryu (2019b) also showed that the springiness, cohesiveness, and chewiness of pea protein extrudates were less than that of soy protein isolate. Protein source and temperature of extrusion are only some of the variables which affect texture. The bulk density of textured pea protein has been found to be

related to the hardness of the product; hardness increases with increasing density of the product off of the extruder (Webb et al., 2020). Schreuders et al. (2019) found that texture properties of pea protein extrudates were similar to that of chicken filets. Even though pea protein isolate gives different textural properties than what is already popular on the market, pea protein gives comparable texture to chicken, making it a viable option for the TVP market.

Wheat has been studied in TVP with inclusion levels ranging from 19.5% to 100% of formulas (Pietsch et al., 2019; Samard & Ryu, 2019a; Schreuders et al., 2019). At 60% of the formula, with the high presence of methionine and cystine in wheat gluten, its use in textured pea protein increases cutting strength, and also has significant impact on other texture quality characteristics such as springiness and hardness (Samard & Ryu, 2019b). The high concentration of sulfur-containing amino acids is thought to make the noteworthy fibrous, dense structure which is most closely comparable to meat.

While mung bean flour has been texturized and formed similar microstructure to textured soy flour (Brishti et al., 2017), texturized mung bean isolate still has low values in springiness, cohesiveness, and chewiness as well as cutting strength when compared to texturized soy, wheat, and pea isolates (Samard & Ryu, 2019b). The properties may be less desirable when using mung bean protein on its own, but it may be a useful ingredient to increase protein content while being able to control the texture. Little, if any, research has been published on textured plant protein extruded from chickpea, lentil, or faba sources. However, research has shown that hardness, gumminess, and cohesiveness of textured lentil and faba protein are similar to textured pea protein (Kim, 2018).

5. Conclusion

Varying methods of extraction and analysis make clear comparison between ingredient types difficult. However, it is clear that even within an ingredient type, major differences in critical qualities exist. This shows that ingredient characterization before extrusion can be very useful to determine the properties that will affect both processing and the final product and give understanding of the quality of protein.

Protein functionality for texturization is dependent on traits of the protein such as denaturation and gelling temperatures. Compared to soy, a higher concentration of pea protein and higher processing temperature is needed to produce the fibrous texture desired to mimic meat. These traits are crucial for the ability to texturize the protein and form meat-like structures. The quality of pea protein TVP is heavily dependent on processing parameters such as SME, moisture, and temperature. These parameters influence the solubility, porosity, and water holding capacity. Thus, both the protein source and processing are factors in the resulting textured protein.

Comparing texturization of pea protein to texturized soy and wheat gluten has shown useful traits to consider and is useful insight to begin texturization work with other pulse proteins like mung bean, lentil, chickpea, and faba bean. With the tools used to change over from soy and wheat gluten to pea, a transition can be made from pea protein to additional pulse proteins. These protein sources have potential to create meat analogs, though raw material characteristics of each isolate should be considered before extrusion, as isolate properties can vary, even when from the same botanical source.

In a rapidly growing area, plenty of research remains. Studies have determined the functionality of pea protein as it applies to its texturization of meat analogs. However, little

information has been gathered regarding how closely pea protein meat analogs from extrusion processing reflect animal meat products. Future work could be done to determine the meat product, i.e. chicken nugget, fish, ground beef, beef fillet, etc., texturized pea protein most closely resembles and if processing parameters or formulation can result in varying texture so as to mimic a specific meat product. In considering different formulations, the effect of a lower concentration of protein could be studied to determine the minimum level of protein needed to achieve texturization. Additionally, extrusion texturization of chickpea, faba bean, lentil, and mung bean have many avenues of research available, especially in relating raw material attributes to processing functionality and final product qualities.

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Chapter 3 - Pulse Flours and Fiber for Control of Texture Characteristics of Texturized Pea Protein

Abstract

Due to health, environment, and animal welfare concerns, some consumers are vying for a “flexitarian” diet, creating a growing movement toward plant-based meat. Pea protein is a becoming a popular ingredient for texturizing and understanding of the role of starch and fiber in the structuring of textured pea protein could lead to the use of co-products from protein isolation, reduced cost, and improved plant-based meat.

A pilot-scale twin-screw extruder was used to extrude pea protein isolate (PPI) and 4 treatments containing 20% pulse flours (desi chickpea flour (CPF), kabuli CPF, commercial precooked CPF, pea flour). An additional 3 treatments were tested to compare to the pea flour treatment, containing 80% PPI, pea fiber at levels 5, 10, or 15%, and pea flour for the remaining percent. The aim was to understand raw ingredient functionality (water absorption, flow temperature, denaturation enthalpy), the impact on processing, and final textured pea protein quality (water holding capacity (WHC), bulk density, texture properties).

Thermal properties were not significantly affected by the addition of any carbohydrate source. Adding pulse flour resulted in low WHC of the extrudates compared to the control of PPI (241-391% and 496%, respectively). However, fiber increased the WHC from the pea flour treatment (428-442%). An increase in instrumental hardness was observed from PPI to all pulse flour treatments (475 g to 837-2333 g) due to the disruption of protein-networking and protein-based expansion. Fiber additions decreased the hardness compared to the pea flour treatment, though the impact was less (1295 g to 534-1050 g). Both WHC and hardness have a strong relationship with the bulk density of the extrudate.

Adding pulse flours to PPI in plant-based meat was shown to increase density and hardness of the extrudate and decrease WHC. However, addition of fiber reduced the impact of starch, and could allow more flexibility in targeting specific qualities while reducing the protein costs for plant-based meats.

1. Introduction

As consumers are gradually shifting to alternative protein sources, production of plant-based meat is increasing. As plant-based meat is catching attention all over the world, consumer curiosity is a driving force leading many to try meat alternatives (Estell et al. 2021). Consumers opting for plant-based diets generally do so for ethical reasons, which may include environmental or animal welfare issues (Estell et al. 2021). Plant-based diets have a perception of being healthier than those which include animal-based products. Plant-based meat alternatives have been shown to decrease environmental impact, while simultaneously increasing the health (Saget et al. 2021)

In addition to the common reasons, COVID-19 catalyzed many consumers to explore the plant-based meat category as scarcity in meat supply resulted and heightened concerns of the spread of zoonotic disease (Attwood and Hajat 2020). At the end of 2020, the plant-based meat market had reached a worth of $1.4 billion, a 45% increase in dollar sales from 2019 (Good Food Institute 2021).

As fast and exciting as the growth of plant-based meat has been, there are admittedly many challenges to overcome to create products that will last in the market. Motivating factors for consumers turning to plant-based meats may include achieving their health goals and improving the environment, but repeat purchases are driven by the taste and other sensory properties (Weinrich 2019). Consumers have also exhibited a more negative perception about

sensory properties in meat alternatives than animal meat, and flexitarians generally find the taste, texture, and price of meat to be more desirable (Michel et al. 2021). Thus, sensory aspects of meat alternatives are a main concern and a continued focus. Minimizing environmental impact is also a focus.

It is well known that the botanical source, cultivar, growing conditions, and processing parameters contribute to the functionality of pulse ingredients (Boye et al. 2010; Lam et al. 2018). In search of creating the ideal texture and having the ability to target finer details of texture and fibrous structure formation, controlling the starch content can be helpful (Webb et al. 2020; Chen et al. 2021). Further study is needed to determine how varietal and sourcing differences of the starch sources may impact the extrudate.

Along with 40-50% starch, dry peas contain an average fiber content of 16% (Chen et al. 2016; Lu et al. 2019). During the isolation process, most of this fiber will be removed and become a co-product. Reducing the purification processing can lead to sustainability increases (van der Weele et al. 2019). Inclusion of fiber in the diet at 15 g/day can also be useful in decreasing calorie intake and thus in weight loss (Lambert et al. 2017). Fiber in the textured protein could help reduce the caloric impact of the extrudate and increase satiety, while also using a co-product of the protein isolation process and creating less waste stream thus increasing sustainability (Zhou et al. 2021). Having the ability to tailor the amount of fiber may also provide opportunity to target specific processing or textural attributes.

The goal of this study was to texturize pea protein with multiple varieties of pulse flours and to texturize pea protein with increasing levels of pea fiber. Even more, the aim was to understand raw ingredient functionality (water absorption, flow temperature, denaturation enthalpy, viscosity), the impact of starch sources and pea fiber level on processing (specific

mechanical energy), and the final textured pea protein quality (water holding capacity, bulk density, texture properties). An understanding of the role of starch and fiber in the structuring of textured pea protein could lead to the use of co-products from protein isolation, reduced cost, and improved plant-based meat.

2. Materials and Methods

2.1 Materials

Commercially sourced pea protein isolate was blended with various pulse ingredients including desi chickpea flour (Hal Ross Flour Mill, Manhattan, KS), kabuli chickpea flour (Hal Ross Flour Mill, Manhattan, KS), precooked chickpea powder (Archer Daniels Midland, Decatur, IL), pea flour (Ingredion, Westchester, IL), and pea fiber (Cosucra, Warcoing Belgium). Ingredients were mixed in ratios shown in Table 3.1 and their composition was calculated using supplier specifications and proximate analysis as shown in Table 3.2. Throughout the paper, legume flour treatments (CPDesi, CPKabuli, CPCooked, and PF00) will be compared to the pea protein isolate treatment (PPI). Treatments with increasing levels of pea fiber (PF05, PF10, and PF15), will be compared to PF00, as the fiber treatments have pea flour inclusions as well. Occasional comparisons of the fiber treatments to the overall control (PPI) may be made as well.

Table 3.1 Treatment formulations (%)

Treatment	PPI	CPDesi	CPKabuli	CPCooked	PF00	PF05	PF10	PF15
Pea isolate	100	80	80	80	80	80	80	80
Chickpea - raw (Desi)		20						
Chickpea - raw (Kabuli)			20					
Chickpea - Commercial precooked				20				
Pea flour					20	15	10	5
Pea fiber						5	10	15

Table 3.2 Estimated composition of each treatment (%)

	PPI	CPDesi	CPKabuli	CPCooked	PF00	PF05	PF10	PF15
Protein	77.1	66.3	66.2	66.3	66.3	65.3	64.3	63.4
Non-Fiber Carbohydrate	4.0	13.6	13.1	16.0	11.1	10.7	10.3	10.0
Fiber	4.0	3.4	3.5	3.2	7.4	9.0	10.6	12.1
Fat	6.0	6.0	6.1	6.2	5.2	5.1	5.0	4.9
Ash	1.6	1.7	1.7	1.8	1.6	1.6	1.6	1.6
Moisture	4.1	6.5	6.8	3.9	5.9	5.7	5.6	5.5
Unclassified mass	3.2	2.6	2.6	2.6	2.6	2.6	2.6	2.5
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

2.2 Raw Material Analysis

2.2.1 Moisture Content

Moisture content was measured for raw ingredients, preconditioned treatments, and extrudates (before drying) using the AACC 44-19.01. Triplicate samples of approximately 2 g were dried at 135ºC for 2 hrs.

2.2.2 Water Absorption Capacity and Oil Absorption Capacity

Water absorption capacity (WAC) was measured as described by (Anderson et al. 1970) with modification. Raw material samples of 2.5 g were placed in centrifuge tubes with 30 ml deionized water. Samples were vortexed until the sample was dispersed and allowed to sit at room temperature for 30 minutes, with 2 additional agitations in that time. Then samples were centrifuged at 3900 RPM for 30 min and the water was carefully decanted. WAC was then calculated using the following equation:

$$\textbf{WAC}\ (\text{g water/g protein}) = \frac{W_f - W_i}{W_i}$$

where W_f is the weight of the sediment and W_i is the initial weight of the sample.

Oil absorption capacity (OAC) was determined using the methods described by Brishti et al. (2017), with some modification. Raw material samples of 2.5 g were placed in centrifuge tubes with 30 ml sunflower oil. Samples were shaken until the sample was dispersed and allowed to sit at room temperature for 30 minutes. Then samples were centrifuged at 3900 RPM for 30 min and the oil was carefully decanted. The tubes were then inverted, allowing excess oil to drain for 20 min. OAC was then calculated using the following equation:

$$\textbf{OAC}\ (\text{g oil/g sample}) = \frac{W_f - W_i}{W_i}$$

where W_f is the weight of the sediment and W_i is the initial weight of the sample.
WAC and OAC were measured in triplicate for each sample.

2.2.3 Rapid Visco Analyzer Viscosity

A rapid visco analyzer (RVA) (RVA 4500, Perten Instruments, Waltham MA) was used to measure the viscosity of each treatment using the AACC Method 76-21.02 STD1. Protein slurries at 15% solids concentration (w/v) were mixed and placed in the RVA within 1 min. Slurries were then heated to 50 ºC, stirred at 960 rpm for 10 seconds. A speed of 160 rpm was used for the remainder of the test. After 1 min at 50 ºC, slurries were heated to 95 ºC at 12 ºC/min, then held at that temperature for 2.5 min. Slurries were then cooled to 50 ºC. Peak and final viscosities were recorded, as well as the temperature and time the peak viscosity was reached.

2.2.4 Phase Transition Analysis

Phase Transition Analysis (PTA) was conducted on preconditioned raw materials in triplicate on the phase transition analyzer (Wenger Manufacturing). PTA gives understanding to the polymer deformation of raw materials by indicating the temperature at which the material

softens and flows in similar pressure conditions to extrusion. PTA was conducted as described in (Webb et al. 2020). Samples taken from the preconditioner during extrusion were used for PTA in order to have the same moisture as during processing (24% wb). A sample of approximately 2 g was compressed in the chamber with a blank die to 120 bars for 15 s, then compressed to 100 bars while the sample was heated at a rate of 8 ºC/min, with a starting temperature between 5-7 ºC. After the material softened, a 2 mm capillary die was placed under the sample and compressed again to 120 bars for 15 sec with 100 bars of pressure thereafter. The temperature of flow (T_f) was measured as the temperature at which the material melted and began to flow through the capillary die, as shown by mm displacement of the compressing rod.

To understand changes in T_f after extrusion, PTA was conducted on each extruded treatment. Extrudate for this test was run on a lab-scale extruder (Micro-18, American Leistritz, Somerville, NJ) to understand protein changes before running at the pilot scale. Raw materials were hydrated to 24% MC before extrusion and run at 3 kg/hr throughput and a screw speed at 550 RPM. Wheat treatments were omitted from lab-scale extrusion due to inability to evenly hydrate the gluten. The material was extruded through barrel sections with temperatures of 30, 40, 55, 95, 120, 140ºC. An oval die with width of 5.5 mm and length of 3.0 mm was used to make ropes of extrudate. For PTA, the frozen extrudate was ground finer than 250 microns with a coffee grinder, hydrated to 24% moisture, and run on the PTA using the parameters described above.

2.2.5 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measures thermal changes the temperature and energy input required to denature a protein (Brishti et al. 2017). DSC was performed using a

Q100 V9.9 Build 303 (TA Instruments, New Castle, DE) and data was analyzed with Universal Analysis Program, V4.5A (TA Instruments).

DSC was conducted according to Brishti et al. (2017) with a few modifications. Raw sample materials (8-10 mg) were weighed into stainless steel, high volume pans, hermetically sealed with an o-ring in the lid. The contents were heated at a rate of 10 ºC/min until reaching 250 ºC with an empty pan as a reference. Nitrogen purge flow was 50.0 mL/min. Temperature of peak denaturation and enthalpy of denaturation were recorded.

2.2.6 Pilot Scale Extrusion Parameters and Calculations

Treatments were mixed for 5 minutes with a batch ribbon blender (Wenger Manufacturing, Sabetha, KS, USA). Treatments were extruded with a pilot-scale, co-rotating twin screw extruder (TX-52, Wenger Manufacturing, Sabetha, KS) with a screw diameter of 52 mm and an L/D ratio of 19.5. The dry material feed rate was 50 kg/hr. The extruder screw speed was 450 RPM. Water was added only in the preconditioner at a rate of 13 kg/hr, and no steam was used leading to a consistent in-barrel moisture (IBM) of 24% for all treatments. Four temperature zones were used at 40, 70, 90, and 110°C from inlet of extruder barrel to the outlet. The screw profile was composed of double flighted elements of decreasing pitch, with two forward kneading blocks and four reverse kneading blocks dispersed throughout the profile, and a conical cut element at the end (Table 3.3). A 3/16” venturi die and an 11” Teflon spacer were placed before the final die plate which had two 3/16” openings (Figure 3.1). A knife with 3 hard blades was used with a knife speed of 410 RPM. Extrudates were dried at 200°C for 12 min and cooled for 8 min in a dual pass drier (4800, Wenger Manufacturing). Dried extrudate was collected at three time points for five minutes and stored at room temperature. Other sample was

taken from off of the extruder and immediately milled to a 0.18" size (Comitrol, Urschel Laboratories Incorporated, Valparaiso, IN) and frozen.

Specific mechanical energy (SME) was calculated using the following formula:

$$\textbf{SME}\ (\text{kJ/kg}) = \frac{\left(\frac{\tau-\tau_0}{100}\right)\times\frac{N}{N_r}\times P_r}{\dot{m}} \qquad (1)$$

where τ is the % torque, τ_o is the no-load torque, N is the measured screw speed in RPM, N_r is the rated screw speed (336 rpm), P_r is the rated motor power (22.4 kW), and $\dot{m}$ is mass flow rate in kg/s.

In-barrel moisture (IBM) content was calculated using the following equation:

$$\textbf{IBM}\ (\%\ \text{wb}) = \frac{m_f \times X_{fw}+m_{pw}+m_{ew})}{m_f+m_{pw}+m_{ew}} \qquad (2)$$

where m_f is the dry feed rate in kg/h, X_{fw} is the moisture content of the dry feed material (expressed as a fraction), m_{pw} is the water injection rate into the pre-conditioner in kg/hr, and m_{ew} is the water injection rate into the extruder in kg/hr.

Table 3.3 Extruder screw configuration

left	1	1	1	1	3	3B	1	1	1	1	4	5	4	5	4	4B	6
right	2	2	1	1	3	3B	1	1	1	1	4	5	4	5	4	4B	6

1	3/4 pitch, double flight
2	¾ pitch, single flight
3	Forward kneading block
3B	forward kneading block, backward
4	reverse kneading block
4B	reverse kneading block, backwards
5	1/2 pitch, double flight, cut flight
6	¾ pitch, double flight, cut flight, cone

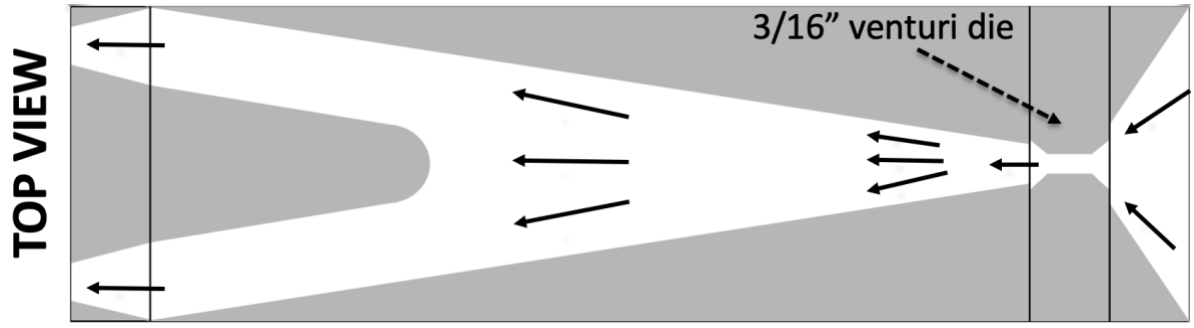

Figure 3.1 Extrusion Die Configuration

2.3 Extrudate Analysis

2.3.1 Water Holding Capacity

Water holding capacity (WHC), the amount of water that the final product can internally hold, was measured according to Kearns, Rokey, & Huber (1989), with modifications. Samples of 20 g were soaked in excess, room temperature, DI water for 20 minutes, then drained on a mesh screen for 5 min. WHC was calculated using the following equation:

$$\textbf{WHC}\ (\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

2.3.2 Texture Analysis

Texture analysis was used to measure hardness, springiness, and chewiness using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Analysis was first performed on loose, ground, rehydrated product to understand the texture qualities without the use of binders. Treatments were hydrated to their water holding capacity by hydrating in excess room temperature water for 5 min, then allowed to drain for 5 min. A back-extrusion cup was utilized to contain 20 g of sample that was lightly filled to 1 cm height in the cup. A two-compression test was completed on 10 replicates for each treatment, compressing to 70% of the total distance with a circular aluminum probe. Texture on patties was done according to guidelines from the American Meat Science Association (AMSA 2015). Patties were made with 91.5 g of each material (each plant-based treatment, ground beef, ground pork, ground chicken,

and a commercial plant-based patty) and pressed to 1 cm thickness. The formula for plant-based patties is shown in Table 3.4. Ground beef (96% lean, 4% fat) (The Kroger Company, Cincinnati, OH), ground pork (The Kroger Company, Cincinnati, OH), ground chicken (Perdue, Salisbury, MD), and Beyond Beef Ground (Beyond Meat, El Segundo, CA) were purchased from local grocery store and frozen until use. Patties were panbroiled with no oil until an internal temperature of 71 ºC was reached. Patties were allowed to cool to room temperature and a 2.5 cm core was taken from the center of 10 patties. A two-compression test was completed on the free-standing, room temperature cores, compressing to 70% of the total distance with a circular aluminum probe.

Table 3.4 Patty formulation for texture analysis

Ingredient	Percentage
Hydrated Textured Pea Protein	88.75
Pea Protein Isolate	1.5
Chickpea Flour	1.5
Sunflower oil	3
Methylcellulose	2.75
Salt	1
Beet Powder	0.75
Spices	0.75

2.4 Experimental Design and Statistical Analysis

Samples were run in triplicate unless otherwise stated. ANOVA was conducted to compare means and differences using SAS software (SAS, Cary, NC). Significance of differences was determined by Tukey's test ($p < 0.05$).

3. Results and Discussion

3.1 Raw Materials and Processing

3.1.1 Water Absorption Capacity and Oil Absorption Capacity

PPI had the highest WAC (3.3 g water/g protein) while the treatment with the lowest WAC were those with 20% flour additions (Figure 3.2). Fiber has been known to increase the WAC of raw ingredients (de Angelis et al. 2020). Pea fiber, as a raw ingredient, was tested alone and had a WAC twice as large as the PPI and 3 to 6 times as large as the legume flours (not shown). When pea fiber was then added to the treatment mixes in minor amounts (5-15%), a distinguishable increase in WAC was measured.

It is important to note that this method shows the amount of water absorbed by insoluble material. Soluble material would be discarded in the supernatant which lowers the WAC measurement since some of the original solids would no longer be present in the sediment (Osen et al. 2014). Thus, some treatments may have a higher WAC than displayed from this testing.

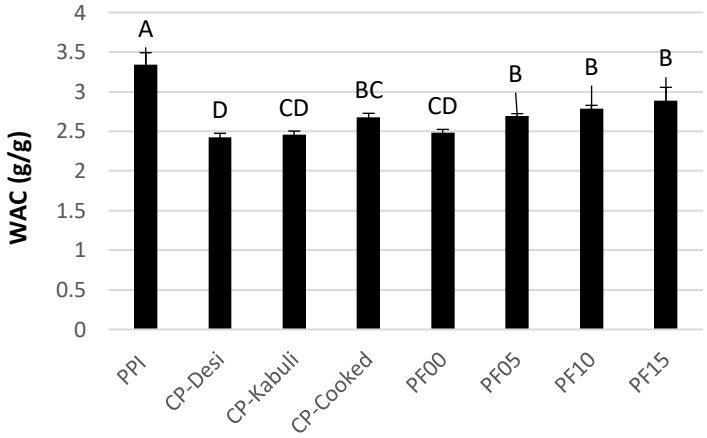

Figure 3.2 Means of water absorption capacity for each treatment raw material mix. Means denoted by the same letter above the bar are not significantly different.

Most treatments had similar OAC; the addition of legume flours to PPI did not impact the OAC of the raw treatments (Figure 3.2). However, levels of fiber addition did prove to impact

the OAC; with PF15 significantly increased the OAC compared to PF00. The absorption of liquids clearly changes with the various treatment mixes, and this has potential to impact other behaviors of the treatments, as will be discussed in the following sections.

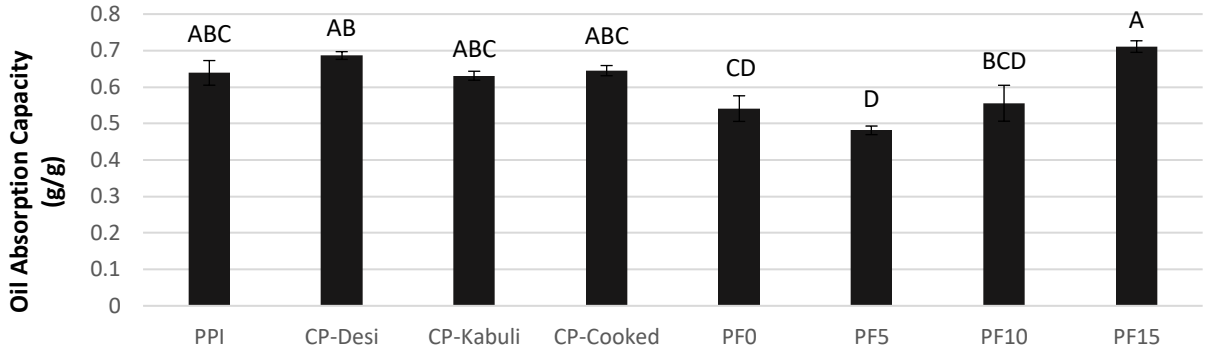

Figure 3.3 Means of oil absorption capacity for each treatment raw material mix Means denoted by the same letter above the bar are not significantly different.

3.1.2 Rapid Visco Analyzer Viscosity

PPI reached peak viscosity without addition of any heat yet took roughly 1 min to fully hydrate at the set temperature of 50 ºC to reach peak viscosity (Figure 3.4 and Table 3.5). With the addition of any legume flour, achieving peak viscosity required time and heating. CPDesi and CPKabuli showed similar peak viscosities, but CPCooked had significantly less viscosity build due to the degraded starch from prior processing of the chickpea flour. The CPCooked showed less ability to create viscosity upon heating since the starch in the flour had already been gelatinized and the starch granules ruptured (Gajula et al. 2009; Martin 2020). PF00 had a lower viscosity from the raw CPDesi and CPKabuli, but a higher viscosity than CPCooked.

With every addition of pea fiber, the viscosity increased. Not only did the peak viscosity increase, but the treatments with fiber also built viscosity faster. With the highest addition of fiber, a distinct additional peak viscosity was formed. The additional peak was less pronounced with lower fiber additions but is still evident. This can be explained by the high WAC that fiber

brings to fiber treatments. WAC and peak viscosity had a moderately strong linear relationship (correlation coefficient of 0.79, $p<0.0001$). Similar to how the PPI developed a cold-peak viscosity, the fiber would add more viscosity without requiring heating. With the ability to absorb more water, the viscosity of the solution is able to increase. Osen et al. (2014) had similar results with protein swelling upon hydration, reflecting the protein's WAC, and then decreasing in viscosity during heating.

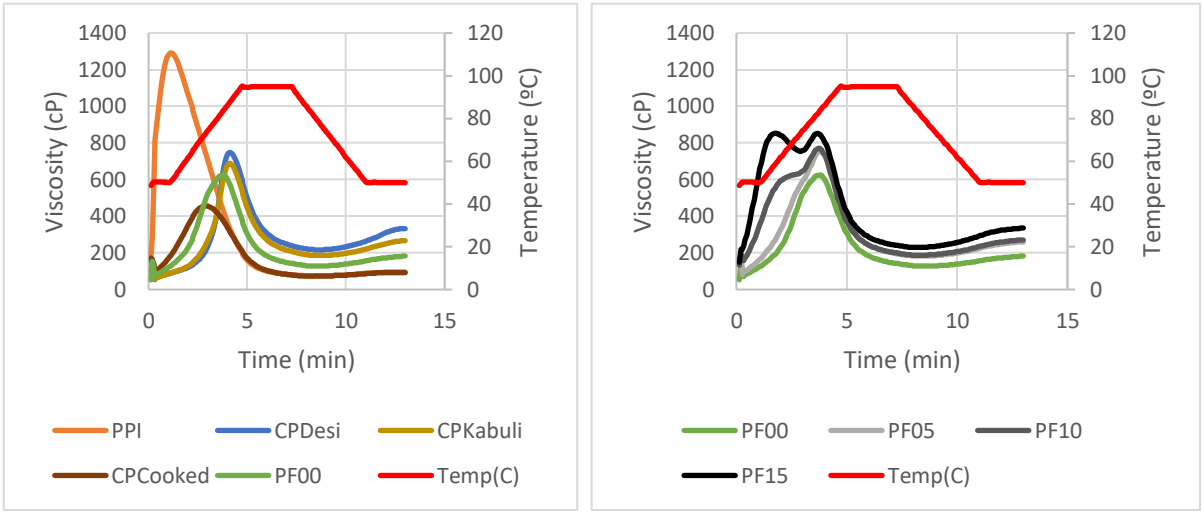

Figure 3.4 Average RVA curves for each treatment at 15% solids

Table 3.5 Peak viscosity, time of peak viscosity, and temperature of peak viscosity as measured by rapid visco analysis.
Means in the same column followed by the same letter are not significantly different.

Treatment	Peak Viscosity (cPa)	Time of Peak Viscosity (s)	Temperature of Peak Viscosity (ºC)	Final Viscosity (cPa)	T_f Raw (ºC)	SME (kJ/kg)
PPI	1303 ± 116^{a}	67 ± 9^{e}	50.82 ± 0.58^{e}	96 ± 7^{e}	62.6 ± 9.5^{a}	213.4 ± 10.7^{ab}
CPDesi	749 ± 52^{bcd}	244 ± 4^{a}	87.15 ± 0.88^{a}	332 ±52^{ab}	56.2 ± 5.1^{a}	179.0 ± 8.1^{c}
CPKabuli	694 ± 36^{cd}	245 ± 6^{a}	87.40 ± 1.26^{a}	265 ±39^{bc}	60.0 ± 1.5^{a}	184.9 ± 4.7^{c}
CPCooked	456 ± 28^{e}	169 ±5^{c}	72.07 ± 0.92^{c}	93 ± 3^{e}	62.0 ± 5.5^{a}	181.7 ± 5.8^{c}
PF00	626 ± 7^{d}	227 ± 2^{b}	83.70 ± 0.52^{b}	182 ± 1^{d}	51.1 ± 3.1^{a}	190.8 ± 5.6^{bc}
PF05	756 ± 9^{bcd}	225 ± 2^{b}	83.40 ± 0.52^{b}	261 ± 5^{c}	59.7 ± 3.1^{a}	221.5 ± 15.2^{a}
PF10	772 ± 15^{bc}	223 ± 2^{b}	82.82 ± 0.49^{b}	270 ± 6^{abc}	62.7 ± 4.7^{a}	217.2 ± 6.1^{a}
PF15	860 ± 21^{b}	105 ± 2^{d}	58.72 ± 0.55^{d}	335 ± 9^{a}	60.4 ± 5.8^{a}	235.5 ± 0.9^{a}

3.1.3 Phase Transition Analysis

The temperature required to reach a flowable state decreased with all pulse flour additions compared to PPI (Figure 3.5). However, adding fiber increased the temperature of flow compared to PF00. Fiber created a mixture that required more thermal energy to go into a plastic state; a similar thermal energy to what PPI requires.

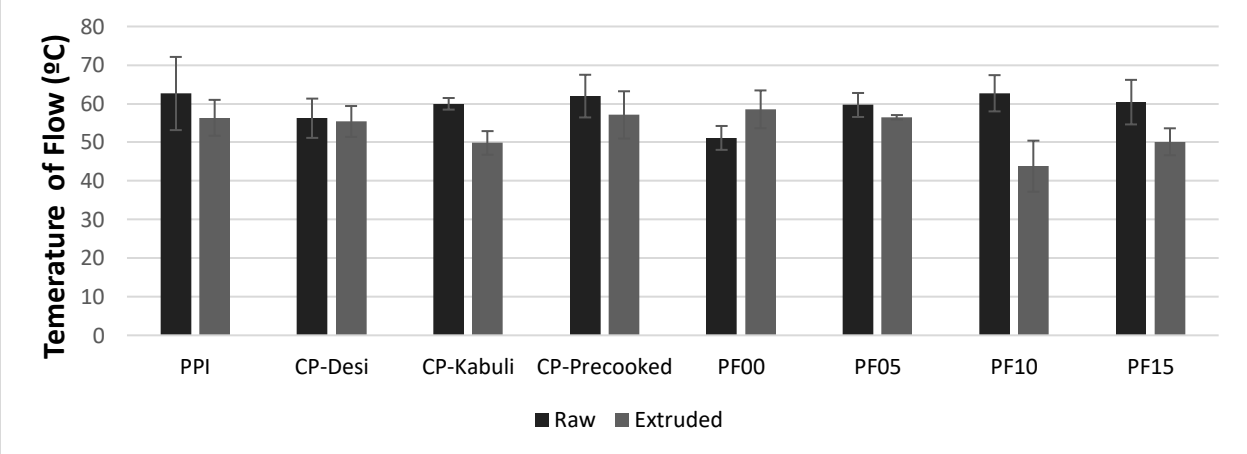

Figure 3.5 Mean temperature required for raw materials and lab-scale-extruded materials to flow through the capillary die of the PTA.

Raw materials with a greater T_f during PTA displayed higher SME during pilot scale processing (Table 3.5). The T_f of raw treatments decreased with starchy additions potentially due to the breakdown of starch and less need for energy to have the molecules flow. When fiber was added into the matrix (while the starchy additions simultaneously decreased and protein remained the same), the T_f increased and was closer to the T_f of PPI. With less starch present in those treatments, less starch breakdown occurred and thus the lowering of T_f was not observed. Fiber also increases the WAC, and thus competes for water in the treatments, allowing less water for protein and starch to absorb, and higher viscosity results, and thus a higher T_f.

Greater thermal energy was required to achieve a flow of PPI after extrusion. This shows that more thermal energy was needed to break the bonds of the solids to create the melt. More thermal energy was needed for extrudates of PF00 and PF15 compared to their raw forms, which may indicate they also had stronger or more bonding after extrusion. Thermal energy breaks hydrogen bonding and hydrophobic interactions. Thus, more energy required to flow may indicate that these bonds were greater in number after extrusion or potentially less accessible due to stronger bonding like disulfide linkages.

3.1.4 Differential Scanning Calorimetry

DSC is essential for understanding a protein's denaturation temperature and thus for understanding how a globular plant protein will transform with shear and temperature to the fibrous structure (McClements et al. 2021). No significant difference was found in enthalpy (15.81-20.53 J/g) (Figure 3.6), starting temperature of denaturation (164.93-169.81 ºC) or peak temperature of denaturation (185.86-187.95 ºC) between the samples (not shown). However, there is a noticeable trend with all flour additions; the enthalpy decreases by 2-3 J/g compared to PPI. With 20% less protein, it is logical that the enthalpy required for denaturation would be

roughly 20% lower. Addition of pea fiber increased the enthalpy by 1 J/g compared to PF00. PF15 required 3.5 J/g more energy than pea flour and pea protein alone (PF00) and 1.5-2.5 J/g more than PF05 and PF10.

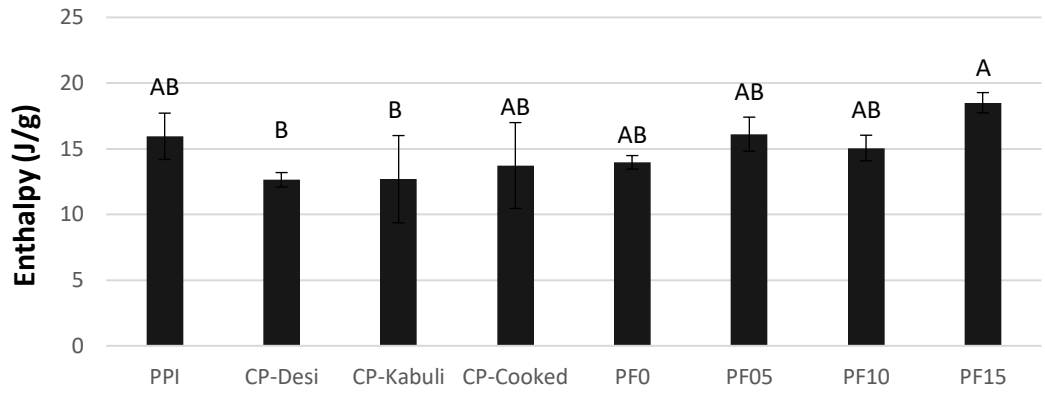

Figure 3.6 Average energy required to denature the raw material.
Means with the same letter above the bar are not significantly different ($p<0.05$).

Though enthalpy is a measure of thermal energy and SME measures mechanical energy, a strong linear correlation showed between the enthalpy measured and the SME required during processing ($R=0.9235$, $p<0.01$). More energy was required for denaturation and more energy was required for processing when fiber increased. Pea fiber has a molecular weight around 625-809 kDa, while pea proteins range in molecular weight from 170-380 kDa (Barac et al. 2010; Cheng et al. 2018). Higher molecular weights can increase the SME during processing as more energy is needed, and with more bonds to break, the thermal energy would increase.

Previous work has shown that fiber addition into a starch matrix causes the endothermic transition to disappear in the DSC thermogram as explained by the competition for water that fiber introduces (Randzio et al. 2003). This is consistent with the results seen here; as the fiber is added into the matrix, the effect of flour is reduced, and the protein effect is made more pronounced.

3.1.5 Specific Mechanical Energy

SME ranged from 166 kJ/kg to 236 kJ/kg (Table 3.5). A decrease in SME occurred from PPI when any pulse flour was added. The flour treatments absorbed less water at room temperature than PPI (Figure 3.2) and had less viscosity (Figure 3.4). Thus the material is easier to flow in the extruder and SME is lower for flour treatments, even though there is some viscosity build due to gelatinization.

SME increased with any addition of fiber compared to PF00. This is also logical with the increased WAC and viscosity that resulted with the addition of fiber. The SME also mirrors the trend of the raw material T_f measured during PTA and peak viscosity from RVA. Most treatments saw a lower T_f and peak viscosity with flour additions, and an increased T_f and peak viscosity with fiber additions. The flow in the extruder was similar to that measured by PTA and RVA, resulting in mostly similar SME trends. SME has been known to be related to the melt and flow properties of the materials (Osen et al. 2014).

3.2 Extrudate Analysis

3.2.1 Visual Analysis

Dried extrudate can be seen in Figure 3.7. To observe the internal structure of the extrudate, samples were cut along the direction of extrusion (longitudinal) and across the direction of extrusion (horizontal) (Figure 3.8.) Images of the longitudinal and horizontal cross sections can be seen in Figure 3.9 and Figure 3.10.

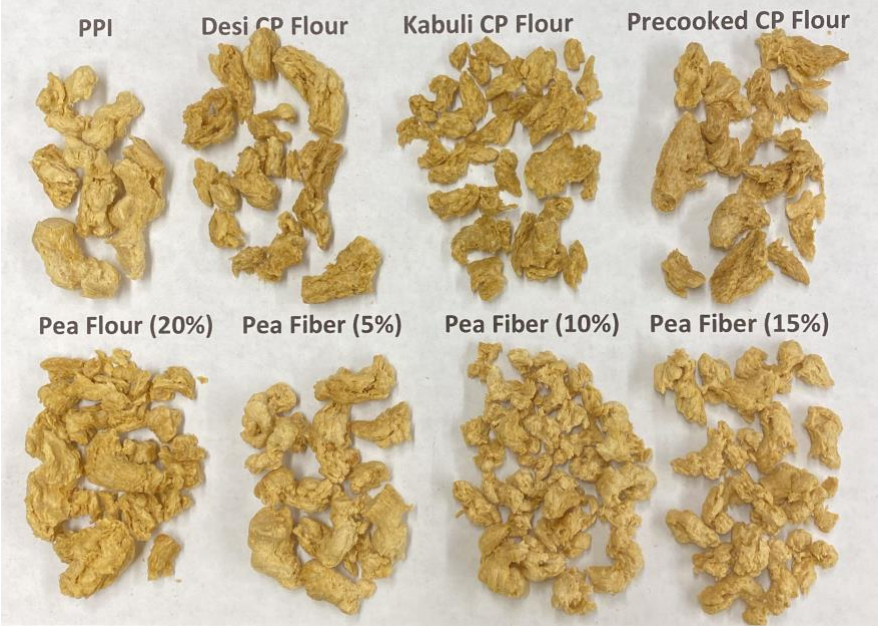

Figure 3.7 Dried extrudate, whole pieces

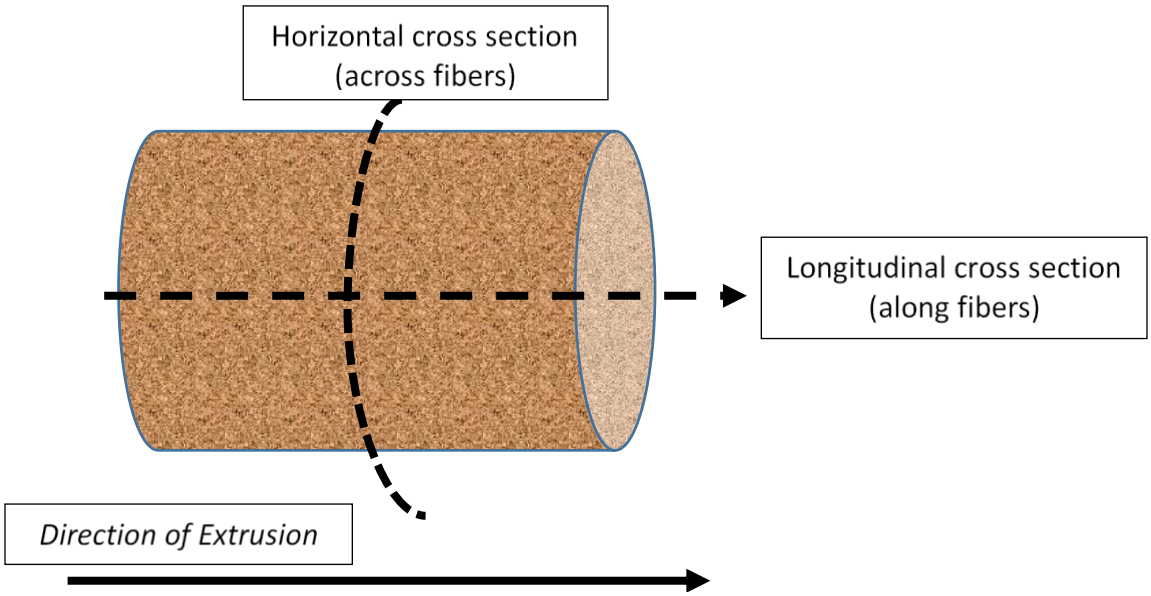

Figure 3.8 Diagram of cross sections for internal visual analysis

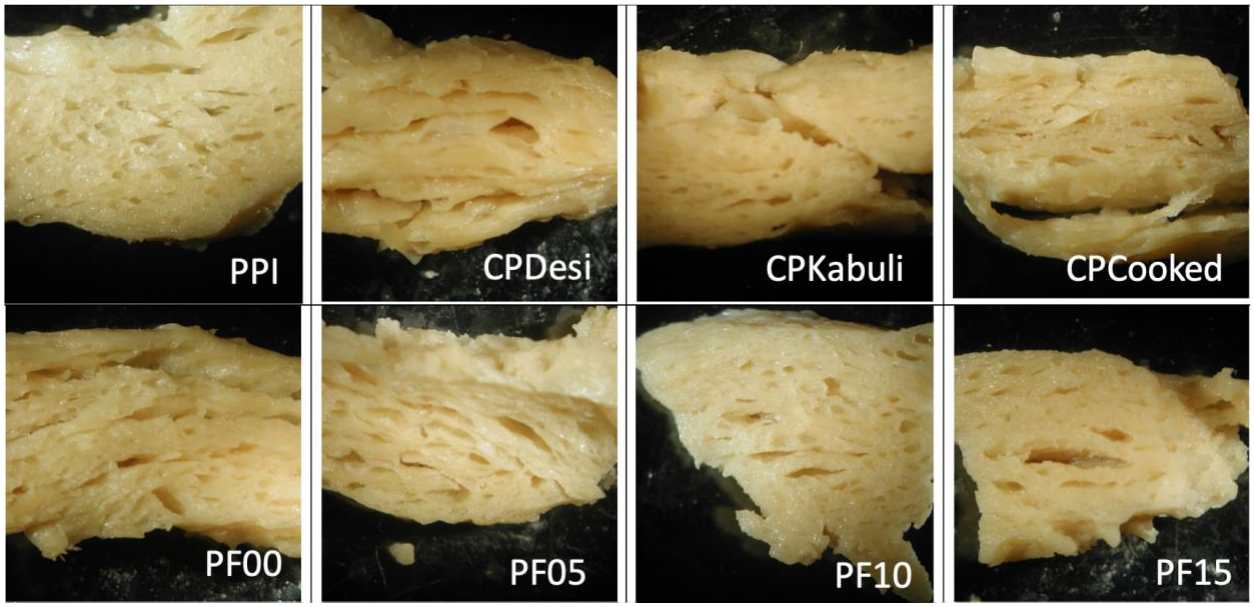

Figure 3.9 Longitudinal cross sections of extruded texturized pea protein with legume flour and/or pea fiber additions

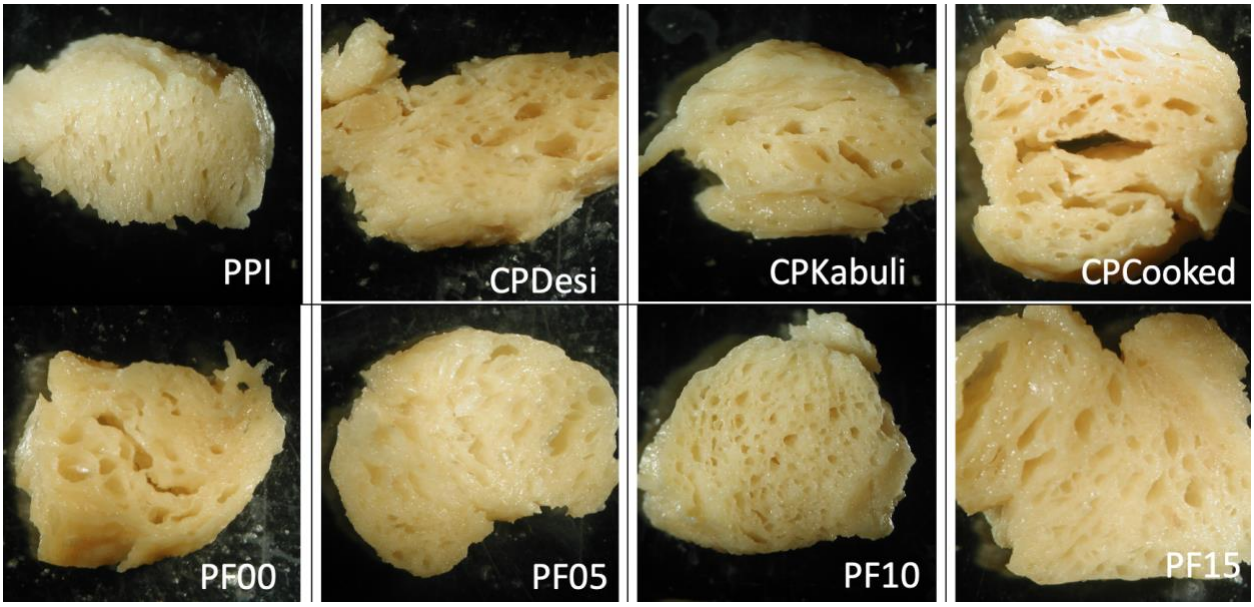

Figure 3.10 Horizontal cross sections of extruded texturized pea protein with legume flour and/or pea fiber additions

Longitudinal cross sections clearly show the anisotropic formation of proteins. PPI shows cells that are elongated in the direction of flow. CPDesi, CPKabuli, CPCooked, and PF00 show very distinct layers. Particularly visible in CPDesi, CPCooked, and PF00, some larger separation

between the layers is visible which is noticeable in the horizontal cross sections discussed below. This is evidence of the protein-polysaccharide phase separation observed and utilized in layering and fiber formation of plant proteins (Dekkers et al. 2018a; McClements and Grossmann 2021). The starch in these treatments may have acted as a laminating material to the proteins, thus the layers easily come apart from each other, rather than being strongly connected in a fibrous way. PF05, PF10, and PF15 show great anisotropic formation. With the fiber addition, the laminated layering subsided, and interacting fibers were able to form (Figure 3.11b).

Research with wheat gluten and SPI mixes for texturization have explored the importance of dominant and dispersed phases in texturization (Dekkers et al. 2018b). Dekkers et al. (2018b) also found that the differences in hydration of the phases is important in determining the phase rheology which in turn governs the fiber structuring.

Similar to an animal muscle myofibril that is a long tube of protein, the ideal extrudate will show long orientation of fibers in the longitudinal direction, but thin fibers in the horizontal direction. The horizontal cross sections of treatments with starch show large pore sizes that are a considerable length of the diameter of the product. This creates layers of protein that do not intersect (see Figure 3.11a) and easily fall apart. Smaller pore sizes in the horizontal direction were noticed as the pea fiber increased. Smaller pore sizes in the horizontal direction allows the layers that form longitudinally to be smaller in diameter, forming strands rather than layers like the starchy products.

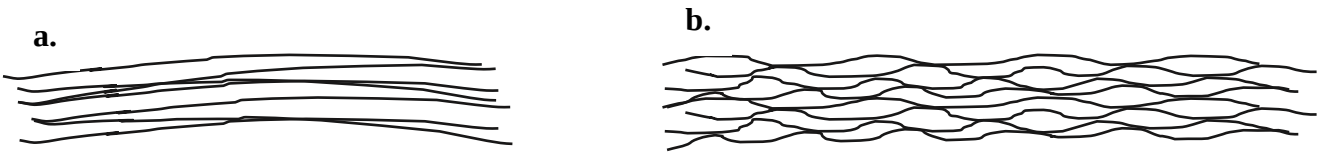

Figure 3.11 Depiction of (a) the lamination seen in treatments with only legume flour additions and (b) the interacting layers that form the fibrous nature in PPI, PF05, PF10, and PF15

Fiber could be interrupting the ability of starch to coat the protein well, thus allowing the protein to interact more and not create layers. This could also be fiber's nucleating effect. Insoluble fiber interrupts the continuous phase of protein and starch, creating more air cells and smaller strands of protein that connect to each other more frequently than the layers formed with only protein and starch ingredients.

3.2.2 Water Holding Capacity

WHC is a measurement of water held within the matrix of a texturized protein. Previous studies have found that the ability of the final product to hold water is impacted by many processing parameters including barrel temperatures, screw speed, and in-barrel moisture (Wang et al. 1999; Osen et al. 2014). In relation to ingredients, it has been found that formulas which lead to greater expansion and porosity allow for greater WHC (Wu et al. 2018; Webb et al. 2020).

In this study, WHC decreased with addition of legume flours (Figure 3.12). With addition of fiber, WHC increased in comparison with PF00. WHC exhibited a high correlation with bulk density (R^2=0.9649, $p<0.05$).

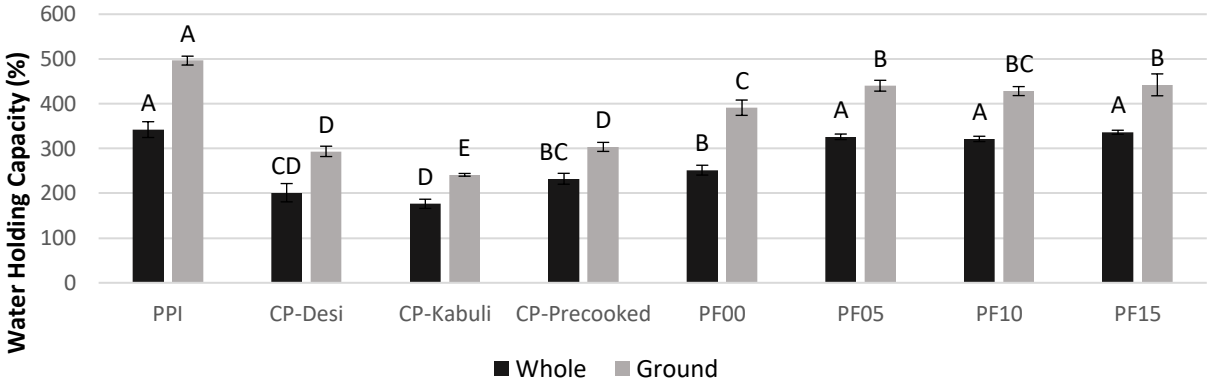

Figure 3.12 Water holding capacity of whole extrudate and ground extrudate
Means of treatments in the same category denoted by the same letter above the bar are not significantly different.

3.2.3 Bulk Density

In the extrusion of puffed snacks, expansion depends on a continuous phase of starch. Starch content is the structure forming component and creates bulk density. The interactions between starch and protein, or the composite morphology of ingredients, dictates the expansion of an extrudate (Kristiawan et al. 2018). In texturizing plant proteins, though, the continuous phase is protein. Addition of pulse flours created a dispersed phase of starch, which disrupted the continuous protein phase. This disruption inhibited protein interaction and increased bulk density (177.5-213.6 g/L) and decreased expansion (Figure 3.13). PPI had a bulk density of 64.6 g/L, meaning that additions of starch nearly tripled the bulk density. The starch was able to create distinct layers of protein, as the starch coated the protein during flow.

Additions of fiber created an additional dispersed phase. In starch-based snacks, fiber has been found to interrupt the continuous phase of starch, increasing the bulk density and decreasing the porosity (Karkle et al. 2012). However, fiber addition of any amount to the protein/starch matrix decreased the bulk density (73.5-80 g/L) compared to the treatment with only pea flour addition (177.5 g/L). Pea fiber (insoluble fiber) has been found to break down protein-starch networks (Tudorică et al. 2002). Rather than disrupting the continuous phase, like starch did and like fiber does in starch snacks, the fiber prohibited starch from interfering with protein networking, allowing the continuous phase to network together. The bulk density of fiber treatments (73.5-80 g/L) is similar to that of PPI (64.6 g/L), showing that the fiber allowed the protein to network and expand almost as if no additives were there. These differences in expansion among different formulations may be due to differences in their rheology and the intermolecular interactions occurring (Beck et al. 2018).

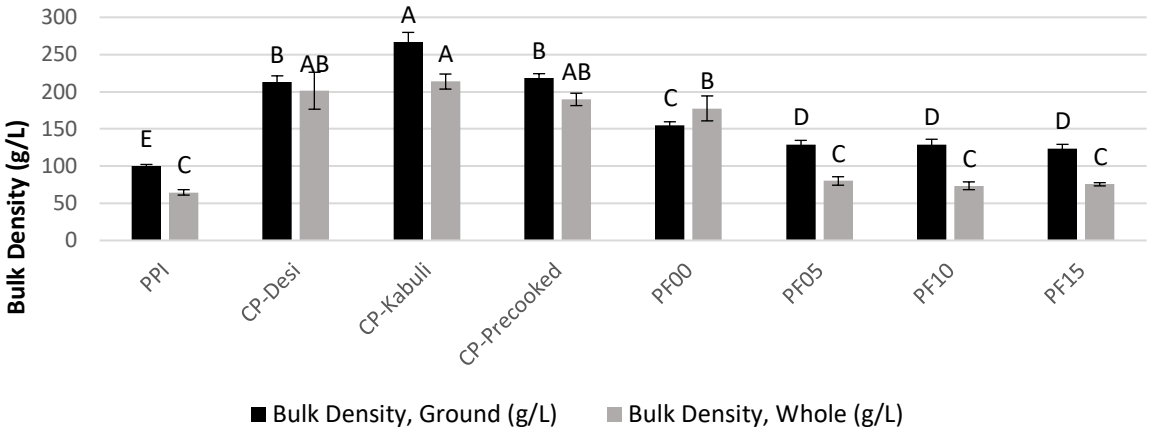

Figure 3.13 Bulk density (g/L) of ground extrudate and whole extrudate after drying. Means in the same classification followed by the same letter are not significantly different ($p<0.05$)

3.2.4 Texture Analysis

Hardness, springiness, and chewiness of ground extrudate and patties were measured (Figure 3.14). The ground product was measured to understand the characteristics of the extrudate in a uniform piece size while patties were measured to determine if differences could be detected in a final product form.

In the ground form, hardness increased with all pulse flour additions. Fiber addition decreased the hardness compared to PF00 but made the hardness more comparable to PPI. Adding the starchy flours decreased protein interaction and interrupted the continuous protein phase, resulting in less expansion and greater hardness. When the bulk density and hardness of the ground product were compared, a strong correlation of 0.82 resulted ($p<0.05$). The inclusion of air cells within the extrudate influences the hardness of the product.

Springiness of the ground extrudate decreased with flour additions. Chewiness increased with CPKabuli and CPCooked. Springiness was relatively unaffected by the addition of fiber, but chewiness increased with increasing fiber.

While hardness varied in the ground form, showing differences in the extrudates, flour additions did not impact the hardness in patty form. The hardness of the plant-based patties (PBP) is impacted by the additives used, and not exclusively the extrudate. The increase in hardness for the patties may also be due to the compacting of the material during the patty formation, which the ground extrudate did not undergo.

Additions of pea fiber created interesting texture results. Hardness was mostly similar and generally hovered around 4000 g. Most meat controls were of similar hardness, as well, meaning that these PBP had good imitation of real meat hardness. However, other attributes of chewiness and springiness varied greatly from the PBP to the meat patties.

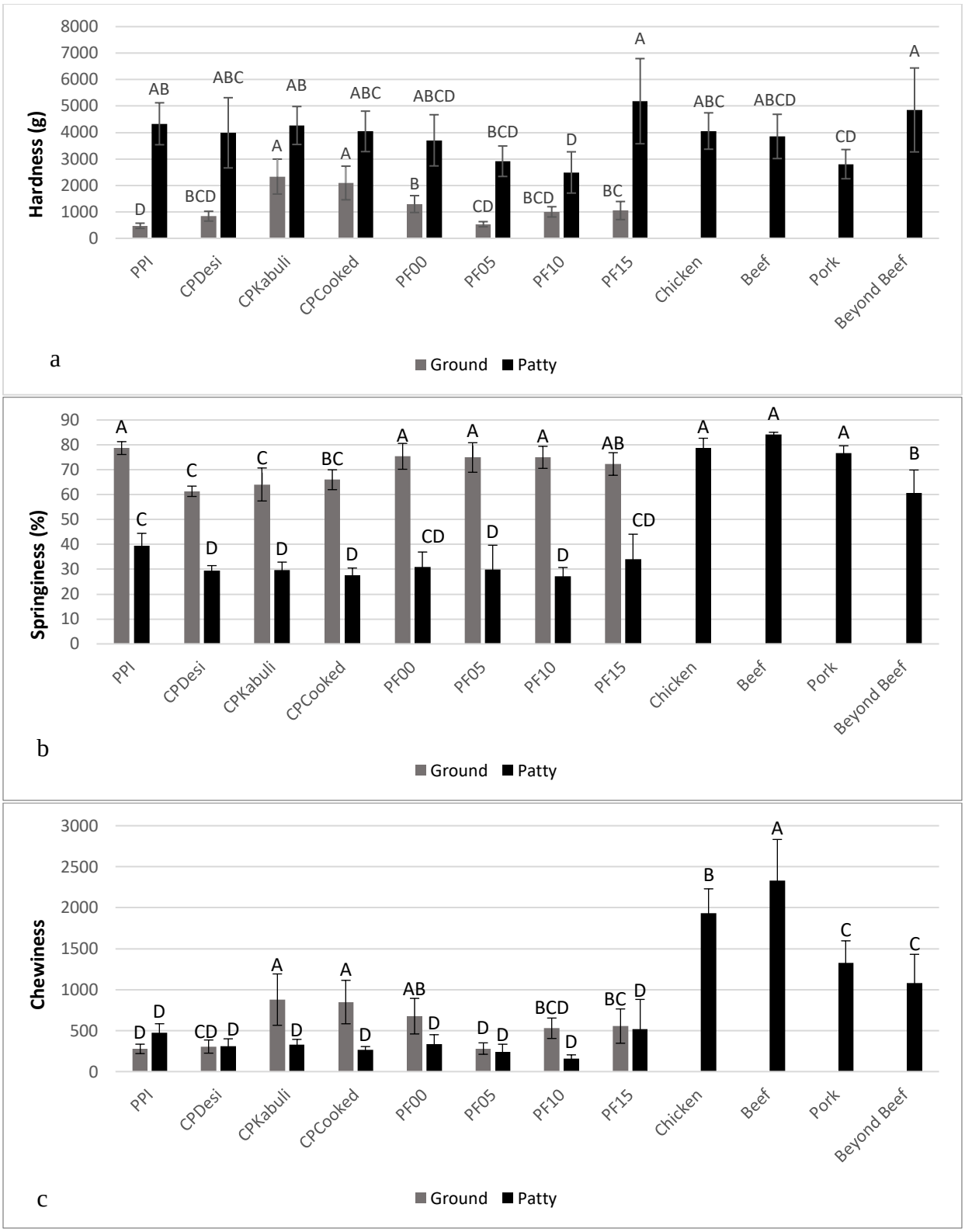

Figure 3.14 Mean (a) hardness, (b), springiness, (c) and chewiness of ground extrudate and patties made from the ground extrudate, as well as patties made with ground meats and commercial plant-based patties. Means of treatments in the same category denoted by the same letter above the bar are not significantly different.

In patties, springiness and chewiness decreased by 10% and 175, respectively, with all flour types. Springiness decreased for all PBP that had flour and/or fiber added compared to the PPI treatment. The PPI PBP was able to give a springier structure while the other treatments that contained carbohydrate were prone to more permanent deformation. Springiness for the PPI PBP was measured at 40%, while the patties with carbohydrate additions were roughly 30%, which was less than half of the springiness of chicken, beef and pork. The commercial PBP was twice the springiness of the PBP of this study, and it showed springiness closer to the meats. It is interesting to note, however, that the springiness for ground extrudate (without binders), was similar to meat anchors, showing again that binders also impact texture. Targeting texture is reliant on both the extrudate and the binding system.

Chewiness was similar to springiness in that there was a decrease from PPI to the remaining treatments, but all chewiness values for PBP were statistically similar. At the addition of 15% fiber, the chewiness was able to increase again to similar values as the PPI, showing that fiber addition can allow for decreased protein usage for texturized pea proteins, yet still allow for similar texture. However, all chewiness values were anywhere from 3 to 5 times lower than the meat comparisons. Since chewiness is derived from hardness and springiness, increasing springiness of the PBPs could also improve chewiness and make it more meat-like.

4. Conclusion

In texturized pea proteins, 20% inclusion of chickpea flour impacts the texture. However, different chickpea cultivars and even pre-treatment did not significantly impact texture. Variations in starch ingredients added in minor amounts may not be a concern for final product attributes. Increasing pea fiber did impact the hardness. Fiber may have acted as a nucleating

agent to increase decrease bulk density and impact texture. Controlling ratios of starchy ingredients and fiber can be a useful way to control texturization and resulting texture.

The greatest indicator of textural properties was the bulk density as air cells made the product less firm. Creation of patties from the ground extrudate changed the textural attributes, and extrusion scientists and formulators should be aware of how their specific binding system may change the extrudate textural properties.

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Chapter 4 - Texturization of pea, wheat, and soy proteins using extrusion and their application in plant-based meat

Abstract

Many protein isolation factors can be controlled to result in different functional protein properties, even when the isolate comes from the same botanical source. Due to variations in processing, pea protein functional properties are different from the commercial suppliers and this creates unique opportunity to understand the functional properties and key advantages they bring to extrusion texturization.

In this study, 4 commercial pea proteins were analyzed in their raw form and extruded on a pilot-scale twin screw extruder in a low moisture environment to create textured pea protein. Wheat and soy treatments were analyzed in addition, with the intent to study difference within the same source (pea) and between sources (pea, wheat, soy).

Proteins that had higher water absorption capacity also had the highest initial viscosity and particle size and were the most soluble according to SDS-PAGE. These proteins required the least specific mechanical energy during processing. Pea protein extrudates displayed similar hardness, chewiness, and springiness, but varied in these traits when formed in a patty. A single trait did not seem to be most impactful in the creation of certain extrudate characteristics, and thus further study should be conducted on understanding the role of multiple factors to create a model which balances the most important factors for desired outcomes.

1. Introduction

Popularity of plant-based meat is soaring. A global interest has been fostered in consuming protein sources that are perceived as ethical (Estell et al. 2021), causing the rising

interest in plant-based meat alternatives. In the US, the plant-based meat market grew to $4.2 billion in 2020, a 24% increase from 2019 (Gaan 2021).

As of 2019, soy-based products made up 48% of the plant-based meat market (Grand View Research 2020). However, with large company investments into pea protein, a McDonald's partnership with Beyond Burger, and other companies entering the unique space, pea protein is a large and growing percentage of the market (Bashi et al. 2019; The Insight Partners 2020). Greenhouse gas emission and land used to make 100 g of pea protein (0.4 kg CO_2eq and 3.4 m^2, respectively) are much lower than what is required for beef (50 CO_2eq and 164 m^2), contributing to environmental ethics considerations and the rise of plant-based meats with pea ingredients (Poore and Nemecek 2018). Additionally, pea protein is attractive for companies to fulfill consumers desires for cleaner labels, as pea protein has low-allergenicity and is non-GMO (Bashi et al. 2019).

Although many companies use pea protein, pea protein from different sources can be vastly different and lead to different product types. Pea protein can be extracted in a wet environment or through dry fractionation and air classification. Generally, pea protein is made by wet extraction: soaking yellow peas in water, crushing them, and separating fiber and starch. The remaining protein is then placed in an alkaline solution for neutralization and extraction through the isoelectric point and then steam sterilized before being spray dried (Boukid et al. 2021; Kornet et al. 2021). Isolating at a pH of 9 increases aggregation of protein, decreases protein solubility, and decreases beaniness of the isolate compared to isolating at a pH of 8.5 (Gao et al. 2020). A small adjustment in processing clearly leads to different protein function.

For functional purposes, isolates may also be hydrolyzed with papain or bromelain enzymes or cross-linked with transglutaminase (Boukid et al. 2021). Some initial steps with

fermentation of pea protein for functional purposes have also been taken, though it is not commercialized yet (García Arteaga et al. 2021). Prolonged heat treatment or exposure to high temperatures denatures the protein (Sirtori et al. 2012). With varying heat and pH treatments, milling parameters, and hydrolysis, it is obvious that the function of pea proteins would vary greatly among suppliers. All of these differences are specific to the isolation process, and are in addition to the cultivar and environmental differences in the proteins that may exist prior to the isolation (Day 2013; Lam et al. 2018).

The goal of this study was to determine raw material characteristics of various commercial pea isolates (water absorption, denaturation qualities, viscosity) and determine how those qualities may create unique opportunities for textured protein traits (water holding capacity, bulk density, hardness, etc) as well as to understand the chemical bonds that make the final structure possible. Soy and wheat proteins were extruded for comparison of pea proteins to other standard proteins used in protein texturization.

2. Materials and Methods

2.1 Materials

A total of 8 treatments were tested in this study; 4 pea treatments 2 wheat treatments, and 2 soy treatments. Treatment formulations can be found in Table 4.1. The purpose of this design was to compare between pea protein sources, as well as compare between types of protein sources. Pea protein isolate was sourced from four separate companies (PP1-PP4). Vital wheat gluten (VWG) (MGP Ingredients, Atchison, KS) was utilized for the wheat treatment. Soy protein isolate and soy protein concentrate (SPC) were also obtained from a commercial source.

Pea protein isolates had a protein content ranging from 80-83% db. One wheat gluten treatment (VWG) and one soy treatment (SPI/SPC) were included to match this level of protein. An additional wheat gluten treatment was diluted to roughly 71% protein with hard red winter wheat flour (Hal Ross Flour Mill, Manhattan, KS) (Dilute VWG) and an additional soy treatment was only concentrate (70% protein) for comparison throughout the paper. Composition of the main treatments of interest is shown in Table 4.2.

Table 4.1 Treatment formulas

Treatment	PPI 1	PPI 2	PPI 3	PPI 4	VWG	Dilute VWG	SPI/SPC	SPC
Pea isolate 1	100							
Pea isolate 2		100						
Pea isolate 3			100					
Pea isolate 4				100				
Vital Wheat Gluten					100	90		
Wheat Flour						10		
Soy Protein Isolate							50	
Soy Protein Concentrate							50	100

Table 4.2 Composition of standardized treatments as determined by proximate analysis and supplier specifications (%)

Component	PP1	PP2	PP3	PP4	VWG	SPI/SPC
Protein	80.3	80.30	82.88	79.2	86.67	77.76
Non-Fiber Carbohydrate	4.0	9.29	7.63	3.2	7.27	9.81
Fiber	4.0	0.2	1.01	2.7	0.2	1.80
Fat	6.0	0.47	0.45	6.0	0.87	0.55
Ash	1.6	4.06	5.33	4.1	0.44	4.64
Moisture	4.1	5.88	3.71	4.8	4.76	5.44
Total	100.0	100.0	100.0	100.0	100.0	100.0

2.2 Extrusion Parameters and Calculations

A ribbon blender (Wenger Manufacturing, Sabetha, KS, USA) mixed the soy and wheat treatments for 5 minutes. A pilot-scale (52 mm diameter, L/D ratio of 19.5), co-rotating twin screw extruder (TX-52, Wenger Manufacturing, Sabetha, KS) was used for texturization. Operating parameters for each treatment can be found in Table 4.3. The dry material feed rate was constant for all pea and wheat protein treatments at 50 kg/hr. The dry feed rate was decreased to 40-45 kg/hr for the soy treatments. The extruder screw speed was 450 rpm for pea and wheat treatments and 200-320 rpm for soy treatments. Water was added at a rate of 8 kg/hr in the preconditioner for all treatments. Pea protein treatments received water in the extruder barrel at 8 kg/hr, but wheat and soy treatments required 12-14 kg/hr. No steam was used. Four temperature zones were used at 40, 70, 90, and 110°C from inlet of extruder barrel to the outlet.

The screw profile was composed of double flighted elements of decreasing pitch, with two forward kneading blocks and four reverse kneading blocks dispersed throughout the profile, and a conical cut element at the end (Table 4.4). A 1/8" (3.172 mm) venturi die was used for all treatments, except soy treatments used a ¼" (6.35 mm) venturi die to prevent burning. After the venturi, a 11" (27.94 cm) Teflon spacer was placed, then the final die plate which had two ¼ in openings. Three hard knives were used with a knife speed of 250 rpm. Sample was taken from the extruder and immediately milled to 0.18" (.46 cm) pieces (Comitrol, Urschel Laboratories Incorporated, Valparaiso, IN) and frozen. Whole extrudate sample passed through a dryer at 200°C for 12 min and cooled for 8 min in a dual pass drier (4800, Wenger Manufacturing). Dried extrudate samples were stored at room temperature.

Specific mechanical energy (SME) was calculated using the following formula:

$$\textbf{SME}\ (\mathrm{kJ/kg}) = \frac{\left(\frac{\tau-\tau_0}{100}\right)\times\frac{N}{N_r}\times P_r}{\dot{m}} \qquad (1)$$

where τ is the % torque, τo is the no-load torque, N is the measured screw speed in RPM, Nr is the rated screw speed (336 rpm), Pr is the rated motor power (22.4 kW), and ṁ is mass flow rate in kg/s.

In-barrel moisture (IBM) content was calculated using the following equation:

$$\textbf{IBM}\ (\%\ \text{wb}) = \frac{(m_f \times X_{fw}) + m_{pw} + m_{ew}}{m_f + m_{pw} + m_{ew}} \qquad (2)$$

where m_f is the dry feed rate, X_{fw} is the moisture content of the dry feed material (expressed as a fraction), m_{pw} is the water injection rate into the pre-conditioner in kg/hr, and m_{ew} is the water injection rate into the extruder in kg/hr. An IBM of roughly 29% was used for pea protein treatments, while 35-38% IBM was used for wheat and soy treatments.

Table 4.3 Extrusion parameters for each treatment.
All parameters remained consistent for pea protein treatments, while optimization based on product outcome was required for wheat and soy treatments

Extrusion Parameter	PP1	PP2	PP3	PP4	Dilute VWG	VWG	SPI/SPC	SPC
Feed Rate (kg/hr)	50	50	50	50	50	50	45	45
IBM (%)	29.9	29.3	28.9	28.7	35.2	34.3	34.7	38.7
Screw Speed (RPM)	450	450	450	450	450	450	320	206
Venturi die size (in)	1/8	1/8	1/8	1/8	1/8	1/8	1/4	1/4

Table 4.4 Extruder screw configuration

left	1	1	1	3	4	4B	1	1	5	5	6	7	6	7	6	6B	8
right	2	2	1	3	4	4B	1	1	5	5	6	7	6	7	6	6B	8

1	Full pitch, double flight, 9U
2	Full pitch, single flight, 9U
3	Full pitch, double flight, 6U
4	Forward kneading block
4B	Forward kneading block, backward
5	¾ pitch, double flight
6	Reverse kneading block
6B	Reverse kneading block, backwards
7	½ pitch, double flight, cut flight
8	¾ pitch, double flight, cut flight, cone

2.3 Moisture Content

Moisture content was measured for raw ingredients, preconditioned treatments, and extrudates (before drying) using the AACC 44-19.01. Triplicate samples of approximately 2 g were dried at 135ºC for 2 hrs.

2.4 Raw Material Analysis

2.4.1 Particle Size Distribution

The particle size distribution of each treatment was determined in duplicate using the Air Jet Sieve e200LS (Hosokawa Alpine Group, Augsburg, Germany). A 100 g sample was placed on the smallest sieve, with the remains continuing to the next largest sieve until all material passed through. Sieves with 32, 53, 75, 106, 125, 150, 180, 212, 250 and 300 microns were used.

2.4.2 Water Absorption Capacity and Oil Absorption Capacity

Water absorption capacity (WAC) was measured as a previous study described, but with modification (Anderson et al. 1970). Samples of 2.5 g were placed in centrifuge tubes with 30 ml deionized water. To disperse the sample, the slurries were vortexed. Samples were then allowed

to sit at room temperature for 30 minutes with 2 additional agitations in that time. Samples were centrifuged at 3000 x g for 30 min and the water was carefully decanted. WAC was calculated using the following equation:

$$\textbf{WAC}\ (\text{g water/g protein}) = \frac{W_f - W_i}{W_i}$$

where W_f is the weight of the sediment and W_i is the initial weight of the sample.

Oil absorption capacity (OAC) was measured similarly, using the methods described by Brishti et al. (2017), with some modification. Samples of 2.5 g were placed in centrifuge tubes with 30 ml sunflower oil. Samples were shaken until the sample was dispersed and allowed to sit at room temperature for 30 minutes. Samples were centrifuged at 3000 x g for 30 min and the oil was carefully decanted. The tubes were then inverted, allowing excess oil to drain for 20 min. OAC was then calculated using the following equation:

$$\textbf{OAC}\ (\text{g oil/g protein}) = \frac{W_f - W_i}{W_i}$$

where W_f is the weight of the sediment and W_i is the initial weight of the sample.
WAC and OAC were measured in triplicate for each sample.

2.4.3 Least Gelation Concentration

Least gelation concentration (LGC) of each treatment was obtained by dispersing different concentrations of pea and soy proteins (12 - 20% w/v) in 10 mL DI water in 1 cm diameter test tubes. The solutions were then heated, uncovered, at 95-100 °C for 1 hr, immediately cooling via a cold water bath, and then keeping at 4 °C for 2 hr. Wheat proteins were not tested since they are hydrophobic in nature and clump upon addition of water. The LGC was determined, after chilling, as the concentration that forms a stable gel that does not drop or run when the test tube is inverted.

2.4.4 Rapid Visco Analyzer Viscosity

A rapid visco analyzer (RVA) (RVA 4500, Perten Instruments, Waltham MA) was used to measure the viscosity of each treatment in triplicate using the AACC Method 76-21.02 STD1. Protein slurries at 15% solids concentration (w/v) were manually mixed so that protein clumps were better dispersed and reduced noise in the plot. Slurries were placed in the RVA in within 1 min of initial mixing. Slurries were heated to 50 ºC and held for 1 min, with initial stirring at 960 rpm for 10 seconds. The remainder of the test, slurries were stirred at 160 rpm. Slurries were then heated to 95 ºC at 12 ºC/min, then held for 2.5 min and cooled again to 50 ºC. Peak viscosity, time and temperature of peak viscosity, and end viscosity were measured and recorded.

2.4.5 Differential Scanning Calorimetry

Protein denaturation, as shown by enthalpy, was measured by differential scanning calorimetry (DSC) with a Q100 V9.9 Build 303 (TA Instruments, New Castle, DE) and analyzed with Universal Analysis Program, V4.5A (TA Instruments). Comparing the DSC results of raw commercial protein isolates can be helpful to understand the impact of isolation processing on the denaturation. DSC was conducted according to Brishti et al. (2017) with a few modifications. Raw samples of 8-10 mg dry matter were weighed into stainless steel, high volume, hermetically sealed pans. Samples were heated to 250 ºC at a rate of 10 ºC/min. An empty pan served as a reference. Nitrogen purge flow was 50.0 mL/min. The start and peak denaturation temperature and enthalpy of denaturation were recorded.

2.4.6 Molecular Weight

Molecular weight of each legume protein (raw and extruded) was qualitatively understood through sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in non-reducing conditions. Extruded proteins were ground to less than ½ mm. Protein was

extracted for an hour with deionized water (15 µg:1 mL) then centrifuged for 5 min at 8000 rcf. The supernatant was then mixed with Laemmli buffer (2 supernatant: 1 buffer) and heated for 10 minutes in a boiling water bath. The 4x Laemmli sample buffer contained 277.8 mM Tris-HCl (pH 6.8), 44.4% (v/v) glycerol, 4.4% LDS, and 0.02% bromophenol blue (Bio-Rad Laboratories, Inc, Hercules, California).

Prepared sample (12 µL) was pipetted into the gel lanes. Precision Plus Protein Standard (Bio Rad Laboratories, Hercules, CA) was added at 5 µL and contained protein markers from 10-250 kDa. Electrophoresis was then conducted at 200 V, 25 mA, and 250 W to separate the proteins by molecular weight with 12% separating gel and 4% stacking gel. After electrophoresis, samples were fixed and stained using Brilliant Blue R concentrate. Samples were then destained overnight with 10% acetic acid, and further destained with deionized water.

2.4.7 Phase Transition Analysis

Phase Transition Analysis (PTA) was used to measure raw material softening and flow point temperatures. The test was conducted as described in (Webb et al. 2020) with samples hydrated to 24%. Raw treatments (2 g) were compressed in the chamber with a blank die to 120 bars for 15 s. Pressure of 100 bars was applied as the sample was heated at a rate of 8 ºC/min, with a starting temperature between 5-7 ºC. After the softening point of the material was measured, a 2 mm capillary die was place under the sample and compressed again to 120 bars for 15 sec with 100 bars of pressure thereafter. Wheat gluten treatments required using lower pressure; 75 bars of consistent pressure was used throughout the test. When material began to flow through the capillary die, the compressing rod displacement changed, showing the flow point, and the temperature was marked as the flow temperature.

Extruded material was also tested to determine changes in flow point temperature compared to the raw material. For this analysis, raw materials were extruded on a lab scale, Micro-18 extruder (Micro-18, American Leistritz, Somerville, NJ). This is to help understand potential for protein networking prior to pilot scale extrusion. Raw materials were hydrated to 24% MC before extrusion and run at 3.3 kg/hr throughput and a screw speed at 550 RPM. The material was extruded through barrel sections with temperatures of 30, 40, 55, 95, 120, 140ºC. An oval die with width of 5.5 mm and length of 3.0 mm was used to make ropes of extrudate. Extrudate was not dried, was ground finer than 250 microns with a wiley mill, hydrated to 24% moisture, and run on the PTA using the parameters described above.

2.5 Extrudate Analysis

2.5.1 Water Holding Capacity

Water holding capacity (WHC) is the measurement of water that is held within the structure of the final product, measured according to Kearns, Rokey, & Huber (1989), with modifications. Milled samples (15 g) were soaked in excess, room temperature, water for 20 minutes, then drained on a mesh screen for 5 min. WHC was calculated using the following equation:

$$\textbf{WHC}\ (\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

2.5.2 Texture Analysis

Hardness, springiness, and chewiness characteristics were measured using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) with a two-compression test. Analysis was performed on ground and rehydrated product to understand the texture qualities of more uniform extrudate pieces. Treatments were rehydrated with room temperature water to 60% moisture. A back-extrusion cup was utilized to contain 20 g of sample that was

filled to 1 cm height in the cup. A two-compression test was completed on the samples, compressing to 70% of the total distance with a circular aluminum probe. Texture properties of patties were measured in accordance to guidelines from the American Meat Science Association (AMSA 2015). Patties were made with 91.5 g of each treatment and pressed to 1 cm thickness. The formula for plant-based patties is shown in Table 4.5. Patties were panbroiled with no oil until an internal temperature of 71 ºC was reached. Patties were allowed to cool to room temperature and a 2.5 cm core was taken from the center of 10 patties. A two-compression test was completed on the room temperature cores, compressing to 70% of the total distance with a circular aluminum probe.

Table 4.5 Patty formulation for texture analysis

Ingredient	Percentage
Textured Pea Protein	59.25
Water	29.6
Pea Protein Isolate	1.5
Chickpea Flour	1.5
Sunflower oil	3
Methylcellulose	2.75
Salt	1
Beet Powder	0.75
Spices	0.65

2.6 Experimental design and Statistical Analysis

A 1-way treatment structure was used. ANOVA was performed to compare means and differences with SAS software (SAS, Cary, NC). ANOVA was followed by Tukey's test to determine significance of differences and control for Type 1 error ($p < 0.05$).

3. Results and Discussion

3.1 Raw Material Characteristics

3.1.1 Particle Size Distribution

Particle size can vary based on industrial processing due to varying temperatures, vaporization, and air-water interface which can cause increased denaturation and aggregation of hydrophobic regions (Arteaga et al. 2021). PP1 and PP3 had a significant portion of their particles under 53 microns, as did the soy treatments (Figure 4.1). PP2 and PP4, however, had a wider particle size distribution with substantial portions ranging from 53 to 150 microns. VWG had 40% under 53 microns, but the remaining portion (about 55%) was spread all the way to 250 microns. Thus, PP1 and PP3 had the most uniform particle size for pea treatments and the SPI/SPC also had relatively uniform particle size.

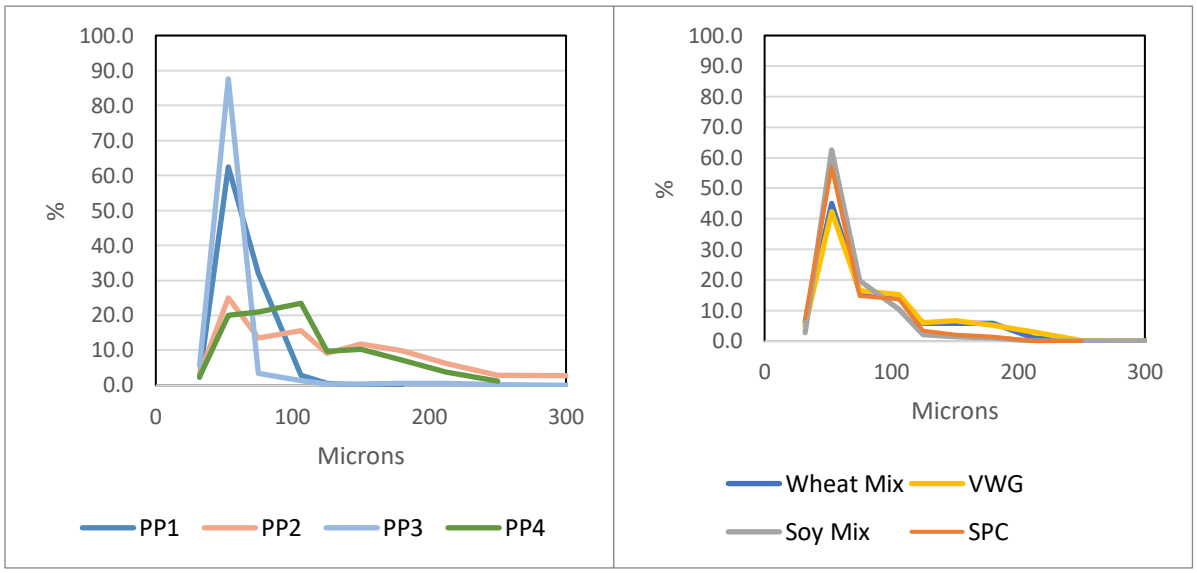

Figure 4.1 Particle size distribution of (a) pea protein and (b) wheat and soy treatments as percentage of particles through each sieve.

3.1.2 Water Absorption Capacity and Oil Absorption Capacity

WAC and OAC of each treatment can be seen in Figure 4.2 and Figure 4.3. The WAC and OAC of each the pea treatments is within the same range as previously reported (Osen et al. 2014). The difference in ability of the pea proteins to absorb water may be partially owed to the variation in particle size. PP1 and PP3 have the lowest WAC of the pea proteins which may be a result of the low particle size and the particles packing together well and not allowing water into the matrix. Additionally, PP2 and PP4 had a greater WAC and had a wider range of particle size that could allow for more water absorption due to less packing of the material. VWG displayed the lowest WAC due to the hydrophobic nature of wheat gluten. Wheat gluten varies from the leguminous proteins in that the major fraction of protein is not albumins or globulins, but prolamins and glutelins which are soluble in alcohol and acid, respectively, rather than water or salt solutions (Urade et al. 2018). The way gluten interacts with water is therefore quite different, and results in the low WAC observed. SPI/SPC displayed a slightly higher WAC than all pea proteins, while SPC was similar. Thus, the SPI in the SPI/SPC treatment contributed to the higher WAC of SPI/SPC compared to SPC.

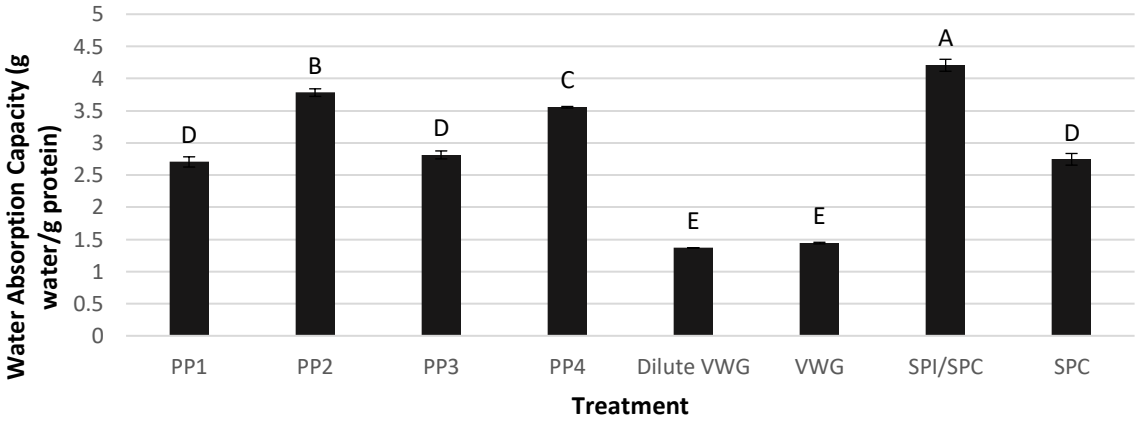

Figure 4.2 Average water absorption capacity of all treatments.
Means of treatments with the same letter above the bar are not significantly different.

PP2 had the highest OAC. Most proteins exhibited similar OAC, but PP1 had a substantially lower OAC. Though gluten is a hydrophobic protein, it did not exhibit a higher OAC. Overall, the OAC of these proteins may be more of an indicator of space between particles than the affinity of the ingredients. Because PP2 had a greater particle size, it would not be as compact and would be able to hold more liquid between the particles, while small particle size PP1 would pack well and not hold much oil.

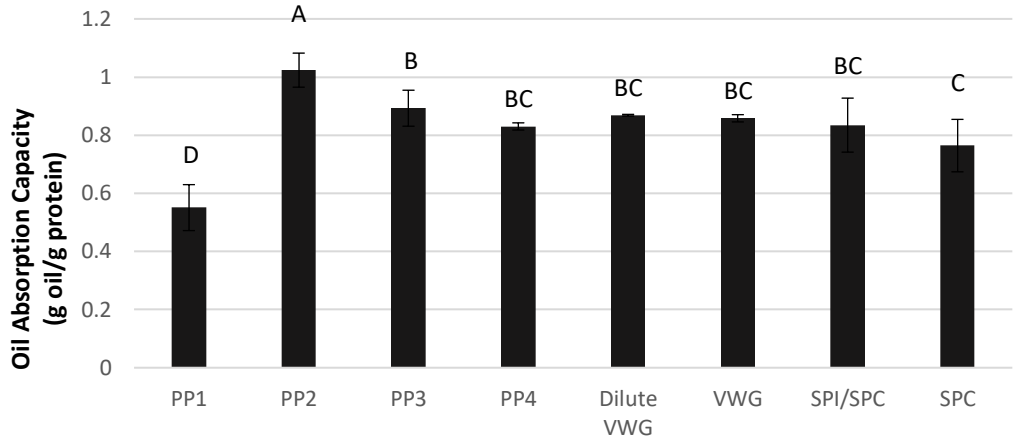

Figure 4.3 Average oil absorption capacity of all treatments
Means with the same letter above the bar are not significantly different.

3.1.3 Least Gelation Concentration

LGC is a test used to determine the gelling properties of proteins and has been used to understand extrusion texturization (Jones 2016; Brishti et al. 2017). Gelation is the ability of protein to form a three-dimensional network through its denaturation and aggregation (Mession et al. 2015). The structure is held by protein-protein interactions of bonds and electrostatic forces. In a gel, the protein also interfaces with a solvent held within the network (Uruakpa 2012). LGC tests specifically for the concentration at which a gel can form and thermogelation, the gelation mechanism utilized within extrusion. Characterizing gelation is helpful since

gelation of proteins after shearing is what helps create and solidify the fibrous and layered structure in meat analogs (McClements and Grossmann 2021).

PP1 gelled at 14% solids concentration, while the remaining PPIs required slightly more protein to gel at 16% solids (Table 4.6). Interestingly, SPI/SPC required a higher solids concentration to create a firm gel. Despite the similar LGCs, extrusion outcomes varied greatly in terms of internal structure and density.

Table 4.6 Least gelation concentrations for pea and soy protein treatments. (Gluten not tested due to its hydrophobicity.)

Treatment	12%	14%	16%	18%	20%
PP1	-	+	+	+	+
PP2	-	-	+	+	+
PP3	-	-	+	+	+
PP4	-	-	+	+	+
SPI/SPC	-	-	-	+	+
SPC	-	+	+	+	+

It is important to note that the protein isolation process manipulates the protein structure and processes vary throughout the industry (Aydemir and Yemenicioĝlu 2013). Thus, the proteins could be exposed to treatments that would allow for various gelation behaviors. The gelation concentration of globular proteins is affected by a number of factors but especially by the pH and ionic strength the protein is exposed to, as well as enzymes and pressure treatment (Renard and Lefebvre 1992; Nicolai and Chassenieux 2019). It is by various combinations of these treatments, too, that different proteins may test at the same LGC but for different molecular level reasons. With the amount of factors that change protein properties, comparison between protein sources is difficult (Nicolai and Chassenieux 2019).

Higher solubility can be achieved through hydrolysis but results in a tradeoff of lower gel strength (Nicolai and Chassenieux 2019). This could be indication that PP2, PP3, and PP4 have

been processed in a way to increase solubility at the expense of gel strength and thus leading to a higher critical concentration of protein.

Stronger gels came from pea proteins that were less fractionated (Kornet et al. 2021). This phenomena is seen with less concentration required for SPC than for SPI/SPC, even though SPI/SPC has a greater protein concentration.

3.1.4 Rapid Visco Analyzer Viscosity

Pea protein solutions displayed different behavior upon hydration, heating, and low shear. Average RVA curves are shown in Figure 4.4. Only one protein, PP1, had an increase in viscosity during heating. PP1 is also the only treatment that formed a gel upon heating in LGC testing at the concentration which RVA was conducted (15%). Due to the gelation and thermal properties of PP1, the heat allows the protein aggregate and increase viscosity. Another protein, PP2, had very high viscosity at the outset, before heating and long exposure to shear, showing that it had instant hydration. PP4 also had a relatively high starting viscosity. Both of these proteins also had high WAC and particle size in comparison to the other two pea proteins. A moderately strong relationship was present between WAC and the peak viscosity (0.78, $p<0.0001$). Similar findings have previously related the WAC and viscosity of pea proteins, which found that a pea protein that was able to absorb more water resulted in a higher viscosity (Osen et al. 2014).

PP4 has the highest end viscosity after heating and cooling. PP2 has the next highest final viscosity, with PP3 and PP1 at the lowest end viscosities. The WAC of each protein supports this finding. Pea proteins that are able to absorb more water (PP2 and PP4) have higher final viscosities and initial cold viscosities. With the same amount of water available, these proteins

absorb more of it, increasing the final viscosity. VWG failed to gain viscosity during the RVA heating due to the low concentration of protein and the hydrophobic nature of gluten.

PP1 gelled at 14% protein while the others gelled at 16%. Since RVA run at 15% solids content would be the only one that would have enough protein to create a gel according to LGC results (Table 4.7). PP1 is also the only protein that displayed a peak viscosity during heating. Due to its thermal properties, PP1 was able to gel and gain viscosity during heating but the viscosity was ultimately diminished from the shear.

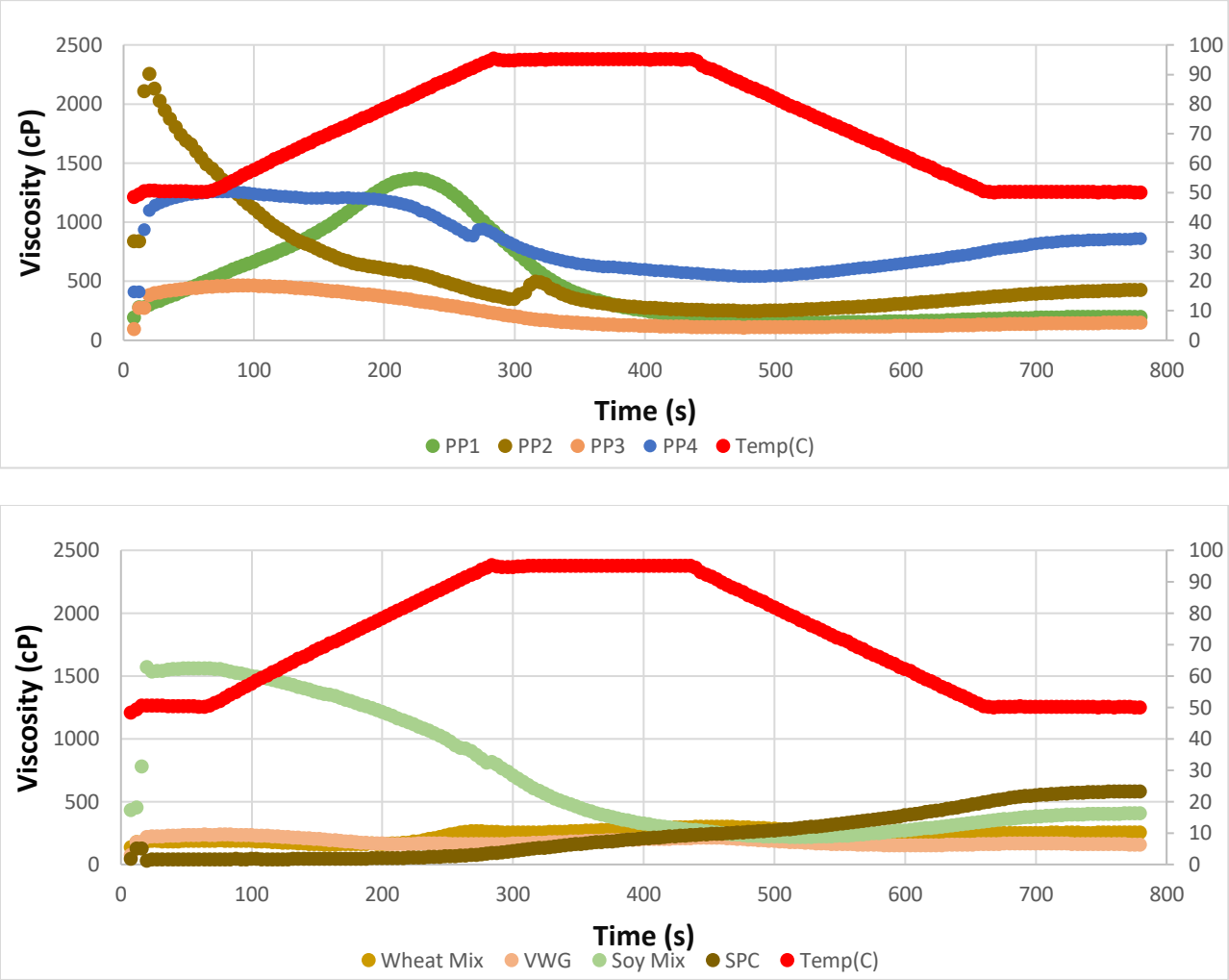

Figure 4.4 Average viscosity curves for (a) pea proteins and (b) wheat and soy proteins using 15% solids concentration of raw materials.

Table 4.7 Means and standard deviations of RVA measurements.

Means in a column followed by the same letter are not significantly different ($p<0.05$).

Treatment	Peak Viscosity (cP)	Time of Peak Viscosity (s)	Temperature of Peak Viscosity (ºC)	Final Viscosity (cP)
PP1	1387 ± 157^{c}	223 ± 14^{c}	83 ± 3^{c}	195 ± 7^{b}
PP2	1947 ± 52^{a}	20 ± 0^{e}	51 ± 0^{e}	299 ± 3^{d}
PP3	460 ± 83^{de}	91 ± 6^{d}	55 ± 1^{d}	144 ± 21^{c}
PP4	1257 ± 38^{c}	75 ± 5^{d}	52 ± 1^{d}	856 ± 25^{d}
Dilute VWG	300 ± 11^{ef}	453 ± 2^{b}	91 ± 0^{b}	251 ± 8^{a}
VWG	236 ± 23^{f}	79 ± 2^{d}	53 ± 1^{d}	156 ± 14^{cd}
SPI/SPC	1626 ± 94^{b}	56 ± 31^{d}	51 ± 1^{d}	403 ± 10^{d}
SPC	580 ± 47^{d}	780 ± 0^{a}	50 ± 0^{a}	580 ± 47^{d}

3.1.5 Differential Scanning Calorimetry

The starting and peak temperature of denaturation were different among the pea proteins, ranging from 146-160 ºC and 179-188 ºC, respectively (Figure 4.5). Wheat treatments were lower than pea proteins in starting and peak temperatures of denaturation, while soy was higher. The enthalpy measured during denaturation varied as well (Figure 4.6). PP3 required the least amount of energy (12.59 J/g), while PP4 required the most (23.36 J/g). PP1 and PP2 required 19.45 and 17.00 J/g, respectively. The soy treatment required less energy to denature (15.07 J/g). A previous study has found that lower denaturation enthalpies are a result of harsher or longer thermal treatments (Sirtori et al. 2012). These results suggest that greater protein denaturation has occurred for PP3 during isolation. PP4 may have the least denatured protein.

PP1 required one of the highest enthalpies to denature, meaning it would require temperature to denature and associate to a gel and build viscosity, as seen with RVA testing. PP4, though it also required a high enthalpy, had a much higher WAC, which created the higher initial viscosity and thus no peak viscosity during heating.

The enthalpy for Dilute VWG is higher than the enthalpy for VWG. With no water during this DSC test, the gelatinization of starch could not occur, and thus melting of the crystalline structure would occur around the same temperature of the denaturation of plant protein, around 170-230 ºC depending on the water content (Donovan et al. 1983, Leon et al. 2003, Takahashi and Yamada 1998). With the overlap of temperatures of melting and denaturation, the larger enthalpy seen in this testing may be a result of protein-starch interactions in a low moisture environment.

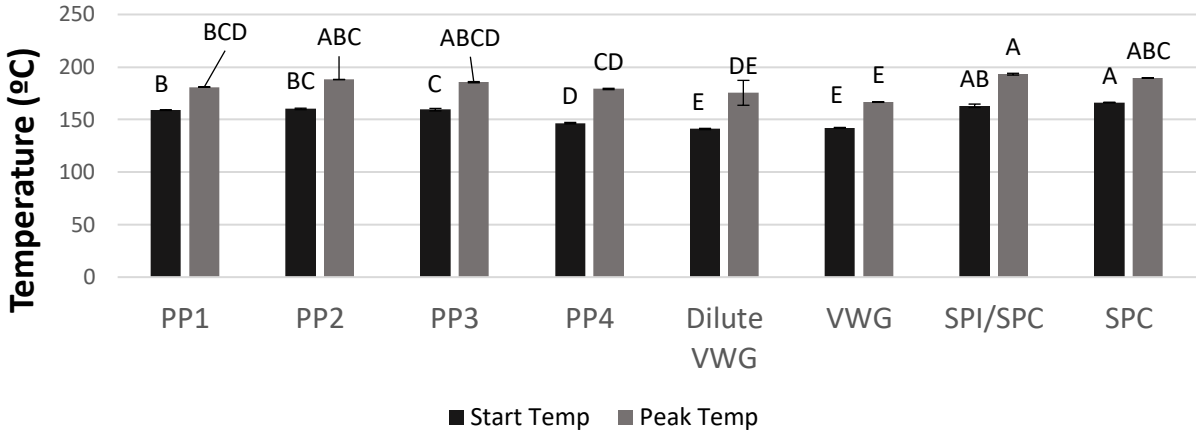

Figure 4.5 Mean starting and peak temperatures of denaturation for each protein treatments.
Means of treatments in the same category denoted by the same letter above the bar are not significantly different.

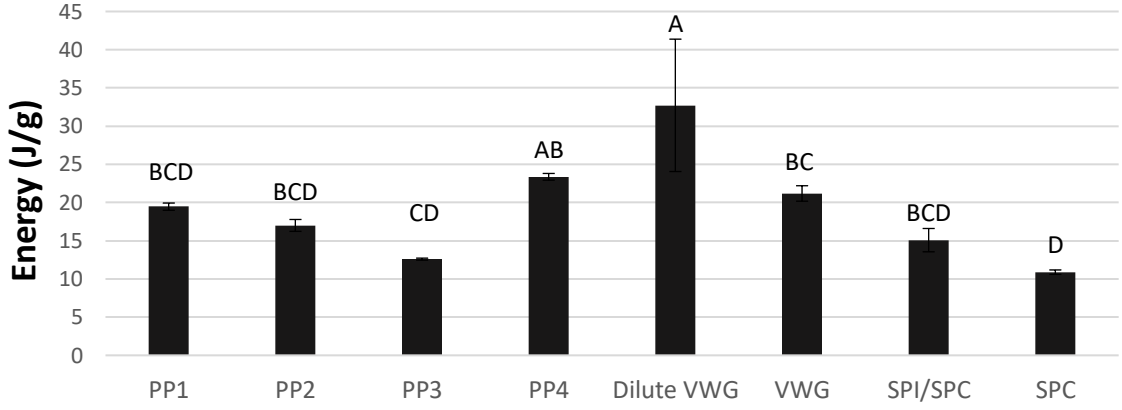

Figure 4.6 Mean energy required to denature the raw material.
Means with the same letter above the bar are not significantly different.

3.1.6 Molecular Weight Analysis

Proteins differ based on their cultivar and extraction methods (Tanger et al. 2020; Arteaga et al. 2021). Having a molecular level understanding of proteins can be useful in understanding their gelation upon heating, and for pea protein this generally means understanding the solubility and the content of legumin, vicilin, and convicilin. Legumin is attributed with disulfide bonding during gelation and texturization due to the greater presence of sufur-containing amino acids, but gelation functionality of legumin can vary by variety (O'Kane 2004). Vicilin lacks the sulfur content of legumin, yet the convicilin subunit of vicilin still contributes to gelling. The core of convicilin is largely the same as vicilin, but convicilin is distinguished from vicilin because of a highly charged end of the protein which allows it to form the gel network (O'Kane 2004).

Due to the lower intensity of bands of PP1, this protein seems to be less soluble in water than the other proteins (Figure 4.7). PP2 is the most soluble as noted by the greatest intensity of the bands. Seeing the great solubility of PP2 gives explanation to its high WAC and the RVA cold viscosity that it displays. After immediate hydration, PP2 is able to take in the water and create viscosity but upon heating and mild shear, it begins to solubilize and viscosity decreases.

The more soluble proteins (PP2-PP4) have a more intense 70 kDa bands, indicating a higher presence of convicilin, and each of these had a slightly higher LGC, which could be due to the electrostatic repulsion preventing gelling and requiring slightly more protein to network. With a higher convicilin content, more N-terminus negative charges and therefore more repulsion and took more protein to make a gel (O'Kane 2004).

SDS-PAGE of select extrudates caused obvious change in the molecular weight and solubility of the proteins (Figure 4.7). No distinct bands were present after extrusion. During low

moisture extrusion, previous studies found that the vicilin protein was unaltered while legumin changed after extrusion, likely by aggregation and increase in molecular weight (Osen et al. 2015; Beck et al. 2017). Similarly, the proteins in this study aggregated and became insoluble, rendering a gel with no distinct bands.

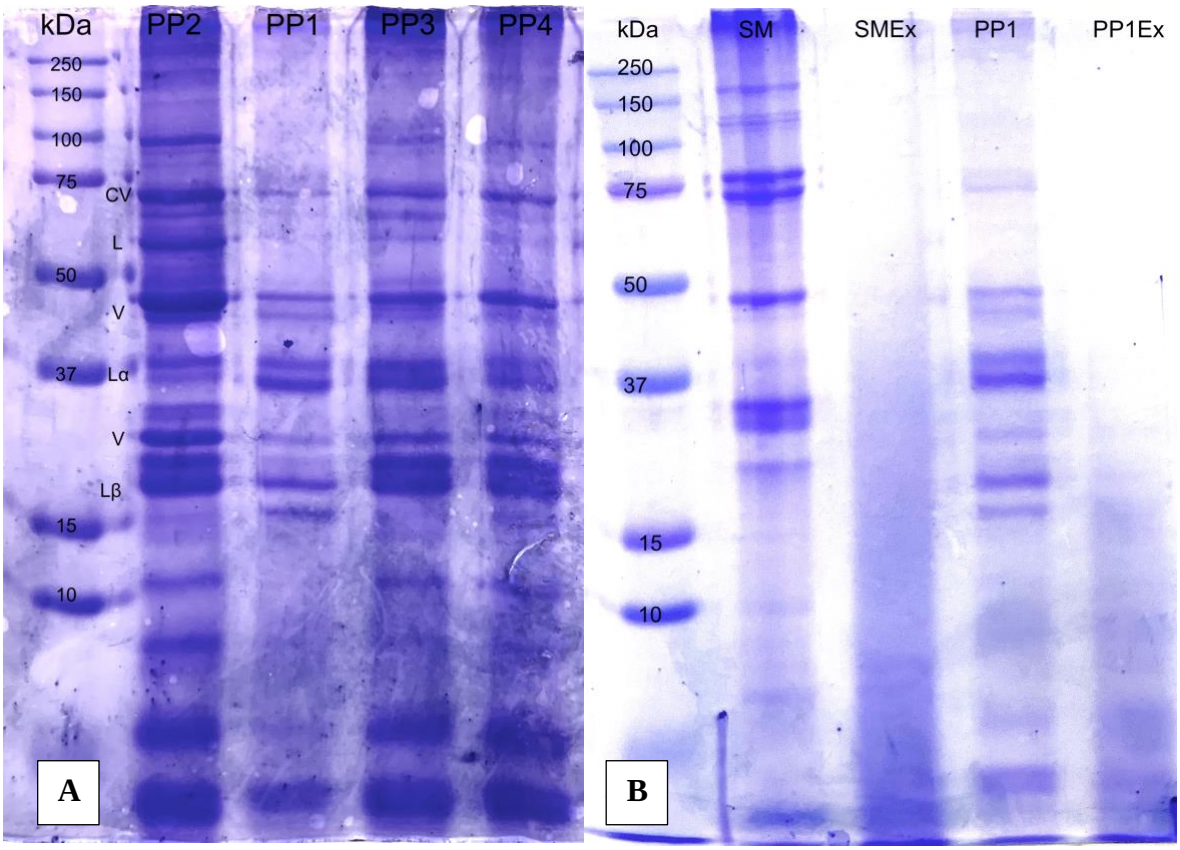

Figure 4.7 Non-reducing SDS-PAGE gel for (a) raw pea proteins and select raw and (b) extruded proteins
Columns in (a) are the standard marker, PP2, PP1, PP3, and PP4. Note that PP2 comes before PP1. Columns in (b) are the standard marker, raw SPI/SPC (SM), SPI/SPC extruded (SMEx), PP1, and PP1 extruded (PP1Ex). CV, Convicilin; L, Legumin; V, Vicilin; Lα, acid subunit of legumin; Lβ, basic unit of legumin

3.1.7 Phase Transition Analysis

The various raw materials showed different phase transition behavior. The temperature at which the material began to flow through the capillary die was highest for PP3 and lowest for PP2. Soy and wheat proteins were the most resistant to flow in the PTA. With a higher

temperature required to achieve a flowable melt, it follows that more energy will be required to process the material in the extruder. The thermal energy required for raw materials to flow in the PTA mirrored the SME required for pilot scale processing. PP2 and PP4 required the least amount of thermal energy to flow in the PTA, and they also saw the least amount of energy for processing in the pilot scale extruder.

RVA gives some viscosity insight of the materials, but there is no relationship between the RVA of 15% solids concentration and the flow in PTA. PTA gives understanding of flow requirements of a protein in the pressure of an extruder, while RVA gives insight to the reactions of the raw materials with water and mild heating. In the future, rheological tests of protein gels could be conducted to relate the viscosity of the proteins to the phase transition measured in PTA.

After extrusion, the temperature to achieve flow increased for PP2 and SPI/SPC. With more thermal energy required for the protein to flow, this may indicate the presence of stronger bonds than before and thus ability for stronger protein networks. The interactions formed in extrusion for the remaining treatments may not be as strong and thus required less energy to form a flowable melt.

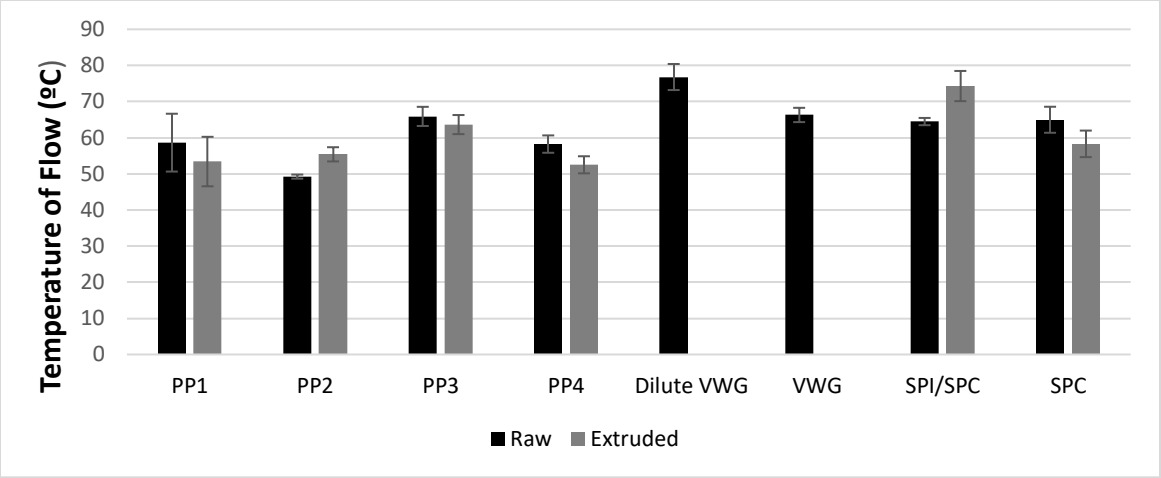

Figure 4.8 Flow temperature for raw material treatments and lab-scale extrudate. *VWG was not able to be hydrated and extruded on the lab scale extruder, thus no extruded PTA was conducted on VWG. Means of treatments in the same category denoted by the same letter above the bar are not significantly different.

3.1.8 Processing

SME was greatest for PP3, with PP2 having the lowest SME (Figure 4.9). Greater SME was required for wheat and soy treatments. T_f seems to be a good indicator of processing SME (Karkle et al. 2012). Both measurements relate measure an aspect of the resistance to flow of raw materials. With a higher T_f, the material requires more thermal energy to flow and on the extruder, this means that the material will resist flowing and require more SME. Thus, PP2 and PP4 are the least resistant to flow both in the PTA and extruder and wheat gluten and soy require greater energy inputs to flow.

For pea proteins, the SME has an inverse trend from their WACs. The more water the protein is able to hold, the less energy it requires to process. During extrusion, each pea protein was processed with the same amount of water. A reason for the low SME for PP2 may be the high solubility the protein has, which means it will not build viscosity as well, and it would require less energy to process. To attain the same SME with a more soluble protein like PP2, a lower IBM may be required so the melt can have greater viscosity.

PP2 and PP4 had the largest particle size and the lowest SME of the pea protein treatments. In corn meal extrusion, larger particle size flows easily and leads to lower SME (Carvalho et al. 2009).

SPC had the most water added to it during extrusion, which would generally plasticize the melt and reduce the viscosity and SME in the extruder. Still, the greatest SME was found in SPC. This may be a result of less harsh fractionation and isolation of the protein; the SPC would have a greater amount of viscosity-building starch as well as more functional proteins. Indeed, SPC has a higher viscosity than most pea proteins and wheat treatments as shown in RVA.

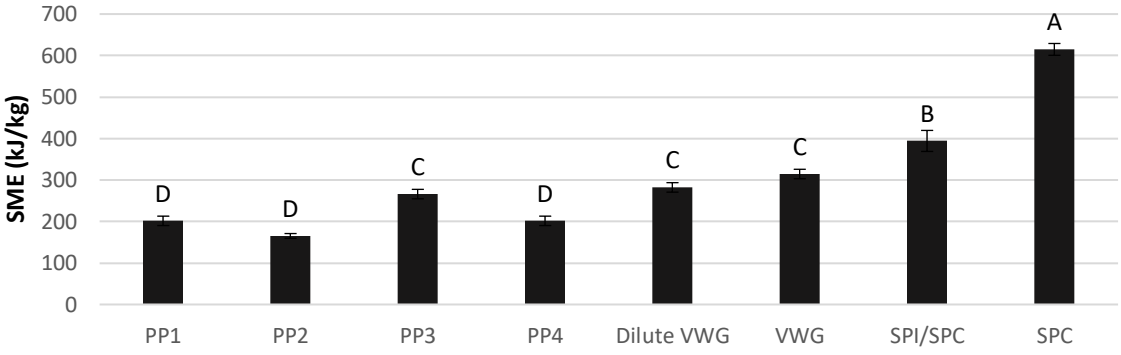

Figure 4.9 Mean SME required by each treatment during processing
Means with the same letter above the bar are not significantly different.

3.2 Extrudate Analysis

3.2.1 Visual Analysis

PP2 displayed a very porous structure, followed by PP4 and PP3, with PP1 showing the least expanded internal structure (Figure 4.10 and Figure 4.11). The longitudinal cross sections show some directional cell formation, mostly present in PP1 and PP3. VWG has the most obvious anisotropic fiber formation in both the horizontal and longitudinal cross sections. Wheat gluten is naturally a fibrous-shaped protein while both pea and soy are globular proteins

(McClements & Grossmann, 2021). Thus, wheat gluten structures into the fibers easier than the other proteins.

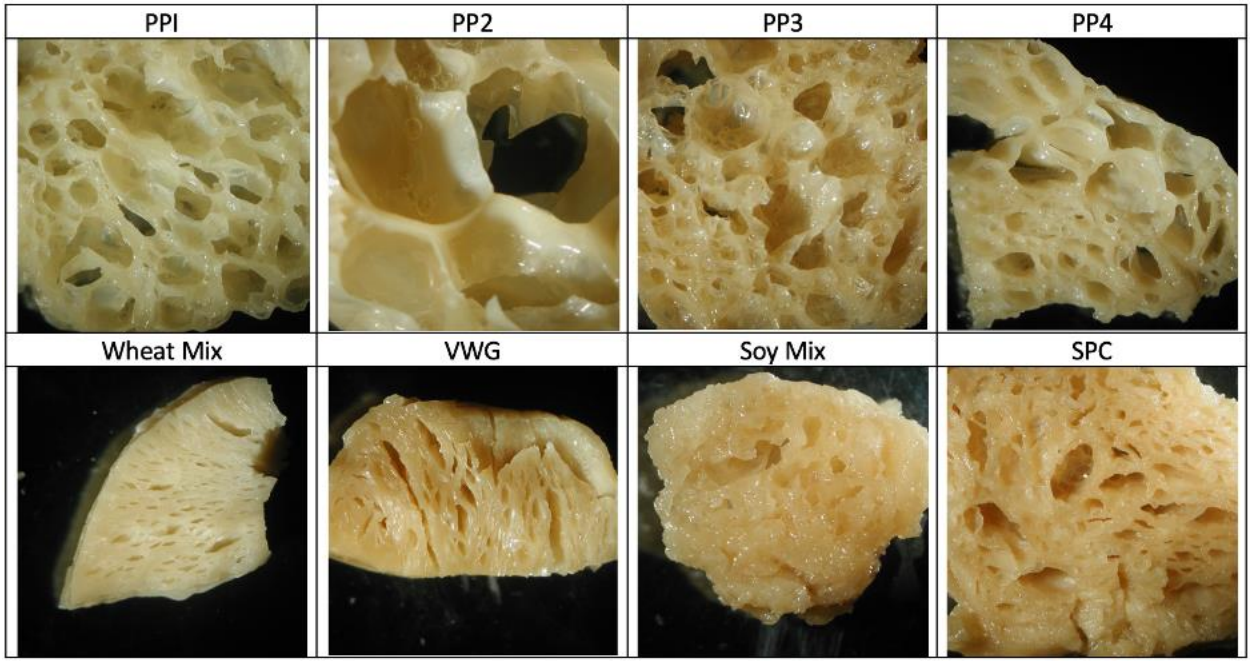

Figure 4.10 Horizontal cross sections of whole extrudate pieces. Horizontal cross sections are cut against the direction of flow from the extruder.

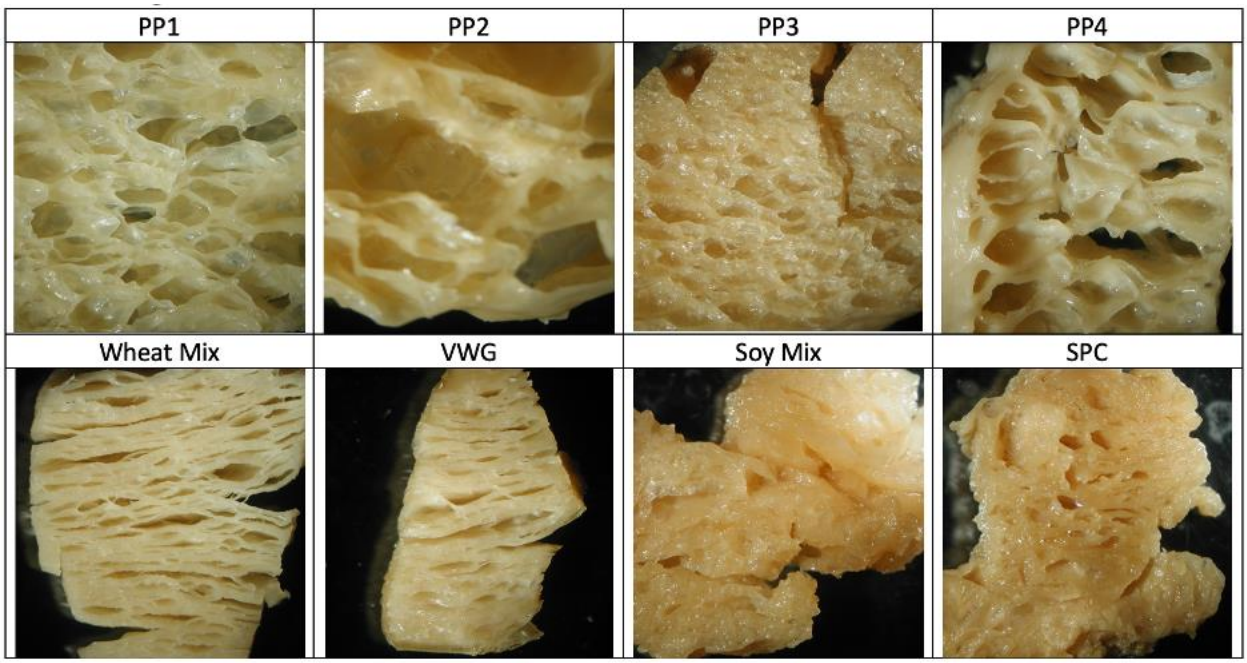

Figure 4.11 Longitudinal cross sections of whole extrudate pieces. Longitudinal pieces were cut along the direction of flow from the extruder

3.2.2 Bulk Density

Bulk density of PP1, PP2, and PP3 extrudates ranged from 58-65 g/L (Figure 4.12). While the densities were similar, the internal structures were very different, as described above. This shows that bulk density is a helpful guideline during extrusion, but it does not differentiate between types of internal expansion, i.e., air cells versus fibrous alignment. PP4 was much denser at 143 g/L and a similar density to the SPI/SPC (146 g/L). Even though the densities were similar, the PP4 has much larger air cells than the SPI/SPC extrudate. VWG had the highest density of all extrudates at 268 g/L. This extrudate was the most fibrous in nature, had the smallest and most elongated air cells, and thus the highest density.

Bulk density mirrored the overall trend of SME. A higher SME resulted in a denser product. Generally, with more energy input, starch-based extrudes cook and are able to expand more, creating lower bulk densities (de Mesa et al. 2009), but that trend is not seen with these protein extrudates. It may be that too much energy was being put into the wheat and soy treatments so as to degrade the protein, even with higher water input and a less restrictive die for soy. Too much degradation would result in the protein not having the ability to hold air within its structure and creating this higher bulk density.

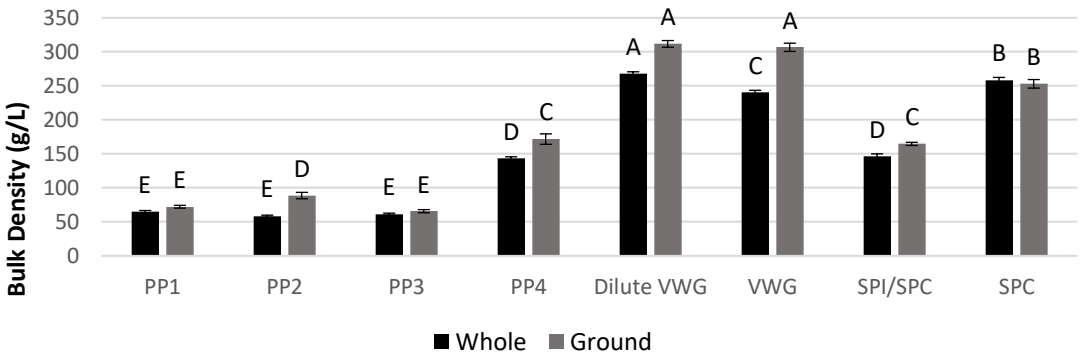

Figure 4.12 Average bulk density of whole extrudate and ground extrudate
Means of treatments in the same category denoted by the same letter above the bar are not significantly different.

3.2.3 Water Holding Capacity

Previous work has shown that the internal structure of extrudates and the bulk density have great impact on the extrudate WHC (Webb et al. 2020). The results in this study confirm this finding (Figure 4.13). With the highest density, VWG showed the least WHC. Extrudates with lower density resulted in higher WHC. Though the bulk densities of PP1, PP2, and PP3 were all similar, the WHC does show differentiation due to the internal structure. Of the three, PP2 has the largest and fewest air cells while also having the lowest WHC. PP1 has a greater number of smaller air cells in a more elongated manner which held more water than the other two extrudates. The soy treatment, which had similar bulk density to PP4, actually held significantly more water in its structure due to the small air cells rather than the large cells PP4 displayed.

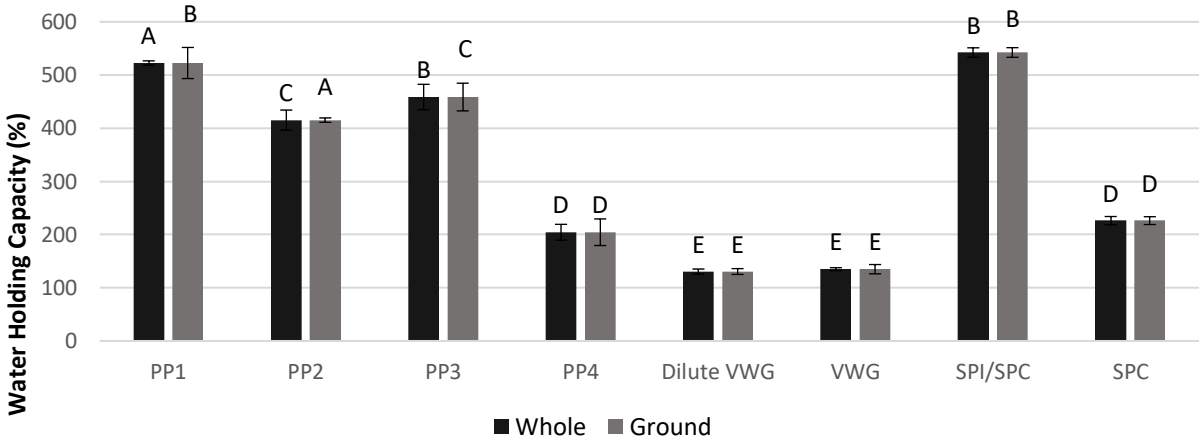

Figure 4.13 Water holding capacity (%) of whole extrudates and ground extrudates. Means of treatments in the same category denoted by the same letter above the bar are not significantly different.

3.2.4 Texture Analysis

Ground pea protein showed very low hardness compared to VWG and their hardness was also lower than soy. Compared to meat anchors, VWG was the closest in hardness in both

ground and pattied forms. PP1, PP2, and PP3 were all softer than PP4 in the ground form which could be attributed to the lower density for these extrudates and their porous structure. However, once binders were added, the PP2 gave a hardness similar to pork.

Chewiness for the textured proteins was substantially less than the meat anchors in both ground and patty forms. Hardness and chewiness of VWG resulted in high values and variation since TPA was conducted on a 20 g samples and it had a very high bulk density compared to the other textured proteins. This meant 20 g of VWG couldn't be standardized to the thickness the other treatments were (it was a thinner layer than the other treatments), resulting in the variation.

Springiness of the ground extrudates was about 10% greater than any meat anchor. PP4 and soy gave texture properties closest to the meat and commercial textured pea protein anchors.

Overall, the texture characteristics measured here were mostly similar for all pea proteins in the ground for and they did not vary in patty form, even though the pea protein extrudates varied greatly in internal structure, water holding capacity, and bulk density. However, this textural testing fails to account for mouthfeel which can critically change the perception of the extrudates and patties. Future sensory analysis is suggested to understand the mouthfeel differences in the products. Attributes such as juiciness, cohesiveness of mass, and geometrical properties (grainy, smooth, fibrous, lumpy, etc) are expected to vary among the pea proteins due to their different WHC and bulk densities. This information could help transfer the knowledge of protein differences and their processing to their final product applications.

4. Conclusion

The properties of protein isolates can vary widely and will result in different textured proteins after low moisture extrusion. Extrudate density and internal structure are very important to the final product texture, yet did not result in significant difference in hardness, springiness or

chewiness in patties. However, the raw ingredient source and functionality can assist in attaining the desired internal structure which may have more impact on sensory properties not studied in this research. Pea proteins are innately different from wheat and soy proteins and thus result in different extrudate properties. Protein characteristics such as WAC, denaturation energy and temperature, and LGC give helpful information to understand the proteins and the resultant products. In the future, this understanding of the proteins can be applied by tailoring extrusion processes to the protein functions and the desired extrudate.

Further understanding of protein viscosities and rheological characteristics and the relationships to other functionalities would give helpful insight to the impact of raw materials on extrusion processing and final product quality.

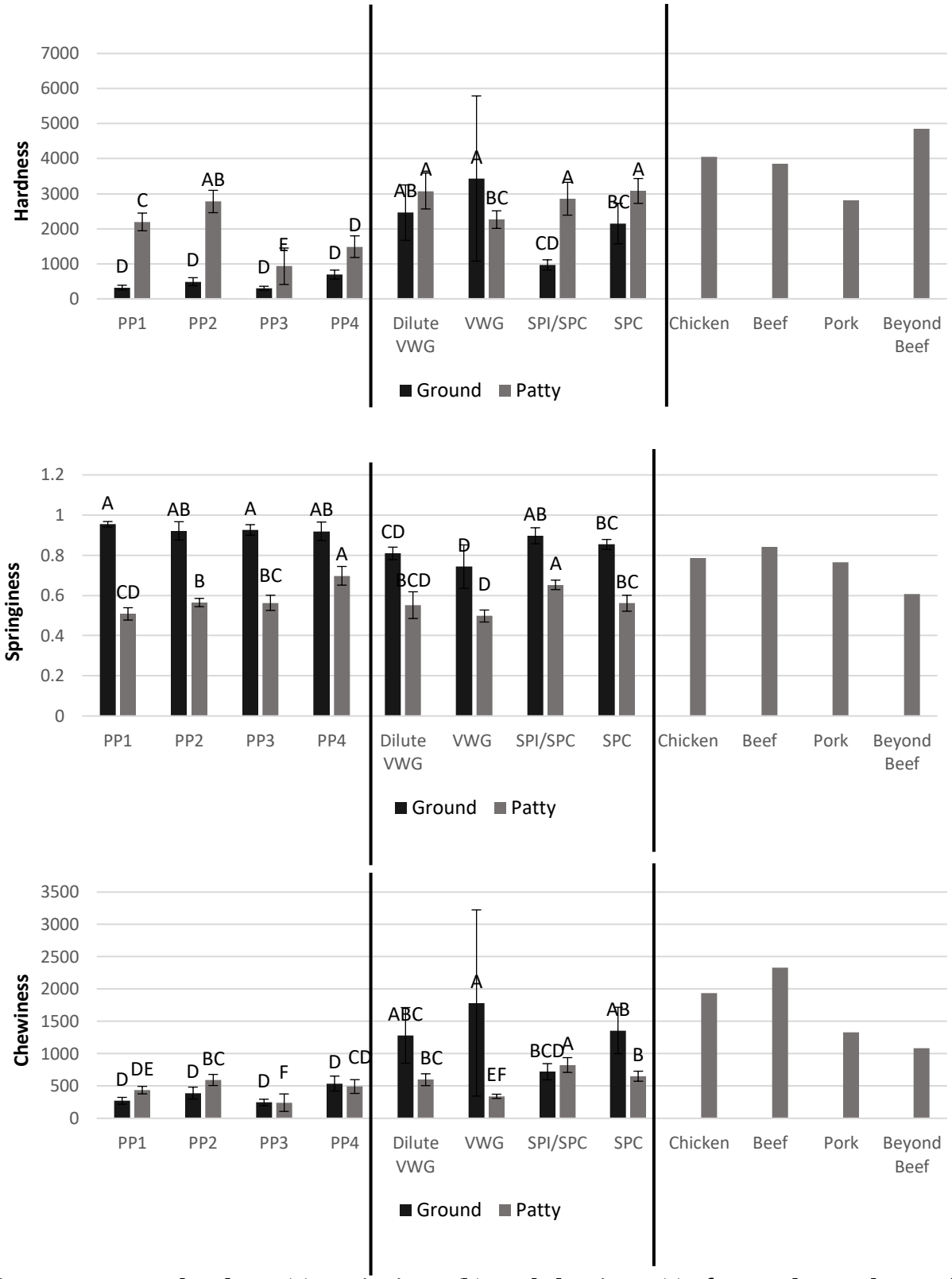

Figure 4.14 Mean hardness (a), springiness (b), and chewiness (c) of ground extrudate and patties made from the ground extrudate.
Means of treatments in the same category denoted by the same letter above the bar are not significantly different.

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Chapter 5 - Conclusions and Future Work

Raw ingredient source, composition, and pre-processing are crucial considerations for the extrusion of plant proteins for plant-based meat. The plant type, whether soy, wheat, or another legume, naturally gives different protein composition and functional traits. Starchy ingredient additions to the protein matrix create a dispersed phase that interrupt the protein phase and allows for the creation of protein layers. Adding fiber to this dispersed phase still interrupts the protein network, but not to the extent of starch alone, and this creates an internal structure of layers which interact—a fibrous internal structure.

Within one plant protein, pea protein, differences in texturization result as a consequence of previous processing. However, with processing steps unknown, it is difficult to make true correlations since same functional traits may be the result of different processing.

In the future of pea protein texturization, further study should be conducted on the rheology and understanding flow within the extruder. While RVA has attempted to explain some rheological properties, it lacks similar parameters seen in extrusion such as solids concentration, temperature, and pressure. Furthermore, a study which utilizes this information to tailor formulations and processes should be developed. Of specific interest would be optimizing the raw ingredient formulation to the streams of protein concentration or isolation in order to optimize the waste streams and resultant functionality. Ideally, as greater understanding of protein functionality and extrusion processing develops, a gold standard textured pea protein could be made from less isolated product, just as textured soy protein can be made from soy flour. The understanding of starch and fiber components in texturization investigated in this study give a great platform for the next steps in this work.