Techno-functional properties of pea protein TVPs and their function as meat extender in burgers

By

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Abstract

An immerging group of consumers are reducing their animal protein intake for environmental, health and animal welfare concerns. TVPs are plant proteins that have been processed to have a fibrous, meat like structure. Majority of TVPs are made from soy and wheat, which can restrict their usage, as soy and wheat carry priority allergen status. This study explored the potential of pea protein based TVPs. In this thesis, the impacts of protein blend formulas (70, 76 and 82% protein, d.b.), screw speed (350, 400, 450 rpm) and feed moisture content (38 and 42% water, d.b.) on nitrogen solubility index and techno-functional properties of raw protein blend formula and TVPs were explored. Increasing the raw material PBF resulted in lower nitrogen solubility index, but increased water holding capacity, emulsion capacity and emulsion stability. After extrusion, the raw material nitrogen solubility index reduced, while oil absorption capacity increased. The TVPs themselves reduced in nitrogen solubility index but increased in hydration time and integrity index as protein blend formula increased. Nitrogen solubility index had strong correlations with hydration time (r=-0.97) and integrity index (r=-0.81), which suggest higher protein blend formulas raises texturization levels. Feed moisture content and screw speed did not have any distinguishing trends on TVP techno-functional or physical properties. Furthermore, a select group of TVPs (i.e., 70, 76, 82% protein, protein blend formula, 38 and 42% water, feed moisture content, at 400 rpm) were tested as meat extenders (20, 30 and 40% w/w extension levels) in ground beef burgers. Increasing the addition of TVP (i.e., extension level) reduced burger total cooking loss but did not impact burger size retention or textural quality attributes. The lower PBF resulted in lower total cooking loss, hardness and chewiness. The 70% protein blend formula extended burgers had similar textural qualities to the negative control. The 70% protein blend formula and 42% feed moisture content have the

greatest potential as a meat extender because they reduced total cooking loss but maintained similar textural quality attributes to the negative control. The lower protein blend formulas TVPs are ideal for industry, as higher protein ingredients increase expenses.

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Preface

The thesis is written in a manuscript style that comprises 6 chapters. Chapter 1 is the introduction of the study, and Chapter 2 is a literature review that covers the background information to set the foundation for the elements researched throughout the thesis. Chapter 3 and 4 are research sections, which are manuscript style written chapters. Chapters 3 was prepared for journal publication with the authorship of Elyssa Chan, Argenis Rodas-Gonzálas, Mehmet Tulbek and Filiz Koksel. Chapter 4 was prepared for journal publication with the authorship of Elyssa Chan, Nasibeh Sinaki, Argenis Rodas-Gonzálas, Mehmet Tulbek, and Filiz Koksel. Chapter 5 summarizes the conclusion, limitations and future recommendation.

Contribution of Authors:

Chapter 3, "Effects of pea protein formulation and extrusion processing parameters on techno-functional properties of pea protein" was prepared for journal publication, authored by Elyssa Chan, Argenis Rodas-Gonzálas, Mehmet Tulbek and Filiz Koksel. Elyssa Chan was responsible for the conduction of research and the manuscript draft. Argenis Rodas-Gonzálas gave critical advice regarding the statistical analysis, while Mehmet Tulbek guided the extrusion processing methods used. Filiz Koksel was the corresponding author and oversaw the research design and the reviewing of the manuscript. All authors were agreed on the final version of the manuscript.

Chapter 4, "Impact of pea protein blend formulations and extrusion conditions on the physical properties of TVPs in extended beef burgers" was prepared for journal publication with authorship by Elyssa Chan, Nasibeh Sinaki, Argenis Rodas-Gonzálas, Mehmet Tulbek, and Filiz Koksel. Nasibeh Sinaki kindly prepared the SEM images. Argenis Rodas-Gonzálas informed the burger cooking quality analysis, while Mehmet Tulbek guided the physical properties studied.

Filiz Koksel was the corresponding author and was in charge of the research design and the reviewing of the manuscript. All authors were agreed on the final version of the manuscript.

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List of Abbreviations

AACCI	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
CBD	Change in burger diameter
d.b.	Dry basis
EC	Emulsion capacity
eq	Equation
ES	Emulsion stability
FMC	Feed moisture content
FP	Forward paddles
HT	Hydration time
IBT	Increase in burger thickness
II	Integrity index
LS	Lead screw
NSI	Nitrogen solubility index
OAC	Oil absorption capacity
PBF	Protein blend formula
PPC	Pea protein concentrate
PPI	Pea protein isolate
SS	Screw speed
TCL	Total cooking loss
TSP	Texturized soy protein
TVP	Texturized vegetable protein
w.b.	Wet basis
WHC	Water holding capacity

Chapter 1: Introduction

Consumers are steadily growing aware of the environmental impacts of the food system, specifically animal production. In conjunction with personal health and animal welfare concerns (Kumar et al., 2017), many consumers are actively reducing their animal protein consumption and are turning to plant protein (McClements, Weiss, Kinchla, Nolden, & Grossmann, 2021). Increasing the consumption of plant protein reduces the risk of cardiovascular disease, colon cancer and type 2 diabetes (Health Canada, 2019). For these health benefits, Canada's dietary guidelines officially recommends regular plant-based protein consumption over animal-based protein (Health Canada, 2019). Despite plant protein sources (e.g., pulses and beans) being widely available, consumers are resistant to changing their dietary habits and desire familiar products (Figueira, Curtain, Beck, & Grafenauer, 2019; Hoek et al., 2011).

Innovative companies are producing a wide variety of plant protein products that mimic popular animal protein products (i.e., meat analogues), such as burgers, meatballs, sausages, and chicken (Beyond Meat, 2021; Impossible, 2022; Lightlife, 2022). For consumer acceptance, meat analogues must seamlessly substitute their animal counterparts by fulfilling consumer expectations (i.e., shape, appearance, texture, flavour) (Elzerman, Hoek, Boekel, & Luning, 2011). To imitate the chewy texture of meat, many meat analogues use texturized vegetable proteins (TVPs) (Dekkers, Boom, & Jan van der Goot, 2018; Samard & Ryu, 2019a).

Despite having several definitions (Riaz, 2011; Shurtleff & Aoyagi, 2016; USDA, 1971), TVP is a trade-mark term for texturized soy protein, produced by Archer Daniel Midland (Riaz, 2011). However, the term TVP has evolved and is used to describe the growing group of plant-protein based products that have a fibrous structure, similar to animal protein (Asgar, Fazilah, Huda, Bhat, & Karim, 2010; Kumar et al., 2017; J. Zhang et al., 2018). TVPs can mimic whole muscle, ground and comminuted meat products (Dekkers et al., 2018; Samard & Ryu, 2019a; Sha & Xiong, 2020). TVP's popularity as a meat analogue is relatively recent. TVPs were initially used as a meat extender (Maningat, Jeradechachai, & Buttshaw, 2022).

Meat extenders are non-meat ingredients added to animal-protein based products to reduce formulation cost and improve the final product's techno-functional (i.e., water holding capacity, emulsifying properties) and physical (i.e., total cooking loss, shape retention) properties (Edmondson & Graham, 1975; Mills, 2014). As TVP's fibrous texture is similar to animal protein, it can be incorporated at higher levels (10-40% extension level) without detracting overall product texture (Bakhsh, Lee, Lee, Hwang, & Joo, 2021). Consumers have previously viewed extended animal products as being a lower quality product (Feiner, 2006b). However, producers like BUMP are reframing the use of TVP extenders. BUMP embraces and advertises their application of TVP extenders in their ground beef and pork sausages, as a means to reduce the environmental footprint and improve nutritional value, without scarifying taste (BUMP, 2022). TVPs' characteristic fibrous texture is commonly developed using extrusion processing (Alam, Kaur, Khaira, & Gupta, 2016; Dekkers et al., 2018).

Extrusion processing is an affordable and efficient processing method (Fellows, 2017). Extrusion cooking is a continuous process that combines mixing, shearing, pressurizing, heating and forming to create numerous products (e.g., breakfast cereal, pasta, pet foods, TVPs) (Fellows, 2017). The extruder imparts high shear and heat to the starting materials. Together, heat and shear denature and unravel the starting material's proteins, degrade starches into a paste, and integrates the starting material components (i.e., protein, starch, dietary fiber, fat) into a homogenous melt (Verbeek & Van den Berg, 2010). The mobile denatured proteins align into a laminar structure, and form disulfide and hydrogen bonds (Liu & Hsieh, 2008; J. Zhang et al., 2018). Upon exiting the extruder, the melt expands, which leads to the final fibrous and porous TVP structure (J. Zhang et al., 2018). To enable texturization, the raw material must have sufficient protein content. Proteins form disulfide bonds, hydrophobic interactions and hydrogen bonds, which are the basis for TVP structure formation (Liu & Hsieh, 2008; J. Zhang et al., 2018).

The most prominent TVP starting materials used are soy and wheat protein (Kumar et al., 2017). The popularity of these starting materials are from their ample availability, affordability, desirable techno-functional properties, and their ability to texturize (Riaz, 2011). Despite these beneficial qualities, soy and wheat are falling out of favour because of their priority allergen statuses (Government of Canada, 2019) and soy's GMO connotations (Vatansever, Tulbek, & Riaz, 2020). Therefore, alternative ingredients are being explored.

Several studies have investigated the use of alternative staring materials, including mung bean protein, black gram by-product, peanut protein and blends of chickpea flour and pea protein, and pea protein (Brishti et al., 2021; Kamani, Luithui, & Meera, 2021; Samard & Ryu, 2019b; Webb et al., 2020). Pea protein is a promising alternative to soy and wheat protein, due to its ample availability, techno-functional properties, and lack of allergenicity or GMO connotations (Maningat et al., 2022). Pea protein based TVPs have demonstrated promising techno-functional and physical characteristics in previous studies (Samard & Ryu, 2019b; N. Wang, Bhirud, & Tyler, 1999). The application of pea protein in TVPs is reflected in industry, as numerous companies, such as Roquette, AGT Foods and Ingredients, Puris, and ETprotein produce and sell pea protein based TVPs (AGT Foods, 2022; ETprotein, 2022; Puris, 2022b; Roquette, 2022) or use pea protein based TVPs in their product (e.g., Beyond meat, Lightlife, BUMP) (Beyond Meat, 2021; BUMP, 2022; Lightlife, 2022).

The objectives of the thesis are (1): Investigate the impact of raw PBF (70, 76 and 82% protein (d.b.)) on its techno-functional properties (i.e., PDI, WHC, OAC, EC and ES); (2) Assess the influence of PBF and extrusion processing parameters (i.e., 350, 400 and 450 rpm; 38 and 42% FMC) on TVP techno-functional (i.e. PDI, WHC, OAC, EC, and ES) and physical properties (i.e., HT and II) and (3) Characterize the impact of TVP parameters (i.e., PBF and FMC) and extension level (i.e., 20, 30 and 40%) on burger cooking properties (i.e., TCL, CBD, IBT) and textural quality properties (i.e., hardness, cohesiveness, springiness and chewiness).

Chapter 2: Literature Review

2.1. Textured Vegetable Protein (TVP)

Textured Vegetable Protein (TVP) is a term trademarked in 1970 by Archer Daniel Midland Co. (ADM) to describe their textured soy products. Later, the term TVP has become synonymous with all textured vegetable products (Riaz, 2004, 2011). In dry form, TVPs do not necessarily mimic the appearance, texture or mouth feel of meat (Riaz, 2004); however, when hydrated, cooked and/or retorted, they have a fibrous, meat-like structure. It is expected for TVPs to maintain their structural integrity and texture throughout processing (Riaz, 2011). There are numerous forms of TVPs, such as chunks, minced and flakes (Riaz, 2004). Flavouring and colourants may also be added to suit specific product applications (Endres, 2001). TVP products fall into the plant-based meat market category; meaning TVPs fulfill the same purposes of animal proteins (Good Food Institute, 2020).

2.1.1. TVP History

The first vegetable protein meat analogue was introduced in 1879 by Dr. John Kellogg in Battle Creek Michigan, USA (Edmondson & Graham, 1975). His meat analogue was made from fresh wheat gluten, and mimicked veal steaks. Despite creating the industry, Kellogg never commercially produced his wheat gluten meat analogues because it lacked a market base (Robinson, 1972; Shurtleff & Aoyagi, 2016). The first record of transforming soy proteins into a fibrous structure was done by Harold Lundgren (1949). Soon after, Robert Boyer submitted a patent in England for simulated meat products that mimic the flavour, colour, taste and 'chewiness' of meat (Shurtleff & Aoyagi, 2016). Boyer also published a patent in America for producing an edible fibrous protein structure, by spinning vegetable proteins (United States Patent No. 2,682,466, 1954). TVPs were not commercially accessible until the 1960's, when production was scalable. In 1960, Boyer joined Ralston Purina as a protein scientist. Using

Boyer's American patent, Ralston Purina launched Textured Edi-Pro in 1962. This was the first commercially produced spun soy protein fiber product. A few years later, General Mills and Worthington foods introduced their spun soy protein fiber meat analogues, which resembled bacon bits and chicken, respectively (Shurtleff & Aoyagi, 2016). Worthington Foods is considered a pioneer for the development of TVPs, especially those produced from spinning soy protein fibers. In 1966, ADM and H.B. Taylor Co., both from Illinois, USA and Hayes Ashdod Ltd from Ashdod, Israel, launched their respective TVP products. Similarly, all companies achieved texturization using spinning fibers (Ralston Purina, General Mills and Worthington Foods), or extrusion technology (ADM, H.B. Taylor Co., and Hayes Ashdod Ltd), and advertised their TVP products as a meat extender or meat analogue (Shurtleff & Aoyagi, 2016). TVP produced from extrusion processing gained significant popularity in the 1970's (Shurtleff & Aoyagi, 2016) and is now the most common texturization method (Riaz, 2011).

2.1.2. International TVP Production

2.1.2.1. Soy TVP

TVPs are produced internationally (Riaz, 2004) by numerous companies. Although based in America, ADM is an international company who produces numerous products, including soy based TVP and crumbles (TVC®) (ADM, 2022c). Cargill, an international competitor, produces textured soy proteins trademarked as Prosante® textured soy flour (Cargill, 2022b). Furthermore, textured soy protein and textured soy flour products are also produced internationally by Dupont™ Danisco® (Dupont, 2022).

Soybean proteins in the forms of soy flours, concentrates and isolates (Pearson, 1976) are internationally the largest ingredient source for producing textured protein products. Soybean popularity is stemmed from its high protein content, ample availability, and inexpensive cost (Riaz, 2004) and superior techno-functionality (Singh, Kumar, Sabapathy, & Bawa, 2008a).

Defatted soy flour contains approximately 47% protein, 38% carbohydrates, 7% moisture, 6% ash and 1% fat (Health Canada, 2018a), while soy concentrate is composed of approximately 58% protein, 30% carbohydrates, 6% moisture, 5% ash and <1% fat (Health Canada, 2018b). Soy protein isolates contain approximately 81% protein, 10% carbohydrates, 5% moisture, 4% ash and <1% fat (Health Canada, 2018c). Furthermore, soy protein concentrates and isolates are capable of numerous techno-functional properties, including emulsion capacity, water holding capacity, and gelation, which are essential for meat extension applications (Mills, 2014).

Despite soy's prevalence in the TVP industry, it has limitations. The greatest concern is soy's priority food allergen status in Canada. Consumers who are allergic to soy must completely avoid it, or otherwise face detrimental health effects (Food Allergy Canada, 2022). Additionally, soy is also associated with genetic modification, and therefore less preferred by some consumers(Zhang, Fan, Chen, Cao, & Pu, 2021).

2.1.2.2. Wheat TVP

Wheat is the most common cereal ingredient used in TVP production. Wheat protein may be used to create a soy-free TVP or used in combination with soy protein (Riaz, 2011). Beneo and Loryma are both German companies who produce textured wheat proteins within Europe. Both companies offer wheat protein TVPs in chunk and flake forms (Beneo, 2022; Loryma, 2022). Within America, MGP produces textured wheat protein, in addition to a variety of fiber, protein and starch ingredients (MGP, 2022).

Wheat protein is a common ingredient in TVPs because it is comparatively less expensive than soy protein (Kumar et al., 2017). Approximately 85% of wheat protein is comprised of gluten (Day, 2011). Gluten proteins have two fractions, glutenin and gliadin, and together they enable a unique elastic and extendible structure (Shewry, Halford, Belton, &

Tatham, 2002). Gluten's physical and techno-functional characteristics easily lend themselves to form thin fibrous filaments during processing. These fiber bundles closely mimic meat structure and texture (Day, 2011). Although wheat protein has numerous assets, it also has several concerns.

Celiac disease is an autoimmune disorder which causes diarrhea, emaciation, aphthous stomatitis and malabsorption to those impacted. Approximately 1% of the global population (both children and adults) have celiac disease (NIDDK, 2020). Those with celiac disease must completely avoid any gluten intake (Health Canada, 2017). Additionally, consumers have been trending away from products containing gluten. The trend is fuelled by the belief that gluten causes adverse health effects, such as weight gain and arthritis, to consumers (Nash & Slutzky, 2014). Due to these restrictions, producers have begun developing TVPs made of alternative ingredients.

2.1.2.3. Pea TVP

TVPs made from pea protein have surged to popularity, especially in Europe. European companies, AM Nutrition and Nakskov Mill Foods, from Norway and Denmark respectively, created a joint venture, NISCO (based in Denmark). NISCO produces textured pea protein concentrate suitable for meat extension (NISCO, 2022). Textured pea protein is also produced in Norway, by Vestkorn (2022b), and in France by Roquette and Union Française d'agriculture biologique (UFAB) (Roquette, 2022; UFAB, 2022). In China, ETprotein produces flaked and granular forms of textured pea proteins (ETprotein, 2022). Pea based TVPs are produced in America as well, by A&B Ingredients, AGT Foods and Ingredients, MGP and Puris^{TM} (A&B Ingredients, 2022; AGT Foods, 2022; MGP, 2022; Puris, 2022b).

2.1.2.4. Other plant protein TVP

TVPs are also being developed from additional sources. Fava bean, for instance, is an emerging TVP ingredient. Norwegian company, Vestkorn, and French company, Roquette UFAB, are currently producing textured fava bean protein (UFAB, 2022; Vestkorn, 2022b). Roquette announced in December 2019 that they are expanding their textured vegetable protein line to include a fava bean protein TVP as well (Roquette, 2019). American company, AGT Foods and Ingredients, is also producing a fava bean TVP, in addition to a lentil protein TVP (AGT Foods, 2022).

2.1.3. International TVP Market

The plant-based meat market is experiencing a significant growth in America. In 2021, the American plant-based meat category was worth $1.4 billion USD. This was equal to 83% three year dollar sales growth (i.e., 2018 to 2021). Interestingly, there was 19% growth from 2018 to 2019, 46% growth between 2019 to 2020, but 0% growth from 2020 and 2021. GFI contributes the lack of growth from the pandemic causing unusual retail activity, paired with ingredient shortage and supply chain disruptions (Good Food Institute, 2021). Furthermore, the plant-based meat products are primarily frozen (59%), followed by refrigeration (40%) and shelf stable (1%), such as TVPs, (Good Food Institute, 2021). In 2019, dollar sales of shelf stable plant-based meat, such as TVPs, were worth $10 million USD. Compared to the previous year, the dollar sales experienced more than 30% growth (Good Food Institute, 2020). Internationally, the 2019 TVP market revenue was worth $1.1 billion USD. By 2029, it is predicted that the valuation will reach $2 billion USD, equalling a compound annual growth rate of ~6% (Transparency Market Research, 2020).

2.1.3.1. TVP Consumers

Within the agricultural supply chain, manufacturers often sell their products to other companies. This is known as a business to business transaction (Chen, 2020). TVPs produced by the previously mentioned companies target other manufactures to use their product as an ingredient in their formulations. Businesses may also directly sell to the end user, known as a business to consumer transaction. This method is increasingly common as online retailing has surged in popularity (Kenton, 2019).

TVP consumers are primarily in North America and Europe (Markets and Markets, 2018; Transparency Market Research, 2020). ADM specifies their textured soy products are sold throughout Brazil, Europe, Africa and the Middle East (ADM, 2022b, 2022a). Cargill also markets their textured soy protein in North America, Asia Pacific, Middle East, Turkey and Africa (Cargill, 2022a).

2.1.3.2. TVP Application

TVP is used in the processing industry to provide several benefits. Firstly, texturizing plant proteins develops a unique texture, making it suitable for various applications, including meat alternatives, meat extenders and snack foods (AGT Foods, 2022). TVPs are also relatively inexpensive, and can decrease overall product cost (Kumar et al., 2017). They are also capable of modifying the product’s functional and sensory characteristics. For instance, as a meat extender, TVPs can increase the oil absorbance and water holding capacities, resulting juicer products such as sausages and burgers (Riaz, 2006). Furthermore, TVPs are high in protein, contain minimal fats and lack cholesterol, making them nutritionally appealing (Riaz, 2004), and attractive for many consumers (de Bakker & Dagevos, 2012).

Consuming high levels of meat and saturated fat contributes to chronic conditions, such as cardiovascular disease and diabetes (Walker, Rhubart-berg, Mckenzie, Kelling, & Lawrence, 2005). Therefore, Canada recommends consuming plant based proteins more frequently than animal based ones (Health Canada, 2019). The incorporation of TVPs can provide high protein and low fat for health-conscious consumers (Riaz, 2011). Consumers are becoming more engaged and conscious of the social and environmental impacts of their food as well (Hartmann & Siegrist, 2017). The movement towards the flexitarian diet (i.e., the conscious decision to reduce of meat from the diet, however, not completely abstain from it) is a growing response from consumers to address these concerns (de Bakker & Dagevos, 2012). Companies like BUMP are targeting these consumers, by advertising their usage of TVPs in their beef or pork based products, to reduce their environmental footprint, without forfeiting taste (BUMP, 2022). Therefore, TVPs pose as a suitable protein supplement or meat substitute for ethically conscious consumers (Kumar et al., 2017).

Industrial and consumer applications of TVPs are broad. For instance, ADM advertises their textured soy proteins to be utilized in meat alternatives, nutrition bars, snacks and cereal products (ADM, 2022c). Roquette claims that their textured pea protein can be applied in baked goods, such as biscuits and cookies, breakfast cereals and cereal bars, and in processed meat and seafood products (Roquette, 2022). Bob's Red Mill (an American company based in Oregon) provides TVP based recipes for their consumers, including TVP tacos, burgers and xiao long bao (Bob's Red Mill, 2022). Furthermore, many producers in the plant-based meat space produce TVPs inhouse to use exclusively in their own products (Beyond meat, personal communication, 2022). Of these applications, the largest market segment for TVP use is meat extension (Riaz, 2004).

2.2. Meat extension

CFIA defines meat product extender as “a food that is a source of protein and that is represented as being the purpose of extending meat products” (CFIA, 2018). Meat extenders must be high protein ingredients and are added to meat systems to reduce product cost (FAO, n.d.; Feiner, 2006a), improve techno-functional properties and product nutrition (Mills, 2014). By enhancing techno-functional properties, such as water holding capacity and oil absorbance capacity, extenders can improve product’s physical and sensory characteristics (Singh et al., 2008). For example, meat products with extenders can increase product structural integrity, decrease cooking loss and decrease shrinkage, relative to without extender usage (Lecomte, Zayas, & Kastner, 1993).

Extender composition can vary, however, they are primarily composed of proteins, followed by carbohydrates (starch and dietary fiber), and low amounts of fat (Mills, 2014). Depending on the ingredient and what techno-functional and physical characteristics needed, an extender may be primarily proteins with low amounts of starch, or a nearly even ratio of proteins and starch (FAO, n.d.). The proteins and/or starches in extenders enable strong gelation. Gel matrices are 3D networks of proteins and/or starches held together by Van der Waals, electrostatic and hydrogen bonds. Within this matrix, fine emulsified meat particles, water and fat are trapped during cooking (Sanjeewa, 2008). When protein gelation properties of an extender are improved, so are product yield and texture (Pietrasik, Jarmoluk, & Shand, 2007). Proteins are also emulsifiers, which further stabilizes the meat emulsion (Damodaran, 1997). Starches can absorb ample amounts of water and create a heat set gel. Starch gelation improves water binding capacity and cooking yield (Comer, 1979).

2.2.1. History of TVPs as meat extenders

Beginning in the 1970's, food prices, specifically meat, were rising in America. As commercial TVP production was timely introduced in the 1960's, TVPs were an opportunity to offset growing food prices. In 1971, the USDA approved textured protein products for federal reimbursement credit used within school lunch programs (Shurtleff & Aoyagi, 2016). American school lunch programs were permitted to replace up to 30% of meat, with hydrated TVPs. TVPs were further proven suitable for school lunch programs as they can be fortified with additional nutrients to ensure they met nutritional specifications (Shurtleff & Aoyagi, 2016). School lunch programs used an estimated 10.4 million kg of TVP from 1971-1972 (Edmondson & Graham, 1975). At that time, ground beef prices were also rising. In response, retailers created extended ground beef with 23-27% TVP to reduce prices. Blended ground beef took over 30-35% of the ground beef market the following year (Degner, Nichols, & Branson, 1974).

2.2.2 Canadian standards and guidelines for meat extenders

There is a set of Canadian government regulations known as standard of identities. These standards indicate the requirements for a product to be considered a specific food item. The regulations for minimum protein content and maximum fat content are dependent on the product. Meat extenders must contain a minimum of 16% total protein and a protein rating (Protein rating = protein in a reasonable daily intake × protein efficiency ratio (PER)) of at least 40 in a rehydrated state (Government of Canada, 2020). Protein ratings are used by the Canadian food regulations to assess overall protein quality. A rating of 40 indicates that the meat extender is considered an excellent source of protein (Bender, 2006). The extender must also meet Food and Drug Regulations for vitamin and mineral nutrient content (CFIA, 2018).

2.2.3. Extended meat burgers

Although the origins of meat burgers and meat patties are unknown, it is generally accepted that the first hamburger was invented in USA. The standard USA burger is 112 g and is popularly made with beef (Feiner, 2006b). In Canada, meat burgers and meat patties have different standard of identities. Meat burgers (uncooked) must contain fresh boneless meat and/or fresh mechanically separated meat, and may contain fillers, sweetening agents, flavour enhancers and water. Meat patties (uncooked) however, must contain fresh boneless meat and may contain seasonings, salts and spices (CFIA, 2018). Meat burgers (uncooked) require meat protein to meet or exceed 11.5% and the total protein must equal or exceed 13%. In contrast, meat patties must contain a minimum of 15% protein (from meat products) and a minimum 16% total protein. Meat burger and meat patties fat content restrictions are not specified (CFIA, 2018). Generally, meat burgers contain 25-30% fat and animal fats are essential for their functional, sensory and nutrition qualities (Feiner, 2006b). Regardless of other additives used, the main component of meat burgers is lean meat (Feiner, 2006b).

2.2.3.1. Lean Meat

Lean meat is comprised of water (~70-75%), crude protein (~19-23%) and fat (~3-5%). The exact composition is dependent on the animal species, age and sex (Kijowski, 2001). Accordingly, the techno-functionality of the lean meat is also dependent on animal species, sex, and age, as well as processing treatments (Kijowski, 2001). From the animal tissue, lean meat's protein is skeletal muscle. Skeletal muscle is made from myofibrillar (55-60%), sarcoplasmic (30-35%) and stromal proteins (5-15%) (Jiménez Colmenero, 2014; Kijowski, 2001). Myofibrillar proteins are salt soluble proteins whereas sarcoplasmic proteins are water soluble. Water soluble proteins, such as sarcoplasmic proteins, can also be extracted with low-ionic solutions. Stromal proteins are insoluble connective tissues (Jiménez Colmenero, 2014). Among these proteins, myofibrillar proteins are essential for meat burgers, as they are effective emulsifying agents, thus enable product cohesion (Feiner, 2006c). These proteins are also crucial for water holding capacity. Like other proteins, the techno-functionality of myofibrillar proteins are impacted by processing conditions (i.e., temperature) and other additives present (i.e., salt and phosphate), which can ultimately change the animal protein's pH and ionic strength (Xiong, 2014).

2.2.3.2. Meat extenders

Selecting a meat extender is dependent on the specific techno-functional properties needed, product compatibility and cost (Mills, 2014). Generally, TVPs are a preferred extenders for coarsely chopped meat products, like meat burgers, because they have a meat like appearance and mouthfeel (Singh et al., 2008b). TVPs can generally absorb double to triple their dry weight in water, and thus enhance burger juiciness (Riaz, 2004). In meat burgers, hydrated TVP is typically used to replace the meat and fat, however, only substituting the lean meat component can further economic benefits. TVPs may replace 20-50% of lean meat and fat (Feiner, 2006b). The added protein from the TVPs improves meat burger's structural integrity and dimension retention. With the use of TVPs, the overall meat burger nutrition is also improved because TVPs contain a high level of protein, and low-fat content. Therefore, by substituting the lean meat and fat components with TVP, the product's fat content is lowered (Lecomte et al., 1993).

Despite the added benefits of using higher extension levels, there are limitations. High extension levels cause the burger appearance to be negatively impacted because the meat patty colour is lightened. Using a caramel colourant additive can remedy this concern (Smith, Marshall, Branson, & Mnnke, 1976). The meat flavour is also diluted as extension level increases (Singh et al., 2008). Therefore, the level of meat extension used is partially limited by sensory results (Shen et al., 2022).

Meat extender starting material substantially impacts the TVP's techno-functional, physical, and sensory characteristics (Riaz, 2004). As previously mentioned, soy and wheat proteins are the dominant starting materials for TVPs. Both TVPs possess the necessary characteristics for meat extension, including water holding capacity, oil absorbance and a meat-like texture (Riaz, 2004). Although these TVPs have been extensively used as meat extenders, they are limited by their priority food allergen statuses (Government of Canada, 2019). Milk proteins, such as non-fat dried milk solids, casein and whey are also common meat extenders (Mills, 2014). Textured whey protein (TWP) is a novel extender, produced from extruding a combination of whey protein isolate (80% protein) and corn starch. Although the composition can vary, TWP has previously been composed of 48-64% protein and 20-40% starch (Walsh, Nam, Pettee, & Carpenter, 2008). TWPs have proven as effective meat extenders by reducing cooking loss and product shrinkage in beef burgers (Hale, Carpenter, & Walsh, 2002). TWP extended beef burgers have also demonstrated superior consumer sensory characteristics (i.e., appearance, texture, flavour, aftertaste and overall acceptability) relative to TVP extended beef burgers (Walsh et al., 2008). The drawback for TWP is that whey proteins are expensive relative to vegetable proteins (Feiner, 2006a; Mills, 2014). In addition, like soy and wheat TVPs, dairy derived ingredients are also priority food allergens in Canada (Government of Canada, 2019). By excluding consumers with dietary issues, the consumer base is decreased. With these restraints, other meat extenders, such as rice protein isolate, common bean flour and legume flours have been investigated (Dzudie, Scher, & Hardy, 2002; Sanjeewa, 2008; Shoaib, Sahar, Sameen, Saleem, & Tahir, 2018). Peas and pea ingredients have gained interest for meat applications because of their suitable techno-functional and nutritional properties (Tulbek, Lam, Wang, Asavajaru, & Lam, 2016).

2.3. Pea and pea protein

2.3.1. Pulses

Pulses belong to the leguminoseae family, commonly known as legumes (Singh, 2017). They are uniquely distinguished by their edible dried seed and low-fat content. FAO recognizes 11 types of pulses grown internationally, four of which are grown in Canada. Canada produces dry beans, chickpeas, lentils and dry peas (Pulse Canada, 2020). In 2018, Canadian pulse production was 6.33 million tonnes, and was composed of 57% dry peas, 33% lentils, >5% dry beans and <5% chickpeas (FAO, 2018). Due to drought conditions, the 2021-2022 production is expected to drop 45% (2.5 million tonnes) for dry peas, 37% (>1.0 million tonnes) for lentils, and 28% (352 thousand tonnes) for dry beans, and 70% (64 thousand tonnes) for chickpeas (Donihee & McDougall, 2022).

2.3.1.1. Composition of peas, pea protein concentrates and pea protein isolates

Dried peas are primarily composed of carbohydrates (60-65%), followed by proteins (23.1-30.9%), minimal lipids (1.5-2.0%) and contain slight amounts of vitamins, minerals and polyphenols (J. Boye, Zare, & Pletch, 2010; Ratnayake, Hoover, & Warkentin, 2002). Although peas contain several antinutrients (i.e., phytic acid, saponins and oxalates) (J. Boye et al., 2010; Ratnayake et al., 2002), they are still very nutritious since these antinutrients can be inactivated through various processing technologies, such as extrusion cooking (van der Poel, Stolp, & van Zuilichem, 1992). Although pea protein is not comprised of many sulphur-containing amino acids, it is an abundant source of essential amino acids, specifically lysine and threonine (Lampart-Szczapa, 2001). Arguably pea protein is the most valuable component of the dry pea (Sandberg, 2011). Therefore, great effort is taken into isolating the pea protein.

Pea protein comes in numerous forms, such as protein concentrates or isolates. Pea protein concentrates and isolates are differentiated by their protein content. Dijkstra et. al. (2003) states that protein concentrates have approximately 60-90% protein, where as protein isolates have higher protein levels (90% protein), however these values are not standardized. For instance, AGT Foods and Ingredients pea protein concentrate contains approximately ≤10.0% moisture, ≥55.0% protein, ≥2.0% starch, ≤15.0% dietary fiber, and ≤4.0% fat, while their pea protein isolate contains ≤10.0% moisture, ≥85.0% protein, ≥2.0% starch, ≥4.0% dietary fiber, and ≥8.5.0% fat (AGT Food and Ingredients, 2022). The protein concentration method is dependent on the protein level required. These methods are generally classified as dry and wet separation. Dry separation is used to create protein concentrates (<60% protein), while wet processing is used for higher protein (>60% protein) concentrates and isolates (Dijkstra et al., 2003). More recently, combinations of dry and wet concentration methods, as well as novel techniques such as electrostatic separation are also utilized for addressing environmental concerns (Schutyser & van der Goot, 2011; Thakur, Scanlon, Tyler, Milani, & Paliwal, 2019).

2.3.2. Pea production, pea protein processing and pea protein techno-functionality

2.3.2.1. Canadian pea production

The United Nations General Assembly announced 2016 as the International Year of Pulses (IYP). IYP’s purpose was to promote the importance of pulses for international food sustainability, food security and nutrition (FAO, 2016). Since Canada is an international leader of pea and lentil production, this campaign significantly impacted Canadian agriculture. In anticipation of IYP, Canada increased total pulse production (i.e., lentils, dry peas, beans and chickpeas) by 28% from 2015 to 2016 (Statista, 2019). After this campaign, Pulse Canada introduced their “25 by 2025” goal in early 2017. Pulse Canada intends to increase pulse production by 25% (equalling 2 million tonnes of additional pulses) to be introduced to new markets and use categories by 2025 (Pulse Canada, 2017). Therefore, total pulse production is expected to exceed 10.5 million tonnes by 2025.

Peas are grown and processed in the Prairie provinces (Alberta, Saskatchewan, and Manitoba) because they have ideal growing conditions (i.e., soil and climate) and industry infrastructure to produce and add values to peas (Field Guide Consulting & Strategic Vision Consulting, 2013). In fact, Canada has been the leading international dry pea producer since 2003 (FAO, 2018). In 2018, Canada produced 3.58 million tonnes of dry peas, equalling approximately 28% of the total global dry pea production (FAO, 2018). The second and third largest international producers are Russian Federation (2.30 million tonnes, equalling 18% of the total global dry pea production) and mainland China (1.53 million tonnes, equalling 12% of the total global dry pea production), respectively (FAO, 2018).

2.3.2.2. Pea protein processing

Pea protein processing is primarily done in China, Europe and North America. In 2017, China had seven pea protein producers, whose total processing capacity was 67,453 tonnes annually. As of 2019, approximately half of the international pea protein processing is housed in China, due to their high production volume capacity and low cost (Siu, 2019). In Europe, companies such as Cosucra, Emsland Group Roquette and Vestkorn process pea protein in Belgium, Germany, France, and Norway, respectively (Cosucra, 2022; Emsland Group, 2022; Roquette, 2022; Vestkorn, 2022a). There are also numerous American pea protein processors, such as AGT Foods and Ingredients, Anchor Ingredients, Ingredion and Puris (AGT Food and Ingredients, 2022; Anchor Ingredients, 2022; Ingredion, 2022; Puris, 2022a). Cargill has invested more than $75 million USD in Puris, in order to more than double Puris' pea protein production. Puris is North America's largest pea protein producer (Cargill, 2019).

Canada is positioned to become an international leader in pea protein processing. The largest Canadian pea protein producer is Nutri-Pea Ltd (Businesswire, 2018; NutriPea, 2020). Numerous companies, including Canadian Protein Innovations, Roquette, Merit Functional Foods and Verdient Foods Inc. have either announced, began building, or are operating multimillion-dollar pea protein fractionation facilities across the Canadian prairies. Roquette's facility will be the largest in not only Canada, but the world. The facility is expected to process up to 250,000 tonnes of peas annually (Pratt, 2017).

2.3.2.3. Pea protein processing methods

As mentioned previously, dry fractionation is used to develop pea protein concentrates. It is referred to as dry processing because water is not used. The pea protein and starches are separated by fractionating them with a dry mill, followed by air classification. When milled, the peas are ground to create pea flour. After milling, the starch granules are mostly intact coarse particles, whereas protein matrix is crushed into fine particles. As the starch and protein particles have different sizes and densities (Sandberg, 2011), air classification utilizes these differences to separate the protein and starch fractions. It is noted that there is still some adhesion of proteins within the starch granules and starches affixed to the protein matrix. By repeatedly milling and air classifying the pea flour, separation between protein and starch is improved (Reichert & Youngs, 1978). Dry processing is a cost-effective method to produce pea protein concentrates, however it is not effective enough to isolate as pure of a protein fraction as wet processing (Sandberg, 2011).

Wet fractionation is an effective method to separate protein and starch and to develop protein isolates. Depending on the conditions used, the protein level in an isolate can vary from 60-90% (Dijkstra et al., 2003). Wet processing relies on initially solubilizing the protein from milled peas using water, alkali or acid, which separates the protein fraction from the starch portion (Sandberg, 2011). Once the proteins are solubilized, the insoluble components are removed. The proteins are recovered by modifying the solubilized protein's pH to reach the protein's isoelectric point, thus cause protein precipitation or using ultra filtration, and collecting the proteins through centrifugation, sieving or spray drying (Dijkstra et al., 2003; Schutyser & van der Goot, 2011).

2.3.2.4. Pea protein techno-functionality

The techno-functionality of protein concentrates and isolates is impacted by intrinsic and extrinsic factors. For instance, intrinsic factors are from the protein itself, including the amino acid composition and sequence, protein size, ratio between hydrophobicity/hydrophilicity, protein conformation and protein reactivity. Extrinsic factors are external factors, which include the environment the protein is in. This includes pH, ionic strength, temperature and method of extraction significantly impact techno-functionality (Barać, Pešić, Stanojević, Kostić, & Čabrilo, 2015). For example, wet fractionation uses harsh conditions (i.e., pH and temperature) to separate the starch and protein components, and to dry the protein components into a powdered form. These processes can negatively impact ingredient functionality (Schutyser & van der Goot, 2011). Stone et al., (2015) compared the techno-functional properties of different protein sources (Table 1). Although pea protein isolates demonstrated slightly inferior techno-functionality relative to some commercial protein isolates, pea protein has great potential for numerous food applications (Barać et al., 2015).

Table 1. The comparison of numerous commercial protein isolates' techno-functional properties. Data excerpted from Stone et al. (2015).

Commercial Protein isolates	WHC (g/g)	OHC (g/g)	Sol (%)	FC (%)	FS (%)	EC (%)	ES (%)
Whey	CD[1]	1.4 ± 0.1	97.0 ± 0.9	276.7 ± 5.8	75.5 ± 2.6	210.4 ± 14.4	100.0 ± 0.0
Wheat	NM[2]	2.8 ± 0.0	0.7 ± 0.0	182.2 ± 10.2	49.2 ± 8.4	106.2 ± 0.0	24.7 ± 3.1
Soy	12.4 ± 0.3	1.8 ± 0.1	14.9 ± 0.8	171.1 ± 16.8	67.7 ± 3.0	172.9 ± 7.2	100.0 ± 0.0
Pea	3.1 ± 0.1	1.0 ± 0.0	5.0 ± 0.1	81.1 ± 17.1	27.1 ± 7.4	177.1 ± 7.2	80.7 ± 3.1

Abbreviations: WHC, water holding capacity; OHC, oil holding capacity; Sol, solubility; FC, foaming capacity; FS, foaming stability; EC, emulsion capacity; ES, emulsion stability.

[1] CD = completely dissolved.

[2] NM = not measurable, remained suspended in water as particulates (not dissolved).

2.3.3. Pea and pea protein market

2.3.3.1. Pea and pea protein consumers

Canada has been the leading international exporter of dry peas since 2003, and far exceeds any other country's dry pea exports. In 2017, Canada exported 3.24 million tonnes of dried peas, which is equivalent to 79% of the Canadian dry pea production and valued at $1.05 billion USD. The second and third leading dry pea exporters were Russian Federation and Ukraine, who exported 1.04 million tonnes and 561 thousand tonnes, respectively (FAO, 2018). That same year, the top 3 countries who imported dry peas were India (3.11 million tonnes worth $1.03 billion USD), China (1.29 million tonnes worth $432 million USD) and Bangladesh (379 thousand tonnes worth $155 million USD) (FAO, 2018).

Prior to 2017, Canada primarily exported their dry peas and lentils to India. When India implemented agricultural tariffs in 2017, trade between Canada and India decreased significantly (T. Der, personal communication, October 20, 2019). Bilateral discussions between Canada and India are currently underway to enable the accessible trade of agricultural commodities

(Government of India, 2018). Due to these barriers, China has emerged as Canada's top dry pea consumer. China adds value to dry peas by fractionating them to produce protein concentrates and isolates and incorporating them as primary ingredients for vermicelli noodle production and animal feed (T. Der, personal communication, October 20, 2019).

Canada primarily exports pea in their dry form, however the Canadian pea protein market is growing. In 2017, the Canadian pea protein market was valued at $6.29 million, and is projected to reach $11.03 million, with CAGR growth of 9.9% in 2023 (Businesswire, 2018). While it is not specified if the pea protein market growth is driven by the Canadian domestic market or from exports, the application of pea protein is expanding both in Canada and globally. The inclusion of pea ingredients in new products is mostly seen in the USA and the UK, followed by Canada and France (T. Der, personal communication, October 20, 2019).

2.3.3.2. International pea and pea protein application

2.3.3.2.1. Peas as livestock feed

Dry peas have traditionally been used towards the feed industry in North America and Europe (USA Pulses, 2020). For example, in Manitoba, peas have been used in animal feed, primarily for hog, poultry and cattle industries. Peas are used for feed in western Canada because they are an economical ingredient that can substitute imported soybean meal and corn from the USA. However, peas are not as popular in eastern Canada because they cannot economically compete with imported soybean meal and corn from the USA's mid-west. American soybean meal and corn is less expensive than Canadian dry peas because the transportation cost is lower (Government of Manitoba, 2011). Although dry pea is established for livestock feed, the human food market is emerging (USA Pulses, 2020). AgriMarketing Program invested approximately $5.8 million CAD in 2019, towards the diversification of the pulse market base by supporting

Pulse Canada and Canadian Special Crops Association (Pulse Canada, 2019). Canada has the unique opportunity to establish as the leading producer, exporter and user of pea products.

2.3.3.2.2. Pea ingredients in the food industry

The global food industry has embraced peas, specifically pea protein. The initial movement towards pea and pea ingredients in meat analogue products was initiated by the Canadian company, Gardein^{TM}. They were able to prove pea would be a cost effective and techno-functional ingredient for meat analogue products (T. Der, personal communication, October 20, 2019). As previously described, dry peas are processed to separate their components (i.e., protein, fiber, and starch) (Puris, 2022a). In 2018, the top 10 pulse ingredients used in internationally launched products included peas, pea protein, pea fiber and pea flour (T. Der, personal communication, October 20, 2019). Pea protein is especially popular, and internationally one of the fastest growing vegetable protein ingredients (Mintel, 2019; T. Der, personal communication, October 20, 2019). For example, meat substitutes, meat products and poultry products have increased the usage of pea protein ingredients (Mintel, 2019; T. Der, personal communication, October 20, 2019).

Pea protein industry has grown, due the demand for non-GMO and non-allergen products. Each company advertises their products' specific functional benefits and possible applications. For instance, Emsland Group states their pea protein has a neutral taste, high protein content, no sugar and high content of amino acids (e.g., lysine and arginine). Their pea protein is suitable for soups, sauces, dairy substitutes, nutritional supplements, meat substitutes and processed meats (Emsland Group, 2022). Vestkorn advertises their pea protein is an emulsifier with good water and fat holding characteristics, and resistant to heat. Vestkorn pea protein can be used as a gluten-free soy protein substitute in numerous products, such as biscuits, meat extenders, emulsified sausages, vegan spreads and to fortify breads and extruded snacks with protein (Vestkorn, 2022a). Roquette is unique because they have several lines of pea protein isolates, each with their own functional properties, lending to specific applications (Roquette, 2022). It is noted that Emsland Group, Vestkorn, and Roquette all state that their pea protein products are non-GMO, non-allergenic and gluten-free (Emsland Group, 2022; Roquette, 2022; Vestkorn, 2022a). These companies advertise their products towards other food processors. However, other produces such as Myprotein and Naked nutrition markets their products directly toward the health-conscious consumer. Both companies advertise their respective products vegan, dairy free and natural (Myprotein, 2022). Naked nutrition further advertises their products as gluten free, GMO free and soy free (Naked nutrition, 2022).

2.4. TVP Extrusion processing

Extrusion cooking technology is a versatile processing method used in food industry, commonly denoted as a high temperature short time (HTST) process (Berk, 2013). It is a one-step, continuous process that combines shear, heat and pressure. Extrusion's mechanical and thermal energy inputs alter the techno-functional and physical properties of food polymers (i.e., proteins and starches) to form numerous products (i.e., extrudates) such as puffed snacks, breakfast cereals, TVPs, etc. (Riaz, 2011; Wang, Bhirud, & Tyler, 1999). The measurement of mechanical energy that has transferred from the extruder's motor per unit mass of food material within the barrel is known as specific mechanical energy (SME, Wh/kg) (Della Valle, Berzin, & Vergnes, 2011). SME is calculated using the following equation (1):

$$SME = \frac{n \times \tau}{\dot{m}} \qquad (1)$$

where n is the screw speed, the number of screw rotations per minute (s^{-1}), τ is torque (N·m) and $\dot{m}$ is the mass flow rate ($kg \cdot s^{-1}$) of the starting material, i.e., the ingredients

(McCarthy, Rauch, & Krochta, 2012). Torque, and therefore SME, is the result of the material's apparent viscosity in the barrel which depends on extrusion parameters, including moisture content, screw speed and barrel temperature (Chen, Wei, Zhang, & Ojokoh, 2010; Osen, Toelstede, Wild, Eisner, & Schweiggert-weisz, 2014). These extrusion conditions can be optimized to produce unique products (Yacu, 2016).

2.4.1. History of extrusion

Prior to 1950, extrusion was used as a non-thermal process to mix and form products, such as pasta. Extrusion cooking was initially introduced using single screw extruders in the 1950s (Berk, 2013). Single screw extruders have one screw extending along the barrel. The screw and barrel surface generate friction with the material inside the barrel. The material moves along the barrel toward a small orifice (i.e., the die) at the end of the barrel. Based on its cross-section (i.e., circular, slit shaped, etc.), the die shapes the extrudate (Berk, 2013). In 1960s, single screw extruders were used to develop the first TVPs. Soon after, the twin screw extruder was introduced in the 1970s (Berk, 2013). These types of extruders are composed of a pair of intermeshed parallel pair of screws that extend along the barrel and rotate (co- or counter-direction) to move the material inside the barrel towards the die. Twin screw extruders can handle the processing of a wider variety of starting materials when compared to single screw extruders. For example, they can work at higher moisture and lipid contents, have superior feeding, mixing, heat transfer and pumping performance (Berk, 2013; Yacu, 2016), and their screw configuration (i.e., order of different screw elements) can be customized to form novel products.

2.4.2. Extrusion methodology

Initially, the starting materials are pretreated using a preconditioner to efficiently hydrate and heat the starting materials using hot water and/or steam. A schematic design of the preconditioning and extrusion process is illustrated in Figure 1. The moisture and heat are evenly distributed within the preconditioner, and thus the starting materials, by rotating paddles (Riaz & Rokey, 2011). The preconditioner feeds the preconditioned starting materials into the extruder feed hopper.

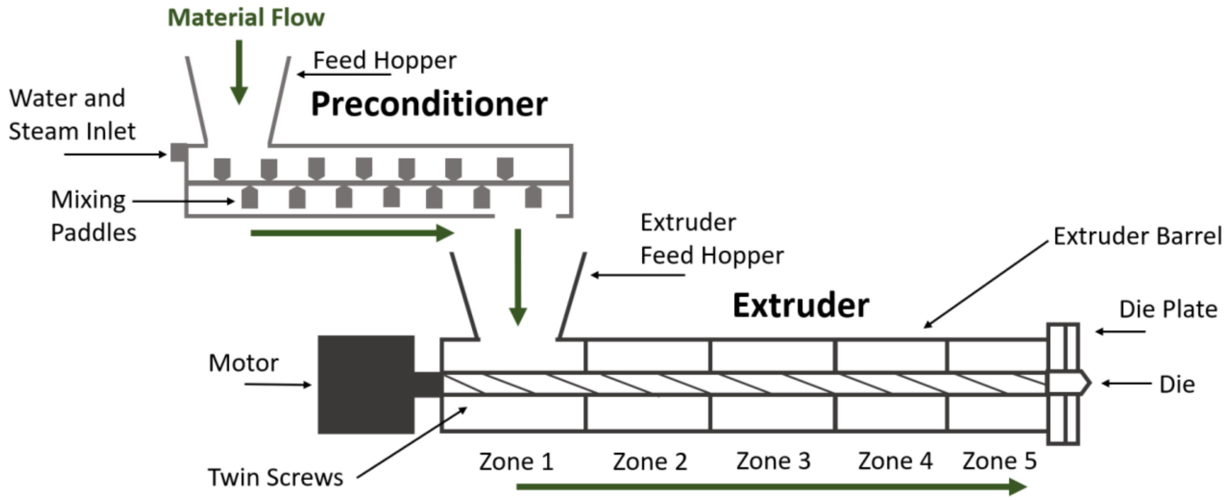


Figure 1. *Schematic diagram of preconditioner and extruder with temperature zones.*

The feed hopper funnels the preconditioned starting materials into the extruder barrel. Extruder barrel temperature is controlled by several heating elements, which can be calibrated to specific temperatures. Generally, the barrel temperature increases towards the end of the extruder to allow for gradual changes in food polymers (starch gelatinization, protein denaturation, etc.). The starting material temperature is further increased by the heat generated by the friction between the starting material and the extruder (i.e., inside surface of the barrel and screws). An even heat distribution is achieved by the rotating screws.

The twin screw configuration divides the extruder barrel (Berk, 2013) into three sections: conveying, compressing and metering (Samard, Gu, & Ryu, 2019). The conveying section of the screw configuration initially accepts the preconditioned starting materials into the barrel. The rotating twin screws move the starting material forward by positive displacement (Fellows, 2017; Verbeek & Van den Berg, 2010; Yacu, 2016). The space between the twin screws and the interior of the extruder barrel is greater than the space where the screws are intermeshed (Yacu, 2016). The material in the screw's flight transfers the starting material to the adjacent screw, in a self-wiping motion (Fellows, 2017). This pattern continues throughout the extruder barrel, moving the starting material in the outline of an infinity pattern (Singh & Heldman, 2014). The starting material structure is not substantially altered while in the conveying section. When the preconditioned starting material reaches the compression section, the heat and shear further denature the starting materials' proteins. Meanwhile, starches partially gelatinize (Berk, 2013; Harper, 1986; Riaz, 2011). The flow of the partially modified starting materials can be restricted by specific screw elements, which cause the pressure within the barrel to increase. When the starting materials reach the final metering section, majority of melting and chemical reactions take place due to increased shear and heat (Berk, 2013; Harper, 1986; Riaz, 2011). The denatured proteins, partially gelatinized/molten starch, fat and dietary fiber form a homogenous viscoelastic mass, known as the "melt" (Verbeek & Van den Berg, 2010). The melt is pushed through the die, shaped, and then sliced using a cutter to achieve the desired size (Berk, 2013; Harper, 1986; Riaz, 2011).

Although extrusion cooking is a streamlined process, preconditioning and extrusion operating parameters (i.e., extrusion's independent variables) need to be customized to create distinct products. The operating parameters for preconditioning include moisture content, temperature and rotating blades' speed, while those for extrusion are extruder feed rate, screw speed, barrel temperature and screw configuration. The numerous variations and combinations of these operating parameters influence extruder's responses (e.g., SME, torque and pressure) which consequentially impact the changes in the starting materials (e.g., starch gelatinization, protein degradation, protein aggregation, intermolecular interactions) and therefore, final product quality (Zhang et al., 2018).

2.4.3. Texturization

Among the wide variety of products that can be manufactured using extrusion cooking, TVPs are unique because they require texturization during processing. Texturization creates a fibrous (Day, 2011) and porous structure (Alam et al., 2016) that mimics meat texture (Riaz, 2004), and thus enhances consumers' sensory experience (Noguchi, 1989). In addition to structural and textural changes during texturization, a vegetable proteins' taste can be improved by eliminating bitter flavours, produced from polyunsaturated fatty acids converting to aldehydes, ketones and alcohols (Swanson, 1990), through the high temperature of extrusion cooking (Kearns, Rokey, & Huber, 2012).

For texturization to be successful, the starting materials must contain a significant level of protein. Lower protein levels (40% protein in the starting material, wet basis) produce soft textured TVPs, medium protein levels (70% protein) create firm textured TVPs, while high protein levels (90% protein) develop rubbery textured TVPs (Riaz, 2004). Protein content is inversely related to the remaining components of the starting material (i.e., starch, fat, dietary fiber) (Kearns et al., 2012).

Texturization is initiated at the preconditioning step, where the starting materials are efficiently and uniformly heated, hydrated and mixed (Riaz & Rokey, 2011). Even moisture distribution is critical for texturization because non-uniform moisture often results in heterogeneous extrudate expansion at the die (Kearns et al., 2012). During preconditioning, proteins are partially and irreversibly denatured, while the starch is partially gelatinized (Kearns et al., 2012; Riaz & Rokey, 2011).

When the preconditioned material enters the extruder barrel, heat, pressure, and mechanical shear are applied (Kearns et al., 2012; Riaz, 2011). As previously mentioned, the preconditioned starting materials are not significantly impacted in the barrel's conveying section. As the preconditioned starting material enter the compression section, mechanical and thermal energy inputs cause the preconditioned food polymers to further lose their native structures (Kearns et al., 2012; Riaz, 2011). Following the compression section, the metering section completes majority of the melting and chemical reactions from intensified shear and heat (Berk, 2013; Harper, 1986; Riaz, 2011). Starch's partially crystalline structure is further broken through shear; causing the starch molecules to degrade and form a paste (Ye et al., 2018). Meanwhile, proteins are generally fully denatured, allowing the protein molecules to further dissociate and unravel (Verbeek & Van den Berg, 2010), exposing their hydrophobic amino acids which were originally enclosed within the 3D structure of the protein molecule (Akdogan, 1999). The unravelled protein chains are mobile and fully integrate with the degraded starch, fat and dietary fiber to form a homogenous viscoelastic melt (Verbeek & Van den Berg, 2010). The melt is moved forward from the positive displacement of the rotating twin screws. The screws' rotation moves the melt in a uniform direction, which aligns unravelled protein chains in a parallel formation (Samard et al., 2019). The proteins maintain the laminated structure with numerous newly formed bonds, such as disulfide bonds and hydrogen bonds. Hydrophobic interactions between proteins, as well as between proteins and carbohydrates also keep the aligned proteins in a laminated structure (Liu & Hsieh, 2008). The continuous matrix of parallel protein chains entraps the dispersed gelatinized starch phase which separate groups of aligned protein chains and help render a fibrous protein structure. This is known as phase separation (Berk, 2013; Tolstoguzov, Grinberg, & Gurov, 1985).

The alignment of the protein molecules in the melt is further enforced when exiting the extruder, as the melt's protein chains continue to be oriented by shear forces from the walls of the die (Berk, 2013). Upon exiting the extruder, the pressure around the melt suddenly decreases, causing extrudate expansion and therefore reduced extrudate density (Berk, 2013; Yacu, 2016). This is due to the barrel temperature being above water's boiling point (i.e., 100°C) which causes the water within the extrudate to evaporate at atmospheric pressure. Expanded extrudate structure creates a desirable chewy texture in TVPs (Riaz, 2011). After exiting the extruder, the extrudate is cooled down to ambient temperature and dried to obtain the final product, i.e., the TVP. The TVP may undergo further size reduction processes (e.g., flaking, mincing, etc.) to form the final TVP shape (Kearns et al., 2012; Riaz, 2004). TVP structure and techno-functional properties drastically differ from its starting materials (Wang, Wang, & Johnson, 2005) allowing it to be used for new applications, such as meat extenders or meat alternatives (Riaz, 2004).

2.4.4. Extrusion operating parameters affecting TVP techno-functional and physical properties

2.4.4.1. Starting materials

Selecting the TVP starting materials depends on their availability, price, techno-functional properties, nutritional quality and consumer acceptance (Riaz, 2011). The selected starting material for TVP production must have suitable amounts of proteins (>50%), lipids (0.5-6.5%), fibers (0.5-7%), and carbohydrates (<40%) (Kearns et al., 2012; Riaz, 2004; Strahm, 2006). Starting materials with high levels of protein require lower energy input for texturization, making them texturize readily. However, high protein can also cause tough and firm textured TVPs (Strahm, 2006) which result in inferior textural integrity and water holding capacity properties (Kearns et al., 2012). Higher levels of fat can act as a lubricant, which reduces the extruder's shearing effects. This can be undesirable, as shear is needed to texturize the starting material. High levels of starch and dietary fiber partially inhibit texturization by reducing the respective protein content and disrupting the textured matrix (Strahm, 2006). Furthermore, the starting materials must have acceptable protein quality (nitrogen solubility index (NSI)) and particle size (Kearns et al., 2012; Riaz, 2004; Strahm, 2006).

2.4.4.2. Moisture content

Low moisture TVPs are preconditioned and extruded with 20-40% moisture content (Akdogan, 1999). After extrusion, the TVPs are dried to approximately 6-10% moisture content (Riaz, 2011). Moisture content added during preconditioning or directly into the barrel, decreases melt viscosity and melt's mean residence time in the barrel. Subsequently, the amount of mechanical energy and shear force transferred to the melt, thus SME, decreases as well (Chen et al., 2010). In line with the changes in extrusion response parameters (i.e., pressure inside the barrel and at the die, residence time distribution of the materials in the barrel, torque and SME), TVP's physical (e.g., porosity, density, integrity index and degree of texturization) and techno-functional properties (e.g., nitrogen solubility index, water holding capacity, oil absorbance capacity, emulsion capacity) are significantly impacted (Chen et al., 2010; Wang et al., 1999). For example, moisture content was positively correlated to pea protein TVP density but negatively correlated to TVP porosity (Wang et al., 1999). Furthermore, an increase in moisture content resulted in lower water holding capacity, oil absorption capacity, but higher emulsion capacity in pea based TVPs (Wang et al., 1999). Due to differences in starting materials, extruders and die design, there have been conflicting reports of the effect of moisture content on texturization (Zhang et al., 2018).

2.4.4.3. Screw speed

Screw speed modifies the food polymers in starting materials by controlling shear forces (Berk, 2013). Increasing screw speed raises shear forces which generates greater friction between the melt and the extruder surfaces, and thus causes the melt temperature to increase. Rising melt temperatures result in lower melt viscosities, and therefore require lower torque values to rotate the screws (Yacu, 2016). As explained in the SME equation (Eqn. 1), screw speed and torque are direct inputs of SME. Although screw speed decreases torque, SME increases overall from raising screw speed (Yacu, 2016).

Higher levels of screw speed also enhances the distribution of starch within the continuous protein matrix (Thiébaud, Dumay, & Cheftel, 1996). It has been reported that increased screw speed reduced TVP density, while increased porosity in low moisture pea-based TVPs (Wang et al., 1999). Wang et al. (1999) also reported that increased screw speed resulted in higher water holding capacity but lower oil absorbance capacity and integrity index for textured pea proteins. Screw speeds raised to too high levels may cause over-shearing which can decrease texturization in TVP products by destroying the formed fibers (Fang, Zhang, & Wei, 2014; Holay & Harper, 1982; B. Zhang, Zhang, Dreisoerner, & Wei, 2015).

2.4.4.4. Barrel temperature

The several separate heating elements within the extruder barrel are individually operated, allowing unique temperature zones. The operator can manipulate these zones to set a unique temperature progression. During extrusion, the starting materials' temperature matches the temperature of the extruder barrel. Therefore, when the barrel temperature increases, the temperature of the starting material does as well (Berk, 2013).

Barrel temperature is an integral component of extrusion because it significantly impacts the properties of the melt. Heat enables the melt to have fluid-like properties even at low moisture conditions (i.e., <40% moisture content) during extrusion. The lower melt viscosity reduces SME and torque, which are reflected in the TVP's physical and techno-functional characteristics (Yacu, 2016). For example, higher barrel temperatures were reported to produce lower density extrudates due to greater expansion at the die exit (Yacu, 2016). A positive correlation was also reported between barrel temperature and TVP porosity for pea protein TVPs. In addition, raising barrel temperature increased water holding capacity, oil absorption capacity and integrity index. Despite extruder barrel temperature's significance, this parameter was not selected for investigation in this M.Sc. project because barrel temperature does not impact techno-functional and physical properties as greatly as moisture content and screw speed do (Wang et al., 1999).

2.4.4.5. Screw configuration

One of the reasons for the versatility of twin-screw extrusion cooking is the operator's ability to customize the screw configuration. Screw configuration is the order and type of screw elements (e.g., conveying screws, lead screws and kneading blocks) on the rotating shafts of a twin-screw extruder. The several screw elements dictate the numerous characteristics, such as mixing, shearing, pressure build-up, screw fill degree and SME (Berk, 2013; Fang et al., 2014; Yacu, 2016). Screw configuration with more shearing screw elements (i.e., kneading elements) causes higher SME input that alters physical properties of TVPs. For example, soy based TVPs produced with higher levels of SME had higher tensile strength, hardness and chewiness, but lower texturization. Screw configuration also impacted extrudate colour (Fang et al., 2014). There are currently no studies analyzing the impact of screw configuration on TVP techno-functionality. Although it is recognized that screw configuration can greatly impact TVP quality (Riaz, 2004), an optimal screw configuration for texturization for our starting materials was selected during preliminary experiments and different screw configurations were not explored in this M.Sc. project.

2.5. Techno-functional properties

2.5.1. Techno-functional properties of starting materials and TVPs

2.5.1.1. Nitrogen solubility index (NSI)

Nitrogen solubility index (NSI), as measured by the percentage of nitrogen dispersed in water, indicates the starting material's protein quality (Riaz, 2006). Starting material NSI is a function of intense processing operations. For example, NSI is reduced due to heat treatments because heat denatures proteins (Deak, Johnson, Lusas, & Rhee, 2008; Riaz, 2004) which changes protein structure, by causing surface-exposed reactive amino acids to aggregate, or exposing previously hidden hydrophobic amino acids (Sikorski, 2001). The newly formed protein aggregates are generally insoluble in water, thus reducing nitrogen solubility. NSI is an important techno-functional property for TVP starting materials because it indicates the starting material's ability to texturize. Starting materials with low NSI require higher levels of SME to become textured. Extrusion operating parameters (e.g., moisture content, screw speed, and temperature profile) can be modified to enable suitable SME values to texturize specific starting materials (Bhattacharya, 2012).

The degree of texturization formed through extrusion processing can be analyzed by comparing the NSI of the starting material, and its extrusion processed counterpart (Qi & Onwulata, 2011). Extrusion processing denatures the protein, resulting in insoluble protein formation and protein texturization (Riaz, 2004; Samard, Maung, Gu, Kim, & Ryu, 2021).

2.5.1.2. Water holding capacity (WHC)

Water holding capacity (WHC) is the quantity of water a food material can absorb without weeping under light centrifugal force, and is reported as the amount of water (g) absorbed per amount of sample (g) (AACC, 2005). WHC is a crucial techno-functional quality for TVPs because TVPs require hydration prior to their use (Riaz, 2011). WHC heavily relies on TVP's available hydrophilic amino acids and porous structure (Mills, 2014). TVP's pores initially absorb water, while it's structural matrix physically entraps it (A. C. Y. Lam, Can Karaca, Tyler, & Nickerson, 2018; Sikorski, 2001). Therefore, more porous TVPs are ideal for quickly absorbing and entrapping water (Ngadi, Kassama, & Raghavan, 2001). When TVPs are applied as meat extenders, high WHC is essential for extended meat burger sensory quality because the TVP matrix releases the absorbed water during chewing, which increases the meat burger's perceived juiciness. Therefore, higher levels of WHC is desirable for TVPs used as meat extenders (Shoaib et al., 2018). High WHC also reduces overall product cost by weight because water is a relatively inexpensive ingredient (Millss, 2014; Warner, 2017).

2.5.1.3. Oil absorption capacity (OAC)

Oil absorption capacity (OAC) is the amount of oil a food material absorbs, and is reported as the amount of oil (g) absorbed per amount of sample (g) (Alonso, Orue, Zabalza, Grant, & Marzo, 2000). A TVP's OAC is improved when its proteins have hydrophobic characteristics because the protein's nonpolar side chains (e.g., for amino acids such as leucine and alanine) interact with the lipid's aliphatic chains (Sanjeewa, 2008; Withana-Gamage, Wanasundara, Pietrasik, & Shand, 2011). Numerous factors, including protein structure, lipid type and emulsion stability (i.e., the ability to maintain immiscible phases as a cohesive emulsion) influence OAC (Hall, 1996). Meat extenders, such as TVPs, need high OAC to enhance meat product sensory quality (Der, 2010); including mouthfeel (Chobert & Haertle, 1997) and structural uniformity. High OAC also prevents fat loss during cooking (Sandberg, 2011).

2.5.1.4. Emulsion capacity (EC)

Emulsions are a two phased system of immiscible liquids; one of which is dispersed into the continuous phase of the other. For example, a water-in-oil emulsions is when water droplets are distributed within a continuous oil phase. Opposingly, an oil-in-water emulsion has oil droplets scattered throughout the continuous water phase (D. McClements, 2005). Emulsions are stabilized with emulsifying agents. Emulsifying agents (or emulsifiers) are amphipathic, meaning they possess hydrophilic and lipophilic characteristics. This allows emulsifiers to surround emulsion droplets (i.e., dispersed phase) and reduce the surface tension between the immiscible phases. Proteins are effective emulsifiers because their side chains have amphipathic properties.

Emulsion capacity (EC) is an emulsion's ability to intake oil or water, prior to inverting (i.e., oil-in-water emulsion transforming into a water-in-oil emulsion, or vice versa) (Varzakas, Polychniatou, & Tzia, 2014), and is reported in the units of gram oil per gram protein (in the emulsion) at the inversion point. To determine the inversion point, and thus to differentiate an oil-in-water emulsion from a water-in-oil emulsion, the conductivity of the emulsion is measured. A relatively high conductivity indicates an oil-in-water emulsion, while a relatively low conductivity represents a water-in-oil emulsion (Stone et al., 2015).

Meat burgers have similar properties of an oil-in-water emulsion, although they are not considered true emulsions because the animal's fat globules are too large and in a solid state (Ugalde-Benitez, 2012; Varzakas et al., 2014). In the meat burger "emulsion," the solid animal fat is distributed within the aqueous phase of the meat system (i.e., myofibrillar proteins, soluble proteins, connective tissue, water) (Varzakas et al., 2014). The aqueous phase also integrates other ingredients, such as meat extenders (Xiong, 2000) including TVPs, which are added to meat burgers for their amphipathic properties (Mills, 2014). Good emulsification properties are integral for meat extenders because high EC reduces product shrinkage (Patel, Merkel, Reynolds, & Youngs, 1980) and improves product texture. When extended meat burgers are cooked, extenders with high EC prevent fat separation and water loss. Meat burgers with high EC extenders also improve product uniformity (Riaz, 2004).

2.5.1.5. Emulsion Stability (ES)

Emulsion stability (ES) is the maintenance of an emulsion's physicochemical properties over a period of time (McClements, 2005; Varzakas et al., 2014; Joseph F. Zayas, 1997). Emulsions' thermodynamic instability (Ladjal Ettoumi, Chibane, & Romero, 2016) leads to emulsion separation into its oil and water phases over time (Varzakas et al., 2014). Emulsion destabilization can be accelerated from flocculation, coalescence, gravitational separation and disproportionation (Ladjal Ettoumi et al., 2016; Varzakas et al., 2014). ES is a significant techno-functional property for meat extenders (Correia & Mittal, 2000) because ES influences the extended meat product's cooking yield (Mills, 2014). EC is dependent on protein shape, charge, and hydrophobicity, while ES relies on the degree of protein interactions (Nakai & Powerie, 1984; Zayas, 1997). Proteins (and surfactants) prevent emulsion separation by acting as emulsifying agents (Damodaran, 1997) and prevent coalesce by forming a protective barrier around the suspended phase's particles. This barrier prevents the break down of an emulsion (Kinsella, 1979; Zayas, 1997). When a meat emulsion is cooked, the emulsion breaks down, and causes fat and moisture loss, and thus lowers yield (Jiménez Colmenero, 2014).

2.6. Physical properties

2.6.1. Physical properties of TVPs

2.6.1.1. Hydration time (HT)

Hydration time is the time (in minutes)necessary to fully hydrate a TVP (Roberts, 2013). Hydration time is an important techno-functional properties because TVPs are hydrated prior to their use (Riaz, 2011) and thereby indicate TVP quality and feasibility in in a processing setting

(Roberts, 2013). Water is absorbed into TVP's pores, which increase TVP volume (Parmer, Wang, Aglan, & Mortley, 2004).The quantity of water TVPs absorb impacts the final extended meat product quality. An excess of absorbed water can negatively impact the final product texture (Roberts, 2013).

2.6.1.2. Integrity index (II)

Integrity Index (II) assesses a TVP's structural resilience to processing treatments, i.e., it is the percentage of TVP that withstood processing (Samard & Ryu, 2019b). Processing conditions used in II determination mimic how a TVP is prepared for meat extender application (Wang et al., 1999). High II TVPs may be hard, lack texturization and do not absorb water readily; which are undesirable characteristics for TVPs (Roberts, 2013). TVP quality and yield are impacted by II (Samard & Ryu, 2019a).

2.6.2. Physical properties of meat burgers extended with TVPs

2.6.2.1. Total cooking loss (TCL)

Total cooking loss (TCL) measures the change in meat burger weight as a result of cooking, and is reported as a percentage (Warner, 2017). During cooking, animal proteins denature and contract, which causes water and oil loss; particularly at the meat burger's surface (Oroszvári, Bayod, Sjöholm, & Tornberg, 2005; Sanjeewa, 2008). While moisture is lost as vapour and drip, while fat is only lost as drip (Oroszvári, Bayod, et al., 2005; Shilton, Mallikarjunan, & Sheridan, 2002). In meat burgers, TCL should be minimized to increase product yield (Mills, 2014). Meat burgers with low cooking loss have superior sensory quality, such as tenderness and juiciness. They are also more advantageous for product economics (Oroszvári, Bayod, et al., 2005). Meat burgers with TVP extension have shown reduced TCL, and thus produced juicer burgers (Feiner, 2006a).

2.6.2.2. Meat burger shape retention

Meat burger shape retention is minimizing the change in meat burger diameter and thickness after cooking (Hale et al., 2002). Change in burger diameter (CBD) is the difference of meat burger diameter before and after cooking, divided by the meat burger diameter before cooking (Oroszvári, Bayod, et al., 2005). Likewise, increase in burger thickness (IBT) is the difference in average meat burger thickness before and after cooking, compared to the meat burger thickness before cooking. CBD and IBT are both reported as a percentage. Meat burger shrinkage is primarily from moisture and fat loss during cooking (Besbes, Attia, Deroanne, Makni, & Blecker, 2008; López-Vargas, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2014; Oroszvári, Sjöholm, & Tornberg, 2005). Retaining meat burger shape eases the mechanization during the manufacture process, and therefore is desirable for institutional, commercial and retail settings (Hale et al., 2002).

2.6.2.3. Texture

Texture is a primary indicator of meat burger quality (Brady & Mayer, 1985). As texture is considered a critical sensory property of food products by consumers (Warner, 2017), it is integral that meat burgers extended with TVPs have desirable texture properties. When meat is cooked, connective tissues, myofibrillar and soluble proteins denature and contract, which changes meat texture (Zayas & Naewbanij, 1986). When meat burgers are extended with TVPs, the texture of the burger is substantially altered (Smith et al., 1976) because extenders interact with the animal protein to increase product cohesion (Mills, 2014).. Texture profile analysis (TPA) objectively quantifies several textural properties (i.e., hardness, cohesiveness, springiness, and chewiness) (Meullenet & Carpenter, 1998). In the consumer's sensory perspective, hardness is the amount of force needed to bite through the product (Ruiz De Huidobro, Miguel, Blázquez, & Onega, 2005), while cohesiveness is the structural uniformity of the product (Ruiz De

Huidobro et al., 2005; Tahmasebi, Labbafi, Emam-Djomeh, & Yarmand, 2016). Springiness is the foods capability to recover to its initial condition after being deformed (Szczesniak, Brant, & Friedman, 1963) and chewiness is the work necessary to chew the product until it reaches a state ready for swallowing (Ruiz De Huidobro et al., 2005). Consumer preference is personal, therefore the impact of TVP extension and its optimum extension level is subjective (Hidayat, Wea, & Andriati, 2017). However, tender (i.e., softer) and less cohesive meat burgers are generally preferred by elderly consumers (Baugreet, Kerry, Botineştean, Allen, & Hamill, 2016). It has been shown that the addition of TVPs modifies the burger structure and produces softer burgers (Kassama, Ngadi, & Raghavan, 2003).

Chapter 3: Effects of pea protein formulation and extrusion processing parameters on techno-functional properties of pea protein.

3.1. Abstract

Pea protein was investigated for its potential for developing texturized vegetable proteins (TVPs). Pea protein concentrate and isolate were blended at three different ratios (protein blend formula (PBF)) of 70, 76 and 82% protein content and extruded as a function of three screw speeds and two feed moisture contents. The nitrogen solubility index (NSI) and techno-functional properties, i.e., water holding capacity (WHC), oil absorbance capacity (OAC), emulsion capacity (EC) and emulsion stability (ES) of the raw materials and TVPs were examined. Raising protein content through manipulating PBF decreased NSI for both the raw formulas and the TVPs. Regardless of the PBF, extrusion processing substantially decreased NSI, but increased OAC. Extrusion's impact on WHC, EC and ES was complex, and might have been impacted by the protein content and source (i.e., PBF). Overall, PBF had a more pronounced impact on the techno-functionality of TVPs than extrusion conditions did. At higher PBF, the NSI, EC and ES values of TVPs were negatively impacted, implying that high protein content does not equate to better TVP quality. Future TVP techno-functionality research should focus on a wider range of feed moisture content and screw speed during extrusion or the effects of protein content and source.

3.2. Introduction

Plant focused diets, such as flexitarian, vegetarian and vegan, have tremendously increased in popularity in recent years (Ismail, Hwang, & Joo, 2020). This shift in consumer diet is generally motivated from health, animal welfare and/or sustainability concerns (Hoek et al., 2011). These changing diet trends are raising the public's interest in meat alternative foods, including plant protein-based products (Sha & Xiong, 2020). As consumers still desire quality, convenience and familiarity in the products they consume (Sadler, 2004), producers must continue to innovate to meet these needs (Ismail et al., 2020).

Texturized vegetable proteins (TVPs) are plant proteins that have been processed to fulfill the role of meat products in everyday meals (Samard & Ryu, 2019a). TVPs can be used to partially or entirely replace animal protein in numerous products (i.e. meat extension and substitution, respectively) such as burgers, sausages and meatballs (Grasso, Smith, Bowers, Ajayi, & Swainson, 2019; Hidayat et al., 2017; Neville, Tarrega, Hewson, & Foster, 2017; Samard & Ryu, 2019a). Extending meat based products with TVP incorporation in the formula can enhance the final product quality by improving techno-functional properties (i.e., water and oil binding and emulsion capabilities of the extended product) (Asgar et al., 2010) while maintaining their fibrous, chewy texture (Riaz, 2011; Vatansever et al., 2020).

The fibrous characteristic structure of a TVPs is often achieved through extrusion processing (Vatansever et al., 2020) which proteins are denatured, aligned and texturized (F. L. Chen et al., 2010). To achieve texturization, high protein content ingredients with desirable techno-functional qualities and specific extrusion operating parameters (e.g., screw configuration, feed moisture content and screw speed) are required (Vatansever et al., 2020; Zhang et al., 2018).

The most common starting materials for TVPs are soy and wheat protein because they are easily processed to form a meat-like fibrous appearance and consistency (Riaz, 2004, 2006). However, soy and wheat protein popularity is declining because of GMO hesitancies (Lam, Warkentin, Tyler, & Nickerson, 2017) and their priority food allergenicity statuses (Government of Canada, 2019). In response, alternative plant protein sources, such as mung bean protein isolate, peanut protein isolate (Samard & Ryu, 2019b), black gram (*Vigna mungo*) by-product

(Kamani et al., 2021) and chickpea flour and pea protein isolate blends (Webb et al., 2020), have been tested as raw materials for TVPs.

Pea protein is a promising TVP starting material because it has similar functional properties to soybean proteins (Samard & Ryu, 2019b), but is non-GMO and a non-priority food allergen (Egbert & Payne, 2009; Riaz, 2011). Pea proteins are also a good source of essential amino acids (Lampart-Szczapa, 2001) as well as vitamins, minerals and polyphenols (Boye, Zare, & Pletch, 2010). Several studies have developed pea protein based TVPs (Alonso, Orue, Zabalza, Grant, & Marzo, 2000; Osen, Toelstede, Wild, Eisner, & Schweiggert-weisz, 2014; Samard & Ryu, 2019b; Wang, Bhirud, & Tyler, 1999), but there is a lack of research reporting the impacts of pea protein based blend formulation and extrusion cooking conditions on TVP techno-functionality. The objectives of this study were to evaluate the impact of protein blend formula (i.e., pea protein concentration and source), extrusion feed moisture content (38 and 42%) and screw speed (350, 400, 450rpm) on nitrogen solubility index and techno-functional properties (i.e., water holding capacity, oil absorbance capacity, emulsion capacity and emulsion stability) of TVP pea protein.

3.3. Materials and methods

3.3.1. Raw materials and proximate analyses

Pea protein concentrate (PPC) and pea protein isolate (PPI) were provided by AGT Food and Ingredients (Minot, ND, USA). The proximate composition of PPC and PPI (Table 2). were measured in triplicate, using AOAC methods 930.15 and 990.03 and AOCS method AM5-04 for moisture, crude protein and ash contents, respectively. The total carbohydrates content was calculated by difference. The protein contents of the PPC and PPI were used to determine their ratios by weight for the preparation of protein blend formulas (PBFs).

Table 2. Proximate composition of the raw materials.

Proximate composition (%, d.b.)	Pea protein concentrate	Pea protein isolate
Protein	54.8 ± 0.0	81.5 ± 0.0
Fat	2.7 ± 0.0	0.4 ± 0.0
Ash	4.7 ± 0.0	4.8 ± 0.0
Carbohydrates	37.8	13.2

3.3.2. Preliminary treatment of materials

Using a mixer (A200, Hobart, Offenburg, Germany) with a paddle attachment, PPC and PPI were blended in specific ratios to create 70% (44.2 PPC: 55.8 PPI) and 76% (21.8 PPC: 78.2 PPI) PBFs (i.e., 70 and 76 g protein/100 g dry PBF. The 82% PBF only consisted of PPI (0 PPC: 100 PPI), (i.e., 82 g protein/100 g dry PBF. Each PBF was preconditioned to 35% moisture (i.e., 35 g water/100 g dry PBF) with boiling water, while being continuously mixed at the lowest speed setting of the mixer. The preconditioned PBFs were stored in an environmental chamber (HPP 260 IPP plus, Memmert, Schwabach, Germany) at 40°C at 65% relative humidity until extrusion.

3.3.3. Extrusion process

The preconditioned PBFs were extruded with a lab-scale, co-rotating twin screw extruder (MPF19, APV Baker Ltd, Peterborough, UK) with a 25:1 screw length-to-diameter (L:D) ratio (D = 19 mm). The screw configuration (starting at the feed input, ending at the die) consisted of 7D twin thread feed screws, 4D single thread lead screws (LS), 2D 60° forward paddles (FP), 4D LS, 1.5D 30° FP and 6.5D LS. The five temperature zones of the extruder were set to 60, 80, 100, 110, and 115°C, from the feeder to die end of the barrel. The extruder was equipped with a 5 mm circular die. The preconditioned PBFs were calibrated to a dry feed rate of 2.75 kg/hr and a final extrusion feed moisture content (FMC) of 38 or 42% (i.e., 38 or 42 g water/100 g dry PBF by water directly into the barrel. The screw speeds used were 350, 400 and 450 rpm. TVPs were produced at their respective PBF, FMC and screw speed in duplicate. The 82% PBF, 42% FMC and 400 rpm treatment was not studied due to the sample being compromised. The TVPs were cooled down to room temperature, then dried (<19 g water/100 g dry extrudate) overnight at room temperature. The following day, TVPs were reduced to 1-2 mm pieces, using a laboratory mill (Mill 3610, Perten Instruments, Turku, Finland). About 100 g of these dried TVPs were ground with an ultracentrifugal mill (ZM 200, Retsch, Haan, Germany) equipped with a 250 or 500 µm sieve and then stored at -40°C until further analysis.

3.3.4. Nitrogen solubility index (NSI)

Nitrogen solubility index (NSI) was measured in triplicate following AACCI method 46-23.01 with minor modifications (AACCI, 1999). Approximately 5 grams of either raw PBF or ground (250 µm) TVP was combined with pre-warmed (30°C) distilled water, until the mixture volume reached 200 mL. The mixture was stirred at 120 rpm in a shaking water bath (SW22, Julabo, Seelbach, Germany) for 2 hr at 30°C to extract the soluble nitrogen. To prevent the sample's particles from settling on the bottom, the mixture was shaken manually for 30 s every 30 min to. Following extraction, 10 mL of the mixture was centrifuged (Sorvall RC6 plus, Thermo Fischer Scientific, Ashville, NC, USA) at 4180 × *g* for 10 min and the supernatant was filtered using Whatman no.2 filter paper. The quantity of water soluble nitrogen in the filtrate and the total nitrogen from the raw PBF (or the TVP) were determined according to AACCI method 46-16.01. NSI, expressed as a percentage, was calculated by dividing the water soluble nitrogen by the total nitrogen measured in the sample (i.e., raw material or ground TVP) using equation (2):

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

3.3.5. Techno-functional properties of the raw materials and TVPs

3.3.5.1. Water holding capacity (WHC) and oil absorbance capacity (OAC)

Water holding capacity (WHC) and oil absorbance capacity (OAC) were measured in triplicate with slight modifications (Stone et al., 2015). To summarize, 0.5 grams of either raw PBF or ground (500 µm) TVP was combined in a 50 mL falcon tube with 5 mL of water or canola oil, for WHC and OAC measurements, respectively. The mixture was vortexed (G-560, Scientific Industries Inc., Bohemia, NY, USA) for 10 s every 5 min for a total of 30 min, followed by centrifugation (Sorvall RC6 plus, Thermo Fisher Scientific, Ashville, NC, USA) at 1200 x *g* for 18 min for WHC and 1100 x *g* for 15 min for OAC. After centrifugation, the sample supernatant was decanted, and the remaining pellet was weighed. WHC and OAC were calculated using equations (3) and (4).

$$WHC\ (g\ water/g\ sample) = \frac{W_f - W_i}{W_i} \tag{3}$$

$$OAC\ (g\ oil/g\ sample) = \frac{W_f - W_i}{W_i} \tag{4}$$

Where W_f is the final weight of the solid pellet after centrifugation and W_i is the initial raw PBF (or TVP) weight.

3.3.5.2. Emulsion capacity (EC) and emulsion stability (ES)

Emulsion capacity (EC) and emulsion stability (ES) were measured in sextuplicate with slight modifications (Brishti et al., 2017). Approximately 3.5 grams of raw PBF or ground (500 µm) TVP was homogenized using a standard lab homogenizer (Silverson Machines LTD, Waterside, Chesham Buckinghamshire, England) at 3200 rpm with 50 mL distilled water for 30 s, followed by 50 mL canola oil for 30 s. The emulsion was divided equally into two 50 mL falcon tubes and centrifuged (Sorvall RC6 plus, Thermo Fisher Scientific, Ashville, NC, USA) at 1100 x *g* for 5 min. EC was calculated by taking the ratio of the height of the emulsion layer to the height of the total contents in the falcon tubes. Following EC measurements, the emulsions were heated at 80°C for 30 min in a circulating water bath (F3, HAAKE, Vreden, Germany) and centrifuged again at 1100 x *g* for 10 min. ES was calculated using the same procedure as EC.

3.3.6. Statistical analysis

The experiment had a completely randomized design, with the PBF, screw speed and FMC combined in single independent variables using 17 treatments in an incomplete factorial structure. Thus, statistical differences among NSI and techno-functional properties were determined with one way ANOVA ($p<0.05$). LSD t-test was used a mean separation ($p<0.05$) (SAS software, version 9.2, SAS Institute Inc., Cary, NC, USA). Correlations between techno-functional tests were calculated using the data analysis function on Microsoft Excel 365 (2021).

3.4. Results and discussion

3.4.1. Nitrogen solubility index (NSI)

Raw PBF NSI can indicate the mechanical energy required to texturize a plant protein. When everything else is kept constant, a plant protein with a low NSI would require relatively greater mechanical energy input to be texturized to the same level (Riaz, 2004). The NSI values of raw PBFs and TVPs as a function of FMC and screw speed are presented in Figure 4. The NSI values decreased as protein content increased either in raw PBFs or TVPs treatments ($p<0.05$). Regardless of the protein content, raw PBF presented higher NSI than TVP treatments; being raw PBF 70% the highest. At the same time, within TVP-PBF treatments, the combination of 70% PBF, 350 rpm and 38% FMC- had higher NSI values than the other TVP-PBF treatments. Overall, the TVP-PBF at 70% regardless of FMC and SS; had higher NSI than the other treatments. A decrease in NSI after extrusion is indicative of texturization (Riaz, 2004; Samard & Ryu, 2019a, 2019b) and therefore expected, as proteins denature and form insoluble protein aggregates, causing NSI to decrease when extruded (Qi & Onwulata, 2011; Samard & Ryu, 2019a). In soy and wheat protein texturization, NSI of their raw material dropped significantly (38%) after extrusion (Samard & Ryu, 2019a). Likewise, extrusion processing decreased NSI in several additional studies (Lin, Huff, & Hsieh, 2002; Samard & Ryu, 2019a). On average, ~22% decrease in NSI values of raw PBFs were observed after extrusion.

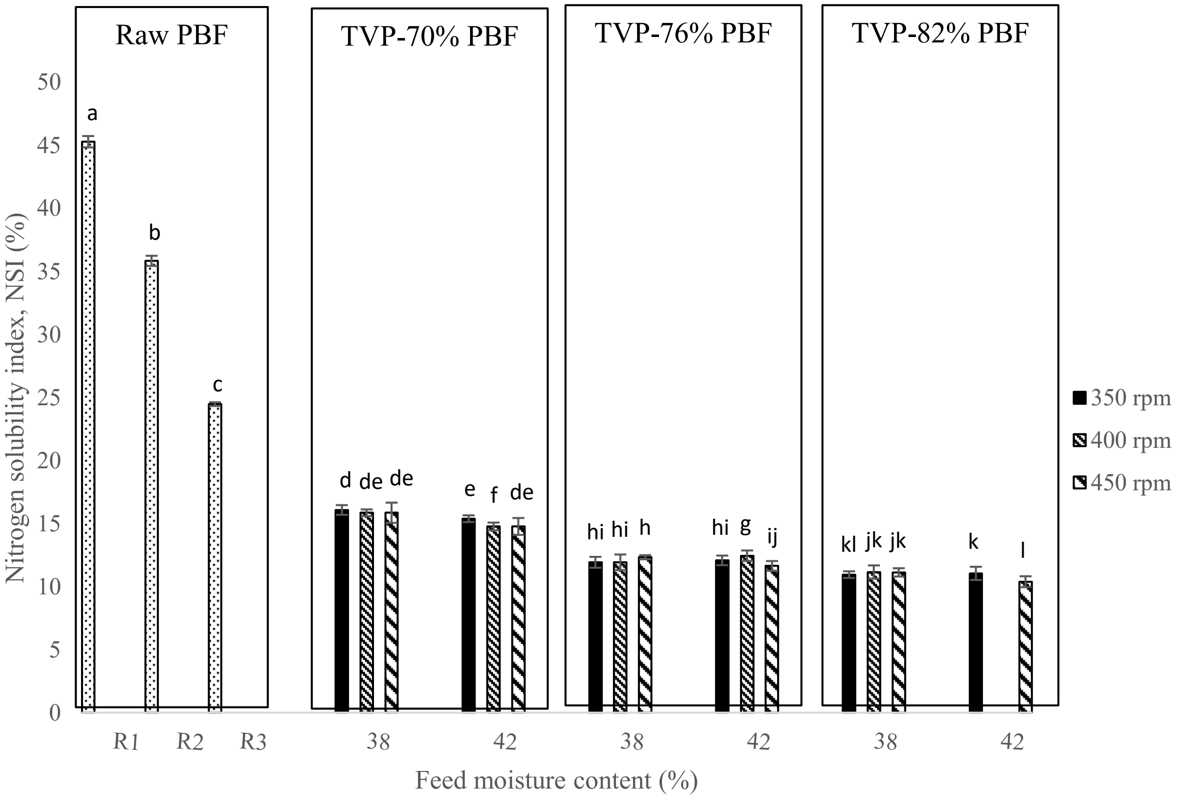


Figure 2. Nitrogen solubility index (NSI) of raw protein blending formulas (PBFs) at 70% (R1), 76% (R2) and 82% (R3) and texturized vegetable proteins (TVPs) that were produced using a combination of protein blend formulas (PBFs), screw speeds and feed moisture contents (FMCs) treatments (n=3 for raw PBFs, n=6 for TVPs). NSI values were not significantly different if designated with the same letter (p<0.05). Error bars represent ± standard deviation.

These findings were expected because concentration of PPI relative to PPC increases as PBF goes from 70% to 76% and then to 82%. PPC is commonly produced using air classification, while PPI undergoes relatively more severe processing conditions (e.g., wet fractionation) to obtain a higher protein concentration (Vatansever et al., 2020). Thus, protein solubility decreases due to the severity of the protein concentration methods used to create PPI (Kiosseoglou & Paraskevopoulou, 2010; Mozafarpour, Koocheki, Milani, & Varidi, 2019) which impacts the surface characteristics of proteins by exposure of hydrophilic and hydrophobic groups of proteins and increasing protein-protein interactions (Karaca, Low, & Nickerson, 2011; Stone et al., 2015).

In TVP-PBF, within each protein content group, screw speed (350-450 rpm) and FMC (38 vs 42%) did not show a discernable trend, indicating a complex interaction between the effects of screw speed, FMC and PBF. Similarly, it was reported that raising screw speed from 150 to 200 rpm did not significantly impact the NSI of TVPs made from soy protein, wheat gluten and corn starch blend (Samard et al., 2019). On the contrary, NSI was reported to increase with raising screw speed from 100 to 200 rpm, for TVPs made from soy protein isolate, wheat gluten, corn starch and green tea (Ma, Gu, & Ryu, 2018) and when increasing screw speed from 135 to 245 rpm for TVPs made from pea protein (Wang, Bhirud, & Tyler, 1999). Regarding to FMC, some researchers have indicated (Ma et al., 2018; Samard et al., 2019), NSI significantly decreased with an increase in FMC (30-70% FMC; 45-55% FMC, respectively) for soy based TVPs and soy and wheat gluten based TVPs, while the opposite effect at relatively lower FMC (24.0-30.5% FMC) was found in TVPs made from pea protein (Wang et al., 1999). These findings indicate that the effects of texturization on NSI are dependent on the complex interactions between raw materials characteristics and the extent of extrusion conditions.

3.4.2. Water holding capacity (WHC)

Table 3 presented the WHC of the raw PBF and TVPs. WHC is an important characteristic for meat extenders. WHC greatly influences end product quality attributes; such as mouthfeel and texture, as well as retention of flavour (Vatansever et al., 2020). WHC of raw PBF increased ($p<.0.05$) as protein content increased. Within the raw PBF and TVP treatments studied the 70% raw PBF had the lowest ($p<0.05$) WHC. WHC is dependent on the accessibility and availability of hydrophilic amino acid side chains, which increased in quantity when protein concentration was raised through PBF, to interact with water (Der, 2010; Lee, Lu, Zhang, Fu, & Huang, 2021; Stone et al., 2015; Toews & Wang, 2013). This trend was also observed in various studies where legume protein concentrates were used (e.g., pea, lentil, navy bean, chickpea and soy) (Toews & Wang, 2013).

Table 3. Techno-functional properties of raw protein blending formulas (PBFs) and TVPs produced with different protein blend formulas (PBF), and at different feed moisture contents (FMC) and screw speeds.

PBF (**% protein**)	**FMC** (**%**)	**Screw speed** (**rpm**)	**WHC** (**g H_2O/g sample**)	**OAC** (**g oil/g sample**)
70 (raw)	N/A	N/A	0.81 ± 0.00^{g}	0.75 ± 0.00^{g}
76 (raw)	N/A	N/A	0.85 ± 0.00^{f}	0.76 ± 0.01^{g}
82 (raw)	N/A	N/A	0.89 ± 0.00^{cd}	0.76 ± 0.02^{g}
70	38	350	0.90 ± 0.01^{abc}	$0.85 \pm 0.02^{b-e}$
		400	0.86 ± 0.01^{ef}	$0.85 \pm 0.03^{b-f}$
		450	0.89 ± 0.02^{abc}	0.86 ± 0.03^{bcd}
	42	350	0.89 ± 0.05^{abc}	0.86 ± 0.02^{bcd}
		400	0.91 ± 0.03^{abc}	0.90 ± 0.01^{a}
		450	0.92 ± 0.01^{a}	0.86 ± 0.01^{bcd}
76	38	350	0.86 ± 0.04^{def}	$0.85 \pm 0.03^{b-f}$
		400	0.89 ± 0.02^{cde}	$0.84 \pm 0.03^{c-f}$
		450	0.89 ± 0.01^{abc}	0.86 ± 0.03^{bcd}
	42	350	$0.89 \pm 0.03^{a-d}$	0.86 ± 0.02^{bc}
		400	0.91 ± 0.02^{abc}	0.87 ± 0.03^{b}
		450	0.90 ± 0.01^{abc}	0.87 ± 0.02^{b}
82	38	350	0.89 ± 0.02^{bcd}	0.83 ± 0.02^{ef}
		400	0.92 ± 0.01^{ab}	$0.84 \pm 0.01^{b-f}$
		450	0.90 ± 0.04^{abc}	0.83 ± 0.02^{def}
	42	350	0.89 ± 0.02^{bcd}	0.82 ± 0.04^{f}
		450	0.90 ± 0.03^{abc}	$0.84 \pm 0.04^{c-f}$

WHC: water holding capacity, OAC: oil absorption capacity. Each value represents the mean ± 1 standard deviation (n=3 for raw materials, n=6 for TVPs). Values within a column are not significantly different if designated with the same letter ($p<0.05$).

Compared to their raw PBF counterparts, extrusion processing improved the WHC of 70% and the majority of 76% PBF TVPs but did not generally impact those of 82% PBF. The 70% raw PBF had the lowest WHC ($p<0.05$) of the raw PBFs as well as all TVP combinations studied. Within the TVP treatment combinations, the TVP produced with 70% PBF, 450 rpm and 42% FMC had the highest WHC ($p<0.05$), while 70% PBF, 400 rpm and 38% FMC had the lowest

WHC ($p<0.05$). The rest of treatments presented intermediate values ($p>0.05$). The increase in WHC after extrusion is in line with the literature. For example, WHC was reported to improve as a result of extrusion processing for pea protein TVPs by 203% (Wang et al., 1999) and black gram by-product TVPs by 127% (Kamani et al., 2021). While this increase is substantially higher compared to the WHC increase in the current study, it should be noted that the previously mentioned studies ingredients had much lower proportion of protein to starch (55% protein and ~33% protein, respectively) (Kamani et al., 2021; Wang et al., 1999).The greater amount of starch in the formulas (~24% starch for 70% PBF and ~19% starch for 76% PBF, respectively) may have positively impacted their WHC due to starch gelatinization during extrusion which improves starch's interaction with water (Webb et al., 2020). As 82% PBF contains relatively the lowest amount of starch, this PBF may not have substantially benefited from starch gelatinization. Additionally, extrusion processing causes protein conformational changes, where the proteins unfold and rearrange themselves into new structures through protein-protein cross linkages (Brishti et al., 2021; Liu & Hsieh, 2008) which may impact WHC (Kamani et al., 2021; Lopes, De Aleluia Batista, Fernandes, & De Andrade Cardoso Santiago, 2012). Proteins may not have been as critical for TVP WHC improvement as heat treatments, like extrusion processing, are known to expose the hydrophobic protein residues (Kannan, Hettiarachchy, Sato, & Marshall, 2012; Osen et al., 2014).It is therefore possible that the gelatinized starch is relatively more hydrophilic than the denatured proteins (Wang, 2018).

In general, the range of FMCs studied had a negligible effect on the WHC of TVPs when PBF and SS was kept consistent. Although some TVP treatments showed a difference between FMC levels, it was not consistent. The intermediate FMC range in the present study may not have been conducive to observe a significant impact, similar to the findings of other studies with slightly lower FMCs (24-30.5% db) (Wang et al., 1999). PBF and screw speed generally did not impact TVP WHC when the other treatment variables were not altered in the present study as well. On average, the WHC values of 70, 76 and 82% PBF TVPs were 0.90 ± 0.02, 0.89 ± 0.02 and 0.90 ± 0.01 g water/g sample, respectively. Although protein content was reported to have a positive correlation with WHC (Toews & Wang, 2013), it is possible that the proteins were denatured prior to extrusion to a state where WHC plateaued (Kaleda et al., 2020; Ma et al., 2018; Zayas, 1997). The lowest PBF TVPs having comparable WHC to the highest PBF TVP is beneficial for industry applications, as purer protein is a relatively expensive ingredient. On average, the WHC values of 350, 400 and 450 screw speed TVPs were 0.89 ± 0.01, 0.90 ± 0.02 and 0.90 ± 0.01 g water/g sample, respectively. In line with these results, screw speed was not a critical factor affecting WHC for soy protein and gluten TVPs (Wu et al., 2018) and pea flour extrudates (Kristiawan et al., 2018).

3.4.3. Oil absorbance capacity (OAC)

OAC is integral for meat extender quality because it enhances meat product juiciness and mouthfeel (Pasqualone, Costantini, Labarbuta, & Summo, 2021; Riaz, 2004). OAC results are summarized in Table 3. OAC was identical ($p<0.05$) between the raw PBFs. Slightly higher OAC values have been reported in literature for pea proteins at higher protein concentrations (Boye et al., 2010; Wang, Maximiuk, Fenn, Nickerson, & Hou, 2020). The variance in OAC values between the current study and previous studies may be attributed to the different protein structure, type of oil used, in addition to the possible presence of emulsifying agents within the mixture(Lam, Can Karaca, Tyler, & Nickerson, 2018).

The raw PBFs had the lowest OAC values ($p<0.05$) of all treatments studied. TVPs had significantly higher ($p<0.05$) OAC compared to their raw PBF counterparts. This is in agreement with the literature for TVPs made from soy protein (Tehrani, Ehtiati, & Azghandi, 2017) and pea protein (Osen et al., 2014). Thermal processing, such as extrusion cooking, causes protein denaturation and aggregation, which may result in a higher level of exposed nonpolar amino acid side chains; thus favoring hydrophobic interactions (Kannan, Hettiarachchy, Sato, et al., 2012; Osen et al., 2014), and thereby increase OAC (Khattab & Arntfield, 2009; Samard & Ryu, 2019b).

When comparing TVPs, OAC seemed to be slightly impacted by the FMC. Under the same PBF and screw speed conditions, the OAC of 38% FMC TVPs were slightly lower than those of 42% OAC, averaging 0.84 ± 0.01 and 0.87 ± 0.02g oil/g sample, respectively. Only TVPs produced with 76% PBF at 350 and 400 rpm screw speed dropped in OAC when FMC increased. PBF and screw speed did not consistently affect the OAC of TVPs when the other treatment variables were maintained. It is beneficial for industrial applications that a wide range of protein levels and processing conditions can maintain similar OAC values.

Among all the extrusion treatments studied, the TVP produced at 76% PBF 38% FMC and 400 rpm had the highest OAC. These results indicate that an optimal FMC and screw speed that maximizes TVP OAC may exist under specific formulation and extrusion conditions. It was observed in pea protein-based TVPs that OAC reduced when both FMC (from 24 to 30.5%) and screw speed was raised (from 135 to 245 rpm) (Wang et al., 1999). The treatment combination of 82% PBF, 350 rpm and 42% FMC had the lowest ($p<0.05$) OAC.

3.4.4. Emulsion capacity (EC)

Meat extenders with high EC and ES prevent cooking loss (i.e., fat and moisture) (Jiménez Colmenero, 2014) and shrinkage (i.e., change in thickness and/or diameter) when applied in products such as burgers and sausages (Lonergan, Topel, & Marple, 2019; Riaz, 2004;

Wong, Corradini, Autio, & Kinchla, 2019), making these properties are integral for TVP quality. When raw PBFs are considered, EC increased significantly ($p<0.05$) as raw PBF increased from 70 to 76, and then to 82% protein (Figure 5). The raw 70% PBF had the lowest EC of all raw PBFs and treatments studied. This is expected as proteins act as the primary emulsifiers in food emulsions (Burger & Zhang, 2019). Generally, EC depends on the protein's capacity to immerse itself into the oil droplet surface and form a densely packed protein layer (Ladjal-Ettoumi, Boudries, Chibane, & Romero, 2016; Lam et al., 2017; Shevkani, Singh, Kaur, & Rana, 2015). Similar EC values were reported for pea protein isolate in other studies (Gharsallaoui, Saurel, Chambin, & Voilley, 2012; Shen & Li, 2021).

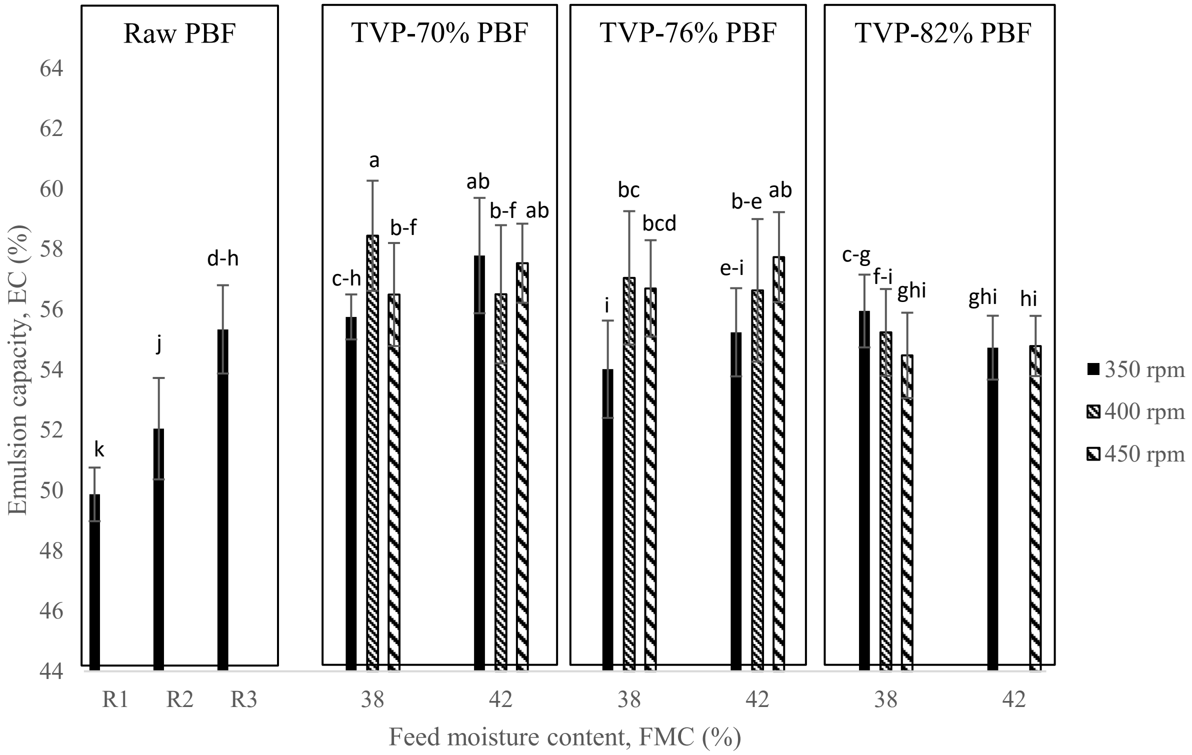


Figure 3. Emulsion capacity (EC) of raw protein blending formulas (PBFs) at 70% (R1), 76% (R2) and 82% (R3) and texturized vegetable proteins (TVPs) that were produced using a combination of protein blend formulas (PBFs), screw speeds and feed moisture contents (FMCs) treatments (n=6 for raw PBFs, n=12 for TVPs). NSI values were not significantly different if designated with the same letter (p<0.05). Error bars represent ± standard deviation.

Extrusion processing improved EC of 70% and 76% PBF TVPs but did not impact 82% PBF compared to their raw counterparts. Of the extrusion treatments studied, the TVP produced with 70% PBF, 400 rpm and 38% FMC had the highest (p<0.05) EC, while the 76% PBF, 350 rpm and 38% FMC treatment had the lowest (p<0.05) EC. Some treatment combinations (i.e., 70% PBF, 42% FMC, at 350 and 450 rpm) had slightly higher EC, with others (i.e., 82% PBF, 38% FMC at 400 and 450 rpm and 42% FMC at 350 and 450 rpm) EC skewed lower. As protein adsorption on an oil-water interface depends on protein solubility and hydrophobicity (Shevkani et al., 2015), the higher NSI values of TVPs at the lower PBFs (Figure 4) might account for their enhanced EC values for these TVPs, with a moderate positive correlation between NSI and EC (r=0.60). Protein solubility was also reported to be positively correlated with EC of legume protein isolates, but other factors, like the surface charge and hydrophobicity of the protein surface and its interaction may also effect EC (Karaca et al., 2011). Higher EC levels are desirable for meat extender applications as it can improve the perceived product juiciness (Wi, Bae, Kim, Cho, & Choi, 2020).

Under the same PBF and FMC conditions, an increase in screw speed (350 to 400 rpm) enhanced EC for only a small number of TVPs (i.e., 70% PBF at 38% FMC and 76% PBF at 38% FMC) but was ineffective for the remaining TVPs. In contrast, a reduction in EC may be due to an increase in screw speed (from 135 to 245) for pea protein-based TVPs (Wang et al., 1999). This difference compared to the results of Wang et al. (1999) may have resulted from molecular breakdown of proteins at faster screw speed (Fang et al., 2014) employed in the current study. Generally, smaller size proteins can more readily move to oil-water interfaces and adsorb on more easily due to their relatively higher flexibility when compared to larger sized proteins. With the PBF and screw speeds studied, FMC's effect on EC was generally negligible. The selected FMC may not have been wide enough to demonstrate a significant difference for this starting material, as seen in previous studies. For instance, an increase in EC as FMC increased (30.5-42% FMC) for pea protein-based TVPs (Wang et al., 1999), while a different study noted that an increase in FMC (20-24%) resulted in higher EC values for chickpea flour and sorghum flour based extrudates, but in lower EC values for corn flour based extrudates (Wang, Ai, Hood-Niefer, & Nickerson, 2019).

3.4.5. Emulsion stability (ES)

For raw PBFs, ES significantly increased ($p<0.05$) as PBF was raised (Figure 6). In fact, 82% raw PBF had the highest ES of all raw PBF and treatments studied. This trend was expected as ES has a positive relationship with protein concentration (Ladjal Ettoumi et al., 2016), because higher protein levels can more easily cover an oil droplet surface and prevent coalescence (Burger & Zhang, 2019; Varzakas et al., 2014). ES depends on the properties (e.g., rheological, protein structure and surface charge) of the absorbed protein layer in itself (Ladjal-Ettoumi et al., 2016;Lam et al., 2017; Shevkani et al., 2015). The average ES value for raw PBFs studied was ~50% (70-82% protein, d.b.) which is relatively lower compared to the literature. The average ES of pea protein (71-88% protein, d.b.) isolated from several cultivars and obtained using different isolation methods was reported as ~99% (Stone et al., 2015), while ES of a laboratory prepared pea protein isolate (70-74% protein, d.b.) was 95.7% (Lam, Warkentin, Tyler, & Nickerson, 2017). To some extent, the difference between the ES values in the current study and previous work can be attributed to the different variety of pea cultivars, protein isolation methods (Stone et al., 2015; Vogelsang-O'Dwyer, Zannini, & Arendt, 2021) and emulsion formation conditions (e.g., homogenization devices, homogenization speeds, type of oil used) (Burger & Zhang, 2019; Friberg, Larsson, & Sjoblom, 2004). It is interesting to note that the raw PBF had lower EC compared to their respective TVPs, but had higher ES compared to their TVP counterparts. It has been reported that EC is impacted by the relationship between protein solubility and surface charge, while ES is affected by the interaction between protein surface charge and surface hydrophobicity (Karaca et al., 2011). Therefore, the intricacies between these characteristics may contribute to the raw PBF EC and ES characteristics.

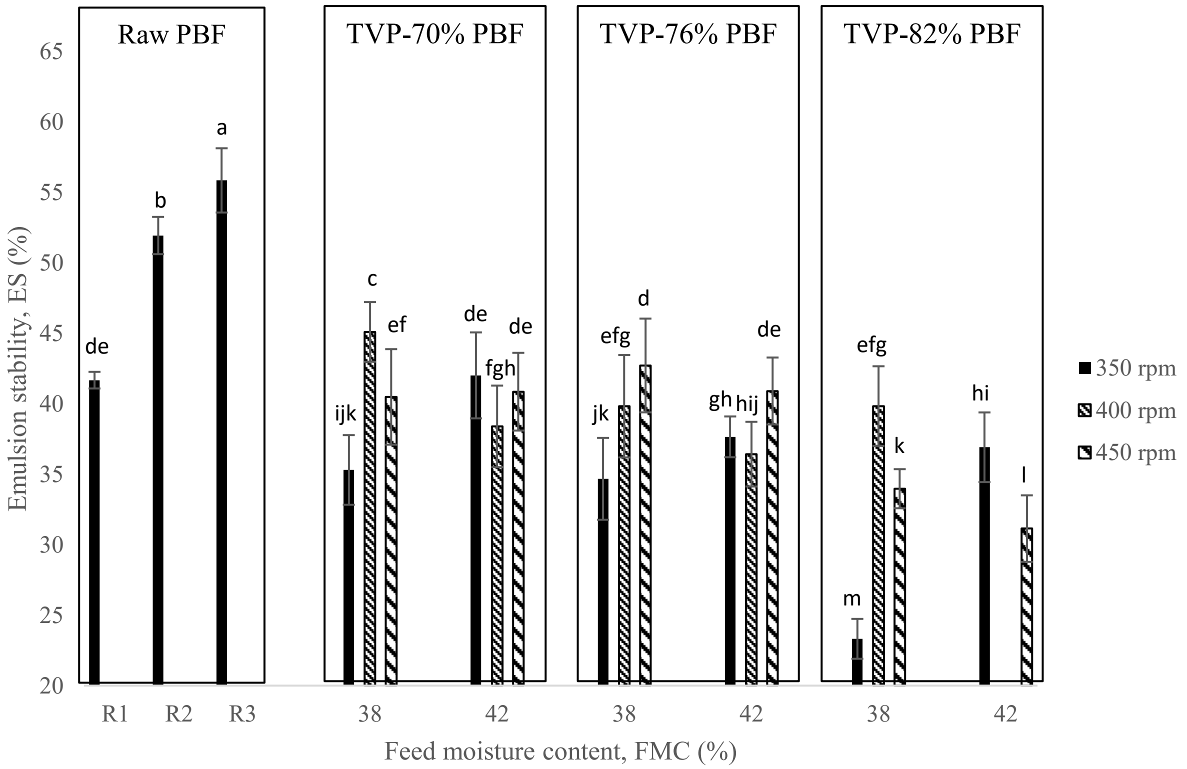


Figure 4. Emulsion stability (ES) of raw protein blending formulas (PBFs) at 70% (R1), 76% (R2) and 82% (R3) and texturized vegetable proteins (TVPs) that were produced using a combination of protein blend formulas (PBFs), screw speeds and feed moisture contents (FMCs) treatments (n=6 for raw PBFs, n=12 for TVPs). NSI values were not significantly different if designated with the same letter (p<0.05). Error bars represent ± standard deviation.

Extrusion processing negatively impacted ES properties for majority of the TVPs. After extrusion ES significantly (p<0.05) decreased for all 76 and 82% PBF TVPs. Although some 70% PBF TVPs decreased (p<0.05) from extrusion processing, some (450 rpm, 38% FMC and 350, 450 rpm at 42% FMC) maintained similar ES values when compared to their raw PBF counterparts. The 70% PBF, 400 rpm and 38% FMC treatment maintained the greatest stability (p<0.05). In contrast, the TVPs produced with 82% PBF, 350 rpm and 38% FMC had the lowest (p<0.05) ES. The remaining treatments had intermediate ES values. In protein stabilized emulsions, denaturing proteins can greatly impact emulsion properties. High protein solubility is essential for proteins to effectively move towards the oil-water interface and cover oil droplets (Burger & Zhang, 2019; Karaca et al., 2011). The drop in NSI from extrusion processing may have contributed to the reduction in ES for higher protein containing PBF TVPs, as solubility was found to positively impact legume protein isolate emulsion stability (Karaca et al., 2011). Due to its higher starch content, the lowest PBF (i.e., 70%) may have been able to generally maintain similar ES levels after extrusion cooking by forming relatively more gelatinized starch and Maillard reaction induced protein-gelatinized starch complexes (Shen & Li, 2021). Gelatinized starch can act as a protective barrier between oil droplets, which reduces the rate of coalescence (Aluko, Mofolasayo, & Watts, 2009; Ma et al., 2011; Sikorski, 2001). Maillard reaction induced protein-polysaccharide interactions can strengthen the absorbed protein layer at the oil-water interface in an emulsion (Lin et al., 2017; Shen & Li, 2021). The 70% PBF EC may have benefited more from these stabilizing interactions, as it has relatively more starch than 76 and 82% PBFs. Similar to the current study, the ES of TVPs made from black gram protein by-product (51% protein, d.b.) significantly decreased after extrusion cooking (Kamani et al., 2021).

On average, ES generally decreased with raising PBF when screw speed and FMC were kept constant. ES values for the 70, 76 and 82% PBF were 0.40 ±0.03, 0.39 ±0.03 and 0.33 ± 0.06%, respectively. Although increasing protein concentration has previously been reported to improve emulsion properties (Peng et al., 2016), other factors such as protein solubility (Figure 4) (Ladjal-Ettoumi et al., 2016) and molecular flexibility (Mozafarpour et al., 2019) may have impacted TVP ES. Similarly, soy protein isolate ES decreased after heat treatment. The authors attributed the reduction in ES to protein aggregation, and thus the proteins becoming less effective at surrounding the oil droplets (Keerati-u-rai & Corredig, 2009).

Increasing shear by increasing screw speed (Zhang, Zhang, Dreisoerner, & Wei, 2015), can modify a protein's ES positively by modifying the protein structure via disruption of protein aggregates and exposure of its hydrophilic or hydrophobic groups (Beck, Knoerzer, & Arcot, 2017). An increase in ES occurred with an increase in screw speed from 350 to 400 rpm was observed for all TVPs produced at 38% FMC, regardless of the PBF. However, when the screw speed was further increased to 450 rpm, it was detrimental to ES of some TVPs (i.e., 70% and 82% PBF). Higher screw speed enhances shear, which may have resulted in greater denaturation of the protein, thus compromising the protein-stabilized emulsion (Lin et al., 2017; Shen & Li, 2021). At 42% FMC, there were some differences between the different screw speed levels, however there was no clear pattern.

At a constant PBF and screw speed, FMC did not generally impact the ES values of TVP. At 38 and 42% FMC, the average ES values of TVPs were 0.37 ± 0.06 and 0.38 ± 0.03%, respectively. However, there was a complex interaction between the effects of FMC and screw speed on ES. For example, the ES of TVPs at the lowest screw speed (i.e., 350 rpm) increased ($p<0.05$) when the FMC increased from 38 to 42%, regardless of the PBF. The lower FMC may have enabled greater protein denaturation which uncovered a greater amount of buried hydrophobic groups of the protein (Chen, Wei, & Zhang, 2011). The dominantly hydrophobic proteins would be more attracted to the oil interface causing the oil droplet size to increase via droplet flocculation, which was shown to cause emulsion destabilization (Mozafarpour et al., 2019; Peng et al., 2016). However, this was not consistent for all TVPs. For example, for TVPs produced with 70% PBF at 400 rpm, EC significantly ($p<0.05$) decreased when FMC increased from 38 to 42%. This may indicate that achieving the balance between hydrophilic and hydrophobic protein molecules favorable for ES (Karaca et al., 2011) can be obtained through different combinations of processing conditions. FMC did not impact the ES of soybean meal extrudates (Singh & Koksel, 2021).

3.5. Conclusion

PPC and PPI were blended and texturized using extrusion processing. The impacts of protein concentration (via PBF) as well as the effects of extrusion processing conditions on NSI and TVP techno-functional properties were investigated and compared with those of raw starting materials. When PBF of the raw starting material increased, it corresponded with a decrease in NSI, along with an increase in WHC, EC and ES, but did not impact OAC. The heat and shear from extrusion cooking likely impacted protein structure, which then may have created new bonds (i.e., protein-protein and protein-starch), and changed the techno-functional properties of the TVPs. At all PBFs, extrusion processing decreased NSI but increased OAC. However, extrusion's impact on WHC, EC, and ES was more complex and possibly interacted with protein concentration via PBF. For instance, TVPs produced with 70% and 76% PBF contained a relatively higher amount of starch, which may have increased the quantity of gelatinized starch after extrusion. This may have improved this TVPs interaction with water, which resulted in an overall improved WHC. Except for NSI reducing from an increase in FMC in some TVPs, the extrusion conditions studied did not show a consistently discernable impact on TVPs for the remaining techno-functional property discussed. The TVP produced with 70% PBF, 450 rpm and 42% FMC may be suitable for burger and non-emulsion products as it has the highest WHC and relatively higher OAC values. However, for emulsified products (e.g., sausages), 70% PBF, 400 rpm and 38% FMC had superior emulsion characteristics, compared to the other treatments studied. These results indicate that future TVP work should be focused on PBF, as this had the greatest impact for NSI and the techno-functional properties. Furthermore, the 70% PBF TVPs may be promising for meat extender applications, as it had generally had higher EC and ES, and similar WHC and OAC compared to the higher PBF TVPs, while also being relatively the least expensive PBF.

Bridge to Chapter 4

Determining the techno-functional qualities of TVPs can indicate their potential as a meat extenders; as certain techno-functional characteristics can enhance extended meat products However, the TVP physical characteristics are also important qualities to determine if they can be applied with animal protein, as they must maintain their fibrous, physical structure after numerous downstream processing steps. Furthermore, the application of TVPs as a meat extender will indicate if differences in techno-functional and physical properties will influence the cooking properties and texture quality attributes of burgers. The following chapter explores the impact of PBF and extrusion processing parameters (screw speed and FMC) on hydration time and integrity index. Furthermore, a selection of TVPs were applied as a meat extender at different extension levels (20, 30 and 40% w/w). The impact of extension level, PBF and FMC were investigated for their impact on burger cooking characteristics (i.e., total cooking loss, change in burger thickness, and increase in burger thickness) and texture quality attributes (hardness, cohesiveness, springiness and chewiness) are discussed.

Chapter 4: Impact of pea protein blend formulations and extrusion conditions on the physical properties of TVPs in extended beef burgers

4.1. Abstract

Texturized vegetable proteins (TVPs) are mainly soy- or wheat-based products that are commonly applied as meat extenders in numerous foods, including burgers. More consumers avoiding soy and wheat gluten, warrants investigation of new TVP ingredients. The objectives of this study were to assess the physical properties of pea protein-based TVPs and TVP function as a meat extender (20, 30 and 40% w/w) in beef burgers. TVPs were produced with varying protein blend formulas (PBF) (70, 76, 82% protein db), extrusion screw speed (350, 400, 450 rpm) and feed moisture content (FMC) (38 and 42%, db). Increasing PBF raised TVP hydration time and integrity index, while FMC and screw speed had no discernable trend on these properties. A select group of TVPs with varying PBF and FMC were applied as meat extenders in beef burgers which were tested for their cooking properties and textural quality (hardness, cohesiveness, springiness and chewiness). TVP PBF had varying impacts on total cooking loss of burgers, but lower PBF levels reduced change in burger diameter, burger hardness and chewiness. Higher FMC lowered total cooking loss for some burgers, but overall did not significantly impact burger cooking properties or texture. Increasing extension level reduced total cooking loss and change in burger diameter but had no discernible trend for texture. TVPs produced with 70% PBF at 42% FMC had the greatest potential in meat extender applications, as they decreased total cooking loss and change in burger diameter, while maintaining comparable textural qualities to burgers with no TVP addition.

4.2. Introduction

Beef burgers are one of the most popular meat products in the world (Bahmanyar, Hosseini, Mirmoghtadaie, & Shojaee-Aliabadi, 2021). Burgers often contain non-meat ingredients (i.e., extenders) to reduce cost, improve their nutritional, functional and sensory qualities (Feiner, 2006b). Popular meat extenders are contain high protein content as the proteins impart high water holding capacity, emulsion stability, and protein-protein interactions (Mills, 2014). Extenders come in numerous forms, such as plant protein isolates (Shen et al., 2022), legume flours (Serdaroğlu, Yildiz-Turp, & Abrodímov, 2005), and texturized vegetable proteins (TVPs) (Feiner, 2006b). TVPs are plant proteins that have been processed to form a fibrous structure and chewy texture (Riaz, 2004). When TVPs are incorporated into meat products, they can improve the product's overall techno-functional properties, without detracting from overall meaty texture (Asgar et al., 2010).

TVP's unique fibrous structure is produced through texturization; a process where proteins are denatured and aligned during extrusion processing (F. L. Chen et al., 2010). To enable texturization, certain starting materials (i.e., high protein content and suitable techno-functional qualities) and extrusion operating conditions (e.g., screw configuration, feed moisture content (FMC), screw speed, etc.) are required (Vatansever et al., 2020; J. Zhang et al., 2018).

The most established ingredients for TVPs are soy and wheat protein; as they are readily available, inexpensive and relatively easy to texturize (Riaz, 2004). However, soy and wheat protein (i.e., gluten) usage is becoming limited due to their priority allergen status' globally, including North America, Central America, European Union, and many South American and Asian countries (FARRP, 2022; FDA, 2022; Food Standards Agency, 2022; Health Canada, 2017) and GMO associations (Webb et al., 2020). Therefore, alternative plant proteins, such as mung bean protein isolate, peanut protein isolate (Samard & Ryu, 2019b) and black gram (*Vigna mungo)* by-product (Kamani et al., 2021), have been investigated for their ability to texturize or function as meat extenders (Shen et al., 2022; Shoaib et al., 2018). However, there is a gap in literature for testing alternative plant protein based TVPs as meat extenders in popular meat products like burgers. Pea is a promising alternative protein to soy and wheat for the manufacture of TVPs, because it is non-allergenic, does not have GMO associations (Maningat et al., 2022) and possesses promising techno-functional and physical characteristics in TVP applications (Samard & Ryu, 2019b; Wang et al., 1999).

The objective of the present study is to assess the influence of protein blend formula (PBF), extrusion screw speed and feed moisture content (FMC) on TVP physical characteristics (i.e., integrity index (II) and hydration time (HT)). Additionally, a group of TVPs were selected to be applied at different extension levels as a meat extender in beef burgers. The impacts of TVP processing conditions and extension levels were tested on burger cooking (i.e., total cooking loss (TCL) and burger shape retention (change in burger diameter (CBD) and increase in burger thickness (IBT)) and textural quality attributes (i.e., hardness, cohesiveness, springiness and chewiness) were also assessed.

4.3. Materials and methods

4.3.1. Materials and proximate analyses

Pea protein concentrate (PPC) and pea protein isolate (PPI) and Texturized Pulse Protein (a commercial pea protein-based TVP with a protein content of 73% (db) as per the information provided by the manufacturer) were sourced from AGT Food and Ingredients (Minot, ND, USA). Fresh medium ground beef was sourced from a local butcher. The proximate composition of the PPC and PPI were measured in triplicate, and the medium ground beef in duplicate following standard methods. Moisture content, crude protein and fat content were measured using AOAC method 930.15, AOAC method 990.03 and AOCS method AM 5-04, respectively. Ash contents of pea protein (PPC and PPI) and medium ground beef were measured following AOAC methods 923.03 and 942.05, respectively. Total carbohydrates were calculated by difference. The PPC and PPI proximate composition (Table 4) was used to calculate the pea protein concentrate to isolate ratios for the protein blend formulas (PBFs).

Table 4. Proximate composition of raw materials.

Proximate composition (%, db)	Pea protein concentrate	Pea protein isolate	Medium ground beef
Protein	54.8 ± 0.0	81.5 ± 0.0	57.1± 0.7
Fat	2.7 ± 0.0	0.4 ± 0.0	40.8± 0.2
Ash	4.7 ± 0.0	4.8 ± 0.0	3.1± 0.3
Total carbohydrates	37.8	13.2	0.0

Data presented is the mean ± standard deviation (n=3 for pea protein concentrate and isolate, n=2 for medium ground beef).

4.3.2. Preliminary treatment of materials

Calculated ratios of PPC and PPI were mixed to create 70% (44.2 PPC: 55.8 PPI) and 76% (21.8 PPC: 78.2 PPI) protein (db) containing PBFs, using a Hobart mixer (model A200, Offenburg, Germany) with a paddle attachment. The 82% PBF was comprised only of PPI. All PBFs were preconditioned to 35% (db) by continuously mixing at a low speed while slowly pouring in boiling water. The preconditioned PBFs were kept warm by storing them in an environmental chamber (HPP 260 IPP plus, Memmert, Schwabach, Germany) at 40°C at 65% relative humidity, prior to being fed into the extruder's feed hopper.

4.3.3. Extrusion process

The preconditioned PBF was extruded using a lab-scale, co-rotating twin screw extruder (MPF19, APV Baker Ltd, Peterborough, UK) with 25:1 length-to-diameter (L:D) ratio (D = 19 mm). The screw configuration (starting at the feed input, ending at the die) consisted of 7D feed screws (twin thread), 4D lead screws (LS) (single thread), 2D 60° forward paddles (FP), 4D LS, 1.5D 30°FP and 6.5D LS. The preconditioned PBFs feed rate was calibrated to 2.75 kg/hr (db). The final feed moisture content (FMC) of 38 and 42% (db) was from additional water being directly injected into the barrel. The screw speeds used were 350, 400 and 450 rpm. The barrel temperature was set to 60-80-100-110-115°C from the feeder to die end of the barrel, based on visual assessment of texturization during preliminary experiments. The extruder was affixed with a 5 mm diameter circular die. Extrudates were produced at their respective PBF, FMC and screw speed, in duplicate, cooled down to room temperature and then dried overnight at ambient conditions (~21-23 °C) to achieve a moisture content <20%. The following day, TVPs were reduced to 1-2 mm pieces, using a laboratory mill (Mill 3610, Perten Instruments, Turku, Finland) and were stored in air-tight plastic bags at -40°C until further analysis. TVP produced at 82% PBF, 400 rpm and 42% FMC was not analyzed due to being compromised during storage.

4.3.4. TVP physical properties

4.3.4.1. Hydration time

Hydration time (HT) was measured following the method reported by Roberts (2013) with slight modifications. Five grams of dried TVP was hydrated in room temperature distilled water (60 mL) and checked every 30 s until it was fully hydrated. TVP was considered completely hydrated when there were no hard areas remaining in its centre. HT was the total time needed to hydrate.

4.3.4.2. Integrity index

Integrity index (II) was measured using a method adapted from Ma et al. (2018). Five grams of dried TVP was soaked in 100 mL of distilled water and autoclaved (SV-120 Scientific Prevacuum Sterilizer, Steris, Mentor, OH, USA) for 15 min at 121°C. The autoclaved TVP was drained and rinsed with distilled water for 15 s, then placed in a 200 mL beaker with 100 mL of distilled water. This mixture was homogenized (Silverson Machines LTD, Waterside, Chesham Buckinghamshire, England) at 3,200 rpm for 1 min. The homogenized extrudate was filtered with a mesh sieve size of 710 µm. The extrudate residue on the sieve was dried at 200°C in an air oven (Isotemp 180L Oven Gravity, Thermo Scientfic, Langenselbold, Germany) for 2 hours. II was calculated according to equation (5):

$$\text{II}\ (\%) = \frac{\text{Dried TVP residue weight (g)}}{\text{Initial TVP weight (g)}} \times 100 \qquad (5)$$

4.3.5. Scanning electron microscopy (SEM)

The microstructure of extrudates was investigated according to Koksel & Masatcioglu (2018). Briefly, a blade was used to cut the extrudates into 5mm thick slices. Then, each extrudate was coated in a cold sputter coater (Denton Vacuum, Desk II, NJ, USA) with Au-Pd alloy. Coated samples were placed in a Scanning Electron Microscope (SEM) (Quanta FEG 650, FEI, Hillsboro, OR, USA) and the microstructural images were obtained at a magnification of 100×.

4.3.6. Cooking properties of extended beef burgers

4.3.6.1. Preparation of burgers

Beef burgers were extended with a select group of TVPs (70, 76 and 82% PBF produced with 38 and 42% FMC at 400 rpm) at 20, 30 and 40% (wb) extension levels. These TVPs were selected as meat extenders because PBF and FMC was shown to have the greatest impact on nitrogen solubility index and techno-functional properties, like emulsion capacity and emulsion stability (Refer to chapter 3). Given the impact of these properties on the quality of the extended product (Jiménez Colmenero, 2014; Lonergan et al., 2019; Wong et al., 2019), it was important to determine if the techno-functional properties of the TVPs bear any impact to the burger cooking properties and textural quality attributes. A commercial pea protein-based TVP was also tested as a positive control and was applied at the same extension levels. TVPs were hydrated with a water: TVP ratio of 1.55: 1 (w/w) which was predetermined in a maximum hydration ratio test (data not shown). The maximum hydration ratio test involved measuring the greatest amount of water the TVP could absorb in its established HT, without leaving any residual water. All TVPs were hydrated according to their previously established HT.

To prepare the burger formulas, the respective amounts of medium ground beef and hydrated TVPs were mixed for 4 min using a mixer (Professional 600, KitchenAid) affixed with a paddle attachment. The burger mixture was weighed to 40 g and compressed between two pieces of parchment paper using a circular patty former (~63.5 mm diameter, ~12.5 mm thickness). Formed burgers were arranged on a single tray and placed in the freezer (-20°C) for 30 min. After partial freezing, burgers were removed from the tray, placed in plastic freezer bags and stored in a -20°C freezer until cooking. The negative control burger (i.e., no burger extension) was prepared similarly, but with no TVP added. The positive control and TVP extended burgers were prepared identically.

4.3.6.2. Burger cooking

Burgers were pan broiled according to the American Meat Science Association research guidelines (2016). Briefly, non-stick pans were preheated to 163°C. A pre-test patty was used to determine the cooking time for each formula. A timer was started as soon as the patty was placed onto the pan. The burger was flipped every minute to ensure even cooking and prevent sticking. A temperature probe was used to monitor the internal burger temperature, until 66°C was reached. The burgers were placed on a cooling rack and reached 71°C after resting for 2 min. The remaining burgers were cooked using the same method to their respective established cooking time. A temperature probe was not used on these patties to preserve the internal structure for future tests.

4.3.7. Cooking characteristics

The weight, diameter, and thickness of 12 burgers from each formulation were measured before and after cooking. The burger cooking properties were measured promptly after the 2 min resting period. After measuring cooking characteristics, the burgers were stored in air-tight plastic bags in the refrigerator until further analysis.

4.3.7.1. Total cooking loss

Total cooking loss (TCL) is the weight loss resulting from cooking. After cooking the burgers, they were blotted with a paper towel prior to being weighed. The burger was weighed before and after cooking, and TCL was calculated as per equation (6) following the method described by Lucas-González et al. (2020).

$$\text{TCL (\%)} = \frac{\text{Raw weight (g)} - \text{Cooked weight (g)}}{\text{Raw weight (g)}} \times 100 \qquad (6)$$

4.3.7.2. Change in burger diameter (%)

The burger diameter was measured with a digital calliper (accuracy 0.01 mm) in 45° intervals around the circumference of the burger (totalling 4 measurements) before and after cooking. The respective measurements were averaged prior to calculating burger diameter shrinkage. Change in burger diameter (CBD) is calculated using equation (7) following the method described by Oroszvári, Bayod, Sjöholm, & Tornberg (2005):

$$\text{CBD (\%)} = \frac{\text{Average raw diameter (mm)} - \text{Average cooked diameter (mm)}}{\text{Average raw diameter (mm)}} \times 100 \qquad (7)$$

Increase in burger thickness (%)

The burger thickness was measured with a digital caliper. The average of 3 measurements before and after cooking was used to calculate the increase in burger thickness (IBT) using equation (8):

$$\text{IBT (\%)} = \frac{\text{Average cooked thickness (mm)} - \text{Average raw thickness (mm)}}{\text{Average cooked thickness (mm)}} \times 100 \qquad (8)$$

4.3.8. Textural quality attributes

The cooked burgers were left at room temperature for 2 hours for temperature equilibration prior to texture measurement. An infrared thermometer was used to confirm that the burgers were at room temperature. Burgers were cut into small pieces (1.5 cm x 1.5 cm x 1.0 cm) following Samard (2021). The textural properties were measured with a texture profile analyzer (TA-XT-plus; Stable Micro Systems, Godalming, UK) equipped with a 30 kg load cell. The burger pieces were placed beneath a 25 mm diameter cylindrical ebonite probe and compressed to 75% of their original thickness. The probe compressed the burger piece twice creating a force-time plot and moved at a constant speed of 100.0 mm/min during the test. The resulting force-time graph had two peaks, corresponding to the two compressions. Textural characteristics including hardness, cohesiveness, springiness and chewiness were determined from the force-time plot. Hardness (N) is defined as the peak force of the initial compression, cohesiveness is the ratio of the area under the second peak to that under the first peak, springiness is the ratio of the height of the second peak to that of the first peak, and chewiness is the product of hardness, cohesiveness and springiness (TTC, 2020).

4.3.9. Statistical analysis

Using a completely randomized design, each TVP was produced using 17 individual treatment combinations (i.e., PBF, screw speed and FMC) in an incomplete factorial structure. The extended burgers were also structured as a completely randomized design with an incomplete factorial structure, with 5 TVP treatment combinations (i.e., PBF and FMC) and 3 extension levels. In addition, one positive (i.e., AGT commercial TVP) and one negative (i.e., no extension) treatment were used. The statistical differences between TVP physical properties (i.e., HT and II) and burger cooking and textural properties (i.e., TCL, CBD, IBT, hardness, cohesiveness, springiness and chewiness) were compared in a 1-way ANOVA ($p<0.05$), followed by mean separation through least square difference (LSD) t-test ($p<0.05$) (SAS software, version 9.2, SAS Institute Inc., Cary, NC, USA). Correlations between techno-functional tests were calculated using the data analysis function on Microsoft Excel 365 (2021).

4.4. Results and discussion

4.4.1. Physical properties of pea TVPs

4.4.1.1. Hydration time

Hydration time (HT) which is the time necessary to fully hydrate a TVP (Roberts, 2013) is an important quality attribute for industry applications, because TVPs are hydrated prior to being used in downstream processing steps (Riaz, 2011). The HT of the positive control and the TVPs are summarized in Figure 1. Among all the PBFs studied, TVPs produced at 70% PBF had the closest HT to that of the positive control (6 min). It has been suggested that HT should be between 5-15 min when hydrating TVPs with room temperature water (Riaz, 2011), in line with our findings for the 70% PBF TVPs. However, it should be noted that it is difficult to compare HT between different TVP products, as their size and surface area can significantly differ (Riaz, 2004). In the present study, TVPs were reduced in size to achieve a similar size range when compared to that of the positive control. Higher HT values for the 76 and 82% PBF TVPs (~18-22 min) were similar to those reported for texturized pea protein (~18 min) that resembled ground meat (Perera, 2016).

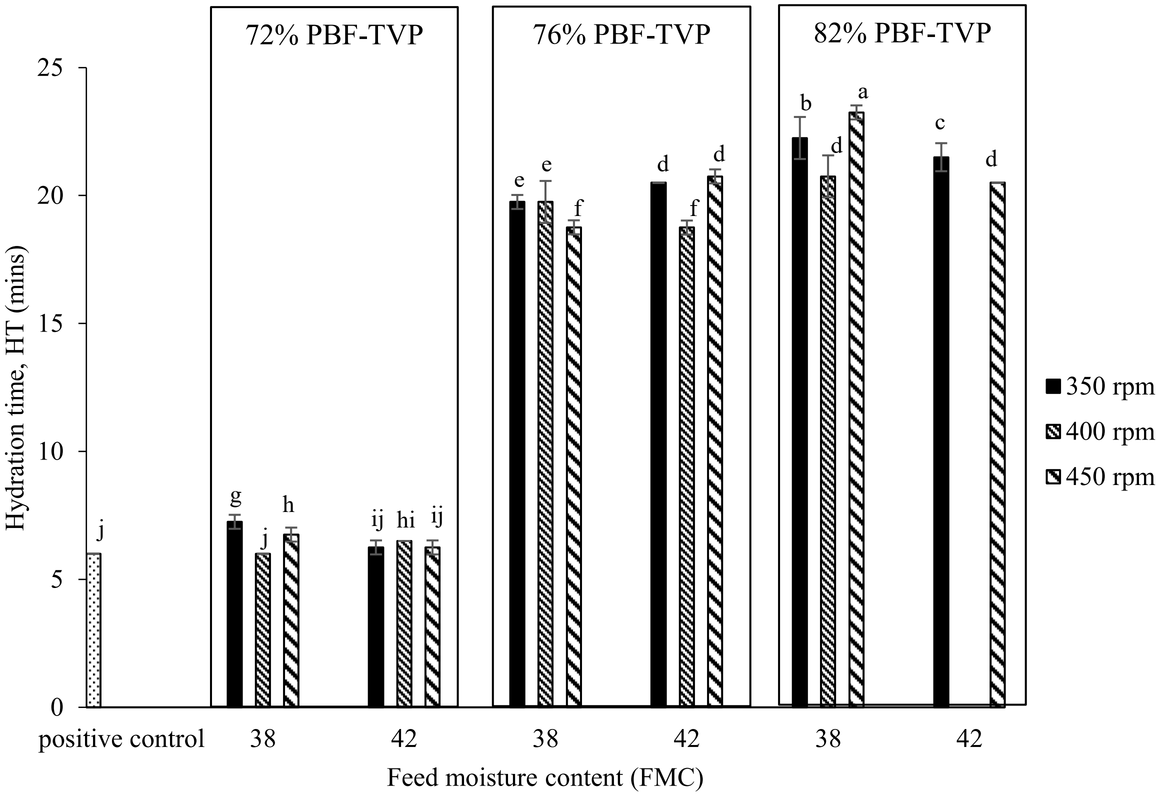
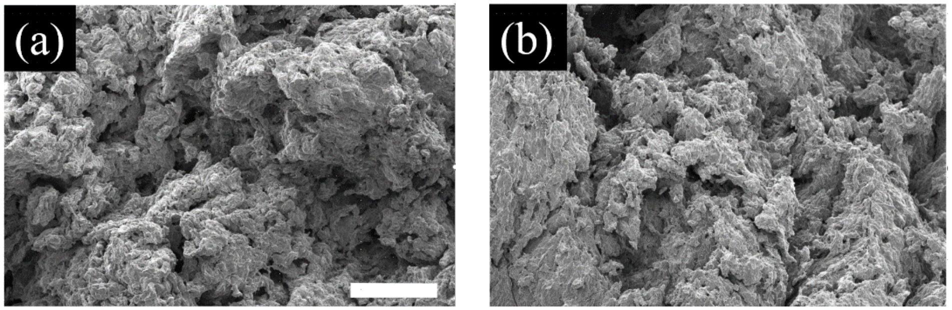


Figure 5. Hydration time (HT) of the positive control (i.e., commercially prepared TVP) (n=3) and texturized vegetable proteins (TVPs) that were produced using a combination of protein blend formulas (PBFs), screw speeds and feed moisture contents (FMCs) treatme treatments (n=6). HT values were not significantly different if designated with the same letter ($p<0.05$). Error bars represent ± standard deviation.

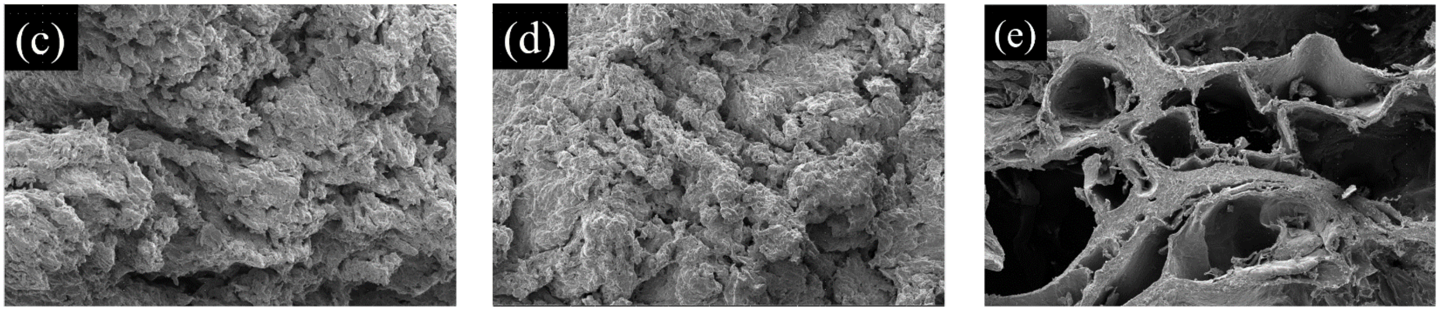
The HT of TVPs produced at the same processing conditions (i.e., FMC and screw speed) significantly increased ($p<0.05$) when PBF was raised from 70 to 76%. A further PBF increase to 82% resulted in a slight but significant rise ($p<0.05$) in HT of all TVPs, except for the TVP produced with 450 rpm and 42% FMC which was not affected by the increase in PBF. The greater protein content at higher PBFs may have increased the viscosity of the melt, due to the increased possibility of protein cross-links at the higher protein concentration (Philipp, Emin, Buckow, Silcock, & Oey, 2018; Verbeek & Van den Berg, 2010). HT's strong negative correlation to NSI (r= -0.97) (Refer to chapter 3) supports the likelihood that greater protein-

protein interactions (Riaz, 2004; Samard et al., 2021) occurred when PBF was increased. The relatively higher melt viscosity at higher PBSs may also inhibit bubble formation in the TVP leaving the extruder die, leading to a denser structure (Mosibo, Ferrentino, Alam, Morozova, & Scampicchio, 2020). Compared to the positive control, all TVPs were denser and lacked a discernable porous structure at the resolutions studied (Figure 2). A dense TVP is unfavorable for water absorption, which increases HT (Roberts, 2013; Webb et al., 2020).



76% PBF, 400 rpm, 38% FMC

76% PBF, 400 rpm, 42% FMC



76% PBF, 350 rpm, 42% FMC

76% PBF, 450 rpm, 42% FMC

Commercially prepared TVP

Figure 6. Scanning electron microscopy (SEM) images of laboratory prepared texturized pea protein produced with 76% protein blend formula (PBF) and varying feed moisture content (FMC) (a, b) and screw speed (c, b, d). The positive control (commercially prepared TVP) (e) is also pictured. The scale bar in (a) is 400 µm. All images are magnified to the same scale.

In general, screw speeds studied impacted the HT values of TVPs, but did not have an overall distinct trend. During extrusion, screw rotation transfers mechanical and thermal (via friction) energy to the melt. Due to this energy input with raising screw speed, melt viscosity may decrease (Moraru & Kokini, 2003), enabling greater extrudate expansion and porosity (Moraru & Kokini, 2003; Philipp et al., 2018). High porosity lends to greater water absorption (Samard et al., 2021; Webb et al., 2020), thus faster HT (Brishti et al., 2021; Roberts, 2013). Accordingly, as screw speed increases a lower HT value is expected. This trend was observed for only some of the TVPs, e.g., 70% and 82% PBF at 38% FMC when screw speed was increased from 350 to 400 rpm.

Raising FMC impacted HT but did not have a clear trend at any particular screw speed. An optimal TVP porosity and water absorption capacity for mung bean protein based TVPs was observed when manipulating FMC, screw speed and barrel temperature (Brishti et al., 2021), which indicated the complex interactions between these extrusion process conditions and their effects on the TVP structure.

4.4.1.2. Integrity index

Integrity index (II) reflects TVP quality and yield by measuring the quantity of TVP residue after downstream processing such as hydration, pressure, homogenization and drying (Samard, Maung, Gu, Kim, & Ryu, 2021; Wang, Bhirud, & Tyler, 1999). II results are summarized in Figure 2. The II value of the positive control was similar to those of 70% PBF TVPs. II results in the present study were comparable to the II of TVPs made from pea protein isolate (Samard & Ryu, 2019b), but lower than those from mung bean protein isolate (Brishti et al., 2021), meaning that TVPs made from pea proteins are likely more susceptible to harsh processing conditions when compared to those from mung bean proteins.

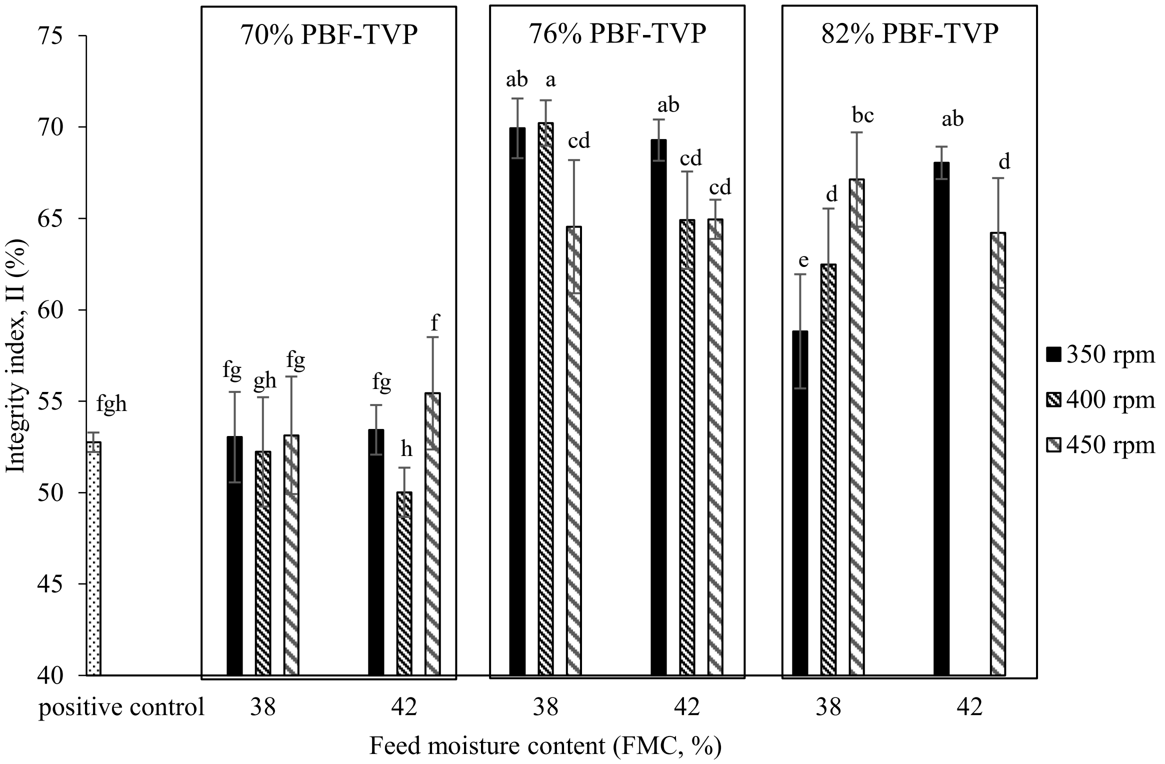


Figure 7. Integrity index (II) of the positive control (i.e., commercially prepared TVP) (n=3) and texturized vegetable proteins (TVPs) that were produced using a combination of protein blend formulas (PBFs), screw speeds and feed moisture contents (FMCs) treatments (n=6). HT values were not significantly different if designated with the same letter (p<0.05). Error bars represent ± standard deviation.

The II of TVPs initially increased when PBF was raised from 70 to 76% PBF. In general, higher II indicates TVPs with greater structural strength which may be due to a higher level of protein texturization (Samard et al., 2021; Samard, Gu, & Ryu, 2019). In the context of TVP production, a higher level of protein denaturation indicates higher protein texturization as well as an increase in insoluble protein formation (Riaz, 2004; Samard et al., 2021). A high degree of texturization is desirable for meat extender products (Brishti et al., 2021) as it gives the product a fibrous, meat like texture. This result corresponds with the strong negative correlation between NSI and II (r=-0.81) (Refer to chapter 3), and is in line with the literature (X. Ma et al., 2018;

Samard et al., 2021; Samard & Ryu, 2019b). Greater amount of starch in the 70% PBF may have caused a lower texturization level (Webb et al., 2020) and thus resulted in a softer structure (Yada, 2004). As texturization relies on the formation of protein-protein interactions, lower protein levels, thus higher starch levels, increase the overall likelihood of protein-starch interactions when compared to that of protein-protein interactions (Webb et al., 2020). A further increase in PBF from 76 to 82% PBF caused the II of some TVPs (i.e., 38% FMC at 350 and 400 rpm) to decrease; however, the remaining treatments were not impacted. It has been reported that greater amounts of mechanical energy is required to achieve the texturization of raw materials with low NSI value (Riaz, 2004). As 82% PBF has a lower NSI than 76% PBF (refer to chapter 3), the 82% PBF based TVPs may have required more mechanical energy to be effectively texturized during extrusion. Not surprisingly, II and HT are strongly and positively correlated with each other (r= 0.89), as both physical tests had strong negative correlations with NSI (r=-0.97 and r=-0.81, respectively) (Refer to chapter 3).

Although extrusion screw speed impacted II, there was no perceptible trend in the present study. Likewise, screw speed did not have a significant effect on the II of soy protein, wheat gluten and corn starch based TVPs (Samard et al., 2019). Similarly, FMC did not generally affect II. Raising FMC caused a decrease in II for soy protein, corn starch and green tea based TVPs and conversely, an increase in II for pea protein-based TVPs (X. Ma et al., 2018; N. Wang et al., 1999).

4.4.2. Cooking properties of extended beef burgers

4.4.2.1. Total cooking loss (TCL)

The cooking properties of extended burgers are summarized in Table 2. The negative control (i.e., no extension, all beef burger) had higher total cooking loss (TCL) than all burgers extended with the commercial TVP (i.e., the positive control), meaning that burgers extended with the commercial TVPs lost less of their weight when compared to the negative control. The same outcome was observed for the TCL values of burgers extended with the laboratory prepared TVPs. Overall, TCL values of the TVP extended burgers were in the range of 15-26% and similar to previous studies (Bakhsh et al., 2021; Walsh et al., 2008). Increasing extension level reduced TCL, with average TCL values of 24.6 ± 1.4 % at 20% extension level, 21.6 ± 2.7% at 30% extension level, and 17.9 ± 2.0% at 40% extension level. Therefore, it can be deduced that increasing the amount of TVP increased the burgers' overall water and oil binding capacity, in line with the literature (Hidayat et al., 2017). It should also be noted that as increasing TVP extension level reduces the overall burger fat content; a higher extension level reduces the overall fat that can be lost during cooking (González-Pérez & Arellano, 2009). During cooking, myofibrillar and collagen proteins denature and shrink, causing liquid (i.e., water and fat) and volatiles losses (Shen et al., 2022). The higher cooking loss for the negative control may be attributed to a relatively weaker emulsion stability of meat proteins; thereby contributing to greater water and fat loss (Samard et al., 2021; Verma, Banerjee, & Sharma, 2015). TVP extension reduces TCL by trapping moisture and fat (Hale et al., 2002; Jiménez Colmenero, 2014), and thus increases cooking yield (i.e., product retained after cooking). Other studies also showed that TVP extension reduced TCL, however these studies required higher extension levels for statistical differences. For example, textured soy protein (TSP) extension of beef patties did not improve cooking yield until >30% extension level relative to all meat control (Wong et al., 2019). Likewise, beef meatball yield was not significantly impacted when extended with 15% TSP, but significantly increased when extension level was raised to 30% (Grasso et al., 2019). The difference between the minimum extension level needed for reducing TCL may be attributed to the different types of TVPs used and texturization conditions explored between the present study and previous studies.

Table 5. Cooking properties of burgers.

Extension Level (%)	PBF (%)	FMC (%)	TCL (%)	CBD (%)	IBT (%)
0	Negative control		28.46 ± 2.0^{a}	23.22 ± 1.4^{a}	$12.14 \pm 3.6^{d\text{-}g}$
20	Positive control		20.63 ± 1.9^{f}	19.75 ± 1.8^{e}	20.41 ± 3.3^{a}
30	Positive control		16.78 ± 1.6^{jk}	16.69 ± 1.6^{h}	15.85 ± 2.7^{b}
40	Positive control		11.69 ± 1.4^{m}	11.94 ± 1.2^{j}	10.46 ± 1.9^{ghi}
20	70	38	25.39 ± 2.0^{b}	22.06 ± 1.4^{b}	13.98 ± 2.9^{bcd}
		42	22.36 ± 1.9^{e}	22.09 ± 1.4^{bc}	13.65 ± 4.3^{bcd}
	76	38	25.77 ± 1.8^{b}	21.48 ± 1.3^{bc}	$11.97 \pm 4.4^{e\text{-}g}$
		42	24.09 ± 2.3^{cd}	21.00 ± 1.5^{cd}	15.62 ± 2.9^{b}
	82	38	25.22 ± 2.3^{bc}	20.99 ± 1.6^{cd}	13.72 ± 4.3^{bcd}
30	70	38	18.51 ± 1.6^{ghi}	20.15 ± 1.6^{de}	14.81 ± 4.4^{bc}
		42	18.18 ± 2.2^{ij}	19.15 ± 2.5^{ef}	14.87 ± 4.8^{bc}
	76	38	24.61 ± 1.8^{bc}	19.26 ± 1.4^{ef}	$11.40 \pm 3.4^{e\text{-}h}$
		42	22.61 ± 2.5^{e}	19.30 ± 2.6^{ef}	14.46 ± 3.9^{bc}
	82	38	23.23 ± 2.6^{de}	18.44 ± 1.5^{fg}	$10.94 \pm 3.1^{f\text{-}i}$
40	70	38	15.24 ± 1.8^{l}	18.57 ± 1.8^{f}	13.40 ± 2.7^{cde}
		42	16.35 ± 1.3^{kl}	18.69 ± 1.5^{f}	$13.10 \pm 2.9^{c\text{-}f}$
	76	38	19.57 ± 1.4^{fgh}	17.07 ± 1.0^{h}	9.62 ± 2.4^{hi}
		42	18.40 ± 1.7^{hi}	17.44 ± 1.1^{gh}	10.40 ± 3.8^{ghi}
	82	38	19.74 ± 2.8^{fg}	15.10 ± 1.1^{i}	9.11 ± 2.9^{i}

PBF: Protein blend formula, FMC: Feed moisture content, TCL: Total cooking loss, CBD: Change in burger diameter, IBT: Increase in burger thickness.

The cooking properties of the negative control (i.e., burger with no TVP extension), positive control (i.e., burgers extended with the commercially produced TVP) (n=12) and experimental burgers (i.e., burgers extended with the laboratory prepared TVP) (n=24) extended burgers. Sharing letters within the same column are not significantly different ($p<0.05$). Values presented are the mean ± standard deviation.

The positive control burgers had lower TCL compared to those of the TVP extended burgers at their respective extension levels ($p<0.05$). The exception to this trend were the burgers extended with TVPs produced with 70% PBF and 42% FMC at 30% extension level, whose TCL was comparable to the positive control. The difference in TCL between the burgers extended with the positive control compared to the laboratory prepared TVPs at their respective extension level may be due to the TVP structure. As can be seen in Figure 2, the positive control had a more porous structure compared to the laboratory prepared TVPs (Figure 2) which would allow for better water and oil absorption (Webb et al., 2020), and thus contribute to the lower TCL values observed for the burgers extended with the positive control. Furthermore, the commercial TVP may have had relatively superior protein-water and starch-water interactions compared to the laboratory prepared TVP, which would have further reduced TCL (Grasso et al., 2019). Overall, 70% PBF based TVPs had lower TCL than 76 and 82% PBF based TVPs.

4.4.2.2. Burger shape retention

4.4.2.2.1. Change in burger diameter (CBD)

The burger shape retention results, i.e., change in burger diameter (CBD) and increase in burger thickness (IBT), are summarized in Table 2. TVP extended burgers had significantly lower ($p<0.05$) CBD than the negative control. These results indicate that 20% extension level may be sufficient to reduce CBD, relative to the negative control. On average, CBD decreased from 21.5 ± 0.44% to 19.3 ± 0.61% and then to 17.9 ± 1.45% as extension level increased from 20% to 30% and then to 40%, respectively. The cooking process induces denaturation of animal proteins (i.e., collagen and myofibrillar matrices) causing them to shorten and shrink in size (Bakhsh et al., 2021). The addition of extenders, such as TVPs, to meat products disrupt the collagen and myofibrillar matrices and reduce dimensional shrinkage (Riaz, 2006). TVP extension also reduced CBD in previous studies (Velioğlu, Velioğlu, Boyaci, Yilmaz, &

Kurultay, 2010; Walsh et al., 2008). In addition, lower PBF generally increased CBD while FMC generally did not influence CBD.

4.4.2.2.2. Increase in burger thickness (IBT)

When comparing the IBT values of the negative control and all extended burgers studied, approximately half of the extended burgers were comparable to the negative control. Only two burger treatments (i.e., laboratory produced TVP treatments of 40% extension level produced at 76% and 82% PBF and 38% FMC at) had lower ($p<0.05$) TCL relative to the negative control. The remaining burgers had significantly higher ($p<0.05$) or similar IBT compared to the negative control. Accordingly, it was concluded that there are minimal benefits for TVP extension in reducing IBT compared to the negative control. Compared to the literature, IBT for beef burgers extended with textured whey protein and TVP was not impacted and reduced, respectively (Walsh et al., 2008).

For laboratory TVPs, IBT decreased ($p<0.05$) as extension level increased from 20 to 40% extension, except for TVPs with 70% PBF. A similar trend was also seen for the burgers extended with the positive control. These results comply with beef burgers extended with textured whey protein (Hale et al., 2002) and soy-based TVPs (Bakhsh et al., 2021), which were extended to similar levels to the present study. These results indicate that higher extension levels should be preferred to reduce the changes in burger thickness. Alternatively, extenders with greater water and oil holding capacities and emulsion stability can be used to mitigate reduction of product shrinkage (Kamani, Meera, Bhaskar, & Modi, 2019; Shen et al., 2022).

The effect of PBF on IBT did not have a clear trend. Similarly, FMC generally did not impact IBT. However, a raise in FMC corresponded with an increase in IBT for some TVPs, namely the ones produced at 76% PBF at 20 and 30% extension level. In the literature, an increase in FMC resulted in an increase in IBT for meatless soy and wheat gluten TVP based burgers (Samard et al., 2021). A greater range of FMC may be required to observe a significant difference in CBD and IBT, as seen in other studies (Samard et al., 2021). This will be explored further in the future.

4.4.3. Textural quality attributes

Burger texture results are summarized in Table 3. Compared to the negative control burger, burgers extended with 76% and 82% PBF TVPs were harder ($p<0.05$) and burgers extended with 70% PBF TVPs were similar in hardness or softer. Furthermore, for all extension levels, TVPs produced at 70% PBF were softer ($p<0.05$) when compared to those at 76 and 82% PBF. It may be possible that the higher protein content in TVPs produced at 76% PBF and beyond may have increased the quantity of interactions (e.g., non-covalent bonds) between the proteins present (i.e., pea proteins in TVPs and myofibrillar proteins in beef), resulting in harder burgers (Bahmanyar et al., 2021; Shen et al., 2022; Youssef & Barbut, 2010). These results suggest that lower PBF values may perform better in terms of obtaining similar hardness values to the negative control. Likewise, beef burgers (with 30% texturized soy protein (TSP) extension, ~50% overall protein content) (Heywood, Myers, Bailey, & Johnson, 2002) and goat meat burgers (with 20% TSP extension) (Gujral, Kaur, Singh, & Sodhi, 2002) reported an increase in burger hardness when comparted to all beef burgers (Heywood et al., 2002). Furthermore, beef burgers extended with pea protein isolate (2.5 and 5% extension level) also increased in hardness relative to the negative control.

Table 6. Textural properties of burgers.

Extension Level (%)	PBF (%)	FMC (%)	Hardness (N)	Cohesiveness (%)	Chewiness (-)
0	Negative Control		119.0 ± 15.2^{de}	40.4 ± 2.4^{a}	48.3 ± 7.8^{ef}
20	Positive control		121.5 ± 14.7^{d}	35.9 ± 2.6^{de}	43.8 ± 7.7^{fg}
30	Positive control		115.0 ± 13.3^{de}	32.8 ± 2.73^{f}	38.0 ± 7.0^{hi}
40	Positive control		99.9 ± 8.5^{g}	28.8 ± 2.8^{g}	28.9 ± 4.2^{j}
20	70	38	106.0 ± 11.7^{fg}	34.5 ± 3.2^{ef}	36.7 ± 6.1^{i}
		42	112.2 ± 12.8^{ef}	36.8 ± 3.3^{cd}	41.4 ± 7.0^{gh}
	76	38	142.1 ± 17.1^{abc}	40.3 ± 3.6^{a}	57.5 ± 10.4^{abc}
		42	136.3 ± 20.6^{bc}	38.9 ± 3.1^{ab}	53.3 ± 10.6^{cd}
	82	38	135.8 ± 12.5^{bc}	39.7 ± 2.5^{ab}	54.1 ± 7.4^{bc}
30	70	38	113.6 ± 12.4^{def}	39.5 ± 2.8^{ab}	44.9 ± 5.8^{fg}
		42	113.4 ± 11.8^{def}	38.1 ± 3.0^{bc}	43.1 ± 5.4^{g}
	76	38	146.3 ± 15.0^{a}	40.1 ± 1.9^{a}	58.8 ± 6.8^{a}
		42	144.0 ± 15.1^{ab}	40.5 ± 2.5^{a}	58.5 ± 8.8^{ab}
	82	38	138.6 ± 17.0^{abc}	38.3 ± 3.1^{bc}	53.3 ± 9.0^{ab}
40	70	38	112.1 ± 9.9^{ef}	36.0 ± 2.7^{de}	40.4 ± 4.7^{ghi}
		42	114.8 ± 8.0^{de}	37.1 ± 2.1^{cd}	42.6 ± 3.8^{g}
	76	38	146.9 ± 10.3^{a}	39.2 ± 2.7^{ab}	57.7 ± 7.1^{abc}
		42	136.8 ± 15.7^{bc}	38.9 ± 2.8^{ab}	53.2 ± 7.7^{cd}
	82	38	133.7 ± 15.4^{c}	37.0 ± 2.0^{cd}	49.5 ± 7.2^{de}

PBF: Protein blend formula, FMC: Feed moisture content.

The textural properties of the negative control (i.e., burger with no TVP extension), positive control (i.e., burgers extended with the commercially produced TVP) (n=12) and experimental burgers (i.e., burgers extended with the laboratory prepared TVP) (n=24) extended burgers. Sharing letters within the same column are not significantly different ($p<0.05$). Values presented are the mean ± standard deviation.

The hardness values of the negative and positive control burgers were similar at 20 and 30% extension levels. Further extension at the 40% level resulted in softer burgers. These results are in line with previous studies for TVPs made from soy protein that a decrease in beef burger and meatball hardness values is seen as extension level was raised (Bakhsh et al., 2021; Grasso et al., 2019). Extension level did not substantially influence burger hardness of TVP extended burgers. Average hardness values at 20, 30 and 40% extension levels were 126.5 ± 16.2, 131.12 ± 16.4 and 128.9 ± 14.9 N, respectively.

Overall, at each extension level, the average cohesiveness of the burgers extended with laboratory prepared TVPs ranged in between the cohesiveness of the negative control burger and the average cohesiveness of the burgers extended with positive control TVPs at the corresponding extension level. In light of these findings, the TVPs studied generally outperformed the positive control in terms of the cohesiveness of the burgers produced, as the cohesiveness of positive control extended burgers was the lowest on average, followed by the laboratory TVP extended burgers. Among different PBFs studied, the 76% PBF TVPs stood out as, regardless of the extension level, their burgers had comparable cohesiveness values to that of the negative control which had the highest cohesiveness overall. It was also shown for TSP extended (10 to 40% extension level) burgers that TSP addition decreased cohesiveness when compared with all beef burgers in beef burgers (Bakhsh et al., 2021). The authors attributed the decline in cohesiveness from the myofibril network disrupted by TVP extension (Bakhsh et al., 2021).

Springiness was generally not impacted by extension level, PBF or FMC with average values ranging between 99.99-100.01% for TVP extended burgers, for burgers extended with the positive control, and for the negative control. Similarly, springiness was not impacted in several studies that tested different extenders (i.e., soy protein, bread crumb, quinoa, buckwheat flour, TVPs) in beef burgers (Bahmanyar et al., 2021; Bakhsh et al., 2021).

Compared to the negative control burger, burgers extended with 70% PBF TVPs generally had less chewiness while the majority of burgers extended with 76% and 82% PBF

TVPs were relatively chewier regardless of the extension level (Table 3). In general, TVP extended burgers were chewier than the positive control, and the positive control was similar or less chewy than the negative control. This may have resulted from the microstructural differences between the positive control and the TVPs (Figure 2). As the positive control burgers had lower TCL (regardless of extension level) compared to the TVPs, it may be deduced that these burgers contained a relatively higher level of moisture and fat. The greater amount of moisture and fat may have reduced burger chewiness (Akwetey & Knipe, 2012). The lower overall chewiness of the burgers made from the positive control TVP may make them more desirable for elderly consumers (Baugreet et al., 2016). Burger chewiness had no discernable trend with TVP extension level. This was not the case for the positive control where a reduction in chewiness was observed as extension level increased. This was also seen for TSP extended beef meatballs (Grasso et al., 2019). In contrast, TSP extension increased the chewiness of goat meat based burgers (Gujral et al., 2002). Chewiness of extended burgers increased as PBF was raised from 70 to 76% PBF but was generally not impacted with a further increase to 82% PBF. The higher PBF level may have developed a stronger protein network, thus increasing chewiness (Samard et al., 2021).

FMC did not substantially impact any textural property studied. The FMC levels studied may not have been wide enough to cause a measurable difference in the various textural properties studied. It was noted that there was no consistent trend for the mechanical and textural properties for soy based meat extenders in the literature; and the inconsistencies were attributed to the different soy ingredient composition, processing conditions (i.e., how the products were prepared) and, to a lesser extent, the texture analysis methods utilized (i.e. compression-relaxation tests vs. texture profile analysis) (Wong et al., 2019). The differences in the present study and literature may also be attributed to the different TVP starting material (i.e., soy protein vs. pea protein), as they have different techno-functional properties (Schreuders et al., 2019).

4.5. Conclusion

The impact of PBF and extrusion processing parameters (i.e., screw speed and FMC) on physical properties (i.e., HT and II) of pea protein-based TVPs were examined. As PBF increased from 70 to 76%, HT and II also increased. Further increase in PBF substantially changed HT and some II results. Both HT and II had strong correlations to NSI (r=- 0.97and r=- 0.81, respectively), indicating that the NSI of TVPs may inform TVP texturization level and signify structural changes. Neither screw speed nor FMC caused any discernable trend in HT or II. The group of TVPs selected were successfully applied as meat extenders, at different extension levels. Raising extension level reduced TCL, CBD, and the IBT of some burgers, but generally did not impact their textural quality attributes. Lower levels of PBF showed a decrease in TCL, burger hardness and chewiness. Raising PBF generally demonstrated a decrease in CBD and IBT. Although higher FMC generally resulted in lower TCL; overall, FMC did not influence any other burger cooking or texture properties. Based on the present study, 70% PBF and 42% FMC based TVPs showed the greatest potential in meat extender applications because they reduced TCL, while maintaining similar hardness to the negative control. From an economic feasibility perspective, the fact that the TVPs made from the lowest PBF performed the best was very promising, as ingredients with high levels of protein (i.e., PPI) are more expensive than those with lower protein levels (i.e., PPC). To further assess the potential of pea protein-based TVPs as meat extenders, future work should include sensory analysis.

Chapter 5: Overall conclusions, limitations, and future recommendations

The first objective of the thesis was to investigate the impact of protein blend formula (PBF: 70, 76 and 82% protein (d.b.)) on the nitrogen solubility index and the techno-functional properties of blends raw pea protein concentrate (PPC) and isolate (PPI). The techno-functional properties studied were nitrogen solubility index (NSI), water holding capacity (WHC), oil absorption capacity (OAC), emulsion capacity (EC) and emulsion stability (ES). The second objective of this thesis was to assess the impact of PBF and extrusion processing parameters on the techno-functional and physical properties of texturized vegetable proteins. The extrusion cooking conditions explored were screw speed (350, 400 and 450 rpm) and feed moisture content (FMC at 38 and 42%). The assessed TVP techno-functional properties were WHC, OAC, EC and ES. NSI values of the TVPs were also measured. The physical properties studied were hydration time and integrity index. Among the TVPs studies, a small group was selected based on their techno-functional properties for the third objective. The third objective of this thesis was to characterize the impact of TVP PBF and FMC on burger properties. Burgers were extended at three extension levels (i.e., 20, 30 and 40 % w/w) and the cooking and textural quality properties of the resulting burgers were studied. The burger cooking properties studied were total cooking loss and burger shape retention (i.e., change in burger diameter and increase in burger thickness), and the textural quality attributes examined were hardness, cohesiveness, springiness and chewiness.

The raw PBFs were comprised of blended PPC and PPI. As protein content of raw PBFs was raised, NSI decreased, reflecting how the different processing methods impacted the protein characteristics (Vatansever et al., 2020). Meanwhile, an increase in PBF may also have increased the quantity of protein, thus the number of accessible hydrophilic protein chains that could bind with water; resulting in higher WHC (Toews & Wang, 2013). EC and ES also increased with

PBF. This was expected as proteins are the primary emulsifiers in food systems. In contrast, raw PBF did not impact OAC.

Extrusion processing imparted heat and shear to the raw PBF. The intense heat and shear modified the raw PBFs which likely induced new bonds (i.e., protein-protein and protein-starch), changed the protein structure and thus the techno-functional properties of the end-product. Extrusion processing reduced NSI, but increased OAC at all PBFs. This was not surprising, as extrusion processing has previously been reported to reduce protein solubility (Qi & Onwulata, 2011; Samard & Ryu, 2019a) and increase protein hydrophobicity (Kannan, Hettiarachchy, & Marshall, 2012; Osen et al., 2014). The increase in protein hydrophobicity with extrusion, and the relatively higher NSI of 70% PBF may have caused an improvement of its EC. In addition, the higher starch content in 70% PBF may have resulted in a higher level of gelatinized starch after extrusion and improve the interaction of TVP made from this PBF with water, thus improving its WHC (Webb et al., 2020). The higher level of gelatinized starch in the 70% PBF TVP may also have maintained ES levels by creating a protective barrier between oil droplets (Aluko et al., 2009; Z. Ma et al., 2011; Sikorski, 2001).

The techno-functional and physical properties of TVPs were primarily dependent on PBF. NSI of TVPs decreased as PBF increased. The reduction in the protein solubility with increasing TVP PBF, may have reduced EC (Shevkani et al., 2015). Although raising protein content may have resulted in higher WHC for the raw material, the proteins in the TVP may have been denatured to the extent of WHC plateauing (Kaleda et al., 2020; Ma, Gu, & Ryu, 2018; Zayas, 1997). Increasing PBF (70 to 76% PBF) also resulted in an increase in HT and II. The greater amount of protein may have increased melt viscosity by raising the likelihood of protein cross-linkage formation (Philipp et al., 2018; Verbeek & Van den Berg, 2010) which would have prevented melt expansion. The lack of bubble formation would have created a dense TVP structure, which was unfavorable for water absorption, which increased HT. While the greater amount of protein eases texturization, it could also lead to tougher and firmer textures (Riaz, 2004), as reflected in II. As TVP NSI had a strong and negative correlation with HT (r=-0.97) and II (r=-0.81), protein solubility may indicate the TVP texturization level (Riaz, 2004; Samard et al., 2021). The change in processing parameters (i.e., SS and FMC), did not demonstrate a consistently discernable trend for any of TVP techno-functional properties; nor impart any impact on physical properties.

Due to the COVID-19 pandemic, access to the pilot lab was limited when beginning preliminary extrusion experiments. Therefore, the ability to further test different processing parameters (e.g., dry feed rate, screw configuration, die shape, PBF, SS and FMC range) and gaining preliminary insight on these impacts on techno-functional properties was restricted. There is potential to develop TVPs with greater porosity to better reflect commercial TVPs.

As seen in the present research, the increase in PBF impacted techno-functional, as well as physical properties. It would be beneficial for future work to focus on understanding the wholistic changes in blend formula proximate composition. As carbohydrates were calculated by difference, the ratio of starch and dietary fiber was not measured. Previous literature has reported that dietary fiber can have a detrimental impact on texturization (Riaz, 2004); specifically when fiber is above 5% of the total proximate composition and larger than 400 µm (Riaz & Rokey, 2012). It also may be interesting to explore the types of dietary fiber, as insoluble fiber may inhibit expansion, while soluble fibre may have the opposite effect (Riaz & Rokey, 2012). There may be an opportunity to determine an optimized proportion of proximate composition to yield specific techno-functional and textural properties.

It is also recommended that future work should include additional tests such as the WHC of the TVP itself, rather than the ground TVP and TVP bulk density. Whole TVP WHC is essential as TVPs are hydrated prior to application, and impacts product texture (Samard & Ryu, 2019a; Webb et al., 2020) and juiciness (Brishti et al., 2021). TVP bulk density indicates porosity. Porosity heavily impacts the TVP's ability to hydrate and absorb liquids (e.g., marination) (Brishti et al., 2021). It would also be interesting to test if there is a relationship between texturization level and bulk density to these techno-functional properties explored in the present work. These properties can indicate the TVP's performance as a meat extender or analogue, and well as the consumer sensory experience (Webb et al., 2020).

TVPs produced with varying PBF (i.e., 70, 76 and 82% protein, d.b.) and FMC (38 and 42%, d.b.) were selected to be tested as meat extenders in medium ground beef burgers because PBF and FMC demonstrated varying impacts on the TVP's techno-functional and physical properties. TVP extension impacted burger cooking and textural properties. TVP extension reduced burger TCL, which may have indicated that the TVPs were superior in water and oil holding properties compared to animal protein. As such, as TVP extension level increased, TCL reduced. Interestingly, at 20% extension level, the 42% FMC based TVPs had lower TCL than 38% FMC. This indicates that higher FMC may be preferable to lower TCL. Furthermore, higher levels of TVP may have disrupted the burger matrix, which prevents the muscle proteins contracting from heat induced denaturation (i.e., cooking) (Riaz, 2006). However, extension level generally did not impact IBT or any textural quality attributes.

TVP PBF impacted TCL, but not CBD or IBT. As average TCL reduced with increasing PBF levels, reducing PBF caused a decrease in burger hardness and chewiness. The greater quantity of protein in higher PBF levels may have increased the non-covalent interactions with the beef myofibrillar proteins, resulting in a harder and chewier burger (Bahmanyar et al., 2021; Shen et al., 2022; Youssef & Barbut, 2010). The 70% PBF based TVPs had generally similar textural qualities to the negative control.

FMC did not impart any influence on burger cooking properties, nor textural quality attributes. The exception was that higher TVP FMC level (i.e., 42% FMC) enabled lower TCL at 20% extension level. This result was not surprising, as FMC levels had an inconsistent and generally negatable impact on TVP techno-functional properties, and no impact on TVP physical properties.

Based on the burger cooking and texture quality attribute tests, 70% PBF and 42% FMC based TVPs showed the greatest promise as a burger meat extender. These conditions reduced TCL but kept similar textural quality attributes to the negative control. This is beneficial for industry applications, as lower protein levels are less expensive.

There is potential to improve the understanding between the TVP properties and the extended burger for future studies. Greater variation between TVP techno-functional and physical properties may have demonstrated the connection between TVP quality and extended burger qualities. Additionally testing the extended burger colour may be valuable, as consumers use colour to perceive raw product freshness and acceptability (Turner et al., 2015). It will also be useful to test the TVP in multiple applications (e.g., sausage extension, plant-based formulas) as product versatility is invaluable for industrial applications. It may also reveal the TVP techno-functional requirements for different product applications.

Finally, obtaining a Safe Food for Canadians Regulations (SFCR) license for Ellis pilot plant would enable the possibility for consumer sensory evaluation. Consumer acceptance of product appearance, taste and texture is a necessity for TVP extended product success (Elzerman et al., 2011; Hartmann & Siegrist, 2017).

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6. Appendix

Appendix 1: Sample of SAS code used for Chapter 1; Effects of pea protein formulation and extrusion processing parameters on techno-functional properties of TVPs.

```
        OPTIONS LS=120 PS=100 NONUMBER NOTES NODATE;

        DATA ELYSSA;

        INPUT NUMBER REP PROTE SRP MOIST TREAT $ NSI;

        CARDS;

        1       1       70      0       0       CONT-1          0.457692

        2       1       70      0       0       CONT-1          0.448872

        3       1       70      0       0       CONT-1          0.451812

        4       1       76      0       0       CONT-2          0.362453

        5       1       76      0       0       CONT-2          0.354419

.

.

.

*repeat for remaining samples and respective measurements*

;

PROC PRINT DATA = ELYSSA;

RUN;


PROC FREQ DATA = ELYSSA;

TABLE TRT;

RUN;


PROC MIXED DATA= ELYSSA;

CLASS REP TREAT;

MODEL NSI=TREAT;

RANDOM REP(TREAT);
```

```
LSMEANS TREAT/PDIFF;
Ods output diffs=ppp lsmenas=mmm;
RUN;
%include‘C:\User\agfood\Desktop\pdmix800.sas’;
%pdmix800(ppp, mmm, alpha = 0.05, slice=, sort=yes)
```

Appendix 2: Correlation table for TVP techno-functional and physical properties.

	NSI	*WHC*	*OAC*	*EC*	*ES*	*HT*	*II*
NSI	1.00						
WHC	0.10	1.00					
OAC	0.45	0.35	1.00				
EC	0.60	0.07	0.40	1.00			
ES	0.51	-0.07	0.40	0.61	1.00		
HT	-0.97	-0.11	-0.44	-0.58	-0.48	1.00	
II	-0.81	-0.18	-0.37	-0.52	-0.21	0.89	1.00

Appendix 3: Correlation table for burger cooking and textural quality attributes.

	TCL	*CBD*	*IBT*	*HARD*	*COH*	*SPR*	*CHEW*
TCL	1.00						
CBD	0.57	1.00					
IBT	0.08	0.71	1.00				
HARD	0.41	-0.32	-0.51	1.00			
COH	0.28	-0.02	-0.05	0.76	1.00		
SPR	0.02	-0.07	-0.15	0.00	0.08	1.00	
CHEW	0.42	-0.23	-0.39	0.98	0.86	0.02	1.00