

Understanding protein physico-chemical properties, functionality, and mechanisms underlying
texturization in extruded plant-based meat – A novel approach for optimum structure and
mouthfeel

by

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Abstract

Plant-based protein is a promising and growing sector of the food industry with retail sales of plant-based meat alone recently surpassing \$5 billion in 2021. This growth is partially due to the rapid increase in “flexitarian” consumers. Flexitarians are people who eat meat but want to reduce their meat consumption due to a variety of reasons. Some common reasons of increased plant-based protein consumption are perceived benefits of plant-based foods including nutrition, sustainability, and animal welfare. One current major challenge restricting the widespread acceptance of plant-based meat by consumers is the texture of the product. Many flexitarians want a product that reminds them of traditional meat products so it can be easily incorporated into their everyday recipes and meals. This is a challenge because real meat structure can be difficult to replicate because of the fibrillar structure of the animal muscle. Many plant-based meat products are made using extrusion to cook and realign plant protein ingredients into a fibrous and layered piece that mimics meat texture. This research will utilize low-moisture extrusion to create texturized vegetable protein (TVP) that can then be combined with water, binders, oil, and seasonings to make a plant-based burger. TVP can be made from many different plant sources but some of the most popular are soy, pea, and wheat.

This research aims to understand several key functionality attributes of plant protein sources, such as solubility and gelling capacity, to determine how they impact the quality of the final plant-based meat product. The results of the first study confirmed that proteins can be characterized into two categories: those that are soluble at room temperature (cold swelling) and those that are more soluble after heating (heat swelling). According to their swelling category, these proteins act differently in the extrusion process and produce meat analogues with different textures as shown by changes in bulk density, visual structure, and water holding capacity.

Results show that bulk density was higher for treatments targeting a firm product (274 g/L, 287 g/L) than those targeting a soft (160 g/L, 223 g/L). Hardness as determined by texture profile analysis (TPA) also showed that soft targeting treatments seemed to show a lower hardness (1154 g, 1595 g) than the firm targeting treatments (2231 g, 1893 g), but there was a large standard deviation for each. More conclusive differences between proteins were found in the following study in Chapter 3 by using cooked plant-based patties for TPA and by confirming these results with a consumer sensory study and focus groups. This study was designed to include 6 treatments with increasing amounts of cold swelling proteins. WAI was highest for the 90% CS treatment at 5.61 g/g. On the other end, the lowest WAI was found to be from the 0% CS treatment at 2.51 g/g. Overall, it was shown that, as hypothesized, cold swelling proteins tend to produce a softer and more sponge-like product while heat swelling proteins create more densely packed layers and a firmer texture.

By gaining a more thorough understanding of the solubility of different protein sources and making connections to how these properties impact the texture of plant-based meat analogues, product developers can use simple solubility information to predict outcomes and qualities of extruded plant protein products. This is vital as new plant-based products are being created with novel textures, and as current products are being improved to be more appealing to consumers. Industry can also benefit by using this swelling categorization method to maintain consistent product quality through the use of simple analytical methods such as WAI, LGC, and RVA to check ingredient consistency and functionality properties.

Table of Contents

List of Figures	viii
List of Tables	xi
Acknowledgements	xiii
Chapter 1 - Introduction.....	1
1.1 Background.....	1
1.2 Overall objectives	2
1.3 Chapter 2 objectives.....	3
1.4 Chapter 3 objectives.....	4
1.5 References.....	5
Chapter 2 - Understanding relationships between protein functionality and texture of extruded plant-based meat analogues	6
Abstract	6
2.1 Introduction.....	8
2.2 Materials and Methods.....	10
2.2.1 Ingredient selection and formulation	10
2.2.2 Extrusion processing.....	10
2.2.3 Raw material analysis	12
2.2.3.1 Least gelation concentration	12
2.2.3.2 Water absorption index	13
2.2.3.3 Rapid visco analysis.....	13
2.2.4 Phase Transition Analysis and lab-scale extrusion	14
2.2.5 Extrudate analysis	15
2.2.5.1 Water holding capacity	15
2.2.5.2 Visual analysis	15
2.2.5.3 Texture analysis	15
2.2.6 Statistical analysis:.....	16
2.3 Results and Discussion	16
2.3.1 Extrusion processing.....	16
2.3.2 Raw material analysis	18

2.3.2.1 Least gelation concentration	18
2.3.2.2 Water absorption index	19
2.3.2.3 Rapid visco analysis.....	20
2.3.3 Phase transition analysis & lab-scale extrusion	21
2.3.4 Extrudate analysis	22
2.3.4.1 Water holding capacity	22
2.3.4.2 Visual analysis	23
2.3.4.3 Texture analysis	24
2.4 Conclusion	26
2.5 References.....	27
2.6 Figures and tables	29
Chapter 3 - Utilizing Protein Functionality Information to Tailor Quality of Plant-Based Meat	
Analogue	37
Abstract.....	37
3.1 Introduction.....	39
3.2 Materials and Methods.....	40
3.2.1 Formulation.....	40
3.2.2 Extrusion processing.....	41
3.2.3 Raw Material Functionality	41
3.2.3.1 Water absorption index	41
3.2.3.2 Least gelation concentration	42
3.2.3.3 Rapid visco analysis.....	43
3.2.3.4 Protein solubility	43
3.2.4 Protein chemical analysis.....	44
3.2.4.1 SDS-PAGE	44
3.2.4.2 SEC-HPLC.....	45
3.2.5 Extrudate analysis	45
3.2.5.1 Water holding capacity	45
3.2.5.2 Visual analysis	46
3.2.5.3 Texture analysis	46
3.2.6 Sensory analysis.....	47

3.2.6 Statistical analysis	48
3.3 Results and Discussion	48
3.3.1 Extrusion Processing.....	48
3.3.2 Raw material functionality	49
3.3.2.1 Water absorption index	49
3.3.2.2 Least gelation concentration	50
3.3.2.3 Rapid visco analysis.....	51
3.3.2.4 Protein solubility	52
3.3.3 Protein chemical analysis	53
3.3.3.1 SDS-PAGE	53
3.3.3.2 SEC-HPLC.....	55
3.3.4 Extrudate analysis	56
3.3.4.1 Water holding capacity & bulk density	56
3.3.4.2 Texture analysis	58
3.3.4.3 Visual analysis	59
3.3.4.4 Sensory analysis.....	60
3.4 Conclusion	63
3.5 References.....	65
3.6 Figures and tables	68
Chapter 4 - Conclusion	80
4.1 Background	80
4.2 Chapter 2.....	81
4.3 Chapter 3.....	81
4.4 Future work.....	82
4.5 References.....	84
Appendix A - Chapter 2 Section 2.2.2.4: Lab-scale extrusion	85
Appendix B - Chapter 3 Section 3.3.2.3: Rapid visco analysis (RVA)	87

List of Figures

Figure 2-1 The screw profile used in the TX-52 pilot-scale twin screw utilized elements such as cut flight screws and kneading blocks to increase mechanical energy input.....	29
Figure 2-2 Specific mechanical energy (SME) represents the amount of mechanical energy used on the material during extrusion processing. A small decreasing trend can be seen from cold to heat swelling. Treatments are labeled cold swelling (CS), intermediate swelling (IS), and heat swelling (HS).....	29
Figure 2-3 Water absorption index (WAI) decreases from cold to heat swelling proteins. Treatments are labeled cold swelling (CS), intermediate swelling (IS), and heat swelling (HS).....	30
Figure 2-4 Rapid visco analysis (RVA) was used to find the peak viscosity and time at which this peak was reached for each treatment. Cold swelling (CS) treatments had a low peak time and high viscosity while heat swelling (HS) had the opposite.	30
Figure 2-5 Compression testing was conducted to determine hardness of each TVP sample both as a whole piece and milled.	31
Figure 2-6 Compression testing was conducted to determine chewiness of each TVP sample both as a whole piece and milled.	31
Figure 2-7 Compression testing was conducted to determine springiness of each sample both as a hydrated whole piece and milled TVP.....	32
Figure 2-8 A microscope camera was used to capture the internal structure of hydrated TVP cut in the horizontal direction (against the flow from extruder).	32
Figure 2-9 A microscope camera was used to capture the internal structure of hydrated TVP cut in the longitudinal direction (with the flow from extruder).	33
Figure 2-10 Water holding capacity (WHC) and bulk density showed an inverse relationship from cold swelling to heat swelling proteins.	33
Figure 2-11 Viscosity over time as it underwent a heating cycle was recorded using RVA on raw materials of each individual protein ingredient to identify differences in swelling properties.	34

Figure 3-1 The screw profile for the pilot-scale extrusion run shows the need for increased mechanical energy through the inclusion of several kneading blocks and cut flight elements.	68
Figure 3-2 Water absorption index (WAI) of each treatment indicates how absorbent the materials are at room temperature. A higher WAI is expected for a cold swelling protein. 68	
Figure 3-3 Water holding capacity (WHC) and bulk density can be used to quantify the expansion and internal structure of the extruded pieces. Bulk density should decrease and WHC should increase as more cold swelling proteins are used.	69
Figure 3-4 The hardness and chewiness of a plant-based patty was recorded for each treatment. More cold swelling proteins led to a decreased hardness and chewiness as expected.	69
Figure 3-5 Springiness was found using texture profile analysis (TPA) for plant-based patties containing increasing amounts of cold swelling proteins.	70
Figure 3-6 A dual compression test was done on hydrated whole TVP pieces for each treatment to determine differences in hardness.....	71
Figure 3-7 A dual compression test was done on hydrated whole TVP pieces for each treatment to determine differences in springiness.....	71
Figure 3-8 The average overall liking scores from the consumer study show that the top plant-based burgers were 90% CS and 30% CS. Similar trends appeared for flavor and texture. Blue indicates soy, orange is soy and wheat, and green is soy and pea.....	72
Figure 3-9 The internal structure of a hydrated whole piece of TVP was captured in the longitudinal directions (with the flow of materials).....	73
Figure 3-10 Results from SDS-PAGE analysis shows that each treatment at different cold swelling (%) levels had identifiable bands for the soy proteins β -conglycinin and glycinin. These bands were still present after extrusion although many other bands disappeared or faded. (S = soy, S+P = soy and pea, S+W = soy and wheat).....	74
Figure 3-11 SDS-PAGE showed the molecular weights of different protein subunits from the raw materials of each ingredient.	74
Figure 3-12 SEC-HPLC determines which proteins are present through peaks at certain molecular sizes. After extrusion, many proteins are cross-linked and bonded so that they no longer show up on the graph because of their large molecular size.	75

Figure 3-13 SEC-HPLC determines which proteins are present through peaks at certain molecular sizes. After extrusion, many proteins are cross-linked and bonded so that they no longer show up on the graph because of their large molecular size.	76
Figure B-3 RVA curves are shown for 0% and 30% cold swelling (CS) protein mixes.....	87
Figure B-4 RVA curves are shown for 40% and 50% cold swelling (CS) protein mixes.....	88
Figure B-5 RVA curves are shown for 60% and 90% cold swelling (CS) protein mixes. Soy and pea are cold swelling and therefore show an earlier and higher viscosity peak.	89

List of Tables

Table 2-1 Raw material properties of each protein source were characterized as cold or heat swelling by analyzing results from WAI, LGC, and RVA. A + indicates a positive result and a – indicates a negative result.	34
Table 2-2 The experiment was designed to include 6 total treatments, 2 treatments each, of cold, heat, and intermediate protein swelling properties identified by CS, HS, and IS respectively. The chart below represents the % of each ingredient used in each of the treatments. Protein content was calculated to be between 70-80% for each treatment.	35
Table 2-3 Raw materials and extruded products for each treatment were analyzed using a Phase Transition Analyzer (PTA) to determine changes in flow temperatures that could provide evidence of crosslinking. When temperatures increase from raw to extruded it indicates crosslinking of proteins.	35
Table 2-4 Pilot-scale extrusion parameters are listed below for each treatment. In-barrel moisture (IBM), die temperature, and screw speed are important in producing an optimal TVP product.	36
Table 3-1 Treatments were formulated to target a range of cold swelling (CS) amounts. This was hypothesized to lead to a decrease in firmness and chewiness as CS increases.	77
Table 3-2 Rapid visco analysis (RVA) was used to determine peak viscosity values for each treatment as it underwent a heating and stirring cycle. A short peak time (< 4 min) indicated cold swelling (blue) and a longer peak time (>4 min) indicates more heat swelling (red) proteins.	77
Table 3-3 Average least gelation concentration (LGC) did not show many statistically significant differences but did decrease slightly as the amount of cold swelling (%) was increased.	78
Table 3-4 Protein solubility was calculated to confirm and determine the cold swelling abilities of each treatment before and after extrusion. Solubility decreased after extrusion indicating cross-linking of proteins.	78
Table 3-5 Pilot-scale extrusion parameters were used to calculate specific mechanical energy (SME) and in-barrel moisture (IBM) for each treatment containing increasing percentages of cold swelling proteins. Die temperature was also important to TVP quality.	79

Table 3-6 Two recipes were developed to target either a plant-based beef or fish patty. Each recipe consists of texturized vegetable protein (TVP), water, oil, binders, and flavors. Both the beef and fish flavored patties were used for the focus groups, and just the beef flavored patty was used for the consumer study and texture analysis.....	79
Table A-1 Extruder parameters were kept constant except for the extruder screw speed which was increased for the rice protein. This was because the pieces were not cohesive at the lower screw speed and needed higher mechanical energy to crosslink.	86
Table A-2 Extruder parameters were kept mostly constant except for the extruder screw speed which was increased for the heat swelling (HS) treatments. This was because the pieces were not cohesive at the lower screw speed and needed higher mechanical energy to crosslink.	86

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Chapter 1 - Introduction

1.1 Background

Plant-based alternatives to many traditional animal products such as meat, milk, cheese, and eggs have grown rapidly in popularity in recent years. In just two years (2017-2019), the total retail sales of plant-based foods in the United States increased 29% (McClements et al., 2021). Plant-based meat is the second largest plant-based food category after plant-based milk and had \$1.2 billion in sales in 2020 (Good Food Institute, 2020). The number of households that regularly purchase plant-based meat also rose to 18% in 2020 from what was previously 14% in 2019 (Good Food Institute, 2020). Plant-based meat is a promising alternative protein option for not only vegetarians, but flexitarian consumers who are trying to lower their meat consumption and supplement their diet with plant-based options (Webb et al., 2020). Reasons for this shift to more plant-based foods include perceived health benefits, animal welfare concerns, and environmental benefits. A diet high in meat emits around twice as much carbon dioxide as a vegan diet, so even small shifts towards plant-based foods can have a significant impact on the environment (McClements et al., 2021). Plant-based meat analogues are different from other traditional vegetarian protein options such as tofu or tempeh, in that they attempt to directly mimic the sensory and visual properties of real meat. This is done through extrusion processing which takes globular plant proteins and realigns them into fibrous layers with thermal and mechanical energy. This process is called crosslinking or polymerization which is the formation of disulfide bonds between proteins that leads to the fibrous texture that is like meat myofibrillar structure (Ryu et al., 2020; Schreuders et al., 2021). This similarity to animal meat can make the transition to eating more plant-based foods easier for consumers who also eat meat.

1.2 Overall objectives

Some of the major hurdles facing the widespread acceptance of plant-based meat products are sensory properties such as taste and texture (Yuliarti et al., 2021). Consumers may be willing to try a new product for health or environmental reasons, but if it does not taste good or have an acceptable texture, they will be unlikely to purchase a second time. This research will focus on improving the texture of plant-based meat products by using protein functional properties such as swelling ability to better understand and control the texture of the final product. By better understanding how raw material properties relate to extrusion processing parameters and physical properties of the final plant-based product, researchers or industry professionals can adjust and optimize formulations more efficiently to reach specific product texture goals. This way, plant-based meat products can have improved texture attributes that more closely mimic traditional animal protein.

With this knowledge, current products on the market can be improved and expanded, or new products with innovative textures can be made. There are also potential applications in the food industry as an aid for product development and quality control. By understanding and categorizing various protein ingredients by their functionality attributes, formulations can be changed with less extensive and expensive research trials. Also, ingredient changes due to cost or supply chain issues can be quickly implemented by switching the ingredient with a similar functional protein based on the categorization determined through this research. With the world's growing population, more and more protein will be needed to support this growth. Plant-based meat has the potential to alleviate the stress on the livestock industry and provide a more sustainable option to diversify protein options for consumers. In the end, everyone is affected by the furthering of research towards improving the quality of plant-based protein options as it has

the potential to have a significant impact on our world through environmental and nutritional benefits.

1.3 Chapter 2 objectives

In Chapter 2, it was hypothesized that the solubility properties of protein raw materials could be characterized as either cold or heat swelling, and that these swelling abilities would have an impact on texture of extruded plant-based meat products. The terms cold and heat swelling were first used by Webb et al. (2023) and this study aims to define them in greater detail by establishing several standard parameters for characterization. Also, the impact of cold and heat swelling proteins from different sources will be investigated to determine and confirm how their swelling characterization can be used to predict and manipulate the finished product texture of plant-based meat analogues. According to preliminary studies, proteins with a high cold-water solubility such as soy have produced a more expanded extruded product. In contrast, heat swelling protein sources such as wheat gluten have produced a denser and firmer product with higher degrees of texturization (Samard et al., 2019). This experiment aims to use swelling ability characterization, as determined through preliminary raw material analysis, to predict and manipulate the final texture attributes of extruded texturized vegetable protein (TVP) made from multiple common plant protein sources. Overall, this research aimed to establish and understand any relationships that can be drawn between cold/heat swelling and texture or structure attributes of plant-based meat analogues made using low-moisture extrusion.

1.4 Chapter 3 objectives

This research investigated the effect of protein swelling ability on the texture of meat analogues and how it can be used to fine-tune and target certain applications that require different texture attributes, such as a plant-based burger or plant-based salmon. This study was intended to dig deeper into the preliminary information obtained in Chapter 2 and in a previous study by Webb et al. (2023) about protein swelling abilities and see if that knowledge could be utilized and applied to manipulate product texture using soy-based formulations. Protein ingredients were first characterized as cold or heat swelling through various raw material analytical tests such as water absorption index (WAI), rapid visco analysis (RVA), and protein solubility (%). Treatments were formulated to have different ratios of cold/heat swelling properties in order to influence product texture. Each treatment was soy-based with pea protein isolate, wheat gluten, and soy flour used to modulate texture. It was hypothesized that there would be a clear relationship between cold swelling (%) and final product physical attributes such as hardness and internal structure of the TVP pieces. Low-moisture extrusion processing was used to produce texturized vegetable protein (TVP) which was later made into patties. Extruded product testing included water holding capacity (WHC), texture profile analysis (TPA), visual analysis, SDS-PAGE, SEC-HPLC, and sensory studies. These results will help to determine if protein swelling properties are a valid and useful parameter that can be used to utilize to control the end-product properties of extruded plant-based meat products and help to identify areas in need of further research and future studies.

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Chapter 2 - Understanding relationships between protein functionality and texture of extruded plant-based meat analogues

Abstract

In 2020, the U.S. market for plant-based foods grew at almost twice the rate of other foods and surpassed \$7 billion (Good Food Institute, 2020). Plant-based meat analogues specifically are growing substantially. Plant-based meat is made from plant protein that is extruded to form texturized vegetable protein (TVP), which is combined with other ingredients to form patties, nuggets, and many more products. A common obstacle faced when producing meat analogues is consumer acceptance, largely based on sensory differences from animal-based counterparts. Different protein sources such as soy, pea, or wheat can create distinctly different products in terms of texture due to several factors such as solubility, protein structure, and presence of specific amino acids that affect texturization. This research aimed to identify these differences and successfully manipulate them to reach a desired texture goal. This research utilized simple and reproducible analytical methods to characterize protein properties as either cold or heat swelling. The terms cold and heat swelling were first used by Webb et al. (2023) and this study aimed to define them in greater detail by establishing several standard parameters for characterization. It was hypothesized that any protein ingredient could be characterized into these categories and that they would have similar and predictable impacts on texture.

Protein functionality was determined through least gelation concentration (LGC), water absorption index (WAI), and rapid visco analysis (RVA). These results were used to create formulations that targeted 3 different texture objectives. To create a meat analogue that is softer in texture, an LGC >16% and WAI >4.0 g/g is desired while an LGC <16% or WAI <4.0 g/g should lead to a firmer product. High WAI and LGC signal a cold swelling peak and lower WAI

and LGC signal a heat induced swelling peak. An RVA peak time less than 4 minutes indicated cold swelling properties while greater than 4 min was considered heat swelling. The bulk density was higher for treatments targeting a firm product (274 g/L, 287 g/L) than those targeting a soft (160 g/L, 223 g/L). The softer treatments exhibited a microstructure that was flaky and gel-like. The firm treatments showed a more laminar microstructure, unlike the medium treatments that had many small air cells. Hardness, as determined by texture profile analysis (TPA), showed that soft targeting treatments seemed to show a lower hardness (1154 g, 1595 g) than the firm targeting treatments (2231 g, 1893 g), but there was a large standard deviation for each. Controlling the texture of the final meat analogue can be a valuable tool when producing a plant-based product. For example, fish has a much softer and flakier texture than chicken breast or steak and would require a very different TVP. By increasing the knowledge of how protein functionality affects meat analogue texture, time and money can be saved when formulating a specific product.

2.1 Introduction

Plant-based meat products have grown in popularity in recent years mostly due to increasing amounts of “flexitarian” consumers and their demand for products that are more sustainable and nutritious. If plant-based meat products are not able to meet consumer expectations of health, cost, appearance, and flavor, they will struggle to be adopted into the daily diet and lifestyle of consumers. Sensory properties such as texture are some of the main drivers of consumer acceptance of plant-based meat products (Sha & Xiong, 2020). Many plant-based meat products on the market are made using extrusion of plant proteins to make texturized vegetable protein (TVP). The extruded TVP is combined with water, binders, and seasonings to make formed products such as plant-based burgers, sausages, and meatballs. The quality and attributes of the TVP is extremely dependent on the source of plant protein. Common plant protein ingredients for plant-based meat include soy, wheat, pea, and fava bean. The chemical makeup and functionality properties of these proteins are very different and play an important role in determining sensory properties of the final meat analogue (Osen et al., 2014; Schreuders et al., 2019). Protein content also varies between ingredient sources, and even between the same plant source as different isolation methods of the protein lead to different protein content and functionality. Solubility has been shown to be a major contributor to TVP texture. For example, proteins with lower solubility at room temperature such as wheat gluten have been shown to lead to a more layered TVP structure and chewy texture (Samard et al., 2019).

The solubility properties of protein raw materials can be characterized as either cold or heat swelling. The terms cold and heat swelling were first used by Webb et al. (2023) and this study aims to define them in greater detail by establishing several standard parameters for characterization. These swelling abilities have been shown to impact the texture of extrudate

products. According to previous studies, proteins with a high cold-water solubility such as soy have produced a more expanded and softer extruded product and texturize easier than pea and wheat proteins at a similar protein content (Hong et al., 2022). In contrast, heat swelling protein sources such as wheat gluten have produced a denser and firmer product (Webb et al., 2023). This experiment aims to use swelling ability characterization, as determined through preliminary raw material analysis, to predict and manipulate the final texture attributes of texturized vegetable protein. By using raw material functionality properties such as swelling ability to control the texture of meat analogue products, improvements can be made at formulation and quality control stages to improve and reduce the time, cost, and effort required to produce a quality plant-based meat product. Companies can better understand and quantify the properties of raw materials that affect functionality so that they can continuously check the qualifications of their ingredients to stay within parameters and maintain a consistent and quality product. There is also potential to create novel textures that have been difficult to achieve in the past due to formulation challenges.

First, several raw material analysis methods will be used to determine functionality and sort each protein type into either cold swelling or heat swelling. These quantified functionality attributes can be used to relate and predict texture and quality of plant-based meat analogues. It is hypothesized that a formulation containing mostly heat swelling proteins will produce a firmer and denser product, while a formulation containing mostly cold swelling proteins would produce a softer and spongier product. If these hypotheses are proven correct, formulation using cold swelling and heat swelling proteins can be utilized to better design and predict product quality of innovative plant-based meat products while potentially reducing ingredient costs and providing more consistent quality control.

2.2 Materials and Methods

2.2.1 Ingredient selection and formulation

Several preliminary experiments were first conducted to determine properties of eight different plant protein concentrates and isolates. These included a soy protein isolate, three soy protein concentrates, pea protein isolate, fava bean protein concentrate, wheat gluten, and rice protein concentrate. The data collected from least gelation concentration (LGC), rapid visco analysis (RVA), and water absorption index (WAI) was then analyzed and used to characterize each protein as heat swelling, cold swelling, or some of both (Table 2-1). This information was used to create six different TVP formulations: two each of cold, heat, and intermediate swelling abilities. Each mix was formulated to target a dry basis protein content of around 70-80% which was chosen because it is an average value for an animal-based protein such as chicken at a similar moisture content (USDA, 2018). Also, a protein content of >50% is needed to texturize and form a fibrous structure during low-moisture extrusion (Hong et al., 2022). Treatments including wheat gluten also included small additions of trisodium phosphate (TKPP) and soda ash to increase the pH of the mixture to promote texturization of the gluten. Treatment formulations are shown in Table 2-2.

2.2.2 Extrusion processing

Prior to extrusion, raw material mixes were prepared using a batch double-ribbon blender (Wenger Manufacturing, Sabetha, KS, USA). These mixes were then extruded using a pilot-scale TX-52 twin-screw extruder (TX-52, Wenger Manufacturing, Sabetha, KS, USA) with a screw diameter of 52 mm and L/D ratio of 19.5 with 4-barrel heads. An aggressive screw profile (Figure 2-1) that produced high levels of mechanical energy and shear was required to fully

texturize each plant protein (Webb et al., 2021). This was done by incorporating screw elements such as cut flight, reverse, and kneading block elements. These elements make it more difficult for the material to be conveyed forward using the screws and cause an increase in shear and fill of material in the barrel. A $\frac{1}{4}$ in. venturi die was used to increase mechanical energy and channel the material flow into 2 $\frac{1}{4}$ in. outlet dies. The product exiting the die was then cut into pieces using 3 hard rotating knives. Some product from each treatment was sent directly from the extruder to the dual pass dryer (4800; Wenger Manufacturing, Sabetha, KS, USA) as chunks, while some product was collected off the extruder and immediately milled using an Urschel mill or hammer mill (Fitzpatrick Co., Westwood, MA). The milled product was then packaged and stored in a freezer until later use in plant-based patties. Extruder parameters during processing were optimized for each treatment to produce TVP that was of the highest quality possible, meaning the most densely layered and smooth surface products. The raw material feed rate was kept constant for each treatment at 50 kg/hr. Steam injection was not used, and the main parameters that were manipulated for each treatment were water injection rate into the preconditioner and barrel, as well as the extruder shaft screw speed.

During extrusion processing, the motor load percentage is recorded which represents how much mechanical energy is needed to turn the screws of the extruder and force the material through the die opening. Using equation 2-1, specific mechanical energy (SME) can be determined using the motor load percentage, throughput, and no-load percentage (the amount of energy needed to turn the screws when there is no material present in the extruder). SME can represent the amount of mechanical energy absorbed by the material which can lead to different levels of cross-linking of proteins and texturization. SME can also be an indicator of viscosity changes as the material should take more energy to move the screws as viscosity of the material

in the extruder barrel increases. Specific mechanical energy was calculated using the equation below where τ is the torque (%), τ_0 is the no-load torque (%), N is the screw speed (rpm), N_r is the rated screw speed (336 rpm), P_r is the rated motor power (22.4 kW), and $\dot{m}$ is the material flow rate (kg/s).

$$SME \left(\frac{kJ}{kg} \right) = \frac{\frac{(\tau - \tau_0)}{100} \times \frac{N}{N_r} \times P_r}{\dot{m}} \quad (2-1)$$

Bulk density was recorded during extrusion processing by using a 1-liter cup and weighing the mass of material that would fill the cup completely. Bulk density can be used to quickly quantify expansion and texturization during processing so that the extrusion parameters can be changed if needed to reach desirable product attributes. A lower bulk density signals a more expanded and therefore softer product.

2.2.3 Raw material analysis

2.2.3.1 Least gelation concentration

Least gelation concentration (LGC) was used to determine the heat swelling properties of protein raw materials. LGC can determine the minimum concentration of a material that is necessary to form a heat-induced gel. A method modified from Wang et al. (2007) was utilized to determine the LGC of eight different plant protein concentrates and isolates. First, 10 mL of distilled water is combined with a series of increasing protein amounts to produce concentrations ranging from 8%-20%. The test tubes are then stirred by hand to break apart any clumps and distribute the protein to form a slurry. Next the test tubes are partially submerged into a heated water bath at 90-100 °C for a duration of 1 hour. Afterwards the test tubes are placed in a cool water bath for 10 minutes followed by 2 hours in the refrigerator to chill. When this is all completed, the test tubes are inverted, and observations are made about the gel formation at each protein concentration. The LGC was determined to be the concentration at which a strong gel

was formed that had no slipping or falling down the sides of the test tube. A low LGC can signal a high heat gelling ability and should theoretically correspond to results found from rapid visco analysis (RVA) showing a late, heat induced peak in viscosity.

2.2.3.2 Water absorption index

Water absorption index (WAI) quantifies the amount of water absorbed and held by the protein at room temperature. It can indicate cold swelling properties if the WAI is high because it represents solubility without the addition of thermal energy. The method used was adapted from the method utilized by Anderson, Conway, and Peplinski (1970). First, 2.5 g of raw materials are mixed and suspended in 30 mL of distilled water using a vortex. Each sample tested was done in duplicate. The samples are then mixed using a shaker table for 30 minutes. After mixing the samples were placed in a centrifuge at 3,000g for 15 min using a Centrifuge 5810 R 15 Amp Version (Eppendorf, Hauppauge, NY, USA). The supernatant was removed using a pipette and placed in trays in the oven for 3 hours at 100 °C to evaporate the water. The dried trays were then weighed to determine the mass of dry solids. The leftover precipitate gel left in the test tubes was also weighed and the mass was recorded. The equation below was used to calculate the WAI.

$$WAI \left(\frac{g}{g} \right) = \frac{Gel\ weight}{Sample\ weight} \quad (2-2)$$

2.2.3.3 Rapid visco analysis

Rapid visco analysis (RVA) is a useful tool in determining the effect of time, heat, and shear on the viscosity of a material. The results should be able to confirm and help elaborate the functionality results from LGC and WAI. This is because it can detect both cold and heat swelling properties by illustrating peaks in viscosity over time and as heat is added. Results include a peak viscosity, final viscosity, and time that it took to reach peak viscosity. RVA was determined using AACC Method 76-21.02 STD1 which utilized a rapid visco analyzer (RVA

4500, Perten Instruments, Waltham MA). Raw material samples were first combined with distilled water to create a 15% suspension on a dry basis. Samples were placed into the RVA and then analyzed to determine their viscosity profile. The testing cycle began with the samples being kept at 50 °C for 1 min and then heated up to 95 °C at 12.2 °C /min and held for 2.5 min at 95 °C. This was followed by cooling to 50 °C at 11.8 C/min and then holding for 2 minutes.

2.2.4 Phase Transition Analysis and lab-scale extrusion

The cross-linking and texturization of protein are often difficult to compare between treatments by methods other than simple visual analysis of layering. Phase transition analysis (PTA) determines the flow and softening temperatures of a material by compressing it with a piston to a constant pressure while the barrel is heated to temperatures up to 160 °C. Samples were analyzed as raw materials and ground extrudates from a preliminary lab-scale extrusion to see if cross-linking did occur. Lab-scale extrusion was completed using a Micro-18 twin screw extruder (Micro-18, American Leistritz, Somerville, NJ). Lab-scale extrusion was used to provide moderate shear to the samples to initiate some crosslinking, but to fully texturize and restructure the proteins into TVP a pilot-scale extrusion process is needed that can provide larger amounts of mechanical energy (Webb et al., 2023). Details about the lab-scale extrusion process can be found in Appendix-A. All samples were hydrated to 30% to mimic the in-barrel moisture during lab-scale extrusion. Tests were conducted on a phase transition analyzer (Wenger Manufacturing, Sabetha, KS). First, 1.5-2g of raw material was loaded into the chamber where they were compressed with a piston to a constant pressure of 100 bars. The sample was then heated at 8 °C/min after reaching a starting temperature of 5-7 °C. After the softening period of the material is determined, a 2 mm capillary die was moved underneath the sample and then the point at which the sample flows through the orifice at a constant pressure of 100 bars was

recorded as the flow temperature (Webb et al., 2023). Tests were done in triplicate on both raw materials and lab-scale extrudates. If cross linking and texturization did occur, then the flow temperatures and softening periods should increase from the raw to extruded material (Schreuders et al., 2019).

2.2.5 Extrudate analysis

2.2.5.1 Water holding capacity

Water holding capacity (WHC) often corresponds with bulk density and is used to quantify the amount of water that can be absorbed and held by the porous texturized extrudate. WHC is determined by placing 15g of whole dried extrudates into excess water and letting them soak for 20 minutes. The hydrated extrudates are then drained and left to set on a mesh metal screen for 5 minutes (Webb et al., 2020). Tests were completed in duplicate. The mass before and after hydration is recorded and used to determine the WHC with the equation shown below:

$$WHC \left(\frac{g}{g} \right) = \frac{m_{wet} - m_{dry}}{m_{dry}} \quad (2-3)$$

2.2.5.2 Visual analysis

An upright light microscope (SMZ1000, Tokyo, Japan) attached to a camera (Coolpix 990, Nikon) was used to capture images of the internal layering and structure of extruded TVP pieces. Dried extruded samples were first hydrated in excess water for 30 minutes and then drained on a screen for 5 minutes to remove excess water. The hydrated pieces were then cut in half both in the longitudinal (with the grain) and horizontal (against the grain) directions and placed on the microscope platform for imaging. Visual analysis can help to identify cross-linking and compare the denseness of layering between products.

2.2.5.3 Texture analysis

Texture analysis of extrudates was completed using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) using a method utilized by Webb, et al. (2020). Each treatment was done in 10 replicates. Both the dried whole chunks of TVP and milled TVP directly off the extruder were hydrated and placed into a small shallow dish slightly larger in diameter than the cylindrical probe (Hong et al., 2022). Samples were compressed twice by a cylindrical probe to 7.00 mm past the first impact with a modified method from Webb et al., 2021 using a 5 g trigger force, 1.00 mm/s pre-test speed, 3.00 mm/s test speed, and a 3.00 mm/s post-test speed. This was done to mimic a two-bite compression with a pause in between to allow the sample to recover and springiness to be measured. Compression force data is collected by the texture analyzer software and hardness, springiness, and chewiness are calculated. Hardness (g) is defined as the peak force during the first compression cycle, chewiness (g) is the $(\text{Area } 2^{\text{nd}} \text{ peak} / \text{Area } 1^{\text{st}} \text{ peak}) * \text{hardness} * \text{springiness}$, and springiness (mm/mm) is the $\text{distance } 1^{\text{st}} \text{ compression} / \text{distance } 2^{\text{nd}} \text{ compression}$ (McClements, 2021).

2.2.6 Statistical analysis:

Statistical analysis was done using SAS software (SAS, Cary, NC, USA) and an ANOVA model with Tukey's test with a significance level of 5% to determine the means and significant differences between experimental treatments.

2.3 Results and Discussion

2.3.1 Extrusion processing

Extruder parameters such as preconditioner water, extruder water, and extruder shaft speed had to be adjusted according to each treatment to reach the optimum product. Extrusion parameters can have a very significant impact on the physical qualities of the final product and

often need to be adjusted for each different protein source or application (Lyu et al., 2022). For most cold and intermediate swelling treatments, the product was of the highest quality at a die temperature between 155 °C and 160 °C. An optimal product was described as having a fibrous structure that can pull apart and shred. This indicates that the protein has been fully denatured and texturized using shear and thermal energy in the extruder barrel to form a new fibrous structure from covalent and non-covalent protein bonds (Hong et al., 2022). The TVP should also be uniform and smooth in appearance and not show any signs of burning such as ununiform dark or hard spots. Heat swelling treatments were optimal at a slightly lower die temperature of 150 °C to 155 °C. Treatments that were mostly soy-based seemed to require more water in the preconditioner (14-19 kg/h) than treatments that were mostly wheat-based. This is because very functional and cold-swelling proteins (ex. soy protein isolate, Arcon S soy concentrate, and pea protein isolate) texturize very well and with less mechanical and thermal energy requirements than other more heat-swelling proteins (Riaz, 2004). Cold-swelling proteins readily absorb water and develop viscosity before heat is added so more water can be absorbed in the preconditioner rather than the extruder barrel. The heat-swelling treatments required the least amount of water overall, especially in the preconditioner. This is because wheat gluten forms a strong protein matrix when combined with water and becomes difficult to flow and convey (Schreuders et al., 2019). That is why water in the preconditioner is minimized for gluten treatments because the paddles in the preconditioner are not able to convey the elastic dough formed from adding too much water to gluten.

Overall, SME exhibited no significant trend as more heat-swelling proteins were incorporated (Figure 2-2). Bulk density on the other hand did show significant differences with a clear increasing trend as more heat-swelling proteins were used. This is as expected and shows

that heat-swelling proteins do cause an increase in density and therefore most likely a more compactly layered TVP chunk (Schreuders et al., 2019). Heat swelling proteins such as gluten, have unique functional properties that allow them to form a more fibrous and dense structure. These include the ability to form thin films and the proclivity for crosslinking through sulfur-containing amino acids that more readily form disulfide bonds during extrusion (Samard & Ryu, 2019). Wheat gluten also has a natural state structure that is more fibrous and different from the globular native proteins of soy and pea that require more structural changes to texturize (Webb et al., 2023). This was confirmed later by additional analysis such as visual and texture analysis.

2.3.2 Raw material analysis

2.3.2.1 Least gelation concentration

LGC is meant to detect heat swelling properties as it determines the lowest concentration at which proteins form a gel after heating. Least gelation concentration (LGC) was difficult to interpret and did not show many expected significant differences due to swelling properties of the proteins. This detection was prevented by the mixture of protein types being tested. For example, some extremely cold swelling proteins such as soy protein isolate still can produce a low LGC because it forms the gel before heating even occurs and is able to maintain this gel structure even after the heating process is done because of its ability to resist shear-thinning (Hong et al., 2022). This is thought to be the reason why the two cold-swelling treatments and the two heat-swelling treatments both had the same LGC values of 16% and 14%. The intermediate swelling treatments showed the highest LGC values of 18% and 20%. Although LGC did not show any clear connections to extruded product texture or structure, it can still be useful to identify differences in protein chemistry and structure. Gelation properties are

important in solidifying the final texture and structure of an extrudate as it undergoes heat and shear. Gelation abilities can also be impacted by the processing method used to isolate the proteins so many commercial ingredients from different companies can have extremely different functional properties. Many commercial pea protein isolates are extensively denatured during the isolation process used during production which decreased their gelation abilities and protein-protein interactions (Mession et al., 2015). LGC can be influenced by many properties that make it harder to analyze and identify trends in cold swelling (%) such as protein bonds, interactions with a solvent, and effects of thermal energy (Webb et al., 2023). More research is needed in the future to understand how LGC can be better utilized to characterize protein functionality and to determine who it can be connected to extruded product texture. LGC has the potential to be a simple way to further categorize heat and cold swelling proteins so that texture of meat analogues can be more closely controlled and predicted through formulation.

2.3.2.2 Water absorption index

Water absorption index (WAI) was intended to identify cold swelling properties as it quantifies the amount of water absorbed by raw materials at room temperature. As expected, the treatments showed a clear decreasing trend in WAI with significant differences as the amount of heat swelling proteins in the mix increased. This is shown in Figure 2-3. As WAI decreased and more heat swelling proteins were used, the bulk density increased signaling a more tightly packed crosslinking structure. The WAI of raw materials is dependent on properties of the proteins such as hydrophobicity which is derived from the subunits and structure that make up each individual protein ingredient (Webb et al., 2023). Hydrophobicity, and therefore WAI, can also be impacted by different processing methods and steps used during isolation of the proteins. Commercial production of protein concentrates and isolates often requires the use of physical

and chemical methods that modify the functionality of the protein, such as solubility (Osen et al., 2014). A low WAI (< 4.0 g/g) was observed for the heat swelling treatments and this is most likely because they contained wheat gluten which is comprised of prolamins and glutelins that are most soluble in alcohols or acids and not in water. The cold swelling treatments contained soy protein isolate (SPI) that is usually very water soluble and pea protein isolate (PPI) that is moderately to highly water soluble depending on the ingredient source (Webb et al., 2023). This is why the cold swelling treatments had the highest WAI results (> 4.0 g/g). These WAI parameters can be used to categorize different protein ingredients or mixes into either cold or heat swelling. WAI can therefore be useful in industry applications as it is a simple and quick test to perform that can be used to quickly characterize the swelling abilities of a new ingredient or check the quality of an existing ingredient source.

2.3.2.3 Rapid visco analysis

The results of RVA include a peak viscosity (cP) and a peak time (min) for each experimental treatment. The results showed a very clear and significant difference between cold and heat swelling protein treatments with a distinct decreasing trend in peak viscosity and increasing trend in peak time (Figure 2-4). This aligns with the hypothesized results as cold swelling proteins should reach peak viscosity much quicker than heat swelling proteins because of their ability to solubilize at room temperature (Figure 2-11). Cold swelling proteins like soy and pea are globular in structure and this allows them to easily form viscosity and gels. This is because as they unfold due to shear or heat and are exposed to water, their non-polar and sulfhydryl groups readily form hydrophobic and disulfide bonds that cause the proteins to aggregate and form an increase in viscosity (McClements et al., 2021). Peaks in viscosity that occurred before 4 minutes into the heating cycle were categorized as having cold swelling

properties while peaks later than 4 minutes were indicative of heat swelling properties. The cold swelling treatments showed much higher viscosity values (166-471 cP) than other treatments which is due to the high inclusion of soy which is known to be very soluble and gels easily without the addition of thermal energy (Hong et al., 2022). Heat swelling proteins had a later peak time (6.6-7 min) and a lower peak viscosity (30-50 cP). This was expected because heat swelling proteins such as fava, wheat gluten, and low functionality soy concentrate require the addition of thermal energy and time to form a strong gel. The relationship between peak viscosity and peak time is illustrated in Figure 2-4.

2.3.3 Phase transition analysis & lab-scale extrusion

PTA was done on raw materials and on lab-scale extruded products to It was hypothesized that the flow temperatures of each treatment should increase from raw materials to extruded product because of the crosslinking of proteins resulting in an increase in molecular size. Crosslinking is the formation of covalent disulfide bonds between molecules that results in texturization of the plant protein into TVP (Riaz, 2004). Of the six total treatments, four were able to show an increase in flow temperature that aligned with this hypothesis and the impact of extrusion processing was confirmed to cause a significant difference in flow temperature. The softening and flow temperatures before and after extrusion are shown in Table 2-3. There were no significant differences in flow temperature between each treatment. The treatments that decreased flow temperature were intermediate swelling (IS1) and heat swelling (HS1). This could be explained by the lab-scale extrusion process not being able to input enough mechanical energy to successfully cross-link and texturize the protein. This aligns with extrusion processing data from the pilot-scale extrusion process showing that treatments IS1 and HS1 required a higher extruder screw speed to create an optimal texturized product than the other treatments.

From this it is inferred that the reason for a decrease in flow temperature is due to the lab-scale extruder being able to provide sufficient mechanical energy required to texturize those protein types. Lab-scale extrusion can be a convenient way to do preliminary testing on raw materials and get an idea of how they would act in a larger extruder, but this shows one example of the limitations of lab-scale extrusion. The HS1 and IS1 treatments were also the only mixes containing fava protein concentrate (FPC). FPC had the lowest protein content and highest carbohydrate content of any of the protein ingredients. The higher amount of starch present in these treatments could have led to more starch degradation which has the opposite effect as crosslinking on PTA flow and softening temperatures (Webb et al, 2021). As the starch is degraded, the molecular size is broken down into smaller pieces that allow it to flow more easily through the orifice. This is opposite of protein crosslinking as disulfide bonds are formed that increase molecular weight and require more thermal energy to break down and flow through the orifice, resulting in an increase in flow temperature from the raw material to extruded sample.

2.3.4 Extrudate analysis

2.3.4.1 Water holding capacity

Water holding capacity (WHC) is the amount of water that can be absorbed and held by TVP and is used to gain insight on the internal structure of the pieces. Overall, WHC decreased consistently as more heat swelling proteins were incorporated. This is shown in Figure 2-10 in relation to bulk density which relates the internal structure and WHC of the TVP pieces. This aligns with the hypothesis that heat swelling proteins will cause a more dense and tightly layered product. The compactness of the layers is most likely what prevents the TVP from absorbing and holding the water in its internal structure. Cold swelling treatments were able to absorb more

water and had a higher WHC. This is confirmed by other studies that described soy and pea protein (cold swelling proteins) as highly expanded and texturized products, especially compared to other treatments containing wheat gluten (Webb et al., 2023; Schreuders et al., 2019). When there are more pores and air cells formed during extrusion, the product often can hold onto more water by trapping it in the spaces between fibers. This means that the cold swelling protein treatments were more expanded and had less of a compact layered structure. This aligns with and is confirmed by bulk density results that showed an increase in bulk density as heat swelling proteins were used. This also is confirmed by visual analysis of hydrated TVP cross sections that show an increase in fibrousness and layering for heat swelling proteins and a more expanded and spongy texture for cold swelling treatments. The differences in structure forming ability between different protein types and swelling abilities arise from differences in protein makeup, structure, and processing (Hong et al., 2022).

2.3.4.2 Visual analysis

Pictures were taken of the longitudinal and horizontal cross sections of hydrated TVP pieces to confirm the internal structure (Figures 2-8 & 2-9). The intermediate swelling treatments had the largest pore size, while the heat swelling treatments had more layered fibrous structure with less open space. The cold swelling treatments formed many smaller pores, like a sponge, which is inferred to relate to a softer texture. This is confirmed by WHC and bulk density results that convey the structure of the cold swelling pieces to be more expanded versus the heat swelling treatments that should be more crosslinked and fibrous.

Soy protein is known to have strong gelling abilities and to texturize very well and form an expanded extrudate. Pea protein also has gel forming abilities but weaker than those of soy (Kyriakopoulou et al., 2021). These gelling and texturization advantages influence the structure

of TVP by allowing the material to stretch and form a strong film so that the final TVP is expanded with a porous internal matrix (Webb et al., 2023). Wheat gluten has an initial structure that is much different than the native globular structure of other legume and cereal proteins such as pea and soy. This unique structure along with the amino acid composition of wheat gluten give it unique functionality properties that lead to a more dense and fibrous structure. The amino acids in wheat gluten containing sulfur or reactive free amino groups such as lysine, cysteine, and glutamic acid are thought to increase the ability of the protein to form disulfide bonds which helps to form the more layered versus expanded texture and structure (Samard & Ryu, 2019).

2.3.4.3 Texture analysis

A dual compression test was utilized to determine and compare the textural attributes of TVP made with cold or heat swelling proteins. Texture analysis results did not show significant differences in hardness between many of the treatments, but the form of the TVP tested (milled vs whole) did cause a significant difference in hardness. The form of the extrudates and varying piece size make them difficult to test and to compress in a consistent manner and so standard deviations were high for most treatments even though 10 replicates were completed. Visually, there is a slight increasing trend in hardness when looking at the cold and heat swelling treatments (Figure 2-5). The heat swelling treatments usually have a higher hardness value than other treatments. Intermediate swelling treatments produced hardness results that were most like cold swelling proteins. The trends stayed mostly the same between the different types of TVP, but the whole pieces produced higher hardness values than the milled overall. This aligns with previous results showing that WHC was higher and bulk density was lower for cold swelling proteins compared to heat swelling. These results were also further confirmed by visual analysis

that illustrated how TVP internal structure was changed as the swelling properties of the formulations were changed. Cold swelling proteins produced a more porous and spongy appearance versus heat swelling that exhibited a more dense, layered, and fibrous structure.

Much like in animal meat, internal structure is critical to determining the texture of a meat analogue because the air spaces in between layers along with the thickness or toughness of the films and layers impact the sensory properties and mouthfeel of a meat or plant-based meat product such as juiciness, chewiness, and tenderness (Schreuders et al., 2021). When looking at chewiness results, there were similar trends shown to hardness (Figure 2-6). This makes sense because hardness is used in the calculation of the chewiness values. Denser, therefore heat swelling treatments were the chewiest. This was expected and could also be due to the inclusion of wheat gluten which can form a stronger protein network and elastic fibrous structure that is more difficult to break down than a cold swelling protein like soy (Webb et al., 2023; Samard & Ryu, 2019). Springiness is thought to relate to the gelation ability of the protein raw materials. Springiness of both the whole TVP and milled TVP showed no significant differences between treatments in this study (Figure 2-7). This aligns with previous results by Lyu et al. (2019). The lack of differences in springiness could be due to the compression methods of analysis not being able to pick up the small differences between treatments.

In the future, texture profile analysis (TPA) testing could be conducted on plant-based patties made from the milled TVP to better characterize the texture in a way that a consumer would experience the product. This was initially avoided to remove the influence of binders and WHC of the different TVP treatments on the texture of the product. Other texture analysis methods such as a Warner-Bratzler cutting test could also be utilized to produce more relevant texture analysis data. In addition to mechanical texture testing, sensory analysis could be utilized

to capture aspects of texture such as juiciness, springiness, and chewiness that are difficult to capture and represent using a texture analyzer (Schreuders et al., 2021).

2.4 Conclusion

Different plant protein sources were categorized according to swelling abilities, and this was used successfully to predict physical properties of plant-based meat analogues. It was hypothesized that proteins with cold swelling ability would lead to a more expanded product that leads to a softer texture. This was confirmed by a low bulk density, high WAI, low WHC, and expanded visual structure produced by cold swelling proteins. Texture analysis of the extrudates themselves didn't show clear significant trends in hardness, but whole TVP pieces had higher hardness and chewiness values than the milled TVP. Springiness did not show any significant differences between treatments for either TVP form. Visual analysis of heat-swelling treatments showed an increase in compact layering and decrease in internal cell size that aligned with an increase in bulk density and decrease in WHC. In the future, further analysis methods such as sensory studies and other methods of texture analysis could be utilized to better understand the quantitative and qualitative effects of protein functionality in texture. Overall, it was confirmed that swelling abilities of plant proteins are an extremely important parameter when formulating a recipe for TVP based plant-based meat analogues. It was shown that cold swelling proteins can best be identified by a $WAI > 0.4 \text{ g/g}$ and an RVA peak that occurred before 4 minutes. This information can be utilized to target certain meat analogue applications based on texture, such as a chewier and firmer product for a beef or chicken analogue versus a soft and flakier texture desired for a fish analogue. Texture and sensory perception of plant-based meat analogues is currently one of the largest obstacles blocking the widespread consumption of plant-based meat.

By continuing to research the effect of protein functionality on texture, this hurdle can begin to be overcome and the quality of plant-based meat products can continue to improve in the future and new novel textures can be created.

2.5 References

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2.6 Figures and tables

#	SCREW PROFILE
1	Full pitch, double, 9U
2	Full pitch, single, 9U
3	$\frac{3}{4}$ pitch, double, 9U
4	$\frac{3}{4}$ pitch, double, 6U
5	Forward KB (5B = backward)
6	$\frac{1}{2}$ pitch, double, cut flight
7	Reverse KB (7B = backward)
8	$\frac{3}{4}$ pitch, double, cut flight, cone

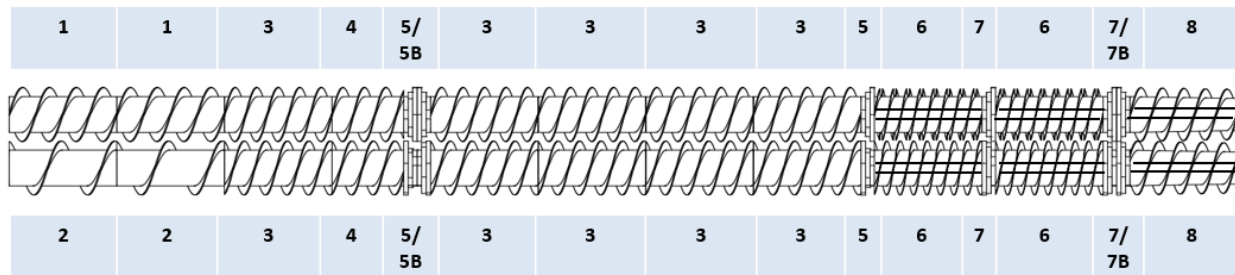


Figure 2-1 The screw profile used in the TX-52 pilot-scale twin screw utilized elements such as cut flight screws and kneading blocks to increase mechanical energy input.

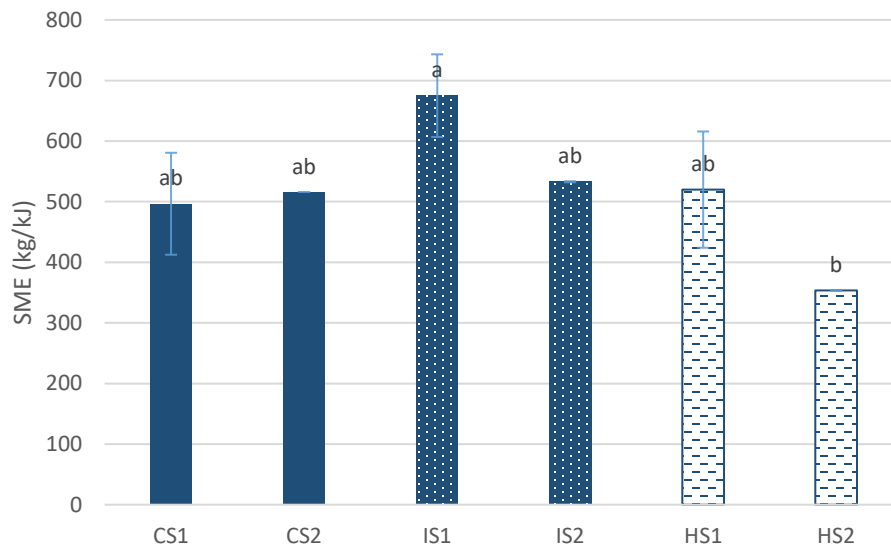


Figure 2-2 Specific mechanical energy (SME) represents the amount of mechanical energy used on the material during extrusion processing. A small decreasing trend can be seen from cold to heat swelling. Treatments are labeled cold swelling (CS), intermediate swelling (IS), and heat swelling (HS).

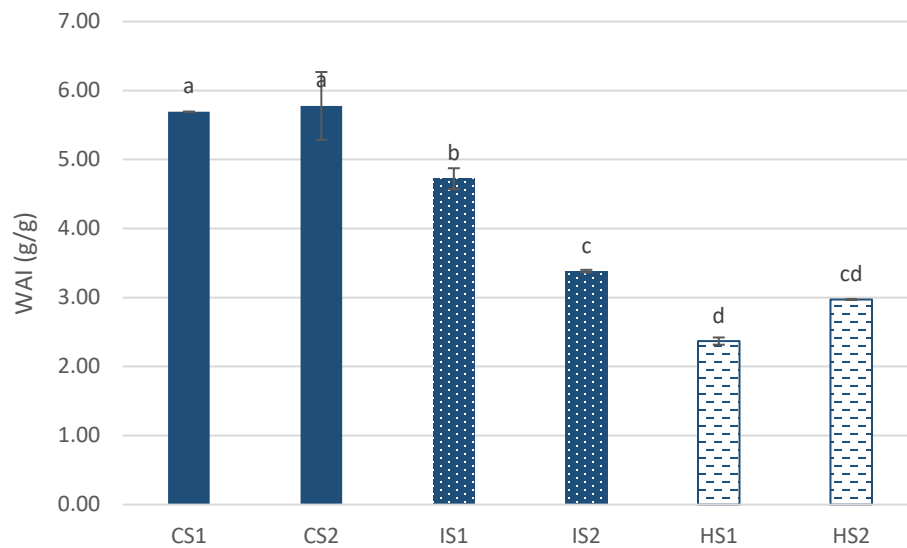


Figure 2-3 Water absorption index (WAI) decreases from cold to heat swelling proteins. Treatments are labeled cold swelling (CS), intermediate swelling (IS), and heat swelling (HS).

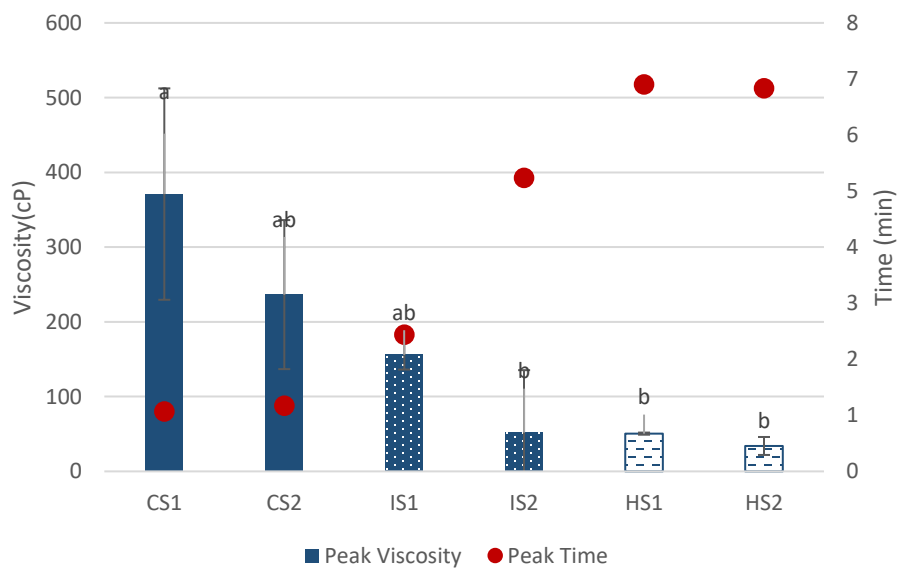


Figure 2-4 Rapid visco analysis (RVA) was used to find the peak viscosity and time at which this peak was reached for each treatment. Cold swelling (CS) treatments had a low peak time and high viscosity while heat swelling (HS) had the opposite.

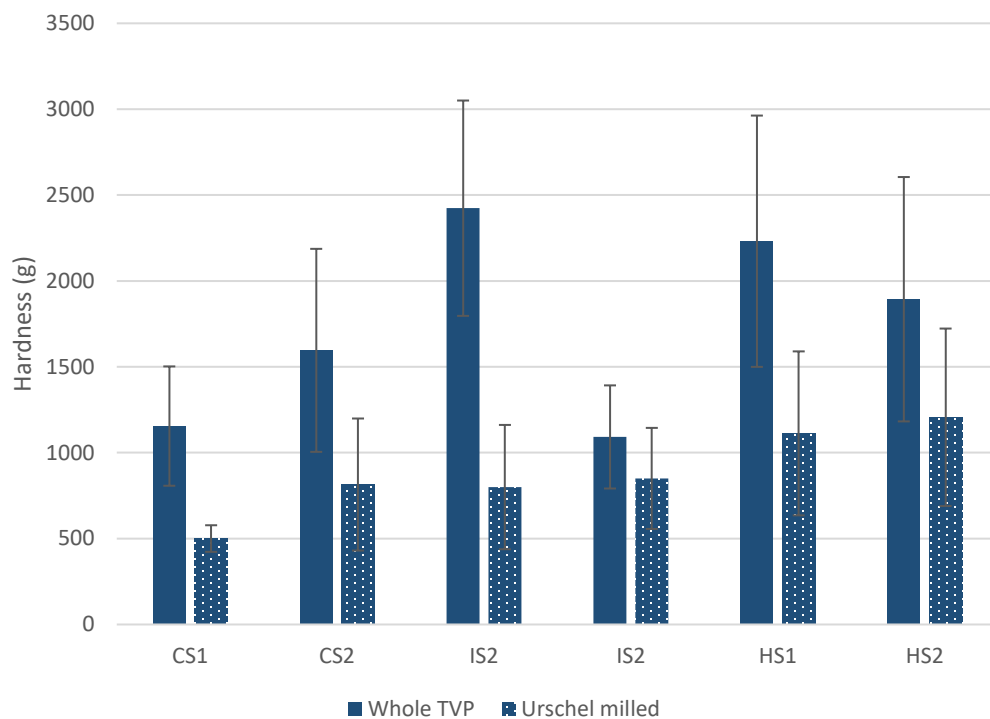


Figure 2-5 Compression testing was conducted to determine hardness of each TVP sample both as a whole piece and milled.

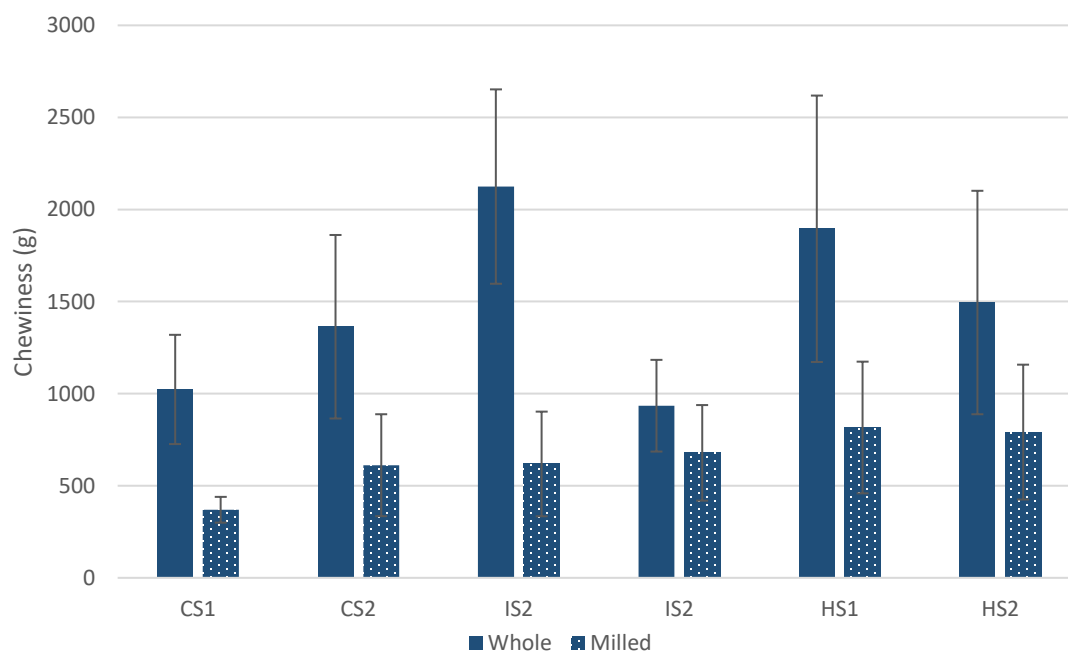


Figure 2-6 Compression testing was conducted to determine chewiness of each TVP sample both as a whole piece and milled.

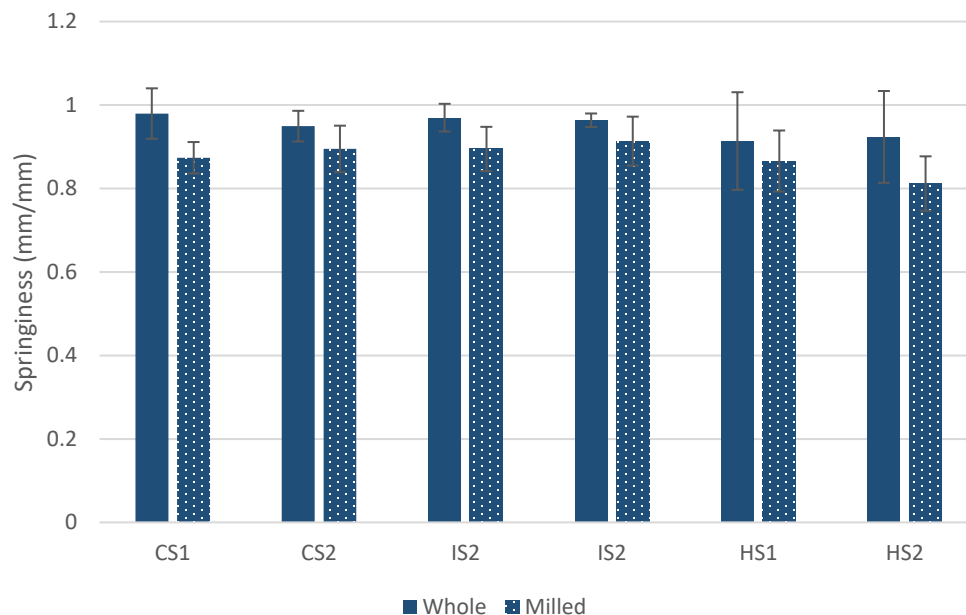


Figure 2-7 Compression testing was conducted to determine springiness of each sample both as a hydrated whole piece and milled TVP.

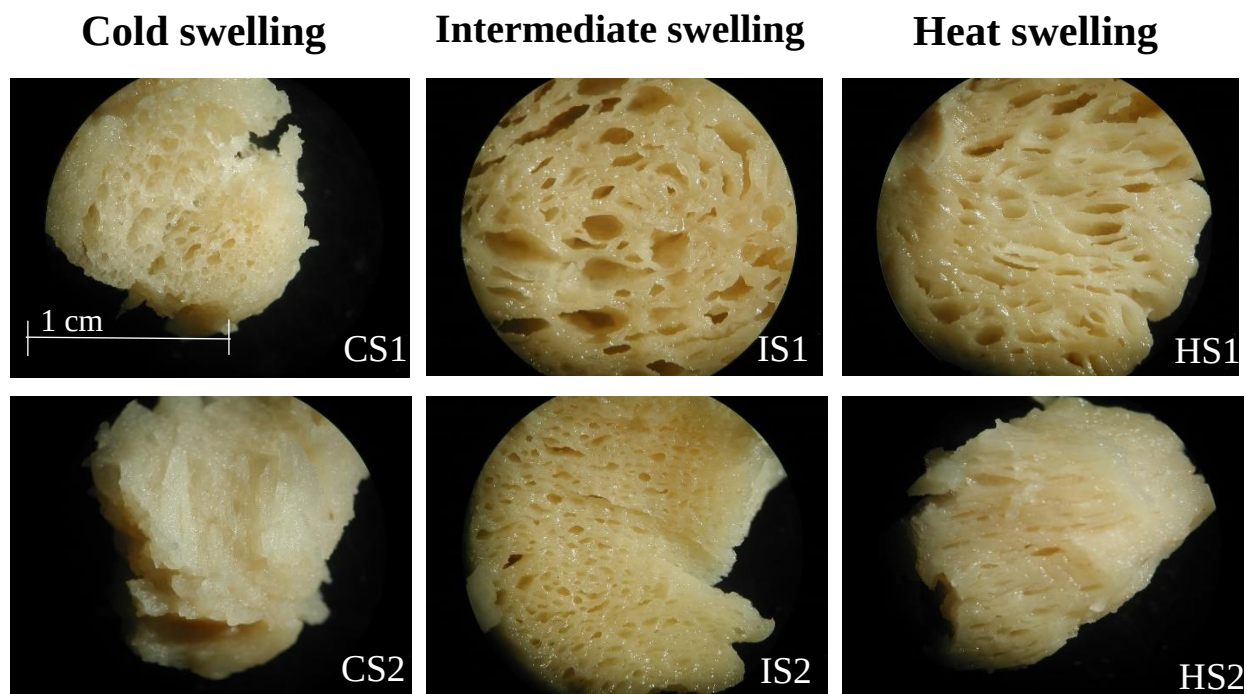


Figure 2-8 A microscope camera was used to capture the internal structure of hydrated TVP cut in the horizontal direction (against the flow from extruder).

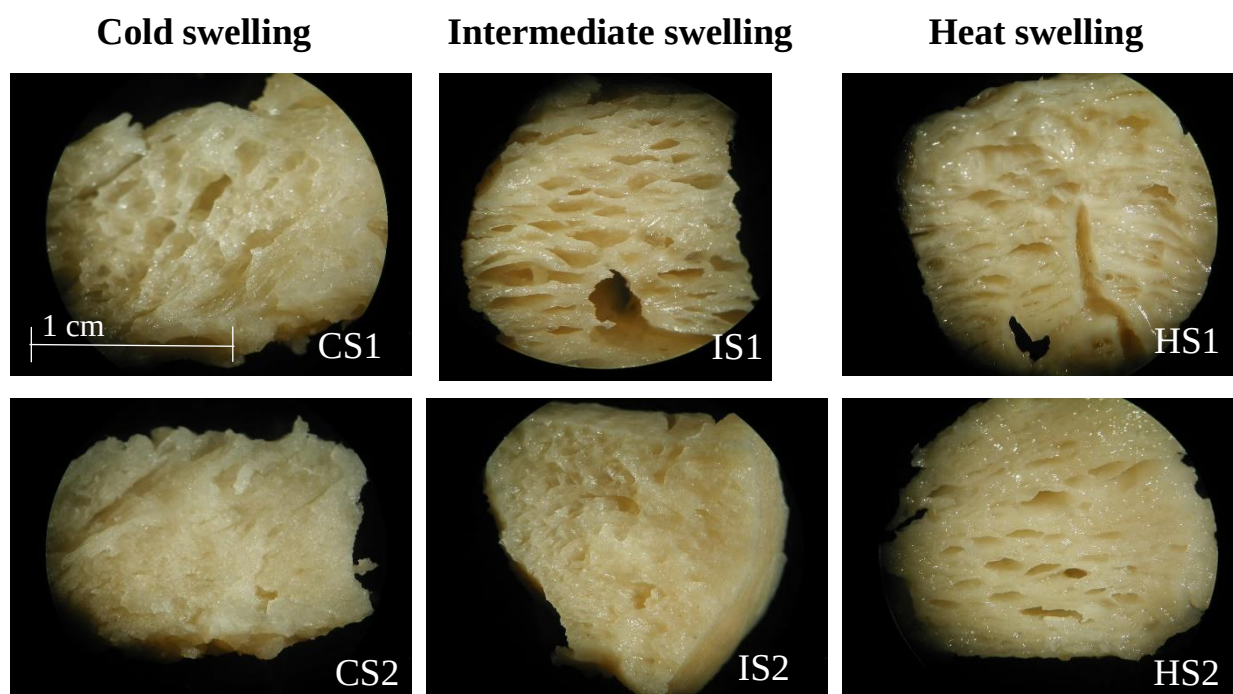


Figure 2-9 A microscope camera was used to capture the internal structure of hydrated TVP cut in the longitudinal direction (with the flow from extruder).

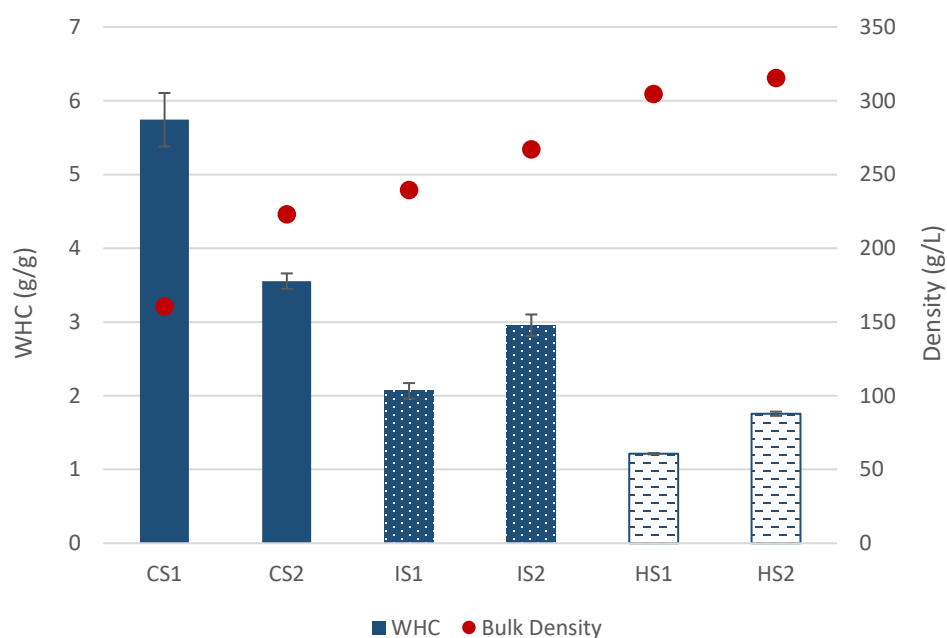


Figure 2-10 Water holding capacity (WHC) and bulk density showed an inverse relationship from cold swelling to heat swelling proteins.

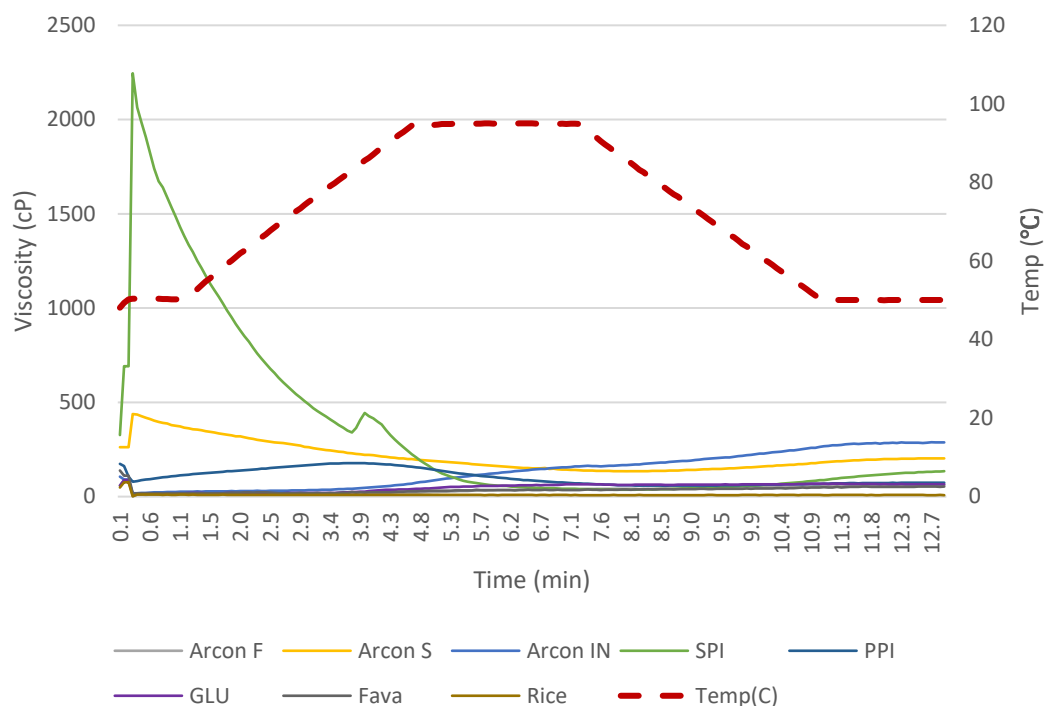


Figure 2-11 Viscosity over time as it underwent a heating cycle was recorded using RVA on raw materials of each individual protein ingredient to identify differences in swelling properties.

Table 2-1 Raw material properties of each protein source were characterized as cold or heat swelling by analyzing results from WAI, LGC, and RVA. A + indicates a positive result and a – indicates a negative result.

Ingredient	Cold	Heat	RVA		WAI (g/g)	LGC (%)
	Swell	Swell	peak time (min)	peak viscosity (Cp)		
Pea Protein Isolate	+	-	3.1	134.5	4.307	16
Soy Protein Isolate	+++	+	1.07	1429.5	8.383	14
Arcon S Soy Protein Concentrate	++	+	1.07	372	5.224	14
Arcon F Soy Protein Concentrate	-	--	6.8	39	3.505	20
Arcon IN Soy Protein Concentrate	+	-	6.9	155	4.516	18
Wheat Gluten	--	-	7	64	2.483	16
Fava Protein Concentrate	--	+	6.3	37.5	2.029	14
Brown Rice Protein	--	---	1.1	11	2.457	-

Table 2-2 The experiment was designed to include 6 total treatments, 2 treatments each, of cold, heat, and intermediate protein swelling properties identified by CS, HS, and IS respectively. The chart below represents the % of each ingredient used in each of the treatments. Protein content was calculated to be between 70-80% for each treatment.

Treatment	ID	SPI	Arcon S	Arcon F	Arcon IN	PPI	GLU	Fava	Total Protein
Cold	CS1	10	70			20			75.7%
Swelling	CS2	40	30		30				79.5%
Mixed	IS1	50					25	25	79.3%
Swelling	IS2		20			20	60		75.4%
Heat	HS1			20			70	10	71.9%
Swelling	HS2			55	10		35		71.1%

Table 2-3 Raw materials and extruded products for each treatment were analyzed using a Phase Transition Analyzer (PTA) to determine changes in flow temperatures that could provide evidence of crosslinking. When temperatures increase from raw to extruded it indicates crosslinking of proteins.

		Start (°C)	Mid (°C)	End (°C)	Flow (°C)
CS1	Raw	6.2	20.5	35.3	75.5
	Extruded	7.0	37.3	67.8	143.1
CS2	Raw	5.7	17.9	29.9	70.4
	Extruded	7.4	39.5	71.8	105.8
IS1	Raw	6.3	28.1	50.1	107.0
	Extruded	13.9	40.0	66.8	95.5
IS2	Raw	12.6	38.9	65.6	77.1
	Extruded	7.3	33.6	60.4	103.7
HS1	Raw	13.9	40.7	67.6	120.3
	Extruded	5.0	26.3	47.4	99.1
HS2	Raw	6.1	12.5	19.2	30.3
	Extruded	6.6	25.2	44.5	86.5

Table 2-4 Pilot-scale extrusion parameters are listed below for each treatment. In-barrel moisture (IBM), die temperature, and screw speed are important in producing an optimal TVP product.

Treatment	In-barrel moisture (%)	Die temperature (°C)	Screw speed (rpm)
CS1	41.5	159	346
CS2	46.8	166	301
IS1	48.8	159	450
IS2	42.9	155	415
HS1	32.7	156	422
HS2	41.6	153	300

Chapter 3 - Utilizing Protein Functionality Information to Tailor

Quality of Plant-Based Meat Analogues

Abstract

This research investigates the effect of protein swelling ability on the texture of meat analogues and how it can be used to target certain applications, such as a plant-based burger. Protein sources were first characterized as cold or heat swelling through various raw material analytical tests such as water absorption index (WAI), rapid visco analysis (RVA), and protein solubility (%). Treatments were formulated to have different ratios of cold/heat swelling properties in order to influence product texture. Extrusion processing was used to produce texturized vegetable protein (TVP) which was later made into patties. Extruded product testing included water holding capacity (WHC), texture profile analysis (TPA), visual analysis, SDS-PAGE, SEC-HPLC, and sensory studies.

WAI decreased as the amount of cold swelling proteins in the formulation decreased (5.61 g/g for 90% CS, 3.79 g/g for 40% CS). WHC also decreased as cold swelling increased with a maximum of 5.25 g/g at 90% CS and a minimum of 2.69 g/g at 40% CS. This aligned with an increase in bulk density that signals that heat swelling proteins successfully created denser and more layered products. Visual analysis also showed an increase in the number of pores and a spongier internal structure as the amount of cold swelling increased. Texture analysis results were not able to identify any differences between treatments when testing the hydrated TVP pieces, but when plant-based patties were tested the 0% CS treatment was significantly harder than the 90% CS treatment, as was hypothesized. The degree of crosslinking and texturization was also measured using protein chemical analysis methods SDS-PAGE and SEC-HPLC. These results produced evidence that treatments containing wheat were the most

crosslinked followed by pea and then soy. By understanding how protein functionality influences structure formation during extrusion, different protein sources can be better utilized to produce consistent and quality meat analogues for a specific product goal. This research provides a strong base of knowledge on how protein swelling abilities can be utilized to manipulate texture of plant-based meat products. With future research, these findings could be solidified and a more thorough understanding of the effects of mixtures of different protein types could be identified.

3.1 Introduction

Plant-based meat analogues and other alternative protein products have become increasingly popular in recent years as the number of “flexitarian” consumers increases. These consumers are demanding a product that is equally or more nutritious, affordable, environmentally friendly, and tastier than their animal derived counterparts. With these goals, challenges arise such as the ability to accurately mimic the texture and other important sensory aspects of a meat product. This research aims to investigate the impact of protein functionality on texture and to see if functionality information can be used to formulate recipes to target certain applications, such as a plant-based burger or fish filet. Previous research, as shown in Chapter 2, has been able to gather knowledge on how protein solubility can affect texture. This study further investigated these relationships by utilizing this information to create formulations with increasing ratios of cold/heat swelling proteins in an attempt to control and fine-tune meat analogue texture using mostly soy-based treatments.

Many plant-based meat analogues such as the ones utilized for this research are made through low moisture extrusion processing. The plant protein concentrates or isolates are extruded to form texturized vegetable protein (TVP) that is milled and later combined with other ingredients such as water, binders, oils, and seasonings to make a plant-based meat product. In 2019, 48% of plant-based meat products were made using soy protein (Webb et al., 2023). Other proteins such as wheat gluten, pea, and fava protein are also found commonly in plant-based meat with the industry beginning to explore a wide variety of protein sources (Webb et al., 2023). Extrusion utilizes a combination of mechanical and thermal energy to texturize the protein, which means that it realigns the native globular structure of the plant protein into fibrous

layers that mimic animal muscle structure. This allows for a more authentic layered structure to be the base of plant-based meat products (Riaz, 2004).

Research on improving the texture of plant-based meat analogues through the manipulation of ingredients with different functionalities is a critical step forward in improving the quality and sensory attributes of current plant-based meat and paves the way for new innovative plant-based products to be made with previously difficult or unattainable texture goals.

3.2 Materials and Methods

3.2.1 Formulation

One very important functionality attribute affecting texture that has been identified in previous research is the functionality and solubility of raw materials (Webb et al., 2020). Protein solubility can be characterized as either cold swelling or heat swelling. Preliminary research has shown that cold swelling proteins are more “functional” and lead to more texturization and expansion of the texturized vegetable protein (TVP) product causing a softer and spongier final texture, while heat swelling proteins are less “functional” and lead to a denser and more layered TVP. Other studies have also shown evidence that pea protein produces a softer texture than soy, and that wheat can improve cross-linking and texturization (Yuliarti et al., 2021; Lee et al., 2023; Chiang et al., 2019). For this experiment, 6 total treatments were tested with varying amounts of cold swelling proteins (0% to 90% CS) as shown in Table 3-1. Treatments were all soy based while pea and wheat were used to modulate texture. Proteins characterized previously in Chapter 1 as heat swelling included wheat gluten and Arcon F soy protein concentrate (SPC) and cold swelling included soy protein isolate (SPI), Arcon S SPC, and pea protein isolate (PPI). Starch

ingredients such as soy flour and tapioca starch were also used to influence texture and have been shown in previous studies to increase layering and decrease texturization by interrupting the protein cross-linking that occurs during extrusion processing (Webb et al., 2020). Protein contents of each of the treatments were kept consistent between 70-80% because a higher protein content can influence texture of the final TVP by making it tougher and chewier (Riaz, 2004). The formulation details for each treatment can be found in Table 3-1.

3.2.2 Extrusion processing

Extrusion processing was done on a TX-52 pilot-scale twin screw extruder (Wenger Manufacturing, Sabetha, KS, USA). A ¼ inch venturi die was used to increase shear and mechanical energy to promote texturization of the plant protein materials. The screw profile used is illustrate in Figure 3-1 and shows the use of aggressive screw elements such as kneading blocks, reverse, and cut flight elements that increase barrel fill and shear. The outlet die contained 2 openings that were each ¼ inch and the product was cut after exiting the die by 3 hard rotating knives. Half of the product was sent directly to a dryer after exiting the extruder and dried, while the other half was taken directly off the extruder and milled using an Urschel mill with a screen size of 0.18 in. Processing information was recorded twice for each treatment at the beginning and end of the collection time and was also collected using a data acquisition system every second. This data along with other cost and resource usage information will be used to conduct an economic study and a consumer survey about plant-based meat analogues.

3.2.3 Raw Material Functionality

3.2.3.1 Water absorption index

Water absorption index (WAI) is used to identify cold swelling proteins by quantifying the amount of water that can be absorbed by a material. WAI was conducted on both raw

materials and ground extruded products for each treatment mix along with the individual ingredient raw materials. WAI represents cold swelling abilities because it detects the water absorbed at room temperature with no thermal energy added. The method used was adapted from the method utilized by Anderson, Conway, and Peplinski (1970). The test begins with 2.5g of sample being mixed with 30 mL of distilled water using a vortex mixer for 10 seconds. The samples are then placed on a shaker table to continue mixing for 30 minutes. Next the samples are centrifuged at 3,000g for 15 minutes using a Centrifuge 5810 R 15 Amp Version (Eppendorf, Hauppauge, NY, USA). The supernatant is then removed from the sample as it should separate out from the gel formed at the bottom of the test tube. The gel mass is then recorded, and WAI is calculated using the equation below. Water absorption index (WAI) is calculated using the weight of the gel divided by the weight of the dry solids.

$$WAI \left(\frac{g}{g} \right) = \frac{W_{gel}}{W_{dry\ solids}} \quad (3-1)$$

3.2.3.2 Least gelation concentration

Least gelation concentration (LGC) was also conducted on the raw materials for every treatment and the individual ingredients. LGC is used to identify heat swelling properties as it measures the materials capacity to form a gel at certain concentrations after undergoing a heat treatment. According to a method modified from Wang et al. (2007), protein concentrations ranging from 8%-20% are made using 10 mL of distilled water and placed in a series of test tubes. These test tubes are then mixed until the material has been completely combined and there are no clumps present. The samples are then placed in a water bath at 90-100 °C for 1 hour followed by a bath of room temperature water for 10 minutes. After this is completed, the samples are placed in a refrigerator for another 2 hours. After refrigeration is complete, the test tubes are inverted, and observations are made to conclude if the sample formed a strong cohesive

gel at each concentration. The smallest concentration at which the sample did not slip or fall down the sides of the test tube indicated the LGC.

3.2.3.3 Rapid visco analysis

Rapid visco analysis (RVA) is also used to determine cold and heat swelling capabilities and is the main way that proteins are characterized into either category. RVA uses a rapid visco analyzer (RVA 4500, Perten Instruments, Waltham, MA, USA) and determines the viscosity of the material over time as it is stirred continuously and undergoes a heating cycle. A peak in viscosity at the beginning of the cycle before heating takes place indicates a cold swelling protein while a later peak indicates a heat swelling protein. The samples are prepared by combining the raw materials with distilled water to create a 15% d.b. suspension. The RVA parameters were determined using AACC Method 76-21.02 STD1 which keeps the sample at 50 °C for 1 min and then heats to 95 °C at 12.2 °C/min where it is held for 2.5 minutes. Next the sample is cooled back to 50 °C at 11.8 °C/min and then held for an additional 2 minutes.

3.2.3.4 Protein solubility

Protein solubility was measured to determine how much water the protein fractions of each ingredient and treatment were able to absorb. This can be used to confirm the results found from RVA, LGC, and WAI to characterize each protein type as cold or heat swelling. To begin 0.5g of a sample was dispersed in 10 mL of deionized (DI) water. Once the sample was thoroughly mixed, the original pH was recorded. The mixture was then stirred for 30 minutes at room temperature to allow for the samples to be fully hydrated. Next, the suspension was centrifuged at 4500 rpm for 30 minutes. The supernatant was then removed, and the remaining precipitate was frozen and freeze dried. The precipitate protein content was then tested and used to calculate the protein solubility using the equation below. Protein content was found using the

combustion method (AACC Method 46-30.01) and a LECO analyzer and nitrogen to protein conversion factor of 6.25 was used. Protein solubility was calculated using the difference between the weight of the original sample and the precipitate, over the weight of the original sample.

$$\text{Protein solubility (\%)} = \frac{\text{wt of protein in original sample (g)} - \text{wt of protein in precipitate (g)}}{\text{wt of protein in original sample (g)}} \times 100 \quad (3-2)$$

3.2.4 Protein chemical analysis

3.2.4.1 SDS-PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the molecular weight of proteins present in each ingredient and treatment before and after extrusion. If a decrease in intensity of bands is seen after extrusion, it could indicate polymerization and cross-linking of the proteins (Pietsch, 2017). First, 100 mg of each sample was suspended in 10 mL of PBS (pH=6.8) buffer containing 2% w/v SDS. The samples were then mixed using a shaker for 1 hr (250 rpm) at room temperature followed by centrifugation at 8000 g for 5 minutes. The supernatant is then collected and used for the SDS-PAGE analysis using non-reducing conditions. Non-reducing conditions were chosen for this application to capture the presence of crosslinking because reducing conditions would break down disulfide bonds and split the protein into subunits and not give an accurate depiction of the state of the protein before and after extrusion. To begin the SDS-PAGE analysis, 30 μ L of each sample was combined with 10 μ L of 4 x Laemmli buffer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The mixture is then heated in boiling water for 5 minutes. Following heating, 15 μ L of each treatment sample was loaded into wells (10 μ L for isolated protein raw materials) of a 4-20% Mini-Protean TGX gel (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and separated at 200 V for around 37 minutes all at room temperature. In order to watch the molecular weight progress,

a Precision Plus Protein Dual Color Standards (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was loaded (5 μ L) parallelly. A Brilliant Blue R Concentrate (Sigma, St. Louis, MO, USA) was then used to stain the gel with gentle shaking for 8 minutes. DI water was then used to repeatedly de-stain the gel until the background was clear and readable.

3.2.4.2 SEC-HPLC

SEC-HPLC was also used to detect crosslinking by looking at presence of certain molecular weights before and after extrusion. Similar to the preparation for SDS-PAGE, 100 mg of each sample was mixed with 20 mL 2% SDS in PBS (pH=6.8) and then vortexed for 1h at room temperature followed by centrifugation at 8000 g for 5 minutes. The supernatant was then collected and diluted to 1 mg/mL using 2% SDS in PBS and then filtered with 0.45 μ m PVDF membrane filter. Next the prepared samples were loaded on a Yarra 3 μ m SEC-4000 column (300 x 7.8 mm, Phenomenex, Torrance, CA, USA) and processed using an Agilent HPLC 1100 system. The elution solvents contained water with 0.1% trifluoroacetic acid (A) and acetonitrile (B). The linear gradient was 0 min at 80% A, 20 min at 70% A, 25 min 65% A, and 30 min 80% A. The detection level was set at 214 nm and the injection volume and flow rate were 20 μ L and 0.7 mL/min respectively. This was conducted at 30 $^{\circ}$ C.

3.2.5 Extrudate analysis

3.2.5.1 Water holding capacity

Water holding capacity (WHC) is simply the amount of water that whole dried extrudate pieces can absorb divided by the initial weight of the sample. First 15g of the whole dried extrudate pieces are added to excess water and allowed to soak for 20 minutes. The extrudates should then be fully hydrated and can be drained over a mesh screen for 5 minutes (Webb et al., 2020).

3.2.5.2 Visual analysis

Visual analysis was conducted using a Nikon D750 SLR digital camera with a Nikkor 105 mm macro lens to capture several images of the internal structure of hydrated whole extrudate pieces cut in two different directions, longitudinal (with the grain) and horizontal (against the grain). Extrudate pieces were hydrated using the same protocol mentioned for WHC. Visual analysis can help to compare and evaluate the changes in texturization and denseness of layering that happens as the material is extruded and cross-linked.

3.2.5.3 Texture analysis

Texture analysis was completed using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) and implemented a method used previously by Webb, et al. (2020). A dual compression test was utilized that measures the peak force needed to compress the sample 7 mm twice with a cylindrical probe. This was chosen because it mimics the biting action of a mouth and has been previously used for a variety of meat and meat analogue products (Schreuders et al., 2021). Hydrated pieces were placed one layer deep in a shallow circular dish slightly larger in diameter than the probe to contain the sample as it was being compressed. The compression cycles were done at 1.00 mm/sec and parameters recorded were used to calculate hardness, chewiness, and springiness (Samard & Ryu, 2019). Tests were done with 10 replicates for each treatment. After testing hydrated whole TVP pieces, plant-based patties were tested using texture profile analysis (TPA). Plant-based patties were cut into 2" x 2" squares to be tested using the same cylindrical probe. A TPA test was then conducted using a modified method from Yuliarti et al. (2021) by compressing the patties twice to 50% of the original height with a speed of 1.00 mm/sec. The patty recipe includes milled TVP, water, oil, seasonings, fava protein concentrate, and methylcellulose as a binder. Data from both types of

compression testing can be used to calculate hardness, springiness, and chewiness. Hardness (g) is defined as the peak force during the first compression cycle, chewiness (g) is the $(\text{Area } 2^{\text{nd}} \text{ peak} / \text{Area } 1^{\text{st}} \text{ peak}) \times \text{hardness} \times \text{springiness}$, and springiness (mm/mm) is the distance 1^{st} compression / distance 2^{nd} compression (McClements, 2021).

3.2.6 Sensory analysis

Two focus groups were conducted to determine consumer perceptions of plant-based meat products and to gain feedback on the texture of several experimental plant-based products targeting either a beef or fish patty. Participants of the study were aged 18-61 and indicated in a screening survey that they were interested at least somewhat in plant-based protein alternatives. Each focus group tasted a plant-based meat product made from one of the experimental treatments, a commercial plant-based equivalent, and an actual meat product. The plant-based patty recipe used for both the consumer study and focus groups can be found in Table 3-6.

A consumer study was also conducted to determine if consumers could perceive a difference in texture between treatments, and to determine their overall liking of the products and the closeness to a real meat product texture. A total of 120 participants aged 18-61 were recruited with the requirements being that they were at least moderately interested in plant-based protein and were the primary grocery shopper for their household. Each person tasted a total of 5 products, which were 4 plant-based treatments and 1 actual beef burger. A completely randomized design was used so each participant tasted each of the 4 plant-based products in a random order but with the real beef patty always being tasted last to avoid any bias. Participants were asked about overall liking, texture, flavor, aftertaste, and overall perceptions of plant-based meat using a combination of 9-point hedonic scale and short answer questions.

3.2.6 Statistical analysis

Statistical analysis was completed using SAS software (SAS, Cary, NC) and one-way ANOVA with Tukey's test to determine p-values and significant differences ($p < 0.05$) and relationships between treatments. For sensory study results, statistical analysis was run using XLSTAT for each attribute using one-way ANOVA with Tukey's test and Fisher's LSD for mean separation.

3.3 Results and Discussion

3.3.1 Extrusion Processing

Through pilot-scale extrusion, native globular plant proteins are hydrated and plasticized in the extruder barrel due to the addition of pressure, shear, and thermal energy. As the material exits the die, the structure of the plasticized material is realigned into a fibrous structure and disulfide bonds are formed between protein molecules. This is called texturization and it can cause changes in the conformation and structure of the proteins along with modifying its functional and physical properties (Hong et al., 2022). During processing one of the most important factors that impacted the quality of the TVP was die temperature (Lyu et al., 2022). Different die temperatures were ideal for each treatment. Overall, the lower % CS treatments required a higher die temperature, which makes sense because they had a higher ratio of heat swelling proteins in their formulation that required more thermal energy to form viscosity. The higher % CS treatments had lower die temperatures, which is most likely due to the high amounts of soy protein isolate (SPI). SPI has been shown in previous studies by Samard et al. (2019) to produce optimal texture SPI-based TVP at a higher moisture content and lower die temperature (50%, 130 °C). Water addition in the preconditioner was lowest for treatments

containing wheat and highest for treatments containing just soy. This was as expected because soy is usually more soluble, especially before heat is added, and wheat is difficult to hydrate in the preconditioner because it immediately forms a strong and sticky protein matrix dough when exposed to water and is best handled in the extruder barrel instead (Riaz, 2004). Extruder screw speed varied for each treatment with the highest being 30% CS at 449 rpm and the lowest 0% CS at 300 rpm. 30% CS most likely required such a high screw speed to provide mechanical energy to texturize the product fully because it had the most water added of any treatment and therefore the material had a lower viscosity. Specific mechanical energy (SME) was calculated for each treatment and showed no significant differences between treatments with the highest being 30% CS at 997.2 kJ/kg and the lowest being 50% CS at 874.8 kJ/kg (Table 3-5).

3.3.2 Raw material functionality

3.3.2.1 Water absorption index

Water absorption index (WAI) was used initially on the raw materials to help characterize the proteins themselves as either cold or heat swelling. A high WAI (> 4.0 g/g) indicates cold swelling abilities, and a low WAI (< 4.0 g/g) indicates heat swelling. Proteins that were clearly cold swelling as indicated by WAI included soy protein isolate (SPI) and the more functional Arcon-S soy protein concentrate (SPC) at 5.92 g/g and 5.70 g/g respectively. WAI was also used to analyze the treatment formulations to further confirm that cold swelling treatments produced a high WAI and to determine any interactions with heat swelling components. As the inclusion of cold swelling proteins in the formulation increased, there was an increasing trend in WAI as well (Figure 3-2). The highest WAI was produced by the 90% CS treatment at 5.61 g/g. On the other end, the lowest WAI was found to be from the 0% CS treatment at 2.51 g/g. This confirms WAI can pick up cold swelling abilities and can be used to

help determine the properties of a protein mixture. The WAI of raw materials is dependent on functionality properties of the proteins which are derived from the subunits and structure that make up each individual protein ingredient. The polarity and availability of different protein residues differ for each source and can impact how the protein interacts with water and its structure. Proteins with lower amounts of polar amino acids may decrease WAI and nonpolar may have the opposite effect (Hong et al., 2022). Hydrophobicity, and therefore WAI, can also be impacted by different processing methods and steps used during isolation of the proteins. Commercial production of protein concentrates and isolates often requires the use of physical and chemical methods that modify the functionality of the protein, such as solubility and folding structure. Low WAI was observed for the low cold swelling treatments, and this is most likely because they contained wheat gluten which is comprised of prolamins and glutelins that are most soluble in alcohols or acids and not in water. The cold swelling treatments contained soy protein isolate (SPI) that is usually very water soluble and pea protein isolate (PPI) that is moderately to highly water soluble depending on the ingredient source and isolation method used (Webb et al., 2023).

3.3.2.2 Least gelation concentration

Least gelation concentration (LGC) was initially utilized to help categorize the raw protein ingredients as either cold or heat swelling. Theoretically LGC should be lower for heat swelling proteins as that means it takes less material to form a strong gel after heating. Overall, the results of LGC were not significantly different between treatments when testing both individual protein ingredients and the treatment mixes (Table 3-3). This is because although LGC undergoes a heating cycle to form a gel, some proteins that easily form a gel at room temperature can maintain this state throughout the entire test until the end when the results are

interpreted. This indicates that cold swelling proteins that produced a low LGC, such as soy protein isolate, are resistant to shear thinning and can maintain a gel after heating and cooling which can be a benefit to final texture of the meat analogue (Hong et al., 2022). Although, some cold swelling proteins do not always have good gelling abilities, such as pea protein isolate, which could be why the treatments were not showing the expected LGC results. Many commercial pea protein isolates are extensively denatured during the isolation process used during production which decreased their gelation abilities and protein-protein interactions (Mession et al., 2015). Production of ingredients could potentially be modified to produce a pea protein isolate with better gelation abilities, but it would take more time with less intensive processing.

Overall, it was concluded that LGC was not the most useful analytical method when determining swelling properties of plant-proteins and other tests should be relied upon for characterization of swelling abilities such as WAI and RVA. Although LGC can still identify differences in protein functionality and the use of LGC to identify chemical and functionality differences other than solubility should be investigated further. Gelation may be separate from swelling ability and is another important parameter that needs to be understood further to control the texture and structure of plant-based meat analogues. Gelation is needed to form the final fibrous structure of a meat analogue so the LGC can represent ability to form layered structures in the final product (Webb et al., 2023).

3.3.2.3 Rapid visco analysis

Rapid visco analysis (RVA) was used to determine viscosity over a heating cycle with continuous stirring. RVA was first used to characterize proteins as cold or heat swelling through peaks in viscosity. Peaks in viscosity that occurred before 4 minutes into the heating cycle were

categorized as having cold swelling properties while peaks later than 4 minutes were indicative of heat swelling properties. Multiple peaks may be present indicating the presence of both the cold and heat swelling ingredients. Cold swelling proteins also had higher peak viscosities which aligns with WAI data as the more water that is absorbed, the higher the viscosity that is formed as was also found in a study by Hong et al., 2022. This is because cold swelling proteins like soy and pea are globular, and this allows them to easily form gels. As they unfold due to shear or heat and are exposed to water, their non-polar and sulfhydryl groups readily form hydrophobic and disulfide bonds that cause the proteins to aggregate and form an increase in viscosity (McClements et al., 2021).

There were limited statistically significant differences between % CS treatments in peak viscosity. The RVA viscosity curves are shown in more detail for each treatment mix in Appendix-B. Peak time was significantly different for the 90% vs 0% CS protein mixes as it was much higher for the heat swelling treatment because it took more time for the protein to solubilize and increase in viscosity as the heating cycle occurred over time (Table 3-2). One factor influencing the lack of significant differences between the peak viscosities of the mixes is that some proteins, such as SPI and Arcon S, are very good at solubilizing and forming a gel as shown by LGC data and confirmed by previous studies (McClements et al., 2021). That means that even when they were incorporated in smaller amounts, they still overpowered the viscosity peak results of the RVA test. The starch in the formulation also could have had an impact on viscosity as starch has a heat induced peak in viscosity as it is gelatinized (Webb et al., 2021).

3.3.2.4 Protein solubility

Protein solubility for each of the raw material ingredients helped to characterize and confirm cold swelling or heat swelling properties. Overall, a protein solubility (%) of greater

than 20% often indicated cold swelling while less than that indicated heat swelling abilities. Solubility has been shown previously to be an indicator of protein texturization with higher solubility leading to more texturization (Hong et al., 2022). Soy protein isolate (SPI) had the highest average protein solubility of 58.86% which was expected and indicates strong cold swelling properties because the material can solubilize well without heating. Wheat gluten had the lowest protein solubility (14.20%) which was also expected and able to confirm previous characterization of the protein as heat swelling based on RVA and WAI data (Table 3-4). Wheat gluten also contains the most sulfur-containing amino acids compared to other ingredients which could result in more disulfide bond formation and cross-linking during texturization that would greatly reduce protein solubility (Hong et al., 2022). These results align with previous solubility data collected by Hong et al. (2022) on commercial TVP samples from different protein sources. According to their research, soy based TVP had the highest protein solubility, followed by pea, and then wheat (Hong et al., 2022). Soy flour had a high protein solubility average of 46.9%, which indicates cold swelling properties, but it has a much lower protein content, so the effect of starch is more relevant. Starch gelatinizes and increases in viscosity as heating occurs so the activity of the starch would be considered similar to heat swelling properties. Previous studies have shown that flour usually increases density and acts more like a heat swelling protein when used in concentrations less than 20% (Webb et al., 2020). Protein solubility overall decreased after extrusion for each treatment (Table 3-4). This is an indicator of cross-linking because of the increased aggregation of proteins after denaturation and unfolding through the shear and thermal energy of extrusion (Osen et al., 2014; Hong et al., 2022).

3.3.3 Protein chemical analysis

3.3.3.1 SDS-PAGE

SDS-PAGE using non-reducing conditions was run on treatment samples before and after extrusion. Non-reducing conditions were chosen to preserve the bond formation that occurred during extrusion so that the aggregation of protein molecules could be observed. Although these conditions were used, the SDS buffer still breaks apart some of the proteins into subunits. The bands on the gel showed a significant decrease in intensity after extrusion. This is an indicator of polymerization and cross-linking as shown by a decrease in solubility (Osen et al., 2014; Pietsch et al., 2017; Samard & Ryu, 2019). Crosslinking occurs during extrusion using high heat and shear to denature the proteins and realign them to form new covalent and non-covalent interactions. When the molecules form disulfide bonds and other non-covalent bonds after extrusion they aggregate to become large enough that they do not pass through the gel and become insoluble, so they are not seen in the same concentrations as before extrusion (Chen et al., 2011).

The only prominent bands seen for every treatment after extrusion are at 75 and 50 kDa which is an indicator of soy β -conglycinin subunits and glycinin subunits as shown in Figure 3-10 (Fukushima, 2004). This could be because each treatment was soy based and contained soy flour so there is a higher concentration of soy proteins left even after extrusion. The treatments containing wheat had the most diminished band intensity overall which means that it most likely had the most crosslinking and polymerization occur followed by pea and then soy. This is because wheat gluten crosslinks very well due to the higher amounts of sulfur-containing amino acids that are available to form disulfide bonds. Pea protein also has sulfur containing amino acids in its legumin subunit that are attributed to disulfide bond formation (Webb et al., 2023). Maillard reaction from the heat and shear during the extrusion process can also cause some crosslinking (Meng & Cloutier, 2014). Heat swelling treatments required a higher die

temperature which could have increased Maillard reactions and therefore decreased the solubility more than the cold swelling treatments as well (Samard & Ryu, 2019). The treatments containing pea protein would also be affected more significantly by the Maillard reaction because it has more lysine that can bond with glucose during extrusion (Samard & Ryu, 2019). The differences in band intensity between different protein sources can also be explained by a difference in protein solubility that was shown previously between the raw ingredients. The SDS-PAGE gel showing the individual protein ingredient solubility according to the intensity of the bands is shown in Figure 3-11. According to previously mentioned protein solubility results, soy protein often has a high solubility and is then followed by pea and then gluten accordingly. This aligns with WAI and RVA results that show a decrease in peak viscosity and water absorption as % CS is decreased. Overall heat swelling proteins should show a lighter band intensity and cold swelling should have a higher band intensity. This aligns with results found previously by Webb et al. (2023) that show a relationship between cold soluble proteins and stronger band intensity.

3.3.3.2 SEC-HPLC

SEC-HPLC was used to confirm the results found through SDS-PAGE, and it also allows for a wider range of molecular weights to be captured and a clearer representation of protein concentration through peak area rather than band intensity, which is subjective. HPLC also doesn't use the same buffer as SDS-PAGE so the proteins are left more in their aggregated native state versus being broken down into their subunits. Peak height represents the concentration of soluble protein present at that molecular weight. When looking at the individual protein ingredients, a peak height greater than 0.8 mAU indicated a cold swelling protein while a peak less than 0.8 mAU indicated heat swelling properties. All the peaks for each treatment were either reduced or completely disappeared after extrusion, which means that all the proteins were

successfully texturized and the proteins were able to aggregate and form disulfide bonds, so they no longer are small enough to be detected (Figure 3-12; Figure 3-13). The extrusion process caused the proteins to be less soluble because of the cross-linking that occurred and therefore the peaks are found to be smaller (Osen et al., 2014). The peaks that did remain after extrusion for each treatment were at the same molecular size of 670 kDa. This is bigger than most protein fractions and the large molecular size is most likely from multiple protein molecules interacting and being aggregated together through protein bonds. The remaining peak after extrusion seen for each treatment at 670 kDa had the largest peak area for cold swelling treatments above 50% CS. This means that the concentration of soluble proteins at this molecular weight is most likely due to remaining cold swelling proteins such as soy and pea protein isolates. As mentioned before and as confirmed by SDS-PAGE results, heat swelling proteins, such as wheat gluten, readily form disulfide bonds and are more inclined to crosslink followed by pea and then soy (Webb et al., 2023; Samard & Ryu, 2019). This inclination for crosslinking and disulfide bond formation comes from the presence of more sulfur-containing amino acids and reactive amino acids such as cysteine, lysine, and glutamic acid (Samard & Ryu, 2019). Since soy and pea are slightly less crosslinked and had such a high solubility to start out with, it would make sense that they would still have some solubility remaining after extrusion and be present in small peaks for each treatment as all contained some soy.

3.3.4 Extrudate analysis

3.3.4.1 Water holding capacity & bulk density

Water holding capacity (WHC) is defined as the amount of water able to be absorbed and held by a material. WHC is impacted by the internal structure of the extruded piece as larger pores and internal open space allows for more water to be absorbed. Other protein functionality

properties such as protein type and ability to interact with water also affect WHC but the main contributor is the structure of the TVP formed during extrusion (Hong et al., 2022). The fibrous structure of TVP is meant to mimic meat texture and be able to trap water inside its internal structure to create juiciness and tenderness like the myofibrillar structure of animal protein (Samard & Ryu, 2019). According to a study by Samard and Ryu in 2019, a soy and wheat based TVP showed a much higher WHC compared to freeze-dried meat from chicken, pork, and beef with WHC decreasing in that same order. The same study also indicated that WHC decreases as animal meat is cooked because the muscle structure is destroyed by thermal energy and the fibrous structure is shrunk and densified so that it absorbed and held less water. Therefore, a challenge arises with TVP texture in that to be similar to actual cooked meat the WHC should be decreased. The WHC of TVP depends on several factors such as the protein source, hydrophobicity, protein conformation structure, and extrusion processing parameters. Therefore, it was hypothesized that cold/heat swelling characterization could be used to manipulate structure of TVP and WHC as less % CS was predicted to lead to a lower WHC and more densely layered product which would be most similar to cooked animal meat. Overall, there was a slight increasing trend in WHC as the amount of cold swelling proteins increased. Although, the significant differences between the intermediate treatments were minimal, and the only concrete differences in WHC existed between 0, 50, and 90% CS. This means that the swelling properties of the proteins did impact WHC, but only at the extreme ends and the impact is minimized when both heat and cold swelling proteins are combined. Bulk density was hypothesized to correlate inversely with WHC, and this was found to be correct (Figure 3-3). There was an overall slight decreasing trend in bulk density as CS% increased. Similarly, to WHC, the bulk density results indicate that the most significant differences exist between the

most extreme treatments, 0 and 90% CS with a slight trend shown for intermediate treatments that would need to be confirmed by further research.

3.3.4.2 Texture analysis

The results from the TPA test on hydrated whole extrudate pieces produced no significant differences in peak force. This is thought to be caused by the variety of piece sizes and shapes that make it difficult to equally test each treatment and compare amongst each other. Also, because the probe was smaller in diameter than the dish used to hold the sample, there were effects from the product escaping on the outer edges of the probe during compression. Compression testing of extruded pieces also is not the best representative of the texture of the actual product, as it is not in its final form. Therefore, plant-based burger patties made using the TVP from each treatment were tested instead to quantify the texture of the final product as would be experienced by consumers while eating. TPA testing done on plant-based patties were able to identify several significant differences and a slight decreasing trend in hardness (peak force) as the amount of cold swelling proteins were increased (Figure 3-4). This aligns with WHC as a lower WHC leads to an increase in hardness of the TVP like results found by Hong et al. (2022). The only outlier to the linear decreasing trend was the 40% CS treatment. This could be caused by the functional soy proteins overpowering the wheat gluten used in the formulation at this ratio, or because this treatment had a higher amount of soy flour (20%) than other mid-range treatments such as the 50% and 60% CS that only used 10% soy flour. This is because soy flour contains higher amounts of starch that gelatinize as the sample is heated and cause an increase in viscosity of the material. There was also a decreasing trend shown for chewiness as the % CS increased that was very comparable to the trend seen for hardness. This aligns with a previous study by Samard & Ryu in 2019 that showed that the integrity (resistance to being destroyed by

high pressure, temperature, and shear) showed an increasing trend as the solubility of the raw material plant proteins decreased (Samard & Ryu, 2019). A study by Hong et al. also exhibited a positive correlation between hardness and chewiness that corresponded with a lower WHC leading to a firmer and chewier texture (Hong et al., 2022). This means that a lower solubility protein source like wheat which is characterized as heat swelling, would cause a tougher and therefore chewier product than a high solubility protein which would most likely have cold swelling functionality properties indicated by a high WAI.

3.3.4.3 Visual analysis

When looking closely at the horizontal cut pieces of TVP, the lower cold swelling treatments such as 0% CS show a more layered appearance with larger air cells as was hypothesized (Figure 3-9). This is thought to be because treatments with more heat swelling proteins, including wheat gluten, have functional properties such as the ability to form thin elastic protein films that are critical to forming fibrous structure along with a native fibrous structure instead of globular like pea or soy (Webb et al., 2023). Gluten contains gliadins and glutenins that form intramolecular and intermolecular disulfide bonds, respectively. The ratio of gliadins to glutenins in wheat gluten can therefore greatly impact functionality and texturization. Protein isolation, modification, extrusion, or other processing steps can modify the gluten subunits and structure and therefore greatly influence the functional properties of the protein that allow it to form a fibrous structure (Kyriakopoulou et al., 2021). In comparison, TVP at treatments larger than 50% CS shift to a more sponge-like structure with smaller pores, and with less visible layering. Treatments higher in % CS contain larger amounts of proteins such as soy protein isolate and pea protein isolate. Soy protein is known to have strong gelling abilities and to texturize very well. Pea protein also has gel forming abilities but weaker than those of soy

(Kyriakopoulou et al., 2021). These gelling and texturization advantages influence the texture and structure of TVP by allowing the material to stretch and form a strong film so that the final TVP is expanded with a porous internal matrix (Webb et al., 2023).

When looking at the longitudinal direction of the cut TVP pieces, the differences are more difficult to differentiate because all the pieces have visible layering because this was the direction that the material was flowing out of the extruder die. Although differences can be identified as the lower % CS treatments show a more densely packed arrangement of layers. The overall piece shape also changes as cold swelling proteins increase with the low cold swelling treatments (0% and 30%) having a more irregular and jagged appearance compared to other treatments that exhibit a more rounded and smooth outer appearance. As % CS increases there is also a slight increase in the paleness of the extrudates. This is because of the higher die temperatures required for heat swelling proteins and the composition of amino acids including increased lysine that impact the degree of Maillard reaction that is taking place (Samard & Ryu, 2019). Although extrusion can form TVP with fibrous structure with many types of plant protein ingredients and sources, there are often still differences between the TVP structure and that of an actual cooked meat product. This is due to the differences in protein structure, gel formation, and amount, shape, and size of air cells. This can lead to visual and sensory differences in internal structure and affect texture parameters such as springiness, juiciness, and chewiness along with WHC and bulk density (Samard & Ryu, 2019).

3.3.4.4 Sensory analysis

Initial sensory results from the focus groups provided valuable feedback used to inform changes in the design of the consumer sensory study. The recipes for both plant-based patties are found in Table 3-3. The focus group that tested the hamburger products had several positive

reactions to the plant-based treatment including the texture and how it holds together and doesn't crumble when chewing. Participants also liked the appearance of the sample because it was brown and crispy like real meat. Negative perceptions of the product were that the flavor was too bland, it had an unidentifiable aroma, and the mouthfeel was lacking and seemed like there was no fat. Overall, the participants liked the product enough that they would purchase it if it was a healthier and affordable option compared to actual meat. Many in the group found the product to be more like a pork sausage and would use the product in a breakfast sandwich application at home.

The soft analogue focus group with a salmon patty control had fewer positive results because the appearance and flavor were not thought to be fish-like. The texture was also described as being chewier and spongier than they would expect for a fish product. Some positive reactions included the aroma, the appearance of being plant-based, and the moistness of the product. Overall, the participants were hesitant to accept a plant-based fish product because fish is thought to already be a healthier alternative to other meats. Based on the feedback from the focus groups, the consumer study would cover only plant-based beef analogues to focus on differences in texture between several treatments. The concentration of seasonings and oils was also increased to improve the flavor and mouthfeel of the plant-based burgers.

The consumer study results for overall liking indicated that the most liked was the softest treatment (90% CS) and the least liked was the firmest treatment (0% CS) (Figure 3-8). Other factors that influence overall liking such as flavor, texture, and appearance were also tested and can be used to help identify the drivers of overall liking scores. The 0% CS treatment contained soy and wheat, the 90% CS contained soy and pea, while the other two intermediate treatments (30% and 60% CS) were only soy based. This could be a reason for the differences in overall

liking because the flavor and aftertaste rankings also placed 90% CS as the most acceptable and 0% CS as the least acceptable. Therefore, there is evidence that the soy and pea flavor combination is more desirable than the soy and wheat combination. The 0% CS treatment also had high percentages of participants (37% and 50%) that thought the flavor was either not enough or too strong, respectively. This indicates an unbalanced flavor profile and is undesirable. When looking at overall scores for texture, the 30% CS treatment is the highest (5.59) of the plant-based burgers and 0% CS was the lowest. This could mean that a firm texture can be desirable, but that 0% CS might be too firm. As shown by TPA, the 0% CS treatment was the chewiest of the products so the dislike of the texture of 0% CS could be because it was too chewy and not necessarily about the firmness. It could also mean that soy only based burgers have a desirable texture, but that the soy flavor is too strong and influences overall perception and liking by consumers and therefore reduces the overall liking score. Further consumer studies are needed in the future focusing on just soy and wheat, soy and pea, and soy only treatments separately to determine if the flavor of the specific ingredients is in fact impacting flavor liking and overall liking scores. Each plant-based treatment also had more participants rank the texture as too soft versus very few who thought it to be too firm.

When participants were asked about their overall perceptions and expectations for plant-based meat products, the most common benefits, or reasons for purchasing included health benefits, affordability, and having a variety of flavor and meal options. The most mentioned concerns about plant-based meat were taste, texture, cost, and being highly processed, in that order.

3.4 Conclusion

Plant-based meat products have the potential to be a sustainable and nutritious protein alternative, but several hurdles need to first be overcome to increase consumer acceptance. According to consumer study and focus group results, texture and flavor are among several of the top concerns consumers have when it comes to plant-based meat. This research aimed to further the understanding of how protein functionality attributes, such as swelling abilities, can be used to manipulate and control product textures. This can then be used to improve the texture and therefore consumer acceptance of plant-based meat products currently on the market, and to develop new products with novel and previously unattainable textures.

The results of this research found that plant proteins can be characterized into two categories, heat swelling or cold swelling, and these can be used to formulate plant-based meat products to target specific textures. It was hypothesized that including more cold swelling proteins in a formulation would increase the softness of the product while increasing heat swelling would cause an increase in firmness of the product. This hypothesis was proven partially correct in that the extreme ends formulation followed these predicted trends, but the intermediate treatments showed inconsistent and nonsignificant differences in many cases.

For example, texture analysis of plant-based burger patties made from each treatment showed a significantly higher peak force needed to compress the sample for the 0% and 30% CS treatments and lower peak force for 60% and 90% CS. WHC and bulk density were also significantly different between the 0% (2.74 g/g, 235.5 g/L) and 90% (5.25 g/g, 157.3 g/L) treatments, but there were little differences between the other intermediate treatments. Protein solubility had similar results where the lowest was for 0% CS and the highest for 90% CS, but the intermediate treatments showed no pattern or trend. WAI showed an increasing trend as cold

swelling percentage increased as was expected. These results indicate that there is a potential relationship between protein swelling abilities and the texture of the final plant-based meat product, but more research and replications are needed to understand how these proteins interact when mixed. Also, further studies could investigate swelling ability using only soy ingredients to see if the type of protein has an impact that potentially overrides some swelling ability effects on texture. Overall, this research contributes to the understanding of protein functionality and proposes a novel technique to manipulate and control plant-based meat texture. This can have potential benefits for industry because it will allow for the creation of unique textures, provide a method for quality control, and allow ingredients with similar functionality to be easily switched out to address cost or supply chain concerns.

3.5 References

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3.6 Figures and tables

#	SCREW PROFILE
1	Full pitch, double, 9U
2	Full pitch, single, 9U
3	$\frac{3}{4}$ pitch, double, 9U
4	$\frac{3}{4}$ pitch, double, 6U
5	Forward KB (5B = backward)
6	$\frac{1}{2}$ pitch, double, cut flight
7	Reverse KB (7B = backward)
8	$\frac{3}{4}$ pitch, double, cut flight, cone

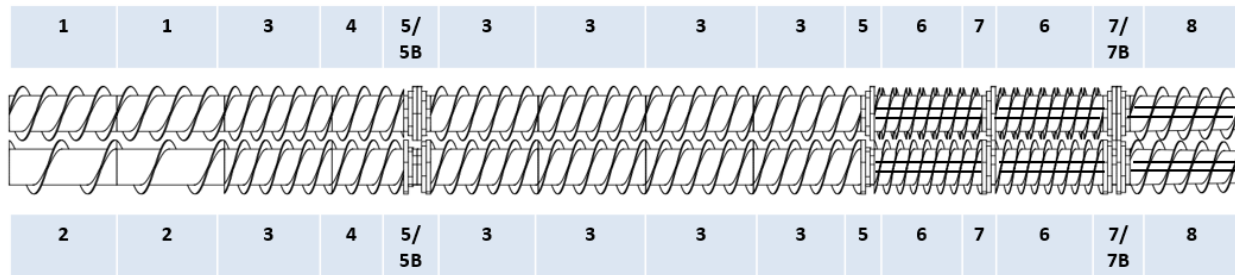


Figure 3-1 The screw profile for the pilot-scale extrusion run shows the need for increased mechanical energy through the inclusion of several kneading blocks and cut flight elements.

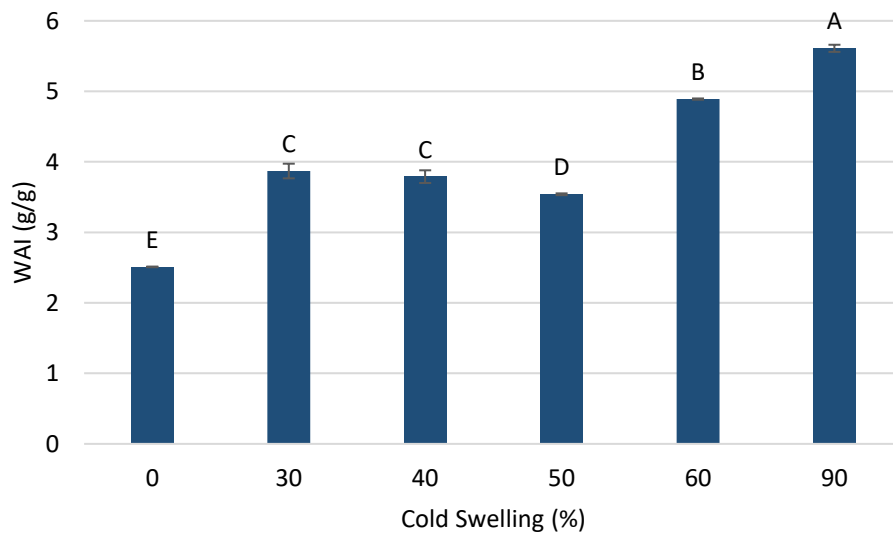


Figure 3-2 Water absorption index (WAI) of each treatment indicates how absorbent the materials are at room temperature. A higher WAI is expected for a cold swelling protein.

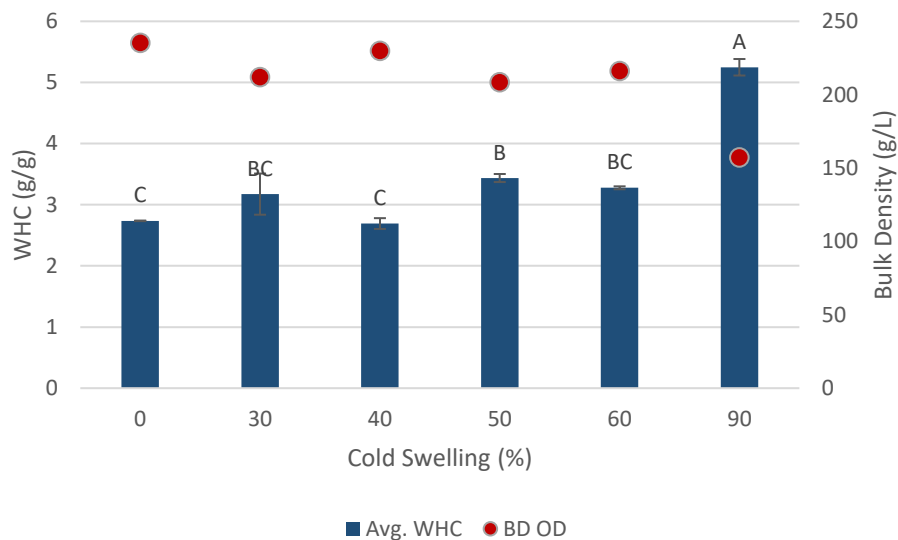


Figure 3-3 Water holding capacity (WHC) and bulk density can be used to quantify the expansion and internal structure of the extruded pieces. Bulk density should decrease and WHC should increase as more cold swelling proteins are used.

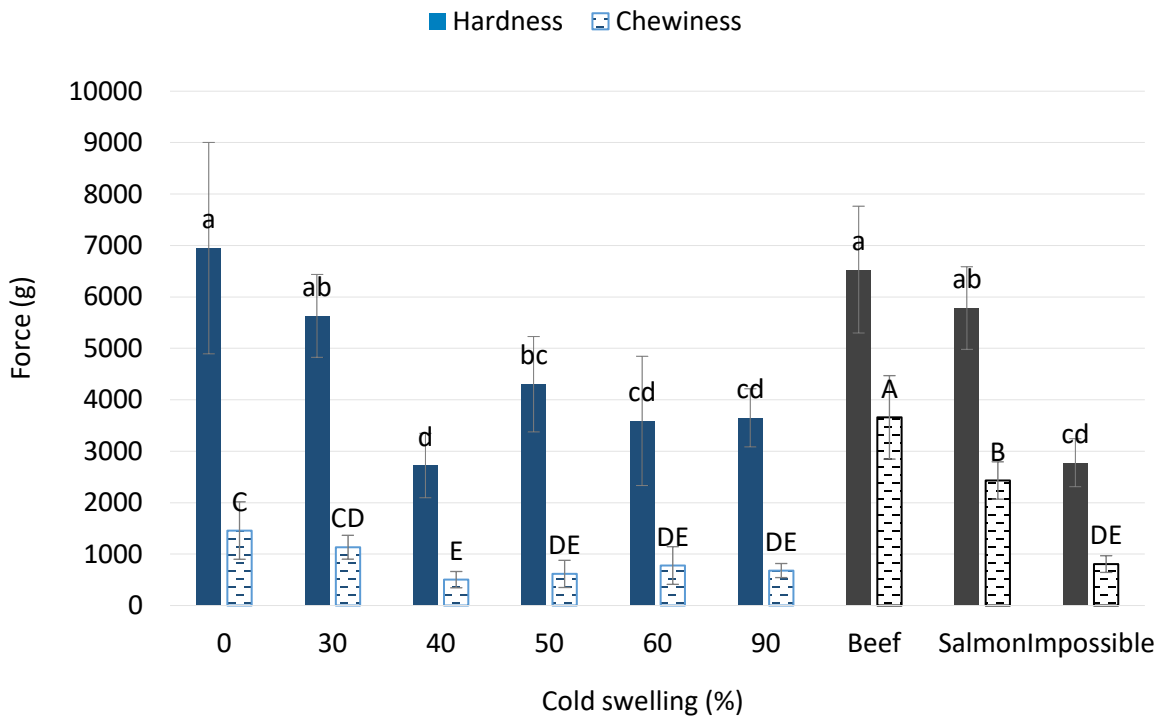


Figure 3-4 The hardness and chewiness of a plant-based patty was recorded for each treatment. More cold swelling proteins led to a decreased hardness and chewiness as expected.

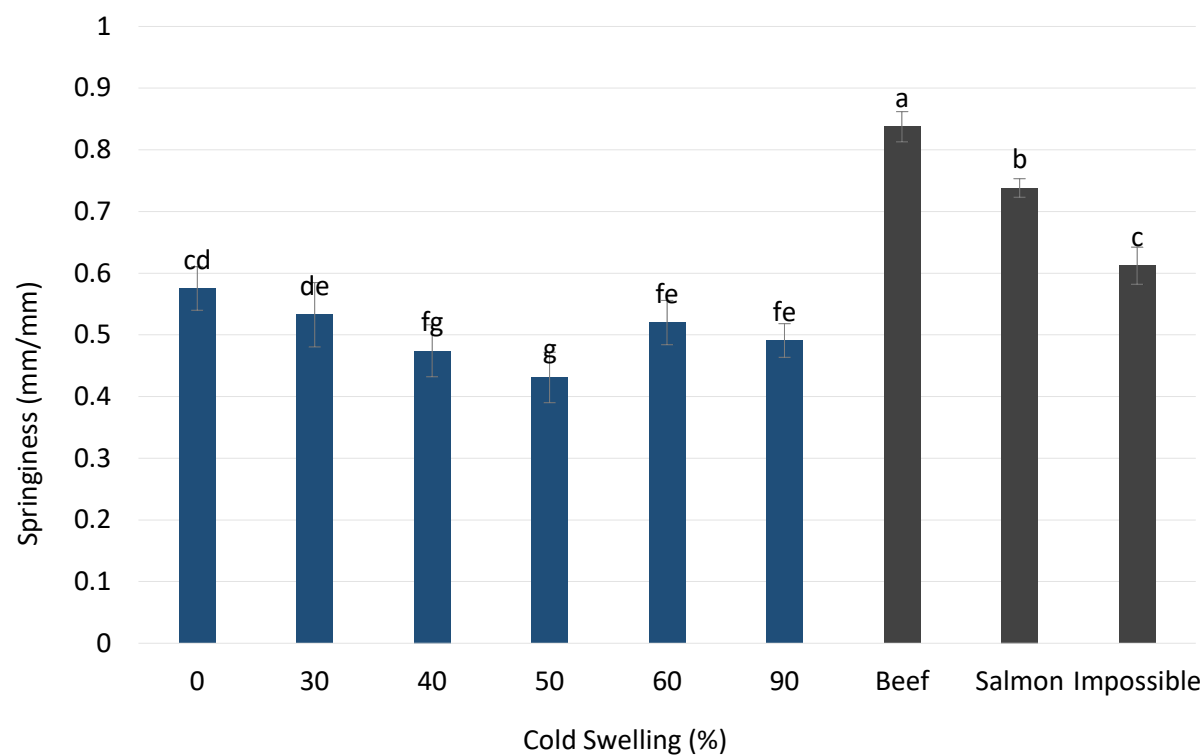


Figure 3-5 Springiness was found using texture profile analysis (TPA) for plant-based patties containing increasing amounts of cold swelling proteins.

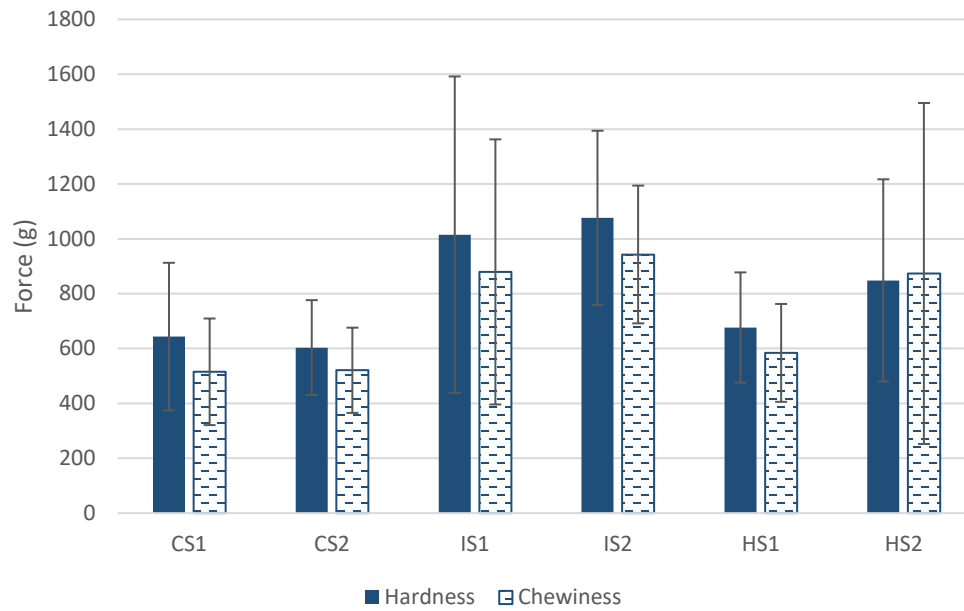


Figure 3-6 A dual compression test was done on hydrated whole TVP pieces for each treatment to determine differences in hardness.

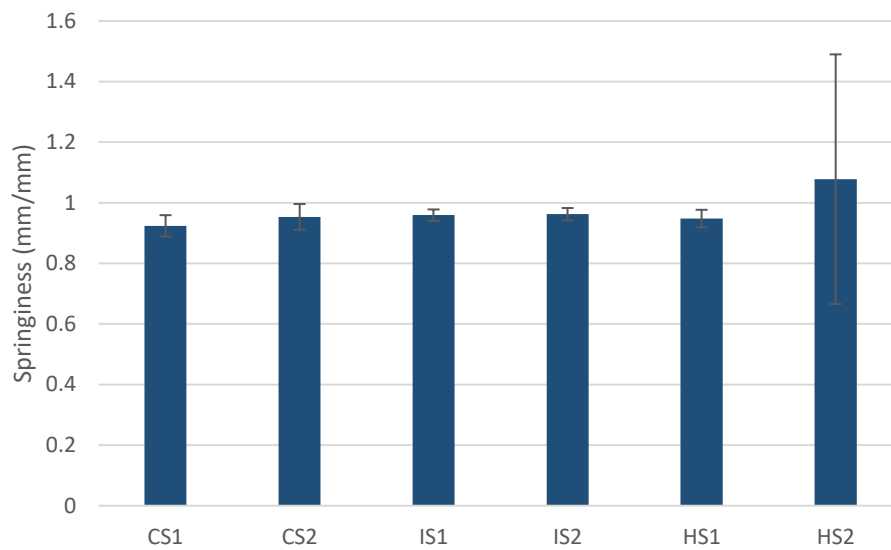


Figure 3-7 A dual compression test was done on hydrated whole TVP pieces for each treatment to determine differences in springiness.

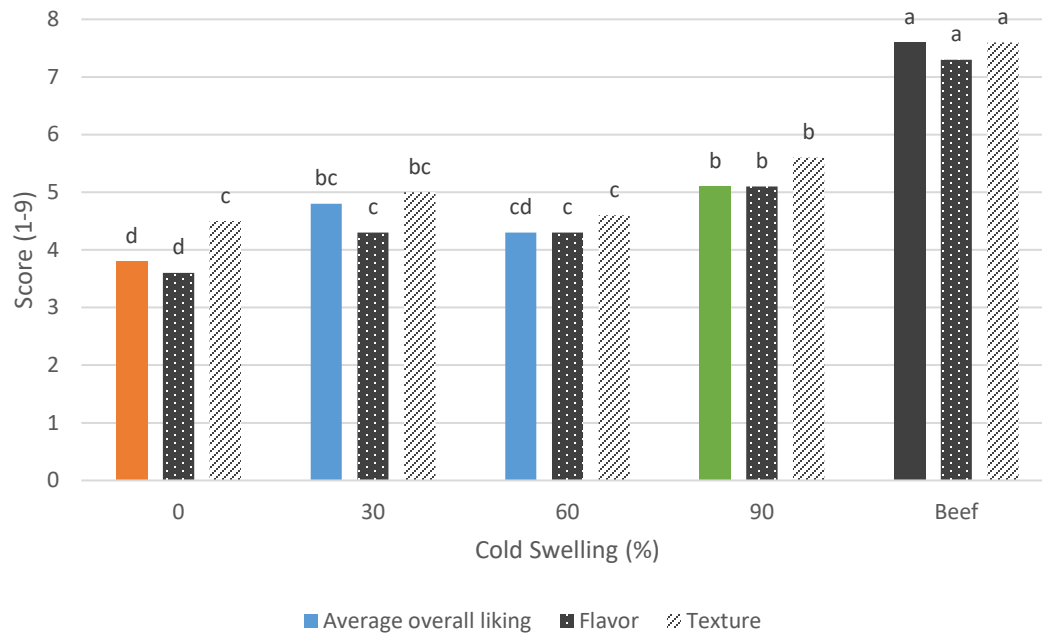


Figure 3-8 The average overall liking scores from the consumer study show that the top plant-based burgers were 90% CS and 30% CS. Similar trends appeared for flavor and texture. Blue indicates soy, orange is soy and wheat, and green is soy and pea.

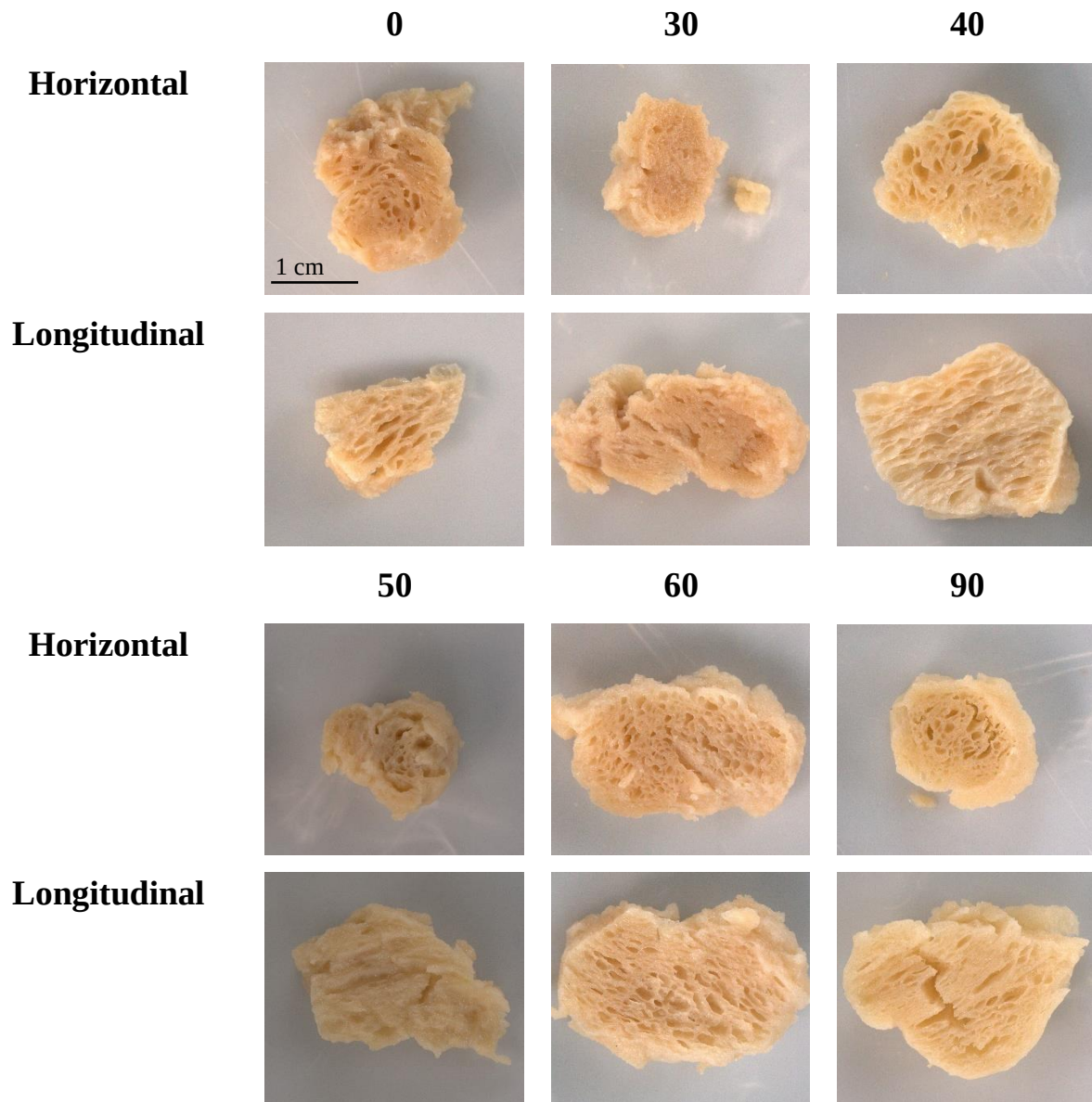


Figure 3-9 The internal structure of a hydrated whole piece of TVP was captured in the longitudinal directions (with the flow of materials).

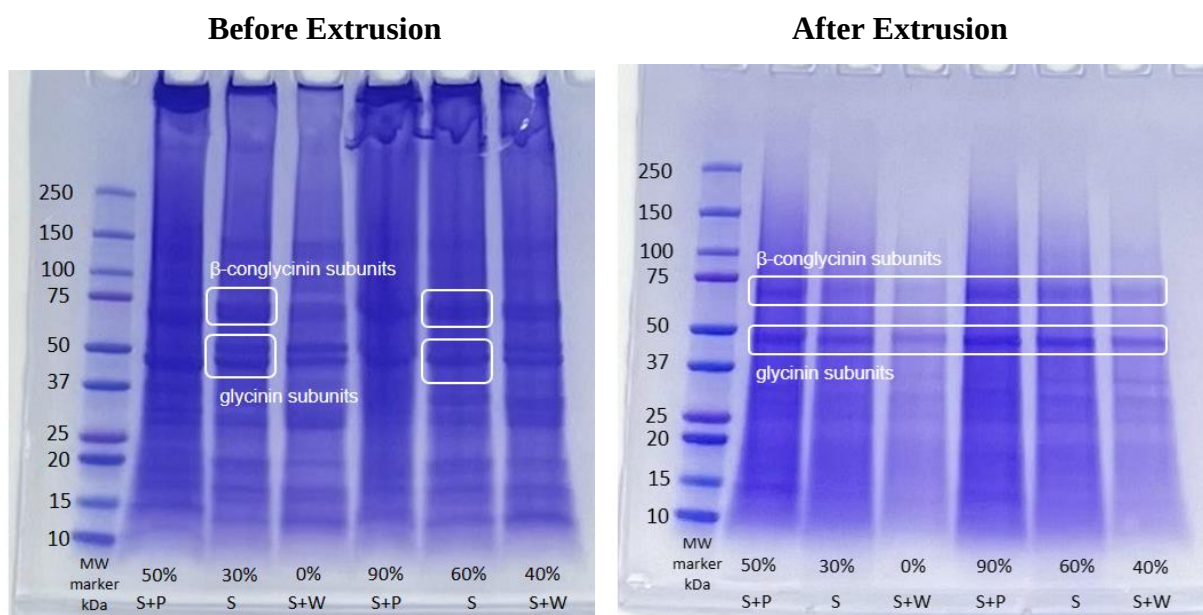


Figure 3-10 Results from SDS-PAGE analysis shows that each treatment at different cold swelling (%) levels had identifiable bands for the soy proteins β -conglycinin and glycinin. These bands were still present after extrusion although many other bands disappeared or faded. (S = soy, S+P = soy and pea, S+W = soy and wheat)

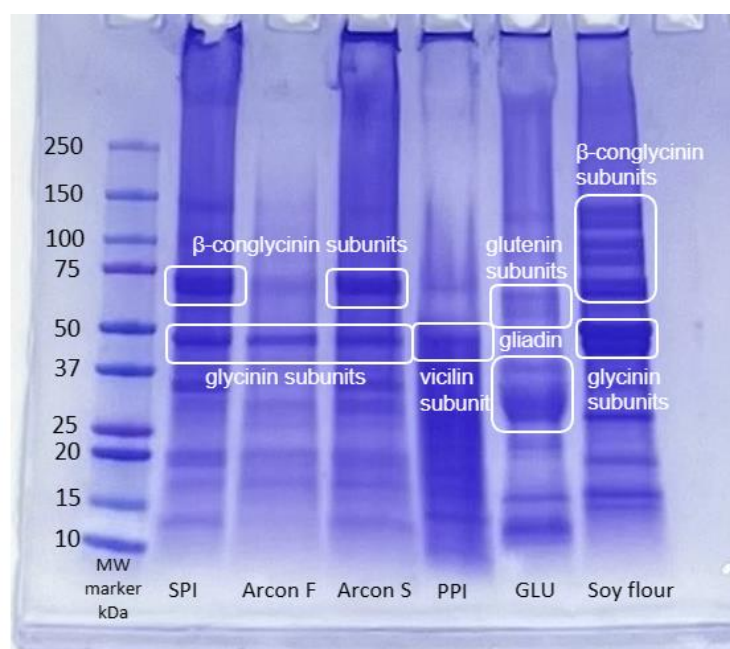


Figure 3-11 SDS-PAGE showed the molecular weights of different protein subunits from the raw materials of each ingredient.

Before Extrusion

After Extrusion

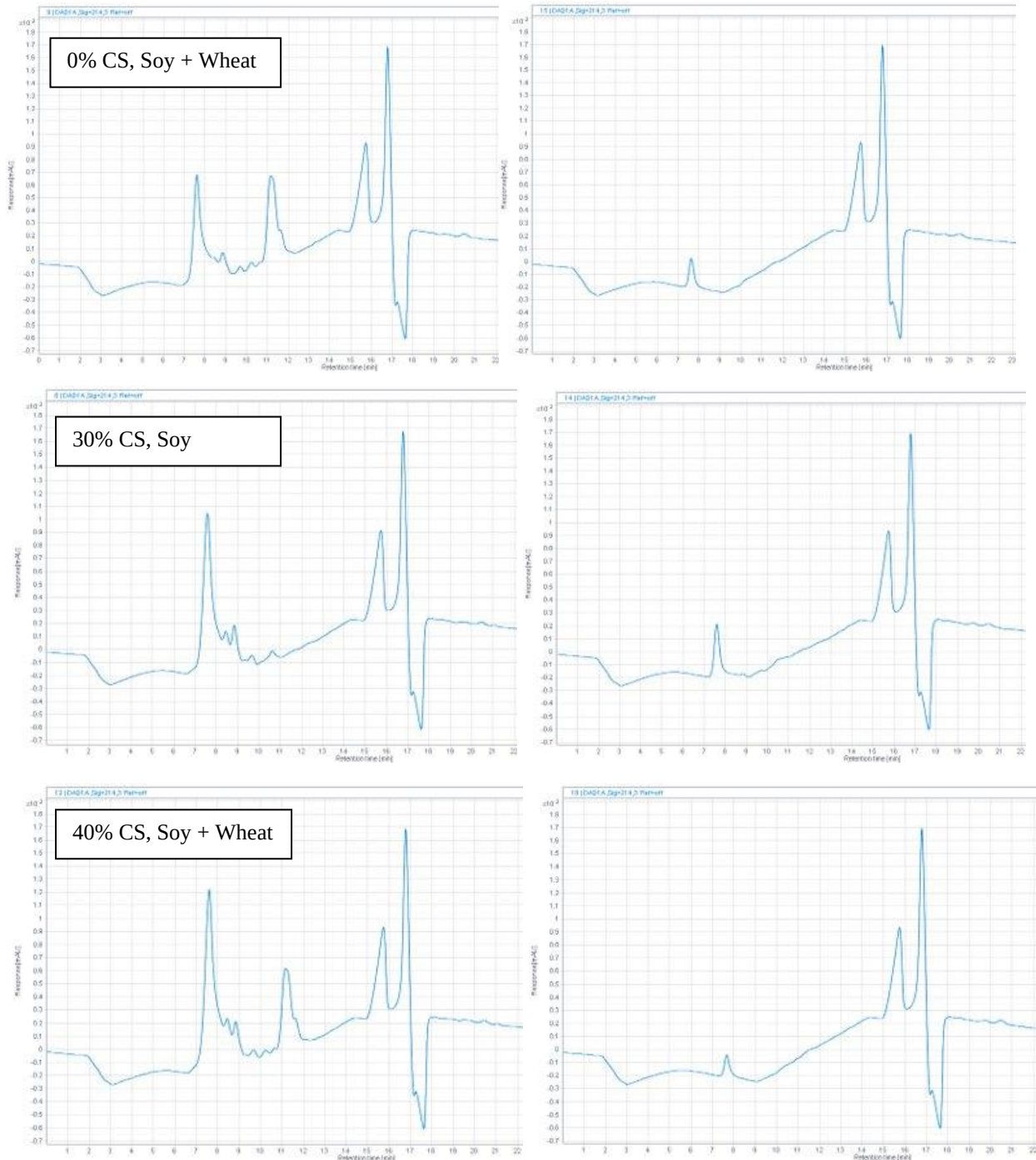
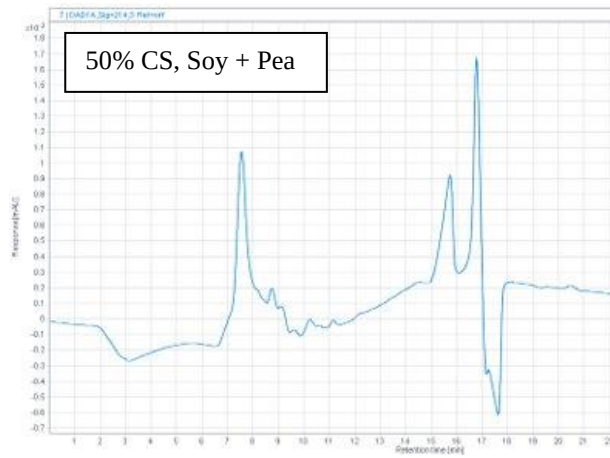


Figure 3-12 SEC-HPLC determines which proteins are present through peaks at certain molecular sizes. After extrusion, many proteins are cross-linked and bonded so that they no longer show up on the graph because of their large molecular size.

Before Extrusion



After Extrusion

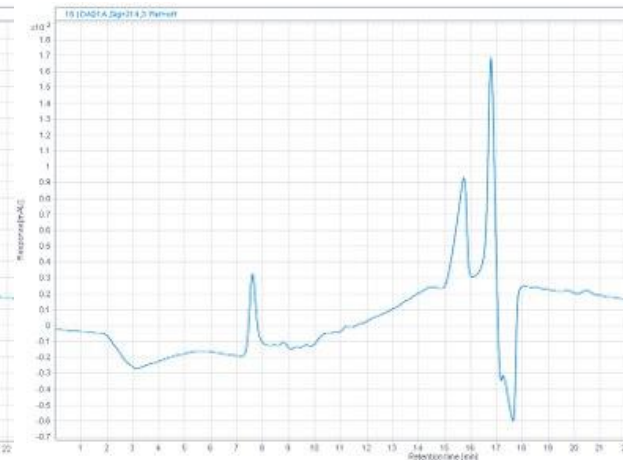
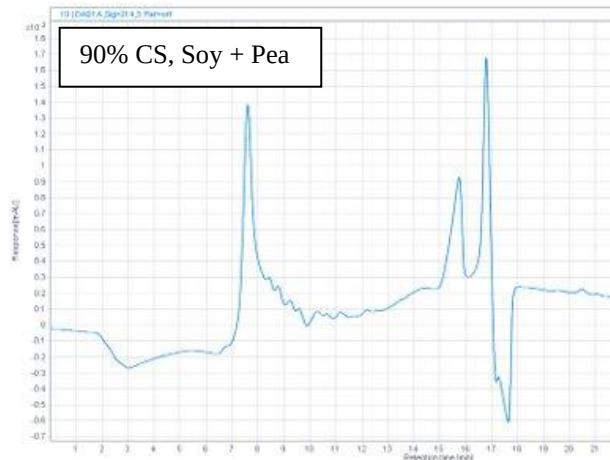
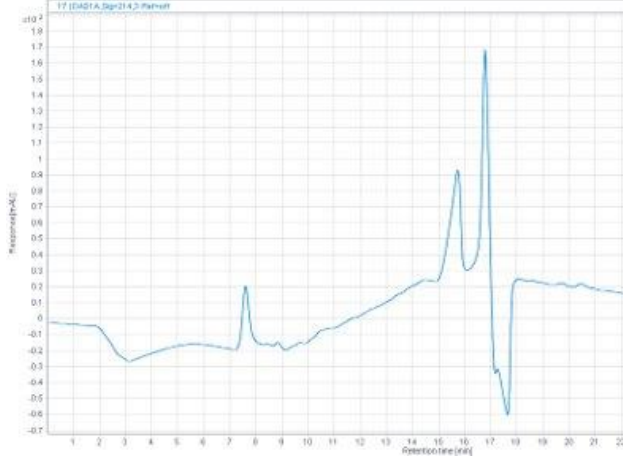
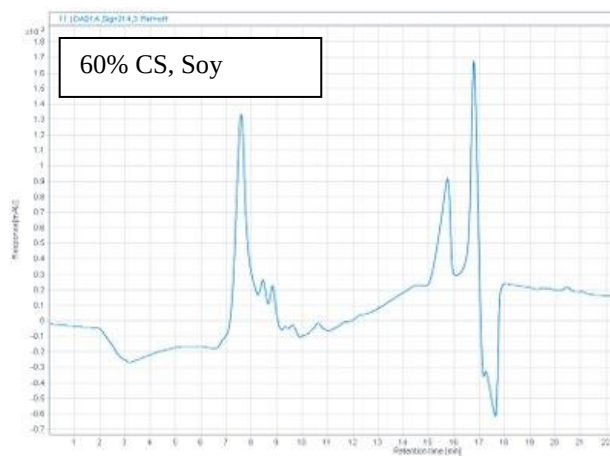
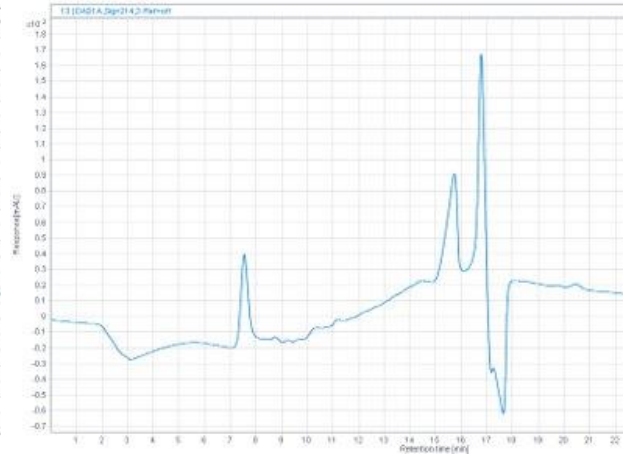


Figure 3-13 SEC-HPLC determines which proteins are present through peaks at certain molecular sizes. After extrusion, many proteins are cross-linked and bonded so that they no longer show up on the graph because of their large molecular size.

Table 3-1 Treatments were formulated to target a range of cold swelling (CS) amounts. This was hypothesized to lead to a decrease in firmness and chewiness as CS increases.

Cold swelling (%)	0% CS	30% CS	40% CS	50% CS	60% CS	90% CS
<i>Soy protein isolate</i>		20	20	10	30	30
<i>Soy protein concentrate (Arcon F)</i>	40	50		30	30	
<i>Soy protein concentrate (Arcon S)</i>		10	20		30	30
<i>Pea protein isolate</i>				40		30
<i>Vital wheat gluten</i>	40		40			
<i>Soy flour</i>	20	20	20	10	10	10
<i>Tapioca starch</i>				10		

Table 3-2 Rapid visco analysis (RVA) was used to determine peak viscosity values for each treatment as it underwent a heating and stirring cycle. A short peak time (< 4 min) indicated cold swelling (blue) and a longer peak time (>4 min) indicates more heat swelling (red) proteins.

Cold Swelling (%)	Peak Viscosity (cP)	Peak Time (min)
0	63.5	6.9
30	52	1.1
40	75	1.47
50	212	5.2
60	115	1.07
90	315.5	1.07
<i>Soy protein isolate</i>	878	1.27
<i>Soy protein concentrate (Arcon S)</i>	395	1.5
<i>Soy protein concentrate (Arcon F)</i>	42.5	6.865
<i>Wheat gluten</i>	51.5	6.265
<i>Pea protein isolate</i>	239.5	3.7
<i>Soy flour</i>	37	1.565

Table 3-3 Average least gelation concentration (LGC) did not show many statistically significant differences but did decrease slightly as the amount of cold swelling (%) was increased.

CS (%)	LGC (%)
0	17 ^{ab}
30	18 ^a
40	17 ^{ab}
50	15 ^{ab}
60	15 ^{ab}
90	14 ^{ab}
<i>Soy protein isolate</i>	11 ^b
<i>Soy protein concentrate (Arcon S)</i>	11 ^b
<i>Soy protein concentrate (Arcon F)</i>	13 ^{ab}
<i>Wheat gluten</i>	13 ^{ab}
<i>Pea protein isolate</i>	15 ^{ab}
<i>Soy flour</i>	17 ^{ab}

Table 3-4 Protein solubility was calculated to confirm and determine the cold swelling abilities of each treatment before and after extrusion. Solubility decreased after extrusion indicating cross-linking of proteins.

% CS	Sample	Protein solubility average (%)
0	<i>Raw</i>	20.036 ^d
0	Extruded	6.680
30	<i>Raw</i>	29.406 ^c
30	Extruded	11.849
40	<i>Raw</i>	38.766 ^b
40	Extruded	13.949
50	<i>Raw</i>	25.972 ^c
50	Extruded	17.870
60	<i>Raw</i>	35.260 ^b
60	Extruded	7.619
90	<i>Raw</i>	46.196 ^a
90	Extruded	7.954

Table 3-5 Pilot-scale extrusion parameters were used to calculate specific mechanical energy (SME) and in-barrel moisture (IBM) for each treatment containing increasing percentages of cold swelling proteins. Die temperature was also important to TVP quality.

CS (%)	SME (kJ/kg)	IBM (%)	Die temp (°C)
0	907.2 ^a	42.5	161
30	997.2 ^a	47.9	166
40	885.6 ^{ab}	43.5	153
50	763.2 ^b	37.9	159
60	936.0 ^a	46.6	162
90	961.2 ^a	39.6	153

Table 3-6 Two recipes were developed to target either a plant-based beef or fish patty. Each recipe consists of texturized vegetable protein (TVP), water, oil, binders, and flavors. Both the beef and fish flavored patties were used for the focus groups, and just the beef flavored patty was used for the consumer study and texture analysis.

Ingredient	Description	(%)
TVP	Texturized vegetable protein (TVP) granules or shred made from soy, wheat, or pea	23.4 - 26.3
Water	Water or broth	55.2 – 58.5
Binder	Methylcellulose, Faba bean protein	8.9 - 9.5
Flavor	Beef patty: spices, yeast flakes, porcini powder, soy sauce, worcestershire sauce, liquid smoke Fish patty: spices, dried roasted seaweed, soy sauce, miso paste, yeast flakes	3 - 6
Color	Beet powder	0 - 0.7
Lipids	Coconut oil, Vegetable oil	2 - 2.3

Chapter 4 - Conclusion

4.1 Background

In recent years there has been an influx of new plant-based products into the market through both retail and restaurant settings. Newer companies focusing solely on plant-based foods seeing success include Beyond Meat, Impossible Foods, and Gardein. However, there is also increasing involvement in the plant-based space by companies that traditionally produce meat such as Tyson and Smithfield (Sha & Xiong, 2020). According to current predictions, the market for plant-based meat products is expected to reach \$85 billion by 2030 (Sha & Xiong, 2020). The explanation for this growth is that many consumers are looking to reduce their consumption of meat for several reasons. The main reasons include health, environmental benefits, and animal welfare (Sha & Xiong, 2020; Sanchez-Sabate & Sabate, 2019). A diet high in meat emits around twice as much carbon dioxide as a vegan diet, so even small shifts towards plant-based foods can have a significant impact on the environment (McClements et al., 2021). Vegans and vegetarians only account for less than 5% of the population in the United States and the United Kingdom.

The goal of this research was to further the understanding of how protein functionality attributes, such as swelling abilities, can be used to control plant-based product textures. This can then be used to improve the texture, and therefore consumer acceptance, of plant-based meat products currently on the market, and to develop new products with novel and previously unattainable textures. The characterization of proteins as cold swelling or heat swelling to predict how they will impact texture is a novel technique that is built upon research by Webb et al. (2023) and is further defined and established through this research.

4.2 Chapter 2

In chapter 2, eight different protein sources were tested using WAI, LGC, and RVA and characterized into one of two categories, heat or cold swelling. Cold swelling treatments were determined to have a WAI < 0.4 g/g and an RVA peak time < 4 min. These labels were then used to formulate a total of six treatments with two treatments targeting either cold, intermediate, or heat swelling proteins only. The results of this study were able to confirm that swelling abilities do have an impact on the physical properties of texturized vegetable protein. Although there were no significant differences found from texture profile analysis, there were clear trends in WHC, bulk density, and visual analysis that led to the conclusion that swelling abilities do have an impact on TVP texture with cold swelling proteins leading to a more expanded and spongy product while heat swelling proteins led to a denser product.

4.3 Chapter 3

The following study in Chapter 3 aimed to investigate further into this understanding to learn if the ratio of cold/heat swelling proteins could be used to fine tune texture of plant-based meat analogues. Each treatment was soy based with wheat and pea protein used to modulate the texture and swelling abilities. New methods for texture analysis along with sensory consumer studies were also utilized to better detect differences in texture between treatments. These results found that at the far ends of formulations (0% CS and 90% CS) there were clear and significant differences in texture identified by WHC, bulk density, visual analysis, and texture analysis of plant-based patties. Although the intermediate treatments (30%, 40%, 60%) showed minimal significant differences for most analysis methods. It was also hypothesized that a firmer texture, predicted to be 0% CS, would be most desirable to consumers when used in a plant-based burger patty. This was shown to be false as the treatments that ranked highest for overall liking, texture,

and flavor were 30% and 90% CS. This could mean that 0% CS was too chewy for a burger analogue and that a more intermediate treatment such as 30% produces a more desirable texture. The consumers could have also been swayed in liking by the flavors of different protein types in the formulation as 0% CS was the only treatment tested containing wheat and 90% CS was the only treatment containing pea. For future studies, a consumer sensory study is needed for an experiment utilizing just one protein type, such as soy, at different levels of CS to determine which texture is most desirable when the flavor is more consistent.

4.4 Future work

Overall, there is great potential in utilizing protein functionality properties such as swelling ability to manipulate and fine tune the texture of plant-based meat products. This research has provided a base of knowledge that can be built upon with future studies. This research provides evidence that cold swelling proteins lead to a more expanded, spongy, and soft TVP while heat swelling leads to a more dense, chewy, and layered product. Results were unclear for some intermediate swelling treatments, but these results should be confirmed by future studies with a larger sample size and a look into the effects of different protein source combinations. Examples of future research include looking more directly at each protein source individually and further utilizing protein chemistry analysis methods to better understand the interactions between different protein sources and ingredients with different swelling abilities. This could help to better understand why intermediate CS % treatments showed minimal differences in texture. Additional methods of detecting texture differences could also be utilized such as a cutting test utilizing the texture analyzer.

By continuing to build upon this research, there is potential for a more efficient and cost-effective method of developing and improving plant-based meat product texture. Simple

analytical methods can be used to characterize any protein quickly and efficiently as heat or cold swelling. This has implications for both research and industry as a quality control method and an opportunity for cost saving if an expensive ingredient can be replaced with a more affordable one with similar swelling properties. This research could also help to reach difficult to replicate meat textures such as seafood or create completely new textures to create a novel plant-based protein product. In conclusion, improving the texture of plant-based meat could lead to an increase in alternative protein consumption that will have beneficial impacts on the environment and public health.

4.5 References

- McClements, D. J., & Grossmann, L. (2021) The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. *Comprehensive Reviews in Food Science and Food Safety*, 20(4), 4049-4100.
- Sanchez-Sabate, R., & Sabate, J. (2019) Consumer attitudes towards environmental concerns of meat consumption: A systematic review. *International Journal of Environmental Research and Public Health*. 16(7), Article 1220.
- Sha, L., & Xiong, Y. (2020) Plant protein-based alternatives of reconstructed meat: Science, technology, and challenges. *Trends in Food Science & Technology*, 102, 51-61.

Appendix A - Chapter 2 Section 2.2.2.4: Lab-scale extrusion

Lab-scale extrusion was completed using a twin-screw Micro-18 extruder (Micro-18, American Leistritz, Somerville, NJ). Lab-scale extrusion was used as a method of preliminary testing to determine if proteins had the potential to be texturized during a pilot-scale extrusion trial. Lab-scale extrusion is able to provide moderate shear energy to initiate the crosslinking of the proteins but does not have the shear capabilities of pilot-scale extrusion that lead to higher levels of texturization and formation of fibrous structure (Webb et al., 2023). Information was collected for extruder parameters such as die temperature, screw speed, and specific mechanical energy (SME) in Table A-1. Several different plant-protein ingredients were tested such as soy protein isolate (SPI), fava protein concentrate (FPC), pea protein isolate (PPI), and several soy protein concentrates (SPC). Each of the formulated treatment mixes was also extruded on a lab-scale. Several parameters were kept constant for each treatment during extrusion such as in-barrel moisture (IBM) at 30% and feed rate which was kept at 1 kg/h. The die used was a single circular die that had a width of 18.85 mm, a length of 19 mm, and an outlet diameter of 4.60 mm.

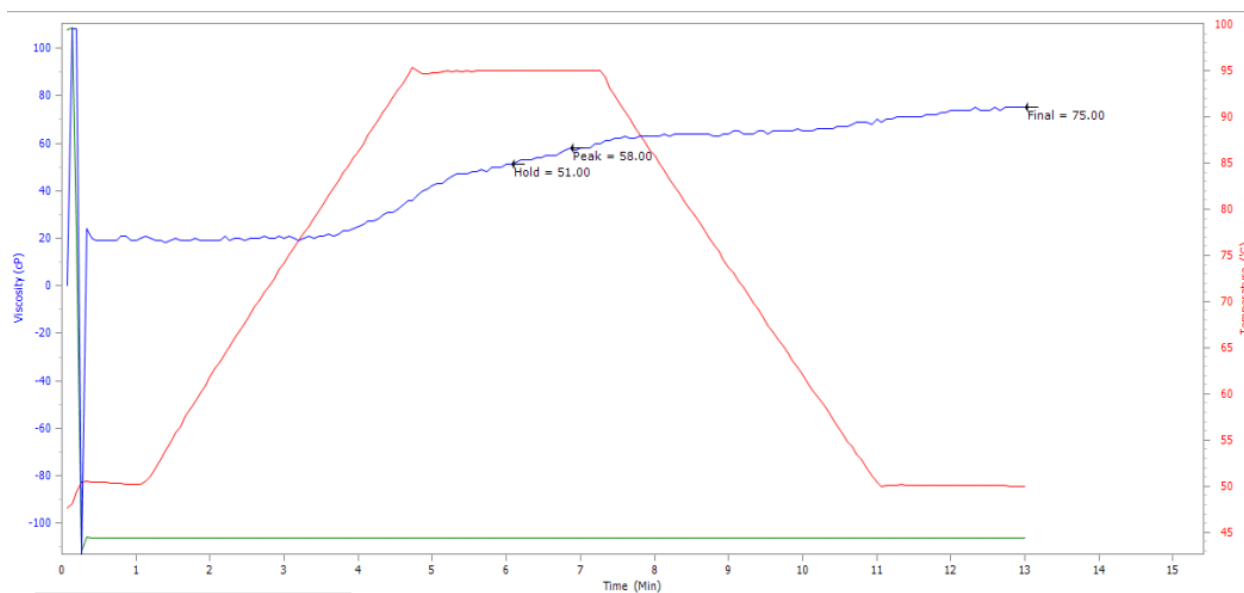
Table A-1 Extruder parameters were kept constant except for the extruder screw speed which was increased for the rice protein. This was because the pieces were not cohesive at the lower screw speed and needed higher mechanical energy to crosslink.

	Arcon S	Arcon F	Arcon IN	SPI	Fava	PPI	Rice
In-barrel Moisture (%)	30	30	30	30	30	30	30
Feed Rate (kg/h)	1	1	1	1	1	1	1
Feeder Screw Speed (rpm)	220	153	182	258	177	150	154
Extruder Screw Speed (rpm)	201	204	203	205	202	200	254
Barrel Sect. 1 (° C)	39	35	27	29	29	28	2
Barrel Sect. 2 (° C)	50	50	50	50	50	50	50
Barrel Sect. 3 (° C)	70	70	70	70	70	70	74
Barrel Sect. 4 (° C)	90	90	90	90	90	90	96
Barrel Sect. 5 (° C)	97	97	95	43	42	41	
Barrel Sect. 6 (° C)	102	100	100	99	92	91	101
Die temp (°C)	129.4	136.1	133.7	121.6	101	95.2	131.3
Die Pressure (psi)	700	850	750	1500	500	700	500
Motor load (%)	32	31	27	42	32	23	31
SME (kJ/kg)	615.9	592.2	458.4	958.8	619.0	322.6	716.9

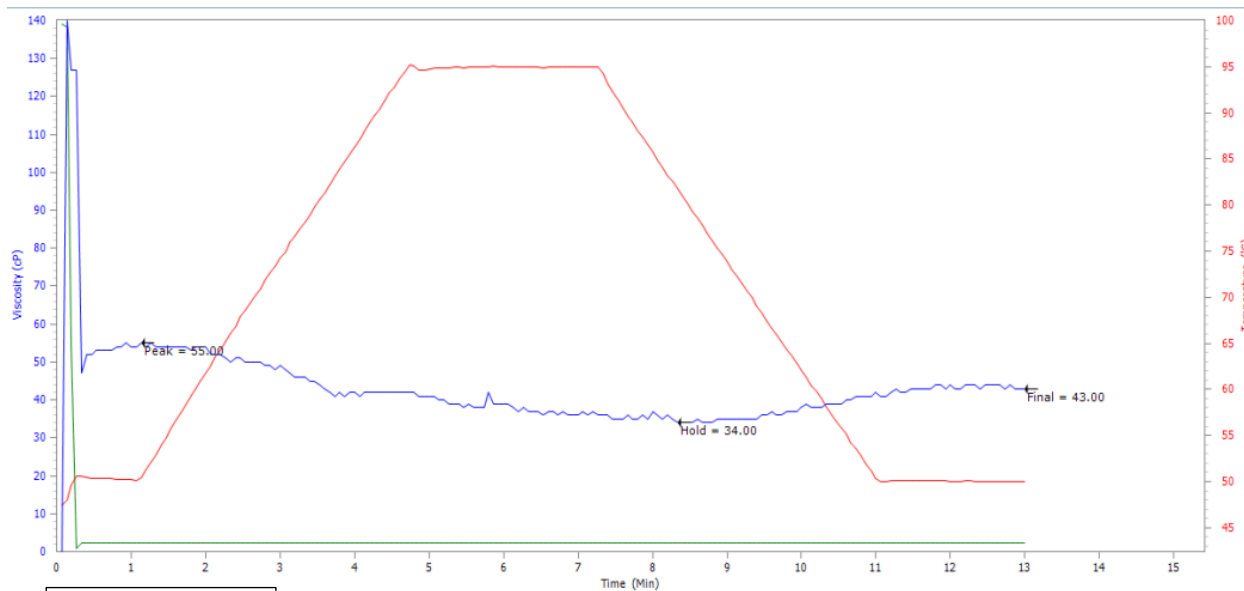
Table A-2 Extruder parameters were kept mostly constant except for the extruder screw speed which was increased for the heat swelling (HS) treatments. This was because the pieces were not cohesive at the lower screw speed and needed higher mechanical energy to crosslink.

	CS1	CS2	IS1	IS2	HS1	HS2
In-barrel Moisture (%)	30	30	30	30	30	30
Feed Rate (kg/h)	1	1	1	1	1	1
Feeder Screw Speed (rpm)	194	175	188	216	209	209
Extruder Screw Speed (rpm)	203	206	202	250	255	255
Barrel Sect. 1 (° C)	32	30	28	29	31	29
Barrel Sect. 2 (° C)	50	50	50	50	50	50
Barrel Sect. 3 (° C)	74	75	75	75	75	75
Barrel Sect. 4 (° C)	98	95	95	95	95	95
Barrel Sect. 6 (° C)	102	101	99	99	100	100
Die temp (°C)	122.4	130.8	118.8	120.8	123.2	123.6
Die Pressure (psi)	850	1200	600	550	500	425
Motor load (%)	39	45	31	28	27	27
SME (kJ/kg)	851.2	1063.2	586.4	584.6	555.2	555.2

Appendix B - Chapter 3 Section 3.3.2.3: Rapid visco analysis (RVA)

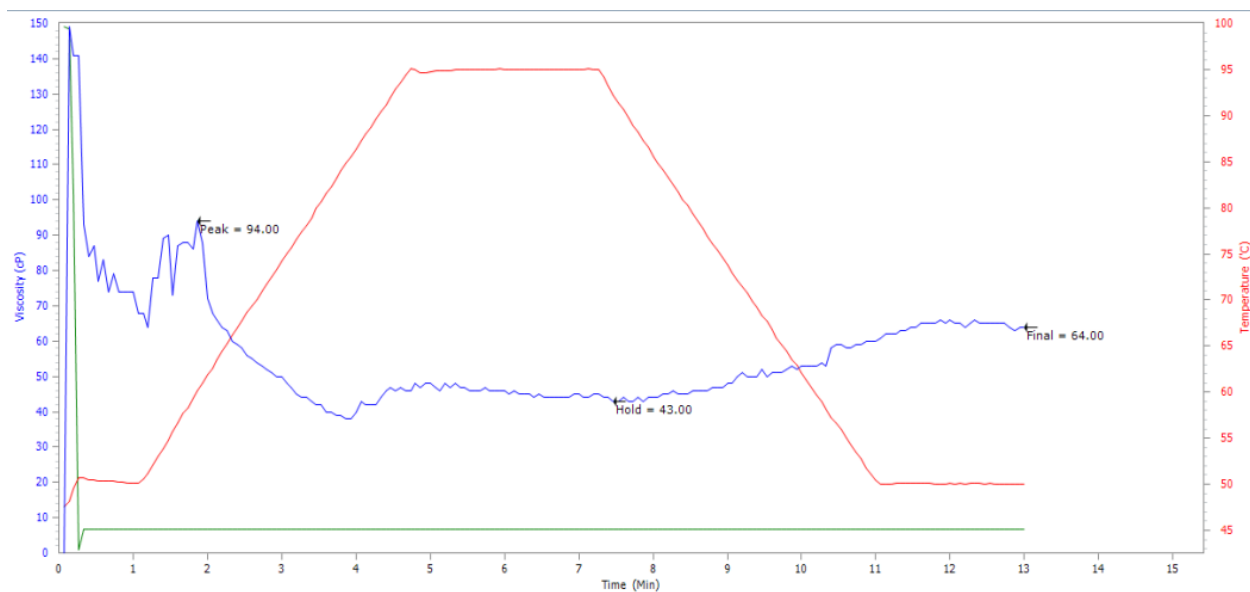


0 % CS, Soy + wheat

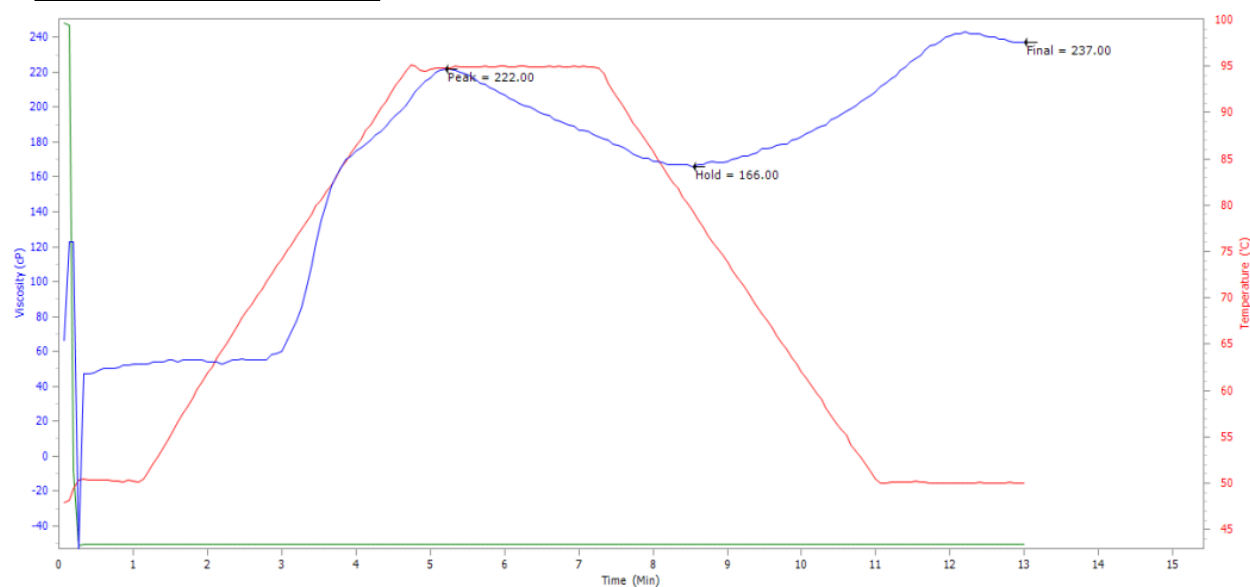


30 % CS, Soy

Figure B-1 RVA curves are shown for 0% and 30% cold swelling (CS) protein mixes.

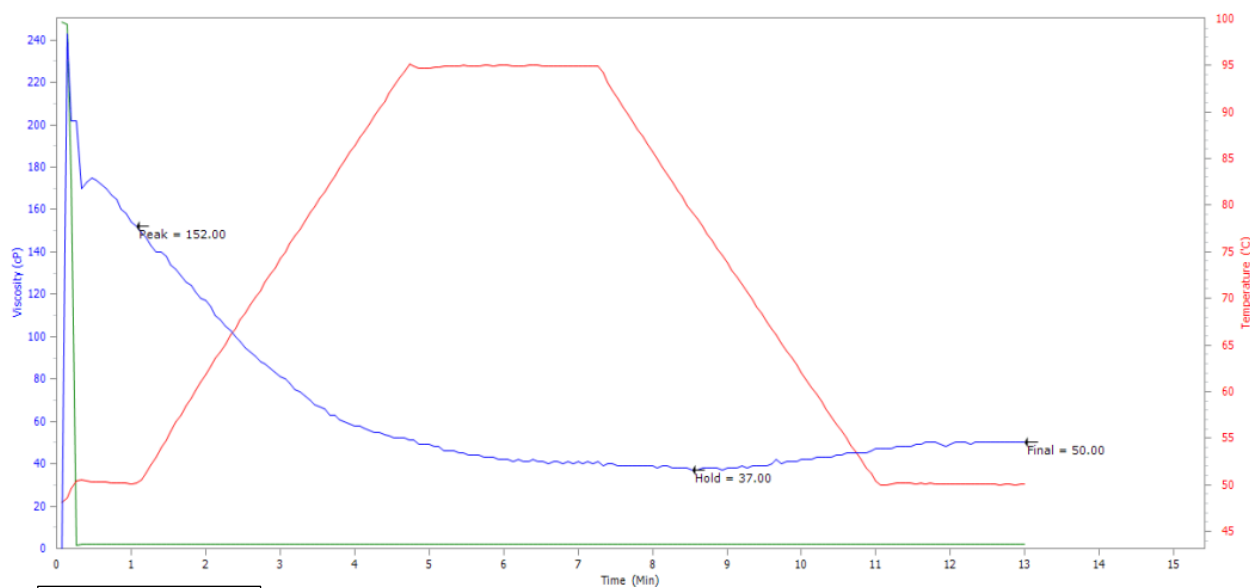


40 % CS, Soy + wheat

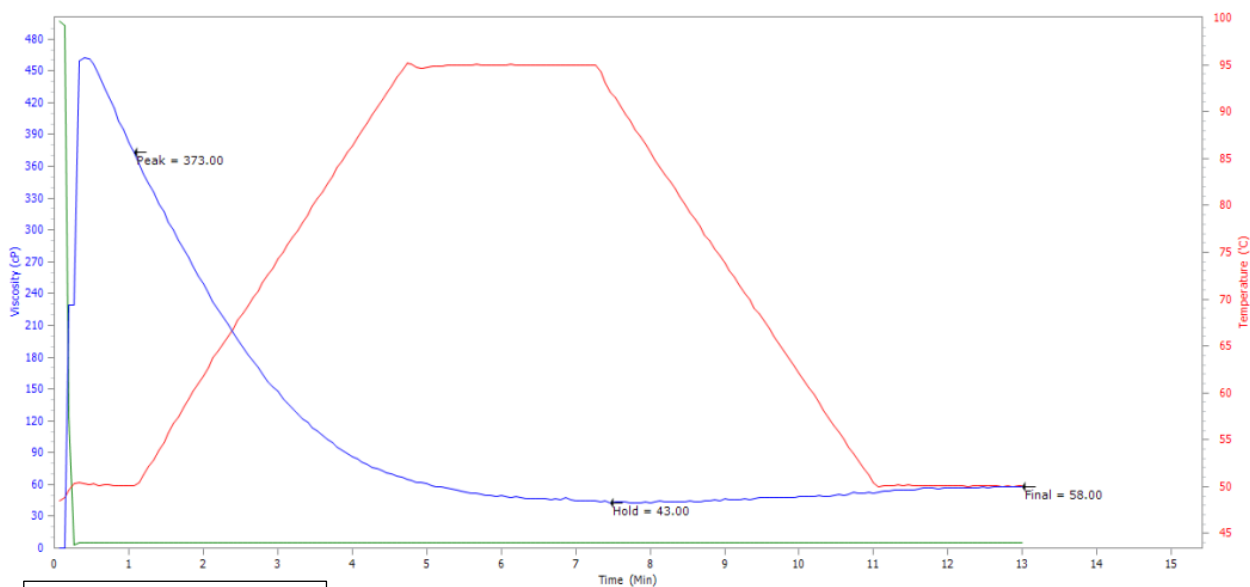


50 % CS, Soy + pea

Figure B-2 RVA curves are shown for 40% and 50% cold swelling (CS) protein mixes.



60 % CS, Soy



90 % CS, Soy + pea

Figure B-3 RVA curves are shown for 60% and 90% cold swelling (CS) protein mixes. Soy and pea are cold swelling and therefore show an earlier and higher viscosity peak.