Impact of plant protein functionality and extrusion conditions on texture of high moisture meat analogs (HMMAs)

by

Blake John Plattner

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College of Agriculture

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Approved by:

Professor
Sajid Alavi

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Abstract

The current food landscape in North America is one in which consumers will pay more for premium, nutritious, and healthy products. Plant-based meat fits under this umbrella, and provides an alternative food option for consumers who do not prefer to eat animal meat, for animal welfare, environmental, or health reasons. Extrusion technology is the primary processing method for the development and production of plant-based meat, also called ‘meat analogs’. Textured vegetable protein (TVP)-based products have been predominately used in the past to make plant-based patties, meatballs, nuggets, and other product forms; but a new version of meat analogs has evolved recently with advances in extrusion technology. High moisture meat analogs, also known as HMMAs, are plant-based meat products designed to mimic the aesthetic and nutritional qualities of whole animal muscle meat cuts, like steak or fillets. The intent of this research was to study various plant protein sources, and also investigate the impact of extrusion equipment design and processing techniques on the overall texture and quality of HMMAs.

In the first part of this study, five different plant protein recipes were processed on a Wenger TX-52 pilot-scale extruder equipped with a long downstream cooling die. Three recipes utilized only single-protein sources, namely soy (S), wheat (W), and pea (P). The other two recipes consisted of blends of soy proteins with wheat (WS) and pea-based (PS) protein sources. In-barrel moisture (IBM) and cooling water injection rate were optimized for each treatment to achieve optimum texturization. Clear differences were observed in rapid-visco analysis pasting profiles of the single-protein recipes, with higher peak viscosity for W (169 cP) and S (167 cP) as compared to P (102 cP). Cutting test on HMMA products using a TA-XT2 texture analyzer demonstrated much higher firmness (5,635 and 5,220 g, respectively) and toughness (36,725 and 37,370 g.sec, respectively) for W and P, as compared to the other three recipes that had soy

proteins (firmness 2,020 – 2,680 g and toughness 11,905 – 16,815 g.sec), while textural profile analysis (TPA) data indicated that the S recipe had highest hardness (10,925 g) and chewiness (6,890) and lowest springiness (0.87) values. The overall conclusion was that soy protein led to the highest quality HMMA, also confirmed based on visual analysis, forming uniform and laminated or layered products. The overall textural quality and consistency of the wheat and pea-based HMMAs were inferior to soy. These products contained thick outer shells, possibly due to inadequate cooling in the die.

The second part of this study focused on pea protein, given its labeling advantages and high demand. Four pea protein types were analyzed to assess differences in their raw material properties and then processed on a twin-screw extruder, using the same die set up as in the first study, to evaluate functionality differences. It was found that the pea protein types exhibiting lower solubility (PPI2 and PPI3), measured using the Biuret method and confirmed with rapid-visco analysis pasting data, led to increased product firmness (10,960-13,550 g) and better overall visual quality. Additionally, complementing pea protein isolate (PPI) with pea protein concentrate (PPC) was found to improve product quality and reduce outer shell formation. When compared to cooked animal meat anchors, using a TA-XT2 texture analyzer, HMMA products displayed similar hardness, firmness, and toughness to that of animal meat.

The third and final part of this study was also conducted with pea proteins, with an emphasis placed on extrusion equipment design and processing parameters. Two recipes, varying only in PPI inclusion amount (50-60%), were processed on a Wenger TX-52 pilot-scale extruder with a thinner, longer cooling die (5/16" diameter) as compared to the previous experiments, at different extrusion process conditions. As feed rate increased from 25 to 35 kg/hr, hardness (21,005-31,230 g) and toughness (30,345-43,285 g.sec) values significantly increased for the

HMMA products. Higher barrel temperatures (150°C versus 130°C) and PPI inclusion (60% versus 50%) also increased texturization, but not as significantly as feed rate.

This research demonstrates the importance of understanding the impact of raw materials, extruder hardware, and processing conditions on final product properties and overall textural quality of HMMAs. Generally, pea proteins exhibit lower functionality compared to soy and wheat proteins, but with specialized recipes and a high-level understanding of extrusion processing parameters and cooling die design, high-quality HMMA products can be developed using peas.

Table of Contents

List of Figures

List of Tables

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Chapter 1 - Introduction

Overview of High Moisture Meat Analogs

The current food landscape in North America is one in which consumers will pay premiums for foods that are minimally processed, clean label, nutritious, and healthy (Bohrer, 2019). This basic framework applies to the wide majority of foods available for consumer purchase, but there are a few unique products that do not follow this trend. Plant-based meat is one well-known example. Although generally being perceived as being “healthier” than animal meat, plant-based meats are very highly processed and often contain high amounts of additives and binders. One reason for their increase in demand stems from the rapidly growing number of vegan, vegetarian, and flexitarian consumers, who avoid animal products for health, environmental, and/or animal welfare reasons (He et al., 2020). Additionally, the ability of plant proteins to be cholesterol-free is another reason plant-based meats might be perceived as healthier (Asgar et al., 2010).

The other aspect that is driving the market demand for plant-based meat is food sustainability. It is predicted by the year 2050 that the world population will reach 9.7 billion (United Nations, 2019). This will put the food supply chain under great stress to feed this growing number of people. There are not sufficient bioavailable resources to meet this growing protein demand using only animal proteins, but plant-based meat is one alternative that can be used to help complement the limited sustainability of the animal meat production system (Lee et al., 2020).

Several forms of plant-based meat, such as tofu, seitan, and textured vegetable protein (TVP), already exist in the market, but demand for more innovative and “less processed” alternatives is increasing. High moisture meat analogs (HMMAs) are the newest form of plant-based meat to enter onto the scene, and may provide a better alternative to the existing options (Riaz, 2011).

A 'high moisture meat analog' is a plant-based meat product designed to mimic both the aesthetic and nutritional qualities of whole animal muscle meat cuts, like steak and fillets (Galanakis, 2019). A variety of plant protein sources can be used to manufacture these products, but soy, wheat, and pea protein are among the most commonly used ingredients. HMMAs are created through high moisture extrusion cooking, which imparts high shear and pressure, to create fresh, "meat-like" products. Following extrusion, products are cut, shaped, seasoned, and stored frozen, similar to that of animal meat.

This research focused on the development of HMMAs, comparing the functionality and behavior differences between plant protein sources, with a primal focus on pea protein. The intent was to build a framework for food developers, bridging together raw material properties and extrusion processing techniques, to understand their combined impact on the texture of HMMA products.

Chapter 2 Objectives

Chapter 2 focuses on identifying raw material differences between three plant proteins, namely soy, wheat, and pea, and assesses their impact on the texture of HMMAs. Given that these proteins are most commonly used as complements to one another for both functional and nutritional purposes, we attempted to understand if they could successfully be extruded as "single-protein HMMAs". Recipes were developed, and analysis was performed on the raw recipes to identify differences in viscosity and solubility. Following extrusion, texture analysis was performed on the extruded HMMAs to quantify textural differences. Texture of ‘milled’ HMMAs was also compared to animal meat anchors (pulled pork and pulled chicken).

Chapter 3 Objectives

Chapter 3 focuses on expanding what was learned about pea protein in Chapter 2. Given the superior labeling advantages that pea protein holds, being both allergen-free and non-GMO, a specific focus was placed on improving the texture of pea-based HMMAs. Four pea protein isolate types were evaluated to identify intrinsic property differences, and their respective impact on the extrusion and texture of HMMAs. Additionally, varying inclusion amounts of pea protein concentrate were added to recipes to understand its impact on product quality. Texture of HMMA products was compared against whole muscle animal meat cuts (beef steak, pork chop, chicken breast, and fish fillet).

Chapter 4 Objectives

The aim of Chapter 4 was to take the knowledge about pea protein ingredients, gained from the previous two chapters, and pair that with systematic changes in equipment design and extrusion processing parameters, to measure their collective impact on the texture and quality of HMMAs. Recipes were evaluated for rheology differences, while texture and sensory analyses were performed on the extruded HMMAs.

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Chapter 2 - Impact of soy, wheat, and pea protein on the texture of high moisture meat analogs

Abstract

The recent development of high moisture meat analogs (HMMAs) has been instrumental in the growth and expansion of the plant-based meat market. HMMAs are manufactured by extrusion, and are designed to mimic the aesthetic and nutritional qualities of whole, shredded, or cubed animal muscle meat. Soybeans, wheat, and peas are the three primary plant proteins utilized in HMMA formulation, and are typically used as complements to one another for nutritional, textural, and functional purposes. In this study, single-protein HMMAs were processed on a twin-screw extruder, equipped with a long cooling die to understand if they could match similar functional and textural attributes to that of their multi-protein HMMA counterparts. Differences in peak viscosity (97-180 cP) and solubility, identified by rapid-visco analysis, provided explanation for the variations in mechanical energy (280-495 kJ/kg) and in-barrel moisture (IBM) (42-61.55%), occurring during extrusion of the recipes. The inclusion of 30% wheat gluten increased mechanical energy by 20% and the firmness and toughness of the extrudates by more than 33%. Texture profile analysis (TPA) showed the hardness (10,925 g), gumminess (7,925), and chewiness (6,890) values of the soy HMMA being greater than each of the other products (5,520-10,875 g). Pea-based HMMA texture results were misleading. Despite showing high firmness values, it was made up of a hard outer shell that encompassed a poorly-texturized interior. This led to overall inconsistent and soft texture, which was exposed after milling the sample. When comparing milled products to animal meat anchors (pulled chicken and pulled pork) on TPA, all but the pea-based HMMA showed higher values. Their ability to mimic pulled animal meat was apparent.

2.1. Introduction

Meat is defined as “the flesh of an animal considered especially for food” (Merriam-Webster Dictionary, n.d.). It is primarily constituted of water, protein, and fat. Protein is arguably the most important component of meat as it provides a rich source of amino acids and vitamins that are essential for optimum physiological human health (Lee et al., 2020). The public health message seems to be actively trying to persuade people to increase their protein intake, as many are avoiding sugar and simple carbohydrates and turning to protein-rich foods, snacks, and supplements. These new high-protein diets are often referred to as “the new low-fat” or “the new low-carb” diet (Egan, 2017). Meeting the growing protein demand solely with animal meat will not suffice for the following reasons: limited land and water resources, consumers' ecological, ethical, religious, social, and health concerns with consuming animal flesh, and the cost consideration. Therefore, the protein demand may need to be compensated with a larger supply of plant-based protein sources (Samard et al., 2019).

A relatively new product form of plant-based meat, invented in Europe and then brought to the United States, is commonly referred to as a high moisture meat analog (HMMA) (Riaz, 2011). HMMAs are manufactured via the extrusion process and are designed to mimic the aesthetic and nutritional qualities of whole or pulled animal muscle meat. Current research, aligned with market trends, points to three primary plant protein sources being used in the production of HMMAs, namely soybeans, wheat, and peas. With further innovation, these ingredients can be used to accelerate the development of HMMAs to help complement the limited sustainability of the traditional animal meat production system (Lee et al., 2020).

Soy proteins are extremely versatile ingredients, and possess a unique ability to create ‘meat-like’ texture. They also provide a complete amino acid profile and exhibit good extrusion functionality, that gives them an edge over wheat and peas (Riaz, 2011). Limitations in application exist due to the presence of allergenic proteins and because most of the commercial soybeans are genetically modified organisms (GMOs) (Samard et al., 2019). These drawbacks have resulted in greater demand for other alternative protein sources like wheat gluten and peas.

Wheat protein, specifically gluten, is also frequently used in the production of plant-based meat applications. Gluten provides many unique properties, such as being able to form anisotropic meat-like structures, exhibiting beneficial viscoelastic properties, and enhancing the polymerization of protein subunits (Samard & Ryu, 2019; Guo et al., 2020). A couple challenges associated with using wheat gluten is its poor water solubility properties and the increasing rise in the number of consumers with celiac disease. These two factors have limited the widespread use of wheat gluten in textured protein applications, but its ability to texturize and embody an appealing flavor profile makes it a valuable ingredient source. Wheat gluten is often accompanied by either soy or pea protein to create the ideal amino acid balance, as well as to mediate the fiber formation capacity of gluten (Akdogan, 1999).

Pea protein provides unique labeling opportunities, as peas are both a “non-GMO” and low allergen crop. The use of peas has been limited primarily because of their low supply and inferior functionality, but pea protein has recently become a coveted ingredient. Pea protein is highly bioavailable for digestion, contains a great amino acid balance, and is a rich source of antioxidants (polyphenols) (Samard & Ryu, 2019; Asgar et al., 2010). Using fiber and starch to dilute the overall protein concentration is used for practical purposes to lower the cost of final products (Webb et al., 2020).

A comprehensive review of the amino acid composition and physicochemical properties of soy, wheat, and pea proteins can be located in Webb (2021). In short, the major fraction of soy protein consists of the common legume proteins, namely 7S vicillin (52%) and 11S legumin (35%). These two salt-soluble proteins are classified as globulins. Wheat gluten is composed primarily of gliadin and glutenin, which account for 85% of the proteins. Gliadin is alcohol-soluble while glutenin is soluble in dilute acid. Peas consists of four protein fractions, namely globulin (55-65%), albumin (18-25%), prolamin (4-5%), and glutelin (3-4%). Vicilin and legumin are the two subunits that make up the bigger globulin fraction.

Many plant-based meat products on the current market use soy, wheat, and pea proteins as complement ingredients to one another to improve their nutritional and functional qualities (Day et al., 2006). It is unknown if these ingredients can be extruded to create high-quality products on their own, as single-protein HMMAs. Therefore, the objective of this study was to determine if soy, wheat, and pea can effectively texturize and create single-protein HMMAs that are comparable in quality and texture to their complementary, multi-protein HMMA counterparts, as well as to animal meat anchors. If proven successful, market expansion for HMMA products could ensue, given the distinct labeling capabilities.

2.2. Materials and Methods

2.2.1. Ingredients and Recipe Preparation

Five recipes were developed to target three single-protein HMMA products and two complementary, multi-protein HMMA products. Complete recipes are detailed in Table 2.1. From left to right, the first recipe, a soy only recipe, denoted as S, contained 25% soy protein isolate (SPI) and 50% soy protein concentrate (SPC). The second recipe, a wheat only recipe, denoted as W, contained 65% wheat gluten (WG). A pea only recipe, denoted as P, was the third recipe containing 80% pea protein isolate (PPI). The fourth recipe, a wheat-soy blend, denoted as

WS, contained 30% (WG) and 55% SPC. Lastly, a pea-soy blend, denoted as PS, contained 40% PPI, 38% SPC, and 2.5% SPI. Ingredients were mixed together for each recipe in 100 lb batches.

Table 2.1. Recipes for each high moisture meat analog (HMMA), containing selected combinations of soy, wheat, and pea-based ingredients.

	Recipes				
Ingredients	**S**	**W**	**P**	**WS**	**PS**
Pea Protein Isolate 1					40
Pea Protein Isolate 2			80		
Soy Protein Isolate	25				2.5
Wheat Gluten		65		30	
SPC – (non-functional)	50			10	13
SPC – (semi-functional)				45	25
Faba Bean Protein					15.5
Wheat Flour		21		11	
Soy Flour	15				
Tapioca Starch	6				
Pea Starch			6		
Wheat Bran		10			
Pea Fiber			10		
Granulated Salt	2	2	2	2	2
Sunflower Oil	2	2	2	2	2
Total	**100**	**100**	**100**	**100**	**100**

To note, these recipes were specially developed for the purpose of creating high-quality HMMA products, that would serve as benchmarks for the next two studies. Recipes were formulated using prior industry, ingredient, and extrusion knowledge, in an attempt to optimize final product texture; for this reason, different combinations of proteins, carbohydrates, fibers, and fats were utilized. The differing protein sources that were studied constituted the majority (65-85%) of the overall recipe, so differences in processing and analytical testing is primarily attributed to the protein source. However, other constituents like starch and fiber do play a role and their effects are seen in prior research by Webb et al. (2020) and Webb (2021).

Nutritional composition of each recipe was estimated from the given specifications provided by the suppliers. Table 2.2 shows the overall nutritional composition for each recipe.

Table 2.2. Total estimated nutritional content of each recipe containing selected combinations of soy, wheat, and pea-based ingredients.

	Recipes				
Nutrients:	**S**	**W**	**P**	**WS**	**PS**
Protein (%)	65.45	51.85	64.70	62.10	72.15
Starch (%)	5.88	26.75	9.48	12.80	0.62
Fiber (%)	12.70	6.53	8.00	11.93	9.85
Fat (%)	2.80	3.56	8.40	3.26	1.86

2.2.2. Moisture Content

Moisture content of the recipes was found according to AACC 44-19.01. Approximately 2 g of each sample was dried at 135 ºC for 2 hrs. Analysis of samples was completed in triplicate.

2.2.3. Phase Transition Analysis

Phase transition analysis was used to predict and assess thermodynamic flow behaviors of the recipes. A phase transition analyzer (PTA™) (Wenger Manufacturing) is a modified capillary rheometer that uses a combination of time, temperature, and pressure to measure the softening (T_s) and flow (T_f) temperatures of a biopolymer sample during thermal processing (Karkle et al., 2012; Plattner et al., 2001). These temperatures are a measure of polymer deformation and flow behavior when under conditions similar to extrusion (Roberts, 2013; Webb et al., 2020). Information gained helps the extrusion operator predict raw material flow properties which allow process adjustments to be made more efficiently during the extrusion operation (Plattner et al., 2001; Webb et al., 2020).

For this trial, IBM could not be simulated on the PTA as it applies too much pressure to handle such high moisture levels. Keeping the moisture content consistent between samples is essential to accurately evaluate raw material flow differences. Therefore, the recipes were hydrated to a standard moisture content of 24%. W recipe was not able to be analyzed on the PTA at this moisture level, due to its high inclusion of gluten. Hydrating W to 24% turned the material into a dough-like consistency, which could not flow through the PTA die orifice. For this reason, W was only hydrated to 15%, but a direct comparison to the other four recipes could not be made.

Analysis was conducted on hydrated samples following the procedures outlined by Webb et al. (2020) and Roberts (2013), with slight modification. A blank die was inserted into the PTA, 1.5-2.5 g of material was weighed and placed into the PTA chamber, and an initial compression force of 75 bars was applied for 15 s. The pressure was then fixed at 75 bars and the sample heated at a rate of 8 °C/min with a starting temperature range between 3-7 °C. T_s was logged as the midpoint of the temperature range of softening (Liu et al., 2011). After the material reached the end of softening, the blank die was replaced with a 2 mm capillary die and heating continued at the same rate and operating pressure from approximately 5 °C below the softening end point. Melting, or flow point (T_f), was defined as the temperature at which the material began to flow through the capillary and was identified by a steep increase in displacement (Karkle et al, 2012; Liu et al., 2011; Webb et al., 2020). All samples were tested in triplicate.

2.2.4. Rapid-Visco Analysis

The viscosity was measured to understand the rheological behavior differences between recipes, when subjected to heat, moisture, and shear, as is similar to conditions found during the extrusion process. Viscosity was quantified according to the standard AACC method 76-21 STD 1, using the Rapid Visco Analyzer (RVA) 4800 (Perten Instruments, Perkin Elmer, NSW, Australia). In short, approximately 3.5 g of recipe was combined with 25 mL of water, to obtain

14% (w/v), dry matter basis; the solids were adjusted accordingly to correct for inherent moisture differences between the recipes. Hydrated samples were heated to 50 ºC while being stirred under a constant shear rate of 960 rpm for 10 s. It was then held at 50 ºC for 50 s and then heated up to 95 ºC under a shear rate of 160 rpm. It was held there for 3 min and then cooled back down to 50 ºC. (Zahari et al., 2020). 3 of the 5 samples had no defined peaks, given the S-shaped curve of the lines, so peak viscosity was determined as the highest viscosity value attained at an operating temperature at or above 90 °C. Pasting time and temperature were also evaluated. Testing was done in triplicate.

2.2.5. Extrusion Processing

Processing was carried out under the following conditions. Each recipe was mixed for 5 min in a batch ribbon blender (Wenger Manufacturing, Sabetha, KS, USA), prior to extrusion, to ensure a homogeneous mix. All five recipes were processed on a pilot-scale twin-screw extruder (TX-52, Wenger Manufacturing), equipped with a differential diameter cylinder preconditioner with a volumetric capacity of 0.056 m^3 (DDC2, Wenger Manufacturing). Recipes were first fed into the preconditioner at 35 kg/hr, and then conveyed into the extruder having a screw speed fixed at 450 rpm. Water was injected into the preconditioner at a rate ranging from 20-37 kg/hr between recipes, and into the extruder at approximately 11 kg/hr, for PS and S. The rates were adjusted accordingly to achieve optimum texturization for each recipe. Steam was not used.

The screw profile, shown in Figure 2.1, consisted of 5 double flighted sections, decreasing in pitch moving from the inlet to the discharge end of the barrel. It also included four forward-conveying kneading blocks, 5 reverse-conveying kneading blocks, and a conical cut flight unit at the discharge end.

Head 1	Head 2	Head 3	Head 4	Head 5

Left Shaft

1	1	2	2	3	4	2	2	3	1	1	3	5	6	5	6	7	5	6	7	8	9	10
11	11	2	2	3	4	2	2	3	1	1	3	5	6	5	6	7	5	6	7	8	9	10

Right Shaft

1: ¾ pitch, double flight, 9 unit
2: Full pitch, double flight, 9 unit
3: Forward kneading block, 3 unit
4: Forward kneading block, backward, 3 unit
5: ½ pitch, double flight, 9 unit
6: Reverse kneading block, 3 unit
7: Reverse kneading block, backward, 3 unit
8: ½ pitch, double flight, cut flight, 9 unit
9: ½ pitch, double flight, cut flight, 6 unit
10: ¾ pitch, double flight, cut flight, cone screw
11: ¾ pitch, single flight, 9 unit

Figure 2.1. Extruder screw profile

The screw diameter was 52 mm with an L/D ratio of 25.5. Five heating zones were utilized to heat the extruder barrel; their respective temperatures were set at 40, 110, 130, 130, and 125 °C, moving from inlet to discharge end of the barrel. A long-barrel cooling slit die, having two sections, was equipped to the end of the extruder to allow for protein alignment and texturization, and enable product cooling. The first section was run without forced cooling to allow the protein to set, align, and cross-link. The second die section was cooled with water, with direct control by a manual throttle valve. Dimensions of the die orifice were ½” x 2¾” (H x W).

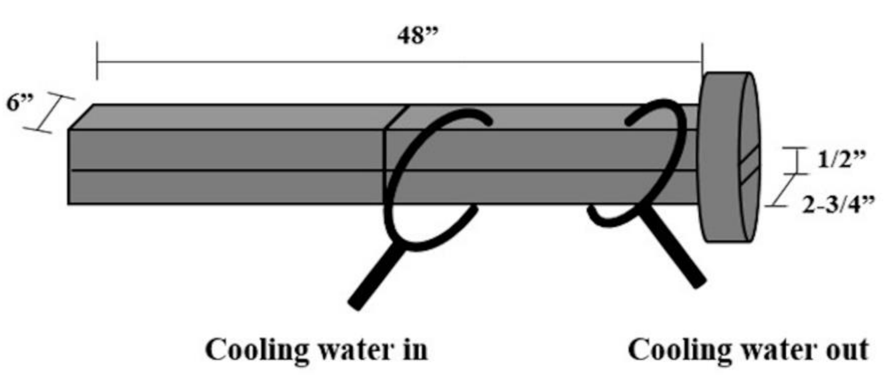


Figure 2.2. Cooling die configuration and dimensions

Product was manually cut at the die exit into approximately 1 ft long pieces with a Chef's knife. Approximately half of the extrudates collected were kept as whole pieces and the rest were shredded or milled using a Marlen Tabletop Meat Shredder (Marlen International Inc., Riverside, MO, USA) and an Urschel Comitrol 3610 mill (Urschel Laboratories, Chesterton, IN, USA), respectively. The Urschel mill was equipped with a ¾ in cutting head. All samples were immediately transferred to resealable plastic poly freezer bags and stored until analysis.

Mechanical energy was measured with a wattmeter. The wattmeter was installed and equipped to the extruder motor, and a direct power reading was given in kW. This was converted to kJ/kg with the following formula:

$$\textbf{Specific Mechanical Energy}\ (\mathrm{kJ/kg}) = \frac{(P-P_0)}{\frac{\dot{m}}{3600}}$$

where P is motor power reading in kW, P_0 is the no load motor power reading, and $\dot{m}$ is mass flow rate in kg/hr.

In-barrel moisture (IBM) and die cooling water injection rate were adjusted for each recipe. A higher IBM (~61% w.b.) was used to process PS and S recipes, compared to the others' (48.8-55.33% w.b.). This was to facilitate flow of the material through the extruder and to optimize texturization and product integrity in the cooling die.

IBM content was calculated using the following equation:

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

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

2.2.6. Texture Analysis

Cutting test and texture profile analysis (TPA) were the instrumental analysis techniques utilized to evaluate the overall texture and physical properties of the extruded products. Evaluation was performed using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Prior to assessing texture, HMMA samples were thawed in a 2% saltwater brine at room temperature for 45 min to prevent product water loss and shrinkage. Former research by (Osen, 2017) confirms no significant effect of freezing or thawing extrudates on the cutting strength.

The cutting strength was measured in both the longitudinal and transverse direction, according to the procedures described by (Zahari et al., 2020). Samples were cut into squares with dimensions of 3 x 3 cm and then analyzed. A guillotine knife blade (70 mm width x 100 mm height x 3 mm thickness), at a test speed of 2 mm/s was used to cut through the entire height of the sample. Longitudinal cutting was done in the direction parallel to the flow and fiber formation of the sample, whereas transverse cutting was done perpendicular to the direction of the flow and fiber formation of the sample. Figure 2.3 provides illustration of the two cutting directions. 10 replicates were measured for each sample in both directions of cut. Data was collected for firmness and toughness values. Firmness is often synonymous with hardness and is measured as the peak force during cutting of the sample. Toughness is calculated as the total positive area under the curve and is defined as the accumulated amount of work it takes to cut the sample.

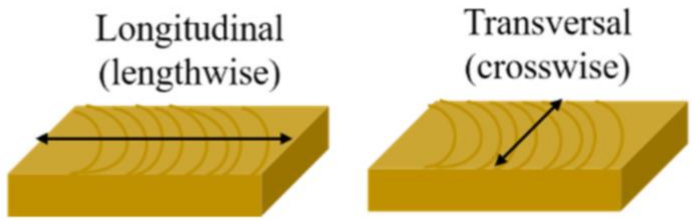


Figure 2.3. Schematic showing cutting directions on high moisture meat analog (HMMA) products, borrowed from Ferawati et al. (2021).

TPA was used to assess both whole and milled HMMA product forms. For whole product analysis, samples were cut into squares with dimensions of 3 x 3 cm as described above for cutting test. A 50 mm diameter aluminum cylinder probe compressed the sample twice to 50% strain, with a contact force of 5 g, at a test speed of 2 mm/s, and a wait time of 2 s between compressions. 15 replicates were measured for each sample. Milled HMMA products were assessed and compared against animal meat anchors. Two meat anchors, namely pulled chicken and pulled pork, were purchased at Hy-Vee Grocery Store (Manhattan, KS, USA). Their specific identifications are as follows: (1) Curly's Sauceless Roasted & Seasoned Pulled White Meat Chicken and (2) Curly's Sauceless Naturally Hickory Smoked & Seasoned Pulled Pork. Both animal meat products were purchased 'already fully cooked' but were not heated prior to texture analysis. The milled HMMA products were thawed in resealable plastic quart bags at room temperature (about 2 hr), prior to analysis. Approximately 20 g of each product was gently packed into an open-faced plastic cylinder (75 mm diameter) and compressed twice to 75% strain, with the same probe, contact force, test speed, and wait time as noted above for whole products. 15 replicates were measured for each sample. Data was collected for the attributes of hardness, springiness, and chewiness for both whole and milled product forms. Hardness is measured as the peak force during the first compression. Springiness is the ratio of the height of the product at the beginning of the second compression over the product's original height. Chewiness is extrapolated as hardness x cohesiveness x springiness (Texture Technologies, n.d.).

2.2.7. Statistical Analysis

All analyses were performed in at least triplicate, unless otherwise stated. Data was evaluated using SAS software (SAS, Cary, NC, USA), with means and pair-wise differences being calculated using 1-way ANOVA and Tukey's test, respectively. Results with significance level of ($\rho < 0.05$) were considered to be statistically significant.

2.3. Results and Discussion

2.3.1. Phase Transition Analysis

The PTA flow temperature (T_f) is an indirect measure of resistance to flow or viscosity (Karkle et al., 2012). Therefore, T_f can be a predictor of mechanical energy in the extruder, if all independent variables, e.g. IBM, are kept constant. A material that is more resistant to flow in the PTA (higher T_f) is likely to generate higher viscosity and mechanical energy during the extrusion process. However, IBM was adjusted during extrusion to optimize product quality, so a direct correlation cannot be made between mechanical energy and T_f. PTA results are shown in Table 2.3. PS and S have the highest T_f values (76.73 and 83.63 °C). WS resulted in lowest T_f value (64.97 °C) and P was intermediate (73.90 °C).

Table 2.3. Softening mid-point (T_s) and flow point (T_f) temperatures, from phase transition analysis, of each high moisture meat analog (HMMA) recipe containing selected combinations of soy, wheat, and pea-based ingredients.

Recipe	T_s m.p. (°C)[1]	T_f (°C)
S	27.67	83.63
P	23.50	73.90
WS	23.93	64.97
[2]PS	26.53	76.73
[3]W	40.57	87.13

The T_f values for S (83.63 °C), PS (76.73 °C), and P (73.90 °C) show the addition of pea protein lowering the melt point. Flow temperatures are presumed to be related to the molecular weight and solubility of recipes (Gropper et al., 2002). It is well-known that wheat gluten has the highest molecular weight, followed by soy protein, and then pea protein (Zhao et al., 2020). Proteins with higher globular molecular weight and solubility are more resistant to flow and would require higher water to process, as confirmed by IBM. The high T_s and T_f of W, shown in the footnote, can be disregarded as it was analyzed at lower moisture (15%) on the PTA, given the hydration limitations of gluten. Decreasing moisture content (%) naturally raises the T_s and T_f temperature of samples (Oterhals et al., 2019; Brownsey et al., 2003). In general, the PTA may not be a good prediction tool for wheat gluten, as gluten, requires shear to generate viscosity (Lindborg et al., 1997). With no shear induced in the PTA, wheat gluten is unable to generate viscosity resulting in low T_f for WS (64.97 °C). Wheat gluten was also confirmed to have lower T_f temperature in study by (Bengoechea et al., 2007). Rapid-visco analysis demonstrates the ability of wheat gluten to generate viscosity, after shear addition.

[1] No significant differences were observed for T_s and T_f. F value: 0.83, $\rho > 0.5142$ for T_s. F value: 1.70, $\rho > 0.2441$ for T_f.

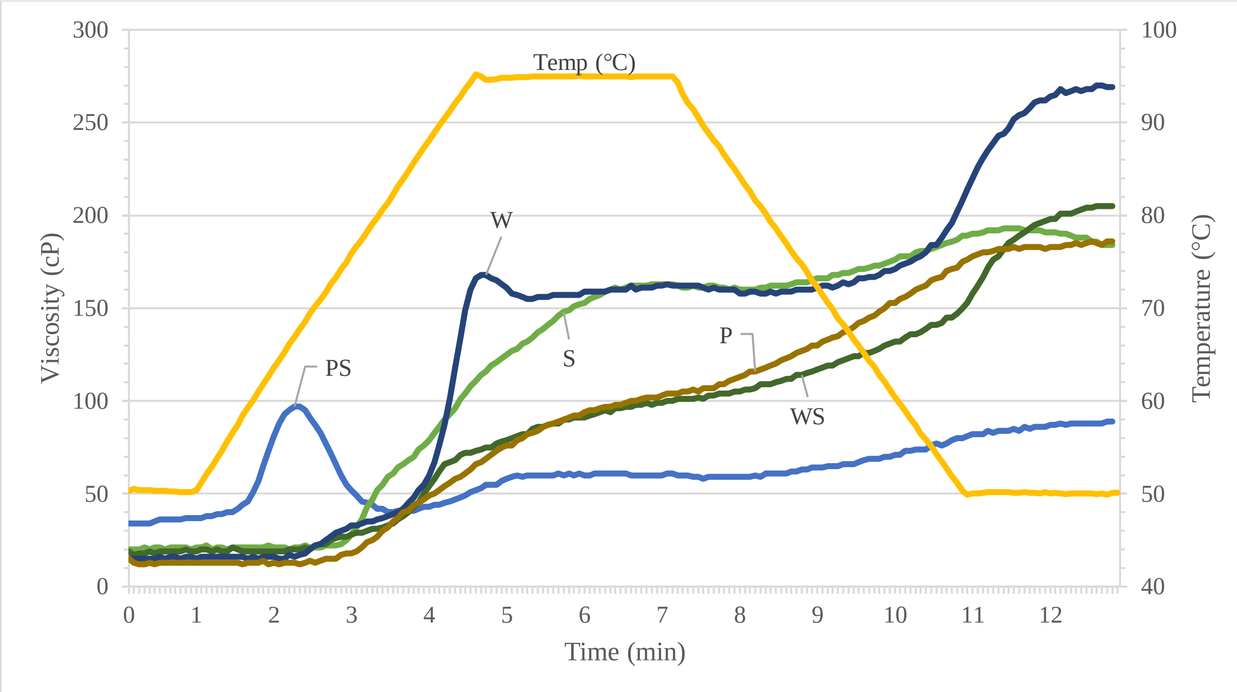
[2] Different pea protein isolate (PPI) types were used in PS and P, contributing to flow and solubility differences.

[3] Softening (T_s) and flow (T_f) temperatures of wheat gluten hydrated to lower moisture content (15%).

For high moisture/high protein extrusion applications, the PTA may not be a good instrument to help predict the functionality of protein polymers; in the case of HMMAs, mechanical energy in the extruder is being generated from the cross-linking of proteins in the cooling die, whereas pressure is creating the mechanical energy in phase transition analysis. Furthermore, IBM was not able to be simulated on the PTA, as it applies too much pressure to handle such high moisture levels. These dynamics inhibit prediction capabilities. For this reason, the PTA was not used in the next two studies to assess raw material properties.

2.3.2. Rapid-Visco Analysis

Major differences in viscosity and flow properties were evident between the soy, wheat, and pea-based recipes. This is depicted in Figure 2.4, a collective graph of one replicate of each recipe.



[4]Figure 2.4. Rapid-visco analysis curves for each high moisture meat analog (HMMA) recipe containing selected combinations of soy, wheat, and pea-based ingredients.

Contrary to PTA results, W generated highest viscosity due to the insolubility and high molecular weight of wheat gluten. This is shown in Table 2.4, as both the peak and final viscosity for W were higher than all other recipes at 169 cP and 270 cP, respectively; SME was also highest for W, shown in Table 2.5, confirming the ability of wheat gluten to induce high viscosity upon shear addition. The peak viscosity of S followed closely behind W at 167 cP while P exhibited lowest at 102 cP. With S demonstrating higher peak viscosity (167 cP) than PS (97 cP) and P (102 cP), results are confirmed from the PTA. The higher solubility and molecular weight of soy protein enables it to generate more viscosity than pea protein (Zhao et al., 2020). Pasting time and temperature follows the same trend as peak viscosity; W had lowest pasting time (293 s), followed by S (429 s), and then P (452 s).

[4] Different pea protein isolate (PPI) types were used in PS and P, contributing to flow and solubility differences.

Final viscosity is higher than peak viscosity for all recipes except PS (Table 2.4). PS demonstrated high cold-paste viscosity (94 cP) that subsequently decreased upon addition of thermal energy and mechanical shear. This was the same result as found in Osen et al. (2014). PS and P contained different PPI types, so flow differences between samples were likely created by solubility differences between the PPIs (Osen et al., 2014). Pasting time (148 s) and temperature (68 °C) were significantly lower for PS, suggesting the higher solubility for the PPI utilized in PS. In general, pea proteins are not able to generate the viscosity that soy proteins can (Zhao et al., 2020), and as a result, lower functionality and texturization is often seen during extrusion of pea proteins (Kyriakopoulou et al., 2018).

Despite the highest viscosity for W (169 cP) and S (167 cP), their combined interaction in WS exhibited relatively lower viscosity (101 cP). Similar to PS, this is primarily created by the differences in formulation between recipes. A lower inclusion of wheat gluten combined with no inclusion of SPI, both being very functional ingredients, likely decreased the viscosity for WS.

Table 2.4. Rapid-visco analysis of each high moisture meat analog (HMMA) recipe containing selected combinations of soy, wheat, and pea-based ingredients. Cells labeled with the same letter are not significant.

Recipe	Peak viscosity (cP)	Final viscosity (cP)	Pasting Time (s)	Pasting Temp (℃)
S	167 ± 10 ^{A}	189 ± 10 ^{B}	429 ± 5 ^{B}	95 ± 0 ^{A}
W	169 ± 6 ^{A}	270 ± 11 ^{A}	293 ± 2 ^{C}	95 ± 0 ^{A}
P	102 ± 10 ^{B}	176 ± 19 ^{B}	452 ± 4 ^{A}	91 ± 1 ^{B}
WS	101 ± 5 ^{B}	204 ± 8 ^{B}	451 ± 6 ^{A}	92 ± 1 ^{B}
[5]PS	97 ± 5 ^{B}	94 ± 8 ^{C}	148 ± 0 ^{D}	68 ± 0 ^{C}

Given the limitations of this specific RVA, denaturation of the proteins could not be achieved with this procedure; however, the RVA profile used in this study still provided useful

[5] Different pea protein isolate (PPI) types were used in PS and P, contributing to flow and solubility differences.

results. This procedure allows mimicking of both cold water swelling tests (such as water binding capacity) and heat gelation tests (such as least gelation concentration), which are discussed more thoroughly in Chapters 3 and 4. For future studies, using a pressurized RVA to analyze recipes under high temperature conditions could induce denaturation of proteins (Onwulata et al., 2013). This would allow for further prediction of recipes during extrusion processing. The combination of the two methods is suggested for future research.

2.3.3. Extrusion Parameters

For typical extrusion of starches, mechanical energy is a relatively simple parameter to evaluate. Material viscosity drives mechanical energy; therefore, as a higher degree of starch gelatinization occurs and increases viscosity, mechanical energy will in turn, also increase (Gropper et al., 2002; Zhu et al., 2017). Protein extrusion is much different, as proteins denature, align, and crosslink rather than gelatinize like starch molecules. Therefore, evaluating mechanical energy can be rather ambiguous. Shown in Table 2.5, SME quantifies the amount of mechanical energy generated in the extrusion system. SME is a factor of the combination of extrusion processing parameters and raw material flow properties; SME alongside other system response parameters like die pressure and die temperature, can be used to evaluate overall mechanical energy produced in an extrusion system.

In this trial, IBM appears to have the biggest impact on SME. Prior research supports evidence that increasing IBM lowers mechanical energy, given the plasticizing effect of water (Zhang et al., 2020; Maung et al., 2020). PS and S, which were processed at highest IBM (61.53 and 61.32%, respectively), had lowest SME values (277.71 and 288.00 kJ/kg, respectively). Likewise, WS, W, and P showed lower IBM (41.98-55.33%) but higher SME (380.57-493.71

kJ/kg), validating this correlation between IBM and mechanical energy. The system response parameters also correspond to SME, as found in Palanisamy et al. (2018). Die pressures were higher for WS, W, and P (500 psig), compared to PS (350 psig) and S (300 psig).

Ingredients also impacted processing. Most notably, the addition of wheat gluten increased mechanical energy. In comparing recipes, S, WS, and W, the SME values (288.00 kJ/kg, 401.14 kJ/kg, and 493.71 kJ/kg, respectively) increased with additions of gluten (30-65%). Wheat gluten builds viscosity quickly, as it develops into a hydrated visco-elastic mass, given its hydrophobic nature and insolubility in water (Biesiekierski, 2017; Day et al., 2006). In addition to its hydrophobicity, wheat gluten is also highest in molecular weight, compared to soy and pea protein (Zhao et al., 2020). This combination led to more mechanical energy, and more potential for protein cross-linking in wheat-based recipes. Soy protein is known to have a greater affinity for water and higher molecular weight compared to pea protein (Zhao et al., 2020; Burger & Zhang, 2019; Webb, 2021); this was confirmed by higher IBM values for PS and S.

Table 2.5. In-barrel moisture (IBM) (%), specific mechanical energy (SME) (kJ/kg), and die pressure (psig) for each high moisture meat analog (HMMA) recipe containing selected combinations of soy, wheat, and pea-based ingredients.

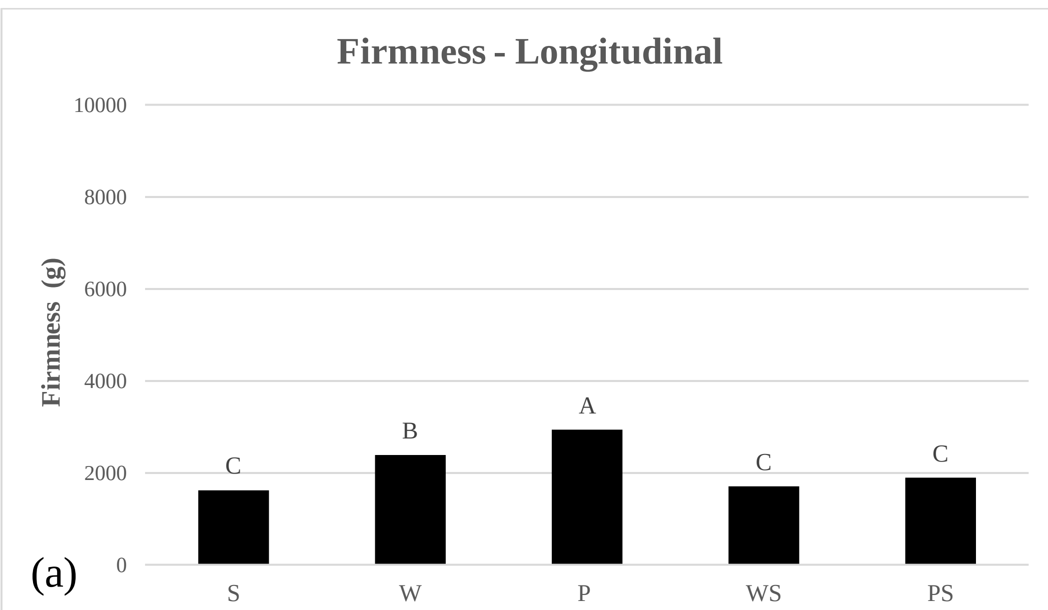
Recipe	IBM (%)	SME (kJ/kg)	Die pressure (psig)
S	61.32	288.00	300
W	41.98	493.71	500
P	48.84	380.57	500
WS	55.33	401.14	500
PS[6]	61.53	277.71	350

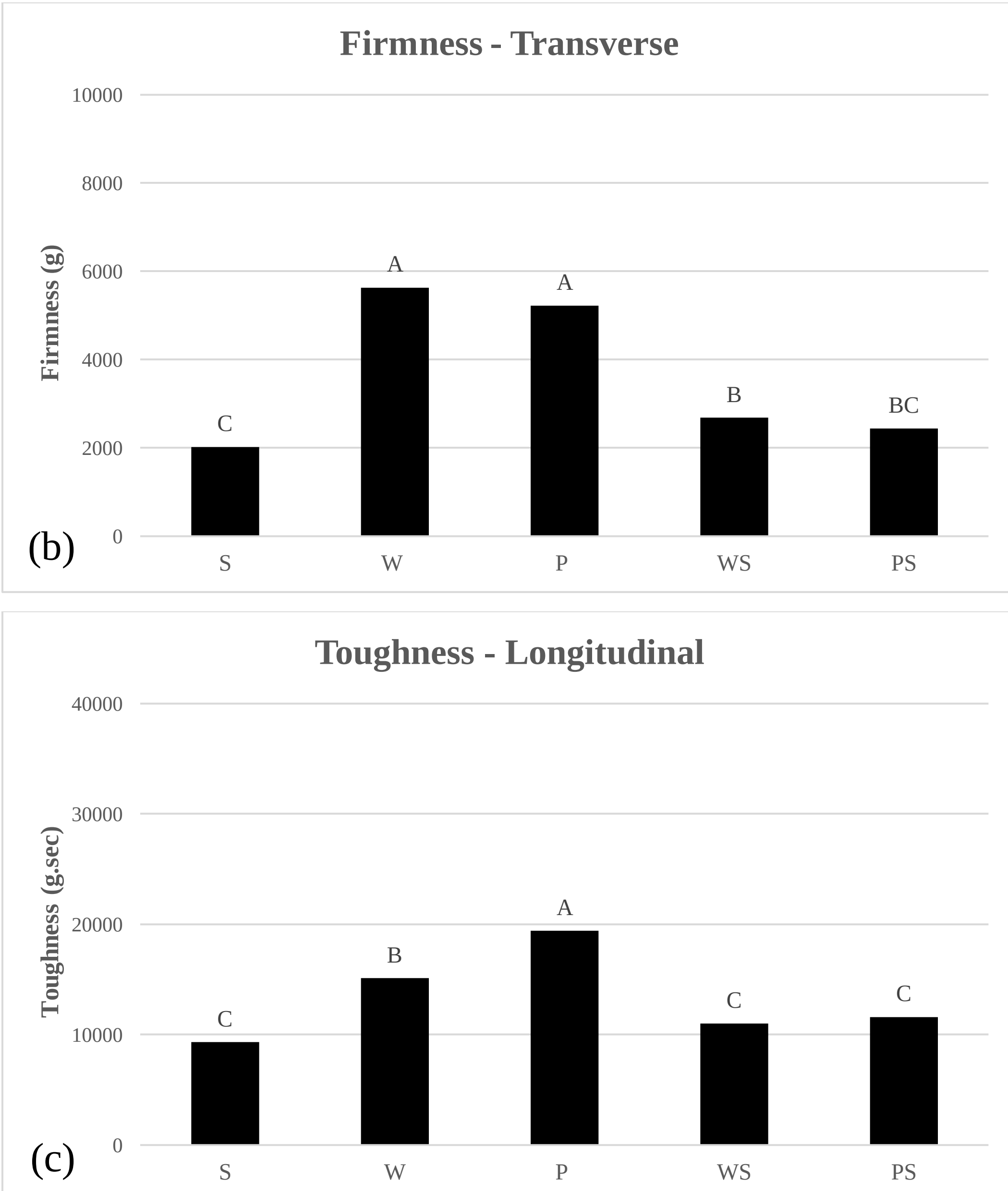
2.3.4. Texture Analysis

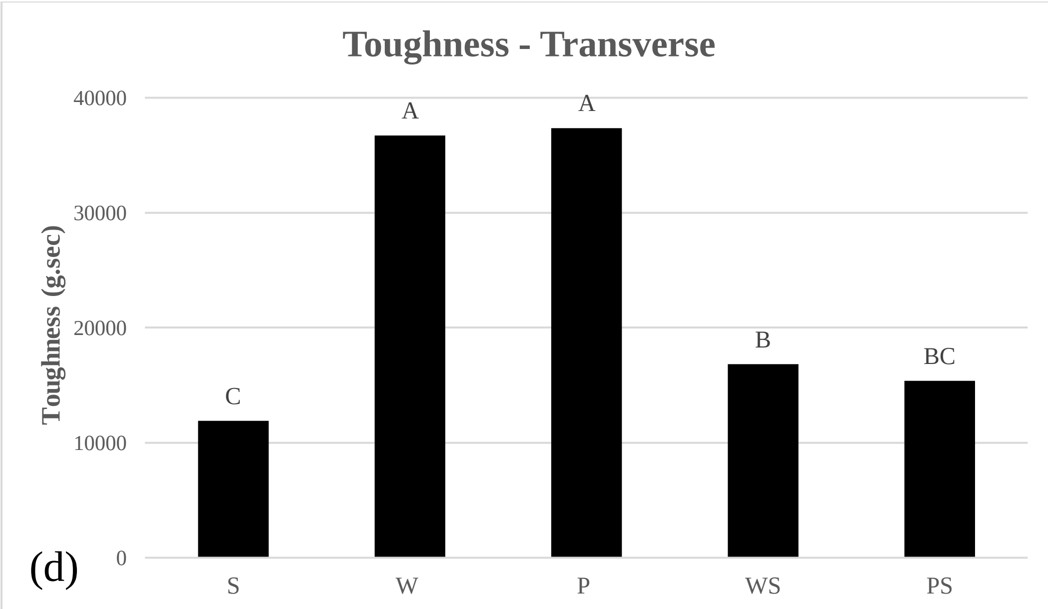
Cutting test results are shown in Figure 2.5. Significant differences were found for W and P. Using high amounts of wheat gluten (65%) led to a highly-texturized, robust product, as in

[6] Different pea protein isolate (PPI) types were used in PS and P, contributing to flow and solubility differences.

Chiang et al. (2019), making the cutting force significantly higher for W. With an abundance of cysteine residues present in wheat, protein cross-linking is prominent, leading to an extremely texturized product (Samard et al., 2019). P showed the highest value for firmness (2,935 g) and toughness (19,395 g.sec), in the longitudinal direction of cut. Physical examination of the products (Figure 2.7) offers a new perspective. A thick, outer shell overlaying a soft, porous interior is identified in P. In contrast, the internal structure of W was densely texturized, but inadequate cooling of the product also led to shell formation. The high cutting force that was required to cut through P was initially perceived to be associated with high amounts of texturization. This notion was nullified after milling the products. The development of the shell is primarily created by inconsistent cooling of the product, in the cooling die; the lack of time for complete heat transfer to occur creates a type of case-hardening (Gulati & Datta, 2015).




Firmness - Transverse
Firmness (g)
10000
8000
6000
4000
2000
0
A
A
B
BC
C
S
W
P
WS
PS
(b)
Toughness - Longitudinal
Toughness (g.sec)
40000
30000
20000
10000
0
A
B
C
C
C
S
W
P
WS
PS
(c)



[7]**Figure 2.5. Cutting test results of soy, wheat, and pea-based high moisture meat analog (HMMA) products for (a) longitudinal firmness, (b) transverse firmness, (c) longitudinal toughness, and (d) transverse toughness. Within each graph, bars labeled with the same letter are not significantly different.**

The transverse to longitudinal cutting test ratios, shown in Table 2.6, offer insight into the mechanism of texturization, that differs between soy, wheat, and pea proteins. A higher ratio indicates a greater degree of isotropic, intramolecular disulfide bonding, that results in a larger degree of texturization seen in the transverse cutting direction. This is noted most prominently for W and P. A lower ratio points to a greater degree of anisotropic, intermolecular disulfide bonding, suggesting a greater amount of texturization seen in the longitudinal direction. This is most eminent in PS and S. Figure 2.6 depicts the differences in disulfide bonding more clearly.

[7] Different pea protein isolate (PPI) types were used in PS and P, contributing to flow and solubility differences.

Table 2.6. Transverse to longitudinal ratios, derived from cutting test values, for soy, wheat, and pea-based high moisture meat analog (HMMA) products.

Recipe	Firmness Ratio	Toughness Ratio
S	1.25	1.28
W	2.36	2.43
P	1.78	1.93
WS	1.57	1.53
PS	1.29	1.33

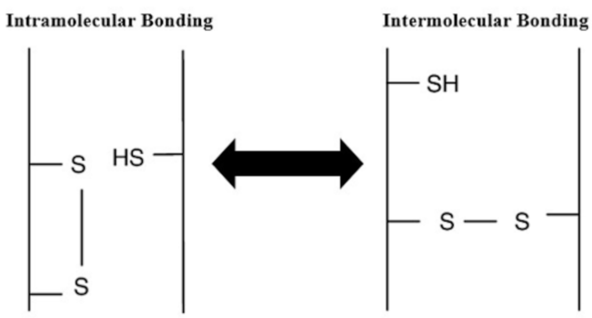
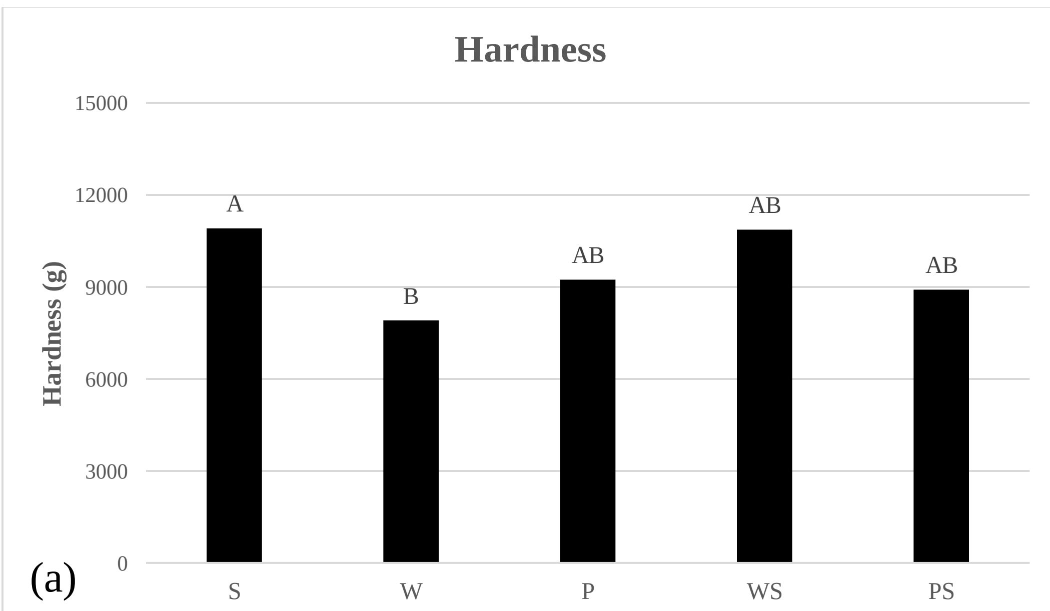
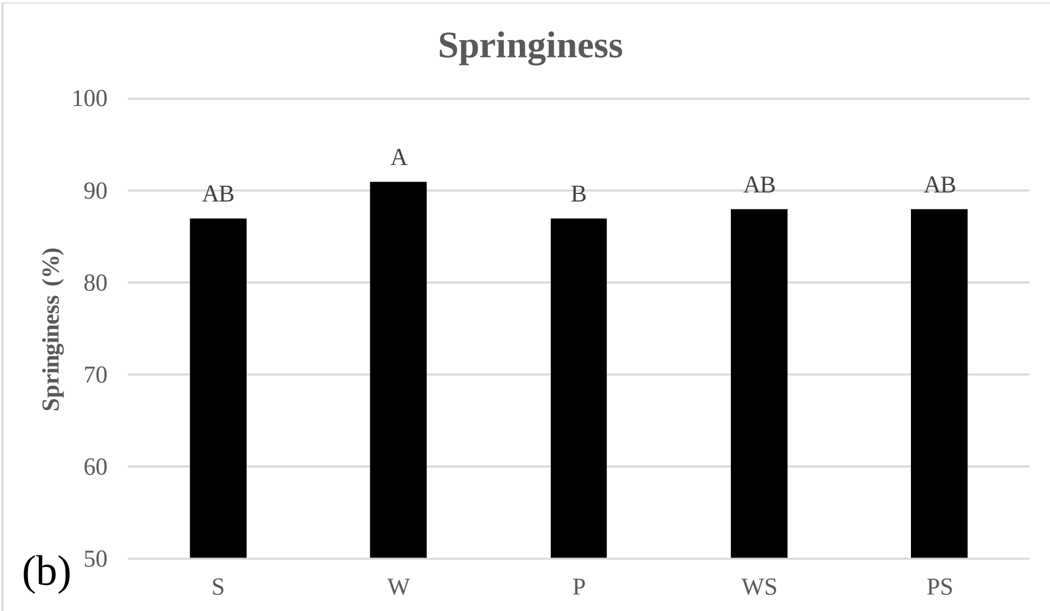


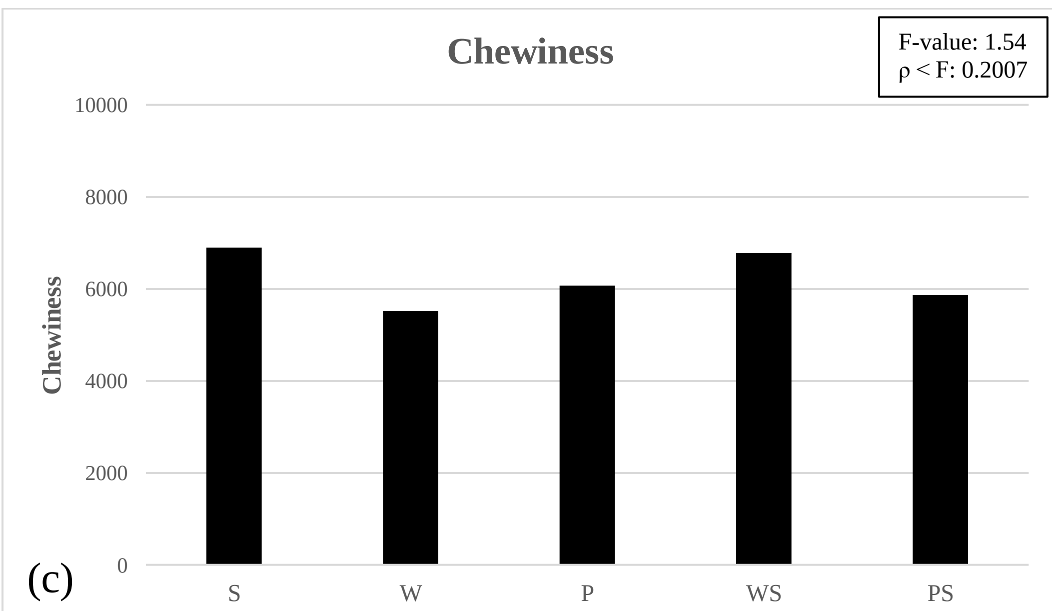
Figure 2.6. Schematic showing intramolecular and intermolecular disulfide bond formation between proteins, revised from Lanier et al. (2005).

TPA results for whole HMMA products are displayed in Figure 2.8. In general, there are not as many significant differences. This was in large part due to high standard deviations from inconsistencies in piece shape and texture. S showed highest values for hardness (10,925 g) and chewiness (6,890), despite having the lowest firmness and toughness values for cutting test. This perceived contradiction exists because of differences in the internal microstructure of the extrudates (Figure 2.7). PS, S, and WS, contain layered, laminar structures that are more apt to withstand a constant force applied over a larger area, expended by TPA. The rigid outer shells of W and P provide more resistance against the knife blade cutting through the sample, as in cutting test; the weak internal structure of P is further revealed after milling. From a visual standpoint, PS, S, and WS appear to be most "meat-like" in texture and appearance.

Figure 2.7. Visual cross-section of soy, wheat, and pea-based high moisture meat analogs (HMMAs).







[8]Figure 2.8. Texture profile analysis (TPA) of soy, wheat, and pea-based high moisture meat analog (HMMA) products for (a) hardness, (b) springiness, and (c) chewiness. Within each graph, bars labeled with the same letter are not significantly different.

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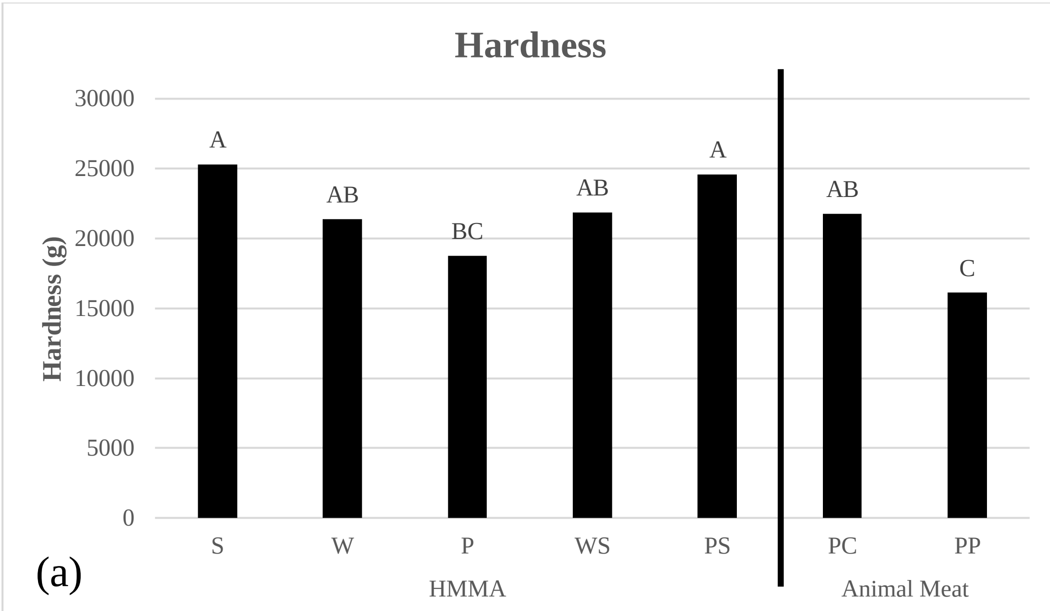
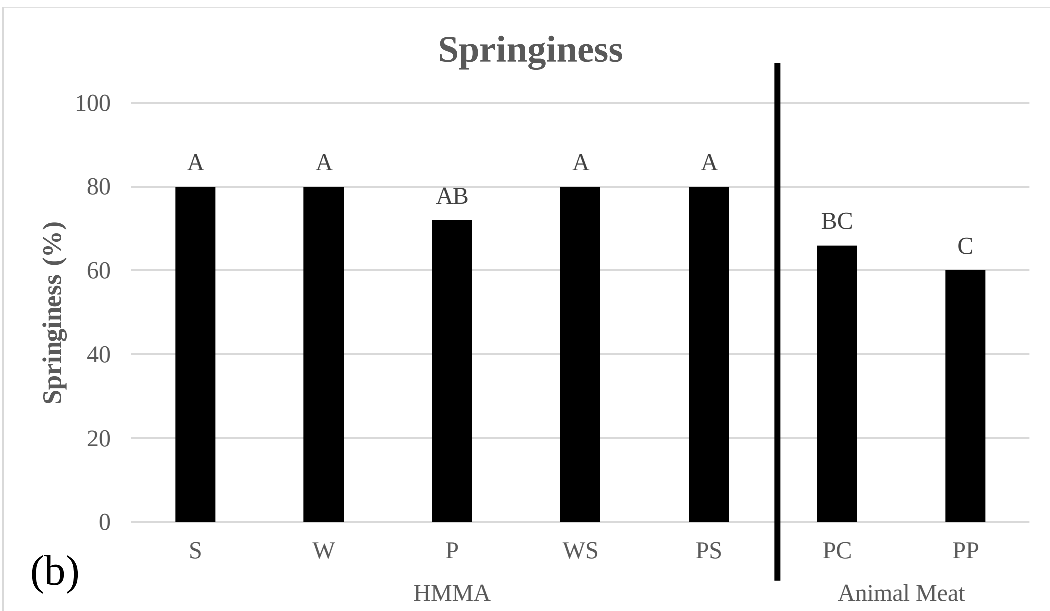
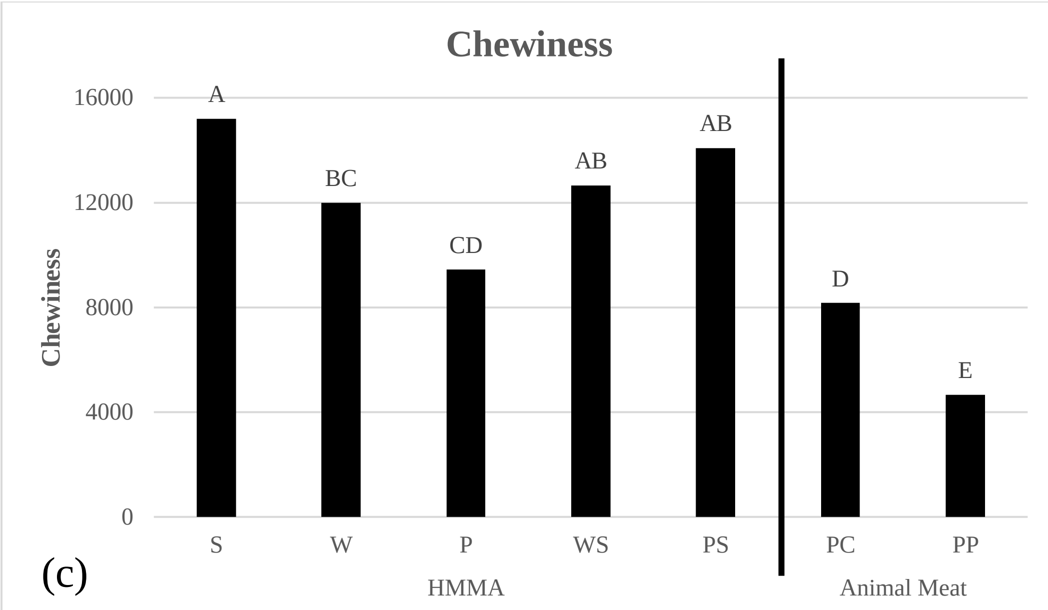
[8] Different pea protein isolate (PPI) types were used in PS and P, contributing to flow and solubility differences.

TPA analysis of milled products (Figure 2.9) showed S having the highest hardness (25,315 g) and chewiness (15,190). This data gives a more accurate representation of the true, overall texturization of each product, as milling destroyed the outer shells. Milled samples were compared against two animal meat anchors, to understand if texture was similar to pulled chicken (PC) or pulled pork (PP). PP was significantly lowest for all measured attributes, compared to the other samples. Similarities, i.e. no significant differences, were found between WS (21,880 g), W (21,375 g), P (18,770 g), and PC (21,765 g) for hardness, while only P showed similar springiness (72%) and chewiness (9,435) values to PC (66% and 8,165, respectively). From these results, soy is confirmed to increase hardness and chewiness of plant-based meat products (Webb, 2021), especially compared to that of pea. Wheat gluten was also found to have lower TPA hardness in (Samard et al., 2019), despite increasing firmness and toughness for cutting test; former research demonstrates the capability of gluten to increase texturization as a complementary protein source (Maung, et al., 2020; Chiang et al., 2019; Webb, 2021), but further research is required to confirm its influence as a single-protein HMMA.

The meat anchors used for comparison were 'fully cooked', and contained flavors, seasonings, fat, and other additives. The cooking process and cold-storage conditions likely reduced the hardness of the animal meat anchors, making them softer than most of the meat analogs. Similarly, if further culinary preparation, e.g. heating, was completed on the meat analogs, texture properties could have been more closely matched to that of the pulled animal meat anchors. As the culinary preparation facet was not a primary focus of this study, future work in this area is needed to provide more definitive results.


Hardness
Hardness (g)
30000
25000
20000
15000
10000
5000
0
A
AB
BC
AB
A
AB
C
S
W
P
WS
PS
PC
PP
HMMA
Animal Meat
(a)


Springiness
Springiness (%)
100
80
60
40
20
0
A
A
AB
A
A
BC
C
S
W
P
WS
PS
PC
PP
HMMA
Animal Meat
(b)



[9]Figure 2.9. Texture profile analysis (TPA) of milled soy, wheat, and pea-based high moisture meat analog (HMMA) products and also animal meat anchors for (a) hardness, (b) springiness, and (c) chewiness. Within each graph, bars labeled with the same letter are not significantly different.

2.4. Conclusion

Extrusion processing and product texture of HMMAs is largely dependent upon the type of plant protein source used. Having the ability to use plant protein sources in complement to one another provides an advantage from both a nutritional and functional point of view. This is not to say that single-protein HMMAs are inferior, but extrusion equipment must be specialized, and processing conditions optimized to reach the product quality level of their multi-protein HMMA counterparts. From this study, milled TPA data confirms the ability of soy, wheat, and pea-based HMMA products to mimic pulled animal meat. With additional culinary preparation, the extruded meat analogs created in this study could closely match the textural attributes of pulled

[9] Different pea protein isolate (PPI) types were used in PS and P, contributing to flow and solubility differences.

animal muscle meat. Soy led to a harder and more uniform texture, making the case that it may have the greatest potential in whole muscle meat applications, like steak. Utilizing pea protein lowered texturization, creating a soft interior structure, enveloped by a hard outer shell. Despite having lower texturization, the pea-only recipe could be improved by diluting with pea protein concentrate, as shown in PS and in (Webb et al., 2020). Wheat gluten should be used in combination with other protein sources or additives, as high amounts of gluten leads to an overly texturized product. In the future, unmilled meat analogs should be compared to whole muscle animal meat anchors, such as beef steaks, pork chops, chicken breasts, and fish fillets, to assess their potential for use in more premium applications. In addition, official consumer sensory panels should be conducted to determine the perception of "meat-likeness" and gauge consumer acceptability of the meat analogs. Finally, formulations should be fine-tuned and extrusion equipment specially designed to improve the processing and overall texture of HMMAs.

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Chapter 3 - Impact of pea proteins on texture of high moisture meat analogs

Abstract

A relatively new form of plant-based meat, known as 'high moisture meat analogs' (HMMAs), is captivating the market because of its ability to mimic fresh, animal muscle meat. Utilizing pea protein in the formulation of HMMAs provides unique labeling opportunities to these products, as peas are a "non-GMO" and low allergen crop. However, many of the pea protein isolate (PPI) types available for purchase differ in functionality, causing variation in product quality. Additionally, overall PPI inclusion level has a major impact on final product texture. To understand the impact of PPI type and its inclusion level on final product texture of HMMAs, two studies were completed. The first study compared four different types of PPI while the second study assessed differences in PPI inclusion amount (30-60%). Both studies were performed on a Wenger TX-52 extruder, equipped with a downstream long-barrel cooling die. Rapid-visco analysis and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) indicated differences in protein solubility and pasting time (0-210 s) among the different PPI types. In general, lower protein solubility, as in the case of PPI2 and PPI3, led to higher product quality with less variation, based on visual evaluation. Cutting strength and texture profile analysis (TPA) showed increasing PPI inclusion level from 30% to 50-60% led to significantly higher product hardness (14,160-16,885 g) and toughness (36,690-46,195 g.sec). PPI4 led to significantly weaker texture, in terms of longitudinal and transverse toughness (26,110 and 33,725 g.sec), compared to the other PPIs (44,620-60,965 g.sec). The heat gelling capacity of PPI4 was also highest among PPI types, by way of least gelation concentration (LGC) and rapid-visco analysis. When compared against animal meat anchors, a higher protein

inclusion level (50-60%) better mimicked the overall texture and firmness of beef steak and pork chops, while lower protein inclusion better represented a softer product like chicken breast.

3.1. Introduction

Textured vegetable protein (TVP) is a food product derived from plant proteins that is experiencing rapid market growth around the world. Currently, it occupies the biggest market share among the different meat alternatives and is projected to reach over $1.5 billion by 2025 (Markets & Markets, 2020). It is manufactured primarily via the extrusion process and is available in the market in several different forms (Plattner, 2009). A relatively new product form invented in Europe and then brought to the United States is commonly referred to as a 'high moisture meat analog' (HMMA) (Riaz, 2011). HMMAs are plant-based meat products designed to mimic the aesthetic and nutritional qualities of whole animal muscle meat cuts (Galanakis, 2019). A specialized extrusion process, using a long cooling die, enables the production of these fresh, premium meat analogs that have the appearance and eating sensation similar to cooked animal muscle meat, while the high protein content offers a similar nutritional value (Samard et al., 2019). HMMA application is specifically targeted at mimicking whole animal muscle meat cuts, but also is capable of taking the form of pulled muscle meats or canned meat chunks.

Soy, wheat, and pea are the three primary plant proteins utilized in HMMA formulation. Despite the prevalence and processing advantages of soy and wheat, the meat alternative market has experienced a recent shift in demand toward clean label ingredients, such as peas. Pea protein provides unique labeling opportunities, as peas are both a "non-GMO" and low allergen crop. The use of peas has been limited primarily because of their high purchasing cost and low supply in the United States, but they have become more popular (Webb et al., 2020). Pea protein is highly bioavailable for digestion, contains a great amino acid balance, and is a rich source of polyphenols, which have high antioxidant activity that improves the body's immune system (Samard & Ryu, 2019; Asgar et al., 2010). The gel forming ability, solubility, and emulsifying capacity of pea protein has been found to be similar to soy protein, but its functionality is generally inferior to soy (Asgar et al., 2010; Vatansever et al., 2020). The three primary challenges barring the widespread use of pea protein is its high cost, bitter flavor, and inferior functionality. With high purchasing cost, due to limited supply, it has not fully become cost-effective to use high amounts of pea protein to manufacture HMMAs for widespread market applications. Using concentrate, fiber, and starch to dilute the concentration of pea isolate is used for practical purposes to lower the cost of final products (Webb et al., 2020). Additionally, due to the physical nature of peas, higher attention must be given to processing parameters to effectively texturize pea protein, as the structure is known to be chemically susceptible to extreme processing conditions which occur during extrusion (Osen, 2017).

Prior research and industry developments have shown the potential to create single-protein HMMAs, using only pea protein (Osen et al., 2014; Osen, 2017; Samard & Ryu, 2019). In some cases, utilizing high amounts of pea isolate (~80%) produces a HMMA with a thick outer shell that encompasses a soft interior center. It is predicted that diluting isolate with concentrate can help counteract the formation of this shell and improve product quality (Ferawati et al., 2021; Webb et al., 2020). Therefore, the first objective of this study was to create a high-quality, texturized HMMA product, using ascending ratios of pea protein isolate to pea protein concentrate.

Differences in growing conditions in the field, combined with varying protein extraction methods utilized throughout industry has led to certain pea proteins being more functional than others (Vatansever et al., 2020). This leads to processing differences, texture inconsistency, and can even alter the taste profile of the extrudates. It is important for processors to understand which commercial pea proteins will create the highest quality HMMA products, and identify which of their respective raw material properties most influences processability and texturization. Therefore, the second objective of this study was to determine which PPI types can texturize well and create HMMAs that are comparable in quality and texture to animal meat anchors. Each PPI type would be evaluated through various analytical methods to understand which physicochemical properties are good indicators of product quality and texturization potential. This can help food developers and manufacturers begin to build a framework that bridges together ingredient selection and processing techniques, with textural properties.

3.2. Materials and Methods

3.2.1. Ingredients and Recipe Preparation

The recipes were prepared, according to information in the tables below. The first set of recipes, shown in Table 3.1, had the same formulation but differed in PPI type. Isolates are denoted as PPI1, PPI2, PPI3, and PPI4, accordingly. The remaining recipes, shown in Table 3.2, used the same PPI type, i.e. PPI1, but had differing inclusion levels of PPI and PPC. For the purpose of distinguishing recipes, each recipe is denoted by PPI type and level of isolate inclusion (%). Recipes in Table 3.1 are denoted as PPI1-40, PPI2-40, PPI3-40, and PPI4-40. The remaining four recipes in Table 3.2 are denoted by PPI1-30, PPI-40, PPI1-50, and PPI1-60.

Table 3.1. Pea-based high moisture meat analog (HMMA) recipes containing different pea protein isolate (PPI) types.

	Recipes			
Ingredients:	**PPI1-40**	**PPI2-40**	**PPI3-40**	**PPI4-40**
Pea Protein Isolate	40	40	40	40
Pea Protein Concentrate	38	38	38	38
Pea Flour	13	13	13	13
Pea Fiber	5	5	5	5
Salt	2	2	2	2
Oil	2	2	2	2
Total	**100**	**100**	**100**	**100**

Table 3.2. Pea-based high moisture meat analog (HMMA) recipes containing different inclusion levels of pea protein isolate (PPI) and pea protein concentrate (PPC).

	Recipes			
Ingredients:	**PPI1-30**	**PPI1-40**	**PPI1-50**	**PPI1-60**
Pea Protein Isolate	30	40	50	60
Pea Protein Concentrate	48	38	28	18
Pea Flour	13	13	13	13
Pea Fiber	5	5	5	5
Salt	2	2	2	2
Oil	2	2	2	2
Total	**100**	**100**	**100**	**100**

All recipes contained equal levels of pea flour (13%) (Ingredion, Westchester, IL), pea fiber (5%) (Cosucra, Warcoing, Pecq, Belgium), granulated salt (2%) (Cargill, Wayzata, MN), and high oleic sunflower oil (2%) (Columbus Vegetable Oils, Des Plains, IL). Ingredients were mixed together for each recipe in 150 lb batches. Nutritional composition of each ingredient was estimated from the specifications provided by the suppliers. Tables 3.3 and 3.4 show the overall nutritional composition of each recipe.

Table 3.3. Total estimated nutritional content of each recipe, differing in pea protein isolate (PPI) type.

	Recipes			
Nutrients:	**PPI1-40**	**PPI2-40**	**PPI3-40**	**PPI4-40**
Protein (%)	54.00	52.88	53.50	52.76
Starch (%)	10.36	10.36	10.36	10.36
Fiber (%)	8.62	8.62	8.62	8.62
Fat (%)	1.65	1.65	1.65	1.65

Table 3.4. Total estimated nutritional content of each recipe, differing in inclusion level of pea protein isolate (PPI) and pea protein concentrate (PPC).

	Recipes			
Nutrients:	**PPI1-30**	**PPI1-40**	**PPI1-50**	**PPI1-60**
Protein (%)	51.33	54.00	56.68	59.36
Starch (%)	10.56	10.36	10.16	9.96
Fiber (%)	10.12	8.62	7.12	5.62
Fat (%)	2.05	1.65	1.25	0.85

3.2.2. Moisture Content

Moisture content of the PPIs, PPC, and raw recipes was found according to AACC 44-19.01. Approximately 2 g of each sample was dried at 135 ºC for 2 hrs. Analysis was completed in triplicate.

3.2.3. Protein Content

The protein content of each PPI and PPC was analyzed, according to AACC method 46-30.01, using a LECO analyzer. A nitrogen to protein conversion factor of 6.25 was used and results were reported on an as-is basis.

3.2.4. Particle Size

Particle size of the raw PPIs and PPC was analyzed using an Alpine Air Jet Sieve E200 LS (Hosokawa Alpine Group, Augsburg, Germany). 100 grams of protein sample was weighed and loaded onto finest mesh screen. A negative pressure of 3400 Pa was applied to the underside of the sieve. Meanwhile, a rotating arm spun counterclockwise to disperse airflow and fluidize material sitting on the screen. This combination of pressure and dispersion removes and transports particles finer than the screen, through the sieve, and into a collecting jar. Nine sieves were used, starting at the finest mesh size and progressively increasing to the largest mesh size. The set of sieves used are as follows: 25, 32, 53, 63, 75, 90, 106, 125, and 250 μm. Times for sieving for each respective sieve are as follows: 4, 3, 3, 3, 3, 3, 3, 3, 2 minutes. Weights of the overs were recorded and transferred to the next consecutive sieve. Cumulative distribution was calculated following analysis, based on recorded weights. Analysis was completed in duplicate.

3.2.5. Protein Solubility

Protein solubility for each PPI and PPC was determined according to the method reported by Shen et al. (2021). 0.4 g of protein was dispersed in 10 mL of water to attain 4% (w/v) solution. The suspensions were adjusted to pH 3 to 11 using either 1 M HCl or NaOH, accordingly. Each suspension was stirred for 30 min at room temperature, followed by centrifugation at 4000 ×g for 30 min to remove insoluble residues. Protein content in supernatants was determined using the Biuret method with BSA as a standard, with analysis being performed using a double beam spectrophotometer (VWR UV-6300PC), at 540 nm absorbance. Total protein content of original samples was measured by dissolving in DI water and adjusting to pH 13. Protein solubility was calculated using the following equation:

$$\textbf{Protein Solubility}\ (\%) = \frac{\text{Protein content in the supernatant}}{\text{Total protein content in the original sample}}$$

3.2.6. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed following the method of Laemmli (1970), under reducing conditions, to determine the protein molecular weight distributions of the PPIs and PPC. Protein samples were diluted with deionized water to 0.015% concentration and then centrifuged at 8000 ×g force for 5 min. The samples were then suspended in treatment buffer, consisting of 277.8 mM Tris-HCl (pH 6.8), 44.4% (v/v) glycerol, 4.4% SDS, and 0.02% bromophenol blue (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and heated for 10 min in boiling water. A 12% separating gel and 4% stacking gel were prepared and used to separate proteins via gel electrophoresis. 5 ul of the molecular weight marker was deposited in the first well and 12 ul of the protein supernatants were deposited in the remaining wells. Electrophoresis was conducted at room temperature under the following conditions: 200 V, 25 mA, 250 W. After protein separation, the gel was stained with Coomassie Brilliant Blue R250 (Bio-Rad Laboratories) and subsequently destained overnight to allow for visualization of the protein distribution.

3.2.7. Rapid-Visco Analysis

The viscosity of the PPIs, PPC, and raw recipes was measured to characterize rheology behavior differences between pea proteins. Evaluation of samples was assessed according to AACC method 76-21 STD 1, using the Rapid Visco Analyzer (RVA) 4800 (Perten Instruments, Perkin Elmer, NSW, Australia). In short, approximately 3.5 g of recipe was combined with 25 mL of distilled water, to obtain 14% (w/v), dry matter basis; the solids were adjusted accordingly to correct for inherent moisture differences between the samples. Hydrated samples were then placed in the RVA chamber and heated to 50 ºC while being stirred under a constant shear rate of 960 rpm for 10 s. It was held at 50 ºC for 50 sec and then heated up to 95 ºC under a shear rate of 160 rpm. It was held there for 3 min and then cooled back down to 50 ºC. Peak and final viscosity values were captured for each sample. Pasting time and temperature were also evaluated. Testing was completed in triplicate.

3.2.8. Least Gelation Concentration

Gelling capability of pea proteins was characterized by least gelation concentration (LGC) for each PPI and PPC, using slight modifications of the method described by Zhu et al. (2017). This was conducted by dispersing different concentrations of each PPI and PPC (12-20% w/v) in DI water, heating at 95-100 °C for 1 hr, immediately cooling in a cold-water bath, and transferring to a refrigerator at 4 °C for 2 hr. LGC was determined, after chilling, as the minimum concentration of protein that forms a stable gel that does not fall or run upon inversion of the test tube.

3.2.9. Differential Scanning Calorimetry

Denaturation of the PPIs and PPC was determined using a Differential Scanning Calorimeter (DSC) Q100 (TA Instruments Inc., New Castle, DE, USA), according to the method of Brishti et al. (2017), with slight modification. The instrument was calibrated using an empty pan as a reference. 5-10 mg of protein was weighed into a stainless-steel pan and hermetically sealed. The pan was heated from 25 °C to 250 °C at a rate of 10 °C/min. Each sample was measured in triplicate. Onset, peak denaturation, and end temperatures were recorded.

3.2.10. Size Exclusion Chromatography by High Performance Liquid Chromatography (SEC-HPLC)

The molecular weight distribution of pea proteins, raw recipes, and extruded recipes was estimated by size exclusion chromatography, using an Agilent 1100 HPLC system (Santa Clara, CA, USA), equipped with a Phenomenex SEC-4000 column (7.8 × 300 mm, Phenomenex, Torrance, CA). To prepare the extruded recipes for analysis, the samples were dried for 48 h at -105 ℃, 0.005 mbar, in a freeze dryer (FreeZone 4.5 Liter Benchtop Freeze Dry System, Labconco, Kansas City, MO, USA). Following, the dried samples were ground into a fine powder using a coffee mill (Casara coffee grinder, Model: SP-7440) for 30 s. To extract the protein, samples were dissolved at 1 mg/mL in 0.05 M sodium phosphate buffer (pH 6.8) containing 2% SDS (w/v). After shaking for 1 h at 250 rpm and centrifugation at 8000 ×g for 5 min, the supernatant was collected and filtered through a 0.45 μm Nylon membrane (Fisher

Scientific, Hampton, NH). Then, 20 μL of each sample was injected into the system for separation. The column temperature was maintained at 30 °C. The mobile phase, including 0.1% trifluoroacetic acid in water (phase A) and acetonitrile (phase B), was set at the following gradient conditions: 20-30% phase B at 0-20 min, 30-35% phase B at 20-25 min, and 35-20% phase B at 25-40 min to elute the residues. The proteins were separated at a flow rate of 0.7 mL/min and detected at 214 nm using a diode array detector (Agilent, Santa Clara, CA, USA). Protein standards, including thyroglobulin bovine (670 kDa), γ-globulins from bovine blood (150 kDa), and chicken egg grade VI albumin (44 kDa), were used as molecular weight references and analyzed at the same chromatography conditions.

3.2.11. Extrusion Processing

Processing was performed similar to Chapter 2. Each recipe was mixed for 5 min using a batch ribbon blender (Wenger Manufacturing, Sabetha, KS, USA). The recipes were processed on a pilot-scale twin-screw extruder (TX-52, Wenger Manufacturing), equipped with a differential diameter cylinder preconditioner with a volumetric capacity of 0.056 m3 (DDC2, Wenger Manufacturing). Water was injected into the preconditioner at a rate ranging from 12-16 kg/hr, and into the extruder at a rate ranging from 2-9 kg/hr, depending on recipe requirements. The recipes were initially fed into the preconditioner at 35 kg/hr, and then subsequently transferred into the extruder having a screw speed fixed at 450 rpm. The screw profile was the same as before, found in Figure 2.1. Five heating zones were utilized to heat the extruder barrel; their respective temperatures were set at 25, 110, 110, 130, and 135 °C, moving from inlet to discharge end of the extruder. The same cooling die was also used as before, displayed in Figure 2.2. The first section was run without cooling while the second die section was cooled with water, directly controlled by a manual throttle valve. Product was cut by hand at the die exit, into approximately 1 ft long pieces, with a Chef's knife. All collected samples were immediately transferred to plastic totes and stored frozen until analysis.

A wattmeter, equipped to the extruder motor, was used to directly measure the mechanical energy required to turn the extruder screws. Specific mechanical energy (SME) was assessed from the power input given in kW. This was converted to kJ/kg with the following formula:

$$\textbf{SME}\ (\mathrm{kJ/kg}) = \frac{(P-P_0)}{\frac{\dot{m}}{3600}}$$

where P is motor power reading in kW, P_0 is the no load motor power reading in kW, and $\dot{m}$ is mass flow rate in kg/hr.

In-barrel moisture (IBM) and die cooling water injection rate were optimized for effective processing of each product. IBM requirement stayed relatively constant between recipes (40-42% w.b.) but was slightly higher (44-45% w.b.) for the two recipes containing highest levels of PPI. This was adjusted to facilitate flow of the material through the extruder and to optimize texturization of each product.

IBM content was calculated using the following equation:

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

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

3.2.12. Texture Analysis

Cutting test and texture profile analysis (TPA) were utilized to evaluate the overall texture and physical properties of the extruded HMMA products. Evaluation of samples was performed using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Prior to analysis, the frozen, extruded products were thawed in a 2% saltwater brine at room temperature for 20 min to prevent desiccation. Former research by Kim et al. (2021) supports this method for maintaining consistency and quality in product texture during thawing.

The cutting strength was measured in both the longitudinal and transverse direction, according to the procedures described by (Zahari et al., 2020). Samples were cut into squares with dimensions of 3 x 3 cm and then analyzed. A guillotine knife blade (70 mm width x 100 mm height x 3 mm thickness), at a test speed of 3 mm/s, was used to cut through the entire height of the sample. Four animal meat anchors (beef steak, pork chop, chicken breast, salmon fillet) were also measured for texture comparison, using the same test procedures. Animal meat was purchased from Dillons Food Store (Manhattan, KS). The meat anchors were thawed in a refrigerator overnight, then cooked on a Proctor Silex nonstick electric griddle at 350 °F to internal temperatures recommended "as safe" by USDA (USDA, 2020). Products were flipped every 10 minutes until done. A list of cook times, cooking losses, and product specifications are detailed in Table 3.5. To calculate the moisture loss from cooking, the equation below was used:

$$\textbf{Total Cooking Loss}\ (\mathbf{g}) = \text{Initial weight (g)} - \text{Weight after cooking (g)}$$

Table 3.5. Product identification, weight, moisture loss, and cooking time of animal meat anchors.

Meat Anchors	Product ID	Initial weight (g)	Weight after cooking (g)	Total cooking loss (g)	Cook Time (min)
Beef Steak	USDA Choice Beef Top Sirloin Steak Boneless	734.50 ± 64.35	611.00 ± 110.31	122.50 ± 45.96	19:41 ± 1:24
Pork Chop	Pork Loin Center Cut Chops Boneless	127.78 ± 18.71	112.78 ± 16.42	15.00 ± 3.50	10:26 ± 1:10
Chicken Breast	Tyson Boneless Skinless Chicken Breasts	403.33 ± 68.17	303.00 ± 57.07	100.33 ± 22.25	58:40 ± 0:56
Salmon	Salmon Atlantic Fresh Farm Raised Fillet Family Pack Color Added	1155.50 ± 79.90	976.50 ± 26.16	179.00 ± 53.74	22:03 ± 0:27

10 replicates were measured for all extruded products and meat anchors for each direction of cut. Data was collected for firmness and toughness values.

TPA was also conducted on both the extruded HMMA products and animal meat anchors. Samples were cut into squares with dimensions of 3 x 3 cm, as described above for cutting test. A 50 mm diameter aluminum cylinder probe compressed the sample twice to 25% strain, with a contact force of 1000 g, at a test speed of 2 mm/s, and a wait time of 2 s between each compression. Meat anchors, cooked following same procedures done for cutting test, were also measured using TPA, for comparison. Fifteen replicates were measured for each sample. Data was collected for attributes of hardness, resilience, cohesiveness, and gumminess for all products. Hardness is denoted as the peak force during the first compression. Resilience is defined as “how hard a product fights to regain its original height." It is calculated by dividing the upstroke energy of the first compression by the downstroke energy of the first compression.

Cohesiveness is calculated as the area of work during the second compression divided by the area of work during the first compression. It demonstrates how well the product withstands a second deformation relative to its resistance under the first deformation. Gumminess is an extrapolated value and is simply calculated as hardness x cohesiveness (Texture Technologies, n.d.).

3.2.13. Statistical Analysis

Data from analysis was evaluated using IBM SPSS Statistics version 25.0 software (SPSS Inc., Chicago, IL, USA) and SAS software (SAS, Cary, NC, USA). All means and pair-wise differences were calculated using 1-way ANOVA and Tukey's test. Results with significance level of ($\rho < 0.05$) were considered to be statistically significant.

3.3. Results and Discussion

3.3.1. Protein Content

As shown in Table 3.6, PPI1 had the highest protein content (80.00%), followed by PPI3 (78.75%), PPI2 (77.19%), and PPI4 (76.88%). The protein content of PPC was significantly lower than the PPIs (53.22%).

Table 3.6. Protein content for different pea protein isolate (PPI) types and pea protein concentrate (PPC).

	Protein Content (%)
PPI1	80.00 ± 0.00 ^A^
PPI2	77.19 ± 0.44 ^C^
PPI3	78.75 ± 0.00 ^B^
PPI4	76.88 ± 0.00 ^C^
PPC	53.22 ± 0.04 ^D^

3.3.2. Particle Size

Particle size is reported to play a significant role in low moisture extrusion, especially in regard to the processing and texture-forming properties of the ingredients (Osen et al., 2014). Hence, the particle size was measured for each of the PPIs and PPC. Their cumulative distributions are shown in Table 3.7. Of the different PPIs, PPI1 had the largest particle size, followed closely by PPI4. At 63 μm, differences in particle size between PPIs and PPC are apparent. Greater than 90% of the particles for PPI2, PPI3, and PPC were smaller than 63 μm, while only 30% of the particles were smaller for PPI1 and PPI4. The variation in particle size is due to differences in pea protein grinding methods (Osen et al., 2014).

Table 3.7. Particle size distribution for pea protein isolate (PPI) types and pea protein concentrate (PPC).

	Cumulative (%)				
Sieves (μm)	**PPI1**	**PPI2**	**PPI3**	**PPI4**	**PPC**
25	1.5	3.9	4.2	2.3	0.3
32	5.2	11.5	4.9	4.8	1.4
53	27.8	81.5	93.4	23.3	71.6
63	33.8	92.1	95.1	33.4	92.3
75	41.4	96.4	96.2	43.2	99.9
90	50.7	98.6	97.1	54.6	100.0
106	57.9	99.5	97.7	63.0	100.0
125	66.0	100.0	98.2	71.4	100.0
250	96.2	100.0	99.9	97.3	100.0

3.3.3. Protein Solubility

As expected (Shen & Li, 2021), minimal solubility was observed for all pea proteins at the isoelectric point around pH 4.5, with solubility increasing when the pH was further increased or decreased beyond the isoelectric point (Figure 3.1). PPC had the highest solubility at each pH value while PPI2 had the lowest solubility. PPI1, PPI3, and PPI4 possessed similar protein solubility trends from pH 3 to 11. According to Osen et al. (2014), protein solubility reflects the heat treatment history of proteins, with a lower solubility following extensive heat treatment from mechanical grinding or high-temperature spray-drying.

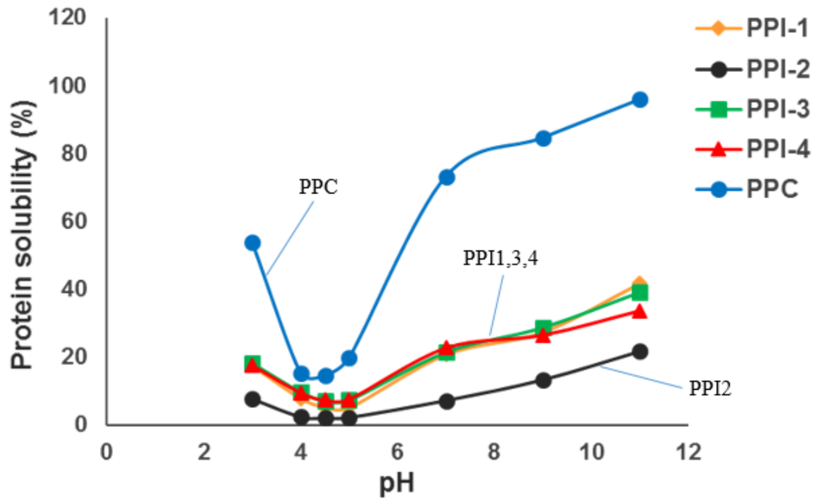


Figure 3.1. Protein solubility curves of pea protein isolates (PPIs) and pea protein concentrate (PPC).

3.3.4. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis

The molecular weight profiles of the PPIs and PPC were analyzed via SDS–PAGE. Results are shown in Figure 3.2. Pea proteins are comprised of two major components, legumin and vicilin, as well as a small amount of convicilin (Tang et al., 2021). All bands show the presence of each of these components, although variation in color intensity is apparent. The intensity of the lines suggests differences in protein solubility. Darker bands indicate higher solubility while lighter bands indicate lower solubility (Hosseinpour et al., 2011; Tang et al., 2021; Webb, 2021).

PPI3 and PPI4 contain almost identical bands, providing inference for their similar processing conditions during extrusion. PPI2 profile is nearly transparent, with darker bands only appearing at Lα and V. The combination of fewer and lighter bands suggests lower protein solubility for PPI2 (Hosseinpour et al., 2011; Webb, 2021), while the darker bands for PPC suggests higher protein solubility; this was confirmed using the Biuret method (see Figure 3.1). Darker bands are also noted in PPI1, indicating higher solubility; the cold-paste solubility identified using RVA (Figure 3.3) provides confirmation for its high solubility. Overall, the differences in SDS-PAGE profiles between the pea proteins are related to the plant species, protein extraction methods, and previous processing history (Tang et al., 2021).

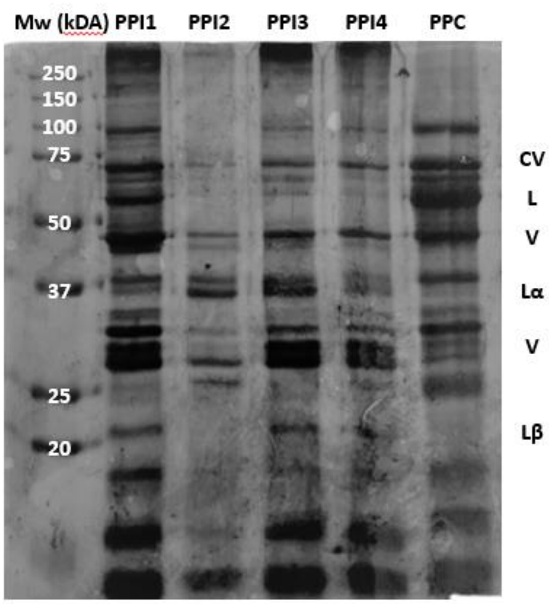


Figure 3.2. Bands of pea protein isolates (PPIs) and pea protein concentrate (PPC) from sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), under reducing conditions. Mw = molecular weight markers. Bands of CV = convicilin, L = legumin, V = vicilin, Lα = Legumin alpha, and Lβ = Legumin beta are identified across lanes.

3.3.5. Rapid-Visco Analysis

The Rapid-Visco Analyzer (RVA) was used to measure rheology differences among the individual pea proteins and raw recipes. Distinctions in flow properties were evident between the various PPIs when subjected to heat, moisture, and shear. The pasting profiles relate to the proteins' functional properties, namely protein solubility, water binding capacity, and heat gelation (Osen et al., 2014). Figure 3.3 is a collective graph including one replicate of each PPI and PPC run on the RVA.

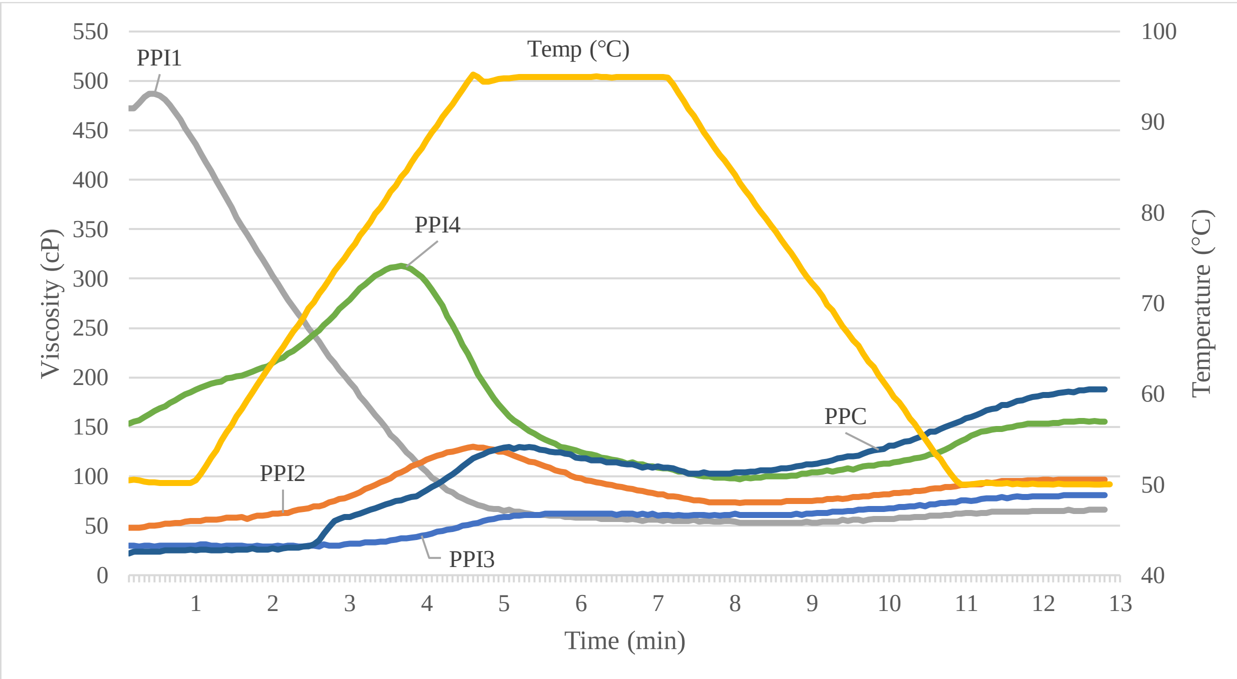


Figure 3.3. Rapid-visco analysis curves for pea protein isolate (PPI) types and pea protein concentrate (PPC).

PPI1 showed high initial cold-water viscosity, with an average peak reaching 489 cP, that immediately decreased upon heating to 95 °C; however, average final viscosity for PPI1 was lowest among all PPIs, at 68 cP. Upon hydration, this protein powder absorbs water and swells, resulting in a high starting viscosity that subsequently decreases with the addition of thermal energy and shear (Osen et al., 2014). It was observed that PPI1 also led to the highest SME among PPI types (Table 3.13). PPI4 demonstrated hot-paste viscosity, with an average peak occurring at 293 cP after heating, which subsequently declined to a final viscosity of 145 cP. In contrast, PPI2 and PPI3 had low starting viscosities, that marginally increased during heating to 95 °C. The low starting viscosity can be attributed to lower protein solubility and particle size (Osen et al., 2014), relative to PPI1 and PPI4. High solubility of PPI1 and low solubility of PPI2 is the same as found in SDS-PAGE. These observations show that the PPI types vary in terms of their functional properties.

Initial viscosity is likely attributed to protein solubility, while sudden increases in viscosity after heating, e.g. PPI4, relate to heat gelation properties. The superior heat-gelling ability of PPI4 is confirmed by LGC. It should be noted that higher protein solubility is desired for better functionality and texturization in low-moisture extrusion of TVP (Vatansever et al., 2020; Riaz, 2004); however, this is not always true for high-moisture extrusion applications, like HMMA. In general, the PPIs with lower solubility led to HMMAs with better visual quality and uniform texture.

Table 3.8. Rapid-visco analysis of pea protein isolate (PPI) types and pea protein concentrate (PPC). Cells labeled within each column with the same letter are not significant.

	Peak Viscosity (cP)	Final Viscosity (cP)	Pasting Time (s)	Pasting Temp (◦C)
PPI1	525 ± 59 A	68 ± 3 D	24 ± 11 E	50 ± 0 C
PPI2	115 ± 14 C	90 ± 6 C	293 ± 5 C	95 ± 0 A
PPI3	63 ± 2 C	83 ± 3 CD	349 ± 0 A	95 ± 0 A
PPI4	293 ± 19 B	145 ± 9 B	232 ± 0 D	85 ± 0 B
PPC	133 ± 5 C	182 ± 5 A	312 ± 0 B	95 ± 0 A

Recipes show quite different RVA curves due to the addition of concentrate, flour, and fiber (Figures 3.4 and 3.5). In general, the addition of these nutrients lowered the solubility and viscosity of the curves, compared to the individual pea proteins. All curves began at a low initial viscosity (~20-30 cP), but upon heat and shear addition, viscosity increased with time. PPI3-40 showed lowest peak viscosity (23 cP), which was consistent with Figure 3.3. The heat gelation property of PPI4 is again identified by a small peak occurring at ~150 s, after heating.

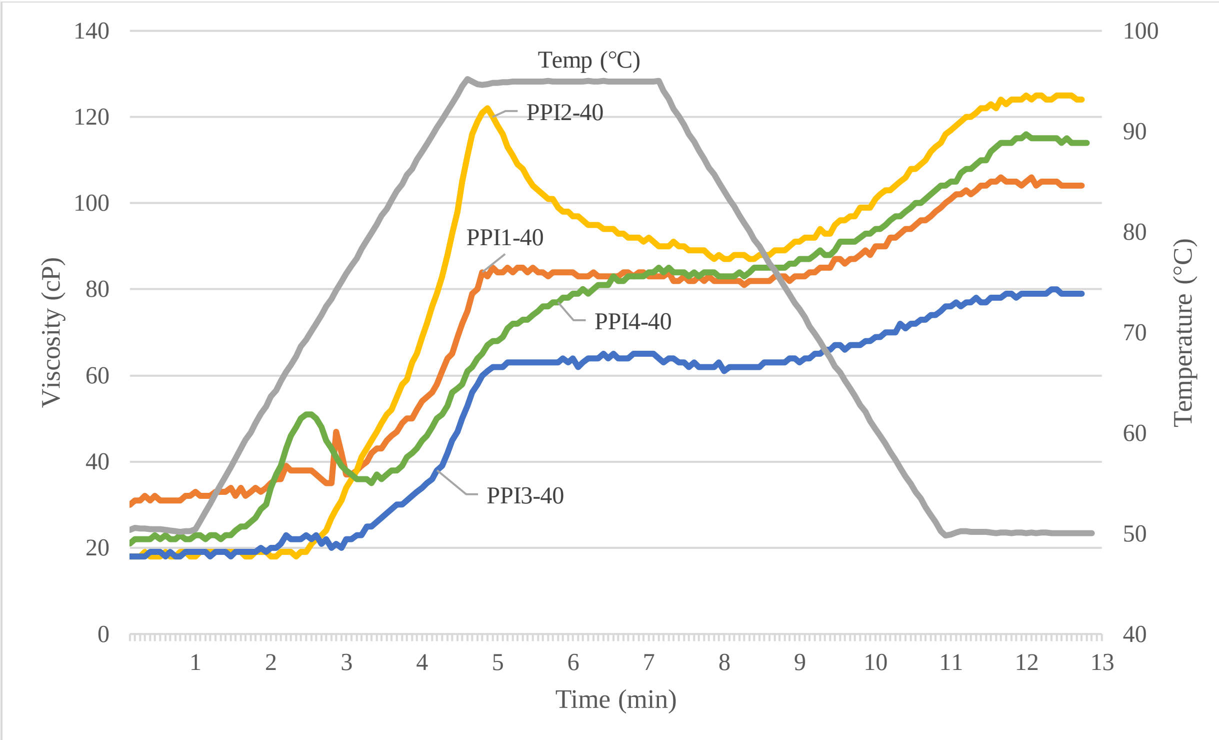


Figure 3.4. Rapid-visco analysis curves of each recipe containing different pea protein isolate (PPI) types.

Peak viscosity increased with higher PPI inclusion (32-91 cP), as found in Table 3.10; this observation was the same as found in Onwulata et al. (2013). Molecular weight of the recipes increases with greater substitution of PPI for PPC, inducing higher viscosity. As concentrate is substituted for isolate, overall molecular weight decreases, and peak viscosity is less. These results pair well with specific mechanical energy (SME) values. Lower viscosity is generated with lower PPI inclusion (30-40%); therefore, higher SME (946-1080.00 kJ/kg), by way of lower water input (40.57-41.99%), is required to achieve optimum product quality. The recipes higher in PPI inclusion (50-60%) generate higher viscosity, so less SME (761-781 kJ/kg) is needed to induce texturization and obtain optimum product quality.

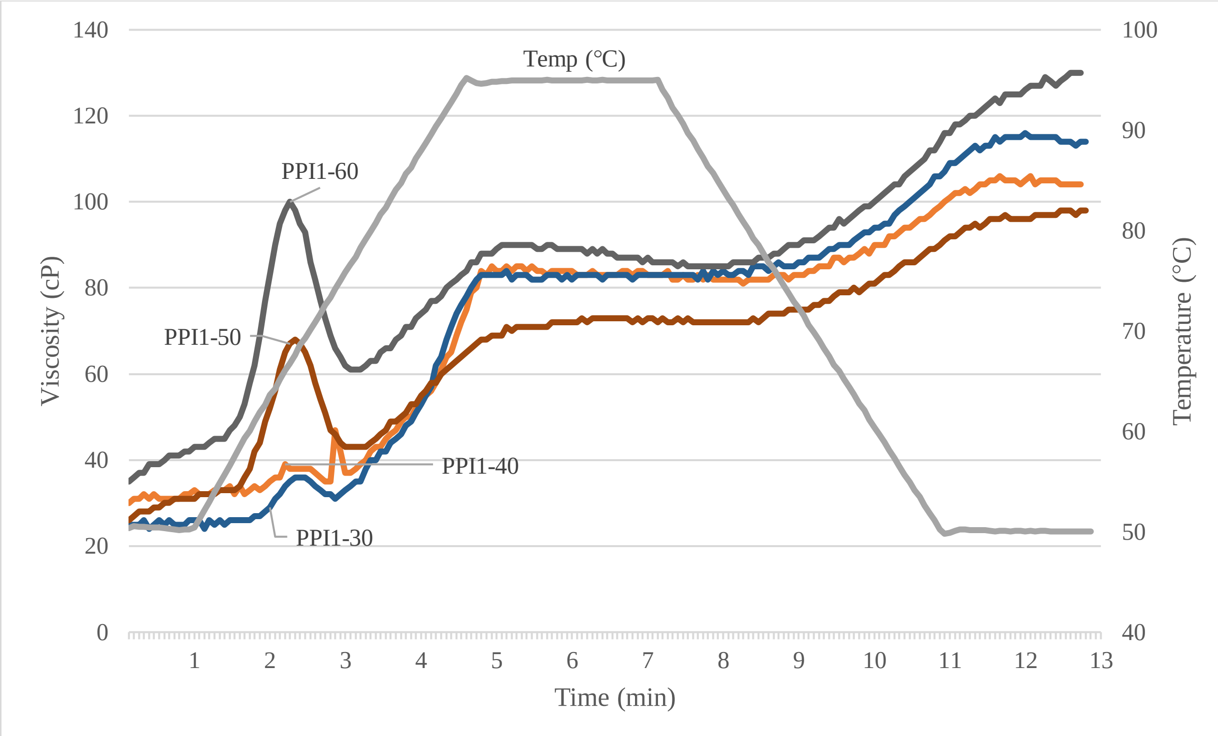


Figure 3.5. Rapid-visco analysis curves of each recipe containing different inclusion levels of pea protein isolate (PPI) and pea protein concentrate (PPC).

Table 3.9. Rapid-visco analysis of each recipe containing different pea protein isolate (PPI) types. Cells labeled with the same letter are not significant.

Recipe	Peak Viscosity (cP)	Final Viscosity (cP)	Pasting Time (s)	Pasting Temp (◦C)
PPI1-40	39 ± 7 BC	106 ± 5 A	153 ± 6 B	69 ± 1 B
PPI2-40	118 ± 9 A	117 ± 8 A	304 ± 4 A	95 ± 0 A
PPI3-40	23 ± 1 C	81 ± 10 B	165 ± 16 B	71 ± 3 B
PPI4-40	52 ± 6 B	119 ± 10 A	161 ± 2 B	70 ± 1 B

Table 3.10. Rapid-visco analysis of each recipe containing different inclusion levels of pea protein isolate (PPI) and pea protein concentrate (PPC). Cells labeled with the same letter are not significant.

Recipe	Peak Viscosity (cP)	Final Viscosity (cP)	Pasting Time (s)[10]	Pasting Temp (◦C)
PPI1-30	32 ± 7 C	118 ± 8 A	155 ± 5	69 ± 1
PPI1-40	39 ± 3 C	106 ± 5 AB	153 ± 6	69 ± 1
PPI1-50	64 ± 3 B	96 ± 6 B	151 ± 2	68 ± 0
PPI1-60	91 ± 8 A	118 ± 12 A	152 ± 0	69 ± 0

3.3.6. Least Gelation Concentration

LGC was performed on all PPIs and PPC. Results are displayed in Table 3.11. (+) symbol indicates a weak gel, while (++) symbol indicates a strong gel. Alternately, (-) symbol signifies no gelling occurred, while (/) symbol denotes an increase in viscosity but no firm gelling. Proteins exhibiting lower LGC are said to have greater heat-gelling capacity (Yang et al., 2021). Based upon the results, PPI1 did not increase viscosity or form a gel until reaching 16% protein concentration (w/v); for this reason, LGC was considered highest for PPI1. PPI2 and PPI3 increased viscosity at 14% w/v but did not fully form rigid gels until 16%. PPI4 and PPC exhibited lowest LGC at 12% protein concentration (w/v).

The low LGC for PPC and PPI4 was primarily caused by their higher heat-gelling capacities (Webb, 2021), which was consistent with RVA. The greater heat gelation capacity of PPI4, relative to the other PPIs, corresponds to its hot peak viscosity in RVA. PPI1 exhibited highest cold peak viscosity, but viscosity decreased sharply upon addition of heat, providing indication of lower heat gelling capacity. Nicolai & Chassenieux (2019) discovered that higher cold-water solubility can be achieved through protein hydrolysis but this results in a tradeoff of

[10] No significant differences were seen for pasting time and temperature. F value: 1.03, ρ > 0.4293 for pasting time. F value: 0.40, ρ > 0.7600 for pasting temperature.

lower gel strength. Thus, PPI1 may have been processed in a way to increase its cold-water solubility but was achieved at the expense of gel strength; this was confirmed by highest LGC found in PPI1. These results demonstrate that LGC, in combination with RVA, can be used to characterize proteins into cold swelling and heat swelling properties (Webb, 2021). In a recent publication by (Tulbek et al., 2017), it is noted that proteins having a lower gelation concentration possess a greater ability to bind water and fat. This could interrupt protein crosslinking, and may have led to the weaker internal texture as seen in PPI4-40 and PPI1-30.

Table 3.11. Least gelation concentration (LGC) of pea protein isolate (PPI) types and pea protein concentrate (PPC).

	LGC				
	12%	14%	16%	18%	20%
PPI1	-	-	+	+	++
PPI2	-	/	+	++	++
PPI3	-	/	+	++	++
PPI4	+	+	++	++	++
PPC	+	+	++	++	++

For future studies, using a quantitative method to assess gel strength could provide more conclusive results. Using a texture analyzer and penetration (needle) probe, the ‘bloom strength’ of gels could be measured to evaluate gel strength. Liu et al. (2019) gives detailed insight into specific methodology and instrumentation that can be used to perform this.

3.3.7. Differential Scanning Calorimetry

DSC was used to measure the denaturation temperature of the pea proteins. It is important to know this value prior to extrusion, as protein cross-linking will only occur above this temperature (Brishti et al., 2017). Denaturation can be identified by a change in heat enthalpy, which takes on the form of a peak. An example of this peak is depicted in Figure 3.6. Results, shown in Table 3.12, were different among PPI types. PPI1 showed significantly highest denaturation and end temperatures (190.82 °C and 206.75 °C, respectively), while PPC showed significantly lowest denaturation temperature (175.79 °C). The denaturation temperatures are also linked to the heat-gelling properties of the proteins. Denaturation temperature was highest for PPI1 and it possessed the poorest heat swelling properties as shown by RVA and LGC. In contrast, PPI4 and PPC have greatest heat swelling capability but lowest denaturation temperatures. The proteins with better heat-swelling capabilities are able to reach peak denaturation temperature much quicker by way of protein aggregation and more efficient heat transfer.

Table 3.12. Differential scanning calorimetry (DSC) of pea protein isolate (PPI) types and pea protein concentrate (PPC).

	Onset Temperature (°C)	Peak Denaturation Temperature (°C)	End Temperature (°C)
PPI1	161.50 ^{A}	190.82 ^{A}	206.75 ^{A}
PPI2	159.59 ^{AB}	183.76 ^{B}	199.32 ^{B}
PPI3	153.93 ^{C}	181.38 ^{BC}	196.04 ^{BC}
PPI4	154.42 ^{BC}	180.20 ^{C}	192.48 ^{BC}
PPC	156.85 ^{ABC}	175.79 ^{D}	190.81 ^{C}

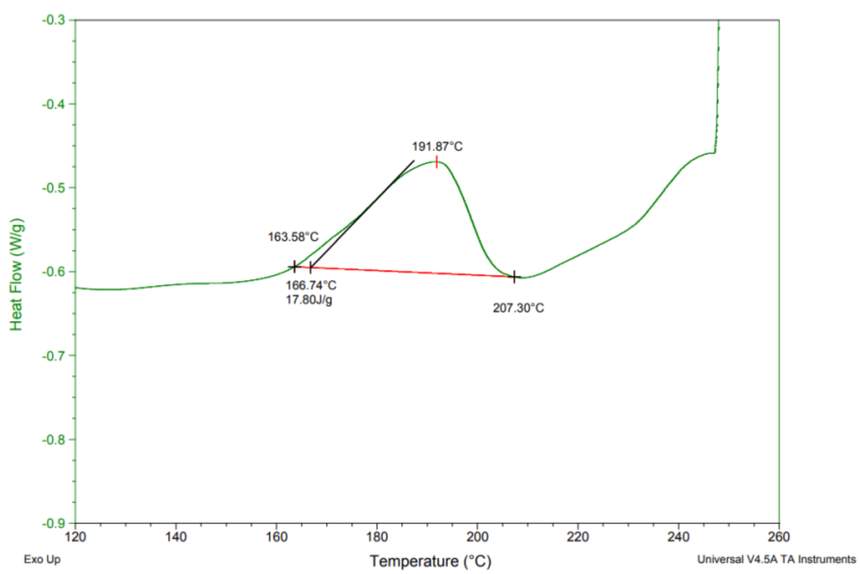


Figure 3.6. Example differential scanning calorimetry (DSC) curve from analysis of pea protein isolate (PPI)

3.3.8. Size Exclusion Chromatography by High Performance Liquid Chromatography (SEC-HPLC)

PPC had a higher proportion of lower molecular size proteins, as indicated by the relatively smaller absorbance peak around 670kDa and larger peak at 150kDa (Figure 3.7). The profile of PPC was comparable with PPI1. PPI2, PPI3, and PPI4 shared similar peak patterns, being composed of larger molecular sized proteins, with a dominant peak around 670kDa. A common trend between protein size and heat-gelling capacity was observed, specifically for PPI1 and PPI4. LGC was observed to be highest for PPI1 and lowest for PPI4; furthermore, PPI1 was more fractionated, i.e. contained more small-sized proteins, while less fractionation was observed in PPI4. Webb (2021) cited that stronger gels are often seen in pea proteins that are less fractionated. The high degree of fractionation found in PPI1, using SEC-HPLC, is a potential indicator for its poor heat-gelling capability.

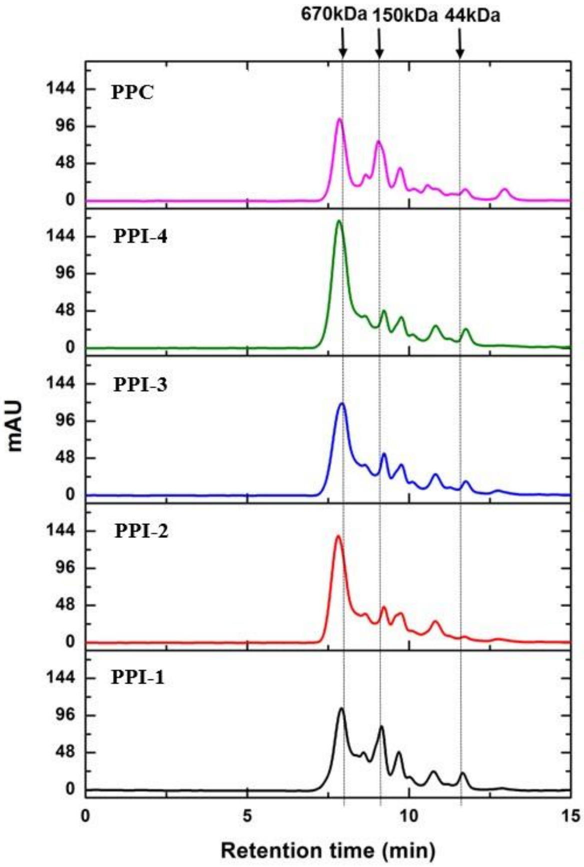


Figure 3.7. Size exclusion high performance liquid chromatography (SEC-HPLC) separation of pea protein isolate (PPI) types and pea protein concentrate (PPC) into peptide fragments.

SEC-HPLC results from the raw recipes, and their respective texturized extrudates, are presented in Figure 3.8. As shown in (A), PPI1-40 raw had a relatively higher proportion of lower molecular size proteins. This is evidenced by a relatively smaller absorbance peak around 670kDa and a larger peak around 150kDa, relative to PPI2-40, PPI3-40, and PPI4-40. This is consistent with results from Figure 3.7. After texturization, protein solubility decreased for all proteins dramatically, with much smaller peaks presented at 670kDa, and disappearance of the majority of the smaller proteins found in the raw recipes. The relatively flat chromatograms, after 670kDa, in the texturized samples, indicate lower solubility of the proteins, which is created by texturization, via cross-linking (Webb, 2021).

Figure (B) shows that, with an increase of PPI1 inclusion, particularly at 50 and 60%, a small peak at 44 kDa appeared after extrusion. This would imply incomplete texturization and/or protein cross-linking with higher amount of PPI in the formulation. The upper separation limit of the Phenomenex SEC-4000 column is around 700 kDa, which may explain why the newly cross-linked proteins in the texturized products were not identified through the SEC-HPLC.

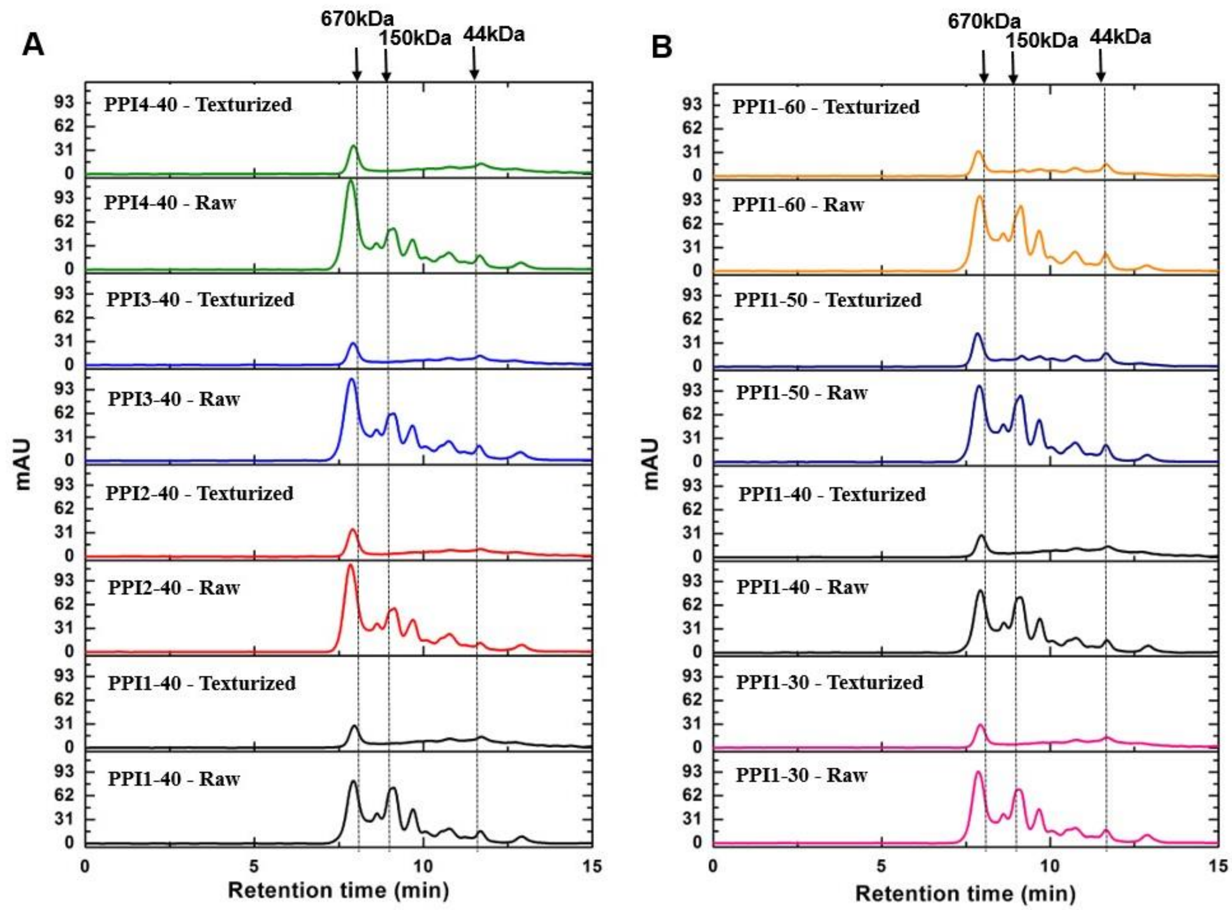


Figure 3.8. Size exclusion high performance liquid chromatography (SEC-HPLC) separation of raw recipes and their respective high moisture meat analog (HMMA) extrudates, into peptide fragments, for recipes differing in (A) pea protein isolate (PPI) type and (B) PPI inclusion level.

3.3.9. Extrusion Parameters

3.3.9.1 Moisture

IBM was optimized between recipes to target high-quality HMMA products, based on visual evaluation. As shown in Table 3.13, no major adjustments in IBM content (%) among the four PPI types were required. Prior research supports evidence that there is a positive correlation between IBM and protein inclusion (Zhang et al., 2020). At the lowest protein inclusion, i.e. PPI1-30, less IBM was required (40.57%). As PPI inclusion level increased, as seen in Table 3.14, IBM was increased as well (40.57-45.31%). Higher protein, by way of substituting PPI for PPC, requires more water for processing, given the increase in molecular weight (Webb et al., 2020).

3.3.9.2 Mechanical Energy

System response variables, such as die pressure, die temperature, and mechanical energy, are indicators of melt viscosity and are usually related to the quality of the final extrudate (Palanisamy et al., 2018; Chen et al., 2021). The outcome of these variables results from the combined interaction of independent process parameters and recipes. Given that the extrusion parameters were kept as constant as possible, apart from IBM, changes in mechanical energy are primarily driven by a three-way interaction between IBM, protein inclusion amount, and inherent differences among the PPI types.

SME is a measurement that allows mechanical energy to be quantified. These values, shown in Tables 3.13 and 3.14, correlate well with IBM and PPI inclusion. Lowest SME, die pressure, and die temperature were shown for PPI-50 and PPI-60, which were (a) processed at highest IBM and (b) contained highest overall protein inclusion. PPI1-30 contained lowest overall protein inclusion (51.33%) and was processed at the lowest IBM (40.57%). More mechanical energy was needed to achieve optimum product quality with lower inclusion of PPI.

As the PPI inclusion level increased, the level of SME needed to reach optimum product quality was lower given the positive effect of PPI on texturization.

SME ranged from 699.43 to 812.57 kJ/kg amongst PPI1-40, PPI2-40, PPI3-40, and PPI4-40. These slight differences are likely related to flow properties caused by differences in protein solubility, denaturation temperature, heat-gelation properties, and protein content of the PPIs. SME was highest for PPI1-40, while die temperature and pressure were lowest for PPI4-40. PPI1 exhibited high cold-paste solubility while PPI4 possessed greatest heat-gelling properties.

Table 3.13. Overall protein content (%), in-barrel moisture (IBM) (%), specific mechanical energy (SME) (kJ/kg), die temperature (°C), and die pressure (psig) for each recipe differing in pea protein isolate (PPI) type.

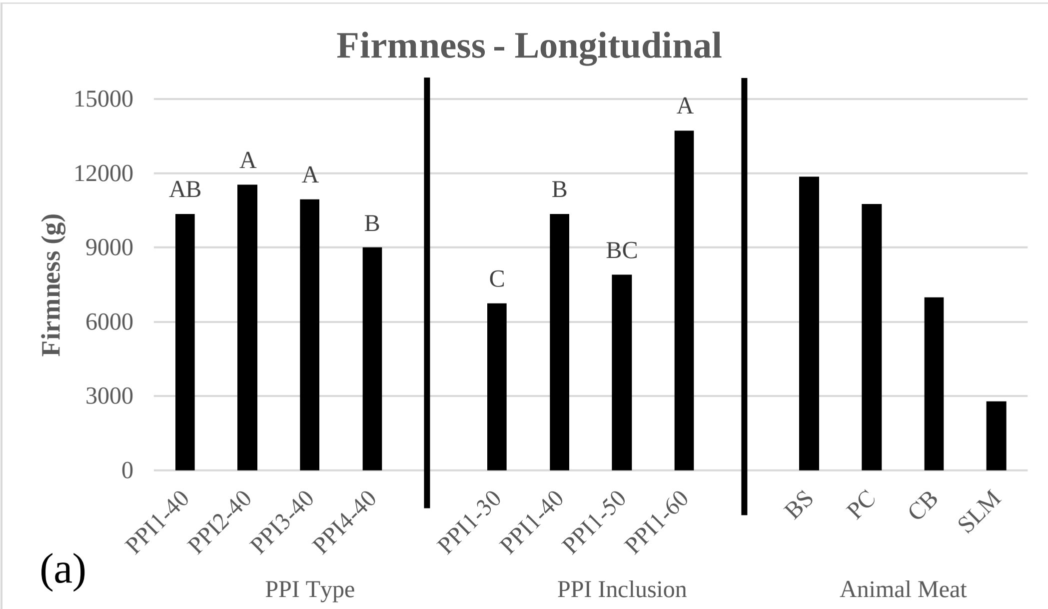
	Recipes			
	PPI1-40	**PPI2-40**	**PPI3-40**	**PPI4-40**
Protein (%)	54.00	52.88	53.50	52.76
IBM (%)	41.99	42.52	41.01	41.12
SME (kJ/kg)	812.57	750.86	699.43	709.71
Die Temperature (°C)	163	161	163	158
Die Pressure (psig)	850	825	650	575

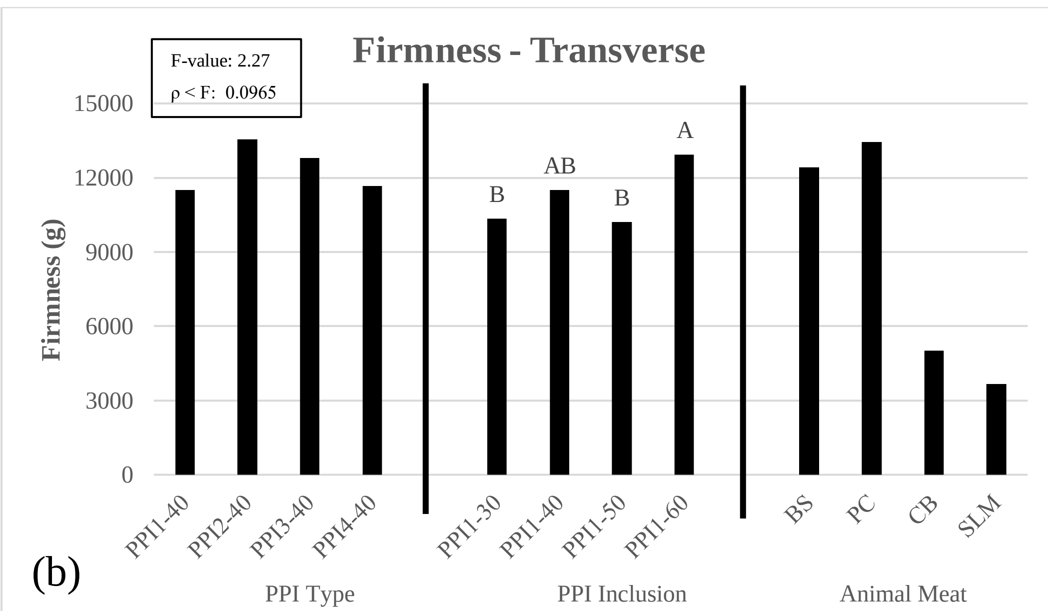
Table 3.14. Overall protein content (%), in-barrel moisture (IBM) (%), specific mechanical energy (SME) (kJ/kg), die temperature (°C), and die pressure (psig) for each recipe differing in the inclusion level of pea protein isolate (PPI) and pea protein concentrate (PPC).

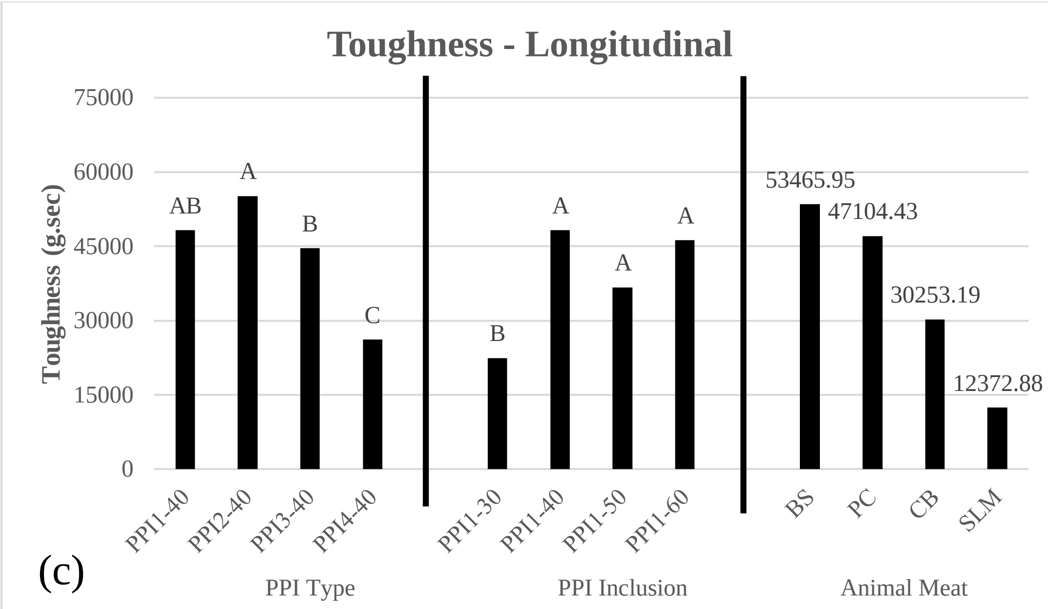
	Recipes			
	PPI1-30	**PPI1-40**	**PPI1-50**	**PPI1-60**
Protein (%)	51.33	54.00	56.68	59.36
IBM (%)	40.57	41.99	44.64	45.31
SME (kJ/kg)	678.86	812.57	514.29	493.71
Die Temperature (°C)	161	163	155	151
Die Pressure (psig)	575	850	400	425

3.3.10. Texture Analysis

Cutting test results are shown in Figure 3.9. Among recipes differing in PPI type, PPI2-40 displayed highest firmness (11,530-13,550 g) and toughness values (55,190-60,965 g.sec), having significantly higher toughness than PPI3-40 (44,620-50,970 g.sec) and PPI4-40 (26,110-33,725 g.sec). PPI4-40 exhibited lowest firmness and toughness compared to all other HMMAs differing in PPI type.







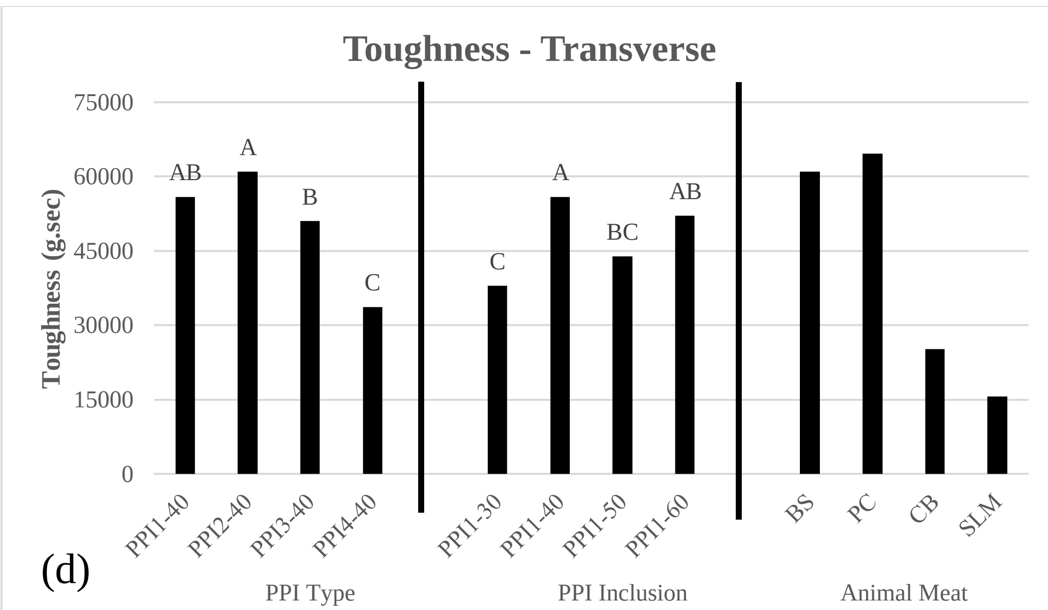


Figure 3.9. Cutting test results of pea-based high moisture meat analog (HMMA) products differing in pea protein isolate (PPI) type and PPI inclusion, and also animal meat anchors for (a) longitudinal firmness, (b) transverse firmness, (c) longitudinal toughness, and (d) transverse toughness. Within each individual bar cluster, bars labeled with the same letter are not significant.

In general, increasing the PPI inclusion level led to significantly higher product firmness, as in (Webb et al., 2020); toughness differences were not as pronounced. With more protein present in the recipe, a higher degree of texturization is able to occur during extrusion.

Almost all recipes, apart from PPI4-40 and PPI1-30, displayed similar firmness and toughness values to the beef steak and pork chop. Given their soft texture, PPI4-40 and PPI1-30 were more similar to the chicken breast, especially in regards to toughness. The HMMAs were too firm and tough to match the softness of salmon. Transverse to longitudinal cutting ratios (Tables 3.15 and 3.16) were much lower than chapter 2, emphasizing an increase in anisotropic, intermolecular bonding achieved by complementing PPI with PPC. A product image of each HMMA can be seen in Figure 3.10; the outer shell observed for P recipe in Chapter 2 was greatly reduced after supplementing PPI with PPC.

Table 3.15. Transverse to longitudinal cutting test ratios for high moisture meat analog (HMMA) recipes differing in pea protein isolate (PPI) type.

Recipe	Firmness Ratio	Toughness Ratio
PPI1-40	1.11	1.16
PPI2-40	1.18	1.10
PPI3-40	1.17	1.14
PPI4-40	1.30	1.29

Table 3.16. Transverse to longitudinal cutting test ratios for high moisture meat analog (HMMA) recipes differing in pea protein isolate (PPI) and pea protein concentrate (PPC) inclusion level.

Recipe	Firmness Ratio	Toughness Ratio
PPI1-30	1.53	1.69
PPI1-40	1.11	1.16
PPI1-50	1.29	1.20
PPI1-60	0.94	1.13

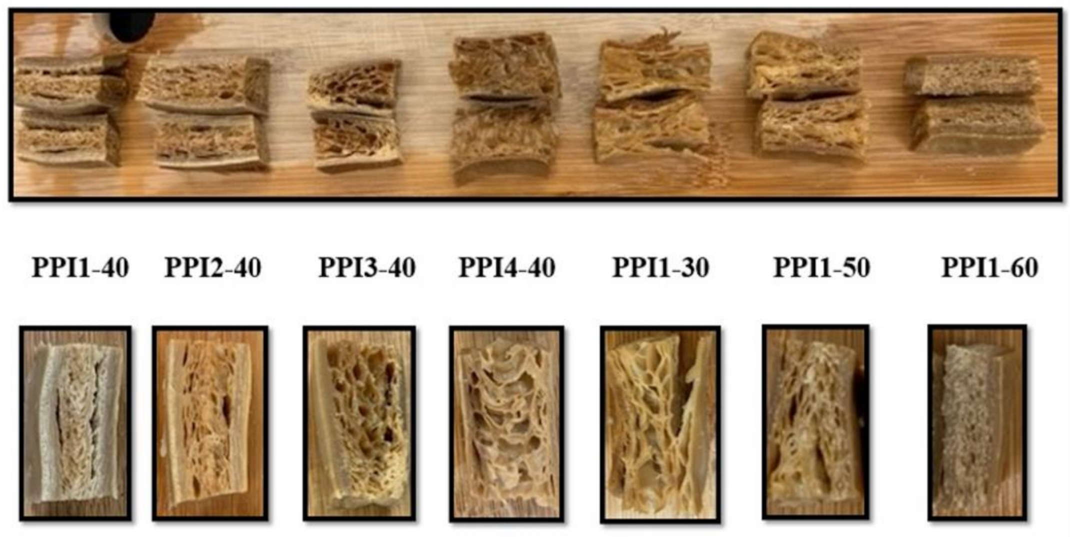
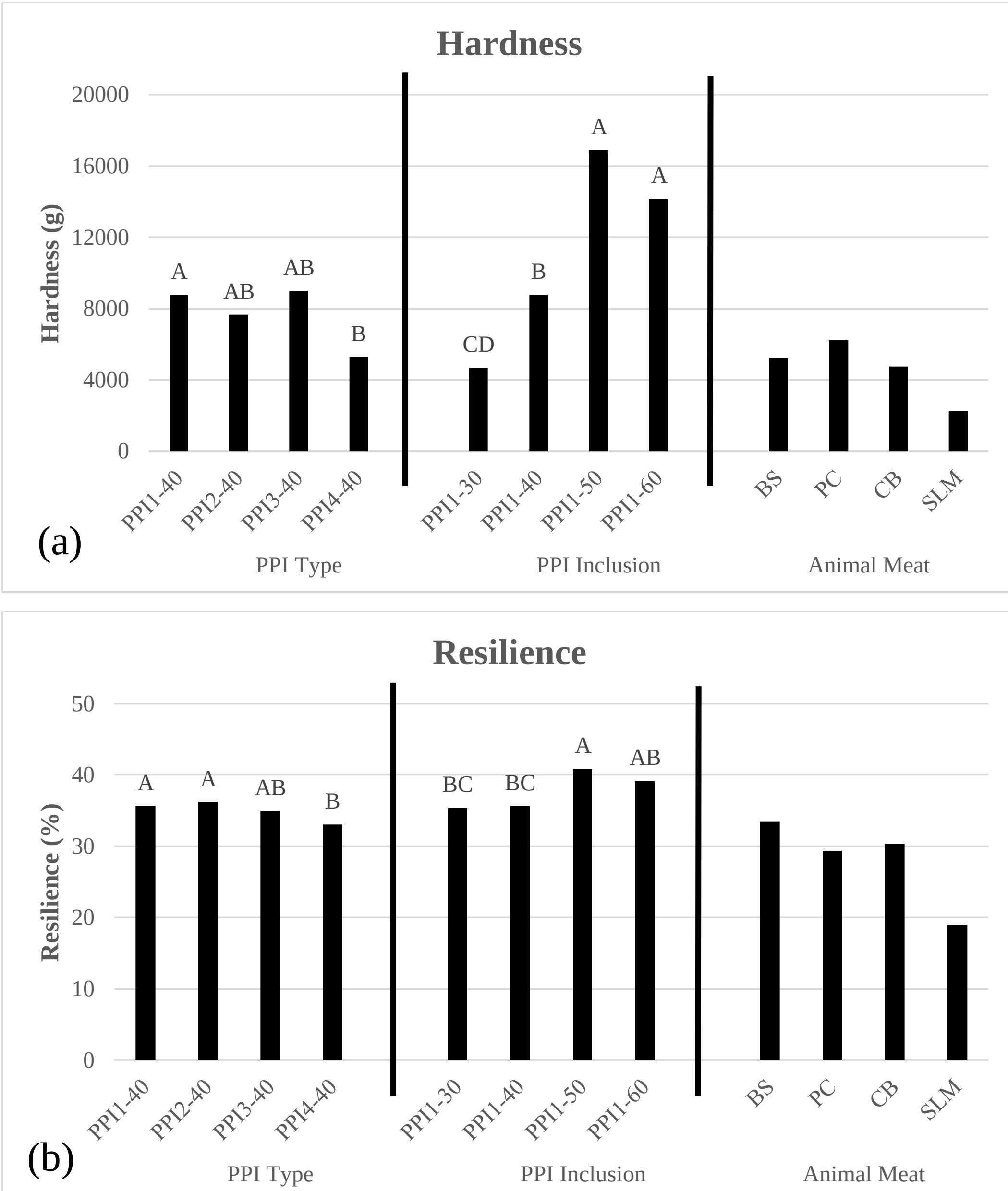
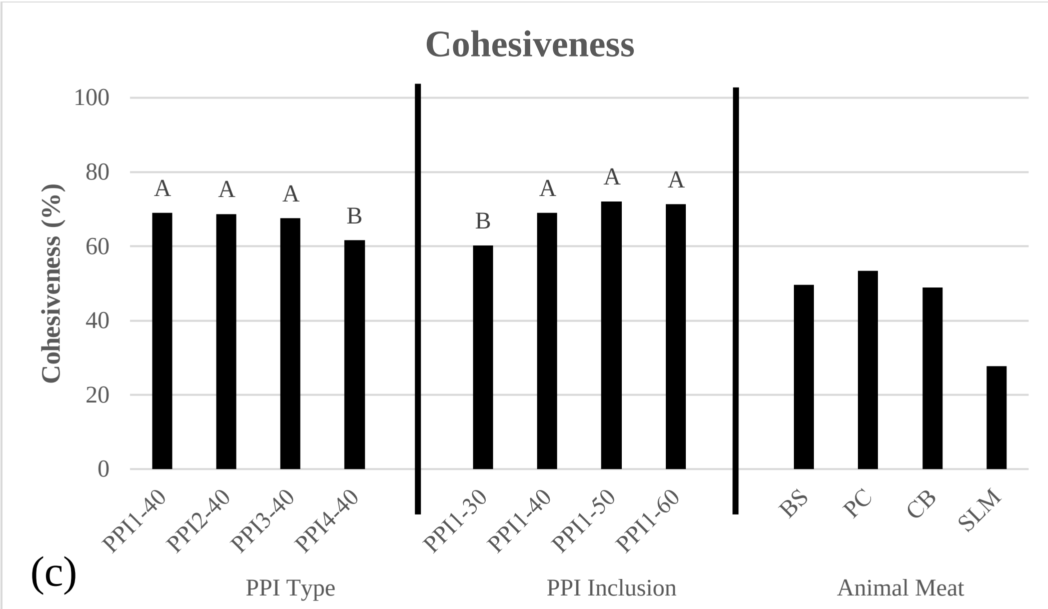


Figure 3.10. Cross-section photographs of extruded high moisture meat analog (HMMA) products.

TPA results for hardness, resilience, cohesiveness, and gumminess are displayed in Figure 3.11. A high contact force (1000 g) was used to assess ‘true’ internal product structure and eliminate irregularities caused by differences in piece thickness and shape. Among the HMMAs using different PPI types, PPI1-40, PPI2-40, and PPI3-40 demonstrated higher hardness, resilience, cohesiveness, and gumminess values than PPI4-40. PPI-2 showed highest resilience (36%) and cohesiveness (69%) compared to PPI1, PPI3, and PPI4, which accords with the high cutting test values. Very few correlations between raw materials, extrusion processing, and texture can be seen, providing indication that functional differences between the pea proteins are primarily related to their respective protein extraction methods and/or cultivate varieties (Shen et al., 2021). To note, PPI2 was lowest in protein solubility and led to highest cutting test and TPA values. PPI4 had greatest heat gelling capacity among PPI types and led to lowest texture values. These properties may be important to keep in mind for future HMMA studies.


Hardness
Hardness (g)
20000
16000
12000
8000
4000
0
A
AB
AB
B
CD
B
A
A
PPI1-40
PPI2-40
PPI3-40
PPI4-40
PPI1-30
PPI1-40
PPI1-50
PPI1-60
BS
PC
CB
SLM
(a)
PPI Type
PPI Inclusion
Animal Meat
Resilience
Resilience (%)
50
40
30
20
10
0
A
A
AB
B
BC
BC
A
AB
PPI1-40
PPI2-40
PPI3-40
PPI4-40
PPI1-30
PPI1-40
PPI1-50
PPI1-60
BS
PC
CB
SLM
(b)
PPI Type
PPI Inclusion
Animal Meat



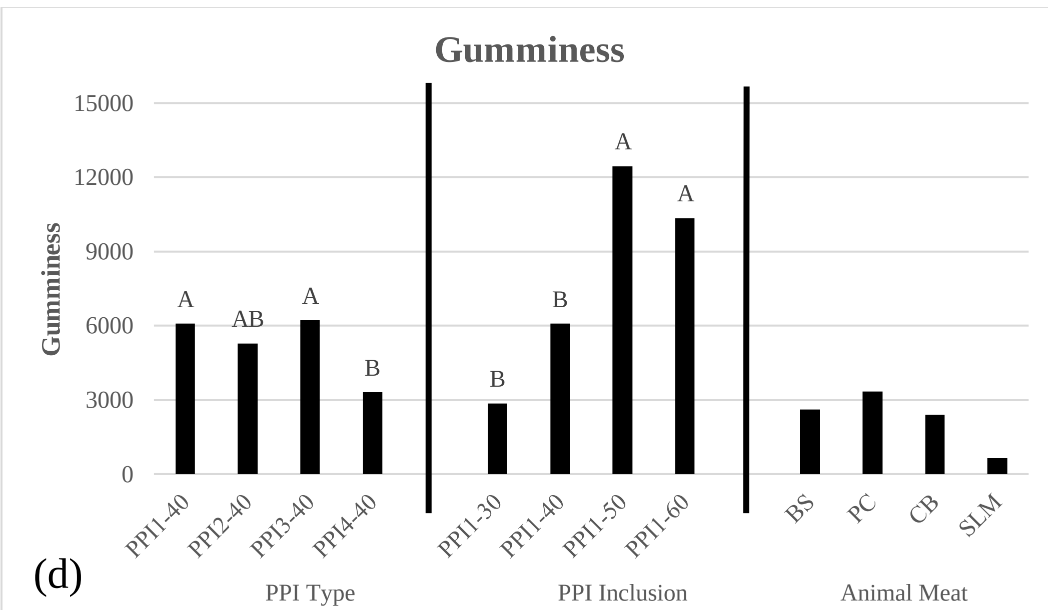


Figure 3.11. TPA results of pea-based high moisture meat analog (HMMA) products differing in pea protein isolate (PPI) type and PPI inclusion, and also animal meat anchors for (a) hardness, (b) resilience, (c) cohesiveness, and (d) gumminess. Within each individual bar cluster, bars labeled with the same letter are not significant.

In general, higher PPI inclusion also led to higher TPA values. PPI1-50 and PPI1-60 showed higher hardness, resilience, cohesiveness, and gumminess values compared to PPI1-30 and PPI1-40. All HMMA products showed statistically similar and/or higher values, compared to the animal meat anchors. PPI1-50 and PPI1-60 values were especially higher, confirming the significant effect that PPI inclusion has on product hardness and overall texturization. Unlike the animal meat anchors, the HMMA products did not contain additives, seasonings, or undergo heating prior to analyzing texture. Therefore, many of the values were significantly higher compared to their animal meat counterparts. With additional culinary preparation, e.g. heating, most of the HMMA products could likely be softened to provide a better texture match to their animal meat counterparts. As the culinary preparation facet was not a primary focus of this study, future work in this area is needed to provide more definitive results. From these results, PPI4-40 and PPI1-30 are more suited to target a softer meat product like chicken breast, whereas the other recipes could be specialized to match a beef steak or pork chop, given their similar textural attributes. PPI1-50 and PPI1-60 are perhaps too hard to mimic animal meat.

3.4. Conclusion

The texture and quality of HMMAs depend heavily on the type of pea protein isolate utilized, as well as its level of inclusion. Intrinsic differences found between the PPIs are likely to be most attributed to the specific plant species and respective protein extraction method. These variables create differences in particle size, protein solubility, and molecular weight of the protein polymers, which changes their functionality. Protein solubility and heat gelation properties seemed to be the biggest differences found among pea proteins. Higher visual product quality and uniform texture was found to be associated to the PPIs with lower protein solubility (PPI2 and PPI3). With regards to overall protein content, a higher inclusion of PPI (50-60%) in the recipe was found to significantly increase product hardness, firmness, and overall texturization, likely created by an increased amount of protein crosslinking. PPI4, which had highest heat-gelling capacity, induced lowest texturization. Conversely, PPI2, which exhibited lowest protein solubility, led to the greatest amount of texturization. In most cases, the HMMA textural attributes were similar to beef steak, pork chops, or chicken breast, but the salmon fillet was too soft to mimic. For future studies, the extrusion process should be assessed to understand its impact on final product texture of HMMAs.

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Chapter 4 - Impact of extrusion conditions and pea protein inclusion level on texture of high moisture meat analogs

Abstract

The meat alternative market has experienced a recent shift in demand toward using clean-label ingredients, like pea protein, given its non-GMO and low-allergenic status. High purchasing cost and lower functionality during extrusion processing has limited the use of pea protein, especially in high moisture meat analog (HMMA) applications. Regarding functionality, there is potential to improve the quality of pea-based HMMAs by pairing adjustments in formulation with extrusion process parameter modifications. Two formulations, differing only in pea protein isolate (PPI) inclusion level (50-60%), were processed on a Wenger twin-screw extruder (TX-52), equipped with a 6-foot cooling die. Three different feed rates (25, 30, 35 kg/hr) and two sets of barrel temperatures (last four heads: 80/110/130/130 °C and 90/120/140/150 °C) were applied to each recipe. Cutting test and texture profile analysis (TPA) were conducted on HMMA products and animal meat anchors, using a TA-TX2 texture analyzer, to assess physical product attributes. Cutting test results indicate that increasing PPI inclusion amount from 50% to 60% led to >48% increase in firmness and toughness values, when processed at low feed rate and barrel temperatures. The recipe containing 60% PPI and processed at the highest feed rate (35 kg/hr), displayed uppermost firmness value (22,600 g). TPA showed the two recipes processed at the highest feed rate (35 kg/hr) had upmost resilience values among treatments (62 and 61%), with the latter one also having the highest hardness value (31,230 g). Barrel temperatures were also shown to increase hardness (26,995-31,000 g) but not as significantly as feed rate (31,230 g). TPA showed all treatments being significantly higher in all quantifiable values compared to the animal meat anchors, but cutting test showed similar

attributes between the HMMAs and animal meat. The targeted animal meat application to be mimicked is contingent on product texture and appearance.

4.1. Introduction

Many consumers are progressively shifting away from consuming animal proteins due to their association with health, environmental, religious, and animal welfare concerns (He et al., 2020). Plant-based diets are perceived to be a healthier food option because of their capability to be cholesterol-free and contain high amounts of protein and fiber. Nutritional advantages, combined with sustainability concerns, are the two long-term driving forces for the plant-based meat market. With the global population expected to reach almost 10 billion people by the year 2050 (United Nations, 2019), the sustainability of the livestock production system may be compromised, due to limited land and water resources. These motivators have created a widespread market demand for alternative protein sources, like plant-based meat, over the past decade. A specialized extrusion process, using a long cooling die, enables the production of fresh, premium plant-based meat products called 'high moisture meat analogs' (HMMAs). This type of meat analog provides a means to mimic whole muscle animal meat products, rather than being used as an extender or primary protein source in ground meat applications, like that of dry textured vegetable proteins (TVPs).

HMMAs are manufactured using isolated and concentrated proteins from three primary crops, namely soybeans, wheat, and peas (Webb, 2021). Within the meat alternative market, there has been a recent shift in demand toward using clean label ingredients, like peas, given their non-GMO, gluten-free, and low-allergen ingredient status. Pea protein is a comparable option to wheat and soy in terms of nutrition and chemical properties, but there are a couple drawbacks to using peas; these include higher purchasing cost and lower functionality experienced during extrusion. High purchasing cost can simply be solved by the economic law of supply and demand, with an increase in supply leading to lower cost (Riaz, 2004). Recent drought and poor growing conditions in Canada has led to a reduction in supply, but production and market share of pea protein is expected to grow at a compound annual rate of 12.7% over the next several years (Market Analysis Report, 2021). From a functionality standpoint, there is potential to improve the quality of pea-based HMMAs by pairing adjustments in formulation with extrusion process modifications.

Therefore, the objectives of this study were to (a) determine the optimum inclusion ratio of pea protein isolate to pea protein concentrate and (b) understand how changing processing parameters, i.e. feed rate and barrel temperatures, and also cooling die design will impact the overall texture of HMMA products. Overall protein content and processing factors affect the physicochemical properties of recipes and can be altered to improve the texture of HMMAs; this will help enable these products to better mimic the flavor, texture, and appearance of whole muscle meat anchors, such as beef steak, pork chops, chicken breast, and salmon fillets.

4.2. Materials and Methods

4.2.1. Ingredients and Recipe Preparation

Two recipes were prepared for this trial, shown in Table 4.1, differing only in the inclusion amount of pea protein isolate (PPI) and pea protein concentrate (PPC).

Table 4.1. Complete recipes for each pea-based high moisture meat analog (HMMA), differing in the inclusion level of pea protein isolate (PPI) and pea protein concentrate (PPC).

	Recipes	
Ingredients:	**PPI-50**	**PPI-60**
Pea Protein Isolate	50	60
Pea Protein Concentrate	28	18
Pea Flour	13	13
Pea Fiber	5	5
Granulated Salt	2	2
Sunflower Oil	2	2
Total	**100**	**100**

For the purpose of discussion, each recipe is denoted by level of isolate inclusion (%). The first recipe is denoted as PPI-50, and the second as PPI-60. Both recipes contained equal amounts of pea flour (13%) (Ingredion, Westchester, IL), pea fiber (5%) (Cosucra, Warcoing, Pecq, Belgium), granulated salt (2%) (Cargill, Wayzata, MN), and high oleic sunflower oil (2%) (Columbus Vegetable Oils, Des Plains, IL). Ingredients were mixed together according to each recipe in 150 lb batches. Nutritional composition of each ingredient was estimated from the given specifications provided by the suppliers. Table 4.2 shows the complete nutritional profile for each recipe.

Table 4.2. Total estimated nutritional content of each pea-based high moisture meat analog (HMMA) recipe, differing in pea protein isolate (PPI) and pea protein concentrate (PPC) inclusion level.

	Recipes	
Composition:	PPI-50	PPI-60
Protein (%)	57.18	59.68
Starch (%)	13.80	12.30
Fiber (%)	9.53	8.33
Fat (%)	7.25	7.65

4.2.2. Moisture Content

Moisture content of the two recipes was found according to AACC 44-19.01. 2 g of each recipe was dried at 135 ºC for 2 hrs. Analysis was completed in triplicate.

4.2.3. Rapid-Visco Analysis

The viscosity of the two recipes was measured according to AACC 76-21.02, STD 1, using the Rapid-Visco Analyzer (RVA) 4800 (Perten Instruments, Perkin Elmer, NSW, Australia). Peak and final viscosity values, in addition to pasting time and temperature, were captured for each sample. Testing was completed in triplicate.

4.2.4. Water Binding Capacity

Water binding capacity (WBC), also known as water holding capacity or water absorption capacity, was performed to understand the degree to which certain concentrations of protein interact with water. The procedure was completed according to Webb (2021). 2.5 g of raw recipe was combined with 30 mL of water, then vortexed for 10 sec. Samples remained still at room temperature for 30 minutes, with 2 additional agitations during that time. They were then centrifuged at 3900 RPM for 30 min and the residual water was decanted. WBC was calculated using the following equation:

$$\mathbf{WBC}\ (\text{g water/g protein}) = \frac{(\mathrm{W_f} - \mathrm{W_i})}{\mathrm{W_i}}$$

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

4.2.5. Differential Scanning Calorimetry

Protein denaturation temperature of the recipes was determined using a Differential Scanning Calorimeter (DSC) (TA Instruments Inc., New Castle, DE, USA). This was done according to the method of Brishti et al. (2017), with slight modification. Onset, peak denaturation, and end temperatures were captured. Each sample was measured in triplicate.

4.2.6. Extrusion Processing

Processing was completed according to the procedures below. Four batches of each recipe were mixed for 5 min each using a ribbon blender (Wenger Manufacturing, Sabetha, KS, USA), for a total of eight treatments. Each treatment was subjected to its own, unique set of processing conditions, to measure the impact of both formulation and extrusion parameters on final product texture. Treatments are denoted with a six-letter descriptor. The first two letters indicate protein inclusion level; LP = low protein, HP = high protein. The next two letters specify feed rate; LR = low rate, MR = medium rate, HR = high rate. The final two letters relate to barrel temperatures; LT = low temperatures, HT = high temperatures. A composite experimental design and treatment structure can be located in Table 4.3.

Table 4.3. Experimental design and treatment structure for each pea-based high moisture meat analog (HMMA) recipe.

Treatments	Factors			
	Protein (%)	Feed Rate (kg/hr)	Barrel Temperatures (°C)[11]	Treatment ID
1	57.18	25	0, 80, 110, 110, 130	LPLRLT
2	59.68	25	0, 80, 110, 110, 130	HPLRLT
3	59.68	30	0, 80, 110, 110, 130	HPMRLT
4	57.18	30	0, 80, 110, 110, 130	LPMRLT
5	57.18	35	0, 80, 110, 110, 130	LPHRLT
6	59.68	35	0, 80, 110, 110, 130	HPHRLT
7	59.68	30	0, 90, 120, 140, 150	HPMRHT
8	57.18	30	0, 90, 120, 140, 150	LPMRHT

For analysis and statistical purposes, treatment structure was divided up into three separate designs to assess individual and interactive impact of each processing variable, i.e. protein inclusion, feed rate, and barrel temperatures. These split designs are shown in Tables 4.4-4.6. Data will be presented in this split-design arrangement throughout the remainder of this chapter.

Table 4.4. 1-way experimental design and treatment structure for each pea-based high moisture meat analog (HMMA) recipe processed at different feed rates (kg/hr).

Treatments	Factors		
	Protein (%)	Feed Rate (kg/hr)	Barrel Temperatures (°C)
HPLRLT	59.68	25	0, 80, 110, 110, 130
HPMRLT	59.68	30	0, 80, 110, 110, 130
HPHRLT	59.68	35	0, 80, 110, 110, 130

[11] Barrel temperatures are listed in order from inlet to discharge end of the extruder

Table 4.5. 2x2 experimental design and treatment structure for each pea-based high moisture meat analog (HMMA) recipe differing in protein inclusion (%) and feed rate (kg/hr).

	Factors		
Treatments	Protein (%)	Feed Rate (kg/hr)	Barrel Temperatures (°C)
LPLRLT	57.18	25	0, 80, 110, 110, 130
HPLRLT	59.68	25	0, 80, 110, 110, 130
LPHRLT	57.18	35	0, 80, 110, 110, 130
HPHRLT	59.68	35	0, 80, 110, 110, 130

Table 4.6. 2x2 experimental design and treatment structure for each pea-based high moisture meat analog (HMMA) recipe differing in protein inclusion (%) and barrel temperatures (°C).

	Factors		
Treatments	Protein (%)	Feed Rate (kg/hr)	Barrel Temperatures (°C)
LPMRLT	57.18	30	0, 80, 110, 110, 130
HPMRLT	59.68	30	0, 80, 110, 110, 130
LPMRHT	57.18	30	0, 90, 120, 140, 150
HPMRHT	59.68	30	0, 90, 120, 140, 150

The treatments were processed on a pilot-scale twin-screw extruder (TX-52, Wenger Manufacturing), equipped with a differential diameter cylinder preconditioner with a volumetric capacity of 0.056 m^3 (DDC2, Wenger Manufacturing). Treatments were first fed into the preconditioner at feed rates ranging from 25-35 kg/hr, and then transferred into the extruder having a screw speed fixed at 450 rpm. Water was injected into the preconditioner at a rate ranging from 12-15 kg/hr and into the extruder at a rate ranging from 4-9 kg/hr; rates were contingent on treatment constraints. The screw profile was the same as previous chapters, shown in Figure 2.1. The screw diameter was 52 mm, with an L/D ratio of 25.5. Five segmented temperature-controlled barrel zones were utilized to heat the barrel. For the first six treatments, their respective temperatures were set at 0, 80, 110, 130, and 130 °C, moving from the inlet to the discharge end of the barrel. Temperatures were raised for the final two treatments according to the experimental design (Table 4.3); therefore, the barrel zones were increased to 0, 90, 120, 140, and 150 °C, respectively. An extrusion gear pump (Polymer Systems Incorporated, Conover, NC, USA), equipped to the final head of the extruder, served as a transition point between the extruder and cooling die to regulate melt flow; the gear pump was fixed at 46 rpm for all treatments. This was followed successively by a 5/16” three-section, cooling slit die (Figure 4.1) to allow for protein alignment, texturization, and product cooling. The first die section was run without forced cooling to allow the protein fibers to set, align, and cross-link. The second and third die sections were cooled with water, directly controlled by a manual throttle valve. Cooling water injection rates were adjusted accordingly, to optimize product texturization, at a rate ranging from 25.6-142.4 kg/hr.

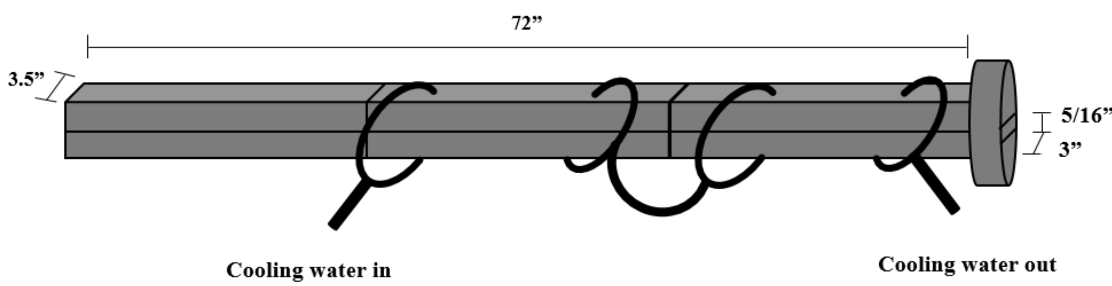


Figure 4.1. Cooling die configuration

Product was manually cut at the die exit into approximately 1 ft long pieces with a Chef’s knife. All collected samples were immediately transferred to plastic totes and stored frozen until analysis.

Mechanical energy was measured with a wattmeter, which was equipped to the extruder motor. A direct power reading was given in kW. This was converted to kJ/kg with the following formula:

$$\textbf{SME}\ (\text{kJ/kg}) = \frac{(P-P_0)}{\frac{\dot{m}}{3600}}$$

where P is motor power reading in kW, P_0 is the no load motor power in kW, and $\dot{m}$ is mass flow rate in kg/hr.

Extrusion process moisture and die cooling water injection rate were optimized for processing of each HMMA recipe. IBM was relatively consistent between treatments (45-47% w.b.) and was only slightly adjusted, as necessary, to optimize texturization of each product.

IBM content was calculated with the following equation:

$$\mathbf{IBM}\ (\%\ \mathrm{wb}) = \frac{(\mathrm{m_f} \times (\mathrm{X_{fw}}) + \mathrm{m_{pw}} + \mathrm{m_{ew}})}{\mathrm{m_f} + \mathrm{m_{pw}} + \mathrm{m_{ew}}}$$

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

4.2.7. Texture Analysis

Cutting test and texture profile analysis (TPA) were once again utilized to evaluate the overall texture of the HMMAs. Evaluation of samples was performed using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Prior to analysis, the frozen HMMAs were thawed in a 2% saltwater brine at room temperature for 20 min to prevent desiccation. After thawing, HMMA samples were fully submerged in vegetable oil and immediately transferred to a Proctor Silex nonstick electric griddle set at 350 °F. HMMAs were heated evenly on each side for 2 min, totaling four min of heating time. Warmed HMMAs were then cut into squares with dimensions of 3 x 3 cm for proper texture evaluation. The cutting strength was measured in both the longitudinal and transverse direction according to the procedures described by (Zahari et al., 2020). A guillotine knife blade (70 mm width x 100 mm height x 3 mm thickness), at a test speed of 3 mm/s, was used to cut through the entire height of the sample. The same four meat anchors used in Chapter 3, see Table 3.5, were used for texture comparison. 10 replicates were measured on both the extruded HMMA products as well as the animal meat anchors in both directions of cut. Data was collected for firmness and toughness values.

TPA was conducted on both the heated HMMAs and cooked animal meat anchors. Samples were prepared following same procedures done for cutting test, and were cut into squares with dimensions of 3 x 3 cm. A 50 mm diameter aluminum cylinder probe compressed the sample twice to 25% strain, with a contact force of 1000 g, at a test speed of 3 mm/s, and a wait time of 2 s between compressions. Fifteen replicates were measured on each product. Data was collected for attributes of hardness, resilience, cohesiveness, and gumminess.

4.2.8. Sensory Analysis

A comprehensive descriptive analysis was performed on a group of selected HMMA products from Chapters 3 and 4, as well as two animal meat anchors. 8 samples were evaluated in total. From Chapter 3, PPI3-40, PPI4-40, PPI1-30, and PPI1-60 products were selected for analysis. LPLRLT and LPHRLT, from Chapter 4, were also chosen for analysis. In addition, beef steak (beef loin, choice, KC strip steak, boneless) and chicken breast (chicken breast, skinless, boneless, 99% fat-free) were purchased from Dillon’s Food Store (Manhattan, KS), for sensory evaluation as well.

Prior to analysis, HMMAs and animal meat anchors were heated and cooked, respectively, using a non-stick skillet on a gas stovetop. HMMA samples were thawed in room-temperature 2% saltwater brine for 30 min, submerged in vegetable oil, and then transferred to a heated skillet. Products were heated for 4 min at 350 °F, with samples being flipped once after 2 min. Animal meat anchors were thawed overnight in a refrigerator, and then transferred to a skillet and cooked to safe temperatures as recommended by USDA (USDA, 2020). After cooking, samples were transferred to microwaveable bowls and sealed with aluminum foil to keep warm. Prior to serving, all samples were cut into ½" cubes and were warmed in a microwave on high power for 30 s. Individual analysis of the samples was done in duplicate using 4 highly trained professional panelists. The panelists were trained 1 day prior to analysis to allow them to become familiar with and understand the characteristics of a HMMA. Eight pieces of each sample were served to each panelist in 3.25 oz containers, each being blindly labeled with a random 3-digit code. The samples were ranked on attribute intensity scores ranging from 0-15 with 0.5 increments. A total of 17 attributes were identified, defined, and referenced. Panelists were provided unsalted crackers and deionized water for palate cleansing between samples. Textural attributes evaluated were springiness, denseness, juiciness, residual particles, firmness, chewiness, fiber awareness, tooth packing, astringent, and starchy. The flavor attributes evaluated were beany, starchy, grain, green, umami, barnyard, and cardboard. Definitions of each attribute can be found in Tables 4.7 and 4.8.

Table 4.7. Texture attributes and references for sensory analysis

Texture Attribute	Definition	Reference
Springiness	Degree to which sample returns to its original height when compressed once partially with molar teeth and slowly released.	Oscar Mayer Uncured Bun-Length Wieners
Denseness	The degree of compactness of the cross section.	Oscar Mayer Uncured Bun-Length Wieners
Juiciness	The amount of liquid expressed from the sample at the maximum intensity from 5 chews.	Hormel Cure 81 Ham
Residual Particles	Particles remaining in mouth after mastication and swallowing. Maybe fibers, flakes and/or granules.	Hormel Cure 81 Ham
Firmness	The force required to bite completely through the meat pieces with the molar teeth.	Hormel Cure 81 Ham
Chewiness	Difficulty with which the sample can be broken down with the molars for swallowing.	Hormel Cure 81 Ham
Fiber Awareness	The perception of filaments or strands of muscle tissue in product during mastication.	Hillshire Farms Lit'l Beef Smokies
Tooth Packing	The amount of sample packed in a between the molar teeth after swallowing.	Wheaties
Astringent	A drying puckering or tingling sensation on the surface and/or edge of the tongue and mouth.	Alum Solution
Starchy	Degree to which the sample mixes with saliva to form a starchy, pasty slurry that coats mouth surfaces after swallowing.	American Beauty Elbo-Roni

Table 4.8. Flavor attributes and references for sensory analysis

Flavor Attribute	Definition	References
Beany	A slightly brown, musty, slightly nutty and starchy flavor associated with cooked beans.	Bush's Best Pinto Beans
Starchy	The dry aromatics associated with starch and starch based grain products such as wheat, rice, oats and other grains.	Bush's Best Pinto Beans
Grain	A general term used to describe the aromatic which includes musty, dusty, slightly brown, slightly sweet and is associated with harvested grains and dry grain stems.	Cereal Mixture (dry)
Green	A green aromatic associated with fresh green peapods. May include beany, increased pungent, musty/earthy, bitter and astringent.	Great Value Frozen Baby Lima Beans
Umami	A general term for aromatics associated with juices from cooked seafood, meat and/or vegetables.	Button Mushroom Broth
Barnyard	Combination of pungent, slightly sour, hay-like aromatics associated with farm animals and the inside of a barn.	White Pepper in water
Cardboard	The flat aromatics that may be associated with cardboard or paper packaging.	Cardboard soaked in water

4.2.9. Statistical Analysis

Statistical data was evaluated using SAS software (SAS, Cary, NC, USA) and XLSTAT (Addinsoft Incorporated, New York, NY, USA). Means and differences were calculated using 1-way and 2-way ANOVA, respectively, followed by Tukey's test for pair-wise comparisons. Results with significance level of ($\rho < 0.05$) were considered to be statistically significant.

4.3. Results and Discussion

4.3.1. Rapid-Visco Analysis

The Rapid-Visco Analyzer (RVA) was used to assess rheology differences between the two recipes. Significant differences in peak and final viscosity were apparent. Results are found in Table 4.9.

Table 4.9. Rapid-visco analysis of each pea-based high moisture meat analog (HMMA) recipe differing in pea protein isolate (PPI) and pea protein concentrate (PPC) inclusion level. Cells labeled with the same letter are not significant.

Recipe	Peak Viscosity (cP)	Final Viscosity (cP)	Pasting Time (s)[12]	Pasting Temp (°C)
PPI-50	67 ± 3 ^{A}	70 ± 5 ^{A}	293 ± 2	94.62 ± 0
PPI-60	54 ± 3 ^{B}	51 ± 2 ^{B}	289 ± 5	94.87 ± 0

Figure 4.2 is a collective graph including one replicate of each recipe run on the RVA. PPI-50 demonstrated higher peak (67 cP) and final viscosity (70 cP), compared to PPI-60 (54 and 51 cP, respectively); the higher hot-paste viscosity providing indication for higher solubility, after heating (Osen et al., 2014). A higher amount of PPI, present in PPI-60, reduced the overall hot-paste viscosity and solubility. Heat gelling properties were superior for PPC compared to PPI in Chapter 3, demonstrating the consistency between results. No significant differences were seen for pasting time or temperature. Based on these results, it was expected that mechanical energy would be greater for PPI-50, given its higher viscosity and solubility; results were confirmed after extrusion with higher SME being generated for PPI-50. Higher peak viscosity being linked to higher SME is consistent with Chapters 2 and 3.

[12] No significant differences were seen for pasting time and temperature. F value: 1.80, $\rho > 0.2508$ for pasting time. F value: 2.92, $\rho > 0.1626$ for pasting temperature.

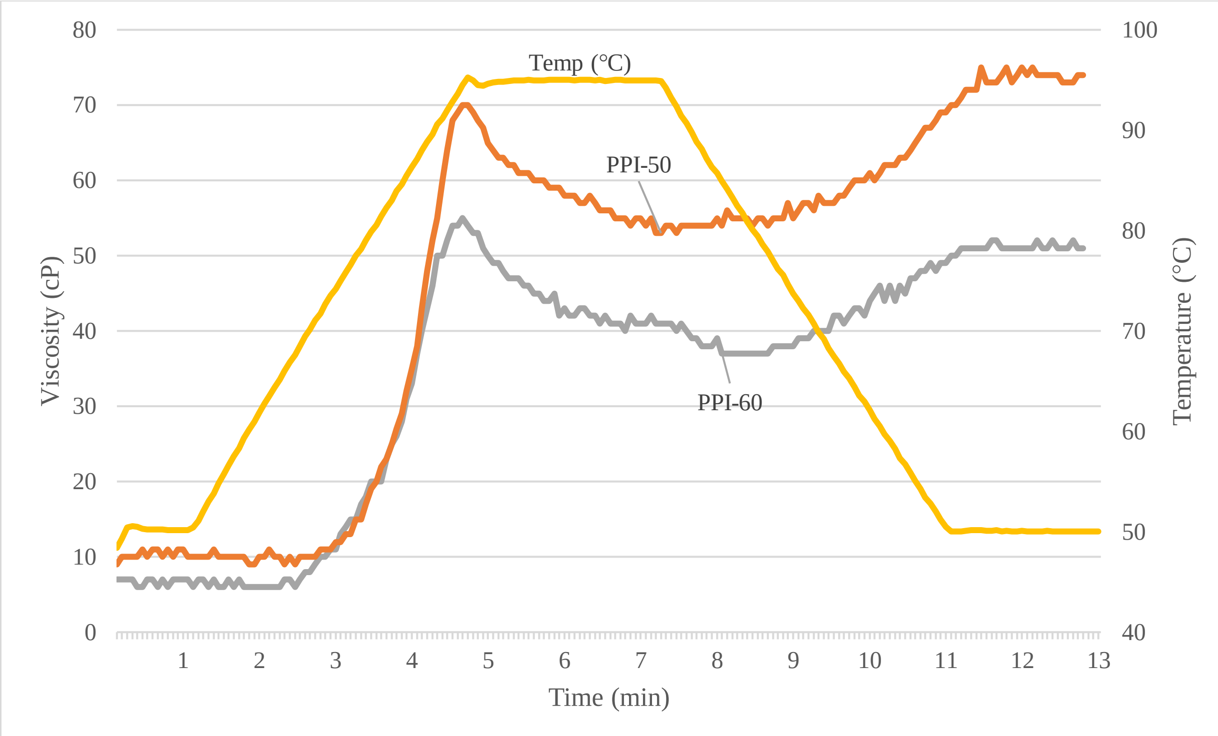


Figure 4.2. Rapid-visco analysis curves for each pea-based high moisture meat analog (HMMA) recipe differing in pea protein isolate (PPI) and pea protein concentrate (PPC) inclusion level.

4.3.2. Water Binding Capacity

The WBC of proteins is related to protein solubility, with higher solubility indicated by greater WBC (Webb, 2021). PPI-60 had slightly higher WBC (1.47 g protein/g water) than PPI-50 (1.41 g protein/g water), implying that substituting PPI for PPC in a recipe will increase overall cold-water solubility. PPI was also shown to have high WBC in Osen et al. (2014). However, as shown by LGC and RVA, the hot-water solubility is much higher for PPC, which is more practical to extrusion. Webb (2021) observed SME having an inverse trend with WBC for pea proteins, concluding that the more water the protein is able to hold, the less energy it requires to process. Higher SME values were observed for PPI-50, confirming her findings.

Table 4.10. Water binding capacity (WBC) of each pea-based high moisture meat analog (HMMA) recipe differing in the inclusion level of pea protein isolate (PPI) and pea protein concentrate (PPC). Cells labeled with the same letter are not significant.

Recipe	WBC (g protein/g water)
PPI-50	1.41 B
PPI-60	1.47 A

4.3.3. Differential Scanning Calorimetry

The DSC was used to determine the denaturation temperature of the proteins in the recipes. This analysis technique helps identify the amount of energy required during extrusion to unravel and denature the protein to induce disulfide cross-linking; cross-linking and texturization will only occur after the proteins are denatured (Brishti et al., 2017). Protein denaturation can be identified by a change in heat enthalpy, which takes on the form of a peak. This can be observed in Figure 4.3. As shown in Table 4.11, denaturation temperatures were observed to be significantly different between recipes, but onset and end temperatures were not significant. The onset and denaturation temperatures of PPI-60 were 152.70 °C and 181.67 °C, respectively, while PPI-50 onset and denaturation temperatures were lower (148.20 °C and 178.75 °C, respectively). As in Chapter 3, lower denaturation temperatures are associated with the recipe higher in PPC. The superior heat-swelling properties of PPC, as determined by LGC, allows PPI-50 to be denatured faster by way of more efficient heat transfer.

Table 4.11. Differential scanning calorimetry (DSC) results for each pea-based high moisture meat analog (HMMA) recipe differing in pea protein isolate (PPI) and pea protein concentrate (PPC) inclusion level. Cells labeled with the same letter are not significant.

Recipe	Onset Temp (°C)[13]	Peak Denaturation Temp (°C)	End Temp (°C)
PPI-50	148.20 ± 1.41^{A}	178.75 ± 1.35^{B}	194.74 ± 2.30^{A}
PPI-60	152.70 ± 3.17^{A}	181.67 ± 1.45^{A}	194.18 ± 1.01^{A}

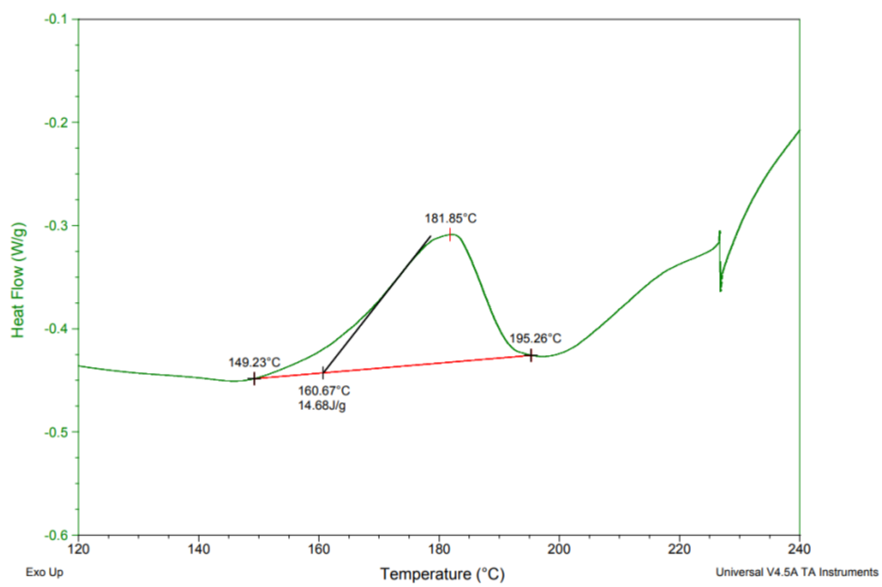


Figure 4.3. Example differential scanning calorimetry (DSC) curve from analysis of pea-based high moisture meat analog (HMMA) recipe

4.3.4. Extrusion Parameters

When all independent extrusion parameters, e.g. IBM, feed rate, barrel temperatures, are kept constant, changes in extruder responses can be directly attributed to differences in formulation. However, as these parameters are changed, extruder responses are not only influenced by changes in formulation, but also from processing factors. Die pressure, die

[13] No significant differences were seen for onset and end temperatures. F-value: 5.05, ρ > 0.0879 for onset temperature. F-value: 0.15, ρ > 0.7191 for end temperature.

temperature, and SME are three measurable extruder responses that provide indication of melt viscosity and degree of cook. These outputs are predictors of texturization, even before visual examination of the final product can be performed (Palanisamy et al., 2018; Chen et al., 2021).

4.3.4.1. Moisture

Prior research supports evidence that higher IBM is necessary to process recipes that are higher in protein (Zhang et al., 2020). The increase in IBM is expected to reduce friction and lower mechanical energy (Lin et al., 2002). However, in this trial, PPI-60, which contained higher protein, did not require higher amounts of water to process and texturize the protein effectively. This might be due to a couple dynamics including (a) increasing the overall protein content from 57 to 59% may not be significant enough to affect IBM and (b) altering the processing variables (feed rate and barrel temperatures) overshadowed the influence of IBM.

A negative correlation between feed rate and IBM is noted; as feed rate increased, IBM decreased. This is documented in Tables 4.12 and 4.13. More compression, induced by the higher feed rate, enables higher efficiency for forward conveyance of the recipe through the extruder, which may have lowered the IBM requirement.

4.3.4.2. Mechanical Energy

The effect of higher protein inclusion, brought in by PPI-60, was seen with a slight reduction in overall mechanical energy (see Tables 4.13 and 4.14). This was noted in all cases except in comparing treatments LPMRHT (324.00 kJ/kg) and HPMRHT (324.00 kJ/kg), where SME did not change. According to Osen et al. (2014), these changes in mechanical energy may be related to protein solubility. PPI has lower solubility than PPC, detected using the Biuret method in Chapter 3, which leads to lower viscosity and less SME.

Effects on mechanical energy primarily result from changing process parameters. As feed rate was increased from 25 kg/hr to 35 kg/hr (Table 4.12), mechanical energy, die temperature, and die pressure all increased in response; this is in agreement with the study by Yeh & Jaw (1999), paired with Riaz (2000). Higher amounts of pressure and mechanical energy result from increased barrel fill, which creates more compression and friction in the screw channels. The longer cooling die utilized for this trial, compared to the previous chapters, increased overall die pressure (500-950 psig) as found in Pietsch et al. (2017). More energy is required to push material through the longer die, creating higher backpressures and barrel fill. When increasing the barrel temperatures (Table 4.14), mechanical energy, die pressure, and die temperature decreased, as found in Ferawati et al. (2021) and Osen et al. (2014). With higher thermal energy introduction, melt viscosity is lowered, leading to a reduction in mechanical energy. This is confirmed in a study by Chen et al. (2010), who found that increasing the cooking temperature from 140 to 160 °C lowered the viscosity of the melt.

Table 4.12. Overall protein content (%), feed rate (kg/hr), in-barrel moisture (IBM) (%), specific mechanical energy (SME) (kJ/kg), die pressure (psig), and die temperature (°C) for pea-based high moisture meat analog (HMMA) recipes processed at different feed rates.

	Factors					
Treatment	Protein (%)	Feed Rate (kg/hr)	IBM (%)	SME (kJ/kg)	Die Pressure (psig)	Die Temperature (°C)
HPLRLT	59.68	25	47.20	187.20	500	136
HPMRLT	59.68	30	45.54	216.00	650	135
HPHRLT	59.68	35	44.69	442.29	800	144

Table 4.13. Overall protein content (%), feed rate (kg/hr), in-barrel moisture (IBM) (%), specific mechanical energy (SME) (kJ/kg), die pressure (psig), and die temperature (°C) for pea-based high moisture meat analog (HMMA) recipes processed at different protein inclusion levels and feed rates.

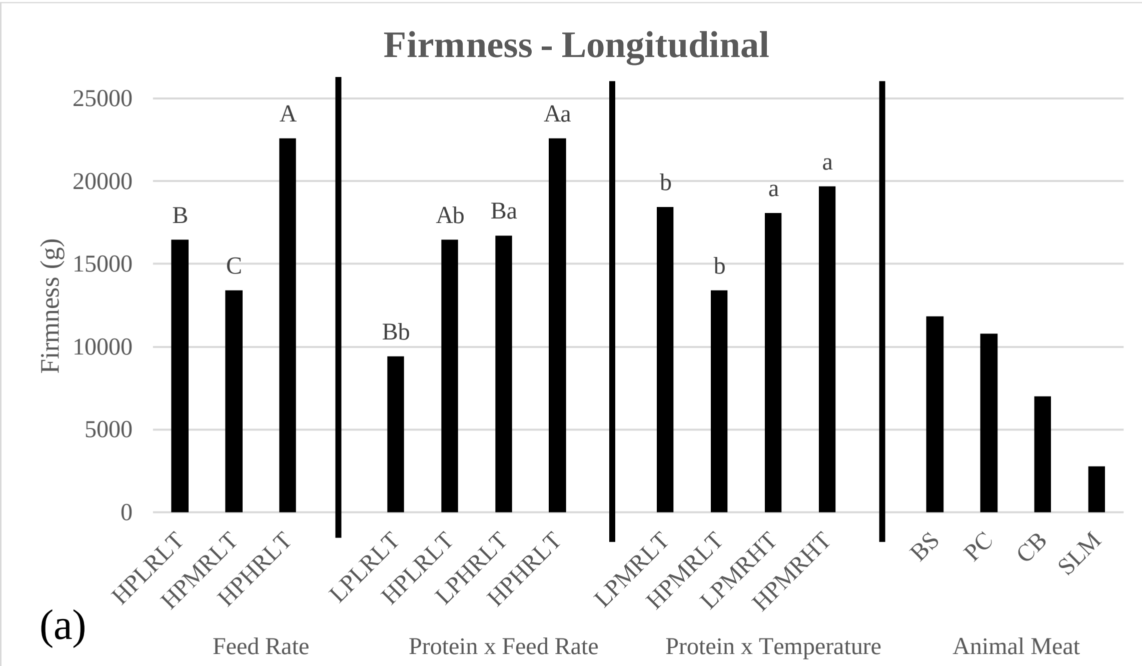
	Factors					
Treatment	Protein (%)	Feed Rate (kg/hr)	IBM (%)	SME (kJ/kg)	Die Pressure (psig)	Die Temperature (°C)
LPLRLT	57.18	25	46.56	331.20	500	145
HPLRLT	59.68	25	47.20	187.20	500	136
LPHRLT	57.18	35	45.66	709.71	950	152
HPHRLT	59.68	35	44.69	442.29	800	144

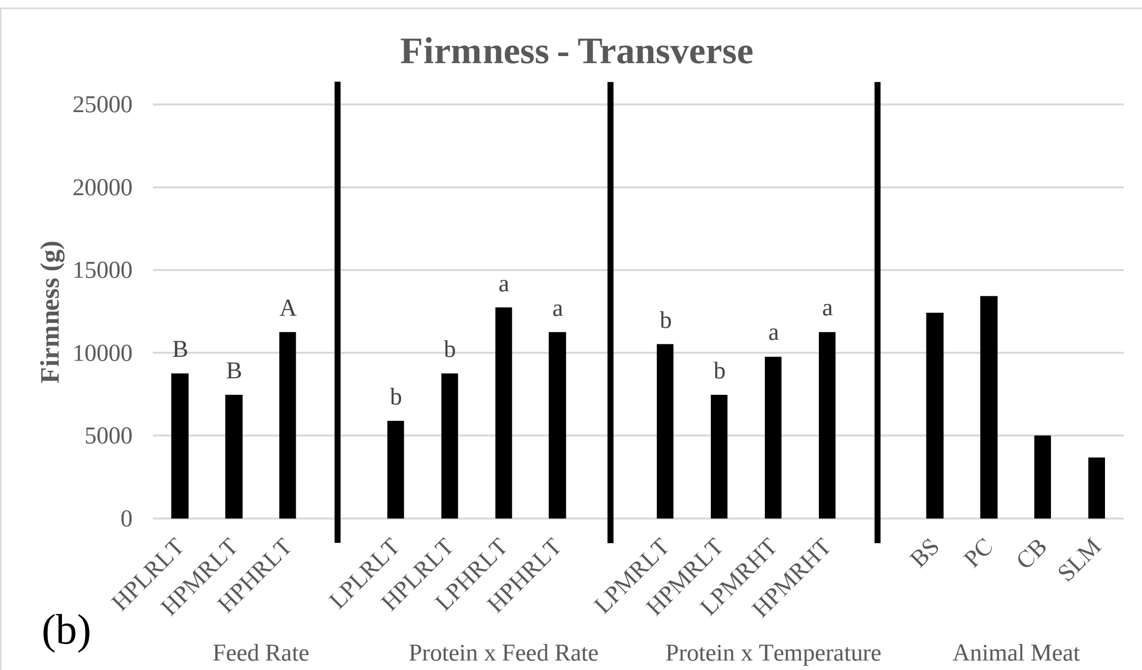
Table 4.14. Overall protein content (%), feed rate (kg/hr), in-barrel moisture (IBM) (%), specific mechanical energy (SME) (kJ/kg), die pressure (psig), and die temperature (°C) for pea-based high moisture meat analog (HMMA) recipes processed at different protein inclusion levels and barrel temperatures.

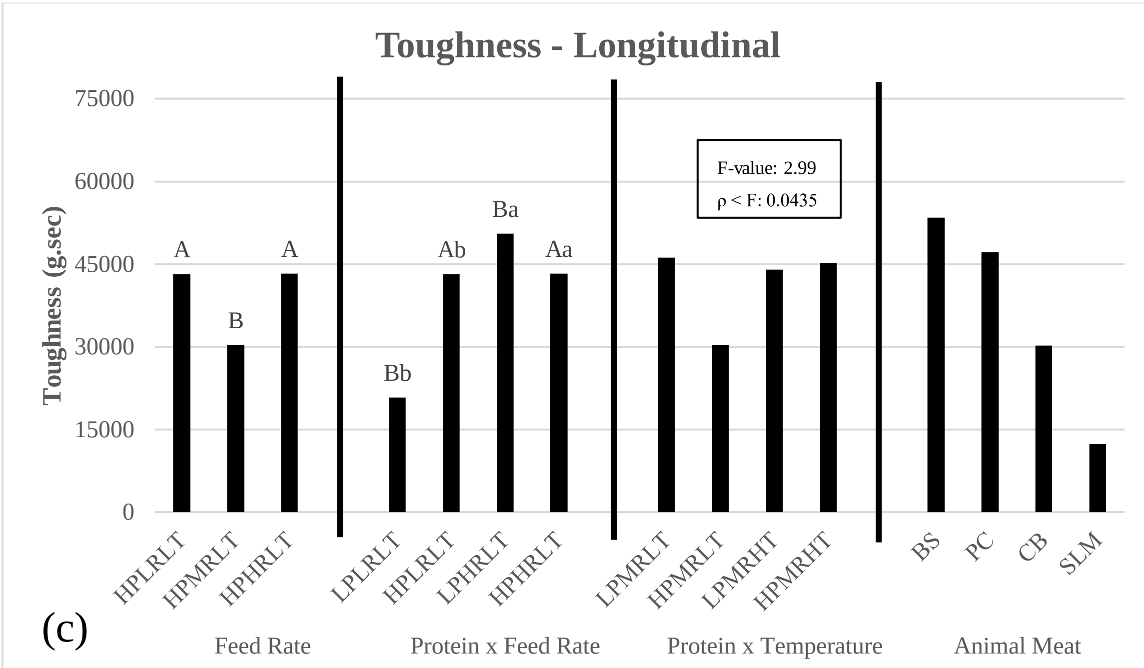
	Factors					
Treatment	Protein (%)	Barrel Temps (°C)	IBM (%)	SME (kJ/kg)	Die Pressure (psig)	Die Temperature (°C)
LPMRLT	57.18	130	46.31	588.00	950	151
HPMRLT	59.68	130	45.54	216.00	650	135
LPMRHT	57.18	150	46.10	324.00	600	147
HPMRHT	59.68	150	46.93	324.00	600	142

4.3.5. Texture Analysis

Product texture was affected by both formulation and processing variables. Cutting test results are shown in Figure 4.4. Based on this data, there is strong evidence proposing feed rate had the most significant impact on texturization. In general, a higher feed rate led to firmer and tougher products, particularly at the highest feed rate (35 kg/hr). The two treatments processed at the highest feed rate, i.e. LPHRLT and HPHRLT, possess either the uppermost firmness or toughness value, in both directions of cut. HPHRLT displayed uppermost firmness value (22,600 g) in the longitudinal direction of cut, while LPHRLT displayed uppermost firmness value (33,430 g) in the transverse direction of cut. The smoothness of the products, seen in Figure 4.4, increases dramatically as feed rate is increased, providing additional indication of higher texturization and protein cross-linking.







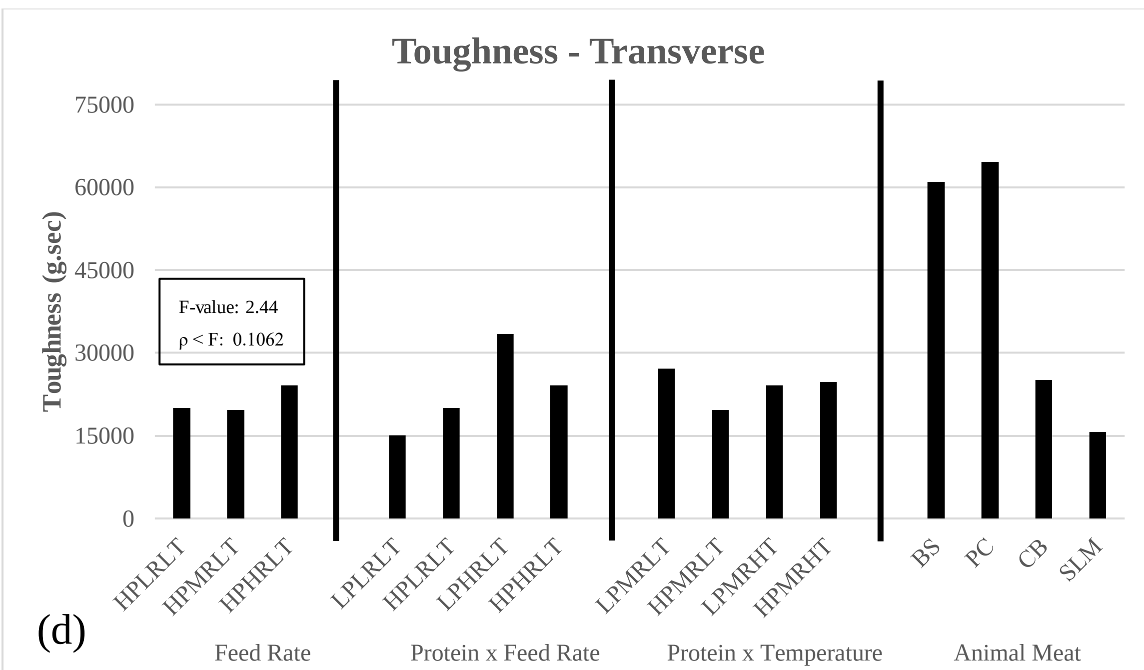


Figure 4.4. Cutting test results of pea-based high moisture meat analog (HMMA) products differing in feed rate, protein content and feed rate, and protein content and barrel temperatures, and also animal meat anchors for (a) longitudinal firmness, (b) transverse firmness, (c) longitudinal toughness, and (d) transverse toughness. Within each individual bar cluster, bars labeled with the same letter are not significant. For the two-way ANOVAs, significant differences for protein content are denoted with capital letters, whereas significant differences for feed rate and barrel temperatures are denoted with lowercase letters.

Like in the study by Ferawati et al. (2021), the fibers were more textured in the longitudinal than transverse direction (see Table 4.15), for all extruded products. The combination of higher PPI inclusion (50-60%) and higher die pressure, induced by the thinner cooling die, enabled a high amount of anisotropic, intermolecular disulfide bonding to occur. Given the ratios were less than 1, it can be concluded that more intermolecular protein crosslinks were formed than intramolecular crosslinks. Figures 4.5 and 4.6 illustrate the effectiveness of the thinner cooling die on product quality, as the outer shell seen in the previous chapters was completely prevented from forming. The longer cooling die increases retention time in the die for adequate product cooling to occur; this eliminates inconsistencies in product texture caused by deficiencies in heat transfer.

Table 4.15. Transverse to longitudinal firmness and toughness ratios of pea-based high moisture meat analog (HMMA) products.

Recipe	Firmness Ratio	Toughness Ratio
LPLRLT	0.63	0.73
HPLRLT	0.53	0.46
HPMRLT	0.56	0.65
LPMRLT	0.57	0.59
LPHRLT	0.76	0.66
HPHRLT	0.50	0.56
HPMRHT	0.57	0.55
LPMRHT	0.54	0.55

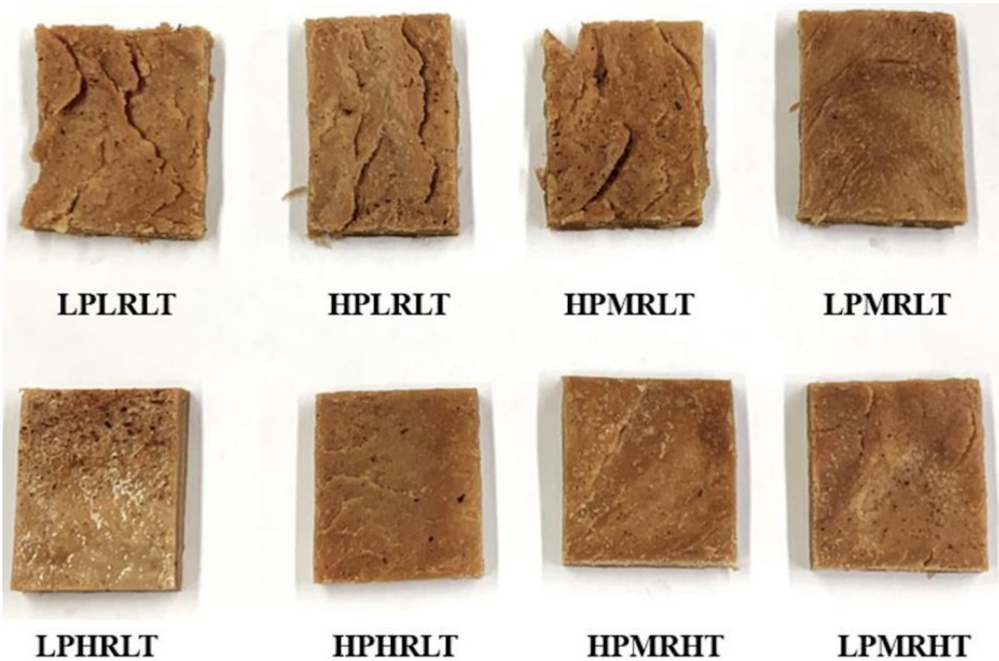


Figure 4.5. Overhead cross-sectional view of pea-based high moisture meat analog (HMMA) products.

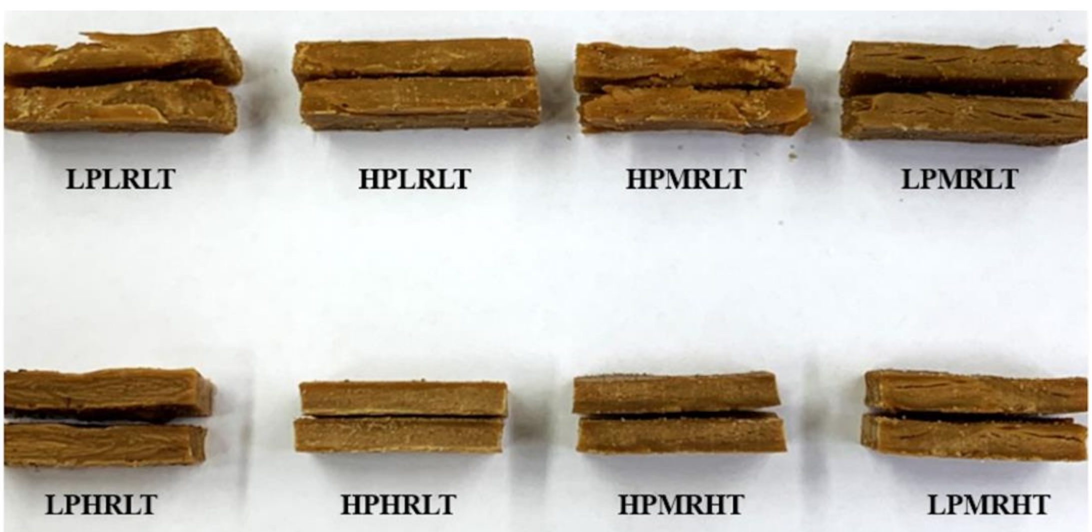


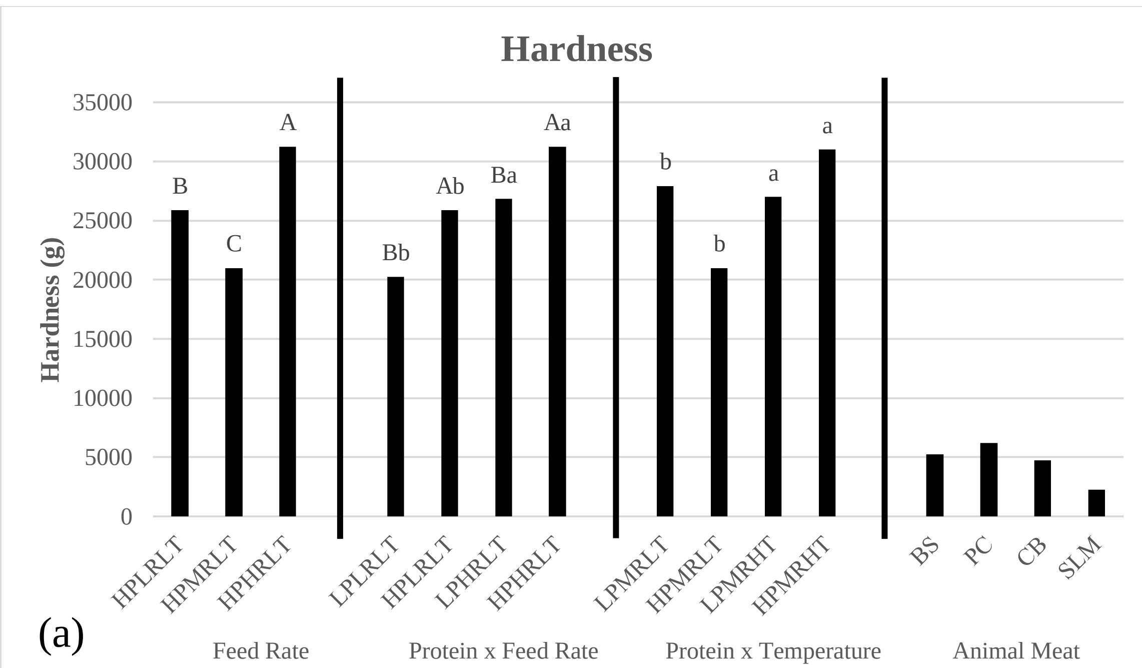
Figure 4.6. Overhead cross-sectional view of the internal structures of pea-based high moisture meat analog (HMMA) products.

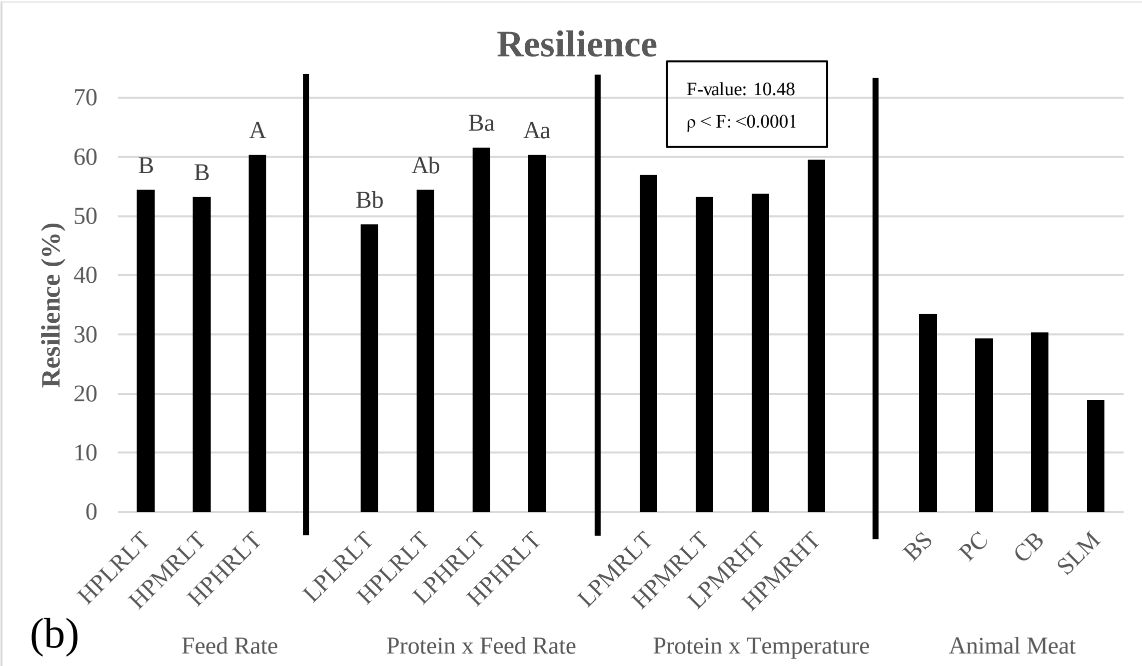
Protein inclusion level also had a significant impact on final product texture, but to a lesser extent than feed rate. In the longitudinal direction of cut, the higher inclusion of PPI significantly increased longitudinal firmness for three out of four comparisons; HPLRLT (16,465 g) compared to LPLRLT (9,420 g), HPHRLT (22,600 g) compared to LPHRLT (16,725 g), and HPMRHT (19,705 g) compared to LPMRHT (18,095 g). Higher protein inclusion creates greater opportunity for more texturization and cross-linking of proteins, which results in firmer products (Webb et al., 2020). A significant interaction ($\rho < 0.05$) between protein and feed rate was seen in all cases, except for longitudinal firmness. At the higher protein inclusion level (59.68%), texture differences created by feed rate were not as noticeable between treatments, compared to larger differences observed at the lower protein inclusion level (57.18%).

As barrel temperatures were raised, firmness and toughness increased from higher thermal energy input. This is confirmed in comparing HPMRLT with HPMRHT, and LPMRLT with LPMRHT. Significant effects were apparent for firmness but not for toughness. Interaction effects between protein and barrel temperatures were significant ($\rho < 0.05$) in all instances except longitudinal toughness. At the higher protein content (59.68%), increasing barrel temperatures does not increase firmness and toughness as effectively as at the lower protein content (57.18%). This was the same interaction effect observed between protein content and feed rate.

Relative to the animal meat anchors, most of the heated HMMA products possess similar firmness and toughness for an appropriate texture match; however, firmness and toughness would need to be increased or decreased in some cases to target specific applications. To match a tougher product, like beef steak or pork chops, treatments processed at the highest feed rate (35 kg/hr) and protein inclusion level (59.68%) would be a closer match; whereas, the treatments processed at lower feed rates (25-30 kg/hr) and protein inclusion level (57.18%) might be more suited to mimic a softer meat like chicken breast. Products were too dense to mimic salmon. Texture could be fine-tuned, accordingly, through more focused culinary preparation methods.

TPA results for hardness, resilience, cohesiveness, and gumminess are displayed in Figure 4.7. Like cutting test, it is evident from TPA results that changing process parameters has a significant impact on texture. Feed rate, once again, was observed to have most significant impact on product texture, confirming the cutting test results. Higher feed rate induced higher mechanical energy, which increased texturization. HPHRLT showed uppermost hardness value (31,230 g) while LPHRLT showed uppermost resiliency (62%) and cohesiveness (90%); both treatments were processed at the highest feed rate (35 kg/hr). In a study by Maung et al. (2020), hardness and cohesiveness were also shown to increase from higher mechanical energy.




Resilience
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Resilience (%)
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60
50
40
30
20
10
0
B
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A
Bb
Ab
Ba
Aa
HPLRLT
HPMRLT
HPHRLT
LPLRLT
HPLRLT
LPHRLT
HPHRLT
LPMRLT
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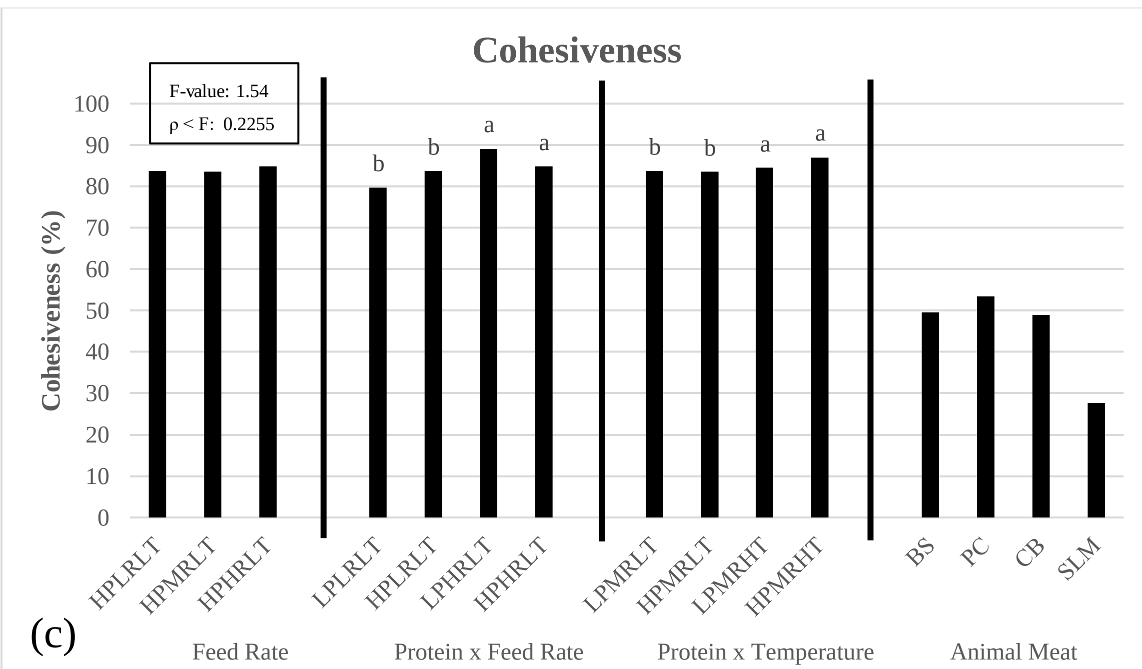
HPMRHT
BS
PC
CB
SLM
(b)
Feed Rate
Protein x Feed Rate
Protein x Temperature
Animal Meat


Cohesiveness
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ρ < F: 0.2255
Cohesiveness (%)
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90
80
70
60
50
40
30
20
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b
a
a
b
b
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a
HPLRLT
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HPHRLT
LPLRLT
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Protein x Temperature
Animal Meat

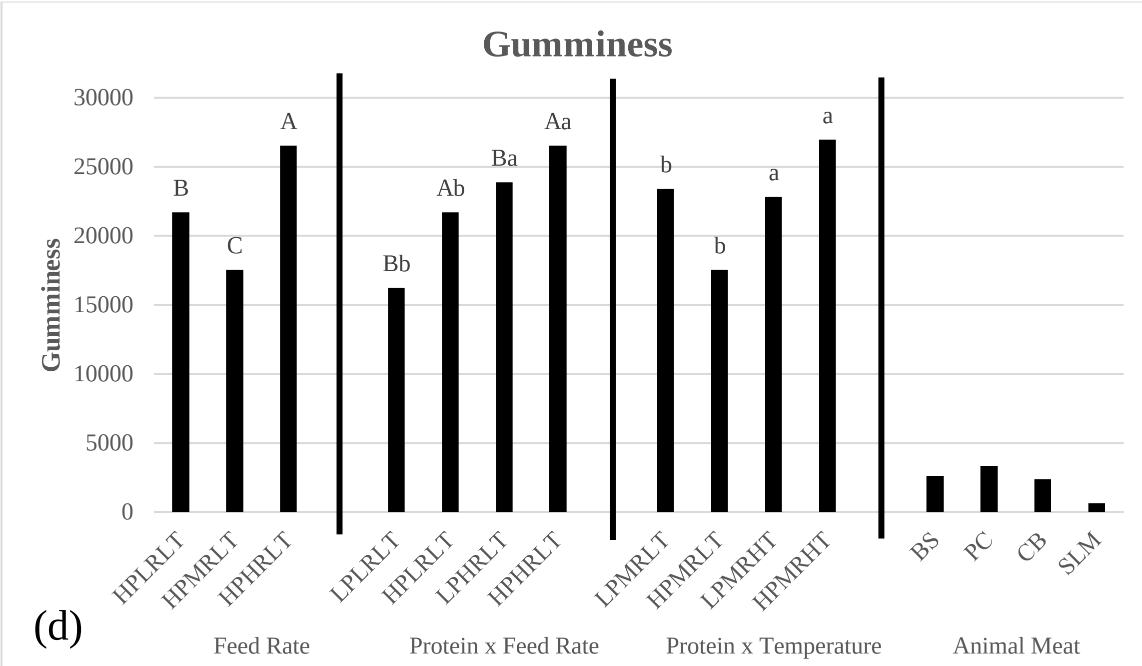

Figure 4.7. Texture profile analysis (TPA) of pea-based high moisture meat analog (HMMA) products differing in feed rate, protein content and feed rate, and protein content and barrel temperatures, and also animal meat anchors for (a) hardness, (b) resilience, (c) cohesiveness, and (d) gumminess. Within each individual bar cluster, bars labeled with the same letter are not significant. For the two-way ANOVAs, significant differences for protein content are denoted with capital letters, whereas significant differences for feed rate and barrel temperatures are denoted with lowercase letters.

Higher barrel temperatures and PPI inclusion were also found to increase TPA values, but not as significantly as feed rate. A higher inclusion of protein significantly increased hardness, resilience, and gumminess of the HMMA products processed at different feed rates; however, this same effect from protein was not seen in conjunction to raising barrel temperatures. An increase in barrel temperatures was found to significantly increase hardness, cohesiveness, and gumminess of the HMMA products. Interactions, at a significant level ($\rho < 0.05$), occurred between protein content and barrel temperatures for all attributes, and also between protein content and feed rate for cohesiveness and resilience. The textural differences, between treatments processed at high (35 kg/hr) versus low feed rate (25 kg/hr), were noticed less at the higher protein content (59.68%). Conversely, the difference between treatments processed at higher versus lower barrel temperatures was more distinct at the higher protein level. As was similar to Chen et al. (2010), a synergism between protein content, feed rate, and barrel temperatures seems to exist.

All treatments had significantly higher hardness, resiliency, cohesiveness, and gumminess values than the animal meat anchors. This is primarily due to the increase in texturization, induced by higher friction (Osen et al., 2014), created in the thinner and longer cooling die. As expected, beef steak and pork chop had significantly highest texture values of the animal meats, in all cases; but, hardness, resilience, cohesiveness, and gumminess would need to be reduced in future experiments to better mimic the animal meat products. This could likely be done by either reducing the overall protein content of the recipe, or increasing the orifice diameter of the cooling die. Sensory analysis helps support this recommendation.

Nutrient analysis of the HMMA products was not performed in these studies, as this was out of the scope of our research. In general, the protein content of HMMAs is higher than animal meat while the moisture and fat content is lower. The typical nutritional composition of animal meat can be found in “Nutritional Composition of Meat” by Ahmad et al. (2018). Fat could certainly create softer texture in the animal meat anchors but it is very difficult to include in HMMA recipes, as it will inhibit mechanical energy and texturization. A focus on protein ingredients, as in this study, is more practical in relation to extrusion.

4.3.6. Sensory Analysis

The trained panelists’ perceptions are detailed in Tables 4.16 and 4.17. For texture attributes, no significant differences ($\rho < 0.05$) were noted for springiness, fiber awareness, tooth packing, or astringent properties. Among the HMMA samples, PPI1-60 was reported to have the greatest chewiness (11.13) and firmness (13.00), while PPI3-40 was highest in springiness (5.19) and juiciness (5.94). The high firmness of PPI-60 correlates well with texture analysis data in Chapter 3, where cutting test showed firmness to be greatest for PPI-60 compared to the other HMMA samples. Denseness was highest for LPLRLT (10.81) and LRHRLT (12.13) among all evaluated samples. This was induced by the higher protein content (57-59%) and thinner cooling die (5/16”) used for the extrusion of HMMAs in Chapter 4. Relative to animal meat (beef steak and chicken breast), the PCA plot shows PPI3-40, PPI4-40, and PPI1-30 being the most similar in texture to animal meat. Each of these products were extruded on the shorter and thicker (1/2”) cooling die in Chapter 3. The shorter cooling die permitted more die expansion, as the products could not be completely cooled down before exiting the die. The high springiness (5.19) for PPI-30 led to the highest juiciness value (5.94) among HMMAs, that was reported to be most similar to beef steak (6.19) and chicken breast (5.63).

Differences in recipe and processing were not reported to affect flavor attributes of the HMMAs nearly as significantly as texture. All HMMA samples were perceived to be significantly more beany than animal meat, while only PPI1-30 was statistically similar to animal meat with regards to starchy, grain, green, and cardboard flavor attributes. Pulse proteins have been known to increase the beany flavor of plant-based meat (Kim et al., 2021), but current industrial practices used to remove beany flavor of legumes is summarized in Wang et al. (2021).

Based on these results, it was perceived that the HMMA recipes containing >40% pea protein isolate led to extruded products that were too firm to appropriately mimic the animal meat anchors. The thinner and longer cooling die, utilized in Chapter 4, led to higher amounts of texturization among HMMA products, but may have created products that were too dense to mimic a beef steak or chicken breast; this was confirmed by the high TPA values. A recommendation to future researchers would be to use the longer cooling die, as in Chapter 4, but increase the orifice diameter, as in Chapter 3; this could provide enough time for adequate cooling to occur, while simultaneously creating products that are less dense.

Table 4.16. Least square (LS) means for texture attributes from sensory analysis of pea-based high moisture meat analogs (HMMAs) and animal meat anchors. Within each column, values labeled with the same letter are not significant.

Recipe	[14]Springiness	Denseness	Juiciness	Residual Particles	Firmness	Chewiness	Fiber Awareness	Tooth Packing	Astringent	Starchy
PPI3-40	5.19	6.94 ^{C}	5.94 ^{A}	4.25 ^{AB}	8.94 ^{BC}	8.25 ^{B}	7.25	2.06	2.81	2.19 ^{BC}
PPI4-40	4.31	7.94 ^{BC}	5.50 ^{AB}	3.81 ^{AB}	9.69 ^{BC}	9.00 ^{AB}	7.19	2.19	2.81	2.69 ^{AB}
PPI1-30	4.56	8.81 ^{ABC}	5.00 ^{AB}	3.63 ^{AB}	10.56 ^{B}	9.31 ^{AB}	6.88	2.19	2.13	1.00 ^{CD}
PPI1-60	3.19	8.31 ^{BC}	3.88 ^{AB}	3.56 ^{AB}	13.00 ^{A}	11.13 ^{A}	6.75	2.00	3.00	3.38 ^{AB}
LPLRLT	3.06	10.81 ^{AB}	3.38 ^{B}	5.50 ^{A}	8.35 ^{C}	8.31 ^{B}	7.88	2.50	3.63	4.19 ^{A}
LPHRLT	3.25	12.13 ^{A}	4.44 ^{AB}	3.88 ^{AB}	10.25 ^{B}	8.81 ^{AB}	6.56	1.81	3.13	2.75 ^{AB}
Beef Steak	3.75	11.00 ^{AB}	6.19 ^{A}	3.56 ^{AB}	9.44 ^{BC}	8.63 ^{AB}	8.31	1.63	2.25	0.25 ^{D}
Chicken Breast	3.31	8.94 ^{ABC}	5.63 ^{AB}	3.19 ^{B}	8.25 ^{C}	7.75 ^{B}	7.88	2.19	1.69	0.25 ^{D}

Table 4.17. Least square (LS) means for flavor attributes from sensory analysis of pea-based high moisture meat analogs (HMMAs) and animal meat anchors. Within each column, values labeled with the same letter are not significant.

Recipe	Beany	Starchy	Grain	Green	Umami	Barnyard	Cardboard
PPI3-40	5.06 ^{A}	3.13 ^{AB}	3.44 ^{A}	2.81 ^{A}	2.13 ^{AB}	2.06	2.56 ^{A}
PPI4-40	4.88 ^{A}	3.00 ^{AB}	3.69 ^{A}	2.63 ^{A}	2.31 ^{AB}	2.13	3.06 ^{A}
PPI1-30	3.69 ^{A}	1.69 ^{BC}	2.19 ^{AB}	1.56 ^{AB}	2.00 ^{AB}	1.50	1.75 ^{AB}
PPI1-60	4.88 ^{A}	3.19 ^{AB}	3.50 ^{A}	2.31 ^{A}	1.81 ^{B}	2.19	2.81 ^{A}
LPLRLT	4.50 ^{A}	4.75 ^{A}	3.56 ^{A}	2.81 ^{A}	2.19 ^{AB}	2.19	2.94 ^{A}
LPHRLT	4.25 ^{A}	3.06 ^{AB}	3.69 ^{A}	2.50 ^{A}	1.88 ^{AB}	2.00	3.19 ^{A}
Beef Steak	0.25 ^{B}	0.00 ^{C}	1.00 ^{B}	0.25 ^{B}	2.63 ^{AB}	0.69	0.50 ^{B}
Chicken Breast	0.25 ^{B}	0.50 ^{C}	0.81 ^{B}	0.50 ^{B}	2.88 ^{A}	1.13	0.25 ^{B}

[14] No significant differences were seen for springiness, fiber awareness, tooth packing, astringent, or barnyard. F value: 2.24, $\rho > 0.044$ for springiness. F value: 1.89, $\rho > 0.089$ for fiber awareness. F value: 0.32, $\rho > 0.942$ for tooth packing. F value: 1.80, $\rho > 0.105$ for astringent. F value: 2.20, $\rho > 0.048$ for barnyard.

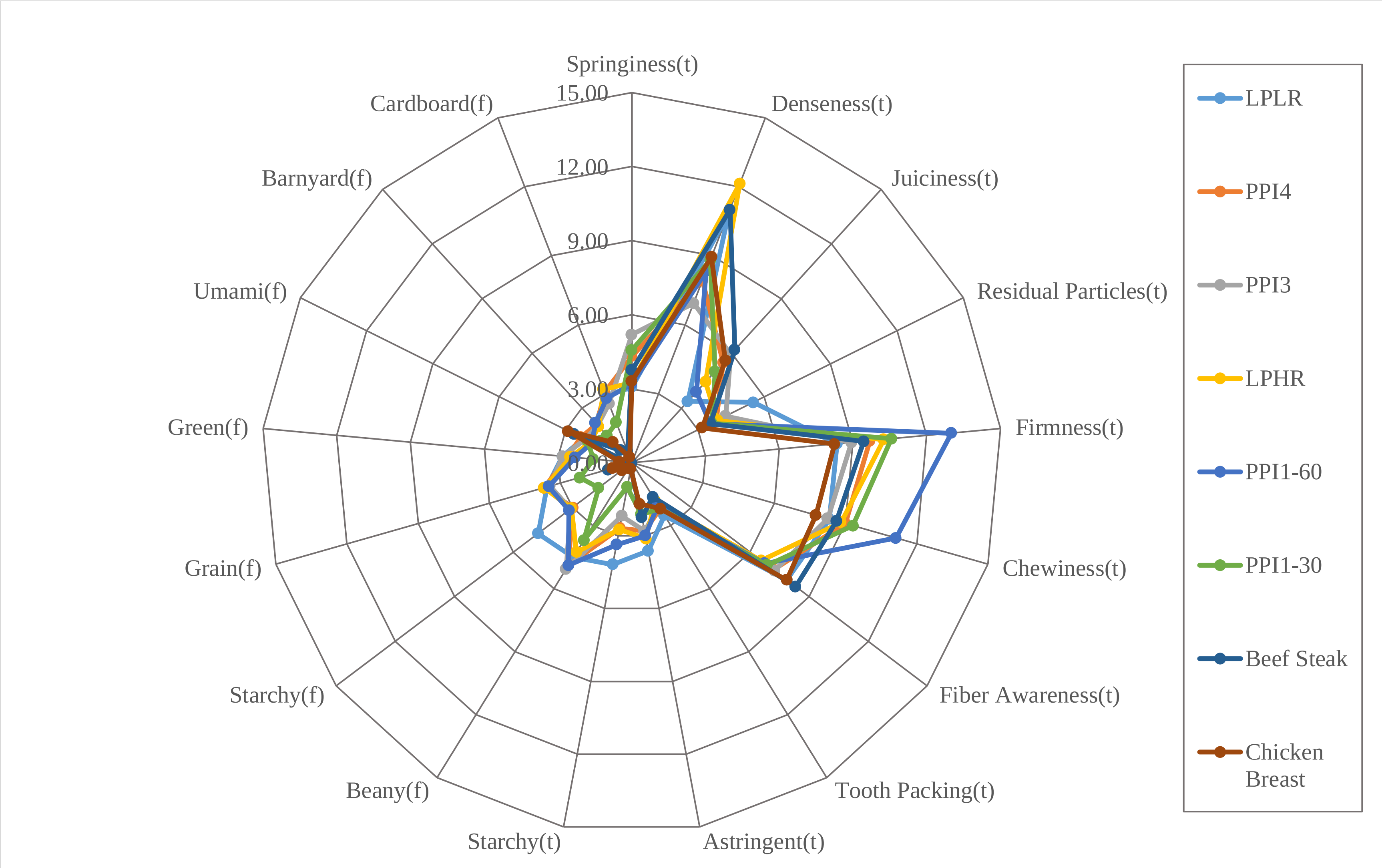

Figure 4.8. Spider plot from least square (LS) means for texture and flavor attributes of pea-based high moisture meat analogs (HMMAs) and animal meat anchors.

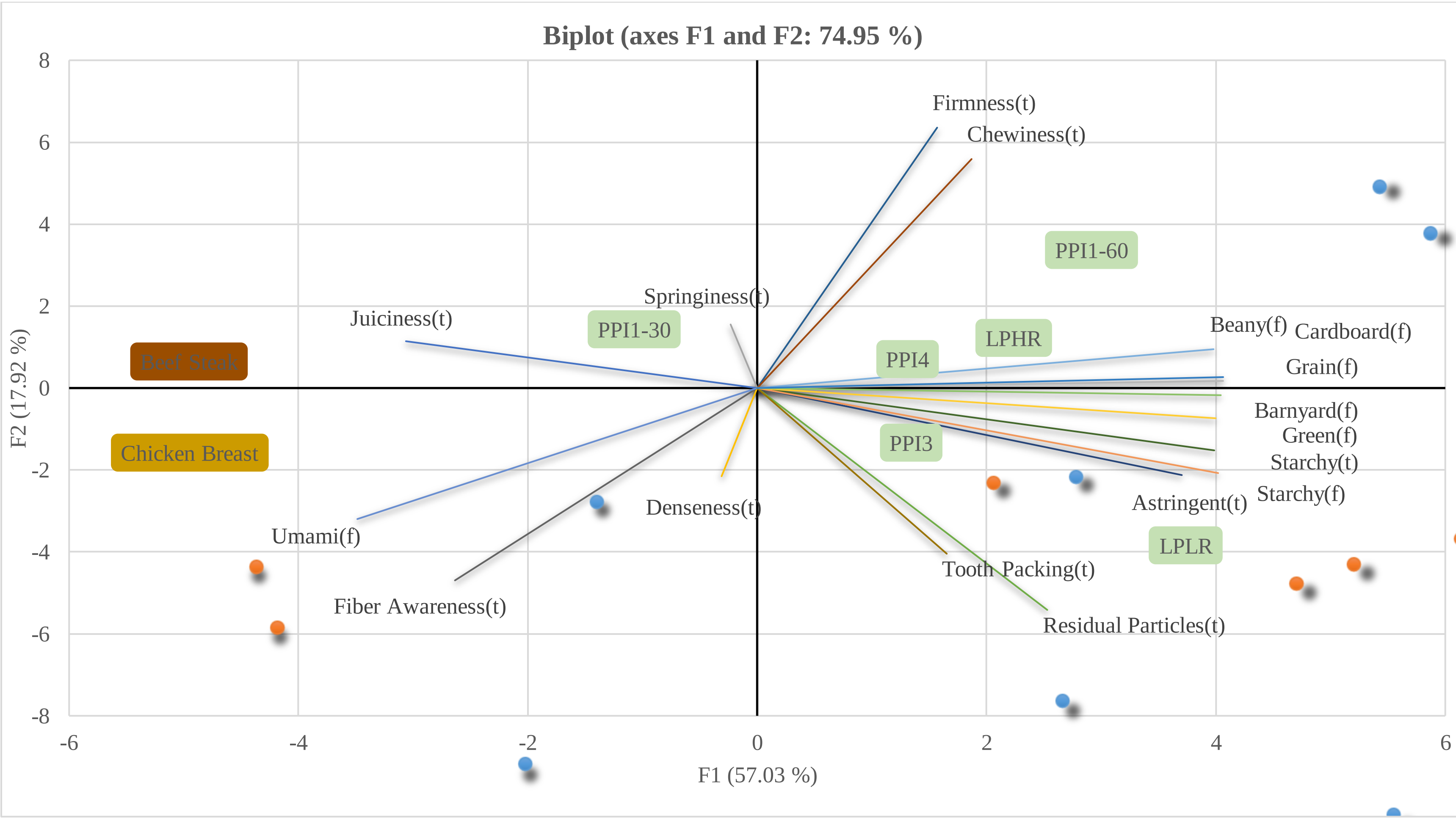

Figure 4.9. Principal component analysis (PCA) plot from least square (LS) means for texture and flavor attributes of pea-based high moisture meat analogs (HMMAs) and animal meat anchors.

4.4. Conclusion

The pathway to creating a plant-based meat product that closely resembles that of whole animal muscle meat is multi-dimensional. Raw material selection, extruder hardware and configuration, as well as extrusion conditions all influence final product properties and overall textural quality. From this research study, results indicate that die dimensions, extruder hardware components, process parameters, and overall protein content all impact the final texture of HMMAs. Increasing the overall protein content was found to decrease mechanical energy during extrusion, by way of lowering viscosity and solubility of the recipe. This was confirmed through RVA work. Process parameters also showed correlation with mechanical energy output. As the feed rate increased, mechanical energy also increased; conversely, as barrel temperatures were increased, mechanical energy decreased, from the drop in viscosity induced by higher thermal energy input. In terms of final product quality, results from texture analysis linked higher feed rate, barrel temperatures, and overall protein content to increased firmness and hardness in the HMMA products. All HMMA products showed higher hardness and similar firmness and toughness values to that of the animal meat anchors, suggesting high amounts of texturization can be achieved using pea protein. Former research has shown pea protein to be an inferior ingredient to soy and wheat protein in terms of functionality and texturization potential, but this study can be used to accelerate new opportunities for pea protein in the plant-based meat market. For future studies, a target meat application can be selected, and the experiment specially tailored to achieve the desired texturization level, based on the information learned in this work. Additional culinary preparation methods should also be studied, to achieve a closer texture match to animal meat.

4.5. References

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Chapter 5 - Conclusions and future work

Ingredient selection, extruder hardware and configuration, as well as extrusion processing conditions all have an impact on final product properties and overall textural quality of HMMAs. Prior research demonstrates the effectiveness of soy or wheat proteins to be used in plant-based meat applications, while pea protein was shown to be an inferior ingredient. However, this research showed that intermediate addition of pea protein concentrate (20-30%), combined with the specialization of cooling die equipment and processing changes, allows pea protein to have great potential within the competitive plant-based meat market.

Although some gaps were bridged between raw materials, extrusion processing, and textural quality, room for further development in this area of research remains. A more intensive focus on protein chemistry is needed to discover or develop new testing mechanisms to be able to quantitatively measure the amount of protein cross-linking (texturization) occurring during extrusion. Texture, visual, and sensory analysis are current practices utilized to quantify texturization, but no established chemical methods of measuring this exist.

Culinary preparation and post-processing of HMMAs could also be another faucet of future work. A bigger emphasis placed on achieving a closer texture, color, and aesthetic match to animal meat anchors, through alternative heating methods, would pair well with this research to provide more definitive results. Additionally, natural or synthetic coloring agents could be added to the extrusion process to better match the visual appearance of animal meat.

The final recommendation involves taking a deeper dive into extrusion equipment design to further understand the impact of extrusion on HMMA texture. Other extrusion parameters or screw profiles, not focused on in this research, could be assessed to understand their respective impact on HMMA texture.

All in all, the basic framework of linking ingredient properties and extrusion processing techniques to product texture of HMMAs, built in this study, gives a great foundation for researchers and processors to further expand this work.

Appendix A

Table A.1. Moisture content (%) of each high moisture meat analog (HMMA) recipe containing selected combinations of soy, wheat, and pea-based ingredients. Cells labeled with the same letter are not significant.

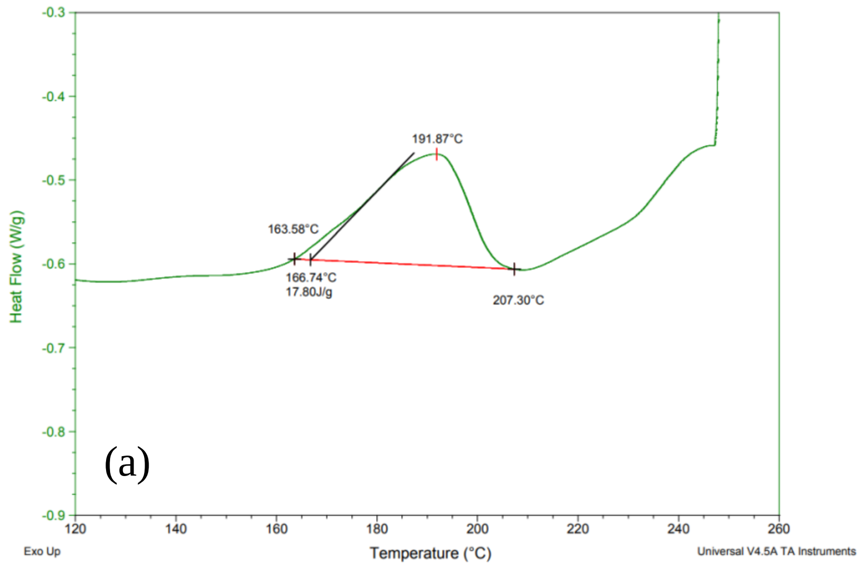
Recipe	Moisture (%)
S	8.38 ± 0.17 ^{BC}
W	9.66 ± 0.16 ^{A}
P	7.62 ± 0.44 ^{D}
WS	9.00 ± 0.18 ^{AB}
PS	7.99 ± 0.27 ^{CD}

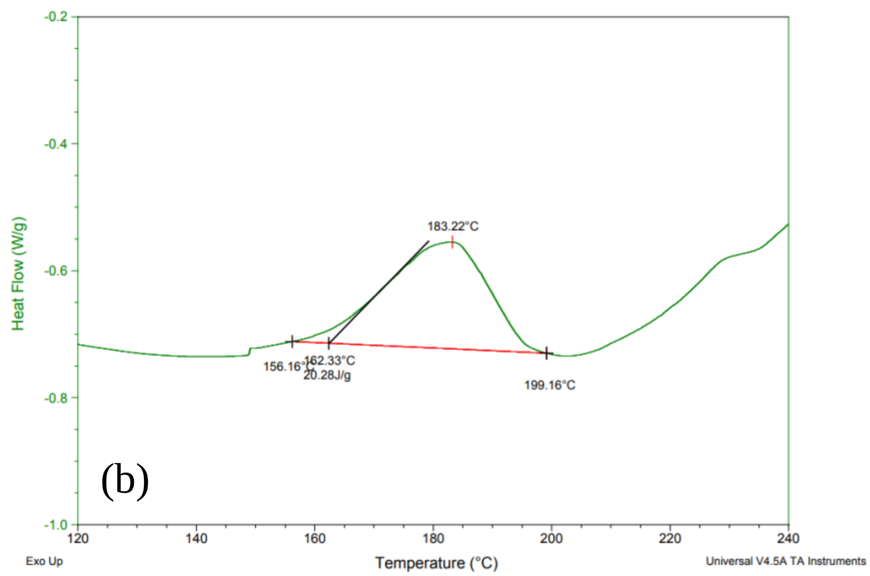
Table A.2. Moisture content (%) of pea protein isolates (PPIs) and pea protein concentrate (PPC). Cells labeled with the same letter are not significant.

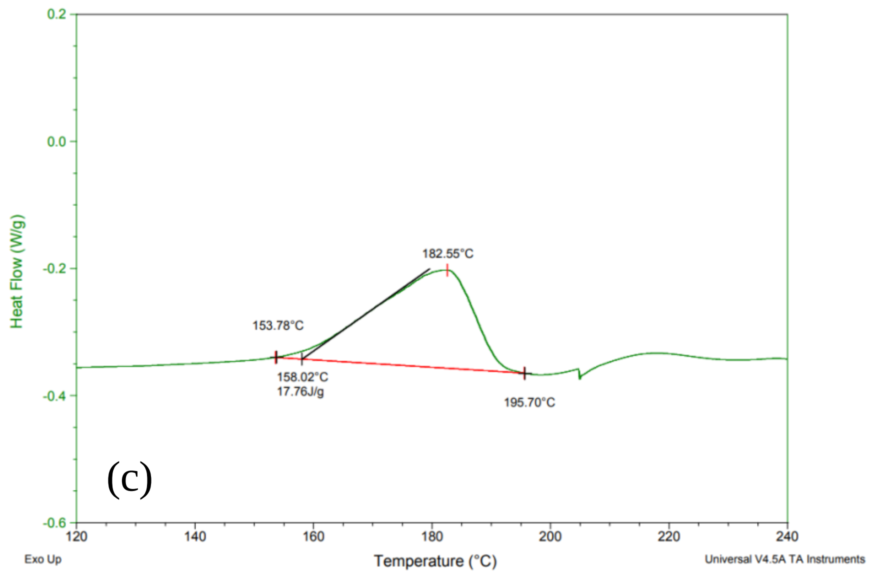
Recipe	Moisture (%)
PPI1	6.30 ± 0.30 ^{C}
PPI2	7.57 ± 0.26 ^{AB}
PPI3	8.07 ± 0.16 ^{A}
PPI4	7.02 ± 0.16 ^{B}
PPC	6.22 ± 0.34 ^{C}

Table A.3. Moisture content (%) of recipes containing differing levels of pea protein isolate (PPI) and pea protein concentrate (PPC). Cells labeled with the same letter are not significant.

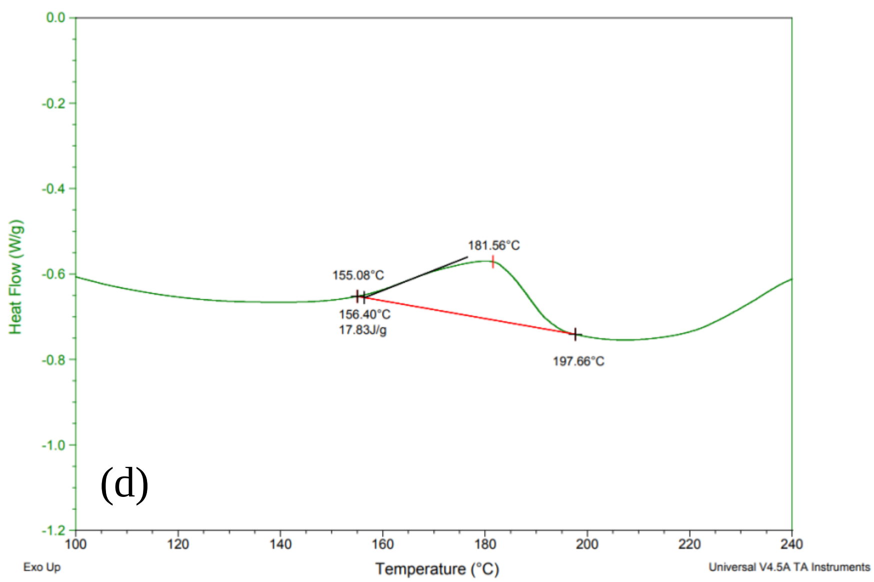
Recipe	Moisture (%)
PPI-50	9.80 ± 0.31
PPI-60	9.56 ± 0.17


-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
Heat Flow (W/g)
191.87°C
163.58°C
166.74°C
17.80J/g
207.30°C
(a)
120
140
160
180
200
220
240
260
Exo Up
Temperature (°C)
Universal V4.5A TA Instruments


-0.2
-0.4
-0.6
-0.8
-1.0
Heat Flow (W/g)
183.22°C
162.33°C
20.28J/g
199.16°C
(b)
120
140
160
180
200
220
240
Exo Up
Temperature (°C)
Universal V4.5A TA Instruments



Heat Flow (W/g)
182.55°C
153.78°C
158.02°C
17.76J/g
195.70°C
(c)
Exo Up
Temperature (°C)
Universal V4.5A TA Instruments



Heat Flow (W/g)
181.56°C
155.08°C
156.40°C
17.83J/g
197.66°C
(d)
Exo Up
Temperature (°C)
Universal V4.5A TA Instruments

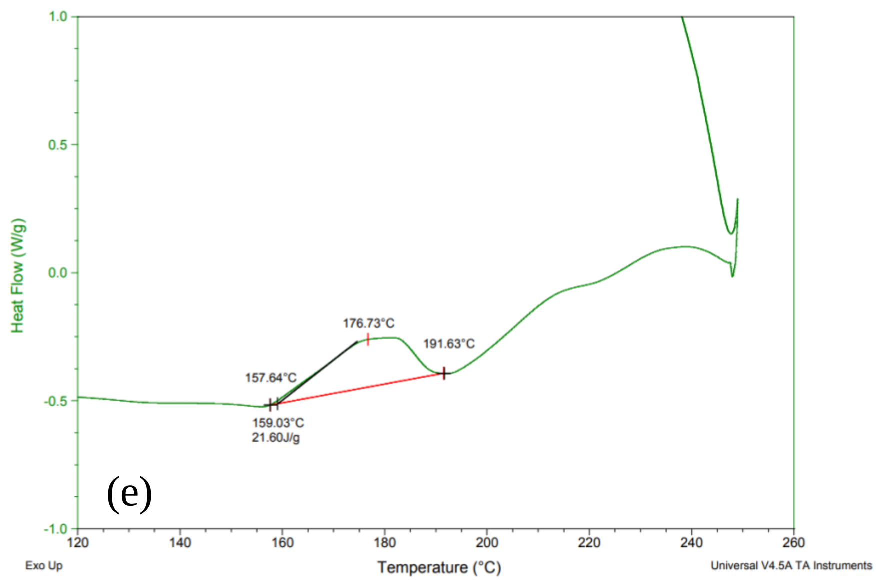
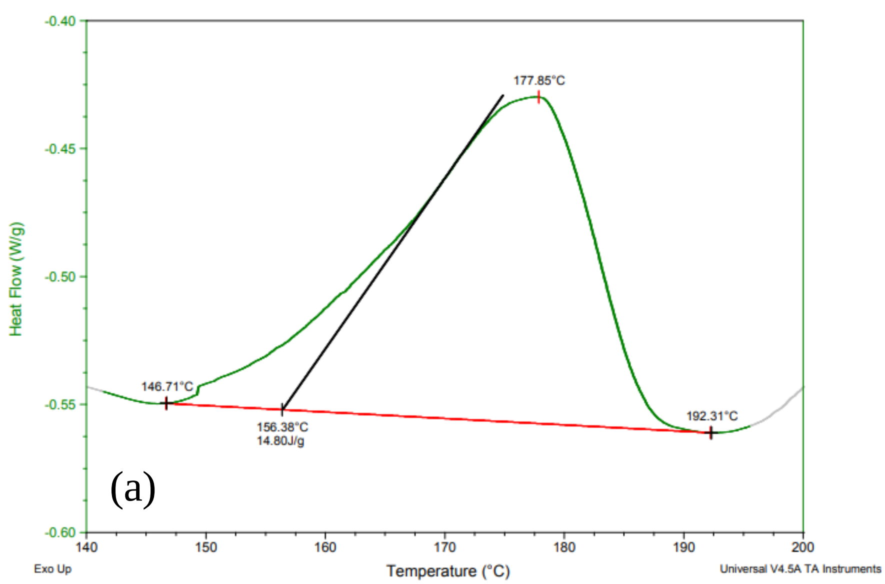


Figure A.1. Differential scanning calorimetry thermograph of one replicate for (a) PPI1, (b) PPI2, (c) PPI3, (d) PPI4, and (e) PPC.



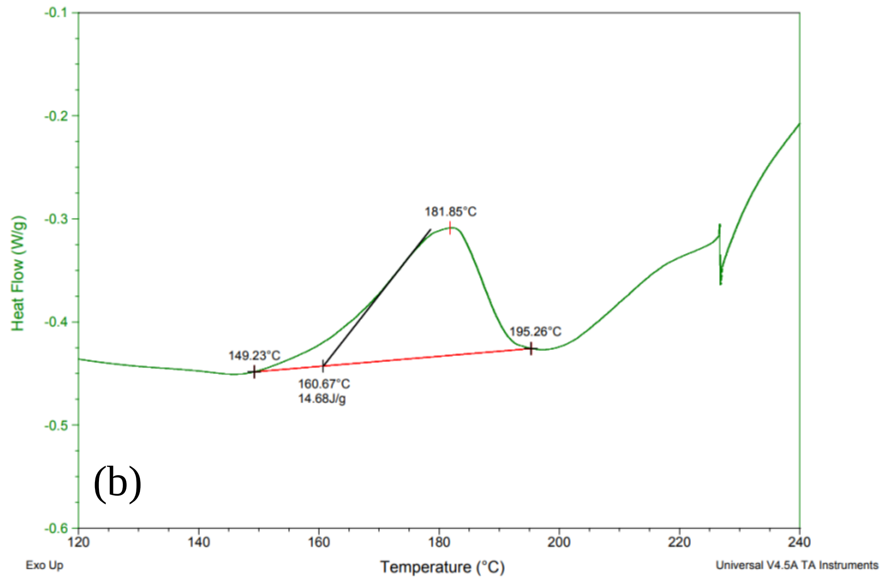


Figure A.2. Differential scanning calorimetry thermograph of one replicate for (a) PPI-50 and (b) PPI-60.