

# TEXTURIZATION OF PULSE PROTEINS: PEAS, LENTILS, AND FABA BEANS

A Dissertation

by

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## ABSTRACT

As interest in alternate sources for meat has increased, pulses have gained attention due to their excellent nutritional profile and few concerns about allergens, gluten, and genetically modified organism issues. The objective of this study was to develop texturized pulse proteins (TPP) and high moisture meat analogs (HMMA) with a twin-screw extruder (TX-52) using pea proteins (PP), lentil proteins (LP), and faba bean proteins (FP) and conduct sensory evaluations with patties containing these products. Commercial PP (55.4% protein), LP (55.4% protein), and FP (61.5% protein) were prepared for production of TPP and HMMA. Soy concentrate (SC, 75.8% protein) was used as a control.

Initially, these pulse proteins (PLP) were extruded to produce TPP using a processing condition of texturized SC. However, they were not texturized as well as did soy. High shear configuration was then applied for the TPP with calcium hydroxide (CH), sodium bisulfite, xanthan gum, and pea isolates (PI). Compared to control, texturized FP had significantly higher water holding capacity (WHC), less brown color, and similar gumminess. CH decreased WSI and increased gumminess in texturized LP, and the addition of PI decreased WSI and improved gumminess. 30% of each TPP were formulated to make meat patties with a beef flavor, and consumer evaluation was conducted. They had similar cooked appearance, overall, flavor, and texture except texturized LP (lower overall and texture) and FP (lower overall and flavor).

Premixed recipes (PLP, PI, wheat gluten, and canola oil) were texturized, followed by cooling in a media, freezing, thawing, and rehydration. The control had the best-

defined fiber orientation. HMMA containing PLP had significantly different parameters (less lightness, yellowness, M.C., and texture and higher redness and WSI) compared to control. Trained panelists observed higher bean-like, salty, sweet, umami, heated-oil and cohesiveness of mass and less soy, green, cardboardy, musty earthy, salty, hardness, and springiness than control. Consumer panelists gave similar scores on vegetable patties containing PL in cooked appearance, overall, and but lower overall texture.

Our findings suggest that PLP can be used in TPP and HMMA as alternative meat sources, and consumers will have more options for choosing alternative products.

## DEDICATION

텍사스 에이엔엠 대학에서 식품영양학으로 박사 학위를 받기까지 몰심 양면으로 함께 해 주신 가족에게 감사를 드립니다. 용기가 필요 할 때, 자신감이 필요 할 때, 친구가 되어 준, 그리고, 학업중에 태어난 세 아이를 돌보며 씩씩하게 견뎌 낸 현모양처, 아내 (조현진)에게 고마움을 전합니다. 집에서 학교에 있는 아빠를 기다리며, 엄마를 돕고 동생들을 돌봐 준 든든한 첫째 딸 (김가온), 귀엽고 상냥한 말씨로 엄마, 아빠에게 웃음을 선사하고, 언니, 동생과 사이좋게 지낸 둘째 딸 (김지은), 그리고, 막내지만 등직한 아들 (김성준)에게도 고마움을 전합니다. 특히, 항상 겸손한 모습으로 세상을 살아가는데 본이 되어주시고, 오랜 유학 생활을 하는 동안 믿고 기다려준, 그리고, 경제적으로도 헌신하신 아버지 (김우영), 어머니 (조찬호)께 감사를 드립니다. 귀한 막내 딸, 머나먼 미국으로 유학생에게 시집 보내 놓고 학업하는 동안 미국을 방문하여 손자, 손녀들 출산과 양육을 도와 주신 장인 (조희완), 장모 (염점순)님께 감사 드립니다. 한국에서 믿고 기다려 주며 부모님을 돌보느라 수고하신, 누님들 (김상희, 김선영, 김선경)과 여동생(김지선), 그리고, 매형 (채영철, 김관녕, 안병기)과 매제 (임명훈)에게 감사를 드립니다. 특별히, 논문 발표때, 놀라운 손재주로 교수님들을 매료 시켰던 처형 (조현정)에게 고마움을 전합니다. 한국에서 막내 동생 (조현진)을 보고 싶어하는 형님들에게도 고마움을 전합니다. 지혜와 용기를 북돋아 주시며, 항상 기도로 함께해 주신, 목사님 (임홍일), 사모님 (임혜경)께 감사를 드립니다. 한 교회에서 형제, 자매로 만난 비전선교교회 성도들에게도 감사를 드립니다.

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## NOMENCLATURE

PLP	Pulse proteins
PI	Peas isolates
SC	Soy concentrates
SI	Soy isolate
PP	Pea proteins
LP	Lentil proteins
FP	Faba bean proteins
TPP	Texturized pulse proteins
TXVP	Texturized vegetable proteins
TXSC	Texturized soy concentrates
TXPP	Texturized pea proteins
TXLP	Texturized lentil proteins
TXFP	Texturized faba bean proteins
TXPLP	Texturized pulse proteins
H-TXSC	Hydrated texturized soy concentrates
H-TXPP	Hydrated texturized pea proteins
H-TXLP	Hydrated texturized lentil proteins
H-TXFP	Hydrated texturized faba bean proteins
H-TPLP	Hydrated texturized pulse proteins
CH	Calcium hydroxide
SB	Sodium bisulfite

XG	Xanthan gum
WAI	Water absorption index
WSI	Water solubility index
WHC	Water holding capacity

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## CHAPTER I

### INTRODUCTION: RESEARCH OBJECTIVES AND LITERATURE REVIEW

#### 1.1 Introduction: Research Objectives and Literature Review

There has been an increased interest in alternative sources of meat. Because of a growing population and an increasing interest in healthy food from a growing middle class, pulses have regained attention as meat extenders due to their excellent nutritional profile and fewer concerns about allergens, gluten, and genetically modified organism issues. Textured vegetable proteins (TVP®) and high moisture meat analogs (HMMA) are good alternatives for meat, and the markets for these products are expected to grow. During this study, using pea, lentil, and faba bean proteins, texturized pulse proteins (TPP) as meat extenders, and HMMA as meat alternatives were produced using a twin-screw extruder (TX-52, Wenger Manufacturing Inc., Sabetha, KS). Optimized processing conditions including feed rate, preconditioning, running temperatures, steam, and extruder rpms for recipes using soy were provided by Wenger Manufacturing Inc. TPP, as a meat extender, were mixed with meat to make hamburger patties for consumer sensory evaluation. In contrast, high moisture meat analogs (HMMA) were evaluated by trained sensory panelists and used as a meat analog to produce vegetable patties for consumer sensory tests.

The intent of this project was to determine if commercially available pea, lentil, and faba bean proteins can be alternative sources for meat proteins since there is no texturized product using these pulse proteins yet. Also, there is a limited number of studies examining texturized pulse proteins compared to soy protein, wheat gluten, and

whey protein. Therefore, this research will help product developers to produce texturized vegetable proteins (TXVP) and HMMA and using pulse proteins.

In this study, the physical and chemical properties and sensory experiences of TPP and HMMA produced from commercially available pulse proteins were evaluated and compared to soy based textured proteins. The final products are allergen free, non-GMO, and if the products go commercial, will give consumers more selections for meat alternatives and meat extenders.

I hypothesized that TPP and HMMA using commercially available pea, lentil, and faba bean proteins would not have significantly different properties compared to the soy-based samples. Therefore, sensory characteristics, in particular appearance, flavor, and texture, would not be affected by the types of TPP proteins used in meat patties as meat extenders and HMMA used in vegetable patties compared to soy-based samples.

The main objectives of this study are to

- 1) Produce texturized pulse proteins (TPP) using pea, lentil, faba bean proteins and evaluate the physical and chemical properties.
- 2) Develop hamburger patties with TPP (30%) and meat (70%) and conduct a sensory evaluation with consumers.
- 3) Produce HMMA using pea, lentil, and faba bean proteins and evaluate the physical and chemical properties.
- 4) Develop vegetable hamburger patties containing HMMA and conduct a sensory evaluation with trained panelists and consumers.

## **1.2 Literature Review**

### **1.2.1 Pulses**

Pulses are the dry edible seeds of plants in the legume family including field peas, dry beans, lentils, chickpeas, and faba beans (Tyler and others 2017). Tyler and others (2017) mentioned that the contemporary definition of pulses excludes oil seed legumes and legumes consumed in immature form. There are many different varieties of pulses that grow all over the world, and they are consumed as staples in many countries. They are used in whole or dehulled form in canned goods, sweets, soups, and pastes, while pulse flours are becoming ingredients in a wide variety of food and pet food products such as baked goods, pasta and noodles, biscuits, and condiments (Tyler and others 2017). Pulses have a high protein content (about 20-40%) and abundant dietary fiber, resistant starch, vitamins, and minerals (Sozer and others 2016). In addition, the Frost and Sullivan Analysis found that pulses are considered non-allergenic, non-GMO, and appeal to vegans. Therefore, pulses can be excellent alternative meat sources.

### 1.2.1.1 Nutritional Profile of Pulses

**Table 1. Chemical Composition of Legumes (dry wt. basis).**

Legume	Proteins, %	Fat, %	Carbohydrates, %	Fiber, %	Ash, %
Peas	22-24	2-3	61-62	9-10	3
Lentils	21-23	2	65-68	6-7	3
Chickpeas	19-21	7	60-62	9-10	3
Faba beans	26-35	1	52-64	6-8	3-4
Cowpeas	22-26	1-2	60-65	4-6	3-4
Soybeans	37-41	18-21	30-40	4-6	4-5
Pigeon peas	15-29	1-3	60-66	5-10	3-4
Lima beans	19-25	1-2	70-75	4-6	3-5

<sup>a</sup> Data adopted from De Almeida Costa and others (2006); Bhatti (1974); Subuola and others (2012).

As seen in Table 1, pulses have a relatively small amount of fat (1-7%) compared to soybeans (18-21%) and oil seed so that they do not require a wet milling process to remove oil. They also have a significant amount of carbohydrates, fiber, and ash.

**Table 2. Essential Amino Acid Profiles of Meat, Cereal, and Legumes (g/16g N) Compared to the FAO/WHO/UNI (1985) Pattern.**

Protein sources	Meat %	Wheat %	Corn %	Rice %	Soy, %	Peas %	Lentils %	Faba beans %	F.R.A %
Histidine	2.8	1.8	2.2	2.2	2.86	2.8	2.8	3.2	1.6
Isoleucine	4.7	3.8	3.6	4.2	3.83	4.9	4.6	3.3	1.3
Leucine	8	6.8	14.1	8.1	7.78	7.5	7.2	7.2	1.9
Lysine	8.5	1.8	1.4	3.3	6.54	7.7	6.8	7.3	1.6
Methionine	2.5	1.4	1.8	2.6	1.7	3.3	2.9	1.1	--
Phenylamine	4.5	3.8	4	4.1	5.76	8.1	7.8	3.6	--
Threonine	4.6	3.2	3.7	4.1	3.66	3.8	3.6	4.1	0.9
Tryptophan	1.1	0.7	0.2	0.8	0.57	0.9	0.7	1.1	0.6
Valine	5.5	4.9	5	6.7	4.71	5.2	5	3.7	1.3

<sup>a</sup> Data adopted from Eastoe and Long (1960), Koehler and Wieser (2013), Vasconcelos and others (1997), Wang and Daun (2004), and Khalil and Mansour (1995). F.R.A = FAO/WHO/UNI recommended for Adult.

Lysine and methionine are the first limiting amino acids in most cereal grains and legumes, respectively, (Sarwar and Peace 1986). Most legumes are also low in



methionine while high amounts of methionine can be found in eggs, nuts, fish, meat and cereal grains. In contrast, peas and lentils have more methionine than meat and cereals, 94% and 53%, respectively, compared to soy (Table 2). These pulses (peas, lentils, and faba beans) are rich in lysine, which is limited in cereals. In addition, they have significantly more essential amino acids than is recommended by the FAO/WHO/UNI (1985). Therefore, the pulse proteins used in this research have excellent essential amino acid profiles.

### 1.2.1.2 Production and Consumption of Pulses

**Table 3. World Production of Pulses in 2014 (1,000 Metric Tons).**

Region	Pulses, Total	Beans, Dry	Peas, Dry	Chickpeas	Lentils
World	77,599	25,093	11,332	14,239	4,885
Asia	35,124	10,665	2,580	12,003	2,098
Africa	16,999	5,927	656	765	181
Americas	15,381	7,744	4,412	478	2,159
Europe	6,986	703	3,370	174	94

a Data adopted from Sozer and others (2016); (FAOSTAT 2016).

Table 3 shows world production of pulses in 2014. Global pulse production was 77 million metric tons in 2014. Asia led by almost half of the global production, and Africa and America, especially Canada, produced 21.9 and 19.8% (FAOSTAT 2016). Global pulse production was 10% of wheat production, but about three times the global oat production (Sozer and others 2016).

**Table 4. Amount of Pulses in the Food Supply in 2011 (kg/year per capita).**

Region of Pulses	Pulses	Beans	Peas
World	6.8	2.5	0.8
Asia	6.3	1.6	0.8
Africa	10.8	3.4	0.5
Europe	2.7	0.7	1.4
Americas	8.3	6.8	0.3

<sup>a</sup>Data adopted from Sozer and others (2016); (FAOSTAT 2016).

The average pulse consumption per capita in 2011 was 2.7-10.8 kg/year (Table 4). When different continents are compared consumption is lowest in Europe and highest in Africa (FAOSTAT 2016). Joshi and Rao (2016) carried out a business-as-usual scenario to estimate a supply and demand projection for total pulse production. The report mentions that Europe, North America, and Latin America will have a surplus that will grow from 2020 to 2050, and North America will have the largest surplus of 8 million tons in 2050, up from 5 million tons in 2020. In contrast, Africa and Asia will face huge deficits, about 11 million tons in Africa and 5.5 million tons in Asia in 2050 (Joshi and Rao 2016). Within Asia, Eastern Asia will have a surplus whereas Southern Asia will have a deficit of 9 million tons by 2050 (Joshi and Rao 2016). Increased use of plant foods is unavoidable owing to the demands of sustainability, food security, and increased population (Sozer and others 2016). In other words, consumption of pulses in near future is expected to increase the production of pulses.

### *1.2.1.3 Protein Enrichment of Pulses*

Commercially, there are various types of purified ingredients from legumes that go through an enrichment process to separate out the unwanted particles. Soy protein concentrate and soy protein isolate containing a minimum of 70% and 90% protein,

respectively, are examples of modified ingredients to increase protein content (Lusas and Riaz 1995). Most pulses have a significant amount of carbohydrates, fiber, and ash as well, and they are used in foods as nutritionally balanced ingredients. There are some commercially available purified forms of pulses such as pea isolates, pea protein concentrate, lentil protein concentrate, and faba bean protein concentrate that are also used as food ingredients (Sozer and others 2016).

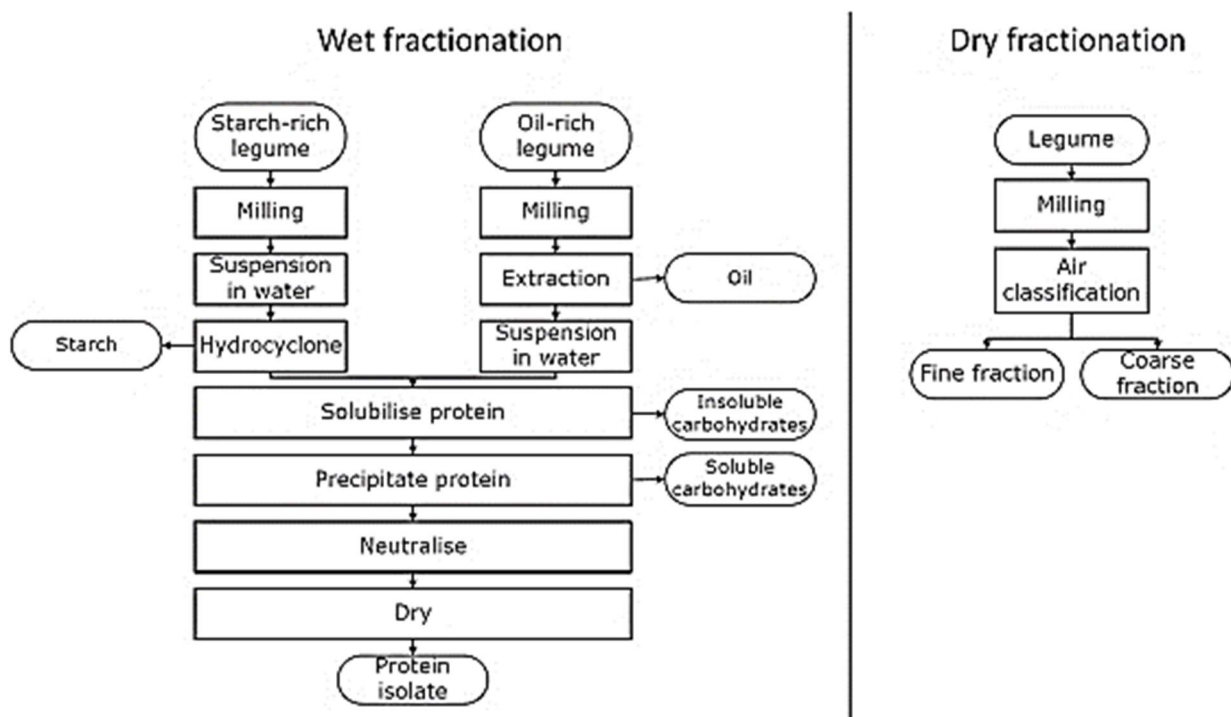


Figure 1. Schematic illustration of wet (left) and dry (right) fraction process (Reprinted with permission from Schutyser and others 2015).

Figure 1 shows a schematic view of the milling process generally used for protein enrichment (Schutyser and others 2015). All legumes can be enriched by wet fraction; however, legumes with low fat can be enriched by dry fraction as well. The dry milled pea, lentil, and faba bean protein flour that are commercially available and will be used

in this study are usually classified by air-flow based on the size of the particles, gravity, or density.

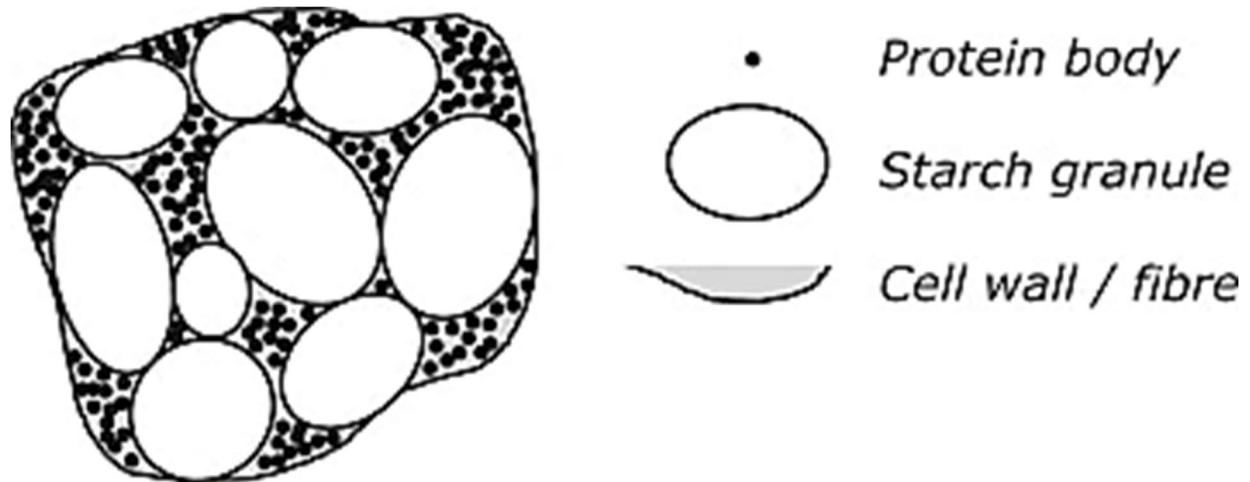


Figure 2. Schematic view of the protein body, starch granule, cell wall, and fiber in a legume (Reprinted with permission from Schutyser and others 2015)

As seen in Figure 2, the size of the protein body is relatively smaller than the size of the starch granules (Schutyser and others 2015). The cotyledon cells consist of starch granules (above 20 $\mu\text{m}$ ) embedded in a matrix of protein bodies (1 to 3  $\mu\text{m}$ ) that are surrounded by a fiber-rich cell wall (Tyler and Panchuk 1982). A milling machine cracks the structure of the protein body, starch granule, cell wall, and fiber of the legumes (Figure 2). In wet fraction, starch-rich legumes such as peas are hydrated and centrifuged to remove the starch after milling while oil-rich legumes require an extraction process using a solvent treatment such as aqueous alcohol, methanol, or hexane before hydration and after the milling process. Insoluble carbohydrates are separated from solubilized proteins by a method such as centrifugation, and soluble carbohydrates are removed from precipitated proteins with HCl or NaOH since the *iso*-electric points of legumes is around pH = 4. They are neutralized and dried to produce a protein isolate.

In contrast, legumes with a low-fat content are mechanically milled using a dry milling process.

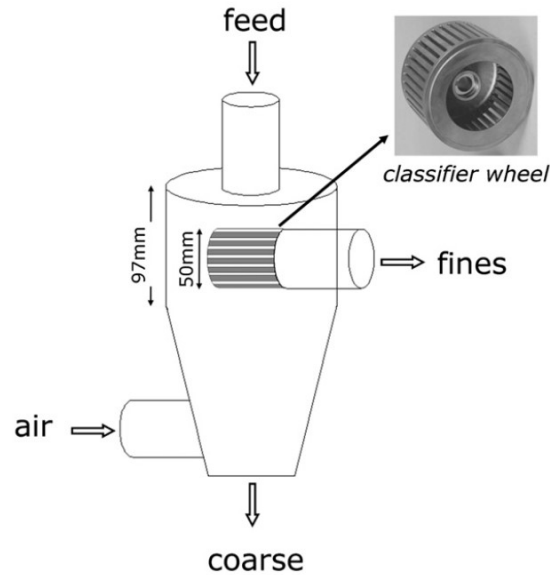


Figure 3. Schematic overview of the air classifier (ATP50) (Reprinted with permission from Pelgrom and others 2013).

Figure 3 show a schematic overview of the classifier for the major ingredients used in this project (Pelgrom and others 2013). Milling before dry fraction should detach protein bodies from other components of the cell such as starch granules. The particle size of a protein body is 1 to 3  $\mu\text{m}$ , starch granules are around 22  $\mu\text{m}$ , and particles larger than 40  $\mu\text{m}$  are whole cells or parts of cells (Pelgrom and others 2013; Pernollet 1978; Vose 1978). The dry milled flour is classified by air-flow based on the size of the particles, gravity or density, and fine ground proteins pass through the classifier wheel. As a result, unwanted particles are removed, and fine ground proteins (above 50% protein) are collected.

**Table 5. Protein Enrichment by Air Classification of Wheat and Several Legumes.**

Legume/grain	Initial protein content (g/100 g dry matter)	Protein content fine fraction (g/100 g dry matter)
Wheat	12.3 ± 1.8	28.3 ± 4.0
Lima beans	23.7 ± 0.4	48.9 ± 0.8
Cowpeas	27.2 ± 0.0	50.9 ± 0.2
Common beans	26.3 ± 1.6	54.7 ± 2.2
Navy beans	27.2 ± 1.6	56.7 ± 6.8
Lentils	23.7 ± 2.1	57.6 ± 4.1
Peas	23.8 ± 1.2	58.5 ± 3.0
Mung beans	27.2 ± 0.4	62.3 ± 1.2
Faba beans	31.0 ± 0.8	69.9 ± 5.2
Lupines	40.4 ± 0.6	59.4 ± 0.6

<sup>a</sup>Data adopted from Schutyser and others (2015).

Table 5 shows the protein enrichment by air classification of wheat and several legumes. As shown in Table 5, most of the legumes and the wheat had at least a 100% increase in protein content with fine fraction after air-classification compared to the initial protein content except cowpeas (87%) and lupines (47%). In other words, to produce protein concentrates, dry-milling with an air-classification significantly improved protein content by removing unwanted particles without any solvent treatment compared to a wet-milling process.

### **1.2.2 Meat**

Meat is an important source of several essential nutrients such as protein and is indispensable for optimal health for human life (Ekmekcioglu and others 2017). Meat contains all of the essential amino acids with no limiting amino acids (Williams 2007). In addition, meat has been widely consumed by humans since pre-historic times due to its ready source of energy, high quality proteins, and palatability as well as its images of strength and power (Fiddes 2004; Latvala and others 2012).

### 1.2.2.1 Meat Consumption and Concerns about Meat Production

The per capita consumption of meat has more than doubled from 1961 to 2007 and has grown rapidly in developing countries compared to developed countries (Kumar and others 2015). Demand for meat is expected to increase by 72% due to increasing population growth, urbanization, industrialization, education, and a rise in incomes. Steinfeld and others (2006) and Fiala (2008) projected animal product production to increase from 229 billion kg to 465 billion kg as the population grows from 6.0 billion in 2000 to 9.1 billion by 2050. Consequently, there will be a 70% increase in the amount of food required by 2050, and there will be a 100% increase in the demand for protein in the coming decades (Kumar et al., 2017).

Meat production has increased 5-13% in last decade as well (Post 2012). However, Post (2012) determined that meat production almost reached its maximum as well. In addition, (Post 2012) suggested three major concerns relate to meat production:

- 1) Environmental issues – such as environmental pollution, deforestation, depletion of natural resources, etc.
- 2) Animal welfare issues – such as cruelty and the unethical treatment of animals during rearing, transportation, and slaughter.
- 3) Public health issues – such as over 1.8 million deaths annually due to the overconsumption of meat resulting in a quarter of all ischemic heart disease (Key and others 1999). Larsson and Wolk (2006) reported that the consumption of 120g of red meat/day or 30 g of processed meat/day would seriously raise the risk of colorectal cancer. The food borne pathogens found in meats, such as *Salmonella*, *Campylobacter*, and *E. coli*, are responsible for the illness of 1 in 6

Americans (or 48 million people), the hospitalization of 128,000, and the death of 3,000 each year (CDC 2012).

In the near future, meat may not be the sole major ingredient supplying protein for humans due to limited land, water, and energy resources. Therefore, the possibility of fabricating palatable protein-rich foods from plant sources has stimulated great interest, and policy makers and scientists must shift their mindset toward the development of suitable alternatives of simulated meat-like products, with controlled texture, flavor, color, and nutritional value (Kumar and others 2017).

### **1.2.3 *Alternative Meat Products***

Alternative meat products are in the infant stage of development and at present account for only 1-2% of the total meat market (De Bakker and Dagevos 2010). However, due to the cheapness of the protein, plus environmental and nutritional factors, the use of vegetable sources is bound to increase as alternatives for expensive meat proteins (Kumar and others 2017). As a result, vegetable proteins such as soya protein, pulses, nuts, cereal proteins, vegetables, and mycoproteins are currently the main sources of material for meat analogues (Kumar and others 2017). At present, soybeans are the main source of meat alternatives due to its competitive price compared to other sources. However, newer ingredients in meat analogs are expected to be introduced due to wider consumer preferences.

In addition, the global substitute meat market is optimistic as well. In 2014, the global substitute meat market was valued at \$3.3 billion and is expected to reach \$5.8 billion by 2022, resulting in a compound annual growth rate (CAGR) of 7.5% from 2015 to 2022 (Grand View Research 2016). TVP® emerged as a leading product



segment and accounted for 43.7% of the total market revenue for global meat substitutes in 2014 (Grand View Research 2016).

Generally, a meat analog is considered a food made from nonmeat ingredients, sometimes without dairy products (Malav and others 2013). Food researchers and processors invented meat analogs, which is food that is structurally similar to meat but differs in composition, to overcome this dilemma and satisfy meat lovers (Malav and others 2013; Sadler 2004). These meat analogs are also called meat substitutes, mock meat, faux meat, or imitation meat (Sadler 2004). The key ingredients used during the preparation of meat analogs are soy protein, mushrooms, wheat gluten, egg albumin, carbohydrates, gum, and flavoring and other miscellaneous compounds such as fiber, caseinate, or carrageenan, as needed (Kumar and others 2017).

#### *1.2.3.1 Textured Vegetable Proteins*

Even though the term meat analog has been mostly used for products based on spun protein filaments, it also includes many other generalized products such as TXVP (Kumar and others 2017). The most commonly known processed food ingredients used as meat alternatives are textured vegetable proteins (TVP®) used as meat extenders and HMMA used as vegetable meats. TXVP is dry food products textured by spinning or by extrusion, and they are popular for use in vegetarian food since they provide a fibrous structure for the product similar to the texture of meat (Kitcharoenthawornchai and Harnsilawat 2015). Usually, TXVP is a shelf stable product due to their low moisture content, and they are hydrated for use as meat extenders to increase the volume of meat in foods. Hydrated TVPs can be formulated to make meat analogs and can be

formed into sheets, disks, patties, strips and other shapes and the finished products taste like chicken, beef, lamb, ham, sausage, seafood, etc. (Malav and others 2013).

### *1.2.3.2 High Moisture Meat Analog*

High moisture meat analogs, HMMA, is a protein product produced by an extrusion process with the addition of moisture (40% to 80%) during the process to prevent expansion of the product in a cooling die attached to the end of the extruder. Unlike low moisture extruded protein products, HMMAs have well defined fiber formations, resemble chicken or turkey breast meat, and therefore have an enhanced visual appearance and taste sensation (Yao and others 2004).

### *1.2.3.3 Texturization*

Proteins in a native state of ingredients are complex compounds folded and assembled with chemical reactions along a polypeptide backbone including hydrogen bonds, hydrophobic interactions, van der Waals interactions, a disulfide bridge, and ionic bonds. Texturization of protein can be explained as a structural change from globular to fibrous shapes during the cooking process.

TVP® is also usually made from soy flour from which fats and soluble carbohydrates are removed (Kitcharoenthawornchai and Harnsilawat 2015). Depending on the purpose of the final products, the contents of the soy flour can be modified to increase the protein content and produce soy protein concentrates (SPC) and soy protein isolates (SPI) containing a minimum of 70% and 90% protein, respectively (Lusas and Riaz 1995). SPC is produced through aqueous alcohol or methanol extraction from defatted soy flakes, which typically contain 65-70% crude protein, and

removes a majority of the phytate, lecithin, and oligosaccharides (Anderson and Wolf 1995; Lusas and Riaz 1995). SPI is produced by a series of aqueous extractions completed at different pH levels (Blaufuss and Trushenski 2012). SPC costs 2 to 2.5 times more than defatted soy flour, and SPI costs normally 5 to 7 times as much (FAO 2013a; FAO 2013b). Raw materials containing higher protein levels are more easily texturized with an extruder at lower levels of energy input, and produce tougher and firmer textures (Riaz 2004). Therefore, the selection of protein levels is important for production of TVP® to provide the best quality in the final products.

#### 1.2.3.4 Production of Texturized Vegetable Proteins and High Moisture Meat Analogs

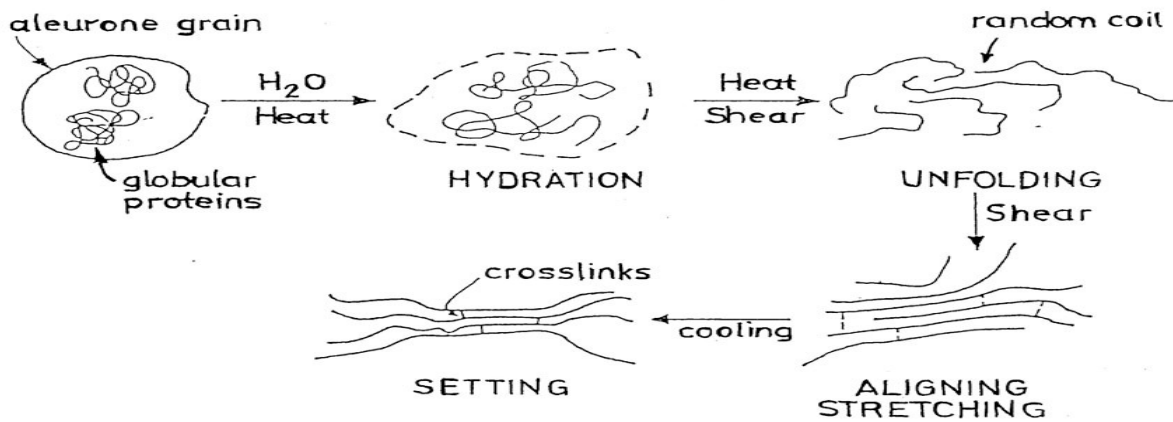


Figure 4. Diagram of the texturization process (Anonymous).

As seen in Figure 4, during the cooking process in the extruder with water and heat, the globular shaped native proteins in flours are unfolded (Anonymous). These unfolded proteins are aligned by shear driven by the screw in the barrel of the extruder. During the cooling process, these aligned proteins are aggregated and texturized by

crosslinking between protein molecules. In other words, during the cooking process in the barrel, sulfhydryl groups are reduced by water and heat, and disulfide bonds are broken by heat and shear. These unfolded and elongated protein molecules are reformed and result in protein aggregation and texturization.

Proteins are the main seed component in all grain legumes, and are the reason for their important nutritional and socio-economic impacts (Duranti and Gius 1997). Most vegetable sources of TXVP are currently limited to soy or wheat although there are various other legumes containing abundant proteins. Plant origin protein is an alternative to animal protein for food applications due to the widespread variety of sources, such as legumes, oilseeds, cereals, and fungi (Kumar and others 2015). Therefore, all the sources have different types of protein which function differently in foods. For example,  $\beta$ -conglycinin is a major protein component of soybeans, and glycinin and vicilin are components of legumes (Kumar and others 2015). Soybean proteins are mainly composed of 2S, 7S, 11S, and 15S. 2S consists of 8,000 to 20,000 daltons, and 7S are heterogeneous with  $\beta$ -conglycinin composing 150,000 daltons. 11S has glycinin as a major component with 320,000-350,000 daltons built of 12 units and associated through hydrogen bonding and disulfide bonds related to their functional properties and particularly to their texturization. 15S are composed of dimers of glycinin. Both 7S and 11S subunits of soy protein and major components for texturization, start to unfold when heated above 100°C and become totally unfold at 140°C (Soeda 1994). The 7S and 11S fractions of soybeans account for about 70% of the total protein content (Saio and Watanabe 1978). The 11S protein in tofu precipitates faster, forms a larger aggregate and higher water holding capacity, has higher tensile values,

hardness, and expands more on heating. Major components of legumes are carbohydrates, but they also contain protein, fiber, ash, and fats (Table 1). More importantly, most legumes contain approximately 20% proteins.

**Table 6. Protein Contents and Amino Acid Profiles of Pulse Proteins (Peas, Lentils, Faba Beans).**

Pulse	Crude protein (%)	Albumins (%)	Globulins (%)	Glutelin (%)	Prolamin (%)
Peas	14-31	15-25	49-70	11	5
Lentils	23-31	17	51	11	4
Faba beans	36	2	74	18	4

a Data adopted from Hall and others (2017).

b Glutelin and prolamin reported together.

In contrast, pulse is mainly composed of 2S (albumin), 7S (vicilin), and 11S (globulin) (Clifford Hall et al., 2016). As seen in Table 6, 11S (globulins) is a major component of pulses (peas, lentils, and faba beans) that are the major ingredients for this study. All peas, lentils, and faba beans have a considerable number of globulins (7S and 11S). Regarding major components (7S and 11S) for the texturization of soy, these pulse proteins are expected to be texturized.

### 1.2.3.5 Market Trend of Alternative Meat Sources

The global market production of pulses (various peas, beans, lupines, and lentils) in 2014 was 77 million metric tons, whereas the global market production of soybeans in 2014 was 278 million metric tons (FAO 2013c). The production of peas and lentils in the United States was approximately 0.9 million metric tons in 2014, and 70% of the production was exported (Asif and others 2013; USDA 2015). The average world consumption was 6.8 kg per person in 2011, and a 23% growth is expected by 2030

compared to current consumption (Sozer and others 2016). Therefore, the projected production and consumption of pulses are optimistic. In addition, the global substitute meat market is optimistic as well. In 2014, the global substitute meat market was valued at \$3.3 billion and is expected to reach \$5.8 billion by 2022, resulting in a compound annual growth rate of 7.5% from 2015 to 2022 (Grand View Research 2016). TVP® emerged as a leading product segment and accounted for 43.7% of the total market revenue for global meat substitutes in 2014 (Grand View Research 2016).

## CHAPTER II

### PRELIMINARY TEST FOR TEXTURIZATION OF PULSE PROTEINS:

#### PEAS, LENTILS, AND FABA BEANS

##### 2.1 Introduction

Pulses are the dry edible seeds of plants in the legume family including field peas, dry beans, lentils, chickpeas, and faba beans (Tyler and others 2017). They are high in dietary fiber, resistant starch, vitamins, minerals, and proteins, such as lysine, and are recognized as nutritious (Udahogora 2012). Thus, they have been widely consumed in many different forms in countries with limited meat consumption.

Meats have been an unbeatable food source due to their nutritional excellence, unique flavor, and availability to people throughout human history. However, they have a negative image, raise questions of animal diseases, and underscore concerns about the shortage of animal protein with the increasing global population. Currently, soy is a major meat substitute in the food industry because of its competitive price, health benefits, and functional properties. Since textured vegetable protein (TVP<sup>®</sup>) also called textured soy protein (TSP<sup>®</sup>) was invented by Archer Daniels Midland (ADM) in the 1960s, soy-based meat alternatives have been widely used in foods such as food toppings, hamburger patties, or vegetarian foods. Along with the global population growth, estimated to be 9.1 billion by 2050, the demand for vegetable protein sources is expected to increase, and consumer's preferences for allergy free, non-genetically modified organisms (GMOs), and organic products are also expected to increase (Steinfeld and others 2006; Bruinsma 2009). Therefore, pulses can be another option to

replace meat, can provide an answer for the strong demand for wholesome and religiously sanctioned foods, and are economical.

In this research, pulse proteins (peas, lentils, and faba beans) were investigated as texturized pulse proteins (TPP) to find alternative sources for meats and soy, which are major proteins in the human diet. Generally, these pulse proteins are produced by a dry milling process followed by air classification. These processes increased protein content up to 2.3 times by separating large starch granules and cell wall fibers (Schutyser and others 2015). In pulses, proteins are in a globular form with complex chemical reactions including hydrogen bonds, hydrophobic interactions, Van der Waals interactions, disulfide bonds, and ionic bonds. However, once they are processed with water in the extruder, through thermal and mechanical energy, they are unfolded, aligned, and stretched. As they cool, they are re-associated, crosslinked, and finally texturized.

Objectives of this research were to develop TPP using commercially available proteins, pea, lentil, and faba bean proteins, and to evaluate the effect of the pulses in the texturization process. I hypothesized that the pulse proteins would texturize as well as soy, and their physical and chemical characteristics would be like soy concentrate. It was expected that if these pulse proteins would texturize as well as soy concentrate, they could be not only excellent meat substitutes, but also potential substitutes for current major meat substitutes such as soy and wheat gluten. As a result, the consumer will have more options when choosing meat extenders. In addition, this study will help product developers produce texturized vegetable protein products using pulse proteins.



## 2.2 Materials and Methods

### 2.2.1 Materials

**Table 7. Chemical Composition of Ingredients.**

Protein	Moisture (%)	Protein (% d.b.)	Carbohydrate (% d.b.)	Fat (% d.b.)	Ash (% d.b.)
SC	9	75.8	20.9	3.3	0
PP	8	55.4	35.9	3.3	5.4
LP	8	55.4	35.9	3.3	5.4
FP	9	61.5	29.7	3.3	5.4

<sup>a</sup> SC=soy concentrate, PP=pea protein, LP=lentil protein, and FP=faba bean protein.

<sup>b</sup> Data provided from Ingredion Incorporated (Westchester, IL) and Alliance Grain Traders (AGT) Food and Ingredients (Regina, Canada).

Ingredients were obtained from Ingredion Incorporated (Westchester, IL) and Alliance Grain Traders (AGT) Food and Ingredients (Regina, Canada) which supplied Vitessence™ Pulse 1550 (pea protein), Vitessence™ Pulse 2550 (lentil protein), and Vitessence™ Pulse 3600 (faba bean protein). Arcon® F (soy concentrate) was obtained from ADM (Decatur, IL). The pH of each raw ingredient was measured using a pH meter (Five Easy Plus, Mettler Toledo, Australia) at room temperature. Before being used, the pH meter was calibrated using buffer solutions of pH  $4.01 \pm 0.02$ ,  $7.02 \pm 0.02$ , and  $9.2 \pm 0.02$ . Table 7 shows the chemical composition of the raw ingredients.

### 2.2.2 Extrusion

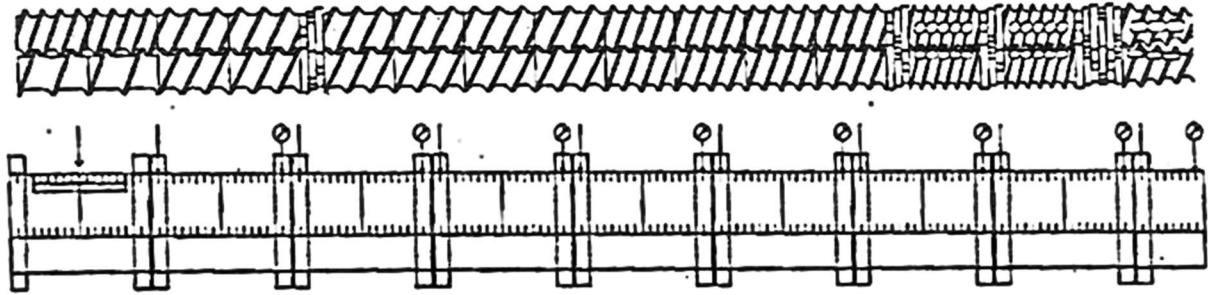


Figure 5. Regular shear configuration with twin screw extruder (TX-52, Wenger) to produce texturized pulse proteins.

For this research, optimized processing conditions including feed rate, preconditioning, running temperatures, steam, and extruder running rpms for recipes with soy were provided by Wenger Manufacturing Inc. (Sabetha, KS). Figure 5 shows a regular shear configuration to produce TPP. For the preliminary test to optimize the processing conditions for pulse proteins, pea protein (PP), lentil protein (LP), and faba bean protein (FP) were texturized using the Wenger twin-screw extruder (TX-52), and soy concentrates (SC) were used as a control for texturization. The ingredients were extruded and cut with a knife at the end of the square dimensional die (9.53 mm x 9.53mm).

**Table 8. Processing Conditions for the Texturization of Soy Concentrates and Pea Proteins for the Preliminary Test.**

Process	Condition	SE1	PE1	PE2	PE3	PE4	PE5
Feeder	Speed, rpm				15		
	Speed, rpm				400		
Preconditioning	Steam flow, kg/hr	14	14	14	14	16	19
	Water flow, kg/hr	15	15	15	15	14	5
	Discharge temp, °C	91	93	94	95	99	99
	Shaft speed, rpm	328	328	355	382	382	522
Extrusion	Steam flow, kg/hr	0	0	0	0	0	10
	Water flow, kg/hr				0		
	Max temp, Z5, °C	114	105	98	98	98	100

<sup>a</sup>SC=soy concentrate, PP=pea protein, LP=lentil protein, FP=faba bean protein, SE=soy concentrates extrudates, PE=pea protein extrudates. The numbers after PE indicates different extrudates with different processing conditions.

Table 8 shows the processing conditions for the pulse protein concentrates (Wenger TX52). During the preliminary test, PE1 was not texturized as well as SE1 so the processing condition was varied. During the texturization, PE1, PE2, and PE3 were processed with 3 different extruder shaft speeds (328 rpm, 355rpm, and 382 rpm, respectively). PE4 (16kg/hr) and PE5 (19 kg/hr) had increased steam in the preconditioner, and PE5 (522 522 rpm and 10 kg/hr) and PE (500 rpm and 5 kg/hr) had a high extruder shaft speed and steam added to the extruder and a decreased water flow in the preconditioner.

**Table 9. Processing Conditions for Texturization of Pulse Protein Concentrates for the Main Test with Modified Processing Conditions from the Preliminary Test.**

Process	Condition	SE	PE	LE	FE
Feeder	Speed, rpm			15	
	Speed, rpm			400	
Preconditioning	Steam flow, kg/hr			13	
	Water flow, kg/hr			2	
	Discharge temp, °C	98	96	98	97
	Shaft speed, rpm			500	
Extrusion	Steam flow, kg/hr	6	5	7	6
	Water flow, kg/hr	3	3	4	3
	Max temp, Z5, °C	114	99	99	101

<sup>a</sup>SE=soy concentrates extrudates, PE=pea protein extrudates, LE=lentil protein extrudates, and FE=faba bean protein extrudates.

For the main test, the processing conditions for PE5 with water and steam added to the extruder were used to produce PE, LE, FE, and SC (Table 9).

### **2.2.3 Drying**

After texturization, the extrudates were dried in a hot air dryer (Wenger Manufacturing Inc. Sabetha, KS) at 105°C until only about 10 % moisture content remained, and were placed into labeled air tight containers for further analysis (AOAC 1990).

### **2.2.4 Water Absorption Index (WAI) and Water Solubility Index (WSI)**

The water absorption index (WAI) and water solubility index (WSI) of the raw ingredients and extrudates were calculated using Equation 2.1 and Equation 2.2, respectively (Anderson 1982). Samples were ground with a cyclone mill (UDY Corp., Fort Collins, CO) (1mm mesh) before testing.

$$WAI = \frac{\textit{Weight of absorbed water into samples}}{\textit{Weight of dry samples}} \times 100 \quad \text{Equation 2.1}$$

$$WSI = \frac{\textit{Weight loss during hydration}}{\textit{Weight of dry samples}} \times 100 \quad \text{Equation 2.2}$$

### **2.2.5 Expansion Ratio (ER)**

The axial and radial dimensions of the extrudates were measured with a digital Vernier caliper. The axial lengths of 10 extrudates for each sample were measured in mm, and the radial expansion ratios were calculated using Equation 2.3. The radial dimension of the die was 9.53 mm.

$$ER = \frac{\textit{Diameter of extrudate}}{\textit{Diameter of die}} \times 100 \quad \text{Equation 2.3}$$

### **2.2.6 Color**

Raw ingredients and extrudates were directly evaluated using a colorimeter (Model CR-310, Minolta, Osaka, Japan). In addition, extrudates were ground using a coffee grinder and were evaluated with the colorimeter. Values were expressed as L\*, a\* and b\*, where L\* values (lightness) vary from black (0) to white (100), chroma a\* values (redness) vary from green (-60) to red (+60), and chroma b\* values (yellowness) vary from blue (-60) to yellow (+60).

### **2.2.7 Statistical Analysis**

A one-way ANOVA was used to determine the significant difference (P<0.05) between different varieties of pulses before and after extrusion. Tukey's HSC (honestly

significant difference) analysis was also conducted for pair comparison. All statistical tests were performed using JMP software (JMP Pro 12.0.1, SAS Institute Inc., Cary, N.C., USA).

## 2.3 Results and Discussion

### 2.3.1 Texturization

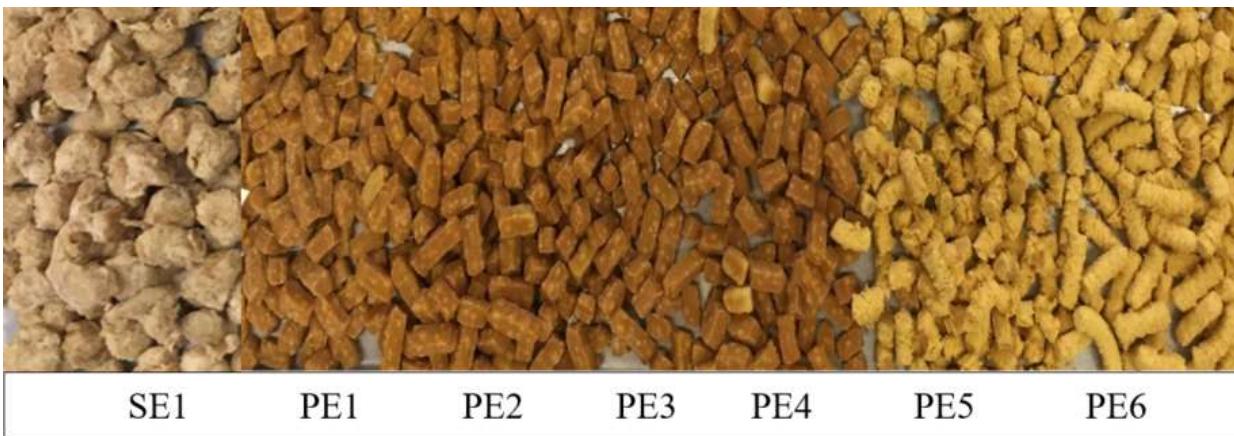


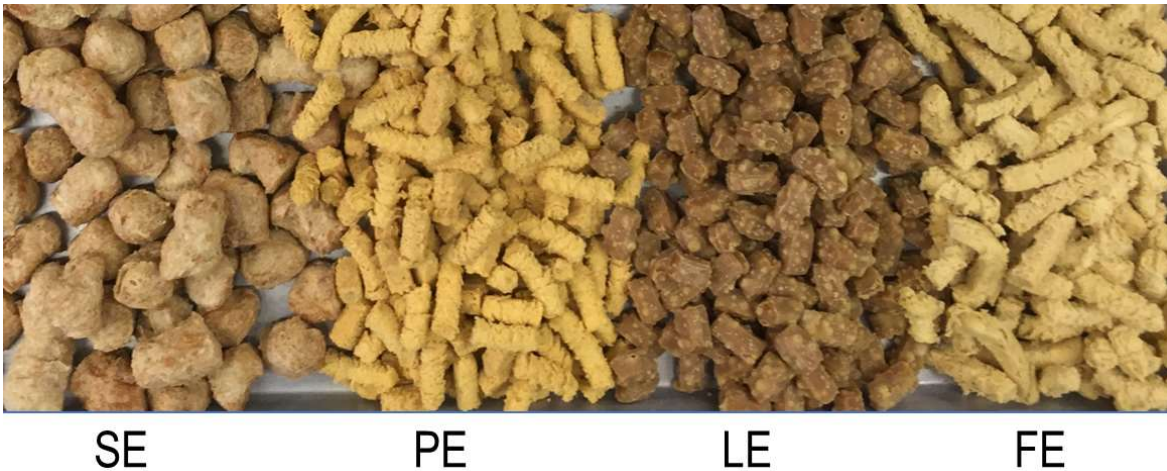
Figure 6. Texturization of soy concentrate (control) and pea proteins (PP) in the regular shear configuration of the extruder SE=soy-concentrate extrudates and PE=Pea protein extrudates. The numbers after PE indicates different extrudates with different processing conditions.

Figure 6 shows the texturized proteins from the preliminary test to find the optimum conditions of texturization for PP with a control (SE1). The soy concentrate was texturized well, but the PP samples were not texturized as well as the soy concentrate (SC). Therefore, PE1, PE2, PE3, PE4, and PE5 did not texturize as well as did the soy concentrate. As seen in Table 8, the shaft speed of the extruder was gradually increased to provide more mechanical energy (PE1, PE2, and PE3), and more steam

and less water were put into the preconditioner (PE4 and PE5). The shaft speed was increased which means that more mechanical energy was applied, and steam was added (PE5). Pea extrudates (PE1, PE2, PE3, and PE4) that were texturized with less steam and more water in the preconditioner and a lower shaft speed of the extruder compared the samples (PE5) had rectangular parallel shapes. In contrast, PE5 texturized with more steam and mechanical energy had cylindrical shapes with a crumbly appearance on the surface. PE5 did not have the degree of texturization as well as SE1, but PE5 had a better texturized appearance than other samples (PE1, PE2, PE3, and PE4).

In addition, PE1, PE2, PE3, and PE4 had different colors and visual textures from PE5. The previous four samples had a light brown color and smoothness with lumps on the extrudate surfaces that indicted moisture might have been trapped inside and released by pressure buildup, while the last two samples (PE4 and PE5) had a gold color and were crumbly with a rough and layered surface that indicted that the steam on the surface of the extrudates might have evaporated while cooling. In other words, mechanical energy and moisture affected the appearance of the extrudates. The color changes can be attributed to a Maillard reaction involving amino acids and reducing sugar in the condition of excessive energy, which resulted in a light brown color in the four samples. In addition, steam might provide more energy to induce the cleavage of the protein body structure and moisture to avoid the Maillard reaction in the ingredients during this process. PP was run with a decreased shaft speed and steam flow compared to processing conditions to produce PE5 because of concerns that there might be structural damage of the ingredients. Water was introduced into the extruder

as well, but PE6 had a similar appearance to PE5. From a texturization of the preliminary test, a processing condition (PE) after testing with the maximized processing conditions of the extrusion system, except for the changing of the screw configuration, was selected for the texturization of SC, LP and FP.



**Figure 7. Texturization of SC (control) and pulse pea proteins in a higher shear configuration of the extruder. SE=soy-concentrate extrudates, PE=pea protein extrudates, LE=lentil protein extrudates, and FE=faba bean extrudates.**

Figure 7 shows texturized vegetable proteins (TXVP) from the main test with the same processing conditions used in the preliminary test (PE). SE had a well-texturized appearance and a more expanded volume than SE1. Other samples, pea protein extrudates (PE), lentil protein extrudates (LE), and faba bean protein extrudates (FE), did not have a well-texturized appearance compared to SE, and they had different color and visual textures. LE had a brown color and was smooth with lumps on the surface like the first four samples (PE1, PE2, PE3, and PE4) of the preliminary test (light brown). During cooling after texturization, the steam on the surface evaporated and



created lumps on the surface to release the steam after a gel formed and pressure built. In contrast, PE and FE did not have lumps and had crumbly textures with a rough and layered surface that suggested that the steam of the extrudates might have evaporated without forming lumps while cooling. LP might need more energy to denaturize the protein structure for texturization than PP and FP that were denaturated less in protein structure for texturization than SC to produce SE. PE had a gold color and FE had a tan color.

### 2.3.2 Color

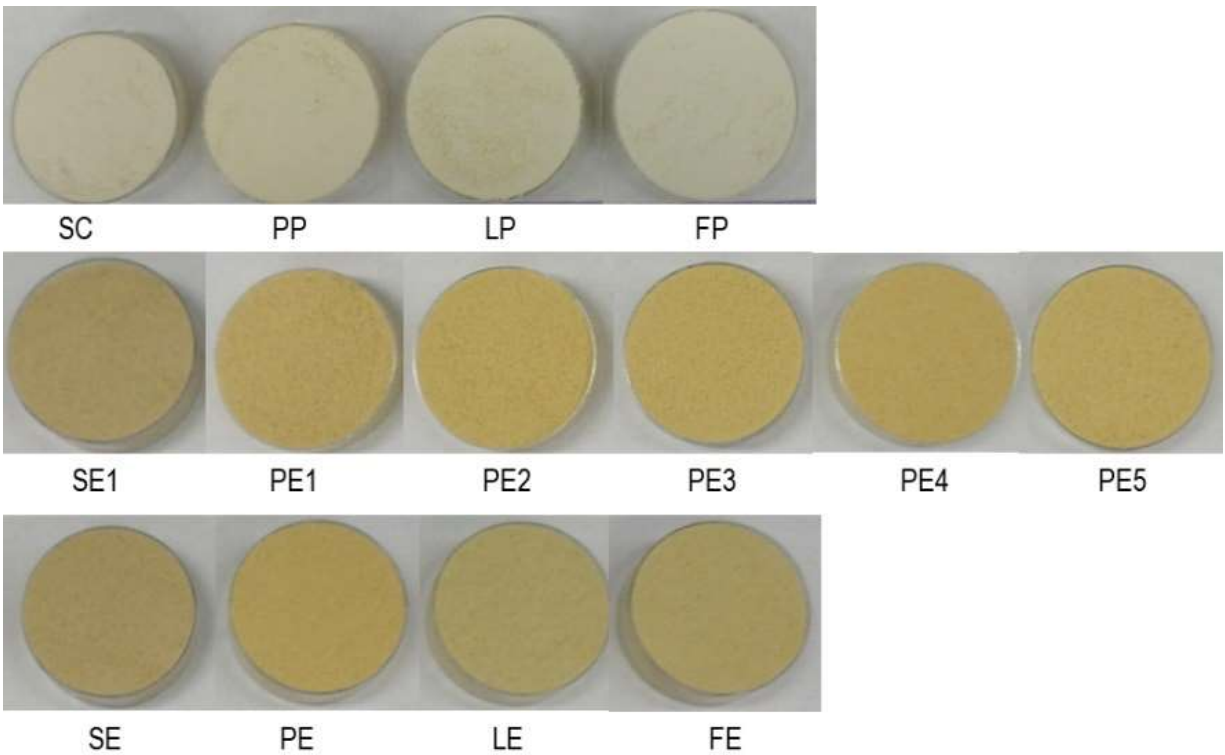


Figure 8. Pulse proteins (top), ground extrudates for the preliminary test (middle), and ground extrudates (bottom). SC=soy concentrates, PP=pea proteins, LP=lentil proteins, FP=faba bean proteins, SE=soy-concentrate extrudates, PE=pea protein extrudates, LE=lentil protein extrudates, and FE=faba bean extrudates. The numbers with sample names indicates different extrudates with different processing conditions.

Figure 8 shows raw materials and ground extrudates. Raw and grinding extrudates resulted in homogenization of particles by deformation of their structure so that raw materials had a lighter color and ground extrudates had a golden color.

**Table 10. Effects of Texturization on Color (L\*, a\* and b\*) of Pulse Proteins, Extrudates, and Ground Extrudates.**

Status	Sample	L*	a*	b*	
Raw material	SC	89.41 ± 0.04 <sup>b</sup>	0.47 ± 0.10 <sup>ij</sup>	13.14 ± 0.02 <sup>k</sup>	
	PP	91.77 ± 1.16 <sup>a</sup>	-0.40 ± 0.44 <sup>jk</sup>	15.52 ± 1.18 <sup>j</sup>	
	LP	92.59 ± 0.12 <sup>a</sup>	-1.37 ± 0.26 <sup>kl</sup>	16.75 ± 0.04 <sup>ij</sup>	
	FP	93.28 ± 0.02 <sup>a</sup>	-1.45 ± 0.10 <sup>l</sup>	12.02 ± 0.02 <sup>k</sup>	
Extrudates	SE1	56.98 ± 0.62 <sup>i</sup>	5.63 ± 0.23 <sup>cd</sup>	20.42 ± 0.91 <sup>h</sup>	
	PE1	52.98 ± 0.71 <sup>j</sup>	7.26 ± 0.34 <sup>b</sup>	19.55 ± 0.66 <sup>h</sup>	
	PE2	48.51 ± 0.41 <sup>kl</sup>	8.98 ± 0.22 <sup>a</sup>	19.88 ± 0.13 <sup>h</sup>	
	PE3	47.22 ± 0.38 <sup>l</sup>	9.06 ± 0.19 <sup>a</sup>	18.28 ± 0.10 <sup>hi</sup>	
	PE4	47.03 ± 0.30 <sup>l</sup>	9.08 ± 0.13 <sup>a</sup>	18.34 ± 0.21 <sup>hi</sup>	
	PE5	65.13 ± 0.11 <sup>g</sup>	5.17 ± 0.28 <sup>de</sup>	30.96 ± 0.28 <sup>cd</sup>	
	SE	59.76 ± 0.38 <sup>h</sup>	6.67 ± 0.44 <sup>bc</sup>	23.6 ± 0.17 <sup>g</sup>	
	PE	65.4 ± 0.40 <sup>g</sup>	5.68 ± 0.13 <sup>cd</sup>	32.35 ± 0.25 <sup>bc</sup>	
	LE	49.37 ± 0.21 <sup>k</sup>	4.42 ± 0.19 <sup>efg</sup>	16.15 ± 0.87 <sup>ij</sup>	
	FE	64.27 ± 0.39 <sup>g</sup>	1.58 ± 0.05 <sup>i</sup>	26.19 ± 0.69 <sup>fg</sup>	
	Ground extrudates	SE1G	72.85 ± 0.24 <sup>ef</sup>	3.79 ± 0.16 <sup>fgh</sup>	26.77 ± 0.60 <sup>f</sup>
		PE1G	74.80 ± 0.10 <sup>de</sup>	3.33 ± 0.02 <sup>h</sup>	33.42 ± 0.09 <sup>b</sup>
PE2G		76.32 ± 0.13 <sup>cd</sup>	3.50 ± 0.10 <sup>gh</sup>	36.61 ± 0.11 <sup>a</sup>	
PE3G		77.19 ± 0.02 <sup>c</sup>	3.19 ± 0.10 <sup>h</sup>	36.17 ± 0.03 <sup>a</sup>	
PE4G		76.96 ± 0.01 <sup>c</sup>	3.37 ± 0.01 <sup>h</sup>	36.45 ± 0.05 <sup>a</sup>	
PE5G		76.67 ± 0.03 <sup>cd</sup>	3.90 ± 0.01 <sup>fgh</sup>	37.60 ± 0.04 <sup>a</sup>	
SEG		71.39 ± 0.1 <sup>f</sup>	4.54 ± 0.02 <sup>ef</sup>	28.08 ± 0.01 <sup>ef</sup>	
PEG		76.57 ± 0.00 <sup>cd</sup>	3.89 ± 0.01 <sup>fgh</sup>	37.31 ± 0.01 <sup>a</sup>	
LEG		76.95 ± 0.04 <sup>c</sup>	0.75 ± 0.01 <sup>i</sup>	29.42 ± 0.04 <sup>de</sup>	
FEG		77.09 ± 0.03 <sup>c</sup>	0.59 ± 0.13 <sup>ij</sup>	32.02 ± 0.03 <sup>bc</sup>	

SC=soy concentrates, PP=pea proteins, LP=lentil proteins, FP=faba bean proteins, SE=soy-concentrate extrudates, PE=pea protein extrudates, LE=lentil protein extrudates, and FE=faba bean extrudates. G indicates ground. The numbers with sample names indicates different extrudates with different processing conditions.

Table 10 illustrates the effect of texturization on the color parameters of pulse proteins, pulse protein extrudates, and ground pulse protein extrudates. All raw materials, extrudates, and ground extrudates of each ingredient had significantly different colors ( $P < 0.05$ ). Extrudates and ground extrudates had decreased lightness and increased redness and yellowness, compared to the raw materials; however, the color parameters of the ground extrudates were placed between the raw materials and

extrudates. In other words, the size of the extrudates are important for the final product in terms of the color due to variation of color attributed to the size of the extrudates. The Maillard reaction might be enhanced on the surface of extrudates due to more energy and a longer contact period on the surface of the extrudates from the extrusion system before discharge or less moisture by evaporation of the steam from the surface of the extrudates after being discharged from the extrusion system.

SC had different color parameters from SE1 and SE, but SE had a significant increase in lightness and yellowness compared to SE1 as SE expanded more as seen in the texturization section mentioned previously. In other words, the expanded structure of soy extrudates contributed to their lighter and yellower color. For pea protein extrudates, mechanical energy at a certain point between 328 and 355 rpm of extrusion shaft speed affected the color of the pea extrudates so that PE1 was lighter and less red compared to the other samples (PE2, PE3, PE4, PE5, and PE).

As PE4 and PE5 had different colors as seen in Figure 7, PE5 had a significant increase in lightness and yellowness and decrease in redness compared to PE4, but PE5 did not have a significant difference from PE. Steam added to the extruder to produce PE5 and PE might inhibit the Maillard reaction and result in an increase in lightness and a decrease in redness.

Ground extrudates resulted in intermediate color parameters between raw materials and extrudates. From the preliminary test, ground pea protein extrudates fluctuated in lightness parameters, and did not show a significantly different red color ( $P>0.05$ ) each other. Only ground PE1 had significantly decreased parameters of yellowness

compared to other ground samples (PE2, PE3, PE4, PE5, and PE). Extrudates had more variables on the surface, such as temperature gradients, with the extrusion system and less moisture by the evaporation of steam than the inside of the extrudates, which resulted in higher lightness and yellowness and less redness. Grinding caused homogenization of particles on the surface and inside of the extrudates in cell walls including pores and protein bodies in the structure. Therefore, grinding is important for the final products since it changes the size of the particles and degree of homogenization. Grinding affects the color of the products, and colorants to be added to the final products can be minimized as needed.

### 2.3.3 WAI and WSI

**Table 11. WAI and WSI of Raw Materials and TPP for Soy (control) and Pulse Proteins.**

Sample	WAI, g/g dry solids	WSI, g/100 g dry solids
SC	4.02 ± 0.03 <sup>a</sup>	8.36 ± 0.03 <sup>ad</sup>
PP	2.26 ± 0.03 <sup>f</sup>	47.93 ± 0.40 <sup>a</sup>
LP	2.40 ± 0.01 <sup>f</sup>	48.10 ± 2.25 <sup>a</sup>
FP	1.88 ± 0.07 <sup>g</sup>	45.96 ± 0.12 <sup>a</sup>
SE1	3.75 ± 0.01 <sup>a</sup>	9.12 ± 1.21 <sup>d</sup>
PE1	3.42 ± 0.01 <sup>b</sup>	19.24 ± 0.20 <sup>bc</sup>
PE2	3.40 ± 0.04 <sup>b</sup>	19.70 ± 0.98 <sup>bc</sup>
PE3	3.19 ± 0.01 <sup>bc</sup>	18.65 ± 0.01 <sup>bc</sup>
PE4	3.06 ± 0.12 <sup>cde</sup>	18.89 ± 0.21 <sup>bc</sup>
PE5	3.14 ± 0.15 <sup>bcd</sup>	22.19 ± 0.27 <sup>b</sup>
SE	3.95 ± 0.01 <sup>a</sup>	12.03 ± 0.31 <sup>c</sup>
PE	2.86 ± 0.01 <sup>de</sup>	23.94 ± 0.03 <sup>b</sup>
LE	2.79 ± 0.03 <sup>e</sup>	21.75 ± 0.05 <sup>b</sup>
FE	2.96 ± 0.03 <sup>cde</sup>	21.67 ± 0.08 <sup>b</sup>

<sup>a-g</sup> Mean WAI and <sup>a-d</sup> Mean WSI values with the same superscripts are not significantly different at  $p < 0.05$ .

Table 11 shows the WAI and WSI of pulse proteins and TXVP. All extrudates except the control (SE1) had a significantly higher ( $P < 0.05$ ) WAI and lower WSI

compared to the raw materials. In contrast, whereas the control (SC) did not have a significantly different WAI from SE1 and SE, the control (SC) had a similar WSI to SE1, but had a significantly different value from SE. Relatively, pulse proteins had a lower WAI and a higher WSI than the control. The WAI indicates the degree of water absorption into the structure, and the WSI shows the degree of particle solubility of the structure. Therefore, texturized products should contain high WAI and a low WSI since the products should absorb water to provide functional properties such as juiciness, but not be solubilized in the water to maintain structural integrity for a meat-like texture. Table 7 indicates that protein and carbohydrates are the major components of the ingredients. During texturization, the protein in the ingredients interacts with other components such as carbohydrates and moisture through energy and pressure. The proteins should be previously solubilized, so they are realigned by the shear in the extruder. This would result in a fiber-like texture after cross-linking during cooling after extrusion.

**Table 12. pHs of Ingredients.**

Sample	pH
SC	7.12 ± 0.02 <sup>a</sup>
PP	6.52 ± 0.01 <sup>c</sup>
LP	6.50 ± 0.02 <sup>c</sup>
FP	6.73 ± 0.01 <sup>b</sup>

<sup>a-c</sup> Mean pH values with the same superscripts are not significantly different at  $p < 0.05$ .

A study (Fan and Sosulski 1974) found that the steep portion of the nitrogen extraction curve for each legume flour occurred between pH 5-7. The pH of the ingredients is an important aspect for texturization and should increase the solubility of

the ingredients. The raw materials were prepared differently (PP, LP, and FP = dry-milled and air-classified, and SC=wet-milled), and they had different pHs (PP=6.52, LP=6.50, FF=6.73, and SC=7.12) (Table 12). In other words, only SC may have already been neutralized to increase the solubility of the protein, and the degree of the folding structure in SC decreased. Therefore, the pulse proteins (PP, LP, and FP) might require more processing such as greater thermal and mechanical energy or additives to increase the solubility of the structure for texturization. Water absorption depends on the availability of hydrophilic groups which bind water molecules on the gel-forming capacity of macromolecules (Gomez and Aguilera 1983). SC with a high pH that was solubilized relatively more than the pulse proteins had more hydrophilic groups to bind water molecules which resulted in a higher WAI compared to PP, LP, and FP. As a result, texturization did not increase active hydroxyl groups in the products (SE1 and SE). In contrast, the pulse proteins were not neutralized to have a higher pH, therefore, they would not have hydrophilic groups to absorb water because their higher degree of folding resulted in less solubility and inhibited the texturization. The texturization of pulse proteins helped increase active hydroxyl groups in extrudates. Therefore, the pulse proteins had a low WAI, and the extrudates had a higher WAI than the pulse proteins. As seen in Table 7 and Table 12, FP (61.5% protein and  $6.73 \pm 0.01$  pH) had a higher protein content and a lower pH than PP (55.4% protein and  $6.52 \pm 0.01$  pH) and LP (55.4% protein and  $6.50 \pm 0.02$  pH) and resulted in a lower WAI and WSI compared to PP and LP. A higher pH could help increase the solubility of FP to create more hydroxyl groups, but the higher protein content of FP inhibited the increase of solubility and resulted in inhibiting an increase in hydroxyl groups. The WSI is a

measure of the solubility of particles into water. The WSI of SC was not significantly different ( $P>0.05$ ) from that of SE1 but was different from SE. SC also had a higher structural integrity compared to other samples. As mentioned earlier, the raw materials were prepared differently. The SC production might have involved a heat treatment during oil extraction. A sulfhydryl and disulfide interchange reaction is reported to be involved in the insolubilization of the 11S soy protein upon heating (Wolf and Tamura 1969). Therefore, SC would have strong disulfide bonds that decrease solubility and result in a lower WSI compared to other pulse samples. Texturization of soy proteins by extrusion is attributed to disulfide bonding (Jeunink and Cheftel 1979). Texturization caused an expanded shape of the extrudates, causing more active chemical sites to be solubilized. As a result, SE had a significant increase in WSI compared to SE1. Conversely, other pulse proteins had no disulfide bond formed by heating, so they had a higher WSI compared the control. However, texturization might cause disulfide bond formation resulting in a lower WSI in pulse protein extrudates compared to the control.



### 2.3.4 Expansion Ratio

**Table 13. Axial Length and Radial Expansion Ratios of Extrudates.**

Sample	Axial length, mm	Radial expansion ratio, mm/mm
SE1	23.51 ± 0.81 <sup>bc</sup>	1.74 ± 0.04 <sup>b</sup>
PE1	21.20 ± 1.09 <sup>c</sup>	1.10 ± 0.01 <sup>d</sup>
PE2	19.62 ± 1.44 <sup>c</sup>	1.10 ± 0.01 <sup>d</sup>
PE3	19.64 ± 1.19 <sup>c</sup>	1.08 ± 0.01 <sup>d</sup>
PE4	19.36 ± 0.91 <sup>c</sup>	1.09 ± 0.01 <sup>d</sup>
PE5	21.48 ± 0.92 <sup>c</sup>	1.07 ± 0.01 <sup>d</sup>
SE	33.89 ± 1.75 <sup>a</sup>	2.35 ± 0.03 <sup>a</sup>
PE	27.67 ± 1.14 <sup>bc</sup>	1.04 ± 0.01 <sup>d</sup>
LE	19.33 ± 0.56 <sup>c</sup>	1.23 ± 0.01 <sup>c</sup>
FE	34.13 ± 2.32 <sup>a</sup>	1.10 ± 0.02 <sup>d</sup>

<sup>a-c</sup> Mean Axial length and <sup>a-d</sup> Mean Radial expansion ratio values with the same superscripts are not significantly different at  $p > 0.05$ .

Table 13 illustrates the axial length and radial expansion ratio of extrudates. Soy was texturized well, while other pulse proteins were not texturized as well as did soy and became pastes. The soy had a good axial and radial expansion ratio, but SE had a significant increase in both the axial and radial expansion ratio compared to SE1. Unlike soy, from the preliminary test, all pea proteins did not have a significant difference in axial and radial direction. From the main test, FE and LE had the greatest expansion in axial and radial direction compared to soy. FE had a larger expansion ratio compared to other samples. In other words, faba bean proteins showed a possibility for being texturized under different processing conditions.

### **2.3.5 Suggested Methods for Texturization**

These results suggested some different conditions and the use of additives. Adjusting the screw to provide more mechanical energy and additions such as calcium hydroxide, sodium bisulfites, and protein isolates could have helped in texturization. It was expected the addition of friction would create more energy and may help break the disulfide bonds in proteins. A modification of pH using calcium hydroxide would increase the solubility of proteins. This indicates an *iso*-electric at which all proteins are coagulated at that point, increases solubility as the pH increases. So, isoelectric points of legumes are similar. An increased pH of other pulses close to the pH of soy (about 7.2) will increase the solubility of proteins and texturization. Also, an increase in protein content can be an option for texturization. Sodium bisulfite will aid in the cleavage of disulfide bonds, which assists in the unraveling of long twisted protein molecules. Therefore, it may help in texturizing other samples.

### **2.4 Conclusion**

The materials prepared differently had different results. SC that was neutralized and heat treated was texturized well, and other pulse proteins were not texturized and became pastes. Therefore, the pH and protein contents of ingredients and thermal and mechanical energy supplied in the extruder are important factors for texturization and modification with these factors were recommended.

## CHAPTER III

### DEVELOPMENT OF TEXTURIZED PULSE PROTEINS

#### (HIGH SHEAR AND ADDITIVES): PEAS, LENTILS, AND FABA BEANS

##### 3.1 Introduction

Pulse proteins (pea, lentil, and faba bean proteins) were texturized with a twin-screw extruder (TX-52, Wenger Manufacturing Inc., Sebetha, KS). However, the pulse proteins were not texturized as well as the soy concentrates during the main test described in Chapter 2 using the same processing conditions provided by Wenger Manufacturing Inc. (Sebetha, KS) in which soy concentrates was successfully texturized. Kearns and others (1989) suggested adjusting the pH of the raw material to increase the solubility of the proteins, enhancing the cleavage of the bisulfide bonds during plasticizing in the extruder, and increasing the protein level of the raw material for the textural integrity of texturized vegetable proteins. Nuno (Sereno and others 2007) suggested that the extrusion melts and aligns xanthan macromolecules, and a network structure is created and maintained by associations involved in ordered regions as a consequence.

In this research, pea proteins (PP), lentil proteins (LP), and faba bean proteins (FP) were texturized with additives and pea isolate with a higher shear screw configuration of the extruder due to their unacceptable degree of texturization as soy. For texturization, 0.06% and 0.12% calcium hydroxide (CH), 0.05% and 0.10% sodium bisulfite (SB), and 0.10% and 0.20% xanthan gum (XG), and enough PI to make 65% and 76% protein were added to each pulse protein. The CH was expected to increase the pH of the raw material, produce more solubilization of the protein in the material,

and the SB was anticipated to enhance cleavage bisulfide bonds during plasticization in the extruder. The XG was used to manipulate the ionic bond between protein molecules in the material and help in texturization. The addition of PI to increase the protein content of the ingredients was simply predicted to help the textural integrity of the final products.

The objectives of this research will be to evaluate the effects of protein type, additive, and additive dose level on the texturization of these pulse proteins. I hypothesized that addition of additives (CH, SB, and XG) and PI in the recipe would significantly promote the texturization of pulse proteins, and these added materials would affect texturization of pulse proteins differently.

## **3.2 Materials and Methods**

### **3.2.1 Materials**

Vitessence™ Pulse 1550 (PP), Vitessence™ Pulse 2550 (LP), and Vitessence™ Pulse 3600 (FP) were obtained from Ingredion Incorporated (Westchester, IL), Alliance Grain Traders (AGT) Food, and Ingredients (Regina, Canada). Calcium Hydroxide Powder FCC (CH) and Sodium Bisulfite Granular FCC (Spectrum Chemical Mfg. Corp.) (BS) were purchased through VWR (Westchester, PA). Xanthan Gum FCC (Keltrol F) NK (XG) was obtained from the Kraft Chemical Company (Meltrose Park, IL). Nutralys®-S85F (PI) was obtained from Roquette America, Inc. (Keokuk, IA).

### 3.2.2 Recipe Preparation

0.05% and 0.10% SB, 0.10% and 0.20% XG was added to each pulse protein, and enough PI to make 65% and 76% protein content for each pulse protein of the recipes were prepared and homogenized in an industrial mixer (G0028, Engineered Systems and Equipment, Inc., Caney, KS). 0.06% (1.2 g CH /1998.8 g water) and 0.12% (2.4 g CH/1997.6 g water) were prepared. The additives and PI were identified as low and high based on the amount added.

### 3.2.3 Extrusion

During the main test described in Chapter 2 following the optimized processing conditions (Figure 5) with soy that Wenger Manufacturing Inc. recommended, PP, LP, and FP were not texturized as well as was soy.

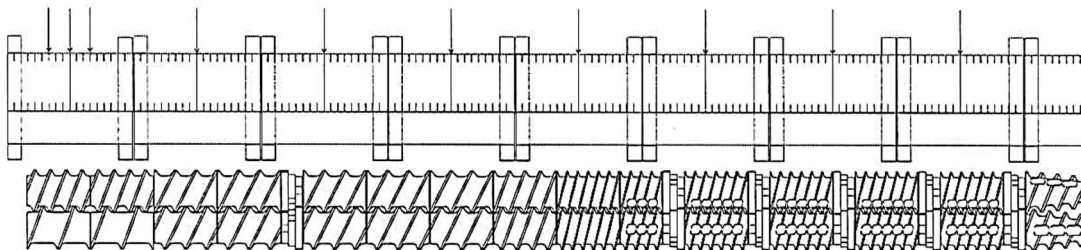


Figure 9. High shear configuration with a twin-screw extruder (TX-52, Wenger) to produce texturized pulse proteins.

Therefore, a higher shear screw configuration was prepared for better texturization (Figure 9), and the processing condition of PE5 in Table 9 in Chapter 2 was used. Each prepared recipe was texturized using the Wenger twin-screw extruder (TX-52). Each pulse protein was supplied to the feeder; the pre-made CH solutions were introduced at

the end of the preconditioner at the rate of 1.68 kg/h through a variable-speed Master flex pump, model L/S easy load (Cole-Parmer, Vernon Hills, IL, USA) and texturized. Other dry premixed recipes were texturized as well. The setting parameters of the extruder during the texturization were kept constant at a screw speed of 495 rpm, a feed rate of 1 kg/min, a water-flow rate of 3kg/hr, and a product exit temperature of 100°C. These parameters were expected to vary based on the characteristics of the premixed recipes. Extrudates were discharged through a square dimensional die (9.53 mm x 9.53 mm) and cut with a knife at the end of the die. After the texturization, the products were dried in a hot-air conveying dryer (Wenger Manufacturing Inc. Sabetha, KS) at 105°C until only about 10% moisture content remained (AOAC 1990). The samples were stored at room temperature in a dark colored air tight container for further analysis.

### **3.2.4 Color**

Dried samples were hydrated at room temperature for 20 mins. The hydrated TPP were drained on a 20-mesh screen for 3 mins and evaluated with the colorimeter. These samples extrudates were directly evaluated using a colorimeter (Model CR-310, Minolta, Osaka, Japan). Values were expressed as L\*, a\* and b\*, where L\* values (lightness) vary from black (0) to white (100), chroma a\* values (redness) vary from green (-60) to red (+60), and chroma b\* values (yellowness) vary from blue (-60) to yellow (+60).

### **3.2.5 Water Holding Capacity (WHC) and Water Solubility Index (WSI)**

The WHC and WSI were measured through a modified method using Equation 3.1 and Equation 3.2 (Crowe and Johnson 2001).

$$WHC = \frac{\text{Weight of absorbed water into TPP}}{\text{Weight of dry TPP}} \times 100 \quad \text{Equation 3.1}$$

$$WSI = \frac{\text{Weight loss during hydration}}{\text{Weight of dry TPP}} \times 100 \quad \text{Equation 3.2}$$

Following the modified method of Crowe and Johnson (2001), 30 g of each TPP was placed in a 400-mL beaker and soaked with 150 mL of water at room temperature for 20 mins. Each hydrated TPP was drained on a pre-weighted 20-mesh screen tilted at a 25° angle, allowed to drain 3 mins, and the juice was collected in a 1000 ml plastic container. The WHC was determined using Equation 3.1. The collected juice was stirred for 5 mins and sampled in a test tube. The sampled juice was homogenized using a vortex (G560, Scientific Industries, Inc., Bohemia, NY). About 10 g of sample from the test tube was dried in an oven for 2 hours at 135°C. The WSI was calculated using Equation 3.2 by interpolating the ratio of purged sample/dried TPP between the collected juice and the sampled juice from the test tube. The WHC and WSI of each sample were measured twice.

### **3.2.6 Texture Analysis**

Texture analysis was performed with a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) using the texture profile analysis measurement. A cork-borer (about 10 mm in diameter) was used to obtain cylindrical testing samples (about 10 mm in diameter and 30 mm in length), and the samples were placed on a

square aluminum plate. A cylindrical probe (76.2 mm in diameter) was used to compress the samples to 50% of their initial thickness with a two-cycle compression test at 1 mm/s. Three attributes, hardness, cohesiveness, and gumminess were recorded. Six samples for each treatment were used to collect data for the analysis.

### **3.2.7 Statistical Analysis**

Data based on each protein type (PP, LP, and FP), additive (CH, SB, XG, and PI), and dose level (none, low, and high) for each parameter were prepared for the statistical analysis of the data. A Tukey's HSC (honestly significant difference) analysis was conducted for pair comparison. All statistical tests were performed using JMP software (JMP Pro 12.0.1, SAS Institute Inc., Cary, N.C., USA).

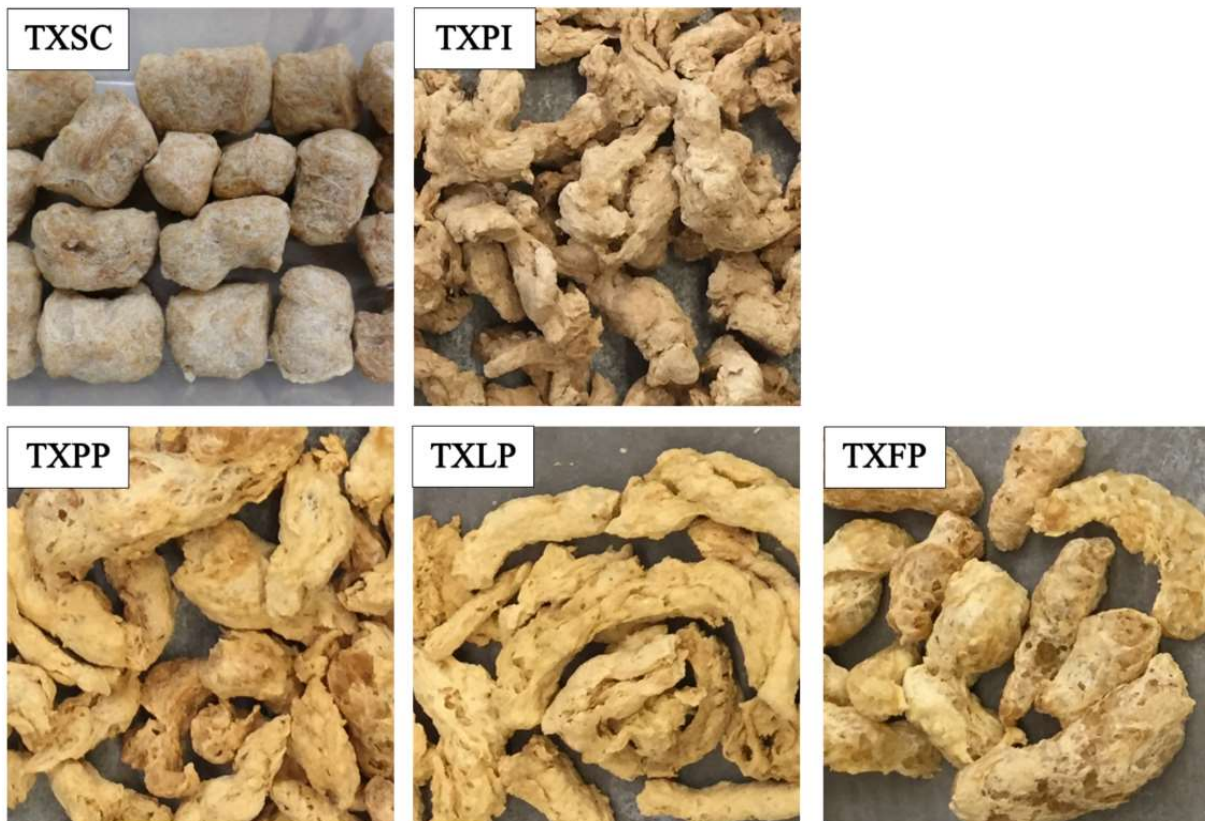
## **3.3 Results and Discussion**

### **3.3.1 Texturization to Produce Texturized Pulse Proteins**

Prior to the drying and cooling after texturization, particle size reduction is recommended based on desired final product size and intended use as the moisture content is high at this point (Kearns and others 1989). However, in this research, the texturized vegetable proteins (TXVP) did not undergo the size reduction process due to their lack of uniform shape and forms as extrudates. During texturization, they had various shapes and forms such as fine or relatively small particles, irregular and honey comb shapes, and a burnt-like brown color. An explosive discharge by a sudden releasing of a pressurized clog at the die of the extruder without a discharge of extrudates was observed at times and resulted in an irregular form of the extrudates.



Therefore, they had to be sorted by hand to find the TPP that were visually acceptable for analysis or products. During size reduction for the final products, based on the intended use, it is recommended that the TXVP be hydrated, drained, and resized before making a formulation with a recipe due to their structural fragility because of their low moisture.



**Figure 10.** Texturized vegetable proteins (TXVP) produced with a twin-screw extruder (TX-52) with a high shear configuration. TXSC=texturized soy concentrates, TXPI=texturized pea isolates, TXPP=texturized pea proteins, TXLP=texturized lentil proteins, and TXFP=texturized faba bean proteins.

As seen in Figure 10, the visual images of TXVP such as shape, color, and texture varied based on protein type. Overall, TPP (TXPI, TXPP, TXLP, TXFP) had a relatively denser, yellower, and irregular shape compared to the TXSC. TXFP had relatively more uniform and radial expanded shapes than other TPP. Soeda (1994) mentioned that both 7S and 11S subunits of soy protein and major components for texturization, start to unfold when heated above 100°C and become totally unfold at 140°C. Also, Hall and others (2017) mentioned that pulse is mainly composed of 2S (albumin), 7S (vicilin), and 11S (globulin), and 11S is a major component of pulses. As seen in Table 6 in Chapter 1, all peas, lentils, and faba beans have a considerable number of globulins (7S and 11S). Regarding major components (7S and 11S) for texturization of soy, these pulse proteins were expected to be texturized, and faba bean proteins would be texturized better than other PLP. As expected, TXFP had a better degree of texturization than any other TPP.

### 3.3.2 Effects of Protein Type

#### 3.3.2.1 Color

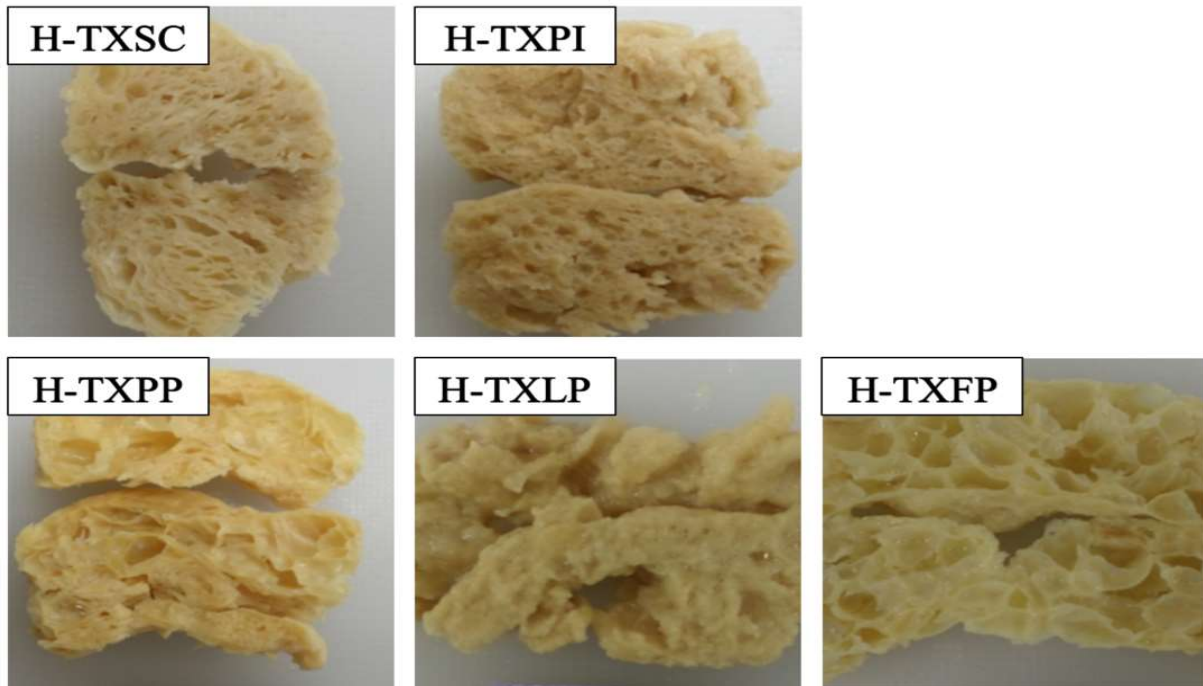


Figure 11. Hydrated and bisected texturized vegetable proteins (TXVP) produced with a twin-screw extruder (TX-52) with a high shear configuration. H-TXSC=hydrated texturized soy concentrates, H-TXPI= hydrated texturized pea isolates, H-TXPP= hydrated texturized pea proteins, H-TXLP= hydrated texturized lentil proteins, and H-TXFP= hydrated texturized faba bean proteins.

For measurement, TXVP were hydrated for 30 mins. Hydrated TXVP including TXSC, TXPI, TXPP, TXLP, and TXFP were denoted H-TXSC, H-TXPI, H-TXPP, H-TXLP, and H-TXFP, respectively. After hydration, each sample was bisected with a knife to observe the degree of texturization visually. As seen in

Figure 11, all the samples had good muscle-like texturized images so that they were easily cut with a knife except hydrated TXLP which had a mushy texture with regularly shaped pores, so it was difficult to cut and retain its original shape while cutting. Hydrated TXFP had a greater pore size than did hydrated TXSC, the control.

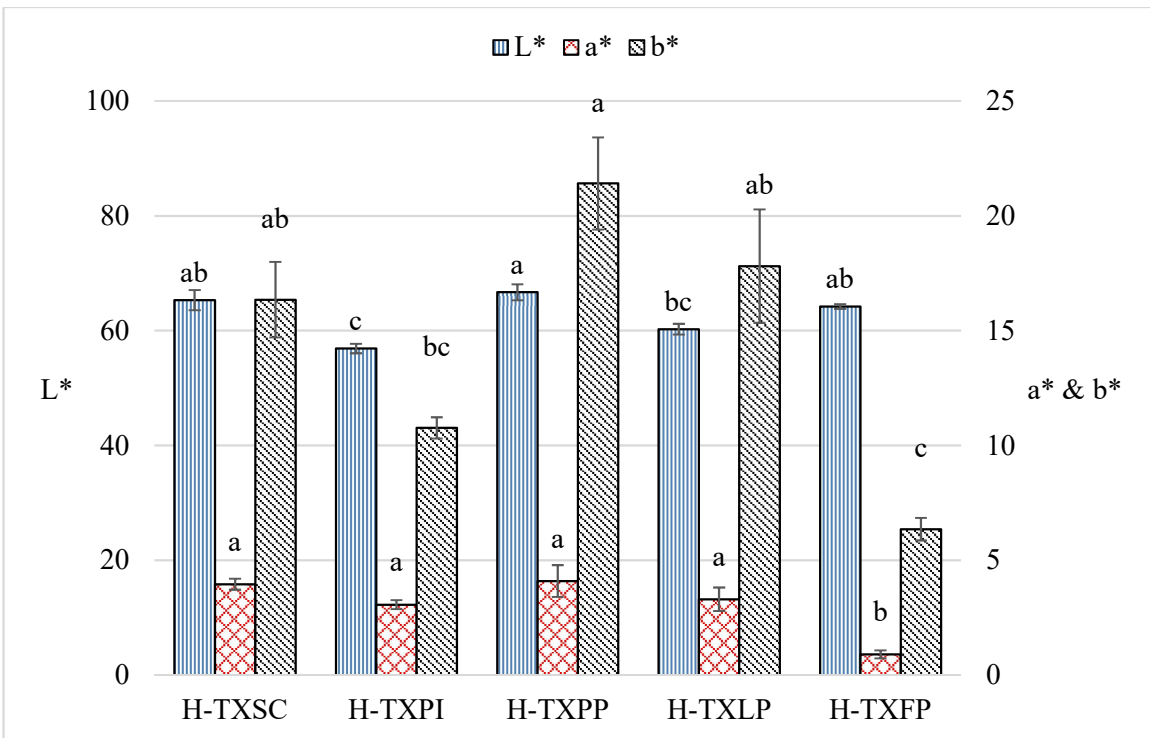


Figure 12. Parameters of color ( $L^*$ ,  $a^*$ , and  $b^*$ ) from hydrated texturized vegetable proteins (TXVP) produced with a twin-screw extruder (TX-52) with a high shear configuration. H-TXSC=hydrated texturized soy concentrates, H-TXPI=hydrated texturized pea isolates, H-TXPP=hydrated texturized pea proteins, H-TXLP=hydrated texturized lentil proteins, and H-TXFP=hydrated texturized faba bean proteins. <sup>a-c</sup> Mean color ( $L^*$ ,  $a^*$ , and  $b^*$ ) values with the same superscripts are not significantly different at  $p > 0.05$ .

Figure 12 shows the parameters of color ( $L^*$ ,  $a^*$ , and  $b^*$ ) from hydrated TXVP. Compared to the control, H-TXSC, all samples had similar color parameters except H-TXPI which was lower in lightness and H-TXFP which was lower in redness and yellowness. Figure 12 shows the parameters of color from hydrated TXVP. H-TXPI was

darker, and H-TXFP was less brown due to their lower redness and yellowness compared to other hydrated TXVP. During texturization, TXFP had a more radial expansion and greater pore size which resulted in less redness and yellowness. In other words, when TXFP is formulated to make a final product, it will have a wider range of available products that require the addition of less colorants to mimic the color of final products compared to other TXVP.

### 3.3.2.2 Water Holding Capacity (WHC) and Water Solubility Index (WSI)

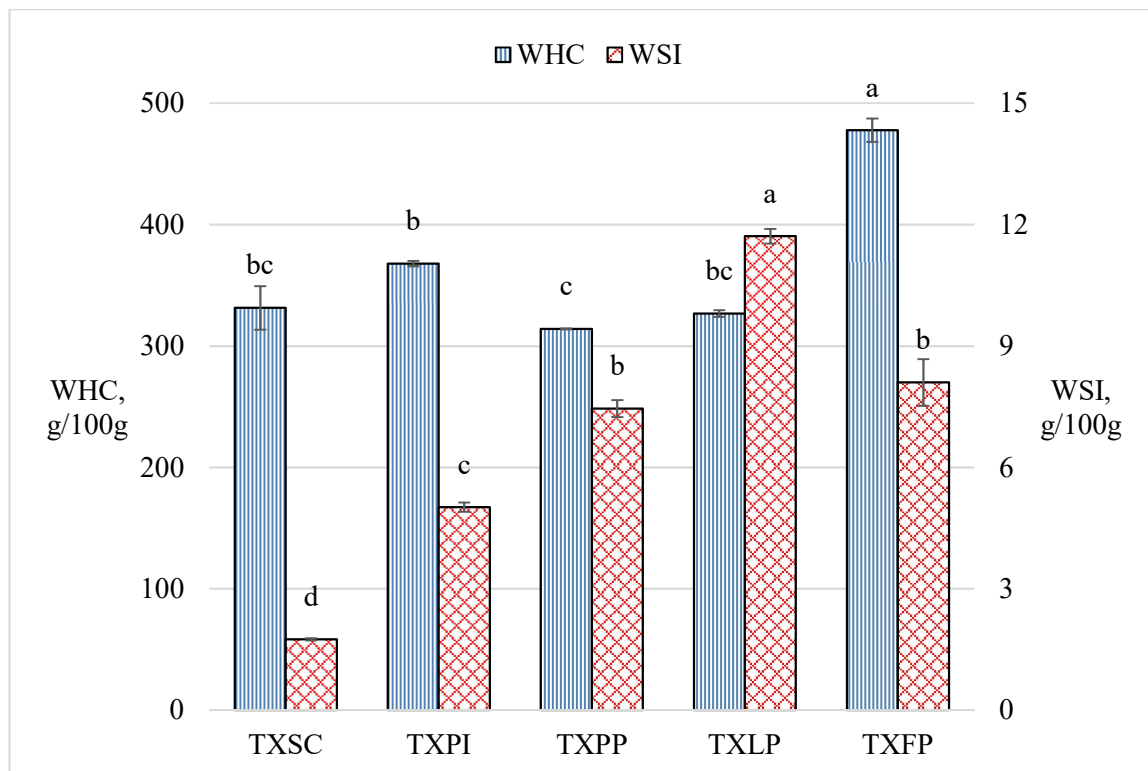


Figure 13. Water holding capacity (WHC) and water soluble index (WSI) of texturized vegetable proteins (TXVP) produced with a twin-screw extruder (TX-52) with a high shear configuration. TXSC=texturized soy concentrates, TXPI=texturized pea isolates, TXPP=texturized pea proteins, TXLP=texturized lentil proteins, and TXFP=texturized faba bean proteins. <sup>a-c</sup> Mean WAI and <sup>a-d</sup> mean WSI values with the same superscripts are not significantly different at  $p > 0.05$ .

Each TXVP was hydrated for measurement of the WHC and WSI, and Figure 13 shows the WHC and WSI of TXVP. Compared to the control, all TXVP had a similar WHC except TXFP, which had the highest WHC indicating that it absorbed more than five times the amount of water compared to the weight of TXFP. As seen in Figure 11 and Figure 12, TXFP had greater pore size causing a greater surface area resulting in holding more water. Usually, meat loses moisture during cooking; however, if these TPP are used in meat products as a meat substitute, they will prevent drip loss resulting in a high cooking yield, especially TXFP.

All TPP had higher WSI compared to the control, and TXPP and TXFP had a similar WSI. In other words, all TPP lost significantly more substance into the water during hydration. TXLP had the highest WSI indicating it lost about 10% its substance in the water during hydration which meant that TXLP had the least textural integrity. As seen in Figure 11, TXLP showed a mushy texture that did not hold its structure and released substance into water during hydration. Compared to other TPP, TXFP showed the unique characteristic of the highest WHC and a relatively low WSI, and it will give more juiciness and sponge-like texture when it is formulated with water as a meat substitute to make final products such as hamburger patties, chicken nuggets, sausage, and pizza toppings.

### 3.3.2.3 Texture

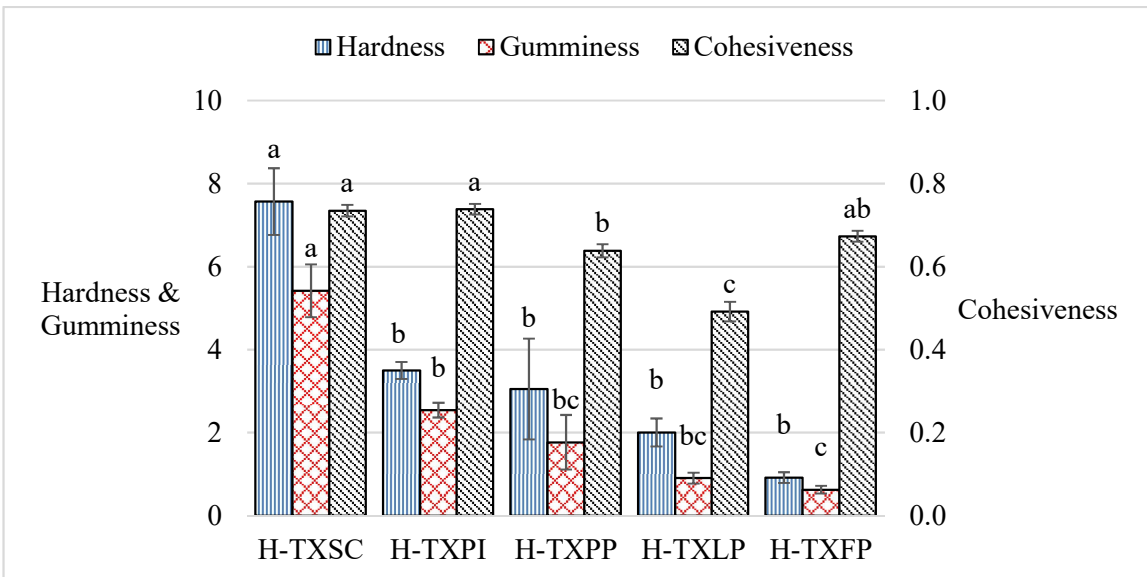


Figure 14. Textural properties (hardness, gumminess, and cohesiveness) of hydrated texturized vegetable proteins (TXVP) produced with a twin-screw extruder (TX-52) with a high shear configuration. H-TXSC=hydrated texturized soy concentrates, H-TXPI= hydrated texturized pea isolates, H-TXPP= hydrated texturized pea proteins, H-TXLP= hydrated texturized lentil proteins, and H-TXFP= hydrated texturized faba bean proteins. <sup>a-b</sup> Mean hardness, <sup>a-c</sup> mean gumminess, and <sup>a-c</sup> mean cohesiveness values with the same superscripts are not significantly different at  $p > 0.05$ .

Textural analysis for hydrated TXVP was conducted, and Figure 14 shows the textural properties of hydrated TXVP. Compared to the control, all hydrated TPP had significantly lower ( $p < 0.05$ ) hardness and gumminess, and H-TXPI and H-TXFP had a similar cohesiveness.

For gumminess, all TPP had similar values except H- which had a significantly ( $p < 0.05$ ) lower value. Therefore, it is necessary that the hardness and gumminess be improved for all TPP to be used for meat substitutes. Fiber is a suitable additional ingredient for cooked meat products to increase the cooking yield due to its water-binding and fat-binding properties and to improve texture (Cofrades and others 2000). Especially, oat fiber increases hardness and sensory toughness for cooked meat products (Steenblock and others 2001). To improve the texture of cooked meat

products such as hamburger patties, sausage, and nuggets, the addition of oat fiber is recommended.

Compared to the control, H-TXPI and H-TXFP had a similar cohesiveness value, but H-TXPP and H-TXLP had a significantly ( $p < 0.05$ ) lower value. Therefore, TXPI and TXFP will have a similar functionality in terms of cohesiveness that will more easily bind with the meat as meat substitutes. In other words, all TPP have enough cohesiveness to be meat substitutes.

Cohesiveness is the strength of the internal bonds making up the body of the product (Breene and Barker 1975). H-TXPI and H-TXFP had the same strength of internal bonds making up the body of the product as H-TXSC, but they had less hardness and gumminess. Therefore, it is recommended that ingredients such as fiber be added to increase these functionalities when they are used in food products as a meat substitute instead of soy concentrate.

### ***3.3.3 Effects of Different Levels of Additives (CH, SB, and XG) and Ingredients (PI) on Pulse Proteins***

During texturization using a twin-screw extruder (TX-52) with a high shear configuration, each pulse protein (PP, LP, and FP) had two different levels of additive additions (CH, SB, and XG) and ingredients (PI). The color and texture of each hydrated TPP were directly measured using each device, and the WHC and WSI was calculated from weight differences between each TPP and each hydrated TPP. TPP with no additives and ingredients were used as a control.



### 3.3.3.1 Pea Proteins

**Table 14. Parameters of color (L\*, a\*, and b\*) and texture (hardness, cohesiveness, and gumminess) from hydrated TXPP and the WHC and WSI from TXPP with different levels of additives (CH, SB, XG, and PI) during texturization produced with a twin-screw extruder (TX-52) with a high shear configuration.**

Additives and ingredients	L*	a*	b*	WHC, g/100g	WSI, g/100g	Hardness, N	Cohesiveness	Gumminess, N
NO	66.69±1.40 <sup>ab</sup>	4.10±0.69	21.41±3.67	314.10±0.45 <sup>abc</sup>	7.45±0.21 <sup>bc</sup>	3.05±1.21 <sup>b</sup>	0.64±0.02 <sup>ab</sup>	1.77±0.66 <sup>b</sup>
Low-CH	64.74±1.09 <sup>ab</sup>	3.14±0.24	16.66±0.62	243.71±2.27 <sup>c</sup>	6.31±0.20 <sup>de</sup>	4.41±0.65 <sup>ab</sup>	0.60±0.03 <sup>b</sup>	2.34±0.26 <sup>ab</sup>
High-CH	68.75±4.00 <sup>a</sup>	3.31±0.29	21.14±4.26	369.26±18.05 <sup>a</sup>	8.04±0.04 <sup>ab</sup>	2.20±0.69 <sup>b</sup>	0.66±0.02 <sup>ab</sup>	1.33±0.34 <sup>b</sup>
Low-SB	63.77±0.73 <sup>ab</sup>	3.84±0.86	16.45±2.99	319.32±10.36 <sup>abc</sup>	7.23±0.07 <sup>bcd</sup>	2.33±0.41 <sup>b</sup>	0.64±0.02 <sup>ab</sup>	1.41±0.22 <sup>b</sup>
High-SB	65.38±0.73 <sup>ab</sup>	2.32±0.25	10.75±0.26	339.20±16.25 <sup>ab</sup>	8.63±0.32 <sup>a</sup>	2.02±0.29 <sup>b</sup>	0.65±0.01 <sup>ab</sup>	1.29±0.18 <sup>b</sup>
Low-XG	64.15±0.51 <sup>ab</sup>	3.18±0.20	13.99±1.23	266.80±31.96 <sup>bc</sup>	8.47±0.36 <sup>a</sup>	2.50±0.34 <sup>b</sup>	0.66±0.02 <sup>ab</sup>	1.51±0.18 <sup>b</sup>
High-XG	61.84±1.98 <sup>ab</sup>	3.57±0.82	14.29±1.11	259.50±3.75 <sup>c</sup>	7.15±0.13 <sup>bcd</sup>	3.26±0.82 <sup>ab</sup>	0.63±0.01 <sup>ab</sup>	1.91±0.44 <sup>b</sup>
Low-PI	59.32±1.08 <sup>b</sup>	3.45±0.48	11.87±1.67	243.27±4.61 <sup>c</sup>	6.56±0.16 <sup>cde</sup>	6.19±0.66 <sup>a</sup>	0.65±0.02 <sup>ab</sup>	3.69±0.31 <sup>a</sup>
High-PI	59.01±1.03 <sup>b</sup>	3.55±0.83	12.64±2.91	342.02±2.02 <sup>ab</sup>	5.77±0.12 <sup>e</sup>	3.77±0.47 <sup>ab</sup>	0.70±0.01 <sup>a</sup>	2.57±0.31 <sup>ab</sup>

<sup>a</sup> Means with different letters of the same style are significantly different (p<0.05).

<sup>b</sup> NO=Neither addition of additive or ingredients, CH=calcium hydroxide, SB=sodium bisulfite, XG=xanthan gum, and PI=pea isolates.

<sup>c</sup> Different levels of additives: CH (0.06% and 0.12%), SB (0.05% and 0.10%), XG (0.10% and 0.20%), and PI (35% and 59%) to make 65% and 76% protein content in recipes indicated as low (Low) or high (High) based on the amount added.

Table 14 shows the parameters of color ( $L^*$ ,  $a^*$ , and  $b^*$ ), WHC, WSI, and texture to see the effects on PP during texturization of additives and ingredients based on their levels. Compared to the control (NO), the addition of additives and the PI ingredient did not significantly ( $p>0.05$ ) affect color ( $L^*$ ,  $a^*$ , and  $b^*$ ), WHC, and cohesiveness of PP during texturization. In other words, WSI, hardness, and gumminess were affected by additives and ingredients and their levels of addition. Low-CH and high-PI significantly ( $p<0.05$ ) decreased WSI while high-SB and low-XG significantly ( $p<0.05$ ) increased WSI. Low-PI significantly ( $p<0.05$ ) increased hardness and gumminess. Even though the additives and PI ingredient did not significantly ( $p>0.05$ ) affect color ( $L^*$ ,  $a^*$ ,  $b^*$ ), WHC, and cohesiveness on PP during texturization, high-CH had a higher  $L^*$  value than both levels of PI, a higher WHC than low-CH, XG, and a low-PI, and greater values of cohesiveness than Low-CH. Even though there were incremental levels applied (NO, Low, and High), each parameter of each property did not consistently increase or decrease due to their competition with other factors with additives or ingredients for texturization such as chemical composition or energy.

### 3.3.3.2 Lentil Proteins

**Table 15. Parameters of color (L\*, a\*, and b\*) and texture (hardness, cohesiveness, and gumminess) from hydrated TXLP and WHC and WSI from TXLP with different levels of additives (CH, SB, XG, and PI) during texturization produced with a twin-screw extruder (TX-52) with a high shear configuration.**

Additives and ingredients	L*	a*	b*	WHC, g/100g	WSI, g/100g	Hardness, N	Cohesiveness	Gumminess, N
NO	60.27±0.93 <sup>cd</sup>	3.31±0.51 <sup>ab</sup>	17.81±2.47 <sup>ab</sup>	326.74±2.84	11.71±0.18 <sup>ab</sup>	2.01±0.34 <sup>ab</sup>	0.49±0.02 <sup>bc</sup>	0.91±0.13 <sup>bc</sup>
Low-CH	62.58±0.50 <sup>abc</sup>	2.28±0.40 <sup>ab</sup>	10.35±0.72 <sup>bc</sup>	343.21±15.31	10.18±0.51 <sup>bc</sup>	2.98±0.54 <sup>a</sup>	0.53±0.02 <sup>bc</sup>	1.47±0.25 <sup>ab</sup>
High-CH	64.07±0.81 <sup>a</sup>	3.26±0.28 <sup>ab</sup>	18.55±1.12 <sup>a</sup>	354.24±3.44	9.11±0.08 <sup>c</sup>	2.57±0.35 <sup>ab</sup>	0.58±0.01 <sup>b</sup>	1.44±0.20 <sup>ab</sup>
Low-SB	63.63±0.74 <sup>ab</sup>	1.85±0.29 <sup>b</sup>	7.36±0.59 <sup>c</sup>	316.14±2.96	10.16±1.12 <sup>bc</sup>	1.18±0.12 <sup>b</sup>	0.50±0.03 <sup>bc</sup>	0.55±0.04 <sup>c</sup>
High-SB	62.17±0.42 <sup>abc</sup>	2.66±0.56 <sup>ab</sup>	9.86±2.89 <sup>c</sup>	372.37±23.62	13.52±0.46 <sup>a</sup>	1.90±0.37 <sup>ab</sup>	0.50±0.03 <sup>bc</sup>	0.83±0.09 <sup>bc</sup>
Low-XG	62.45±0.44 <sup>abc</sup>	2.58±0.20 <sup>ab</sup>	7.17±1.17 <sup>c</sup>	342.97±9.11	10.80±0.07 <sup>bc</sup>	2.13±0.18 <sup>ab</sup>	0.50±0.02 <sup>bc</sup>	1.00±0.06 <sup>bc</sup>
High-XG	59.12±0.79 <sup>d</sup>	3.84±0.06 <sup>a</sup>	13.59±0.82 <sup>abc</sup>	335.01±21.91	8.73±0.20 <sup>c</sup>	2.08±0.24 <sup>ab</sup>	0.48±0.02 <sup>c</sup>	0.93±0.10 <sup>bc</sup>
Low-PI	60.84±0.07 <sup>bcd</sup>	2.90±0.08 <sup>ab</sup>	10.29±0.98 <sup>bc</sup>	343.55±15.16	5.93±0.31 <sup>d</sup>	1.94±0.18 <sup>ab</sup>	0.69±0.01 <sup>a</sup>	1.28±0.09 <sup>abc</sup>
High-PI	61.31±0.04 <sup>abcd</sup>	3.42±0.24 <sup>ab</sup>	11.79±1.11 <sup>abc</sup>	330.32±18.40	3.82±0.14 <sup>d</sup>	2.80±0.61 <sup>ab</sup>	0.69±0.01 <sup>a</sup>	1.87±0.42 <sup>a</sup>

<sup>a</sup> Means with different letters of the same style are significantly different (p<0.05).

<sup>b</sup> NO=Neither addition of additive or ingredients, CH=calcium hydroxide, SB=sodium bisulfite, XG=xanthan gum, and PI=pea isolates.

<sup>c</sup> Different levels of additives added: CH (0.06% and 0.12%), SB (0.05% and 0.10%), XG (0.10% and 0.20%), and PI (35% and 59%) to make 65% and 76% protein content in a recipe indicated as low (Low) or high (High) based on the amount added.

Table 15 shows parameters of color ( $L^*$ ,  $a^*$ , and  $b^*$ ), WHC, WSI, and texture to find the effects of additives and ingredients based on their addition levels on LP texturization. Compared to the control (NO), the addition of additives and the PI ingredient did not significantly ( $p>0.05$ ) affect the color of  $a^*$  (redness), WHC, and hardness of LP during texturization. In contrast, each additive and ingredient influenced the color in lightness, yellowness, WSI, cohesiveness, and gumminess. A high-CH and low-SB had an increased L value. Both levels of SB and low-XG lowered the b value, and high-CH and XG had lower WSI. Both levels of PI greatly decreased WSI and increased cohesiveness. However, high-PI increased gumminess. Although the addition of additives and the PI ingredient did not significantly ( $p>0.05$ ) affect the color of  $a^*$  (redness), WHC, and hardness on LP during texturization, low-SB had lower  $a^*$  value than high-XG, and low-CH had higher hardness than low-SB.

### 3.3.3.3 Faba Proteins

**Table 16. Parameters of color (L\*, a\*, and b\*) and texture (hardness, cohesiveness, and gumminess) from hydrated TXFP and WHC and WSI from TXFP with different levels of additives (CH, SB, XG, and PI) during texturization produced with a twin-screw extruder (TX-52) with a high shear configuration.**

Additives and ingredients	L*	a*	b*	WHC, g/100g	WSI, g/100g	Hardness, N	Cohesiveness	Gumminess, N
NO	64.20±0.40	0.90±0.18	6.36±0.49 <sup>c</sup>	477.78±9.57 <sup>a</sup>	8.10±0.58 <sup>abc</sup>	0.92±0.13 <sup>b</sup>	0.67±0.01	0.63±0.10 <sup>b</sup>
Low-CH	62.67±0.22	2.44±0.66	15.08±1.97 <sup>ab</sup>	477.75±9.54 <sup>a</sup>	8.19±0.69 <sup>abc</sup>	1.25±0.20 <sup>b</sup>	0.70±0.01	0.89±0.14 <sup>b</sup>
High-CH	62.60±0.94	0.25±0.04	13.83±2.10 <sup>abc</sup>	441.72±14.34 <sup>ab</sup>	7.27±0.22 <sup>bc</sup>	0.97±0.06 <sup>b</sup>	0.69±0.01	0.67±0.04 <sup>b</sup>
Low-SB	62.05±0.55	1.47±0.10	9.07±0.81 <sup>bc</sup>	413.37±2.59 <sup>b</sup>	10.51±0.69 <sup>a</sup>	0.90±0.15 <sup>b</sup>	0.69±0.01	0.63±0.10 <sup>b</sup>
High-SB	59.94±1.50	2.07±0.44	13.98±0.53 <sup>ab</sup>	409.51±12.58 <sup>b</sup>	8.82±0.32 <sup>ab</sup>	1.61±0.46 <sup>b</sup>	0.66±0.01	1.02±0.24 <sup>b</sup>
Low-XG	62.89±1.53	1.14±0.63	11.02±1.41 <sup>bc</sup>	416.92±14.68 <sup>bc</sup>	8.20±0.12 <sup>abc</sup>	1.14±0.09 <sup>b</sup>	0.69±0.01	0.77±0.04 <sup>b</sup>
High-XG	63.48±0.70	1.80±0.73	14.42±0.40 <sup>ab</sup>	420.42±1.98 <sup>bc</sup>	9.61±1.04 <sup>ab</sup>	0.74±0.23 <sup>b</sup>	0.67±0.01	0.52±0.15 <sup>b</sup>
Low-PI	64.25±2.72	2.47±0.85	18.70±2.06 <sup>a</sup>	366.94±6.81 <sup>c</sup>	6.21±0.10 <sup>cd</sup>	1.19±0.12 <sup>b</sup>	0.69±0.02	0.83±0.07 <sup>b</sup>
High-PI	61.76±2.38	2.57±0.12	14.32±2.24 <sup>ab</sup>	367.72±7.20 <sup>c</sup>	4.63±0.04 <sup>d</sup>	3.46±0.35 <sup>a</sup>	0.70±0.01	2.36±0.24 <sup>a</sup>

<sup>a</sup> Means with different letters of the same style are significantly different (p<0.05).

<sup>b</sup> NO=Neither addition of additive or ingredients, CH=calcium hydroxide, SB=sodium bisulfite, XG=xanthan gum, and PI=pea isolates.

<sup>c</sup> Different levels of additives: CH (0.06% and 0.12%), SB (0.05% and 0.10%), XG (0.10% and 0.20%), and PI (35% and 59%) to make 65% and 76% protein content in a recipe indicated as low (Low) or high (High) based on the amount added.

Table 16 shows the parameters of color ( $L^*$ ,  $a^*$ , and  $b^*$ ), WHC, WSI, and texture to determine the effects of additives and ingredients based on their addition levels on FP during texturization. Compared to the control (NO), addition of additives and the PI ingredient significantly influenced yellowness and WSI and did not significantly ( $p>0.05$ ) affect the color of  $L^*$  (lightness) and  $a^*$  (redness), WHC, and cohesiveness on FP during texturization. Low-CH, high-SB and XG, and both levels of PI increased  $b^*$  value. High-PI decreased WSI and increased hardness and gumminess.

#### ***3.3.4 Effects of Additions of Additives (CH, SB, and XG) and an Ingredient (PI)***

Each parameter for each level (low and high) of additives (CH, SB, and XG) and ingredient (PI) used in each PLP were combined and compared to the control (NO) to generalize how each additive and ingredient worked on the parameters of each PLP during texturization.

**Table 17. Parameters of color (L\*, a\*, and b\*) and texture (hardness, cohesiveness, and gumminess) from hydrated TPP and WHC and WSI from TPP with different levels of additives (CH, SB, XG, and PI) during texturization produced with a twin-screw extruder (TX-52) with a high shear configuration.**

Additive & ingredient	L*	a*	b*	WHC, g/100g	WSI, g/100g	Hardness, N	Cohesiveness	Gumminess, N
TXPP								
NO	66.69±0.88a	4.10±0.44	21.41±2.32 <sup>a</sup>	314.10±0.26	7.45±0.14 <sup>a</sup>	3.05±0.82 <sup>ab</sup>	0.64±0.01	1.77±0.44 <sup>b</sup>
CH	66.74±2.06 <sup>a</sup>	3.23±0.17	18.90±2.17 <sup>ab</sup>	306.49±37.00	7.17±0.34 <sup>ab</sup>	3.31±0.56 <sup>ab</sup>	0.63±0.02	1.84±0.25 <sup>b</sup>
SB	64.58±0.58 <sup>a</sup>	3.08±0.52	13.60±1.85 <sup>b</sup>	329.26±9.74	7.93±0.31 <sup>a</sup>	2.17±0.25 <sup>b</sup>	0.65±0.01	1.35±0.14 <sup>b</sup>
XG	63.00±1.05 <sup>ab</sup>	3.37±0.39	14.14±0.74 <sup>ab</sup>	263.15±13.30	7.81±0.31 <sup>a</sup>	2.88±0.44 <sup>ab</sup>	0.64±0.01	1.71±0.23 <sup>b</sup>
PI	59.17±0.67 <sup>b</sup>	3.50±0.43	12.26±1.51 <sup>b</sup>	292.64±28.58	6.17±0.18 <sup>b</sup>	4.98±0.53 <sup>a</sup>	0.67±0.01	3.13±0.27 <sup>a</sup>
TXLP								
NO	60.27±0.59 <sup>C</sup>	3.31±0.32	17.81±1.56 <sup>A</sup>	326.74±1.64	11.71±0.12 <sup>AB</sup>	2.01±0.23 <sup>AB</sup>	0.49±0.02 <sup>C</sup>	0.91±0.09 <sup>C</sup>
CH	63.33±0.54 <sup>A</sup>	2.77±0.31	14.45±1.93 <sup>AB</sup>	348.72±7.16	9.64±0.31 <sup>C</sup>	2.77±0.31 <sup>A</sup>	0.56±0.01 <sup>B</sup>	1.46±0.15 <sup>AB</sup>
SB	62.90±0.50 <sup>AB</sup>	2.26±0.34	8.61±1.43 <sup>B</sup>	344.26±18.92	11.84±0.85 <sup>A</sup>	1.54±0.21 <sup>B</sup>	0.50±0.02 <sup>BC</sup>	0.69±0.06 <sup>C</sup>
XG	60.79±0.84 <sup>BC</sup>	3.21±0.30	10.38±1.57 <sup>B</sup>	338.99±9.96	9.76±0.40 <sup>BC</sup>	2.10±0.14 <sup>AB</sup>	0.49±0.01 <sup>C</sup>	0.97±0.06 <sup>BC</sup>
PI	61.07±0.11 <sup>ABC</sup>	3.16±0.16	11.04±0.74 <sup>B</sup>	336.94±10.45	4.87±0.43 <sup>D</sup>	2.37±0.33 <sup>AB</sup>	0.69±0.01 <sup>A</sup>	1.58±0.22 <sup>A</sup>
TXFP								
NO	64.20±0.26	0.90±0.11 <sup>b</sup>	6.36±0.31 <sup>c</sup>	477.78±5.52 <sup>a</sup>	8.10±0.38 <sup>ab</sup>	0.92±0.09 <sup>b</sup>	0.67±0.01	0.63±0.06 <sup>b</sup>
CH	62.64±0.43	1.35±0.57 <sup>ab</sup>	14.46±1.32 <sup>ab</sup>	457.93±12.28 <sup>a</sup>	7.73±0.38 <sup>b</sup>	1.11±0.11 <sup>b</sup>	0.70±0.01	0.78±0.08 <sup>b</sup>
SB	61.00±0.86	1.77±0.24 <sup>ab</sup>	11.52±1.18 <sup>b</sup>	418.67±3.87 <sup>b</sup>	9.66±0.48 <sup>a</sup>	1.25±0.25 <sup>b</sup>	0.68±0.01	0.82±0.14 <sup>b</sup>
XG	63.19±0.76	1.47±0.45 <sup>ab</sup>	12.72±1.00 <sup>ab</sup>	411.44±8.86 <sup>b</sup>	8.90±0.55 <sup>ab</sup>	0.94±0.13 <sup>b</sup>	0.68±0.01	0.65±0.09 <sup>b</sup>
PI	63.01±1.71	2.52±0.38 <sup>a</sup>	16.51±1.68 <sup>a</sup>	367.33±3.54 <sup>c</sup>	5.42±0.30 <sup>c</sup>	2.32±0.38 <sup>a</sup>	0.70±0.01	1.59±0.26 <sup>a</sup>

<sup>a</sup> Means with different letters of the same style are significantly different (p<0.05).

<sup>b</sup> TXPP=texturized pea proteins, TXLP=texturized lentil proteins, and TXFP=texturized faba bean proteins

<sup>c</sup> NO=Neither addition of additive or ingredients, CH=calcium hydroxide, SB=sodium bisulfite, XG=xanthan gum, and PI=pea isolates.

Table 17 shows the parameters of color ( $L^*$ ,  $a^*$ , and  $b^*$ ), WHC, WSI, and texture (hardness, cohesiveness, and gumminess) to understand effects of additives (CH, SB, and XG) and an ingredient (PI) on TPP. Compared to the control, the addition of additives (CH, SB, and XG) and an ingredient (PI) to the vegetable proteins (SC, PP, LP, and FP) for texturization did not significantly ( $p>0.05$ ) affect WHC on all TPP.

However, the addition of PI to the recipe for texturization significantly decreased the color of  $b^*$  (yellowness), decreased WSI, and increased gumminess for all TPP. There was a tendency for PI to decrease WSI and increase gumminess while there was no tendency for PI to decrease  $b^*$  (yellowness) on TXPP and TXLP but increased the yellowness on TXFP. In other words, the addition of PI might help the unfolded and aligned protein structure form chemical bonds like cross-linking during texturization resulting in decreased WSI and increased gumminess. In contrast, the pigment of PI provided a strong equilibrated color in yellowness to balance between  $12.26\pm 1.51$  to  $16.51\pm 1.68$  and resulted in decreased yellowness in TXPP and TXLP but increased the yellowness in TXFP.

SB had a similar tendency as PI in that it significantly decreased the yellowness in TXPP and TXLP, but it increased the value in TXFP. PI decreased lightness in TXPP and increased redness in TXFP. Compared to the control, XG did not significantly influence color, WHC, WSI, and texture for any TPP except TXLP. CH improved cohesiveness and gumminess for TXLP. As seen in



Figure 11, TXLP did not show an integrity of texturization so that CH helped LP texturize during the texturization process and resulted in improved texture (cohesiveness and gumminess).

### **3.4 Conclusion**

High shear screw configuration significantly improved texturization of pulse proteins. All TPP had inferior WSI and texture, but hydrated TXPI and TXFP had a similar cohesiveness. TXFP had less brown color, higher WHC, relatively less WSI compared to other pulse proteins and similar gumminess to the control. Compared to the control, TXSC, the samples used in this study had a similar color ( $L^*$ ,  $a^*$ , and  $b^*$ ), but TXPI had a lower value in lightness, and TXFP had lower values in redness and yellowness. Calcium hydroxide influenced the parameters to define the characteristics of PLP during texturization once they were not texturized with the processing condition such as with TXLP. However, the addition of PI for texturization improved the texture (gumminess) although each PLP had a well-defined textural integrity after texturization. All additives influenced the quality of TPP depending on protein types.

## CHAPTER IV

### BEEF HAMBURGER PATTIES WITH TEXTURIZED PULSE PROTEINS (TPP)

#### USING PEAS, LENTILS, AND FABABEANS

##### 4.1 Introduction

For sensory evaluation with consumer panelists, three texturized pulse proteins (TXPP, TXLP, TXFP) with 0.12% calcium hydroxide (CH), one texturized pea isolate (TXPI) produced in the tests described in Chapter 3 and one texturized protein (TXSC, control) produced in the main test described in Chapter 2 were selected. TXLP had relatively less defined fiber structure visually (Figure 11) and less water holding capacity (WHC) and higher water solubility index (WSI) (Figure 13). However, TXLP with 0.12% calcium hydroxide (CH) had relatively less WSI and higher cohesiveness (Table 17) that might help bind better in meat patty as a meat substitute to give a better meat-like texture. TXPP and TXFP were selected as well for consistency of the processing conditions. TXPI without any addition of additives were selected to observe how they work as meat substitutes with higher protein content in the texturized protein. The meat patties with these TXPLP were expected to have less solubilized materials and good textural parameter during hydration.

Each 30% rehydrated TPP (1:2.7 TPP to water) was mixed with 70% meat to make a typical fast-food-style ground-beef patty. Therefore, five treatments were prepared for a consumer central location test in Dr. Miller's sensory lab of the Kleberg Building at

Texas A&M University. Eighty consumers recruited through a flyer and email and participated in this sensory test approved by the IRB (IRB2017-0362M).

The objective of this study was to understand consumer perceptions of hamburger patties in which TPP was used as meat substitute. I hypothesized that the qualities of the TPP used in hamburger patties would not be significantly different from the control soy-based products. Therefore, this study will contribute to developing soybean-free products as alternative sources of meat-like products, for which the consumer demand is rising steadily worldwide.

## **4.2 Materials and Methods**

### ***4.2.1 Preparation of Ground-Beef Patties***

80/20 coarse ground beef, frozen within 10 days of slaughter, was purchased from Ruffino Meats in Bryan, TX. TPP produced as described in Chapter 3 were screened based on the WSI and textural property (cohesiveness) and TXPLP (TXPP, TXLP, and TXFP) with 0.12% CH added to the preconditioner of the extruder during texturization were selected. TXPI were selected as well to measure the consumer acceptance as meat substitutes. TXSC processed with a regular high shear screw profile as described in Chapter 2 was used as a control.

Following the modified method of Heywood and others (2002), the meat was mixed with 30% hydrated TPP (1:2.7 TPP to water) to produce a typical fast-food-style ground-beef patty. The meat was ground with a meat grinder fitted with a 0.64-cm (1/4 in.) plate and stored in the refrigerator (4°C) until used. Each TPP was soaked in water (1:2.7 TPP to water) for 30 min and it was confirmed that the TPP was thoroughly well

hydrated. The hydrated TPP was ground with the meat grinder fitted with a 0.64-cm (1/4 in.) plate and stored in the refrigerator (4°C) until used. The ground TPP was mixed in a Hobart mixer (Hobart mixer, Model N50, Canada) at a speed of ~61 rpm for 1 min, and 0.5% salt and 0.2% black pepper were sprinkled slowly over the TPP during mixing. The ground meat was added to the mixer and the TPP and ground meat were mixed for 30 seconds. The ground samples were ground with a meat grinder fitted with a 0.32-cm (1/8 in.) plate. Patties for each treatment (113g) were formed with a patty maker (Supermodel 54 Food Portioning Machine, Hollymatic Corporation, Countryside, IL) with a 2.54 cm plate. Patty paper was placed on the top and bottom of the patties and they were placed in a single lay on trays, placed in a -40°C freezer, crust frozen for 20 min, vacuum packaged, and stored in the -40°C freezer until the sensory test.

#### **4.2.2 Cooking Protocols**

Approximately 24 hours prior to testing, the frozen samples were removed from the freezer and placed on racks in a single layer to thaw in a cooler (4°C). One hour prior to testing, patties were organized by cooking order on the trays, removed from their vacuum packaged bags and patty paper, and raw weights (g) were taken. Patty trays were covered with plastic wrap and held in the cooler until it was time for them to be cooked. Prior to cooking, five temperature readings of the surface of the grill were taken using an infrared temperature reader (MS6530H Infrared Thermometer, Commercial Electric Products Corporation, Cleveland, OH) with a target temperature of 162°C. Samples were cooked on a commercial flat-top grill to an end temperature of 71°C, with a flip temperature at 27°C. Internal temperatures were monitored using thermocouple probes (Model SCPSS-040U-6, Type T, 0.040 Sheath Diameter, 15.24

cm length Ungrounded Junction Thermocouple, Omega Engineering, Stamford, CT) pushed into the geometric center of the patty periodically during cooking and the temperatures were observed with a thermometer (Omega HH501BT Type T, Omega Engineering, Stamford, CT). Raw temperatures and the time when the patties were put on the grill were recorded, along with the end temperature, the time the patties were taken off the grill, and the final cooked weights. They were wrapped in foil and placed in a holding oven (Model 750-TH-II, Alto-Shaam, Menomonee Falls, WI) for no longer than 20 min until served.

#### **4.2.3 Consumer Sensory Evaluation**

Previously, 80 consumer panelists were recruited by emails and advertisements. They were also asked to provide demographic information and sign a consent form through a survey website ([www.tamuag.az1.qualtrics.com](http://www.tamuag.az1.qualtrics.com)). Based on their answers regarding the time they were available for the test, they were divided into four different sessions (20 consumer panelists each) for 1-hour intervals. In each session, they were assigned to one of five groups since each patty was divided into four wedges. Four consumer panelists were randomly assigned to each group and had the same treatment in the same order (APPENDIX C). Before the test, the consent forms for the test were collected from the panelists. In the booths, they were presented with a packet containing testing procedures, palate cleansers of distilled water and saltless saltine crackers, a demographic ballot, and five individual sample ballots. Consumer demographic questions included: gender, age, ethnicity, household income, household population, employment level, protein sources and location where they were consumed, frequency of protein consumption, preferred cooking method for ground beef, degree of

doneness desired for ground beef, type of ground beef typically purchased, desired fat percentage of ground beef, and types of cuisines consumed (APPENDIX G). Opinions of cooked appearance, overall appearance, overall flavor, and overall texture were included on each sample ballot measured with a 9-point hedonic scale. Open-ended questions, “Please write any words that describe what you LIKE about this meat patty” and “Please write any words that describe what you DISLIKE about this meat patty” were also included on each ballot (APPENDIX H).

Each sample was served in a plastic cup marked with a random three-digit code. Samples consisted of a quarter of a patty each, and consumers were given a new transparent plastic fork and transparent plastic knife to evaluate each sample. Consumer panelists were provided with five random samples over the course of a one-hour session (APPENDIX H).

#### **4.2.4 Cooking Yield and Cooking Time**

Cooking yield was calculated using Equation 4.1 .

$$\text{Cooking yield} = \frac{\text{Cooked patty (g)}}{\text{Raw patty (g)}} \times 100 \quad \text{Equation 4.1}$$

Cooking time of each patty in minutes was measured as well.

#### **4.2.5 Color Measurement**

Frozen hamburger patties were thawed for 24 hours in a cooler (4°C), and at room temperature for about 20 mins after removing their vacuum bags and patty paper. They

were directly evaluated using a colorimeter (Model CR-200, Minolta Co., Ramsey, NJ, USA). Cooked hamburger patties were measured as well. Values were expressed as  $L^*$ ,  $a^*$  and  $b^*$ , where  $L^*$  values (lightness) vary from black (0) to white (100), chroma  $a^*$  values (redness) vary from green (-60) to red (+60), and chroma  $b^*$  values (yellowness) vary from blue (-60) to yellow (+60). The color of three locations on each patty were measured, and color measurements were performed with three samples for each treatment.

#### **4.2.6 Texture Analysis**

A texture analysis of the meat patties with TPP was conducted with a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) using the texture profile analysis measurement. According to the modified method by Ganhão and others (2010), a cylindrical sample (2.54 cm diameter) from the center of each patty was taken and subjected to a two-cycle compression test. The samples were compressed to 70% of their original height with a cylindrical probe of 7.25 cm diameter and a cross-head speed of 1 mm/s. Texture profile parameters were evaluated following descriptions by Bourne (1978). All analyses were performed with five samples for each treatment.

#### **4.2.7 Statistical Analysis**

A one-way ANOVA was used to determine the significant differences ( $P < 0.05$ ) between each beef hamburger patty mixed with 30% TPP. Tukey's HSC (honestly significant difference) analysis was also conducted for pair comparison. All statistical tests were performed using JMP software (JMP Pro 12.0.1, SAS Institute Inc., Cary, N.C., USA).

## 4.3 Results and Discussion

### 4.3.1 Consumer Demographics

Table 18. Demographic Frequencies for Meat Patty Consumers (n = 80).

Question	Number of Respondents	Percentage of Respondents
Sex		
Male	26	32.5
Female	54	67.5
Age		
20 years or younger	34	42.5
21 – 25 years	31	38.8
26 – 35 years	8	10.0
36 – 45 years	2	2.5
46 – 55 years	2	2.5
56 – 65 years	3	3.8
66 years and older	0	0
Ethnicity		
African-American	5	6.3
Asian/Pacific Islanders	8	10.0
Caucasian (non-Hispanic)	50	62.5
Latino or Hispanic	14	17.5
Native American	1	1.3
Other	2	2.5
Household income		
Below \$25,000	26	32.5
\$25,001 - \$49,999	13	16.3
\$50,000 - \$74,999	5	6.3
\$75,000 - \$99,999	15	18.8
\$100,000 or more	21	26.3
Household size including yourself		
1	11	13.8
2	13	16.3
3	17	21.3
4	22	27.5
5	13	16.3
6 or more	4	5.0



**Table 18. Continued.**

Employment level				
Not employed	43		53.8	
Part-time	25		31.3	
Full-time	12		15.0	
Proteins consumed at home or at a restaurant (away from home)				
At Home	Do not consume	Consume	Do not consume	Consume
Chicken	0	80	0.0	100.0
Beef (steaks)	8	72	10.0	90.0
Ground Beef	9	71	11.3	88.8
Pork	23	57	28.8	71.3
Fish	15	65	18.8	81.3
Lamb	66	14	82.5	17.5
Egg	1	79	1.3	98.8
Soy Based Products	59	21	73.8	26.3
Away from Home/Restaurant	Do not consume	Consume	Do not consume	Consume
Chicken	1	79	1.3	98.8
Beef (steaks)	3	77	3.8	96.3
Ground Beef	9	71	11.3	88.8
Pork	22	58	27.5	72.5
Fish	16	64	20.0	80.0
Lamb	52	27	65.8	34.2
Eggs	9	71	11.3	88.8
Soy Based Products	56	24	70.0	30.0

**Table 18. Continued.**

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Weekly consumption of protein		
	Beef	
0	10	12.5
1 – 2	59	73.8
3 – 4	7	8.8
5 – 6	3	3.8
7 or more	1	1.3
	Ground Beef	
0	9	11.3
1 – 2	56	70.0
3 – 4	11	13.8
5 – 6	3	3.8
7 or more	1	1.3
	Pork	
0	26	32.9
1 – 2	50	63.3
3 – 4	2	2.5
5 – 6	1	1.3
7 or more	0	0.0
	Lamb	
0	55	79.7
1 – 2	13	18.8
3 – 4	1	1.4
5 – 6	0	0.0
7 or more	0	0.0

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Table 18. Continued.

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	Chicken	
0	0	0.0
1 – 2	17	21.3
3 – 4	38	47.5
5 – 6	17	21.3
7 or more	8	10.0
	Fish	
0	15	20.8
1 – 2	41	56.9
3 – 4	14	19.4
5 – 6	1	1.4
7 or more	1	1.4
	Soy Based Products	
0	46	68.7
1 – 2	15	22.4
3 – 4	4	6.0
5 – 6	1	1.5
7 or more	1	1.5

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**Table 18. Continued.**

What cooking method do you prefer to use when cooking ground beef?				
	Do not use	Use	Do not use	Use
Pan-frying or skillet on the Stove	13	67	16.3	83.8
Grilling outside	44	36	55.0	45.0
Oven baking	62	18	77.5	22.5
Electric appliance (George Forman Grill or Electric grill)	68	12	85.0	15.0
Stir fry	66	14	82.5	17.5
	73	7	91.3	8.8
	75	5	93.8	6.3
Degree of doneness preference for ground beef				
Rare		2		2.5
Medium Rare		16		20.0
Medium		21		26.3
Medium Well		18		22.5
Well		16		20.0
Very Well		7		8.8

**Table 18. Continued.**

What percentage of fat do you normally buy when purchasing ground beef?				
	Do not	Purchase	Do not	Purchase
4%	63	17	78.8	21.3
7%	59	21	73.8	26.3
10%	59	21	73.8	26.3
15%	67	13	83.8	16.3
20%	74	6	92.5	7.5
27%	80	0	100.0	0.0

What flavor or types of cuisines do you like?				
	Do not eat	Eat	Do not eat	Eat
American	6	74	7.5	92.5
Chinese	19	61	23.8	76.3
French	45	35	56.3	43.8
Barbeque	11	69	13.8	86.3
Greek	42	38	52.5	47.5
Thai	44	36	55.0	45.0
Mexican/Spanish	5	75	6.3	93.8
Japanese	30	50	37.5	62.5
Lebanese	61	19	76.3	23.8
Indian	53	27	66.3	33.8
Italian	6	74	7.5	92.5

Demographic information for consumers (n = 80) who participated in this study are reported in Table 18. More females (67.5%) participated in the study compared to males and the majority of participants (91.3%) fell into the 21 - 35 age range with a slightly heavier representation of the 20 - younger age range (42.5%). The majority of consumers represented the Caucasian (non-Hispanic) ethnicity (62.5%), followed by Latino or Hispanic (17.5%), Asian/Pacific Islanders (10.0%), and African-American (6.3%). Household incomes were distributed with 32.5% below \$25,000, 26.3% in the \$100,000 or more group, 18.8% in the \$75,000-\$99,999 group, 16.3% in the \$25,001 - \$49,999 group, and 6.3% in the \$50,000 - \$74, 999 group of income brackets.

Household size was fairly evenly represented by a majority of four-person households (27.5%), followed by three-person (21.3%), and two and five-person (16.3%) households. Most of the consumers were not-employed (53.8%) or employed part-time (31.3%).

When asked about proteins consumed at home, over 70% of consumers reported consuming chicken, beef (steaks), ground beef, pork, fish, and eggs. The top three proteins consumed at home included chicken (100%), eggs (98.8%), and beef (steaks) (90.9%). When asked about proteins consumed away from home or at restaurants, over 70% of consumers reported consuming chicken, beef (steaks), ground beef, fish, and eggs as well. The top proteins consumed away from home included chicken (98.8%), beef (steaks) (96.3%), ground beef and eggs (88.8%), and fish (80.0%). Interestingly, 26.3% and 30.0% of consumers reported consuming vegetable sources, soy-based products, at home and away from home/restaurants, respectively.

Consumers were asked to report how many times a week they consumed each protein source. The majority of consumers reported consuming beef (steaks) 1 to 2 times per week (73.8%), followed by 0 times per week (12.5%) and 1-2 times per week (13.8%). For ground beef consumption, the majority of consumers reported eating it 1 to 2 times per week (70.0%), followed by 3 to 4 times per week (13.8%), and 0 times per week (11.3%). For pork consumption, consumers reported 1 to 2 times per week (63.3%), followed by 0 times per week (32.9%). For lamb consumption, the majority of consumers reported 0 times per week (79.7%) followed by 1 to 2 times (18.8%). For chicken consumption, the majority of consumers consumed chicken 3 to 4 times per week (47.5%), followed by both 1 to 2 times per week and 5 to 6 times (21.3%). For

fish consumption, the majority of consumers reported eating fish 1 to 2 times per week (56.9%), followed by either 0 times per week (20.8%) or 3 to 4 times per week (19.4%). Finally, for soy-based products, consumers reported eating soy-based products 0 times per week (68.7%) followed by 1 to 2 times per week (22.4%).

Consumers were asked what methods they preferred when cooking ground beef. The majority of consumers preferred to pan-fry/skillet on the stove (83.8%). Some consumers grilled outside (45.0%), oven baked (22.5%), stir-fried (17.5%), or used an electric appliance (George Forman Grill; 15.0%), and even fewer used oven broiling (8.8%), or a microwave (6.3%).

When asked for preferences on the degree of doneness for ground beef, the majority of consumers responded with medium (26.3%), followed by medium well (22.5%) and both medium rare and well (20.2%). Few consumers preferred the extremes with only 2.5% reporting rare and 8.8% for very well done.

When consumers were asked what fat level they typically purchased, the top two percentages were 7% (26.3%) and 10% (23.6%), followed by 4% (21.3%), 15% (16.3%), and 20% (7.5%).

Consumers were asked what types of cuisines they liked to purchase. Over 90% reported enjoying American, Mexican/Spanish, and Italian cuisines, followed by Barbeque (86.3%), Chinese (76.3%), and Japanese (62.5%). Lebanese, Indian, French, Thai, and Greek were among the lowest typically consumed. These results indicate that consumers in this study were an acceptable population to test meat patties containing 30% texturized vegetable proteins (SC, PI, PP, LP, and FP).

### 4.3.2 Consumer Perception of Beef Patties with TPP

Table 19. Consumer Liking for Meat Patties with TPP by hedonic test.

Attribute	P-value	SC	PI	PP	LP	FP	<sup>b</sup> RMSE
Cooked appearance	0.02	5.4 <sup>ab</sup>	6.3 <sup>a</sup>	5.9 <sup>ab</sup>	5.4 <sup>b</sup>	6.1 <sup>a</sup>	1.87
Overall	<0.0001	5.6 <sup>a</sup>	5.4 <sup>ab</sup>	5.0 <sup>abc</sup>	4.4 <sup>bc</sup>	4.2 <sup>c</sup>	2.06
Overall flavor	0.0001	5.5 <sup>a</sup>	5.0 <sup>a</sup>	5.0 <sup>a</sup>	4.8 <sup>ab</sup>	3.9 <sup>b</sup>	2.19
Overall texture	<0.0001	5.7 <sup>a</sup>	5.5 <sup>a</sup>	4.7 <sup>a</sup>	3.5 <sup>b</sup>	5.2 <sup>a</sup>	2.29

<sup>a</sup> Means within a row and effect followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>b</sup> RMSE = root mean square error, SC = texturized soy concentrate, PI = texturized pea isolate, PP = texturized pea protein, LP = texturized lentil protein, and FP = texturized faba bean protein.

<sup>d</sup> Consumer liking measured with 0 = extremely dislike and 9 = extremely like.

Consumer perception scores are reported in Table 19. Different protein sources in meat patties containing TXVP significantly affected all perceptions, liking the cooked appearance ( $P = 0.02$ ), overall ( $P = <0.0001$ ), overall flavor ( $P = 0.0001$ ), and overall texture ( $P = <0.0001$ ). Liking the cooked appearance ( $P = 0.02$ ) was similar for all samples compared to SC, but PI had a higher score than FP. The overall flavor was similar for all samples compared to SC, but higher than FP. Overall texture was similar for all samples compared to SC, but higher than LP.



Figure 15. Consumer liking (a) or disliking (b) descriptors for meat patties containing SC.



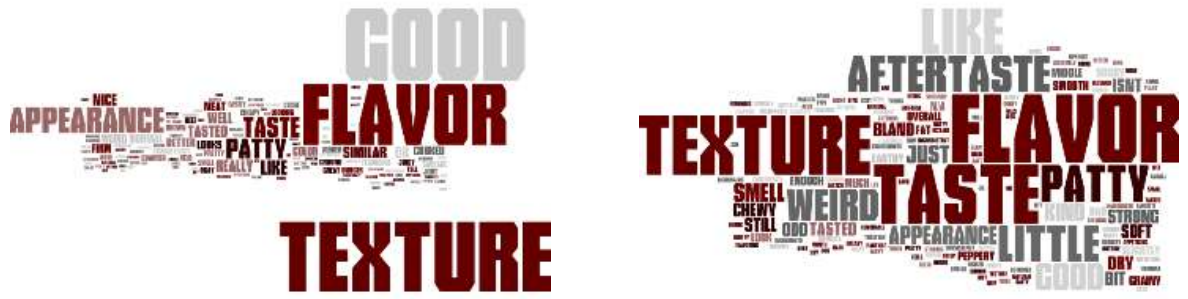


Figure 16. Consumer liking (a) or disliking (b) descriptors for meat patties containing PI.

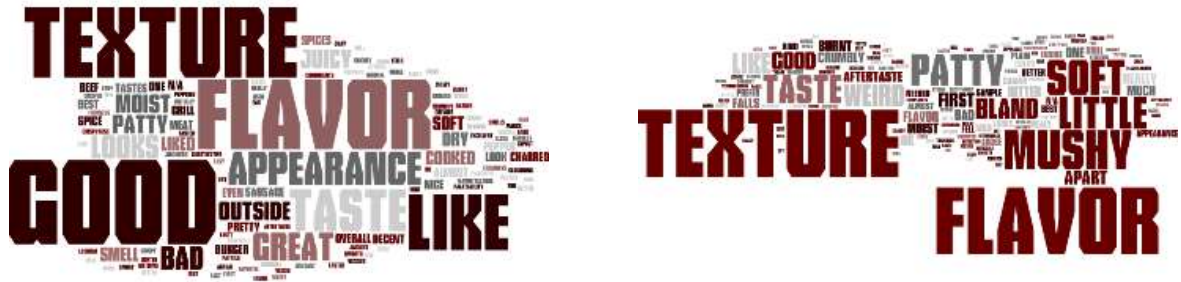


Figure 17. Consumer liking (a) or disliking (b) descriptors for meat patties containing PP.



Figure 18. Consumer liking (a) or disliking (b) descriptors for meat patties containing LP.



Figure 19. Consumer liking (a) or disliking (b) descriptors for meat patties containing FP.

Word clouds were produced using the comments from consumer panelists answering the like and dislike open-ended question. Figure 15 to 19 show the consumer’s responses separated by meat patties containing different legume proteins (30%). The size of the words illustrates how frequently the consumers used the words. For SC, the most commonly used words for liking the meat patties were texture, good, and flavor (Figure 15) and for disliking them the words were flavor, texture, little, taste, and bland. Consumers used more terms to describe what they did not like than what they liked about SC. More words are presented on the dislike word cloud compared to the like word cloud. For PI, the most frequently used words for liking the patties were texture, flavor, good, appearance, and taste (Figure 16) and the words for disliking the patties were flavor, texture, taste, patty, like, aftertaste, and weird. More words to describe disliking were used than liking. The most commonly used descriptors for meat patties with PP were texture, flavor, appearance, good, like taste, great while the most commonly used descriptors for dislike descriptors were texture, flavor, mush, little, and soft. More positive words to describe the quality of patties were used for meat patties containing PP than negative words. The most commonly used words for liking patties

containing LP were good, flavor, and taste. The most frequently used words for disliking them were texture, mush, soft, taste, and flavor. For patties containing FP, the most commonly used words for liking them were texture, good, and flavor, while the most frequently used words for disliking them were flavor, taste, bitter, aftertaste, texture, and weird. For all treatment including texturized vegetable proteins, more descriptive words were used when the consumer panelists responded to describe disliking points of the sample compared to like descriptors. Across all the words clouds, texture and flavor were most consistently used for describing whether a consumer liked a sample.

#### 4.3.3 Color of Raw and Cooked Meat Patties with TPP

Table 20. Color of Raw and Cooked Meat Patties Made with TPP.

Attribute	P-value	SC	PI	PP	LP	FP	<sup>b</sup> RMSE
<u>Raw</u>							
L*	0.0017	59.8 <sup>a</sup>	56.6 <sup>b</sup>	60.5 <sup>a</sup>	61.2 <sup>a</sup>	60.1 <sup>a</sup>	0.97
a*	0.0283	9.6 <sup>ab</sup>	8.5 <sup>bc</sup>	11.0 <sup>a</sup>	9.7 <sup>ab</sup>	6.8 <sup>c</sup>	1.31
b*	<0.0001	13.6 <sup>c</sup>	13.5 <sup>c</sup>	18.4 <sup>a</sup>	17.4 <sup>a</sup>	15.1 <sup>b</sup>	0.71
<u>Cooked</u>							
L*	0.0036	53.2 <sup>ab</sup>	47.2 <sup>c</sup>	51.7 <sup>ab</sup>	54.0 <sup>a</sup>	50.3 <sup>bc</sup>	1.65
a*	0.0447	5.5 <sup>b</sup>	6.6 <sup>ab</sup>	7.2 <sup>a</sup>	5.7 <sup>ab</sup>	5.8 <sup>ab</sup>	0.65
b*	0.0019	15.5 <sup>bc</sup>	14.7 <sup>c</sup>	18.3 <sup>ab</sup>	17.8 <sup>a</sup>	17.4 <sup>ab</sup>	0.87

a Means within a row and effect followed by the same letter are not significantly different ( $P > 0.05$ ).

b RMSE = root mean square error, SC = texturized soy concentrate, PI = texturized pea isolate, PP = texturized pea protein, LP = texturized lentil protein, and FP = texturized faba bean protein.

Table 20 shows the color of raw and cooked meat patties with texturized vegetable proteins. Protein sources in meat patties containing TPP significantly affected all parameters in both raw and cooked meat patties. The perceptions of the consumers for liking the cooked appearance ( $P = 0.02$ ), overall ( $P = <0.0001$ ), overall flavor ( $P = 0.0001$ ), and overall texture ( $P = <0.0001$ ). Liking the cooked appearance ( $P = 0.02$ )

was similar for all samples compared to SC, but PI had a higher score than FP. Overall flavor was similar for all samples compared to SC, but higher than FP. Overall texture was similar for all samples compared to SC, but higher than LP.

#### 4.3.4 Cooking Properties and Texture of Meat Patties containing TPP

Table 21. Cooking Yield, Cooking Time, and Texture of Cooked Meat Patties with TPP.

Attribute	P-value	SC	PI	PP	LP	FP	<sup>b</sup> RMSE
Cooking parameters							
Cooking yield, %	<0.0001	88.4 <sup>c</sup>	83.7 <sup>a</sup>	86.7 <sup>bc</sup>	86.5 <sup>b</sup>	88.4 <sup>c</sup>	2.07
Cooking time, min	<0.0001	5.8 <sup>b</sup>	7.8 <sup>a</sup>	6.1 <sup>b</sup>	5.7 <sup>b</sup>	6.1 <sup>b</sup>	1.07
TPA							
Hardness, N	<0.0001	66.8 <sup>a</sup>	49.4 <sup>b</sup>	35.5 <sup>c</sup>	28.9 <sup>c</sup>	43.5 <sup>b</sup>	3.59
Cohesiveness	<0.0001	0.4 <sup>a</sup>	0.4 <sup>b</sup>	0.3 <sup>d</sup>	0.3 <sup>d</sup>	0.4 <sup>c</sup>	0.02
Gumminess, N	<0.0001	29.3 <sup>a</sup>	20.0 <sup>b</sup>	11.4 <sup>d</sup>	8.6 <sup>d</sup>	16.2 <sup>c</sup>	1.74

<sup>a</sup> Means within a row and effect followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>b</sup> RMSE = root mean square error, SC = texturized soy concentrate, PI = texturized pea isolate, PP = texturized pea protein, LP = texturized lentil protein, and FP = texturized faba bean protein, TPA = texture profile analyzer.

Table 21 shows cooking properties (cooking yield and cooking time) and texture (hardness, cohesiveness, and gumminess) of meat patties containing 30% of different TPP. The protein source in meat patties containing TPP significantly ( $P = <0.0001$ ) affected all cooking parameters. Cooking yield was the lowest for PI compared to other samples, and PP and FP had a similar cooking yield to SC and LP had a similar cooking yield to PP. Cooking time was the most for PI compared to other samples, and other samples required a similar cooking time.

The protein source in meat patties containing TPP significantly ( $P = <0.0001$ ) affected all textural parameters as well. Hardness was the highest for SC compared to other samples, followed by PI and FP that were not significantly different. The hardness of LP was not significantly different from PP and lower than for other samples. Cohesiveness and gumminess showed the same pattern. SC had the highest cohesiveness and gumminess, followed by PI, FP, PP, and LP.

#### **4.4 Conclusion**

Consumer panelists (n = 80) conducted a sensory analysis to evaluate their preferences (cooked appearance, overall, overall flavor, and overall texture) of meat patties containing 30% of different TPP to compare to the control, texturized soy proteins. Different protein sources in meat patties significantly influenced all the perceptions of the consumer panelists. Compared to the control, LP had a significantly lower cooked appearance, overall, and liking of the overall texture, and FP had significantly lower overall and liking the overall flavor as well. The most frequently used words from consumer panelists on the like and dislike open-ended question about whether or not a consumer liked a sample was texture and flavor.

Raw and cooked patties with PI had a lower lightness and redness for the raw patty compared to the control. PP and FP had similar cooking yield compared to the control, and all samples containing TPP did not have significantly different cooking time except PI compared to the control. Hardness, cohesiveness, and gumminess were higher for SC than other samples. Therefore, PLP can be an alternate source of soy since consumers scored a similar liking of the samples containing the TPP, especially PI and PP, as SC. In addition, cooking yield and cooking time were similar to each other for meat patties containing PLP except LP for cooking yield and PI for cooking time. Even though the TPA gave lower textural properties for meat patties containing TPP, these proteins might provide a unique combination of attributes and attract the consumers.

## CHAPTER V

### HIGH MOISTURE MEAT ANALOGS (HMMA) WITH PULSE PROTEINS: PEAS, LENTILS, AND FABA BEANS

#### 5.1 Introduction

High moisture meat analog (HMMA) is a meat-like product produced by a high moisture extrusion that has an additional cooling die at the end of the extruder. The cooling die prevents expansion of the product, and reduces viscous dissipation of energy during gelation and restructuring of protein and fat emulsification (Cheftel and others 1992). This process is capable of producing a wide range of cooked foods with a highly fibrous texture simulating meat, poultry, or fish muscle (Roussel 1996). Most of the research on HMMAs was limited to focusing on soy or wheat-based products. These studies did not discuss proper handling methods for HMMA products after production such as cooling before freezing for storage and thawing and rehydration after freezing.

In this research, each pea protein (PP), lentil protein (LP), and faba bean protein (FP) was premixed with pea isolate (PI) and constant ingredients (canola oil and wheat gluten) and texturized to produce HMMA using a Wenger twin-screw extruder (TX-52). Soy concentrate (SC) and pulse proteins were premixed with constant ingredients, and the recipe was used as a control. Before freezing for storage, each HMMA was cooled by one of four different methods in the media: air, water, or brine solutions (2% and 4%). Frozen samples were thawed at room temperature for three hours and rehydrated by one of three methods: normal rehydration, blanching, or boiling.

The objectives of this research were to produce HMMA made from SC, PP, LP, and FP and evaluate the different characteristics of these samples and the effects of

different cooling and rehydration methods. I hypothesized that the HMMAs made from these SC and pulse proteins would not have different characteristics (color, moisture content, density, and texture), and the quality of the HMMAs would not be significantly influenced by the cooling treatments and rehydration methods.

## 5.2 Materials and Methods

### 5.2.1 Materials

Vitessence™ Pulse 1550 (PP), Vitessence™ Pulse 2550 (LP), and Vitessence™ Pulse 3600 (FP) were obtained from cooperating companies, Ingredion Incorporated (Westchester, IL) and Alliance Grain Traders (AGT) Food and Ingredients (Regina, Canada). Arcon® F (SC) and Pro-Fam® 974 (soy isolate) was obtained from Archer Daniels Midland (ADM Decatur, IL), and Nutralys ®-S85F (PI) and Provim Esp® (wheat gluten) were obtained from Roquett America, Inc. (Keokuk, IA). In addition, Crisco pure canola oil (JM Smucker Co., Orrville, OH) was purchased from a local grocery store (College Station, TX).

**Table 22. Premixed recipes to produce HMAA.**

Ingredients, %	C1	T1	T2	T3
Soy concentrate	69	0	0	0
Soy isolate	10	0	0	0
Pea isolate	0	63	63	59
Pea protein	0	16	0	0
Lentil protein	0	0	16	0
Faba bean protein	0	0	0	21
Wheat gluten	15	15	15	15
Canola oil	6	6	6	6

With a constant ingredient (canola oil and wheat gluten), soy concentrate (C1) premixed with soy concentrates (SI) and each of pea proteins (PP), lentil proteins (LP), and faba bean proteins (FP) premixed with pea isolates (PI) before texturization and denoted C1, T1, T2, and T3, respectively.



The recipes are identified as C1 (control), T1, T2, and T3 in Table 22, which also includes the recipes. Three recipes (T1, T2, and T3) with pea isolate (PI) were premixed with the constant ingredients (5% canola oil and 15% wheat gluten) for the HMMA. C1 mixed with soy isolate (SI) and constant ingredients (5% canola oil and 15% wheat gluten) were used as a control. Each recipe had approximately 73.4% protein. The recipes were homogenized in an industrial mixer (G0028, Engineered Systems and Equipment, Inc., Caney, KS).

### 5.2.2 Texturization

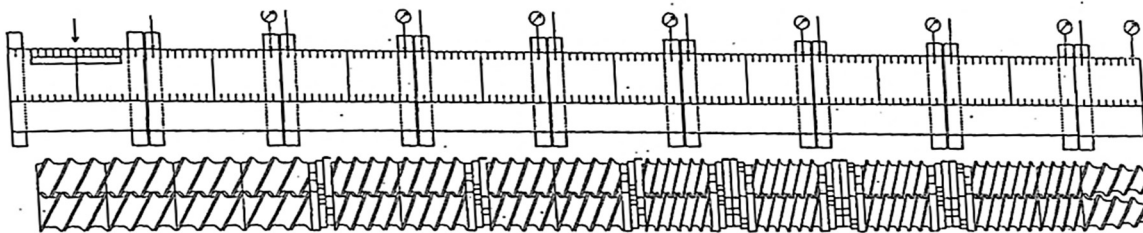


Figure 20. Shear configuration with a twin-screw extruder (TX-52, Wenger) to produce high moisture meat analogs.

Figure 20 shows the screw configuration used in this research. Each premixed recipe was fed into the feeder and extruded using a Wenger twin-screw extruder (TX-52). The optimized processing conditions provided by Wenger Manufacturing Inc. (Sabetha, KS) including feed rate, preconditioning, running temperatures, steam, and extruder rpms for recipes made with SC were used.

**Table 23. Processing Conditions for Texturization to Produce High Moisture Meat Analogs (HMMA).**

Process	Processing	Values
Feeding	Speed (rpm,)	9
	Speed (rpm)	377
Preconditioning	Water flow (kg/hr)	24
	Screw speed (rpm)	403
Extrusion	Motor load (%)	32
	Steam flow (kg/hr)	15
	Water flow (kg/hr)	10
	Temperature zone 1 (set) °C	50
	Temperature zone 2 (set) °C	70
	Temperature zone 3 (set) °C	85
	Temperature zone 4 (set) °C	85
	Temperature zone 5 (set) °C	95
Temperature zone 6 (set) °C	95	
Cooling	Cooling head 1 pressure (psi)	90
	Cooling head 2 pressure (psi)	250

The setting parameters during the texturization were kept constant for the experiment as seen in Table 23. Fresh HMMA was cooled in two segmented dies. The first segment (15.24 cm wide, 2.86 cm high, and 60.96 cm long) did not have a water circulation system, but the second segment (15.24 cm wide, 2.54 cm high, and 60.96 cm long) had a water circulation system for cooling. As the product came out of the opening (7 cm wide, 2 cm high, rectangular with curves in the corners) of the cooling die, they were cut with a knife. Each HMMA was further cooled for 10 min with one of four different extra cooling methods: at room temperature with a non-cooling (NC), in water (Water), in 2% brine solution (2B), and in 4% brine solution (4B), and stored in Ziploc bags in the freezer (-18°C) for further analysis.

### **5.2.3 Rehydration**

Frozen samples stored in Ziploc bags were thawed at room temperature for 3 hours (control) and resized into about 12 mm cubes using a stainless-steel handheld cutter (Internetbest.com). About 25 g of each thawed sample was prepared for three different rehydration treatments (soaking, blanching, and boiling). In the first treatment, each thawed sample was rehydrated in distilled water for 2 hours. Following the modified method of Lin and others (2002), each thawed sample was blanched in distilled water at 50°C for 12 hours for the second treatment. For the third treatment, each thawed sample was soaked in boiling water for 2 minutes (Lin and others 2002). These samples were drained on a 20-mesh screen for 3 min. The samples, after the water was drained, were used to measure the water absorbing capacity, and the drained water was collected to measure the cooking loss.

### **5.2.4 Color**

These samples extrudates were directly evaluated using a colorimeter (Model CR-310, Minolta, Osaka, Japan). Values were expressed as L\*, a\* and b\*, where L\* values (lightness) vary from black (0) to white (100), chroma a\* values (redness) vary from green (-60) to red (+60), and chroma b\* values (yellowness) vary from blue (-60) to yellow (+60).

### **5.2.5 Water Absorbing Capability (WAC) and Water Solubility Index (WSI)**

The WAC was measured using a modified method.(Lin and others 2002). The WAC was recorded as gram of water retained per gram of dried sample and calculated using Equation 5.1.

$$WAI = \frac{\textit{Weight of absorbed water into HMMA}}{\textit{Weight of HMMA}} \times 100 \quad \text{Equation 5.1}$$

After rehydration, the drained solution was collected, stirred for 5 min, and sampled in a test tube. The solution was homogenized using a vortex (G560, Scientific Industries, Inc., Bohemia, NY). About 10 g of the sample from the test tube was dried in an oven for 24 hours at 105°C (AOAC 1990) . The weight of the purged sample was calculated using the weight difference between the collected solution and the sampled solution from the test tube. The WSI was calculated by the interpolation of the ratio using Equation 5.2

$$WSI = \frac{\textit{Weight loss during rehydration}}{\textit{Weught of HMMA}} \times 100 \quad \text{Equation 5.2}$$

The WAI and WSI were measured twice.

### **5.2.6 Moisture**

The moisture content was determined by the AOAC (1990) method.

### **5.2.7 Density**

Fourteen cube-sized (12 mm) samples for each treatment was prepared to measure the density of the samples. The volume of the samples (12 mm cubes) was estimated to be  $1.728 \times 10^{-6}$  L. The weight of the samples for each treatment was recorded for density. Density was calculated by the estimated volume of the samples divided by the weight of the samples.

### **5.2.8 Texture Analysis**

A TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) was used for texture analysis using the texture profile analysis measurement. The samples were placed on a square aluminum plate. A cylindrical probe (40 mm in diameter) compressed the samples to 50% of their initial thickness at 1mm/s of the cross-head speed. Three attributes, hardness, cohesiveness, and gumminess were recorded. Five samples for each treatment were collected and used in the analysis.

### **5.2.9 Statistical Analysis**

Data based on each protein type (C1, T1, T2, and T3) for each parameter were prepared for the statistical analysis of the data. A Tukey's HSC (honestly significant difference) analysis was also conducted for pair comparison. All statistical tests were performed using JMP software (JMP Pro 12.0.1, SAS Institute Inc., Cary, N.C., USA).

Results and Discussion

## **5.3 Results and Discussion**

### **5.3.1 Texturization to Produce High Moisture Extrusion**

After discharging HMMA from the cooling die, blister formations on the surface of most samples were observed, and the blisters disappeared as the vapor pressure and temperature on the surface dropped. As soon as the products came out of the cooling die, the products had an extra cooling in a media which were water, or 2% or 4% brine solutions (Water, 2B, or 4B, respectively). Initially, all products (C1, T1, T2, and T3) soaked in the 4% brine solution and the C1 soaked in the 2% brine solution floated in

the middle of the cooling media. As they cooled, and the products absorbed the solution in the media, the density of the product increased, and finally the floating products sank.

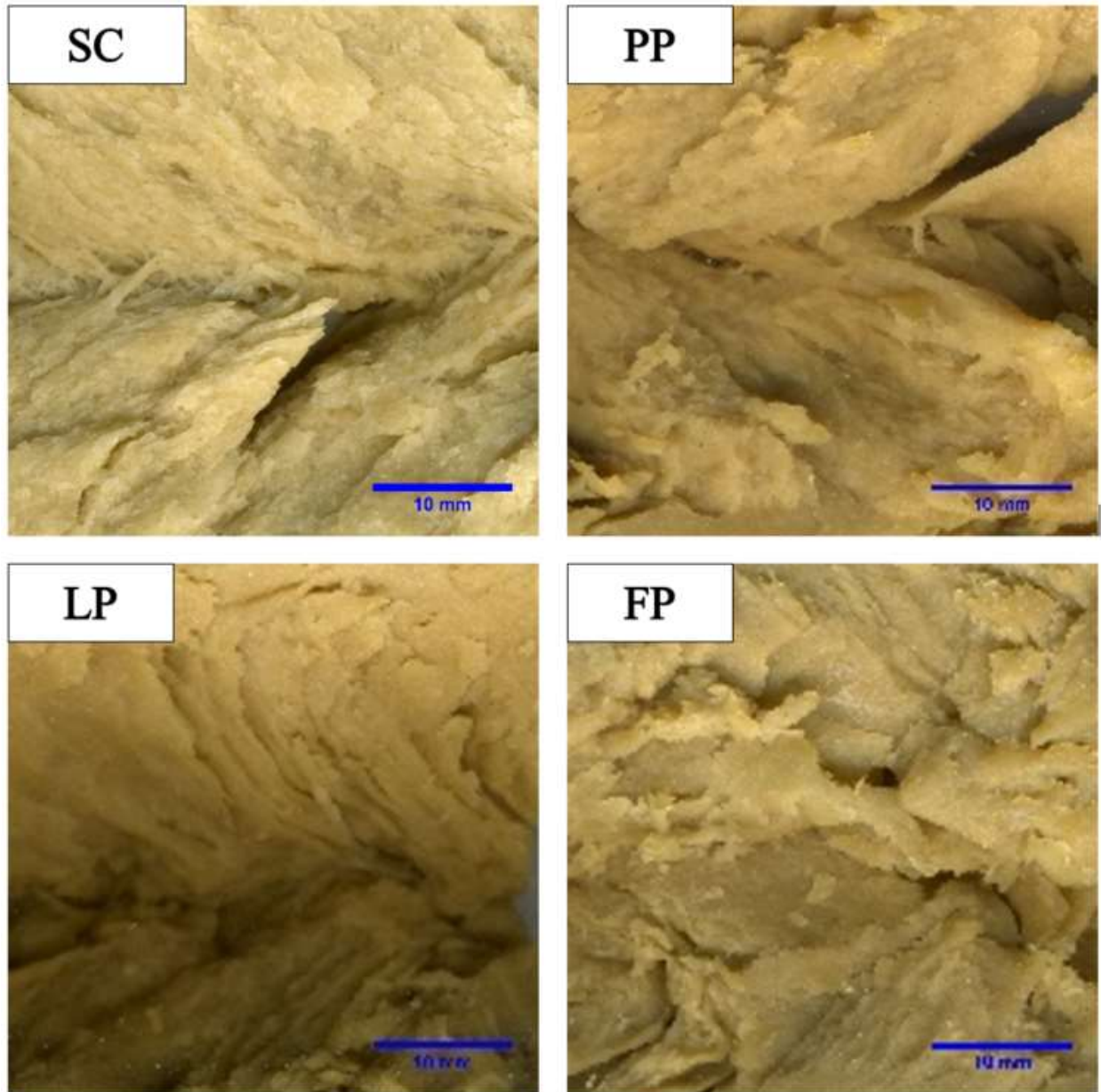


Figure 21. Images of a high moisture meat analog (HMMA) using a twin-screw extruder (TX-52). With a constant ingredient (canola oil and wheat gluten), soy concentrate (SC) premixed with soy concentrates (SI) and each of pea proteins (PP), lentil proteins (LP), and faba bean proteins (FP) premixed with pea isolates (PI) before texturization and denoted C1, T1, T2, and T3, respectively.

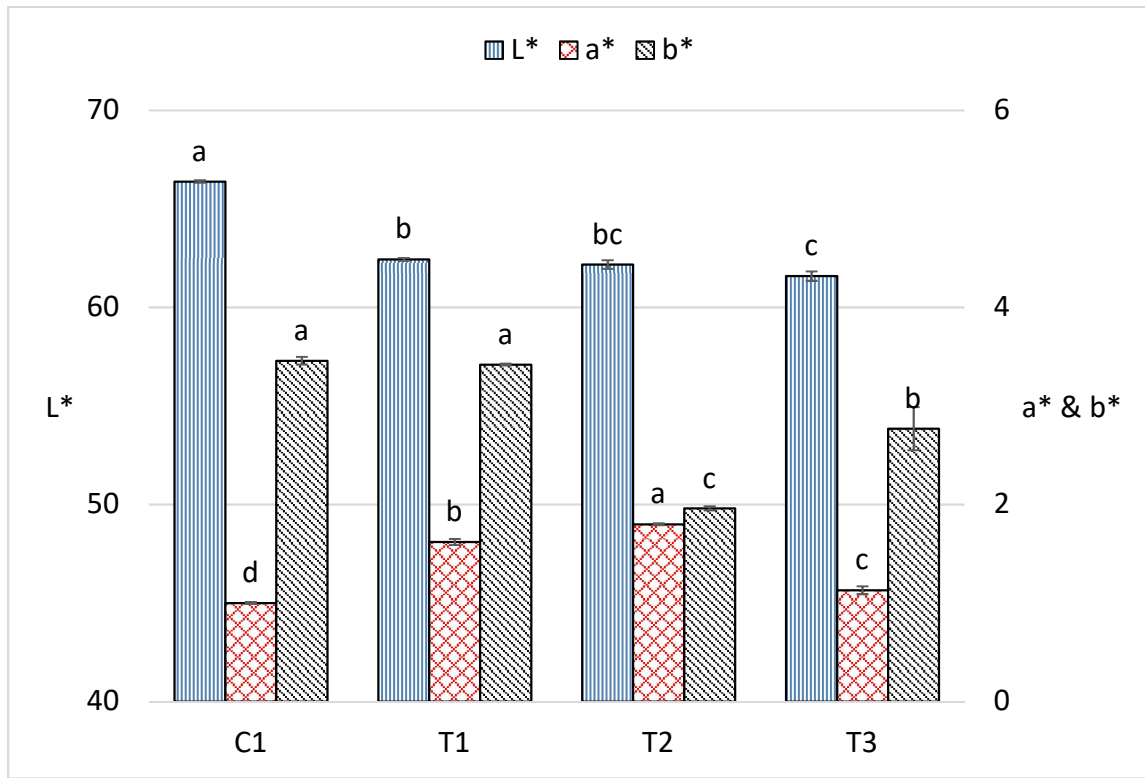
For visual images, each frozen HMMA was cooled in a 2% brine solution, thawed for 3 hours in a Ziploc bag, and was rehydrated in water for 2 hours. The samples were dissected and peeled. Figure 21 shows the images of the products. Visual examination is subjective; however, it is an important method for the product developers since they can make an immediate judgement of the product as soon as it comes out. C1 showed a well-defined fiber orientation. The other samples did not have as defined a fiber orientation as C1 but did have an acceptably-defined fiber orientation.

### ***5.3.2 Effect of Protein Type with Different Pulse Proteins on a High Moisture***

#### ***Meat Analog***

HMMA were cooled in the water, stored in the freezer (-40°C), thawed for 3 hours in a plastic bag at room temperature, and rehydrated for 2 hours at room temperature to find the effects of protein type with different pulse proteins on HMMA.

### 5.3.2.1 Color



**Figure 22. Parameters of color ( $L^*$ ,  $a^*$ , and  $b^*$ ) from HMMA with a combination of cooling (water) and rehydration (soaking) methods for HMMA cooled in water, stored in a freezer, thawed for 3 hours in a plastic bag at room temperature, and rehydrated for 2 hours. Recipes for texturization, C1 (control): soy concentrate and soy isolate, T1, T2, and T3: pea proteins, lentil proteins, and faba bean proteins premixed with a constant ingredient (canola oil and wheat gluten).**

Figure 22 illustrates the parameters of color from HMMA with a combination of cooling (water) and rehydration (soaking) methods. Compared to the control, C1, each treatment had significantly different parameters ( $P < 0.05$ ) in ( $L^*$ ,  $a^*$ , and  $b^*$ ), except T1,



The protein source in meat patties containing TPP significantly ( $P = <0.0001$ ) affected all textural parameters as well. Hardness was the highest for SC compared to other samples, followed by PI and FP that were not significantly different. The hardness of LP was not significantly different from PP and lower than for other samples. provided the same lightness, and the colors (redness and yellowness) of each TPP varied based on the second major ingredients (PP, LP, and FP).

### 5.3.2.2 M.C. and Density Water Holding Capacity and Water Solubility Index

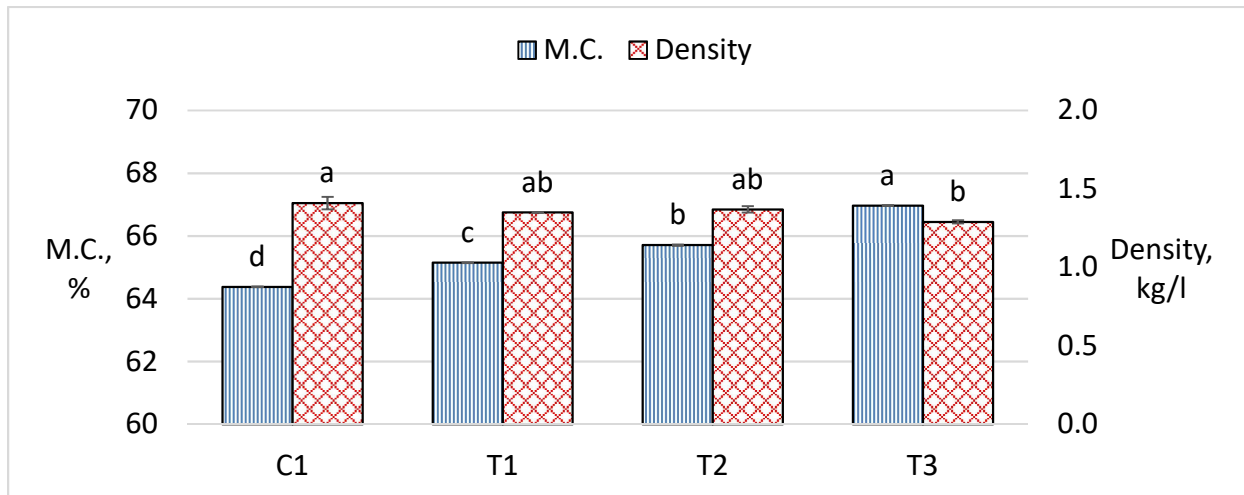


Figure 23. Moisture content and density from HMMA with a combination of cooling (water) and rehydration (soaking) methods for HMMA cooled in water, stored in a freezer, thawed for 3 hours in a plastic bag at room temperature, and rehydrated for 2 hours. Recipes for texturization, C1 (control): soy concentrate and soy isolate, T1, T2, and T3: pea proteins, lentil proteins, and faba bean proteins premixed with a constant ingredient (canola oil and wheat gluten).

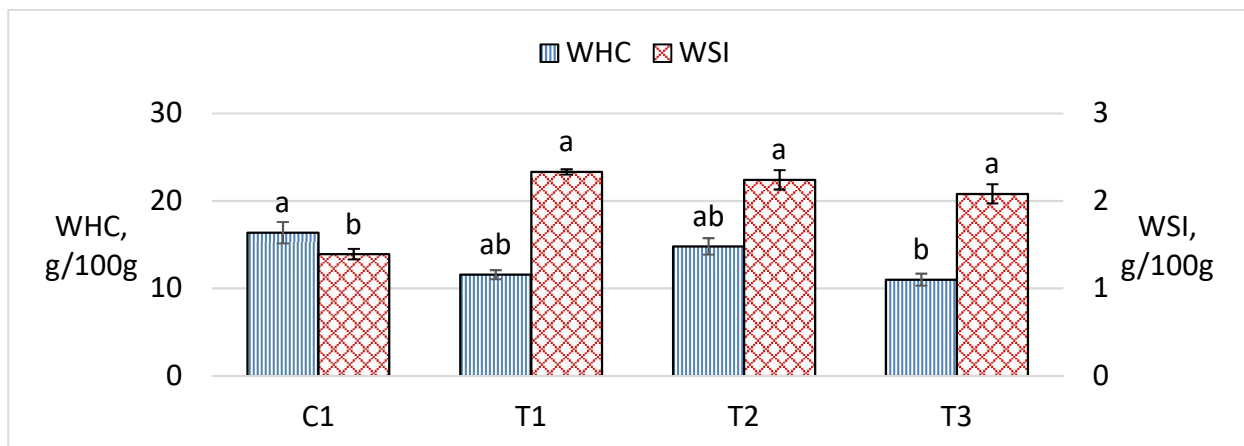


Figure 24. Water holding capacity and water solubility index from HMMA with a combination of cooling (water) and rehydration (soaking) methods for HMMA cooled in water, stored in a freezer, thawed for 3 hours in a plastic bag at room temperature, and rehydrated for 2 hours. Recipes for texturization, C1 (control): soy concentrate and soy isolate, T1, T2, and T3: pea proteins, lentil proteins, and faba bean proteins premixed with a constant ingredient (canola oil and wheat gluten).

All samples had different moisture content, and C1 had the lowest following T1, T2, T3, respectively while all samples had a similar density except T3 that has a significantly lower density than C1 (Figure 23). In other word, T3 following T2, T1, and C1 in order had the highest water absorption from texturization to final products, and they were saturated and resulted in the similar density except C1 and T3 that were significantly different ( $P<0.05$ ). In addition, T3 had lower WHC than C1 although T3 had higher M.C. than C1 due to higher moisture content before soaking in water for rehydration. Therefore, C1 imparted the firmest texture, and T3 provided relatively floppy texture compared to C1. All samples had a similar WAI except T3 that was like T1 and T2 and lower that C1. In Figure 23 and Figure 24, density and WAI shows the same pattern, and it explains C1 absorbed more water than T3 until maximum amount of water taken up. All samples including pulses had significantly lower WSI than C1, but they had a similar WSI each other (Figure 24). During rehydration, C1 released more substances into water than other samples.

### 5.3.2.3 Texture

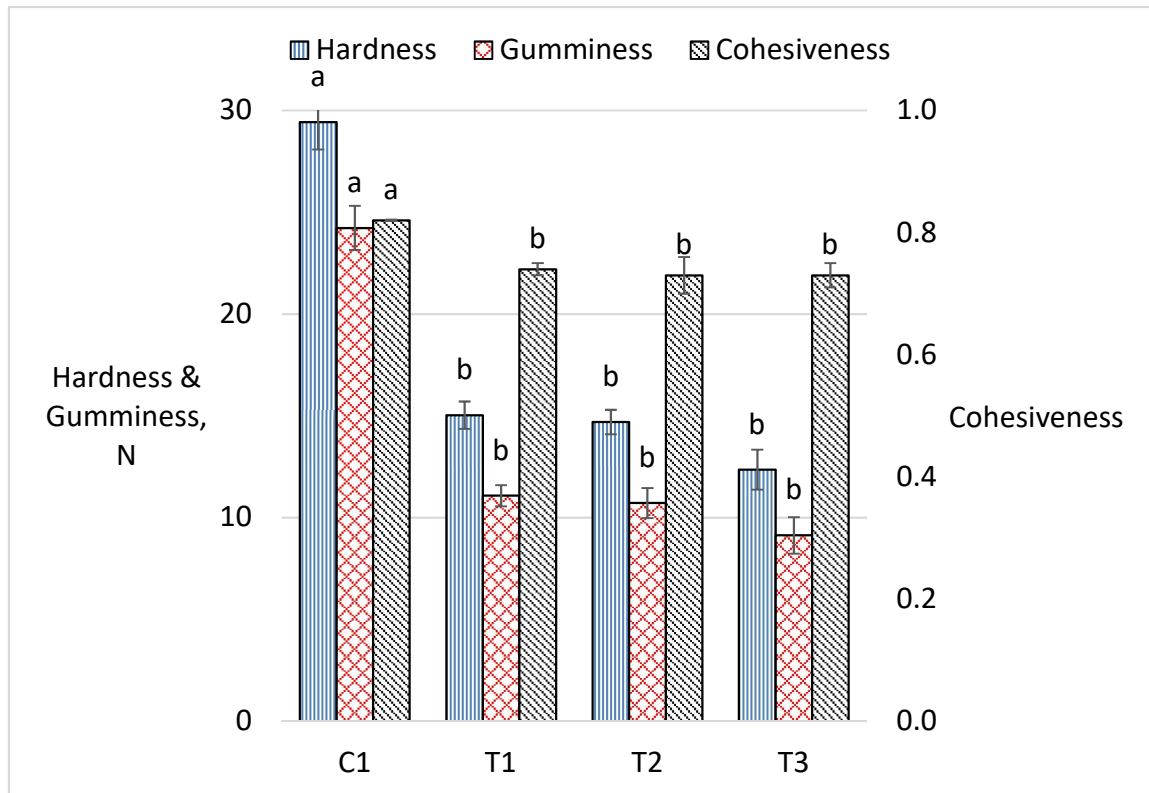


Figure 25. Texture (hardness, gumminess, and cohesiveness) from HMMA with a combination of cooling (water) and rehydration (soaking) methods for HMMA cooled in water, stored in a freezer, thawed for 3 hours in a plastic bag at room temperature, and rehydrated for 2 hours. Recipes for texturization, C1 (control): soy concentrate and soy isolate, T1, T2, and T3: pea proteins, lentil proteins, and faba bean proteins premixed with a constant ingredient (canola oil and wheat gluten).

C1 had higher textural values (hardness, gumminess, and cohesiveness) than other samples including pulse proteins that had a similar texture to each other (Figure 25). As mention above, C1 gave a firmer texture than other samples, which is accordance with the pattern of hardness. Therefore, HMMA with PLP required less force to break it than did C1 corresponding to 49% - 58%. Cohesiveness of the samples had the same pattern as hardness and gumminess. In other words, C1 provided more water binding capacity resulting in a higher cohesiveness of C1. However, the rate of reduction of cohesiveness was much less than hardness, and HMMA with PLP had less

cohesiveness than C1 corresponding to 10% - 11%. The gumminess of the samples was similar to the hardness. Gumminess refers to semisolid materials and organoleptically indicates a denseness that persists throughout mastication (Szczesniak and others 1963). In addition, gumminess defines the energy required to disintegrate the sample to a state ready for swallowing (Dubost and others 2003). Therefore, C1 was deformed more than other samples including PLP rather than sheared during measurement.

### **5.3.3 Effects of Different Media during Cooling with Different Pulse Proteins on HMMA**

HMMA were cooled in different media (Water, 2B, and 4B), stored in a freezer (-40°C), thawed for 3 hours in a Ziploc bag at room temperature, and rehydrated for 2 hours at room temperature to find the effect of media on HMMA during cooling with different pulse proteins.

**Table 24. Properties of HMMA cooled in different media with different pulse proteins.**

Cooling	L*	a*	b*	M.C., %	Density, kg/l	WAI, g/100g	WSI, g/100g	Hardness, N	Cohesiveness	Gumminess, N
C1										
NC	66.47±0.03a	1.13±0.01a	3.78±0.04a	62.10±0.05c	1.40±0.01a	22.84±0.1a	1.43±0.06a	34.79±1.43a	0.82±0a	28.51±1.24a
Water	66.39±0.06a	1.00±0.01ab	3.46±0.04a	64.38±0.01b	1.41±0.04a	16.36±1.23bc	1.39±0.06a	29.42±1.34b	0.82±0a	24.23±1.09ab
2B	66.42±0.16a	1.03±0.01ab	3.51±0.1a	64.74±0.00a	1.43±0.03a	18.95±0.17b	1.47±0.05a	28.17±0.80b	0.83±0a	23.28±0.76b
4B	66.45±0.22a	0.95±0.05b	3.49±0.09a	64.30±0.01b	1.37±0.01a	13.82±0.19c	1.53±0.06a	29.26±1.42b	0.82±0a	23.92±1.23b
T1										
NC	62.48±0.13a	1.72±0.00a	3.12±0.14a	60.15±0.08c	1.30±0.01a	10.73±0.49c	1.91±0.03b	17.99±1.05a	0.75±0.01a	13.46±0.79a
Water	62.44±0.07a	1.62±0.03ab	3.42±0.01a	65.15±0.01b	1.35±0.00a	11.56±0.52bc	2.33±0.03a	15.03±0.67ab	0.74±0.01a	11.07±0.52ab
2B	62.41±0.24a	1.52±0.03c	3.38±0.08a	65.21±0.01b	1.37±0.03a	12.97±0.19ab	1.93±0.11b	16.76±0.71a	0.75±0.01a	12.64±0.58a
4B	61.76±0.02b	1.58±0.02bc	3.36±0.03a	67.40±0.03a	1.36±0.01a	14.09±0.14a	2.06±0.06ab	12.57±0.79b	0.73±0.02a	9.25±0.78b
T2										
NC	60.83±0.07b	1.6±0.06b	2.98±0.16a	61.95±0.01d	1.34±0.01b	14.29±0.07a	1.64±0.06b	21.03±0.64a	0.75±0.01a	15.87±0.42a
Water	62.18±0.22a	1.80±0.01a	1.96±0.02b	65.71±0.02c	1.37±0.02ab	14.80±0.94a	2.24±0.11a	14.69±0.6b	0.73±0.03a	10.71±0.74b
2B	61.23±0.16b	1.22±0.05c	3.05±0.1a	66.64±0.02b	1.40±0.01a	16.89±1.07a	1.95±0.07ab	15.30±0.86b	0.76±0.01a	11.66±0.58b
4B	60.76±0.09b	1.40±0.00c	2.97±0.14a	66.88±0.01a	1.37±0.01ab	18.19±0.21a	2.10±0.04a	12.96±1.25b	0.75±0.02a	9.80±1.12b
T3										
NC	60.24±0.12b	1.33±0.04a	2.67±0.17a	61.53±0.03a	1.33±0.02ab	14.21±0.79ab	1.63±0.07b	25.24±1.37a	0.78±0.01a	19.62±1.13a
Water	61.59±0.24a	1.13±0.04b	2.77±0.22a	66.97±0.01a	1.29±0.01b	10.99±0.69b	2.08±0.11a	12.35±0.98b	0.73±0.02a	9.12±0.90b
2B	59.81±0.4b	1.12±0.06b	2.69±0.17a	63.79±2.39a	1.37±0.01a	15.59±0.20a	1.98±0.08ab	14.54±0.65b	0.73±0.01a	10.59±0.59b
4B	59.50±0.10b	1.12±0.02b	2.56±0.06a	66.62±0.03a	1.36±0.01a	12.53±0.5ab	2.01±0.04ab	12.47±0.82b	0.73±0.02a	9.21±0.81b

<sup>a</sup> Means with different letters of the same style are significantly different (p<0.05).

<sup>b</sup> Recipes for texturization, C1 (control): soy concentrate and soy isolate, T1, T2, and T3: pea proteins, lentil proteins, and faba bean proteins premixed with a constant ingredient (canola oil and wheat gluten).

<sup>c</sup> NC=cooling in air, Water=cooling in water, 2B=cooling in 2% brine solution, and 4B=cooling in 4% brine solution.

Table 24 shows the properties of HMMA cooled in different media with different pulse proteins. Compared to the control, NC, the cooling media did not significantly change yellowness, density, and cohesiveness on all samples (C1, T1, T2, and T3) except for a lower value of yellowness with water on T2 and a greater density with 2B on T2. 4B decreased lightness on T1, and water increased lightness on T2 and T3. The brine solutions decreased redness on all samples.

The cooling media did not significantly affect the WSI of the control, C1, while cooling in water significantly increased the WSI of other samples (T1, T2, and T3) except cooling in 4% brine solution on T2 that had a greater value for WSI than any other except the treatment cooled in water. Due to building an ionic bridge using sodium chloride in the brine solution to hold the protein structure between proteins in HMMA, the particles from HMMA did not dissolve much in water. Commercial soy proteins (SC and SI) used in C1 were neutralized (around pH=7.2) and had higher pH values (pH=6.5-6.7) than pulse proteins (PP, LP, and FP) used in T1, T2, and T3, respectively. Their higher pH value imparted higher protein solubility with mechanical and thermal energy driven by the screw in the extruder, had a better alignment of muscle-like protein structure by crosslinking, and had a strong structure. Therefore, C1 had a limited WSI with variations of cooling media. In contrast, other samples (T1, T2, and T3) with lower pH might not give a strong bonding between protein structures. There might be a competition between the ions in the water from sodium chloride and the ions in the protein structure in HMMA. Therefore, 2B as cooling media and modification of the recipe using additives for texturization is recommended.



Based on the cooling media compared to NC, C1 and T2 had a significantly different WAI but T2 and T3 did not have significantly different WAI. All cooling treatment increased M.C., and the brine solutions greatly increased the moisture content of C1, T1, and T2 while T3 did not have significantly different values from NC.

As expected, all samples had lower values of hardness and gumminess with cooling treatments compared to NC. 2B on samples including pulse proteins had the lowest degree of decrease in hardness and gumminess from NC resulting in the highest values in hardness and gumminess after NC.

#### ***5.3.4 Effects of Different Rehydration Methods with Different Pulse Proteins on***

##### ***HMMA***

HMMA were cooled in water, stored in a freezer (-40°C), thawed for 3 hours in a plastic bag at room temperature, and rehydrated (soaking, blanching, and boiling) to find the effects of rehydration methods on HMMA with different pulse proteins.

**Table 25. Properties of HMMA rehydration with different methods with different pulse proteins.**

Rehydration	L*	a*	b*	M.C., %	Density, kg/l	WAI, g/100g	WSI, g/100g	Hardness, N	Cohesiveness	Gumminess, N
C1										
NR	66.66±0.14 <sup>b</sup>	1.15±0.01 <sup>a</sup>	2.77±0.03 <sup>b</sup>	59.03±0.00 <sup>d</sup>	1.21±0.02 <sup>c</sup>	N/A	N/A	27.79±1.50 <sup>a</sup>	0.80±0.02 <sup>a</sup>	22.16±1.32 <sup>a</sup>
Soaking	66.39±0.06 <sup>b</sup>	1.00±0.01 <sup>b</sup>	3.46±0.04 <sup>a</sup>	64.38±0.01 <sup>b</sup>	1.41±0.04 <sup>b</sup>	16.36±1.23 <sup>b</sup>	1.39±0.06 <sup>a,b</sup>	29.42±1.34 <sup>a</sup>	0.82±0.00 <sup>a</sup>	24.23±1.09 <sup>a</sup>
Blanching	67.53±0.20 <sup>a</sup>	0.85±0.02 <sup>c</sup>	3.23±0.04 <sup>a</sup>	69.17±0.03 <sup>a</sup>	1.56±0.02 <sup>a</sup>	27.92±0.15 <sup>a</sup>	2.67±0.39 <sup>a</sup>	20.06±1.46 <sup>b</sup>	0.82±0.00 <sup>a</sup>	16.36±1.16 <sup>b</sup>
Boiling	66.65±0.22 <sup>b</sup>	1.12±0.04 <sup>a</sup>	2.42±0.09 <sup>c</sup>	63.08±0.06 <sup>c</sup>	1.24±0.01 <sup>c</sup>	10.85±0.57 <sup>c</sup>	0.70±0.01 <sup>b</sup>	23.91±2.4 <sup>ab</sup>	0.79±0.00 <sup>a</sup>	19.00±1.93 <sup>ab</sup>
T1										
NR	65.47±0.05 <sup>B</sup>	1.73±0.03 <sup>A</sup>	2.77±0.12 <sup>B</sup>	59.59±0.03 <sup>C</sup>	1.21±0.01 <sup>C</sup>	N/A	N/A	15.43±1.12 <sup>A</sup>	0.74±0.01 <sup>A</sup>	11.45±0.97 <sup>A</sup>
Soaking	62.44±0.07 <sup>D</sup>	1.62±0.03 <sup>A</sup>	3.42±0.01 <sup>A</sup>	65.15±0.01 <sup>B</sup>	1.35±0.00 <sup>B</sup>	11.56±0.52 <sup>B</sup>	2.33±0.03 <sup>B</sup>	15.03±0.67 <sup>A</sup>	0.74±0.01 <sup>A</sup>	11.07±0.52 <sup>A</sup>
Blanching	63.10±0.22 <sup>C</sup>	1.44±0.02 <sup>B</sup>	3.41±0.19 <sup>A</sup>	69.93±0.00 <sup>A</sup>	1.48±0.01 <sup>A</sup>	21.34±1.60 <sup>A</sup>	4.48±0.29 <sup>A</sup>	11.56±0.65 <sup>A</sup>	0.71±0.01 <sup>A</sup>	8.21±0.51 <sup>A</sup>
Boiling	66.49±0.14 <sup>A</sup>	1.66±0.03 <sup>A</sup>	2.77±0.05 <sup>B</sup>	59.79±0.09 <sup>C</sup>	1.08±0.03 <sup>D</sup>	-0.36±0.17 <sup>C</sup>	1.10±0.04 <sup>C</sup>	14.01±1.29 <sup>A</sup>	0.71±0.02 <sup>A</sup>	10.01±1.11 <sup>A</sup>
T2										
NR	65.34±0.13 <sup>a</sup>	1.50±0.04 <sup>b</sup>	2.62±0.16 <sup>b</sup>	59.38±0.02 <sup>d</sup>	1.19±0.00 <sup>b</sup>	N/A	N/A	13.91±1.17 <sup>ab</sup>	0.77±0.01 <sup>a</sup>	10.72±0.99 <sup>a</sup>
Soaking	62.18±0.22 <sup>b</sup>	1.80±0.01 <sup>a</sup>	1.96±0.02 <sup>c</sup>	65.71±0.02 <sup>b</sup>	1.37±0.02 <sup>a</sup>	14.8±0.94 <sup>a</sup>	2.24±0.11 <sup>b</sup>	14.69±0.60 <sup>a</sup>	0.73±0.03 <sup>a</sup>	10.71±0.74 <sup>a</sup>
Blanching	61.83±0.17 <sup>b</sup>	1.26±0.03 <sup>c</sup>	3.20±0.14 <sup>a</sup>	68.93±0.04 <sup>a</sup>	1.45±0.05 <sup>a</sup>	18.83±3.45 <sup>a</sup>	4.38±0.28 <sup>a</sup>	11.18±0.65 <sup>b</sup>	0.69±0.02 <sup>a</sup>	7.67±0.42 <sup>a</sup>
Boiling	66.05±0.14 <sup>a</sup>	1.53±0.04 <sup>b</sup>	2.40±0.05 <sup>bc</sup>	60.06±0.06 <sup>c</sup>	1.14±0.03 <sup>b</sup>	1.77±0.62 <sup>b</sup>	1.24±0.03 <sup>b</sup>	12.70±0.71 <sup>ab</sup>	0.71±0.03 <sup>a</sup>	9.05±0.77 <sup>a</sup>
T3										
NR	65.15±0.10 <sup>B</sup>	1.19±0.03 <sup>AB</sup>	2.12±0.07 <sup>AB</sup>	62.04±0.01 <sup>D</sup>	1.17±0.01 <sup>C</sup>	N/A	N/A	15.31±0.77 <sup>A</sup>	0.75±0.01 <sup>A</sup>	11.56±0.7 <sup>A</sup>
Soaking	61.59±0.24 <sup>C</sup>	1.13±0.04 <sup>AB</sup>	2.77±0.22 <sup>A</sup>	66.97±0.01 <sup>B</sup>	1.29±0.01 <sup>B</sup>	10.99±0.69 <sup>B</sup>	2.08±0.11 <sup>B</sup>	12.35±0.98 <sup>AB</sup>	0.73±0.02 <sup>A</sup>	9.12±0.90 <sup>AB</sup>
Blanching	60.22±0.18 <sup>D</sup>	1.05±0.04 <sup>B</sup>	2.72±0.18 <sup>AB</sup>	71.46±0.00 <sup>A</sup>	1.41±0.01 <sup>A</sup>	19.84±0.44 <sup>A</sup>	4.47±0.13 <sup>A</sup>	9.65±0.81 <sup>B</sup>	0.70±0.01 <sup>A</sup>	6.77±0.64 <sup>B</sup>
Boiling	66.12±0.06 <sup>A</sup>	1.23±0.01 <sup>A</sup>	2.09±0.03 <sup>B</sup>	62.99±0.04 <sup>C</sup>	1.06±0.03 <sup>D</sup>	1.58±0.33 <sup>C</sup>	1.22±0.02 <sup>C</sup>	10.53±1.05 <sup>B</sup>	0.70±0.02 <sup>A</sup>	7.45±0.95 <sup>B</sup>

<sup>a</sup> Means with different letters of the same style are significantly different (p<0.05).

<sup>b</sup> Recipes for texturization, C1 (control): soy concentrate and soy isolate, T1, T2, and T3: pea proteins, lentil proteins, and faba bean proteins premixed with a constant ingredient (canola oil and wheat gluten).

<sup>c</sup> NR=non-rehydration, Soaking=soaked in water for 2 hours, Blanching=blanched in distilled water at 50°C for 12 hours, and boiling= boiled in water for 2 minutes.

Soaking and blanching decreased lightness and boiling increased lightness for the samples containing pulse proteins while this rehydration did not influence the lightness, except for blanching. Blanching decreased redness in C1, T1, and T2. Blanching increased yellowness in C1, T1, and T2, and soaking increased yellowness in C1 and T2 and decreased yellowness in T2. Major ingredients of C1 and among T1, T2, and T3 are soy concentrate (lemon color), and pea isolate (a more orange color), respectively (Table 25). The strongest determinate of the color of products against rehydration methods is the color of the ingredients. Therefore, it is recommended to select major ingredients that fit the color of the final products.

Compared to the control, NR, the rehydration method did not significantly change cohesiveness on every sample. Texture (hardness, cohesiveness, and gumminess) of T1 and T2 were not affected by rehydration methods. Blanching significantly decreased the hardness and gumminess on C1 and T3 and boiling decreased the hardness and gumminess on T3 as much as did boiling.

As expected, all rehydration treatments increased M.C., density, WAI and WSI for every sample. Blanching significantly increased M.C, density, WAI, and WSI for every sample. Compared to soaking for density, WAI, and WSI, boiling decreased density (12%, 20%, 17%, and 18%), WAI (34%, 103%, 88%, and 86%) and WSI (50%, 53%, 45%, and 41%) on C1, T1, T2, and T3, respectively. In other words, compared to soaking, blanching caused more absorption of more water resulting in increasing M.C., density, WAI, and WSI. In contrast, boiling inhibited absorption of water into HMMA at room temperature and resulted in decreasing M.C., density, WAI, and WSI.

### **5.3.5 Effects of Combined Cooling and Rehydration Methods with Different Pulse Proteins on HMMA**

The combined effect of cooling and rehydration methods on proteins was also investigated. Each sample had different values from the combined effect of cooling and rehydration methods, and it was difficult to find a trend due to various competing factors. Therefore, for understanding these effect, the data analysis can be found in the APPENDIX B.

## **5.4 Conclusion**

Soy based HMMA (C1) had the best-defined fiber orientation, and other recipes had relatively well-defined fiber orientation. Samples with PLP were texturized well and had low texture compared to C1. For a better quality HMMA with the other recipes, a high-shear screw configuration for the extruder and inclusion of additives are recommended. A 2% brine solution for cooling after production of HMMA was recommended for higher WAI and less WSI. For better quality in terms of WAI, WSI, and texture, blanching, boiling, and soaking, respectively, for rehydration of HMMA was recommended before producing the final product.

## CHAPTER VI

### VEGETABLE HAMBURGER PATTIES WITH HIGH MOISTURE MEAT ANALOGS (HMMA) USING PULSE PROTEINS: PEAS, LENTILS, AND FABA BEAN PROTEINS

#### 6.1 Introduction

For the trained panelist tests and consumer tests of vegetable patties, HMMA were developed using a Wenger twin-screw extruder (TX-52) as described in Chapter 5 using pulse proteins which were pea proteins (PP), lentil proteins (LP), and faba bean proteins (FP). The recipes to produce the HMMA were identified as C1 (control), T1, T2, and T3 as seen in Table 22 of Chapter 5. As soon as the HMMA samples came out of the cooling die of the extruder, they were cooled in 2% brine solution and stored in the freezer at -40°C. These samples were selected due to their higher water absorption index (WAI) and less water solubility index (WSI) and used in this project for both the trained panelists descriptive tests and the consumer central location tests.

For the trained panelist tests frozen HMMA was thawed for 24 hours in the cooler at 4°C, boiled for 2 minutes, stored in the oven at 72°C, served. Each sample was cut into a 2 cm cubic size before serving. Nine trained panelists participated in the test and they had training sessions for 4 days to learn about the beef lexicon and attributes of the samples following a training panel test for 3 days to evaluate the samples. For the consumer test, the frozen HMMA was thawed for 24 hours in the cooler at 4°C, boiled for 2 minutes, and resized using a size reducer with a 9-mm blade (Comitrol 3500, Urschel Laboratories, Inc., Valparaiso, IN). For this study, vegetable hamburger patties with HMMA were produced with the addition of spices, binders, and so on. Eighty

consumers participated in the tests at the sensory lab of the Kleberg Building at TAMU to provide consumer perceptions of the patties.

The objectives of this study were to understand the trained panelist's perceptions of HMMA, the consumers' perceptions of vegetable hamburger patties with HMMA, and the relationships between the trained panelists' tests and the consumers' tests. The hypothesis was that the qualities of the HMMA used in the trained panelists' tests and the consumers' tests would not be significantly different from the control, C1, and both tests would have a strong relationship in evaluating the quality of the products. Therefore, this study will contribute to developing a vegetable patty as a meat substitute, for which the consumer demand is rising steadily worldwide.

## **6.2 Materials and Methods**

### **6.2.1 *Trained Panelist Test***

#### **6.2.1.1 *Sample Preparations***

Frozen HMMs (C1, T1, T2, and T3) were thawed in the refrigerator for 24 hrs. Samples for each treatment were randomly selected. Before being served to trained panelists, they were boiled for 2 mins and stored in an oven at 80°C covered with aluminum foil. The samples in the oven were cut into 2 cm cubes and placed in randomized plastic cups to be served.

### 6.2.1.2 *Sensory Evaluation with Trained Panelists*

The treated samples were evaluated by 9 trained panelists from Texas A&M University who have been trained to evaluate beef flavor descriptive attributes. They were also trained for 3 days to help them become familiar and understand the attributes of a vegetable patty. Panelist training and testing was approved by the Institutional Review Board for the Protection of Human Subjects in Research (IRB) protocol IRB2017-0362M. Each trained panelist had a packet including the attributes of the food, double-distilled deionized water, sparkling water, and saltless saltines. On the first day, panelists learned about basic tastes, cardboardy, grainy, musty-earth/hummus, malt-like, hay-like, buttery, and heated oil. On the second day, the panelists learned about greens, lentils, vegetable IDs, celery, carrots, roots, starches, faba beans, peas, and soy (APPENDIX F). Next day, the panelists learned texture including cohesiveness, hardness, springiness, particle size, and slipperiness (APPENDIX F). On the last day, the training on the third day will be repeated to help in understanding (APPENDIX F). Screened lexicons and remaining lexicons such as flavors (starchy, grainy, bean-like, soy-like, green, salty, sweet, umami, cardboardy, musty-earthly, malt-like, buttery, heated oil, cohesiveness of mass (COM)) and texture attributes (hardness, and springiness) were tested with trained panelists (APPENDIX F). At the end of each training day, each sample in a randomized plastic cube was given to the trained panelists to determine the appropriate lexicons applicable to describe the characteristics of the samples.

Panelists received a warm-up sample to calibrate each sensory day, and the warm-up was individually evaluated by each panelist and discussed. Panelists came to

consensus for all attributes prior to testing. Each sample was served in a plastic cup marked with a random three-digit code. Double-distilled deionized water was prepared as a mouth cleanser between samples. Each panelist was given a tablet (iPad Air 1, Apple Inc., Cupertino, CA) to record their individual data using an electronic spreadsheet (Microsoft Excel, One Drive, Microsoft Corporation, Redmond, WA), and samples were evaluated independently. Four random samples over the course of a two-hour session were evaluated each sensory day.

## 6.2.2 Consumer Test

### 6.2.2.1 Materials

**Table 26. Recipe for Producing a Vegetable Hamburger Patty with a High Moisture Meat Analog.**

Ingredients	g/100g
HMMA	53.28
Chilled Water, g	28.61
Minced Dried Onion	0.90
Egg White Powder (non-whipping)	5.39
Carrageenan (Kappa)	0.49
Beef Flavor	2.69
Black Pepper	0.20
Natural Flavor Enhancer	0.45
Lactic Acid	0.45
Citric Acid	0.05
Methylcellulose	1.24
Shortening	6.26
Total	100.00

Commercially available, minced dried onion and black pepper (Member's Mark Minced Onion by Tone's, ACH Food Companies, Inc., Memphis, TN), lactic acid (Druids Grove Lactic Acid, Modernist Pantry LLC, Eliot, ME), and citric acid (Millard Citric Acid,



Millard Brands, Lakewood, NJ) were purchased. Non-whipping egg white power (Spray Dried Standard Egg Whites) was obtained from Sonstegard Foods Co. (Sioux Falls, SD), and beef flavor (TasteEssentials™ Nat Beef Vegetarian Flavor Type) was obtained from Givaudan (Cincinnati, OH). Natural flavor enhancer was obtained from Kikkoman (San Francisco, CA), and methylcellulose (Methocel SG A16M Food Grade Modified Cellulose) was obtained from The Dow Chemical Company (Midland, MI). Shortening (SanTrans™ 39) was obtained from Loders Corklaan USA, LLC (Channahon, IL).

Frozen HMMA samples were stored for 24 hours in the refrigerator at 4°C. The HMMA samples were boiled for 2 min as was done in the trained panelists test, resized using a size reducer (Comitrol 3500, Urschel Laboratories, Inc., Valparaiso, IN) with a 9-mm blade, and stored in the refrigerator for further experiments. Table 26 shows the recipe used to produce a vegetable hamburger patty for the consumer test. The spice mixture including minced onion, dried egg white, beef flavor, carrageenan, and flavor enhancers were prepared in the mixer, and lactic acid and citric acid were mixed to form a homogenous dry ingredient mixture. Shortening was chilled, ground through a 3.18 mm grinder plate and frozen. The frozen strings were broken into fat pellets and stored in the freezer until they were added to the mixture to make a vegetable patty.

#### *6.2.2.2 Making Patties*

For the consumer test, the HMMA (0-2°C) chilled with ice in a container were mixed in a Hobart mixer (Hobart mixer, Model N50, Canada) at 20 rpm controlled by a rheostat (Type 3PN1010, Staco Energy Products Co., Dayton, OH) as water (0-2°C) chilled with ice in a container was added slowly. During mixing, methylcellulose was sprinkled in slowly for long enough to ensure a uniform methylcellulose coating of the

HMMA particles. The dry ingredient mixture was added slowly to ensure uniform distribution of all ingredients followed by the addition of the shortening until the mix was uniform.

Patties for each treatment were formed with a patty maker (Supermodel 54 Food Portioning Machine, Hollymatic Corporation, Countryside, IL) with a 2.54 cm plate. The patties were placed with patty paper on top and bottom in a single layer on trays, placed in a -40°C freezer, crust frozen for 20 min, vacuum packaged, and stored in the -40°C freezer until the sensory test.

#### 6.2.2.3 *Cooking Protocols*

Approximately 24 hours prior to testing, samples were removed from the freezer and placed on racks in a single layer to thaw in a cooler (4°C). One hour before testing, patties were organized by cooking order on the trays. Their vacuum packaged bags and patty paper were removed, patty trays were covered with plastic wrap and held in the cooler until time to cook. Prior to cooking, five temperature readings of the surface of the grill were checked using an infrared temperature reader (MS6530H Infrared Thermometer, Commercial Electric Products Corporation, Cleveland, OH) with a target temperature of 162°C. As seen in APPENDIX D, the weights and temperatures of the raw samples and the time they were put on the grill were recorded, along with the end temperature, time they were taken off the grill, and final cooked weights.

Samples were cooked on a commercial flat-top grill to an end temperature of 71°C, with a flip temperature at 27°C. Internal temperatures were monitored using thermocouple probes (Model SCPSS-040U-6, Type T, 0.040 Sheath Diameter, 15.24 cm length Ungrounded Junction Thermocouple, Omega Engineering, Stamford, CT) by

inserting them into the geometric center of each vegetable patty periodically during cooking. The temperature was displayed using a thermometer (Omega HH501BT Type T, Omega Engineering, Stamford, CT). Each sample was prepared on a clear plastic plate (clear 15.88 cm plastic plates premium quality, Members Mark, Sam's Club, Bentonville, AR) marked with a random three-digit code. Each patty was cut into four equal pieces and a quarter of a patty was served to each consumer. Consumers were given a new transparent plastic fork and transparent plastic knife to use for each sample as well.

After patties were taken off the grill and weighed, they were wrapped in foil and placed in a holding oven (Model 750-TH-II, Alto-Shaam, Menomonee Falls, WI) for no longer than 20 min, until served.

#### *6.2.2.4 Sensory Test*

In advance, 80 consumers were recruited by emails and advertisements. They provided demographic information and signed a consent form through a survey website ([www.tamuag.az1.qualtrics.com](http://www.tamuag.az1.qualtrics.com)). Depending on their answers of available time for the test, they were assigned to one of four different sessions (20 consumer panelists each) for a 1-hour interval. In each session, they were divided into five groups since four wedges were cut from each patty. Four consumer panelists in each randomized group had the same treatment in the same order (APPENDIX D). Before the test, a consent form from each panelist was collected again. When they were seated in the booth under a red light, they were given a packet containing testing procedures, palate cleansers of distilled water and saltless saltine crackers, a demographic ballot, and five individual sample ballots. Demographic information from each panelist including gender, age,

ethnicity, household income, household population, employment level, protein sources consumed, and location consumed, frequency of protein consumption, preferred cooking method for ground beef, degree of doneness desired for ground beef, type of ground beef typically purchased, desired fat percentage of ground beef, and types of cuisines consumed was received (APPENDIX G). Cooked appearance, overall appearance, overall flavor, and overall texture were evaluated by the panelists on each sample ballot utilizing a 9-point hedonic scale. Open-ended questions, “Please write any words that describe what you LIKE about this meat patty” and “Please write any words that describe what you DISLIKE about this meat patty” were included on each ballot (APPENDIX H).

#### 6.2.2.5 *Cooking Yield and Cooking Time*

Cooking yield was calculated by using Equation 6.1.

$$\text{Cooking yield (\%)} = \frac{\text{Cooked patty weight (g)}}{\text{Raw patty weight (g)}} \times 100 \quad \text{Equation 6.1}$$

Cooking time of each patty in minutes was measured.

#### 6.2.2.6 *Color Measurement*

Frozen vegetable hamburger patties were thawed for 24 hours in a cooler (4°C) and remained at room temperature about 20 mins after their vacuum packaged bags and patty paper were removed. Three locations on each patty were directly evaluated using a colorimeter (Model CR-200, Minolta Co., Ramsey, NJ, USA). Values were expressed as L\*, a\* and b\*, where L\* values (lightness) vary from black (0) to white (100), chroma a\* values (redness) vary from green (-60) to red (+60), and chroma b\* values

(yellowness) vary. Cooked hamburger patties were measured as well. Color measurements were made with three samples for each treatment.

#### **6.2.2.7 Texture Analysis**

A texture analysis of the vegetable hamburger patty was performed with a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) using the texture profile analysis measurement. According to the modified method of Ganhão and others (2010), a cylindrical sample (2.54 cm diameter) from the center of each patty was sampled. A two-cycle compression test was conducted to compress the sample to 70% of the original height with a cylindrical probe of 7.25 cm diameter and cross-head speed of 1 mm/s. Texture profile parameters were evaluated following descriptions by Bourne (1978). All analyses were performed with five samples for each treatment.

#### **6.2.3 Statistical Analysis**

Data based on each protein type (C1, T1, T2, and T3) for each parameter were prepared for the statistical analysis of the data. The trained panel descriptive flavor and texture attributes, consumer preferences, color (raw and cooked), cooking yield, and cooking time of the samples were analyzed using the general linear mode procedure in SAS (9.4, SAS Institute, Cary, NC) with a predetermined alpha of 5%. For the trained panel results, data were averaged across panelists, order was defined as a random variable, and replicate was included in the model as a fixed effect. A full model was calculated where main effect of protein types was included. A one-way ANOVA was used to determine the significant difference ( $P < 0.05$ ) between vegetable hamburger patties containing HMMA with different protein sources. Color ( $L^*$ ,  $a^*$ , and  $b^*$ ) for raw

and cooked, cooking yield, and cooking time of the beef hamburger patties data were analyzed similarly. Least square means were calculated and differences between least squares means were determined using the pdiff function when differences were significance ( $P < 0.05$ ) in the Analysis of Variance table.

## 6.3 Results and Discussion

### 6.3.1 Trained Descriptive Flavor and Texture Perception

**Table 27. Trained Descriptive Flavor and Texture Perception of Vegetable Patties Containing HMMA with Pulse Proteins.**

Attribute	P-value	C1	T1	T2	T3	b RMSE
Flavor						
Starchy	0.58	3.7	3.8	3.9	4.0	0.34
Grainy	0.87	3.0	3.1	3.1	3.1	0.4
Bean-like	<0.0001	1.9a	3.1b	3.2b	3.5b	0.33
Soy	0.01	4.4a	3.7b	3.8b	3.6b	0.44
Green	0.65	0.0	0.0	0.0	0.0	0.05
Salty	<0.001	1.4a	2.5b	2.5b	2.6b	0.29
Sweet	<0.0001	1.3a	2.4b	2.3bc	2.2c	0.16
Umami	<0.0001	2.1a	3.9b	4.0b	3.9b	0.41
Cardboardy	<0.0001	3.8a	2.5b	2.6b	2.6b	0.36
Musty Earthy	0.0063	1.7a	2.0b	2.0b	2.1b	0.22
Malt-Like	<0.0001	1.6a	2.4b	2.4b	2.2b	0.26
Buttery	0.41	0.0	0.0	0.0	0.0	0.02
Heated Oil	<0.0001	1.9a	2.6b	2.7b	2.8b	0.24
Texture						
Cohesiveness of Mass	<0.0001	4.0a	6.8b	7.0b	7.2b	0.74
Hardness	<0.0001	8.3a	4.7b	4.0bc	3.9c	0.67
Springiness	<0.0001	8.0a	4.9b	4.7b	4.3b	0.58

<sup>a</sup> Means within a row and effect followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>b</sup> RMSE = root mean square error.

<sup>c</sup> Recipes for texturization: C1 (control) = soy concentrate and soy isolate, T1, T2, and T3 = pea proteins, lentil proteins, and faba bean proteins, respectively, premixed with a constant ingredient (canola oil and wheat gluten).

The trained panelists' perceptions are reported in Table 27. Protein sources in vegetable patties containing HMMA did not significantly affect flavor attributes for starchy ( $P = 0.58$ ), grainy ( $P = 0.87$ ), green ( $P = 0.65$ ), and buttery ( $P = 0.41$ ). Trained panelists could not perceive green and buttery in all samples.

Compared to the control, C1, the samples (T1, T2, and T3) containing pulse proteins were scored higher for flavor attributes that were bean-like, salty, sweet, umami, musty earthy, and malt-like and heated oil indicated extremely small values ( $P < 0.0001$ ). In contrast, these samples were lower for soy ( $P = 0.01$ ) and cardboardy ( $P < 0.0001$ ). However, these pulses did not have significantly different flavor attributes from each other except the sweetness attribute for T1 was higher than T3.

Pulse proteins in vegetable patties containing HMMA significantly affected texture attributes of cohesiveness of mass, hardness, and springiness resulting in extremely small values ( $P < 0.0001$ ) compared to C1. The samples including pulse proteins (T1, T2, and T3) were significantly higher for cohesiveness of mass and lower for hardness and springiness compared to the control. T1 was higher for hardness compared to T3.

Pulse proteins in the samples did not affect the flavor including starchy, grain, green, and buttery attributes compared to the control.

### 6.3.2 Consumer Demographics

**Table 28. Demographic Frequencies for Vegetable Patty Consumers (n = 80).**

Question	Number of Respondents	Percentage of Respondents
Sex		
Male	21	26.6
Female	58	73.4
Age		
20 years or younger	54	67.5
21 – 25 years	21	26.3
26 – 35 years	3	3.8
36 – 45 years	2	2.5
46 – 55 years	0	0
56 – 65 years	0	0
66 years and older	0	0
Ethnicity		
African-American	5	6.3
Asian/Pacific Islanders	14	17.7
Caucasian (non-Hispanic)	43	54.4
Latino or Hispanic	13	16.5
Native American	1	1.3
Other	3	3.8
Household income		
Below \$25,000	23	29.1
\$25,001 - \$49,999	6	7.6
\$50,000 - \$74,999	10	12.7
\$75,000 - \$99,999	16	20.3
\$100,000 or more	24	30.4
Household size including yourself		
1	3	3.8
2	9	11.3
3	18	22.5
4	33	41.3
5	12	15.0
6 or more	5	6.3



**Table 28. Continued.**

Employment level				
Not employed	48		60.0	
Part-time	29		36.3	
Full-time	3		3.8	
Proteins consumed at home or at a restaurant (away from home)				
At Home	Do not consume	Consume	Do not consume	Consume
Chicken	6	74	7.5	92.5
Beef (steaks)	8	65	11.0	89.0
Ground Beef	9	67	11.8	88.2
Pork	23	54	29.9	70.1
Fish	15	59	20.3	79.7
Lamb	66	16	80.5	19.5
Egg	1	72	1.4	98.6
Soy Based Products	59	29	67.0	33.0
Away from Home/Restaurant	Do not consume	Consume	Do not consume	Consume
Chicken	1	73	1.4	98.6
Beef (steaks)	3	67	4.3	95.7
Ground Beef	9	60	13.0	87.0
Pork	22	57	27.8	72.2
Fish	16	65	19.8	80.2
Lamb	52	26	66.7	33.3
Eggs	9	72	11.1	88.9
Soy Based Products	56	33	62.9	37.1

Table 28. Continued.

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Weekly consumption of protein		
	Beef	
0	20	26.3
1-2	46	60.5
3-4	8	10.5
5-6	1	1.3
7 or more	1	1.3
	Ground Beef	
0	14	18.2
1-2	51	66.2
3-4	12	15.6
5-6	0	0.0
7 or more	0	0.0
	Pork	
0	28	38.4
1-2	41	56.2
3-4	4	5.5
5-6	0	0.0
7 or more	0	0.0
	Lamb	
0	64	92.8
1-2	5	7.2
3-4	0	0.0
5-6	0	0.0
7 or more	0	0.0

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Table 28. Continued.

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	Chicken	
0	3	3.8
1-2	18	23.1
3-4	39	50.0
5-6	17	21.8
7 or more	1	1.3
	Fish	
0	21	27.6
1-2	49	64.5
3-4	4	5.3
5-6	2	2.6
7 or more	0	0.0
	Soy Based Products	
0	45	64.3
1-2	15	21.4
3-4	8	11.4
5-6	1	1.4
7 or more	1	1.4

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**Table 28. Continued.**

What cooking method do you prefer to use when cooking ground beef?				
	Do not use	Use	Do not use	Use
Pan-frying or skillet on the Stove	18	62	22.5	77.5
Grilling outside	44	36	55.0	45.0
Oven baking	62	18	77.5	22.5
Electric appliance (George Forman Grill or Electric grill)	68	12	85.0	15.0
Stir fry	66	14	82.5	17.5
Oven broiling	73	7	91.3	8.8
Microwave	75	5	93.8	6.3
Degree of doneness preference for ground beef				
Rare		2		2.5
Medium Rare		16		20.0
Medium		21		26.3
Medium Well		18		22.5
Well		16		20.0
Very Well		7		8.8

**Table 28. Continued.**

What percentage of fat do you normally buy when purchasing ground beef?				
	Do not	Purchase	Do not	Purchase
4%	63	17	78.8	21.3
7%	59	21	73.8	26.3
10%	59	21	73.8	26.3
15%	67	13	83.8	16.3
20%	74	6	92.5	7.5
27%	80	0	100.0	0.0

What flavor or types of cuisines do you like?				
	Do not eat	Eat	Do not eat	Eat
American	6	74	7.5	92.5
Chinese	19	61	23.8	76.3
French	45	35	56.3	43.8
Barbeque	11	69	13.8	86.3
Greek	42	38	52.5	47.5
Thai	44	36	55.0	45.0
Mexican/Spanish	5	75	6.3	93.8
Japanese	30	50	37.5	62.5
Lebanese	61	19	76.3	23.8
Indian	53	27	66.3	33.8
Italian	6	74	7.5	92.5

Demographic information for consumers (n = 80) participating in this study are reported in Table 28. More females (73.4%) participated in the study compared to males and the majority of participants (67.5%) fell in the 20 years or younger age group, followed by 21 to 25 (26.3%). The rest of the age groups that participated in this study were in the 26-35 age range (3.8%) and 36-45 age range (2.5%). The majority of consumers represented the Caucasian (non-Hispanic) ethnicity (54.4%), followed by Asian/Pacific Islanders (17.7%), Latinos or Hispanics (16.5%), and African-Americans (6.3%). Household incomes were distributed with 29.1% falling into the below \$25,000 group, 7.6% falling into the \$25,001 - \$49,999 group, 12.7% falling into the \$50,000 -

\$74, 999 group, and the \$75,000 - \$99,999 group, and 30.4% falling in the \$100,000 group. Household size was represented by a majority of four-person households (41.3%), followed by three-person (22.5%), five-person (15.0%), and two-person (11.3%) households. The majority of participants (60%) were not-employed, followed by participants who were employed part-time (36.3%).

When asked about proteins consumed at home, over 80% of consumers reported consuming chicken, beef (steaks), ground beef, and eggs, followed by fish (79.7%) and pork (70.1%). The top two proteins consumed at home included eggs (98.6%) and chicken (92.5%). When asked about proteins consumed away from home or at restaurants, over 80% of consumers reported consuming chicken, beef (steaks), ground beef, fish, and eggs. The top proteins consumed away from home included chicken (98.6%), ground beef (95.7%), eggs (88.9%), and ground beef (87.0%).

Consumers were asked to report how many times a week they consumed each protein source. The majority of consumers reported consuming beef (steaks) 1 to 2 times per week (60.5%), followed by 0 times per week (26.3%) and 3 to 4 times per week (10.5%). For ground beef consumption, the majority of consumers reported eating it 1 to 2 times per week (66.2%) followed by 0 times per week (18.2%) and 3 to 4 times per week (15.6%). For pork consumption, consumers reported eating it 1 to 2 times per week (56.2%) followed by 0 times per week (38.4%). For lamb consumption, the majority of consumers reported 0 times per week (92.8%) followed by 1 to 2 times (7.2%). For chicken consumption, the majority of consumers consumed chicken 3 to 4 times per week (50.0%), followed by 1 to 2 times per week (23.1%) and 5 to 6 times per week (21.8%). For fish consumption, the majority of consumers reported eating fish 1

to 2 time per week (64.5%) followed by 0 times per week (27.6%). Finally, for soy-based products, consumers reported eating soy-based products 0 times per week (64.3%) followed by 1 to 2 times per week (21.4%).

Consumers were asked what methods were preferred when cooking ground beef. The majority of consumers preferred to pan-fry/skillet on the stove (77.5%). Some consumers grilled outside (45.0%) and oven baked (22.5%), and even fewer used stir frying (17.5%), an electric appliance (George Forman Grill; 15.0%), oven broiling (8.8%), or a microwave (6.3%).

When asked for preferences on degree of doneness, consumers reported fairly evenly distributed between medium rare to well. They reported medium (26.3%), medium well (22.5%), and both medium rare (20%) and well done (20%). Few consumers preferred the extremes with only 2.5% reporting rare and 8.8% for very well done.

When consumers were asked what fat level they typically purchased, consumers responded with both 7% and 10% with a 20% fat level, followed by 4% (21.3%), 15% (16.3%), and 20% (7.5%).

Consumers were asked what types of cuisines they liked to purchase. Over 80% reported enjoying American, Barbeque, Mexican/Spanish, and Italian cuisines, followed by Chinese (76.3%) and Japanese (62.5%). Lebanese (23.8%), Indian (33.8%), French (43.8%), and Greek (47.5%) were among the lowest typically consumed. These results indicate that consumers in this study were an acceptable population to test vegetable patties containing (C1, T1, T2, and T3).

### 6.3.3 Consumer Perception of Vegetable Patties Containing HMMA

Table 29. Consumer Liking for HMMA Vegetable Patties.

Attribute	P-value	C1	T1	T2	T3	<sup>b</sup> RMSE
Cooked appearance	0.89	5.5	5.2	5.4	5.3	2.05
Overall	0.11	6.0 <sup>a</sup>	5.3 <sup>ab</sup>	5.4 <sup>ab</sup>	5.0 <sup>b</sup>	2.03
Overall flavor	0.24	6.0	5.3	5.4	5.1	2.09
Overall texture	0.003	6.0 <sup>a</sup>	4.9 <sup>b</sup>	4.8 <sup>b</sup>	4.7 <sup>b</sup>	2.16

<sup>a</sup> Means within a row and effect followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>b</sup> RMSE = root mean square error.

<sup>c</sup> Recipes for texturization: C1 (control) = soy concentrate and soy isolate, T1, T2, and T3 = pea proteins, lentil proteins, and faba bean proteins, respectively, premixed with a constant ingredient (canola oil and wheat gluten).

<sup>d</sup> Consumer likes were measured with 0 = extremely dislike and 9 = extremely like.

Consumer perception scores are reported in Table 29. Protein sources in meat patties containing HMMA did not significantly affect the number of consumers who liked the cooked appearance ( $P = 0.89$ ) and overall flavor ( $P = 0.24$ ). However, C1 was more desirable for overall liking and overall texture than T3.



Figure 26. Consumer liking (a) or disliking (b) descriptors for vegetable patties containing HMMA with C1.





Word clouds were created using the comments from consumer panelists answers to an open-ended question about whether or not they liked or disliked vegetable patties containing HMMA. Figure 26 to 29 demonstrate the consumer's responses separated by vegetable patties containing HMMA with different protein sources (C1, T1, T2, and T3). The size of the word illustrates how often the consumers used the words. For C1, the most commonly used words for liking were texture, flavor, good, like, and taste (Figure 26) and for disliking most commonly used words were texture, flavor, bad, little, dry, and bland. Flavor and texture were the most frequently used words for the like and dislike descriptors. For T1, the most frequently used words for liking were flavor, good, and texture (Figure 27) and for disliking the most commonly used words were texture, taste, and flavor. As for the like descriptors, the most commonly used for T2 were flavor, good, and texture while for dislike descriptors, the most commonly used word was texture. More positive words to describe the quality of the patties were used for T2 than negative words. As for like descriptors, the most commonly used words for T3 were flavor, texture, good, like, and the most frequently used word for disliking was texture. More positive words to describe the quality of the patties were used for T3 than were negative words.

For all vegetable patty samples, more descriptive words were used when the consumer panelists responded to describe liking points of the sample compared to dislike descriptors. Across all the words clouds, texture was most consistently used for describing whether or not a consumer liked a sample except for T1 for the like descriptors.

### 6.3.4 Color of Raw and Cooked Vegetable Patties

Table 30. Color of Raw and Cooked, HMMA Vegetable Patties.

Attribute	P-value	C1	T1	T2	T3	<sup>b</sup> RMSE
Raw						
L*	0.09	57.4	56.1	56.6	57.1	0.55
a*	0.001	4.4 <sup>c</sup>	6.4 <sup>a</sup>	5.8 <sup>ab</sup>	5.5 <sup>b</sup>	0.38
b*	0.004	20.0 <sup>b</sup>	23.5 <sup>a</sup>	23.1 <sup>a</sup>	22.8 <sup>a</sup>	0.87
Cooked						
L*	0.27	57.5	57.6	55.3	55.8	1.63
a*	0.05	6.8 <sup>b</sup>	8.5 <sup>ab</sup>	9.2 <sup>a</sup>	8.9 <sup>a</sup>	0.96
b*	0.42	22.8	24.7	23.6	24.3	1.49

<sup>a</sup> Means within a row and effect followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>b</sup>RMSE = root mean square error. <sup>c</sup> Recipes for texturization: C1 (control) = soy concentrate and soy isolate, T1, T2, and T3 = pea proteins, lentil proteins, and faba bean proteins, respectively, premixed with a constant ingredient (canola oil and wheat gluten).

Table 30 shows the color of raw and cooked vegetable patties with HMMA. The protein source did not affect the color of raw and cooked patties for lightness ( $L^*$ ,  $P = 0.09$ ) and cooked patties for yellowness ( $P = 0.42$ ) compared to C1. However, the protein source significantly affected the redness of raw patties ( $P = 0.0012$ ) and yellowness ( $P = 0.0044$ ) and redness in cooked patties ( $P = 0.05$ ). Redness and yellowness were higher for these samples of raw patties containing pulse proteins compared to the control, redness was similar for T2 to T1 and T3, but higher for T1 compared to T3. Yellowness was similar for these samples of raw patties to each other containing PLP. Redness was similar for these samples to each other.

### 6.3.5 Cooking Properties and Texture of Vegetable Patties containing HMMA with PLP

Table 31. Cooking Yield, Cooking Time, and Texture of Cooked HMMA Vegetable Patties with PLP.

Attribute	P-value	C1	T1	T2	T3	<sup>b</sup> RMSE
Cooking parameters						
Cooking yield, %	<0.0001	92.5 <sup>c</sup>	93.6 <sup>b</sup>	94.1 <sup>ab</sup>	96.2 <sup>a</sup>	1.62
Cooking time, min	<0.0001	5.1 <sup>a</sup>	4.9 <sup>a</sup>	4.6 <sup>ab</sup>	4.1 <sup>b</sup>	0.80
TPA						
Hardness, N	0.1	67.3	52.0	59.4	57.5	9.05
Cohesiveness	0.002	0.4 <sup>a</sup>	0.3 <sup>b</sup>	0.3 <sup>b</sup>	0.3 <sup>b</sup>	0.02
Gumminess	0.009	24.9 <sup>a</sup>	16.8 <sup>b</sup>	19.6 <sup>ab</sup>	18.2 <sup>b</sup>	3.37

<sup>a</sup> Means within a row and effect followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>b</sup> RMSE = root mean square error.

<sup>c</sup> Recipes for texturization: C1 (control) = soy concentrate and soy isolate, T1, T2, and T3 = pea proteins, lentil proteins, and faba bean proteins, respectively, premixed with a constant ingredient (canola oil and wheat gluten).

Table 31 shows cooking properties (cooking yield and time) and texture (hardness, cohesiveness, and gumminess) of vegetable patties containing HMMA with different PLP. The protein source in vegetable patties containing HMMA significantly ( $P = <0.0001$ ) affected cooking yield and cooking time. Cooking yield was the highest (96.20%) for T3 and lowest for C1 (92.53%) compared to other samples, followed by T3 which was not significantly different from T2 which was like T1. The cooking time of C1 was not significantly different from T1 and T2, but C1 required more cooking time than T3, which was like T2. The more the patties cooked, the more water evaporated and resulted in a decrease in cooking yield.

The protein source in vegetable patties containing HMMA did not significantly ( $P = 0.1$ ) affect hardness but did significantly affected cohesiveness ( $P = 0.002$ ) and gumminess ( $P = 0.009$ ). C1 had the highest hardness, cohesiveness, and gumminess compared to other samples except T2 in which gumminess was not significantly

different from C1. Other patties containing PLP did not have significantly different cohesiveness and gumminess.

## 6.4 Conclusion

Trained panelists (n=9 or 10) evaluated the flavor and texture of HMMA containing different PLP to compare to C1, a soy based HMMA. Bean-like, salty, sweet, umami, heated-oil and cohesiveness of mass were significantly higher for HMMA containing PLP than C1 while soy, green, sweet, cardboardy, musty earthy, malt-like, hardness, and springiness were significantly lower than C1.

Consumer panelists (n = 80) conducted a sensory evaluation to evaluate their preferences (cooked appearance, overall, overall flavor, and overall texture) of vegetable patties made with HMMA containing different PLP compared to the control containing soy-based protein. Different protein sources in vegetable patties did not significantly influence the consumers' liking of the cooked appearance, overall, and overall flavor except for T3, which had a lower overall liking compared to C1. In contrast, overall texture was lower for vegetable patties containing PLP. The most frequently used words from consumer panelists in response to the open-ended question about liking or disliking a sample was texture.

The protein source did not affect the color of raw and cooked patties for lightness and cooked patties for yellowness compared to C1. However, the protein source significantly affected the redness and yellowness of raw patties and redness for cooked patties. Cooking yield was higher and cooking time was lower for vegetable patties containing PLP compared to the control. They did not have significantly different hardness, or significantly lower cohesiveness or gumminess compared C1.

Therefore, PLP can be an alternate source of soy to produce HMMA since consumers scored a similar liking of vegetable proteins containing different PLP. In

addition, the cooking yield of the samples containing PLP was higher than C1, and they needed relatively less cooking time. Although the TPA gave lower textural properties in cohesiveness and gumminess for the vegetable patties containing PLP, these proteins might provide a unique combination of attributes and attract consumers.

## CHAPTER VII

### SUMMARY AND CONCLUSION

#### 7.1 Summary

This study demonstrates that PLP (PP, LP, and FP) can be texturized using a twin-screw extruder (TX-52) to produce TX-PLP and HMMA, which are used as alternative meat sources. For production of TXVP with PLP, a higher screw profile, additives, and an ingredient affect the degree of texturization resulting in different quality parameters of the product. For production of HMMA, different types of protein, cooling methods, and rehydration methods affected the quality of the final product. A 2% brine solution used as a cooling media after production of HMMA improves WHC and WSI. Blanching, boiling, or soaking for rehydration improves WHC, WSI, and texture, respectively. Both TPP used in meat patties and HMMA used in vegetable patties are competitive with soy-based samples, currently the dominant alternative meat source, according to both the trained panelist descriptive test and the consumer panel test.

This study provides evidence for the potential use of PLP in alternative meat products as new ingredients to produce commercial products like meat patties with TXVP and vegetable patties from the responses of the trained panelists and consumer panelists. It also demonstrates that every PLP has different parameters to define their own characteristics and can be used in various products as needed for things such as patties, nuggets, or sausage.

This study was conducted to produce TPP and HMMA using processing conditions for soy and only meat patties containing TXVP and beef flavored vegetable patties. The



mechanisms for texturization of PLP and processing conditions were also not fully elucidated.

## **7.2 Recommendation for Further Research**

Further studies are needed to demonstrate the successful application of PLP in alternative meat products using texturization and their functional benefits. Follow up studies could focus on:

1. The optimum processing conditions of each PLP for texturization.
2. The application of each TXPLP in alternative meat products such as nuggets or sausage.
3. The application of other ingredients or additives to improve the quality of the final products.

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## APPENDIX A

### Combined Effect of Cooling and Rehydration for Color on HMMA with Pulse Proteins

Sample	Cooling	Rehydration	L*	a*	b*
C1	NC	NR	66.65±0.14 <sup>ab</sup>	1.18±0.02 <sup>ab</sup>	2.48±0.13 <sup>b</sup>
		Soaking	66.47±0.03 <sup>bc</sup>	1.13±0.01 <sup>b</sup>	3.78±0.04 <sup>a</sup>
		Blanching	67.07±0.15 <sup>a</sup>	0.93±0.03 <sup>c</sup>	3.42±0.09 <sup>a</sup>
		Boiling	66.07±0.02 <sup>c</sup>	1.32±0.06 <sup>a</sup>	2.41±0.25 <sup>b</sup>
	Water	NR	66.66±0.14 <sup>b</sup>	1.15±0.01 <sup>a</sup>	2.77±0.03 <sup>b</sup>
		Soaking	66.39±0.06 <sup>b</sup>	1.00±0.01 <sup>b</sup>	3.46±0.04 <sup>a</sup>
		Blanching	67.53±0.20 <sup>a</sup>	0.85±0.02 <sup>c</sup>	3.23±0.04 <sup>a</sup>
		Boiling	66.65±0.22 <sup>b</sup>	1.12±0.04 <sup>a</sup>	2.42±0.09 <sup>c</sup>
	2B	NR	66.58±0.05 <sup>b</sup>	1.1±0.02 <sup>b</sup>	2.53±0.06 <sup>b</sup>
		Soaking	66.42±0.16 <sup>b</sup>	1.03±0.01 <sup>b</sup>	3.51±0.10 <sup>a</sup>
		Blanching	67.83±0.16 <sup>a</sup>	0.77±0.03 <sup>c</sup>	3.5±0.21 <sup>a</sup>
		Boiling	66.34±0.09 <sup>b</sup>	1.22±0.04 <sup>a</sup>	2.57±0.12 <sup>b</sup>
	4B	NR	66.83±0.17 <sup>a</sup>	1.11±0.01 <sup>ab</sup>	2.70±0.08 <sup>b</sup>
		Soaking	66.45±0.22 <sup>a</sup>	0.95±0.05 <sup>bc</sup>	3.49±0.09 <sup>a</sup>
		Blanching	66.62±0.22 <sup>a</sup>	0.80±0.02 <sup>c</sup>	3.07±0.13 <sup>ab</sup>
		Boiling	66.34±0.26 <sup>a</sup>	1.22±0.05 <sup>a</sup>	2.56±0.17 <sup>b</sup>

APPEDIX A. CONTINUED

T1	NC	NR	65.02±0.08 <sup>b</sup>	1.74±0.05 <sup>a</sup>	2.6±0.12 <sup>b</sup>
		Soaking	62.48±0.13 <sup>c</sup>	1.72±0.00 <sup>a</sup>	3.12±0.14 <sup>a</sup>
		Blanching	62.54±0.06 <sup>c</sup>	1.58±0.02 <sup>b</sup>	3.31±0.06 <sup>a</sup>
		Boiling	66.08±0.04 <sup>a</sup>	1.74±0.01 <sup>a</sup>	2.39±0.01 <sup>b</sup>
	Water	NR	65.47±0.05 <sup>b</sup>	1.73±0.03 <sup>a</sup>	2.77±0.12 <sup>b</sup>
		Soaking	62.44±0.07 <sup>d</sup>	1.62±0.03 <sup>a</sup>	3.42±0.01 <sup>a</sup>
		Blanching	63.1±0.22 <sup>c</sup>	1.44±0.02 <sup>b</sup>	3.41±0.19 <sup>a</sup>
		Boiling	66.49±0.14 <sup>a</sup>	1.66±0.03 <sup>a</sup>	2.77±0.05 <sup>b</sup>
	2B	NR	65.68±0.10 <sup>a</sup>	1.68±0.03 <sup>ab</sup>	2.70±0.12 <sup>b</sup>
		Soaking	62.41±0.24 <sup>b</sup>	1.52±0.03 <sup>b</sup>	3.38±0.08 <sup>a</sup>
		Blanching	62.64±0.22 <sup>b</sup>	1.51±0.06 <sup>b</sup>	3.32±0.18 <sup>a</sup>
		Boiling	65.6±0.04 <sup>a</sup>	1.74±0.04 <sup>a</sup>	2.52±0.13 <sup>b</sup>
	4B	NR	65.68±0.03 <sup>a</sup>	1.69±0.03 <sup>ab</sup>	2.56±0.11 <sup>b</sup>
		Soaking	61.76±0.02 <sup>b</sup>	1.58±0.02 <sup>bc</sup>	3.36±0.03 <sup>a</sup>
		Blanching	62.14±0.15 <sup>b</sup>	1.48±0.04 <sup>c</sup>	3.25±0.08 <sup>a</sup>
		Boiling	65.93±0.08 <sup>a</sup>	1.78±0.04 <sup>a</sup>	2.77±0.15 <sup>b</sup>

## APPEDIX A. CONTINUED

T2	NC	NR	64.6±0.08 <sup>b</sup>	1.63±0.02 <sup>a</sup>	2.36±0.05 <sup>c</sup>
		Soaking	60.83±0.07 <sup>c</sup>	1.60±0.06 <sup>a</sup>	2.98±0.16 <sup>ab</sup>
		Blanching	61.27±0.20 <sup>c</sup>	1.30±0.02 <sup>b</sup>	3.06±0.15 <sup>a</sup>
		Boiling	65.63±0.10 <sup>a</sup>	1.70±0.02 <sup>a</sup>	2.48±0.04 <sup>bc</sup>
	Water	NR	65.34±0.13 <sup>a</sup>	1.50±0.04 <sup>b</sup>	2.62±0.16 <sup>b</sup>
		Soaking	62.18±0.22 <sup>b</sup>	1.80±0.01 <sup>a</sup>	1.96±0.02 <sup>c</sup>
		Blanching	61.83±0.17 <sup>b</sup>	1.26±0.03 <sup>c</sup>	3.20±0.14 <sup>a</sup>
		Boiling	66.05±0.14 <sup>a</sup>	1.53±0.04 <sup>b</sup>	2.40±0.05 <sup>bc</sup>
	2B	NR	64.91±0.10 <sup>a</sup>	1.60±0.02 <sup>a</sup>	2.72±0.09 <sup>b</sup>
		Soaking	61.23±0.16 <sup>b</sup>	1.22±0.05 <sup>b</sup>	3.05±0.10 <sup>a</sup>
		Blanching	60.98±0.24 <sup>b</sup>	1.30±0.01 <sup>b</sup>	3.02±0.02 <sup>ab</sup>
		Boiling	65.28±0.19 <sup>a</sup>	1.53±0.06 <sup>a</sup>	2.31±0.04 <sup>c</sup>
	4B	NR	65.25±0.14 <sup>a</sup>	1.54±0.05 <sup>a</sup>	2.56±0.12 <sup>ab</sup>
		Soaking	60.76±0.09 <sup>b</sup>	1.40±0.00 <sup>b<sup>c</sup></sup>	2.97±0.14 <sup>a</sup>
		Blanching	60.53±0.17 <sup>b</sup>	1.30±0.03 <sup>c</sup>	2.91±0.09 <sup>a</sup>
		Boiling	65.6±0.09 <sup>a</sup>	1.49±0.01 <sup>ab</sup>	2.35±0.10 <sup>b</sup>

## APPEDIX A. CONTINUED

T3	NC	NR	64.28±0.06 <sup>b</sup>	1.35±0.06 <sup>a</sup>	2.00±0.13 <sup>c</sup>
		Soaking	60.24±0.12 <sup>c</sup>	1.33±0.04 <sup>a</sup>	2.67±0.17 <sup>ab</sup>
		Blanching	60.12±0.13 <sup>c</sup>	1.12±0.03 <sup>b</sup>	2.86±0.17 <sup>a</sup>
		Boiling	65.28±0.16 <sup>a</sup>	1.44±0.02 <sup>a</sup>	2.11±0.09 <sup>bc</sup>
	Water	NR	65.15±0.10 <sup>b</sup>	1.19±0.03 <sup>ab</sup>	2.12±0.07 <sup>ab</sup>
		Soaking	61.59±0.24 <sup>c</sup>	1.13±0.04 <sup>ab</sup>	2.77±0.22 <sup>a</sup>
		Blanching	60.22±0.18 <sup>d</sup>	1.05±0.04 <sup>b</sup>	2.72±0.18 <sup>ab</sup>
		Boiling	66.12±0.06 <sup>a</sup>	1.23±0.01 <sup>a</sup>	2.09±0.03 <sup>b</sup>
	2B	NR	65.41±0.17 <sup>a</sup>	1.15±0.02 <sup>b</sup>	2.01±0.08 <sup>b</sup>
		Soaking	59.81±0.40 <sup>b</sup>	1.12±0.06 <sup>b</sup>	2.69±0.17 <sup>a</sup>
		Blanching	60.16±0.14 <sup>b</sup>	1.04±0.03 <sup>b</sup>	2.88±0.08 <sup>a</sup>
		Boiling	65.10±0.44 <sup>a</sup>	1.36±0.05 <sup>a</sup>	2.13±0.05 <sup>b</sup>
	4B	NR	65.11±0.20 <sup>a</sup>	1.20±0.03 <sup>a</sup>	1.98±0.04 <sup>c</sup>
		Soaking	59.50±0.10 <sup>b</sup>	1.12±0.02 <sup>ab</sup>	2.56±0.06 <sup>ab</sup>
		Blanching	59.83±0.24 <sup>b</sup>	1.05±0.04 <sup>b</sup>	2.77±0.19 <sup>a</sup>
		Boiling	64.79±0.08 <sup>a</sup>	1.22±0.03 <sup>a</sup>	2.16±0.04 <sup>bc</sup>

<sup>a</sup> Means with different letters of the same style are significantly different (p<0.05).

<sup>b</sup> Recipes for texturization, C1 (control): soy concentrate and soy isolate, T1, T2, and T3: pea proteins, lentil proteins, and faba bean proteins premixed with a constant ingredient (canola oil and wheat gluten).

<sup>c</sup> NC=cooling in air, Water=cooling in water, 2B=cooling in 2% brine solution, and 4B=cooling in 4% brine solution.

## APPENDIX B

### Combined Effect of Cooling and Rehydration for M.C., Density, WHC, WSI, Texture on HMMA with Pulse Proteins

Sample	Cooling	Rehydration	M.C., %	Density, kg/l	WHC, g/100g	WSI, g/100g	Hardness, N	Cohesiveness	Gumminess, N
C1	NC	NR	52.82±0.05 <sup>d</sup>	1.14±0.01 <sup>c</sup>	N/A	N/A	43.74±1.64 <sup>a</sup>	0.82±0.00 <sup>a</sup>	35.79±1.43 <sup>a</sup>
		Soaking	62.10±0.05 <sup>b</sup>	1.40±0.01 <sup>b</sup>	22.84±0.10 <sup>b</sup>	1.43±0.06 <sup>b</sup>	34.79±1.43 <sup>b</sup>	0.82±0.00 <sup>a</sup>	28.51±1.24 <sup>b</sup>
		Blanching	66.62±0.01 <sup>a</sup>	1.59±0.04 <sup>a</sup>	36.02±1.57 <sup>a</sup>	3.31±0.09 <sup>a</sup>	26.93±0.48 <sup>c</sup>	0.82±0.00 <sup>a</sup>	22.09±0.41 <sup>c</sup>
		Boiling	57.97±0.01 <sup>c</sup>	1.19±0.01 <sup>c</sup>	11.11±0.95 <sup>c</sup>	0.71±0 <sup>c</sup>	35.20±2.49 <sup>b</sup>	0.78±0.01 <sup>b</sup>	27.59±2.11 <sup>bc</sup>
	Water	NR	59.03±0.00 <sup>d</sup>	1.21±0.02 <sup>c</sup>	N/A	N/A	27.79±1.50 <sup>a</sup>	0.80±0.02 <sup>a</sup>	22.16±1.32 <sup>a</sup>
		Soaking	64.38±0.01 <sup>b</sup>	1.41±0.04 <sup>b</sup>	16.36±1.23 <sup>b</sup>	1.39±0.06 <sup>ab</sup>	29.42±1.34 <sup>a</sup>	0.82±0.00 <sup>a</sup>	24.23±1.09 <sup>a</sup>
		Blanching	69.17±0.03 <sup>a</sup>	1.56±0.02 <sup>a</sup>	27.92±0.15 <sup>a</sup>	2.67±0.39 <sup>a</sup>	20.06±1.46 <sup>b</sup>	0.82±0.00 <sup>a</sup>	16.36±1.16 <sup>b</sup>
		Boiling	63.08±0.06 <sup>c</sup>	1.24±0.01 <sup>c</sup>	10.85±0.57 <sup>c</sup>	0.70±0.01 <sup>b</sup>	23.91±2.4 <sup>ab</sup>	0.79±0.00 <sup>a</sup>	19.00±1.93 <sup>ab</sup>
2B	NR	58.72±0.02 <sup>d</sup>	1.20±0.03 <sup>b</sup>	N/A	N/A	32.29±1.32 <sup>a</sup>	0.80±0.01 <sup>a</sup>	25.89±1.15 <sup>a</sup>	
	Soaking	64.74±0.00 <sup>b</sup>	1.43±0.03 <sup>a</sup>	18.95±0.17 <sup>b</sup>	2.59±0.05 <sup>a</sup>	28.17±0.80 <sup>ab</sup>	0.83±0.00 <sup>a</sup>	23.28±0.76 <sup>ab</sup>	
	Blanching	68.54±0.05 <sup>a</sup>	1.58±0.03 <sup>a</sup>	27.06±2.04 <sup>a</sup>	1.47±0.40 <sup>ab</sup>	20.70±1.26 <sup>c</sup>	0.81±0.02 <sup>a</sup>	16.84±1.20 <sup>c</sup>	
	Boiling	61.00±0.04 <sup>c</sup>	1.22±0.02 <sup>b</sup>	10.67±0.95 <sup>c</sup>	0.65±0.01 <sup>b</sup>	25.83±0.86 <sup>b</sup>	0.79±0.01 <sup>a</sup>	20.39±0.67 <sup>bc</sup>	
4B	NR	58.44±0.02 <sup>d</sup>	1.21±0.01 <sup>c</sup>	N/A	N/A	35.44±1.18 <sup>a</sup>	0.82±0.00 <sup>a</sup>	27.76±2.07 <sup>a</sup>	
	Soaking	64.30±0.01 <sup>b</sup>	1.37±0.01 <sup>b</sup>	13.82±0.19 <sup>b</sup>	1.53±0.06 <sup>ab</sup>	29.26±1.42 <sup>b</sup>	0.82±0.00 <sup>a</sup>	23.92±1.23 <sup>ab</sup>	
	Blanching	67.62±0.03 <sup>a</sup>	1.55±0.03 <sup>a</sup>	28.14±1.54 <sup>a</sup>	2.64±0.45 <sup>a</sup>	21.34±1.34 <sup>c</sup>	0.82±0.00 <sup>a</sup>	17.53±1.05 <sup>c</sup>	
	Boiling	61.37±0.04 <sup>c</sup>	1.22±0.01 <sup>c</sup>	9.42±0.37 <sup>b</sup>	0.70±0.01 <sup>b</sup>	27.07±0.93 <sup>b</sup>	0.80±0.00 <sup>b</sup>	21.67±0.79 <sup>bc</sup>	

**APPENDIX B. CONTINUED**

T1	NC	NR	54.86±0.04 <sup>d</sup>	1.17±0.01 <sup>c</sup>	N/A	N/A	26.07±0.35 <sup>a</sup>	0.77±0.00 <sup>a</sup>	20.00±0.25 <sup>a</sup>
		Soaking	60.14±0.07 <sup>b</sup>	1.30±0.01 <sup>b</sup>	10.73±0.48 <sup>b</sup>	1.90±0.02 <sup>b</sup>	17.99±1.05 <sup>b</sup>	0.75±0.01 <sup>a</sup>	13.46±0.79 <sup>b</sup>
		Blanching	63.24±0.04 <sup>a</sup>	1.43±0.02 <sup>a</sup>	17.43±0.68 <sup>a</sup>	3.34±0.27 <sup>a</sup>	20.99±1.65 <sup>b</sup>	0.74±0.02 <sup>a</sup>	15.62±1.55 <sup>b</sup>
		Boiling	58.35±0.09 <sup>c</sup>	1.21±0.00 <sup>c</sup>	7.15±1.01 <sup>b</sup>	1±0.01 <sup>b</sup>	27.30±1.38 <sup>a</sup>	0.73±0.01 <sup>a</sup>	20.09±1.22 <sup>a</sup>
	Water	NR	59.59±0.03 <sup>c</sup>	1.21±0.01 <sup>c</sup>	N/A	N/A	15.43±1.12 <sup>a</sup>	0.74±0.01 <sup>a</sup>	11.45±0.97 <sup>a</sup>
		Soaking	65.14±0.00 <sup>b</sup>	1.35±0.00 <sup>b</sup>	11.56±0.52 <sup>b</sup>	2.33±0.03 <sup>b</sup>	15.03±0.67 <sup>a</sup>	0.74±0.01 <sup>a</sup>	11.07±0.51 <sup>a</sup>
		Blanching	69.93±0.00 <sup>a</sup>	1.48±0.01 <sup>a</sup>	21.33±1.6 <sup>a</sup>	4.48±0.29 <sup>a</sup>	11.56±0.65 <sup>a</sup>	0.71±0.01 <sup>a</sup>	8.21±0.51 <sup>a</sup>
		Boiling	59.79±0.09 <sup>c</sup>	1.08±0.03 <sup>d</sup>	-0.36±0.16 <sup>c</sup>	1.1±0.04 <sup>c</sup>	14.01±1.29 <sup>a</sup>	0.71±0.02 <sup>a</sup>	10.01±1.11 <sup>a</sup>
	2B	NR	58.64±0.04 <sup>d</sup>	1.21±0.02 <sup>c</sup>	N/A	N/A	18.39±0.65 <sup>a</sup>	0.77±0.01 <sup>a</sup>	14.20±0.41 <sup>a</sup>
		Soaking	65.21±0.01 <sup>b</sup>	1.37±0.02 <sup>b</sup>	12.96±0.19 <sup>b</sup>	1.92±0.11 <sup>b</sup>	16.76±0.71 <sup>a</sup>	0.75±0.01 <sup>a</sup>	12.64±0.58 <sup>a</sup>
		Blanching	68.38±0.02 <sup>a</sup>	1.51±0.02 <sup>a</sup>	21.37±0.81 <sup>a</sup>	4.83±0.09 <sup>a</sup>	12.71±0.64 <sup>b</sup>	0.74±0.01 <sup>a</sup>	9.40±0.48 <sup>b</sup>
		Boiling	59.21±0.00 <sup>c</sup>	1.11±0.01 <sup>c</sup>	1.22±0.80 <sup>c</sup>	1.02±0.02 <sup>c</sup>	13.10±1.05 <sup>b</sup>	0.73±0.02 <sup>a</sup>	9.62±0.92 <sup>b</sup>
	4B	NR	60.81±0.01 <sup>d</sup>	1.19±0.00 <sup>c</sup>	N/A	N/A	15.18±0.51 <sup>a</sup>	0.76±0.00 <sup>a</sup>	11.49±0.38 <sup>a</sup>
		Soaking	67.40±0.03 <sup>b</sup>	1.36±0.00 <sup>b</sup>	14.09±0.14 <sup>b</sup>	2.05±0.05 <sup>b</sup>	12.57±0.80 <sup>ab</sup>	0.73±0.02 <sup>a</sup>	9.25±0.78 <sup>ab</sup>
		Blanching	70.24±0.00 <sup>a</sup>	1.47±0.01 <sup>a</sup>	21.50±0.45 <sup>a</sup>	3.92±0.33 <sup>a</sup>	10.38±0.72 <sup>bc</sup>	0.72±0.01 <sup>a</sup>	7.47±0.63 <sup>bc</sup>
		Boiling	61.51±0.00 <sup>c</sup>	1.10±0.00 <sup>d</sup>	2.51±1.24 <sup>c</sup>	1.31±0.01 <sup>b</sup>	8.39±0.75 <sup>c</sup>	0.73±0.02 <sup>a</sup>	6.10±0.61 <sup>c</sup>



**APPENDIX B. CONTINUED**

T2	NC	NR	55.28±0.00 <sup>d</sup>	1.17±0.00 <sup>d</sup>	N/A	N/A	21.81±1.59 <sup>ab</sup>	0.76±0.01 <sup>a</sup>	16.67±1.39 <sup>ab</sup>
		Soaking	61.95±0.01 <sup>b</sup>	1.34±0.00 <sup>b</sup>	14.29±0.07 <sup>b</sup>	1.63±0.06 <sup>b</sup>	21.03±0.64 <sup>ab</sup>	0.75±0.01 <sup>a</sup>	15.87±0.42 <sup>ab</sup>
		Blanching	65.32±0.01 <sup>a</sup>	1.44±0.02 <sup>a</sup>	22.48±0.82 <sup>a</sup>	3.59±0.45 <sup>a</sup>	17.06±1.22 <sup>b</sup>	0.75±0.02 <sup>a</sup>	12.82±1.03 <sup>b</sup>
		Boiling	60.20±0.03 <sup>c</sup>	1.25±0.01 <sup>c</sup>	9.10±0.95 <sup>c</sup>	1.09±0.01 <sup>b</sup>	24.53±1.55 <sup>a</sup>	0.74±0.01 <sup>a</sup>	18.21±1.21 <sup>a</sup>
	Water	NR	59.38±0.01 <sup>d</sup>	1.19±0.00 <sup>b</sup>	N/A	N/A	13.91±1.17 <sup>ab</sup>	0.77±0.01 <sup>a</sup>	10.72±0.98 <sup>a</sup>
		Soaking	65.71±0.02 <sup>b</sup>	1.37±0.01 <sup>a</sup>	14.80±0.94 <sup>a</sup>	2.24±0.11 <sup>b</sup>	14.69±0.60 <sup>a</sup>	0.73±0.03 <sup>a</sup>	10.71±0.74 <sup>a</sup>
		Blanching	68.93±0.04 <sup>a</sup>	1.45±0.05 <sup>a</sup>	18.83±3.45 <sup>a</sup>	4.38±0.27 <sup>a</sup>	11.17±0.65 <sup>b</sup>	0.69±0.02 <sup>a</sup>	7.67±0.42 <sup>a</sup>
		Boiling	60.06±0.05 <sup>c</sup>	1.14±0.03 <sup>b</sup>	1.77±0.62 <sup>b</sup>	1.23±0.02 <sup>c</sup>	12.70±0.71 <sup>ab</sup>	0.71±0.03 <sup>a</sup>	9.05±0.77 <sup>a</sup>
	2B	NR	58.58±0.01 <sup>d</sup>	1.19±0.01 <sup>c</sup>	N/A	N/A	16.03±0.44 <sup>a</sup>	0.74±0.02 <sup>ab</sup>	11.91±0.55 <sup>a</sup>
		Soaking	66.64±0.02 <sup>b</sup>	1.40±0.00 <sup>b</sup>	16.89±1.07 <sup>b</sup>	1.95±0.07 <sup>b</sup>	15.30±0.86 <sup>ab</sup>	0.76±0.01 <sup>a</sup>	11.66±0.58 <sup>ab</sup>
		Blanching	69.17±0.04 <sup>a</sup>	1.47±0.01 <sup>a</sup>	23.78±0.00 <sup>a</sup>	4.33±0.39 <sup>a</sup>	11.94±0.11 <sup>c</sup>	0.68±0.02 <sup>b</sup>	8.14±0.26 <sup>c</sup>
		Boiling	60.46±0.01 <sup>c</sup>	1.14±0.00 <sup>d</sup>	2.80±1.56 <sup>c</sup>	1.29±0.02 <sup>b</sup>	12.83±0.9 <sup>bc</sup>	0.74±0.01 <sup>ab</sup>	9.48±0.76 <sup>bc</sup>
	4B	NR	59.64±0.02 <sup>d</sup>	1.16±0.00 <sup>b</sup>	N/A	N/A	15.73±0.87 <sup>a</sup>	0.77±0.01 <sup>a</sup>	12.05±0.64 <sup>a</sup>
		Soaking	66.87±0.01 <sup>b</sup>	1.37±0.00 <sup>a</sup>	18.18±0.21 <sup>a</sup>	2.09±0.04 <sup>b</sup>	12.96±1.25 <sup>ab</sup>	0.75±0.02 <sup>a</sup>	9.80±1.12 <sup>ab</sup>
		Blanching	68.48±0.04 <sup>a</sup>	1.51±0.06 <sup>a</sup>	21.06±2.29 <sup>a</sup>	4.12±0.14 <sup>a</sup>	11.80±1.08 <sup>b</sup>	0.71±0.03 <sup>a</sup>	8.53±1.08 <sup>b</sup>
		Boiling	62.22±0.04 <sup>c</sup>	1.15±0.01 <sup>b</sup>	6.40±2.21 <sup>b</sup>	1.30±0.02 <sup>c</sup>	9.79±0.45 <sup>b</sup>	0.69±0.01 <sup>a</sup>	6.77±0.39 <sup>b</sup>

## APPENDIX B. CONTINUED

T3	NC	NR	55.15±0.01 <sup>d</sup>	1.16±0.00 <sup>c</sup>	N/A	N/A	23.80±0.14 <sup>a</sup>	0.77±0.01 <sup>a</sup>	18.31±0.30 <sup>a</sup>
		Soaking	61.53±0.03 <sup>b</sup>	1.33±0.01 <sup>b</sup>	14.20±0.79 <sup>b</sup>	1.63±0.06 <sup>b</sup>	25.24±1.37 <sup>a</sup>	0.78±0.01 <sup>a</sup>	19.61±1.13 <sup>a</sup>
		Blanching	65.01±0.03 <sup>a</sup>	1.47±0.02 <sup>a</sup>	23.29±0.17 <sup>a</sup>	4.07±0.22 <sup>a</sup>	18.74±0.52 <sup>b</sup>	0.74±0.01 <sup>a</sup>	13.93±0.46 <sup>b</sup>
		Boiling	60.50±0.00 <sup>c</sup>	1.26±0.02 <sup>b</sup>	13.74±0.79 <sup>b</sup>	1.14±0 <sup>b</sup>	23.07±0.96 <sup>a</sup>	0.76±0.01 <sup>a</sup>	17.51±0.61 <sup>a</sup>
	Water	NR	62.04±0.01 <sup>d</sup>	1.16±0.00 <sup>c</sup>	N/A	N/A	15.31±0.77 <sup>a</sup>	0.75±0.01 <sup>a</sup>	11.56±0.7 <sup>a</sup>
		Soaking	66.97±0.00 <sup>b</sup>	1.29±0.01 <sup>b</sup>	10.99±0.69 <sup>b</sup>	2.08±0.11 <sup>b</sup>	12.35±0.98 <sup>ab</sup>	0.73±0.02 <sup>a</sup>	9.12±0.9 <sup>ab</sup>
		Blanching	71.46±0.00 <sup>a</sup>	1.41±0.01 <sup>a</sup>	19.83±0.44 <sup>a</sup>	4.47±0.13 <sup>a</sup>	9.65±0.81 <sup>b</sup>	0.70±0.01 <sup>a</sup>	6.77±0.64 <sup>b</sup>
		Boiling	62.99±0.04 <sup>c</sup>	1.06±0.03 <sup>d</sup>	1.57±0.32 <sup>c</sup>	1.22±0.02 <sup>c</sup>	10.53±1.05 <sup>b</sup>	0.70±0.02 <sup>a</sup>	7.45±0.95 <sup>b</sup>
	2B	NR	59.16±0.03 <sup>b</sup>	1.18±0.00 <sup>c</sup>	N/A	N/A	13.98±0.83 <sup>ab</sup>	0.74±0.01 <sup>a</sup>	10.38±0.73 <sup>ab</sup>
		Soaking	63.78±2.39 <sup>ab</sup>	1.37±0.00 <sup>b</sup>	15.59±0.19 <sup>b</sup>	1.98±0.08 <sup>b</sup>	14.54±0.65 <sup>a</sup>	0.73±0.01 <sup>a</sup>	10.59±0.58 <sup>a</sup>
		Blanching	68.72±0.02 <sup>a</sup>	1.48±0.01 <sup>a</sup>	21.43±1.54 <sup>a</sup>	4.78±0.21 <sup>a</sup>	11.45±0.22 <sup>bc</sup>	0.69±0.02 <sup>a</sup>	7.87±0.30 <sup>b</sup>
		Boiling	60.94±0.02 <sup>b</sup>	1.12±0.01 <sup>d</sup>	1.91±0.06 <sup>c</sup>	1.06±0.02 <sup>c</sup>	10.90±0.91 <sup>c</sup>	0.72±0.02 <sup>a</sup>	7.91±0.85 <sup>b</sup>
4B	NR	60.51±0.01 <sup>d</sup>	1.21±0.00 <sup>c</sup>	N/A	N/A	15.66±1.49 <sup>a</sup>	0.73±0.01 <sup>a</sup>	11.47±1.18 <sup>a</sup>	
	Soaking	66.61±0.02 <sup>b</sup>	1.36±0.01 <sup>b</sup>	12.53±0.5 <sup>b</sup>	2.01±0.04 <sup>a</sup>	12.47±0.82 <sup>ab</sup>	0.73±0.02 <sup>a</sup>	9.21±0.81 <sup>ab</sup>	
	Blanching	69.17±0.00 <sup>a</sup>	1.44±0.00 <sup>a</sup>	19.97±0.87 <sup>a</sup>	3.76±0.6 <sup>a</sup>	9.75±0.24 <sup>bc</sup>	0.74±0.01 <sup>a</sup>	7.23±0.23 <sup>bc</sup>	
	Boiling	62.50±0.05 <sup>c</sup>	1.18±0.00 <sup>d</sup>	3.84±0.35 <sup>c</sup>	1.55±0.3 <sup>a</sup>	8.07±0.31 <sup>c</sup>	0.71±0.02 <sup>a</sup>	5.68±0.24 <sup>c</sup>	

<sup>a</sup> Means with different letters of the same style are significantly different ( $p < 0.05$ ).

<sup>b</sup> Recipes for texturization, C1 (control): soy concentrate and soy isolate, T1, T2, and T3: pea proteins, lentil proteins, and faba bean proteins premixed with a constant ingredient (canola oil and wheat gluten).

<sup>c</sup> NC=cooling in air, Water=cooling in water, 2B=cooling in 2% brine solution, and 4B=cooling in 4% brine solution.

## APPENDIX C

### Cook Sheet for Meat Patty with TPP

Group	TRT	Session	Order	Code	RawWt	TempOn	TimeOn	TempOff	TimeOff	CookWt
1	PI	1	1	812						
2	PI	1	1	283						
3	PI	1	1	400						
4	PP	1	1	244						
5	SC	1	1	335						
1	LP	1	2	524						
2	SC	1	2	951						
3	PP	1	2	306						
4	SC	1	2	712						
5	PI	1	2	614						
1	SC	1	3	112						
2	LP	1	3	466						
3	SC	1	3	212						
4	PI	1	3	251						
5	PP	1	3	595						
1	FP	1	4	326						
2	FP	1	4	469						
3	LP	1	4	235						
4	FP	1	4	373						
5	FP	1	4	897						
1	PP	1	5	379						
2	PP	1	5	522						
3	FP	1	5	169						
4	LP	1	5	740						
5	LP	1	5	333						

**APPENDIX C. CONTINUED**

1	FP	2	1	982
2	FP	2	1	792
3	PP	2	1	422
4	PI	2	1	941
5	PP	2	1	741
1	PI	2	2	888
2	PP	2	2	431
3	FP	2	2	291
4	LP	2	2	648
5	PI	2	2	928
1	SC	2	3	849
2	PI	2	3	314
3	PI	2	3	403
4	PP	2	3	544
5	SC	2	3	254
1	PP	2	4	243
2	LP	2	4	318
3	SC	2	4	870
4	SC	2	4	392
5	LP	2	4	777
1	LP	2	5	671
2	SC	2	5	141
3	LP	2	5	233
4	FP	2	5	950
5	FP	2	5	486

## APPENDIX C. CONTINUED

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1	SC	3	1	946
2	LP	3	1	819
3	LP	3	1	256
4	LP	3	1	780
5	SC	3	1	953

---

1	PI	3	2	878
2	FP	3	2	895
3	PP	3	2	942
4	PP	3	2	464
5	LP	3	2	230

---

1	FP	3	3	498
2	SC	3	3	131
3	FP	3	3	971
4	PI	3	3	731
5	FP	3	3	496

---

1	LP	3	4	589
2	PI	3	4	931
3	PI	3	4	171
4	SC	3	4	297
5	PP	3	4	644

---

1	PP	3	5	867
2	PP	3	5	440
3	SC	3	5	270
4	FP	3	5	369
5	PI	3	5	999

---

## APPENDIX C. CONTINUED

---

1	LP	4	1	691
2	SC	4	1	381
3	PP	4	1	321
4	PI	4	1	816
5	PP	4	1	890

---

1	PP	4	2	711
2	LP	4	2	619
3	FP	4	2	162
4	PP	4	2	109
5	FP	4	2	331

---

1	PI	4	3	238
2	PI	4	3	208
3	SC	4	3	103
4	FP	4	3	716
5	LP	4	3	747

---

1	FP	4	4	516
2	PP	4	4	730
3	LP	4	4	938
4	SC	4	4	121
5	SC	4	4	686

---

1	SC	4	5	650
2	FP	4	5	974
3	PI	4	5	447
4	LP	4	5	351
5	PI	4	5	538

---

## APPENDIX D

### Cook Sheet for Trained Panel Test of Vegetable Patties Containing HMMA

Trt	Code	RawWt	TempOn	TimeOn	TempOff	TimeOff	CookWt
T3	772						
C1	919						
T2	291						
T1	105						
T2	445						
T1	830						
T3	599						
C1	259						
T2	760						
T3	159						
T1	417						
C1	158						
T2	875						
C1	287						
T1	839						
T3	164						
C1	598						
T3	473						
T2	718						
T1	781						
T3	944						
C1	989						
T2	344						
T1	933						

## APPENDIX E

### Cook Sheet for Vegetable Patties with HMMA

Group	TRT	Session	Order	Code	RawWt	TempOn	TimeOn	TempOff	TimeOff	CookWt
1	T2	1	1	382						
2	C1	1	1	809						
3	T1	1	1	117						
4	T1	1	1	821						
5	C1	1	1	385						
1	C1	1	2	984						
2	T2	1	2	811						
3	C1	1	2	112						
4	C1	1	2	975						
5	T1	1	2	958						
1	T3	1	3	150						
2	T3	1	3	222						
3	T2	1	3	580						
4	T3	1	3	179						
5	T3	1	3	347						
1	T1	1	4	149						
2	T1	1	4	810						
3	T3	1	4	268						
4	T2	1	4	274						
5	T2	1	4	317						



## APPENDIX E. CONTINUED

---

1	T3	2	1	769
2	T3	2	1	283
3	T1	2	1	173
4	T2	2	1	587
5	T1	2	1	153

---

1	C1	2	2	772
2	T1	2	2	555
3	T3	2	2	927
4	T1	2	2	698
5	C1	2	2	543

---

1	T1	2	3	170
2	T2	2	3	260
3	C1	2	3	907
4	C1	2	3	956
5	T2	2	3	226

---

1	T2	2	4	606
2	C1	2	4	425
3	T2	2	4	108
4	T3	2	4	322
5	T3	2	4	110

---

APPENDIX E. CONTINUED

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1	C1	3	1	350
2	T2	3	1	351
3	T2	3	1	400
4	T2	3	1	262
5	C1	3	1	711

---

1	T3	3	2	200
2	T3	3	2	554
3	T1	3	2	721
4	T1	3	2	892
5	T2	3	2	973

---

1	T2	3	3	243
2	C1	3	3	169
3	T3	3	3	739
4	C1	3	3	515
5	T3	3	3	270

---

1	T1	3	4	727
2	T1	3	4	189
3	C1	3	4	455
4	T3	3	4	233
5	T1	3	4	303

---

## APPENDIX E. CONTINUED

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1	T2	4	1	338
2	C1	4	1	539
3	T1	4	1	545
4	T1	4	1	138
5	T1	4	1	572

---

1	T1	4	2	586
2	T2	4	2	953
3	T3	4	2	879
4	T3	4	2	339
5	T3	4	2	250

---

1	T3	4	3	287
2	T1	4	3	454
3	C1	4	3	815
4	C1	4	3	788
5	T2	4	3	843

---

1	C1	4	4	982
2	T3	4	4	621
3	T2	4	4	473
4	T2	4	4	746
5	C1	4	4	281

---

## APPENDIX F

### References and Cook Sheet for Trained Panel with HMMA

#### Day 1: Tuesday, October 31, 2017 References and Cook Sheet

- **Try meat analog**
- **Basic Tastes** - recognize intensity levels across attributes
  - Salty - The fundamental taste factor of which sodium chloride is typical.
    - 0.15% sodium chloride solution = 1.5 (flavor)<sup>[L]<sub>SEP</sub></sup>
    - 0.25% sodium chloride solution = 3.5 (flavor)
  - Sweet - The fundamental taste factor associated with sucrose.
    - 2.0% sucrose solution = 2.0 (flavor)
  - Umami - Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids and other molecules called nucleotides.
    - 0.035% Accent Flavor Enhancer Solution = 7.5 (Tasted)
      - 350 mg of Accent Flavor Enhancer in 1 L of deionized water.  
Serve in 1 oz cups.
- **Cardboard:** Aromatic associated with slightly oxidized fats and oils, reminiscent of wet cardboard packaging
  - Dry Cardboard = 5.0, aroma 3.0
    - Place a small 1in square of cardboard in 1 oz cups.
  - Wet Cardboard = 7.0, aroma 6.0
    - Soak squares of dry cardboard in 1 cup of water for 30 minutes.  
Place in 1 oz cups.
- **Grain:** The light brown, dusty, musty, sweet aromatic associated with grains.
  - Mixture of General Mills Rice Chex, General Mills Wheaties and Quaker Quick oats cereal = 8.0
- **Musty-Earthy/Humus:** Musty, sweet, decaying vegetation.
  - Mushrooms
  - Miracle-Gro Potting Mux Soil = 9.0 (Smelled)
    - Fill a 2-ounce glass jar half full with potting soil and seal tightly with screw-on type lid. Prepare one jar for every three panelists.
  - Le Nez du Café no. 1 “earthy” = 12 (Smelled)
- **Malt:** The light brown, dusty, musty, sweet, sour and or slightly fermented aromatic associated with grains.
  - Post Grape-Nuts cereal = 8.0 (f)
    - Serve cereal in a 1-ounce cup. Cover with a plastic lid.
- **Haylike:** Brown/green dusty aromatics associated with dry grasses, hay, dry parsley and tea leaves.
  - McCormick Dry parsley in medium snifter = 5.0 (Smelled)
    - ¼ teaspoon of dry parsley in 1 oz cups.

## APPENDIX F. CONTINUED

- **Buttery:** Sweet, dairy-like aromatic associated with natural butter.
  - Land O'Lakes Unsalted Butter = 7.0 (Tasted)
    - ½ tablespoon of butter in 1 oz cups.
- **Heated Oil:** The aromatics associated with oil heated to a high temperature.
  - Wesson vegetable oil = 7.0, Aroma 7.0
    - Microwave ½ cup oil on high power for 3 minutes. Let cool and pour into 1 oz cups.
  - Lay's Potato Chips = 4.0 (Smelled)
    - Place 4 whole potato chips in a large sniffer. Cover.
- Samples
  - 135 - 1
  - 745 - 3
  - 246 - 2
  - 621 - 4

### Day 2: Thursday, November 1, 2017 References and Cook Sheet

- **Green:** Sharp, slightly pungent aromatics associated with green/plant/vegetable matters such as parsley, spinach, pea pod, fresh cut grass, etc.
  - Fresh parsley water = 9.0 (Tasted)
    - 25 g of fresh parsley, rinse, chop, and add 300 ml of water. Let sit for 15 min. Filter and serve ½ oz of the liquid part in 1 oz cups.
- **Lentils:** The aromatics associated with Lentils
  - Lentils
- **Vegetable ID:** A general term that describes the aromatic of vegetables, in general.
  - Mixed vegetable medley
- **Celery:** Bitter aromatic, slightly astringent feeling factor, slightly salty taste, associated with celery.
  - Hearts: heart of celery from a fresh bunch of celery
    - Cut pieces and put in 2 oz cups.
  - Stalk: chopped raw celery
    - Clean celery and cut into small pieces.
- **Carrots:** Sweet, earthy aromatic characteristic of raw carrots.
  - Sliced carrots
    - Rinse carrots, peel with peeler or scrape outer skin, cut and discard carrot tops and root-ends, cut carrots in half.
- **Rooty:** The aromatics associated with plant roots.

## APPENDIX F. CONTINUED

- **Starchy:** The aromatics associated with the starch of a particular grain source.
  - Corn starch
    - Starch to water ratio 1:10 heat in microwave until boiling (160°F, 71°C) and cool.
  - Wheat starch
    - Gold medal all-purpose flour mixed half and half with water
- **Faba beans:** The aromatics associated with faba beans.
  - Faba Beans
- **Pea:** The aromatics associated with peas.
  - Peas
- **Soy:** The aromatics associated with extruded soy in water
  - Extruded soy in water
- **Sugar Snap Pea:** the aromatics associated with sugar snap peas.
  - Sugar snap peas
- Samples
  - 610 - 3
  - 733 - 1
  - 409 - 4
  - 530 - 2

### Day 3: Friday, November 2, 2017 References and Cook Sheet

- **Textures:**
  - **Cohesiveness of Mass:** The degree to which chewed sample holds together in a mass. **Technique:** chew sample with molars until phase change.
    - Shoestring Licorice = 0.0
      - Serve 1 piece in a 2 oz soufflé cup
    - Carrots = 2.0
      - Uncooked, fresh, unpeeled, serve ½ in slice in a 2 oz soufflé cup.
    - Mushrooms = 4.0
      - Uncooked, fresh, serve ½ in slice in a 2 oz soufflé cup.
    - Hebrew National Beef Frankfurter = 7.5
      - Boiled 5 minutes, cut into ½ in slice, served in 2 oz soufflé cup.
    - Land O' Lakes Yellow American Cheese = 10.0
      - ½ in cubes served in a 2 oz soufflé cup
    - Nabisco Fig Newtons = 14.0
      - Serve a whole newton in a 2 oz soufflé cup.

## APPENDIX F. CONTINUED

- **Hardness:** The force to attain a given deformation, such as: force to compress with the molars, force to compress between tongue and palate or force to bite through with incisors; **Technique:** force to compress between tongue and palate; force to bite through with incisors.
  - Philadelphia Cream Cheese = 1.0
    - ½ inch cube in 2 oz soufflé cup
  - Egg White = 2.5
  - Land O' Lakes Yellow American Cheese = 4.5
    - ½ in cubes served in a 2 oz soufflé cup
  - Hebrew National Frankfurter = 7.0
    - Boiled 5 minutes, cut into ½ in slice, served in 2 oz soufflé cup.
  - Planters Cocktail Peanuts, in vacuum tin = 9.5
    - Serve a few nuts in a 2 oz soufflé cup
  - Shelled Planters or Blue Diamond Almonds
    - Serve a few nuts in a 2 oz soufflé cup
  - Life Savers – 14.5
    - Serve 3 pieces, one color
- **Springiness:** The degree to which sample returns to original shape or the rate with which sample returns to original shape. **Technique:** place sample between molars; compress partially without breaking the sample structure; release.
  - Philadelphia Cream Cheese = 0.0
    - ½ inch cube in 2 oz soufflé cup
  - Hebrew National Beef Frankfurter = 5.0
    - Boiled for 5 minutes, cut into ½ in slice, served in a 2 oz soufflé cup
  - Kraft Miniature Marshmallow = 9.5
    - Serve 3 pieces in 2 oz soufflé cup.
  - Jell-O/Knox Gelatin dessert = 15.0
    - One package Jell-O and one package Knox gelatin are dissolved in 1 ½ cup hot water and refrigerated for 24 hours. Cut into ½ inch cube and serve in 2 oz soufflé cup.
- **Particle Size:** The degree to how large or small the particle is.
  - Small pearly tapioca = 4.0
  - Boba tea tapioca = 8.0
  - Large tapioca balls = 15.0

## APPENDIX F. CONTINUED

- **Slipperiness:**
  - Sabra Classic hummus = 2
  - Beechnut Stage 2 Baby food – peas = 3.5
  - Jello Chocolate pudding, instant, made with whole milk = 7.5
  - 
  - Breakstone Sour cream, full fat = 12.0
  
- **Samples**
  - 483 - 4
  - 670 - 2
  - 385 - 3
  - 714 - 1



## APPENDIX G

### Questionnaire for Consumer Panels for Meat Patty with TPP and Vegetable Hamburger Patties with HMMA

Date January 24, 2018

Panelist #

#### INSTRUCTIONS

Thank you for your participation in this study. Your assistance is very much appreciated. The objective of this study is to evaluate vegetable protein patty samples of pea, lentils, and faba bean proteins and pea isolates. Please take your time and evaluate the samples given to you carefully. Please proceed at your own rate.

This sampling will take you about 45 minutes and you will be eating 8 total samples. Please answer the following questions as completely as possible. If you have any questions, please ask the monitor for assistance.

Begin by filling out the basic demographic questions on the first page. This information is confidential and will not be used to solicit advertising nor will this information be published with your name associated with it.

After filling out the demographic information you are ready to start the evaluation. **BOLD LETTERS** throughout the questionnaire will give you directions on how to complete the evaluation.

Thank you very much for your help and opinions.

#### DEMOGRAPHIC INFORMATION

**Please circle each appropriate response.**

1. Please indicate your gender.

- Male  Female

2. Which of the following best describes your age?

- 20 years or younger  46 - 55 years  
 21 - 25 years  56 - 65 years  
 26 - 35 years  66 years and older  
 36 - 45 years

3. Please specify your ethnicity.

- African-American  Latino or Hispanic  
 Asian/Pacific Islanders  Native American  
 Caucasian (non-Hispanic)  Other

**APPENDIX G. CONTINUED**

4. Which of the following best describes your household income?  
 Below \$25,000                       \$75,000 - \$99,999  
 \$25,001 - \$49,999                 \$100,000 or more  
 \$50,000 - \$74,999
5. How many people live in your household including yourself?  
 1                       2                       3                       4                       5                       6 or more
6. Please indicate your employment level.  
 Not employed                       Part-time                       Full-time
7. Please circle any of the following proteins that you eat either at home or at a restaurant (away from home).

**At Home**

**Away from Home/Restaurant**

- |                                                        |                                                        |
|--------------------------------------------------------|--------------------------------------------------------|
| <input type="checkbox"/> Chicken                       | <input type="checkbox"/> Chicken                       |
| <input type="checkbox"/> Beef (steaks, roasts, strips) | <input type="checkbox"/> Beef (steaks, roasts, strips) |
| <input type="checkbox"/> Ground Beef                   | <input type="checkbox"/> Ground Beef                   |
| <input type="checkbox"/> Pork                          | <input type="checkbox"/> Pork                          |
| <input type="checkbox"/> Fish                          | <input type="checkbox"/> Fish                          |
| <input type="checkbox"/> Lamb                          | <input type="checkbox"/> Lamb                          |
| <input type="checkbox"/> Eggs                          | <input type="checkbox"/> Eggs                          |
| <input type="checkbox"/> Soy Based Products            | <input type="checkbox"/> Soy Based Products            |

8. How many times a week total do you consume the following protein sources?
- |                                           |                            |                              |                              |                              |                                    |
|-------------------------------------------|----------------------------|------------------------------|------------------------------|------------------------------|------------------------------------|
| <b>Beef cuts (steaks, roasts, strips)</b> | <input type="checkbox"/> 0 | <input type="checkbox"/> 1-2 | <input type="checkbox"/> 3-4 | <input type="checkbox"/> 5-6 | <input type="checkbox"/> 7 or more |
| <b>Ground beef</b>                        | <input type="checkbox"/> 0 | <input type="checkbox"/> 1-2 | <input type="checkbox"/> 3-4 | <input type="checkbox"/> 5-6 | <input type="checkbox"/> 7 or more |
| <b>Pork</b>                               | <input type="checkbox"/> 0 | <input type="checkbox"/> 1-2 | <input type="checkbox"/> 3-4 | <input type="checkbox"/> 5-6 | <input type="checkbox"/> 7 or more |
| <b>Lamb</b>                               | <input type="checkbox"/> 0 | <input type="checkbox"/> 1-2 | <input type="checkbox"/> 3-4 | <input type="checkbox"/> 5-6 | <input type="checkbox"/> 7 or more |
| <b>Chicken</b>                            | <input type="checkbox"/> 0 | <input type="checkbox"/> 1-2 | <input type="checkbox"/> 3-4 | <input type="checkbox"/> 5-6 | <input type="checkbox"/> 7 or more |
| <b>Fish</b>                               | <input type="checkbox"/> 0 | <input type="checkbox"/> 1-2 | <input type="checkbox"/> 3-4 | <input type="checkbox"/> 5-6 | <input type="checkbox"/> 7 or more |
| <b>Soy Based Products</b>                 | <input type="checkbox"/> 0 | <input type="checkbox"/> 1-2 | <input type="checkbox"/> 3-4 | <input type="checkbox"/> 5-6 | <input type="checkbox"/> 7 or more |

9. What cooking method do you prefer to use when cooking ground beef? Circle any that apply.
- |                                                                                                 |                                        |
|-------------------------------------------------------------------------------------------------|----------------------------------------|
| <input type="checkbox"/> Pan-frying or using a skillet on the stove                             | <input type="checkbox"/> Stir Fry      |
| <input type="checkbox"/> Grilling Outside                                                       | <input type="checkbox"/> Oven Broiling |
| <input type="checkbox"/> Oven Baking                                                            | <input type="checkbox"/> Microwave     |
| <input type="checkbox"/> Electric Appliance<br>(George Foreman Grill or another electric grill) |                                        |

## APPENDIX G. CONTINUED

10. What degree of doneness do you prefer your ground beef to be cooked to?  
 Rare    Medium-rare    Medium    Medium-well    Well    Very-well
11. What percentage of fat do you normally buy when purchasing ground beef?  
 4%    7%    10%    15%    20%    27%
12. What flavor or types of cuisines do you like, please circle all that apply?  
 American    Barbeque    Mexican/Spanish    Indian  
 Chinese    Greek    Japanese    Italian  
 French    Thai    Lebanese

APPENDIX H

**Ballot for Consumer Panels for Meat Patty with TPP and Vegetable Hamburger Patties with HMMA**

Sample Number \_\_\_\_\_

Order 1

Please take a bite of cracker followed by a sip of water prior to evaluating the vegetable protein patty. Place a mark in the box that represents your answer for each of the following questions.

1. How much do you like or dislike the COOKED APPEARANCE of the patty?

Dislike  
Extremely

Neither  
Like or Dislike

Like  
Extremely

2. How much do you like or dislike this patty OVERALL?

Dislike  
Extremely

Neither  
Like or Dislike

Like  
Extremely

3. How much do you like or dislike of the OVERALL FLAVOR of this patty?

Dislike  
Extremely

Neither  
Like or Dislike

Like  
Extremely

4. How much do you like or dislike of the OVERALL TEXTURE of this patty?

Dislike  
Extremely

Neither  
Like or Dislike

Like  
Extremely

5. Please write any words that describe what you LIKE about this patty.

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**APPENDIX H. CONTINUED**

6. Please write any words that describe what you DISLIKE about this patty.

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