Physiochemical Properties of Pulse Protein Isolates Based on Isoelectric Focused Precipitation

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Abstract

Pulse crops are growing in popularity for consumption of sole ingredients alone, but also as a source of protein in plant-based protein isolates. This growth is related to in part to the wide accessibility, positive environmental impacts and health benefits, and to increased versatility in food cropping systems. Gaining a more thorough understanding of pulse crops and their protein isolates will lay the groundwork for further developments in the field of plant-based protein food products. Currently, pulse proteins can be isolated by mixing milled flour into deionized water, dissolving the protein in an alkali pH and precipitating with an acidic pH. The practice of changing a solutions' pH to isolate protein is referred to as an isoelectric focused protein extraction. The key functional properties pulse protein isolates provide to a food product or system include forming gels, binding other ingredients, forming emulsions, or high solubility leading to high digestibility and forming foams. Through the investigation of testing different isoelectric focused pH's in protein extraction, the optimal protocol was determined for the greatest usage of pulse protein isolates across different applications.

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List of Abbreviations

AACC	American Association of Clinical Chemistry
ANOVA	Analysis of Variance (Used a one-way analysis of variance)
ESI	Emulsion Stability Index
FC	Foaming Capacity
FS	Foaming Stability
HCl	Hydrochloric Acid
LSM	Least Squares Mean
N	Nitrogen
NaOH	Sodium Hydroxide
N/R	Not Reported
OHC	Oil Holding Capacity
SDS-PAGE	Sodium dodecyl sulfate Polyacrylamide Gel Electrophoresis
SEM	Standard Error of Mean
TFA	Trifluoracetic Acid
RP-HPLC	Reversed-Phase High Performance Liquid Chromatography
WHC	Water Hydration Capacity

Review of Literature

1.1 Introduction

As the world population continually increases toward 8 billion people, the ability to feed a growing population is of great importance (Selcuk, 2016). Consequentially there is a growing interest in plant-based protein and plant-based protein products (Singh, 2017). Among the fastest growing plant-based proteins are pulse crops. Pulse crops are members of the legume family. Pulse crops are annual crops that produce one to twelve seeds, within a pod (Hall, Hillen and Robinson, 2017). A unique factor of pulses is that these crops provide both human food and animal feed. There are numerous reasons pulses are in-demand and being consumed at a high rate, such as soil health, low prices, and protein content (Niu et al., 2018; Pannell, 2003).

Lentils, a member of the pulse crop family, fix Nitrogen and require less water than wheat. Lentils produce positive health benefits to the soil. Lentils and other pulse crops are reducing the overall impact on soil and resources needed to produce them (Das and Ghosh, 2012). Legumes are advantageous to soil health because of to the ability to release organic acids from the root system which solubilizes phosphate and thus increases the nitrogen content in the soil (Niu et al., 2018; Das and Ghost, 2012). The more nitrogen in the soil the less fertilizer is needed. The implementation of legume crops provides an inexpensive protein source and restores soil health (Hall, Hillen and Robinson, 2017).

Another crucial reason pulse crops gained consumer acceptance is a growing movement of vegetarians and vegan consumers; individuals who do not eat meat or consume any products created by a living animal, respectively (Kaur et al., 2017). The

meat industry provides most of the average consumer's protein requirements. When switching to or increasing plant-based food in a consumer's diet, protein is still required. Pulse crops are extremely high in protein and production of pulse crops require less land than their animal-based counter parts (Raphaely, 2015), and are often more economical than most meat products. Comparatively to white and brown rice that have 13.1-14.7% protein pulse crops can have up to 30% protein (Gruss, 2019). Another key advantage of consuming pulse crops is the affordability that makes plant-based proteins sources vital in countries with widespread poverty.

On a global scale, related to ubiquity and cost-effective solution of consuming plants and plant-based protein products there is a need to increasingly more adept in processing these products. Through optimization of the protein extraction protocol it is possible to capitalize on the functional properties of the protein isolates which can be used in different applications in the food industry. Soy is the most comparable model to pulse crops in terms of applications in the food industry, but soy is characterized as an oilseed and is not a pulse crop. Some of the most common applications of soy are in beverages, meat analogs, breads, cakes, soups, gravies, cheeses, bologna, whipped toppings and chiffon desserts. Through the implementation of different extraction techniques, it is possible for pulse crop protein isolates to be used in similar applications to soy protein isolates (Lusas and Riaz, 1995).

Pulse crops provide a sustainable solution for a burgeoning population (Rubiales and Mickic, 2015; Chien, Prochnow and Cantarella, 2009). Furthermore, the investigation of the physiochemical properties of pulse proteins based on isoelectric extraction is needed to further develop the quality of these protein isolates.

1.2 Nutritional Value of Pulse Crops

1.2.1 Health Benefits of Pulse Crops

There are many health benefits from consuming pulse and legume proteins. Some of the key health benefits are improved digestion, increased satiety and decreased protein malnutrition (Hall, Hillen and Robinson, 2017). Furthermore, legumes and pulse crops are also high soluble and insoluble fiber (Iqbal et al., 2006).

Pulse crops provide small peptides, which are correlated to protecting cardiovascular health, reduced inflammation, cancer risks, weight control and increased insulin sensitivity (Carbonaro, Maselli and Nucara, 2015). The bioactive proteins and peptides derived from pulse crops have physiological, hormone-like beneficial effects in humans (Hall, Hillen and Robinson, 2017). Bioactive domains and sequences are encrypted in proteins with stable structural properties, characterized by a high percentage of beta-sheet conformation and high hydrophobicity (Singh et al., 2016). The formation of intermolecular beta sheet structures increase structural stability and resistance to gastrointestinal digestion of proteins from legume seeds (Carbonaro, Maselli and Nucara, 2015). This stability and resistance to gastrointestinal digestion is crucial to digesting proteins in the body (Butt and Batool, 2010).

Legumes and pulse crops contain a substantial amount of protein and fiber-both soluble and insoluble fiber. This is advantageous because proteins are needed to fulfill many different biological processes and metabolic reactions. Dietary protein is a necessity to maintain and rebuild muscles in the body and increasing one's intake is a viable option for treating obesity (Kaur et al., 2007). High protein diets are believed to help individuals feel full throughout the day and thus feel less hungry for longer periods of time. The

implementation of a high protein diet could allow overweight and obese individuals to be able to feel full while also consuming a lower number of calories throughout the day, thus aiding in weight loss (Carbonaroo, Maselli and Nucara, 2015; Messina, 2020). The soluble and insoluble fiber present promotes a sense of satiety (Erkkilä and Lichtenstien, 2006).

In addition to increasing the feeling of satiety, fiber is linked to a decreased risk of cardiovascular disease later in life (Hall, Hillen and Robinson, 2017). Insoluble fiber is credited with increasing the rate of digestion and decreasing the likelihood of colon cancer (Erkkilä et al., 2007; Hall, Hillen and Robinson, 2017). Soluble fiber reduces the rate of cardiovascular disease and can regulate sugar levels in many patients (Erkkilä and Lichtenstein, 2006).

Pulse crops also contain antioxidants, most commonly phenolic acid, flavonoids, anthocyanins and procyonids (Singh et al., 2016). Antioxidants are molecules that bind free radicals which decreases the oxidative stress on the body and are linked to decreasing one's risk of developing cancer (Hall, Hillen and Robinson, 2017).

1.2.2 Plant-Based Proteins' Effect on Protein Malnutrition

Malnutrition is a widespread problem in many areas of the world where the most severe form is protein energy malnutrition (PEM). The most afflicted countries have the lowest socioeconomic statuses. For example, some regions in India have an estimated 23% to 70% of the population suffering from PEM and 17% to 54% suffer from chronic energy deficiency (Das and Ghosh, 2012).

Pulses crops can assist in overcoming PEM because of the low-cost protein option sources (Carbonaro, Maselli and Nucara, 2015). Pulse crops are abundant in protein which complements meat consumption or be consumed in place of meat to allow individuals to

reach the recommended daily intake of protein, 50 grams of protein per day (Singh et al., 2016; Das and Ghosh, 2012). The protein content of legumes and pulse crops in their seeds are as follows: 25%, 22.1%, 20.1%, 22.5%, 27% and 40% for Rice beans, French beans, Cowpeas, Peas, Jack beans and Winged beans, respectively (Hall, Hillen and Robinson, 2017; Das and Ghosh, 2012).

1.3 Protein Composition of Pulse Crops

1.3.1 Pulse Proteins

There are multiple distinct applications of pulses and pulse protein isolates. The standard identity of a protein isolate must be defined. To be defined as a protein isolate, a substance must contain at least 90% protein. The application of pulse protein isolates is dependent on the physical and chemical characteristics of the crop itself. For instance, there are some differences in the protein content, composition, and extractability for each individual pea variety (Barac et al., 2010). Within each plant there are variances in the protein composition related to genetic variability and environmental conditions during growing season (Barac et al., 2010). There are no treatments that can be done to change the environmental conditions and genetic variability between each plant grown.

Proteins from pulse crops have a multitude of characteristics unique to them. One of the most essential functional characteristics is the solubility of the proteins. Due to the importance of solubility, many proteins are characterized based on their solubility (Gbadamosi, Abiose and Aluko, 2012; Argos, Narayana and Nielson 1985). The protein isolated from pulses and legumes are classified based onsolubility of the protein isolate in different solutions. Solubility of proteins is heavily influenced by the amino acids that makeup the protein. Furthermore, the solubility of a protein is altered by the environment a

protein molecule is in. For example, the pH of a solution the protein isolate is in greatly influences the solubility (Liu and Hung, 1998).

Solubility is critical when working with protein isolates because it is directly related to its digestibility in the body and solubility can be indicative of what forms of processing can be used on specific proteins. Solubility also demonstrates how a protein will react with other ingredients in the final product. A commonly used scheme for defining protein solubility is the Osborne fractionations– a method of determining the major proteins present based on what solvents the proteins solubilize into. The four major classes in this scheme are: water-soluble albumins, salt-soluble globulins, alkali-soluble glutelins and alcohol-soluble prolamins (Hall, Hillen and Robinson., 2017).

In pulses most of the protein is comprised of albumins and globulins, where albumins are soluble in water and globulins are soluble in salt solutions (Esen, 1986). The globulin protein fraction in pulse crops is rich in arginine, phenylalanine, leucine, and isoleucine (Ma et al., 2011; Hall, Hillen and Robinson, 2017). Globulins are the most widely distributed group of storage proteins and can be divided into 7S vicilin-type proteins and 11S legumin-type globulins (Chereau et al., 2016). Compared to globulins, pea albumins primarily consist of the essential amino acids: tryptophan, lysine, threonine, cysteine, and methionine (Kornet et al., 2020; Boye et al., 2015b). Globulins comprise approximately 70% of the legume seed proteins and have primarily 7S, 11S, 2S and 15S proteins with most legume seed proteins consisting of 7S and 11S (Millerd, 1975).

The numbering system of the proteins originates from the sedimentation coefficient (Stone et al., 2019). The sediment coefficient characterizes the sedimentation during centrifugation and is the ratio of a particle's sedimentation velocity to the applied

acceleration causing the sedimentation. The sedimentation coefficient ranges from 10.5-13 versus the vicilin-like globulins of the 7S family with a sedimentation coefficient values range from 7-9 (Millerd, 1975). There are four families of seed storage proteins that are classified and those four seed storage proteins in legume seeds are: 11S globulin, 7S globulin (which is also known as vicilin and convicillin),7S basic globulin and 2S sulfur-rich albumins (Millerd, 1975). There are some 2S albumins present in pea proteins and those 2S albumin proteins have small globular proteins with some cysteine residues present (Chereau et al., 2016).

When looking at the protein subunits in pulse crops the major storage proteins are quantified (Millerd, 1975). Pulse crop proteins consist of two major polypeptide units, the 11S legumins and 7S vicilin subunits, which function as the storage fractions (Chao and Aluko, 2018). While pulse crop proteins consist of mainly 11S and 7S proteins, the crops also have some 2S prolamin proteins in some pulse crops but in smaller quantities. The 11S is a globulin family of storage proteins. The subunits of the 11S are hydrophobic and inside the globulin. The major storage protein in peas are legumins, vicilin and convicilin, which are all globulins (Hall, Hillen and Robinson., 2017). Legumin and vicilin have sulfur in the amino acids functional groups (methionine and cysteine). The presence of amino acids with sulfur in the functional group is significant because it allows for the presence of disulfide bonds to form. These are strong chemical bonds that contribute to the functional properties and characteristics. The intermolecular forces that bind the subunits together during protein extraction and dehydration steps of the protein extraction changes based on the surface hydrophobicity (Taherian et al., 2011). The low solubility of commercial pea proteins is modified by the hydrophobic interactions between the peptides

and the acidic regions of the side chains in the functional groups. The isolation method used for extracting the pulse crop proteins affects characteristics of the isolated protein. An isoelectric focused method or using strong acidic buffers as a chemical treatment could be used. Depending on the method of protein extraction, the protein content isolated varies from 16.7 to 25.8% for yellow peas, but for other pulse crops the protein content is similar (Ma et al., 2011). Using isoelectric precipitation and ultrafiltration the protein concentrates isolates range from 63.9% to 88.6%. Ultrafiltration had a higher protein concentrate compared to isoelectric precipitation (Ma et al., 2011)

There are a multitude of factors that manipulate protein quantity that can be extracted among crops. Along with the variation related to the extraction method there are differences in the protein content between crops (Uken, Soestrisno and Holmes, 1992). Additionally, there are inherent differences in proteins that are available to be extracted within a sample. Based on current research the protein extraction methods that contribute the highest protein content is an isoelectric focused approach (Uken, Soestrisno and Holmes, 1992; Hall, Hillen and Robinson, 2017). Even with using an isoelectric focused methodology to extract the highest amount of protein, the volume of protein varies amount pulse crops.

In addition to protein solubility, protein activity or functionality is also a crucial characteristic. The activity of a protein depends on its conformation. Molecular forces within a protein dictate the folding of a given protein, which determines the activity or function of a protein. When proteins are in a structure that allows them to be chemically active, proteins are in a “native” conformation (Esen, 1986). Protein conformation is vital because for a protein to possess activity, potentially an enzyme function, it must be in in

the native form. Protein and amino acid structure are determined by forces within the molecule, but the structure is also greatly influenced by the physical environment. Proteins become denatured or lose their structure when undergoing extreme acidic and alkali conditions, physical shear, and high temperatures (Hall, Hillen and Robinson, 2017).

1.3.2 Amino Acid, Vitamin and Mineral Concentration, and Presence in Pulses and Legumes

Amino acids are the building blocks of proteins and are widely used in the body for numerous functions. There are essential and non-essential amino acids. Essential amino acids are amino acids that cannot be synthesized in the human body and need to come from dietary sources. Conversely, non-essential amino acids are amino acids that the human body can independently synthesize. For a food to be considered a “complete protein” it must comprise all of the essential amino acids present (Fernandez-Quintela 1997; Selcuk, 2016).

While pulses are not “complete proteins”, chickpeas, lentils and peas are high in lysine, leucine and arginine (Hall, Hillen and Robinson, 2017). This fulfills all essential amino acid needs of a diet except for sulfur-containing amino acids (Carbonaro, Maselli and Nucara, 2015). The major amino acid composition of chickpeas, peas, navy beans and lentils are shown in Table 1.1.

Lentil proteins lack amino acids cysteine and methionine from a nutritional standpoint but are still present (Fernandez-Quintela et al., 1997). Cereal proteins are also deficient in certain essential amino acids, particularly lysine. This makes legumes a great supplement to diets rich in cereal proteins because the legumes are high in lysine, but it is

still important to note that neither pulse crops nor legumes are “complete proteins”, but by mixing two proteins to balance the amino acid profile is balanced (Iqbal et al., 2006).

Along with amino acids in the body, the human body also requires vitamins and minerals. The uses for vitamins in the body vary from cofactors for enzymatic reactions to building bone tissue. Pulse crops generally lack vitamin A and C but have vitamin B present in multiple forms (Aslam, Horwath and VanderGheynst, 2008). Pulses have vitamin B in the forms of vitamin B1, B2 B3, B5 and B6 (Aslam, Horwath and Vander Gheynst, 2008). All pulses that have been studied are high in the following minerals: potassium, phosphorus, calcium, copper, iron, and zinc. Constituents of minerals in chickpeas and lentils are in Table 1.2 (Hall, Hillen and Robinson, 2017).

1.3.3 Anti-Nutritional Factors in Pulses

As discussed above, there are many health benefits of consuming pulse crops, including – increased sense of satiety, a multitude of vitamins and essential amino acids (Singh et al., 2017). However, one of the main concerns with the consumption of pulses is the presence of anti-nutritional factors. Anti-nutritional factors have detrimental effects in the body. The most common of these are protease inhibitors, phytates and oxalate. The severity of the side effects from consuming anti-nutritional factors varies (Messina, 2020). However, there are many different food processing techniques that can mitigate the effects of anti-nutritional factors including soaking, milling, fermenting and heating.

Anti-nutritional factors are plant compounds that mitigate the body’s ability to digest and absorb essential nutrients (Messina, 2020). The anti-nutritional factors in pulses include protease inhibitors, phytate, oxalate, tannins, saponins, polyphenols, amylase inhibitors and oligosaccharides. Tannins specifically mitigate the body’s ability to absorb

iron and have a bitter taste. The presence of anti-nutritional factors may lead to malnutrition if present during consumption. Protease inhibitors inhibit enzymes that hydrolyze proteins by decreasing the digestive tracts' ability to break down proteins which decreases the digestibility of proteins (Aviles-Gaxiola, Chuck-Hernandez and Saldivar, 2018). The most common protease inhibitors in pulses are trypsin inhibitors; trypsin is another enzyme in the body that breaks down proteins from large polymers into amino acids during digestion in the small intestine. These inhibitors in pulse crops can be mitigated almost entirely by heating, milling and proper storage. Although, the inhibitors present a challenge because it may require an alteration of the isolation process. This would lead to significantly lower protein yields (Chereau et al., 2016).

A shortage of dietary protein is an important public health issue and pulse crops are marketed as an effective plant-based protein to address the shortage. Therefore, it is important that the trypsin inhibitors do not reduce the digestion and absorption of proteins of pulse crops (Aviles-Gaxiola, Chuck-Hernandez and Saldivar, 2018). Thermal treatment inactivates the trypsin inhibitors in legumes and pulse crops (Messina, 2020).

Other common anti-nutritional factors include phytate and oxalate. Phytate can be converted into phytic acid, which can form insoluble complexes when exposed to minerals in the upper digestive tract, rendering them indigestible. In addition, high consumption of oxalate increases the likelihood of developing calcium-oxalate kidney stones. Phytate and oxalate also decrease calcium absorption (Messina, 2020). Decreased calcium absorption can lead to lower bone densities and early onset of osteoporosis (Carbonaro, Maselli and Nucara, 2015).

1.4 Protein Extraction Methods-Isoelectric Focused Protein Extraction

The process used to extract the protein from pulses is closely related to many of their functional and physiochemical properties. There are a few different parameters of the extraction process that are commonly changed including the pH, temperature, chemical modifications, and drying method used (Jarpa-Parra, 2018).

When completing a protein extraction from pulses, there are many factors to be taken into consideration, such as environmental growing conditions, protein treatment, as well as physiochemical and structural properties that influence protein properties (Niu et al., 2018; Jarpa-Parra, 2018; Stone et al., 2015a). Plant protein isolates are extracted through an alkaline extraction and precipitation at the isoelectric point (Lee, Htoon and Patterson, 2007). The precipitated pellet is then washed and dried and the drying can occur with freeze drying, spray drying or drum drying (Nielsen, Sumner and Youngs, 1981). The extraction method used needs to take into consideration the molecular and chemical makeup of the protein subunits (Jarpa-Parra, 2018; Jiang, Xong and Chen, 2010). A common concern with protein isolation is the partial unfolding of the protein (Tzitzikas et al., 2006; Shevkani et al., 2019).

The overall quality of the protein isolate is largely determined by the extraction method employed to isolate the protein. The overall quality of a protein isolated is decreased by the isolate being subjected to harsh environmental conditions including temperatures, alkaline pH, or long treatment times (Chang et al., 2011). The degree of denaturation and the composition of pea concentrate mixtures greatly impacts the functionality. The most common functional characteristics measured in proteins are emulsion stability, foaming stability, droplet size, solubility, water holding capacity, and

fat absorbing capacity (Aluko, Moflasayo and Watts, 2009; Karaca, Low and Nickerson, 2011).

There are multiple methods used to extract the protein from pulse crops, but isoelectric-focused is the most researched. Extracting protein from pulse crops are through using the isoelectric focused method (Karaca, Low and Nickerson, 2011). Isoelectric focused protein extraction relies on an alkali pH that solubilizes the sample and then an acidic pH to precipitate out the protein from the solution (Lee, Htoon and Patterson, 2007). Protein levels of pulse crop isolates ranges from 81-89% with isoelectric points occurring between pH 4.7-4.9 (Jarpa-Parra, 2018; Stone et al., 2015a). When pulse protein isolates were extracted at a pH 2.0 to 6.0 it showed an increased solubility of the protein isolates compared to protein isolates extracted with an acidic pH below 2.0 and between 6.0 to 7.0 (Mune and Sogi, 2015).

pH shifting is the process of inducing protein structural unfolding and following it by refolding the protein and inducing the unfolding with changes in the pH (Liu and Hung, 1998; Chang and Satterlee, 1981; Sathe and Salunkhe, 1981). Based on research with soy protein isolates, it is postulated that 11S globulin protein are more responsive to pH-shifting than the 7S globulins (Reddy, 2009). Additionally, the 11S subunits cause the functionality improvements induced by the pH-shifting treatments (Jiang, Xiong and Chen, 2011). Figure 1.1 is the method for aqueous extraction for pea protein (Kornet et al., 2020).

1.4.1 Drying Methods

In addition to the extraction method used the drying method also impacts the final quality of the protein isolate. The drying method used in the protein isolation is a key component in the protein extraction process. Drying method may change many properties

of the protein isolates. Some different characteristics that can be altered by the drying process are: the surface morphology, particle size distribution, protein subunit composition and secondary structures such as conformation (Chen et al., 2015). The process used to extract the protein isolate from pulses is closely related to many of their functional and physiochemical properties. A common concern with protein isolation is the partial protein unfolding of the protein (Lapidus, 2017).

1.4.2 Drying Methods with Varying Drying Methods and Temperatures

Physiochemical and functional properties of pulse protein isolates differ depending on the drying method(Suliman et al., 2006). The most common methods of drying are freeze drying, spray drying, vacuum drying, and drum drying (Joshi et al., 2011). Freeze drying is a method to remove moisture that uses low temperature dehydration with products that have been frozen before beginning the drying process. Freeze drying relies on temperatures typically around -60°C and extremely high vacuum pressure. Spray drying uses a slurry that coats the object being dried with an atomizer into a chamber with hot air. The slurry dries rapidly using hot gas and the moisture falls to the bottom of the drying chamber. Spray drying works well with heat-sensitive materials because of how quickly the moisture evaporates (Aberkane, Roudaut and Saurel, 2014; Gharsallaoui et al., 2009). Vacuum drying works by having a high vacuum pressure that causes the water to boil off at room temperature. Drum drying is a method of drying that produces a liquid or slurry material and applies it as a thin layer on the surface of moving drums heated with steam. As the drums move in a circle the product is dried and water evaporates (Suliman et al., 2006). Freeze drying is often used with protein isolates because it accomplishes the drying without using heat.

In a 2015 study, it was determined that spray dried lentil protein isolate powders had the highest solubility and better gelling properties but had less successful water absorption capacity compared to lentil protein isolates that were freeze and vacuum dried (Joshi et al., 2011).

When looking at the functional properties of drum-dried chickpeas and soybeans that were later milled into flour, the drum drying and chemical treatments the flours underwent prior to going into the drier changed them. The chemical treatments on the chickpea flour looked at a citric acid and sodium bicarbonate chemical treatment. The citric acid-treated flours had higher oil-absorption capacity and viscosity than the sodium bicarbonate treated flours. There were no significant differences between the soy and chickpea flours between the two chemical treatments in terms of water absorption and gelation capacity. However, the citric acid treatment of chickpea flours reduced the intensity of the bean flavor (Bencini, 1986).

When comparing soy and pea protein isolates using both freeze-drying and drum-drying techniques, pea isolates had higher fat absorptions compared to that for soy isolates. The fat absorption is influenced by the lipid-protein complexes and protein content. Spray dried pea protein isolates had the lightest color and a similar appearance as the soy protein isolates. The freeze and drum dried protein isolates had the darkest color. It is suggested that the dark color of the pea protein isolates is related to the oxidation of polyphenols (Nielsen, Sumner and Youngs, 1981). The color of the protein isolates is significant because color is associated with the quality and is an indicator of rancidity.

A 2015 study was conducted to determine the differences in the characteristics of cowpea and Bambara bean using vacuum, cabinet and freeze drying (Mune and Sogi,

2015; Kalapathy, Hettiararchychy and Rhee, 1997). The method of drying did not have a significant impact on the protein extraction yield (Mune and Sogi, 2015; Chagam, Haripriya and Suriya, 2013).

1.4.3 Drying Method with Varying Temperatures

Both the drying method and drying temperature impact the final protein isolate (Zhong et al., 2003). The drying methods and treatments had soy protein isolate slurry poured onto and dried in an air oven at the following temperatures: 30, 50, 70 and 90 °C. The different drying treatments did not cause further denaturation of soy protein isolate, but the viscosity increased. Furthermore, the drying treatments impacted the adhesion performance of the soy protein isolate adhesives (Hall, Hillen and Robinson, 2017). This suggests drying treatments enhance the solubility of soy protein isolates into water. The heat-treated soy protein isolates were easier to disperse into water and had an increased viscosity (Stone et al., 2015a). This suggests drying treatments may enhance the hydrophobic interactions between soy protein isolates. Drying methods influence the functional properties of all pulse protein isolates (Hu et al., 2009).

Soy protein isolates are the most researched plant-based protein isolates. By looking at the behavior of soy protein isolates it allows for similar conclusions to be inferred with other pulse protein isolates. The soy protein isolates obtained from spray-drying lead to less denaturation of the protein, less free sulfhydryl groups, smaller particles, and better solubility than vacuum and freeze drying (Hu et al., 2009). In addition, there are no significant differences in molecular sizes of unfolded molecules of spray-dried and freeze-dried proteins (Kalapathy, Hettiarachyand Rhee, 1997). The proteins in soy protein isolates and pea protein isolates are commonly compared. The spray-drying

method produces microcapsules with different surface morphologies and different internal structures depending on the composition of the emulsion of pea protein isolate that enter the spray dryer. The particle size and distribution of the structural integrity of pea protein layer is not compromised by the spray-drying process when high dextrose equivalent (19 and 28) carbohydrates were used (Gharsallaoui et al., 2009).

1.5 Functional Properties of Pulse Protein Isolates

1.5.1 Gel Strength

Protein isolates can be used to make gels (Pillai et al., 2019). In a gel, the protein molecules trap water into the spaces between the protein molecules (Hall, Hillen and Robinson, 2017). There are several different methods that can be used to form a gel, but regardless of the method the protein is heated with water and then cooled. The heating and cooling of the protein partially denatures the protein and allows the water in the solution to become trapped between the protein molecules. (Pillai et al., 2019; Pietraski, Jarmoluk and Shand, 2007). The strength of the gel can be indicative of the protein's strength when it is denatured and then attempted to renature into their native structure. In gelling procedures, the heating temperature, time, and pH of the gel are often modified to create the strongest gel (Liu, Low and Nickerson, 2009). The gelling point increases with increased heating rates (Arntfield and Sun, 2011). The gel strength of pea protein isolate was tested in the pH ranges of 3.5, 7 and 9, but no significant differences were found (Taherian et al., 2011).

When comparing pea protein isolates extracted using different techniques, the salt-extracted pea protein isolates were much stronger than the commercial pea protein isolates, but both pea protein isolates were weaker than the soy protein isolates in gelling (Arntfield, Ismond and Murray, 1990).

Globular proteins in protein-protein interactions are strengthened after denaturation during heating. The heating temperature and rate greatly impact globular protein gel network formation (Sandberg, 2011). A slower heating rate has a negative effect on the strength of the gel formed. Conversely, the slower cooling of legumin samples increases the strength of the gel. The gel forming properties of pea protein isolates are related to the environmental stress and genetic variety between different types of peas (O'Kane et al., 2004). Hydrogen bonds, which are favored at low temperatures, are one of the main stabilizing forces involved in gel structure of pea and legumin proteins (O'Kane et al., 2004). Hydrophobic amino acids have polar side groups and form hydrogen bonds that provide links for unfolded proteins and increase the gel strength. Non-polar amino acids are exposed from the globular protein interior during protein denaturation. The weaker gels at higher cooling rates are related to the lack of bond formation within and between pea protein molecules (Arntfield and Sun, 2011). Gel strength of pea protein isolates have previously been studied (Arntfield and Dong, 2011). However, gel strength of chickpea, Great Northern Bean, and lentil isolates have not been evaluated.

1.5.2 Water Holding Capacity

The water holding capacity is a measurement of how much water a protein or powder can absorb (Stone et al., 2015a). The water holding capacity is important because it allows for uniform mixing in a solution. The water holding capacity varies depending on the pulse or legume protein in question, but typically the water holding capacity varies from 0.6 to 2.7g/g (Hall, Hillen and Robinson 2017). There are significant differences in the water holding capacity for the pulse protein (Ma et al., 2011). Yellow pea protein isolate extracted through isoelectric precipitation had the highest water-absorbing capacity

while chickpea protein isolate had the lowest. The water holding capacity reported by Fernandez-Quintela et al. (1997) closely resembled the water holding capacity of soy protein isolate (Parades-Lopez, Ordorica-Falomir and Olivares-Vazquez, 1991; Fernandez-Quintela et al., 1997; Lee, Htoon and Paterson, 2007;). Samples with higher protein content had smaller particles and more starch fragments that may have contributed to a higher value for the water holding capacity (Pelgrom et al., 2013).

For each pulse protein isolate, the protein extract produced from isoelectric precipitation had a slightly higher water holding capacity than those produced using ultrafiltration, but the differences were not statistically significant water holding capacity can be used as a measurement of the functional properties of pulse flours (Toews and Wang, 2013; Toews and Wang, 2011). The water-holding capacity and other functional properties of pulse flours is influenced by the milling technique used (Hespell, 1998).

Air-classified mills use a dry process and are used to mill a variety of different products from applications in wheat to sand and gravel (Challa, Srinvasan and To, 2010). Air-classifier mills separate particles based on the density and use internal air currents that separate products based on their weight. Lighter particles are often separated in air-classified milled due to the low density. Dry milling grinds seeds using either a roller mill or a grindstone (Uken, Soetrisno and Holmes, 1992). Dry milling is often used because it can separate out the different parts of a seed. The different parts are then separated based on the product's end use which is determined by its composition. For example, with maize the endosperm is made up primarily of starch and is used to make flour and the embryo is made up primarily of lipids and produces corn oil (Uken, Soetrisno and Holmes 1992).

Wet milling is a process that uses different chemicals, typically sulfur dioxide to assist in breaking up seeds into different components (Hespell, 1998).

1.5.3 Fat Absorption Capacity

Much like water holding capacity, depending on the pulse protein isolate, there are significant differences among the pulse isolates in fat absorption capacity (Hall, Hillen and Robinson, 2017). The fat absorption capacity is the measurement of the amount of oil that is absorbed into a powder or protein (Hall, Hillen and Robinson 2017). The fat absorption capacity is extremely important in the context of flavor compounds because flavor compounds are fat soluble and there are multiple fat-soluble vitamins- Vitamins A, D, E and K (El-Adawy, 2000). Having a high fat absorption capacity allows for greater uniformity in each food product and prevents having visible portions of fat, which is unappealing to consumers and fat absorption capacity allows for better texture in batters and emulsions. Red lentils and yellow pea protein isolates extracted using ultrafiltration had the highest fat absorption capacity compared to protein isolates extracted using an isoelectric focused protein extraction (Adebiyi and Aluko, 2011). It is postulated that differences in the fat absorption capacity among pulse crops relate to the specific pulse crop, specific variety tested and processing conditions (Boye and Barbana, 2010).

1.5.4 Solubility

Solubility is how well one substance can dissolve into a solvent (Hall, Hillen and Robinson, 2017). Solubility can be determined by many different factors-including the physical properties of the substance being dissolved and the method used to isolate the protein. Solubility is significantly higher in isolates produced through isoelectric

precipitation than compared to ultrafiltration and of protein isolation (Fernandez-Quaintela, Macarula et al., 1997).

Solubility, much like many other functional properties of protein isolates, is dependent on protein-protein and protein-solvent interactions (Taherian et al., 2011). The hydrophobicity of both the protein and the solvent is a determining factor of the protein-protein and protein-solvent interactions. Protein-protein interactions are determined by hydrophobic interactions while protein-solvent interactions can cause water absorption and solubilization (Hespell, 1998).

The solubility of the proteins varies based on the pH of the protein isolate. Higher pH values were more effective at solubilizing the proteins compared to more acidic solutions. The pH-protein solubility profiles are strongly dependent on the pH value (Taherian et al., 2011). The highest solubility for pulse protein isolate ranges from pH 1.0 to 3.0 and from 7.0 to 10.0. Other studies have proven the lowest solubility for pulse and legume protein isolates to be between pH 4.0 and 6.0 and to be the highest between pH 8.0 to 9.0 (Parades-Lopez, Ordorica-Falomir and Olivares-Vazquez, 1991; Fernandez-Quaintela, 1997).

Occurrence of minimum solubility near the isoelectric point is primarily related to the net charge of peptides, which increase as pH moves away from the isoelectric point. This pH shift promotes the aggregation and precipitation via hydrophobic interactions leading to insolubility (Gbadamosi et al., 2012). The decrease in protein solubility at a pH lower than the isoelectric point is caused by predominant electrostatic screening of the positively charged protein and by adsorption of chloride ions by the protein (Jarpa-Parra, 2018).

1.5.5 Emulsifying Ability

Emulsions are immiscible mixtures of at least two different components that are hydrophobic and hydrophilic (Joshi et al., 2011). The most effective emulsions have emulsifying agents or emulsifier with a portion of the molecule that is hydrophobic and one that is hydrophilic (Ladjal-Ettoumi et al., 2016). With both a hydrophobic and hydrophilic portion, the emulsifier decreases the surface tension between the two repelling constituents and reduces any time-sensitive separation of the two constituents (Karac, Low and Nickerson, 2011). Greater integration of the oil and lower surface tension leads to higher emulsion capacities

There are many factors that contribute and impact the emulsifying ability of protein isolates (Kornet et al., 2020). The emulsifying activity of chickpeas, faba beans, lentil and pea protein isolates vary based on the isoelectric precipitation and salt extractions used to isolate the protein (Naczk, Rubin and Shadhidi, 1986). Protein isolates that were isolated using isoelectric precipitation had a greater charge compared to the protein isolates that were isolated using a salt extraction (Papalamprou, Doxastakis and Kiosseoglou 2010; Liu, Low and Nickerson, 2009).

The source of the pulse protein isolate and the method of isolation also impact the emulsifying ability (Karac, Low and Nickerson, 2011; Swanson, 1990). Some of the other key factors that influence emulsifying activity are molecular size of the emulsifier, surface hydrophobicity, net charge, steric hindrance and molecular flexibility. Typically, the process to isolate protein from the pulse and the specific variety of pulse tested did not have a significant impact on the emulsifying ability. Proteins generally have emulsion activity at the respective isoelectric pH. The poor emulsion capacity is related to their low

solubility, poor hydration and lack of electrostatic repulsive forces (Karac, Low and Nickerson, 2011). High electrostatic repulsion between the individual oil droplets causes better emulsion stability. However, near the isoelectric point there is an increase in the ionic strength of the droplets which increases droplet flocculation and leads to poor emulsion stability (Karac, Low and Nickerson, 2011).

Previous research measured the emulsifying ability of different pea flours and, determined the higher the protein content of the pea flour the lower the surface tension (Joshi et al., 2011). Increasing the formation of small oil droplets stabilizes the emulsion and makes a more effective emulsifier (Adebiyi and Aluko, 2011). This research measured the stability of emulsion using a volume weight mean as an indication of stability over time.

Previous research has shown a link between the emulsifying ability of pea protein and the pH of the pea protein. The lowest emulsifying ability occurs with pea protein at a pH of 5.0 while the emulsifying ability greatly increases at, above and below pH 5.0. The highest emulsifying ability for pea proteins occurs at pH 8.0 (Barac et al., 2010). A theory explaining the low emulsifying activity of pea protein at pH 5.0 is related to increased protein-protein interactions and reduced solubility, which caused an increase in the surface tension.

The emulsion stability of lentil protein isolate was tested (Ma et al., 2011). The emulsion stability increased by increasing the sample concentration from 10mg/ml to 50mg/ml. The emulsions were more stable and had lower droplet sizes when in a solution with a pH higher than pH 3.0, 5.0 and 7.0 (Aluko, Mofolasayo and Watts, 2009). When looking at the emulsion stability of a single protein isolate some researchers measured the

emulsion stability by using multiple pulse protein isolates to find the most successful combination (Ma et al., 2011; Liu and Hung, 1998).

Proteins have a multitude of uses in the food industry (Shevkani and Singh, 2014). Proteins are the major emulsifying agent in many foods and allow for more stable emulsions in food products. The better an emulsifying agent a protein is, the more likely the isolate is to be integrated into the food systems successfully (Joshi et al., 2011).

1.5.6 Foaming Ability

A foam is a liquid that trapped air bubbles into small pockets within itself through mechanical force or a chemical reaction (Uken, Soetrisno and Holmes, 1992). Foams have a higher volume than the liquid portion alone. The ideal foam-forming and foam-stabilizing protein is a protein with low molecular weight, high surface hydrophobicity, food solubility, and a small net charge for the pH of the food (Barac et al., 2010). The increased formation of the small oil droplets led to more surface contact with the hydrophilic or the liquid portion of the emulsion. The higher contact with the small oil droplets and the liquid portion led to increased solubility of the liquid portion which led to a higher foaming capacity (Adebiyi and Aluko, 2011).

At a higher pH, pea proteins had a structural conformation more suitable for interfacial membrane formation (Taherian et al., 2011). The foaming ability of pea proteins is closely related to the emulsifying ability. As the pH increases, the net charge of the proteins increases, that leads to more protein unfolding and flexibility allowing more stable foams to form (Barac et al., 2010). The foaming ability of a pea protein is entirely dependent on the pH. The pea protein isolates with the best foaming ability were associated with an increased net charge of proteins and surface hydrophobicity of the

highly charged areas (Taherian et al., 2011). The foaming capacities of the protein fractions were higher at pH 9.0 than at pH 4.0 or 7.0 (Abayomi and Aluko, 2011).

The research suggests the interactions of pea starch with soybean and pea protein led to successful foams. The emulsions and foams made with multiple pulse proteins were dependent on the pea protein levels in pea flours. The interfacial membranes in the emulsions with mixed protein isolates were dependent on the oil-water and water-air interfaces and were determined by the protein-protein interactions to provide good emulsion and foaming stabilities (Aluko et al., 2009).

1.5.7 Chemical Modifications

There are different chemical modifications that can be done during protein extractions. The main chemical modifications previously performed on pulses and legumes are succinylation, acetylation and deamination. These modifications do not modify the nutritional properties of the proteins. Because of the presence of lysine in lentil proteins, it is possible for the lentil proteins to undergo succinylation. This process adds a succinyl group to the lysine of the amino acid's side group with the objective of shifting the isoelectric point to improve the solubility of the protein. Succinylation of lentil globulin proteins shifted the isoelectric point from 4.5 to 3.5 pH and improved the solubility above a 4.0 pH (Esen,1986). However, below pH 4.0, the solubility of succinylated globulins decreased. The water absorption capacity and viscosity of the succinylated proteins increased (Bora, 2002). Another type of chemical modification done to proteins during extraction is deamination. Deamination removes an amine group from the protein being isolated (Ma et al., 2011).

Chemically modifying the protein subunits of different pulses and legumes is done on many other crops as well. In a study done with oat protein isolates, there were effects on the functional properties based on the chemical treatment of the isolates, either deamination or succinylation (Hall, Hillen and Robinson, 2017). With either method, the water and oil binding capacity were higher than activities in unaltered protein isolates. Succinylation and deamination increased the solubility, foaming capacity, and emulsifying activity of the native oat protein. The water-binding capacity increased more for the succinylated proteins, but the fat-binding capacity and foaming capacity increased more for the deamidated proteins (Hall, Hillen and Robinson, 2017).

Another potential chemical modification to proteins to change the functional properties is through acetylation, which involves binding an acetyl group to the side chain of an amino acid (Bora, 2002).

Chemical modifications are performed on a wide range of pulse crops. When comparing succinylated to acetylated mung bean protein isolates succinylation increased the solubility (El-Adawy, 2000). Whereas acetylation increased foam capacity and stabilities. However, both acetylation and succinylation increased the water and oil absorption capacities. Acetylation is more effective at improving the in-vitro protein digestibility compared to succinylation (El-Adawy, 2000; Bora, 2002).

1.5.8 Future Opportunities for Pulse Crops

Pulse crops are rapidly expanding, but there are a multitude of future opportunities surrounding them. While soy is used as a systematic model, soy is an oilseed and is in a different classification of crops. Soy is the model when designing research questions and experiments about pulse crops because it is abundantly used in the food industry and more

widely implemented than pulse crops (Karaca, Low and Nickerson, 2011). In addition to providing a model for pulse crop research, soy replacement in products provides the greatest opportunity for pulse crops to gain market share. While soy has led the research in plant-based protein soy is made up of a lipid component of approximately 20%. From the high lipid component whenever soy is processed it requires a defatting step that pulse crops discussed do not (Chereau et al., 2016). There are numerous methods used to extract proteins from pulse crops. The isolation procedure used greatly influence the functional properties of the proteins after extraction.

By optimizing the extraction protocol for pulse crops, it is possible to capitalize on the functional properties of the protein isolates which can be used in different applications in the food industry. Some of the most common applications of soy are beverages, meats, breads, cakes, soups, gravies, cheeses, bologna, whipped toppings, and chiffon desserts. Through the implementation of different extraction techniques, it is possible for pulse crop proteins to be used in similar applications to soy protein (Lusas and Riaz, 1995).

In meat products, soy proteins emulsify the water and fats and to improve the texture of processed meat. The same techniques are also used with poultry and fish. Soy proteins absorb several times their weight in water. In bakery products, the enzyme-activated soy flours are up to 0.5% of flour-weight basis and often used in standardized baked goods. In most bakery flours, there are lipoxidase enzymes used to bleach the carotenoid pigments in the flour and strengthen the gluten in the flour. Some of the other frequent uses of soy protein isolates include to replace dry skim milk and in sauces and low-fat spreads (Lusas and Riaz, 1995). The addition of soy protein isolates is significant

because soy protein isolates are commonly used as the model for plant-based protein isolates in the food industry.

Soy is currently used in the food industry, but pulse crops could easily be used as a replacement. Soy is included in the top eight Allergen List and even though only 0.3% of Americans have a soy allergy, soy is extremely prevalent in many different foods (Stone et al., 2015b). Pulse protein isolates have extremely close functional characteristics to soy protein isolate but are not on the 'Big 8' allergen list.

1.6 Conclusion

There are a multitude of methods that can be used in order extract protein from flour and change the functionalities of the protein extracted from pulse crops. With the growing global population and popularity of plant-based proteins there is a need for plant-based protein to be extracted in an efficient way. By adjusting the pH treatments in protein extractions, it is possible to first solubilize the protein from a solution and then precipitate out the protein. Changing the pH treatments can increase the overall protein yield from pulse crops and enhance the final physical and chemical properties of the pulse protein isolates.

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Tables and Figures

Table 1.1: Amino Acid Concentrations in Chickpeas, Lentils, Peas and Navy Beans

Amino Acids[c]	Chickpeas (g/16g N)	Lentils (g/16g N)	Peas (g/16g N)	Navy Beans[b] (g/16g N)
Alanine	4.4[d]	4.2[d]	4.5[d]	4.5[d]
Arginine	10.3	7.2	7.9	7.2
Aspartic Acid	11.4	11.3	11.9	13.2
Cysteine	1.3	N/R[a]	N/R[a]	1.1
Glutamic Acid	17.3	15.1	16.5	16.3
Leucine	7	7.2	7.5	6.7
Lysine	7.7	6.8	7.7	7.1
Proline	4.6	6.8	4.2	N/R[a]
Serine	4.9	4.3	4.1	6.8
Threonine	3.6	3.6	3.8	4.7
Tyrosine	3.7	N/R[a]	N/R[a]	3.8

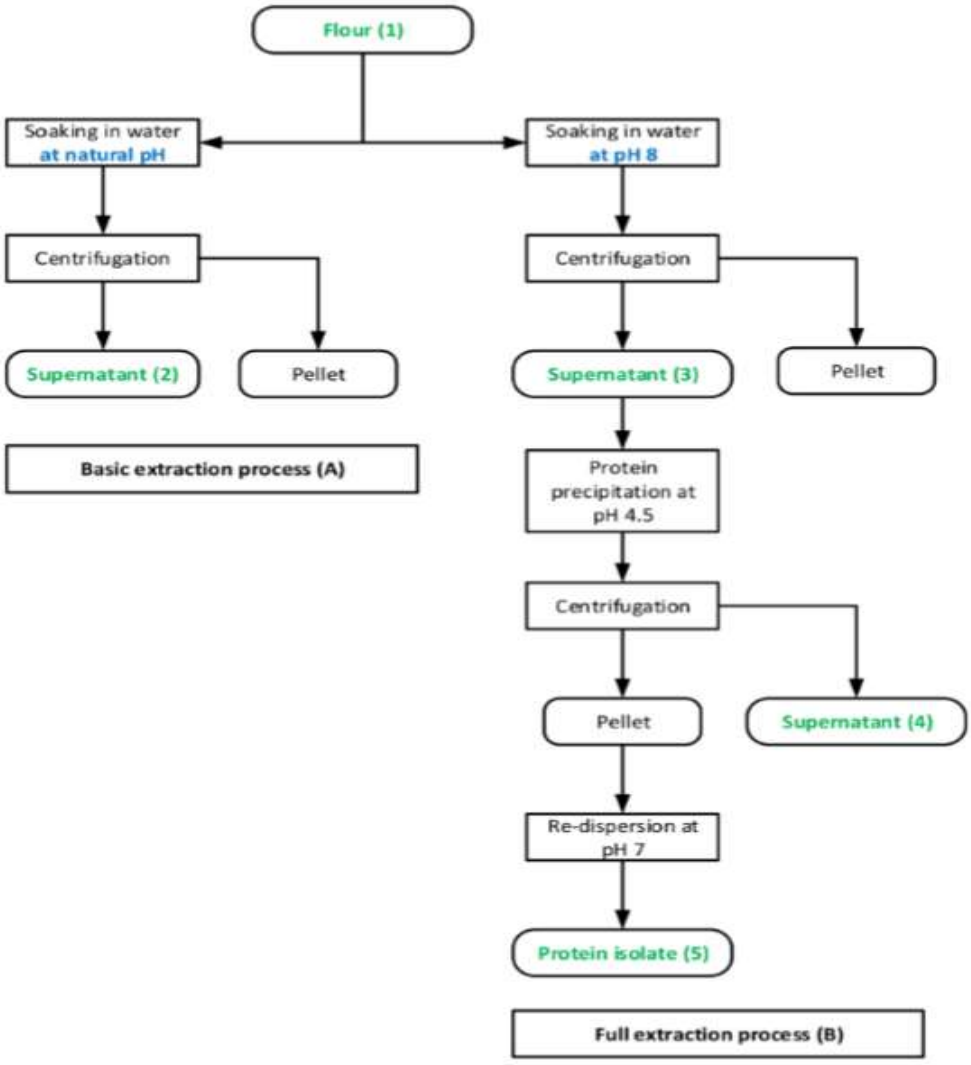
[a]Value is not reported (N/R)
[b]Navy beans are very close in composition to Great northern beans
[c](Hall, Hillen and Robinson, 2017).
[d] All measurements were made in g/16g N

Table 1.2: Minerals in Chickpeas, Great Northern Beans, Peas and Lentils

Minerals [b]	Chickpeas (mg/100g)	Great Northern Beans (mg/100g)	Peas (mg/100g)	Lentils (mg/100g)
Sodium	101[c]	N/R[a]	N/R	79
Potassium	115	1,207-1,759	876-1,463	874
Calcium	82–272	146–176	6–11	59–463
Phosphorus	251	388-603	279-291	294
Iron	4.6–7.5	2.8-7.6	1.9-8.0	6.3-9.2
Copper	11.6	N/R	N/R	9.9
Zinc	3.4–4.4	1.9–3.0	3.0–3.4	2.6–3.8
Magnesium	147-195	149-230	130-172	99-726

[a]Value is not reported (N/R)
[b](Amjad, Khalil and Shah, 2006; Hall, Hillen and Robinson, 2017)
[c] Measured in mg/100g

Figure 1.1: Diagram of Isoelectric Focused Protein Extraction Method of Pulse Protein Extraction



(Adapted from Lee, Htoon and Paterson, 2007)

Chapter 2: Physiochemical Properties of Pulse Protein Isolates Based on Isoelectric Focused Precipitation

2.1 Abstract

There is an increasingly growing interest in plant-based proteins and one of the most popular type of crops used to in protein extractions are pulse crops. Using an isoelectric focused protein extraction process with yellow peas, red lentils, chickpeas and great northern beans the optimal isoelectric pH conditions were determined awith the isoelectric conditions used that led to the highest protein content and greatest physical and chemical properties of the protein isolates. The physiochemical properties tested at the yield weight of the protein isolates, total protein content, protein subunits present determined by SDS-PAGE, protein solubility in buffers with pH values from 3.0 to 9.0, oil holding capacity, water holding capacity, foaming capacity and emulsion stability index.

The objective of this project was to determine the ideal conditions when working with an isoelectric-focused protein extraction process in pulse crops. In the testing completed, it was determined there is a significant interaction between the alkali solubilizing pH and the precipitating acidic pH. There are significant interactions between the conditions used to extract the protein isolate and the overall protein yield for each of the crops yellow pea, red lentil, chickpea and great Northern bean. There were similar trends when looking at the protein content changing with the acidic pH regardless of the alkali pH in both yellow pea and red lentil protein isolates. When looking at the different protein isolates, the sample with the highest level of protein typically had the highest values in the physiochemical properties tested. In the yellow pea, great Northern bean and

red lentil crops, all of the protein isolates had higher protein content when solubilized at pH 8.0 instead of at pH 9.0. In all the crops. For all pulse crops tested, extraction pH of 9.0 resulted in higher yields but poorer solubility. Out of the pulse crops tested, the yellow pea protein isolates had the best overall profile of the crops and the yellow pea protein isolates extracted at pH 4.4/8.0 had the highest quantity of protein extracted.

2.2 Introduction

Pulse crops have become continuously more popular with consumers due to a shift in a more heavily based plant-based diet without decreasing protein intake (Selcuk, 2016; Shevkani and Singh, 2014). One of the best types of crops to use in a plant-based protein extraction are pulse crops (Reddy, 2009; Swanson, 1990). Pulses are a group of plants that are annual crops that yield one to twelve grains or seeds within a pod (Hall, Hillen and Robinson, 2017). Pulse crops can be used for both animal and human consumption (Singh, 2017). Key examples of pulse crops are peas, lentils, navy beans, garbanzo beans and kidney beans (Ma et al., 2011; Erkkila and Lichtenstein, 2006). Not only are pulses cost-effective for human consumption, but pulse crops also improve the overall health of the soil and have numerous health benefits for consumers who regularly eat them (Messina, 2020; Carbonaro, Maselli and Nucara, 2015; Raphaely, 2015; Pannell, 2003). Pulse ingredients are linked in preventing and managing diabetes, preventing obesity and assisting in digestion because of their fiber content (Hall, Hillen ad Robinson 2017,). One of the key concerns of consuming a plant-based diet is consuming enough protein (Das and Ghosh, 2012). Unlike many other fruits and vegetables, pulse crops have a high amount of protein and a high amino acid content (Jarpa-Parra, 2018; Selcuk, 2016). Pulse crops typically have a protein content between 15 to 30% but Great Northern beans are 23-27%, yellow peas 20-24%, red lentils 24% and 21% in chickpeas (Hall, Hillen and Robinson 2017).

While pulses are continually growing in popularity and beginning to be used in plant-based protein products, there is still research needed to produce higher quality isolates to be used in a multitude of applications (Hu et al., 2009; Bencini, 1986). The

method used to extract the protein portion of pulse crops is closely tied to the functionalities of the protein isolates (Jiang, Xiong and Chen 2011; Jiang, Xiong and Chen, 2010; Gharsallaoui et al., 2009). The most popular method to extract protein from pulse crops and make protein isolates is through an isoelectric focused extraction process (Lee, Htoon and Patterson, 2007; Kalapathy, Hettiarchychy and Rhee, 1997). In this, the crop is milled into a flour and then chemically treated first with an alkali pH to solubilize the protein and then with an acidic pH to precipitate out the protein (Hespell, 1998; Uken, Soetrisno and Holmes, 1992). This experiment tested a multitude of physical and chemical properties of protein isolates extracted from yellow pea, chickpea, red lentil and Great Northern bean crops. The objective is to determine which isoelectric protein extraction methodology produces the highest yield in weight, protein content and had the highest values in the physiochemical properties measured.

The physiochemical properties tested are protein content, foaming stability, emulsion stability, oil holding capacity and water holding capacity protein solubility, as well as running SDS-PAGE to identify and quantify the protein subunits. Through the implementation of different physiochemical tests, the objective is to determine which protein extractions allows for a final product with the highest protein content while also being the most adaptive and advantageous to be used as an ingredient in food products.

2.3 Materials and Methods

Yellow pea, Great Northern beans, red lentils and chickpeas seeds were acquired from Palouse Brand based out of Palouse, Washington. Samples from each pulse crop were ground using a flour mill (Double Disc Grinding Flour Machine, Thomas, Bolingbrook,

Illinois) and stored in wide mouth glass Mason jars at room temperature until subsequent analysis. All analyses were run in triplicates.

2.3.1 Pulse Protein Wet Extraction Method

Figure 2.1 highlights the protein wet extraction method. Briefly, a 1:10 mixture of flour to water was prepared using 100 g of ground pulse flour (Palouse Brand, Palouse, Washington) and deionized water. The pH of the solution was adjusted to either 8.0 or 9.0 (Seven Compact pH Meter S210-Uni-Kit, Mettler-Toledo, Columbus, Ohio) with 6N NaOH and stirred for 1 hour on a stir plate (Corning Model PC-420, Toledo, Ohio) at 220 RPM. Following being stirred, the solution was centrifuged at 12,298 *x g* using a large capacity high-speed centrifuge (ScanSpeed 1736R, Labogene, Lillerod, Denmark) for 10 min. The supernatant was then removed. The pH of the supernatant was adjusted to 4.0, 4.1, 4.2, 4.3, 4.4 or 4.5 using 6N HCl. After this pH adjustment, it was centrifuged under the same conditions described above. Then the supernatant was removed and discarded. The remaining pellet was washed with deionized water and then centrifuged under the same conditions. The pH of the precipitant was adjusted to 7.0 (Seven Compact pH Meter S210-Uni-Kit, Mettler-Toledo, Columbus, Ohio) using 6N NaOH, froze at -20°C for 24 h (l7182 Chest Freezer, Kenmore, Chicago, Illinois) and then freeze dried for a minimum of 24 hours in a (Labcono Freezone 4.5 Liter Freeze Dry System, Marshall Scientific, Hampton, New Hampshire) (Tian, Kyle and Small 1999).

2.3.2 Protein Isolate Yield

The protein yield was measured by weighing the final protein isolate after being freeze-dried following the isoelectric focused protein extraction.

2.3.3 Protein Content Determination using High Performance Liquid Chromatography-Reverse Phase (RP-HPLC)

Ten g of each protein isolate was weighed and added to a 2 ml microcentrifuge tube. One ml of Guanidine and 20 µl of β-Mercaptoethanol were then added to the tube. The solution was vortexed (SI-0236 Vortex-Genie 2 Mixer 120V, Genie, Bohemia, New York) for 15 min, then centrifuged for 5 min at 241 *x g*. The isolate supernatant was then diluted in a 1:3 ratio of protein isolate or flour: water. Two more tubes were prepared each with 1 ml of Guanidine and 20 µl of β-Mercaptoethanol. 330 µl of the solution with the protein isolate was transferred to each of the other two tubes and vortexed (SI-0236 Vortex-Genie 2 Mixer 120V, Genie, Bohemia, New York). Following the isolate dilution, 1 ml of the solution was removed with a 1 ml syringe and then filtered into an HPLC vial. The HPLC vial had approximately 500 µl following filtering and 10 µl of the HPLC solution was injected per sample (Taghvaie and Smith, 2020).

The RP-HPLC (Agilent 1200 Series Quaternary Pump, Agilent Technology, Waldbronn, Germany) separation of each protein isolate was performed using 0.089% Trifluoracetic acid (TFA) in water (mobile phase A) and acetonitrile (mobile phase B) with a gradient of 20% B to 30% B for 10 min, 30% B to 39% B for 20 min, 39% B to 60% B for 10 min and keeping 60% B for the last 5 min of the run (Taghvaei and Smith, 2020). A C18 column (Agilent Technology, Waldbronn, Germany) at 55.5°C was used for separation. The quantification of total protein content in all samples was done using commercial pea protein isolate as a standard ($R^2 = 0.9985$ for the standard curve), and values obtained using this method were comparable with those obtained through nitrogen combustion (Taghvaie and Smith, 2020).

2.3.4 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE) Protein Analysis

Ten mg of protein isolate was mixed with 1 ml 1% SDS and 2% β-Mercaptoethanol solution and then vortexed for 10 min at full speed (SI-0236 Vortex-Genie 2 Mixer 120V, Genie, Bohemia, New York) and centrifuged (5415D Centrifuge with Rotor F45-24-11 Microcentrifuge 120 V, Eppendorf, Hamburg, Germany) for 10 min at 1,398 *x g* to prepare the solutions. The samples were then pipetted the protein supernatant 4 µl with a denaturing solution 2 µl and then heated up the solution with the ladder at 95 °C to 100 °C for 5 min. Tubes were then vortexed and 84 µl of deionized water was added to both samples and again vortexed (SI-0236 Vortex-Genie 2 Mixer 120V, Genie, Bohemia, New York). The samples 6 µl, ladder 6 µl and destaining solution 12 µl were then loaded into an Agilent SDS PAGE chip and ran it on the Agilent 2100 bioanalyzer (Agilent Technology, 2016, Waldbronn, Germany) (Holzmuller and Kulozik, 2016).

2.3.5 Protein Solubility

Protein isolate or flour samples were prepared with buffer solutions at pH solutions at 3, 4, 5, 6 ,7, 8 and 9 (Seven Compact pH Meter S210-Uni-Kit, Mettler-Toledo, Columbus, Ohio); if the buffer solutions did not have their correct pH the solutions were adjusted with 6 N NaOH or 6N HCl. For this measurement there was only one sample tested with three replicates and the samples chosen were those with the highest protein content per pulse crop. The buffer solutions were prepared prior to the analysis and the chemical formula of each buffer solution is in Appendix A. Protein solutions were then stirred for one hour at 16 at 220 RPM Corning Model PC-420, Toledo, Ohio). The 0.5% protein solution was transferred to 50ml plastic Falcon tubes and centrifugated for 15 min

at 1,467 *x g* (5415D Centrifuge with Rotor F45-24-11 Microcentrifuge 120 V, Eppendorf, Hamburg, Germany). The supernatant was then transferred into HPLC glass vials.

Using the values of the three replicates of the yellow pea protein isolates, the protein value was averaged. The amount of protein that solubilized into the buffer at pH 3 was divided by the assumed value of the protein in the entire sample. Because of using an average as an assumed value, the protein content in all the replicates may not have been equal, which would allow for the percentage of solubility to be over 100%.

2.3.6 Protein Isolate Yield

Samples ran through the HPLC (Agilent 1200 Series Quaternary Pump, Agilent Technology, Waldbronn, Germany) and protein content of the supernatant was measured using a C18 column (Agilent Technology, Waldbronn, Germany). The HPLC-RP (Agilent 1200 Series Quaternary Pump, Agilent Technology, Waldbronn, Germany) separation of each protein isolate was performed using 0.089% Trifluoracetic acid (TFA) in water (mobile phase A) and acetonitrile (mobile phase B) with a gradient of 20% B to 30% B for 10 min, 30% B to 39% B for 20 min, 39% B to 60% B for 10 min and keeping 60% B for the last 5 min of the run. A C18 column (Agilent Technology, Waldbronn, Germany) at 55.5°C was used for separation. The quantification of total protein content in all samples was done using commercial pea protein isolate as a standard (R^2 of 0.9985 for the standard curve). and values obtained using this method were comparable with those obtained through nitrogen combustion.

When calculating the protein solubility, yellow pea protein isolates were used as a standard and produced a standard curve with $R^2 = 0.9826$ using the equation y=3037.8x-5076. The protein solubility was calculated by measuring the (protein content in the

supernatant/ total protein in the initial sample) × 100 to get a percentage of protein solubility (Taghvaei and Smith, 2020).

2.3.7 Oil Holding Capacity Measurement (OHC)

One half g of each sample was measured (W2) and transferred to a Falcon 50 ml screw cap centrifuge tube (W1). W2 is the weight of the sample and W1 is the weight of the tube alone. Five g of canola oil (Great Value Canola Oil) was added to each centrifuge tube. The tubes were vortexed (SI-0236 Vortex-Genie 2 Mixer 120V, Genie, Bohemia, New York) for 10 s every 5 min for a total of 30 min. The samples were then centrifuged for 15 min at 492 *x g* (Centrifuge 5804, Eppendorf, Hamburg, Germany). The supernatant was decanted and then the final weight of the tube was measured (W3; Toews and Wang, 2013). The OHC was calculated using the following equation:

$$Oil\ holding\ capacity = \frac{W3 - W1 - W2}{W2}\ x\ 100\%$$

2.3.8 Water Hydration Capacity Measurement (WHC)

The water hydration capacity (WHC) was measured according to AACC method 56-30.01 (AACC 2009) with modification. One g of dry sample was weighed into a pre-weighed 15 ml centrifuge screw cap test tubes. Deionized water was added in small increments to the tubes and stirred with small metal spatulas until the sample was saturated. The sample was then mixed for 1 min. When the sample was saturated the tube was centrifuged (Centrifuge 5804, Eppendorf, Hamburg, Germany) for 10 min at 492 *x g*. The supernatant was discarded and the tube was weighed.

Calculate the WHC using the following equation:

$$Approximate\ WHC, \frac{ml}{g} = [(Weight\ of\ tube + sediment) - (\ Weight\ of\ tube + 1)]$$

To determine the WHC into each of the 4 tubes, weigh the quantity of material as calculated where 15 is desired total weight of sample and water.

$$Weight\ of\ material = \frac{15}{Approximate\ WHC + 1}$$

2.3.9 Foaming Protein

A 1% solution of a protein isolate in water dispersion with 50ml deionized water r4 prepared and stirred for one hour at 220 RPM using a stir plate (Corning Model PC-420, Toledo, Ohio). The pH was verified to be between 6.5 to 7.5 (Seven Compact pH Meter S210-Uni-Kit, Mettler-Toledo, Columbus, Ohio). Fifteen ml of the dispersion was pipetted into a 50 ml Falcon plastic tube and homogenized for 5 min using an Omni Macro Homogenizer at 3075 x *g* (Omni International, Marietta, GA, USA). The sample was then transferred into a graduated cylinder and the height of the foam in mm was measured immediately after homogenization and then ten min after homogenization (Stone et al., 2015). The following equations were used to calculate the values for foaming properties in protein isolates:

$$\%FC=\left(\frac{V_0}{15}\right)x\ 100\%$$

$$\%FS=\frac{V_{30}}{V_0}\ x\ 100\%$$

2.3.10 Emulsifying Properties

A solution of 0.5% flour or protein isolate dispersion was prepared with deionized water and then stirred for 1 hour at 220 RPM on stir plate (Corning Model PC-420, Toledo, Ohio). After the pH was standardized between 6.5 to 7.5 using 6N NaOH or 6N HCl(Seven Compact pH Meter S210-Uni-Kit, Mettler-Toledo, Columbus, Ohio), the sample was transferred into canola oil in a ratio of 3:1. This solution was then

homogenized using a homogenizer (Ultra-Turrax TP 18/10S1, Information and Knowledge Advantage, Staufen, Germany) on the 3075 x *g* for 1 min. Fifty µl of the solution was pipetted and diluted into 10ml of 0.1% SDS solution (Stone et al., 2015). Absorbance of the emulsion was measured at 500 nm with UV/Visible Spectrophotometer (Ultraspec 4000, Pharmacia Biotech Braunschweig, Germany) (Pearce and Kinsella,1978).
The Emulsion Stability Index (ESI) is calculated using the following equation:

$$\text{ESI (min)} = \frac{A_o}{\Delta A} \cdot t$$

2.3.11 Statistical Analysis

The experimental design was a completely randomized design with the treatment design of 2×6 factorial arrangement of treatments for each of the four crops. A mixed model was used with the alkali pH, the acidic and the interactions between the alkali and acidic pH as fixed effects and replications as a random effect. An ANOVA is used to determine if there are significant differences among treatments. To determine which treatments are significantly different a means separation test, Tukey's Honest Significant Difference, was used. Tukey's Honest Significant Different is a single step procedure that can be used to determine which means are significantly different from each other. Significance was determined at $P < 0.05$.

2.4 Results

2.4.1 Protein Isolate Yield

There were no significant interactions between the alkali and acidic pH for protein isolate yield for chickpea ($P = 0.93$), great Northern bean ($P = 0.15$), red lentil ($P = 0.48$), or yellow pea samples ($P = 0.90$; Table 2.1). The average yield of chickpea samples at pH

8 was 14.07 ± 1.41 g and at pH 9.0 was 20.92 ± 1.41 g. The average yield of great Northern bean samples at pH 8 was 13.82 ± 0.24 g and at pH 9 was 12.71 ± 0.24 g. The average yield of red lentil samples at pH 8 was 18.74 ± 0.93 g and at pH 9 was 12.72 ± 0.93 g. The average yield of yellow pea samples at pH 8 was 12.78 ± 0.58 g and at pH 9 was 11.57 ± 0.58 g.

All the great Northern bean samples had differences in the average yield of the samples ($P < 0.001$). In the great Northern bean protein samples, the samples isolate with an alkali pH of 8 had an equal or higher yield compared to the samples isolated with alkali pH 9.0 (Table 2.1). The sample with the highest yield was 16.0 g from the sample isolated at pH 4.3/8.0. All the red lentil samples have significant yields with the highest yield of 20.6 grams isolated at 4.4/8.0. All the red lentil samples, the yields were higher for samples isolated with alkali pH 8 compared to samples isolated with alkali pH 9. The sample with the highest yield was the isolates at pH 4.2/8.0. The samples isolated at the acidic pH of 4.0, 4.1, 4.2, 4.4 and 4.5 had a higher yield when isolated with the alkali pH of 8.0 compared to an alkali pH of 9 (Table 2.1).

2.4.2 Measured Protein Content in Pulse Protein Isolates Analysis

An analysis of variance showed all pulse crops are significantly different from each other in all the alkali and acidic pH values tested ($P < 0.001$). In the overall protein content, all the pulse protein samples had significant interactions for the alkali and acidic pH. The significant interaction between the alkali and acidic pH indicates that the different treatments used to isolate the protein isolates had significant differences within each crop $P = 0.001$; Table 2.2).

2.4.3 SDS-PAGE Protein Analysis

The yellow pea protein isolates had significant differences in the vicilin to legumin ratios (Table 2.3). The yellow pea sample with the highest vicilin: legumin ratio was the sample isolated at pH 4.0/9.0. The yellow pea protein had significant interactions between the acidic and alkali pH values in the percentage of albumins and globulin proteins present ($P < 0.001$; Table 2.4). The great Northern bean samples had significant differences in the percentage of albumins and globulins present in the protein isolates (Table 2.5). The chickpea protein samples had significant interactions between the alkali and acidic pH values for the overall percentage of vicilin ($P < 0.001$), percentage of legumin ($P < 0.001$) and percentage of glutelins ($P < 0.001$; Table 2.6). There were significant differences and interactions between the acidic and alkali pH and the percentage of prolamins, glutelins, legumins, albumins, and trimers present in the red lentil protein isolates ($P < 0.001$; Table 2.7).

2.4.4 Protein Solubility

There were significant interactions in protein solubility between the alkali and acidic pH in all the pulse protein isolate- yellow pea, chickpea, red lentil and great Northern bean samples ($P < 0.001$; Table 2.8). Out of all the samples tested, the yellow pea samples had the highest protein solubility-a measured value of 108% (Table 2.8).

2.4.5 Oil Holding Capacity

The oil holding capacity, the chickpea samples had a significant interaction between the acidic and alkali pH ($P = 0.01$), the great Northern bean samples also lacked a significant interaction ($P = 0.51$), while both red lentil ($P < 0.001$) and yellow pea ($P < 0.001$) had significant interactions (Table 2.9). For the chickpea samples the average oil

holding capacity at pH 8.0 was 0.1993 ± 0.0008 and at pH 9.0 was 0.1836 ± 0.0008 (Table 2.9). The great Northern bean samples had an average oil holding capacity at pH 8.0 was at 4.35 ± 0.27 and at pH 9.0 was 4.19 ± 0.27 (Table 2.9).

2.4.6 Water Hydration Capacity Analysis

There were no significant interactions between the alkali and acidic pH in the chickpea samples ($P = 0.08$) or yellow pea samples ($P = 0.08$), but there were significant interactions for great Northern bean ($P < 0.001$) and red lentil samples ($P < 0.001$; Table 2.10). The chickpea samples isolated at pH 8.0 had an average WHC value of 1.67 ± 0.05 and at pH 9.0 was 1.93 ± 0.05 g/g (Table 2.10). The yellow pea samples isolated at pH 8.0 had an average WHC value of 2.02 ± 0.05 g/g and at pH 2.02 ± 0.05 g/g (Table 2.10).

2.4.7 Foaming Capacity

There are statistically significant interactions between the alkali and acidic pH in all the pulse crops- yellow pea, red lentil, great Northern bean and chickpea samples in the foaming stability values (Table 2.11). The chickpea sample with the highest foaming stability was 77.6% with the sample isolated at pH 4.4/9.0 (Table 2.11). When looking at the foam stability values the yellow pea protein isolate samples solubilized at pH 9.0 had higher foam stability values compared to that for samples isolated at alkali pH 9.0 (Table 2.11). The average of foam stability values in great Northern bean protein isolates at alkali pH was 58.8% compared to at alkali pH 9 of 81.3% (Table 2.11). In the foam stability in great Northern bean samples, the samples isolated at alkali pH of 9.0 were higher compared to that for the samples isolated at alkali pH 8. The highest foaming stability in great Northern bean samples was 90.6% found in sample isolated at pH 4.1/9.0 (Table 2.11). The highest foaming stability in red lentils was 91.4% isolates at 4.0/8.0, which was

closely followed behind a foaming stability value of 89.4% isolated at pH 4.5/8.0, which itself was closely followed behind at 89.3% isolated at pH 4.3/8.0 (Table 2.11).

There were significant interactions between the alkali and acidic pH for the foaming capacities for chickpea ($P = 0.001$), great Northern bean ($P < 0.001$), red lentil ($P = 0.006$) and for yellow pea ($P = 0.001$; Table 2.12).

The highest value of all the chickpea samples was 4.2/9.0 which had a foaming capacity value of 6.7 while the average value of samples isolated with an alkali pH of 9.0 was 3.8 (Table 2.12).

2.4.8 Emulsion Testing Analysis

All the pulse protein samples tested for emulsion lacked a significant interaction between the acidic and alkali pH; chickpea ($P = 0.78$), great Northern bean ($P = 0.54$), red lentil ($P = 0.06$) and yellow pea ($P = 0.24$). In the chickpea samples the average ESI at pH 8.0 was 134.94 ± 44.06 and at pH 9 114.45 ± 20.74 (Table 2.13). The great Northern bean samples had an average ESI at pH 8.0 were 24.51 ± 2.29 and at pH 9.0 were 214.42 ± 33.33 (Table 2.13). In the yellow pea samples, the samples solubilized at pH 8.0 had an average ESI value of 132.01 ± 22.92 and at pH 9 were 136.98 ± 21.10 (Table 2.13).

2.5 Discussion

Pulse crops have greatly grown in popularity and have a multitude of applications in the protein isolates of pulse crops (Chereau et al., 2016; Chien Prochnow and Cantarella, 2009; Nielsen, Sumner and Youngs, 1981). The pulse protein isolates were extracted through an isoelectric focused protein extraction (Kornet et. al, 2020; Papalamprou, Doxastakis and Kiosseoglu, 2010). The protein content is an extremely important parameter to look at because many of the physiochemical properties are closely tied to the

protein content (Ladjal-Ettoumi et al., 2016; Parades-Lopez, Ordorica-Falomir and Olivares-Vazquez, 1991).

In all the pulse protein isolates investigated, the yellow pea and red lentil samples had higher protein levels reported when solubilized in an alkali of pH 8.0. Chickpeas had the sample with the highest protein content solubilized at pH 9.0, but overall, the other samples averaged a higher protein content when solubilized at pH 8.0. Based on previous research it is documented that pH 8.0 is commonly used because it caused a high protein content level (Lee, Htoon and Patterson, 2007).

Excluding great Northern bean samples, the other pulses had the highest concentration of protein content at pH 4.3, 4.4 or 4.5 with the protein content increasing from 4.0 to 4.1 slightly decreasing at acidic pH 4.2 and increasing at 4.3 and 4.4 and peaking except for chickpeas and then decreasing at higher than 4.4. Based on previous research, yellow peas should have the highest protein content out of the pulse crops tested which is consistent with the findings of having a protein content of 88% with studies finding pea protein content levels ranging from 63.9% to 88.6% (Berghout, Boom and van der Goot A. 2014.; Ma et al., 2011). The expected protein content levels varied from 81% to 89% when precipitated out at an acidic pH range of 4.7 to 4.9 (Stone et al., 2015).

The chickpea protein isolates had the highest yield out of the pulse protein isolates extracted in this study but had the lowest protein content. Therefore, the chickpea protein isolate had other components including flour in it. Chickpea protein isolates isolated at pH 9 had higher yields than samples extracted at a pH of 8.

The overall protein content is important information, but the subunits of the protein present is also valuable information to collect (Lam and Nickerson, 2013). The

approximate percentage of protein subunits is valuable because different protein subunits have different solubilities and functionalities (Toews and Wang, 2011; Naczk, Rubin and Shaidi, 1986). According to the literature available about the protein content on pulse crops, typically these crops have between 15 to 30% of a crude protein content (Berghout, Boom and van der Goot, 2014). Chickpeas have approximately 8-12% albumins, 53-60% globulins, 18-24% glutelins and 3-7% prolamins in their protein, which there is 19-27% crude protein (Toews and Wang, 2011). The chickpea protein isolates had the expected globulin levels in the samples isolated at pH 4.0/9.0, 4.2/9.0, 4.3/9.0. The chickpea protein isolates at pH conditions 4.2/8.0, 4.4/8.0, 4.3/8.0, 4.1/9.0 and 4.4/9.0 were within the expected range of 18 to 24% glutelins. Great Northern beans have approximately 24 to 27% of crude protein and 16-21% albumins, 59-73% globulins and currently are not reported for having glutelins or prolamins (Toews and Wang, 2011; Chang and Satterlee, 1981). The samples isolated at pH 4.1/8.0, 4.2/8.0, 4.0/9.0 and 4.4/9.0 are within the expected range of albumins being approximately 20% of the proteins made up in great Northern bean subunits (Chang and Satterlee, 1981). Lentils have approximately 23-31% crude protein, with 17% of that as albumins, 51% as globulins and 11% as glutelins and 4% as prolamins (Hall, Hillen and Robinson, 2017*)*. In the red lentil protein samples, isolates extracted at pH 4.0/8.0, 4.4/8.0, 4.5/8.0 and at pH 4.4/9.0 fit with having approximately 50% of the protein subunits being globulins and all the extracted samples had under 10% of the protein isolate subunit being prolamins. For peas, albumins typically make up 15-25%, globulins make up 49-70% and glutelins are 11% and prolamins are around 5% of the proteins (Chereau et al., 2016). Another source that more clearly generalized the protein subunits in yellow pea plants claimed that around 80% of yellow

pea protein subunits are globulins such as legumin, convicilin and vicilin with the remaining 20% being made up of prolamins (Chereau et al., 2016; Argos, Narayana and Nielsen, 1985). All the samples present fall within the guidelines of having 80% globulins made up of vicilin, legumin and convicilin.

Protein solubility is an extremely important measurement to take of the protein isolates (Bora, 2002). Protein solubility is highly dependent on protein-protein interactions and protein-solvent interactions (Parades-Lopez, Ordorica-Falomir and Olivares-Vasquez, 1991). Protein-protein interactions are determined by the hydrophobic interactions while protein-solvent interactions can cause the protein to absorb water and solubilize (Lusas and Riaz, 1995). One key application of protein solubility is to look at how well the protein isolates dissolve into solution at a given pH (Suliman et al., 2006). This is important because this can look at if the protein isolates would dissolve well at the pH of the human stomach, which could correlate to how well the isolates are digested (Taherian et al., 2011).

Regardless of the pulse sample tested, all the samples were significantly different in their solubility depending on the pH of the buffer solution the samples were dissolved into. All the findings, when looking at protein solubility of the pulse crops, are consistent with previous research. For chickpea samples, the highest protein solubility was at pH 9.0, which is consistent with previous findings of chickpeas having the highest protein solubility in the pH range of 8.0 to 9.0 (Chang, et al., 2011). The level of protein solubility increased with the pH of the buffer solutions increasing except for at pH 6.0. In great Northern bean samples, the same trend followed including a brief drop in protein solubility at pH 6.0 (Fernandez-Quantiela, Marcarula, Del Barrio and Martinez, 1997). The great

Northern bean samples went from 60.25 % ± 0.91 to 99.05% ± 1.56 when moving from pH 3.0 to 9.0.

Water hydration capacity is a functional property of pulse protein isolates because it allows for the protein isolate to completely solubilize into solution and form a completely homogenous solution. The typical values of WHC range from 0.6 g/g to 2.7 g/g with yellow peas typically having the highest WHC value of all pulse crops (Hall, Hillen and Robinson, 2017; Liu et al., 2015). These findings slightly vary from previous ones in that the great Northern bean protein isolates average at 2.5 g/g with the highest WHC values with most the values around 2.5 g/g (Hall, Hillen and Robinson, 2017). All the pulse crops have a WHC that varies from 0.9 to 2.5 g/g. There are significant differences between chickpea samples isolated at alkali pH 8.0 and at alkali pH 9.0 with the highest WHC value of 2.1 for samples isolated at pH 4.2/9.0 and pH 4.5/9.0. Comparing the WHC values of all the chickpea samples, the samples isolated with a pH 9.0 were generally higher than those with pH 8. The existing research provides resources with chickpeas having the lowest WHC values, which is consistent with present findings (Parades-Lopez, Ordorica-Falomir and Olivares-Vasquez, 1991; Pelgrom et al., 2013). The chickpea samples varied from 1.5 to 2.1 g/g where the great Northern bean samples varies from 1.6 to 2.5 g/g. The WHC values correlate to having higher values of samples with higher protein contents because samples with higher protein content have smaller particle sizes and more starch fragments that are correlated with a higher WHC value (Papalamprou Doxastaski and Kiosseoglou, 2010; Pelgrom et al., 2013). There are not many clear patterns when comparing the WHC values of samples isolated at the two different alkali

pH values, but the two highest WHC values come from samples isolated at pH 4.2/9.0 and 4.3/9.0 (Table 2.10).

While WHC is used to solubilize the protein isolates into solution, the oil holding capacity is a measurement of how well the protein can mix into a solution with oil (El-Adawy, 2000). Oil holding capacity also has many important applications in food production-especially when working with batters or any type of flavor compound. Almost all flavor compounds are fat soluble, so if there is a high OHC values it means that the protein isolate can easily solubilize into an oil-based solution (Joshi et al., 2011; 2012).

Much like the WHC values, the OHC values are higher in protein isolates with higher levels of protein content. For example, chickpea protein isolates had extremely low protein percentages compared to the other isolates and all the other pulse crops have high OHC values compared to the chickpea protein isolates There were no clear trends between the OHC in samples isolated at alkali pHs 8.0 and 9.0. There were significant differences in the yellow pea samples in the OHC values. For the samples isolated at pH 4.0, 4.1, 4.2 and 4.3 the samples extracted with pH 9 have higher OHC values compares to alkali pH 8.

There are not many clear patterns when comparing the WHC values of samples isolated at the two different alkali pH values, but the two highest WHC values come from samples isolated at pH 4.2/9.0 and 4.3/9.0. There are differences in the WHC values in the red lentil protein isolate samples with the highest WHC being 2.0 from samples isolated at pH values 4.4/9.0. Most of the samples with the higher WHC are samples isolated with the alkali pH of 9.0. There are significant differences in the WHC of samples isolated at different pH conditions with the highest WHC of 2.2 g/g from yellow pea samples isolated at pH 4.5/9.0.

The foaming capacity is a measurement of the height of the foam formed (Kornet et al., 2020; Niu et al., 2018). When a foam is formed, the mixture of water and protein isolate was homogenized to form a foam (Ma et al., 2015). The foaming capacity measures the volume achieved with the air bubbles entrapped in the pockets of the sheared mixture (Aluko, Mofolasayo and Watts, 2009). The highest FC would be indicative of a foam-stabilizing protein with low molecular weight, high surface hydrophobicity, high solubility and a negligible charge (Barac et al., 2010). Of all the pulse crops, the yellow pea samples had the highest FC value and both of those occurred in the samples with the highest protein content. This trend was also true in red lentil, great Northern bean and chickpea protein samples. The foaming ability is closely related to the emulsifying properties of proteins and the foaming ability is correlated with higher protein content values (Stone et al., 2015; Karaca, Low and Nickerson, 2011).

The FC in great Northern beans had statistically significant differences in the samples isolated at different alkali pH values whether it was at pH 8.0 or pH 9.0. When comparing the least squares mean for the alkali and acidic pH the samples isolated at pH 9.0 had higher foam stability compared to samples extracted at pH 8.0. The average of foam stability values in great Northern bean protein isolates at alkali pH is 58.8% compared to at alkali pH 9.0 of 81.3%.

In chickpea samples, there were significant differences in the samples isolated with an alkali pH of 8.0 or 9.0. The chickpea samples isolated with alkali pH 9.0 and acidic pH solutions at pH 4.1 and above 4.5 9.0 had greater foam stability. The great Northern bean samples have different foam stability values depending on the isoelectric method used in the protein extraction isolated at alkali pH 8.0 and 9.0 and between the samples. The

yellow pea samples with the highest foaming stability were 81.7%, which was obtained from samples isolates at pH 4.4/8.0 which also had the highest level of protein. When forming a foam, the protein isolates were mixed with water and then homogenized which provided physical shear to the solutions. The shear in the protein allowed for air to enter the pockets in this solution. The stability of the protein solutions is indicative of how well the protein can entrap the air bubbles over time. Generally, the samples isolated at pH 8 had higher foaming stability compared to samples isolated at pH 9.0 in the red lentil samples.

Emulsions can either be a lipid in water or water in a lipid-based system. To have a high level of emulsion stability, there needs to be an emulsifier that has both a hydrophobic and a hydrophilic portion to decrease the surface tension between the water and canola oil used in the experiment. Another key factor in having a high ESI is having a higher protein content because it lowers the surface tension because the small oil droplets of the emulsions are more stable and make stable emulsions (Hall, Hillen and Robinson, 2017). Out of all the crops tested, the great Northern bean samples with an alkali pH of 214.42 ± 33.33 had the highest ESI of all the samples tested. This indicates that the protein subunits present in samples isolated from great Northern beans have both hydrophobic and hydrophilic portions in the protein isolate (Chao and Aluko, 2018; Sathe and Salunkhe ,1981).

A low ESI at a high acidic pH indicates strong protein-protein interactions and decreases the solubility and increases the surface tension (Liu, Liu and Nickerson, 2009). Yellow pea samples had low ESI values and chickpeas had the highest ESI, which may be indicative of having more of a neutral charge on the amino acids present than in the overall

protein content (Jiang, Liu, Xiong and Zhu, 2014). In chickpeas, there are the highest concentrations of hydrophobic amino acids compared to the other pulse crops when looking at the amount of alanine, cysteine, leucine, proline, serine, threonine and tyrosine (Gbadamosi, Abiose and Aluko 2012; Millerd, 1975). Comparatively, when looking at peas, peas have the highest concentration of acidic amino acids compared to the other pulse crops (Adebiyi and Aluko, 2011; Taherian et al., 2011). Chickpeas having a more balanced charge with neutral net charges but having both hydrophobic and amihydrophilic amino acids allows for the highest ESI (Pillai et al., 2019; Selcuk, 2016).

2.6 Conclusion

This project determines the ideal conditions when working with an isoelectric-focused protein extraction process in pulse crops. Yellow pea, great Northern bean and red lentil isolates had higher protein content when solubilized at pH 8. Furthermore, subsequent pH adjustment to 4.1, 4.2 or 4.3 resulted in more functional isolates. The samples with the highest levels of protein typically had the most desirable values for the physiochemical properties including oil holding capacity, foaming capacity and protein solubility. Out of all the pulse crops tested, the yellow pea protein isolates had the best overall profile of all the crops and the yellow pea protein isolates extracted at pH 4.4/8.0. Further research needs to be conducted to test these isolates in practical food applications.

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2.8 Tables and Figures

Table 2.1: Average Yield of Pulse Protein Isolates Extracted through Isoelectric Focused Protein Extraction Procedure

pH Treatment	Yellow Pea	Red Lentil	Great Northern Bean	Chickpea
4.0*/8.0^	13.4[#]	22.2	13.9	15.0
4.1/8.0	14.3	17.2	12.5	15.7
4.2/8.0	15.7	18.4	12.8	15.6
4.3/8.0	10.4	19.4	16.0	14.4
4.4/8.0	10.8	20.6	12.5	13.5
4.5/8.0	12.1	14.7	15.1	10.3
4.0/9.0	12.4	13.8	13.8	22.1
4.1/9.0	11.3	12.6	12.6	22.2
4.2/9.0	13.6	11.8	11.8	20.8
4.3/9.0	10.7	12.4	13.0	19.3
4.4/9.0	9.8	11.6	11.5	20.4
4.5/9.0	11.6	14.2	13.6	20.7
SEM	1.4	2.2	0.6	3.4

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as grams of protein yield

Table 2.2: Averaged Total Protein Content of Pulse Protein Isolates Determined by RP-HPLC Method

pH Treatment	Yellow Pea	Red Lentil	Great Northern Bean	Chickpea
4.0*/8.0^	70.3[#b]	69.1[c]	44.7[e]	56.3[bc]
4.1/8.0	71.8[b]	48.8[d]	38.7[f]	61.8[b]
4.2/8.0	50.9[c]	71.7[bc]	87.6[a]	59.9[bc]
4.3/8.0	59.4[b]	81.8[a]	45.9[e]	36.6[cd]
4.4/8.0	88.0[a]	74.6[b]	59.9[d]	23.1[d]
4.5/8.0	60.1[b]	73.1[b]	27.6[h]	41.4[c]
4.0/9.0	51.0[c]	75.2[b]	30.2[g]	65.7[b]
4.1/9.0	49.4[c]	8.9[e]	72.2[b]	64.1[b]
4.2/9.0	23.4[d]	48.3[d]	65.2[c]	61.1[b]
4.3/9.0	58.7[bc]	78.1[ab]	60.4[d]	56.8[bc]
4.4/9.0	86.4[a]	54.7[d]	73.1	40.7[c]
4.5/9.0	13.7[e]	62.9[c]	48.9[e]	73.2[a]
SEM	0.9	1.1	0.9	0.8

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as the percentage of protein ± the SEM (standard error of mean);
[a-h] Statistically different ($P < 0.05$)

Table 2.3: Vicilin: Legumin Ratio of Yellow Pea Protein Isolates Determined by SDS-PAGE

pH Treatment	Vicilin:Legumin Ratio
4.0*/8.0^	$2.7 \pm 0.02^{\#c}$
4.1/8.0	0.7 ± 0.08^{e}
4.2/8.0	4.3 ± 0.17^{bc}
4.3/8.0	2.3 ± 0.14^{c}
4.4/8.0	0.4 ± 0.02^{e}
4.5/8.0	1.7 ± 0.1^{cd}
4.0/9.0	21.2 ± 2.0^{a}
4.1/9.0	7.8 ± 0.5^{b}
4.2/9.0	1.2 ± 0.7^{d}
4.3/9.0	0.4 ± 0.01^{e}
4.4/9.0	0.8 ± 0.1^{e}
4.5/9.0	2.5 ± 0.09^{c}

*Supernatant pH adjustment
^Initial pH adjustment
[a-e]Statistically different ($P < 0.05$)
[#]Expressed as the ratio of vicilin to legumin ± the SEM (standard error of mean)

Table 2.4: Percentage of Vicilin, Legumin and Convicilin Protein Subunits in Yellow Pea Protein Isolate Samples Determined by SDS-PAGE

pH Treatments	Vicilin (%)	Legumin (%)	Covicilin (%)
4.0*/8.0^	29.9 ± 6.4[#d]	28.5 ± 7.1[d]	30.2 ± 7.4[b]
4.1/8.0	33.4 ± 0.3[d]	62.1 ± 3.3[bc]	7.05 ± 2.8[d]
4.2/8.0	75.4 ± 2.2[a]	18.0 ± 0.3[e]	2.4 ± 0.8[e]
4.3/8.0	42.6 ± 6.8[c]	32.1 ± 1.9[d]	22.1 ± 1.6[b]
4.4/8.0	12.9 ± 2.2[e]	79.7 ± 3.3[a]	17.1 ± 1.8[bc]
4.5/8.0	56.2 ± 3.2[b]	33.3 ± 3.3[d]	10.5 ± 1.5[d]
4.0/9.0	33.9 ± 1.2[d]	4.5 ± 0.4[e]	61.1 ± 5.9[a]
4.1/9.0	60.1 ± 2.8[b]	9.8 ± 1.3[e]	13.1 ± 0.6[c]
4.2/9.0	45.3 ± 2.2[bc]	42.7 ± 2.2[c]	6.6 ± 0.2[d]
4.3/9.0	22.6 ± 3.6[d]	67.5 ± 0.7[b]	12.7 ± 1.0[c]
4.4/9.0	46.2 ± 0.8[bc]	61.7 ± 4.5[bc]	1.0 ± 0.2[e]
4.5/9.0	69.7 ± 2.2[a]	28.3 ± 3.2[e]	0.70 ± 0.1[e]

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as the percentage of given protein subunit± the SEM (standard error of mean)
[a-e] Statistically different ($P < 0.05$)

Table 2.5: Percentage of Albumin and Globulin Protein Subunits in Great Northern Beans Determined by SDS-PAGE

pH Treatment	Albumin (%)	Globulins (%)
4.0*/8.0^	$80.7 \pm 2.6^{\#ab}$	22.0 ± 0.8^{de}
4.1/8.0	71.7 ± 3.5^{b}	28.4 ± 3.3^{d}
4.2/8.0	73.5 ± 2.8^{b}	26.5 ± 3.0^{d}
4.3/8.0	39.4 ± 3.4^{d}	58.5 ± 6.0^{b}
4.4/8.0	44.8 ± 4.8^{cd}	45.0 ± 1.5^{c}
4.5/8.0	83.9 ± 4.8^{a}	15.6 ± 4.5^{f}
4.0/9.0	80.6 ± 2.3^{ab}	18.8 ± 3.9^{e}
4.1/9.0	88.3 ± 1.2^{a}	10.1 ± 1.2^{g}
4.2/9.0	9.5 ± 0.5^{e}	90.5 ± 0.5^{a}
4.3/9.0	58.7 ± 2.3^{c}	19.4 ± 4.5^{e}
4.4/9.0	80.0 ± 5.6^{ab}	19.4 ± 4.6^{e}
4.5/9.0	87.6 ± 2.1^{a}	12.4 ± 2.1^{f}

*Supernatant pH adjustment
^Initial pH adjustment
$^{\#}$Expressed as the percentage of given protein subunit ± the SEM (standard error of mean)
$^{a-g}$ Statistically different ($P < 0.05$)

Table 2.6: Percentage of Vicilin, Legumin and Glutetins in Chickpea Protein Isolates Determined by SDS-PAGE

pH Treatments	Vicilin (%)	Legumin (%)	Glutetins (%)
4.0*/8.0^	$30.5 \pm 5.7^{\#d}$	47.6 ± 2.1^{b}	12.7 ± 2.0^{b}
4.1/8.0	45.6 ± 2.0^{bc}	47.7 ± 1.5^{b}	3.1 ± 0.8^{e}
4.2/8.0	50.9 ± 2.8^{b}	31.0 ± 5.0^{c}	10.2 ± 0.8^{c}
4.3/8.0	34.5 ± 2.7^{d}	48.1 ± 0.6^{b}	16.7 ± 2.7^{a}
4.4/8.0	47.8 ± 3.3^{b}	44.3 ± 2.1^{b}	10.2 ± 2.4^{c}
4.5/8.0	40.9 ± 1.1^{c}	49.6 ± 1.4^{b}	12.1 ± 0.9^{b}
4.0/9.0	67.3 ± 1.6^{a}	32.3 ± 1.2^{c}	0.81 ± 0.2^{e}
4.1/9.0	41.7 ± 1.4^{c}	36.1 ± 0.9^{c}	19.3 ± 0.7^{a}
4.2/9.0	71.9 ± 2.4^{a}	12.7 ± 4.9^{d}	5.9 ± 0.6^{d}
4.3/9.0	64.7 ± 0.7^{a}	27.9 ± 0.9^{c}	5.9 ± 0.6^{d}
4.4/9.0	42.2 ± 0.3^{c}	4.5 ± 1.5^{e}	12.4 ± 1.4^{b}
4.5/9.0	29.4 ± 0.6^{d}	69.2 ± 1.2^{a}	1.9 ± 0.1^{e}

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as the percentage of a given protein subunit ± the SEM (standard error of mean)
[a- e] Statistically different ($P < 0.05$)

Table 2.7: Percentage of Prolamins, Glutelins, Legumins, Albumins and Trimers in Red Lentil Protein Isolates Determined by SDS-PAGE

pH Treatments	Prolamins (%)	Glutelins (%)	Legumins (%)	Albumins (%)	Trimers (%)
4.0*/8.0^	19.5^{c}	23.1^{c}	45.8^{a}	N/R[$]	9.6^{b}
4.1/8.0	36.6^{a}	16.8^{cd}	23.6^{c}	21.6^{b}	0.8^{e}
4.2/8.0	17.6^{cd}	28.2^{b}	23.3^{c}	28.9^{a}	1.1^{e}
4.3/8.0	34.3^{a}	24.3^{c}	23.2^{c}	N/R	N/R
4.4/8.0	29.9^{ab}	40.8^{a}	28.4^{b}	N/R	1.4^{e}
4.5/8.0	28.2^{ab}	9.3^{d}	59.8^{a}	N/R	2.7^{d}
4.0/9.0	27.5^{b}	28.1^{b}	14.4^{d}	27.5^{a}	0.1^{e}
4.1/9.0	22.3^{c}	18.4^{cd}	34.1^{b}	1.5^{e}	18.0^{a}
4.2/9.0	22.4^{c}	35.6^{ab}	41.9^{a}	N/R	4.5^{c}
4.3/9.0	21.2^{c}	39.8^{a}	27.8^{b}	6.3^{d}	4.3^{c}
4.4/9.0	16.0^{d}	22.2^{bc}	42.3^{a}	22.0^{b}	4.3^{c}
4.5/9.0	31.0^{a}	24.4^{b}	34.3^{b}	0.2^{e}	4.5^{c}
SEM	1.9	2.1	2.8	0.4	0.4

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as the percentage of a given protein subunit ± the SEM (standard error of mean)
[a- e] Statistically different ($P < 0.05$)
[$]Value is not reported

Table 2.8: Pulse Protein Solubility Based on Buffer Solution pH

Buffer Solution pH	Great Northern Bean[w]	Yellow Pea[x]	Red Lentil[y]	Chickpea[z]
3.0	65.2 ± 0.5[c]	108.0 ± 3.3[a]	70.5 ± 0.54[c]	62.1 ± 1.9[b]
4.0	60.3 ± 0.9[c]	106.6 ± 1.4[a]	70.8 ± 0.06[c]	66.6 ± 0.6[b]
5.0	76.1 ± 0.7[b]	82.5 ± 0.8[c]	93.2 ± 1.7[a]	66.3 ± 1.5[b]
6.0	54.2 ± 0.7[d]	92.2 ± 1.0[b]	92.7 ± 1.7[a]	63.5 ± 1.5[b]
7.0	80.4 ± 0.6[b]	92.2 ± 1.7[b]	77.2 ± 0.7[b]	68.8 ± 2.9[b]
8.0	78.0 ± 1.3[b]	90.3 ± 1.7[b]	81.4 ± 5.4[b]	71.4 ± 0.2[a]
9.0	99.5 ± 1.6[a]	59.8 ± 0.17[d]	50.0 ± 2.1[d]	73.7 ± 1.1[a]

[#]Expressed as the percentage of protein present measured in RP-HPLC ± the SEM (standard error of mean)
[w]Great Northern Bean samples were protein isolates isolated at pH 4.2/8.0
[x]Yellow pea protein samples were protein isolates isolated at pH 4.4/8.0
[y]Red lentil samples were protein isolates isolated at pH 4.3/9.0
[z]Chickpea samples were protein isolates isolated at pH 4.5/9.0
[a- d] Statistically different ($P < 0.05$)

Table 2.9: Pulse Protein Isolates Oil Holding Capacity

pH	Yellow	Great Northern		
Treatments	Pea	Red Lentil	Bean	Chickpea
4.0*/8.0^	1.9[a]	6.8[a]	3.8	0.14
4.1/8.0	1.8[b]	6.7[a]	5.5	0.20
4.2/8.0	1.5[c]	5.3[b]	4.1	0.22
4.3/8.0	1.6[c]	4.8[bc]	4.3	0.20
4.4/8.0	1.3[d]	5.5[b]	3.9	0.19
4.5/8.0	2.0[a]	5.5[b]	4.4	0.24
4.0/9.0	1.9[ab]	5.3[b]	2.3	0.22
4.1/9.0	1.8[b]	5.3[b]	4.6	0.19
4.2/9.0	2.1[a]	5.0[b]	4.6	0.17
4.3/9.0	1.9[a]	5.6[b]	4.8	0.19
4.4/9.0	1.8[b]	6.6[a]	4.2	0.15
4.5/9.0	2.1[b]	4.3[c]	4.6	0.21
SEM	0.1	0.2	0.6	0.02

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as the oil holding capacity (OHC) ± the SEM (standard error of mean)
[a- d] Statistically different ($P < 0.05$)

Table 2.10: Pulse Protein Water Hydration Capacity

pH Treatments	Yellow Pea	Red Lentil	Great Northern Bean	Chickpea
4.0*/8.0^	1.6	1.6[b]	1.6[b]	1.9
4.1/8.0	1.5	1.5[b]	2.3[a]	1.8
4.2/8.0	1.9	1.9[a]	2.2[a]	1.5
4.3/8.0	1.8	1.8[b]	1.4[b]	1.6
4.4/8.0	1.9	2.2[a]	1.9[b]	1.3
4.5/8.0	2.2	1.9[a]	2.2[a]	2.0
4.0/9.0	1.8	1.8[b]	1.8[b]	1.9
4.1/9.0	1.8	1.9[a]	2.2[a]	1.8
4.2/9.0	1.9	1.5[b]	2.5[a]	2.1
4.3/9.0	1.5	1.5[b]	2.5[a]	1.9
4.4/9.0	2.0	2.0[a]	2.5[a]	1.8
4.5/9.0	1.7	1.7[b]	1.8[b]	2.1
Flour	1.2	0.9[c]	1.8[b]	1.5
SEM	0.1	0.1	0.2	0.1

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as the WHC value ± the SEM (standard error of mean);
[a- c] Statistically different ($P < 0.05$)

Table 2.11: Foam Stability (FS) of Pulse Protein Isolates

pH Treatment	Yellow Pea	Red Lentil	Great Northern Bean	Chickpea
4.0*/8.0^	55.5^{d}	91.4^{a}	48.5^{e}	71.1^{a}
4.1/8.0	72.2^{b}	60.1^{c}	57.1^{cd}	54.9^{c}
4.2/8.0	77.8^{a}	77.5^{b}	76.8^{b}	72.8^{a}
4.3/8.0	66.3^{c}	89.3^{a}	60.4^{cd}	51.8^{c}
4.4/8.0	52.1^{d}	79.6^{b}	56.2^{de}	62.7^{bc}
4.5/8.0	73.1^{b}	89.4^{a}	59.9^{cd}	28.8^{e}
4.0/9.0	74.9^{ab}	87.0^{a}	68.3^{c}	68.2^{b}
4.1/9.0	61.7^{bc}	79.5^{b}	90.6^{a}	75.9^{a}
4.2/9.0	55.1^{c}	81.6^{a}	83.1^{a}	71.8^{a}
4.3/9.0	66.2^{b}	78.3^{b}	87.9^{a}	66.9^{b}
4.4/9.0	81.7^{a}	64.3^{c}	79.8^{b}	77.6^{a}
4.5/9.0	66.2^{c}	81.1^{b}	79.9^{b}	47.9^{d}
SEM	1.2	1.6	1.8	2.4

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as the percentage of FS ± the SEM (standard error of mean);
$_{i}$-μ)^{2}/N]/}
[a- e] Statistically different ($P < 0.05$)

Table 2.12: Pulse Protein Isolate Foaming Capacity (FC)

pH			Great Northern	
Treatments	Yellow Pea	Red Lentil	Bean	Chickpea
4.0*/8.0^	2.9[b]	4.5[a]	2.7[b]	3.3[b]
4.1/8.0	3.3[b]	2.8[b]	2.6[b]	2.0[b]
4.2/8.0	5.1[a]	2.8[b]	5.8[a]	4.1[a]
4.3/8.0	2.3[c]	3.6[ab]	3.9[b]	2.0[b]
4.4/8.0	2.3[c]	3.9[ab]	2.4[b]	1.9[b]
4.5/8.0	2.3[c]	2.9[b]	2.4[b]	2.0[b]
4.0/9.0	2.2[c]	2.7[b]	2.4[b]	2.2[b]
4.1/9.0	4.2[ab]	3.5[ab]	3.7[b]	2.2[b]
4.2/9.0	6.7[a]	3.0[b]	2.7[b]	2.3[b]
4.3/9.0	3.9[b]	4.4[a]	3.3[b]	1.8[b]
4.4/9.0	3.4[b]	2.5[bc]	2.9[b]	2.1[b]
4.5/9.0	2.2[c]	4.4[a]	3.8[b]	1.6[bc]
Flour	1.2[d]	2.9[b]	3.0[b]	1.2[c]
SEM	0.5	0.4	0.2	0.2

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as FC ± the SEM (standard error of mean)
[a-d] Statistically different ($P < 0.05$)

Table 2.13: Pulse Protein Isolate Emulsion Stability Index (ESI)

pH Treatment	Yellow Pea	Great Northern Red Lentil	Great Northern Bean	Chickpea
4.0*/8.0^	12.2[#]	72.3	23.6	125
4.1/8.0	9.4	130.2	20.9	107.2
4.2/8.0	8.0	113.8	21.2	166.1
4.3/8.0	7.3	87.1	28.8	116.8
4.4/8.0	3.9	97.5	25.9	142.1
4.5/8.0	12.9	84.0	72.9	103.3
4.0/9.0	4.0	150.2	48.8	151.7
4.1/9.0	17.1	136.2	59.0	167.5
4.2/9.0	22.1	103.2	14.9	104.2
4.3/9.0	26.4	120.1	12.3	123.5
4.4/9.0	16.8	148.3	13.2	130.0
4.5/9.0	28.0	109.7	14.5	132.0
SEM	1.4	9.2	3.2	12.7

*Supernatant pH adjustment
^Initial pH adjustment
[#]Expressed as the ESI ± the SEM (standard error of mean)
$_i-\mu)^2/N]/\}$

Figure 2.1: Diagram of Method Used in Isoelectric Focused Pulse Protein Isolate Protein Extraction Procedure

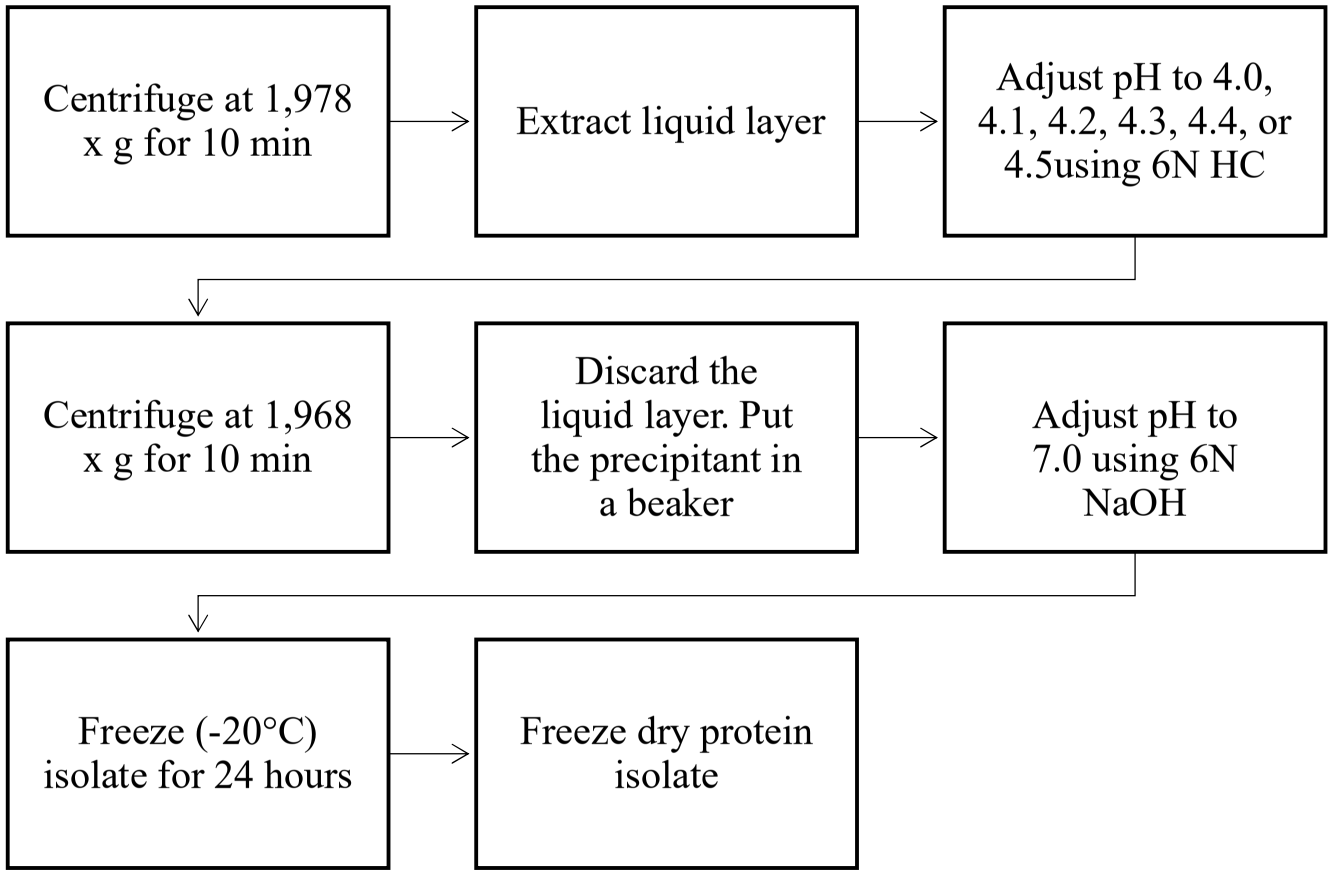
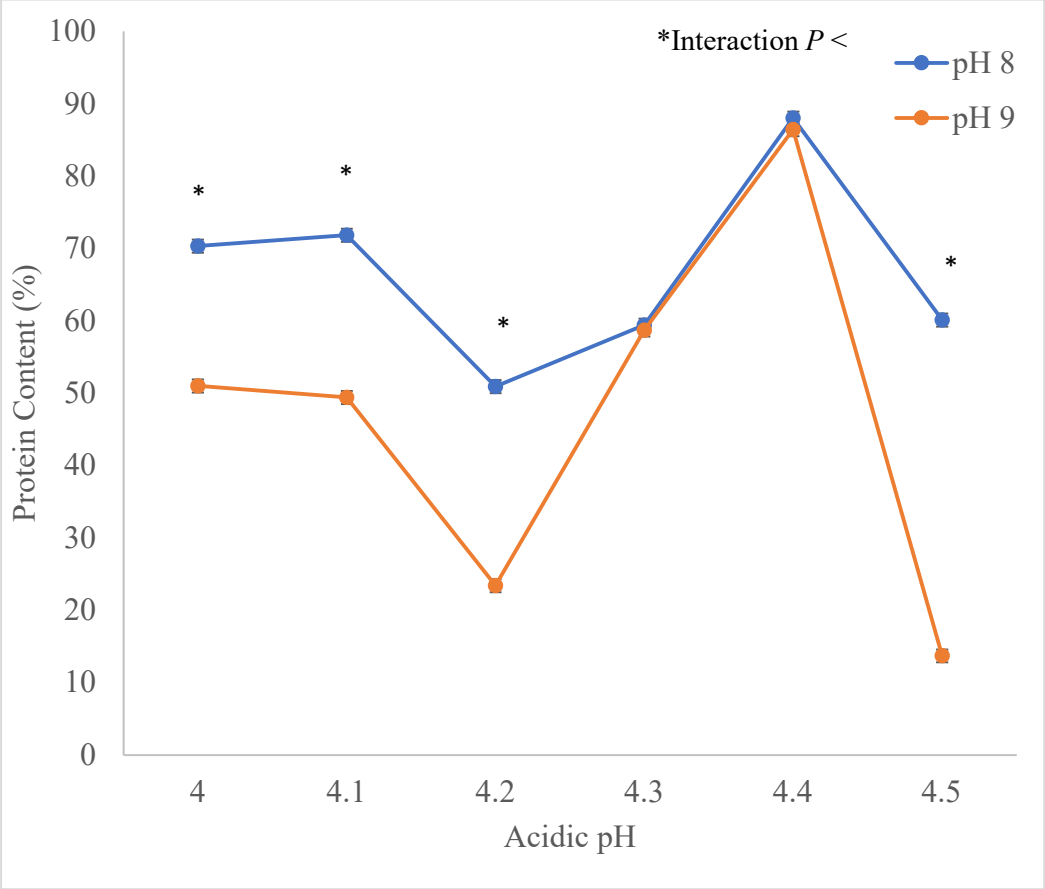
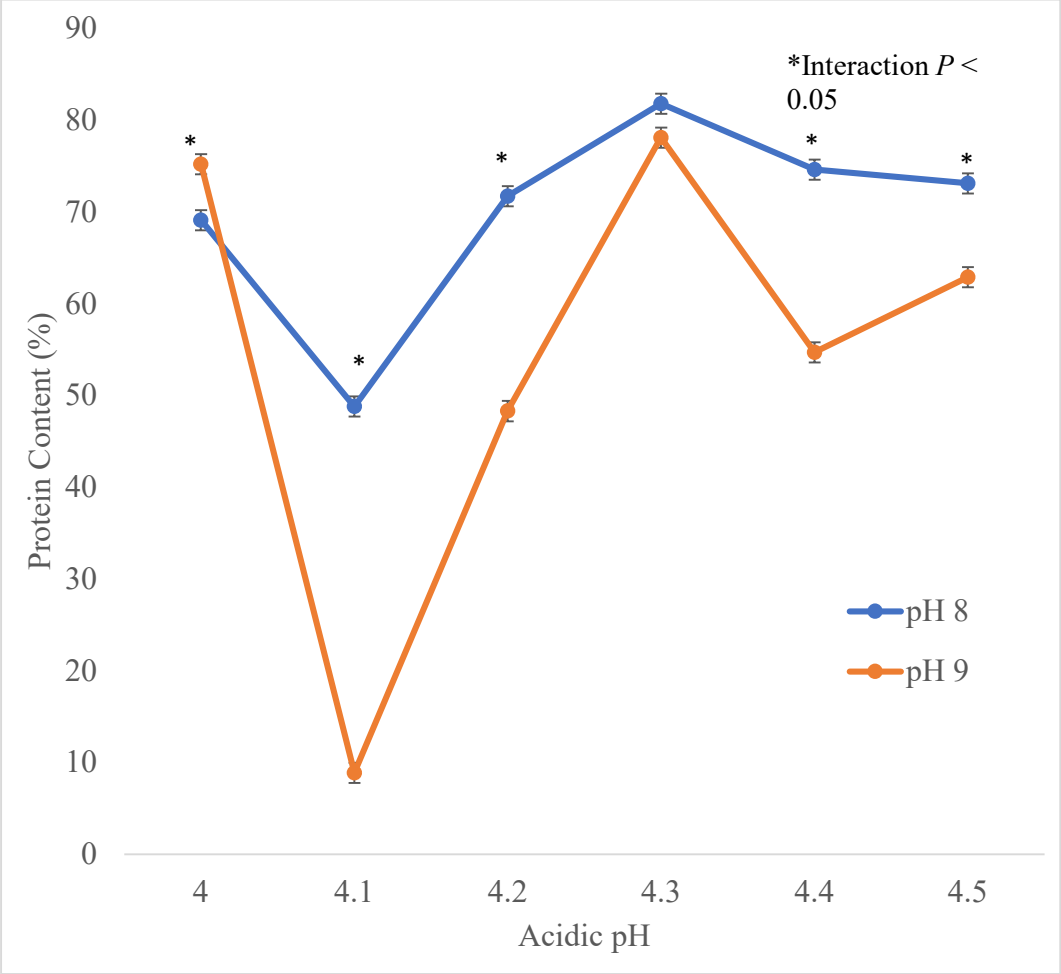


Figure 2.2: Acidic and Alkali Interactions in Total Protein Content in Yellow Pea Protein Isolate Samples.



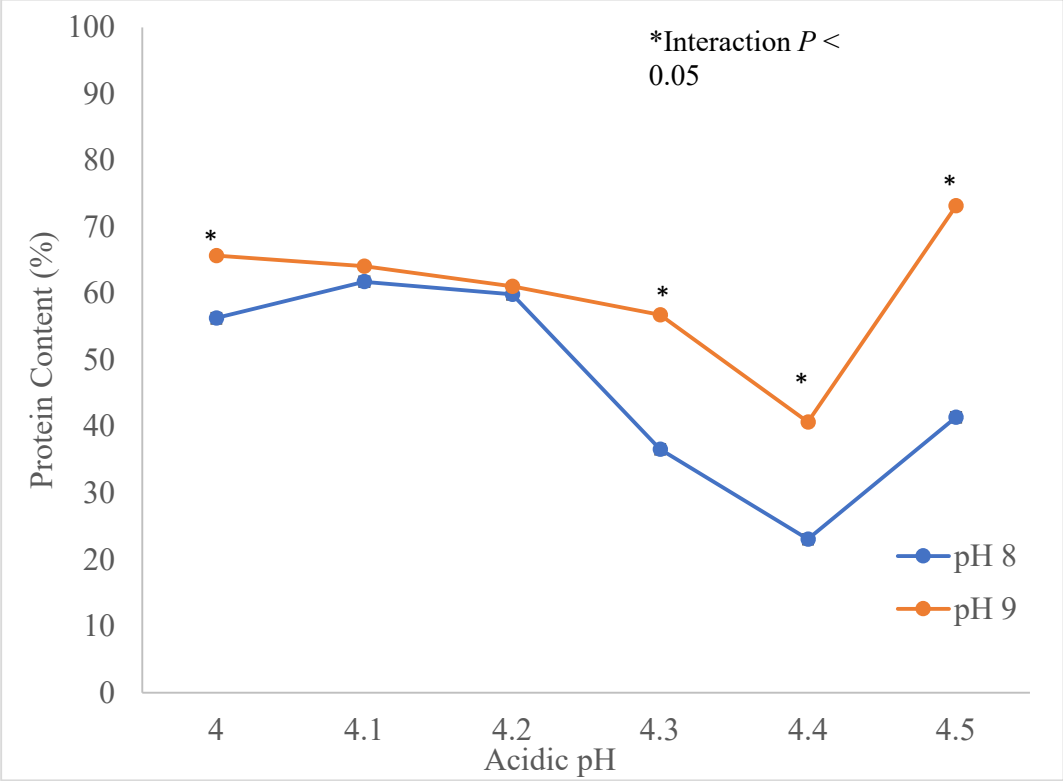
Interaction of Alkali (Solubilizing) and Acidic (Precipitating) pH in yellow pea protein isolates were analyzed. The protein isolates were solubilized into a solution using an alkali pH, either 8.0 or 9.0 and then precipitated out of solutions using an acidic pH varying from 4.0 to 4.5 in 0.1 increments. The values displayed are LSM ± SEM with the SEM. The asterisks denote significant differences between alkali pH values ($P < 0.05$).

Figure 2.3: Acidic and Alkali Interactions in Total Protein Content in Red Lentil Protein Isolate Samples.



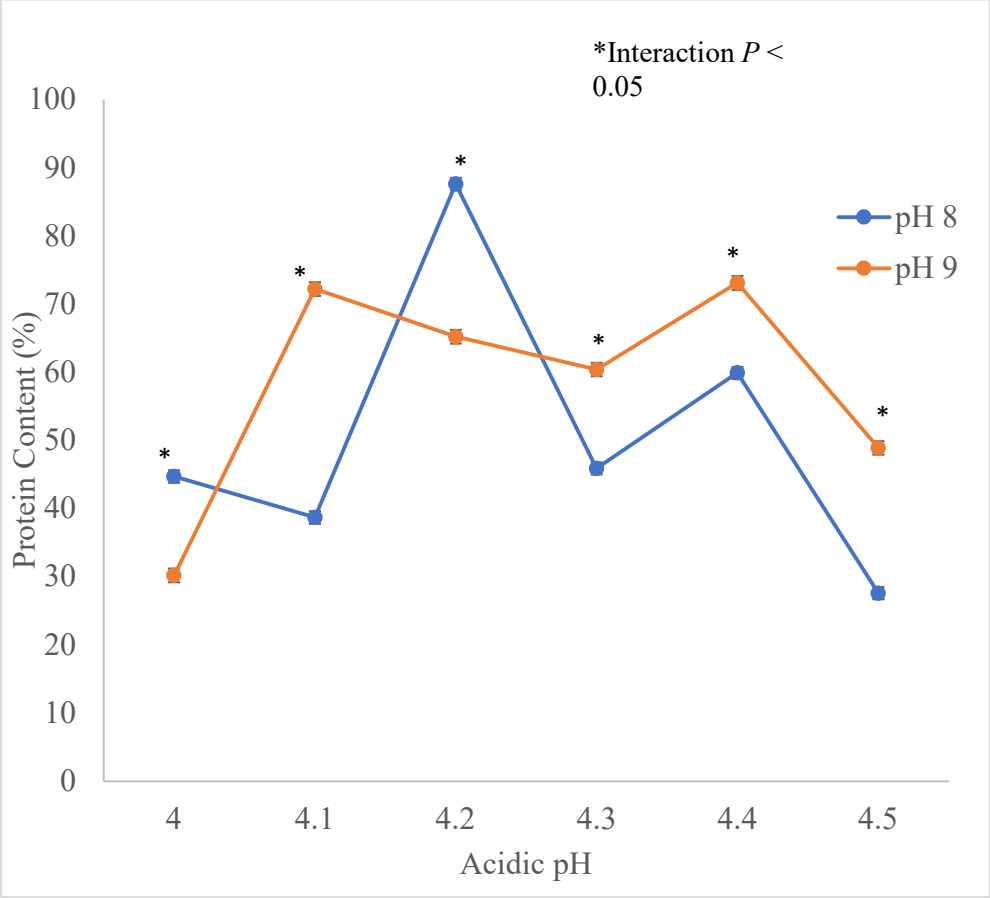
Interaction of Alkali (Solubilizing) and Acidic (Precipitating) pH in red lentil protein isolates were analyzed. The protein isolates were solubilized into a solution using an alkali pH either 8.0 or 9.0 and then precipitated out of solutions using an acidic pH varying from 4.0 to 4.5 in 0.1 increments. The values displayed are LSM ± SEM. The asterisks denote significant differences between alkali pH values ($P < 0.05$).

Figure 2.4: Acidic and Alkali Interactions in Total Protein Content in Chickpea Protein Isolate Samples.



Interaction of Alkali (Solubilizing) and Acidic (Precipitating) pH in chickpea protein isolates were analyzed. The protein isolates were solubilized into a solution using an alkali pH either 8.0 or 9.0 and then precipitated out of solutions using an acidic pH varying from 4.0 to 4.5 in 0.1 increments. The values displayed are LSM ± SEM. The asterisks denote significant differences between alkali pH values ($P < 0.05$).

Figure 2.5: Acidic and Alkali Interactions in Total Protein Content in Great Northern Bean Protein Isolate Samples.



Interaction of Alkali (Solubilizing) and Acidic (Precipitating) pH in great Northern bean protein isolates were analyzed. The protein isolates were solubilized into a solution using an alkali pH either 8.0 or 9.0 and then precipitated out of solutions using an acidic pH varying from 4.0 to 4.5 in 0.1 increments. The values displayed are LSM ± SEM. The asterisks denote significant differences between alkali pH values ($P < 0.05$).

Appendix A: Protein Solubility Analysis

Adapted from Stone et al., 2015

Procedure:

1. Weigh out approximately 250mg of the protein or flour samples into a 150ml glass beaker-record the exact weight of the sample. When weighing out the samples there will be 7 samples taken.
2. Add approximately 45ml of the selected buffer to each sample. Each sample will be adjusted to a specific pH. The pH of the samples to be tested are: 3,4,5,6,7,8
3. and 9. Below are the concentrations needed to prepare the pH of the solutions to test assuming a 1L solution is being made.

pH of Buffer Solution	Citric Acid anhydrous (g)	Sodium Citrate Dihydrate (g)	Deionized Water (ml)
3.0	8.21 g	2.13 g	993 ml
4.0	5.99 g	5.55 g	988 ml
5.0	3.52 g	9.32 g	987 ml
6.0	1.11 g	12.84 g	986 ml
	Sodium phosphate monobasic (g)	Sodium phosphate dibasic (g)	Deionized Water (ml)
7.0	2.92 g	7.73 g	989 ml
8.0	0.47 g	12.49	987 ml
9.0	0.05 g	13.30 g	986 ml

4. Adjust the pH if needed with 1M NaOH and 1M HCl.

Stir the protein solutions for 1 hour.

5. Transfer the protein solutions to volumetric flasks and fill the flasks with deionized water to the line in the glassware.

6. Transfer the protein solution of 0.5% to a 50 ml plastic Falcon centrifuge tubes.
7. Centrifuge the Falcon tube for 15 minutes at 1,467 *x g*.
8. Transfer 1ml of supernatant to the glass test tubes.
9. Now you have the protein in the solution so the samples can be tested on in an HPLC.

Appendix B: Appendix B: Pulse Protein Wet Extraction Procedure

Adapted from Tian, Kyle and Small, 1999 with minor modifications

Procedure:

1. Create a 1:10 mixture of flour: deionized water; weigh 100 g of flour and 900ml deionized water. Place the mixture into 1,500 ml beaker.
2. Place the beaker on a stir plate and stir at 220 RPM until the solution is well mixed.
3. Calibrate the pH meter using the standard solutions at pH 5,7 and 9.
4. Adjust the pH of the solution to 8.0 or 9.0 using 6 Normal NaOH.
5. Put a stir rod into the beaker and allow the mixture to stir for one hour at 220 RPM.
6. Put the mixture into 3 of the centrifuge plastic jars and label the jars.
7. Centrifuge the plastic jars at 1968 *x g* for 10 minutes after transferring the solution.
8. Pour the supernatant into a beaker.
9. Place a stir rod into the beaker and stir at 220 RPM and use a pH meter to measure the pH of the solution. Use the 6N HCl to adjust the pH to 4.0, 4.1, 4.2, 4.3, 4.4 and 4.5, respectively.
10. Put the pH-adjusted solution into plastic centrifuge jars and centrifuge at 19678 *x g* for 10 min.
11. Decant the supernatant.
12. Wash the pellet with deionized water and centrifuge for 10 min at 19678 *x g*.
13. Remove the pellet and place it into a beaker. Add a stir rod and stir at 220 RPM Adjust the pH to 7.0 using 6 N NaOH.
14. Place the contents of the beaker into one-gallon freezer bags which should be less than 2.54 cm thick.
15. Place the bags in the freeze drier and allow to freeze for at least 24 hours.
16. Remove the samples from the bag and freeze dry the protein isolate.

Appendix C: Appendix C: Protein Content of Pulse Protein Isolates Using RP-HPLC

Adapted from Taghvaei and Smith 2020

Procedure:

1. Weigh out 10mg of the protein isolate or the flour.

2. Add the protein isolate or flour to a 2ml microcentrifuge and clearly label the tubes.

3. Add 1ml of Guanidine and 20µl of β-Mercaptoethanol to the tube with the dry sample.

4. Vortex the solution for 15 min then centrifugate the tube for 5 minutes at 241 x g.

5. Dilute the supernatant into a 1:3 ratio where two more tubes are prepared each with 1ml of guanidine and 20µl of β-Mercaptoethanol.

6. Pipette 330 µl of the solution with the protein isolate was transferred into the other two tubes.

7. Following the dilution, 1ml of the solution is removed with a 1ml syringe and filtered into a labeled HPLC vial.

8. The HPLC vial has approximately 500 µl of the solution following being filtered and 10 µl of the HPLC solution is injected per each sample.

9. To run the samples through the HPLC-RP-prepare the solvents for the mobile phases.

10. The solvents that need to be prepared are 0.089% Trifluoracetic acid (TFA) in water for (mobile phase A) and acetonitrile for (mobile phase B).

11. For mobile phase A measure out 1,000ml of deionized water and pipette in 890 µl Trifluoracetic acid (TFA).

12. The HPLC-RP separation of protein content is performed with 20% of mobile phase B to 30% of mobile phase B for 10 min, 30% of mobile phase B to 39% mobile phase B for 20 min. Then adjust the mobile phase gradient from 39% mobile phase B to 60% mobile phase B for 10 min and keep it at 60% mobile phase B for 5 the last 5 min of the run.

13. The HPLC-RP should be run on a C18 column at 55.5°C was used for separation.

The quantitation of total protein content in pulse protein isolate samples was done using commercial pea protein isolate as a standard (R^2 of 0.9985 for the standard curve) and values obtained using this method were comparable with those obtained through nitrogen combustion.

Appendix D: Appendix D: SDS PAGE Protein Analysis

Adapted from Stone et al., 2015

Procedure:

Preparing the Gel-Dye Mix:

1. Add 25µl of the protein 230 dye concentrate to one protein 230 gel matrix.
2. Vortex well and spin down the tube for 15 sec.
3. Transfer to a spin tube.
4. Centrifuge at 2,500 g for 15 min.
5. Label the tube with the date and use within 4 weeks.

Preparing the Destaining Solution:

1. Pipette 650 µl of the gel matrix into a new spin filter and label the tube and include the date of preparation. The solution needs to be used within 4 weeks.

2. Store the solution; the solution can be used for 25 chips.

Running SDS-PAGE:

1. Weigh out 10mg of flour or protein samples into a 2ml tube.
2. Add 1ml of 1% SDS and 2% β-Mercaptoethanol solution. Vortex for 15 minutes and centrifuge at the highest speed.
3. Centrifuge at the highest speed for 5 min.
4. Pipette 4 µl of the protein or sample to a 0.5ml minicentrifuge tube with 2 µl of the denaturing solution into a 0.5ml microcentrifuge tube.
5. Place the sample tubes and tube with 6 µl of the protein 230 ladder at 95◦ C to 100 ◦C for 5 min. Allow the tubes to cool down afterward.
6. Spin the tubes for 15 s.

7. Add 84 µl of deionized water to the samples and ladder. Vortex the samples.

Loading the Gel-Dye Mix, Samples and Ladder:

1. Adjust the baseplate of the chip priming station to position A and the syringe clip to its new middle position.
2. Put a new protein chip to the chip priming solution.
3. Pipette 12 µl of the gel-dye mix into the well-marked for it.
4. Put a plunger at 1ml and close chip priming station.
5. Press the plunger until it is held by the clip and wait 60 s and then release the clip.
6. Wait for 5 s and then slowly pull the plunger back to the 1ml position.
7. Remove the gel-dye mix solution from the well marked for it.
8. Pipette 12 µl of the gel-dye mix into the wells marked for it.
9. Pipette 12 µl of the destaining solution in the well-marked for it.
10. Pipette 6 µl of the samples into the sample wells.
11. Pipette 6 µl of the ladder in the well-marked for the ladder.
12. Place the chip in the Agilent 2100 bioanalyzer (Agilent Technology, 2016, Waldbronn, Germany) and start immediately.

Appendix E: Oil Holding Capacity (OHC) Analysis

Adapted from Stone et al., 2015

Procedure:

1. Record the weight of the tube, tare. (W1)
2. Weight 0.5 g of flour/protein in a 50 ml screw cap centrifuge tube. (3 replicates per sample). Record weight of sample (W2).
3. Add 5.0 g of oil.
4. Vortex sample for 10 s every 5 min for a total of 30 min.
5. Centrifuge samples at 1000 $\times g$ for 15 min.
6. Decant the supernatant carefully to the waste container.
7. Record the weight of the tube. (W3)
8. Calculate oil holding capacity using the following equation:

$$Oil\ holding\ capacity = \frac{W3 - W1 - W2}{W2}\ x\ 100\%$$

Appendix F: Water Hydration Capacity (WHC) Analysis

Method taken from AAC 56-30.01

Procedure:

1. Weigh 1.0g of the test material into a pre-weighed 15-ml centrifuge tube.
2. Add distilled water in small unmeasured increments and stir with a glass rod after each addition of water is added until the dry material is saturated.
3. Wipe the stirring rods on the sides of the tube.
4. Centrifuge 2000 *x g* for 10 min.
5. Discard the slight amount of supernatant and weigh.

Calculate the WHC using the following equation:

$$Approximate\ WHC, \frac{ml}{g} = [(Weight\ of\ tube + sediment) - (\ Weight\ of\ tube + 1)]$$

To determine the WHC into each of the 4 tubes, weigh the quantity of material as calculated where 15 is desired total weight of sample and water.

$$Weight\ of\ material = \frac{15}{Approximate\ WHC + 1}$$

Appendix G: Foaming Properties Analysis

Procedure:

1. Prepare 1.0% (w/w) flour or protein dispersion with distilled water in a 50ml glass beaker with 3 replicates per sample: measure 0.15 g of sample and 15ml of distilled water.
2. Stir the solution for 1 hour at 220 RPM
3. Measure the pH of the sample and ensure it is between 6.5-7.0.
4. Transfer 15ml of the solution into a Falcon 50ml plastic centrifuge tube.
5. Homogenize the samples for 5 min using an Omni Macro Homogenizer at speed 4.
6. Transfer the sample to a 50ml glass beaker.
7. Measure the foam volume at time zero (V_0) and measure the foam after 30 min (V_{30}).

Calculate the foaming capacity and foam stability using the following equations:

$$\%FC=\frac{V_0}{15} \text{ x } 100\%$$

$$\%FS=\frac{V_{30}}{V_0} \text{ x } 100$$

Appendix H: Emulsion Properties Analysis

Procedure:

1. Prepare solutions with 0.5% (weight/weight) flour/protein dispersion with distilled water into 100ml of small glass beakers with three replicates per sample.

2. For each solution weigh out 0.25 g of the flour or protein sample and prepare with 50ml of deionized water.

3. Stir the flour or protein dispersions for one hour at room temperature at 220 RPM.

4. Turn on the spectrophotometer and allow the spectrophotometer to warm up for at least 15 min before taking any measurements.

5. Prepare a 50ml plastic Falcon centrifuge tube with 2ml of canola oil.

6. Transfer 6ml of the flour/protein dispersion into the tube with canola oil in it

7. Homogenize sample using Ultra-Turrax TP 18/10S1 at the highest setting for 1 min.

8. Take 50 µl emulsion sample from the bottom of the tube at 0 and 10 min after homogenization.

9. Prepare 15ml Falcon tubes with 10 mL 0.1% sodium dodecyl sulfate (SDS) solution.

10. Pipette 50 µl of the emulsion sample from the bottom of the Falcon tubes.

11. Measure the absorbance of the emulsion at 500 nm with UV/Visible Spectrophotometer (Ultrospec 4000, Pharmacia Biotech Braunschweig, Germany) using plastic cuvettes (1 cm path length).

12. Set a reference and zero the baseline for the spectrophotometer using 0.1% SDS solution.

Calculate ESI were calculated by using the following equation:

$$\text{ESI (min)} = \frac{A_o}{\Delta A} \cdot t$$

where, A_o is the absorbance of the diluted emulsion immediately after homogenization, DF is the dilution factor (x 200), c is the concentration of protein (g/mL) in aqueous phase before emulsion formation, φ is oil volume fraction of the emulsion, ΔA is the change in absorbance between 0 and 10 min and t is the time period (10 min).