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The Comparison of Functional and Physical Properties of Commercial Pulse Proteins to Soy Protein

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THE COMPARISON OF FUNCTIONAL AND PHYSICAL PROPERTIES OF COMMERCIAL PULSE PROTEINS TO SOY PROTEIN

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to soy protein

A thesis presented

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ABSTRACT

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COMMERCIAL PULSE PROTEINS TO SOY PROTEIN

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There has been growing interest in the utilization of plant-derived proteins as functional ingredients in many food and beverage applications because they are perceived as being more sustainable, healthy, and ethical than animal-derived proteins by many consumers. Traditionally, soy proteins have been the most widely employed plant protein in the food industry. However, a number of alternative plant-based protein sources have recently become available, with pulse proteins being one of the most popular. In this study, the physicochemical properties and functional attributes of various commercially available pulse protein isolates were compared with those of soy protein isolate to evaluate their potential application in foods and beverages. The water holding capacity, oil holding capacity, gelation properties, emulsifying properties, and color of faba bean (FPI), pea (PPI), lentil (LPI), and soy (SPI) protein isolates were therefore measured. SPI had a significantly higher water holding capacity (7.6 g/g) than the pulse protein isolates (2.2-5.1 g/g). Among the plant protein isolates, PPI had a significantly lower oil holding capacity and gelling property. LPI was more effective at producing small oil droplet sizes during homogenization than the other protein isolates.

Nevertheless, all of the plant proteins were capable of forming relatively small oil droplets (D_{32} = 1-3 mm) at a protein-to-oil ratio of 1:10. As expected, droplet size decreased with increasing protein concentration for all plant protein isolates, which increased their resistance to creaming. These results suggest that pulse proteins may have similar or better techno-functional properties than soy proteins for certain applications. In particular, lentil proteins were more effective emulsifiers, whereas faba bean proteins were more effective gelling agents. These proteins may therefore be suitable for application in plant-based milks, eggs, cheese, or meats where emulsifying or gelling properties are required.

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LITERATURE REVIEW

1.1 Introduction

There has been an increasing trend of shifting to a more sustainable and healthy diet in recent years, including vegetarian and vegan diets. Different organizations such as the Good Food Institute and the EAT-Lancet Commission have been promoting plant-based alternatives for meat, dairy, and eggs to promote sustainability and improved health. While plant-based alternatives are commonly accepted among vegetarians and vegans (e.g., tofu, seitan, tempeh), companies are now creating meat analogs that are intended to taste and smell more like real meat products, marketed towards meat-eaters to increase their acceptability.

Besides traditional plant-based ingredients such as soybeans and wheat gluten, there are emerging plant proteins that are used in meat analogs. In the US, among households avoiding certain food or ingredients, 39% of these consumers avoid food containing wheat or gluten and 22% of consumers avoid food or food ingredients containing soy due to allergic reactions (Srivastava, 2020). Although the leading plant protein in meat alternatives is still soy protein, the percentage of soy protein fell from 17% in 2015 to 14% in 2019 among new plant-based products (Srivastava, 2020). Pulse proteins, proteins from leguminous seeds, are emerging as they are contributing to 9% distribution of active patents for food & drink disclosing plant-based protein source (Srivastava, 2020). Among pulse proteins, the application of pea protein in plant-based products has increased to 11% from 2015 to 2019 (Srivastava, 2020). This diversification of plant-based proteins provides a wide range of physiochemical properties that may offer

advantages over traditional plant-proteins, which can ultimately impact the functional properties of food products.

Recent developments of this emerging plant proteins had led to more research on applying them in different food applications. Therefore, it is important to understand their functional properties to be applied in these applications. This article provides an overview of the factors impacting the functional properties of different plant-based ingredients, focusing on emerging plant proteins like chickpea, pea, lentil and faba bean proteins and also soy protein which is the most popular plant protein ingredient in the market. Previous studies investigating plant proteins have focused on individual plant-based proteins, with limited information on the factors impacting the functional properties thus, making it challenging to compare functionality within the pulse ingredient category. Moreover, there is a lack of consistency in the methods used to assess each functional property, which hinders the ability to analyze data from different papers critically. Common methods used to test functional properties are also discussed to understand which of the methods should be used for better comparisons between studies. Performance of emerging plant proteins on their functional properties are also shown in comparison to their protein content so that we would know what form of pulse proteins should be used in different applications.

1.2 Factors affecting functional properties of plant proteins

A review of the literatures has a research emphasis on plant protein ingredients including cereals, legumes, oil seeds, algae etc. (Loveday, 2019). Among these plant protein ingredients, pulses (peas, chickpeas, lentils, and beans), which has a high initial protein content (>20 g protein/100 g dry matter), is focused in many publications on their

functional properties to be applied in different applications (Schutyser, Pelgrom, van der Goot, A. J., & Boom, 2015). Current extraction methods are able to purify the pulses to ingredients with different levels of protein content from low to high including flour, concentrates and isolates. From the cultivar type to processing method, there are many factors that can affect the functional properties of plant protein ingredients, which would be discussed below.

1.2.1 Cultivars and genotypes

In general, the majority of plant proteins are composed of albumin and globulin fractions (Singhal, Karaca, Tyler, & Nickerson, 2016). Different cultivars and genotypes innately have different ratio of these protein components, which can influence the functional properties of the extracted plant protein concentrates and isolates (Singhal et al., 2016). There are several studies that suggest cultivar and genotypes have a significant impact on their functional properties.

Among lentil proteins, the water-soluble protein contents of the lentil proteins are reported to be significantly different among cultivars, where red lentil Fırat and green lentil Pul II have the highest water-soluble protein content of around 0.7g/g (Aydemir & Yemenicioglu, 2013). The influence on cultivar type is also supported by Boye et al. (2010) reporting that red lentil concentrate has a higher protein solubility than that of green lentil concentrate. The gelling properties of lentil proteins also varied among cultivars, where the gelling property of Ciftci and Kafkas red lentils are not strong enough to form a hard gel with a protein concentration of 14% while other cultivars (e.g.Ali dayı, Fırat) are able to (Aydemir & Yemenicioglu, 2013). The oil absorption capacity, foaming capacity, and foaming stability showed some statistically significant differences by cultivar, where Firat red lentil performed best in these functional

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properties. Firat red lentil also shows a significantly high foaming capacity that is even higher than that of soy protein isolate. Common Blaze red lentil concentrate produced by ultrafiltration also was shown to have higher fat absorption capacity than Grandora green lentil concentrate (Boye, J. I. et al., 2010).

Among chickpea proteins, the most common types Kabuli and Desi are being compared on their functional properties by many publications. Within the Kabuli type, different cultivars have shown differences in their water absorption capacity and foaming properties. Sarı-98 chickpea protein has a higher water absorption capacity than other cultivars of 23% higher than average (7.94g/g> 6.46g/g) and higher foaming capacity of approximately 18% higher than average (13ml>11ml) (Aydemir & Yemenicioglu, 2013). Another cultivar Cevdetbey-98 is also able to have high water-soluble protein content, gelling property and oil absorption capacity as Sarı-98, suggesting they both have advantages over other cultivars (Canıtez, Gökçe). Boye et al. (2010) studied desi chickpea compared to Xena kabuli chickpea resulting in similar overall functionality (including protein solubility, water holding capacity, gelling property and emulsifying property). Kaur et al. (2007) also looked at comparing 5 genotypes of desi chickpea protein isolates to one type of kabuli chickpea protein isolate. Kabuli chickpea protein isolate held a lower water absorption capacity but a higher oil absorption capacity than that of desi chickpea protein isolates. Current works indicate that the difference might be due to the presence of more non-polar amino acids in kabuli chickpea protein, which can help bind to fats. The kabuli chickpea also showed highest foaming stability after 120 minutes of storage. Therefore, when considering using Kabuli or Desi chickpeas, it is

important to consider the foaming property needed in the application as Kabuli has demonstrated better foaming ability.

Comparing pea cultivars, the isoelectric points of the pea protein isolates are all similar in the range of 4.6-4.9 (Stone, Avarmenko, Warkentin, & Nickerson, 2015). Their water and oil holding capacity are also similar among the cultivars. CDC Dundurn isolate was found to have a significantly higher protein solubility of 75.9% than the other isolate (66%). The higher solubility of the CDC Dundurn isolate compared to the other isolates was probably due to the lower surface hydrophobicity of CDC Dundurn isolate. Cooper and CDC Dundurn isolates showed significantly lower emulsifying capacity than the other 5 cultivar isolates although there is no significant difference found among the cultivars for emulsifying stability. This pointed out that CDC Dundurn isolates might associate poorly with the oil-water interface, thus might not be considered in an emulsion-based food product. Moreover, this study suggests there is a synergistic effect of extraction methods and cultivar on the functional properties of the pea proteins, including water holding capacity, foaming capacity, foaming stability, and emulsifying properties. For example, CDC Meadow isolates had the highest water holding capacity when extracted by salt extraction, but lower water holding capacity than others when extracted by micellar precipitation. This shows that the effect of cultivar-type has less of an impact on differences in functional properties than the extraction method.

But not all pulse proteins show variations among their genotypes, for example faba bean *Vicia faba* L. genotypes have similar functional properties among each other. Comparing the 7 genotypes' protein isolates, the zeta potential, hydrophobicity, protein solubility, oil holding capacity and emulsion capacity, creaming stability, emulsification

activity, and stability indices are all not significantly different (Singhal, Stone, Vandenberg, Tyler, & Nickerson, 2016). As the differences between the genotypes are small, there is less concern on which genotype to choose for different food applications.

1.2.2 Extraction methods

Extraction of protein from pulses and legumes includes removing the starch fractions to increase their functional properties. There are two main categories of protein extraction methods, dry and wet processing. The most common methods of wet extraction method include isoelectric precipitation (with alkali or acid extraction) and salt extraction. Isoelectric precipitation (IEP) is used by first mixing the plant proteins in alkaline or acid solution to solubilize most protein into the solution. The pH of the solution is then adjusted to the isoelectric point to precipitate out the protein. The other popular method of salt extraction (SE) is by dispersing plant flours in a salt solution with high ionic strength like ammonium sulphate and sodium chloride solution, where the salt concentration is high enough to promote proteins to aggregate and precipitate. Micellar precipitation (MP) also uses the principle of salting-in but the last step of precipitation is done by diluting the salt concentration to lower the ionic strength instead of dialysis in SE. Ultrafiltration (UF) is a type of membrane filtration method where hydrostatic pressure is applied to separate materials from water and salts using a semipermeable membrane. By using different extraction methods, it has been shown that the plant proteins from the same cultivar can perform differently in their functional properties.

In general, wet processing is an efficient process for extraction of protein, extracting a minimum of 70% of the total protein content. While this is desirable, there is variation in the reported effect of different wet processing methods on the protein content

on plant proteins, yet there are inconsistencies in these findings. IEP isolates of chickpea, faba bean, lentil and pea proteins were reported to have a higher protein content (81.9% - 88.2%) whereas those produced by salt extraction had a lower range (72.6%-81.6%) (Karaca, Low, & Nickerson, 2011), although Paredes-López et al. (1991) reported differently for chickpeas having higher protein content for SE (87.8%) than IEP (84.8%). The IEP soy protein isolate also was reported to have a similar protein content of 82.3-86% (Brishti et al., 2017) (Sosulski & McCurdy, 1987).

With the different extraction methods, the functional properties of plant proteins are significantly different from each other. MP chickpea protein isolates had a higher than that of IEP protein isolates (Karaca, Low, & Nickerson, 2011). Moreover, Stone et al. (2015) reported that MP pea proteins had the highest water holding capacity (3.2-3.6g/g), followed by IEP isolates (2.4-2.6g/g), and SE isolates (0.34–2.6 g/g). The author suggested that MP may have exposed more polar groups on the protein, allowing better hydrogen bonding with water, whereas the isoelectric technique results in proteins with a structure that limits the ability of the proteins to interact with and absorb water. For emulsifying activity, IEP pulse protein isolate is significantly higher than that of SE pulse protein isolates (Karaca et al., 2011). The IEP pulse emulsion droplet size was also found to be significantly smaller (~1.6μm) than that of SE protein isolates (Karaca et al., 2011). This is because the protein isolates that are produced by IEP had slightly higher surface charge, and surface hydrophobicity than that of SE (Karaca et al., 2011) (Stone et al., 2015). As surface hydrophobicity of globulin are reported to be higher than albumins, it is predicted that isoelectric precipitation may extract out more globulins than albumins (Stone et al., 2015). The emulsifying stability, creaming stability and foaming expansion

are also higher for protein isolates produced by IEP than SE as the creaming stability is positively correlated with surface charge and solubility of protein. This may imply that IEP protein isolates should be used for emulsion-based application, which requires high emulsion stability.

By using UF instead of IEP, the step for lowering the pH to the isoelectric point can be opted; therefore, it suggests that the UF proteins can be less denatured by this process and result in higher functional properties. Boye et al. (2010) reported that UF with diafiltration results in a slightly higher protein content but similar protein yields compared to that of IEP. UF pulse protein concentrates was also found to have higher oil holding capacity and better gelling property than isoelectric precipitated protein concentrates, meeting the hypothesis. But for some IEP pulse protein including green lentil and chickpea concentrates have higher foaming stability than that of UF, which might show the protein type had a greater effect than that of extraction method. But besides that, UF pulse protein concentrates had no significant difference of its water holding capacity, emulsifying property and foaming capacity than that of IEP.

The lesser used method, dry processing (air classification) is not able to extract proteins with high purity (<50%) as it separates the protein and starch fractions using an air stream based on their particle sizes. However, this method can provide advantages over IEP even though the protein extracted has a lower protein value than that of wet processed proteins. (Vogelsang-O'Dwyer et al. (2020) reported the faba bean protein extracted using air classification resulted in a 64.1% protein concentration compared to wet processed isoelectric precipitated isolates with 90.1% protein due to the inherent limitations of air classification. However, it was found that air classified faba bean

protein demonstrated superior functionality in many attributes including significantly higher protein solubility (85%), compared to isoelectric precipitated isolates (32%) at pH 7. This is supported by the higher surface hydrophobicity of faba bean isolates due to denaturation that might have occurred during the drying process required after wet extraction. Moreover, the air classified faba bean protein also performed better in foaming capacity and gelling ability compared to isoelectric precipitated isolates, with significantly higher gel strength of air classified faba bean protein gels at 15% concentration than that of faba bean protein isolate. The author suggested that the higher carbohydrate in air classified faba bean protein might have contributed to the difference. As the dry processed protein can perform well in different functional properties, it is also worthwhile to understand how to apply them in food applications.

1.2.3 Drying methods

After wet protein extraction, proteins are usually dried so that it becomes shelf stable prior to packing and shipping. The most common commercial method is spray drying (SD), which is a quick way to directly convert a liquid to dried powder by rapidly drying with hot gas. Freeze drying (FD) on the other hand, usually done in research studies, converts water from wet protein to vapor by sublimation using pressure and reduced temperature. Commercially, spray drying is more common, as freeze drying is a more expensive and slower method. There are other novel drying methods that lower the processing temperature, which are vacuum drying (VD) and refractance window drying (RWD). VD in comparison to the other two methods has a faster drying rate, lower drying temperature, and uses an oxygen-deficient processing environment. Similarly, RWD uses low temperatures and has a short drying time. Different drying process are

known to impact the functional properties of the extracted protein due to variations in heating temperatures and duration of drying time.

Joshi et al. (2011) has directly compared SD, FD, and VD of lentil protein isolates. Among the three methods, SD was previously thought to have lower functional properties than others because the heating process can reach 80℃ or above. However, SD showed comparable high solubility as FD protein isolates because the solvent evaporation creates a self-cooling effect that prevents the temperature of the protein from reaching too high (Abdul-Fattah, Kalonia, & Pikal, 2007). The high solubility of SD powders may be due to their smaller and more uniform particle size distribution. As SD and FD of lentil protein isolates are found to have significantly different lentil protein isolates, the common use of FD in laboratory-based setting can lead to functional differences than SD, which is more common in commercial production. VD protein isolates showed significantly lower solubility than that of other methods, which may be due to the longer drying time (up to 48 hours) allowing more proteins to be denatured. VD soy protein isolate is also reported to be more denatured than that dried by the other two methods (Hu et al., 2010). With lowest solubility, VD lentil protein isolates also show significantly lowest gelling property, forming the weakest gel. Although having a high solubility, SD protein isolate has the lowest water holding capacity than that of other drying methods. This might be due to spray drying can create very thin and highly moisture resistant skin on the protein powders during the drying process.

Comparing FD to the novel method of RWD for chickpea protein isolates, the maximum protein solubility of RWD (74.5%) is significantly lower than FD (94.2%) (Tontul, Kasimoglu, Asik, Atbaken, & Topuz, 2018). The lower solubility of the RWD

may be due to higher degree of denaturation as the temperature of processing is higher than freeze drying. However, RWD protein isolates have higher water holding capacity than that of FD protein isolates. The RWD protein isolate has also higher performance in emulsifying activity and stability as their surface hydrophobicity is 50% higher than that of FD protein isolates. But in terms of foaming and gelling property, FD protein isolates shows better foaming stability and can form stronger gels than RWD protein isolates. Therefore, RWD protein isolates are more suitable to be applied in high-fat emulsion products as its surface hydrophobicity and emulsifying activity is high. On the other hand, FD protein isolates can be applied in applications that requires better foaming and gelling properties.

1.2.4 Different forms of plant proteins

Plant proteins are usually in the form of flour, concentrate, or isolate based on the overall protein concentration which is attributed to the overall extraction processed used. Researchers have noted variations between functional properties across different forms, due to the increasing protein content from flour to isolate. With higher protein content, protein isolates usually exhibit higher water holding capacity as compared to their respective flour form. This is attributed to the additional carbohydrate and other components present in flours may act as barrier to hold water. Aryee et al. (2017) also suggest lower lipid content and smaller particle size of lentil protein isolates may contribute to its higher water holding capacity compared to flours. Oil holding capacity of chickpea protein isolates ranged between 2.08 and 3.96 g/g which were significantly higher than those observed for their corresponding flours ranging 1.05 g/g –1.24 g/g (Kaur & Singh, 2007). However, the gelation property of great northern bean and

chickpea protein isolates are significantly lower than their corresponding flour as their least gelation concentration is higher than that of flour (Sathe & Salunkhe, 1981) (Kaur & Singh, 2007). In this case, the gelling property may not only be influenced by the protein content alone but also dependent on the type of protein, as their sulfhydryl groups suppress the intermolecular bonding between proteins through disulphide bridge formation (Berghout, Boom, & van der Goot, A. J., 2015). Sosulski et al. (1987) also found when comparing pea and faba bean protein isolates to their respective flours, protein isolates had a lower nitrogen solubility index of only 38-40% soluble at pH 6.6 where the pea and faba bean flours were 80-86% soluble. The lower protein solubility of isolates can reduce other functional properties like emulsifying and foaming properties. Regarding foaming properties, different chickpea protein isolates were observed to have foaming capacity ranging 30.4% to 44.3 %, which were significantly higher than their corresponding chickpea flours of approximately 15-20% (Kaur & Singh, 2007). The emulsifying capacity was also reported to be higher for pulse protein isolates (pea, faba bean and great northern bean) as these two functional properties are positively corresponded to solubility (Sathe & Salunkhe, 1981) (Sosulski & McCurdy, 1987).

1.2.5 Commercial or laboratory processed plant proteins

Many of the past published works that have reported the functional properties of plant protein isolates and/or concentrates that were prepared starting with flour and further concentrating the proteins with bench extraction methods. While this has helped to indicate overall functions of new and emerging proteins, evidence suggests significant differences in the overall property performance exist between benchtop and commercially processed proteins.

Shen (1976) reported the commercial soy protein isolate had lower solubility than that of the laboratory processed soy protein, with great difference especially in the pH 6-10 range where commercially processed soy protein isolate has 30% lower solubility. Similarly, Stone et al. (2015) reported higher solubility of pea protein isolates prepared in the laboratory. The average solubility of laboratory prepared pea protein isolate (65.7%) is much greater than that of the commercial processed one (5%). This indicates that commercial processing has and additional effect on insolubilizing proteins, which may affect their functional properties as many functional properties depends on the protein solubility. Although the author mentioned that there was still variability in solubility between different commercial soy isolates, but this finding is also shown in other studies (Aydemir & Yemenicioglu, 2013) (Tang, Wang, Yang, & Li, 2009) (Wagner, Sorgentini, & Añón, 2000). For instance, although both commercial soy protein isolate and their soy protein extract have similar total protein content (0.90-0.92g/g), their water soluble protein content are significantly different, where that of soy protein extract is 0.57g/g compared to 0.21g/g of commercial protein isolate (Aydemir & Yemenicioglu, 2013). This might be due to the fact that plant proteins are easily denatured under acid precipitation and higher and longer temperature exposure, which occurs more often in large-scale industrial production (Tang et al., 2009). Moreover, common practices in commercial production for example calcium hydroxide addition during neutralization instead of calcium chloride can lower solubility as chloride anion helps weakening of electrostatic interactions of polypeptides and hydrophobic interactions (Wagner et al., 2000). However, others have demonstrated that additional processes such as combining

homogenization and ultrasonic treatment can reduce protein denaturation (or improve functionality) in commercial production (Tang et al., 2009).

As mentioned that commercial and laboratory processed plant proteins may have different denaturation levels, the thermal denaturation temperature of pea protein isolate that is laboratory processed is reported to be higher (Td = 82.61-94.28 °C) than did the commercial pea protein isolate (Td = 72.83-72.92 °C) (Sun & Arntfield, 2010). The laboratory processed pea protein isolate had a higher heat flow (Td 15.81-17.84J/g protein) than did the commercial pea protein isolate (Td = 0.033-0.036J/g protein), where a higher heat flow indicates that the pea protein was less denatured before heat treatment. Furthermore, when the commercial pea protein isolates are heat treated to about 86°C, there is a lack of a transition peak, which means that most of the protein isolates are already denatured during processing. As expected, the least gelation concentration test showed that commercial pea protein isolates need significantly higher concentration (14.5%) to gel than that of laboratory processed pea protein isolates (5.5%). Laboratory processed pea protein isolates also has better gel strength than that of commercial pea protein isolates as they may be less denatured.

Añón et al. (2001) reported commercial soy protein isolates had higher water holding capacity and lower solubility than laboratory processed soy protein isolates. And compared to the intentionally thermally treated soy protein isolates, their water holding properties are very similar to that of commercial soy protein isolates, showing that denaturation may have occurred in commercial soy protein isolates. Therefore, higher solubility of soy protein isolates did not guarantee good hydration properties. Moreover, the commercial soy protein isolate also shows higher water holding capacity of

commercial soy protein isolate (7.94 gH$_2$O/g) than that of soy protein extract (1.69

gH$_2$O/g), but lower oil holding capacity of 1.16 g oil/g than 8.23 g oil/g of soy protein

extract (Aydemir & Yemenicioglu, 2013). The apparent viscosity of commercial soy

protein isolate is also higher because of its better hydration property (Añón et al., 2001).

Therefore, it is prudent to use commercial processed protein isolates for product

development due to the overall functional differences. c.

1.2.6 Structure of plant proteins

Although many reviews have explained in detail of the structures of the plant

proteins, we would still like to highlight the main points of how the structure of plant

proteins have essential impact on their functional properties. Most plant proteins mainly

consist of salt-soluble globulin and water-soluble albumin in a ratio of approximately 70

to 20 depending on the type of plant proteins (Singhal et al., 2016). Legumin (11S) and

vicilin (7S) are the main globulins in plant proteins (Boye et al., 2010). Other minor

proteins in plant protein include convicilin, prolamins and glutelins, which consists of

different amino acids (Boye et al., 2010). The ratio of legumin and vicilin in plant

proteins varies depending on the type of plant proteins and can affect the functional

properties of plant proteins. Barac et al. (2010) reported that a low legumin to vicilin ratio

in pea proteins can increase the functional properties including emulsifying and gelling

property of the plant protein because of higher protein extractability. The amino acid

compositions in plant proteins also varies due to the type of protein and genotype (Hall,

Hillen, & Garden Robinson, 2017). This difference in amino acid compositions can affect

the functional properties because the difference in ratio of polar and non-polar amino

acids can affect the surface hydrophobicity of the plant proteins. Therefore, the structure

of the plant proteins is also another factor that can influence their functional property

performance.

1.3 Characterization of plant protein functional properties

1.3.1 Water and oil holding capacity/absorption

Water and oil holding capacity (WHC) is the measure of how much water and oil

can the protein hold on a per gram basis. This is especially important to consider for food

applications as it can affect the juiciness of one's product. The common method based on

Beuchat (1977) and Lin et al. (1974) is to disperse a known portion (g/g) of the protein

in distilled water or vegetable oil followed by vigorous mixing. The solution is then

centrifuged, and excess water or oil are removed. The difference in weight between the

protein before and after centrifugation is calculated to determine how much water or oil

the protein can hold (expressed as gH_2O/g protein or g oil/g protein). Among the past

studies that have measured the water and oil holding capacity, different concentrations of

protein solution were used, and some had longer mixing time than others. The mixing

time for protein to dissociate in water is an important factor as protein powder takes time

to absorb the water surrounding it which may affect the final water holding capacity. And

after decanting the supernatant, some studies have put the centrifuge tube upside down

for removing the excess water or oil and this additional step can cause variation in the

result as some studies uses small amount of protein, which may cause bigger error.

In general, the plant protein isolates have increasing reported values for both

WHC and OHC with increasing protein content, shown in **Table 1.1**. For example, with

increasing protein content from approximately 20% to 90%, there is increasing trend for

the WHC of chickpea protein. Although there is a high WHC value of 4.90-7.94 gH2O/g

for approximately around 75% protein content, this sudden increase can be attributed to factors such as cultivars and extraction methods, as discussed above, where there is stronger impact on WHC than protein content. The plant proteins OHC also increases with protein content. Looking at pea protein, its OHC also increases with protein content (25% to 82%), but the impact for OHC is smaller than that of WHC. This shows that protein content has impact on WHC and OHC to different extent. Among all plant proteins, the highest reported WHC is from soy protein with 90-92% protein content of 4.52-7.94 gH2O/g, followed by chickpea, pea and lentil protein in decreasing rank order.

As these plant proteins have good water and oil holding capacity, they are being applied as meat extenders and in meat analogs. By adding 2.5% common bean flour as extender in beef sausage, the water holding capacity, which is measured by water the sausage can hold when compressed with 1kg weight, is reported to be significantly higher than control (Dzudie, Scher, & Hardy, 2002) . Sanjeewa et al. (2010) reported that the addition of chickpea and pea flour applied in low-fat pork bologna model resulted in higher cooking yield than the control, where cooking yield for chickpea flour is the highest of 97.2%. The purge loss, the percentage of weight loss of the sample after storage, was also significantly lower for plant flour added bologna than the control. This shows that the addition of plant proteins help maintains the quality of the product during storage.

Pea protein isolates was also added as meat extenders in chicken nuggets as they are able to increase the water holding capacity as the concentration level increases from 3% to 12% (Shoaib, Sahar, Sameen, Saleem, & Tahir, 2018). The overall product cook loss also decreases as more pea protein isolate was added, with the lowest cook loss of

5.01% compared to the control of 12.43% with no plant protein added. This is due to more water and oil being retained in the product by the plant proteins. Although the cook loss is lowered, the overall moisture content of the chicken nuggets decreased with concentration more than 3% pea protein isolates added. Therefore, more water might be needed in product formulation as protein isolate powders can make the product dry.

Plant protein concentrates and isolates has also been applied as textured vegetable proteins (TVP) by extrusion to be applied in meat analogs, which also demonstrates high water holding capacity. The water holding capacity of the TVP can highly influence the porosity and air cell size of TVP structure (Samard & Ryu, 2019). TVP are usually made from soy protein isolates as it is the most popular and common plant protein, but emerging proteins such as pea, mung bean, peanut are also applied as TVP in recent years. Pea based TVP can be produced by high (55%) moisture or low (26-35%) moisture extrusion (Schreuders et al., 2019). Comparing to other plant based TVP, pea protein based TVP has a higher water holding capacity than mung bean, peanut, gluten based TVP and higher oil holding capacity than soy protein and mung bean protein based TVP (Samard & Ryu, 2019).

In the application of chicken sausage analog application, a plant protein-based formulation of SPI, gluten and chickpea flour is able to reduce the cooking loss and shrinkage of the product (2019). Either complete replacement of meat in sausage or sausage with only 20% of chicken meat is able to reduce the cooking loss to 0 from 8.72% of the 60% chicken meat sausage. Therefore, it is showing, the plant proteins combination in the meat analog can greatly help with binding water and oil in the product.

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Gelling property is an essential functional attribute for plant proteins when the food application requires the gel for the structure and texture of the product. The most common method for measuring gelling property is called least gelation concentration (LGC), where the protein solution forms a gel that does not slide from the tube when inverted (Sathe, Deshpande, & Salunkhe, 1982). This method is based on Sathe et al. (1982) and is widely used in past publications. This method requires a series of plant protein solutions usually from 2% to 20% prepared by heating at around 100℃, to facilitate heat gelation. After heating for an hour, it is allowed to cool and then the tubes are inverted for observation. Although the least gelation concentration method can compare across plant proteins, it does not provide information on the hardness of the gel that is formed. Therefore, some studies have also added methods to test textural properties of gels made with plant proteins by using compression tests. For example, texture profile analysis (TPA), can measure hardness, adhesiveness, springiness, cohesiveness, gumminess, and resilience of gels. Using this method, Makri et al. (2006) reported that lupine protein gel has higher hardness than that of pea and faba bean protein gels, showing better gelling property of lupine protein. Dynamic rheological measurements with the change in temperature using a rheometer, shows kidney bean protein has higher gel strength and thermal stability than pea protein (Shevkani, Singh, Kaur, & Rana, 2015).

In general, the protein content of plant proteins does not affect their gelling property. See values in **Table 1.1** for pea, faba bean, chickpea, lentil and mung bean protein. The least gelation concentration found for most plant proteins falls in the range

of 10-18%, which shows great gelling properties as the maximum test concentration is 20%. Among all plant proteins, the highest gelling property is chickpea protein with lowest LGC of 5-7%, followed by faba bean and green lentil protein.

Therefore, these plant proteins are added as gelling agents to provide textural integrity to meat products as meat extenders (Asgar, Fazilah, Huda, Bhat, & Karim, 2010). Many studies have evaluated the textural properties of the products added with plant proteins as meat extenders. Motamedi et al. (2015) has reported that the addition of chickpea and lentil flour in hamburger resulted in higher hardness. Addition of chickpea protein concentrate in Merguez" sausages also shows significant difference on the texture properties to control as it can form a stronger protein gel (Mokni Ghribi et al., 2018). Kamani et al. (2019) did a comparison of only adding 20% or 60% chicken meat in soy-based sausage and found no significant difference in their gel strength. This shows that the main formulation of soy protein isolate, gluten and also chickpea flour can produce a very strong gel. The textural properties including cohesiveness, chewiness, stiffness, adhesiveness and gumminess are not significantly different between the 20% and 60% chicken meat formulated sausages. Although the chicken meat free version of the sausage has a significantly lower gel strength than the hybrid sausages, it might be due to the higher amount of water added into the formulation. Therefore, there is great potential in applying plant proteins in making not only hybrid meat products to reduce meat consumption, but also meat-free products with more research on its formulation proportions.

Žˇugcˇic et al. (2018) compared the addition of soy, pea, lentil and bean protein as meat extenders in beef patties and found that soy protein added beef patties resulted the

highest hardness, gumminess and chewiness among the plant proteins, which may be due to formation of harder gel. Although pulse proteins added beef patties have lower textural properties, it may be resulted in the lower protein content (55-60%) compared to that of soy protein (90%). Faba bean flour can also be applied in producing protein-based emulsion gel foods, including yogurt and tofu analogue products (Jiang, Wang, Stoddard, Salovaara, & Sontag-Strohm, 2020). With starch removed from the faba bean flour, which increases the protein content in the flour, the tofu analogue resulted in higher gel texture and water holding capacity.

1.3.3 Protein solubility

Protein solubility can have an impact on other functional properties, especially for the emulsification and foaming process to help facilitate plant proteins' migration to the oil–water or air–water interface (Johnston, Nickerson, & Low, 2015). A common method of protein solubility is referenced from Morr et al. (1985), where protein is dispersed in a buffer solution and the pH is adjusted by the addition of 0.1M NaOH or HCl. Then the solution is centrifuged, and the supernatant is removed for evaluation of its protein content. There are different modifications of this method and slight differences across studies, where some disperse proteins in NaOH directly, or in water prior to adjusting the pH. With buffer added first, the adjustment for the desired pH should be more stable. Moreover, the stand time that allows the protein to dissociate in the solution varies from 30 minutes to overnight. It was found that IEP soybean, faba bean, and pea protein isolates had a higher protein solubility at pH 7 in Karaca et al. (2011) than that of Fernández-Quintela et al. (1997) which might be caused by difference in mixing time of protein solution of 30 minutes compared to stirring overnight. Lastly, the final

measurements of protein solubility are usually done by micro Kjeldahl method, Bradford Assay and Lowry method.

In general, the protein solubility of plant proteins is the lowest from pH 4-6, which is lower than approximately 20% because their isoelectric points are in this pH range, shown in **Figure 1.1**. High solubility of above 80% are reported for soy, chickpea, faba bean, pea and lentil proteins when reaching pH 8, while the majority of plant proteins are in the range of 40-60% at pH 3. Therefore, it is at recommended that pH levels are held at 8 or above for optimal solubility. The pH of meat products like hamburgers and sausages are usually at pH 5-7 depending on the type of meat, which intercepts the range of isoelectric points of the plant proteins. If small amount of plant proteins is added as meat extenders (3%), the pH of plant proteins including soy, bean, lentil, broad bean proteins added chorizo sausage is reported at around 5.8, which is within the isoelectric point range (Thirumdas et al., 2018). However, with the application of total replacement of plant proteins in meat analog, the pH was able to be increased to around pH7, therefore increasing the solubility of the plant proteins (Kamani et al.,2019). Therefore, plant proteins have potential in the application in meat analogs as the the high concentration of plant protein can increase the pH, therefore increase the protein solubility.

1.3.4 Emulsifying property

Emulsifying property is usually classified by emulsifying capacity and emulsifying stability. Emulsifying capacity is the ability of the dispersed protein solution to emulsify oil and emulsion stability is the ability to stabilize the emulsion over time. As emulsifying properties can be affected by multiple factors of the protein molecules for

example the shape, charge, and hydrophobicity of the protein molecules, there are a variety of ways of measuring the emulsifying property reported among past published works. The two most common methods of measuring emulsion capacity and stability, according to Yasumatsu et al. (1972) and Pearce & Kinsella (1978) is measured as the term emulsion activity (EA) or emulsion activity index (EAI). Both methods suggested to prepare the emulsion by dispersing protein in buffer solution or water and then homogenize with vegetable oil. However, Yasumatsu et al. (1972) uses centrifugation after the emulsion is made, measuring the emulsion activity by the ratio of emulsion layer volume to total volume after centrifugation. As pointed out by McClements (2007) these techniques are greatly affected by the type of blender and blending conditions used in the test, which make it difficult to compare between studies as these conditions are different among studies. This is because the amount of emulsifier required to stabilize the emulsion depends on the oil-water interfacial area rather than on the oil concentration. However, it is still useful for comparing the efficiency of different emulsifiers under the same experimental conditions.

On the other hand, the emulsion stability test, according to (Yasumatsu et al., 1972) requires first incubating the emulsion in 80℃ for 30 minutes before centrifugation to accelerate the breakdown of emulsion. The emulsion stability is measured by the deduction by 100 of the ratio emulsion layer volume/initial emulsion layer volume. For Pearce & Kinsella (1978) method, an aliquot of the emulsion is diluted with 0.1% SDS solution and the absorbance at 500nm is measured. For the emulsifying stability test, an additional sample of emulsion is diluted in 0.1% SDS solution 10 minutes after homogenizing. The result of emulsion activity index and emulsion stability index is

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calculated by the equation provided below in **Figure 1.2**. But this method can be overly simple to demonstrate the complex relationship between emulsion turbidity and particle size (McClements, 2004).

Other methods can be used to assess emulsifying capacity. For example, measuring the maximum amount of oil that is emulsified in protein solution before the emulsion breaks, which is usually expressed in gram or milliliter of oil per gram of protein (Sosulski & McCurdy, 1987) and the turbidity (NTU) of plant protein emulsions (Aydemir & Yemenicioglu, 2013). With these different methods and terms indicating emulsifying properties, the results cannot be compared between different methods. In more recent studies on the emulsifying properties of plant protein concentrates, few use particle size distributions to understand the emulsion capacity of plant proteins and emulsion stability is measured by the change under environmental stress for example pH, ionic strength, temperature (Gumus, Decker, & McClements, 2017) (Johnston et al., 2015). Additional tests on plant-based emulsions' droplet characteristics, include zeta potential, surface hydrophobicity, and interfacial tension, as the physicochemical properties of food emulsions are strongly influenced by the characteristics of the droplets that they contain.

The emulsifying properties of plant proteins using three of the methods (Yasumatsu et al., 1972) (Pearce & Kinsella, 1978) (Sosulski & McCurdy, 1987) are reported in **Table 1.2**, which is divided into three table because the results using different methods cannot be compared. There are different trends in the impact of protein content on emulsifying capacity. The emulsifying capacity of mungbean proteins decrease while that of faba bean and soybean proteins increases when protein content increases. This

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shows potential in choosing higher protein content of faba bean and soybean when higher emulsifying capacity is needed in applications like meat analogs. Chickpea is reported to have highest emulsifying stability among the pulse proteins with similar protein contents. **Table 1.2c** shows differences in both emulsifying activity and emulsifying stability between studies, which can be attributed to different homogenizing speeds and blending time. However, within each of the studies, chickpea had the highest reported emulsifying activity and stability index among other plant proteins, showing more potential in emulsifying applications.

These plant proteins can be used to emulsify and bind fat in meat products such as frankfurters and patties. Leonard et al. (2019) reported that the addition of lupin flour can enhance the emulsion stability in beef sausage. With increasing addition of lupin flour in beef sausage, the fluid released, fat released and water released decreased, therefore a higher cooking yield. Pulse proteins also helps maintain emulsion in the application of salad dressings reducing the addition of egg yolk as emulsifier (Ma, Boye, & Simpson, 2016). The results showed that the addition of lentil, chickpea and pea protein isolates supplemented salad dressing have similar physical properties as commercial ones. This might be due to good emulsifying property of these proteins.

1.3.5 Foaming property

Plant proteins can stabilize foam by adsorbing at the interface and form a stabilizing film around the air bubbles. This is important for food applications, such as cakes and ice cream, for creating their creamy and fluffy texture. The foaming property consists of measuring both foaming capacity and foaming stability. Foaming capacity is the measure of how much foam a protein solution can create by vigorous mixing, while

foam stability is the measure of how long protein can stabilize the foam created for a period of time. The common method to create foams is by whipping the protein solution using a homogenizer or a blender. After the foam is created, the volume of the foam is recorded by immediately pouring into a graduated cylinder where the volume of foam is measured over a period of time to observe the volume change in the foam. In different studies, large variations are reported for mixing speed and time. Therefore, the volume of foam might be lower for the ones using low speed mixers, causing indirect comparisons between studies.

The foaming properties of plant proteins are reported in **Table 1.3**, where there is a wide range of reported foaming capacity. This might be attributed to the different blending methods used in each study to create the foam. The highest reported foaming stability is from soy, followed by green lentil, pea, kidney bean proteins, which are higher than 90%. These higher foaming stability values are reported from proteins that have a relative higher protein content of at least 90%, showing the higher protein content may be attributed to higher foaming stability. Other studies have also used specific volume (mL/g) as a measurement for foaming property, which is the ratio of the volume of whipped protein solution to the weight of the whipped solution, but this method is seldomly used as the volume increase in foam can demonstrate the foam capacity in a more direct way. (Gupta, Chhabra, Liu, Bakshi, & Sathe, 2018) (Sathe & Salunkhe, 1981).

As these plant proteins demonstrate good foaming properties, they are being applied to many baked goods. Lentil protein has been used to replace egg white and milk protein in angel food cake and muffin (Jarpa-Parra et al., 2017). The final product volume

for both muffins and angel food cakes did not significantly change when egg white and milk protein are replaced by lentil protein. Moreover, this lentil protein replacement had lower baking loss than the control. Lupin flour has also been reported to be good bread additive as the structure and height of bread did not significantly change up to 5% of substitution of wheat flour (Pollard, Stoddard, Popineau, Wrigley, & MacRitchie, 2002). This result would help reduce time in the batter mixing process.

1.4 Prediction of plant protein functional properties

As discussed in previous sections, functional properties of plant protein are affected by a large number of factors including intrinsic factors such as cultivars, genotypes, and chemical structures and extrinsic factors such as environmental conditions (product pH, ionic strength etc.) and processing conditions (pressure and temperature etc.) (Damodaran, 1994). In order to facilitate the application and modification of the plant protein in modern food processing, one of the challenges is to develop models that describe quantitative relationships between functional properties and pertaining factors. *In silico* approaches in predicting functional properties of plant protein is the same as predicting the functionality of any other proteins and can essentially be classified in two major techniques: 1) statistical based quantitative structural activity relationship (QSAR) modelling and 2) physical based particle-based simulations.

The QSAR aims to develop quantitative expressions to corelate molecular features to activity and functionality of proteins (Roy, Kar, & Das, 2015). Models are developed using a wide range of statistical modelling techniques ranging from regression such as partial least square method and response surface method (Nakai & Li-chan, 1985) (Mune, Sogi, & Minka, 2018) to modern machine learning techniques such as artificial neural

networks (Arteaga & Nakai, 1993) (Liu, 2017) (Rifaioglu, Doğan, Martin, Cetin-Atalay, & Atalay, 2019), depending on the dimensionality of the descriptors. Chemical attributes of a protein at all levels have been used as descriptors in predicting functionalities of a protein. These descriptors include amino acids sequence (Fetrow & Skolnick, 1998), amino acids composition (Siebert, 2003), physicochemical properties (Arteaga & Nakai, 1993), conformational characteristics of proteins, molecular geometries (Chen, 2006) and combinations of the aforementioned (Lee, Redfern, & Orengo, 2007) (Mune et al., 2018)

QSAR models can be predictive even the underlying biophysical mechanisms are elusive, however, quality and availability of the data determine the reliance of the model. Therefore, it is of critical importance to develop standard methods to characterize plant protein functionalities, which is currently lacking (c.f. section 2). The other limitation of QSAR models is that they do not account for the conformational change of plant proteins under the influence of extrinsic factors such as heat and pressure exposure during extraction and drying processes, which has a repercussion on their functionalities. The dynamics of protein conformational changes during processing can be resolved by particle-based simulations.

While particle-based simulations include a family of techniques such as coarse-grained, Brownian dynamic and molecular dynamics simulations, molecular dynamic simulations are commonly employed as a suitable approach that addresses the length-scale and time-scale of the three dimensional conformation changes of proteins such as folding and denaturation under external processing conditions such as thermal and electric fields (Singh, Orsat, & Raghavan, 2013) (Vagadia, Vanga, Singh, & Raghavan, 2016) (Singh, Vanga, Orsat, & Raghavan, 2018) (Chen, 2006). A molecular dynamic simulation involves

numerically solving Newton's equation of motion of all the particles under stimulations. In addition, molecular dynamic simulation also used in the homology modelling in simulating protein three dimensional geometries based on amino acid sequence information, which can be used as an additional layer of descriptor in QSAR models (Kuhlman & Bradley, 2019) (Barroso da Silva, Fernando Luís et al., 2020). We believe that combining physical based molecular dynamic simulations with statistical based QSAR as a hybrid modelling approach will be a promising future trend in predicting functionalities of plant proteins with higher accuracy and sensitivity.

1.5 Conclusion/Future looks

Plant proteins are continuously being explored, especially pulse proteins, as the change in diet to vegan and vegetarian have gained popularity and the need to increase food sustainability. The identification of new plant proteins allows for a diversification of products due to the variability in physiochemical properties, which is advantageous in producing alternative meat substitutes. Understanding and characterizing their properties have been challenging but understanding the functionality is important for future food applications Many factors from cultivars to processing methods of plant proteins has found significant in impacting the different functional properties of the plant proteins. Most commercial processed protein isolates have reported lower solubility but higher water holding capacity than that of laboratory extracted protein, which are usually reported in published works. Therefore, further studies should focus on the functional properties of commercial plant protein isolates, as most published works mostly reported on laboratory extracted proteins. In general, protein contents of plant proteins have been found to increase the water and oil holding capacity and foaming stability. Common

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methods for measuring the water and oil holding capacity, gelling property and protein

solubility are utilized in many papers, but there are different methods have been found for

measuring the emulsifying property and foaming property, which makes it difficult to

compare between studies. Therefore, the methods of measuring the emulsion droplet

sizes and the changes of protein under environmental stress for example pH, ionic

strength, temperature are suggested to compare the emulsifying property between plant

proteins. With good functional properties, plant proteins are applied as meat extenders, in

meat analogs, baked goods and also salad dressings. To be more accurate in predicting

the protein functional properties, physical based particle-based simulations and QSAR

should be done in future study on plant proteins.

Forms of protein	Protein type	Protein content* (%)	WHC (gH2O/g)	OHC (g oil/g)	LGC (%)	References
Flour	Chickpea	20.60-26.70	1.40-1.50	1.05-1.24	10-14	(Kaur & Singh, 2007)
	Pea	25.00	0.78	0.41	-	(Sosulski & McCurdy, 1987)
	Green lentil	27.29	1.00	1.70	-	(Aryee & Boye, 2017)
	Faba bean	29.20	0.72	0.47	-	(Sosulski & McCurdy, 1987)
	Pea	47.20	1.09	0.59	-	(Sosulski & McCurdy, 1987)
	Soybean	48.20	1.75	0.56	-	(Sosulski & McCurdy, 1987)
	Faba bean	63.30	1.03	0.65	-	(Sosulski & McCurdy, 1987)
Concentrate	Faba bean	64.10	-	-	7	(Vogelsang-O'Dwyer et al., 2020)
	Chickpea	63.90-76.50	2.50-3.10	1.20-1.40	10-14	(Boye et al., 2010)
	Soybean	70.00	4.52	1.73	>14	(Aydemir & Yemenicioglu, 2013)
	Chickpea	71.00-77.00	4.90-7.94	10.93-14.59	5-7	(Aydemir & Yemenicioglu, 2013)
	Red lentil	78.20-82.70	3.70-4.10	1.10-2.30	10-12	(Boye et al., 2010)
	Green lentil	79.10-88.60	3.40-3.90	1.20-1.35	8-12	(Boye et al., 2010)
	Pea	80.30	2.52	0.98	-	(Sosulski & McCurdy, 1987)

	Pea	80.60-89.00	1.91-2.37	1.10-1.40	-	(Stone et al., 2015)
	Faba bean	81.20	1.80	1.60	14	(Fernández-Quintela et al., 1997)
	Mung bean	81.53	3.33	3.00	12	(Brishti et al., 2017)
	Pea	81.70-83.90	3.90-4.50	1.20-1.75	12-14	(Boye et al., 2010)
	Soybean	82.20	1.30	1.10	16	(Fernández-Quintela et al., 1997)
	Soybean	82.30	2.65	1.03	-	(Sosulski & McCurdy, 1987)
	Pea	83.60	1.52	1.40	18	(Butt & Batool, 2010)*
	Chickpea	84.80-87.80	2.40-4.90	1.70-2.00	-	(Paredes-López et al., 1991)
	Pea	84.90	1.70	1.20	18	(Fernández-Quintela et al., 1997)
	Mung bean	85.46	1.63	1.13	16	(Butt & Batool, 2010)*
	Soybean	86.00	3.00	3.45	14	(Brishti et al., 2017)
	Faba bean	86.30	2.16	1.78	-	(Sosulski & McCurdy, 1987)
Isolate	Green lentil	87.00-95.00	1.04-1.47	6.90-10.40	12-14 ,>14	(Aydemir & Yemenicioglu, 2013)
	Chickpea	89.90-94.40	2.34-3.50	2.08-3.96	14-18	(Kaur & Singh, 2007)
	Soybean	90.00	7.94	1.16	10	(Aydemir & Yemenicioglu, 2013)*

	Faba bean	90.10	-	-	12	(Vogelsang-O'Dwyer et al., 2020)
	Green lentil	90.15	2.70	2.20	-	(Aryee & Boye, 2017)
	Green lentil	90.20-91.90	0.43-0.48	-	11-14	(Joshi et al., 2011)
	Soybean	92.00	4.52	8.23	10	(Aydemir & Yemenicioglu, 2013)*
	Faba bean	92.14-99.36	-	4.64-4.81	-	(Singhal et al., 2016)

Table 1.1 Water and oil holding capacity (WHC and OHC) and least gelation concentration (LGC) of plant proteins presented in order of overall protein concentration as reported by the respective publication source.

- = not reported

All reported in dry basis except *, which are not reported in literature or reported in wet basis
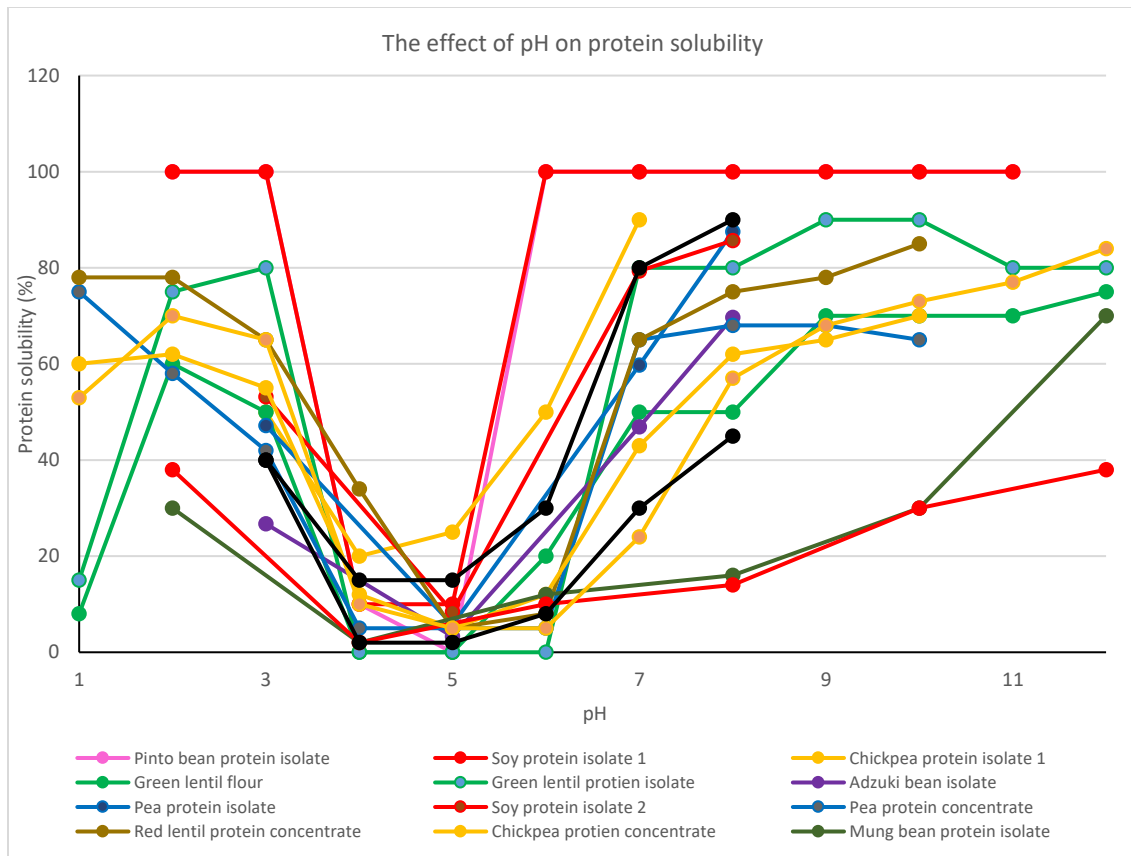
Figure 1.1 The effect of change in pH on protein solubility of plant proteins reported in published works.

Data was extracted or estimated from (Tan, Ying-Yuan, & Gan, 2014), (Kaur & Singh, 2007), (Aryee & Boye, 2017), (Barac et al., 2015), (Boye et al., 2010), (Brishti et al., 2017), (Tontul et al., 2018), (Vogelsang-O'Dwyer et al., 2020).

Note: Soy and chickpea protein isolate 1,2,3 are values reported in different references

(a)

Protein type	Protein* content (%)	Emulsifying activity (%)	Emulsifying stability (%)	References
Mungbean	81.53	63.2	62.8	(Brishti et al., 2017)
Pea	83.60	21.0	43.2	(Butt & Batool, 2010)
Chickpea	84.80	63.7	94.3	(Paredes-López et al., 1991)
Mungbean	85.46	41.1	45.5	(Butt & Batool, 2010)
Soybean	86.00	74.5	81.2	(Brishti et al., 2017)
Chickpea	87.80	72.9	85.0	(Paredes-López et al., 1991)

(b)

Protein type	Protein content* (%)	Emulsifying capacity (mL oil/0.1g protein)	Reference
Faba bean	25.00	34.6	(Sosulski & McCurdy, 1987)
Pea	25.00	34.6	(Sosulski & McCurdy, 1987)
Faba bean	47.20	35.7	(Sosulski & McCurdy, 1987)
Pea	47.20	37.2	(Sosulski & McCurdy, 1987)
Soybean	48.20	37.2	(Sosulski & McCurdy, 1987)
Pea	80.30	36.6	(Sosulski & McCurdy, 1987)
Soybean	82.30	45.1	(Sosulski & McCurdy, 1987)
Faba bean	86.30	38.6	(Sosulski & McCurdy, 1987)

(c)

Protein type	Protein content* (%)	Emulsifying Activity Index (m2/g)	Emulsifying Stability Index (min)	Reference
Chickpea	63.90-76.50	5.70	19.70	(Boye et al., 2010)
Soybean	72.64-87.59	43.35-44.20	25.04-85.97	(Karaca et al., 2011)
Green lentil	74.71-81.90	37.17-44.51	11.02-86.79	(Karaca et al., 2011)
Red lentil	78.20-82.70	5.20	18.10	(Boye et al., 2010)
Green lentil	79.10-88.60	5.00	17.80	(Boye et al., 2010)
Pea	80.6-89.0	31.09-39.05	10.97-11.26	(Stone et al., 2015)
Pea	81.09-88.76	42.73-42.87	10.89-12.40	(Karaca et al., 2011)
Chickpea	81.63-85.40	33.83-47.90	10.92-82.94	(Karaca et al., 2011)
Faba	81.98-84.14	37.11-44.29	10.97-62.39	(Karaca et al., 2011)
Pea	84.90	4.60	18.00	(Boye et al., 2010)
Kidney bean	90.8-94.7	21.30	46.00	(Shevkani et al., 2015)
Kidney bean	92.5	23.70	30.90	(Tang et al., 2009)
Pea	92.8	13.10	78.10	(Shevkani et al., 2015)

Table 1.2 The emulsifying property of plant proteins reported in published works using

different methods (a) The emulsifying activity (%) is the ratio of the height of emulsified

layer to the height of total contents in the tube and the emulsifying stability (%) is the

ratio of the height of emulsified layer after heated at 80°C for 30min to the height of

emulsified layer before heating (b) The emulsifying capacity (g oil/g protein) is the

amount of oil the protein can emulsify. (c) Pearce and Kinsella's method of emulsifying

activity index and emulsifying stability index.

all reported in dry basis except (Aydemir & Yemenicioglu, 2013), (Brishti et al., 2017), (Butt & Batool,

2010) not reported while (Karaca et al., 2011) and (Tang et al., 2009) are reported in wet basis

$$EAI(m^2/g) = \frac{2 \times 2.303 \times A_0 \times DF}{c \times \phi \times (1 - \theta) \times 10000}, \; ESI(min) = \frac{A_0}{A_0 - A_{10}} \times 10,$$

where DF is the dilution factor (100), c the initial concentration of protein (g/ml), Φ the optical path (0.01 m), θ the fraction of oil used to form the emulsion (0.25), and A_0 and A_{10} the absorbance of diluted emulsions at 0 and 10 min respectively. Measurements were performed in triplicate.

Figure 1.2 Equation for emulsifying activity index (EAI) and emulsifying stability index

(ESI) (Tang et al., 2009)

	Protein type	Protein content * (%)	Foaming capacity or expansion[1] (%)	Foaming stability[2] (%)	Reference
Flour	Pea	25.00	150.0[#]	70.0[#]	(Sosulski & McCurdy, 1987)
	Faba bean	25.00	110.0[#]	86.4[#]	(Sosulski & McCurdy, 1987)
	Pea	47.20	282.5[#]	76.1[#]	(Sosulski & McCurdy, 1987)
	Faba bean	47.20	220.0[#]	83.0[#]	(Sosulski & McCurdy, 1987)
	Soybean	48.20	185.0[#]	77.0[#]	(Sosulski & McCurdy, 1987)
	Soybean	70.00*	32.0[#]	43.7[#]	(Aydemir & Yemenicioglu, 2013)
	Chickpea	71.00-77.00*	43.9[#]	64.8[#]	(Aydemir & Yemenicioglu, 2013)
Concentrate	Pea	80.30	157.5[#]	73.0[#]	(Sosulski & McCurdy, 1987)
	Pea	80.60-89.00	81.1	27.1	Stone et al. (2015)
	Faba bean	81.2	15.0	77.0	(Fernández-Quintela et al., 1997)
	Mungbean	81.53*	89.7	78.3	(Brishti et al., 2017)

	Soybean	82.20	22	93	(Fernández-Quintela et al., 1997)
	Soybean	82.30	60.0[#]	87.5[#]	(Sosulski & McCurdy, 1987)
	Pea	83.60	78	X	(Butt & Batool, 2010)
	Chickpea	84.80-87.80	43.3-47.5	59.2-66.6	(Paredes-López et al., 1991)
	Pea	84.90	15.0	94.0	(Fernández-Quintela et al., 1997)
	Mungbean	85.46*	110.0	X	(Butt & Batool, 2010)
	Soybean	86.00*	68.7	100.0	(Brishti et al., 2017)
	Faba bean	86.30	100[#]	72.5[#]	(Sosulski & McCurdy, 1987)
	Green Lentil	87.00-95.00*	34.8[#]	96.7[#]	(Aydemir & Yemenicioglu, 2013)
Isolate	Chickpea	89.90-94.40	30.4-44.3	X	(Kaur & Singh, 2007)
	Soybean	90.00*	24.0[#]	66.7[#]	(Aydemir & Yemenicioglu, 2013)
	Kidney bean	90.8-94.7(db)	83.0-121.0	90.0-95.0	(Shevkani et al., 2015)
	Soybean	92.00*	36.0[#]	88.9[#]	(Aydemir & Yemenicioglu, 2013)

		92.14-99.36	143.3-183.3	55.9-71.59	(Singhal et al., 2016)
	Faba bean	92.14-99.36	143.3-183.3	55.9-71.59	(Singhal et al., 2016)
	Kidney bean	92.5	244.9	87.8	(Tang et al., 2009)
	Pea	92.8	87.0-132.0	94.0-96.0	(Shevkani et al., 2015)

Table 1.3 The foaming property of plant proteins reported in published works using

different methods

All reported in dry basis except *, which are not reported, while (Tang et al., 2009) is reported in wet basis

(Brishti et al., 2017)(Butt & Batool, 2010)(Tang et al., 2009)# Calculated using the initial foam volume and

foam volume after standing for thirty minutes reported.

1 Foaming capacity was expressed as the volume (%) increase due to whipping.

2 Foaming stability was estimated as the percentage of foam remaining after 30 min.

CHAPTER 2

THE COMPARISON OF FUNCTIONAL AND PHYSICAL PROPERTIES OF COMMERCIAL PULSE PROTEINS TO SOY PROTEIN

2.1 Introduction

The utilization of plant-based food proteins is rapidly increasing in the food industry as techno-functional ingredients in a variety of plant-based meat, dairy, and egg products (Formanski, 2019). This is due to increasing consumer concern about the sustainability of livestock production, health concern on the consumption of processed meat, and increasing interest in consuming more plants (Srivastava, 2020). Indeed, the livestock sector has been reported to be responsible for 15% of global greenhouse gas emissions, as well as a major cause of pollution, land use, water use, biodiversity loss, and deforestation (Abbasi, Abbasi, & Abbasi, 2016) (Gerssen-Gondelach et al., 2017). Moreover, the demand for food is predicted to grow by 70% by 2050, as it is predicted that the global population will increase by 2.3 billion in the same timeframe (Le Mouël, 2017). A recent study reported that replacing beef in a typical American diet with plant-based derivatives can reduce land use by 90%, greenhouse gas emissions by 96%, and nitrogen fertilizer use by 94% (Eshel, Shepon, Noor, & Milo, 2016). By replacing a portion of meat products with plant-based alternatives, it is possible to reduce water and land waste while also producing a more abundant food supply with additional nutritional benefits.

Traditionally, soy protein has been the most popular protein for constructing plant-based meat analogues because it has techno-functional properties that can mimic many of those associated with real meat products, such as a high water holding capacity,

ability to form semi-solid textures, and ability to stabilize emulsions (Singh, Kumar, Sabapathy, & Bawa, 2008). But its functional attributes are also useful for other applications, such as baked goods, snacks, and functional beverages, as well as plant-based milks, cheeses, and eggs (Singh et al., 2008). While soy is an established plant protein that provides a range of useful functionalities, it does have some limitations due to concern about its allergenicity (Srivastava, 2020) . It has been claimed that the high phytoestrogen content of soy may cause hormone and ovulatory cycle disruption (Cederroth, Zimmermann, & Nef, 2012). There is also some evidence of adverse long-term health consequences associated with consuming soy infant formulas (Patisaul & Jefferson, 2010). Furthermore, soybean cultivation is reported to be a leading cause of accelerated deforestation, especially in the Amazon rainforest (USDA, 2019). Indeed, the USDA reported that the Amazon forest has lost more than 792,000 square kilometers in the past 50 years, where the production of soybean crops in Brazil has grown and is forecasted to reach around 123 million metric tons by 2050. The massive deforestation caused by soybean cultivation may therefore contribute to global warming.

In the past 20 years, the functional properties of various pulse proteins have been explored as potential plant-based alternatives to soy protein, including pea, chickpea, lentil, and faba bean proteins (Singhal, Karaca, Tyler, & Nickerson, 2016). Researchers have reported that pulse proteins have potential in numerous food applications including plant-based meat, pasta and baked goods because of their good functional properties (Singhal et al., 2016). Ideally, it is useful for food formulators to understand how the functional attributes of soy proteins compare to those of pulse proteins for different applications. The majority of previous studies have used highly purified pulse proteins

extracted in the laboratory rather than commercially-available pulse protein ingredients (Burger & Zhang, 2019). For food formulators, it is more important to understand how commercial pulse protein ingredients perform against commercial soy protein ingredients, rather than highly purified ingredients because they may behave differently (Añón, Sorgentini, & Wagner, 2001). Commercial ingredients may behave differently to purified ingredients in a number of ways that can impact their functionality, including their composition, aggregation state, and denaturation state (Aydemir & Yemenicioğlu, 2013). Recently, there have been considerable advances in the extraction and purification techniques used by protein ingredient suppliers, which have led to the availability of higher quality plant protein ingredients. However, there is limited data that characterizes and compares the functionality of different commercial pulse proteins and compares their properties with those of soy protein.

The aim of this study was therefore to compare the physicochemical and functional properties of commercial pulse protein isolates to those of soy protein isolate. In particular, differences in the water holding capacity, oil holding capacity, gelling properties, emulsifying properties, and color of the commercial ingredients were measured. This information may help food formulators create a new generation of plant-based food and beverage products using commercially available pulse ingredients.

2.2 Materials and methods

2.2.1 Materials

Commercial pea protein isolate (PPI) (Product name: FYPP-85, Total protein content > 0.85 g/g), faba bean protein isolate (FPI) (Product name: FFBP-90-C, Total protein content > 0.88 g/g) and lentil protein isolate (LPI) (Product name: FYLP-80, Total

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protein content > 0.80 g/g) were provided by AGT Foods (Regina, Canada). Soy protein

isolate (SPI) (Product name: SUPRO EX 45, Total protein content > 0.90 g/g) was

provided by Solae, LLC (St. Louis, MO, USA). The protein isolates were reported to be

mechanically milled and wet fractionated by the providers. Proximate composition of all

protein isolates was performed according to AOAC Official methods: Moisture (AOAC

930.15), Protein (AOAC 990.03), Fat (AOAC 945.16).

2.2.2 Functional properties

2.2.2.1 Water and Oil holding capacity

The water holding capacity (WHC) and oil holding capacity (OHC) were

measured according to methods described previously (Tan, Ying-Yuan, & Gan, 2014). In

brief, 0.1g of protein isolate was mixed with 1.5 ml of distilled water (density of 1.00

g/ml) or soybean oil (density of 0.912 g/ml) in a pre-weighed microcentrifuge tube and

vortexed for 1 min. Samples were incubated at room temperature (25 ˚C) for 30 minutes

to hydrate the protein isolates. The samples were then centrifuged at 5000 g for 30 min

(accuSpin Micro 17 Microcentrifuge, Fisher Scientific, Waltham, MA, USA). The

resulting supernatant was carefully decanted and the sample was weighed. The WHC and

OHC were expressed as grams of water or oil bound per gram of sample.

2.2.2.2 Gelling properties

The least gelation concentration (LGC) was measured according to a method

described previously (Aydemir & Yemenicioğlu, 2013). Test tubes containing protein

isolate suspensions (6% - 20% w/v) in 5 ml distilled water were heated for 1 hour in a hot

water bath (> 90°C) followed by rapid cooling under cold running water. Then, the tubes

were further cooled at 4°C for 2 h. The LGC corresponded to the lowest concentration of

protein required to form a gel, *i.e.,* the sample did not flow to the bottom of the test tubes after they were inverted.

2.2.2.3 Emulsion preparation and droplet size distribution

Emulsions were prepared by homogenizing a 90% aqueous protein solution (0.1-5.0%) with 10% canola oil. The initial emulsions were prepared using a high-shear mixer (M133/1281–0, Biospec Products Inc., Bartlesville, OK) with a 1.4 cm probe for 2 minutes at 10,000 rpm. The droplet size was then reduced by sonication for 3 minutes (M FB505, Fisher Scientific, Pittsburgh, PA) with a half inch horn, a 2 s on/off setting, and an amplitude of 70%. We measured the particle size distribution using a particle size analyzer (Mastersizer S, Malvern Panalytical, Westborough, MA). The surface-weighted mean diameter (D_{32}) and volume-weighted mean diameter (D_{43}) of the emulsions were then calculated from the particle size distribution.

2.2.2.4 Creaming stability

The creaming index was measured according to a method described previously (Kong, Jia, Zhang, Hua, & Chen, 2017) with slight modifications. Freshly prepared 10% oil-in-water emulsions (20 mL) were prepared by sonicating (as previously described) 63 mL of protein solution and 7 mL of canola oil. Samples of these emulsions (20 mL) were then poured into 30 mL sample vials (height = 3.75 inches; diameter = 1 inch) immediately after preparation. The creaming stability of the emulsions was determined by using a graduated ruler to measure the height of the clear serum layer (H_S) formed at the bottom of the emulsions after the droplets moved upwards, as well as the total height of the emulsions (H_T). The creaming index was then calculated as follows: CI (%) =

(H_S/H_T) × 100. Measurements were made over a 2 week period when the emulsions were stored at room temperature (25 ˚C).

2.2.2.5 Color measurement

      Six grams of powdered protein isolate was weighed into a petri dish (60mm × 15mm) and then the color coordinates were measured using an instrumental colorimeter (ColorFlex EZ, M 45/0, Hunterlab, Sunset Hills Road Reston, VA). The instrument was calibrated using standard black and white tiles before sample analysis. The L^*-, a^*-, and b^*-values of the samples were then determined by the colorimeter.

2.2.2.6 Statistical Analysis

      All experiments were performed in triplicate with 3 replicates and are reported as means and standard deviations. A two-way analysis of variance (ANOVA) with Duncan's New Multiple Test and Dunnett's Test was done to measure the statistical differences in all functional properties using SAS program (SAS 9.4, Cary, NC).

2.3 Results and Discussion

2.3.1 Water and oil holding capacity

      The water (WHC) and oil (OHC) holding properties of many foods are important for determining their desirable quality attributes. The WHC of the plant protein isolates ranged from 2.20 to 7.57 g/g depending on protein type (**Table 1**). SPI (7.57 g/g) had the highest water holding capacity among all plant proteins, while PPI (5.14 g H_2O/g) had the highest amongst the pulse protein isolates. A Dunnett's test showed that all the pulse proteins had significantly lower WHC than the soy proteins. The Duncan's test also showed all of the pulse proteins had significantly different water holding capacities to each other. The OHC of the plant protein isolates were much lower than the water holding capacity, ranging from 0.86 to 1.43 g/ g sample. This would be expected because

the proteins used are predominantly hydrophilic molecules. LPI (1.43 g oil/ g sample) had the highest oil holding capacity, while SPI (1.36 g oil/ g sample) had the second highest. The OHC among the plant protein isolates were not significantly different, with the exception of PPI (0.86 g oil/ g sample), which was significantly lower than the others. The differences in the water and oil holding properties of the different proteins may be due to differences in their surface chemistries or powder porosities.

Boye et al. (2010) reported that the WHC values of pea protein concentrates were around 3.9-4.5 g/g, which is slightly lower than the value found in our study (5.14 g/g) The slightly higher values found in our study may be due to the slightly higher protein content of the powder used (85% versus 82-84%). Tan et al. (2014) also reported that SPI has a relatively high water holding capacity (6.13 g/g), which is in agreement with our study. The same authors reported that the WHC of pinto bean protein isolate was relatively low (1.65 g/g), which can be attributed to the relatively high surface hydrophobicity of the pinto bean proteins limiting protein–water interactions.

Sosulski & McCurdy (1987) reported that the OHC values of soybean, pea, and faba bean proteins were significantly lower than their WHC values, which agrees with our findings. The OHC value of the PPI protein used in our study (0.86 g/g) was considerably less than that reported in some other studies (5-7 g/ g) (Shevkani, Singh, Kaur, & Rana, 2015) (Joshi, Adhikari, Aldred, Panozzo, & Kasapis, 2011), which suggests that the protein ingredient we used was more hydrophilic. It is possible that the protein isolates prepared in the laboratory by these researchers retained more fat. Other researchers have reported that SPI prepared in their laboratory had a higher OHC value

(8.3 g/g) than a commercial SPI ingredient (1.16 g/ g) (Aydemir & Yemenicioğlu, 2013).

These results highlight the importance of the form of the ingredient used.

2.3.2 Gelling property

The ability of plant proteins to form gels is one of their most important functional

attributes for creating meat, egg, cheese, and yogurt analogues. The least gelation

concentration (LGC) is used to determine the minimum amount of protein required to

form a gel. The LGC values for the plant proteins used in this study were between 12-

15% (**Table 2.1**). SPI, FPI, and LPI had no significant difference to each other, but PPI

had a significantly higher LGC, meaning more protein is required to form a hard gel,

thereby increasing the cost of production. Other researchers have reported fairly similar

LGC values for pea protein (12-14%) and lentil protein (8-12%) concentrates (Boye et

al., 2010). It has been reported that the 7S globulin fraction of pulse proteins is mainly

responsible for their thermal gelation, rather than the 11S globulin fraction (Singhal et al.,

2016). The higher LGC of PPI observed in our study may therefore be because it has a

higher 11S-to-7S ratio than the other pulse protein isolates tested (Singhal et al., 2016).

2.3.3 Emulsion droplet size

Plant protein isolates can act as emulsifiers in foods and beverages, such as plant-

based sausages, cakes and soups (Singhal et al., 2016). In these applications, the proteins

adsorb to the surfaces of the lipid droplets and form a protect coating that can prevent the

droplets from aggregating with each other. In many cases, it is important that the

emulsifiers are capable of forming small uniform droplets during homogenization. For

this reason, we measured the influence of protein type and concentration on the mean

droplet diameter of the emulsions. This type of information is commercially important

because it determines how much emulsifier must be added to a product to prevent phase separation.

As anticipated, the mean droplet diameters (D_{32} and D_{43}) of the emulsions decreased as the protein concentration increased for all plant protein isolates, which can be attributed to the fact that there was more emulsifier available to cover the oil droplet surfaces during homogenization. Nevertheless, the droplet sizes in the emulsions did depend on the type of protein used, which may have been due to differences in molecular weight, surface chemistry, and aggregation state. For instance, researchers have reported that FPI has a much lower surface hydrophobicity than SPI and LPI (Johnston et al., 2015), which would be expected to lead to a lower surface activity. This finding is consistent with the fact that the SPI and LPI used in our study resulted in smaller oil droplet sizes than the FPI (**Figure 2.1**).

From a commercial perspective, it is often desirable to be able to produce small oil droplets using a low protein concentration, so as to reduce ingredient costs. At relatively low protein levels, there were major differences in the ability of the protein isolates to form small droplets. The minimum protein concentration required to form relatively small droplets ($D_{32} < 3$ mm) was around 0.1, 0.5, 1.0, and 2.0 for LPI, PPI, SPI, and FPI, respectively. This result suggests that the legume proteins were the most effective emulsifiers in this system. The fact that the D_{43} values were much higher than the D_{32} values indicates that the emulsions contained a wide range of different-sized particles. Other researchers have also reported that plant proteins lead to broad particle size distributions in emulsions (Roesch & Corredig, 2002).

A number of other researchers have compared the emulsifying properties of various plant-based proteins. PPI has been reported to form smaller droplets than LPI by some researchers (Ladjal-Ettoumi, Boudries, Chibane, & Romero, 2016), which is different from observed in the current study. In another study, the droplet size in oil-in-water emulsions was reported to be fairly similar for SPI and PPI (Fernandez-Avila, Arranz, Guri, Trujillo, & Corredig, 2016). A comparison of the emulsifying properties of lentil, faba bean and pea proteins found that they all had fairly similar abilities to reduce the droplet size in fish oil emulsions (Gumus, Decker, & McClements, 2017).

2.3.4 Creaming stability

The creaming velocity in emulsions is known to increase with increasing droplet size, increasing density contrast, and/or decreasing aqueous phase viscosity (Phillips & Williams, 2009). Phase separation due to this mechanism is therefore particularly rapid in oil-in-water emulsions where the oil droplet size is large and the aqueous phase viscosity is low (Lucassen-Reynders, 1966). Therefore, it is important in product development to ensure that plant-based proteins can maintain good creaming stability to prevent phase separation, which can usually be achieved by ensuring they produce small droplets during homogenization. For this reason, we measured the creaming stability of 10% canola oil-in-water emulsions containing different protein types and concentrations (0.1-5.0%) during storage under ambient conditions for 14 days (**Figure 2.2**).

The creaming index (CI) increased rapidly during the first few days, but then increased more slowly and reached a plateau after about the first week (**Figure 2.2**). The creaming index depended significantly on plant protein type (p=0.0017) and concentration (p<0.0001) at a 95% confidence interval. As expected, the CI value decreased as the protein concentration increased for all plant protein isolates, which can

be attributed to a decrease in emulsion droplet size, as well as a slight increase in aqueous phase viscosity. The creaming stability all depended somewhat on protein type. Around 5% or greater PPI, FPI, and LPI was required to stop creaming, whereas only 2% SPI was required (**Table 2.2**). The fact that rapid creaming occurred in the LPI emulsions at relatively low protein concentrations was surprising because they had relatively low mean droplet diameters (**Figure 2.1**). This effect may have been because the droplets in these emulsions were weakly flocculated in the concentrated emulsions, but these flocs broke down when the emulsions were diluted to analyze their particle size.

2.3.5 Color

Color is the one of the initial cues that a consumer uses to evaluate the quality of a food product and so it important to assess the potential impact of different plant proteins on food appearance. Therefore, instrumental colorimeter values (L*a*b*) were measured to quantify differences in the optical properties of the emulsions (**Table 2.3**). The lightness (L*) of the LPI was significantly higher than the other protein isolates. The redness (a*) of all the pulse protein isolates were significantly different to that of the soy protein isolate. Specifically, PPI was slightly redder (higher a*) whereas FPI and LPI were slightly less red (lower a*). There was also a significant difference in the yellowness (b*) of the protein isolates. The PPI had the strongest yellow color (b*), followed by FPI, SPI and then LPI. These significant differences in color may have important implications when incorporating the proteins into different food products, such as yogurts, beverages, meat analogs, *etc*. For example, adjustments are often needed to formulate meat analogues to recreate meat-like colors. Moreover, significant differences in the color of guava juice have been reported after the addition of soy protein (Granato & Masson, 2010).

2.4 Conclusion

With plant-based proteins finding increasing popularity various food applications, this study compared the functional properties of commercial soy and pulse protein isolates. The pea, faba bean, lentil, and soy protein isolates used had significantly different functional attributes. The water holding capacities were higher than the oil holding capacities for all the proteins. The soy protein had the highest water holding capacity, which suggests that it was more hydrophilic than the pulse proteins. It may therefore have advantages over pulse proteins for applications where water retention is important, such as in meat analogs. All of the proteins could form oil-in-water emulsions, with the mean droplet diameter decreasing with increasing protein concentration. Nevertheless, there were differences in the ability of the different proteins to form and stabilize the emulsions. The lentil proteins produced the smallest droplets, whereas the faba bean proteins produced the largest ones. The pea protein isolates had the lowest gelling properties among the plant protein isolates. The colors of the plant protein isolates were significantly different to that of soy protein isolate, suggesting color adjustments may need to be made, for example red colorings might need to be added in meat analog applications to counteract the yellow color from the plant proteins.

In summary, we have shown that the plant proteins studied had fairly similar functional attributes to soy proteins, which may be important for their more widespread application in foods. In the future, it will be important to examine the functionality in actual food products, as well as to carry out sensory analysis of their quality attributes.

2.5 Acknowledgements

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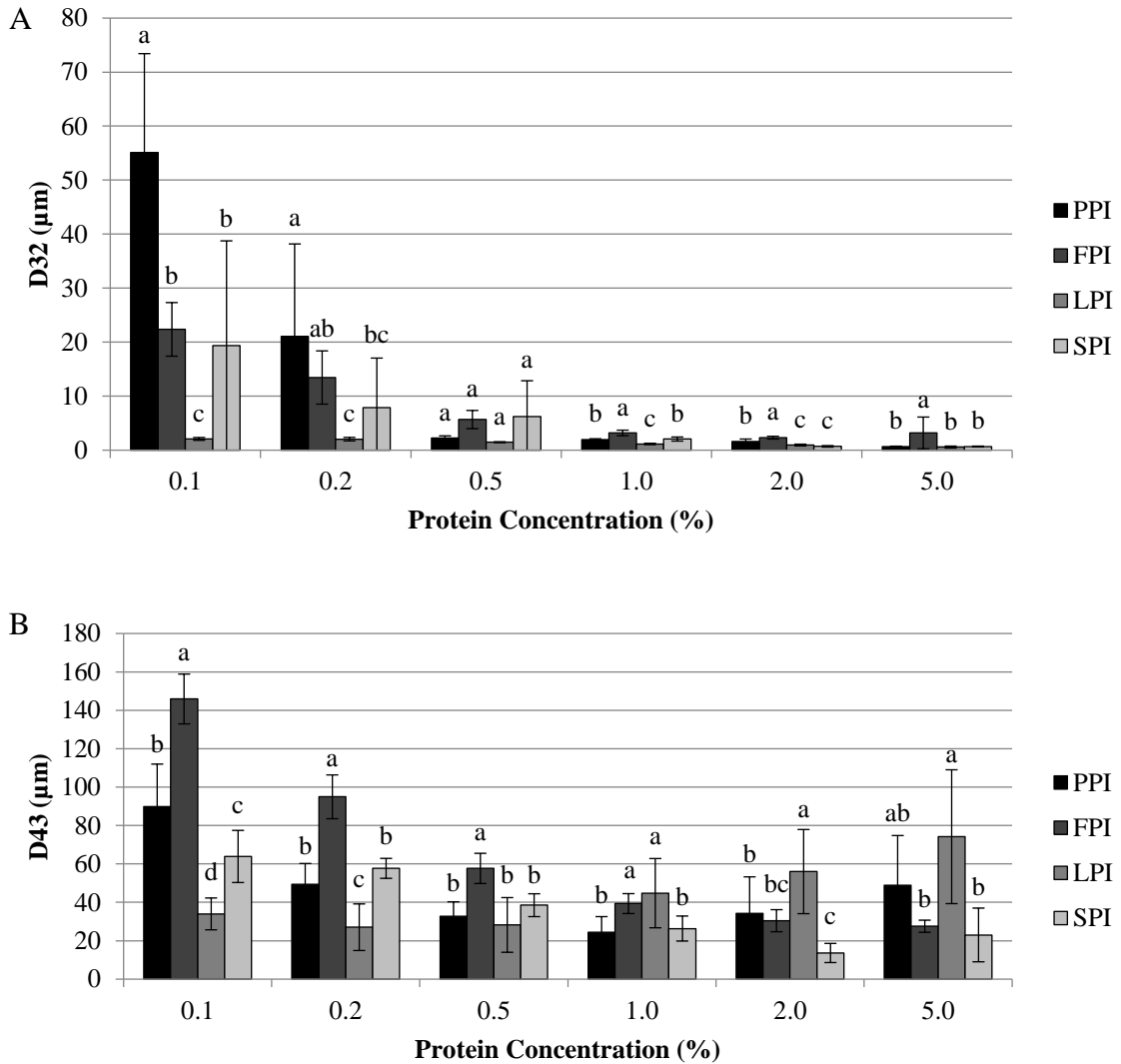
Figure 2.1 (A) D32 of 4 plant protein isolate emulsion droplets in 6 concentrations (0.1,0.2,0.5,1.0,2.0,5.0%). (B) D43 of 4 plant protein isolate emulsion droplets in 6 concentrations.

[a,b,c,d] *Means within each protein concentration followed by different letters are the Duncan groupings from highest to the lowest showing significant difference (p<0.05).*
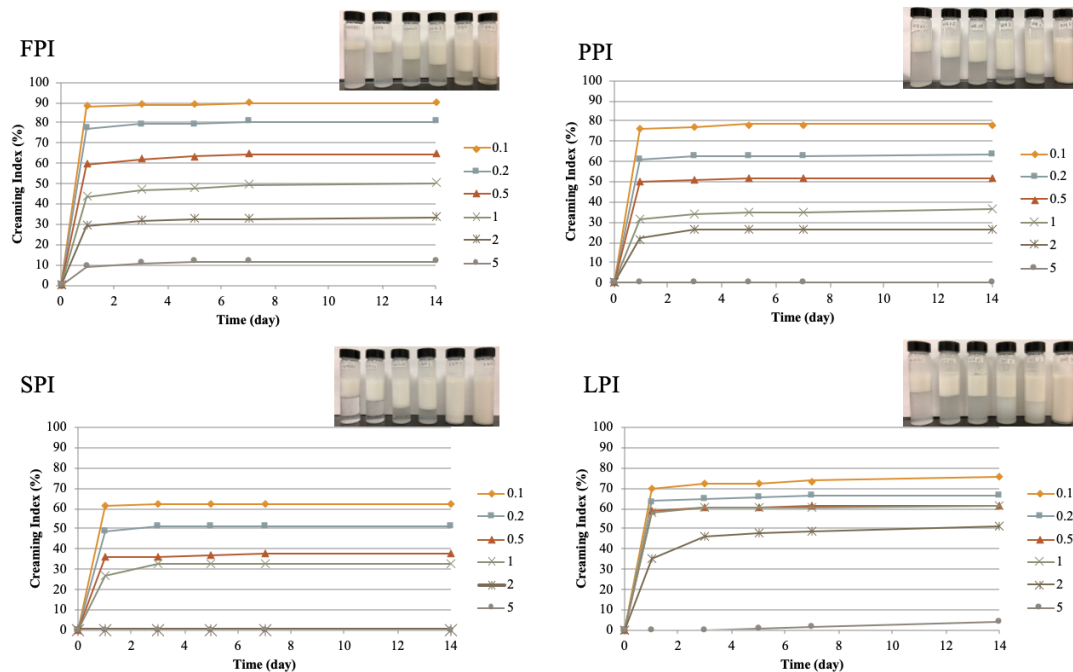
Figure 2.2 The change in creaming index values of soy, pea, faba bean and lentil protein isolate in 6 different concentrations (0.1, 0.2, 0.5, 1.0, 2, 5%) emulsion prepared with 10% canola oil during 14 day period after emulsion was made. The photographs in the upper right corner show images of the 6 concentration of emulsions from lowest to highest from left to right on the 14th day.

Type of plant protein isolate	WHC (g H$_2$O / g)	OHC (g oil/ g)	LGC (%)
SPI	7.57±0.30[a]	1.36±0.17[a]	12.00±0.00[b]
PPI	5.14±0.27[b]*	0.86±0.16[b]*	15.00±0.01[a]*
FPI	3.20±0.09[c]*	1.24±0.06[a]	12.00±0.00[b]
LPI	2.20±0.11[d]*	1.43±0.09[a]	13.00±0.01[b]

Table 2.1 Impact of protein type on water holding capacity (WHC), oil holding capacity (OHC), and least gelling concentration (LGC) for soy, pea, faba bean and lentil protein isolate ingredients.

[a,b,c,d] Means in each column followed by different letters are the Duncan groupings from highest to the lowest showing significant difference (p<0.05).

∗ Means in each column were significantly different in Dunnett's test with SPI as the control with 95% Confidence Interval (p<0.05)

Acronyms presented above are WHC: water holding capacity, OHC: oil holding capacity, and LGC: least gelling concentration.

Protein/ Concentration	0.1%	0.2%	0.5%	1.0%	2.0%	5.0%
SPI	62.0 ± 2.8^c	50.943 ± 0.00^b	38.1 ± 2.3^c	32.7 ± 0.0^c	0.0 ± 0.0^d	0.0 ± 0.0^b
PPI	$78.3\pm2.8^{b*}$	63.806 ± 0.35^{ab}	$64.2\pm0.0^a*$	36.6 ± 0.7^c	$26.7\pm0.7^c*$	0.0 ± 0.0^b
FPI	$89.6\pm0.9^{a*}$	$80.189\pm0.94^{a*}$	$51.9\pm1.9^b*$	$50.5\pm1.4^{b*}$	$33.7\pm1.0^b*$	$11.8\pm2.0^{a*}$
LPI	70.1 ± 0.3^{bc}	61.32 ± 0.94^{ab}	$59.3\pm1.1^a*$	$58.3\pm0.9^a*$	$48.1\pm0.9^a*$	2.9 ± 1.0^b

Table 2.2 Impact of protein type and concentration on the creaming index of emulsions stabilized by different plant protein isolates (measured on 14th day of storage under ambient conditions).

[a,b,c,d] Means in each column followed by different letters are the Duncan groupings from highest to the lowest showing significant difference (p<0.05).

∗ Means in each column were significantly different in Dunnett's test with SPI as the control with 95% Confidence Interval (p<0.05)

Protein type/ Color	L	A	B
PPI	48.8 ± 0.6^b	$2.27^*\pm0.18^a$	$16.2^*\pm0.5^a$
FPI	49.1 ± 0.5^b	$1.12^*\pm0.08^c$	$12.9^*\pm0.3^b$
LPI	$51.9^*\pm1.1^a$	$1.0^*\pm0.11^c$	$9.2^*\pm0.3^d$
SPI	49.4 ± 0.2^b	2.08 ± 0.06^b	12.4 ± 0.2^c

Table 2.3 The Lab color values of plant protein isolates measured using an instrumental colorimeter.

[a,b,c,d] Means in each column followed by different letters are the Duncan groupings from highest to the lowest showing significant difference (p<0.05).

∗ Means in each column were significantly different in Dunnett's test with SPI as the control with 95% Confidence Interval (p<0.05)

Acronyms: SPI: soy protein isolate, PPI: pea protein isolate, FPI: faba bean isolate, LPI: lentil protein isolate.

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