**The effect of infrared heating on the functional and nutritional qualities of green lentil and yellow pea flours**

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By Emma Laing
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## ABSTRACT

The effect of seed tempering moisture (20 vs. 30%) and infrared heating temperature (120 vs 140ºC) on the nutritional and functional properties of the resulting flours from green lentil and yellow pea were evaluated. For both pulses, proximate composition remained unchanged relative to the unprocessed pulses, although seeds became a little darker in colour. The damaged/gelatinized starch content of the control flours steadily increased as both tempering moisture and infrared heat applied to the seeds prior to milling increased. For all processing conditions, surface hydrophobicity (SH) increased relative to the control, whereas surface charge (zeta potential (mV)) remained unchanged. The secondary protein structure of both pulse types transitioned from a more ordered state composed of β-sheet and α-helix structures, to state with a higher relative percentage of random coil structures as processing conditions increased. Functional properties of the flours were mildly affected as a result of tempering and infrared heating, and in most cases were correlated with the SH and damaged/gelatinized starch content of the flours. Protein solubility at pH 5 was unchanged in response to processing, however at pH 7 a slight processing effect was seen, which led to lower solubility. The water and oil holding capacities (WHC, OHC) of the processed flours were improved in comparison to the control group flours, although OHC tended to decline as infrared heat temperatures increased from 120 to 140ºC. Poor foaming capacities (FC) and relatively stable foaming stabilities (FS) were observed for both pulse types. Intensifying processing of the pulse seeds improved emulsion activity (EA) and emulsion stability (ES) up until a critical point, where it then significantly declined. The oil emulsion capacity (OEC) declined with processing relative to the control for both pulse types. The peak and final viscosities of the flours decreased, and pasting temperature increased, as processing temperature and moisture increased, relative to the control group flours. In-vitro protein digestibility (IVPD) of processed flours increased relative to the control group flour, with the exception of yellow pea flours, where a slight decrease in IVPD was found for flours tempered to 30% moisture and heated to 140ºC. Amino acid contents remained unchanged between control and processed flours in both pulse types, and the limiting amino acid (LAA) was found to be tryptophan in both yellow pea and green lentil flours. The in-vitro protein digestibility corrected amino acid scores (IV-PDCAAS) of the flours were not significantly altered by processing.

Rapidly (RDS) and slowly (SDS) digestible starches increased with processing, whereas the amount of resistance starch (RS) declined. RDS and SDS values increased with increased temperature, and with increased moisture when processed. In contrast, RS decreased with increasing temperature and increased moisture. The overall conclusions in this study are that the combined effect of tempering moisture and infrared heat as a pre-milling treatment either did not significantly affect, or slightly reduced, the studied functional properties of the treated flours in comparison to the control group (with the exception of WHC). It was also found that flours that were tempered and infrared heated prior to milling had improved levels of protein and starch digestibility, but not protein quality.

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| | |
|---|---|
| a* | Redness |
| A/W | Air-water interface |
| A1, A2 | Amino acid side chain |
| AA | Amino acid(s) |
| AACC | American Association of Cereal Chemists |
| AAS | Amino acid score |
| ANOVA | Analysis of variance |
| AOAC | Association of Official Analytical Chemists |
| b* | Yellowness |
| CDC | Crop Development Centre |
| cP | Centipoise |
| D [4,3] | Weighted mean particle size |
| d.b. | Dry basis |
| D0 | Abnormally dry soil conditions |
| D4 | Exceptional drought conditions (1 in 50-year event) |
| DIAAS | Digestible indispensable amino acid score |
| DSC | Differential scanning calorimetry |
| EA | Emulsion activity |
| EAA | Essential amino acid |
| EAI | Emulsion activity index |
| ER | Electromagnetic radiation |
| ES | Emulsion stability |
| ESI | Emulsion stability index |
| FA | Foaming activity |
| FAO | Food and Agriculture Organization of the United Nations |
| FS | Foaming stability |
| FT-MIR | Fourier transform mid-infrared |
| g | Gravitational force |
| GL | Green lentil |
| HPLC | High performance liquid chromatography |
| IV-PDCAAS | In vitro protein digestibility corrected amino acid score |
| IVPD | *In vitro* protein digestibility |
| kDa | Kilodalton |
| kDa | Kilodaltons |
| L* | Lightness |
| LAA | Limiting amino acid |
| MM | Molecular mass |

| | |
|---|---|
| MT | Metric tonnes |
| Mt | Million tonnes |
| mV | Millivolts |
| O/W | Oil-water interface |
| OEC | Oil emulsion capacity |
| OHC | Oil holding capacity |
| PDCAAS | Protein digestibility corrected amino acid score |
| PER | Protein efficiency ratio |
| pH | Acidity in logarithmic scale |
| pI | Isoelectric point |
| PR | Protein rating |
| RC | Random coil |
| RDS | Rapidly digestible starch |
| RS | Resistant starch |
| RTE | Ready to Eat |
| RVA | Rapid viscosity analyzer |
| SCAA | Sulphur containing amino acid(s) |
| SDS | Slowly digestible starch |
| SFA | Saturated fatty acids |
| SH | Surface hydrophobicity |
| TPA | Texture profile analysis |
| $U_E$ | Electrophoretic mobility |
| WHC | Water holding capacity |
| WHO | World Health Organization |
| YP | Yellow pea |
| β-A | Anti-parallel β-sheet structures |
| $^oC$ | Degree Celsius |

# 1. INTRODUCTION

## 1.1 OVERVIEW

Pulses are defined by the Food and Agriculture Organization (FAO) as the edible seeds of leguminous plants that can be utilized for both human and animal feed consumption. Common examples of pulses include peas (*Pisum sativum* L.), lentils (*Lens culinaris* Medik), chickpeas (*Cicer arietinum* L.), dried beans such as faba (*Vicia faba* L.), and common beans (e.g., kidney and navy beans) (*Phaseolus vulgaris* L.) (1991). Pulse crops thrive in cool, dryer climates and have the ability to fix nitrogen in the soil. For this reason, pulses crops are commonly planted in rotation with other crop species, as the replenishment of nutrients helps to maintain optimal soil health and promote plant growth. In Canada, the climate has proven to be ideal for the farming of pulses, due to the continental climate experienced across the majority of the country. While growing regions span the nation, the most highly concentrated volumes of pulses are being grown in the prairie provinces of Alberta, Saskatchewan and Manitoba. Together, these three provinces produce and export over 80% of the Canadian pulse supply to the rest of the world (Pulse Canada, 2022). The largest crops that are exported are lentils and peas, which are primarily grown in Saskatchewan and Alberta (StatsCAN Plus, 2022). While Canada has a long history of producing pulses, in the last decade, pulse production has continued to grow and diversify due to increased demand globally for sources of plant protein. Chickpea production increased by three-fold to 214,000 metric tonnes (MT) and faba bean production increased by two-fold to 125,000 MT, between the years of 2016- 2020 (StatsCAN Plus, 2022). In 2021, Canadian farmers in the Prairie Provinces experienced drought conditions that ranged from D0 (abnormally dry), to D4 (exceptional drought; a 1 in 50-year event), as classified by Agriculture and Agri-Food Canada (Donihee & McDougall, 2022). Considering these conditions, pea and lentil crops production tonnage decreased by ~45-55% from 4.9 million tonnes (Mt) and 3.2 Mt, respectively, in comparison to the 2020 crop year. The most recent difficult harvest year has not affected demand for peas and lentils though, as prices continue to remain strong for Canadian farmers (Donihee & McDougall, 2021, 2022).

As established, pulse production is a growing and integral pillar of the Canadian agricultural economy. Canadian pulse producers and food manufacturers continue to face the obstacle that is the lack of familiarity with pulse foods and ingredients by the average Canadian consumer. In 2010, IPSOS reported that one-in-five Canadians expressed they have not consumed any form of pulses/pulse products in the past six months. The most common reason of avoidance was unpleasant taste, gastrointestinal discomfort post consumption, lack of knowledge of preparation, and difficulty of preparation (IPSOS, 2010). This is an ageing statistic, and has presumably changed in the twelve years hence, especially considering the rise of 'plant-based' food trend that has been exploding in the food industry over the past 3-5 years. Even with the knowledge gap between producers and consumers beginning to close slightly as an increasing amount of food companies (large and small) are beginning to formulate with pulses and pulse ingredients for 'plant-based', 'meatless', and vegan food items (e.g., A&W and Burger King releasing plant-based vegan burgers in Canadian restaurant locations in 2020), many food labels do not call out pulses specifically on the front packaging, and products of this nature are still relatively novel in comparison to grocery staples. The potential, however, for plant-based food products formulated with pulses is limitless. The Good Food Institute predicts that if innovation in plant-based meats alone continues to increase at the current rate, they could obtain a 6% share of total meat consumption by 2030 (Troya et al., 2021). In summary, while Canada is a top producer of pulses, the utilization of the crops domestically is still well below potential levels and is required to grow substantially to meet producer and consumer demand alike. This gap has presented an opportunity for research and innovation within the Canadian pulse sector to increase the knowledge base surrounding pulse functionality and nutrition, and therefore, increase utilization both domestically and internationally.

Pulses are increasingly popular for producers to choose to formulate innovative food products with as whole pulses and pulse ingredients impart both functional and nutritional benefits to food products, especially when processed in some manner prior to use. Pulses are high in protein, complex carbohydrates, fibre, B-complex vitamins, folic acid, potassium, calcium and iron, while being low in fat (with the exception of chickpeas) (Bellido et al., 2013; Mudryj et al., 2014). They have been often coined as a 'poor man's meat' by researchers as they provide a source of all essential amino acids, with the general exception of the sulfur containing amino acids (e.g., cysteine and methionine) and in certain cases, aromatic amino acids (e.g., tryptophan). In addition,

they are affordable to the majority of consumers in comparison to more traditional protein sources such as meat or dairy products (Tharanathan & Mahadevamma, 2003). Challenges associated with pulse utilization and consumption includes the presence of antinutritional factors that have the ability to negatively impact the digestion of pulse food items, as well as, poor inherent functional properties due to their compact, nutritionally dense seed structure (in comparison to animal proteins, such as casein and whey, and in some cases, cereal/tuber proteins) (Loveday, 2020). Compounds such as tannins, phenolics compounds, amylase inhibitors, chymotrypsin/trypsin inhibitors, lectins, phytates and oxalates are present in raw flours, but researchers have shown that through pre-treatment processing of pulse seeds, these compounds can be effectively reduced or eliminated (Campos-Vega et al., 2010; Shi et al., 2017). It should be noted, that these antinutritional factors also possess some health promoting properties. Pre-processing methods such as germination (López-martínez et al., 2017), extrusion (Frohlich et al., 2014; Wang et al., 2020), physical approaches (e.g. milling of seeds, dehulling) (Scanlon et al., 2018), roasting (Ma et al., 2011), and tempering and infrared heating (Bai et al., 2018a, 2018b; Guldiken et al., 2022; Liu et al., 2020) have all been utilized as pre, and post-processing methods in recent years. To varying degrees, they have all proven effective at improving both nutritional quality (e.g., protein quality, protein and starch digestibility) and functional properties (e.g., protein and carbohydrate solubility, emulsification and foaming abilities, water hydration and oil holding capacities). Particularly interesting is the use of tempering moisture and infrared heat as a pre-milling processing technique for pulse seeds. The combined effect of elevated moisture levels plus the high intensity heat from shorter wavelengths characteristic of infrared heating, allows for reduced energy inputs, less time required for processing and lowers the overall costs in comparison to other thermal pre-treatments (Rastogi, 2012).

Considering the operational benefits, this research focuses on the use of both moisture tempering and infrared heating of two commercially important pulses: green lentils and yellow peas. Tempering was performed to facilitate a temperature-moisture equilibrium to increase the susceptibility of the pulse seeds to infrared heat treatment. Infrared heating (electromagnetic radiation with a wavelength region of 1.8-3.4 μm) was used to facilitate a rise in the vapour pressure inside of the seeds, effectively leading to rapid internal heating. Once treated, the seeds were milled into flours and evaluated. The overarching goal of this research was to improve the nutritional and functional properties of these pulses, in order to increase their utilization in

common whole foods (e.g. pasta, pan breads, soups, dips/spreads), as ingredients (e.g. analogues and extruded products).

## 1.2 HYPOTHESES

Hypotheses to be tested within this project include:

- The combined effect of increased tempering moisture and seed temperature, to a certain critical level, will significantly alter the overall functionality of the milled pulse flours due to partial protein denaturation and increase in damaged/gelatinized starch content.
- The combined effect of increased tempering moisture and seed temperature, to a certain critical level, will improve the nutritional value of the pulse flours by increasing the protein and starch digestibility due to partial protein denaturation and increase in damaged/gelatinized starch content

## 1.3 OBJECTIVES

This research project focus was on using tempering and infrared heat technology to adjust the moisture equilibrium and seed temperature of two pulse varieties: yellow peas and green lentils. Once the seeds have been appropriately processed, the treated seeds will be milled into flours. The objectives of this project are:

- To examine the effect of tempering moisture and surface seed temperature of yellow peas and green lentils on the physicochemical and functional (i.e., solubility, pasting, foaming and emulsifying) properties of each respective milled pulse species.
- To examine the effect of tempering moisture and surface seed temperature of yellow peas and green lentils on the nutritional properties (i.e., protein quality and protein and starch digestibility) of each respective milled pulse species.

# 2. LITERATURE REVIEW

## 2.1 Composition of pulses

*Proteins*

Pulse proteins contain all of the essential amino acids needed to support growth and development, however, pulses tend to be rich in lysine and deficient in the thiol-containing amino acids (methionine and cysteine) and/or tryptophan (Boye et al., 2010). In contrast, cereals tend to be rich and deficient in the opposite amino acids. In developing countries where access to animal protein sources may be limited, pulses and cereals tend to be consumed as part of a complementary diet. Pulses are considered to be high in protein (18-30 g/100 g) and contain predominantly globulin type proteins (salt-soluble, 50-72% of the total protein) comprised of 11S legumin and 7S vicilin proteins, with 'S' representing a Svedberg Unit determined from the sedimentation coefficient (Oomah et al., 2011). Legumin is a hexameric protein with a molecular mass (MM) of 350-400 kDa, comprised of subunits held together by non-covalent forces. Each subunit is comprised of an acidic α-chain (MM – 40 kDa) and a basic β-chain (MM – 20 kDa), held together by covalent bonds. Vicilin is a trimeric protein with a MM of 150-190 kDa, held together only by non-covalent bonds as it lacks cysteine and therefore does not form disulfide bonds to stabilize the protein's structure (Oomah et al., 2011). Depending on the pulse market class and the variety, the ratio of 7S/11S can vary, which can have a subsequent impact on protein nutritional value and functional properties (Singh & Jambunathan,1982). Water-soluble albumin proteins have a MM ranging between 5- 80 kDa, and make up 10-20% of total protein content and include enzymatic, protease and amylase inhibitors, as well as lectins (Boye et al., 2010; Jarpa-Parra, 2018). Other minor proteins found in pulse seeds include prolamins (alcohol soluble, 0.2-3% of total protein content) and glutelins (5-15% of total protein content) (Boye et al., 2010). Pulse proteins are less bioavailable in comparison to protein sources such as meat or dairy products/ingredients. This is due to the tightly associated structure of storage proteins in the seeds that are resistant to proteolysis (Tiwari & Singh, 2012). In contrast, casein micelles present in dairy products and human breast

milk are highly charged structures and loosely associate together, allowing for superior rates of enzymatic breakdown and digestion in the gastrointestinal system (Lönnerdal, 2003).

*Carbohydrates*

Pulses contain between 37-53 g/100 g of carbohydrates (Tharanathan & Mahadevamma, 2003). Pulse carbohydrates consist of monosaccharides, disaccharides, oligosaccharides and polysaccharides (Oomah et al., 2011). The main storage component of pulses is the starch fraction, which makes up most of the carbohydrate portion in the seed. Starch is stored in the form of granules in pulse seeds that tend to be smooth, oval in shape, and lacking fissures or pin holes (Hoover et al., 2010). Pulse starches consist of amylopectin and amylose. The former structure consists of linear chains of α(1→4)-D-glucopyranosyl residues connected through α(1→6) linkages, which are branching points of the molecule (Hoover et al., 2010). Amylopectin molecules have a large MM and contain both crystalline and amorphous regions with varying chain lengths that are able to form clusters and double helices as a form of ordered structure within the starch granule. In contrast, amylose is a linear polymer comprised of α(1→4)-D-glucopyranosyl with very few branch points [α(1→6)] (Oomah et al. 2011). The macromolecules associate with one another to form helices and can create strong complexes with iodine, fatty acids and other monoglycerides through non-polar interactions inside the helices (Hoover et al., 2010). A common oligosaccharide present in pulses is α-galactoside, which contains galactose in α-D-1→6-linakges. α-Galactosides are sucrose derivatives such as raffinose, stachyose and verbascose (Oomah et al. 2011). These small chain carbohydrates are typically a target of elimination during processing of pulse products as they cause gastrointestinal pain when they are metabolized by microbes in the intestines. Microbes release gas upon metabolization of α-galactosides, causing discomfort, bloating, and pain for the consumer (Devindra & Aruna, 2016).

Pulses are also a rich source of soluble and insoluble dietary fibres (Tosh & Yada, 2010). Insoluble fibre facilitates fecal bulk movement through the digestion tract to improve laxation and soluble fibre acts to regulate blood cholesterol and blood glucose levels (Tosh & Yada, 2010). The consumption of dietary fibre has been related to many health benefits such as reducing/reversing cardiovascular disease, diabetes mellitus, certain cancers as well as being able to catalyze weight loss (Tharanathan & Mahadevamma, 2003). Pulses have between 14-32 g/100 g fibre depending on the market class and variety, with varying levels of insoluble and soluble fibre components.

For instance, dry pea contains 14-26 g/100 g total fibre with 10-15 g/100 g being insoluble and 2-9 g/100 g being soluble (Borowska et al., 1998). For lentils, the total dietary fibre ranges between 18-20 g/100 g, with 11-17 and 2-7 g/100 g coming from insoluble and soluble fibre, respectively (Dalgetty and Baik, 2003; Perez-Hidalgo et al., 1997).

*Lipids*

Typically, pulses are considered to be low in crude lipids (1-2 g/100 g), with the exception of chickpeas which contain about 5-6 g/100 g ( FAO, 2016; Tharanathan & Mahadevamma, 2003). Pulses are high in free unsaturated fatty acids, including linoleic (21-53%) and α-linolenic (4-22%) acids (Tiwari & Singh, 2012). Another major fatty acid common in pulses is oleic acid, which is found in chickpeas, garden peas and lentils (Tiwari & Singh, 2012). Pulse lipids are comprised of three main lipid categories: neutral lipids, phospholipids and glycolipids. Neutral lipids in the seeds include triacylglycerols as well as small portions of di- and monoacylglycerols. Phospho- and glycolipids are present in the cell membranes of the seed (Tiwari & Singh, 2012). Palmitic acid is the most prevalent saturated fatty acid (SFA) seen in pulses (Baptista et al., 2017). Oxidative damage of pulse seeds leads to the production of undesirable by-products such as aldehydes, ketones and esters, all of which are produced via the breakdown of the nutrients (ex. carbohydrates, amino acid, vitamins, minerals) present in pulse seeds (Tiwari & Singh, 2011). Protein-lipid co-oxidation is seen interdependently in pulse varieties due to the interaction of interfacial proteins and lipid molecules in food emulsions (Gürbüz et al., 2018). The production of harmful lipid oxidation by-products, such as hyperoxides, react with proteins to alter the structure and resulting functional properties (e.g. conformational changes leading to agglomeration and precipitation) (Gürbüz et al., 2018). Advertently, pulse proteins may also work as anti-oxidants by acting as chelating and free radical scavengers (Chen et al., 2012; Gürbüz et al., 2018). The presence of lipoxygenase in pulse seeds is also a contributing factor to lipid oxidation in pulse foods and ingredients. The enzyme de-oxygenizes polyunsaturated fatty acids, resulting in the production of hydroperoxides over time, which lead to the presence of off-flavour compounds that decrease the palatability and shelf-life of pulse foods/ingredients (Sanz et al.,1992).

*Minor components of pulses*

Pulses are a source of bioactive compounds that have both beneficial and negative effects on human health. Examples of common antinutritional factors (ANFs) found in pulse seeds include protease inhibitors, amylase inhibitors, phytates, oxalates and tannins (Champ, 2002). For instance, protease (e.g., trypsin and chymotrypsin inhibitors) or amylase inhibitors act to inhibit digestive enzymes needed to breakdown proteins and starch, respectively. On the contrary, these inhibitors also have been shown to be anti-carcinogenic by retarding malignant growth, whereas amylase inhibitors may reduce blood glucose levels and increase blood insulin levels by inhibiting starch decomposition (Champ, 2002). Polyphenols (which include tannins) act to form cross-links with proteins in the presence of oxygen to limit their digestibility, however they are widely known for their antioxidant effects. Oxalates and phytates chelate metal ions to limit mineral absorption; lectins cause diarrhea, vomiting and red blood cell agglutination; whereas saponins interact with bile acid and cholesterol leading to the excessive accumulation of cholesterol in the blood (Sreerama et al., 2012). In balance, these compounds also may offer health benefits: phytic acid and polyphenols work as antioxidants to protect cells from the damaging effects of free radicals produced during oxidation and have been cited as possible preventative agents against diseases associated with abnormal cellular replication (e.g., cancers, inflammation, some neurodegenerative disorders) (Campos-Vega et al., 2010; Zhivotovsky & Orrenius, 2010). Oligosaccharides, also found in pulses in small amounts, are known to cause gastrointestinal distress through the production of short chain fatty acids (e.g. butyric, propionic and acetic acid) during microbial fermentation in the gut that produce uncomfortable volumes of gas (Slavin, 2013). Oligosaccharides also have some prebiotic effects that benefit gut health, as the production of short chain fatty acids work to lower the pH of the colon, which has been shown to possibly reduce the risk of colon cancer (Meyer & Stasse-Wolthuis, 2009; Wong et al., 2006). In addition, for faba bean only, the glycosides vicine and convicine are present, which cause favism (the deficiency of glucose-6-phosphate dehydrogenase) in certain individuals (Cappellini & Fiorelli, 2008). Irrefutably, minor bioactive compounds found in pulses contribute both health promoting and adverse health effects.

## 2.2 Infrared heating

Pulses are cooked using some form of heat treatment such as boiling (Khattab et al., 2009), roasting (Sharma & Gujral, 2011), drum drying (Bencini, 1986), microwave heating (Jogihalli et al., 2017), extrusion (Alonso et al., 2000) or infrared heating (Bai et al., 2018a, 2018b). Heat treatments, depending on the process and the conditions, can act to reduce levels of bioactive compounds within milled flours or protein ingredients (Lee et al., 2006). They also may be used to improve the nutritional value or functional properties of said flours or protein ingredients as well as gelatinize the starches (Žilić et al., 2014). Pulses are well known for their long cooking times, and pre-cooking methods can help to reduce the cooking time for consumers or product developers (Walker & Kochhar, 1982). Infrared heating, also known as micronization, is the heating processes that exposes pulses to electromagnetic radiation (ER) in the wavelength region of 1.8-3.4 μm (Fasina et al., 2001).

Heating is achieved through the application of infrared rays causing water molecules within the grain to vibrate at a frequency of 60,000 to 150,000 MHz. This causes a rise in water vapour pressure and results in rapid internal heating of the seed (Fasina et al., 2001). In the food industry, infrared heating is utilized for several purposes such as drying/dehydration, enzyme inactivation, and pathogen elimination (Riadh et al., 2015). It also is used in the elimination of bioactive compounds (e.g., protease and amylase inhibitors) and improving the functionality of milled flours. The general schematic of a lab scale microionizer is given in Figure 2.1. Grain is fed from the hopper onto the vibrating conveyor to ensure all surfaces of the sample are evenly heated by the infrared emitter which is fueled via the line attached to the propane gas tank (Bai, 2018a, 2018b). Samples are monitored to the desired surface temperature (between 100°C to 150°C typically) using a handheld thermometer. Optimal end surface temperatures will vary depending on the pulse market class and the desired end use, and, can be controlled by adjusting the speed of the conveyor and the subsequent exposure time to the infrared radiation. Typically, prior to infrared heating, the pulses are tempered to a specific moisture (from ~8% up to ~20%) (Bai, 2018a, 2018b; Fasina et al., 1999). Tempering, or holding, is the process of allowing a sample (e.g., cereal grains or pulse seeds) to equilibrate moisture contents (Alsaffar, 2011). Through this process the resulting physical and functional properties may be improved, such as, more sensitive response to heat treatment (e.g., increase water molecule vibration during infrared heating) and

improved textural properties (e.g. lowered enthalpy of gelatinization, increased water adsorption capacity) (Whalen et al., 2000).

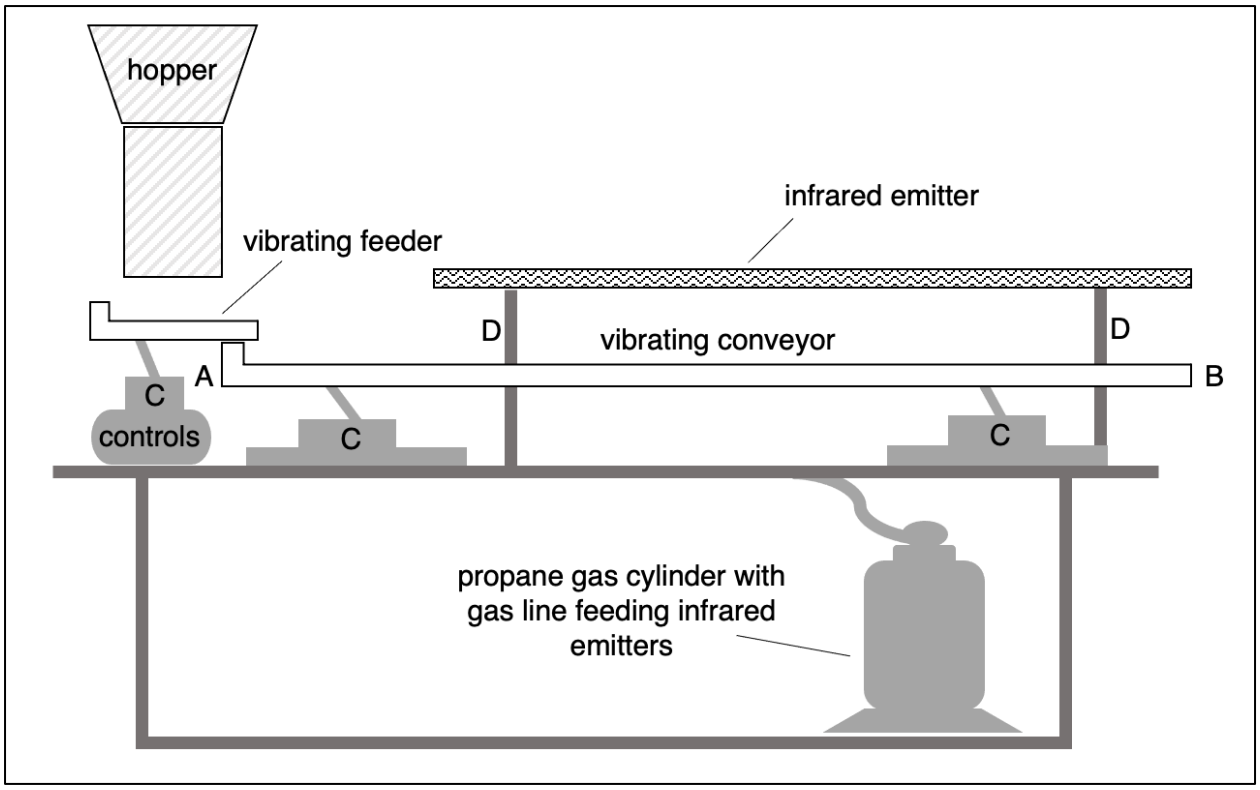


Figure 2.1 Schematic diagram of a lab scale microionizer where (AB) is the vibrating conveyor, (C) is the vibrating feeder, and (D) is the stand supporting the infrared emitters and side plates (Adapted from Fasina et al., 2001).

## 2.3 Tempering

Tempering of plant material has been shown to provide many benefits for both consumers and industry who utilize whole pulses and/or pulse foods or ingredients. Tempering is the process of soaking plant material, such as pulse seeds, in a selection of aqueous solutions (e.g., salt, acidic, basic) (Bai et al., 2018b). While salt solutions have been proven to be effective at solubilizing pulse proteins (globulin type, salt soluble), water remains the most widely used tempering solution as it does not affect the colour or taste of the treated seeds (Bellido et al., 2006). Tempering of pulse seeds has been shown to increase protein solubility by weakening protein-protein and protein-starch non-covalent, hydrogen and hydrophobic bonds (Arntfield et al., 1997). Tempering can also lower the energy input needed for dehulling and milling by facilitating increased ease of

bran removal (Hoesney & Delcour, 2010), decrease cooking times (Scanlon et al., 2005) and improve the nutritional value of pulse flours by decreasing the concentration of ANFs present (Wang et al., 2003). It has been shown by Khattab and Arntfield (2009) that the combination effect of tempering with infrared heating results in superior levels of ANF reduction, specifically tannins and trypsin inhibitors, in comparison to either method on its own.

### 2.4 Milling of pulses

During the milling of pulses, particle size is reduced to a certain threshold (depending on the mill and sieve size). For fine flours that are utilized for desserts, particle sizes between 19-21 μm are desirable, while coarser flours typically contain particle sizes between 45-150 μm (Tiwari & Singh, 2011). Consequently, during milling, the surface area of the particle is increased, allowing for enhanced interactions with other ingredients in solution, faster heat and mass transfer for faster cooking times, improved digestibility and more efficient mixing and dispersion of flours (Bienvenido, 1984; Wood & Malcolmson, 2011; Vishwakarma et al., 2018). Pulses may be either wet or dry milled. The latter creates flour that can be utilized for extruded snacks, breakfast cereals, pasta and other baked and/or fried foods (Velu et al., 2006). There is an array of machinery that can be utilized for the dry milling of pulses. Impact mills, roller mills, chakki (stone) mills, hammer mills, attrition-disc mills and cyclone mills are all commonly used throughout both research and industry applications (Wood & Malcolmson, 2011). The choice of mill type is a determinant of end flour quality and functionality; therefore, the choice needs to be carefully researched prior to the milling process.

For pulse milling, the removal of the seed coat and the cleavage of the two cotyledons present within the seed is very important to obtain the desired starting material for milling (unless whole flours are desired) (Vishwakarma et al., 2018). Removal of the hull prior to milling helps with further processing and commercial use as well as to minimize loss of product in powder form or broken seeds in certain varieties (Wang, 2005). The issue that pulse processers face is that pulse seed milling is not as straightforward as conventional cereal grain processing. Vishwakarma et al. (2018) outlined several challenges processors can face while milling. [1] The operating condition of milling equipment differs between market classes and varieties making standardized conditions difficult. [2] The physical properties (e.g., size and surface roughness) differs between pulses and varieties. [3] The presence of gums in-between the hull and cotyledon of the seed coats, may

require the need for multiple passes through de-hulling machinery. [4] The presence of pectin and lignin molecules impart rigidity to the seed coat further slowing de-hulling. [5] Finally, the power requirements of the mill chosen is an important factor as the cost of energy needed to mill the product, both monetarily and time-wise, is crucial for the final quality of the milling application (Smejtkova & Vaculik, 2018; Vishwakarma et al., 2018).

To try to minimize mechanical, power and time costs, it is common that two types of mills are used to further refine pulse flours. For example in a laboratory setting, the use of an attrition-disc mill for initially coarse flour, followed by a cyclone mill to create a finer flour with a smaller particle size is performed (Bai et al., 2018a, 2018b; Pelgrom et al., 2014). Another method to reduce the various costs associated with pulse milling is to incorporate pre-treatments of seeds to loosen the husk of seeds, increase ease of milling, reduce breakage of seeds, and improve the quality of the split (Tiwari & Singh, 2011). For instance, chemical, enzyme and hydrothermal pre-treatments are all methods that can be utilized in research and industrial applications to improve milling outcomes (Vishwakarma et al., 2018). Tempering of seeds before milling is a common pre-treatment of pulse seeds that are to be heat treated. The process works to loosen the hull, allowing for easier removal of hulls and therefore improves dehulling yield (Tiwari & Singh, 2011). In lentils, a good dehulling yield result is achieved from a combination of long tempering times, following by short drying times. These parameters result in the shrinkage of the cotyledon which effectivity reduced the energy input needed for milling operations (Erskine et al., 1991; Velu et al., 2001).

### 2.5 Functionality of flours from infrared heated seeds

*Protein Solubility*

Solubility of a pulse ingredient typically refers to the amount of protein (or nitrogen) in the liquid phase relative to the amount of protein in the ingredient but is also heavily influenced by other compositional components such as starch and lipids. The protein (nitrogen) solubility of an ingredient tends to be a precursor to other important functional attributes, such as emulsification, foaming and gelation, and can be used as an indication of the quality (or level of denaturation) of a protein ingredient. Solubility of pulse flours varies significantly depending on the variety and corresponding cultivar market class, and the overall composition of nutrients within the pulse. An ingredients is solubilized when protein-solvent interactions are more favored than protein-protein

interactions (Du et al., 2014; Sosulski & McCurdy, 1987). In response to pH, pulse proteins tend to follow a typical U-shaped solubility profile with a minimum occurring at its isoelectric point (pH 4-6 depending on the protein) (Boye et al., 2010; Chang et al., 2015; Joshi et al., 2012). At the isoelectric point (pI), the protein molecule has no net charge, causing aggregation of molecules due to the lack of repulsive forces (Kiosseoglou & Paraskevopoulou, 2011). For most pulses, solubility is increased at <pH 3 or >pH 7, as electrostatic repulsion between neighboring proteins is high. As a general rule, thermal treatment of pulse seeds reduces protein solubility of the resulting flours (Fernandez-Quintela et al., 1997). Bai et al. (2018b) investigated the effect of infrared heating and tempering on the solubility of desi chickpeas at pH 7. The authors found the un-tempered, unheated control flour had a solubility of 69%, which then lowered slightly to 66% and 56% upon heating to 115°C and 135°C, respectively. The addition of tempering to 20% moisture, plus heating to the same temperatures resulted in further declines in solubility to 48% and 43%, respectively. The authors reasoned that the heating process (along with tempering to allow for easier dehulling) resulted in the partial protein denaturation of the chickpea proteins to expose hydrophobic groups which then facilitated protein aggregation and protein-lipid complexation, both of which lead to reduced solubility. Fasina et al. (2001) also reported reduced solubility at all pH values (except at the pI) of green peas, lentils, black, kidney and pinto beans that were infrared heated to 140°C (untempered, moisture content ranged from ~5% (pinto beans) to ~10% (green peas)) over the pH range of 2-12 relative to unheated flour. Reduced protein solubility may negatively alter some functional aspects of pulse flours in food matrices (e.g., sedimentation of protein fractions may lead to lowered quality perceptions by consumers), but protein aggregation resulting from heat treatment may also work to optimize protein isolation for specialized protein ingredients through precipitation and ultrafiltration methods (Fuhrmeister & Meuser, 2003). While reduced solubility of proteins due to aggregation of molecules is seen for infrared heated pulse seeds, the increased solubility of the carbohydrate fraction of the seeds is observed. Infrared heat treatment works to partially gelatinize starch and increase the solubility of gums, such as pectin, allowing for improved starch digestibility, palatability and reduced cooking times (Deepa & Hebbar, 2016).

*Emulsification*

Food emulsions are the mixture of two immiscible liquids (the continuous and discontinuous phase), such as water and oil. Emulsions in foods are commonly observed in everyday foods such as salad dressings, condiments, dairy products (e.g., butter, margarine, milk), and bottled beverages (e.g., smoothies, flavoured coffees). The stability of an emulsion is an indication of quality, as consumers are unlikely to find drinks or sauces separated into two phases appetizing in most situations. The stability of the emulsion is determined by the activity of emulsifiers (also called surfactants), that reduce interfacial tension at the water/oil interface. Pulse proteins have been shown to impart the needed stability to food emulsions to maintain quality by acting as emulsifiers at the interface to reduce tension when in their isolated form (>80% protein d.b.), as well as when isolated proteins are complexed with certain polysaccharides (e.g., ι-carrageenan) (Chang et al., 2015; Lam et al., 2018; Lam & Nickerson, 2013; Wang et al., 2019). The emulsifying properties of pulse flours have also been studied and have been shown to promote emulsion stability in food systems. For example, it has been reported that lentil flour can act to stabilize mineral encapsulation emulsions, although the stability is thought to be caused by an increase in viscosity when baked, not primarily by the surface activity of the proteins in the flours (Kabakci et al,, 2021).

Emulsions are formed using mechanical forces to disperse one immiscible phase (i.e., dispersed phase) into another (i.e., continuous phase) (Lam & Nickerson, 2013). Proteins act to stabilize emulsions by acting as emulsifiers, the ability of which are measured through two main indices, emulsion activity index (EAI) and emulsion stability index (ESI). EAI correlates the ability of a protein to form an emulsion, whereas ESI is the ability of a protein to form and maintain emulsion stability in solution during storage (Ghribi et al., 2015). As emulsifiers, proteins are amphiphilic in nature as they have both hydrophilic and hydrophobic moieties (Lam & Nickerson, 2013). During emulsion formation, proteins migrate or diffuse to the oil-water interface (Figure 2.2a), where they denature and rearrange to position their hydrophobic groups towards the oil phase and their hydrophilic groups towards the water phase (Figure 2.2b). Proteins then aggregate at the interface to form a viscoelastic film that encases the oil droplet and lowers the interfacial tension to keep the droplets stable (Figure 2.2c). The protein film carries a charge depending on the pH and is most stable away from its pI where charge repulsion between emulsified oil droplets helps prevent coalescence from occurring (Karaca et al., 2011). Furthermore, the rearranged

hydrophilic moieties within the film (depending on their primary sequence) can create a 'hairy' surface to give steric stabilization that also prevent coalescence. Emulsions are least stable near the pI of the protein or if salts are present, as electrostatic repulsive forces are significantly reduced.

Emulsifying properties can be improved through protein extraction methods, modification of proteins towards a more highly charged state for greater solubility, as well as, a reduction in emulsion droplet sizes (Karaca et al., 2011;Chang et al., 2015; Joshi et al., 2012; Primozic et al., 2018). Heat treatments may be used to induce changes in emulsifying properties of pulse proteins. A consensus among researchers conclude that the application of heat (e.g., roasting, boiling and convectional drying) partially denatures globular proteins which can increase emulsion activity (EA) by increasing protein adsorption at the O/W interface, but decreases emulsion stability (ES) by decreasing solubility through the exposure of hydrophobic amino acids (Ghribi et al., 2015; Ma et al., 2011; Peng et al., 2016). The effect of the application of infrared heat treatment on EA and ES of pulse proteins is limited. However, Bai et al. (2018b) reported a 44 and 47% increase in EA values for infrared heated chickpea flour (115 or 135°C, respectively) compared to the control/non-processed chickpea flour.

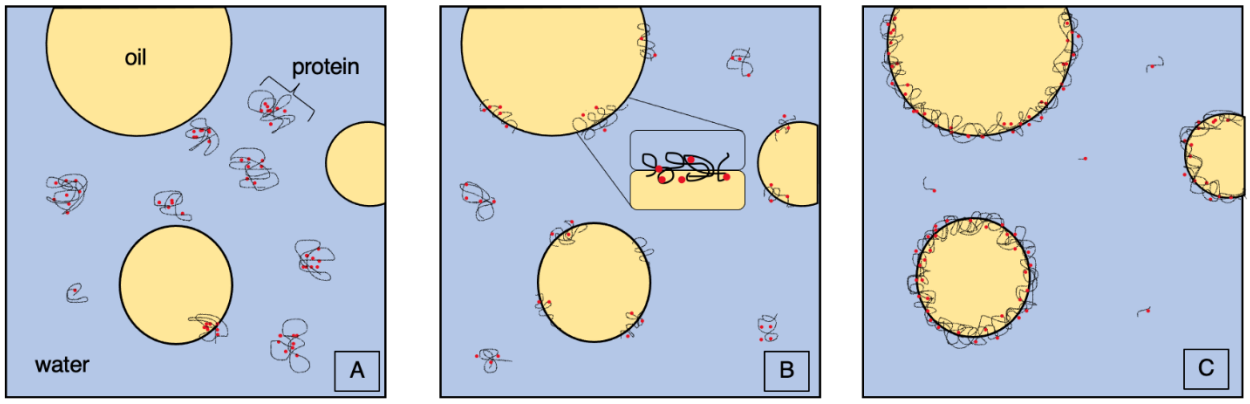


Figure 2.2 Schematic showing steps during emulsion formation involving a protein, including: (A) the migration of proteins to the oil-water interface from the bulk solution; (B) rearrangement of the proteins at the interface to position hydrophobic and hydrophilic moieties towards the oil and water phases, respectively; and (C) formation of a viscoelastic stabilizing film that encases the oil droplet.

*Foaming*

Protein stabilized foams are important for the tenderization of foods (e.g., bread), improvement of mouthfeel (e.g., sponge cakes, ice cream), decrease in overall density of products (e.g., 'lightness') and the marking of visual quality in food products (e.g., beer) (Boye et al., 2010). Foaming capacity (i.e., the percentage of gas entrapped, describing the amount of foam formed) and foam stability (i.e., volume of liquid retained in foam during storage) are used to measure the quality of foams and both depend on the interfacial film formed by proteins around the air-water (A/W) interface (Boye et al., 2010; Du et al., 2014). Researchers have found that foaming capabilities vary across pulse varieties, as nutrient make-up differs slightly between varieties and within cultivars, leading to different protein chemistry/profiles which ultimately may alter functional properties, such as foaming (Adebiyi & Aluko, 2011; Boye et al., 2010; Du et al., 2014; Sosulski & McCurdy, 1987). Generally speaking, pulse proteins are able to produce efficient foams due to the presence of highly surface-active proteins in the soluble continuous water phase (Du et al., 2014). Solubility and flexibility of protein molecules are important factors to enhance the unfolding of the protein to create films at the A/W interface, effectively encapsulating air bubbles (Adebiyi & Aluko, 2011). To increase foaming capacity (FC) and stability (FS), similar methods used to improve other functional properties (e.g., emulsification) may be utilized. The change in pH of solution to improve solubility and extraction methods to purify proteins (fat and carbohydrate molecules impede foam formation) have all been shown to prove effective ( Boye et al., 2010; Du et al., 2014; Sosulski & McCurdy, 1987).

A variety of heat treatments are viable methods to improve the foaming ability of pulse proteins. Ghribi et al. (2015) found that the heating of chickpea protein concentrates through convective methods lowered interfacial tension, which promoted foam formation, but not stability. The researchers suggest that the heating of pulse protein fractions may impart stability by increasing the viscosity of the continuous phase, but further studies are needed to ensure this attribute is attainable. Bai et al. (2018b) reported that the foaming properties of infrared heated desi chickpea proteins correlated positively to solubility and negatively correlated with both lipid content and gelatinized starch content. The same authors reported FC to increase from 181% (untempered, unheated) to 217% at 115°C, but only to 186% at 135°C (untempered). When tempered to 20% moisture and heated to 115°C or 135°C, FC declined to 130%. FS increased from 85% to 93% by heating to 115°C, however with tempering to 20% moisture at the same

temperature FS decreased to 84% and further to 79% when heated to 135°C with tempering. These results correspond with the trend seen for other functional properties of heated, tempered seeds—the decrease is related to the decline in solubility, which limits the amount of foam formed via protein adsorption at the air-water interface. A reduction in FC and FS for chickpea flour also has been observed for roasted samples (in microwave oven, 450-900 W), with FC declining from ~26% down to ~4% and FS declining from ~20% down to 0% (Jogihalli et al., 2017). Similar results have also been cited for roasted peanut flour, where FC reduced from 0.06 mL of foam generated per gram of flour, down to 0.03 mL/g (Yu et al., 2007).

*Water holding capacity*

Water holding capacity (WHC) describes the ability for a food matrix or ingredient (e.g., flour or protein product) to entrap water such that it impedes the bulk flow of water within a food/ingredient (Kumar et al., 2016; Wang et al., 2017). Water is present in three forms: (a) free water: which is the most abundant form, is bound via weak hydrogen bonds, and is readily removed during the drying process; (b) absorbed/structural water: which is associated water to the protein/starch's surface through strong bonding (hydrogen bonds, water-dipole interactions, water-ion) to hydrophilic compounds, and is more difficult to remove via drying; and (c) bound water: which has strong bonds that associate water and hydrophilic molecules in the matrix, and is nearly impossible to remove (Charely, 1982). The amount of water absorbed, or the WHC, of a protein depends on the amount of exposed hydrophilic groups that are able to form bonds with water (Kumar et al., 2016). For pulse flours, the WHC has been shown to vary significantly between cultivars due to varied protein structures (i.e., the amount of hydrophilic amino acid residues exposed) of different pulse types (Boye et al., 2010; Du et al., 2014; Sosulski & McCurdy, 1987). Values range from 0.6-2.7 g/g for pulse protein concentrates (Boye et al., 2010) to 1.12-1.89 g/g for pulse flours (Du et al., 2014). Pulse flours have higher WHC due to the presence of starches/damaged starch which also can hold water (Kumar et al., 2016). When heat treatments are implemented on pulse seeds before being milled into flours, the WHC of the samples increases substantially (Fasina et al., 2001; Ma et al., 2011). This increase can be attributed to the partial denaturation of the predominantly hydrophilic proteins (i.e. globulin proteins, 50-72% of the total protein) during heat treatment, as well as the gelatinization of the starch fraction of the pulse flours (Fasina et al., 2001; Ma et al., 2011). Prinyawiwatkul et al. (1997), found that the WHC of cowpea

flour increased with soaking (control = 1.82 g/g; soaked seed flour = 2.0 g/g) and increased even further with a combined treatment of soaking and boiling (~2.5 g/g). The same authors conclude that the increase in WHC was due to swelling of starch (soaking alone), partial starch gelatinization and the structural conformational changes that exposed further hydrophilic groups on the main pulse protein fractions, all of which collectively contributed to the increased affinity for binding water. For microwave treated chickpea flour, again, the WHC increased steadily with time and increased power inputs (Jogihalli et al., 2017). As with boiling, microwave roasting is thought to increase WHC due to the presence of damaged starch which is a result of gelatinization that is induced by heat treatments. Additionally, the porous surface of the heat-treated seeds (hull is broken or removed) is likely to facilitate the movement of water inside the flour particles through capillary action, effectively increasing the interaction between hydrophilic amino acid residues and water molecules (Sharma et al., 2011).

*Oil holding capacity*

Oil holding capacity (OHC) is similar to WHC, as it is the amount of oil absorbed per gram of sample (Ghribi et al., 2015). The OHC of a sample contributes to the shelf stability and quality of a food product as the oil/lipids absorbed directly relates to the mouthfeel, appearance and flavour of the product, as well as the likelihood of oxidation (Kumar et al., 2016). As with WHC, protein structure plays a large role in the results for OHC of pulse flours. The surface hydrophobicity (SH) of proteins has been shown to correlate to OHC, with proteins containing a higher concentration of hydrophobic amino acid residues exposed, exhibiting superior OHC activity (Ashraf et al., 2012; Sosulski, 1976). Raw pulse seeds contain mainly globulin type proteins, which are soluble in dilute salt solutions, but also contain a small amount of alcohol soluble prolamin proteins. Pulse varieties differ in specific nutrient make-up; therefore, some pulses may have a higher concentration of prolamins as surface proteins, effectively increasing OHC (Kiosseoglou & Paraskevopoulou, 2011). An increase in OHC can be attributed to the processing of pulse seeds, such as milling with or without pre-treatments of seeds. Milling alone works to physically disrupt the structure of seeds by dramatically reducing particle size and removing the compact seed hull. With these changes, conformational protein structures are affected/exposed and surface proteins that once contained hydrophilic surface amino acid residues (globulins/albumins), may now have a greater affinity to binding with lipid molecules (e.g., prolamins now at surface) (Ma et al., 2011).

Untreated, whole legume flour has an approximate OHC of 0.9-1.4 g/g (Du et al., 2014). When heat pre-treatments are applied, the OHCs of pulse flours have been shown to exhibit varied binding capacities for different forms of heat. Ma et al. (2011) reported dry heat treatments (e.g., roasting in oven) decreased OHC slightly in comparison to raw flour and for wet heat treatments (e.g., boiling), OHC increased by 22.5% for chickpea flour and by 70.7% for green lentil flour. The researchers suggest that the boiling of the flours exposed internal hydrophobic groups enhancing OHC, as well as imparting greater porosity of the flours allowing an increased ability to hold fat in the flour matrix. Similar to the reduction in OHC for oven roasted flours, infrared heat treatment has been shown to either not significantly change OHC (Bai et al., 2018b) or slightly reduce it (Jogihalli et al., 2017). Researchers hypothesize that infrared heating and/or roasting does not affect conformational structure as greatly as does boiling, which is thought to solubilize hydrophilic surface proteins, create more highly porous particles and cause greater denaturation of overall protein structure. The latter effectively exposes further hydrophobic amino acid residues to bind with oil (Ma et al., 2011).

*Protein gelation*

Gels in food can be defined as a continuous 3D matrix containing two phases: (1) the solid phase constructed of polymers or particulates and (2) the liquid phase that is entrapped throughout the solid phase (Clark, 1992). Common food gels present in everyday products include meat and vegetarian sausages, puddings and yogurt (Shevkani et al., 2015). As previously discussed, pulse proteins are predominantly salt-soluble globulin proteins with both vicilin (7S) and legumin (11S) subunits and therefore form particulate gels largely stabilized via hydrophobic interactions. Pulse proteins create gels by cross-linking polypeptide chains through various molecular forces such as hydrogen bonds, ionic attractions, disulphide bonds and hydrophobic interactions (one bond type may be utilized in gel formation or a combination of types) (Sun & Arntfield, 2012). To be able to create the opportunity for these bonds/forces to actualize into food gels, first globular proteins must be partially denatured in order to expose the necessary amino groups that will participate in gelation interactions to create a 3D gel network (Mession et al., 2015). Partial denaturation of the proteins can be achieved through heat application, salt addition, pH change or the addition of cross-linking agents to kickstart the interactions that form the gel: (1) flocculation**,** (2) coagulation and

lastly, (3) gelation (Mession et al., 2015; Shevkani et al., 2015; Sun & Arntfield, 2011, 2012). To understand the thermal energy of gel formation, differential scanning calorimetry (DSC) technology is utilized to understand the temperatures of initial globular protein unfolding ($T_{onset}$) and peak denaturation ($T_d$) as well as the proportion of non-denatured proteins before heat treatment ($\Delta H_d$) (Mession et al., 2015). Once the gel is formed, characterization testing is performed such as, large deformation testing (e.g., texture profile analysis (TPA)) and small deformation testing (e.g., oscillatory shear rheometry) to determine gel strength against stress. Rheological testing determines two main parameters of gels: the dynamic storage modulus, or the elastic/solid-like phase (G′), the dynamic loss modulus, or the viscous/fluid-like phase (G″) and the loss tangent, or the measure of energy lost due to viscous flow compared to the energy stored in the elastic phase ($\tan \delta = G''/G'$) (Sun & Arntfield, 2011).

Diverse gelation properties have been reported for pulse flours and isolated pulse protein fractions. In the study of Shevkani et al. (2015), the gelling ability of kidney bean and field pea protein isolates relied on the secondary protein structures of each respective pulse type. It was found that higher gelation temperatures were necessary for kidney bean isolates, as the $G_{reinforcement}$ (the increase seen for G′ and G″ that occurs upon cooling that indicates formation of gel network) was higher than that of field pea isolates. The researchers concluded that this result is due to the presence of a larger ratio of β-sheets compared to α-helices in the secondary structure of kidney bean isolates (in comparison to field pea isolates). β-sheets have a relatively larger surface area and anti-parallel configuration that is more advantageous for intermolecular H-bonding, which resulted in superior junction zone stabilization in the gels (Shevkani et al., 2015a). Additional reseachers examining the gelling properties of pulses include the use of temperature dependent gelation techniques such as heat-induced and cold-set gelation (Mession et al., 2015; Sun & Arntfield, 2012). Heat-induced gelation has been shown to denature pea proteins past the point of being able to form efficient gels, and therefore, this method requires the use of additional reagents in solution such as salts or an extreme change in solution pH away from the pI of the pea proteins (Sun & Arntfield, 2010, 2011, 2012). Mession et al. (2015) evaluated cold-set gelation, and while the method avoided heat-related denaturation, it also was ineffective unless in the presence of acid (glucono-δ- lactone (GDL)). The researchers explain that the use of acid works to de-stabilize the main interactions (i.e., covalent bonds) between initially weak pea-protein aggregates and promotes re-organization of the proteins into larger aggregates, effectively increasing gel strength.

Ma et al. (2011) observed oven roasting of pulse flours caused a slight increase gelling ability of flours at lower concentrations than raw flours (firm gel formed with least gelation concentration of 15% (w/v) vs. 20% (w/v) for raw). The researchers also boiled pulse flours and found that treated pulse flours could form weak gels at 10% (w/v), as well as firm gels at 15% (w/v), showcasing that boiled flours exhibited superior gelling ability at lower least gelation concentrations. The same authors stated that the application of heat to pulse flours, and not pulse seeds before milling (which have been shown to increase least gelation concentration (Abbey & Ibeh, 1988; Prinyawiwatkul et al., 1997)), results in superior gels at lower concentrations (w/v) due to the close association that occurs between the starch and protein molecules in pulse flour during boiling. The researchers claim that the synergistic relationship between protein and starch molecules result in the formation of potentially stabilizing networks as result of increased opportunities for cross-linking between protein and starch, as well as the occurrence of partial starch gelatinization which contributes to a stronger gel matrix through an increase in viscosity (Ma et al., 2011). Researchers studying the effect of infrared heat have cited that the high temperature, short time process is likely to partially denature proteins and cause partial gelatinization of starch in the seeds, but the formation of protein aggregates upon cooling of gelatinized solutions has not been widely examined (Bai et al., 2018b; Fasina et al., 2001; Jogihalli et al., 2017).

### 2.6 Starch digestibility

The increased consumption of foods rich in simple carbohydrates, such as simple sugars and/or refined flours, has been cited as one of the main drivers for elevated obesity rates throughout developed populations (De Spiegeleer et al., 2021; Ferretti & Mariani, 2017). In light of this ever present challenge, researchers have discovered that by adding and/or substituting foods containing a high amount of simple carbohydrates for foods made from pulses or pulse-based ingredients has a positive impact on health overall (Curran, 2012; Mudryj et al., 2014). Native pulse starches have a lower glycemic impact than many cereal or tuber starches and as a result, have been connected to an array of health-related benefits. Said benefits include the prevention and/or reduction of glycemic control issues for individuals at risk for type-2 diabetes, reduced risk of obesity through improved satiety for diets rich in pulses, lowered risk of some cancers (e.g. colorectal cancer) due to anti-proliferative activity of non-digestible fractions of pulse starch and pulse fibre, and

management and/or prevention of cardio-vascular disease via reduction in blood cholesterol levels and overall inflammation (Campos-Vega et al., 2013; Mudryj et al., 2014, 2012; Papanikolaou & Fulgoni, 2008).

Starch makes up the most significant portion of carbohydrates within a pulse seed (37-53 g/100 g). Pulse starch can be classified into three distinctive groups as it relates to starch *in vitro* digestibility—rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). These fractions are defined according to the rate at which they are digested; RDS is completely enzymatically digested within 20 min of consumption and strongly correlated to an individual's glycemic response, SDS is amorphous starch that is not easily physically accessed by digestive enzymes so complete digestion is slowed (~ 120 min for full digestion into simple sugars), and RS is unable to be hydrolyzed by $\alpha$-amylase or pullulanase treatment *in vitro* at >120 min within the small intestine and is instead fermented in the colon (Englyst et al., 1982, 1992; Raigond et al., 2015). RS is divided into five subgroupings: RS1, RS2, RS3, RS4 and RS5. RS1 is classified as starch that is physically inaccessible for digestion (e.g., starch trapped within the seed or cell wall of plant matter) (Raigond et al., 2015). RS2 is related to enzymatic hydrolysis resistance due to the granular morphology of raw/uncooked starch (e.g., granule shape, size and surface characteristics, starch crystallinity, and pore size) (Leszczynski, 2004). RS3 is categorized as retrograded starch that is thermally stable up to 150°C (Raigond et al., 2015). RS4 pertains to starches that are chemically modified, such as etherized, esterified or cross-bonded starches, that are able to physically block enzymatic digestion of $\alpha$ -1,4 and $\alpha$ -1,6 linkages of amylose and amylopectin molecules (Kim et al., 2008). Lastly, RS5 pertains to high amylose starch, where amylose molecules are complexed with lipid molecules and become resistant to digestion (Raigond et al., 2015).

The fractions of RDS, SDS and RS vary across pulse types and within pulse cultivars due to inconsistencies regarding granular morphology, ratio of amylose to amylopectin, and degree of physical or chemical modification. Starch fractions from raw field peas varieties have reported RDS levels by weight between 8-24%, SDS levels between 30-49%, and RS levels between 30-65% (Raghunathan et al., 2017). Common bean cultivars (e.g., dark red kidney beans, light red kidney beans, and navy beans) have reported raw starch fractions by weight between ~12% RDS, 63-65% SDS, and 17-22% RS (Chung et al., 2008). Raw flour fractions (based on total starch) of lentil cultivars can range by weight between 14-23% RDS, 32-41% SDS, and 36-51% RS (Lu et

al., 2018). As most foods are consumed in their cooked/processed state, it is important to note the change imparted to starch digestibility when isolated starches or milled flours are subjected to a form of cooking prior to their use as a food ingredient. In most cases, cooking methods will increase starch digestibility (i.e., increase RDS and decrease SDS and RS) by altering the starch molecules within a food or ingredient, as the more disordered structure is more easily accessed by amylolytic digestive enzymes (Bishnoi & Khetarpaul, 1993). As an example, wet method cooking via extrusion at 90°C can significantly increase the content of RDS by ~12%, while effectively decreasing the content of SDS and RS by ~3% and ~12%, respectively, in pea flours (Qi et al., 2021). Boiling flours in water has also been shown to substantially increase RDS contents of lentil flour by ~70%, whilst decreasing SDS and RS significantly by ~30-37% and ~30-39%, respectively (Lu et al., 2018a). In contrast to wet cooking, popular dry heating methods (e.g., roasting or infrared heating) have been shown to impart negligible change to the digestibility of starch fractions. Lu et al. (2018b) examined the use of dry oven and microwave roasting as processing methods to combat the loss of RS and increase in RDS in pea flours when cooked, to maintain the low glycemic impact of raw pea flour, but with the improved flavour, texture, and appearance of roasted pea flour. The researchers found that RS contents of ~58-60% did not change significantly from the control group in pea flours after oven roasting at 160°C for 30 min and microwave roasting at 1.1 kW for 2 min (Lu et al., 2018b). When dry heating methods are utilized, the material is often tempered to a certain moisture level in solution prior to heating to improve desired functional and nutritional properties (e.g., water and/or holding capacity, solubility, reduction of ANFs, etc.). However, when elevated moisture levels are introduced via tempering, in conjunction with dry heating methods, researchers have reported significant increases in RDS contents and decreases in SDS and RS contents due to starch gelatinization facilitated by the moisture and heat combination (Deepa & Hebbar, 2016; Stone et al., 2021).

### 2.7 Protein quality

Protein quality, over quantity, in the diet is of importance as humans are unable to store protein in body tissues as with other nutrients such as fats (Butts et al., 2012). Proteins are integral biological molecules as they are involved in almost every biological activity in the human body such as tissue growth, hormone synthesis, fluid production (e.g., blood), and enzyme production (Ur Rehman et al., 2017). Some amino acids can be synthesized in a healthy body and are therefore

deemed non-essential, whereas others are not, and must be consumed within our diet. Indispensable or essential amino acids (EAA) include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Animal-derived proteins are a source of all nine EAA and are considered 'complete' proteins, whereas plant proteins typically are lacking in one or more EAA (with the exception of soy protein) and may need to combined to create a 'complete' protein source in one meal (e.g., pulses and cereals) (Boye et al., 2010). To ensure that protein quality is regulated in a way that consumers are able to make informed diet choices, global organizations, such as the World Health Organization (WHO) and Food and Agricultural Organization (FAO), have worked together to create guidelines and recommendations for assessing protein quality (Table 2.1) (FAO, 2013; FAO/WHO, 1991).

Current methods of protein quality evaluation have advantages and disadvantages. All methods work to provide a fair estimate of protein quality in consumer products, while also having to overcome time restraints and ethical hurdles that prevent researchers from being able to accurately assess protein digestion in each unique individual *in vivo*. By accounting for the quantity of EAA as well as protein digestibility, the protein digestibility corrected amino acid score (PDCAAS) is an efficient method for determining protein quality. A limitation of the method is that protein claims are based off a set serving size of 90 g, where the standard serving for pulses is likely closer to 175 mL (121 g) in reality. This can cause protein contents to be underestimated in comparison to real-life serving sizes (Nosworthy et al., 2017). The Digestible indispensable amino acid score (DIAAS), while providing more relevant results (as digestibility is measured in humans, not rats), has logistical considerations yet to be optimized. Nosworthy et al. (2017) stated that the DIAAS acknowledges that the use of ileal digestibility is not yet realistic as there is no data set to accompany results, therefore, fecal digestibility should be used instead. The same authors showcase that the limitations set upon protein quality values may be too high (>0.75) and may discredit the potential for pulses to be deemed good sources of protein. The protein efficiency ratio (PER) and protein rating (PR), adopted in Canada, contains a simple calculation requiring only the measurement of protein intake and resulting growth. A major limitation of the PER/PR is that it does not consider endogenous protein losses or gains (e.g., protein for metabolized growth, enzymes sloughed from intestinal tract during defecation) that ultimately affect the obtained results (Nosworthy et al., 2017). Additionally, the PER does not differentiate between EAA and non-essential amino acids in protein sources.

Table 2.2 gives PDCAAS, DIAAS and PR for commonly cooked Canadian pulses (Nosworthy et al., 2017). No pulse varieties in the study of Nosworthy et al. obtained a protein quality evaluation that would deem the sample an '*Excellent source of protein'*, however all beans, yellow peas and chickpeas received the claim that they are a '*Good source of protein'* as per Canadian standards using the Protein Rating system (2017). Only Navy beans could be deemed a '*Good source of protein'* in the USA/International markets according to the PDCAAS evaluation scheme. None of the pulses evaluated using the DIAAS method could make a protein claim. Bai et al. (2018a) used infrared heat pre-treatment and tempering of desi chickpea and found there to be an increase in the *in vitro* protein digestibility corrected amino acid score (IV-PDCAAS). While the IV-PDCAAS is not currently accepted as a method of protein determination for label claims, it is used in research studies to estimate protein digestibly and quality without the need for loss of animal life. The *in-vitro* method not only side steps potential issues for companies who wish to avoid questioning of ethical practices and/or infractions to vegan claims or certificates, it has also been shown to be strongly correlated to *in vivo* protein determination methods (Marinangeli et al., 2017). Nosworthy et al. (2017, 2018), found a strong correlation between the PDCAAS and IV-PDCAAS for pea, faba bean and lentil protein isolates ($R^2$ = 0.9898), and for baked, extruded and cooked red and green lentil flour ($R^2$ = 0.9971). Bai et al. (2018a) found that the most significant increase in IV-PDCAAS resulted from desi chickpea samples that were tempered to 20% moisture and heated at 135°C, with values rising from 0.65 to 0.71. The researchers explain that the processing of chickpea flours using infrared heating did not change the amino acid content of the sample but worked to eliminate ANFs from chickpea flour (trypsin/chymotrypsin inhibitors, condensed tannins), which increased the *in vitro* digestibly of the proteins, translating to increased PDCAAS values. These results show that while amino acid profiles may remain unchanged with infrared heating, the bioavailability/digestibility of pulse proteins may be significantly improved with infrared heat treatments.

Table 2. 1 Common in vivo testing methods for assessing protein quality (adapted from Nosworthy et al., 2017; FDA, 2013; FAO, 2013; Government of Canada, 2018).

| Method | Calculation | Claims | Country of Adoption |
|---|---|---|---|
| **Protein digestibility corrected amino acid score (PDCAAS)** | [(mg of limiting amino acid in 1 g of test protein) / (mg of the same amino acid in 1 g of reference protein)] x True fecal digestibility (Df %)<br><br>Df %= (N1-N2)/N1 x 100; where N1: nitrogen intake (g protein/6.25) and N2: fecal nitrogen of a diet containing the protein minus the fecal nitrogen of diet containing no protein | Quality protein: >0.2 (non-infant foods); >0.4 (infant foods) | USA<br><br>Current international standard for assessing protein quality recommended by the WHO/FAO |
| **Digestible indispensable amino acid score (DIAAS)** | [(mg of digestible indispensable amino acid in 1 g of dietary protein) / (mg of the same indispensable amino acid in 1 g of reference protein)] x 100 | No nutrition claim to be allowed for protein sources scoring less than 0.75 | Being recommended by the WHO/FAO to replace PDCAAS. |
| **Protein efficiency ratio (PER) and Protein rating (PR)** | PER: weight gain / protein intake over 28 days (*in vivo* laboratory rat studies); adjusted by dividing PER of casein protein (complete protein) of 2.5 by PER of sample protein<br><br>PR: adjusted PER x Protein in the reasonable daily intake (RDI)<br><br>RDI: Varies for proteins sources. | Source of Protein: >20 PR<br><br>Excellent source of protein: >40 PR | Canada<br><br>Canada |

Table 2. 2 Protein quality assessments by PDCAAS, DIAAS and Protein Rating for a range of cooked Canadian pulses (Nosworthy et al., 2017).

| **Pulse Type** | **PDCAAS** | **DIAAS** | **Protein Rating** |
|---|---|---|---|
| Red kidney bean | 0.549 | 0.51 | 23.93* |
| Navy bean | 0.667* | 0.65 | 25.43* |
| Whole green lentil | 0.628 | 0.58 | 18.29 |
| Split red lentils | 0.538 | 0.50 | 18.31 |
| Split yellow peas | 0.643 | 0.73 | 20.01* |
| Split green peas | 0.500 | 0.46 | 13.17 |
| Black beans | 0.534 | 0.49 | 24.55* |
| Chickpeas | 0.519 | 0.67 | 30.44* |
| Pinto beans | 0.590 | 0.60 | 23.27* |
| Casein | 1.00 | 1.31 | n/a |

*Can make a 'Good source of protein' claim

# 3. MATERIALS AND METHODS

## 3.1 Materials

Green lentil (CDC Greenstar; 72.20 $\pm$ 0.7 g per 1000 seeds) and yellow pea (CDC Spectrum; 241.63 $\pm$ 0.9 g per 1000 seeds) were harvested in the 2018 crop year and were obtained from Reisner Farm Ltd. (Limerick, SK). All chemicals used in this study were of reagent grade and purchased from Sigma-Aldrich (Oakville, ON, Canada), unless specified otherwise. All water used throughout both research studies was taken from a Millipore Milli-Q$^{TM}$ water purification system (Millipore Corp., Millford, MA, USA), unless otherwise stated.

## 3.2 Preparation of flour materials

*Tempering*

Yellow pea and green lentil seeds were divided into three groups: untempered (control), tempered to 20% moisture, and tempered to 30% moisture. The AACC approved method of analysis 26-95.01 (AACC, 1999) for tempering was followed:

$$W(H_2O) = \frac{W_S[M_E - M_O]}{100 - M_O} \qquad \text{[Eq. 1]}$$

where $W_s$ refers to the weight of samples prior to tempering, $M_O$ refers to the original moisture content (%) of the seeds and $M_E$ refers to the end moisture content (%) of the seeds. Tempering of seeds was performed at room temperature and atmospheric pressure. To temper, ~8 kg of each seed type was placed in separately sealed polyethylene bags containing Millipore Milli-Q water in amounts determined through the solving of eq. 1. Preliminary moisture uptake experiments were performed for each seed type to understand the time needed for the moisture content of each seed to reach moisture equilibrium. The tempering process took ~1 hr, as predicted by Bai (2018b).

*Infrared Heating*

Heating of seeds was performed at InfraReady Products (1998) Ltd. (Saskatoon, SK, Canada) using a laboratory scale microionizer (Model A 156379- B0, FMC Syntron ® Bulk Handling Equipment, Homer City, PA, USA). The heating mechanism (microionizer) consists of a heat generating burner (Model type R 1603-2 pat, Rinnai, Japan), a Syntron feeder (Model F010, Riley Automatic Ltd., Derby, England) to control speed and volume of passing seeds, and a Syntron magnetic feeder (Mode BF2 A, FMC Corporation, Homer City, PA, USA) to convey seeds along the heating passageway containing the microionizing burners. Burners were situated 19 cm above the 152 cm long feeder and conveyor. Temperatures of seed surface were recorded using a hand-held IR thermometer (Oakton, Vernon Hills, IL, USA). For each pulse seed type (yellow pea and green lentil), ~2 kg of seeds were heated independently and under the same conditions in triplicate, to 120°C and 140°C. The control for this study was the unheated, untempered seeds for both yellow pea and green lentil seeds.

*Milling*

The control seeds and each tempered/heat-treated seed type were initially ground using a standard kitchen blender to reduce particle size by splitting the seeds in roughly quarter sized pieces. From here, seeds were milled using a laboratory hammer type cyclone mill with a 0.8 mm sieve (Perten Laboratory Mill 3100, Perten Instruments, AB, Hagërsten, Sweden). All milled flours were then kept in cold storage (4°C), contained inside of polyethylene Ziploc bags.

**3.3 Physicochemical properties**

All data was collected in duplicate on the triplicate processing runs. Data is presented as the mean ± one standard deviation (n=3).

*(a) Proximate analysis*

Standardized methods established by the Association of Analytical Chemists (AOAC) were followed for moisture (925.10), ash (923.03) and crude fat (920.85) (AOAC, 2012). Protein/nitrogen content was determined using a LECO combustion unit (CN628, LECO Corp., St. Joseph, MI, U.S.A), for nitrogen/protein content in accordance with the AACC approved method of analysis 46-30.01 (AACC, 2000). The nitrogen content was converted to protein by

using a factor of 6.25 (%N x 6.25). Total starch and damaged/gelatinized starch content of both yellow pea and green lentil flours was determined by AACC approved method of analysis 76-13.01 using the Megazyme Total Starch Kit, and the Megazyme Starch Damage Assay Kit following AACC approved method of analysis 76-31.0, respectively (AACC, 2000). All proximate data collected for the flours has been reported on a dry basis (d.b.).

*(b) Colour*

The colour of the control and treated flours was measured using the Hunterlab MiniScan XE colourimeter (Hunter Associates Laboratory, Inc., Reston, VA). Coordinates measured included L*, a* and b*, where L* represents the lightness (0 = black, 100 = white); a* indicates the degree of red-green (-a = greenness, +a = redness); and b* represents the degree of yellowness (-b = blueness, +b = yellowness). The colourimeter was first standardized using a white tile Ls = 92.81, as = -1.25, bs = 1.04 prior to analysis of the flours.

*(c) Surface Charge (zeta potential)*

The method of Chang et al. (2015) was used to determine the surface charge of the flours. In brief, 25 mg of the flour was weighed and dispersed in water to make a total weight of 50 g (0.05% w/w). The initial solution was adjusted to pH 7.0 with 0.1 N of HCl or NaOH, and then stirred overnight in cold storage (4 ºC). In the morning the pH of the 0.05% (w/w) solutions were brought to room temperature and then adjusted to pH 7.0 before determination. The electrophoretic mobility (UE) of the flours was measured using a Zetasizer Nano- EZ90 (Malvern Instruments, Westborough, MA, USA) at room temperature (21-23ºC). The surface charge (ξ) was calculated through Henry's equation:

$$U_E = \frac{2\varepsilon \times \xi \times f(\kappa\alpha)}{3\eta} \qquad \text{[Eq. 2]}$$

where ε (Farad/m) refers to the permittivity, $f(\kappa\alpha)$ is Smoluchowski approximation (set to 1.5 in this study relating to the Debye length (κ) and the ratio of particle radius (α)) and η (mPa·s) is the dispersion viscosity.

*(d) Surface hydrophobicity*

The surface hydrophobicity of the pulse flours was determined by 1-anilino-8-naphthalene sulfonate (ANS) binding method described by Kato and Nakai (1980). Initially, 0.025% w/w (based on protein weight) of each flour sample was dissolved in 10 mM sodium phosphate buffer (pH 7.0) and stirred overnight (~12 h) in cold storage (4°C). The next day, the sample was taken out of cold storage and left to sit for ~1-2 h (to equilibrate to room temperature (21-23ºC)). Once at room temperature, a range of solution concentrations were created for each sample (0.005%, 0.010%, 0.015% and 0.025%) by diluting with 10 mM sodium phosphate buffer (pH 7.0). From here, 20 µL of 8 mM 8-Anilino-1-naphthalenesulfonic acid (ANS) solution (dissolved in 10 mM sodium phosphate buffer (pH 7.0)) was added to 1.6 mL of each flour solution concentration and vortexed for 10 s and stored in the dark for 5 min. The fluorescence intensity (FI) was measured using a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA) with excitation and emission wavelengths of 390 and 470 nm, respectively. ANS blank and protein blanks were subtracted from the FI of the protein solutions containing ANS. Linear regression was used to measure the initial slope (S0) of FI against protein concentration and was used as an index of the protein surface hydrophobicity.

*(e) Fourier transform mid-infrared (FT-MIR) spectroscopy*

All yellow pea and green lentil flours were prepared for analysis by FT-MIR spectoscopy at the Canadian Light Source, Saskatoon, Canada. To begin, pellets containing 1.1-1.3% of each flour sample by weight and potassium bromate (KBr) were formed. The pellet forming process began by taking 5 mg (± 0.03 mg) of each flour sample and homogenizing with 400 mg (±0.07-1.14 mg) KBr by use of a cryogenic Geno/Grinder 2010 grinder (SpexSample Prep, 65 Liberty Street, Metuchen, NJ 08840). Next, 98 mg (±0.06-1.0 mg) of the flour/KBr mixture was pressed into 13 mm pellets by use of an automated hydraulic press (AutoCrushIR PIKE Technologies Inc., Madison, WI, USA). Once pellets were formed, the FT-MIR microscope equipped with a bulk analysis accessory and thermoelectrically cooled Deuterated Lanthanum α-Alanine-doped Tri-Glycine Sulphate (DLaTGS) detector at the mid-IR beamline (Cary 670 series, Agilent Technologies Inc., CA, USA) was prepared for analysis by purging the sample chamber with dry nitrogen to minimize any water vapor or carbon dioxide interference with the sample reading.

Roughly 32 scans of MIR data in the spectral range of 4000-600 $cm^{-1}$ wavenumber with a spectral resolution of 2 $cm^{-1}$ was reported for each flour sample. Background spectrum of the instrument was managed by analyzing a pure KBr pellet every 18 samples. All results were analyzed by Quasar software (version 0.5.6) (10. 5281/zenodo.4287478). The second derivate spectra were calculated using a Savitzky–Golay algorithm with 9-point smoothing to determine peak position for peak fitting. The amide I region (1600–1700 $cm^{-1}$) was deconvoluted through peak fitting utilizing the LMFIT python package (version 1.0.1).

*(f) Particle size distribution*

The distribution of particle sizes of the green lentil and yellow pea flours was determined using a Malvern 2000 Mastersizer (Malvern Panalytical, Saint- Laurent, QC, Canada). Briefly, ~2 g of flour was suspended in 40.0 mL of water to create a 5% (w/w) flour suspension. The suspension was then stirred using a magnetic stir bar (400 rpm) for 5 min prior to analysis. Samples were then added dropwise to the dispersion cell, using distilled water as a dispersant. Sample data was recorded after an optimum laser obscuration of 10-20% was reached. Data is reported as the volume weighted mean particle size (D [4,3]) of the flours.

### 3.4 Functional properties

*(a) Protein solubility*

Protein solubility was measured at described by Wu et al. (1998). In short, 20 mg of protein solutions were dispersed in 20 mL Milli-Q water and stirred at 400 rpm for ~30 min. Next, pH was adjusted to 5.0 or 7.0 (depending on which pH solubility level was being determined) using 0.1 N HCl/NaOH. Samples were then centrifuged at 12,100 × *g* for 10 min. A standard curve was created using the BIO-RAD Quick start Bradford protein assay kit (Catalog number—500-0201), using BSA as a standard. Protein solubility was calculated as:

$$Solubility\ (\%) = \frac{(protein\ content\ in\ supernatant)}{(total\ protein\ content\ in\ sample)} \times 100 \qquad \text{[Eq. 3]}$$

*(b) Water and oil holding capacity*

Water holding capacity (WHC) and oil holding capacity (OHC) were both determined using the modified AACC approved method of analysis 56-30.01 (AACC, 2000) by Nidhina & Muthukumar (2015). To begin, 0.5 g of the flours sample and 5 mL/g water or oil was measured into a 10 mL centrifuge tube. The tubes were then vortexed for 10 s every 5 min for a total of 30 min to ensure that the sample was thoroughly wetted. After 30 min, the tubes were centrifuged at 1000 x *g* for 15 min. Lastly, the supernatant was decanted of any remaining water (or oil) and the resulting tube weight was recorded. The WHC and OHC was then calculated using the following equation:

$$WHC/OHC\ (g/g) = \frac{Wet\ Sample\ Wt - Dry\ Sample\ Wt}{Dry\ Sample\ Wt} \quad \text{[Eq. 4]}$$

*(c) Oil emulsion capacity (OEC)*

The OEC of the yellow pea and green lentil flours were determined according to the method described by Wang and Maximiuk (2015), where OEC is determined at the point at which an oil-in-water emulsion turns into a water-in-oil emulsion, as indicated by a sudden drop in electrical conductivity. To begin, a 0.40% (w/v) flour suspension was prepared in 75 mL deionized water. The flour suspension was then transferred to a 500 mL glass beaker and homogenized with a PowerMax AHS 250 10 x 105 mm saw tooth generator probe at speed 1 for 30 s (VWR, Thorofare, NJ). Next, the beaker holding the homogenized flour solution was attached to a BF-30 homogenizer (Montreal Biotech Inc, Dorval, QC). At a rate of 1.0 mL/s, whilst the mixture was being homogenized and blended at ~6000 rpm, 25 mL of commercial canola oil was added to the flour suspension via a Masterflex pump (Cole-Parmer, Vernon Hills, IL) over a time period of 30 s. A digital multimeter was used to measure the sudden increase in electrical resistance, which indicated the phase inversion of the emulsion (or emulsion break point). At the break point, oil addition was stopped, and the total amount of oil emulsified was calculated. OEC was expressed as milliliters of oil per gram of sample.

*(d) Emulsion activity (EA) and stability (ES)*

Emulsion activity (EA) and emulsion stability (ES) were determined according to Yasumatsu et al. (1972). To begin, 4.25 g of flour was dispersed in 75 mL of deionized water, adjusted to pH 7.0, and stirred overnight at room temperature (21-23ºC). The following morning, the aqueous flour solution was re-adjusted back to pH 7.0 prior to adding in 50 mL commercial canola oil and homogenizing at speed 4 for 1 min using an Omni Macro Homogenizer (Omni International, Marietta, GA, USA). Next, the emulsion was transferred into two 50 mL centrifuge tubes and centrifuged at 1,300 x *g* for 5 min using an Allegra X-22 Series (Beckman Coulter Inc., Mississauga, ON) centrifuge. To understand the resulting emulsifying activity (EA) of the flour, the height of the emulsified layer and the entire emulsion in the tube was recorded using a caliper measuring device. EA was determined using the following equation:

$$\%EA = \frac{Height\ of\ the\ emulsified\ layer\ (cm)}{Height\ of\ the\ entire\ layer\ in\ the\ tube\ (cm)} \times 100\% \qquad \text{[Eq. 5]}$$

The ES of the flours was determined by heating the formed emulsion in an 80°C water bath for 30 min, followed by cooling to room temperature (21-23°C). The heat-treated emulsion was then divided into two tubes (~12.5 mL) and again centrifuged under the same conditions for EA. The ES of the flours was calculated as:

$$\%ES = \frac{Height\ of\ emulsified\ layer\ (cm)\ after\ heating}{Height\ of\ the\ entire\ layer\ in\ the\ tube\ (cm)} \times 100\% \qquad \text{[Eq. 6]}$$

*(e) Foaming capacity and stability*

Foam capacity (FC) and stability (FS) of each pulse flour sample was determined in accordance with the method proposed by Wilde and Clark (1996). To begin, 1% (w/w) of pulse flour solutions was prepared and adjusted to pH 7.0 using 1 N NaOH. The solutions were stirred overnight (~16 h) at room temperature (21-23°C). Following the overnight stirring, adjustment back pH 7.0 occurred prior to analysis. Fifteen mL of this solution was then transferred into a 400 mL beaker and homogenized using an IKA homogenizer (IKA Works, Inc., Wilmington, NC, USA) equipped with a saw tooth probe situated slightly below the air-water interface. Homogenization was performed at speed 2 for 3 min for each flour sample. After 3 min, the sample

was quickly transferred into a 50-mL graduated cylinder and the volume of the foam was recorded as $V_1$. After waiting 30 min, the volume of the foam was measured again and recorded as $V_2$. FC and FS was calculated based on the following equations:

$$\%FC = \frac{V_1}{Inital\ Volume\ (15\ mL)} \times 100\% \quad \text{[Eq. 7]}$$

$$\%FS = \frac{V_2}{V_1} \times\ 100\% \quad \text{[Eq. 8]}$$

*(f) Pasting properties*

The pasting properties of the yellow pea and green lentil flours were determined according to the method described by Ai et al. (2017). Flour suspensions with 8% dry solids were created by suspending the flour samples in distilled water (total volume 28.0 g). The flour suspensions were then measured by a Rapid Visco-Analyzer (RVA Super 3, Newport Scientific, Sydney, Australia) and then analyzed by Standard Method 2 in the Thermocline Software.

### 3.5 Nutritional properties

#### 3.5.1 Protein quality

*(a) Amino acid composition*

Amino acid composition was analyzed at the University of Manitoba, under supervision of Dr. Jim House. In brief, the amino acid composition of pulse flour samples was performed using the Pico-tagTM amino acid analysis system (Waters Corporation, Milford, MA, USA) and a high-performance liquid chromatography system (HPLC). In total, 18 amino acids were determined in the assay. For the determination of 15 amino acids, the method reported by Bidlingmeyer et al. (1987) was followed. To summarize, each pulse flour sample was prepared and mixed with 15 mL 6 N hydrochloride acid in Pyrex tubes, followed by flushing with $N_2$. The tubes were then capped and kept at 110°C for 20 h to hydrolyze the proteins into amino acids for separation in the HPLC. The determination of tryptophan followed the AOAC method 988.15 (2005). The samples were first hydrolyzed by 10 M NaOH and kept in a boiling water bath for 20 min. They were then put in the oven at 110°C for 16 h followed by HPLC determination. Tryptophan was determined by reverse phase liquid chromatography with UV detection. The concentration of sulphur-containing

amino acids, methionine and cysteine, were determined following AOAC method 985.28 (2005), using ion- exchange chromatography with modification. The 1-octanol was not included in the procedure. Cold performic acid was used for cysteine and methionine oxidation and were kept for reaction at 4°C overnight. The sulphur amino acids were oxidized with performic acid and hydrolyzed with 6 M HCl at 110°C for 18 h. The amino acid profile was then run once on the composite material for each treatment.

*(b) Determination of the amino acid score*

The amino acid score of the pulse flours in this study represents the most limiting essential amino acid in the flour. Amino acid score was determined according to FAO/WHO recommendations in Table 3.1:

Table 3.1 FAO/WHO recommendations for amino acid composition of the reference protein for the amino acid requirement for children 2 to 5 years of age (1991).

| Essential Amino Acid | Amino Acid Requirement (mg/g protein) |
|---|---|
| Histidine | 19 |
| Isoleucine | 28 |
| Leucine | 66 |
| Lysine | 58 |
| Methionine + Cysteine | 25 |
| Phenylalanine + Tyrosine | 63 |
| Threonine | 34 |
| Tryptophan | 11 |
| Valine | 35 |

*(c) In-vitro protein digestibility*

*In-vitro* protein digestibility of each pulse flour sample was determined by the pH drop of the multi-enzyme digested solution in accordance with the method described by Tinus et al. (2012). The enzyme solution was 31 mg chymotrypsin, 16 mg trypsin and 13 mg protease in 10 mL of water, and was kept at 37°C. The pH was adjusted to 8.0 ± 0.05 with 0.1 M NaOH and HCl. This

solution was prepared fresh daily. To follow, ~0.2 g of each pulse flour was mixed with 8 mL of water. Next, the mixture was stirred for 1 h at 37°C. The pH of the solution was adjusted to 8.0 ± 0.05 with 0.1 M NaOH and HCl before adding 1 mL of the fresh multi- enzyme solution. The pH of the protein solution was recorded every 30 s for 10 min and the in vitro protein digestibility (IVPD) was calculated in accordance with Eq. 9:

$$IVPD\ (\%) = 65.66 + 18.10 \times \Delta pH_{10min} \quad \text{[Eq. 9]}$$

Where, $\times\ \Delta pH_{10min}$ is the change in pH from intial level (pH 8.0) to the level obtained after 10 min.

*(d)* In-vitro Protein Digestibility Corrected Amino Acid Score (IV-PDCAAS)

The IV-PDCAAS was calculated as the product of the amino acid score and *in vitro* protein digestibility.

$$IV - PDCASS = Amino\ Acid\ Score * IVPD\ (\%). \quad \text{[Eq. 10]}$$

### 3.5.2 Starch digestibility

*In-vitro starch digestibility*

*In vitro* starch digestibility in infrared treated pulse flours was determined according to the method described by Englyst et al. (1992) and was performed at the Canadian Grain Commission Laboratories (Winnipeg, MB). The analysis was done under controlled enzymatic hydrolysis followed by colorimetric measurement of the glucose released. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured following incubation with porcine pancreatic alpha-amylase and amyloglucosidase at 37°C in a water bath. RDS is the glucose released after 20 min and SDS is the glucose released after a further 100 min incubation. RS was measured indirectly by calculating the starch that is not hydrolysed after 120 min incubation.

### 3.6 Statistics

The above experiments were repeated in duplicate on triplicate processing runs and reported as the mean ± one standard deviation. Statistical differences ($p<0.05$) of all treatments

were analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey's Post-hoc test.

# 4. RESULTS AND DISCUSSION

## 4.1 The effect of tempering moisture and seed surface temperature on the physicochemical and functional properties of yellow pea and green lentil flour

### 4.1.1 Compositional and colour properties of raw and treated flours

The compositional data gathered for both yellow pea and green lentil flour samples is presented in Table 4.1.1, with all values being reported on a dry basis. Although, some statistical differences were seen among the treatments, differences were not substantial. The protein content of control and heated/tempered yellow pea and green lentil flours ranged between 20.29-21.9% and 22.0-23.1% respectively, which is comparable to reported data for protein levels in peas and lentils (Hall et al., 2017; Millar et al., 2019; Rathod & Annapure, 2016; Stone et al., 2021; Tharanathan & Mahadevamma, 2003). Total starch levels ranged between 46.8-49.5% and 46.8 – 50.3% for yellow pea and green lentil flours, respectively. Overall starch levels were comparable with total starch values reported in the other studies (Stone et al., 2021). Ash contents ranged between 2.4-2.7% for yellow pea flours and between 2.4-2.6% for green lentil flours, whereas

Table 4.1. 1 Compositional analyses and colour properties of yellow pea and green lentil flours.[1]

| **Process Conditions** | **Moisture (%)** | **Protein (%) (d.b.)** | **Total Starch (%) (d.b.)** | **Ash (%) (d.b.)** | **Lipid (%) (d.b.)** | **Damaged/ gelatinized starch (%)** | ***L**** | ***a**** | ***b**** |
|---|---|---|---|---|---|---|---|---|---|
| ***Green Lentil*** | | | | | | | | | |
| Control | 7.18± 0.08$^{ab}$ | 22.71± 0.17$^{bc}$ | 48.07± 0.60$^{ab}$ | 2.36± 0.07$^{a}$ | 0.81± 0.04$^{a}$ | 1.15± 0.08$^{a}$ | 84.34± 0.01$^{b}$ | 0.41± 0.01$^{bc}$ | 17.69± 0.11$^{bc}$ |
| 120°C, 20% | 9.40± 0.42$^{bc}$ | 22.36± 0.02$^{ab}$ | 47.76± 0.20$^{ab}$ | 2.41± 0.11$^{a}$ | 1.18± 0.03$^{b}$ | 3.41± 0.53$^{b}$ | 84.59± 0.01$^{b}$ | -0.001± 0.01$^{a}$ | 17.49± 0.01$^{a}$ |
| 140°C, 20% | 6.81± 0.18$^{ab}$ | 22.93± 0.03$^{bc}$ | 46.79± 0.77$^{a}$ | 2.49± 0.18$^{a}$ | 1.39± 0.04$^{c}$ | 6.95± 0.21$^{c}$ | 84.18± 0.01$^{b}$ | 0.01± 0.02$^{ab}$ | 15.90± 0.01$^{ab}$ |
| 120°C, 30% | 11.47± 0.09$^{c}$ | 21.98± 0.03$^{a}$ | 50.31± 1.95$^{b}$ | 2.50± 0.03$^{a}$ | 1.30± 0.16$^{bc}$ | 9.61± 0.39$^{d}$ | 82.45± 0.24$^{a}$ | -0.11± 0.14$^{a}$ | 19.31± 1.08$^{a}$ |
| 140°C, 30% | 6.24± 0.17$^{a}$ | 23.1± 0.08$^{c}$ | 47.51± 0.49$^{a}$ | 2.60± 0.01$^{a}$ | 1.31± 0.02$^{bc}$ | 14.46± 0.54$^{e}$ | 82.38± 0.02$^{a}$ | 0.46± 0.01$^{c}$ | 15.72± 0.03$^{c}$ |
| ***Yellow Pea*** | | | | | | | | | |
| Control | 8.55± 0.23$^{A}$ | 21.37± 0.20$^{BC}$ | 47.43± 0.19$^{AB}$ | 2.66± 0.09$^{AB}$ | 0.99± 0.05$^{A}$ | 1.01± 0.03$^{A}$ | 86.47± 0.25$^{C}$ | 1.99± 0.01$^{C}$ | 19.78± 0.12$^{A}$ |
| 120°C, 20% | 9.54± 0.07$^{A}$ | 21.09± 0.04$^{B}$ | 47.00± 0.47$^{AB}$ | 2.40± 0.31$^{A}$ | 1.15± 0.19$^{AB}$ | 2.29± 0.03$^{B}$ | 85.83± 0.02$^{C}$ | 1.77± 0.02$^{C}$ | 20.97± 0.03$^{AB}$ |
| 140°C, 20% | 8.77± 0.08$^{A}$ | 21.92± 0.25$^{C}$ | 46.76± 0.53$^{A}$ | 2.70± 0.04$^{B}$ | 1.54± 0.04$^{D}$ | 8.47± 0.29$^{D}$ | 84.25± 0.57$^{B}$ | 2.04± 0.02$^{B}$ | 22.02± 0.31$^{BC}$ |
| 120°C, 30% | 15.03± 0.22$^{B}$ | 20.29± 0.05$^{A}$ | 49.47± 1.04$^{C}$ | 2.49± 0.04$^{AB}$ | 1.27± 0.08$^{BC}$ | 6.55± 0.35$^{C}$ | 83.79± 0.01$^{B}$ | 1.51± 0.01$^{B}$ | 28.15± 0.10$^{D}$ |
| 140°C, 30% | 9.79± 0.08$^{A}$ | 21.35± 0.20$^{BC}$ | 48.38± 0.39$^{BC}$ | 2.67± 0.03$^{B}$ | 1.41± 0.02$^{CD}$ | 14.87± 0.67$^{E}$ | 81.52± 0.002$^{A}$ | 2.39± 0.01$^{A}$ | 23.26± 0.03$^{C}$ |

[1]Means within a column followed by the same letter (lowercase or uppercase) are not significantly different ($p<0.05$).

crude lipids ranged between 1-1.5% and 0.8-1.4%, respectively. Values were comparable to those found in literature (Stone et al., 2021; Tharanathan & Mahadevamma, 2003).

Damaged/gelatinized starch levels in yellow pea and green lentil flours were significantly impacted by the level of infrared heat and tempering moisture applied as a pre-treatment. In the case of both pulse types, the damaged/gelatinized starch levels steadily and significantly increased from 1.0 to 14.9% and 1.1 to 14.2% for yellow pea and green lentil, respectively, when comparing the control group flours and flours heated to 140°C and tempered to 30% moisture. During the milling process, the starch fractions of seeds and kernels can become damaged, resulting in increased levels of damaged starch within the resulting flour product. Increased levels of damage starch have been directly correlated to increased water absorption, increased fermenting yeast activity (gassing power), and dough handling properties such as stickiness and deformation resistance (Price et al., 2021). Other researchers who have examined the use of tempering moisture and infrared heat to increase seed surface temperature have reported similar data trends. Liu et al., (2020) tempered lentil seeds of varied size (large and small green, small red) to 25% moisture under different factors of tempering time to reach the same moisture level (raw, 24 h, 48 h and 96 h), and found comparable data to the results of the current research study. The seeds were then infrared heated to a surface temperature of 130°C or 150°C. The researchers found that seed size, tempering time, and seed surface temperature all significantly impacted the damaged/gelatinized starch content of the lentils. Large lentil seed flours (CDC Greenstar (identical to the seeds examined in this study)) had increased in damaged/gelatinized starch content from 1.2% of total starch content in the control group flours to 10.4-23.0% as tempering time increased from 24 h to 96 h in seeds heated to 130°C prior to milling. A similar trend was seen in seeds heated to 150°C, but the increase in damaged/gelatinized starch was less (7.3% to 10.4% of total starch for flours tempered between 24 h – 96 h). The researchers hypothesized that the larger seed size of the CDC Greenstar lentils (68.4 ± 0.6 g per 1000 seeds in study of Liu et al., 2020) impacted the content of damaged/gelatinized starch, as the smaller seeds they examined (CDC Invincible; 33.9 ± 0.4 g per

1000 seeds & CDC Maxim; 32 ± 0.3 g per 1000 seeds) had damaged/gelatinized starch contents between roughly 13-15% higher than the larger seeds. The smaller seeds were hypothesized to have a greater content of damaged/gelatinized starch as they had a larger surface area for more rapid moisture migration when tempering, which likely facilitated faster and more efficient heat transfer when exposed to infrared radiation, which in turn increased the degree of starch gelatinization. Damaged/gelatinized starch content increases to a lesser extent in other forms of pre-milling treatments with heat. For example, Revtech roasted yellow peas to 120°C, 140°C and 160°C (injected with 10% steam during roasting) resulted in flour with initial damaged/gelatinized starch content, as a percent of total starch, that was comparable to results found in the current study for control yellow pea flours (1.46%), and actually decreased significantly by ~0.2-0.6% in processed flours (Young et al., 2020). This allows for the conclusion that tempering moisture plays am important role in the content of damaged/gelatinized starch in a flour that is produced from seeds that are pre-treated with heat and/or moisture prior to milling, as the increased moisture content facilitates a higher degree of heat transfer, and therefore a higher degree of starch gelatinization and damaged starch in the flours. This is important to note, as the increase in damaged/gelatinized starch has a significant impact on the functional properties of a flour in a food system, which may be desirable or undesirable depending on the application. This will be discussed further in Section 4.1.5.

End product (e.g., breads, pasta, crackers) consumer acceptability is affected by colour changes within the pulses flours as the pre-treatment conditions vary. Colour parameters were significantly impacted by both heat and tempering moisture applied to the seeds prior to milling. L* values, or the measure of brightness of a flour, steadily decreased as pre-treatment with heat and moisture increased. Control flours for both yellow pea and green lentil samples had the 'lightest' L* values (L*=86.47 and L*=84.34 respectively), and seeds infrared heated to 140°C and tempered to 30%, had the 'darkest' L* values at L*=81.52 and L*=82.38, respectively. The a* values (+redness and/or -greenness) varied between pulse types. Seeds heated to 140°C and

tempered to 30% were the reddest in saturation, and while no yellow pea samples had negative a* values (greenness), the more mildly treated green lentil flours did have various levels of greenness, which can be explained by the inherent color of green lentils. The b* values (+yellowness/-blueness) for yellow pea flours had higher overall b* values in comparison to the green lentil flours, but both pulse types did follow similar trends. For both types of flours, the yellowness score steadily increased as pre-treatment with heat and moisture increased, with the peak reading occurring for flours pre-treated with 120°C and tempering to 30% (b*= 28.15 and b*= 19.31 for yellow pea and green lentil, respectively). When the pre-treatment heat was increased to 140°C and also tempered to 30% moisture, the b* value again decreased for both flour types. Young et al. (2020) found similar results when using roasting heat as a pre-treatment for yellow pea flour. The study reported that the flour had a decreased b* value when higher heating temperatures were applied to the seeds prior to milling resulted in flours with lower b* values (Young et al., 2020). Other researchers have also concluded that using a combination of, or independently, infrared heating and tempering of seeds prior to milling alone can significantly impact the color of seeds and/or flours, which may be a desirable or undesirable effect on consumer acceptance of a food item, depending on the end application of the pulse flour (Emami et al., 2011; Frohlich et al., 2021; Ma et al., 2011; Mwangwela et al., 2007)

### 4.1.2 Surface properties

The surface charge (zeta potential) and surface hydrophobicity (SH) of the protein molecules in the pulse flour treatment groups are presented in Figure 4.1.1 and Figure 4.1.2. The surface properties of the flours are related to important functional attributes of the flours such as the solubility, oil and water holding capacities, emulsifying properties, and foaming properties. The zeta potential (mV) of the flours was measured at pH 7.0 for both yellow pea and green lentil flours (Figure 4.1.1). All flours, regardless of pre-treatment with varying degrees of moisture or

heat, were negatively charged, with no significant differences found between control and treatment groups for either pulse type. The stability of a protein system is considered to be moderate when surface charge results are greater that 30 mV (-30 mV or +30 mV) (Guldiken et al., 2021). In the case of the yellow pea and green lentil flours, surface charges ranged between -32 mV to -41 mV, meaning that most of the protein molecules were negatively charged at the protein surface, with the like charges repelling against each other in solution, maintaining a discrete, suspended solution at pH 7.0. Similar studies that also examined the use of tempering moisture and/or a form of heat as a pre-treatment for pulse seeds have comparable results. In alike tempering moisture and infrared heat conditions to the current study, researchers found the zeta potential for navy beans to range between -38.1 mV to -42.5 mV at pH 7.0 (Guldiken et al., 2022). Bai et al., (2018b) also found that when tempered and infrared heated chickpeas were analyzed, zeta potential ranged between -35 mV to -41.45 mV, with little to no significant difference between control and treatment groups. It is expected that at pH levels closer to the proteins isoelectric points (pI) (between pH 4-5 for both yellow pea and green lentil), the surface charge on the proteins would become less uniform (i.e., increase in attractive forces) causing aggregation of proteins and precipitation out of solution (Joshi et al., 2017; Wongsagonsup et al., 2005; Wu et al., 2015). The fact that the surface charge for both yellow pea and green lentil flours did not significantly differ may be an indication that the tempering moisture and level of infrared heat applied in this study is mild enough pre-treatment combination to improve certain functional and nutritional characteristics, without effecting the zeta potential of surface proteins, which would effectively alter the solubility on protein solutions, which may be desirable or undesirable in application.

The SH of the flours significantly differed between pre-treatment groups for both yellow pea and green lentil flours (Figure 4.1.2). For green lentil flours, SH steadily increased, with the control flours having the lowest SH at 5.96 arbitrary units (a.u.), SH between 14.58-15.47 a.u. for flours tempered to 20% moisture and heated to 120°C and 140°C, and SH between 37.48-39.84 a.u. for flours tempered to 30% moisture and heated to 120°C and 140°C. There were statistical

differences between tempering levels, but no differences reported within tempering groups at the differing heat levels. For yellow pea flours, the trends were not isolated to tempering levels. The control group flours again had the lowest reported SH for the pulse type at 12.61 a.u. The next highest statistically grouped pair were flours heated to 140°C and tempered to 20% and flours heated to heated to 120°C and tempered to 30%, with SH values between 22.02-25.96 a.u. Lastly, the flours with the highest reported SH values were flours heated to 120°C and tempered to 20% and flours heated to 140°C and tempered to 30% with values reported between 34.65-38.79 a.u. As mentioned previously, Guldiken et al. (2022) studied pulses that were pre-treated with tempering and infrared heat conditions that are comparable to the present study. The researchers looked at the change in SH, for navy beans and chickpeas. They found that as tempering moisture and infrared heat increased to a certain degree, the SH of the proteins in the flour also increased from 21.9 a.u to 64.6 a.u (navy beans) and 21.8 a.u. to 73.4 a.u. (chickpeas). They found that the highest degree of SH occurred in samples that were tempered to 30% moisture for both pulse types, but that navy beans were more sensitive to a decline in SH when the temperature was increased from 120°C to 140°C in comparison to chickpeas, which followed a steep increase in SH when higher levels of infrared radiation were applied. The researchers hypothesize that the navy beans may be more sensitive to the structural collapse and macromolecule aggregation when exposed to excessive moisture and increasing heat than chickpeas. What could be interesting is to examine the role of seed size differences between navy beans and chickpeas (not reported), as the overall size and thickness of a seed can affect the efficacy of infrared heating (due to differences in depth/ general surface area that infrared rays are able to penetrate). In the current study, green lentil samples were more responsive to an increase in tempering moisture and increasing infrared heat applied, while yellow peas were more sporadic in change of SH, with seeds tempered to 20% moisture and heated to 120°C and seeds tempered to 30% moisture and heated to 140°C having the highest SH level, with no significant differences between the two processing conditions. As green lentil seeds used in this study are roughly 3x smaller than yellow peas (GL= 72.20 $\pm$ 0.7 g

per 1000 seeds; YP= 241.63 ± 0.9 g per 1000 seeds), they are likely to have more even and efficient heat transfer into the seed due to their larger surface area that may catalyze a greater degree of protein denaturation within the seed. Other researchers have also studied changes in SH in pulse ingredients as a result of milling pre-treatments. Hall & Moraru (2021) examined the effect of heating powdered pulse protein concentrates in a 95°C water bath and found that SH of the pulse concentrates all significantly increased in comparison to the control group. As expected, the degree of SH for the protein concentrates was much higher than those reported in the current study (in the range of 250-300 a.u.), as the proteins were concentrated prior to cooking, and heat was applied to the powdered form, not the seeds themselves prior to concentration. Change in protein SH as a result of heat treatment has also been studied for pulse based food systems, such as lentil 'milk' (lentil protein emulsions) that have been pasteurized at 85°C for 2 min (Jeske et al., 2019). As with protein powders, the lentil protein emulsions were much more suspectable to increases in SH with applied heat, which resulted in more active proteins between phases within the emulsion, which can be beneficial or problematic in application depending on the polarity of the newly exposed surface proteins.

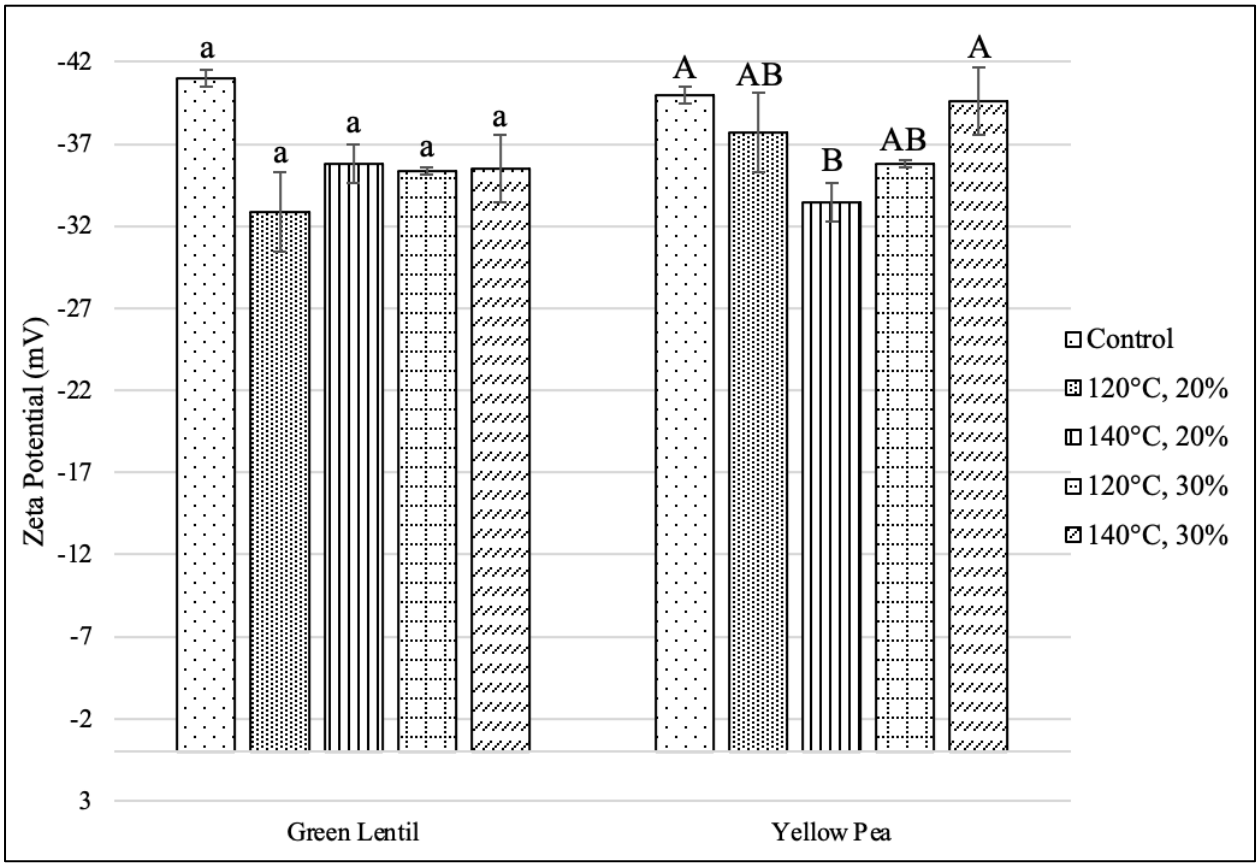


Figure 4.1. 1 Zeta potential (mV) (surface charge) of yellow pea and green lentil flours at differing pre-treatment levels of tempering moisture and applied infrared heat. Means with a column followed by the same letter (lowercase or uppercase) are not significantly different ($p<0.05$). The standard error is indicated for each treatment group.

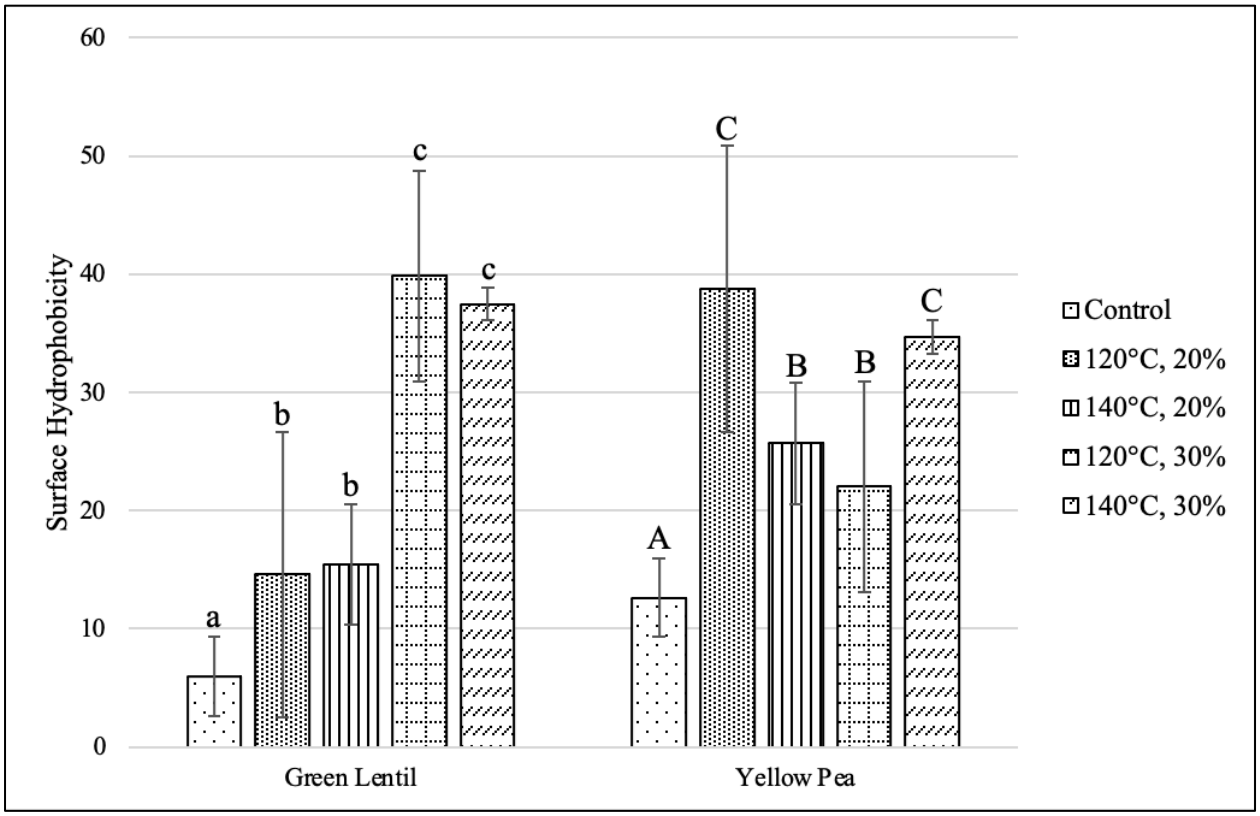


Figure 4.1. 2 Surface hydrophobicity of yellow pea and green lentil flours at differing pre-treatment levels of tempering moisture and applied infrared heat. Means with a column followed by the same letter (lowercase or uppercase) are not significantly different ($p<0.05$). The standard error is indicated for each treatment group.

### 4.1.3 Fourier transformed mid-infrared spectroscopy (FT-MIR)

Changes imparted to the secondary structure of the Gaussian spectral deconvolutions inside the amide I band region, indicating N—H bending (1600–1700 $cm^{-1}$), of the pulse flours were examined by Fourier transformed mid-infrared spectroscopy (FT-MIR). Previously in literature, it has been determined that for legumes and cereals, the Gaussian bands in the following regions

reported via FTIR relate to these corresponding secondary protein structures: amino acid side chains (A1) within 1610–1615 $cm^{-1}$, β-sheet within 1630–1638 $cm^{-1}$, α-helix within 1650–1660 $cm^{-1}$, β-turn (T) within 1660–1680 $cm^{-1}$, and amino acid side chains (A2) within 1690–1695 $cm^{-1}$ (Carbonaro et al., 2012; Guldiken et al., 2021). The relative percent of spectral weights of the amide I band of the flours can be seen in Table 4.1.2. As similarly seen in literature, the largest component of the amide I band was the proportion of β-sheet structures, with 17.3-27.8% and 16.2-26.3% being present in green lentil and yellow pea, respectively (Guldiken et al., 2021; Gunes & Karaca, 2022; Stone et al., 2021). In other types of pulse flours, including lentil and chickpeas, β-sheets contribute to at least 30% of secondary structure weight of raw flours, which is within range for both the yellow pea (26.3% β-sheets) and green lentil (27.8% β-sheets) control flour samples in this study (Carbonaro et al., 2012; Shevkani et al., 2019; Stone et al., 2021). The percentage of β-sheets detected via FT-MIR was significantly ($p<0.05$) impacted by both tempering moisture and infrared heat applied for green lentil flours, with application of moisture and heat significantly reducing the number of β-sheets detected between ~3-10% from the control group flour. The largest drop in detected β-sheet structures was in samples heated to 120°C and tempered to 20% moisture. For yellow pea flours, impact of tempering moisture followed similar trends to green lentil, with flours heated to 120°C and tempered to 20% moisture resulting in the lowest percent of β-sheet structures (16.2%), which indicated a significant reduction (-10.1%) in comparison to the control group. Alternatively, to green lentil, distribution of secondary structure was not significant among processed yellow pea flours and the control flours. The change in β-sheet structures as a result of processing with heat and/or moisture has been documented in literature, although the form of heat used has been shown to impact secondary structural changes differently. For example, conventional oven roasting of chickpeas tempered to 30% moisture at 160°C for 30 min has been shown to significantly increase the percent of β-sheet structures from 21.46% to 26.68% (Stone et al., 2021). In the same study, the use of roasting and tempering together did not impart any significant changes to β-sheet structures in green lentils, navy beans, or yellow peas.

Autoclaving has also been used to examine changes to β-sheet structures, and has been shown to eliminate the β-sheet in legume species (common bean, chickpea, lentil), and instead create a new intermolecular β-sheet aggregates within the spectral band of 1620–1630 $cm^{-1}$ (Carbonaro et al., 2012).

Anti-parallel β-sheet structures (β-A) for green lentil and yellow pea flours did not vary significantly between treatment groups, with spectral weights reported between 10.7-13.5% and 10.6-13.8% for each pulse type, respectively. The percentage of β-turns in the flours was between 11.3-15.8% and 12.2-16.5% for green lentil and yellow pea flours respectively, which are within range of reported data for legumes (Shevkani et al., 2015). For yellow pea flours, significant differences in percent β-turns was seen between control group flours (16.5%) and all treatment groups, with the exception of flours heated to 120°C and tempered to 20% moisture, although no significant differences were reported between processed flours. In green lentil flours, the prevalence of significant changes in percent β-turns was more apparent than in yellow pea flours, with all treatment groups showing a significant change from the control group flours and supporting further change between treatment groups as well. Flours tempered to 20% moisture indicated significant change, with percent β-turns dropping by 3.2% with a temperature increase from 120°C to 140°C. Flour tempered to 30% moisture had a drop in percent β-turns from control group flours, but further significant changes were less apparent. Data for similar changes imparted to the percent β-turns specifically in legume flours as a result of heat processing is limited, although it has been generally determined that the β-band is the most effected by heat treatments, as it the largest component of legume protein secondary structure (Carbonaro et al., 2008; 2012; 2015).

The α-helix, amino acid side chains (A1), amino acid side chains (A2) and random coil (RC) percent spectra weight were between 13.1- 22.3%, 9.8-15.7%, 4.9-14.3%, and 14.7-16.7%, respectively, for green lentil flours, and 13.9-23.2%, 9.7-16.1%, 4.4-15% and 15.2-16%, respectively, for yellow pea flours. Similarly to the reduction in β-sheets in processed samples in comparison to the control group flours, tempered and infrared heated flour samples had a

significantly reduced content of α-helix structures. In the control group flours, α-helix structure contents agreed with those reported in literature for raw pulses at roughly 22% and 23% for green lentil and yellow pea flours, respectively (Guldiken et al., 2021; Shevkani et al., 2015; Stone et al., 2021). In comparison to the control group, all processed flours had significantly reduced α-helix structure contents, with both yellow pea and green lentils showing a ~7-9% decrease (no significant difference between processing conditions for either pulse type). Processing with heat has altered effects on the content of α-helix structures in pulses. Certain dry heat treatments such as oven roasting and thermostatic heating within the range of 120°C to 160°C for <30min, have not been shown to significantly change the percent of α-helix structure contents in common beans, lentils, yellow peas, or chickpeas, while wet heat treatments such as autoclaving and heating in a water bath at 95°C for >30min has been shown to significantly reduce said structural contents (Carbonaro et al., 2008; Stone et al., 2021; Tang & Ma, 2009). While there were some significant differences reported between A1 and A2 content for both green lentil and yellow pea flours, the literature is not conclusive as to what A1 and A2 values are indicative of, as aggregate proteins are not common in raw pulses and legumes. This can lead to the assumption that A1 and A2 contents are a result of amino acid side chain structures being absorbed in raw flours, and are indications of proteins aggregation as a result of denaturation in the processed flours, or a combination of the two (Carbonaro et al., 2012; Guldiken et al., 2021; Stone et al., 2021). For both green lentil and yellow pea flours, random coil structures were not detected for the control group flours and minimal, if at all, significant change between RC content was determined between treatment groups for both pulse flour types.

In conclusion, the movement from a more ordered structure to a less ordered structure, i.e., a reduction in β-sheets and α-helix structures, and the introduction of RC and increase in aggregate structures (A1, A2), has been shown to be induced by processing pulses with heat and moisture, as the secondary structures become compromised and are subject to unfolding and aggregation (Carbonaro et al., 2008; 2012; Shevkani et al., 2019). It is also possible to impart changes to the

secondary structure of pulse proteins when other processing methods are used, such as when a shift in pH is facilitated, and when pulses are soaked in salt solutions (Law et al., 2008; Shevkani et al., 2019; Tang & Ma, 2009). Some benefits of a more disordered secondary structure in processed pulse flours is the increased digestibility of the flour, and surface proteins may become more active for certain functional properties (Carbonaro et al., 2012; Shevkani et al., 2019, 2015; Tang & Ma, 2009). Not examined in this study, but likely very important to consider, would be the effect that the decomposed starch fraction may have on changes to the relative spectral weights of the secondary structure components of green lentil and yellow peas flours in the amide I band. For example, Guerrero et al., (2012) found that when soy protein and various sugars (e.g., sucrose or lactose) were extruded, that both the amide I and amide II (spectral band indicating C==O stretching, typical of sugars) were significantly altered as a result of processing. It would be interesting to understand further effects/interactions between the altered protein and starch structures and the resulting effect on functional or nutritional properties in a future study.

Table 4.1. 2 Relative spectral weights of the secondary structure components of green lentil and yellow peas flours in the amide I band.[1]

| Processing Conditions | A1 | β-sheet | RC | α helix | Turns | β-A | A2 |
|---|---|---|---|---|---|---|---|
| *Green Lentil* | | | | | | | |
| Control | $15.7 \pm 0.3^{A}$ | $27.8 \pm 1^{A}$ | - | $22.3 \pm 1.3^{A}$ | $15.8 \pm 0.3^{A}$ | $13.5 \pm 2^{A}$ | $4.9 \pm 0.9^{C}$ |
| 20%, 120°C | $9.8 \pm 0.3^{C}$ | $17.3 \pm 1.3^{D}$ | $16.7 \pm 0.5^{A}$ | $15.7 \pm 0.1^{B}$ | $13.5 \pm 0.3^{B}$ | $12.6 \pm 0.4^{AB}$ | $14.3 \pm 0.3^{A}$ |
| 20%, 140°C | $10.7 \pm 0.2^{B}$ | $26.2 \pm 0.8^{AB}$ | $14.7 \pm 0.0^{B}$ | $13.1 \pm 0.2^{C}$ | $11.3 \pm 0.2^{D}$ | $10.7 \pm 0.3^{B}$ | $13.3 \pm 0.2^{AB}$ |
| 30%, 120°C | $10.5 \pm 0.1^{B}$ | $23.0 \pm 1.0^{C}$ | $15.2 \pm 0.2^{B}$ | $14.3 \pm 0.3^{BC}$ | $12.2 \pm 0.1^{C}$ | $11.3 \pm 0.2^{AB}$ | $13.4 \pm 0.2^{AB}$ |
| 30%, 140°C | $10.8 \pm 0.1^{B}$ | $24.8 \pm 0.5^{BC}$ | $14.9 \pm 0.1^{B}$ | $13.6 \pm 0.2^{C}$ | $11.9 \pm 0.2^{CD}$ | $11.0 \pm 0.1^{B}$ | $13.0 \pm 0.0^{B}$ |
| *Yellow Pea* | | | | | | | |
| Control | $16.1 \pm 0.1^{a}$ | $26.3 \pm 0.1^{a}$ | - | $23.2 \pm 0.2^{a}$ | $16.5 \pm 0.0^{a}$ | $13.4 \pm 0.2^{ab}$ | $4.4 \pm 0.0^{c}$ |
| 20%, 120°C | $9.7 \pm 1.1^{b}$ | $16.2 \pm 4.0^{b}$ | $15.7 \pm 0.5^{a}$ | $15.1 \pm 0.6^{b}$ | $14.5 \pm 1.7^{ab}$ | $13.8 \pm 1.6^{a}$ | $15.0 \pm 0.8^{a}$ |
| 20%, 140°C | $10.8 \pm 1.0^{b}$ | $24.3 \pm 0.8^{a}$ | $15.2 \pm 1.1^{a}$ | $13.9 \pm 1.3^{b}$ | $12.4 \pm 1.5^{b}$ | $12.1 \pm 1.7^{ab}$ | $13.6 \pm 0.1^{b}$ |
| 30%, 120°C | $11.3 \pm 0.5^{b}$ | $23.1 \pm 1.4^{a}$ | $15.9 \pm 1.6^{a}$ | $14.9 \pm 1.9^{b}$ | $12.8 \pm 1.7^{b}$ | $11.2 \pm 0.2^{ab}$ | $14.3 \pm 0.1^{ab}$ |
| 30%140°C | $11.4 \pm 0.5^{b}$ | $25.3 \pm 1.5^{a}$ | $16.0 \pm 2.2^{a}$ | $14.1 \pm 1.5^{b}$ | $12.2 \pm 1.1^{b}$ | $10.6 \pm 0.4^{b}$ | $13.6 \pm 0.1^{b}$ |

[1]Means within a column followed by the same letter (lowercase or uppercase) are not significantly different ($p<0.05$).

### 4.1.4 Particle size

The volume weighted mean particle size (D [4, 3]) of the green lentil and yellow pea flours can be seen in Table 4.1.2. Green lentil flours reported particle sizes ranging between ~ 101- 138 μm (±11-18 μm), with little significant difference in values between treatment groups. Yellow pea flour particle sizes tended to skew larger overall, with values ranging between ~107- 225 μm (±11-23 μm). The overall larger particle size of the yellow pea flours may be a result of the larger inherent seed size of yellow peas in comparison to green lentils, which is approximately a three-fold difference (GL= 72.20 ± 0.7 g per 1000 seeds; YP= 241.63 ± 0.9 g per 1000 seeds). In

contrast to green lentil flours, a significant increase was observed in mean particle size for yellow pea flours tempered to 30% moisture prior to infrared heating in comparison to the control group flours. The largest mean particle size was reported for flours heated to 120°C (tempered to 30% moisture), with a mean particle size of 224.90± 11.78 μm. Liu et al. (2020) also evaluated the combined effect of tempering and infrared heating of pulse seeds and explained that this roughly 2x increase in mean particle size in comparison to the control group flours may be caused by the increase in denaturation of protein and alteration in starch molecules that can facilitate aggregation to form larger particles. Also to consider as a cause for larger particle sizes may be the increase in starch gelatinization that can cause starch granules to swell, or the effect of increased moisture in the seed acting as a plasticizer. The researchers also explained that plausible reasons as to yellow pea flours heated to 140°C (within same 30% moisture tempering group) reported a significantly smaller particle size mean in comparison to those flours heated to 120°C, is that the higher temperatures can facilitate fragility within the flour matrix to allow for ease of division into smaller particles during milling (Liu et al., 2020). Similar results were also found by Young et al. (2020), where the mean particle size of roasted pea flour decreased as roasting temperature increased from 120°C to 140°C, and to 160°C. Unique to this study are the larger standard deviations tied to the data points. The swing up or down by 11-20 μm for volume weighted means of the particle sizes for both yellow pea and green lentil flours is likely a result of the milling method utilized for this study. All samples were milled using a laboratory sized hammer mill (0.8 mm screen), which has been shown by researchers to result in wider range of particle sizes in comparison to other mill types, such as roller or pin mills (Bourré et al., 2019). For future studies, it may be beneficial to control particle size more closely through use of a roller or pin mill and/or implement the use of sieves to control variation in particle size.

Table 4.1. 3 Particle size distribution of green lentil and yellow pea flours.[1]

| Process Conditions | D [4, 3] – Volume weighted mean (µm) |
|---|---|
| ***Green Lentil*** | |
| Control | 101.05± 18.16$^{ab}$ |
| 120°C, 20% | 106.61±18.22$^{ab}$ |
| 140°C, 20% | 97.73± 11.45$^{b}$ |
| 120°C, 30% | 137.71± 16.61$^{a}$ |
| 140°C, 30% | 108.07± 11.65$^{ab}$ |
| ***Yellow Pea*** | |
| Control | 106.62± 11.75$^{C}$ |
| 120°C, 20% | 117.94± 23.09$^{BC}$ |
| 140°C, 20% | 129.15± 12.89$^{BC}$ |
| 120°C, 30% | 224.90± 11.78$^{A}$ |
| 140°C, 30% | 146.97± 15.93$^{B}$ |

[1]Means within a column followed by the same letter (lowercase or capital) are not significantly different ($p<0.05$)

### 4.1.5 Functional properties

*Solubility*

The solubility of a protein is a key factor for governing functionality, such as foaming capacity and stability, emulsification properties and water and oil holding capacities. The solubility of the green lentil and yellow pea flours were measured at two pH levels, pH 5 and pH 7, to understand how the flours would perform in common food systems, such as beverages, breads, crackers, cookies, and pasta. Solubility results are reported in Table 4.1.4. At pH 5, the solubility

ranged from 22.6-24.7% and 21.9-23.1% for yellow pea and green lentil flours, respectively. In the case of both pulse flour types, the flours heated to 120°C and tempered to 30% moisture had the highest solubilities, and flours heated to 140°C typically had solubilities at the lower end of the range, although the tempering moisture used was less significant when comparing yellow pea and green lentil flours. Overall, while some significant differences were found between processing conditions, the differences were minimal, as changes in solubility ranged below 3% for each pulse type. The low solubility presented at pH 5 is to be expected, as this level is within range of both yellow pea and green lentil isoelectric points (pI). Similar results have been reported by researchers who have studied solubility. Chang et al., (2015) found that solubility of lentil and yellow pea flours both showed minimum solubility at pH 5 (<20%).

The solutions tested at pH 7 resulted in slightly higher protein solubility percentages for both yellow pea and green lentil flours with values ranging between 23.8-28.5% and 22.0-24.9%, respectively. As with flours measured at pH 5, yellow pea flours had slightly higher solubility than green lentil flours overall. In the case of both flours, the most soluble flours were the control group flours, and least soluble were those heated to 140°C, with flours tempered to 20% moisture reporting the lowest overall solubility for both green lentil and yellow pea. As seen with flour samples measured at pH 5, significant differences were minimal (<5%), and no correlations were found between solubility at pH 7 and surface properties. Similar declining trends have been reported in literature for infrared heated pulse flour samples, with infrared heating reducing solubility overall for treated samples, with the addition of tempering prior to heating exacerbating the decline in solubility in the aqueous phase. Interesting to note though, is that the results of the current study show less significance difference in changes to solubility as effected by tempering moisture and seed surface temperature increase. For example, Bai et al., (2018b) found that chickpeas that were tempered to 20% moisture and heated to 135°C had a ~26% decline in solubility at pH 7, which is a more significant drop than in the current study where solubility dropped by <5%. Guldiken et al., (2022), as mentioned previously, had a similar experimental

design to the current study, but instead evaluated functionality changes within chickpea and navy bean flours. The researchers found similar results to Bai et al., (2018b), where chickpea flour solubility at pH 7 declined from 63.8% for the control group flours, down to the lowest solubility of 6.6-7.2% for flours heated to 140°C at either tempering moisture level. In the same study, the solubility of the control group navy bean flours had similar solubility values as yellow pea and green lentil flours in the current study (~ 30%, ± 5% at pH 7). Also in comparison to the current study, as the navy beans were processed, solubility at pH 7 declined, with a minimum solubility (0.9-6.1%) for navy beans flours when seeds were heated to 140°C at either tempering moisture level. In the literature, a decline in solubility is commonly a result of protein denaturation, which leads to the exposure of buried hydrophobic groups. This in turn causes the promotion of protein-protein, protein-starch, and protein-lipid cross linking that can result in aggregation and precipitation out of solution (Aryee & Boye, 2017; Bai et al., 2018b; Fasina et al., 2001). As the yellow pea and green lentil flours did not have a drastic change in solubility at pH 7, it may be inferred that the processing conditions used in this study were not intense enough to facilitate high levels of protein denaturation that would lead to the exposure of said buried hydrophobic groups inside the pulse seeds. The relative stability of the solubility characteristics of the raw and processed green lentil and yellow pea flours may be beneficial in industrial food applications due to the fact that processing parameters could be altered to optimize other functional characteristics, but the solubility of the flour will remain consistent to a certain critical level.

Table 4.1. 4 Functional properties of yellow pea and green lentil flours. Values are reported on a flour basis.[1]

| Process Conditions | Solubility (%) pH 5 | Solubility (%) pH 7 | WHC (g/g) | OHC (g/g) | FC (%) | FS (%) |
|---|---|---|---|---|---|---|
| ***Green Lentil*** | | | | | | |
| Control | 22.16±0.10$^{ab}$ | 24.93±1.14$^{c}$ | 0.58±0.01$^{a}$ | 0.45±0.04$^{a}$ | 116.78±3.34$^{a}$ | 98.78±1.43$^{a}$ |
| 120°C, 20% | 22.39±0.11$^{b}$ | 23.67±0.29$^{bc}$ | 0.63±0.01$^{b}$ | 0.47±0.02$^{a}$ | 112.3±3.84$^{ab}$ | 98.04±1.01$^{a}$ |
| 140°C, 20% | 22.07±0.13$^{ab}$ | 21.98±0.06$^{a}$ | 0.68±0.01$^{c}$ | 0.44±0.07$^{a}$ | 111.00±2.03$^{ab}$ | 97.37±0.39$^{a}$ |
| 120°C, 30% | 23.06±0.15$^{c}$ | 23.92±1.22$^{c}$ | 0.71±0.01$^{d}$ | 0.55±0.02$^{b}$ | 105.33±1.45$^{bc}$ | 96.95±2.08$^{a}$ |
| 140°C, 30% | 21.91±0.10$^{a}$ | 22.17±0.05$^{ab}$ | 0.72±0.01$^{e}$ | 0.49±0.01$^{a}$ | 101.44±1.64$^{c}$ | 98.71±1.38$^{a}$ |
| ***Yellow Pea*** | | | | | | |
| Control | 23.65±0.26$^{B}$ | 28.55±0.14$^{D}$ | 0.54±0.01$^{B}$ | 0.48±0.01$^{B}$ | 117.22±3.47$^{A}$ | 96.75±1.10$^{A}$ |
| 120°C, 20% | 23.84±0.06$^{B}$ | 25.99±0.33$^{C}$ | 0.64±0.01$^{C}$ | 0.53±0.02$^{C}$ | 101.67±3.18$^{B}$ | 98.96±1.77$^{A}$ |
| 140°C, 20% | 22.56±0.21$^{A}$ | 23.77±0.06$^{A}$ | 0.70±0.01$^{D}$ | 0.42±0.05$^{A}$ | 106.44±1.68$^{B}$ | 98.96±1.09$^{A}$ |
| 120°C, 30% | 24.66±0.10$^{C}$ | 26.15±0.23$^{C}$ | 0.48±0.01$^{A}$ | 0.61±0.01$^{D}$ | 100.94±1.64$^{B}$ | 99.00±1.46$^{A}$ |
| 140°C, 30% | 23.40±0.04$^{B}$ | 24.43±0.04$^{B}$ | 0.76±0.01$^{E}$ | 0.54±0.04$^{C}$ | 100.33±0.88$^{B}$ | 98.89±0.38$^{A}$ |

[1]Means within a column followed by the same letter (lowercase or capital) are not significantly different ($p<0.05$).

*Water & oil holding capacity*

The water holding capacity (WHC) of a food ingredient is defined as the amount of water that can be absorbed per gram of a powered ingredient. The WHC of yellow pea and green lentil flours ranged between 0.48-0.76 g/g and 0.58-0.72 g/g, respectively (Table 4.1.4). The unique processing conditions significantly increased the WHC of the flours as tempering moisture and infrared heat applied to the seeds prior to milling increased, which aligns with data reported in the literature for thermally treated pulse flours. For example, the boiling of dehulled yellow pea flours resulted in a WHC of 1.38 g/g, and 1.79 g/g for boiled dehulled Kabuli chickpea flour, which was approximately a two-fold increase in comparison to the respective WHC of the raw flours in the study (Ma et al., 2011). Infrared heat treatments (in combination with tempering to a desired

moisture levels) also has been shown to increase the WHC of pulse flours (Bai et al., 2018b; Fasina et al., 2001; Guldiken et al., 2022; Liu et al., 2020). For both green lentil and yellow pea, the control group flours had the lowest overall WHC, and flours milled from seeds tempered to 30% moisture and heated to 140°C had the highest overall WHC. Ma et al., (2011) explained that the trend of these results agree with the theory in the literature, that as heat and moisture increase, the porosity of the flours is increased, polar internal amino acid side chains are exposed to solution as a result of protein denaturation (effectively increasing the proteins' affinity to bind water), and an increase in the content of damaged/gelatinized starch is observed. In the current study there was a positive correlation with the surface hydrophobicity (which is an indicator of protein denaturation due to processing) of the protein in the flours and the WHC values (GL $r$=.869; $p$<0.01; YP $r$=.624; $p$<0.01). A positive correlation was also seen for WHC and the damaged/gelatinized starch content for both green lentil (GL) and yellow pea (YP) samples (GL $r$=.942; $p$<0.01; YP $r$=.655; $p$<0.01). The increased content of damaged/gelatinized starch in the flours likely allowed for the binding of water by starch molecules, in addition to protein-water interactions, effectively increasing WHC overall, as cited by Ma at al., (2011) and other researchers (Aguilera et al., 2009). As green lentil flours had stronger correlations than yellow pea flours between WHC and surface hydrophobicity, and WHC and damaged/gelatinized starch content, it again may be inferred that the starch molecules and surface proteins present in the green lentil seeds were more highly susceptible to denaturation as the green lentil seeds were roughly 3x smaller in size than yellow peas, which would mean the lentils had a larger surface area for the infrared rays to penetrate into the seed and alter starch and protein structure (Liu et al., 2020).

One interesting result for WHC that did not follow the expected trend of the rest of the data was that yellow pea flours milled from seeds tempered to 30% moisture and heated to 120°C (WHC= 0.48 g/g) experienced a steep decline in WHC in comparison to flours milled from seeds tempered to 30% moisture and heated to 140°C (WHC= 0.76 g/g). Green lentil flours did not follow a similar sudden decline, and instead had a steady increase in WHC as tempering moisture

increased, and infrared heat applied increased within each tempering group. This outlier result may be due to the fact that at these processing conditions for yellow peas, the polarity of the exposed protein sidechains were more hydrophobic in nature. This is a limitation of the method used to measure SH, as while SH measures the increase in hydrophobicity of a protein solution (indicative of protein unfolding/denaturation), it does not give the distribution of hydrophobic patches along a protein surface, which also does not allow for quantification of hydrophilic patches. So while SH and WHC were positively correlated overall, it may be that seeds heated to 120°C and tempered to 30% moisture had a higher relative proportion of hydrophobic surface proteins exposed as result of processing. Further studies are needed to understand this in detail. Also to consider is that the content of damaged/gelatinized starch was significantly lower for yellow pea milled from seeds tempered to 30% moisture and heated to 120°C than for those flours milled from seeds tempered to 30% moisture but heated to 140°C (Table 4.1.1). The lower content of damaged/gelatinized starch could also restrict the flour matrix from retaining larger amounts of water. Lastly, the drop in WHC may be due to the higher overall moisture content of flours heated to 120°C and tempered to 30% moisture (Table 4.1.1), as the increased amount of water present may already be bound within the matrix, and therefore would impede water binding during the assay. Overall, the moderate ability for the yellow pea and green lentil flours to hold water within the flour matrix may be beneficial for certain food applications where a moderate water content it desired, such as flatbreads, crackers, pasta or cookies.

The oil holding capacity (OHC) of a food ingredient is similar to WHC, but instead measures the grams of oil held per gram of material. The OHC were between 0.45-0.55 g/g and 0.48-0.61 g/g for green lentil and yellow pea flours, respectively, which were in line with results from similar studies, where change in OHC was found to be minimal in flours treated with infrared heat (Bai et al., 2018b; Guldiken et al., 2022; Mwangwela et al., 2007; Ribéreau et al., 2018). In the case of both pulse flour types, samples tempered to 30% moisture and heated to 120°C prior to milling had the significantly highest OHC, although significant differences were minimal. As

with WHC, OHC was observed to increase as tempering moisture increased, but in contrast to WHC, as infrared heat applied increased within each tempering group, the OHC of the flours decreased. Again, in contrast to WHC results, little significance was found between different processing conditions for the flours, least so for green lentil flour samples, where the only significantly different OHC result was found for samples tempered to 30% moisture and heated to 120°C (the highest overall OHC reported for green lentil samples). A similar conclusion can be drawn for OHC as with WHC, where an increase in tempering moisture and infrared heat applied to the pulse seeds prior to milling resulted in (1) protein denaturation of the surface proteins and, (2) an increase in damage starch content that may impede binding of oil, due to the hydrophilic nature of starch molecules (Du et al., 2014; Jogihalli et al., 2017). These conclusions are confirmed by correlation between OHC and protein surface hydrophobicity, which was again positively correlated for green lentil flour samples ($r$=.793; $p$<0.01), although less strongly correlated than WHC and surface hydrophobicity. No correlation was found between protein surface hydrophobicity and OHC for yellow pea samples. Lastly, no significant correlations were found between OHC and damaged starch content for either yellow pea or green lentil flours, not surprisingly, as carbohydrates have little affinity for hydrophobic bonding. This result leads to the assumption that while partial protein denaturation increases as tempering moisture and infrared heat applied increases, the internal amino acids that are exposed as a result, are likely more hydrophilic in nature rather than hydrophobic. Again, as with WHC, it can be assumed that green lentil surface proteins are more susceptible to denaturation than yellow pea surface proteins, as a result of their smaller seed size. The stability in OHC of the processed flours is beneficial in industry as any volatility or changes to surface temperature during pre-processing is unlikely to affect the OHC of the resulting flour. This can help minimize any negative changes to the mouthfeel and/or flavour, which are two important factors that are related to fat content within a food product.

*Foaming properties*

Food products that are aerated (i.e., have foams incorporated) are enjoyed in a number of forms in our diets each day. Examples of foams in foods that are visible to consumers are carbonated beverages (e.g., soft drinks, fizzy water, beer, sparkling wine), barista style coffees (e.g. lattes, cappuccinos, cortados), meringues, and whipped cream (Deotale et al., 2020). Foams also play a key role in the tenderization of foods that are less obvious to the eye, such as providing sponginess and lightness in ice cream, cakes, breads, and other baked goods. In the literature, data pertaining to the performance of pulse proteins as foaming agents is well documented (Amagliani et al., 2021; Amagliani & Schmitt, 2017; Jarpa-Parra, 2018; Lam et al., 2018; Primozic et al., 2018; Romano et al., 2021). In general, pulse proteins are adequate at both foam forming and foam stability and exhibit improved foaming properties when proteins are extracted under controlled conditions or modified by some means (e.g., enzymatic, physical or chemical) (Amagliani et al., 2021). Published data pertaining to the foaming properties of pulse flours as an ingredient, instead of the protein fraction alone, is more limited but does provide some insight into how whole and split flours contribute to food foam structures. Typically whole and/or split pulse flours create adequate foams in their raw flour form, but are negatively impacted by the inherent lipid content and thermal pre-treatments (Bai et al., 2018b; Jogihalli et al., 2017; Mwangwela et al., 2007; Stone et al., 2021). The foaming properties of the green lentil and yellow pea flours did not deviate from the expected trends reported in the literature, and exhibited poor foaming properties, which were exacerbated by processing with increased tempering moisture levels and infrared heat. The foaming results of the flours can be seen in Table 4.1.3. The foaming capacity (FC) of the samples ranged between ~101-117% and ~100-117% for green lentil and yellow pea samples, respectively. The foam stability (FS) of both pulse flour types were very similar (ranged between 96-99%, with no significant differences between processing condition groups for either pulse type, including the control group flours). The processing of the pulse seeds resulted in a progressive decline in FC and FS as the processing conditions intensified, with samples tempered to 30% moisture and infrared

heated to 140°C resulting in the poorest FS and FC out of all treatment groups for both yellow pea and green lentil samples. Similar foaming property results have been reported in the literature for infrared heated pulse flours. Guldiken et al., (2022) found that tempered and infrared heated navy bean flours exhibited a decline in FC and FS of ~150% and ~75%, respectively, in comparison to the control flours. In the same study, chickpea flour samples were not able to produce any foam once processed. Mwangwela et al., (2007) also reported similar results for microionized cowpea flour, where processed flours had a decline in FC of ~180-190% in comparison to control flours (FS results not reported).

It has been reported in the literature that FC and FS is typically positively associated with increasing solubility in the aqueous phase and increasing SH, as it is an indication of more highly denatured proteins (Amagliani et al., 2021; Dombrowski et al., 2017). In the case of solubility at pH 7, both pulse types did in fact show the expected positive correlation for FC, although only significantly for yellow pea FC ($r$=.622; $p$<0.05). The lack of positive correlation for green lentil samples and the weaker significant correlation for yellow pea samples is not surprising, as the flours exhibited poor solubility in aqueous solutions at pH 7 (Table 4.1.3), but it is likely that the proteins that were able to solubilize did promote the limited foam forming abilities of the flours. In contrast to the expected positive correlation between foaming properties and SH, a significant negative correlation was seen for both yellow pea and green lentil flours when looking at the SH and FC of the samples (YP: $r$=-.740; $p$<0.01; GL: $r$=-.864; $p$<0.01). The increase in SH due to increasing processing conditions (Figure 4.1.2) is likely to have led to the exposure of buried amino acid side chains within the pulse proteins. The exposure of these internal amino acid side chains within the proteins sitting at the air-water interface would have likely changed polarity of the proteins, and in this case limited the interaction of the proteins at the air-water interface of the foam. Another factor that is likely to have impeded the FC is the amount of short chain carbohydrate molecules, as these compounds can compete with the surface proteins to stabilize the air-water interface of the protein foams. As seen in Table 4.1.1, as processing conditions

intensified, the % damage starch also increased. For both green lentil and yellow pea, a strong negative correlation between damaged starch content and FC was observed (YP: $r$=-.547; $p$<0.05; GL: $r$=-.909; $p$<0.01), which allows the conclusion that short chain carbohydrates were likely to have a higher affinity to bind water at the air-water interface than the surface proteins of the flours, or may have physically blocked the functionally important reactions of the surface proteins at the interface. Also likely to contribute to a decreased FC, but relatively stable FS, is the gelatinized starch content of the flours. The gelatinized starch content has been shown to impart stability by increasing the viscosity of the continuous phase, but may impede surface protein interaction at the air-water interface due to physical bulkiness and protein-starch vs. protein-air interactions (Ghribi et al., 2015). In conclusion, it is imperative to think of the pulse flour matrix as a whole, as there are many compositional components that can improve or negatively impact foaming properties, particularly in comparison to the purified protein content of concentrated or isolated pulse protein ingredients (Amagliani et al., 2021). It is also interesting to think of poor foaming properties as a particular benefit in the food industry where foaming can cause considerable process flow disruptions, such as during initial ingredient addition, or when ingredient slurries are required to be pumped through flow systems within a facility (Stone et al., 2021).

*Emulsifying properties*

In the current study, the emulsion activity (EA), emulsion stability (ES), and oil emulsion capacity (OEC) were studied. The differences between the assays are as such; EA relates to the ability of an emulsifying agent (i.e., protein) to form an initial emulsion, ES relates to the stability of the emulsion after heating at 80°C for 30 min, and OEC is defined as the amount of oil (mL) than can be emulsified by a protein before phase inversion or the collapse of an emulsion is observed (Ning Wang & Maximiuk, 2015). For simplicity, EA and ES results will be discussed together, followed by the results for the OEC of the flours. The EA of the green lentil and yellow pea flours ranged between 7.5-40.7% and 8.1-42.8%, respectively (Table 4.1.5). For both pulse

flours types, processing of the pulse seeds with tempering moisture and infrared heat improved EA up until a critical point. Green lentil flours milled from seeds that were tempered to 20% moisture and heated to 120°C prior to milling had the significantly ($p > 0.05$) highest EA, at ~41% (+~6% increase in comparison to the control group). Yellow pea flours also exhibited higher EA at the same pre-processing conditions as the green lentil flours and continued to show increasing EA as the tempering moisture increased to 30% for the same seed surface temperature (120°C). In contrast to the green lentil samples, the improvement in EA for yellow pea seeds heated to 120°C and tempered to both moisture levels prior to milling did not result in any significant ($p > 0.05$) differences from the control group flour EA. For both pulse type samples, those seeds that were tempered to 30% moisture and heated to 140°C prior to milling had the significantly ($p > 0.05$) lowest EA, with seeds treated at these conditions resulting in Eas roughly 12-30% lower than the other processing condition groups. The emulsion stability (ES) of the flour samples ranged between 8.9-43.4% and 8.3-42.6% for yellow pea and green lentil flours, respectively. As with the EA, flours milled from seeds tempered to 30% moisture and infrared heated to 140°C prior to milling resulted in the poorest ES out of all the treatment groups. The flours milled from seeds tempered to 20% moisture, and again heated to 140°C had the next poorest ES. This allows for the conclusion that as the intensity of infrared heat applied to the seeds prior to milling increases, a negative effect on both EA and ES is observed and is exacerbated by increased tempering moisture. Benmeziane-Derradji et al., (2020) found that when lentil seeds were roasted at 150°C for 30 min the EA of the flours increased from ~28% to ~43%, but that the ES of the flours declined from 120% to ~67%. Aguilera et al., (2009) found that the EA of raw flours were ~23% for chickpea and ~47% for lentils. After soaking and boiling the seeds prior to milling, the EA of the flours declined by ~16% and ~45%, respectively for chickpea and lentil, respectively.

Similar correlations between the solubility at pH 7, the SH of the flours, and the content of damaged/gelatinized of the flours that were observed for foaming properties, were also observed for the EA and ES of the flours. The solubility at pH 7 was positively correlated to both EA ($r$=

.716, $p<0.05$) and the ES ($r= .782$, $p<0.01$) of the green lentil flours. For yellow pea flours, the correlation was only found to be significant between solubility at pH 7 and the ES of the flours ($r= .836$, $p<0.01$). This positive correlation with solubility likely explains that the proteins present in the flours were able to solubilize to a point and arrange at the water/oil interface. For both green lentil and yellow pea flour samples, negative correlations were not deemed significant between the SH of the flours and EA and ES. The strongest correlation for EA and ES was with the damaged/gelatinized starch content of the flours, which was found to have a significant negative impact on the emulsifying properties of the flours (GL→ EA: $r= -.830$, $p<0.01$; ES: $r= -.815$, $p<0.01$/ YP→ EA: $r= -.817$, $p<0.01$; ES: $r= -.857$, $p<0.01$). The increase in gelatinized starch may increase the viscosity and slow the movement of the emulsion droplets in solutions, which may stabilize the emulsion to a degree, but the increase in gelatinized starch may also physically impede the interaction between the surface proteins and continuous oil phase limiting initial EA, and ES. Few researchers have examined the relationship between gelatinized starch and EA and ES as proteins are the primary driver behind emulsion properties, but Ma et al. (2011) cite that the interaction between proteins and carbohydrates within emulsion systems are likely to have effects to different extents on both EA and ES.

The OEC of the flours (Table 4.1.4) generally declined as the pulse seeds were processed prior to milling, with a drop in roughly 20-25% in OEC (mL oil/g) from the control group flours to the processed flours for both yellow pea and green lentil samples. Between different processing condition groups for yellow pea flours, all processed flours had a significantly ($p >0.05$) lower OEC in comparison to the control group. Beyond this, no significant differences were found for yellow pea samples, between processing conditions. The yellow pea flours had similar correlation trends as EA and ES, although in this case, a significant negative correlation between OEC and SH ($r= -.770$, $p<0.01$) was found, as well as a significant positive correlation with solubility at pH 7 ($r= .661$, $p<0.01$). For green lentil samples, a significant ($p >0.05$) drop in OEC from control group flours was seen in comparison to the flours milled from the processed seeds. Flours tempered

to 20% moisture and heated to 120°C had the significantly ($p$ >0.05) lowest overall OEC (202.7 mL oil/g). As with yellow pea samples, the green lentil samples were negatively correlated with protein surface properties, except instead of a correlation with SH, samples were instead negatively correlated with the surface charge of the flours ($r$= -.521, $p$<0.05). Green lentil flours did not show a positive correlation between OEC and solubility at pH 7. Similar results to this study were obtained by Guldiken et al. (2022), where the OEC of tempered and infrared heated navy bean and chickpea flours decreased from ~333 mL/g oil (d.b.) to between ~216-223 mL/g oil, and from ~277 mL/g oil (d.b.) to between ~209-219 mL/g oil (d.b.), respectively. While limited data regarding the OEC of heat treated pules flours is present in the literature, it is expected that the similar theory applies for the previously discussed functional properties in this study. That theory being that as the further denaturation of proteins in the flours occurs as a result of processing, the exposure of internal amino acid residues alter the affinity of the surface protein moieties, which in turn will negatively impact interactions in solution. In contrast to previously discussed functional properties, no significant correlation between the OEC of the flours and the damaged/gelatinized content of the flour was found. This may be due to the fact the starch is not likely to hold oil in solution, as it is composed of mostly hydrophilic sugars. Further studies are necessary to explain this relationship more clearly.

Table 4.1. 5 Emulsion properties of yellow pea and green lentil flours.[1]

| Process Conditions | OEC (mL oil/g, dry matter) | EA (%) | ES (%) |
|---|---|---|---|
| ***Green Lentil*** | | | |
| **Control** | 271.67± 9.67$^{c}$ | 34.94±0.95$^{c}$ | 38.56±1.4$^{d}$ |
| 120°C, 20% | 202.7±6.32$^{a}$ | 40.71±1.23$^{d}$ | 42.59±2.01$^{d}$ |
| 140°C, 20% | 221.03± 5.00$^{b}$ | 20.49±0.72$^{b}$ | 13.85±1.04$^{b}$ |
| 120°C, 30% | 220.67± 1.25$^{b}$ | 31.17±1.03$^{c}$ | 28.55±1.85$^{c}$ |
| 140°C, 30% | 223.6± 5.72$^{b}$ | 7.50±0.7$^{a}$ | 8.63±0.77$^{a}$ |
| ***Yellow Pea*** | | | |
| Control | 266.13± 8.81$^{B}$ | 36.24±1.97$^{BC}$ | 43.14±1.83$^{D}$ |
| 120°C, 20% | 210.53± 9.09$^{A}$ | 39.43±3.02$^{BC}$ | 35.6±2.52$^{C}$ |
| 140°C, 20% | 223.13± 4.76$^{A}$ | 32.66±1.94$^{B}$ | 16.65±0.66$^{B}$ |
| 120°C, 30% | 219.1± 5.20$^{A}$ | 42.78±1.72$^{C}$ | 42.41±2.43$^{D}$ |
| 140°C, 30% | 225.77± 4.70$^{A}$ | 8.08±1.27$^{A}$ | 8.91±0.82$^{A}$ |

[1]Means within a column followed by the same letter (lowercase or capital) are not significantly different ($p<0.05$)

## Pasting Properties

The yellow pea and green lentil samples exhibited dilatant (shear thickening) pasting properties (Figures 4.1.3 and 4.1.4). In both pulse flour types, the control samples exhibited the significantly ($p$ <0.05) highest peak and final viscosities at 822 cP and 1362 cP for green lentil control flours, and 668 cP and 1204 cP for yellow pea control flours. In processed green lentil flour samples, as tempering moisture and infrared heat applied to seeds prior to milling increased, a general decline in peak and final viscosities was observed. The only statistical difference between treatment groups and the control group was observed for flours milled from seeds tempered to 30% moisture and heated to 140°C, where the peak and final viscosities declined to 480.33 and 800.17, respectively. For yellow pea flour samples, the trend was not quite as obvious as with

green lentil samples, as infrared heat applied prior to milling resulted in significant ($p$ <0.05) differences between treatment groups. Yellow pea samples milled from seeds heated to 140°C prior to milling resulted in the lowest overall peak viscosities (195- 317 cP), with no significant differences distinguished between level of tempering utilized prior to heating. Control samples and samples heated to 120°C (at either tempering level) were also not significantly different from one another. The final viscosity of the processed yellow pea flours for flours milled from seeds heated to 120°C (at either tempering level) were not significantly different from one another (904-1000 cP), but did exhibit significantly higher final viscosities than those samples heated to 140°C. In contrast to peak viscosity, in the case of samples heated to 140°C, tempering moisture significantly impacted final viscosity of the yellow pea flours, with samples tempered to 30% moisture having a significantly lower final viscosity (385 cP) than those heated to 140°C and tempered to 20% moisture (1000 cP). Not surprisingly, the initial pasting temperature of the green lentil samples exhibited the opposite properties to those seen for peak and final viscosities, with pasting temperatures gradually and significantly increasing as processing conditions intensified. Control group samples exhibited pasting temperatures of around 74°C, and as the amount of tempering moisture and infrared heat was applied, the pasting temperature increased by roughly 1°C, with samples tempered to 30% moisture and heated to 140°C exhibiting pasting temperatures of about 78-79°C. Yellow pea control samples exhibited pasting temperatures of roughly 75°C, with processed samples exhibiting significantly higher pasting temperatures between ~78-80°C, although pasting temperature increases within the processed samples were not found to be significant.

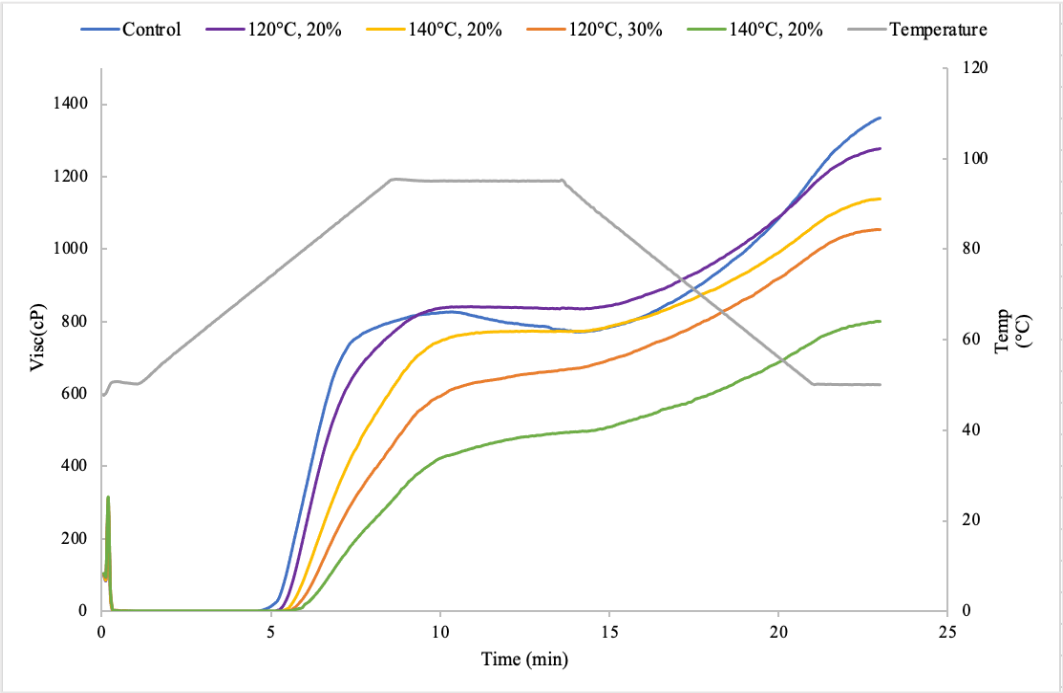


Figure 4.1. 3 Pasting profiles of green lentil flours as determined by RVA.

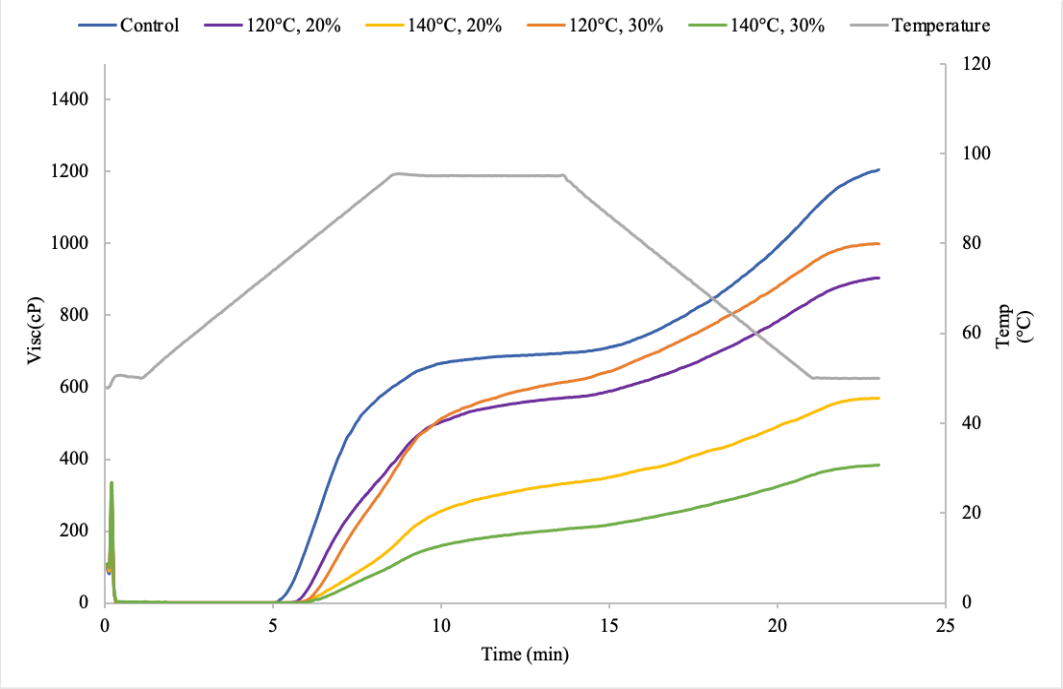


Figure 4.1. 4  Pasting profiles of yellow pea flours as determined by RVA.

The decrease in overall pasting properties and increase in pasting temperature correlates well to data in the literature for heat treated flours, where similar results were found for infrared heated green lentil flours (Liu et al., 2020) and cowpea flour (Mwangwela et al., 2007), dehulled and boiled Mucana beans (Mang et al., 2015), and extruded pea flours (Qi et al., 2021). The pasting properties of flours are closely connected to the compositional fractions that make up the flour matrix. Starch is the structural fraction that demonstrates pasting functional properties within the flour but protein, lipids, shorter chain carbohydrates (e.g., polysaccharides, oligosaccharides), and damaged/gelatinized starch can inhibit the interactions required for starch pasting (Qi et al., 2021; Raghunathan et al., 2017). The relationship between the compositional structure and pasting properties may then be explained by correlations found between peak viscosity, final viscosity and pasting temperate with the SH and damaged/gelatinized starch content of the flours (Table 4.1.6). Both peak and final viscosities are negatively and significantly correlated with SH and damaged/gelatinized starch contents for green lentil samples, while the pasting temperature of the samples were positively and significantly correlated with SH and damaged/gelatinized starch contents. Yellow pea samples exhibited similar trends to green lentil flours, although the correlation between peak viscosity and surface hydrophobicity was not significant. The difference in a correlation with SH between green lentils and yellow peas is likely related to the protein content and the seed size of the pulses, as green lentils contained more protein within a smaller structure, allowing for a greater degree of protein denaturation in response to tempering and infrared heat applied (Section 3.1; Table 4.1.1). The greater degree of denatured protein molecules within the flour matrix may have resulted in protein—starch interactions that impeded the ability of the starch granules to swell and impart pasting viscosity. The significant correlations between damaged/gelatinized starch contents and pasting properties for both pulse flour types has also been reported in literature, where Liu et al (2020) found similar negative correlations between infrared heated lentil flour pasting peak and final viscosities and damaged/gelatinized starch contents ($r$ =

−0.848, $p < 0.01$ and $r = -0.837$, $p < 0.01$, respectively). The researchers explained that higher amount of damaged starch molecules (inferred from both the increased amounts of damaged/gelatinized starch and amylose contents in the processed flours) restricted the swelling and decreased the ability of the starch molecules present to impart pasting viscosity to the flour slurries.

Table 4.1. 6 Pearson Correlations between pasting properties and surface hydrophobicity and damaged starch content.

| **Pasting parameter** | **Surface hydrophobicity** | **Damaged/gelatinized starch content** |
|---|---|---|
| ***Green Lentil*** | | |
| Peak Viscosity (cP) | -.682** | -.794** |
| Final Viscosity (cP) | -.661** | -.800** |
| Pasting Temperature (°C) | .835** | .884** |
| ***Yellow Pea*** | | |
| Peak Viscosity (cP) | NS | -.890** |
| Final Viscosity (cP) | -.584* | -.885** |
| Pasting Temperature (°C) | .574* | .717** |

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
NS- not significant.

## 4.2 The effect of tempering moisture and seed surface temperature on the nutritional properties of yellow pea and green lentil flour

### 4.2.1 Protein quality

The amino acid content (g/100 g) of yellow pea and green lentil flours is reported in Table 4.2.1. Overall, minimal, if it all, significant difference ($p$ <0.05) was found between the control group flours and the flours milled from tempered/infrared heated seeds. An exception was found only in green lentil flour samples, where tempering + infrared heating resulted in a slight increase in glutamate, valine and leucine contents. It has been shown in the literature that certain cooking methods may increase protein content, and therefore the content of certain amino acids, by reducing the amount of starch within the cooked pulse (Candela et al., 1997). Trends between increasing amino acid contents and cooking methods are difficult to confirm though, as changes to specific amino acids vary across both the cooking method used and the unique pulse variety and cultivars (Candela et al., 1997; Nosworthy et al., 2018). The content of essential amino acids (EAA) per mg/g protein are given in Table 4.2.2. The FAO/WHO (1991) nutrition reference pattern for children (2–5 years) was given in Section 3 (Table 3.1). No significant differences were found between mg/g protein contents of EAA in control and processed flours. All flours contained equal or higher amounts of EAA (outside of the limiting amino acid (LAA)) as required by the FAO/WHO (1991), with the exception of threonine (~33 mg/g protein) and methionine + cysteine (~21-24 mg/g protein) in green lentil flours. The LAA was tryptophan in both pulse types with levels of ~7 mg/g protein and 9 mg/g protein for green lentil and yellow pea flours, respectively. In green lentil flours, threonine and methionine + cysteine were just below the FAO/WHO threshold of 34 mg/g protein and 25 mg/g protein, respectively. Pulses tend to be low in methionine + cysteine overall, so this deficiency was expected for green lentil flour samples. Tryptophan being the LAA in both pulse flour types was not expected, nor were depleted levels of

threonine found in green lentil samples. These unusual deficiencies are uncharacteristic of pulse crops, although not completely out of the ordinary. Researchers have reported concurrent results for chickpeas citing threonine as the LAA (Bai et al., 2018a; Wang et al., 2020) and tryptophan as the LAA for yellow peas and green lentils (Guldiken et al., 2022; Stone et al., 2021). There are a two possible reasons as to why tryptophan was found to be the LAA in this study for both pulse varieties, and why lower-than-normal levels of threonine in the green lentil samples were reported. [1] The use of sulfur-based fertilizers to assist rotational crop growth (e.g., canola) likely contributed to elevated levels of sulfur in the soil, which would be metabolized into sulfur containing amino acids (SCAA) (e.g., methionine + cysteine) by the pulse crops, effectively increasing contents of SCAA and reducing the concentration of tryptophan (Bai et al., 2018a; Guldiken et al., 2022). In this case, it would be plausible to expect to see methionine + cysteine above the levels outlined by the FAO/WHO guidelines, which was observed for yellow pea samples in this study, but not for green lentil samples. [2] Difficult growing conditions can affect the metabolism of amino acids within a crop and effect the levels of certain amino acids, such as threonine (Wang et al., 2020). When plants are under environmental stress, some normal cellular biosynthetic processes such as the production of bacteroids from branched chain amino acids can deviate from regular expected outputs (Morneau et al., 2013; Prell et al., 2010). For example, under drought conditions a lentil plant will prioritize synthesis of branched chain amino acids such as isoleucine, which is synthesized from threonine and methionine synthases (threonine synthase and cystathionine $\gamma$-synthase, respectively) (Wang et al., 2020). Galili et al. (2005) explain that there is an inherent preference for the use of cystathionine $\gamma$-synthase to synthesize methionine over threonine synthase/threonine production, which may explain the lower-than-normal levels of threonine in the lentil samples. Both circumstantial reasons for the unexpected, depleted levels of tryptophan and threonine (in lentils only) likely could have had a cumulative effect, particularly considering the crop reports for the 2018 growing season in surrounding areas of Limerick, SK

(growing region for the pulse samples studied here) indicated that topsoil moisture levels were affected by dry conditions (Ministry of Agriculture, 2018).

The combined effect of infrared heat + tempering moisture did not impart any significant changes to the amino acid score of the pulse flours (Table 4.2.3). As mentioned previously, the LAA was tryptophan for both green lentil and yellow pea flours. The amino acid score of the LAA for both pulse type flours was essentially identical, ranging between ~0.64-0.65 for green lentils, and ~0.62-0.65 for yellow pea flours. This is within range of the typical amino acid score for a LAA in pulses (Nosworthy & House, 2017). The *in-vitro* protein digestibility (IVPD) results of green lentil and yellow pea flours are also presented in Table 4.2.3. The IVPD of green lentil flour samples fell between 73.47 and 81.74%. All processed samples resulted in significantly ($p$<0.05) better IVPD when compared to the control samples, although significant differences between varying processing conditions were not apparent until moisture and infrared heat level reached the maximum for this study (30% moisture and 140°C). Yellow pea flour samples had slightly higher IVPD results overall when compared to green lentil, with IVPD values of 78.48-82.56%. Again, as with green lentil samples, all processed samples had significantly higher IVPD results than those of the control group flours. When comparing the level of IVPD between treatment groups, less obvious significant differences were observed. Generally, IVPD increased by 1-2% as processing conditions increased, but yellow pea samples tempered to 30% moisture and heated to 140°C had a slight decline of ~2% in IVPD when compared to samples tempered to the same moisture but heated to only 120°C. As discussed in the previous section regarding functionality, the level of SH is considered an indicator of protein denaturation in the flours. Green lentil flours had a significant and positive correlation between IVPD and SH ($r$= .749, $p$<0.01), which allows for the conclusion that as the flours were further denatured via protein unfolding and dissociation between compositional fractions, digestive enzyme could more easily access the protein molecules resulting in elevated IVPD levels. While a similar significant correlation was not seen between SH and IVPD for yellow pea flour samples, both pulse flour types had IVPD significantly and positively

correlated to damage starch content (GL $r$= .877, $p$<0.01; YP $r$= .549, $p$<0.05), which has also been considered another indicator of compositional changes to the flour matrix as a result of increasing tempering moisture and infrared heat applied prior to milling. The current results agree with those reported in the literature, where it is generally accepted that as the denaturation and gelatinization of the protein and starch fractions increase to a certain point, the IVPD of the flour will also increase, as digestive enzymes may more easily access and digest the nutrients present (Bai et al., 2018a; Qi et al., 2021; Sánchez-Velázquez et al., 2021; Stone et al., 2021). Not evaluated in this study, but still an important factor to consider, is the effect that heat treatments have on the content of inherent ANFs in pulse flours. Researchers have found that thermal treatments such as boiling, roasting, microwaving and infrared heating have all been found to reduce ANFs present in the flours, correlating to an improved IVPD (Bai et al., 2018a; Guldiken et al., 2022; Hefnawy, 2011; Millar et al., 2019; Nosworthy et al., 2018; Yang, et al., 2014). The in-vitro protein digestibility corrected amino acid score (IV-PDCAAS) can also be seen in Table 4.2.3. The IV-PDCAAS ranged between 0.47- 0.53 for green lentil flours and between 0.51- 0.53 for yellow pea flours, which is aligned with reported IV-PDCAAS and PDCAAS values in literature for common pulses (Nosworthy et al., 2017). No significant ($p$ >0.05) differences were observed in IV-PDCAAS levels between control group flours and flours milled from tempered and infrared heated seeds. The results of the current study are not as hypothesized, as it was expected that processing seeds with increasing levels of tempering moisture and infrared heat would lead to the increase in IV-PDCAAS as seen in similar studies (Bai et al., 2018a; Guldiken et al., 2022). The lack of the expected increase in IV-PDCAAS is likely due to the fact that although the IVPD of the flours increased, this increase was a factor of increased damaged/gelatinized starch content (Table 4.1.1) and increased SH (Figure 4.1.2), not changes to the amino acid composition of the proteins present in the flours. This allows for the assumption that the pre-processing of pulse seeds with tempering + infrared heat at the conditions used resulted in partial protein denaturation (i.e., increased SH), but not at high enough levels to change to the exposed amino acid profile within

the flours. That being said, the processed green lentil and yellow pea flours did contain levels of EAA such as histidine, isoleucine, leucine, lysine, phenylalanine + tyrosine, and valine, above the FAO/WHO (1991) guidelines for children 2-5 years. This makes tempered and infrared heated pulse flours an excellent candidate to blend with cereal flours, which may be deficient in one or more EAA, such as lysine (Guldiken et al., 2020).

Table 4.2. 1 Amino acid content (g/100 g) of green lentil and yellow pea flours.[1] Data is reported as the mean between duplicate processing runs.

| Processing Condition | AMMONIA | ASP | THR | SER | GLU | PRO | GLY | ALA | CYS |
|---|---|---|---|---|---|---|---|---|---|
| ***Green Lentil*** | | | | | | | | | |
| Control | 0.22±0.00$^{a}$ | 2.54±0.03$^{a}$ | 0.74±0.01$^{a}$ | 1.02±0.01$^{a}$ | 3.53±0.03$^{c}$ | 0.91±0.00$^{a}$ | 0.83±0.00$^{a}$ | 0.9±0.01$^{a}$ | 0.23±0.03$^{a}$ |
| 120°C, 20% | 0.21±0.01$^{a}$ | 2.52±0.02$^{a}$ | 0.75±0.02$^{a}$ | 1.05±0.02$^{a}$ | 3.54±0.03$^{bc}$ | 0.91±0.1$^{a}$ | 0.84±0.03$^{a}$ | 0.90±0.02$^{a}$ | 0.21±0.01$^{a}$ |
| 140°C, 20% | 0.22±0.01$^{a}$ | 2.6±0.04$^{a}$ | 0.76±0.01$^{a}$ | 1.05±0.02$^{a}$ | 3.63±0.04$^{ab}$ | 0.93±0.02$^{a}$ | 0.83±0.02$^{a}$ | 0.92±0.00$^{a}$ | 0.20±0.01$^{a}$ |
| 120°C, 30% | 0.21±0.01$^{a}$ | 2.5±0.07$^{a}$ | 0.74±0.02$^{a}$ | 1.02±0.03$^{a}$ | 3.53±0.04$^{c}$ | 0.91±0.01$^{a}$ | 0.82±0.02$^{a}$ | 0.90±0.01$^{a}$ | 0.23±0.03$^{a}$ |
| 140°C, 30% | 0.22±0.00$^{a}$ | 2.58±0.02$^{a}$ | 0.76±0.02$^{a}$ | 1.04±0.01$^{a}$ | 3.64±0.01$^{a}$ | 0.94±0.01$^{a}$ | 0.83±0.01$^{a}$ | 0.93±0.01$^{a}$ | 0.20±0.01$^{a}$ |
| ***Yellow Pea*** | | | | | | | | | |
| Control | 0.19±0.13$^{A}$ | 2.37±0.14A | 0.71±0.05$^{A}$ | 0.90±0.06$^{A}$ | 3.45±0.21A | 0.84±0.06$^{A}$ | 0.81±0.06$^{A}$ | 0.87±0.05$^{A}$ | 0.30±0.01$^{A}$ |
| 120°C, 20% | 0.20±0.01$^{A}$ | 2.50±0.07A | 0.75±0.02$^{A}$ | 0.96±0.03$^{A}$ | 3.64±0.10A | 0.89±0.02$^{A}$ | 0.86±0.03$^{A}$ | 0.91±0.03$^{A}$ | 0.30±0.02$^{A}$ |
| 140°C, 20% | 0.20±0.01$_{A}$ | 2.55±0.05A | 0.77±0.03$^{A}$ | 0.99±0.03$^{A}$ | 3.72±0.09A | 0.92±0.03$^{A}$ | 0.88±0.03$^{A}$ | 0.94±0.03$^{A}$ | 0.29±0.01$^{A}$ |
| 120°C, 30% | 0.19±0.01$^{A}$ | 2.44±0.01A | 0.72±0.00$^{A}$ | 0.94±0.01$^{A}$ | 3.53±0.02A | 0.86±0.00$^{A}$ | 0.83±0.01$^{A}$ | 0.90±0.01$^{A}$ | 0.29±0.00$^{A}$ |
| 140°C, 30% | 0.19±0.01$_{A}$ | 2.48±0.05A | 0.75±0.01$^{A}$ | 0.97±0.01$^{A}$ | 3.64±0.04A | 0.90±0.02$^{A}$ | 0.87±0.02$^{A}$ | 0.92±0.01$^{A}$ | 0.27±0.01$^{A}$ |

[1]Means within a column followed by the same letter (lowercase or capital) are not significantly different ($p<0.05$).

*Abbreviations*: ASP, aspartate; THR, threonine; SER, serine; GLU, glutamate; PRO, proline; GLY, glycine; ALA, alanine; CYS, cysteine.

Table 4.2.1 (continued) Amino acid content (g/100g) of green lentil and yellow pea flours.[1] Data is reported as the mean between duplicate processing runs.

| Processing Condition | VAL | MET | ILE | LEU | TYR | PHE | HIS | LYS | ARG | TRP |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Green Lentil*** | | | | | | | | | | |
| Control | 1.03±0.00$^{bc}$ | 0.3±0.03$^{a}$ | 0.91±0.00$^{ab}$ | 1.56±0.01$^{b}$ | 0.57±0.01$^{a}$ | 0.97±0.04$^{a}$ | 0.67±0.08$^{a}$ | 1.59±0.02$^{a}$ | 1.52±0.03$^{a}$ | 0.16±0.01$^{a}$ |
| 120°C, 20% | 1.03±0.01$^{bc}$ | 0.28±0.01$^{a}$ | 0.91±0.03$^{b}$ | 1.55±0.02$^{b}$ | 0.59±0.05$^{a}$ | 0.99±0.03$^{a}$ | 0.64±0.02$^{a}$ | 1.51±0.15$^{a}$ | 1.57±0.05$^{a}$ | 0.16±0.00$^{a}$ |
| 140°C, 20% | 1.05±0.01$^{ab}$ | 0.27±0.00$^{a}$ | 0.93±0.00$^{ab}$ | 1.57±0.02$^{ab}$ | 0.56±0.02$^{a}$ | 0.97±0.01$^{a}$ | 0.66±0.04$^{a}$ | 1.61±0.08$^{a}$ | 1.56±0.00$^{a}$ | 0.16±0.01$^{a}$ |
| 120°C, 30% | 1.02±0.01$^{c}$ | 0.31±0.04$^{a}$ | 0.91±0.01$^{ab}$ | 1.55±0.01$^{b}$ | 0.59±0.03$^{a}$ | 0.97±0.01$^{a}$ | 0.74±0.08$^{a}$ | 1.55±0.07$^{a}$ | 1.52±0.02$^{a}$ | 0.15±0.01$^{a}$ |
| 140°C, 30% | 1.06±0.01$^{a}$ | 0.28±0.00$^{a}$ | 0.94±0.01$^{a}$ | 1.60±0.01$^{a}$ | 0.59±0.01$^{a}$ | 1.00±0.00$^{a}$ | 0.62±0.10$^{a}$ | 1.62±0.08$^{a}$ | 1.55±0.01$^{a}$ | 0.15±0.00$^{a}$ |
| ***Yellow Pea*** | | | | | | | | | | |
| **Control** | 0.94±0.06$^{A}$ | 0.30±0.01$^{A}$ | 0.85±0.06$^{A}$ | 1.44±0.10$^{A}$ | 0.62±0.05$^{A}$ | 0.90±0.07$^{A}$ | 0.62±0.01$^{A}$ | 1.64±0.10$^{A}$ | 1.47±0.11$^{A}$ | 0.21±0.00$^{A}$ |
| 120°C, 20% | 1.00±0.02$^{A}$ | 0.31±0.02$^{A}$ | 0.90±0.02$^{A}$ | 1.51±0.04$^{A}$ | 0.64±0.01$^{A}$ | 0.95±0.03$^{A}$ | 0.60±0.03$^{A}$ | 1.73±0.04$^{A}$ | 1.54±0.04$^{A}$ | 0.21±0.01$^{A}$ |
| 140°C, 20% | 1.03±0.03$^{A}$ | 0.30±0.01$^{A}$ | 0.93±0.04$^{A}$ | 1.57±0.05$^{A}$ | 0.66±0.01$^{A}$ | 0.98±0.03$^{A}$ | 0.61±0.01$^{A}$ | 1.76±0.08$^{A}$ | 1.60±0.06$^{A}$ | 0.21±0.00$^{A}$ |
| 120°C, 30% | 0.98±0.00$^{A}$ | 0.30±0.01$^{A}$ | 0.87±0.01$^{A}$ | 1.47±0.01$^{A}$ | 0.63±0.02$^{A}$ | 0.92±0.01$^{A}$ | 0.55±0.03$^{A}$ | 1.67±0.04$^{A}$ | 1.50±0.01$^{A}$ | 0.20±0.00$^{B}$ |
| 140°C, 30% | 1.00±0.01$^{A}$ | 0.30±0.01$^{A}$ | 0.91±0.01$^{A}$ | 1.53±0.01$^{A}$ | 0.64±0.02$^{A}$ | 0.97±0.02$^{A}$ | 0.55±0.08$^{A}$ | 1.69±0.05$^{A}$ | 1.54±0.01$^{A}$ | 0.21±0.00$^{A}$ |

[1]Means within a column followed by the same letter (lowercase or capital) are not significantly different ($p<0.05$).

*Abbreviations*: VAL, valine; MET, methionine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; ARG, arginine; and TRP, tryptophan.

Table 4.2. 2 Protein content (g/100 g on a dry basis) and essential amino acid content (mg/g protein) of green lentil and yellow pea flours.[1] Data is reported as the mean between duplicate processing runs.

| Processing Condition | Protein content (g/100 g)[2] | THR | VAL | M+C[3] | ILE | LEU | P+T[4] | HIS | LYS | TRP |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Green Lentil*** | | | | | | | | | | |
| Control | 22.71± 0.17$^{bc}$ | 32.74±0.02$^{a}$ | 45.31±0.07$^{b}$ | 23.46±2.55$^{a}$ | 40.09±0.16$^{a}$ | 68.5±0.30$^{b}$ | 67.55±0.31$^{a}$ | 30.23±3.47$^{a}$ | 69.84±0.88$^{a}$ | 6.99±0.23$^{a}$ |
| 120°C, 20% | 22.36± 0.02$^{ab}$ | 33.57±0.97$^{a}$ | 45.96±0.48$^{ab}$ | 21.97±0.82$^{a}$ | 40.5±1.24$^{a}$ | 69.24±0.87$^{ab}$ | 70.64±3.19$^{a}$ | 28.61±1.03$^{a}$ | 67.55±6.62$^{a}$ | 7.02±0.12$^{a}$ |
| 140°C, 20% | 22.93± 0.03$^{bc}$ | 32.98±0.26$^{a}$ | 45.62±0.21$^{b}$ | 20.8±0.48$^{a}$ | 40.38±0.13$^{a}$ | 68.64±0.68$^{b}$ | 66.89±1.33$^{a}$ | 28.69±1.61$^{a}$ | 70.02±3.32$^{a}$ | 7.07±0.50$^{a}$ |
| 120°C, 30% | 21.98± 0.03$^{a}$ | 33.78±0.85$^{a}$ | 46.58±0.52$^{a}$ | 24.34±2.81$^{a}$ | 41.21±0.40$^{a}$ | 70.52±0.53$^{a}$ | 70.90±1.69$^{a}$ | 33.71±3.61$^{a}$ | 70.47±3.16$^{a}$ | 6.86±0.21$^{a}$ |
| 140°C, 30% | 23.1± 0.08$^{c}$ | 32.82±0.07$^{a}$ | 45.76±0.24$^{ab}$ | 20.81±0.64$^{a}$ | 40.84±0.49$^{a}$ | 69.43±0.56$^{ab}$ | 69.03±0.58$^{a}$ | 26.67±4.27$^{a}$ | 69.92±3.26$^{a}$ | 6.49±0.11$^{a}$ |
| ***Yellow Pea*** | | | | | | | | | | |
| Control | 21.37± 0.20$^{BC}$ | 32.23±2.28$^{A}$ | 43.29±2.90$^{A}$ | 27.39±1.30$^{A}$ | 38.83±2.57$^{A}$ | 66.02±4.62$^{A}$ | 69.67±5.74$^{A}$ | 28.80±0.37$^{A}$ | 75.19±4.60$^{A}$ | 9.69±0.12$^{A}$ |
| 120°C, 20% | 21.09± 0.04$^{B}$ | 35.17±0.97$^{A}$ | 47.03±1.06$^{A}$ | 28.53±1.59$^{A}$ | 42.50±0.71$^{A}$ | 71.54±1.86$^{A}$ | 75.46±1.57$^{A}$ | 28.76±1.27$^{A}$ | 81.42±1.94$^{A}$ | 9.76±0.24$^{A}$ |
| 140°C, 20% | 21.92± 0.25$^{C}$ | 35.24±1.27$^{A}$ | 47.14±1.44$^{A}$ | 28.67±0.78$^{A}$ | 42.81±1.60$^{A}$ | 71.43±2.33$^{A}$ | 75.00±1.22$^{A}$ | 27.99±0.25$^{A}$ | 81.57±3.50$^{A}$ | 9.68±0.16$^{A}$ |
| 120°C, 30% | 20.29± 0.05$^{A}$ | 35.12±0.19$^{A}$ | 47.11±0.21$^{A}$ | 28.22±0.81$^{A}$ | 42.41±0.54$^{A}$ | 71.58±0.37$^{A}$ | 75.17±1.34$^{A}$ | 27.11±1.54$^{A}$ | 81.47±2.12$^{A}$ | 9.58±0.11$^{A}$ |
| 140°C, 30% | 21.35± 0.20$^{BC}$ | 35.39±0.30$^{A}$ | 47.34±0.47$^{A}$ | 27.23±0.62$^{A}$ | 42.35±0.44$^{A}$ | 71.70±0.57$^{A}$ | 75.98±0.90$^{A}$ | 27.67±3.68$^{A}$ | 80.39±2.25$^{A}$ | 9.59±0.17$^{A}$ |

[1]Means within a column followed by the same letter (lowercase or capital) are not significantly different ($p<0.05$)

*Abbreviations*: THR, threonine; VAL, valine; ILE, isoleucine; LEU, leucine; HIS, histidine; LYS, lysine; and TRP, tryptophan.

[2]Data was collected in duplicate on the triplicate processing runs and is presented as the mean ± one standard deviation (n=3).

[3]Methionine+Cysteine; [4]Phenylalanine + Tyrosine

Table 4.2. 3 Amino acid score of essential amino acids and IV-PDCAAS of green lentil and yellow pea flours.[1] Data is reported as the mean between duplicate processing runs.

| | **Amino Acid Score** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Processing Condition** | **THR** | **VAL** | **M+C[2]** | **ILE** | **LEU** | **P+T[3]** | **HIS** | **LYS** | **TRP*** | **IVPD (%)[4]** | **IV-PDCAAS** |
| ***Green Lentil*** | | | | | | | | | | | |
| Control | 0.96±0.01$^{a}$ | 1.29±0.00$^{b}$ | 0.94±0.1$^{a}$ | 1.43±0.01$^{a}$ | 1.04±0.01$^{b}$ | 1.07±0.01$^{a}$ | 1.59±0.18$^{a}$ | 1.20±0.02$^{a}$ | 0.64±0.02$^{a}$ | 73.47± 0.47$^{a}$ | 0.47±0.01$^{a}$ |
| 120°C, 20% | 0.99±0.03$^{a}$ | 1.31±0.01$^{ab}$ | 0.95±0.03$^{a}$ | 1.45±0.04$^{a}$ | 1.05±0.01$^{ab}$ | 1.08±0.05$^{a}$ | 1.64±0.05$^{a}$ | 1.22±0.11$^{a}$ | 0.64±0.01$^{a}$ | 77.88± 0.21$^{b}$ | 0.50±0.01$^{a}$ |
| 140°C, 20% | 0.97±0.01$^{a}$ | 1.30±0.01$^{b}$ | 0.92±0.02$^{a}$ | 1.46±0.00$^{a}$ | 1.05±0.01$^{b}$ | 1.08±0.02$^{a}$ | 1.58±0.08$^{a}$ | 1.22±0.06$^{a}$ | 0.65±0.05$^{a}$ | 79.72± 0.09$^{b}$ | 0.51±0.03$^{a}$ |
| 120°C, 30% | 0.99±0.02$^{a}$ | 1.30±0.01$^{a}$ | 0.88±0.11$^{a}$ | 1.45±0.01$^{a}$ | 1.05±0.01$^{a}$ | 1.12±0.03$^{a}$ | 1.51±0.19$^{a}$ | 1.16±0.05$^{a}$ | 0.64±0.02$^{a}$ | 79.54± 0.43$^{b}$ | 0.51±0.02$^{a}$ |
| 140°C, 30% | 0.97±0.00$^{a}$ | 1.30±0.01$^{ab}$ | 0.87±0.03$^{a}$ | 1.43±0.02$^{a}$ | 1.04±0.01$^{ab}$ | 1.10±0.01$^{a}$ | 1.47±0.22$^{a}$ | 1.14±0.06$^{a}$ | 0.65±0.01$^{a}$ | 81.74± 0.3$^{c}$ | 0.53±0.01$^{a}$ |
| ***Yellow Pea*** | | | | | | | | | | | |
| Control | 0.97±0.07$^{A}$ | 1.31±0.08$^{A}$ | 0.85±0.05$^{A}$ | 1.43±0.09$^{A}$ | 1.04±0.07$^{A}$ | 1.10±0.09$^{A}$ | 1.50±0.02$^{A}$ | 1.13±0.08$^{A}$ | 0.65±0.01$^{A}$ | 78.48± 0.04$^{A}$ | 0.51±0.01$^{B}$ |
| 120°C, 20% | 1.04±0.03$^{A}$ | 1.32±0.03$^{A}$ | 0.83±0.06$^{A}$ | 1.44±0.03$^{A}$ | 1.04±0.03$^{A}$ | 1.06±0.02$^{A}$ | 1.51±0.07$^{A}$ | 1.21±0.03$^{A}$ | 0.64±0.02$^{A}$ | 81.44± 0.21$^{B}$ | 0.52±0.02$^{AB}$ |
| 140°C, 20% | 1.03±0.04$^{A}$ | 1.33±0.04$^{A}$ | 0.84±0.03$^{A}$ | 1.45±0.06$^{A}$ | 1.05±0.04$^{A}$ | 1.09±0.02$^{A}$ | 1.58±0.01$^{A}$ | 1.23±0.06$^{A}$ | 0.62±0.01$^{A}$ | 83.49± 0.21$^{CD}$ | 0.52±0.01$^{AB}$ |
| 120°C, 30% | 1.05±0.01$^{A}$ | 1.33±0.01$^{A}$ | 0.89±0.03$^{A}$ | 1.46±0.02$^{A}$ | 1.06±0.01$^{A}$ | 1.12±0.02$^{A}$ | 1.64±0.08$^{A}$ | 1.21±0.04$^{A}$ | 0.62±0.01$^{A}$ | 84.57± 0.64$^{D}$ | 0.53±0.01$^{A}$ |
| 140°C, 30% | 1.04±0.01$^{A}$ | 1.32±0.01$^{A}$ | 0.97±0.02$^{A}$ | 1.47±0.02$^{A}$ | 1.07±0.01$^{A}$ | 1.13±0.04$^{A}$ | 1.77±0.19$^{A}$ | 1.22±0.04$^{A}$ | 0.62±0.02$^{A}$ | 82.56± 0.51$^{BC}$ | 0.51±0.01$^{AB}$ |

[1]Means within a column followed by the same letter (lowercase or capital) are not significantly different ($p<0.05$). *Abbreviations*: THR, threonine; VAL, valine; ILE, isoleucine; LEU, leucine; HIS, histidine; LYS, lysine; and TRP, tryptophan.

[2]Methionine+Cysteine; [3]Phenylalanine + Tyrosine

(*) Indicates first limiting amino acid.

[4]Data was collected in duplicate on the triplicate processing runs and is presented as the mean ± one standard deviation (n=3).

### 4.2.2 Starch digestibility

The *in vitro* enzymatic digestion of the rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) fractions of green lentil and yellow pea flours is presented in Table 4.2.4. For both pulse flour types, similar starch digestibility trends were observed. The RDS content of the flours increased significantly ($p<0.05$) as both tempering moisture and infrared heat applied prior to milling occurred. The RDS content of the green lentil samples tempered to 30% moisture resulted in the highest content of RDS (21.69-22.74%), with infrared heat intensity not significantly ($p>0.05$) impacting RDS within the tempering group. For yellow pea flours, the samples tempered to 30% again had the highest RDS content (20.35-24.26%), but in contrast to green lentil samples, the infrared heat intensity was significant ($p<0.05$), with flours heated to 140°C having significantly higher RDS contents than those heated to 120°C within the same tempering group (30% moisture). The contents of SDS between the differing processing conditions for both pulse flour samples did not greatly vary as tempering moisture and heat increased. Green lentil samples had SDS contents between 8.9-14.8% with a significant difference only found between control group flours (SDS= 8.89%) and flours tempered to 30% moisture and heated to 140°C (SDS=14.8%). Yellow pea flours had SDS contents between 9.13-14.28%, and significant difference was found between control samples and processed samples only when at least 20% moisture and 140°C heat was applied to the seed prior to milling. As processing conditions intensified beyond this point, no further significance between groups was found. Similarly to RDS contents, the RS contents of the flours were significantly impacted by intensity of both tempering moisture and infrared heat. In contrast to RDS, as processing conditions intensified, the RS content decreased. Green lentil flour RS contents steadily decreased from 22.74% (control group), to 8.78% (flours tempered to 30% moisture and heated to 140°C). Statistical significance was found as tempering moisture increased to 20%, then to 30%, but no significance was found between temperature within each respective tempering group. The conclusion may be made that for green

lentil flours, tempering moisture had the most significant impact on RS content. Yellow pea RS contents also decreased, beginning with 23.61% for control group flours, and declining to 8.42% for samples tempered to 30% moisture and heated to 140°C. In contrast to green lentil flours, the yellow pea samples were more responsive to infrared heat within tempering groups, with samples heated to 140°C resulting in significantly lower RS contents than those heated to 120°C at the same tempering level. It may be concluded that yellow pea samples require both increased tempering and infrared heat levels to digest the starch fraction of the seed in comparison to green lentil flours, where tempering moisture is the most important factor.

Similar trends of increasing contents of RDS and SDS, and declining RS contents, for cereal and legume flours that have been pre-processed in some capacity has been reported. It is thought that any pre-processing method that alters metabolic and/or structural changes within the flour composition (e.g., germination, roasting, cooking or baking) will facilitate the breakdown of the starch fraction of the flour, increasing its susceptibility to a digestive enzymatic attack (Chung et al., 2012; Li et al., 2019; Lu et al., 2018a). Du et al. (2014) found that when a variety of pulse and cereal starches were cooked the RDS contents increased by ~76-80%, SDS contents decreased by ~11-16%, and RS contents dropped by ~60-68% for pinto bean, red kidney bean, black bean, and navy bean starches in comparison to raw starches. The increase/reduction in RDS, SDS, and RS is on a larger scale than found in this study as the researchers evaluated isolated pulse starches, not whole flours as in the current study, therefore the enzymatic digestion of the pulse starches was not hindered by protein, lipids, and other minor compositional fractions that could have slowed digestion rates of the starch present. In closer relation to this study, Qi et al. (2021) evaluated the effect that cooking of pea flour via extrusion had on the digestibility of the starch fraction within the flours. The researchers found significant ($p<0.05$) changes to the starch digestibility when the pea flour was extruded at 50°C, 70°C, and 90°C. In comparison to the native control group flour (16.26% RDS), RDS increased by 2.44%, 6.57%, and 12.08%, as temperatures increased from 50°C to 70°C and 90°C, respectively. The content of SDS varied slightly from the

control group (18.94% SDS), with extrusion cooking temperatures of 50°C and 70°C resulting in an increase of 3.76% and 1.64%, respectively, while flours extruded at 90°C had a decline of 2.92% SDS in comparison to the control group. All extruded flours had reduced RS contents in comparison to the control group (16.79% RS), with a decline of 7.38%, 11.28%, and 12.08%, as temperature increased from 50°C to 70°C and 90°C , respectively. As seen throughout the present research studies, it has been shown that as tempering moisture and infrared heat applied to the pulse seeds prior to milling increases, the structural integrity of the main compositional/functional fractions (i.e. protein and starch) are compromised. In the case of digestible starch fractions, significant positive correlation between damaged starch content and RDS and SDS can be seen for both green lentil (RDS; $r$= .893, $p$<0.01/ SDS; $r$= .803, $p$<0.01) and yellow pea flours (RDS; $r$= .898, $p$<0.01/ SDS; $r$= .788, $p$<0.01). Logically, a significant negative correlation is then seen for damaged starch content and RS for both green lentil ($r$= -.886, $p$<0.01) and yellow pea flours ($r$= -.895, $p$<0.01). An increase in damaged starch indicates the breakdown of the starch molecule as a result of starch gelatinization and decomposition caused by increased tempering moisture and applied infrared heat.

Table 4.2. 4 Digestibility of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) of green lentil and yellow pea flours.[1]

| **Process Conditions** | **RDS (%, dry matter)** | **SDS (%, dry matter)** | **RS (%, dry matter)** |
|---|---|---|---|
| ***Green Lentil*** | | | |
| Control | 8.52± 0.04$^{a}$ | 8.89± 1.08$^{a}$ | 22.74± 1.17$^{c}$ |
| 120°C, 20% | 14.00± 0.35$^{b}$ | 10.50± 0.45$^{ab}$ | 17.49± 0.68$^{b}$ |
| 140°C, 20% | 16.78± 0.62$^{c}$ | 10.63± 3.63$^{ab}$ | 16.73± 3.51$^{b}$ |
| 120°C, 30% | 22.74± 0.87$^{d}$ | 13.23± 0.34$^{ab}$ | 9.66± 1.90$^{a}$ |
| 140°C, 30% | 21.69± 1.65$^{d}$ | 14.80± 0.45$^{b}$ | 8.78± 1.92$^{a}$ |
| ***Yellow Pea*** | | | |
| Control | 8.27± 0.22$^{A}$ | 9.13± 0.29$^{A}$ | 23.61± 0.27$^{D}$ |
| 120°C, 20% | 14.49± 0.56$^{B}$ | 10.34± 0.67$^{A}$ | 18.05± 0.04$^{C}$ |
| 140°C, 20% | 19.44± 1.73$^{C}$ | 14.23± 1.33$^{B}$ | 11.75± 1.11$^{B}$ |
| 120°C, 30% | 20.35± 1.51$^{C}$ | 14.17± 0.164$^{B}$ | 11.39± 0.44$^{B}$ |
| 140°C, 30% | 24.26± 0.48$^{D}$ | 14.28± 0.57$^{B}$ | 8.42± 0.78$^{A}$ |

[1]Means within a column followed by the same letter (lowercase or capital) are not significantly different ($p<0.05$).

# 5. OVERALL CONCLUSIONS

Plant based foods and feed formulated with pulses/pulse ingredients, such as green lentils and yellow peas, are gaining popularity in the market as a result of the benefits imparted to consumers and producers alike. In Canada, there is a marked agricultural benefit to producers who choose to plant pulses, as pulse crop yields remain fair in less-than-ideal growing conditions and retain their value in market due increasing demand. In the consumer market, pulses/pulse ingredients are an increasingly popular choice to formulate with, as they impart both functional and nutritional benefits to food/feed products. As a result, products containing whole pulses/pulse ingredients provide greater value to the consumer (e.g., cleaner label, plant-based, high protein & fibre) and to the producer (e.g., less filler ingredients required, multi-functional benefits, lower cost of formulation). Although the attributed benefits to formulating with (and consuming) pulses are becoming more mainstream, the scientific knowledge database pertaining to pulse functionality and nutrition as a result of pre-processing is still in the growth phase. The objectives of this research study was to examine the change imparted to the functional and nutritional properties of green lentil and yellow peas flour milled from seeds that were pre-treated with varying levels of seed tempering moisture (20 vs. 30%) and infrared heating temperature (120 vs 140$^{o}$C). In the first study, the physicochemical and functional properties of the flours were examined. Minimal change was imparted to the proximate composition of the flours, with the exception of the damaged/gelatinized starch content, which increased significantly as a result of processing with tempering moisture and infrared heat. The protein fractions of the flours were denatured as a result of processing, as the SH of the flours increased as processing conditions intensified, and the secondary structure of the flours transitioned from a more ordered to a less ordered state. Tempering and infrared heating increased the WHC and OHC of the flours, but overall either had insignificant or detrimental effects on the other functional properties examined in this study. In

most cases, the functional properties of both pulse flour types were significantly correlated to the SH of flours (indicative of protein denaturation) and/or the damaged/gelatinized starch content of the flours (indicative of starch denaturation). As the major compositional fractions of the flours were altered as a result of processing, the exposure of buried amino acids and the increased presence of shorter chain carbohydrates in solution impeded functional properties either by altering bonding affinities, or by physically blocking functionally important interactions. It was also found that seed size is likely to affect the response to pre-processing treatments, as green lentil flour samples tended to indicate a more sensitive response (i.e., stronger correlations between studied parameters, higher prevalence of significant changes between control and treatment groups) to tempering moisture and infrared heat treatments in comparison to yellow pea flours due to their smaller seed size (i.e., increased surface area).

In study two, the nutritional properties of the flours were examined. It was found that the combined effect of tempering moisture and infrared heat applied increased the protein and starch *in vitro* digestibility of the flours, but did not improve the protein quality. The increase in IVPD and starch digestibility (i.e., increase in RDS, SDS, and decline in RS) of the green lentil and yellow pea flours was positively correlated to the content of damaged/gelatinized starch content in the flours. A positive correlation between SH and IVPD was only observed in green lentil flours. This may lead to the conclusion that the starch and protein digestibility of the flours were increased as a result of the general denaturation/loosening of the compositional structure as a result of processing, which allowed for digestive enzymes to access the protein and starch fractions of the flours more easily. The protein quality of the flours were not improved as a result of pre-processing with tempering moisture and infrared heat, and tryptophan presented as the LAA in both green lentil and yellow pea flours. The IV-PDCAAS remained within the range of 0.47- 0.53 for green lentil flours and 0.51- 0.53 for yellow pea flours. Although the partial protein denaturation (i.e., increased SH) found in study one was found to significantly effect some functional properties, the

level of denaturation is presumed to not have exposed a significantly different amino acid profile within the flours.

Based on this study, green lentil and yellow peas flours milled from seeds that were pre-treated with varying levels of seed tempering moisture (20 vs. 30%) and infrared heating temperature (120 vs 140$^{o}$C) exhibited generally mild changes in functional and nutritional properties. Depending on the end use application of the tempered and infrared heated flours, these changes, or lack thereof, may be desirable where sudden functional and nutritional changes of a formula are not beneficial. For example, it could be inferred that swapping out raw flours for tempered/heated flours on a 1:1 basis in the same formula with the same processing conditions could lead to a product with the same ingredient list and nutrition facts table, but with improved protein and starch digestibility characteristics. The lack of change in amino acid profile, and resulting IV-PDCAAS also has perceived benefits, as the tempered and infrared heated flours retain a similar amino acid profile and can be blended with cereal flours to create a product with a balanced protein content, while also imparting slightly improved functional properties such as WHC and OHC, and protein and starch digestibility.

# 6. FUTURE STUDIES

In this study, the effect of seed tempering moisture (20 vs. 30%) and infrared heating temperature (120 vs 140°C) on the functional and nutritional properties of green lentil and yellow flours were examined. Generally speaking, as the seeds were tempered to a higher moisture level and then infrared heated, a higher degree of protein and starch denaturation was observed, which in turn effected the functional and nutritional properties to varying degrees. What would be beneficial to examine in future studies is the effect that tempering moisture and time, together, have on the green lentil and yellow pea seeds prior to infrared heating. In similar studies, prolonged tempering time (24 h, 48 h, 96h) was shown to impart a greater impact on content of damaged/gelatinized starch. As the damaged/gelatinized starch content of the flours in this study was often correlated to the functional and nutritional properties, it could be presumed that increasing the tempering time from 1 h to upward of 24 h would result in more significant functional and nutritional changes between the control group and processed flours than were found in this study. It may also be beneficial to include both the quantitative and qualitative changes imparted to the seed morphology of the pulse seeds as a result of tempering moisture and time and applied infrared heat, and how this these changes may affect the milling process, and the resulting functional and nutritional properties of the flours.

In future studies it would also be beneficial to conduct a more controlled experimental design of the milling process of the control and tempered/infrared heat pulse seeds. In this study, the pulse seeds were milled in their whole form and were not further sieved to a uniform particle size prior to analysis. This does not correlate well to what is generally done in industry, where it is much more common to de-hull and split pulse seeds prior to milling to save on energy inputs, increase yields of flour fractions, and create value from the efficiently separated flour fractions. It

would be interesting to understand the effect of the milling process for whole vs. split pulse seeds on the benchtop version of mills commonly utilized in industry (e.g., roller mills, hammer mills), and the related post-milling sieving steps to control/standardize flour particle size, to better understand how tempered/infrared heated seeds would perform on an industrial scale. The control of particle size is also likely to impart differences to the functionality of the tempered/infrared flours, as larger vs. smaller particle sizes in the same flour suspension will interact differently in solution.

In terms of protein quality, it would be interesting to further understand the correlation between *in vitro* digestibility and protein quality assessments (i.e., IVPD, IV-PDCAAS) and *in vivo* methods (i.e., PDCAAS, PER, DIAAS) utilized in both animal and human studies. The standardization of one method, preferably *in vitro* for ease of assessment, by the WHO would prevent the over/under estimation of protein quality in food products. This is particularly important for the assessment of protein quality in plant-based foods, which are entering the market at a dizzying speed, as they will play an integral role in feeding the growing population as alternative sources of protein. Another factor that could be included in a future study is the presence of, or lack of, common ANFs in the pulse flours as a result of pre-treating with tempering moisture and infrared heating, and how this may have affected the lack of change in protein quality in the current study. Also interesting to further examine would be the role that pulse type variety and plant bioengineering has on the metabolic pathways of amino acid synthesis in pulses. In this study, it was unexpectedly discovered that TRP was found to be the LAA in the flours, when it is instead more common to see SCAA (e.g., MET+CYS) to be limiting in nature. This was found to likely be related to the crop reports from the green lentil and yellow pea harvest year (2018), where crop soil moisture was below optimal levels, which may have contributed to amino acid level differences in the plants as a result of stress. In the face of climate change, it is becoming more imperative than ever to understand how pulse crops will respond to stress and change in growing conditions, and how this in turn may affect protein quality of the plant.

Lastly, it would be interesting to understand what effect tempering moisture and infrared heat have on the flours in actual food applications, and how the pre-processing treatment could add value in the market to the resulting ingredient. Attributes such as the flavour/aromatic profiles of pulse flours after processing, and how the presumed 'toasted' taste may have the ability to reduce or eliminate the need for flavour masking ingredients in certain applications. It would also be beneficial to assess the effect tempering and infrared heat have on the microbial load of the flours. If an experiment was designed to understand the necessary tempering and infrared heat parameters required to produce a ready-to-eat (RTE) ingredient, the processed pulse flours may have the ability to be incorporated into formulas for applications where a further cooking/heat treatment step is not required (e.g., beverages, novel treats such a 'raw' cookie dough, vegan liquid eggs, etc.).

## 7. REFERENCES


AACC (1999). *Approved Methods of Analysis (9th Ed*.). St. Paul, MN: The American Association of Cereal Chemists.

AACC (2000). *Approved methods of analysis, (10th Ed.).* St. Paul, MN: The American Association of Cereal Chemists.

Abbey, B., & Ibeh, G. (1988). Functional properties of raw and heat processed cowpea (*Vigna unguiculata, Walp*) flour. *Journal of Food Science*, 53(6) 1975–1791.

Adebiyi, A. P., & Aluko, R. E. (2011). Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum L*.) seed protein isolate. *Food Chemistry*, *128*(4), 902–908.

Aguilera, Y., Esteban, R. M., Benítez, V., Mollá, E., & Martín-Cabrejas, M. A. (2009). Starch, functional properties, and microstructural characteristics in chickpea and lentil as affected by thermal processing. *Journal of Agricultural and Food Chemistry*, *57*(22), 10682–10688.

Ai, Jin, Y., Kelly, J. D., & Ng, P. K. W. (2017). Composition, Functional Properties, Starch Digestibility, and Cookie-Baking Performance of Dry Bean Powders from 25 Michigan-Grown Varieties. *Cereal Chemistry*, *94*(3), 400–408.

Alsaffar, A. A. (2011). Effect of food processing on the resistant starch content of cereals and cereal products – a review. *International Journal of Food Science & Technology,* 46: 455-462.

Alonso, R., Aguirre, A., and Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. Food Chemistry, 68(2), 159-165.

Amagliani, L., Costa Silva, J. V., Saffon, M., & Dombrowski, J. (2021). On the foaming properties of plant proteins: Current status and future opportunities. *Trends in Food Science &*

*Technology*, *118*(PA), 261–272.

Amagliani, L., & Schmitt, C. (2017). Globular plant protein aggregates for stabilization of food foams and emulsions. *Trends in Food Science and Technology*, *67*, 248–259.

AOAC (2012). *Official Methods of Analysis of AOAC International* (*19th Ed*.). Gaithersburg, MD: Association of Official Analytical Chemists, Inc.

Arntfield, S. D., Scanlon, M. G., Malcolmson, L. J., Watts, B., Ryland, D., and Savoie, V. (1997). Effect of tempering and end moisture content on the quality of micronized lentils. *Food Research International*, *30*(5), 371-380.

Aryee, A. N. A., & Boye, J. I. (2017). Comparative study of the effects of processing on the nutritional, physicochemical and functional properties of lentil. *Journal of Food Processing and Preservation*, *41*(1).

Ashraf, S., Saeed, S. M. G., Sayeed, S. A., & Ali, R. (2012). Impact of microwave treatment on the functionality of cereals and legumes. *International Journal of Agriculture and Biology*, 14(3), 365–370.

Bai, T., Nosworthy, M. G., House, J. D., & Nickerson, M. T. (2018). Effect of tempering moisture and infrared heating temperature on the nutritional properties of desi chickpea and hull-less barley flours, and their blends. *Food Research International*, *108*, 430–439.

Bai, T., Stone, A. K., & Nickerson, M. T. (2018). Effect of tempering moisture and infrared heating temperature on the functionality of Desi chickpea and hull-less barley flours. *Cereal Chemistry*, *95*(4), 508–517.

Baptista, A., Pinho, O., Pinto, E., Casal, S., Mota, C., & Ferreira, I. M. P. L. V. O. (2017). Characterization of protein and fat composition of seeds from common beans (Phaseolus vulgaris L.), cowpea (Vigna unguiculata L. Walp) and bambara groundnuts (Vigna subterranea L. Verdc) from Mozambique. *Journal of Food Measurement and Characterization*, *11*(2), 442–450.

Bellido, G., Arntfield, S. D., Cenkowski, S., and Scanlon, M. (2006). Effects of micronization pretreatments on the physicochemical properties of navy and black beans (*Phaseolus vulgaris L.*). *LWT-Food Science and Technology*, *39*(7), 779-787.

Bencini, M. C. (1986). Functional properties ofdrum-dried chickpea (*Cicer arietinum L.*) flours. *Journal of Food Science,* 51(6), 1518−1521.

Benmeziane-Derradji, F., Djermoune-Arkoub, L., Ayat, N. E. H., & Aoufi, D. (2020). Impact of roasting on the physicochemical, functional properties, antioxidant content and microstructure changes of Algerian lentil (*Lens culinaris*) flour. *Journal of Food Measurement and Characterization*, *14*(5), 2840–2853.

Bienvenido, O.J. (1984). Rice starch: production, properties and uses. In R.L. Whistler, J.N. Bemiller and E.F. Paschall (Ed's.), Starch: Chemistry and Technology (pp 510-513). Orlando, FL: Academic Press, INC.

Bishnoi, S., & Khetarpaul, N. (1993). Effect of domestic processing and cooking methods on in-vitro starch digestibility of different pea cultivars (*Pisum sativum*). *Food Chemistry*, *47*(2), 177–182.

Borowska, J., Zadernowski, R., Borowski, J., & Swiecicki, W. K. (1998). Intravarietal differentiation of pea (*Pisum sativum* L. subsp. sativum*)* seeds – Their chemical composition and physical properties. *Plant Breeding and Seed Science*, 42(2), 75–85.

Bourré, Frohlich, P., Young, G., Borsuk, Y., Sopiwnyk, E., Sarkar, A., Nickerson, M. T., Ai, Y., Dyck, A., & Malcolmson, L. (2019). Influence of particle size on flour and baking properties of yellow pea, navy bean, and red lentil flours. *Cereal Chemistry*, *96*(4), 655–667.

Boye, J. I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., & Rajamohamed, S. H. (2010). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International*, *43*(2), 537–546.

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional

properties and applications in food and feed. *Food Research International*, *43*(2), 414–431.

Butts, Monro, J. A., & Moughan, P. J. (2012). In vitro determination of dietary protein and amino acid digestibility for humans. *British Journal of Nutrition*, *108*(S2), S282–S287.

Campos-Vega, R., Loarca-Piña, G., & Oomah, B. D. (2010). Minor components of pulses and their potential impact on human health. *Food Research International*, *43*(2), 461–482.

Campos-Vega, R., Oomah, B. D., Loarca-Piña, G., & Vergara-Castañeda, H. A. (2013). Legumes leading the war against "diabesity-the obesity-diabetes epidemic": An overview. *Legumes: Types, Nutritional Composition and Health Benefits* (pp. 93–123).

Canadian Pulses. (2022). *What is a Pulse?* Pulse Canada.

Candela, M., Astiasaran, I., & Bello, J. (1997). Cooking and warm-holding: Effect on general composition and amino acids of kidney beans (*Phaseolus vulgaris*), chickpeas (C*icer arietinum*), and lentils (*Lens culinaris*). *Journal of Agricultural and Food Chemistry*, *45*(12), 4763–4767.

Cappellini, M., & Fiorelli, G. (2008). Glucose-6-phosphate dehydrogenase deficiency. *The Lancet*, *371*(9606), 64–74.

Carbonaro, M., Maselli, P., & Nucara, A. (2012). Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: A Fourier transform infrared (FT-IR) spectroscopic study. *Amino Acids*, *43*(2), 911–921.

Carbonaro, Marina, Maselli, P., Dore, P., & Nucara, A. (2008). Application of Fourier transform infrared spectroscopy to legume seed flour analysis. *Food Chemistry*, *108*(1), 361–368.

Carbonaro, Marina, Maselli, P., & Nucara, A. (2015). Structural aspects of legume proteins and nutraceutical properties. *Food Research International*, *76*(P1), 19–30.

Chang, C., Tu, S., Ghosh, S., & Nickerson, M. T. (2015). Effect of pH on the inter-relationships between the physicochemical, interfacial and emulsifying properties for pea, soy, lentil and canola protein isolates. *Food Research International*, *77*, 360–367.

Champ, M. M. (2002). Non-nutrient bioactive substances of pulses. *British Journal of Nutrition,*

*88*(S3), 307-319.

Chen, B., McClements, D. J., Gray, D. A., & Decker, E. A. (2012). Physical and oxidative stability of pre-emulsified oil bodies extracted from soybeans. *Food Chemistry*, *132*(3), 1514–1520.

Chung, H. J., Cho, D. W., Park, J. D., Kweon, D. K., & Lim, S. T. (2012). In vitro starch digestibility and pasting properties of germinated brown rice after hydrothermal treatments. *Journal of Cereal Science*, *56*(2), 451–456.

Chung, H. J., Liu, Q., Peter Pauls, K., Fan, M. Z., & Yada, R. (2008). In vitro starch digestibility, expected glycemic index and some physicochemical properties of starch and flour from common bean (Phaseolus vulgaris L.) varieties grown in Canada. *Food Research International*, *41*(9), 869–875.

Curran, J. (2012). The nutritional value and health benefits of pulses in relation to obesity, diabetes, heart disease and cancer. *British Journal of Nutrition*, *108*(S1), S1–S2.

Dalgetty, D. D., & Baik, B.-K. (2003). Isolation and characterization of cotyledon fibers from peas, lentils, and chickpeas. Cereal Chemistry, 80(3), 310–315.

De Spiegeleer, M., De Paepe, E., Van Meulebroek, L., Gies, I., De Schepper, J., & Vanhaecke, L. (2021). Paediatric obesity: a systematic review and pathway mapping of metabolic alterations underlying early disease processes. *Molecular Medicine (Cambridge, Mass.)*, *27*(1), 145–145.

Deepa, C., & Hebbar, H. U. (2016). Effect of high-temperature short-time 'micronization' of grains on product quality and cooking characteristics. *Food Engineering Reviews*, *8*(2), 201–213.

Deotale, S., Dutta, S., Moses, J. A., Balasubramaniam, V. M., & Anandharamakrishnan, C. (2020). Foaming characteristics of beverages and its relevance to food processing. *Food Engineering Reviews*, *12*(2), 229–250.

Devindra, S., & Aruna, T. (2016). Effect of chemical soaking, toasting and crude α-galactosidase enzyme treatment on the oligosaccharide content of red gram flour. *Journal of Food*

*Processing and Preservation, 41*(3), 1-8.

Dombrowski, J., Gschwendtner, M., & Kulozik, U. (2017). Evaluation of structural characteristics determining surface and foaming properties of β-lactoglobulin aggregates. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *516*, 286–295.

Donihee, L., & McDougall, T. (2021). Canada: Outlook For Principal Field Crops. *Agriculture and Agri-Food Canada*.

Donihee, L., & McDougall, T. (2022). Canada: Outlook For Principal Field Crops. *Agriculture and Agri-Food Canada*.

Du, Jiang, H., Yu, X., & Jane, J. (2014). Physicochemical and functional properties of whole legume flour. *Food Science & Technology*, *55*(1), 308–313.

Emami, S., Meda, V., & Tyler, R. T. (2011). Effect of micronisation and electromagnetic radiation on physical and mechanical properties of Canadian barley. *International Journal of Food Science and Technology*, *46*(2), 421–428.

Englyst, H. ., Kingman, S. ., & Cummings, J. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46*(2), S33-S50.

Englyst, H., Wiggins, H. S., & Cummings, J. H. (1982). Determination of the non-starch polysaccharides in plant foods by gas - Liquid chromatography of constituent sugars as alditol acetates. *The Analyst*, *107*(1272), 307–318.

Erskine, W., Williams, P.C., & Nakkou, H. (1991). Splitting and dehulling lentil: effects of seed size and different pre-treatments. J. Sci. Food. Agric. 57, 77-84.

FAO/WHO. (1991). *Protein quality evaluation. Report of the joint FAO/WHO expert consultation. Food and nutrition paper No. 51*. Rome: Food and Agriculture Organizations and the World Health Organization.

FAO/WHO. (2013). *Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. Food and nutrition paper No. 92*. Rome: Food and Agriculture

Organizations and the World Health Organization.

Fasina, O. O., Tyler, R. T., Pickard, M. D., & Zheng, G. H. (1999). Infrared heating of hulless and pearled barley. *Journal of Food Processing and Preservation*, *23*, 135–151.

Fasina, O., Tyler, B., Pickard, M., Zheng, G. H., & Wang, N. (2001). Effect of infrared heating on the properties of legume seeds. *International Journal of Food Science and Technology*, *36*(1), 79–90.

Fernandez-Quintela, A., Macarulla, M. T., Barrio, A. S. del, & Martinez, J. A. (1997). Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. *Plant Foods for Human Nutrition*, *51*(4), 331–342.

Ferretti, F., & Mariani, M. (2017). Simple vs. complex carbohydrate dietary patterns and the global overweight and obesity pandemic. *International Journal of Environmental Research and Public Health*, *14*(10).

Frohlich, P., Boux, G., & Malcolmson, L. (2014). Pulse ingredients as healthier options in extruded products. *Cereal Foods World*, *59*(3), 120–125.

Frohlich, Peter, Young, G., Borsuk, Y., Sigvaldson, M., Bourré, L., & Sopiwnyk, E. (2021). Influence of premilling thermal treatments of yellow peas, navy beans, and fava beans on the flavor and end-product quality of tortillas and pitas. *Cereal Chemistry*, *98*(3), 802–813.

Fuhrmeister, H., & Meuser, F. (2003). Impact of processing on functional properties of protein products from wrinkled peas. *Journal of Food Engineering*, *56*(2–3), 119–129.

Galili, G., Amir, R., Hoefgen, R., & Hesse, H. (2005). Improving the levels of essential amino acids and sulfur metabolites in plants. *Biological Chemistry*, *386*(9), 817–831.

Ghribi, A. M., Gafsi, I. M., Blecker, C., Danthine, S., Attia, H., & Besbes, S. (2015). Effect of drying methods on physico-chemical and functional properties of chickpea protein concentrates. *Journal of Food Engineering*, *165*, 179–188.

Guerrero, P., Beatty, E., Kerry, J.P., & de la Caba, K. (2012). Extrusion of soy protein with gelatin and sugars at low moisture content. *Journal of Food Engineering*, 110(1), 53-59.

Guldiken, Konieczny, D., Franczyk, A., Satiro, V., Pickard, M., Wang, N., House, J., & Nickerson, M. T. (2022). Impacts of infrared heating and tempering on the chemical composition, morphological, functional properties of navy bean and chickpea flours. *European Food Research & Technology*, *248*(3), 767–781.

Guldiken, B., Konieczny, D., Wang, N., Hou, A., House, J. D., Tu, K., … Nickerson, M. T. (2021). Effect of variety and environment on the physicochemical, functional, and nutritional properties of navy bean flours. *European Food Research and Technology*, *247*(7), 1745–1756.

Guldiken, B., Yovchev, A., Nosworthy, M. G., Stone, A. K., House, J. D., Hood-Niefer, S., & Nickerson, M. T. (2020). Effect of extrusion conditions on the physical properties of desi chickpea-barley extrudates and quality attributes of their resulting flours. *Journal of Texture Studies*, *51*(2), 300–307.

Gunes, Z. S., & Karaca, A. C. (2022). Examining the amino acid composition, secondary structure, and physicochemical and functional properties of proteins isolated from local lentil landraces of Anatolia. *Cereal Chemistry*, *99*(1), 78–89.

Gürbüz, G., Liu, C., Jiang, Z. qing, Pulkkinen, M., Piironen, V., Sontag-Strohm, T., & Heinonen, M. (2018). Protein–lipid co-oxidation in emulsions stabilized by microwave-treated and conventional thermal-treated faba bean proteins. *Food Science and Nutrition*, *6*(4), 1032–1039.

Hall, A.E & Moraru, C. I. (2021). Structure and function of pea, lentil and faba bean proteins treated by high pressure processing and heat treatment. *Food Science & Technology*, *152*, 112349.

Hall, C., Hillen, C., & Robinson, J. G. (2017). Composition, nutritional value, and health benefits of pulses. *Cereal Chemistry*, *94*(1), 11–31.

Hefnawy, T. H. (2011). Effect of processing methods on nutritional composition and anti-nutritional factors in lentils (*Lens culinaris*). *Annals of Agricultural Sciences*, *56*(2), 57–61.

Hoseney, R., and Delcour, J. (Eds.). (2010). *Principles of cereal science and technology* (3rd ed.). St. Paul, Minn.: AACC International.

Hoover, R., Hughes, T., Chung, H. J., & Liu, Q. (2010). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International*, *43*(2), 399–413.

Ipsos Reid (2010). Factors Influencing Pulse Consumption in Canada: Final Report. *Alberta Agriculture and Rural Development, with the direction and collaboration of Alberta Pulse Growers Commission and Pulse Canada.* Calgary, AB, Canada.

Jarpa-Parra, M. (2018). Lentil protein: a review of functional properties and food application. An overview of lentil protein functionality. *International Journal of Food Science and Technology*, *53*(4), 892–903.

Jeske, S., Bez, J., Arendt, E. K., & Zannini, E. (2019). Formation , stability , and sensory characteristics of a lentil - based milk substitute as affected by homogenisation and pasteurisation. *European Food Research and Technology*, *245*(7), 1519–1531.

Jogihalli, Singh, L., & Sharanagat, V. S. (2017). Effect of microwave roasting parameters on functional and antioxidant properties of chickpea (*Cicer arietinum*). *Food Science & Technology*, *79*, 223–233.

Joshi, M., Adhikari, B., Aldred, P., Panozzo, J. F., Kasapis, S., & Barrow, C. J. (2012). Interfacial and emulsifying properties of lentil protein isolate. *Food Chemistry*, *134*(3), 1343–1353.

Joshi, Matina, Timilsena, Y., & Adhikari, B. (2017). Global production, processing and utilization of lentil: A review. *Journal of Integrative Agriculture*, *16*(12), 2898–2913.

Kabakci, C., Sumnu, G., Sahin, S., & Oztop, M. H. (2021). Encapsulation of magnesium with lentil flour by using double emulsion to produce magnesium enriched cakes. *Food and Bioprocess Technology*, *14*(10), 1773–1790.

Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food*

*Research International*, *44*(9), 2742–2750.

Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochimica et Biophysica Acta (BBA)-Protein Structure, 624(1), 13-20.

Khattab, R. Y., Arntfield, S. D., & Nyachoti, C. M. (2009). Nutritional quality of legume seeds as affected by some physical treatments, part 1: Protein quality evaluation. *LWT- Food Science and Technology*, 42(6), 1107–1112.

Kim, M. J., Choi, S. J., Shin, S. I., Sohn, M. R., Lee, C. J., Kim, Y., … Moon, T. W. (2008). Resistant glutarate starch from adlay: Preparation and properties. *Carbohydrate Polymers*, *74*(4), 787–796.

Kiosseoglou, V. & Paraskevopoulou, A. (2011). Functional and physiochemical properties of pulse proteins. In Tiwari, B.K., Gowen, A. & McKenna, B. (Ed.), Pulse Foods: Processing, Quality and Nutraceutical Applications (pp. 57-90). London, UK, Burlingtion, MA, San Diego, CA: Elsevier Inc.

Kumar, K. A., Sharma, G. K., Khan, M. A., & Semwal, A. D. (2016). A study on functional, pasting and micro-structural characteristics of multigrain mixes for biscuits. *Journal of Food Measurement and Characterization*, *10*(2), 274–282.

Lam, A. C. Y., Can Karaca, A., Tyler, R. T., & Nickerson, M. T. (2018). Pea protein isolates: Structure, extraction, and functionality. *Food Reviews International*, *34*(2), 126–147.

Lam, R. S. H., & Nickerson, M. T. (2013). Food proteins: A review on their emulsifying properties using a structure-function approach. *Food Chemistry*, *141*(2), 975–984.

Law, H. Y., Choi, S. M., & Ma, C. Y. (2008). Study of conformation of vicilin from Dolichos lablab and Phaseolus calcaratus by Fourier-transform infrared spectroscopy and differential scanning calorimetry. *Food Research International*, *41*(7), 720–729.

Lee, S. C., Jeong, S. M., Kim, S. Y., Park, H. R., Nam, K. C., & Ahn, D. U. (2006). Effect of far-infrared radiation and heat treatment on the antioxidant activity of water extracts from peanut

hulls. *Food Chemistry,* 94(4): 489–493.

Leszczynski, W. (2004). Resistant starch- classification, structure, production. *Polish Journal of Food and Nutrition Sciences*, *13*(54), 37–50.

Li, Yuan, T. Z., Setia, R., Raja, R. B., Zhang, B., & Ai, Y. (2019). Characteristics of pea, lentil and faba bean starches isolated from air-classified flours in comparison with commercial starches. *Food Chemistry*, *276*, 599–607.

Liu, Yin, H., Pickard, M., & Ai, Y. (2020). Influence of infrared heating on the functional properties of processed lentil flours: A study focusing on tempering period and seed size. *Food Research International*, *136*, 109568–109568.

Lönnerdal, B. (2003). Nutritional and physiologic significance of human milk proteins. *The American Journal of Clinical Nutrition*, *77*(6), 1537S-1543S.

López-martínez, L. X., Leyva-lópez, N., Gutiérrez-grijalva, E. P., & Heredia, J. B. (2017). Effect of cooking and germination on bioactive compounds in pulses and their health benefits, *38*, 624–634.

Loveday, S. M. (2020). Plant protein ingredients with food functionality potential. *Nutrition Bulletin*, *45*(3), 321–327.

Lu, Z. H., Donner, E., & Liu, Q. (2018). Effect of roasted pea flour/starch and encapsulated pea starch incorporation on the in vitro starch digestibility of pea breads. *Food Chemistry*, *245*, 71–78.

Lu, Z. H., Donner, E., Tsao, R., Ramdath, D. D., & Liu, Q. (2018). Physicochemical and digestion characteristics of flour and starch from eight Canadian red and green lentils. *International Journal of Food Science and Technology*, *53*(3), 735–746.

Ma, Z., Boye, J. I., Simpson, B. K., Prasher, S. O., Monpetit, D., & Malcolmson, L. (2011). Thermal processing effects on the functional properties and microstructure of lentil, chickpea, and pea flours. *Food Research International*, *44*(8), 2534–2544.

Mang, D. Y., Abdou, A. B., Njintang, N. Y., Djiogue, E. J. M., Bernard, C., Scher, J., & Mbofung,

M. C. (2015). Effect of dehulling and boiling on the physico-chemical, functional and pasting properties of two varieties of Mucuna bean (*Mucuna pruriens L.*) flours. *Journal of Food Measurement and Characterization*, *9*(3), 435–447.

Marinangeli, C. P. F., Foisy, S., Shoveller, A. K., Porter, C., Musa-Veloso, K., Sievenpiper, J. L., & Jenkins, D. J. A. (2017). An appetite for modernizing the regulatory framework for protein content claims in Canada. *Nutrients*, *9*(9), 1–19.

Mession, J. L., Chihi, M. L., Sok, N., & Saurel, R. (2015). Effect of globular pea proteins fractionation on their heat-induced aggregation and acid cold-set gelation. *Food Hydrocolloids*, *46*, 233–243.

Meyer, D., & Stasse-Wolthuis, M. (2009). The bifidogenic effect of inulin and oligofructose and its consequences for gut health. *European Journal Of Clinical Nutrition*, *63*, 1277.

Millar, K. A., Gallagher, E., Burke, R., McCarthy, S., & Barry-Ryan, C. (2019). Proximate composition and anti-nutritional factors of fava-bean (*Vicia faba*), green-pea and yellow-pea (*Pisum sativum*) flour. *Journal of Food Composition and Analysis*, *82*, 103233.

Ministry of Agriculture. (2018). *Crop report - For the Period August 21 to 27, 2018*. Government of Saskatchewan.

Morneau, D. J. K., Jaworski, A. F., & Aitken, S. M. (2013). Identification of cystathionine γ-synthase and threonine synthase from *Cicer arietinum* and *Lens culinaris*. *Biochem. Cell. Biol.*, *91*, 95–101.

Mudryj, A. N., Yu, N., & Aukema, H. M. (2014). Nutritional and health benefits of pulses. *Applied Physiology, Nutrition, and Metabolism*, *39*(11), 1197–1204.

Mudryj, A. N., Yu, N., Hartman, T. J., Mitchell, D. C., Lawrence, F. R., & Aukema, H. M. (2012). Pulse consumption in Canadian adults influences nutrient intakes. *British Journal of Nutrition*, *108*(S1), S27–S36.

Mwangwela, A. M., Waniska, R. D., McDonough, C., & Minnaar, A. (2007). Cowpea cooking characteristics as affected by micronisation temperature: a study of the physicochemical and

functional properties of starch. *Journal of the Science of Food and Agriculture*, *87*, 399–410.

Mwangwela, A. M., Waniska, R. D., & Minnaar, A. (2007). Effect of micronisation temperature (130 and 170 °C) on functional properties of cowpea flour. *Food Chemistry*, *104*(2), 650–657.

Nidhina, N., and Muthukumar, S. P. (2015). Antinutritional factors and functionality of protein-rich fractions of industrial guar meal as affected by heat processing. *Food Chemistry*, 173, 920- 926.

Nosworthy, M. G., & House, J. D. (2017). Factors influencing the quality of dietary proteins: Implications for pulses. *Cereal Chemistry*, *94*(1), 49–57.

Nosworthy, M.G., Medina, G., Franczyk, A. J., Neufeld, J., Appah, P., Utioh, A., Frohlich, P., & House, J. D. (2018). Effect of processing on the in vitro and in vivo protein quality of beans (*Phaseolus vulgaris* and *Vicia Faba*). *Nutrients*, *10*(6), 671.

Nosworthy, M. G., Neufeld, J., Frohlich, P., Young, G., Malcolmson, L., & House, J. D. (2017). Determination of the protein quality of cooked Canadian pulses. *Food Science and Nutrition*, *5*(4), 896–903.

Oomah, B.D., Patras, A., Rawson, A., Singh, N. & R. Compos- Vegas (2011). Chemistry of pulses. In Tiwari, B.K., Gowen, A. & McKenna, B. (Ed.), Pulse Foods: Processing, Quality and Nutraceutical Applications (pp. 9-56). London, UK, Burlingtion, MA, San Diego, CA: Elsevier Inc.

Papanikolaou, Y., & Fulgoni, V. L. (2008). Bean Consumption Is Associated with Greater Nutrient Intake, Reduced Systolic Blood Pressure, Lower Body Weight, and a Smaller Waist Circumference in Adults: Results from the National Health and Nutrition Examination Survey 1999-2002. *Journal of the American College of Nutrition*, *27*(5), 569–576.

Pelgrom, P. J. M., Boom, R. M., & Schutyser, M. A. I. (2014). Functional analysis of mildly refined fractions from yellow pea. *Food Hydrocolloids*, *44*, 12–22.

Peng, W., Kong, X., Chen, Y., Zhang, C., Yang, Y., & Hua, Y. (2016). Effects of heat treatment

on the emulsifying properties of pea proteins. *Food Hydrocolloids*, *52*, 301–310.

Prell, Bourdès, A., Kumar, S., Lodwig, E., Hosie, A., Kinghorn, S., White, J., & Poole, P. (2010). Role of symbiotic auxotrophy in the Rhizobium-legume symbioses. *PloS One*, *5*(11), e13933–e13933.

Price, C., Kiszonas, A. M., Smith, B., & Morris, C. F. (2021). Roller milling performance of dry yellow split peas: Mill stream composition and functional characteristics. *Cereal Chemistry*, *98*(3), 462–473.

Primozic, M., Duchek, A., Nickerson, M., & Ghosh, S. (2018). Formation, stability and in vitro digestibility of nanoemulsions stabilized by high-pressure homogenized lentil proteins isolate. *Food Hydrocolloids*, *77*, 126–141.

Prinyawiwatkul, W., Beuchat, L. R., McWatters, K. H., & Phillips, R. D. (1997). Functional Properties of Cowpea (*Vigna unguiculata*) Flour As Affected by Soaking, Boiling, and Fungal Fermentation. *Journal of Agricultural and Food Chemistry*, *45*(2), 480–486.

Qi, M., Zhang, G., Ren, Z., He, Z., Peng, H., Zhang, D., & Ma, C. (2021). Impact of Extrusion Temperature on In Vitro Digestibility and Pasting Properties of Pea Flour. *Plant Foods for Human Nutrition*, *76*(1), 26–30.

Raghunathan, R., Hoover, R., Waduge, R., Liu, Q., & Warkentin, T. D. (2017). Impact of molecular structure on the physicochemical properties of starches isolated from different field pea (*Pisum sativum L.*) cultivars grown in Saskatchewan, Canada. *Food Chemistry*, *221*, 1514–1521.

Raigond, P., Ezekiel, R., & Raigond, B. (2015). Resistant starch in food: A review. *Journal of the Science of Food and Agriculture*, *95*(10), 1968–1978.

Rastogi, N. K. (2012). Recent trends and developments in infrared heating in food processing. *Critical Reviews in Food Science and Nutrition*, *52*(9), 737–760.

Rathod, & Annapure, U. S. (2016). Effect of extrusion process on antinutritional factors and protein and starch digestibility of lentil splits. *Food Science & Technology*, *66*, 114–123.

Riadh, M. H., Ahmad, S. A. B., Marhaban, M. H., & Soh, A. C. (2015). Infrared Heating in Food Drying: An Overview. *Drying Technology*, *33*(3), 322–335.

Ribéreau, S., Aryee, A. N. A., Tanvier, S., Han, J., & Boye, J. I. (2018). Composition, digestibility, and functional properties of yellow pea as affected by processing. *Journal of Food Processing and Preservation*, *42*(1).

Romano, A., Gallo, V., Ferranti, P., & Masi, P. (2021). Lentil flour: nutritional and technological properties, in vitro digestibility and perspectives for use in the food industry. *Current Opinion in Food Science*, *40*, 157–167.

Sánchez-Velázquez, O. A., Ribéreau, S., Mondor, M., Cuevas-Rodríguez, E. O., Arcand, Y., & Hernández-Alvarez, Á. J. (2021). Impact of processing on the in vitro protein quality, bioactive compounds, and antioxidant potential of 10 selected pulses. *Legume Science*, *2*(2), n/a-n/a.

Sanz, L. C., Perez, A. G., Rios, J. J., & Olias, J. M. (1992). Physico-chemical properties of chickpea lipoxygenases. *Phytochemistry*, *31*(10), 3381–3384.

Scanlon, M. G., Cenkowski, S., Segall, K. I., and Arntfield, S. D. (2005). The physical properties of micronised lentils as a function of tempering moisture. *Biosystems Engineering*, *92*(2), 247- 254.

Scanlon, M.G., Thakur, S., Tyler, R. ., Milani, A., Der, T., & Paliwal, J. (2018). The critical role of milling in pulse ingredient functionality. *Cereal Foods World*, *63*(5), 201–206.

Sharma, P., & Gujral, H. S. (2011). Effect of sand roasting and microwave cooking on antioxidant activity of barley. *Food Research International,* 44(1), 235–240.

Shevkani, K., Singh, N., Chen, Y., Kaur, A., & Yu, L. (2019). Pulse proteins: secondary structure, functionality and applications. *Journal of Food Science and Technology*, *56*(6), 2787–2798.

Shevkani, K., Singh, N., Kaur, A., & Rana, J. C. (2015). Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study. *Food Hydrocolloids*, *43*, 679–689.

Shi, L., Mu, K., Arntfield, S. D., & Nickerson, M. T. (2017). Changes in levels of enzyme inhibitors during soaking and cooking for pulses available in Canada. *Journal of Food Science and Technology*, *54*(4), 1014–1022.

Singh, U. & Jambunathan, R. (1982). Distribution of seed protein fractions and amino acids in different anatomical parts of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.). *Plant Foods for Human Nutrition,* 31(4): 347-354.

Slavin, J. (2013). Fiber and prebiotics: Mechanisms and health benefits. *Nutrients*, *5*(4), 1417–1435.

Smejtkova, & Vaculik, P. (2018). Comparison of power consumption of a two-roll mill and a disc mill. *Agronomy Research*, *16*(2), 1486–1492.

Sosulski, F.W., (1976). Funtional properties of rapseed flours, concentrates and isolate. *Journal of Food Science*, *41*, 1349–1352.

Sosulski, F. W., & McCurdy, A. (1987). Functionality of Flours, Protein Fractions and Isolates from Field Peas and Faba Bean. *Journal of Food Science*, *52*(4), 1010–1014.

Sreerama, Y. N., Sashikala, V. B., Pratape, V. M., & Singh, V. (2012). Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. *Food Chemistry*, *131*(2), 462–468.

StatsCAN Plus (2022). *Taking the pulse of pulses on World Pulses Day*. Statistics Canada.

Stone, A. K., Parolia, S., House, J. D., Wang, N., & Nickerson, M. T. (2021). Effect of roasting pulse seeds at different tempering moisture on the flour functional properties and nutritional quality. *Food Research International*, *147*, 110489.

Sun, X. D., & Arntfield, S. D. (2010). Gelation properties of salt-extracted pea protein induced by heat treatment. *Food Research International*, 43, 509e515.

Sun, X. D., & Arntfield, S. D. (2011). Gelation properties of salt-extracted pea protein isolate catalyzed by microbial transglutaminase cross-linking. *Food Hydrocolloids*, *25*(1), 25–31.

Sun, X. D., & Arntfield, S. D. (2012). Molecular forces involved in heat-induced pea protein

gelation: Effects of various reagents on the rheological properties of salt-extracted pea protein gels. *Food Hydrocolloids*, *28*(2), 325–332.

Tang, C. H., & Ma, C. Y. (2009). Heat-induced modifications in the functional and structural properties of vicilin-rich protein isolate from kidney (*Phaseolus vulgaris L.*) bean. *Food Chemistry*, *115*(3), 859–866.

Tharanathan, R. N., & Mahadevamma, S. (2003). Grain legumes - A boon to human nutrition. *Trends in Food Science and Technology*, *14*(12), 507–518.

Tinus, T., Damour, M., Van Riel, V., and Sopade, P. A. (2012). Particle size-starch-protein digestibility relationships in cowpea (*Vigna unguiculata*). Journal of Food Engineering, 113(2), 254-264.

Tosh, S. M., & Yada, S. (2010). Dietary fibres in pulse seeds and fractions: Characterization, functional attributes, and applications. *Food Research International*, *43*(2), 450–460.

Troya, M., Kurawadwala, H., Byrne, B., Dowdy, R., & Weston, Z. (2021). *Plant based meat: Anticipating 2030 production requirements.* The Good Food Institute.

Tiwari, B., & Singh, N. (2012). Major Constituents of Pulses. In *Pulse Chemistry and Technology* (pp. 34-49). Cambridge, UK: RSC Publishing.

Ur Rehman, H., Azam, N., Yao, J. T., & Benso, A. (2017). A three-way approach for protein function classification. *PLoS ONE*, *12*(2), 1–30.

Velu, V., Nagender, A., Prabhakara Rao, P. G., & Rao, D. G. (2006). Dry milling characteristics of microwave dried maize grains (*Zea mays L.*). *Journal of Food Engineering*, *74*(1), 30–36.

Vishwakarma, R. K., Shivhare, U. S., Gupta, R. K., Yadav, D. N., Jaiswal, A., & Prasad, P. (2018). Status of pulse milling processes and technologies: A review. *Critical Reviews in Food Science and Nutrition*, *58*(10), 1615–1628.

Walker, A. F., & Kochhar, N. (1982). Effect of processing including domestic cooking on nutritional quality of legumes. *Proceedings of the Nutrition Society*, *41*(01), 41–51.

Wang, N., Daun, J. K., and Malcolmson, L. J. (2003). Relationship between physicochemical and cooking properties, and effects of cooking on antinutrients, of yellow field peas (*Pisum sativum*). *Journal of the Science of Food and Agriculture*, *83*(12), 1228-1237.

Wang, N., Wood, J., Panozzo, J., Argansoa, G., Hall, C., Chen, L., Singh, M., & Nickerson, M. (2017). AACCI approved methods technical committee report: Collaborative study on a method for determining the water holding capacity of pulse flours and their protein materials (AACCI method 56-37.01). *Cereal Foods World*, *62*(5), 227–230.

Wang, N. (2005). Optimization of a laboratory dehulling process for lentils (*Lens culinaris*). *Cereal Chemistry*, 82(6), 671–676.

Wang, N., & Maximiuk, L. (2015). Development of an improved electrical resistance method for determining emulsifying capacity of pulse and soy materials. *Cereal Chemistry*, *92*(3), 253–257.

Wang, S., Nosworthy, M. G., House, J. D., Niefer, S. H., & Nickerson, M. T. (2020). Effect of barrel temperature and feed moisture on protein quality in pre-cooked Kabuli chickpea, sorghum, and maize flours. *Food Science and Technology International*, *26*(3), 265–274.

Wang, Y., Ghosh, S., & Nickerson, M. T. (2019). Effect of pH on the formation of electrostatic complexes between lentil protein isolate and a range of anionic polysaccharides, and their resulting emulsifying properties. *Food Chemistry*, *298*, 125023–125023.

Whalen, P.J., DesRochers, J.L. & Walker, C.E. (2000). Ready-to-eat Breakfast Cereals. In: *Handbook of Cereal Science and Technology* (edited by K. Kulp & J.G. Ponte). Pp. 615–646. New York, USA: Marcel Dekker Inc.

Wilde, P. J., and Clark, D. C. (1996). Foam formation and stability. In G. M. Hall (Ed.), Methods of testing protein functionality (pp. 110-152). London, England: Blackie Academic and Professional.

Wong, J. M. W., de Souza, R., Kendall, C. W. C., Emam, A., & Jenkins, D. J. (2006). Colonic health: fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology*, *40*,

253–43.

Wongsagonsup, R., Shobsngob, S., Oonkhanond, B., & Varavinit, S. (2005). Zeta potential (ζ) analysis for the determination of protein content in rice flour. *Starch/Staerke*, *57*(1), 25–31.

Wood, J.A. & Malcolmson, L.J. (2011). Pulse milling technologies. In Tiwari, B.K., Gowen, A. & B. McKenna (Ed.), Pulse Foods: Processing, Quality and Nutraceutical Applications (pp. 9-56). London, UK, Burlington, MA, San Diego, CA: Elsevier Inc.

Wu, Y., Eskin, N. A. M., Cui, W., & Pokharel, B. (2015). Emulsifying properties of water soluble yellow mustard mucilage: A comparative study with gum Arabic and citrus pectin. *Food Hydrocolloids*, *47*, 191–196.

Wu, W. U., Hettiarachchy, N. S., & Qi, M. (1998). Hydrophobicity, solubility, and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. *Journal of the American Oil Chemists' Society*, 75(7), 845–850.

Yang, H. W., Hsu, C. K., & Yang, Y. F. (2014). Effect of thermal treatments on anti-nutritional factors and antioxidant capabilities in yellow soybeans and green-cotyledon small black soybeans. *Journal of the Science of Food and Agriculture*, *94*(9), 1794–1801.

Yasumatsu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T., & Ishii, K. (1972). Whipping and emulsifying properties of soybean products. *Agricultural and Biological Chemistry*, 36, 719–727.

Young, G., Bourré, L., Frohlich, P., Borsuk, Y., Sarkar, A., Sopiwnyk, E., Jones, S., Dyck, A., & Malcolmson, L. (2020). Effect of roasting as a premilling treatment on the functional and bread baking properties of whole yellow pea flour. *Cereal Chemistry*, *97*(2), 183–195.

Yu, J., Ahmedna, M., & Goktepe, I. (2007). Peanut protein concentrate: Production and functional properties as affected by processing. *Food Chemistry*, *103*(1), 121–129.

Zhivotovsky, B. and Orrenius, S. (2010). Cell cycle and cell death in disease: past, present and future. *Journal of Internal Medicine,* 268: 395-409.

Žilić, S., Mogol, B. A., Akillioğlu, G., Serpen, A., Delić, N., & Gökmen, V. (2014). Effects of

extrusion, infrared and microwave processing on Maillard reaction products and phenolic compounds in soybean. *Journal of the Science of Food and Agriculture,* 94(1), 45–51.