Comparative evaluation of starch damage measurement methods and effects of heat-moisture treatment on protein structure and functional properties of pulse flours

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Abstract

Pulses, such as lentils, yellow pea and chickpea, are an important dietary staple worldwide, particularly in regions where plant-based proteins are central to nutrition. They are rich in proteins, vitamins, minerals, and carbohydrates such as dietary fiber and resistant starch (RS), which makes them beneficial for managing chronic diseases. This study aimed to (1) compare two methods for measuring damaged starch (DS) in pulse flours with different particle sizes, and (2) evaluate the effects of heat-moisture treatment (HMT) on protein structure and functionality in pulse flours.

During dry milling to produce flours, starch granules are structurally altered, resulting in damaged starch. The level of damaged starch affects flour quality. The objectives of the first part of the study were to investigate how roller dry milling affected damaged starch content in pulse flours with different particle sizes and compare the damaged starch content measured by two methods: the amperometric (SDmatic) method (AACC 76-33.01) and the enzymatic method (AACC 76-31). For all flour types and sizes, the amperometric method consistently yielded higher damaged starch content values than the enzymatic method. For fine flours, damaged starch ranged from 3.31% (chickpea) to 4.37% (yellow pea) using the amperometric method versus 0.95–1.52% by the enzymatic method. The iodide absorption (AI%) decreased as the flour particle size increased, but higher damaged starch content was obtained from the SDmatic instrument when 1 g of flour was used. To improve the accuracy of the amperometric method when using the SDmatic instrument, a larger amount (2 g) of pulse flour was used to ensure that AI% value is greater than 86% during the amperometric measurement. After adjusting the iodide-to-damaged starch ratio, a new calibration equation

was established between AI% and damaged starch content. By using the newly established equation, the amperometric method can be used to measure and obtain the damaged starch content that is correlated strongly with the enzymatic method.

In addition, investigation was conducted to evaluate the effects of HMT on protein behavior in pulse flours.

HMT is a physical method that can be used to modify the properties of flours without destroying starch granules. This method involves incubation of flours at low moisture content (<35% water w/w) and heating for a certain period. While much of the literature emphasizes starch behavior, fewer studies have addressed how HMT alters the protein–starch matrix. The thermal properties of pulse flours at various moisture contents (as-is, 20%, 25%, 30%, 40%, 50%, 60% and 75%) were analyzed by differential scanning calorimetry (DSC). The onset temperature was plotted against moisture content to obtain the phase diagram for the pulse flours. Pulse flours with different moisture levels of as-is, 20%, 25% and 30% were treated at 3, 6, 9, or 12 °C below the onset gelatinization temperature (based on the plotted phase diagram) for 4 hours. This part of the study systematically investigates the effects of HMT on the structural and functional properties of pulse proteins, focusing on protein solubility after pepsin treatment, hydrophobicity, and extractability. Results revealed a marked increase in non-soluble protein content after HMT, rising from 18.24% to 43.61% in yellow pea, 7.28% to 45.33% in lentil, and 12.25% to 84.94% in chickpea. Protein hydrophobicity increased for all pulse flours, reflecting structural changes induced by HMT. Additionally, HMT significantly decreased protein extractability in yellow pea and chickpea, with most treated samples falling below 70% compared to untreated controls, whereas lentil maintained high

extractability (>84%) across all HMT conditions. Understanding the changes in protein during HMT would enable us to target optimization of HMT conditions to enhance the nutritional and functional performance of pulse-based ingredients in food systems.

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Chapter 1 - Introduction

General Background

Pulses, the dry seeds of starchy leguminous crops, are widely recognized as an excellent source of essential nutrients and are highly recommended by health organizations and nutritionists as a fundamental component of a balanced diet due to their rich nutritional profile (Sadohara et al., 2022). They are particularly valued for their high content of protein, vitamins, minerals, and carbohydrates, making them essential for human nutrition(Aguilera et al., 2009; Argel et al., 2020; Kaale et al., 2023; Xu et al., 2019). Beyond their nutritional benefits, pulses are gaining increasing global recognition for their role in promoting sustainable agriculture and environmental preservation. They rank second only to cereals as a primary food source for both humans and animals (S. Du et al., 2014; Ma et al., 2017).

Pulses are frequently utilized in the manufacture of pulse flours, which are widely incorporated into food products and provide the previously mentioned nutritional advantages. (Setia et al., 2020). Globally, there has been an increase in demand for pulses to be incorporated into regular diets as people become more aware of its nutritional advantages. Lentils (Lens culinaris Merr.), yellow pea (Pisum sativum) and chickpea (Cicer arietinum) are the most significant crops among these pulses, and also the oldest crop species in the world (Kaale et al., 2023), which are widely grown grains and major staple food in many Middle eastern, European, Asian countries (e.g. India), and Africa (Pal et al., 2017). The most widely cultivated pulse crops include common bean, pea, chickpea, cowpea, and lentil, with global production exceeding 71 million tons, based on a five-year average from 2016 to 2020. In the United States, the annual production of common bean, pea, chickpea, and lentil

averages approximately 2.9 million tons over the same period (Sadohara et al., 2022). Between 2003 and 2006, lentils accounted for almost 6% of total global dry legume production, ranking sixth after dry beans, peas, chickpeas, faba beans, and cowpeas (*The Lentil*, 2009). In terms of environmental impact, pulse farming emits only 0.66 kg/kg of CO2, much less than living stocks production (4-29 kg/kg) (Ferawati et al., 2021).

According to Lu et al., 2018, consuming pulses has a negative correlation with the rates of cancer, heart disease, and cardiovascular disease. Among the main staple meals, it also has one of the lowest glycemic index (GI) foods (Lu et al., 2018), which has led to numerous studies highlighting their role in managing type 2 diabetes due to the benefits of low GI foods with improved blood sugar control (Ahmed et al., 2016; Aider et al., 2012). The high protein and dietary fiber content in pulses, ranging from 15% to 30% each, makes their flours an excellent ingredient for enhancing the nutritional quality of traditionally wheat flour-based products. Wheat flour alternatives, particularly pea and chickpea flours, are gaining increasing attention due to rising consumer interest in the nutritional and health benefits of pulse-based ingredients (Sadohara et al., 2022). In the study by Aguilera et al., 2009, both in vitro and in vivo research has revealed that pulses exhibit slower starch digestion rates than cereals. Present perspectives on nutritious intake may encourage the utilization of pulse starches as substitutes for cereals(Ma et al., 2017). In areas where animal proteins are costly or where consumption has a long history, these pulses, along with other legumes like cowpeas, are essential components of the diet. In line with numerous studies, pulses—including lentils, yellow pea and chickpea—have the potential to provide a complete dietary source for those suffering from micronutrient deficiency (Benayad & Aboussaleh, 2021).

Despite these numerous benefits, there are some challenges associated with using pulses in food products. Their strong flavors, sometimes described as "beany" or "grassy," can be off-putting to some consumers, limiting their acceptance by the public (Sadohara et al., 2022). Additionally, the absence of gluten in pulse flours means that they behave differently from wheat flour in baking and other food applications and may not be suitable for certain food applications but popular for gluten-free products. However, the positive impact of pulses on both human health and environmental sustainability has brought interest and overcame these drawbacks, making pulse flours a promising ingredient for the future of food production. And there is study by Aider et al., 2012 showed that while pulse proteins can be used in place of wheat flour when creating bread, the mass, volume, color, and texture of the finished product may be impacted.

Structure and Composition of Pulse Flours

Pulse seeds are recognized for their optimal balance of macronutrients and micronutrients and rich in starch, protein and fiber, which contribute to their high nutritional value (Hoover et al., 2010; Lastras et al., 2021). They serve as a substantial source of carbohydrates, with compositional variations depending on species and environmental conditions. In lentils, starch content ranges from 44.5% to 50.1%, dietary fiber from 10% to 17%, and resistant starch from 36.7% to 50.6% (Barbana et al., 2011; Shevkani et al., 2024); Similarly, in peas, starch content varies between 45.7% and 51.2%, while dietary fiber ranges from 14.2% to 20.0% (Ren et al., 2021). The total carbohydrate composition is influenced by both genetic and environmental factors. The study by (Brummer et al., 2015) found that lentil contains higher total soluble fiber than peas and chickpeas, and higher content of dietary fiber

than beans. A large amount of protein content (21-25%) was found in pulse (Wang & Daun, 2006), which has a high nutritional value (Kraska, 2018), proteins contribute to solubility, emulsification, foaming ability, gelling characteristics, and oil absorption, influencing functional properties of final products (Argel et al., 2020). Current studies indicate that lentils contain a small amount of fat (about 0.7-3.4%) and is majorly polyunsaturated fatty acids (Carbonaro & Nucara, 2022). It also served an excellent source of vitamins (thiamine, niacin, biotin, riboflavin, folic acid), antioxidant compounds(Setia et al., 2020) and minerals and main minerals in pulses are magnesium, potassium and iron (Lastras et al., 2021).

However, it is well-known that pulses contain certain anti-nutrients, such as trypsin inhibitor, tannins and phytic acid (De Almeida Costa et al., 2006; Kraska, 2018; Pal et al., 2017; Xu et al., 2019). Trypsin inhibitors are small molecular weight proteins and considered antinutrients since they interfere with the digestive process, specifically by inhibiting the activity of digestive enzyme trypsin (Pal et al., 2017). Trypsin is a crucial protease in the digestive system that breaks down proteins into smaller peptides and amino acids, which can then be absorbed by the body. Tannins are known as anti-nutrients since it could form complexes with proteins/digestive enzymes through hydrogen binding or hydrophobic interactions, which consider to be responsible for low protein digestibility since the complexes are resistant to enzymatic breakdown and decreased amino acid availability and digestibility. Phytic acid, reduces the bioavailability of minerals by binding with nutritionally important mineral cations (e.g., Ca, Fe and Zn) in guts (Thavarajah et al., 2009; Wang & Daun, 2006).

Protein Structure and Properties of Pulse Flours

Pulses are normally high in protein content as talked in previous section, they accumulate proteins during the growth stage and development stage (Carbonaro & Nucara, 2022). They are located in the cotyledon in the form of small (2-4 mm) spherules and part of protein matrix embedded in starch granules (Shevkani et al., 2021).

Amino acid profile

Pulse proteins serve as an excellent complement to cereal proteins, such as wheat, which are deficient in lysine but contain adequate levels of sulfur-containing amino acids. One of the most notable characteristics of pulse proteins is their high lysine content, an essential amino acid, while they are relatively deficient in sulfur-containing amino acids (Aider et al., 2012). The nutritional profile of pulse proteins is particularly significant, as they provide most essential amino acids, ranging from 32 to 39 g/100 g (Shevkani et al., 2024). Additionally, they contain higher levels of methionine and cysteine compared to other plant proteins, making them an important source of these essential amino acids for human nutrition.

This unique composition enhances the overall nutritional quality of pulses, underscoring their importance in supplying a complete amino acid profile essential for human health. The health benefits of pulses have been associated with cholesterol- and lipid-lowering effects, as well as a reduced risk of colon cancer and type 2 diabetes (Roy et al., 2010). Furthermore, pulse proteins present a promising alternative to conventional protein

sources, contributing to the growing global demand for sustainable and health-promoting protein options.

Major protein fractions in pulse proteins

The protein present in pulse seeds are mainly storage proteins which function to provide N, C and S (Shevkani et al., 2024). It could be classified according to their solubility as albumin, globulin, and gluten, of which salt-soluble globulin is the principal constituent of legumes(Jarpa-Parra, 2018; Roy et al., 2010).

Albumin, which accounts for just 10-20% of total protein in pulses, stands out as a water-soluble protein having a considerable impact on overall nutritional value. This class of globular proteins is well-known for adding significant nutritional value to legumes. remarkably, albumin has a high concentration of essential amino acids, notably sulfur-containing amino acids such as tryptophan, threonine, and lysine. The vital role of albumin in seeds cannot be overstated, as this family of globular proteins encompasses a majority of the enzymatic and metabolic proteins and inhibitors essential for fostering pulse growth. Albumins are capable of enhancing the foaming properties of pulses, and they can easily interact with the starch component. Beyond its nutritional contributions, albumin also contains certain malnutritional components, notably amylase and trypsin inhibitors. Although these components are less abundant compared to globulins, their presence is noteworthy. Some articles may not extensively report on these malnutritional components due to their relatively lower abundance compared to other constituents like globulins.

Globulins function as storage proteins representing a source of energy, carbon, and reduced nitrogen for germination and seedling growth (Vioque et al., 1999). Pulses are predominantly composed of globulin, constituting about 70% of total protein content, serving as a storage protein. The two primary types of globulins present in pulses are (11S) legumin and (7S) vicilin (Carbonaro & Nucara, 2022; Jarpa-Parra, 2018; Shevkani et al., 2024). Legumin, the major globulin, is a hexametric protein where subunits are connected by disulfide bonds. Acidic chains are positioned on the surface, while hydrophobic basic units are inside which limits the contact with water and results in a lower foaming ability. In contrast, vicilin is a trimeric protein with monomers linked by non-covalent hydrophobic bonds. Due to their structural characteristics, globulins exhibit lower foaming capacity than albumins, as they have a reduced ability to unfold and trap air. However, this structure plays a crucial role in other functional properties such as emulsification and water absorption capacity (WAC).

Glutelins, belonging to the proline protein class, constitutes a significant portion of the protein complex known as glutenin, which consist of about 11% in pulse protein. Notably, it stands out within the pulse seeds category due to their relatively high in gluten content, ranging from 19% to 25%. This distinctive feature sets pulse apart in terms of protein composition.

Prolamins, comprising a minor fraction of pulses protein at approximately 3% (Jarpa-Parra, 2018). These proteins are characterized by substantial quantities of glutamine and proline, exhibiting heightened solubility in strong alcohols (70-80%), as well as weak acids and alkaline solutions. While prolamin presence is typically abundant in cereal flours, the

unique properties of these proteins in legume proteins remain underrepresented and insufficiently elucidated.

Secondary structure of pulse protein

The secondary structure of pulse protein are majorly comprised of β-sheets (parallel and antiparallel), α-helix and β-turns. According the review by (Shevkani et al., 2024) pulse protein isolates contain a broad range of α-helix content (10.1–32.8%) and β-sheet content (24.1–37.6%). The protein fractions like albumin, globulin, legumin, and vicilin are characterized by relatively low α-helix percentages (17.5–23.2%), with globulin and legumin showing especially high β-sheets content (50.2–55.6%), indicative of their more stable, rigid structure. Antiparallel β-sheets are more prominent in legumin (10.1%), while β-turns are relatively consistent across the fractions. Aggregated structures, suggesting a degree of protein-protein interaction, are more noticeable in globulin and legumin. The proportion of β-sheets was reported as a factor contributing to the lower digestibility of pulse proteins by limiting access to proteolytic enzymes (Shevkani et al., 2019). Pulse protein with a higher amount of β-sheets and lower amount of α-helix were reported to be more thermally stable and would be denatured at higher temperature since greater intermolecular associations (Parmar et al., 2017).

Functional properties of pulse protein

Functional properties refer to the physicochemical properties that determine how proteins behave in processed foods used for both storing and serving. These properties are therefore of great significance for the development of new types of food products. Amino acid profiles play a key role in influencing the structure and conformation of proteins and are also related to processing conditions such as pH, temperature and interactions with other compounds. The most important key properties for food applications include solubility, water absorption capacity (WAC), as well as emulsification, foaming and gelling properties.

Solubility

Solubility is the crucial functional property that centrally defines the amount of protein in a sample capable of dissolving into a solution. Extensive research has explored the solubility of pulse proteins, consistently finding high solubility levels between pH 1 to 3 and pH 7 to 10. The lowest solubility is observed at the isoelectric point, approximately at pH 4 (Grasso et al., 2022).

Water Absorption Capacity (WAC)

Water Absorption Capacity (WAC) of proteins refers to their capacity to retain water against gravity through physicochemical interactions. The ability to bind water molecules depend on the protein's structure and conformation. Studies on pulse protein isolates and

flours reveal variations in WAC, with isolates exhibiting a higher minimum gelling concentration compared to flours. Jukanti et al. (2012) reported higher WAC levels for pulse protein isolates, attributing it to their greater ability to swell, dissociate, and unfold, exposing additional binding sites. Process choice also plays a role, as defatted protein concentrates demonstrated higher WAC than non-defatted concentrates, emphasizing the impact of processing on functional properties.

Emulsifying

The emulsifying performance of food proteins, crucial for various food industry applications, depends on intrinsic properties like amino acid profile, molecular weight, and environmental factors. Plant proteins, notably soy, are recognized as effective emulsifiers. Differences in intrinsic properties between 7S and 11S globulin protein fractions contribute to superior emulsifying properties. In emulsion systems, proteins form films around oil droplets, preventing undesirable outcomes like coalescence and sedimentation. Pulse protein isolates exhibit varied emulsification properties, with soy isolates showing the highest index. Chickpea protein concentrates demonstrate higher emulsifying stability and ability indices than yellow pea or lentil concentrates, with "Kabuli" types slightly outperforming "desi."

Forming property

Food proteins play a crucial role in stabilizing foams, adsorbing onto air/water interfaces, reducing surface tension, and forming robust membranes through protein-protein

interactions. Foaming properties, characterized by foam capacity and stability indices, vary among protein fractions, with albumin typically exhibiting superior foaming capacity and stability compared to globulin. Pulse protein isolates demonstrate foaming capacities ranging from 30% to 44%, while concentrates display values between 26% and 48%. Defatted protein concentrates, though lower than some pulses, still exhibit foaming capacities of 201%–228%. Flours produce low-volume foams with high stability, indicating the surface activity of native soluble proteins in water.

Gelling property

The minimum gelling concentrations of pulse protein isolates ranged from 14% to 18%, beyond the values observed in the corresponding flours (10%-14%). These differences can be attributed to differences in the protein profile of the ingredients, as well as differences in the profile of the non-protein components (Grasso et al., 2022). Papalamprou et al. (2009) observed the formation of gels within the concentration range of 4.5% to 11.5% for chickpea protein isolates obtained through various extraction methods, such as wet extraction followed by isoelectric precipitation or ultrafiltration. Their findings underscored the noteworthy influence of the extraction technique on the gelation characteristics of the protein isolates.

Starch Structure, Functional and Nutritional Properties of Pulse Flours

Starch is one of the main components of cereals and tubers and provides metabolic energy for people's daily activities. Starch acts one of the three essential macronutrients

needed for human nutrition, along with proteins and fats, which serve as the primary source of energy for the body, providing fuel for various cellular processes and physical activities. Key functional properties of starch, such as pasting and gelation, develop only after starch is gelatinized. This process involves granule swelling, loss of crystallinity, uncoiling and dissociation of double helix structure, and ultimately, the disruption and destroy of the starch granules. In chemical aspect, starches are polysaccharides of glucose molecules linked together through glycosidic bonds. The two main types of linkages found in starch are alpha-1,4-glycosidic bonds and alpha-1,6-glycosidic bonds (Sajilata et al., 2006). Amylose and amylopectin are two major components of starch, which are polysaccharides and glucose is the monosaccharide unit that makes up both amylose and amylopectin. Amylose is essentially a linear polymer of glucose units linked together by α (1→4) glycosidic bonds. Amylopectin, on the other hand, is a branched polymer of glucose units. Like amylose, it also consists of α (1→4) glycosidic bonds, but it additionally contains α (1→6) glycosidic bonds at branching points which creates a highly branched structure. Due to the difference in structures, amylose and amylopectin have distinct properties and functions within plants and in the foods derived from them. For example, amylose tends to form a gel-like substance when heated in water, contributing to the texture of foods like rice and pasta.

Morphology of pulse starch granules

Pulse starch granules are predominantly oval in shape; however, other morphological forms, including spherical, round, elliptical, and irregularly shaped granules, are also observed. The surface of pulse starch granules is typically smooth, exhibiting no visible

voids, and confocal laser scanning microscopy (CLSM) showed that pulsed starch particles have fewer porous structures and no internal voids than normal maize and waxy maize starches(Hoover et al., 2010). According to the review by Ren et al., 2021, the average particle size of pulse starch ranges from 15 to 28 microns. Specifically, lentil starch granules typically exhibit widths between 6 and 32 micrometers and lengths ranging from 6 to 37 micrometers. In contrast, yellow pea starch granules have widths of 2 to 34 micrometers, while chickpea starch granules range from 9 to 30 micrometers in width (Ma et al., 2017; Shevkani et al., 2021). Chung et al. (2008) reported the scanning electron microscopy (SEM) of pulse starches, the granule size followed by the order of pea> Lentil >chickpea, and the exact granule size depends on the biogenetic and growing conditions.

Physicochemical properties of pulse starches

The physicochemical properties of pulse starch is much different from those of corn starch in terms of amylose content, amylopectin chain length distribution, amylose leaching, granular swelling, gelatinization transition temperatures and enthalpy, and RDS and RS levels (Chung et al., 2009).

The amylose content in pulse starch is relatively high, with reported ranges of 23.5%–32.3% in lentil, 30.4%–35.0% in chickpea, and 24.0%–49.0% in yellow pea (Hoover et al., 2010). The observed variability in amylose content is influenced by the measurement methodology employed, including affinity separation techniques such as the Megazyme Amylose/Amylopectin Assay Kit, as well as iodine-based methods like colorimetry and

potentiometric titration, which may further contribute to variations in reported values Recent studies of pulse starch mainly focused on the chemical composition, granule morphology and molecular weight, crystallinity and gelatinization (Joshi et al., 2013). All pulse starches exhibited a characteristic C-type diffraction pattern, which is a combination of A-type and B-type crystallinity and with relative crystallinity ranging between 27.2% and 33.5% (Sandhu & Lim, 2008). Lentil starch ranges from 18.7-32.3%, yellow pea starch ranges from 20.4-30.3%, and chickpea starch ranges from 23.0-27.6% (Hoover et al., 2010; Ren et al., 2021). When heated in excess water, starch granules undergo a phase transition from order to disorder, which is known as gelatinization. Heating the starch with water causes the disruption of double helix structure, eventually causing the loss of its crystallinity. During gelatinization, water molecules diffuse into starch granules and cause swelling. Important gelatinization parameters include gelatinization temperatures (onset, To; peak, Tp; and conclusion, Tc) and enthalpy change (ΔH), which are usually determined using differential scanning calorimetry (DSC). Gelatinization temperatures indicate the thermal stability of double helical crystallites in starch, and ΔH shows the amount of energy required to melt the crystallites and it is an indication of how strong the crystallite is. When the lentil starch exposed to water at a ratio of 1:3, the gelatinization parameters determined by DSC have an onset temperature of 57.8–68.4℃, peak temperature of 66.0–76.1℃, conclusion temperature of 71.0–82.0℃, delta H of 3.0–13.3 J/g; yellow pea starch have an onset temperature of 60.8-63.9℃, peak temperature of 67.4–70.6℃, conclusion temperature of 73.4-80.1℃, delta H of 9.9–13.8 J/g; chickpea starch have an onset temperature of 57.9-64.8℃, peak temperature of

63.5-72.5℃, conclusion temperature of 69.8-81.5℃, delta H of 1.2–17.6 J/g (Chung, Liu, Donner, et al., 2008; Hoover et al., 2010; Joshi et al., 2013; Sandhu & Lim, 2008).

Functional properties of pulse flours

The digestibility of pulse flours appears to be relatively lower since several factors, such as high amylose content present in pulse flours, amylose is a linear molecule, which tends to form helical structures that pack tightly together. This tight packing makes it harder for digestive enzymes (like amylase) to access and break down the starch molecules. What's more, the starch granules exhibit a less porous structure as we talk in last paragraph which observed by CLSM which inhibit the penetration of the enzyme and so more resistant to the enzymatic hydrolysis.

In the food industry, pulses are widely recognized for their low glycemic index and their potential to promote health by regulating blood sugar levels and improving insulin sensitivity. Additionally, their consumption has been associated with a reduced risk of chronic diseases such as type 2 diabetes, cardiovascular disease, and certain types of cancer (Brummer et al., 2015; Chung, Liu, Donner, et al., 2008; Chung, Liu, Hoover, et al., 2008; Ren et al., 2021; Sandhu & Lim, 2008). Pulses also contain a significant amount of resistant starch (RS) (Lu et al., 2018). According to the review by Hoover et al. (2010), the digestibility of pulse starch varies depending on the analytical method used. Using the Englyst method, lentils exhibit a digestible starch content ranging from 5.2% to 14.8%, with resistant starch accounting for 29.7% to 41.5% and rapidly digestible starch (RDS) ranging

from 43.7% to 65.2%. However, when analyzed using the AACC method, the digestible starch content increases to 16.0%–16.9%, with resistant starch making up 58.3%–62.2%, while RDS content decreases to 13.0%–13.2%. Similarly, yellow pea starch contains 40.5–58.0 g/100 g of RS when analyzed using the Englyst method, but only 8.1–12.6 g/100 g when assessed by the AACC method. Chickpea starch follows a similar trend, with RS content ranging from 33.5–54.3 g/100 g in the Englyst method and 8.14–18.4 g/100 g in the AACC method.

Heat-Moisture Treatment

Starch is a polysaccharide primarily made up of glucose. It naturally exists in a granular form, is abundant in nature, and functions as an energy storage molecule in plants. The starch granules contain crystalline areas, amylopectin branches, and are amorphous in alternating layers; when exposed to polarized light, this arrangement becomes birefringent. These chains' appearance may differ depending on factors such as botanical origin and plant maturation stage. Native starches have inherent features that include high viscosity, limited solubility, and susceptibility to thermal disintegration and retrogradation (Schafranski et al., 2021) That may not be ideal for a wide range of applications; however, in order to meet technological requirements, particularly in the food industry, starch can be changed, modifying its properties to be acceptable for certain applications and enhancing functional properties. And more essentially, in order to meet the health related issues, food ingredients with lower rapidly resistant starch and higher slowly digestible starch and resistant starch are needed (Chen et al., 2017). These limitations can be overcome by starch modification.

Modified starches are essential in food systems, providing a wide range of functional benefits that enhance product quality and performance. For instance, they serve as binding agents, ensuring structural integrity in items like processed meats and baked goods, while also managing moisture levels to achieve desired textures in various foods. By creating smooth or pulpy textures, modified starches are integral in products like soups, sauces, and desserts, often mimicking the creamy mouthfeel of fats for low-calorie alternatives. Additionally, they contribute to the formation of soft or crispy coatings in fried or baked foods and play a crucial role in stabilizing emulsions, such as salad dressings, by preventing oil and water separation (Bharti et al., 2019). The modification processes, such as physical, chemical, enzymatic or combination of these, enhance specific properties like solubility, viscosity, and resistance to heat or acidity, broadening the application of starches not only in food but also in pharmaceuticals, cosmetics, and textiles. In the context of green technologies for modifying starches, physical methods have become more widespread. The key benefits of these treatments include easy access, high yield, low cost, and no chemical residue. They are also less expensive than enzymatic and chemical treatments and do not have any harmful consequences. Irradiation, annealing, pre-gelatinization, osmotic pressure, pulsed electric fields, and moisture and temperature treatment, often known as heat-moisture treatment (HMT), are some of the physical modifications that can be made to starch.

HMT is the common physical method of which the properties of starch get modified without destroying the granules. It involves incubation of samples at low moisture level (<35% water w/w) and heating for a certain period (15min-16hours) and heating temperature above glass transition temperature but below gelatinization temperature (Chung et al., 2009).

Currently, HMT has an important role to play in the food industry, especially due to its thermal stability and a lower tendency to retrograde. HMT disrupts the crystalline structure by promoting stronger interactions between starch chains, which leads to the separation of the double helical arrangement. The broken crystals then reorganize themselves (Schafranski et al., 2021). HMT influences starch digestibility, gelatinization, pasting properties, amylose content, morphological properties, crystallinity, molecular interactions of rice (Puncha-arnon & Uttapap, 2013), chickpea (M. Du et al., 2023), sorghum (Vu et al., 2017), highland barely (Lv et al., 2022) and mango kernels (Bharti et al., 2019) based on the recent studies. Based on the study of Chung et al., 2009, HMT had a greater impact in RDS, SDS and RS content than annealing on pea and lentil samples. These transformations make starches highly attractive to the food, pharmaceutical and cosmetic industries. HMT starch is widely used in a variety of foods such as noodles, pasta, frozen and canned food products. Although there has been a great deal of work on the changes in starch digestibility and physicochemical properties before and after HMT, few studies have investigated the effects of HMT on starch and protein structure and digestibility and the relationship between them.

Objectives

Modification has been widely studied in starches from various botanical sources; however, limited information is available on the effects of such treatments on pulse flour, starch, and especially pulse proteins. To meet growing health demands, it is important to develop foods with higher contents of slowly digestible starch (SDS) and resistant starch (RS). This study focuses on the impact of Heat-moisture treatment (HMT) on the protein

components of pulse flour, aiming to enhance both nutritional and functional properties. Specifically, the study examines how protein functionality contributes to increased RS content, and investigates the effects of HMT on protein digestibility, surface hydrophobicity, and protein extractability. By modifying protein structure and its interactions within the flour matrix, HMT may also improve textural and processing characteristics, particularly when pulse flour is used alone or in combination with wheat flour in products such as pasta or Asian noodles. This research aims to provide deeper insight into the strategic application of HMT to optimize protein quality in pulse-based ingredients for industrial food applications.

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Chapter 2 - Comparison of Amperometric and Enzymatic Methods to Measure Damaged Starch Content in Pulse Flours with Different Particle Sizes

Abstract

Pulses such as chickpea, yellow pea, and lentil are nutrient-dense and increasingly utilized in foods and gluten-free products. During dry milling to produce flours, starch granules are structurally altered, resulting in damaged starch. The level of damaged starch affects flour quality. The objectives of this study were to investigate how roller dry milling affected damaged starch content in pulse flours with different particle sizes and compare the damaged starch content measured by two methods: the amperometric (SDmatic) method (AACC 76-33.01) and the enzymatic method (AACC 76-31). For all flour types and sizes, the amperometric method consistently yielded higher damaged starch content (SD%) than the enzymatic method. For fine flours, damaged starch ranged from 3.31% (chickpea) to 4.37% (yellow pea) using the amperometric method versus 0.95–1.52% by the enzymatic method. The iodide absorption (AI%) decreased as the flour particle size increased, but higher damaged starch content was obtained from the SDmatic instrument when 1 g of flour was used. To improve the accuracy of the amperometric method when using the SDmatic instrument, a larger amount (2 g) of pulse flour was used to ensure that AI% value is greater than 86% during the amperometric measurement. After adjusting the iodide-to-damaged starch ratio, a new calibration equation was established between AI% and damaged starch content. By using the new established equation, the amperometric method can be used to

measure and obtain the damaged starch content that is correlated strongly with the enzymatic method.

Keywords

Damaged starch; Amperometric method; Enzymatic method; Pulse flours

Introduction

Pulses, such as chickpeas, yellow peas, and lentils, are widely considered nutrient-dense foods, providing substantial amounts of vitamins, minerals, carbohydrates, proteins, and dietary fiber (Jeong et al., 2019). They are often recommended by health organizations and nutritionists as staple foods due to their numerous health benefits. An important nutritional benefit of pulses is their potential to modulate postprandial blood glucose levels. Their high dietary fiber content and low starch digestibility which slow down the absorption of glucose, making them particularly beneficial for individuals with diabetes (Gao et al., 2019). The higher dietary fiber content also promotes weight management by delaying gastric emptying and promoting prolonged satiety, making pulses an ideal and suitable dietary choice for individuals managing blood glucose level or seeking to maintain a healthy weight (Jeong et al., 2019; Sivakumar et al., 2023). Globally, there is a growing demand for incorporating pulses into diets, not only because of their health benefits but also by their affordability and accessibility, particularly in regions where animal-based protein sources are cost-prohibitive (Drewnowski & Conrad, 2024). In such contexts, pulses play a critical nutritional role by providing a cost-effective source of protein and essential nutrients.

Moreover, due to their rich micronutrient profile, pulses have the potential to serve as a complete dietary component for populations affected by micronutrient deficiencies (Drewnowski & Conrad, 2024; Sivakumar et al., 2023).

Pulses are often dry milled into flours and used in foods. During dry milling, starch granules may be physically broken or altered by mechanical forces involved in grinding the grains into flour form, producing damaged starch (Wang et al., 2020). The severity of the damage can vary based on factors such as the hardness of the kernel, the intensity of the grinding process, and pre-treatment such as tempering of the kernels (León et al., 2006). Grains with a hard-textured endosperm typically require greater mechanical energy during milling due to their increased resistance to fragmentation. Therefore, the milling of harder grains tends to cause a higher degree of starch granule damage compared to softer varieties. Starch is a major component in pulses and the degree of starch damage is a critical indicator of flour quality, as damaged starch has a higher water absorption capacity. In the case of wheat flour, damaged starch can retain approximately 2 to 4 times its weight of water, whereas native wheat starch absorbs only about 0.4 times its weight (Dubat & Sas, 2003; McAllister et al., 2011). This property significantly impacts the physicochemical characteristics of wheat flour, such as water absorption capacity, viscosity, gelatinization behavior, retrogradation tendencies, and baking performance (Bojanić et al., 2024; Putri et al., 2020) and consequently affects the quality of final products. A moderate level of starch damage can be advantageous, as it enhances water absorption, supports dough formation, and promotes moisture retention during baking. Adequate water absorption prevents the production of dry and stiff bread, which is associated with undesirable texture and mouthfeel

(Dubat & Sas, 2003). Furthermore, increased water absorption has economic advantages by enhancing product yield, given that water is an inexpensive ingredient (Putri et al., 2020). On the other hand, excessive starch damage may negatively affect baking processes and compromise product quality. For instance, wheat flour with over-damaged starch may lead to sticky dough, weakened dough structure, reduced gas retention, extended baking time, and undesirable textural changes in final products (Ma et al., 2016). During fermentation, such dough may exhibit limited volume expansion, while baking time may increase due to the additional water retention, delaying proper setting and structural development of products like cookies and bread (León et al., 2006).

Starch damage also plays a significant role in determining product shelf life, particularly through its influence on retrogradation and bread staling. Retrogradation refers to the reassociation of starch molecules into more ordered/crystalline structures, which results in the firming of bread crumb and a progressive decline in texture, flavor, and overall quality over time (León et al., 2006). Higher levels of damaged starch have been linked to degradation of starch molecules and accelerate amylopectin retrogradation, thereby promoting staling in bread (Tao et al., 2024). In this aspect, the damaged starch content indirectly contributes to the shelf life of baked products. Consequently, maintaining damaged starch within an optimal range is essential for quality control and shelf-life management in the food processing industry (Putri et al., 2020).

Particle size is a key parameter influencing the functional properties of flour, including pasting profile, gelation, and other physicochemical characteristics (Dziki et al., 2024). The process of roller dry milling, which determines the particle size, also affects the extent of

starch damage in the flour. In general, flours with larger particle sizes experience less milling force, resulting in lower levels of starch damage. In contrast, finer particles are subjected to more intense milling, leading to a higher degree of starch disruption (Wang et al., 2020). The extent of starch damage is particularly important because it directly impacts the functional properties of flour, as previously discussed. Understanding the relationship between particle size and starch damage is essential for optimizing flour performance in specific applications. By controlling these parameters, manufacturers can achieve desired product textures, structures, and overall quality while ensuring consistency to meet consumer expectations. Two primary methods have been developed to measure damaged starch content in flours, particularly in wheat flours (Wilson et al., 2016). These include the enzymatic method, originally devised by Farrand (1964) and later refined by Gibson et al. (1992) and the amperometric method, first introduced by and improved by Medcalf and Gilles (1965).The enzymatic method is commercially available through using an assay kit from Megazyme (Bray, Ireland), while the amperometric method can be performed using the SDmatic instrument from Chopin Technologies (Villeneuve la Garenne, France). A detailed comparison of these two methods is presented in Table 1. The enzymatic method is based on the principle that damaged starch granules are more susceptible to enzyme hydrolysis. This method begins with the hydrolysis of damaged starch granules using fungal alpha-amylase, which breaks down the starch molecules into shorter maltosaccharides and dextrins. After hydrolysis, the maltosaccharides and dextrins are further degraded into glucose molecules by amyloglucosidase, which cleaves the remaining α-1,4 and α-1,6 glycosidic bonds, producing glucose. The glucose molecules are then quantified spectroscopically using a glucose

oxidase/peroxidase reagent mixture. Glucose oxidase catalyzes the oxidation of glucose to gluconic acid, while peroxidase catalyzes the reaction with a chromogenic substrate to produce a colored product. The intensity of the color is directly proportional to the concentration of glucose in the sample, allowing precise quantification. To ensure accurate results, the time allocated for treating damaged starch with fungal alpha-amylase must be carefully controlled. The treatment aims to achieve near-complete solubilization of damaged granules while minimizing the breakdown of intact granules. This method is widely regarded as the most useful way for quantitatively determining damaged starch content due to its high accuracy and reproducibility (Medcalf and Gilles, 1965; McAllister et al., 2008). Morgan and Williams (1995) noted that the enzymatic method produced the best overall correlations when compared to the other established reference methods, including amperometric method. However, the enzymatic method requires a high level of operator expertise and extensive training to minimize errors (Dubat & Sas, 2003, McAllister et al., 2011). In addition, the method is relatively time-consuming for small number of test samples (Wilson et al., 2016). In contrast, the amperometric method, which was originally designed and calibrated to assess starch damage in dry-milled wheat flour (Wilson et al., 2016), relies on the principle that damaged starch granules absorb more iodine than intact starch granules (Monnet et al., 2019, Dubat & Sas, 2003). This method is simpler and less time-consuming than the enzymatic approach, making it more accessible and cost-effective for a wider range of laboratories and applications (Monnet et al., 2019). It is particularly useful in settings where rapid and economical assessments are required.

Several studies (Bojanić et al., 2024, Guldiken et al., 2022) have examined the effects of damaged starch content in relation to different milling techniques and parameters, particularly for wheat flours. These investigations have focused on understanding how damaged starch further influences the physicochemical properties, rheological behavior, and bread-baking performance. Several studies, including those by Morgan and Williams (1995) and McAllister et al. (2011) have compared and analyzed the relationships between the two standard methods for measuring starch damage in wheat flours of varying hardness. Wilson et al. (2016) extended these comparisons to sorghum flours, examining the damaged starch content using these two methods.

Although most of the existing research has focused on damaged starch in wheat flours, a few studies have explored starch damage in pulse flours. Monnet et al. (2019) explored starch damage in pea and wheat flours, focusing on the influence of particle size distribution on starch damage during milling. Although previous findings have provided valuable insights, significant gaps remain in understanding the relationship between particle size and starch damage in pulse flours. Scanlon et al. (2018) emphasized the critical importance of understanding starch damage during milling to produce high-quality pulse flours. Despite these efforts, research on pulses, particularly on how milling methods and parameters affect starch damage, remains limited. The relationship between particles size and starch damage in pulse flours is still not well understood.

Therefore, the objectives of this study were to investigate how roller dry milling affects the damaged starch content in pulse flours with different particle sizes and compare the two commonly used methods for determining damaged starch content in pulse flours – the

amperometric method (AACC 76-33.01) and the enzymatic method (AACC 76-31). By applying both methods to chickpea, yellow pea, and lentil flours with different particle sizes, the study aimed to establish a quantitative correlation between the two methods. Establishing such a relationship would allow researchers to use the simpler and faster amperometric method, while maintaining consistency with the widely accepted enzymatic method.

Materials and methods

Materials

Chickpea and lentil kernels were purchased from the Amazon online Food to Live store, USA. Yellow pea kernels were purchased from the Amazon online store, D'allesandro Gourmet Ingredients, USA. Commercial chickpea flour (CCF) was purchased from the Amazon online store Anthony's, USA. Soft wheat flour (SWF) and hard wheat flour (HWF) were provided by Siemer Milling Company (Teutopolis, KY, USA) and Ardent Mills (Denver, CO, USA), respectively. Control wheat flour (CWF) in Starch Damage Assay Kit, was from Megazyme International Ireland Ltd (Wicklow, Ireland), bottle six. High amylose hard red spring wheat flour (HAWF) and normal wheat flour (NWF) were obtained from Bay State Milling (Quincy, MA, USA). Waxy wheat flour (WWF) (Cultivar Mattern) was provided by Dr. Robert Graybosch (USDA/ARS, University of Nebraska). Yellow pea starch (YPS, ~40% amylose) was obtained from Roquette America, Inc. (Geneva, IL, USA).

Milling of pulse flours

Chickpea, lentil, and yellow pea kernels were milled using a laboratory-scale Ross roller mill (Model 915, Ross Machine and Mill Supply, Oklahoma City, OK, USA). The rolls gap opening and sieve arrangement were adjusted accordingly in order to produce different particle size (small, medium, and coarse) samples.

For yellow pea (YP) and chickpea (CP) fine flours, five breaks were used with roller gap ranges from 0.126 to 0.006 inch for YP and 0.310 to 0.006 inch for CP, followed by two reductions (0.003 inch). YP and CP flours with small and medium particle size were produced using 3–4 breaks (from 0.061 to 0.006 inch) and 0–1 reduction (0.003 inch), while coarse flours of YP and CP were obtained using 2–3 breaks with wider gaps (from 0.061 to 0.012 inch) and no reductions.

Lentil required fewer break passes than CP and YP due to its smaller kernel size. Fine lentil flour was produced using three breaks (from 0.025 to 0.06 inch) and two reductions (0.003 inch). Small and medium lentil flours were prepared using three break passes (from 0.025 to 0.006 inch) and 0–1 reduction (0.003inch). Coarse lentil flour was obtained using two break passes with wider gaps (0.012–0.036 inch) and no reductions.

Methods

Composition of the pulse flours

Moisture content was determined using AACC Method 44-15.02 (AACC, 2000). Nitrogen content was measured by the Dumas combustion method using a Nitrogen/Protein

Analyzer (FP628, LECO Corp., MI, USA) which was converted to protein content (% N × 6.25) for pulse flours in accordance with AOAC Method 992.15 (1995). Ash content was measured by AACC Method 08-01.01 (AACC, 2000). Starch content was quantified by using a Total Starch Assay Kit from Megazyme following "protocol method e" (Suggested procedure for the determination of starch in samples which contain D-glucose and/or maltodextrins - removal of D-glucose and maltodextrins with alcohol washing). The crude fiber content of the sample was analyzed using the AOAC 962.09 method. The crude fat content was determined by the AOCS Method Ba 3-38. Amylose content was quantified using Amylose/Amylopectin Assay Kit from Megazyme.

Starch Isolation of wheat flours

Waxy wheat starch (WWS, ~ 0% amylose), normal wheat starch (NMS, ~ 28% amylose), and high-amylose wheat starch (HAWS, ~ 70% amylose) were isolated from NMF, WWF and HAWF, respectively, using modified hand-washing technique based on AACC International. (1999) and Guan et al. (2009) with slightly modifications. Briefly, dough was prepared at optimal absorption, rested in water for 60 min, then washed manually until rinse water was clear. The wash liquid was filtered through 100- and 75-mesh sieves, and the filtrate was centrifuged at 2500 × g for 20 min. The grey upper layer was removed, and the starch pellet was washed, recentrifuged, and dried at 40 °C for 24 hours. Dried starch was equilibrated at room temperature, ground, and stored for analysis.

Particle size distribution

Particle size distribution was analyzed by Malvern Morphologi G3SE (Malvern instruments, Malvern, UK). A standard sample of 19 mm^3 was used for each duplication and 10000 particles were imaged at 5X magnification for small, medium and coarse particle size pulse flours, 10X magnification for fine pulse flours.

Measurements of Damaged starch content

Amperometric Method (AACC 76-33.01)

The damaged starch content was determined by using a Chopin SDmatic instrument (Chopin Technologies, Villeneuve la Garenne, France) following AACC Method 76-33.01 (Figure 1) with slight modifications. Citric acid was used instead of boric acid due to concerns about the reproductive toxicity with boric acid. Analysis conducted by Chopin Technologies demonstrated that citric acid-based methods were verified to be accurate, repeatable, and reproducible (Chopin Technologies, 2011). Briefly, a reaction mixture was prepared in a glass reaction vessel by combining 1.5 grams of citric acid, 3 grams of potassium iodide, and one drop of sodium thiosulfate with 120 mL of distilled water. This solution was mixed thoroughly before use. To conduct the test, 1 gram of flour was automatically introduced into the reaction vessel. When the flour entered the vessel, the starch in the sample absorbed iodine from the solution. Damaged starch granules absorbed more iodine, leading to a decrease in electrical current and the formation of an intense blue-black color. During the reaction, the maximum current value (Im) was recorded, followed by

the residual current value (Ir) after the reaction was complete. The iodide absorption is calculated as this formula:

Iodine absorption (AI%) = 1 - Ir/Im.

The calculated AI% was then automatically converted into the damaged starch content (SD%) by the instrument's installed software and displayed on the touch screen at the end of the test.

To verify and confirm the hypothesis, the iodide-to-damaged starch ratio was adjusted for further investigation. Specifically, the flour amount was increased from 1 g to 2 g to increase the damaged starch amount present in samples while keeping all other test parameters unchanged.

Enzymatic method

The AACC 76-31 enzymatic method (Figure 2) was applied by using the Megazyme assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). For this method, each flour (100mg) and a fungal alpha-amylase solution (50 U/mL) were equilibrated at 40 °C for 5 min before reaction. The equilibrated fungal alpha-amylase solution was then added to each sample, and the mixture was incubated in a water bath at 40 °C for exactly 10 min to hydrolyze the damaged starch. The reaction was terminated by adding diluted sulfuric acid (0.2% v/v), followed by centrifugation at 3,000 rpm for 5 minutes to separate the hydrolyzed starch. From the resulting supernatant, 100-microliter aliquots were transferred into new tubes, and amyloglucosidase solution (2 U) was added to further hydrolyze the starch into

glucose units. Finally, the glucose content in the hydrolyzed solution was determined and quantified using the glucose oxidase-peroxidase (GOPOD) reagent.

Statistical analysis

All experiments were done in triplicate. Mean values and standard deviations were reported. Analysis of variance (ANOVA) was used to determine the significant difference between the results.

Results and Discussion

Composition and particle size distribution of pulse flours

This composition of yellow pea (YP), lentil (L) and chickpea (CP) flour is presented in Table 2. Yellow pea flour has the highest protein content (30.8%), followed by lentil flour (28.1%) and chickpea flour (22.3%). For carbohydrates, lentil has the highest total starch (54.4%), with yellow pea slightly lower (51.0%) and chickpea the lowest (47.5%). In contrast, chickpea has the highest crude fiber content (1.8%) and crude fat content (7.3%), significantly higher than yellow pea (0.8% fiber and 1.2% fat) and lentil (1.2% fiber and 1.1% fat). Amylose content is similar across the flours, ranging from 31.3% in chickpea to 32.5% in yellow pea.

The protein contents (Table 2) are within the typical range reported for pulse flours (21.3–31.5%) in Ren et al. (2021). The starch contents in pulse flours are also consistent with the values reported in the literature; the starch content in lentil flour has been reported between 44.5% and 50.1% (Shevkani et al., 2024), and starch content in yellow pea flour

between 45.7% and 51.2% (Ren et al., 2021). Amylose content ranges match the reported values of 23.5%–32.3% in lentil, 30.4%–35.0% in chickpea, and 24.0%–49.0% in yellow pea (Hoover et al., 2010).

Table 2 also shows particle size distributions of fine, small, medium, and coarse flours of lentil, chickpea, and yellow pea, focusing on the D10, D50, and D90 parameters, which represented the largest particle in 10, 50, and 90% of the samples, respectively. For lentil flours, the D50 values for the fine, small, medium, and coarse flours were 163.7, 262.7, 438.2 and 466.9 μm, respectively. In chickpea flours, the corresponding D50 values were 157.9, 184.0, 272.2 and 386.5 μm, while for yellow pea flours, they were 172.1, 180.0, 288.8 and 441.4μm. Significant differences in particle size distributions were observed between these flours within each flour type. Moreover, comparisons between different flour types at the same particle group fractions show similarities, particularly at the D90 parameter. However, the chickpea medium flour stands out, as it shows no significant difference compared to lentil small flour and yellow pea small flour in D50 parameters. This observation suggests that chickpea flour tends to have finer particles compared to lentil and yellow pea flours, especially in the small and medium particle size flours, when subjected to the same milling process.

Damage starch content determined by the amperometric and enzymatic methods

Using 1 g of flour in the amperometric method in comparison with the enzymatic method

To validate the accuracy of the amperometric method (AACC 76-33.01), two standard reference wheat flours (1 SD-100/A and 1 SD-100/B) with certified AI% values of 92.3±0.5

and 94.8±0.4, respectively, provided by KPM Analytics North America Corp (Westborough, MA, USA) were tested. The measured AI% values were 91.6 and 94.3 (Table 3), with minimal deviations of 0.2% and 0.1%, respectively, indicating that the SDmatic instrument used in this study was functioning properly and providing reliable results.

The damaged starch content of pulse flours as well as wheat flours measured by the amperometric and enzymatic methods is shown in Table 3. In general, for normal wheat flours (CWF, SWF and HWF), the damaged starch content measured by the two methods is well correlated. The normal hard wheat flour (HWF) sample had a damaged starch content of 7.3%, and 7.1% as measured by the amperometric method and enzymatic method, respectively. Similarly, the control wheat flour (CWF) in the Megazyme assay kit had a damaged starch content of 7.3% and 7.5% as measured by the amperometric and enzymatic methods, respectively. A small difference was noted for the normal soft wheat flour (SWF); 5.5% damaged starch content was obtained by the amperometric method, whereas 3.9% damaged starch content was obtained by the enzymatic method. In a previous study, McAllister et al. (2011) analyzed 65 normal wheat flour samples (including both soft wheat and hard wheat) for damaged starch content using both the enzymatic method and the SDmatic instrument. Their study revealed a close agreement between the two methods, with average damaged starch content measured at 5.8% using the enzymatic method and 6.3% using the SDmatic method.

In contrast, the damaged starch contents measured by the amperometric and enzymatic methods are different for pulse flours. The damaged starch content of fine flours from lentil, chickpea and yellow pea was 3.7, 3.3, and 4.4% by the amperometric method and 1.5, 1.0,

and 1.5% by the enzymatic method, respectively. The results indicated that the SDmatic method consistently gave higher damaged starch content compared with the enzymatic method across chickpea, yellow pea, and lentil flours. This discrepancy can be attributed to the distinct underlying principles and mechanisms in the two methods and the SDmatic calibration was originally developed using wheat flours, which could limit its applicability when applied to pulse flours due to compositional and structural differences in starch (McAllister et al., 2011; Monnet et al., 2019). The amperometric method (AACC 76-33.01) is based on the principle that the intact and damaged starch granules exhibit different iodine binding capacities. This method relies on an electrochemical process where free iodine is generated using a probe. Since iodide itself is not highly soluble in water, potassium iodide is added in this method to provide a source of iodide ions for the reaction. Citric acid is introduced to create an acidic environment, which is necessary for the reaction to proceed efficiently and generates the triiodide ions (Monnet et al., 2019), as shown in reaction (a). The generated linear triiodide ions are soluble and give a yellow color to the reaction solvent, producing a current which is captured by the probe (Figure 1b).

$$I_2 + I^- \longrightarrow I_3^- \qquad \text{(a)}$$

The iodine is absorbed to a greater extent by damaged starch granules due to disruption of the granular structure, which increases iodine accessibility (Dubat, A. 2007). In contrast, intact starch granules, with their compact structure, bind iodine less readily. In this method, the amylose content in pulse flours should also be considered, as iodine binds with the single-helix structure of amylose to form a complex. This interaction can potentially

affect the iodine binding capacity, thereby influencing the results obtained through this method.

Careful examining the AI% and SD% obtained by the amperometric method reveals that when 1 g of flour was used, an unexpected correlation of AI% and SD% was observed (Table 3). According to the principles of the amperometric method, a higher level of damaged starch content should theoretically lead to greater absorption of iodide. This would result in a greater decrease in the amount of iodide present in solvent, leading to a higher AI%. However, the experimental results are different. In lentil flour, an increase in particle size was associated with a decrease in AI% from 90.6% (small) to 74.0% (coarse), but SD% increased from 3.6% (small) to 9.8% (coarse). Those results suggest that using 1 g of pulse flours in the amperometric method does not give a damaged starch content that is correlated with the damaged starch content by the enzymatic method.

The same trend was observed for the pulse flours with different particle sizes (Table 3). Overall, the amperometric method consistently gave higher of damaged starch content compared to the enzymatic method. For pulse flour samples of different particle sizes, the enzymatic method consistently gave a damaged starch content below 2.0% (Table 3), which is similar to a previous study by Guldiken et al. (2022) where all damaged starch content for all samples obtained by different milling methods were lower than 3.0%. There was a clear trend of decreasing damaged starch content as particle size increased across all flour types, which agrees with the study by Lee et al. (2024) who measured the damaged starch content of pea, lentil and faba bean. In contrast, the amperometric method gave a general trend of higher damaged starch content with increasing particle size, except for medium-sized yellow pea

flour. This observation is inconsistent with findings from a previous study by Nkurikiye et al. (2023), who reported the least damaged starch content in coarse flour samples of lentil, chickpea, and yellow pea when using the amperometric method.

To understand the discrepancies observed in pulse flours by the two methods, we tested a high-amylose wheat flour (HAWF), which has a much higher amylose content than normal wheat flours, wheat starches with different amylose contents [waxy wheat starch (WWS), normal wheat starch (NWS), and high-amylose wheat starch (HAWS)] and a commercial yellow pea starch (YPS) (Table 3). We postulate that amylose content in a starch affects the AI% due to the formation of amylose-iodine complex and in turn affects SD% values from the SDmatic instrument. For HAWF, the damaged starch content (7.4%) measured by the amperometric method (SDmatic instrument) was higher than the value (3.9%) by the enzymatic method. A similar trend was observed for high-amylose wheat starch (8.5% by amperometric method and 2.1% by enzymatic method). For all other starches, the damaged starch content measured by the two methods were not correlated either; in all cases, the amperometric method gave higher damaged starch contents. For waxy wheat starch, which had no amylose, AI% was low (60.9%) because of the lack of amylose-iodine complex but a high SD% (35.4%) was obtained from the SDmatic instrument. In contrast, the damaged starch content of WWS was 6.2% as measured by the enzymatic method. It is interesting to note that the yellow pea starch (YPS) had a higher amylose content than the NWS. Yet the AI% for the YPS (92.0%) was lower than that for the NWS (95.0%), indicating that in addition to amylose content, morphology, molecular organization, and compactness of starch granules affect the AI%. All these results highlighted the need to better understand the

relationship between AI% and SD% and to better understand how the SDmatic instrument calculates the starch damage from AI% values.

Increasing pulse flour to 2 g in the amperometric method and establishing equation between AI% and SD%

In the amperometric method, the iodine-to-damaged starch ratio may fall outside the optimal range for pulse flours when 1 g of pulse was used. To adjust the ratio of the iodine-to-damaged starch, we increased pulse flours from 1 g to 2 g in the amperometric test. After increasing the pulse flour amount (from 1 g to 2 g), the trend of damaged starch content measured using amperometric method showed significant changes compared to previous data. The SD% values reported in Table 3 are calculated by dividing the SD% value obtained by the SDmatic instrument by the sample weight since the tests were conducted using 2 g of flours. In all three flour types, AI% decreased markedly as particle size increased, indicating that smaller particles provide a larger surface area for amylose-iodide complex formation. These results were more consistent with those obtained using the enzymatic method. As particle size increased, the damaged starch content decreased consistently: for lentil flour, it dropped from 4.6% (small) to 1.9% (coarse); for chickpea flour, from 3.8% (small) to 1.2% (coarse); and for yellow pea flour, from 4.9% (small) to 1.9% (coarse). For the enzymatic method (Table 3), flour with small particle size had the highest damaged starch content, with lentil flour at 1.5% and yellow pea flour at 1.6%. These results were consistent with the amperometric method (using 2 g of flour), which showed 4.9% damaged starch for small

particle size yellow pea flour and 4.6% for lentil flour. Across all particle sizes and both methods, chickpea flour gave the lowest damaged starch content.

Several studies (McAllister et al., 2011; Monnet et al., 2019; Wilson et al., 2016) have indicated that the conversion from AI% to SD% can be described by a quadratic equation (b):

$$SD\% = a*AI\%^2 + b*AI\% + c \quad \text{(b)}$$

Using our experimental data, the following quadratic equation (c) could be obtained and plotted (Figure 3(a)):

$$SD\% = 0.0533*AI\%^2 - 9.1514*AI\% + 395.03 \quad \text{(c)}$$

This equation had an R^2 value of 1, indicating a perfect fit to the dataset. This equation reveals that the correlation between AI% and SD% is dependent on whether the AI% is above or below a critical point, defined by the axis of symmetry at 85.85%. When AI% is below 85.85%, there is a negative correlation, where a lower AI% corresponds to a higher SD%. Conversely, above 85.85%, AI% and SD% exhibit the expected positive correlation which align with the anticipated relationship where higher AI% correspond to increased SD%.

This quadratic relationship helps explain why coarser flour samples, which generally have minimal starch damage, exhibited disproportionately high SD% values (based on 1 g of test flours). As seen in Figure 3(a), these coarser samples, tested with 1 g of flour, fall on the left side of the symmetry axis of the quadratic curve. In this range, the low iodide absorption (AI%) results in a negative correlation, leading to an overestimation of SD% when computed using the quadratic equation embedded in the SDmatic instrument. Consequently, the actual starch damage in coarser flour samples may be misrepresented. By increasing the sample weight to 2 g, the absorption shifts to higher AI% values, moving these flour samples to the

right side of the symmetry axis. Dividing the SDmatic output by the actual sample weight further refines the measurement, producing values more consistent with enzymatic assessments of starch damage. The adjusted amperometric method demonstrated a strong correlation with the enzymatic method, confirming its reliability in starch damage quantification.

Applying the Calibration Model for Pulse Flours

To further validate the correlation between AI% from amperometric method and starch damage content (mg) measured by the enzymatic method, we selected all pulse flour samples with AI% values greater than 85.85%. For these samples, the amount of damaged starch (mg) was calculated using the enzymatic method and paired with the corresponding AI% values from the amperometric method. Using these data points, a quadratic equation (d):

$$SD(mg) = 0.1811*AI\%^2 - 31.907*AI\% + 1416.9 \quad \text{(d)}$$

This equation was used to generate a calibrated correlation curve, as shown in Figure 3(b). The quadratic nature of the equation aligns with findings from the previous section and suggests that the relationship between AI% and SD is not strictly linear but exhibits a parabolic trend. The curve captures the transition between two distinct trends: at lower AI% values, starch damage content appears to be overestimated due to improper iodide-to-damaged starch ratios, while at higher AI% values, the correlation follows the expected positive trend.

To assess the practical applicability of this calibration, Figure 3(c) compares damaged starch content measured using the enzymatic method with values obtained from the newly

calibrated quadratic equation (d), with addition of fine flour samples (both 1 g and 2 g) and 2 g of commercial chickpea flour (CCF) (the 1 g CCF sample was excluded as its AI% was below 85.85%). Strong correlation was obtained between the damaged starch contents measured by the two methods.

For the commercial chickpea flour, the enzymatic method indicated a starch damage content of 1.3%. When analyzed using the amperometric method using 1 g of flour, the AI% was 85.1%, corresponded damaged starch content of 2.5%. Increasing the flour amount to 2 g raised the uncalibrated damaged starch content to 3.5%. After applying the calibration model, the adjusted value decreased to 1.0%, which more closely aligns with the enzymatic result, supporting the applicability of the calibrated model for commercial flour types.

The improved correlation between the calibrated amperometric values and enzymatic measurements confirms that the quadratic equation effectively eliminates the initial discrepancies observed in uncalibrated results for pulse flours. The amperometric method overestimated starch damage in coarser pulse flour samples (Table 3), likely due to an improper iodide-to-damaged starch ratio. However, after applying the calibration model, the adjusted values closely match enzymatic method results. This refinement enhances the reliability of the amperometric method for quantifying starch damage in pulse flours. These findings emphasize the importance of proper calibration when using the amperometric method to assess starch damage, ensuring its broader applicability to pulse flours and other grains.

Conclusion

The amperometric (SDmatic) method (AACC 76-33.01) gave a higher damaged starch content than the enzymatic method (AACC 76-31) when 1 g of flour was used to measure damaged starch content in pulse (chickpea, yellow pea, and lentil) flours. To improve the accuracy, we recommend using a larger amount (greater than 1 g) of pulse flour to ensure that AI% value is greater than 86% during the amperometric measurement using the SDmatic instrument. After adjusting the iodide-to-damaged starch ratio in this study, a new calibration equation was established between iodine absorption (%) and damaged starch content. By using the new established equation , the amperometric method can be used to measure and obtain the damaged starch content in pulse flours that is correlated strongly with the enzymatic method. In all pulse types, damaged starch content increased as particle size decreased, with fine/small flours exhibiting the highest levels of damaged starch and coarse flours the lowest, confirming that milling intensity directly affects starch damage.

CRediT authorship contribution statement

Bei Shen: Investigation, Methodology, Formal analysis, Writing – excel and original draft. **Jing Qi**: Writing – review & editing. **Yonghui Li:** Writing – review & editing**; Kaliramesh Siliveru:** Writing – review & editing**; Yong-Cheng Shi:** Conceptualization, Methodology Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

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Table 2.1 Comparison of amperometric method (AACC 76-33.01) and enzymatic method (AACC 76-31) used to measure damaged starch.

	Amperometric Method (AACC 76-33.01)	*Enzymatic method (AACC 76-31)*
Principle	Determination of the kinetics of iodine absorption (Dubat & Sas, 2003). The intact starch and damaged starch have different iodine binding capacity (Medcalf &Gilles, 1965, Monnet et al., 2019). It is enzyme-free.	Damaged starch granules are more susceptible to fungal alpha-amylase hydrolysis, and hydrolyzed starch is converted to D-glucose by amyloglucosidase (Megazyme, 2018).
Parameters Measured	Relies on iodine absorption and formation of an amylose-iodine complex with the damaged starch (Monnet et al., 2019, Dubat & Sas, 2003).	The damaged starch granule (both amylose and amylopectin) is digested by alpha-amylase (McAllister et al., 2011; Monnet et al., 2019).
Time Efficiency	High; fully automated processes significantly reduce analysis time, often completing fewer than 10 minutes for one sample (Morgan & Williams, 1995).	Moderate; it can take a few hours and relatively time-consuming and labor-intensive (Wilson et al., 2016).
Data Analysis	With built-in software for analysis and reporting, automated data collection.	Need further calculation. Final glucose concentration is then converted into a damaged starch content.

User-Friendliness/ Target user	Much easier and simpler to the operators who firstly use this instrument. Widely used in the industry.	Require well-trained operator and is more complicated (Dubat & Sas, 2003; J. T. McAllister et al., 2011). Often used by lab test.
Reproducibility	Calibration is needed, against a suitable primary reference method such as the Enzymatic method (AACC 76-31).	Highly accurate and reproducible (Medcalf &Gilles, 1965; Morgan & Williams, 1995; McAllister et al., 2011).
Cost	Higher initial investment for the SDmatic device but potentially lower ongoing chemical costs.	Typically lower equipment cost but may require multiple reagents.

Table 2.2 Composition and particle size distribution of lentil (L), chickpea (CP) and yellow pea (YP) flours.

Samples	Particle size	Protein	Ash	Crude fiber	Crude fat	Total starch	Amylose content
L	fine	28.12±0.07[b]	2.63±0.05[a]	1.21±0.01[a]	1.09±0.20[a]	54.40±0.48[c]	32.37±0.01[a]
CP	fine	22.25±0.16[a]	2.80±0.01[b]	1.78±0.80[a]	7.25±0.26[b]	47.50±0.65[a]	31.32±0.43[a]
YP	fine	30.78±0.23[c]	2.58±0.05[a]	0.74±0.02[a]	1.20±0.22[a]	50.94±0.13[b]	32.47±0.21[a]

Particle size distribution				
		D_{10}(μm)	D_{50}(μm)	D_{90}(μm)
L	fine	38.91±2.17 d	163.7±28.14 c	243.4±33.80 h
	small	147.9±7.21 bf	262.7±13.01 b	363.6±9.40 g
	medium	139.6±52.47 abf	438.2±36.20 a	472.5±57.42 b
	coarse	174.5±50.56 abf	455.9±69.51 a	671.4±118.65 a
CP	fine	22.2±0.03 e	157.9±44.55 c	294.0±21.07 f
	small	38.4±1.97 d	184.0±5.80 c	287.5±11.60 ef
	medium	36.0±8.61 d	272.2±21.14 b	412.0±86.69 bd
	coarse	151.9±4.40 b	386.5±27.37 d	678.3±33.40 a
YP	fine	70.6±7.81 c	172.1±5.02 c	250.8±17.18 ch
	small	64.7±9.28 c	180.0±9.97 c	301.1±38.18 cef
	medium	129.4±30.48 b	288.8±21.21 b	493.4±48.01 bd
	coarse	179.4±3.75 a	441.5±31.54 a	633.6±23.41 a

D_{10}-largest particle in 10% of the sample

D_{50}-largest particle in 50% of the sample

D_{90}-largest particle in 90% of the sample

* Values are means ± SD. Means not sharing a common superscript letter in a column are significantly different at $p \leqslant 0.05$.

Table 2.3 Damaged starch content (SD%) of pulse and wheat flours and starches determined by the enzymatic method (AACC 76-31.01), iodide absorption (AI%) and SD% by the amperometric method (AACC 76-33.01).

	Particle size	Enzymatic method (SD%)	Amperometric method				
			1 gram of flours		2 grams of flours		
			AI%	SD%	AI%	SD%	SD% (Calibrated)
Pulse flours							
Lentil	Fine	1.5±0.0 [a]	91.2±0.3	3.7±0.2	97.8±0.3	5.0±0.2	1.4±0.1
	Small	1.5±0.0 [a]	90.6±0.2	3.6±0.2	97.0±0.1	4.6±0.1	1.3±0.1
	Medium	0.8±0.0 [de]	80.9±0.1	3.7±0.1	92.4±0.2	3.4±0.1	0.7±0.1
	Coarse	0.7±0.0 [de]	74.0±0.0	9.8±0.0	90.9±0.1	1.9±0.1	0.6±0.1
Chickpea	Fine	1.0±0.0 [bc]	90.4±0.2	3.3±0.1	96.2±0.2	4.1±0.1	1.2±0.1
	Small	1.1±0.0 [bc]	89.7±0.1	3.2±0.2	95.7±0.2	3.8±0.1	1.1±0.1
	Medium	0.8±0.0 [cde]	78.9±0.2	5.0±0.2	92.2±0.1	2.3±0.1	0.7±0.1
	Coarse	0.7±0.0 [e]	66.8±0.3	21.7±0.3	86.7±0.1	1.2±0.0	0.6±0.0
Yellow pea	Fine	1.5±0.0 [a]	92.2±0.4	4.4±0.2	97.4±0.2	4.8±0.1	1.4±0.1
	Small	1.6±0.1 [a]	92.4±0.2	4.7±0.2	97.5±0.2	4.9±0.1	1.4±0.1
	Medium	1.1±0.1 [b]	81.7±0.1	3.3±0.0	95.1±0.2	3.5±0.1	1.0±0.1
	Coarse	0.9±0.2 [bcd]	76.5±0.1	7.0±0.2	91.0±0.1	1.9±0.0	0.7±0.0
CCF*	-	1.3±0.0 [f]	85.1±0.0	2.5±0.1	95.2±0.1	3.5±0.1	1.0±0.1

Wheat flours							
CWF*	-	7.5±0.1	95.6±0.1	7.3±0.1	-	-	-
SWF*	-	3.9±0.1	93.7±0.2	5.5±0.2	-	-	-
HWF*	-	7.1±0.4	95.7±0.0	7.3±0.0	-	-	-
HAWF*	-	3.9±0.1	95.5±0.0	7.4±0.0	-	-	-
Starches							
WWS*	-	6.2±0.0	60.9±0.0	35.4±0.1	-	-	-
NWS*	-	3.8±0.1	95.0±0.0	6.9±0.0	-	-	-
HAWS*	-	2.1±0.1	96.5±0.0	8.5±0.0	-	-	-
YPS*	-	1.0±0.0	92.0±0.2	4.5±0.2	-	-	-

Reference flours	Target AI% value	Actual AI% value
Reference Flour sample 1	92.3±0.5	91.59
Reference Flour sample 2	94.8±0.4	94.33

Note: The SD% of 2 grams of flour was calculated by dividing the SD% value obtained from the Chopin SDmatic instrument by the sample weight.

*Abbreviations: CCF: Commercial chickpea flour; CWF: Control wheat flour in Megazyme assay kit; SWF: Soft wheat flour; HWF: Hard wheat flour; HAWF: High-amylose wheat flour; WWS: Waxy wheat starch; NWS: Normal wheat starch; HAWS: High-amylose wheat starch; YPS: Yellow pea starch.

* Values are means ± SD. Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$.

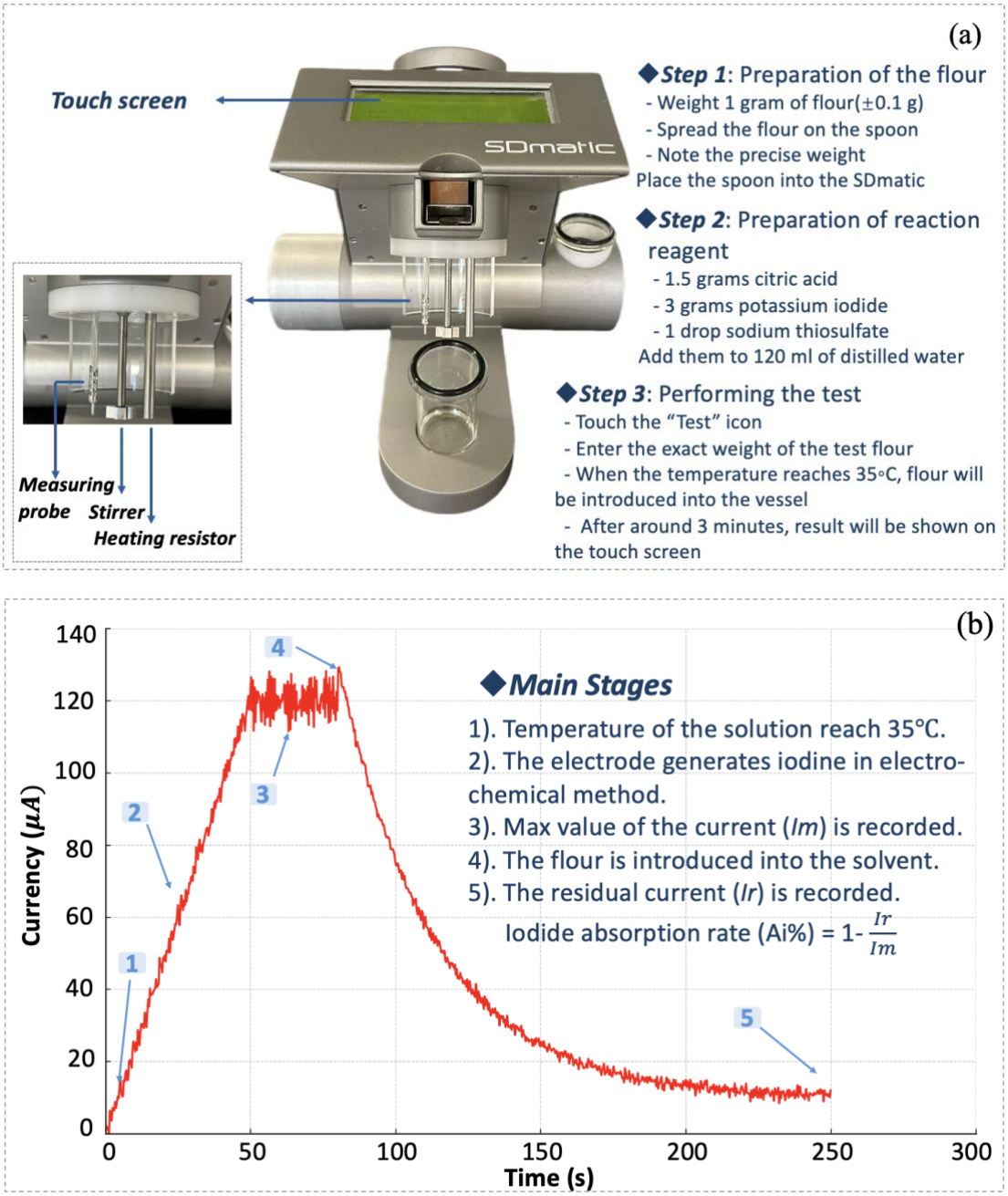

Figure 2.1 (a)Chopin SDmatic operating procedures AACC 76-33.01; (b)Changes in currency (I,μA) during the SDmatic analysis (Adapted from KPM Analytics, SDmatic Automated Damaged Starch Analyzer).

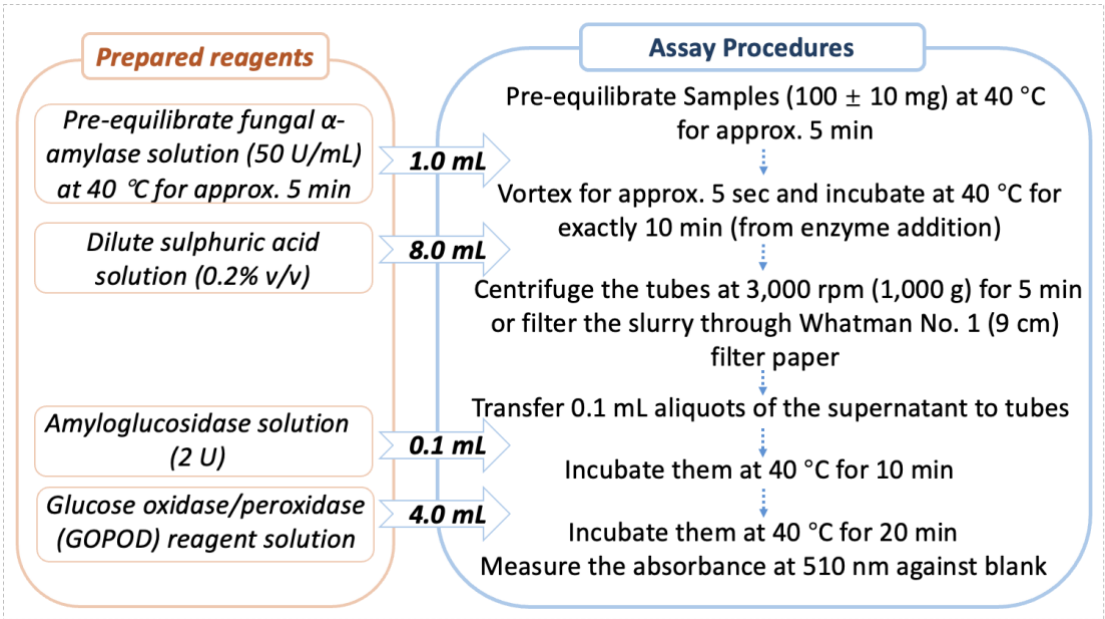

Figure 2.2 Starch damage assay procedure based on AACC 76-31.01 method.

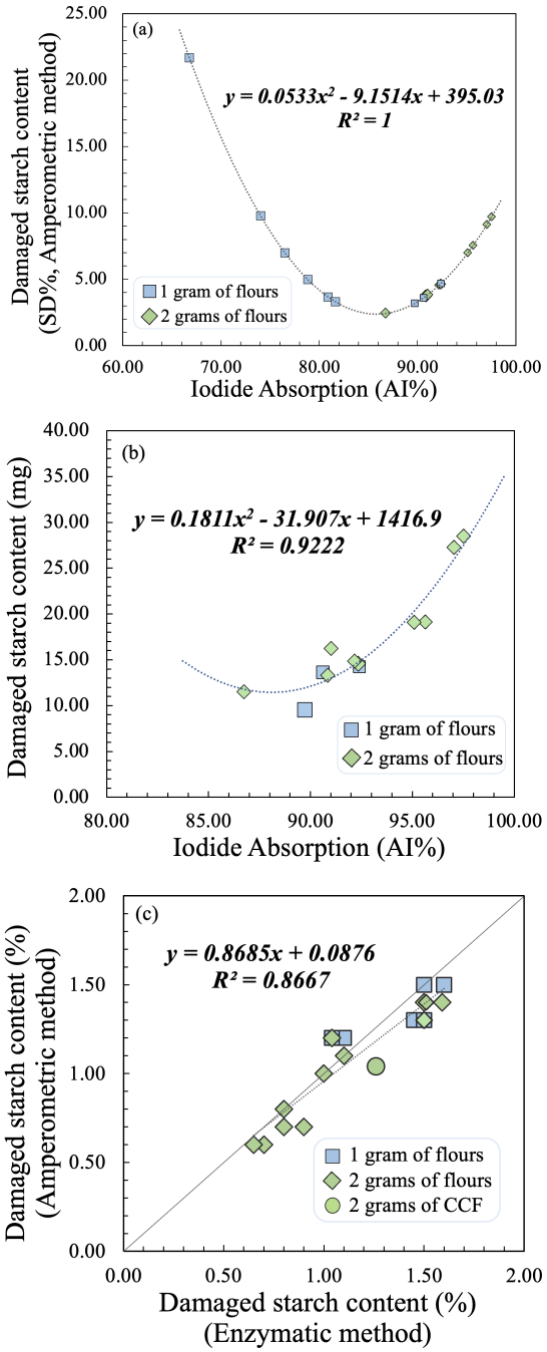

Figure 2.3 (a)Correlation curve of iodine absorption (AI%) and damaged starch content (SD%) of different particle size pulse flours (based on 1 gram and 2 grams of flours for each test); (b)Correlation curve of iodide absorption (AI%) and damaged starch content (mg) using enzymatic method; (c)Comparison of damaged starch content% using our calibrated equation.

Chapter 3 - Effects of Heat-Moisture Treatment on Protein Structure and Functional Properties in Pulse Flours

Abstract

Pulses such as yellow pea, chickpea, and lentil are nutrient-rich crops valued for their high protein content, essential micronutrients, and functional versatility in food systems. As the second most important food source globally after cereals, pulses offer recognized health benefits, including the potential to reduce chronic disease risk. Pulse proteins, varying from 16–32% across different species, are critical contributors to functional properties such as solubility, emulsification, and gelling. Structurally, their proteins—comprising mainly globulins like legumin (11S) and vicilin (7S)—are functionally important but often limited in digestibility due to β-sheet-rich structures that hinder enzymatic access. While various heat treatments have been studied to modify protein functionality, the specific effects of heat-moisture treatment (HMT) on pulse proteins remain insufficiently understood. This study systematically investigates the effects of HMT on the structural and functional properties of pulse proteins, focusing on protein solubility after pepsin treatment, hydrophilicity, and extractability. Results revealed a marked increase in non- soluble protein content after HMT, rising from 18.24% to 43.61% in yellow pea, 7.28% to 45.33% in lentil, and 12.25% to 84.94% in chickpea. Protein hydrophobicity increased for all pulse flours, reflecting structural changes induced by HMT. Additionally, HMT significantly decreased protein extractability in yellow pea and chickpea, with most treated samples falling below 70% compared to untreated controls, whereas lentil maintained high extractability (>84%) across

all HMT conditions. These findings provide new insights into the structure–function relationship of pulse proteins under HMT and offer a foundation for optimizing pulse-based ingredients in thermally processed food applications.

Introduction

Pulses, such as yellow pea, chickpea, and lentil, are recognized as an excellent source of essential nutrients and are widely recommended by health organizations and nutritionists as dietary staples. Ranked as the second most important food source for humans after cereals (Shevkani et al., 2019), pulses are rich in proteins, vitamins, minerals, and carbohydrates, making them vital role to human nutrition (Guldiken et al., 2021; Roy et al., 2010). The consumption of pulses has been linked to numerous health benefits, including reducing the risk of chronic diseases and enhancing overall well-being (Setia et al., 2020). Therefore, there is a growing global demand for incorporating pulses into diets, especially in the form of pulse flours.

Among the nutrients in pulses, protein stands out as a key nutrient in pulses, typically second in abundance after carbohydrates. Protein content in pulses can varying depending on genetic factors and environmental conditions (Hood-Niefer et al., 2012) . The protein content is 21.2-32.9% in yellow pea (Dahl et al., 2012), and 16.1-26.7% “Desi” chickpea and 19.9-25.5% in “Kabuli” chickpea, respectively (Grasso et al., 2022). Lentil also exhibits high protein content, ranging from 21-30.2% (Wang & Daun, 2006), and are recognized for their exceptional nutritional value (Kraska, 2018). Proteins contribute significantly to functional

properties such as solubility, emulsification, foaming ability, gelling characteristics, and oil absorption, influencing functional properties of final products (Argel et al., 2020). Moreover, they are highly complementary to other well-known cereal proteins like wheat. Cereal proteins are typically rich in sulfur-containing amino acids but lack lysine, an essential amino acid. In contrast, pulse proteins are abundant in lysine but are deficient in sulfur-containing amino acids (methionine and cysteine) and tryptophan (Aider et al., 2012; Bou et al., 2022; Keskin et al., 2022; Setia et al., 2020). This complementary amino acid profile makes combining pulses with cereals in food formulations an effect wat to achieve a more balanced and complete protein source.

The protein present in pulse seeds are primary storage proteins, which play a critical role in supplying nitrogen (N), carbon (C) and sulfur (S) (Shevkani et al., 2024). These proteins could be classified according to their solubility as albumin, globulin, and prolamins and glutelins (Jarpa-Parra, 2018; Roy et al., 2010). Pulses are predominantly composed of globulin, constituting about 70% of total protein content, serving as a storage protein (Roy et al., 2010; Shevkani et al., 2019; Shrestha et al., 2023). The two primary types of globulins present in pulses are (11S) legumin and (7S) vicilin (Carbonaro & Nucara, 2022; Jarpa-Parra, 2018; Shevkani et al., 2024).

The secondary structure of pulse proteins is primarily composed of β-sheets, β-turns, and α-helices. β-sheets were found to be the predominant secondary structures in legume proteins, followed by those in cereal, dairy, and chicken meat proteins (Carbonaro et al., 2012). Proteins with a greater proportion of β-structures, such as parallel and antiparallel β-sheets and β-turns, tend to exhibit higher thermal stability, as they denature at higher

temperatures compared to proteins with a higher α-helix content (Carbonaro et al., 2012; Parmar et al., 2017). However, this dominance of β-structures negatively impacts protein digestibility. The dense network of β-sheets creates a structural barrier, limiting the accessibility of digestive enzymes and resulting in reduced protein digestibility (Shevkani et al., 2019). Carbonaro et al., 2012 also reported that β-sheets in legumes, as well as the formation of intermolecular β-sheet aggregates during thermal treatment, were primarily contributors to their reduced digestibility. Generally, heating has a dual impact on protein digestibility, offering both advantages and drawbacks. On the positive side, heat can inactivate digestive enzyme inhibitors and cause proteins to unfold, making them more accessible to enzymatic breakdown (Tang et al., 2009). However, it can also result in negative effects, such as the aggregations of denatured proteins, which reduces their solubility and limits enzyme access. This process usually was recognized as three steps: thermally denaturation, aggregate formation and interaction into networks (Tang, 2008). The overall influence of thermal processing on protein digestibility depends on factors such as the heating conditions and the specific properties of the protein. Cooking and food processing can modify protein structure, exposing enzyme cleavage sites and improving digestibility. However, certain treatments (e.g., excessive heating) can lead to protein aggregation, reducing digestibility (Ajomiwe et al., 2024).

The pulse proteins are primarily located in the cotyledon, either as small (2–4 mm) spherules or as part of a protein matrix embedded within starch granules (Shevkani et al., 2019, 2024). The poor digestibility of native pulse storage proteins, particularly vicilin, has been attributed to their tightly packed structures and the structural stability by their

carbohydrate moieties (Tang et al., 2009). Due to this distinct structure, the digestibility of protein and starch in pulse flour are closely related. Heat–moisture treatment (HMT) is the common physical modification method for both flours and starches. It involves incubation of samples at low moisture level (<35% water w/w) and heating for a certain period (15min-16hours) and heating temperature above glass transition temperature but below gelatinization temperature (Chung et al., 2009). Currently, HMT has an important role to play in the food industry, especially due to its thermal stability and a lower tendency to retrograde. Numerous studies showed that HMT influences starch digestibility, gelatinization, pasting properties, amylose content, morphological properties, crystallinity, molecular interactions of different flours, including rice (Puncha-arnon & Uttapap, 2013), sorghum (Vu et al., 2017), mango kernels (Bharti et al., 2019), wheat (Li et al., 2020), quinoa flour (Dong et al., 2021), highland barely (Lv et al., 2022), chickpea (Du et al., 2023), sweet potato (Zhang et al.,2023) and so on. The study by Puncha-arnon & Uttapap (2013) indicated that HMT had a more pronounced effect on digestibility, pasting properties, and crystallinity in rice flour compared to rice starch. This suggested that other components, such as proteins, play an important role in modifying the structural and functional properties during HMT. Moreover, the study by Dong et al. (2021) found that protein and starch interactions were observed using confocal laser scanning microscopy (CLSM), revealing increased protein-starch complex formation after treatments. Similarly, Wu et al. (2024) examined how proteins from different sources (white kidney bean, soybean, casein, whey protein) influence corn starch structure, physicochemical properties, and digestibility under HMT. Their findings revealed that the addition of proteins during HMT significantly decreased starch digestibility by forming a

physical barrier that inhibited enzymatic breakdown. Additionally, most proteins effectively inhibited α-amylase activity, reducing the breakdown of starch into glucose.

While the effects of HMT on pulse flours especially focused on starch-related changes (Ambigaipalan et al., 2014; Mathobo et al., 2021; Xiong et al., 2019) and heat treatments on protein structure and digestibility (Beliciu & Moraru, 2011; Halabi et al., 2020) were well-documented. Limited studies have indicated how HMT affect the protein part within the flour, especially for pulses. Similar research such as those by Vu et al. (2017) observed that HMT decreased digestibility and solubility of sorghum proteins while inducing structural changes in protein fractions. Based on these existing findings, this study aims to analyze the impact of HMT on the structural properties of yellow pea, chickpea and lentil proteins and its subsequent effects on protein digestibility, hydrophilicity, and extractability in pulse flours.

Materials and methods

Materials

Commercial yellow pea (*Pisum sativum* L.) kernels were purchased from Amazon (D'allesandro gourmet ingredients, Washington, IL, USA). Commercial chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* L.) kernels were purchased from Amazon (Food to Live store, Brooklyn, NY, USA). Chickpea, red lentils, and yellow pea kernels were milled by a laboratory scale Ross roller mill. (Model 915, Ross Machine and Mill Supply, Oklahoma City, OK, USA). The yield of dehulled yellow pea, lentil and chickpea flour from milling were 83.42%, 78.84% and 82.61%, respectively.

Proximate analyses

Moisture content was determined using AACC Method 44-15.02 (AACC, 2000). Nitrogen content was measured by the Dumas combustion method using a Nitrogen/Protein Analyzer (FP628, LECO Corp., MI, USA) which was converted to protein content (% N × 6.25) in accordance with AOAC Method 992.15 (1995). Ash content was measured using AACC Method 08-01.01 (AACC, 2000). Starch content was quantified using Total Starch Assay Kit following protocol method e (Suggested procedure for the determination of starch in samples which contain D-glucose and/or maltodextrins - removal of D-glucose and maltodextrins with alcohol washing.) The crude fiber content of the sample was analyzed using the AOAC 962.09 method. The determination of crude fat was carried out using the AOCS Method Ba 3-38. Damaged starch content was quantified using Damaged Starch Assay Kit by Megazyme (AACC Method 76-31.01. Amylose content was quantified using Amylose/Amylopectin Assay Kit by Megazyme.

Heat-moisture treatment

Appropriate amounts of distilled water were added to pulse flours to adjust moisture content (wet basis) to around 20%, 25% and 30% and the samples were mixed thoroughly distribute the water into the flour by mixograph. Pulse flours with moisture content of as-is, 20%, 25% and 30% were sealed in canning jars and equilibrate overnight. After measuring

the exact moisture content, the flour samples were sealed in pressure tubes (38mL, 150psi, Ace Glass Incorporated, Vineland, NJ) to prevent moisture loss and heated at 3, 6, 9, or 12 °C below the onset gelatinization temperature for 4 hours based on a previous study. The onset gelatinization temperatures of yellow pea at as-is, ~20%, ~25%, and 30% moisture contents were 131°C, 118°C, 111°C, and 106°C, respectively. For chickpea, the temperatures were 137°C, 121°C, 110°C, and 105°C. Lentil showed onset temperatures of 134°C, 120°C, 112°C, and 104°C at the same moisture levels. After heat-moisture treatment, flour samples were transferred to Petri dishes for drying in vacuum dry for 2 hours and then equilibrate at room temperature overnight.

Protein solubility after pepsin treatment

Protein solubility was measured by a modified in vitro Englyst method (Englyst et al, 1992). Briefly, flour samples (~500mg d.b.) with 30 glass beads were placed in a centrifuge tube (50ml) with 5mL water for 30 minutes to hydrate the flours. Freshly prepared pepsin solution (50 mg pepsin and 50mg guar gum in 5 ml 0.02M HCl) was added to the tube, and the mixture was incubated for 30 minutes at 37°C. After pepsin treatment, sodium acetate solution (0.25M) was added to the mixture to stop pepsin digestion process, and centrifuge at 3220g for 10 minutes, decan the supernatant and wash the pellet by 5mL of the same sodium acetate solution and centrifuge one more time. The pellet was then filtered and washed by distilled water; suction was applied. The sample, together with filter paper was combusted

using a Nitrogen/Protein Analyzer (FP628, LECO Corp., MI, USA), the nitrogen mass against blank was recorded and for the calculation of non- soluble protein content.

Protein surface hydrophobicity

Pulse flour samples with different HMT were subjected to protein hydrophobicity test using the bromophenol blue (BPB) method (Chelh, Gatellier, & Santé-Lhoutellier, 2006). Flour (15 mg) was weighed in 15 ml centrifuge tube. 1 ml of 20mM sodium phosphate buffer (pH 6.0) was added to the tube followed by 600µl of 1mg/ml BPB in distilled water. The tubes were vigorously stirring for 10 min. After stirring, sample tubes were centrifuged for 15 min at 2000 g. Supernatant of each sample was transferred to a new glass tube and diluted 10 times using 20mM sodium phosphate buffer. The diluted samples were mixed and read at 595 nm against a blank (sodium phosphate buffer) on a spectrophotometer. The amount of BPB bound representing the protein surface hydrophobicity of samples was calculated as: BPB bound (µg)=600µg*(Absorbance of blank–Absorbance of sample)/Absorbance of blank.

Protein extractability and molecular size distribution

Protein extraction and size-exclusion chromatography (SEC) were carried out following the method described by Ioerger et al. (2007) with slight modification. One milliliter of 12.5 mmol L^{-1} sodium borate buffer (pH 10) with 2% sodium dodecyl sulfate (SDS) was added to 20 mg of pulse flours and vortexed for 30 min. After centrifuging, the

supernatant (non-reduced soluble protein) was collected and heated at 80 ∘C for 2 min to deactivate proteases. The remaining pellet was then extracted with 1 mL of 12.5 mmol L^{-1} sodium borate buffer pH 10.0 with 2% SDS plus 2% β-mercaptoethanol and vortexed for 30 min followed by centrifugation at 3220 × g for 10 min. The supernatant (reduced soluble protein) was collected and heat-deactivated as described above.

SEC analysis of the protein fractions were performed as described in Ioerger et al. (2007) with slight modifications. Proteins were separated by Agilent 1100 HPLC system (Agilent Technologies, Inc.) equipped with a Yarra 3 μm SEC-4000 column (7.8 × 300 mm^2, Phenomenex), with a 50 mM sodium phosphate, pH 7.0, buffer containing 1% SDS as mobile phase with a flow rate of 0.5 mL/min. Total protein extractability was calculated based on the protein that was extractable from untreated pulse flour: protein extractability (%)=100 × (total area of treated flour/total peak area of untreated flour).

Results and Discussion

Compositional analysis

This proximate composition of yellow pea (YP), lentil (L) and chickpea (CP) flour are presented in Table 1. Yellow pea flour has the highest protein content (30.78%), followed by lentil flour (28.12%) and chickpea flour (22.25%). For carbohydrates, lentil has the highest total starch (54.40%), with yellow pea slightly lower (50.94%) and chickpea the lowest (47.50%). In contrast, chickpea leads in crude fiber content (1.78%) and crude fat content(7.25%), significantly higher than yellow pea (0.74% fiber and 1.20% fat) and lentil

(1.21% fiber and 1.09% fat). Damaged starch content is the highest in lentil (1.52%), followed closely by yellow pea (1.46%), while chickpea has the least (0.95%). Amylose content is relatively uniform across the flours, ranging from 31.32% in chickpea to 32.47% in yellow pea.

The protein contents observed align with the typical range reported for pulse flours (21.3–31.5%) in Ren et al. (2021). The starch contents are also consistent with literature, where lentil starch has been reported between 44.5% and 50.1% (Barbana et al., 2011; Shevkani et al., 2024), and pea starch between 45.7% and 51.2% (Ren et al., 2021). Amylose content ranges match the reported values of 23.5%–32.3% in lentil, 30.4%–35.0% in chickpea, and 24.0%–49.0% in yellow pea (Hoover et al., 2010).

Protein solubility after pepsin treatment

The data in Figure 1 presented the non- soluble protein content after pepsin treatment of yellow pea (a), lentil (b), and chickpea (c) flours before and after HMT. The untreated flours showed the lowest non-soluble protein levels, with lentil (7.28%) being the most soluble, followed by chickpea (12.25%) and yellow pea (18.24%). After HMT, non-soluble protein content increased in all flours, reflecting structural changes such as protein unfolding, denaturation, and aggregation that reduce enzymatic accessibility.

Among these three flours, chickpea consistently showed the highest increase in non-soluble protein after HMT compared to the other two pulse flours, particularly at as-is moisture content, where its content peaked at 84.94% at eTo-6°C and remained 83% at eTo-

3°C. Even at milder heating condition (eTo-12°C), chickpea still exhibited a large increase (62.37%). This was significantly higher than the peaks for yellow pea (43.30%) and lentil (45.33%), which indicates that chickpea protein tended to have greater protein aggregation and cross-linking upon heating. Across all moisture levels, chickpea retained high non-soluble protein levels across all moisture conditions. Even at ~20% and ~30% moisture content, chickpea maintained high values (74.45–75.40% and 65.19–77.51%, respectively), which indicates its proteins are more resistant to hydrolysis after HMT. This is different with lentil flours, where higher moisture levels generally led to better digestibility. Moreover, lentil had the lowest non-soluble protein content among the three flours in untreated and certain HMT-treated conditions. At as-is moisture, lentil showed its highest non-soluble protein content (45.33%) at temperatures of eTo-3°C. At higher moisture levels (~20–30%), lentil displayed lower non-soluble protein values compared to as-is moisture, with lower non-soluble protein content. For example, at ~30% moisture, the lowest value (13.83%) occurred at eTo-12°C. For yellow pea, non-soluble protein content also increased with temperature at as-is moisture, peaking at 43.30% before decreasing at higher temperatures (27.69% at eTo°C). At ~20% moisture, yellow pea exhibited similar trends, with a maximum of 43.61% at eTo-3°C, while at ~25% and ~30% moisture, the highest values were relatively lower (32.67% and 37.22%, respectively), where showed a similar trend compared to lentil, with its highest non-soluble protein content (45.33%) occurring at eTo-3°C for as-is moisture, but lower values were observed at higher moisture levels (~20–30%), showing enhanced digestibility as moisture increased.

The clear increase in non-soluble protein content (%) after HMT for all three pulse flours suggests that thermal processing under moisture environment could lead to substantial protein structural changes, probably involving protein denaturation, hydrophobic interactions, and disulfide bond formation, which promote aggregation and reduce susceptibility to enzymatic hydrolysis. Chickpea proteins appeared to have more tendency to aggregate and more resistant to the hydrolysis after HMT, even at higher moisture levels, suggesting a greater degree of inter- or intra- molecular interactions. While lentil proteins were the most soluble, particularly under higher moisture conditions that seemed to reduce heat-induced aggregation. Yellow pea showed similar behavior with lentil flours, with aggregation occurring at low moisture but reduced at higher moisture levels. The greater susceptibility of chickpea to heat-induced aggregation can be attributed to its unique composition. Chickpea proteins consist predominantly of globulins, which are more prone to aggregation due to their higher hydrophobicity and sulfhydryl content (Boye et al., 2010; Stone et al., 2015), while lentil proteins may contain more soluble albumins or globulins with less reactive thiol groups (O'Kane et al., 2004), leading to fewer cross-links upon heating. The ratio of vicilin to legumin, protein secondary structure, and amino acid profile could also contribute to these differential behaviors. Chickpea contains a higher proportion of 11S legumin, a cysteine-rich globulin capable of forming covalent disulfide bonds during heating. These proteins are more prone to irreversible aggregation under thermal stress. In contrast, lentils are dominated by 7S vicilin, a cysteine-free protein that resists disulfide bond formation and aggregates primarily via weaker hydrophobic interactions, which may be more reversible under high-moisture conditions (Boye et al., 2010; O'Kane et al., 2004). This structural difference supports lentil's

enhanced solubility (which will be discussed in the following sections) and lower non-soluble protein levels after HMT.

Amino acid composition further contributes to chickpea's behavior. It contains higher levels of cysteine and lysine than lentil and yellow pea (Stone et al., 2015), making it more reactive during thermal treatment. Cysteine facilitates disulfide cross-linking, while lysine may participate in Maillard-type cross-linking, especially under lower moisture levels. These interactions result in the formation of high-molecular-weight aggregates, as reflected in the higher non-soluble protein levels observed.

Moisture content during HMT also plays a crucial role. At low moisture (as-is), protein mobility is restricted, favoring aggregation and intermolecular cross-linking (Chávez-Murillo et al., 2018; Lefèvre et al., 2022). At higher moisture (~25–30%), water promotes thermal mobility and reduces aggregation potential by allowing more complete unfolding and rearrangement. This is especially evident in lentil and yellow pea, where higher moisture levels led to lower non-soluble protein content, suggesting improved protein digestibility and reduced structural rigidity.

These findings are consistent with the previous researches. Chávez-Murillo et al. (2018) attributed reduced protein digestibility to aggregation and structural modifications, while Genovese and Lajolo (1996) demonstrated that heating promotes the aggregation of dry bean albumins and enhance the formation of high-molecular mass protein aggregates. These results suggest that the negative effects related to aggregation of denatured proteins seem to dominate over the combined influences of heating on digestibility. Similarly, Collar et al. (2020) reported that HMT (15% moisture content, 120°C for 1 hour) altered the protein

structure in wheat and non-wheat flour blends, promoting protein aggregation and forming a more compact network. More recently, Perraulta Lavanya et al. (2021) also found that HMT decreased crude protein content in all tested sorghum varieties, likely due to heat-induced protein denaturation, which affected solubility and digestibility. These studies can conclude that HMT reduces protein digestibility primarily through aggregation and structural reorganization.

The effect of HMT on the hydrophobicity of pulse protein

Figure 2 illustrates the protein surface hydrophobicity (measured by bound bromophenol blue, BPB) of untreated and HMT-treated yellow pea (a), lentil (b), and chickpea (c) flours. Among the untreated samples, lentil showed the highest protein hydrophobicity (67.47), followed by yellow pea (47.76) and chickpea (30.03). After HMT, all samples exhibited increased hydrophobicity, indicating structural changes.

For yellow pea flours, the most significant increase was observed at the condition of eTo-3°C, ~30% moisture (111.03), where the non-soluble protein increased from 18.24% to 37.22% under the same condition, reflecting the exposure of hydrophobic residues and reduced enzymatic accessibility due to protein unfolding. Generally, at the same treating moisture content, with the increase of heating temperature, the protein hydrophobicity increase with the exception of the condition at eTo°C, as-is moisture, both hydrophobicity (50.18) and non-soluble protein content (27.69%) declined compared to the untreated yellow pea flour.

Lentil flour showed a highest protein hydrophobicity among three untreated pulse flours and showed a more complex pattern after HMT. At the as-is moisture level, the highest hydrophobicity (108.07) was observed at eTo-9°C, while further heating to eTo-3°C caused a sharp drop in hydrophobicity (87.40). This decrease likely reflects protein aggregation, as hydrophobic residues become buried, consistent with the highest non-soluble protein content (45.33%) observed at this temperature (eTo-9°C). At ~20% moisture, hydrophobicity reached its peak at eTo-6°C (142.62). This increase consistent with a moderate non-soluble protein content of 22.30% from Figure 1, suggesting that structural changes improved enzyme accessibility to some extent. At higher moisture levels, such as ~30%, hydrophobicity increased steadily with temperature, reaching a maximum at eTo-3°C (133.33), while the corresponding non-soluble protein content remained moderate (24.07).

For chickpea flours, where the untreated sample has the lowest hydrophobicity (30.03%) among all samples, indicating the native protein structure with minimal exposure of hydrophobic residues. After HMT, hydrophobicity increases significantly under most conditions, particularly at higher moisture levels. At as-is moisture, hydrophobicity peaks at eTo-6°C (58.92), suggesting that heating partially unfolds the proteins, exposing hydrophobic groups. Moreover, under the same temperature, Figure 1 shows the highest non-soluble protein content (84.94%), indicating that aggregation accompanies the exposure of hydrophobic groups, reducing enzyme accessibility. Beyond eTo-6°C, hydrophobicity drops sharply (33.03% at eTo°C), which consistent with a slight reduction in non-soluble protein content (67.97%) and may be due to excessive aggregation could hinder hydrophobic residues. At ~25% moisture, hydrophobicity is highest at eTo-12°C (76.77), reflecting

significant protein unfolding with maximal exposure of hydrophobic groups. This corresponds to the lowest non-soluble protein content (55.16%) at this moisture level, indicating improved enzymatic access. As temperature increases (e.g., eTo-9°C to eTo-3°C), hydrophobicity decreases steadily (70.58% to 66.21%), while non-soluble protein content rises, suggesting aggregation limits enzyme accessibility.

Overall, HMT induced protein unfolding across all pulse flours, leading to greater surface hydrophobicity as more hydrophobic residues became exposed. In this study, protein surface hydrophobicity was assessed by measuring the binding of bromophenol blue (BPB) to proteins. It is important to note that this method primarily captures the hydrophobicity of the insoluble protein fraction and aggregated structures, rather than soluble proteins. During the assay, BPB binds preferentially to hydrophobic regions exposed on protein-protein or protein–starch complexes. After incubation, the unbound BPB remaining in the supernatant (which primarily contains soluble proteins) is measured. The final hydrophobicity value is calculated based on the difference between the total BPB added and the BPB detected in the supernatant, thus the assay specifically highlights the behavior of less soluble, aggregated, and structurally altered proteins or matrixes. Therefore, changes in surface hydrophobicity detected by BPB binding also provide insights into the extent of protein unfolding, aggregations, and insolubility induced by HMT. Chickpea proteins, with the lowest initially protein hydrophobicity, showed the most significant increase after HMT, suggesting greater unfolding and aggregation potential. This structural disruption was also accompanied by the largest reduction in protein digestibility. In contrast, yellow pea and lentil proteins showed more moderate increases in hydrophobicity and correspondingly smaller reductions in

digestibility. When higher heating was applied, especially at lower moisture levels, hydrophobic residues may have become buried within aggregates, resulting in decreased surface hydrophobicity. These suggested that protein unfolding and the exposure of hydrophobic groups occurred and potentially leading to increased aggregation and reduced enzymatic accessibility.

These findings align well with prior reports. Ajomiwe et al. (2024) observed that proteins with higher surface hydrophobicity generally exhibited reduced digestibility, primarily due to their poor solubility. Previous studies by Schellman (1997) and Baldwin (1986) provided similar insights into protein stability, emphasizing that while the hydrophobic effect plays a significant role, it does not act alone. Other factors, such as conformational changes and enthalpic interactions, also contribute to the unfolding process and influence the overall protein stability. More recently, Han et al. (2023) demonstrated that cooking (water bath at 100°C for approximately 26 minutes) increases surface hydrophobicity by exposing hydrophobic residues. To investigate the effect of different degrees of doneness on protein structure and digestibility, pork samples were divided into outer, middle, and inner layers. Their findings revealed that the higher hydrophobicity in the outer layers was associated with reduced solubility and lower digestibility, further supporting the idea that hydrophobic proteins are more resistant to enzymatic breakdown. Similarly, Silva et al. (2017) applied HMT to paddy rice grains at 13%, 16%, and 18% moisture levels and autoclaved at 121°C for 30 and 60 minutes. The treatment increased the non-soluble protein content, leading to reduced digestibility, likely due to protein aggregation and denaturation. High molecular weight protein fractions became less extractable, likely due to

increased hydrophobic interactions and disulfide bond formation. These findings collectively suggest that thermal processing-induced changes in protein structure, particularly hydrophobicity and aggregation, significantly in turn impact digestibility.

The effect of HMT on the extractability/solubility of pulse proteins

HMT induced significant variations in protein extractability for pulse flours, indicating structural modifications in protein organization. Figure 3,4 and 5 contains protein extractability (a), HPLC chromatographs of non-reduced protein fraction (b) and reduced protein fraction (c) trends for yellow pea, chickpea and lentil flours under different treating moisture and temperature conditions, respectively. While protein extractability results are shown for all treatment conditions, representative HPLC chromatograms illustrate structural differences in selected samples treated at ~eTo-9 °C across all moisture levels. These chromatographic profiles reflect typical patterns, demonstrating protein unfolding, aggregation, or dissociation of different pulse types and treatment conditions.

For yellow pea flour, protein extractability (Figure 3a) was highest in the untreated sample. However, after HMT, the extractability declined across all conditions, suggesting heat and moisture induced structural alterations. At as-is moisture content, extractability varied with temperature, with values of 65.91% (eTo-12°C), 69.64% (eTo-9°C), 64.51% (eTo-6°C), and 68.25% (eTo-3°C). The lowest extractability was observed in the eTo°C treatment (52.50%) over all treating conditions, suggesting that heating played an important role in protein modifications. When moisture levels were increased to ~20%, extractability

followed a similar decreasing trend, with values of 67.96% (eTo-12°C), 76.44% (eTo-9°C), 70.73% (eTo-6°C), and 62.14% (eTo-3°C). At ~25% moisture, extractability was highest at eTo-12°C (74.10%) but showed reductions at 67.45% (eTo-9°C), 66.83% (eTo-6°C), and 67.30% (eTo-3°C), indicating that higher temperatures resulted in greater protein aggregation and reduced solubility.

At ~30% moisture, the highest extractability of treated samples was observed at eTo-12°C (85.09%), followed by eTo-9°C (72.76%), eTo-6°C (66.93%), and eTo-3°C (61.22%). HPLC chromatograms (Figures 3b and 3c) supported these results. In non-reduced fractions, peak intensity decreased significantly after HMT, especially under low moisture condition, along with peak broadening and loss of sharpness, indicating aggregation. In reduced fractions, a single dominant peak (~20–22 min) appeared across all samples. The large changes observed in non-reduced chromatograms but not in reduced ones suggested that disulfide bonds and hydrophobic interactions as key mechanisms in aggregation.

The decline in extractability after HMT reflects increased protein aggregation and reduced solubility, driven by heat-induced denaturation. At low moisture levels, protein mobility is restricted, favoring aggregation by disulfide bonding and hydrophobic interactions, which reduce extractability. The lowest extractability observed at eTo°C under as-is moisture highlights the effects of higher heating temperature at limited water content. At higher moisture levels (~30%), yellow pea proteins exhibited improved extractability, especially at eTo-12°C. This suggests that additional moisture could enhance protein flexibility and preventing irreversible aggregation. This moisture-enhanced mobility allows for partial unfolding and reorganization of protein structures. The non-reduced

chromatograms highlight the aggregation of high-molecular-weight protein complexes under heating at low moisture, while the reduced profiles confirm that these aggregates are primarily stabilized by disulfide linkages and hydrophobic forces. The persistence of a single dominant peak in reduced conditions suggests that no major fragmentation occurred and that aggregation likely involved reorganization.

These findings align with prior studies. Zhou et al. (2008) demonstrated that higher moisture improves protein molecular mobility, allowing reversible unfolding and preventing aggregation. Similarly, Hood-Niefer and Tyler (2010) highlighted the importance of residual moisture in stabilizing protein structure during thermal processing of pea flour. Our results support these observations, with higher moisture (especially at ~30%) preserving extractability despite thermal exposure. The HPLC trends also support the role of aggregation mechanisms involving disulfide and hydrophobic interactions, consistent with behavior reported in other pulse proteins.

The protein extractability and HPLC chromatograms and of lentil protein fractions (Figure 4) indicate that HMT induces moderate changes in protein solubility and structure, with lentil proteins showing greater thermal stability than those from chickpea and yellow pea. Lentil flour maintained high extractability, ranging from 94.7% (eTo-12°C, ~30% moisture) to 84.8% (eTo-3°C, ~25% moisture), indicating limited loss of solubility.

In the non-reduced chromatograms (Figure 4b), the untreated lentil sample showed a dominant peak eluting at ~18–21 minutes, accompanied by several small early-eluting peaks (~8–14 min), which likely represent loosely associated or weakly aggregated high-molecular-weight proteins. After HMT, particularly under as-is and ~20% moisture conditions, these

early-eluting minor peaks decreased in intensity, while the main peak retained its shape and position, and overall peak intensity declined slightly. In the reduced protein fraction (Figure 4c), all samples (untreated and treated) displayed a single, consistent peak near ~21 minutes, with no observable splitting or additional peaks. This pattern suggested that disulfide bonding played only a minor role in lentil protein aggregation during HMT, and that the proteins were primarily held together by weaker interactions, such as hydrophobic forces, which may have been reversible or insufficient to drive strong insolubility. The extractability data (Figure 4a) supports this conclusion, with lentil flour maintaining high solubility across all treatment conditions, ranging from 94.7% (eTo-12°C, ~30% moisture) to 84.8% (eTo-3°C, ~25% moisture).

Compared to chickpea and yellow pea, where extractability declined more dramatically under the same treatments, lentil proteins exhibited greater resistance and thermal stability to heat-induced insolubility and aggregation. The sustained extractability and consistent HPLC profiles across HMT conditions suggest that lentil proteins resisted extensive unfolding or aggregation. The disappearance of early-eluting peaks likely reflects the dissociation of weakly bound complexes, while the preservation of dominant peaks suggests minimal formation of large, insoluble aggregates.

The unique stability of lentil proteins may partly be due to the difference in their amino acid composition and storage protein composition. Literature reports suggest that lentils generally have lower cysteine content (1.1–1.3 g/100 g protein) compared to chickpea (1.5–2.0 g) and yellow pea (1.6–1.9 g) proteins (Boye et al., 2010; Nosworthy et al., 2017; Stone et al., 2015), which may reduce their propensity for disulfide-mediated aggregation during

HMT. Cysteine residues, due to their reactive thiol groups, could promote disulfide bond formation and aggregation upon heating (Shevkani et al., 2024). A lower abundance of cysteine could reduce the extent of covalent cross-linking in lentil proteins during thermal processing, supporting the observed minor role of disulfide bonds in reduced chromatograms (Figure 4c). Lentils also have slightly lower lysine levels (6.9–7.6 g/100 g protein) than chickpea (7.5–8.2 g) and yellow pea (7.7–8.0 g) (Boye et al., 2010; Nosworthy et al., 2017; Stone et al., 2015), which may further limit non-enzymatic cross-linking under heat. Lysine tends to participate in Maillard reactions upon heating, lower lysine levels could further mitigate aggregation. Differences in major storage protein composition may also contribute. The major storage proteins in pulses are globulins, primarily divided into two subfamilies: 11S legumins and 7S vicilins. Lentils have a higher proportion of vicilin-like proteins, which have fewer cysteine residues compared to 11S legumins abundant in chickpea and yellow pea (Boye et al., 2010; Shevkani et al., 2024; Stone et al., 2015). Vicilins are trimeric proteins that lack cysteine residues, making them less prone to disulfide-mediated aggregation during thermal processing. In contrast, legumins are hexametric proteins rich in cysteine, which facilitates disulfide bond formation, promoting irreversible aggregation and insolubility upon heating (Carbonaro et al., 2015; O'Kane et al., 2004). Specifically, studies report that lentil seed proteins contain a vicilin-to-legumin ratio of around 1.6:1, whereas chickpea and yellow pea are often legumin-dominant (Shevkani et al., 2024). This compositional difference is critical under HMT, where legumin-rich parts tend to unfold and aggregate through covalent (disulfide) and non-covalent (hydrophobic) interactions, leading to reduced extractability. In contrast, the vicilin-rich lentil protein matrix retains higher solubility, even under high

temperature and low moisture. The higher vicilin content and lower cysteine levels in lentil proteins likely contribute to their higher thermal stability during HMT.

These findings are consistent with thermal behavior reported in previous lentil protein studies. Lefèvre et al. (2022) provided a thermal modeling framework showing that lentil proteins undergo two partially overlapping denaturation transitions (7S vicilin and 11S legumin), with aggregation closely tied to moisture-dependent unfolding behavior. Furthermore, Bresciani et al. (2022) reported that industrial-scale thermal treatment of red lentils induced partial protein denaturation and aggregation, primarily stabilized by hydrophobic interactions, with minimal involvement of disulfide bonds—a mechanism echoed in our reduction data. In a separate study, Cimini et al. (2022) found that lentil protein extractability decreased after pasta processing, but emphasized that structural changes were relatively mild, and overall functionality was preserved. Our observation of small early-eluting peak loss and sustained extractability (>84% across all HMT conditions) aligns with these findings, indicating the reorganization of loosely bound proteins rather than extensive aggregation.

For the protein extractability of chickpea flour (Figure 5a), a comparable but more pronounced decline trend was observed under HMT. At as-is moisture content, protein extractability did not exhibit a statistically significant difference across different temperature conditions, however, under higher moisture conditions, clear trends emerged. At the lowest treatment temperature (eTo-12°C), protein extractability remained highest across all tested moisture levels, with values reaching 69.19% (as-is), 63.13% (~20%), 60.31% (~25%), and 51.27% (~30%). In contrast, as the temperature increased to eTo-3°C, a notable decline in

extractable protein was observed, with values decreasing to 53.05% (as-is), 50.61% (~20%), 54.32% (~25%), and 49.15% (~30%), respectively. The strong negative correlation was observed between chickpea non-soluble protein content and protein extractability ($r = -0.85$, $p < 0.0001$) indicates that as the proportion of non-soluble protein increases, protein extractability significantly decreases. This suggests that HMT induces modifications of proteins that strongly impact protein extractability, as more protein becomes non-soluble, extractability significantly decreases. The HPLC chromatograms of chickpea protein fractions further demonstrate that HMT significantly influences protein solubility, aggregation, and structural properties. The non-reduced fraction (Figure 5b) showed a substantial reduction in peak intensity following HMT, particularly under as-is and ~20% moisture conditions, indicating enhanced protein aggregation and decreased extractability. the untreated sample displayed a dominant peak around ~20–22 minutes, with a minor peak at ~10 minutes, likely representing a population of high-molecular-weight protein aggregates. After HMT, overall peak intensity markedly declined, and the ~10-minute peak decreased in height but remained present, suggesting the persistence of aggregated or hydrophobically bound protein species. Unlike yellow pea, the chickpea chromatograms exhibited more distinct peak separations, suggesting that HMT induced specific protein fractionation and selective aggregation rather than broad structural unfolding. The broader peak formations and altered retention times further imply the changes in protein conformation, likely due to by hydrophobic interactions and disulfide bonding. The untreated chickpea flour showed one peak in the reduced fraction (Figure 5c), whereas HMT-treated samples exhibit two peaks. This suggested that HMT induced structural modifications in chickpea proteins, leading to structural reorganization from

larger aggregates into smaller protein fractions. The more pronounced aggregation in chickpea were in line with its higher non-soluble protein content, lower extractability, and more pronounced aggregation behavior.

Among the three pulse flours studied, chickpea showed the most dramatic reduction in protein extractability after HMT, suggests that chickpea proteins are particularly susceptible to thermal denaturation, aggregation, and cross-linking, especially under conditions of higher heating temperatures and moisture. These structural changes may be driven by disulfide bonding, hydrophobic interactions, and possibly Maillard-type cross-links, which collectively reduce solubility. Unlike lentil and yellow pea, chickpea exhibited distinct HPLC peak separations and broader shapes after treatment, implying more selective aggregation and structural remodeling. The persistence of early-eluting peaks, along with fragmented profiles in reduced samples, showing a deeper transformation of chickpea protein structure under thermal conditions.

These findings are consistent with previous reports. Vu et al. (2017) found that HMT significantly reduced protein extractability in sorghum flour, particularly at higher treatment temperatures and moisture levels. Similarly, Gasparre et al. (2022) and Liu et al. (2021) noted that heat processing can induce extensive aggregation through Maillard reactions and disulfide cross-linking. Monahan et al. (1995) and Van Der Plancken et al. (2005) also reported reduced solubility and altered protein structure due to covalent modifications during heat treatment. Ma et al. (2011) and Pennells et al. (2024) observed that thermal stress increases protein rigidity, thereby decreasing aqueous solubility, which is consistent with chickpea's behavior after HMT in our study

Conclusion

This study systematically evaluated the effects of heat-moisture treatment (HMT) on the structural and functional properties of proteins in yellow pea, lentil, and chickpea flours. The results showed that HMT significantly modified protein solubility after pepsin treatment, hydrophobicity, and extractability with distinct variations across different pulse types and treatment conditions. Notably, chickpea proteins were the most sensitive to HMT, exhibiting the greatest increase in non-soluble protein content (from 12.25% to 84.94%), accompanied by the strongest decline in extractability (from ~100% to as low as 49.15%) which indicated strong aggregation and resistance to enzymatic hydrolysis. This behavior is likely driven by chickpea's higher proportion of cysteine-rich legumin (11S) proteins and reactive amino acid residues, which tends to have disulfide cross-linking and hydrophobic interactions during HMT. Lentil proteins, on the other hand, displayed greater thermal stability, minimal disulfide interactions, and consistently higher extractability even under higher HMT conditions, likely due to their vicilin-rich, low-sulfhydryl protein composition. Yellow pea exhibited intermediate behavior, with both non-solubility and extractability moderately impacted by HMT. Additionally, increasing moisture content during HMT seemed to decrease the protein aggregation in lentil and yellow pea but was less effective in chickpea, suggesting the role of protein structure in moisture sensitivity.

These findings highlight the importance of intrinsic protein composition, especially amino acid profile and storage protein type, in determining pulse protein responses to thermal processing. Understanding these molecular interactions enable us to target optimization of

HMT conditions to enhance the nutritional and functional performance of pulse-based ingredients in food systems.

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Table 3.1 Proximate composition of yellow pea (YP), lentil (L) and chickpea (CP) flours (% dry basis).

	Protein	Ash	Crude fiber	Crude fat	Total starch	Damaged starch	Amylose content
YP	30.78±0.23^{c}	2.58±0.05^{a}	0.74±0.02^{a}	1.20±0.22^{a}	50.94±0.13^{b}	1.46±0.01^{b}	32.47±0.21^{a}
L	28.12±0.07^{b}	2.63±0.05^{a}	1.21±0.01^{a}	1.09±0.20^{a}	54.40±0.48^{c}	1.52±0.04^{c}	32.37±0.01^{a}
CP	22.25±0.16^{a}	2.80±0.01^{b}	1.78±0.80^{a}	7.25±0.26^{b}	47.50±0.65^{a}	0.95±0.00^{a}	31.32±0.43^{a}

* Values are means ± SD. Means not sharing a common superscript letter in a column are significantly different at $p \leqslant 0.05$.

Table 3.2 Non-soluble protein content after pepsin treatment of untreated and HMT-treated pulse flours.

	Treatment	Yellow pea	Lentil	Chickpea
Untreated	-	18.24±1.22 h	7.28±1.20 g	12.25±0.50 h
As-is	eTo-12°C	31.40±2.36 cdefg	26.62±0.77 cd	62.37±1.20 f
	eTo-9°C	37.31±2.93 acd	32.11±1.49 c	60.73±0.38 f
	eTo-6°C	43.30±3.40 a	23.73±2.64 de	84.94±2.51 a
	eTo-3°C	40.79±3.16 ab	45.33±2.52 a	83.45±0.40 a
	eTo°C	27.69±4.54 efg	39.68±0.22 b	67.97±0.09 de
~20%	eTo-12°C	38.84±1.66 abcd	20.47±2.61 de	74.45±0.30 bc
	eTo-9°C	34.06±0.46 bcde	26.96±0.90 cd	75.40±0.14 bc
	eTo-6°C	36.07±0.83 abcde	22.30±0.60 de	70.28±0.80 cde
	eTo-3°C	43.61±3.43 a	23.92±1.82 de	72.89±0.66 bcd
~25%	eTo-12°C	32.67±0.54 bcdef	18.57±0.91 ef	55.16±0.22 g
	eTo-9°C	22.93±4.33 gh	20.77±0.48 de	70.05±1.32 cde
	eTo-6°C	24.15±3.82 fgh	24.25±2.43 de	73.02±1.70 bcd
	eTo-3°C	29.28±1.94 defg	23.97±1.38 de	60.66±1.57 f
~30%	eTo-12°C	32.90±1.19 bcdef	13.83±0.73 f	74.33±2.67 bc
	eTo-9°C	30.01±1.98 cdefg	23.47±1.57 de	70.42±3.67 cde
	eTo-6°C	32.70±2.01 bcdef	19.85±1.40 de	77.51±2.28 b
	eTo-3°C	37.22±2.34 abcd	24.07±2.95 de	65.19±1.91 ef

* Values are means ± SD. Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$.

Note: The estimated onset gelatinization temperatures (eTo °C) of pulse flours at as-is, ~20%, ~25%, and 30% moisture contents were as follows: Yellow pea: 131°C, 118°C, 111°C, and 106°C;Chickpea: 137°C, 121°C, 110°C, and 105°C;Lentil: 134°C, 120°C, 112°C, and 104°C

Table 3.3 Protein hydrophobicity of untreated and HMT-treated pulse flours.

	Treatment	Yellow pea	Lentil	Chickpea
Untreated	-	47.76±1.71 f	67.47±0.22 i	30.03±2.09 h
As-is	eTo-12°C	76.02±1.07 e	99.95±0.45 e	55.05±4.65 ef
	eTo-9°C	78.59±1.28 e	108.07±0.48 d	54.32±0.54 ef
	eTo-6°C	87.66±0.43 d	96.78±0.45 ef	58.92±0.83 e
	eTo-3°C	87.96±2.69 d	87.40±0.65 g	33.43±0.91 h
	eTo°C	50.18±1.28 f	60.62±0.80 j	33.03±1.55 h
~20%	eTo-12°C	99.62±0.69 c	122.44±0.71 c	60.71±1.51 de
	eTo-9°C	91.04±1.83 d	130.12±0.77 b	56.98±0.25 e
	eTo-6°C	88.55±3.91 d	142.62±1.74 a	45.09±2.01 g
	eTo-3°C	101.40±2.12 bc	124.80±0.87 c	28.22±0.75 h
~25%	eTo-12°C	104.54±2.16 bc	79.00±1.63 h	76.77±2.37 a
	eTo-9°C	105.94±0.46 abc	108.30±1.85 d	70.58±1.64 abc
	eTo-6°C	103.67±0.92 bc	145.08±0.87 a	69.05±0.75 bc
	eTo-3°C	107.02±3.18 ab	131.86±2.17 b	66.21±0.75 cd
~30%	eTo-12°C	99.78±0.46 c	93.66±0.46 f	73.03±2.55 ab
	eTo-9°C	80.10±1.28 e	99.19±0.92 e	71.60±1.14 abc
	eTo-6°C	91.28±0.85 d	93.98±0.46 f	49.70±1.98 fg
	eTo-3°C	111.03±1.36 a	133.33±2.13 b	56.98±0.25 e

* Values are means ± SD. Means not sharing a common superscript letter in a column are significantly different at $p \leqslant 0.05$.

Note: The estimated onset gelatinization temperatures (eTo °C) of pulse flours at as-is, ~20%, ~25%, and 30% moisture contents were as follows: Yellow pea: 131°C, 118°C, 111°C, and 106°C;Chickpea: 137°C, 121°C, 110°C, and 105°C;Lentil: 134°C, 120°C, 112°C, and 104°C

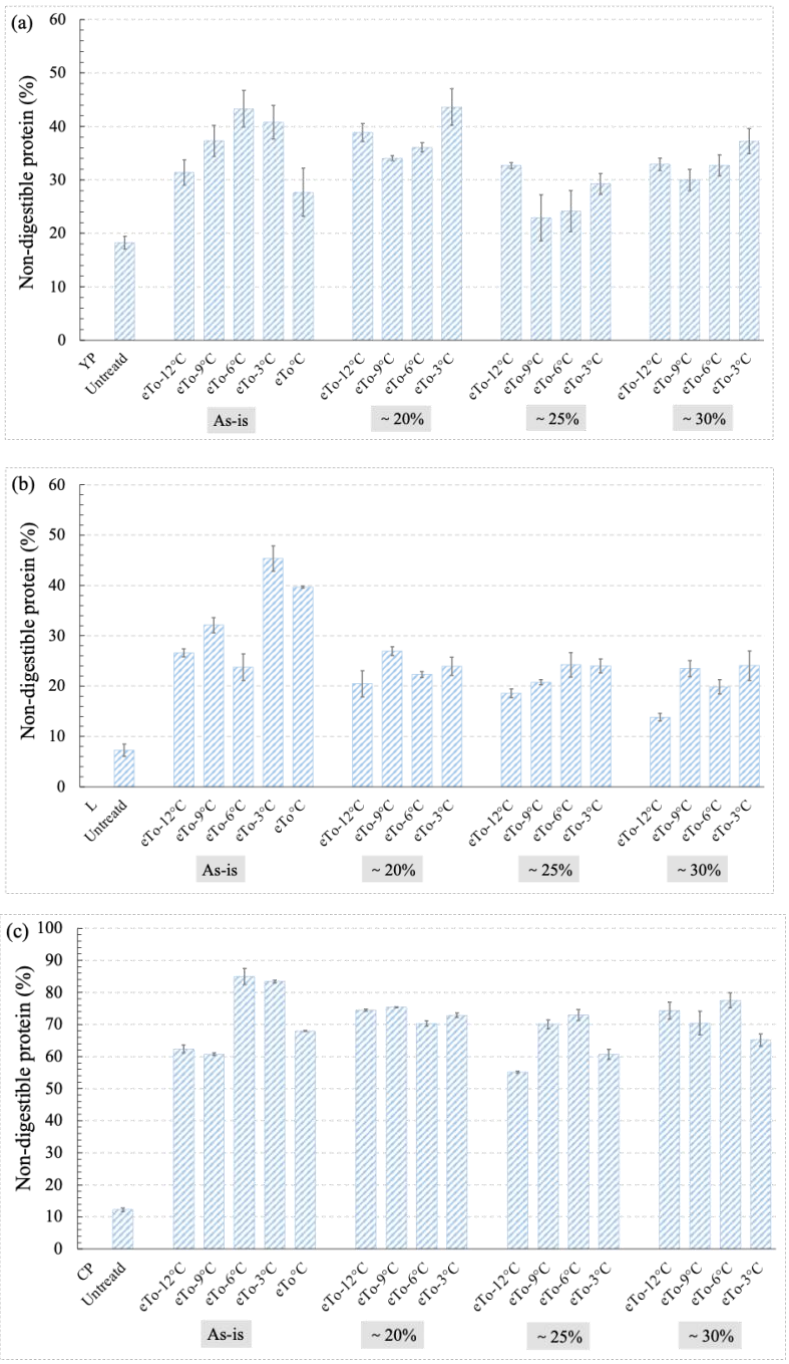

Figure 3.1 Non-soluble protein content (%) after pepsin treatment of untreated and HMT-ed (a)yellow pea (YP), (b)lentil (L) and (c)chickpea (CP) flours. Note: The estimated onset gelatinization temperatures (eTo °C) of pulse flours at as-is, ~20%, ~25%, and 30% moisture contents were as follows: Yellow pea: 131°C, 118°C, 111°C, and 106°C;Chickpea: 137°C, 121°C, 110°C, and 105°C;Lentil: 134°C, 120°C, 112°C, and 104°C

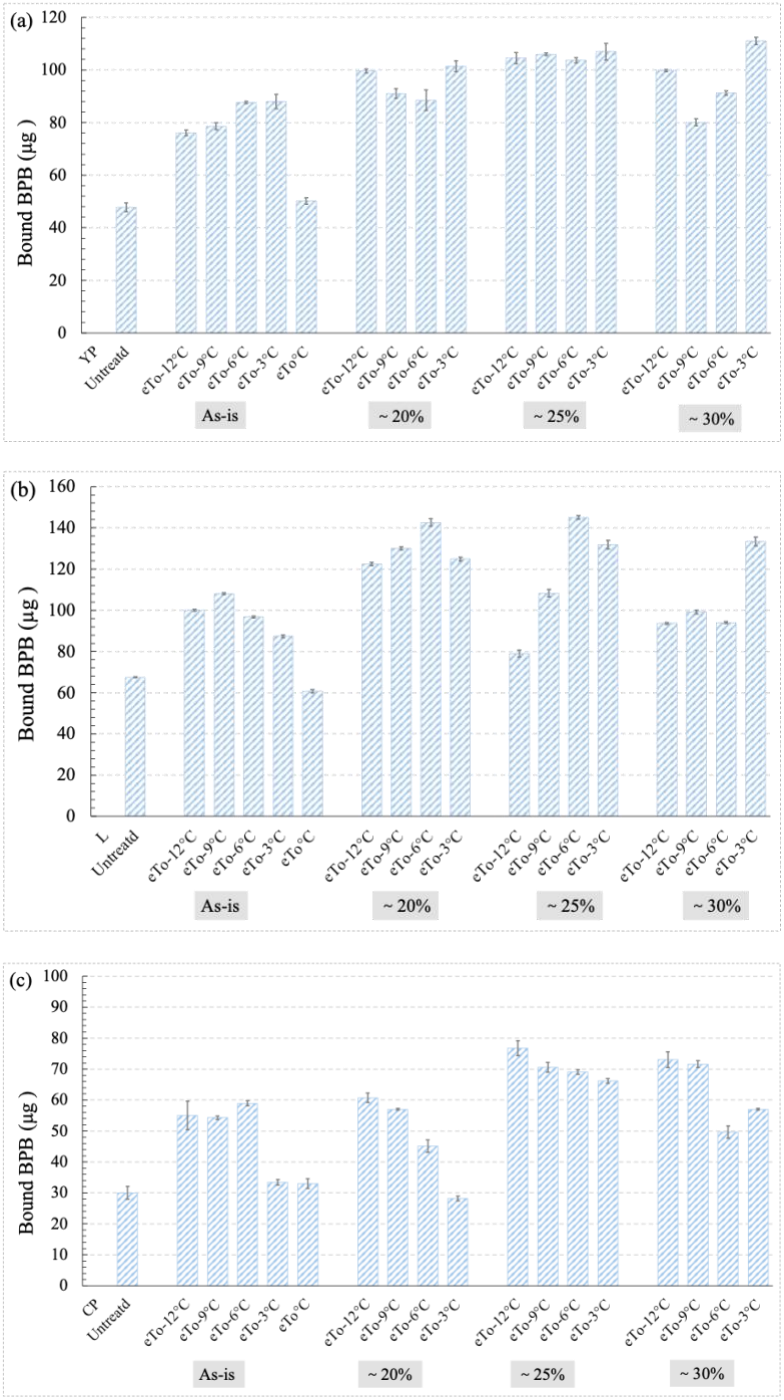

Figure 3.2 Protein hydrophobicity (Bound BPB/µg) of untreated and HMT-ed of (a)yellow pea (YP), (b)lentil (L) and (c)chickpea (CP). Note: The estimated onset gelatinization temperatures (eTo °C) of pulse flours at as-is, ~20%, ~25%, and 30% moisture contents were as follows: Yellow pea: 131°C, 118°C, 111°C, and 106°C; Chickpea: 137°C, 121°C, 110°C, and 105°C; Lentil: 134°C, 120°C, 112°C, and 104°C

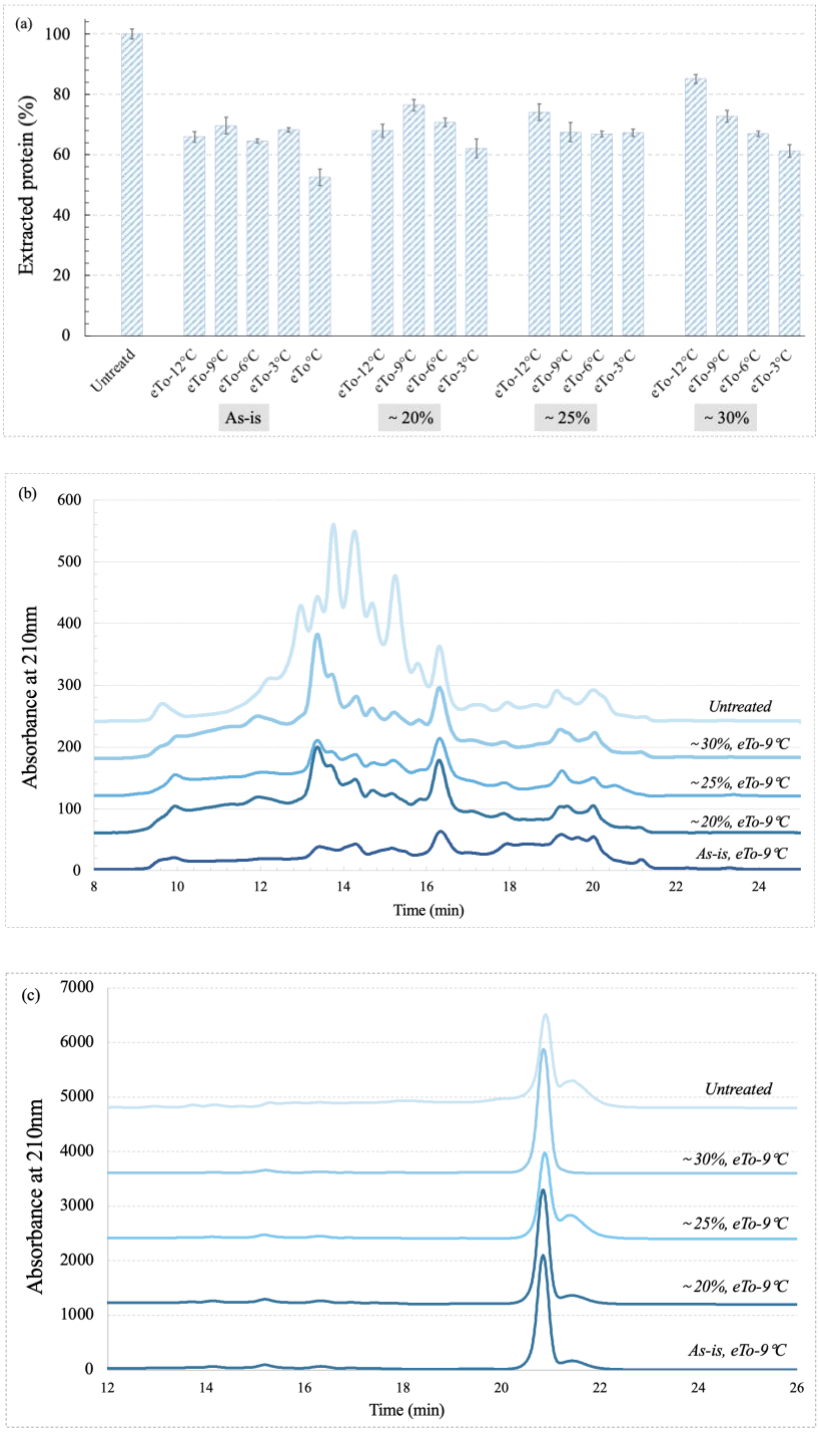

Figure 3.3 Protein extractability (a), HPLC chromatographs of non-reduced protein fraction (b) and reduced protein fraction (c) of yellow pea at different temperature and moisture content. Note: The estimated onset gelatinization temperatures (eTo °C) of yellow pea flours at as-is, ~20%, ~25%, and 30% moisture contents were: 131°C, 118°C, 111°C, and 106°C.

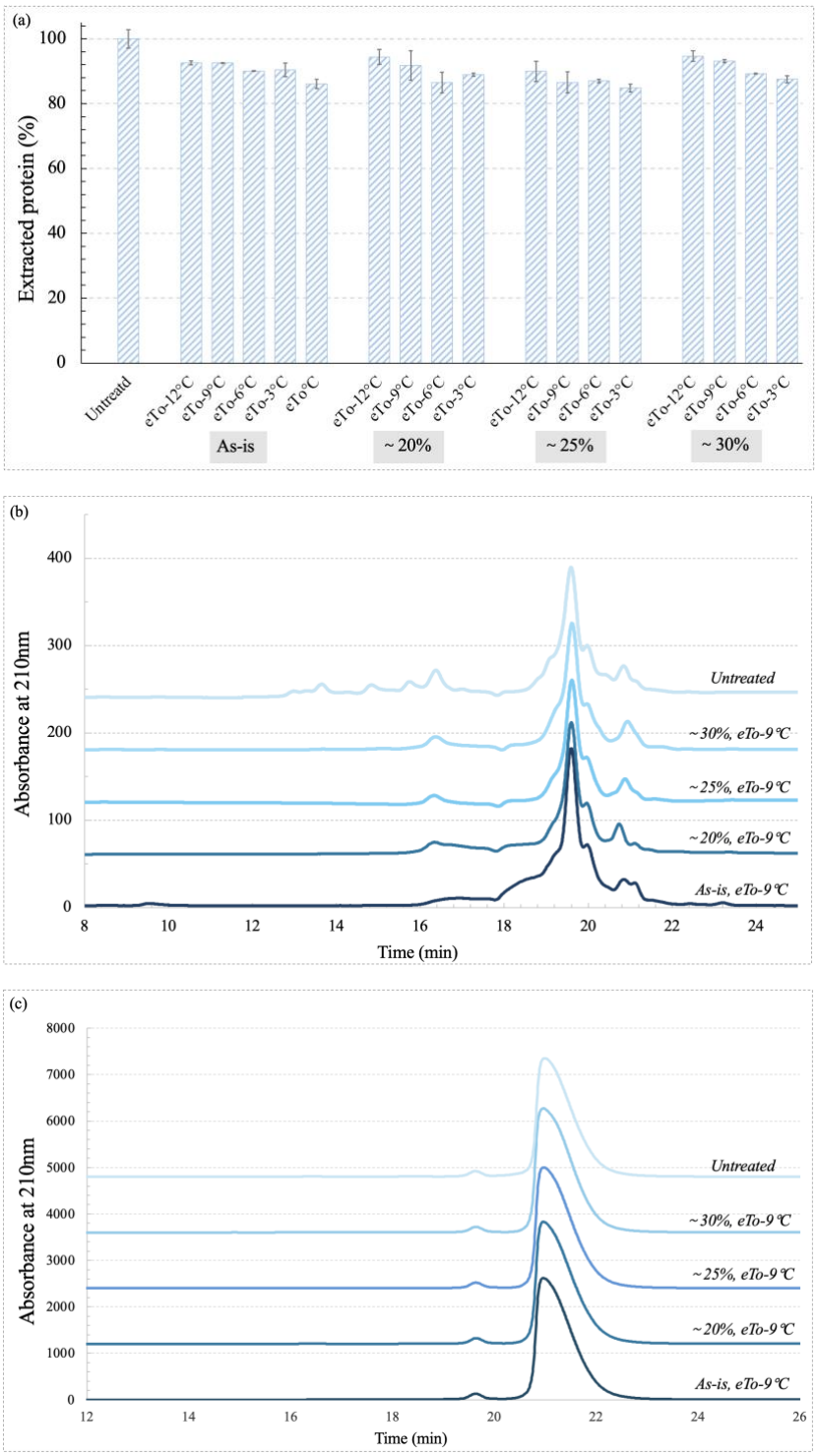

Figure 3.4 Protein extractability (a), HPLC chromatographs of non-reduced protein fraction (b) and reduced protein fraction (c) of lentil at different temperature and moisture content.

Note: The estimated onset gelatinization temperatures (eTo °C) of lentil flours at as-is, ~20%, ~25%, and 30% moisture contents were: 134°C, 120°C, 112°C, and 104°C

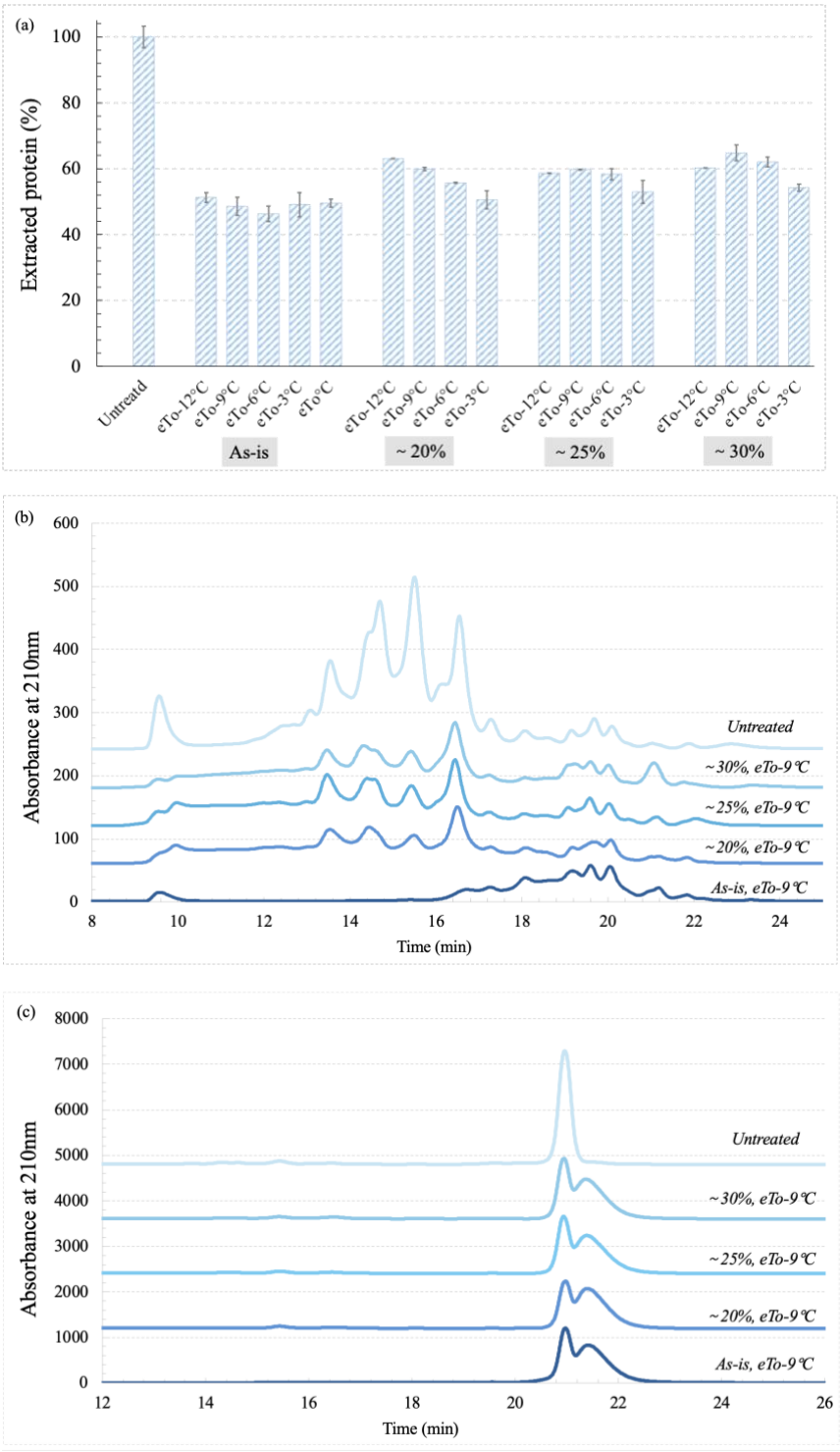

Figure 3.5 Protein extractability (a), HPLC chromatographs of non-reduced protein fraction (b) and reduced protein fraction (c) of chickpea at different temperature and moisture content.

Note: The estimated onset gelatinization temperatures (eTo °C) of chickpea flours at as-is, ~20%, ~25%, and 30% moisture contents were: 137°C, 121°C, 110°C, and 105°C.

Chapter 4 - Conclusions and Perspectives

Chapter 2 focused on evaluating and comparing two widely used analytical methods, amperometric (AACC 76-33.01) and enzymatic (AACC 76-31), for measuring damaged starch (DS) content in pulse flours of varying particle sizes. It was found that the amperometric method consistently reported higher DS values than the enzymatic method across all flour types and sizes, with especially large discrepancies in coarse samples. By developing a calibrated equation to relate iodide absorption (AI%) to enzymatically measured DS content, the study improved the reliability and applicability of the amperometric approach for pulse-based flours. This calibrated method offers a faster, more practical alternative to enzymatic assays for pulse based flours, especially in high output or industrial settings. Understanding DS levels is critical, as damaged starch directly influences water absorption, dough properties, and baking performance. These findings are highly relevant for pulse milling and processing industries aiming to optimize flour quality and performance.

Chapter 3 demonstrated that heat-moisture treatment (HMT) is an effective and clean-label physical modification method to improve the nutritional and functional properties of lentil, chickpea, and yellow pea flours. In addition to its effects on starch, HMT significantly altered protein structure and functionality. Treated samples exhibited reduced protein non-solubility after pepsin treatment and extractability, along with decreased surface hydrophobicity, suggesting protein unfolding, aggregation, and enhanced protein-protein or protein-starch interactions. These changes indicate a strengthened protein-starch matrix, which contributes to thermal stability in food systems. Notably, HMT led to a shift in protein conformation—potentially limiting enzyme access due to the formation of larger aggregates.

These structural changes may enhance resistance to protein hydrolysis but also influence solubility and emulsifying properties, which are critical for food processing. HMT-treated pulse flours may be blended into wheat-based formulations such as noodles, improve dough handling, pasting properties, and final texture, and have potential to make functional ingredients for developing high-protein, gluten-free, or low-glycemic index foods.

Building on the current findings, preliminary work has already been initiated to incorporate HMT-treated pulse flours into wheat-based noodle formulations. Early observations suggest promising improvements in dough handling and noodle structure, supporting the potential of these modified flours in commercial food applications. Further investigation is ongoing to evaluate optimal formulation ratios, textural properties, and digestive attributes.

Future research could also focus on optimizing HMT parameters, such as moisture content, temperature, and treatment duration, aiming to specific pulse types. Since different pulses exhibit unique starch and protein compositions, enhance these variables could selectively enhance desirable properties such as RS content, protein network strength, and thermal stability. Such optimization would enable the targeted development of HMT-treated flours for various food applications. Due to the absence of gluten and the enhanced RS content, these flours are ideal candidates for the formulation of products such as low-GI products, gluten-free breads, high-protein snacks, pasta, protein bars, and dairy alternatives. Future studies should evaluate not only their functional performance (e.g., dough rheology, baking quality, water absorption, emulsification) but also their effects on product shelf life and sensory attributes, such as texture and flavor. The ability of HMT to stabilize protein-

starch interactions may further contribute to product stability and extended shelf life. Moreover, given their naturally high RS and protein content, HMT-treated pulse flours are well-suited for developing health-oriented foods that support glycemic management, digestive health, and satiety. Their use aligns with the growing consumer demand for plant-based, clean-label, and nutrient-dense food products, making them a valuable ingredient in the next generation of functional food innovation.

Appendix A - Onset Gelatinization Temperature-Guided Heat Moisture Treatment: A Novel Approach to Modifying Functional and Nutritional Qualities of Pulse Flours

Abstract

This study introduces an innovative Onset Gelatinization Temperature-Guided Heat Moisture Treatment (HMT) approach to enhance the functional and nutritional properties of pulse flours from yellow pea (YP), chickpea (CP), and lentil (L). The effects of moisture content (10%-30%) and treatment temperatures to the onset gelatinization temperature (eTo) of each sample were systematically evaluated. HMT significantly influenced the color parameters of pulse flours, reducing brightness and inducing a brownish hue due to Maillard reactions and oxidation. Differential Scanning Calorimetry (DSC) analysis revealed increased thermal stability and narrowed gelatinization transition ranges, attributed to the reorganization of starch structures. Starch digestibility was altered, with resistant starch (RS) content increasing in YP and L flours at higher moisture levels, while CP flour exhibited a general RS reduction. HMT enhanced crystallinity, particularly the A-type polymorphs, and modified pasting properties by reducing viscosities and eliminating breakdown peaks, likely due to strengthened crystal structure and starch-protein interactions. Scanning electron microscopy (SEM) and microscopic images showed protein aggregation and unchanged starch granule morphology, further supporting these structural changes. Additionally, RS content increased in cooked sample and total dietary fiber (TDF) content increased in higher moisture treatment, reflecting improved molecular stability and restricted enzyme

accessibility. These findings underscore the potential of HMT to optimize the functional and nutritional profiles of pulse flours, offering new opportunities for their application in food systems demanding enhanced thermal stability, modified starch digestibility, and improved dietary fiber content.

Introduction

Pulses, that include peas, chickpeas and lentils, have garnered significant attention as affordable and nutrient-dense food sources. Rich in protein, dietary fiber, slowly digestible starch (SDS), resistant starch (RS), vitamins, minerals, and other nutraceutical compounds, pulses provide a range of health benefits (Chávez-Murillo, Veyna-Torres, Cavazos-Tamez, de la Rosa-Millán, & Serna-Saldívar, 2018; Du, Jiang, Yu, & Jane, 2014; Jeong, Han, Liu, & Chung, 2019; Ma, Boye, Azarnia, & Simpson, 2016; Singh, Manickavasagan, Shobana, & Mohan, 2021; Sivakumar, Findlay, Karunakaran, & Paliwal, 2023). The consumption of pulses has been associated with the prevention of chronic diseases such as diabetes, cardiovascular disease, and certain cancers (Anderson & Major, 2002; Tharanathan & Mahadevamma, 2003). This is largely attributed to their high content of dietary fiber, SDS, and RS, which contribute to improved glycemic control, delayed gastric emptying, and increased satiety after meals (Singh, et al., 2021; Tharanathan, et al., 2003; Vaz Patto, et al., 2015).

Starch, the main carbohydrate in pulses, is categorized into three types based on in vitro digestion rates: rapidly digestible starch (RDS), SDS, and RS (Englyst, Kingman, & Cummings, 1992). RDS is rapidly broken down into glucose, causing quick spikes in blood

glucose and insulin levels, which can contribute to health complications such as diabetes and cardiovascular diseases. Conversely, SDS provides a sustained release of glucose, stabilizing blood sugar levels and offering critical health benefits such as enhanced glycemic control, improved satiety, and better mental performance. RS as one kind of dietary fiber, resists digestion in the small intestine and ferments in the colon producing short-chain fatty acids that supports colonic health and reduces the risks of diabetes, heart disease, and obesity (Lehmann & Robin, 2007; Lockyer & Nugent, 2017; Miao, Jiang, Cui, Zhang, & Jin, 2015; Slavin, 2008; Zhang, Wang, Cao, & Wang, 2018). Given these health benefits, numerous studies have aimed to increase RS and dietary fiber content through physical, enzymatic, chemical, genetic modifications, and their combinations (Zhang, et al., 2018; Zia-ud-Din, Xiong, & Fei, 2017).

HMT is one physical modification method involves treating starch or flour at low moisture levels (<30%) and high temperatures (80–140 °C), no residual chemical reagents are involved making it an environmentally friendly and cost-effective modification method (Piecyk & Domian, 2021; Zavareze & Dias, 2011).

The effects of HMT on various pulse starches and flours have been widely studied. Researches on pea starch (Cheng, Ren, Warkentin, & Ai, 2024; Cheng, Sun, Li, Warkentin, & Ai, 2025), lentil, navy bean, and normal corn starches (Chung, Liu, & Hoover, 2009, 2010), as well as faba bean, black bean, and pinto bean starches (Ambigaipalan, Hoover, Donner, & Liu, 2014; Xiong, Zhang, Dhital, Huang, & Fu, 2019), have demonstrated significant functional and nutritional modifications. Additionally, HMT has been applied to flours from adzuki bean, pea, and white kidney bean (S. Li, et al., 2024), yellow pea

(Waduge, et al., 2017), and intact cotyledon cells from pinto bean (Xiong, et al., 2019). Results have shown that HMT caused structural changes in both the amorphous and crystalline regions of starch, altering its crystalline structure, swelling behavior, thermal properties, pasting characteristics, and enzyme susceptibility. For example, HMT has been reported to transform C-type pea starch into Ac-type crystals, with a decrease in relative crystallinity (RC) from 27.8% to 18.6% (Cheng, et al., 2025). Similarly, faba bean and black bean starches transformed from C-type to A-type crystals with increased RC after HMT (Ambigaipalan, et al., 2014). The A-type crystalline structure is more thermally stable, and stronger amylose–amylopectin interactions may help RS retain its resistance to digestion after cooking.

However, the effects of HMT on starch within flours or intact cells are less pronounced. In some cases, HMT does not increase RS content and may even reduce it. For instance, Waduge, et al. (2017) reported an 11% decrease in RS in pea flour after HMT. Xiong, et al. (2019) observed that HMT increased the digestibility of uncooked starch and intact cells in pinto beans but had controversial effects on cooked samples. These variations suggest that studying materials, processing conditions—particularly moisture content and temperature relative to the flour's gelatinization properties—are critical in determining HMT outcomes.

Peas, lentils and chickpeas are the major pulses grown throughout the world (Asif, Rooney, Ali, & Riaz, 2013; Chelladurai & Erkinbaev, 2020; Venkidasamy, et al., 2019). Despite their nutritional advantages, their applications in food systems are limited by poor

thermal stability, weak pasting and gelation properties, and the presence of anti-nutritional factors. Overcoming these limitations could expand their use in diverse food products.

The objective of this study is to enhance the RS content and thermal stability of starch in yellow pea (YP), chickpea (CP), and lentil (L) flours using HMT. By improving these properties, the RS content is expected to be better retained after cooking, making pulse flours more suitable for food products aimed at promoting health benefits.

To achieve this, HMT was applied at moisture levels of approximately 10%, 20%, 25%, and 30%, and at temperatures 0, 3, 6, 9, and 12 °C below the starch gelatinization onset temperature for each corresponding moisture content. For a deeper analysis of HMT's effects, treatments at 9 °C below eTo were further evaluated across all moisture levels. This study introduces an innovative onset gelatinization temperature-guided HMT method, offering a precise and systematic approach to optimizing the functional and nutritional properties of pulse flours. Findings from this research will provide valuable insights for expanding the use of pulse flours in food systems requiring enhanced thermal stability and controlled starch digestibility.

Materials and methods

Materials

Commercial yellow pea (Pisum sativum L.) kernels were purchased from Amazon (D'allesandro gourmet ingredients, Washington, IL, USA). Commercial chickpea (Cicer arietinum L.) and lentil (Lens culinaris L.) kernels were purchased from Amazon (Food to

Live store, Brooklyn, NY, USA). The grains underwent milling using a roller mill with meticulous optimization of the milling conditions to maximize the quantity of whole grain flour produced. The yield of yellow pea, lentil and chickpea flour from milling were 83.42%, 78.84% and 82.61%, respectively.

Proximate analyses

Moisture content was determined using AACC Method 44-15.02 (AACC, 2000). Nitrogen content was measured by the Dumas combustion method using a Nitrogen/Protein Analyzer (FP628, LECO Corp., MI, USA) which was converted to protein content (% N × 6.25) in accordance with AOAC Method 992.15 (1995). Ash content was measured using AACC Method 08-01.01 (AACC, 2000). The crude fiber content of the sample was analyzed using the AOAC 962.09 method. The determination of crude fat was carried out using the AOCS Method Ba 3-38. Starch content was quantified using Total Starch Assay Kit following protocol method e (Suggested procedure for the determination of starch in samples which contain D-glucose and/or maltodextrins - removal of D-glucose and maltodextrins with alcohol washing.) The damaged starch content was determined by using Megazyme Starch Damage Assay Kit following AACC Method 76-31.01. The amylose content was determined by using Megazyme Amylose/Amylopectin Assay Kit. The data were reported on a dry basis (db).

Heat-moisture treatment

Yellow pea, lentil, and chickpea flours with ca. 20% moisture content were prepared using a water absorption process within a controlled humidity mixer. Target water contents of ca. 25% and 30% were achieved by introducing water through a spraying and mixing process using a Mixograph (National Manufacturing Co., NE, USA). The samples were then sealed in jars at room temperature for 4 h to allow for equilibration before the actual moisture content (MC) was determined. The estimated onset temperature (eTo) of pules flours with specific moisture content was calculated based on the equations reported by (refs.).

For yellow pea: eTo = -1.4509*MC + 147.22

For chickpea: eTo = -1.6119*MC + 151.79

For lentil: eTo = -1.5569*MC + 149.32

5 g of each sample were placed in a 38 mL pressure tube (864809, Ace Glass, Inc.), purged with nitrogen for 1.5 min, and then loaded into a Mechanical Convection Oven (51028124, Thermo Scientific) at 0 (only for as-is samples), 3, 6, 9 12°C below eTo for 4 h. After this, the sample was transferred to a petri dish and vacuum dried for 2 h. Sealed in a glass bottle overnight, then opened the lid and covered it with gauze at ambient temperature for 6 hours.

Color parameters

The color parameters were measured using a Konica Minolta CR-31D (Japan). Any large particles were broken down using mortar and pestle.

Thermal properties

The thermal properties of pulse flours were analyzed using a differential scanning calorimeter (DSC) (TA Q5000, TA Instruments, New Castle, DE, USA) equipped with an RCS 90 cooling system and a nitrogen purge gas, following the procedure described in (Qi, Mao, & Shi, 2025). Briefly, samples (8–10 mg, db) were prepared with 25% solid content, sealed in stainless-steel pans, and heated from 10°C to 180°C at 5°C/min. Onset (To), peak (Tp), conclusion (Tc) temperatures, and enthalpy (ΔH) were calculated using TRIOS 5.1.1. An empty pan served as the reference.

In vitro *starch digestibility in uncooked and cooked pulse flour*

The digestibility of starch in untreated and treated pulse flour samples was measured using a modified Englyst method (H. N. Englyst, et al., 1992; Sang & Seib, 2006). Briefly, samples (500 mg, db) were soaked in 2.5 mL of distilled water for 30 min. Large visible particles were broken down with a spatula, which was then rinsed with an additional 2.5 mL of distilled water. The samples, free of large particles, were vortexed for 10 seconds to maintain consistency. (For the test of cooked sample, the samples were cooked in boiling

water for 30 min and then cooled to room temperature for 6 min.) The samples were then hydrolyzed in two stages: first with pepsin for 30 min, followed by further digestion with pancreatin and amyloglucosidase (AMG) for 20 and 120 min, respectively. After hydrolysis, 0.25 mL of the mixture was transferred into 10 mL of 66% ethanol (v/v) and centrifuged. The supernatant was collected to determine the digested starch content. RDS, SDS and RS contents were calculated following the method described by H. N. Englyst, et al. (1992); Sang, et al. (2006).

Morphology of flours

Light microscopy

The samples were observed by an Olympus BX51TF microscope attached with a retardation plate (Olympus Optical Co. Ltd., Shinjuku-ku, Tokyo, Japan) as described (Qi, et al., 2025).

Scanning electron microscopy (SEM)

The samples were coated with a gold-palladium alloy and examined using a scanning electron microscope (S-3500N, Hitachi Science System, Ltd., Japan). Imaging was performed at an accelerating voltage of 20 kV, with observations conducted at magnifications of 100× and 500×.

X-ray diffraction (XRD)

The treated pulse flours were ground using a mortar and pestle to pass through a 50 mm screen. Both untreated and heat-moisture treated pulse flour samples were equilibrated to approximately 20% moisture in a sealed desiccator, maintained at 100% relative humidity and around 25°C. X-ray diffraction (XRD) patterns were analyzed using a PANalytical Empyrean diffractometer (PANalytical, Almelo, The Netherlands). The diffraction analysis was performed over a 2 theta range of 3° to 32° with increments of 0.05°, using Cu Kα radiation source (λ = 0.15405 nm). The relative crystallinity was calculated using PeakFit 4.12 software (Systat Software, San Jose, CA), following the method described by Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008).

Pasting properties

The treated pulse flours were ground as described in section 2. 8. The pasting properties of each sample were analyzed using a Rapid Visco Analyzer (RVA 4500, Perten Instruments, Waltham, MA, USA), following AACC Method 76-21.01 with minor modifications.

Flour samples (2.5 g, dry basis) were suspended in distilled water at a 1:10 solid-to-water ratio, manually mixed, and allowed to sit for 3 min to improve clump dispersion. The resulting slurry was stirred at 960 rpm for 10 s, followed by continuous stirring at 160 rpm for the remainder of the analysis. The temperature profile involved holding the slurry at 50 °C for 1 min, followed by heating to 95°C. The slurry was maintained at 95°C for 2.5 min before cooling back to 50 °C, where it was held for 2 min. Peak viscosity, the time and temperature at peak

viscosity, and final viscosity were recorded. All RVA measurements were performed in duplicate.

Total dietary fiber

The treated pulse flours were ground as described in section 2. 8. Total dietary fiber content was determined using a Total Dietary Fiber Kit from Megazyme (Wicklow, Ireland), following AOAC Method 991.43 (AOAC, 1991).

Statistical analysis

The analyses of variance (ANOVA) were performed by the SPSS 24.0 Statistical Software Program (SPSS, Inc., Chicago, IL, USA). Mean values and standard deviations from the duplicated experiments were reported.

Results and discussion

The effect of HMT on the color of pulse flours

The effects of HMT on the color parameters of pulse flours were evaluated, and the results are presented in Figure 1. The parameters L*, a*, and b* represent brightness, redness, and yellowness, respectively. Untreated yellow pea and chickpea flours displayed a yellowish hue with brightness, while lentil flour exhibited a reddish hue with brightness. Compared to

untreated pulse flours, the brightness (L*) of all treated samples decreased, accompanied by an increase in the a* value (except for lentil, which showed a decrease in the a* value), particularly in samples treated at approximately 10% moisture content. The observed brownish coloration and reduced brightness in treated flours were attributed to the Maillard reaction and oxidation, even though purging with nitrogen significantly mitigated the color change. Overall, higher treatment temperatures led to more pronounced color changes, while the presence of moisture appeared to limit these changes.

The effect of HMT on thermal properties of pulse flours

The thermal properties of pulse flours (with and without treatment) were evaluated using DSC, and the results were summarized in Table 2 and Figure 2. The onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), and enthalpy of gelatinization (ΔH) were calculated from the DSC curves (Figure 2). For untreated flours, the To, Tp, and Tc values were as follows: YP flour (65.91°C, 76.03°C, 93.85°C), CP flour (61.51°C, 69.42°C, 84.99°C), and L flour (66.36°C, 75.69°C, 103.70°C). The ΔH values for untreated YP, CP, and L flours were 11.55, 9.61, and 11.15 J/g, respectively. HMT generally enhanced the thermal stability of pulse flours, as evidenced by increased To and Tp values in most treated samples. However, CP flour with 9.10% moisture content treated at 6–12°C below eTo exhibited a decrease in To. Tc values decreased in YP and L flours after HMT, except for YP treated at 6–12°C below eTo with 10.75% moisture content, which showed an increase. Conversely, CP flour exhibited an increased Tc following HMT. HMT narrowed the

gelatinization transition range (ΔT = Tc - To) for YP, CP, and L flours in most cases, except for CP with 9.10% moisture content. This narrowing was reflected in sharper thermal transition peaks for most treated samples. After HMT, the ΔH of all samples decreased, suggesting the disruption of intrahelical hydrogen bonding within double helices due to the melting of crystallites in flours (Ahmed, Thomas, Taher, & Joseph, 2016; Chen, He, Fu, Zhang, & Huang, 2017).

The improved thermal stability was likely attributable to the disruption of "imperfectly" arranged double helices during HMT, leaving the thermally stable crystalline structures intact. Upon cooling, these disrupted regions reorganized into more thermodynamically stable arrangements, enhancing structural stability (Cheng, et al., 2025; Huang, Zhou, Jin, Xu, & Chen, 2016; H.-Y. Kim, Ye, & Baik, 2023b). The formation of amylose-lipid complexes (V-type crystals) during heating may have further contributed to the increased stability (H.-Y. Kim, et al., 2023b). The narrowed gelatinization peak indicated a more uniform structure in the treated samples. The reduction in ΔH may also reflect protein denaturation during HMT. The disappearance or reduction of the shoulder between 90–120°C (indicated by a black arrow in Figure S 1) supports this, as denatured proteins no longer contribute to the thermal transitions of treated flours. Protein denaturation could also explain the decreased Tc in some samples. The increased Tc observed in YP flour treated at 6–12°C below eTo with 10.75% moisture content might result from residual protein crystalline structures overlapping with the starch gelatinization peak during DSC heating. This overlap may have affected the slope of the thermal transition, as the Tc is defined as the intersection of the tangent line fitted to the stable region after the transition and the baseline.

The effect of HMT on the **in vitro** *digestibility of starch in uncooked pulses flours*

The effects of HMT on the contents of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) in uncooked pulse flours are presented in Figure 3. The initial RDS, SDS, and RS contents for the yellow pea (YP), chickpea (CP), and lentil (L) flours were 18.5%, 39.2%, and 42.3%; 21.7%, 53.7%, and 24.7%; and 24.1%, 37.9%, and 38.0%, respectively.

For YP flours (Figure 3 A) with 10.72% moisture content, treatments at 122°C and 128°C (3°C and 6°C below the onset temperature, eTo) did not result in significant changes in starch digestibility. However, treatments at 119°C and 131°C (12°C and 0°C below eTo) led to increases in RDS and SDS, while decreased in RS content, with SDS showing a more pronounced increase. In contrast, treatment at 125°C (9°C below eTo) caused a reduction in RDS, while RS increased, with the most notable decrease observed in RDS content. For YP flour with 20.33% moisture content, all treatments reduced RS and increased RDS and SDS, except for the treatment at 115°C (3°C below eTo), which did not affect the RDS content. In the case of YP flour with 25.14% moisture content, all treatments resulted in an increase in RS and a decrease in RDS and SDS, except for the treatment at 108°C (3°C below eTo), which did not affect RDS or SDS. These results suggested that higher moisture content influences the gelatinization process, leading to different trends in starch digestibility. When YP flour contained 28.74% moisture, similar trends with 25.14% moisture content were observed, but with some differences. The treatment at 100°C (6°C below eTo) increased

SDS, while treatment at 103°C (3°C below eTo) decreased RS and RDS but increased SDS. These variations highlight the importance of moisture content in determining the thermal behavior of starch and its digestibility during HMT.

In CP flours (Figure 3 B) no treatment significantly increased RS content. In fact, most treatments led to a decrease in RS, except for the treatments with 9.10% moisture content at 125°C and 128°C (12°C and 9°C below eTo), where RS content remained unaffected. Similarly, treatments with 25.62% moisture content at 98°C and 101°C (12°C and 9°C below eTo) showed no significant impact on RS content. Moreover, the treatments with 9.10% moisture content at 125°C and 128°C (12°C and 9°C below eTo) did not significantly alter the *in vitro* digestibility of starch in CP flour. However, treatments with 9.10% moisture content at 134°C and 137°C increased SDS. In the cases of CP flour with 19.14% moisture content, RDS content increased, but SDS decreased, with treatments at 115°C and 118°C (6°C and 3°C below eTo) having no significant effect on SDS content. This suggested that the treatments at higher temperatures or with certain moisture contents can affect the digestibility of starch differently in CP flour compared to YP. For CP flour with 25.62% moisture content, SDS increased while RDS decreased, except for treatment at 107°C (3°C below eTo), which led to a significant increase in RDS. In contrast, treatments with 29.09% moisture content resulted in an increase in RDS and a decrease in SDS, except for the treatment at 93°C (12°C below eTo), where SDS increased. The treatment at 99°C (6°C below eTo) did not change the SDS content. These observations further highlight the complex interactions between moisture content, treatment temperature, and the digestibility of starch.

In L flours (Figure 3 C), all treatments with moisture content below 20% led to a decrease in RS, except for the treatment with 9.98% moisture content at 125°C and 128°C (9°C and 6°C below eTo), which had no significant effect on RS content. Furthermore, these treatments did not significantly alter the *in vitro* digestibility of starch in L flours. In fact, all treatments with 9.98% moisture content did not significantly affect RDS. However, treatments with 18.99% moisture content increased both RDS and SDS, indicating that higher moisture content may enhance the digestibility of starch in L flour during HMT. For L flours with higher moisture content (above 25%), RS content increased, except for the treatment with 24.08% moisture content at 103°C (9°C below eTo), which showed no change in RS. Additionally, the treatment with 29.21% moisture content at 101°C (3°C below eTo) decreased RS. In terms of RDS content, higher moisture levels generally led to a decrease in RDS, except for the treatments with 29.21% moisture content at 95°C and 101°C (9°C and 3°C below eTo), where no significant changes in RDS were observed. Regarding SDS, the treatments with 24.08% moisture content did not affect SDS content, except for the treatment at 106°C (6°C below eTo), which decreased SDS. Similarly, treatments with 29.21% moisture content decreased SDS, except for the treatment at 101°C (3°C below eTo), which increased SDS.

Although numerous studies have demonstrated that HMT can increase RS content in isolated starches through structural changes in both the amorphous and crystalline regions (Chung, et al., 2009; Park, et al., 2018; Wang, Zhang, Chen, & Li, 2016), its effectiveness in flours appeared to tell a different story. In this study, HMT consistently decreased RS content in CP flour across almost all treatment conditions, while RS content increased in YP and L

flours at higher moisture treatment conditions. However, the variations in starch digestibility with respect to moisture content and treatment temperature did not follow a consistent trend. These findings indicated that starch digestibility in flours is influenced by more intricate factors compared to isolated starches. Beyond the crystalline structure of starch, changes in the integrity of the cell wall and protein matrix also play critical roles in changing starch digestibility in the flour. Xiong, et al. (2019) reported that intact cell walls act as physical barriers, restricting the interaction between digestive enzymes and starch. When HMT damages the integrity of cell walls, it may increase enzyme accessibility, thereby increasing starch digestibility, as observed in intact pinto bean cells. Similarly, surface proteins can act as protective barriers, inhibiting hydrolytic enzyme access to starch granules (Jenkins, et al., 1987; Lu, Donner, Yada, & Liu, 2016). The interplay of these structural and compositional factors likely contributes to the varied digestibility responses observed in flours subjected to HMT.

In the subsequent sections of this study, treatments at eTo-9°C for each moisture content were selected to further explore the effects of HMT on the morphology, crystallinity, and the nutritional and functional properties of YP, CP, and L flours.

The effect of HMT on the morphology of pulses flours

The light microscopic images of untreated YP, CP, and L flours, along with those treated at approximately 10%, 20%, 25%, and 30% moisture content at 9°C below eTo, were shown in Figure 4. Treatments with moisture contents exceeding 20% resulted in noticeable

aggregations, as evidenced by an increase in particle size compared to the untreated counterparts. The observed aggregations likely resulted from protein hydration, which raised protein viscosity. Hydrated proteins interacted through non-covalent bonds, forming networks that resisted flow and enhanced viscosity (J. Li, Cheng, Chen, & Zheng, 2019). Upon HMT, the viscosity of denatured proteins further increases (Bull & Breese, 1967). Heating denatured the proteins but did not alter starch granule morphology. Strong birefringence remained visible under polarized light microscopy. The Maltese cross observed in starch granules indicated an ordered molecular arrangement, where amylose and amylopectin molecules extended radially from the hilum and aligned perpendicularly to the granule surface (French, 1984; Z. Sun, Shi, & Shi, 2022).

SEM analysis provided additional insights into protein changes induced by HMT. Small protein particles (indicated by red arrows) were visible on the starch granule surfaces in untreated flours and the sample treated with 10% moisture content. However, in flours treated with higher moisture contents, these small protein particles were barely visible, likely due to structural changes in the proteins caused by hydration. Samples treated with 25% and 30% moisture content showed significantly larger particles, suggesting aggregation. In untreated flours, starch granules appeared embedded within surrounding matrix. In contrast, flours treated with 10%–25% moisture content showed weaker connections between starch granules and the surrounding matrix. At 30% moisture content, starch granules again appeared embedded within the protein matrix, indicating that hydration and HMT had altered the protein network.

The effect of HMT on the XRD pulses flours

The XRD patterns and relative crystallinity (RC) of untreated and HMT-treated pulse flours were presented in Figure 5. Untreated YP, CP, and L flours exhibited a characteristic C-type crystalline pattern, as evidenced by strong diffraction peaks near 2θ values of 15.5°, 17.2°, and 23.3°, alongside weaker reflections at 5.8° and 18.3°. This C-type pattern, typical of pulse starches, reflects a combination of A- and B-type crystalline polymorphs (R. Hoover & Ratnayake, 2002). HMT did not alter the crystalline pattern of the pulse flours; however, the peak intensity at 2θ = 18.3° increased compared to the untreated samples, suggesting an enhancement of A-type crystalline polymorphs. Such changes are consistent with previous reports indicating that HMT promotes A-type crystallinity, likely due to the dehydration of water molecules in the central chains of starch granule. This dehydration may cause rearrangements in the double-helix pairs, disrupting starch crystallites and/or modifying their crystalline orientation (Gunaratne, 2018; Schafranski, Ito, & Lacerda, 2021). The unchanged reflection at 2θ = 20° further confirmed that starch gelatinization did not occur, aligning with the strong birefringence observed in Figure 4.

The RC (Figure 5) of untreated pulse flours followed the order: YP (38.3%) = CP (38.3%) > L (37.8%). After HMT, the RC of all pulse flours increased, indicating enhanced the order of starch molecule. This enhancement was attributed to the reorganization of starch chains in both amorphous and crystalline regions (Ratnajothi Hoover, 2010; Zavareze, et al., 2011). Furthermore, HMT likely disrupted the less stable regions of the starch, facilitating the formation of more ordered helical structures and crystallites within the granules (Vermeylen, Goderis, & Delcour, 2006; Xiao, Liu, Wei, Shen, & Wang, 2017).

The effect of HMT on the pasting properties of pulses flours

The pasting profiles of untreated and HMT-treated YP, CP, and L flours were presented in Figure 6. Untreated YP and CP flours exhibited typical RVA pasting patterns with peak viscosity, breakdown, and setback. In contrast, HMT significantly altered the RVA pasting curves; most HMT-treated pulse flours did not exhibit breakdown or setback. For both CP and YP flours, the pasting curves of HMT-treated samples consistently showed reduced viscosities compared to their untreated counterparts. Among the HMT-treated samples of CP and YP, flours treated at 20% moisture content displayed the highest viscosities, followed by those treated at 10%, 25%, and 30% moisture content. A similar trend was observed in HMT-treated L flours (20% > 10% > 30% > 25%). However, unlike CP and YP flours, the peak viscosity of untreated L flour was lower than that of L flours treated at 20% and 10% moisture content. The final viscosity of L flours followed the order: 20% > untreated > 10%. Notably, neither the HMT-treated nor the untreated L flours exhibited breakdown or setback, consistent with findings by Xu, et al. (2019), which indicated that untreated L flour is more resistant to shear thinning than YP and CP flours.

Overall, HMT-treated samples exhibited minimal viscosity development after pasting at 95 °C. This behavior was likely due to enhanced interactions between amylose–amylose and amylose–amylopectin, as well as the strengthened packing of starch molecules through molecular rearrangement (Cheng, et al., 2024; H.-Y. Kim, Ye, & Baik, 2023a; Sharma, Yadav, Singh, & Tomar, 2015; Tan, et al., 2017). Moreover, the denatured protein created packed matrixes which limited the swelling of starch granules (Figure 4). These structural

modifications reflected on the increased thermal stability (Table 2) and RC (Figure 5) of the HMT-treated flours.

The effect of HMT on the **in vitro** *digestibility of starch in cooked pulses flours*

The digestibility profiles of pulse flours, both untreated and treated under varying moisture conditions, were evaluated using the Englyst digestion method (Figure 7 for cooked samples, Figure 3 for uncooked samples).

Untreated YP flour, after cooking, exhibited an RDS of 45.7%, SDS of 30.3%, and RS of 24.1%. HMT at lower moisture levels (10% and 20%) did not significantly alter the RDS of cooked YP flour. However, treatments at 25% and 30% moisture levels significantly increased RS. At 25% moisture, the increase in RS was accompanied by a significant decrease in SDS. At 30%, the increase in RS coincided with a significant decrease in RDS. In uncooked YP flour, the 10% moisture treatment had no impact on digestibility. However, the 20% treatment significantly reduced RS, with corresponding increases in RDS and SDS. At 25% moisture, RS was significantly enhanced, while both SDS and RDS decreased. The 30% treatment also significantly increased RS, with a reduction in RDS.

For CP flour, the untreated sample showed higher digestibility compared to untreated YP flour, with an RDS of 62.0%, SDS of 22.4%, and RS of 15.6% after cooking. HMT had limited effects on the digestibility of cooked CP flour, with most treatments causing no significant changes. The only exception was the 25% moisture treatment, which significantly increased RS compared to the untreated sample. In uncooked CP flour, the 10% moisture treatment caused no significant changes. Treatments at 20% and 30% moisture levels

significantly decreased RS and SDS, with corresponding significant increases in RDS. Conversely, the 25% treatment significantly increased SDS while significantly reducing RDS.

L flour exhibited the highest digestibility among the three pulse flours in its untreated state, with an RDS of 78.9%, SDS of 8.3%, and RS of 12.9% after cooking. The effect of HMT varied with moisture levels. At 10% moisture, SDS significantly increased, while both RS and RDS slightly decreased. At 20%, RS significantly increased, with a significant decrease in RDS. The 25% treatment significantly reduced RDS, while RS and SDS increased slightly. At 30% moisture, RS increased significantly, with a decrease in RDS, while SDS remained stable. In uncooked L flour, the 10% treatment caused no significant changes, while the 20% treatment significantly decreased RS and increased RDS and SDS. Similarly, the 25% treatment significantly reduced RDS, with slight increases in RS and SDS. At 30% moisture, RS significantly increased, accompanied by reductions in RDS and SDS.

Overall, HMT induced varying changes in starch digestibility across the pulse flours, with the effects depending on the moisture content and flour type. YP and L flours exhibited the most significant changes, particularly at higher moisture levels (25% and 30%), which enhanced the RS content after cooking. In contrast, CP flour showed minimal changes, with only the 25% treatment significantly increasing RS. Notably, HMT did not reduce RS content in cooked samples, regardless of their uncooked digestibility profile. Similar findings have been reported in rich starch (Xie, et al., 2020), waxy maize starch (Park, et al., 2018) and rice starch and flour (Chen, et al., 2017). This may be attributed to the increased thermal stability of starch (Table 2), which likely inhibited complete starch gelatinization during cooking.

Starch digestibility is influenced by granule swelling, as swollen granules allow easier access for digestive (Chung, Shin, & Lim, 2008). However, portions of starch that remain intact after gelatinization may partially restrict enzyme access to the starch chains, contributing to the observed differences in digestibility.

The effect of HMT on the total dietary fiber in pulses flours

The total dietary fiber (TDF) content of untreated and HMT-treated pulse flours is presented in Figure 7. The untreated YP, CP, and L flours contained 5.9%, 8.5%, and 6.7% TDF, respectively. Notably, although the RS content in uncooked flours followed the order YP > L > CP, after cooking, the order shifted to YP > CP > L. Despite having the highest RS content in cooked samples, YP flour had the lowest TDF content, while CP flour had the highest, a trend consistent with crude fiber results (**Table 1**: CP 1.8% > L 1.2% > YP 0.7%), though crude fiber values were lower overall. This discrepancy arises from differences in fiber measurement methods. Crude fiber, determined using the AOAC 962.09 method, is based on acid/alkaline hydrolysis and only accounts for insoluble fiber, often underestimating total fiber counted (Hell, Kneifel, Rosenau, & Böhmdorfer, 2014). In contrast, TDF, measured by enzymatic hydrolysis (AOAC Method 991.43), includes both soluble and insoluble fiber (Ferjančič, Skrt, Korošec, & Bertoncelj, 2022). The differences between RS and TDF trends stem from the fundamental distinctions in their analytical definitions. The Englyst method quantifies RS1 and RS2, which represent physically inaccessible and natural starch particles (Brewer, Cai, & Shi, 2012; Kraithong, et al., 2022), whereas AOAC 991.43 measures total fiber, including RS, resistant oligosaccharide and non-starch polysaccharides

such as cellulose, hemicellulose, and pectins (Englyst, et al., 2013). Thus, the higher TDF content in CP flour suggests a greater presence of non-starch polysaccharides beyond RS1 and RS2.

HMT significantly increased the TDF content in YP flours. For CP flours, increases in TDF were observed only in samples treated at 25% and 30% moisture contents, while for L flours, HMT did not result in significant changes to TDF levels. The increase in TDF content in certain HMT-treated flours may be attributed to the following reasons: 1) enhanced thermal stability and molecular packing: The improved thermal stability (Table 2) and strengthened packing of starch molecules through molecular rearrangement (Figure 5) restricted starch granules swelling during heating, as observed in pasting profiles (Figure 6) (Cheng, et al., 2024; Chung, et al., 2009; Kim, et al., 2023b; Sun, et al., 2023); 2) strengthened protein-starch interaction: In HMT-treated flours, starch granules were encased within a packed protein matrix, which restricted penetration of enzyme and limited the accessibility of starch chains to hydrolytic enzymes (Figure 4) (E. H. J. Kim, et al., 2008). Consequently, TDF increases were more pronounced in samples treated with higher moisture content.

However, while HMT enhanced thermal stability, reinforced starch crystalline structures, and strengthened protein-starch interaction, the plant cell wall also plays a critical role in starch digestibility in flour form. Changes in plant cell wall strength, porosity, and stiffness may explain why some samples did not exhibit increased TDF content despite their higher thermal stability, compact structure, and reduced swelling during heating. Xiong, et al. (2019) reported that HMT has a more pronounced impact on isolated pinto bean starch compared to

starch embedded within intact cells, showing contrasting effects. In untreated samples, the digestibility of starch in intact cells was much lower than that of isolated starch, likely due to the limited permeability of plant cell walls to macromolecules, which restricts enzyme access to intracellular starch (Tepfer & Taylor, 1981; Xiong, et al., 2019). However, after HMT, disruptions in plant cell wall strength, porosity, and stiffness may facilitate enzyme access to starch granules within cells, potentially reducing the TDF content in some cases (Ormerod, Ralfs, Jackson, Milne, & Gidley, 2004). This observation suggests that the digestibility of starch in flour form is not solely influenced by the properties of starch itself; other components in the flour, such as the cell wall and protein matrix, also play significant roles.

Conclusion

This study demonstrates that HMT significantly alters the physicochemical, thermal, and nutritional properties of pulse flours, with variations depending on treatment condition and flour type. HMT improved thermal stability across all samples, as indicated by higher gelatinization temperatures. Starch digestibility was notably affected, with RS increasing in YP and L flours at higher moisture levels, while CP flour generally showed a reduction in RS.

Morphological analysis revealed protein aggregation and modified starch-protein interactions, which contributed to changes in pasting properties. The decline in viscosity and setback suggested restricted starch swelling, supported by XRD results showing increased crystallinity. The rise in RS content after cooking may be attributed to enhanced thermal stability, a more ordered crystalline structure, and increased protection from aggregated proteins.

Additionally, total dietary fiber (TDF) increased in certain treated samples, particularly in YP and CP flours, indicating improved fiber retention due to structural modifications induced by HMT. These findings underscore the intricate relationship between thermal processing, starch structure, and digestibility, providing valuable insights for optimizing the functional and nutritional properties of pulse flours in food applications.

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Table A.1 Proximate composition of yellow pea (YP), lentil (L) and chickpea (CP) flours (% dry basis).

	Protein	Ash	Crude fiber	Crude fat	Total starch	Damaged starch	Amylose content
YP	30.78±0.23^{c}	2.58±0.05^{a}	0.74±0.02^{a}	1.20±0.22^{a}	50.94±0.13^{b}	1.46±0.01^{b}	32.47±0.21^{a}
CP	22.25±0.16^{a}	2.80±0.01^{b}	1.78±0.80^{a}	7.25±0.26^{b}	47.50±0.65^{a}	0.95±0.00^{a}	31.32±0.43^{a}
L	28.12±0.07^{b}	2.63±0.05^{a}	1.21±0.01^{a}	1.09±0.20^{a}	54.40±0.48^{c}	1.52±0.04^{c}	32.37±0.01^{a}

All data are means ± standard deviations, n = 2. Different superscript letters in the same column indicate a significant difference ($p < 0.05$) detected between the samples.

Table A.2 Thermal properties of untreated and HMT pulse flours

Samples	Treatment		To(°C)	Tp (°C)	Tc (°C)	ΔT* (°C)	ΔH (J/g)
Yellow pea							
Control		-	65.9±0.1a	76±0.5a	93.9±0.3def	27.9±0.2j	11.5±0.1h
As-is (10.72%)	eTo-12	119°C	67.4±0.2c	78.6±0.7c	94.7±0.7ef	27.3±0.5j	9.5±0.6cdef
(eTo: 131°C)	eTo-9	122°C	68.4±0d	79.5±0de	95.1±0f	26.6±0j	9±0.3cde
	eTo-6	125°C	66.9±0.1bc	78.8±0.8cd	94.4±0.8ef	27.5±0.7j	9.2±0.5cdef
	eTo-3	128°C	66.3±0.2ab	77.7±0.4b	93.6±0.3de	27.4±0.1j	10.3±0.3g
	eTo	131°C	69.7±0.3e	79.7±0.3ef	92.9±0d	23.3±0.3f	8.8±0.1bcd
20.33%	eTo-12	106°C	72.4±1f	79.6±0de	91.5±0.2c	19.1±1.2e	9.4±0.1cdef
(eTo: 118°C)	eTo-9	109°C	71.7±0.3f	79.5±0de	91.2±0.3c	19.6±0e	9.4±0.3cdef
	eTo-6	112°C	71.8±0.3f	80.2±0.2ef	91.5±0.5c	19.6±0.2e	9.9±0fg
	eTo-3	115°C	75.4±0.1g	81.4±0g	89.7±0.1ab	14.3±0.1d	8.8±0bcd
25.14%	eTo-12	99°C	76.2±0h	80.5±0.4f	89.1±0.5a	12.9±0.6bcd	9.6±0.4defg
(eTo: 111°C)	eTo-9	102°C	77.7±0.5i	82.1±0.2g	90.1±1.6abc	12.4±1.2abc	8.2±0.2ab
	eTo-6	105°C	79.1±0.3g	83.4±0.2h	90.1±0abc	11.1±0.3a	8.9±0.1bcde
	eTo-3	108°C	80.1±0.1k	85.4±0.1i	93.6±0.4de	13.6±0.3cd	7.6±0.1a
28.74%	eTo-12	94°C	77.1±0.2i	81.9±0.2g	91.2±0.2c	14±0d	9.6±0.5efg
(eTo: 106°C)	eTo-9	97°C	78.8±0.5g	83.1±0.6h	90.9±0.8bc	12.1±1.3ab	9.4±0.3cdef
	eTo-6	100°C	80.6±0.3k	85.2±0.3i	93.6±0.8de	13±1.1bcd	9.1±0.2cde
	eTo-3	103°C	80.8±0.5k	85.3±0i	92.9±0.8d	12.1±0.2ab	8.8±0.7bc
Chickpea							

Control		-	61.5±0.0b	69.4±0.0a	85.0±1.0a	23.5±0.9j	9.6±0.1i
As-is (9.10%)	eTo-12	125°C	60.6±0.1a	70.6±0.5b	88.9±0.1fg	28.3±0.1i	8.1±0.5efgh
(eTo: 137°C)	eTo-9	128°C	60.7±0.1a	70.0±0.2b	89.3±0.9gh	28.6±0.8i	8.4±0.7gh
	eTo-6	131°C	60.8±0.0a	71.1±1.1b	89.1±0.9gh	28.3±0.8i	8.3±0.3fgh
	eTo-3	134°C	63.1±0.0c	73.4±0.4c	91.4±0.3i	28.4±0.3i	8.1±0.3efg
	eTo	137°C	64.5±0.9d	74.9±0.3de	90.3±0.3hi	25.8±1.2h	6.3±0.4bc
19.14%	eTo-12	109°C	64.9±0.0de	72.7±0.1c	85.4±0.2a	20.6±0.2f	7.4±0.0def
(eTo: 121°C)	eTo-9	112°C	65.4±0.1e	73.4±0.0c	86.6±0.1bcd	21.2±0.1f	8.2±0.2fgh
	eTo-6	115°C	66.6±0.2f	74.7±0.1d	87.6±0.1def	21.1±0.1f	7.3±0.4def
	eTo-3	118°C	66.2±0.2f	74.6±0.0d	87.7±0.4def	21.5±0.6f	7.8±0.1efg
25.62%	eTo-12	98°C	72.2±0.1g	77.5±0.1e	86.8±0.7bcd	14.6±0.6e	8.9±0.4hi
(eTo: 110°C)	eTo-9	101°C	74.0±0.2h	79.2±0.9f	87.3±0.9de	13.4±0.7de	7.5±0.5def
	eTo-6	104°C	74.9±0.1i	80.5±0.1g	87.3±0.1de	12.4±0.2cd	7.4±0.5def
	eTo-3	107°C	75.4±0.1i	80.9±0.3g	87.0±0.0cde	11.7±0.1bc	6.7±0.2cd
29.09%	eTo-12	93°C	74.1±0.1h	78.7±0.2f	85.0±1.1a	10.9±1.3b	8.5±0.5gh
(eTo: 105°C)	eTo-9	96°C	75.3±0.6i	78.6±0.4f	85.8±0.8abc	10.6±0.2ab	8.0±0.4efg
	eTo-6	99°C	79.4±0.4k	84.1±0.3h	88.9±0.5fg	9.5±0.1a	5.8±0.3bcd
	eTo-3	102°C	77.7±0.3j	81.3±1.1g	88.4±0.6efg	10.7±0.3ab	4.1±0.0a
Lentil							
Control		-	66.4±0.6a	75.7±0.2a	103.7±0.1i	37.3±0.7j	11.1±0.3i
As-is (9.98%)	eTo-12	122°C	66.8±0.1ab	76.8±0bc	90.3±0.5fg	23.4±0.6i	10.1±0.5efghi
(eTo: 137°C)	eTo-9	125°C	67±0ab	76.3±0.5ab	89.8±0.4efg	22.9±0.4i	10.4±0ghi

	eTo-6	128°C	67.2±0.2b	76.7±0.2bc	89.7±0.1efg	22.6±0.3i	9.3±1abcdefg
	eTo-3	131°C	66.6±0.5ab	76.7±0.3bc	89.4±0.3def	22.8±0.2i	9.4±0.5bcdefg
	eTo	134°C	68.5±0.1c	77.5±0.3cd	89.8±0.3efg	21.3±0.2h	8±0.1a
18.99%	eTo-12	108°C	71.6±0d	78.2±0.1d	88.1±0abc	16.5±0f	10.5±0.1ghi
(eTo: 120°C)	eTo-9	111°C	73.5±0.4f	79.4±0.5ef	87.9±1ab	14.4±0.5cd	9.6±0.1cdefgh
	eTo-6	114°C	73.5±0.1f	79.4±0.2e	87.3±0.4a	13.9±0.3bc	9.3±0.3abcdefg
	eTo-3	117°C	74.9±0.1gh	80.6±0.2g	90.6±0.4g	15.8±0.3ef	8.9±0.4abcde
24.08%	eTo-12	100°C	74.5±0g	79.9±0.3efg	88.2±0.5abc	13.6±0.5bc	9±1.1abcdef
(eTo: 112°C)	eTo-9	103°C	72.4±0.3e	77.8±0.6d	88.8±0.3bcde	16.4±0.6f	9.9±0.7defghi
	eTo-6	106°C	76.9±0.5j	81.9±0.6h	89.2±1.1cdef	12.3±0.5a	8.3±0.6ab
	eTo-3	109°C	77.4±0.1j	82.6±0.1h	89.6±0efg	12.2±0.1a	8.7±0.4abcd
29.21%	eTo-12	92°C	75.3±0.5hi	79.7±0.2ef	88.3±0.1abcd	13±0.4ab	10.9±0.9hi
(eTo: 104°C)	eTo-9	95°C	75.7±0.5i	80.2±0.1efg	87.9±0.7ab	12.2±0.1a	10±0.7defghi
	eTo-6	98°C	75.4±0.1hi	80.3±0.1fg	92.9±0h	17.6±0.1g	10.3±0fghi
	eTo-3	101°C	77.2±0.0j	82.0±0.8h	92.1±0.4g	15.0±0.4de	8.5±0.3abc

Table A.3 Comparison of starch digestibility of HMT pulse flours and isolated starches

	Flours			***Starches***		
	RDS(%)	SDS(%)	RS(%)	RDS(%)	SDS(%)	RS(%)
Untreated yellow pea	16.12±0.28	41.27±1.49	42.61±1.44	22.38±0.71	48.76±0.83	28.86±0.29
As-is, -9℃	21.89±0.16	36.63±1.44	41.48±1.51	20.25±0.72	51.12±2.13	28.62±1.86
~20%, -9℃	20.41±0.37	54.91±0.12	24.57±0.34	23.39±0.30	53.46±0.77	23.15±0.80
~25%, -9℃	12.04±0.26	32.94±0.82	55.01±0.68	23.13±0.07	53.63±0.58	23.24±0.54
~30%, -9℃	8.73±0.81	41.39±0.51	49.88±0.78	29.96±0.96	50.54±2.61	19.49±1.65
Untreated chickpea	19.44±1.45	52.53±4.48	28.03±3.44	28.38±0.71	37.84±0.60	33.78±0.35
As-is, -9℃	22.39±0.20	55.35±2.54	22.26±2.70	26.16±1.60	57.18±4.71	17.48±3.67
~20%, -9℃	37.88±1.00	50.20±1.58	11.92±2.50	34.11±2.23	56.47±2.21	8.61±0.25
~25%, -9℃	19.40±0.53	59.44±1.83	21.15±1.78	39.30±0.40	41.15±0.30	19.54±0.55
~30%, -9℃	42.90±1.74	42.06±4.04	15.05±3.31	42.59±0.56	40.22±1.21	17.19±071
Untreated Lentil	22.53±1.45	41.32±0.52	36.15±0.92	20.93±0.11	50.47±0.60	28.54±0.55
As-is, -9℃	22.88±0.17	39.15±1.15	37.96±1.01	22.27±0.26	54.32±0.06	23.40±0.30
~20%, -9℃	30.00±0.40	57.56±0.87	12.44±0.87	25.55±0.42	46.94±0.55	27.52±1.15
~25%, -9℃	21.80±0.30	36.22±1.59	41.98±1.84	29.19±2.71	54.30±0.33	16.88±0.39
~30%, -9℃	19.36±0.38	36.02±0.07	44.62±0.44	33.11±0.83	44.15±0.70	22.74±0.41

Table A.4 Comparison of starch digestibility of HMT pulse flours and cooked flours

	Flours			***Cooked Flours***		
	RDS/%	SDS/%	RS/%	RDS/%	SDS/%	RS/%
Untreated yellow pea	16.12±0.28	41.27±1.49	42.61±1.44	58.74±2.45	27.37±2.76	13.88±0.31
As-is, -9°C	21.89±0.16	36.63±1.44	41.48±1.51	59.62±2.71	22.59±2.65	17.79±0.65
~20%, -9°C	20.41±0.37	54.91±0.12	24.57±0.34	62.22±0.60	22.95±1.16	14.83±0.56
~25%, -9°C	12.04±0.26	32.94±0.82	55.01±0.68	59.24±0.78	23.51±1.13	17.24±1.86
~30%, -9°C	8.73±0.81	41.39±0.51	49.88±0.78	58.22±0.75	25.40±0.55	16.38±1.30
Untreated chickpea	19.44±1.45	52.53±4.48	28.03±3.44	65.91±2.42	26.97±2.96	7.12±0.54
As-is, -9°C	22.39±0.20	55.35±2.54	22.26±2.70	71.25±0.44	19.60±0.54	9.16±0.10
~20%, -9°C	37.88±1.00	50.20±1.58	11.92±2.50	72.33±0.49	19.41±1.21	8.26±1.26
~25%, -9°C	19.40±0.53	59.44±1.83	21.15±1.78	71.76±0.36	17.44±0.30	10.80±0.07
~30%, -9°C	42.90±1.74	42.06±4.04	15.05±3.31	71.67±0.11	19.80±0.30	8.53±0.34
Untreated Lentil	22.53±1.45	41.32±0.52	36.15±0.92	63.22±0.71	31.13±0.48	5.63±0.23
As-is, -9°C	22.88±0.17	39.15±1.15	37.96±1.01	58.40±1.10	38.04±0.86	3.56±1.21
~20%, -9°C	30.00±0.40	57.56±0.87	12.44±0.87	64.39±1.00	30.38±0.85	5.23±0.15
~25%, -9°C	21.80±0.30	36.22±1.59	41.98±1.84	65.7±1.53	31.43±1.23	2.87±0.30
~30%, -9°C	19.36±0.38	36.02±0.07	44.62±0.44	66.01±0.23	31.09±0.01	2.90±0.22

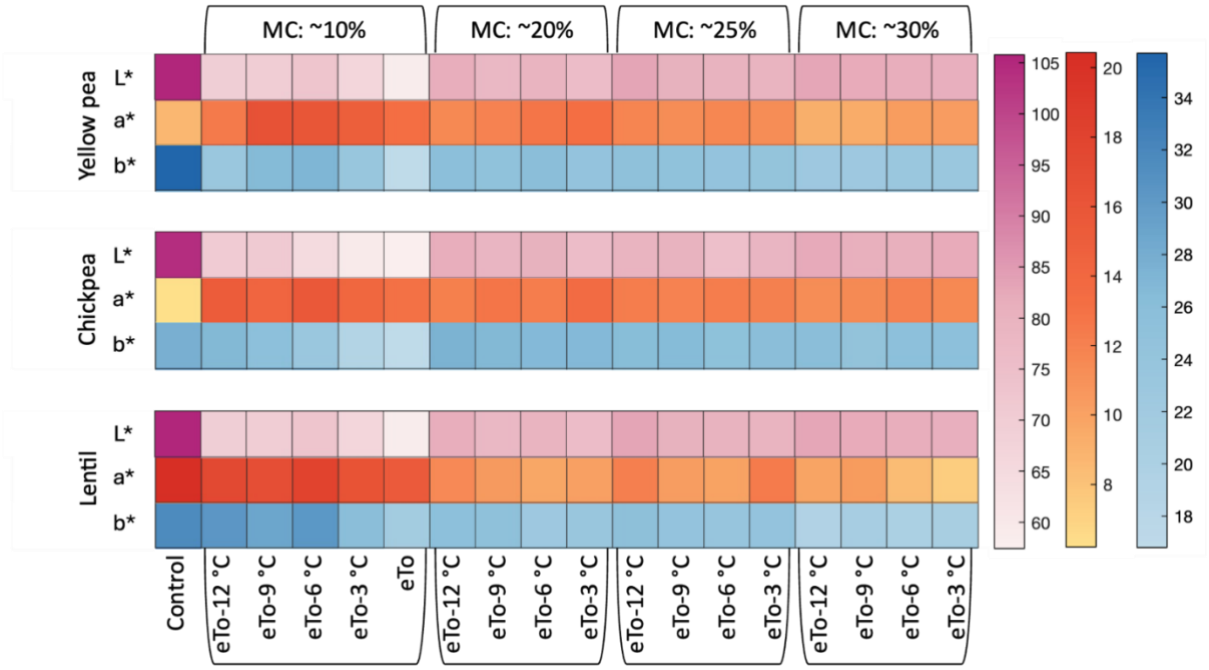


Figure A.1 Color parameters of untreated and heat-moisture-treated flours of yellow pea, chickpea, and lentil

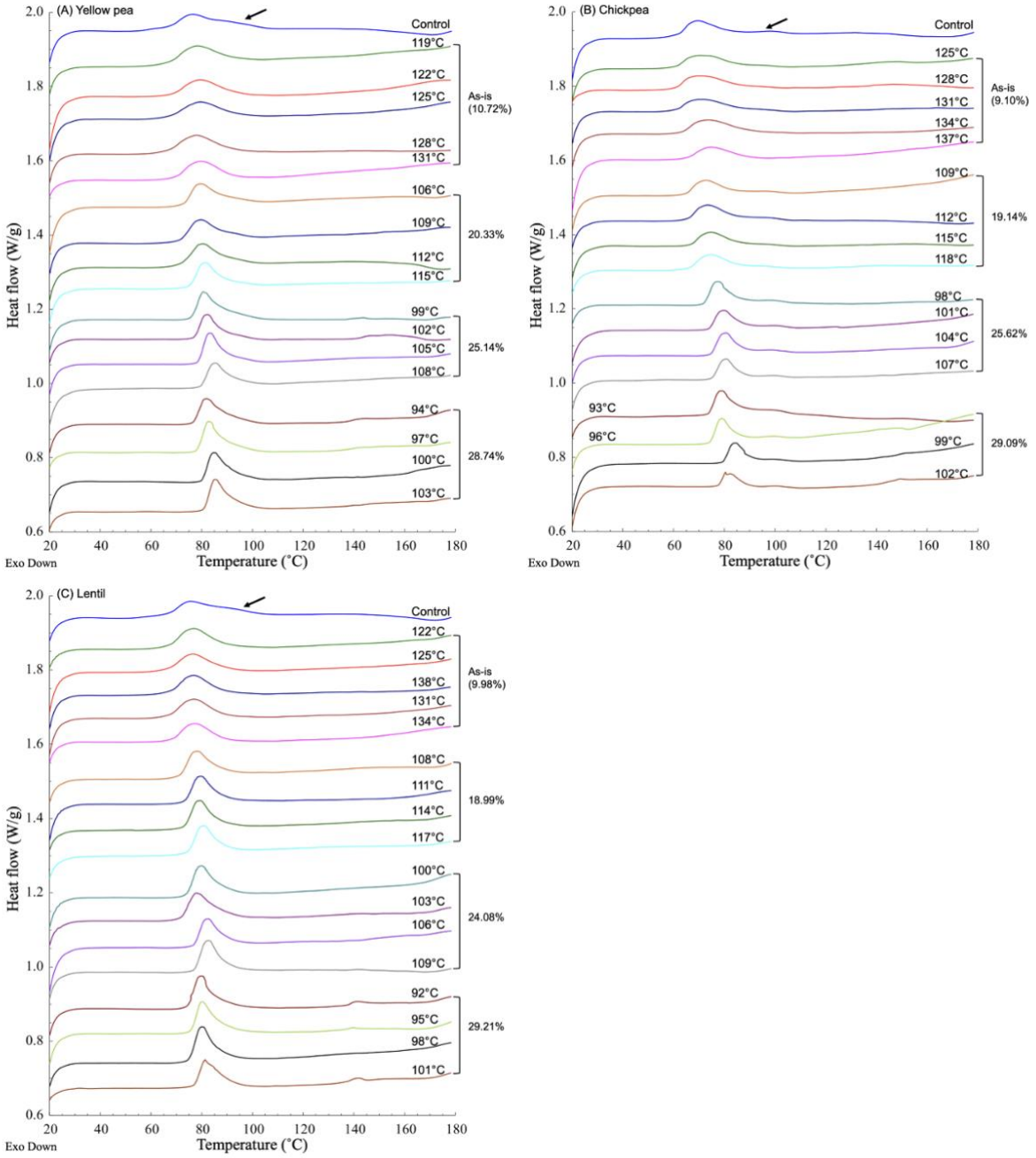


Figure A.2 DSC curve of untreated and heat-moisture-treated flours of yellow pea (A), chickpea (B), and lentil (C).

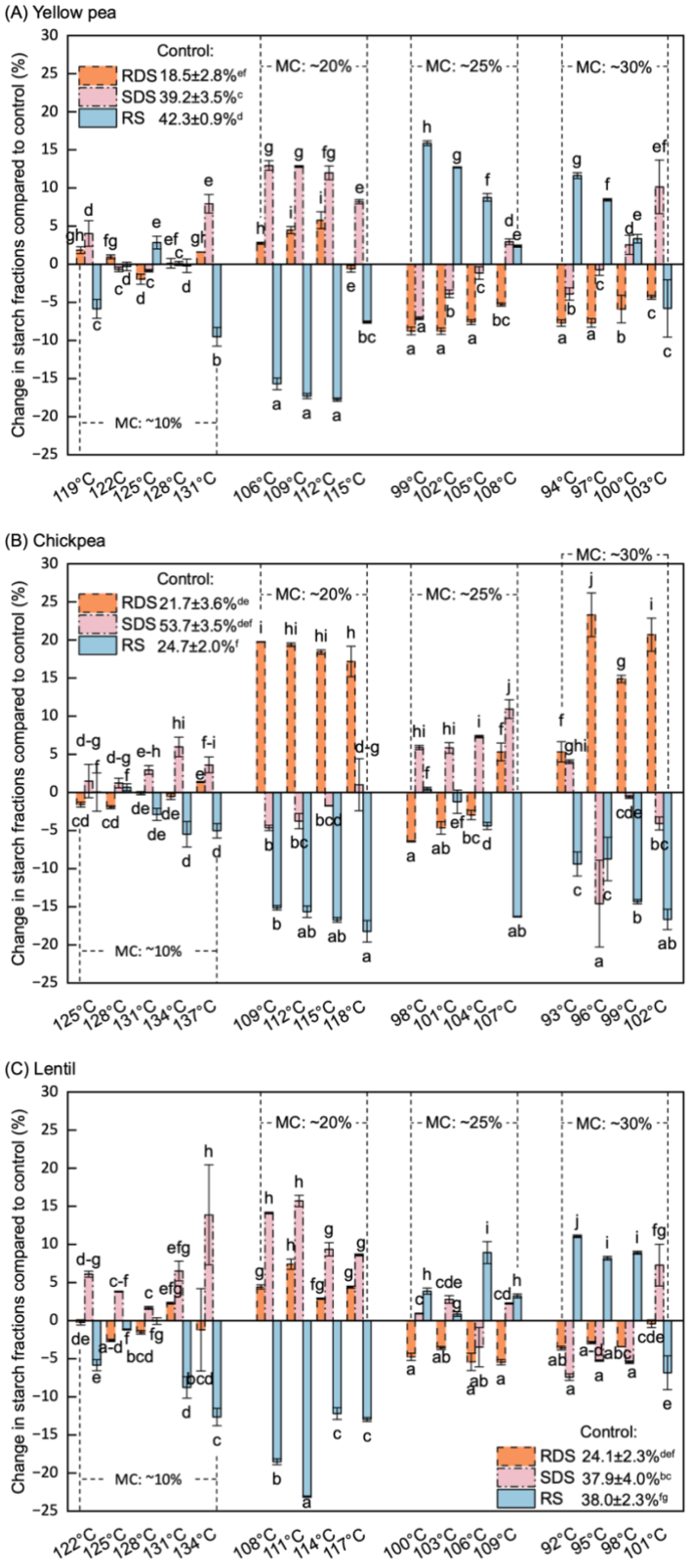
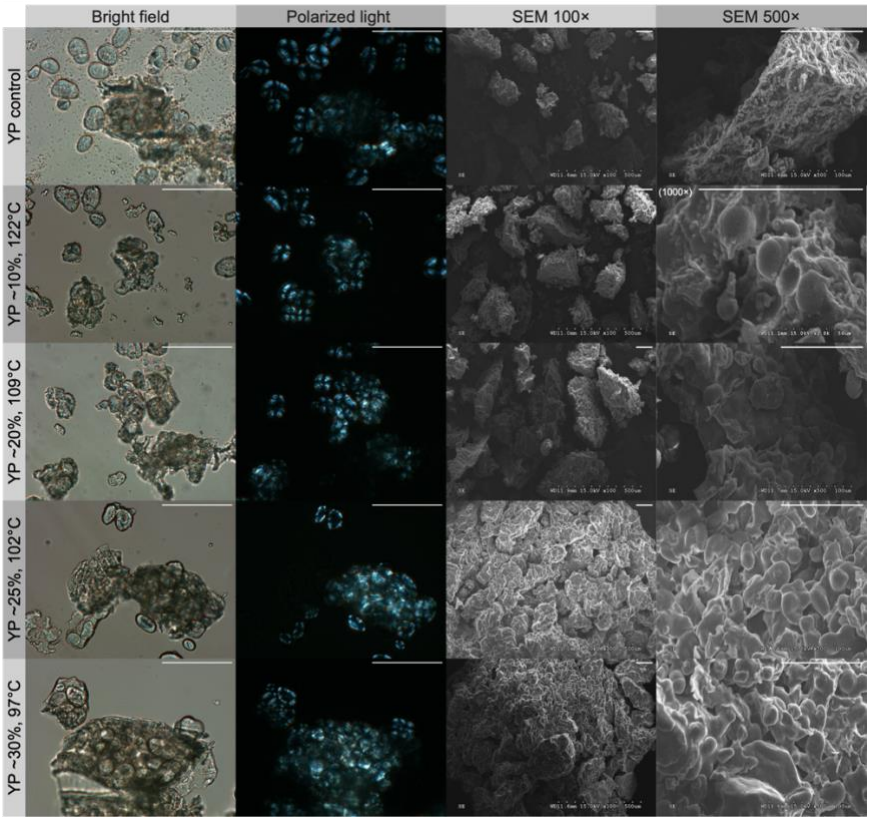
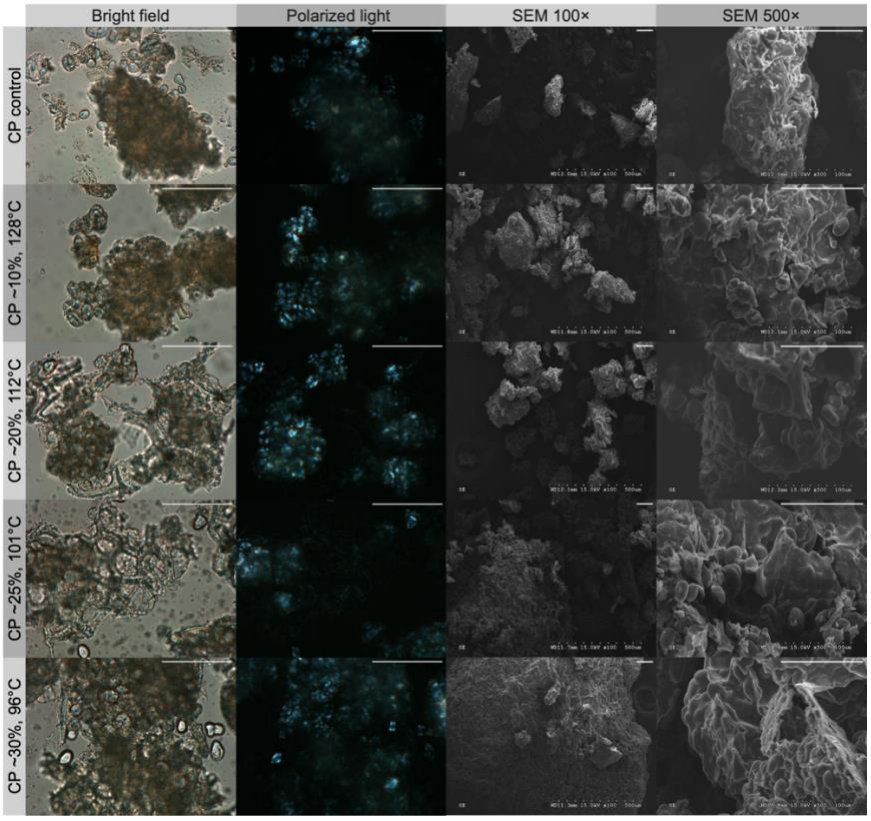


Figure A.3 Starch digestibility fractions—rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS)—in uncooked untreated and heat-moisture-treated flours of yellow pea (A), chickpea (B), and lentil (C).


Bright field
Polarized light
SEM 100×
SEM 500×
YP control
YP ~10%, 122°C
YP ~20%, 109°C
YP ~25%, 102°C
YP ~30%, 97°C
(1000×)


Bright field
Polarized light
SEM 100×
SEM 500×
CP control
CP ~10%, 128°C
CP ~20%, 112°C
CP ~25%, 101°C
CP ~30%, 96°C

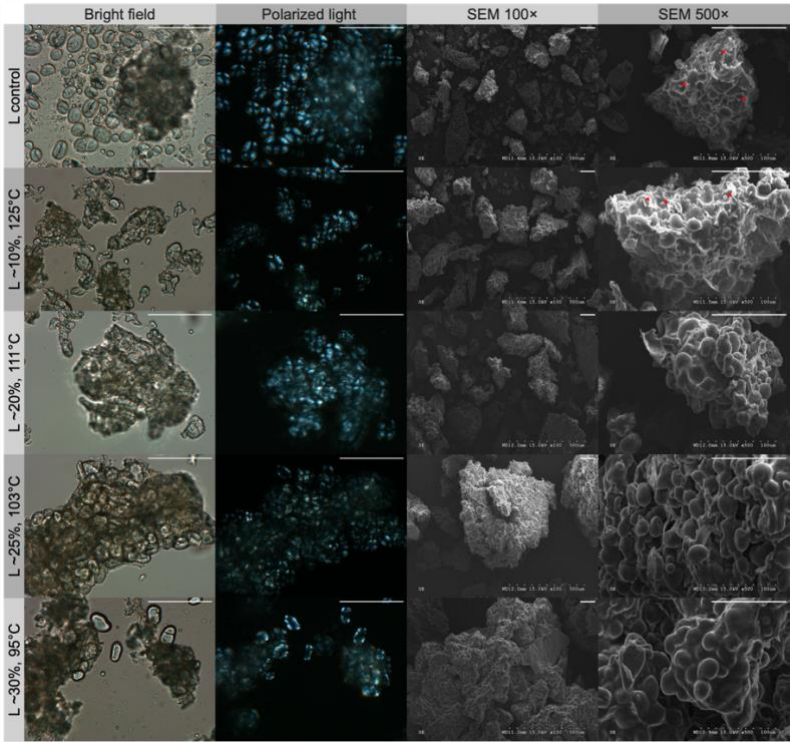


Figure A.4 Light microscopy (bright field and polarized light) and scanning electron microscopy (SEM) images of untreated and HMT flours from yellow pea (YP), chickpea (CP), and lentil (L).

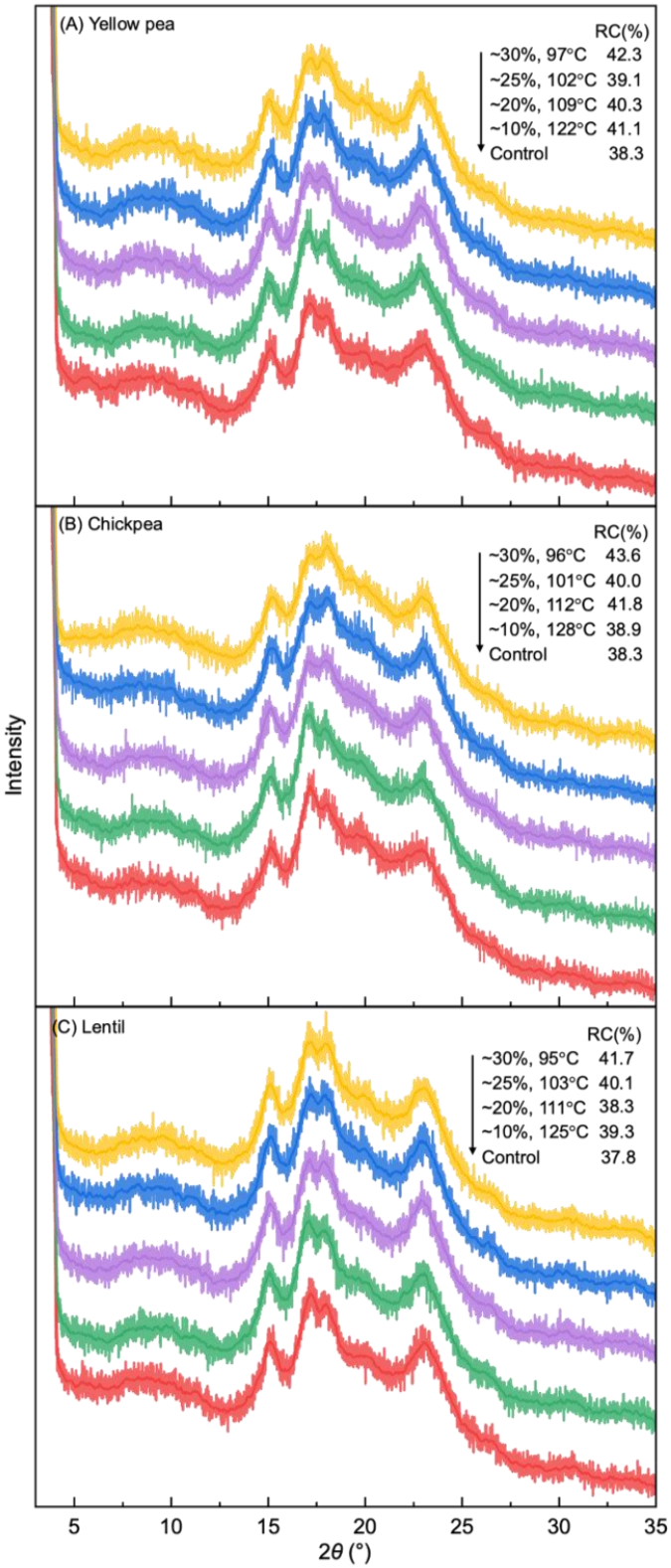


Figure A.5 X-ray diffraction patterns and relative crystallinity (RC) of untreated and heat-moisture-treated flours of yellow pea (A), chickpea (B), and lentil (C).

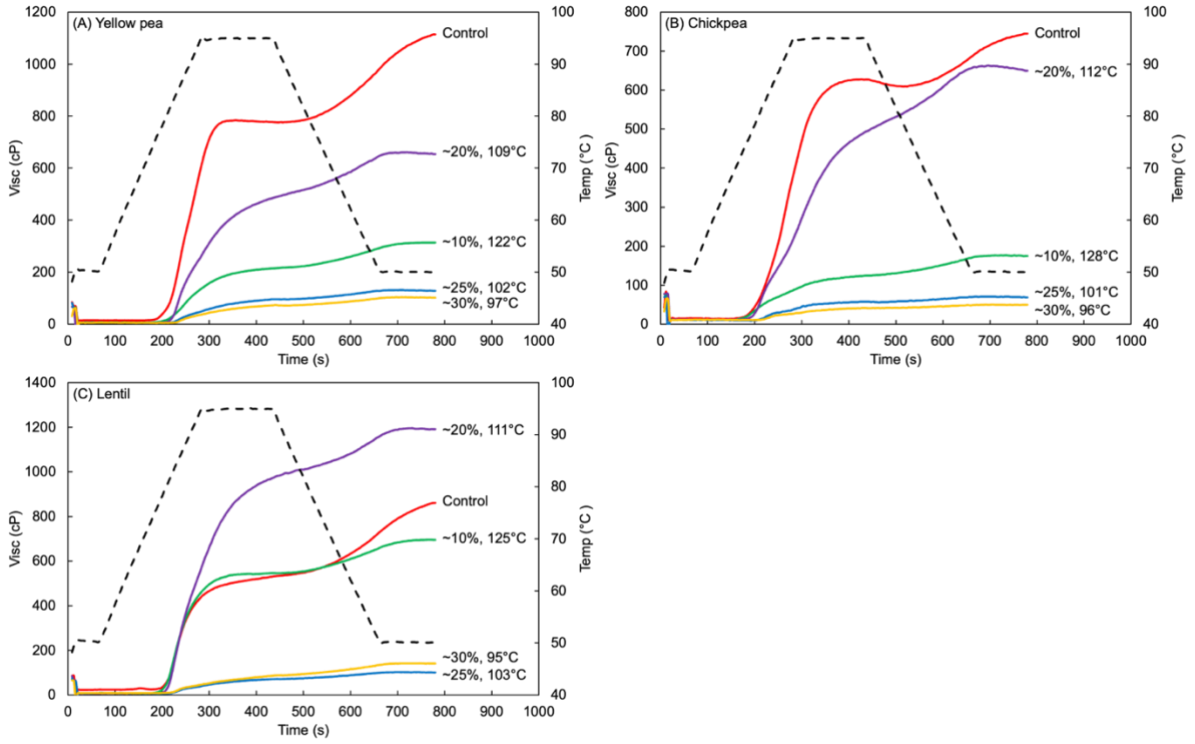


Figure A.6 Rapid viscosity profiles of untreated and heat-moisture-treated flours of yellow pea (A), chickpea (B), and lentil (C).

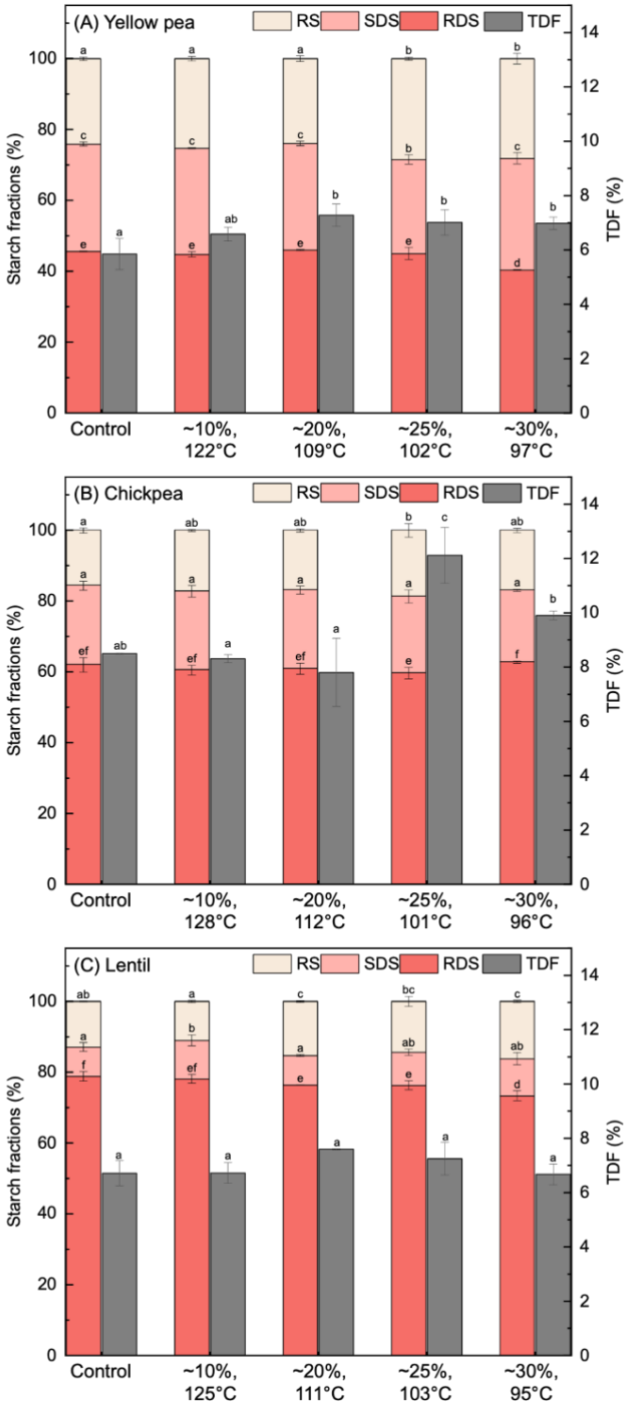


Figure A.7 Starch digestibility fractions (resistant starch (RS), slowly digestible starch (SDS), and rapidly digestible starch (RDS)) from cooked samples (left Y-axis) and total dietary fiber (TDF) from uncooked samples (right Y-axis) in (A) yellow pea, (B) chickpea, and (C) lentil starches under different heat-moisture treatment conditions.