IMPACTS OF GERMINATION ON THE PHYSICOCHEMICAL PROPERTIES, NUTRITIONAL QUALITY AND BREAD MAKING PERFORMANCE OF YELLOW PEA AND FABA BEAN FLOURS

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ABSTRACT

The overarching goal of this thesis research was to examine the impacts of germination on the chemical composition, functionality and nutritional properties of pea and faba bean flours, and their application value in a model dough/bread system. The first study aimed to investigate the changes in the proximate composition, functional properties and nutritional attributes of yellow pea (CDC Amarillo variety) and faba bean (CDC Snowdrop variety) flours as a function of germination time (0, 24, 48 and 72 h). Alpha-amylase activity in the yellow pea and faba bean flours increased gradually during germination, whereas negligible changes were found in their chemical composition. Soaking (0-h germination) and 24-h germination showed a noticeable increase in the pasting viscosities of the pulse flours, whereas longer germination times led to a decrease in their viscosities due to the increased endogenous alpha-amylase activity. Germination was found to enhance the emulsifying and foaming properties of both pulse flours. With respect to their nutritional value, improvements were observed in the *in vitro* digestibility of starch and protein of the flours after germination; germination, however, did not enhance the *in vitro* protein digestibility corrected amino acid scores (IV-PDCAAS).

In the second study, raw and germinated (72-h) yellow pea and faba bean flours were used to replace hard wheat flour at 10% and 20% levels for bread making. In comparison to the 100% wheat flour control, the incorporation of germinated pulse flours at both levels decreased the falling numbers and pasting viscosities. The composite flours tended to require a shorter mixing time to reach optimum dough development, and the generated doughs were stickier than the control dough. Farinograph and rheology results indicated that the doughs formed with the addition of raw/germinated pulse flours were less elastic and possessed a weakened gluten network as compared to the control wheat dough. In addition to the changes observed in flour and dough properties, bread baked from the composite flours displayed reduced loaf volume and decreased slice area, but increased firmness. This study revealed the influence of short-term germination on the functional characteristics and nutritional profiles of pulse flours, and showed the potential of using the modified pulse flours in bread and other bakery goods.

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LIST OF SYMBOLS AND ABBREVIATIONS

AA	Amino acid
ANOVA	Analysis of variance
BU	Brabender unit
BWP	Bandwidth at peak dough resistance
CWRS	Canada Western Red Spring
Da	Dalton
d.b.	Dry basis
DDT	Dough development time
DSC	Differential scanning calorimetry
EA	Emulsion activity
ES	Emulsion stability
ETP	Energy at peak dough resistance
FAB	Farinograph water absorption
FB	Faba bean
FC	Foaming capacity
FN	Falling number
GPI	Gluten performance index
G′	Storage modulus (elastic modulus)
G″	Loss modulus (viscous modulus)
G*	Complex modulus
IVPD	*in vitro* protein digestibility
IV-PDCAAS	*in vitro* protein digestibility corrected amino acid score
J_{el}	Relative elasticity
J_{max}	Maximum creep compliance
MDT	Mixograph development time
MTI	Mixing tolerance index
OBC	Oil-binding capacity
PKH	Peak height value at maximum dough resistance
RDS	Rapidly digestible starch
RS	Resistant starch

RVA	Rapid visco-analyzer
SDS	Slowly digestible starch
SEM	Scanning electron microscope
SRC	Solvent retention capacity
tan δ	Loss tangent
TEG	Total energy required throughout the mixograph curve
T_c	Conclusion gelatinization temperature
T_o	Onset gelatinization temperature
T_p	Peak gelatinization temperature
WHC	Water-holding capacity
YP	Yellow pea
α	Alpha
τ_0	Constant shear stress
ΔH	Enthalpy change of the observed endothermic peak

1. INTRODUCTION

1.1 Overview

Pulses are the dry edible seeds of legume crops that include pea, bean, lentil and chickpea (Padhi and Ramdath, 2017). They are globally popular crops valued for their nutritional and health attributes as they are rich in dietary fibre, protein, resistant starch, minerals (*e.g.*, iron, zinc, and phosphorous), as well as folate and other B-vitamins (Lopez-Martinez et al., 2017). Pulses have a low glycemic index and are typically low in fat (with the exception of chickpea – 6-7% crude fat). The consumption of pulses has been linked to numerous health benefits, including reducing the risks of cancer, diabetes, osteoporosis, hypertension and gastrointestinal disorders, along with having the ability to reduce the levels of low-density lipoprotein (LDL) cholesterol (Boye et al., 2010, Padhi and Ramdath, 2017). Recently, pulses have been gaining interest in human nutrition, especially in underdeveloped and developing countries, where animal protein is not available or limited in availability because of high cost, religious beliefs or cultural habits. Pulses, being a good source of plant proteins, therefore can serve as economical and vegetarian/vegan alternatives to animal-based protein sources (Padhi and Ramdath, 2017). Pulses also are recognized as ecologically sustainable, as they fix atmospheric nitrogen and have lower carbon and water footprints as compared to animal-derived protein sources. Moreover, pulses are excellent sources of the aforementioned bioactive compounds that play metabolic roles in human health, which thus increases their potential to be used in the development of a wide variety of healthy food products (Boye et al., 2010). Hence, blending of pulses with locally grown grains to combat protein malnutrition is of tremendous interest globally.

Despite their nutritional importance, the widespread use of pulses in human diets has been hindered in the marketplace for several important reasons. Pulses contain anti-nutritional factors such as trypsin/chymotrypsin and amylase inhibitors, saponins, phenolic compounds, oligosaccharides, lectins, tannins and phytic acid that are known to negatively affect their nutritional value (Vidal-Valverde et al., 2002; Lopez-Martinez et al., 2017). They also are difficult

to cook and often require pre-soaking of the seeds in order to shorten the cook time. Finally, the flavour profiles of pulses are quite strong and unpalatable to many consumers (Boye et al., 2010). Processing (*e.g.*, germination, extrusion, boiling, roasting, infrared heating, microwave heating, fermentation, enzymatic treatments *etc.*) has been shown to effectively eliminate or reduce the levels of anti-nutrients present, reduce strong flavours, and shorten cook times. The magnitude of the improvement is dependent upon both the method(s) used and the processing conditions employed.

Germination has been suggested as an inexpensive and effective process for improving the overall quality of pulses, and as such is the focus of the current research. It has been used for centuries to soften the seed structure, decrease the cooking time, improve the nutritional value and reduce the impact of anti-nutritional factors in pulses (Vidal-Valverde et al., 2002; Singh et al., 2015). Moreover, it has been used for enhancing the level of bioactive compounds in pulses, improving their organoleptic qualities, and contributing to fine-tuning of perceived flavour. Germination is also relatively simple and environment-friendly, which is becoming an important trend in the food sector to produce functional germinated seeds and sprouts.

Research efforts also have been made to determine the effects of germination on the functional properties and nutritional value of pulse flours and to evaluate the performance of the resultant flours in various food products. Urbano et al. (2005) germinated peas to enhance the organoleptic and nutritional properties of the flours. Zafar et al. (2015) prepared bread using a flour blend at a ratio of chickpea:wheat of 35:65, and the bread obtained showed acceptable taste and texture and, more importantly, a lower glycemic response after being consumed by human subjects. Marengo et al. (2017) found that germination enhanced the functional and nutritional characteristics of chickpea flour in comparison to the ungerminated counterpart, which could offer insights for producing highly nutritious foods for low-income consumers.

The overarching goal of my thesis project was to examine the effects of germination of yellow pea and faba bean on the physicochemical and nutritional value of their resulting flours, as well as their performance in pan bread at 10 and 20% flour substitution levels. Various functional and nutritional properties of the pulse flours, including dietary fibre, protein, starch, lipid and ash contents, starch and protein digestibility, protein solubility, amylose content of starch, water-holding and oil-binding capacity, thermal properties, pasting profiles, and foaming and emulsifying properties, were determined. The resultant germinated pulse flours also were used to

replace 10 and 20% of hard wheat flour for the baking of bread. The flour quality, dough strength and stability, and rheology, as well as the loaf volume, cell structure and crumb texture of the bread baked from the pulse-wheat flour blends, were measured to assess their performance in bread making.

Results from this project not only provided useful information about the technological characteristics of germinated pulse flours, but also will lead to the promotion of Canadian pulses in domestic and international markets based on their intrinsic quality attributes. Insights from this research will allow the domestic pulse industry to market their products more competitively in world markets and will provide information to food processors about the nutritional and functional advantage of incorporating raw and germinated pulses into their product formulations. This approach of replacing wheat flour with raw and germinated pulse flours offers an opportunity to the pulse industry to identify novel food uses of pulses that can enhance the quality of the finished products.

1.2 Objectives

- To assess the influence of germination for different time periods on the structural and functional properties of yellow pea and faba bean flours.
- To investigate the impact of germination for different time periods on the nutritional profiles of yellow pea and faba bean flours.
- To evaluate the flour quality and dough properties of composite flours (wheat flour:raw/germinated pulse flour ratios of 9:1 and 8:2, db) in comparison with those of a 100% control wheat flour.
- To prepare bread using the composite flours and to characterize its quality attributes in comparison to those of a 100% control wheat bread.

1.3 Hypotheses

The following hypotheses will be tested in this project:

- Longer germination times result in enhanced protein solubility, water and oil binding capacities, and emulsifying and foaming properties, and lead to changes in thermal and pasting properties as compared to the ungerminated control flours. Germination will

increase the hydrolysis of macromolecules and also enhance the surface activity of proteins to affect the structural and functional properties of the germinated pulse flours.

- Germination of yellow pea and faba bean seeds will increase their starch and protein digestibilities as compared to the corresponding ungerminated control flours. The breakdown of macromolecules into their simpler forms during germination will render them more susceptible to digestion by hydrolytic enzymes in comparison to their raw counterparts.
- The composite flours (wheat flour:raw/germinated pulse flour ratios of 9:1 and 8:2, db) will affect the flour quality and will develop doughs with varied strength, stability, and handling and rheological properties in comparison with wheat flour. The partial replacement of wheat gluten with pulse proteins will weaken the gluten network and thus affect the aforementioned properties.
- The use of composite flours for bread making will affect the loaf volume, texture, and cell structure as compared to that of the control bread. The partial substitution of wheat flour with pulse flours will influence the gluten structure formed during baking, which will subsequently cause noticeable differences in the baking performance of the bread made from the composite flours in comparison to bread made from 100% wheat flour.

2. LITERATURE SURVEY

2.1 The economics of pulses in Canada

Pulses are the dry edible seeds of legume crops which include pea, bean, lentil, chickpea and faba bean (Tosh and Yada, 2010). Canada's vast and varied agricultural land is suitable for growing different varieties of pulse crops, which makes it the second largest producer and leading exporter of pulses across the globe (Hoover et al., 2010). Canada's pulse production is about 4.5 to 5 million tonnes per annum and it contributes to 41% of the global pulse trade followed by Australia, Myanmar, USA, China, Russia and India (Saskatchewan Pulse Growers, 2017. Among the major pulse producers in Canada, Saskatchewan pulses are in high demand around the world (Saskatchewan Pulse Growers, 2017). The province is the largest producer of peas, lentils and chickpeas with a small bean industry, making up 90% of Canada's total pulse exports. In 2016, over 5 million tonnes of pulses were exported from Saskatchewan, which were valued at $3.4 billion.

2.2 Benefits of pulses

Pulses are globally popular crops primarily due to their excellent nutritional value. They are rich in protein and complex carbohydrates (*e.g.*, fibre, resistant starch) and have high levels of minerals (*e.g.*, iron, zinc, and phosphorous) and vitamins (*e.g.*, folate) (Lopez-Martinez et al., 2017; Tosh and Yada, 2010). Moreover, they are known to be low in fat (except for chickpea and lupin) and are considered to be low-glycemic foods (Boye et al., 2010; Tosh and Yada, 2010). Pulses also have amino acid profiles complementary with those of cereal grains (Boye et al., 2010). They have much greater levels of lysine, leucine, aspartic acid, glutamic acid and arginine which provide well-balanced amino acid profiles when consumed with cereals rich in sulphur-containing amino acids and tryptophan (Yousif and Safaa, 2014; Mohammed et al., 2014). Pulses are also high in antioxidants such as phenolic acids, polyphenols and flavonoids, which can potentially benefit human health (Lopez Martinez et al., 2017; Mamilla and Mishra, 2017). The proximate

composition (*e.g.*, protein, carbohydrates, fibre, lipid and ash) of pea, lentil, chickpea and navy bean is presented in Table 2.1.

As crops widely grown and consumed, pulses are an economical source of essential nutrients to low-income populations. Moreover, pulses are considered gluten free and thus they can be consumed safely by celiac patients (Yousif and Safaa, 2014; Melini et al., 2017; Foschia et al., 2017). Because of these nutritional advantages, pulses have been included as an important part of human diets to combat various diseases such as diabetes, cancer, coronary heart diseases, obesity and digestion problems (Hall et al., 2017).

Table 2.1 Proximate composition of various pulses[a,b]

Pulse	**Crude protein (%)**	**Carbohydrates (%)**			**Dietary Fibre[c] (%)**			**Lipid (%)**	**Ash (%)**
		Total	**Sugars[d]**	**Starch[e]**	**Total**	**Insoluble**	**Soluble**		
Pea	14-31	55-72	5 - 12	30-49 (23-49)	3 - 20	10 - 20	2 - 9	1 - 4	2.3-3.7
Lentil	23-31	42-72	5 - 6	37-59 (19-25)	7 - 23	11 - 19	1 - 7	1 - 3	2.1-3.2
Chickpea	19-27	52-71	3 - 5	30-56 (23-28)	6 - 15	10 - 18	1 - 8	2 - 7	1.8-3.5
Navy Bean	19-27	67-75	6 - 8	21-40 (28-41)	14-25	15-18	3 - 5	2	4-4.9

[a] Hall et al. (2017).
[b] Values are expressed as dry weight basis.
[c] Tosh and Yada (2017).
[d] Total sugar content includes mono- and oligosaccharides.
[e] Values in parentheses represent the percentage (%) of amylose in the starch.

2.3 Factors limiting the utilisation of pulses

In many developing regions and countries, pulses have a long history of consumption (Noorfarahzilah et al., 2014). In some parts of Asia, pulses have traditionally been used in the preparation of foods such as dhal (cooked split chickpea flour), phutna (roasted grains), pakora (oil fried), kadi (boiled in buttermilk), roti (composite of chickpea and wheat flour), dhokla (fermented product), and satu (roasted chickpea flour with other cereal flours) (Boye et al., 2010). More recently, pulses also find new applications in food products such as bread, cookies, biscuits, and pasta (Sozer et al., 2017). Gomez et al. (2012) utilized starch- or protein-rich fractions from pea to prepare cake. Research efforts have also been undertaken to incorporate lentil, chickpea and green pea flours in the formulation of bread baked from composite flours (Kohajdova et al., 2013; Angioloni and Collar, 2012).

Despite their applicability as food ingredients, their widespread use in the food industry has been limited due to the presence of: a) anti-nutritional compounds (*e.g.,* trypsin inhibitors, chymotrypsin inhibitors and α-amylase inhibitors, oligosaccharides, phytates, phenolics, tannins, lectins and saponins; b) undesirable flavour compounds; and c) functionality (Patterson et al., 2017). To further promote their utilization, research has focused on overcoming these challenges. Studies have shown that processing, such as extrusion (Albarracin et al., 2015), fermentation, (Hemalatha et al., 2007; Khanam and Platel, 2016; Singh et al., 2013), cooking (Xu et al., 2017; Roland et al., 2017), autoclaving (Chitra et al., 1995), roasting (Xu et al., 2017; Roland et al., 2017; Chitra et al., 1995), dehulling (Guajardo Flores et al., 2017; Ghavidel and Prakash, 2007), and germination (Singh et al., 2013; Roland et al., 2017; Frias et al., 1995; Ghavidel and Prakash, 2007) can reduce the contents of anti-nutrients in pulses, thus enhancing their acceptability. Processing has also been shown to alter the functional properties of the resulting flours/fractions (Ghavidel and Prakash, 2006; Xu et al., 2017; Marengo et al., 2017) and reduce flavour compounds (Ronald et al., 2017). Among all these methods, germination is found to be a conventional, inexpensive and relatively simple process, which not only decreases the anti-nutritional factors and enhances the bioavailability of nutrients in pulses but also improves their physicochemical and organoleptic properties simultaneously (Tharanathan and Mahadevamma, 2003; Marengo et al., 2017; Vidal-Valverde et al., 2002). Germination is a natural process that triggers the activity of hydrolytic enzymes (*e.g.*, proteases and amylases), which can break down large molecular compounds in pulses (*e.g.*, proteins and carbohydrates) into simpler forms for enhanced

digestibility. Recently, there is a growing interest in incorporating germinated legumes into cereal-based formulations to enhance the quality of food products (Sattar et al., 2017; Marengo et al., 2017; Ouazib et al., 2016; Yousif and Safaa, 2014; Mahmood et al., 2015).

2.4 Germination

2.4.1 Seed germination

A pulse seed is a mature ovule formed after fertilization, which has an outer protective coating (known as testa or seed coat) and micropyle. The interior of seeds contains embryo, consisting of hypocotyl, radicle and plumule (Szewinska et al., 2016). Various metabolic processes and cell growth take place in seeds germinated under desirable environmental conditions (Szewinska et al., 2016). The germination of seeds consists of four stages: (1) uptake of water by the seed (known as imbibition); (2) activation of hydrolytic enzymes; (3) metabolism of storage reserves and transport of the products from endosperm to embryo; and (4) emergence of the radicle and growth of the seedling. When the radicle grows out of the covering seed layers, the process of seed germination is completed. After reaching a specific sprout length, the accumulation of some nutrients (*e.g.*, digestible carbohydrates and proteins, folate, tocopherols, vitamin-C, polyphenols and GABA) in the seeds reaches the maximum level (Patil and Khan, 2011). During germination, increased activities of endogenous enzymes are observed in the seeds, which lead to significant changes in biochemical and physical properties of the seeds (Xu et al., 2017). The hydrolytic enzymes can break down large molecular substances, such as starch, non-starch polysaccharides and proteins, into smaller molecular compounds (Albarracin et al., 2015). The degraded reserve substances (*e.g.*, starch, protein and lipids) of the seeds are used partly for respiration and partly for the synthesis of new cell constituents of the developing embryo during germination. Germination can also increase the levels of bioactive components such as ascorbic acid, tocopherols, tocotrienols and polyphenols, contributing to enhanced antioxidant activity (Gan et al., 2017; Lopez Amoros et al., 2006). The germination of seeds is influenced by both internal and external factors. Some of the critical internal factors include seed vitality, genotype, seed maturation and seed dormancy (Acosta et al., 2012; Chauhan et al., 2006). Important external factors include incubation temperature, moisture content of seeds, the presence of oxygen or air and the exposure to light. The germination process of seeds can be controlled by varying the external factors.

2.4.2 Germination efficiency of seeds

Germination percentage and seedling growth are two important variables in determining the extent of seed germination (i.e., germination efficiency). Germination efficiency is an estimate of seed viability, which refers to the ability of seeds to germinate, and is determined as the ratio of the number of germinated seeds to the total number of seeds (Cáceres et al, 2014). Germinated seeds are considered as those with the radical emerging out from the embryo. Another important variable is sprout length, which is defined as the length of the radicle from the seed's root to the base of its cotyledon (Albarracin et al., 2015; Lee et al., 2007). The sprout length is often used as a determinant of the duration of germination process.

2.4.3 Factors affecting germination

For germination to occur, the seed must not be in a state of dormancy and the environmental requirements for the germination of seed must be met (Butler et al., 2015). These conditions include temperature and time, seed moisture content, exposure to light, and presence of oxygen or air (Olivier and Annandale, 1997; Butler et al., 2015; Sravanthi et al., 2013; Aguilera et al., 2014; Swieca et al., 2012). These variables can favour or inhibit the germination of seeds and thus alter their nutritional and functional characteristics.

2.4.3.1 Temperature

Temperature is one of the important factors that play a significant role in seed germination (Olivier and Annandale, 1997; Butler et al., 2015). Depending upon the seed type, an optimum temperature is required for germination to occur with the initiation of metabolic activities. By implementing germination under a wide range of temperatures, early seedling emergence can be achieved. Three "cardinal temperatures" generally characterize germination response to temperature: the minimum, optimum, and maximum. The minimum (or base, T^b) and maximum (or ceiling, T^c) temperatures define the range, below or beyond which germination will not occur. The optimum temperature (T^o) is the one at which germination is the most rapid (Covell et al., 1986; Raveneau et al., 2011; Barbosa et al., 2006). The cardinal temperatures for pea, lentil, chickpea and navy bean are presented in Table 2.2. Butler et al. (2015) found that germination rate for warm-season legume seeds (*e.g.*, cowpea, soybean) generally increased with increasing

temperature and then decreased at temperatures greater than the optimal temperature range (range in which maximum seeds can germinate most rapidly).

Table 2.2 Estimates of minimum (T^b), optimum (T^o), and maximum temperature (T^c) for pea, lentil, chickpea and navy bean to germinate.

Species	Cardinal Temperatures (°C)		
	T^b	T^o	T^c
Pea [a]	-2 to 0	20 - 27	29 - 40
Lentil [b]	2.5	24 -24.4	31.8 - 34.4
Chickpea [b]	0	31.8 - 33	48 - 60.8
Navy Bean [a]	5.1 – 9.6	30 - 36	46 - 50

[a] Raveneau et al. (2011)

[b] Covell et al. (1986)

2.4.3.2 Moisture content of seed

Moisture can significantly affect germination percentage by softening the seed coat, improving its permeability and, most importantly, rehydrating the seed to levels that can support increased respiratory activity, the breakdown of complex reserve materials into simpler forms and the synthesis of new components for growth. For germination to take place, the moisture content varies among different species due to difference in their chemical composition. An optimum of 30-40% moisture is required for germination to take place (50-60% in case of soybean) (Delouche, 1980; Delouche, 2016).

2.4.3.3 Presence of oxygen

Another factor influencing seed germination is the presence or absence of oxygen. Although oxygen is present in sufficient amount in the environment, its availability to the seed can be limited because of inherent seed conditions and excessive moisture in the soil. Excessive moisture can displace oxygen in the pores of seed and decrease its availability (Delouche, 1980; Delouche, 2016). Oxygen is required for embryo respiration to provide energy for an effective germination process. Al Ani et al. (1985) concluded that germination of starchy grains (pea, rice, wheat, sorghum and maize) require much lower partial pressure of oxygen (pO_2) (with exception

of lentil) than the pO_2 of air (21 kPa). Pea seeds require less than 0.1 kPa pO_2 to germinate completely while increasing the pO_2 to 0.9 kPa and 36 kPa can slightly enhance the germination rates to 50% and 100% of their maximum value, respectively. Stimulation of germination by increased oxygen concentration could be attributed to increased oxygen diffusion, which results in an overall increase in respiration and metabolism.

2.4.3.4 Exposure to light

Light is also an important environmental signal that regulates seed germination. Although light can prevent or delay germination in some species, but it can also enhance seed germination in other species (*e.g.*, lentils and beans) (Aguilera et al, 2014). However, there are species in which light has negligible effect on germination percentage. The same study reported that bean sprouts subjected to 12 h light/12 h dark period showed 100% germination on the eighth day while the beans with germination under 24 h dark period showed 96% germination on the eighth day. Moreover, lentil sprouts subjected to 12 h light/12 h dark period exhibited 92% (maximum) germination on the third day and 83% germination for 24 h dark period for Day 3. Hence, light can considerably enhance the percent germination in beans and lentils.

2.5 Effect of germination on the nutritional properties of pulses

2.5.1 Anti-nutritional factors

Anti-nutritional factors such as phytate (phytic acid), enzyme inhibitors (trypsin inhibitors, chymotrypsin inhibitors and α-amylase inhibitors), oligosaccharides, polyphenols, tannins, lectins and saponins can cause deleterious effects (Patterson et al., 2017; Ghavidel and Prakash, 2007; Albarracin et al, 2015). However, there exists a misperception of the term anti-nutrient as these compounds can be beneficial for human health by reducing the risks of chronic diseases (Lopez-Martinez et al., 2017). They are termed as anti-nutrients because they tend to reduce the bioavailability of nutrients in the human body when consumed in an uncooked form. Phytates and saponins function to chelate micronutrients impacting their absorption (Thompson, 1993). Protease and amylase inhibitors act to inhibit digestive enzymes from breaking down protein and starch, respectively (Champ, 2002). Total phenolics and tannins act to cross link proteins, inhibiting their digestion (Thompson, 1993; Champ, 2002). Lectins function as carbohydrate binding proteins which reduce the activity of hydrolytic enzymes and hinder the breakdown of

starch into simpler forms (Shi et al., 2007; Champ, 2002; Thompson. 1993). Chitra et al. (1996) used germination (25^{o}C for 48 h) to lower the phytic acid content in kabuli chickpea from 920 mg/100g in control (ungerminated) to 330 mg/100g in germinated chickpea. A study by Hemalatha et al. (2006) observed a significant decrease in tannin (215.2 mg/100g to 104.3 mg/100g) and phytate content (180.8 mg/100g to 164.2 mg/100g) of chickpeas germinated at 25^{o}C for 48 h. Contrary to the previous reports by Chitra et al. (1996) and Hemalatha et al. (2006), Guajardo Flores et al. (2017) found an increase in trypsin inhibitor activity while germinating black beans at 20^{o}C for 5 d (0.35 TIU/mg in raw to 0.37 TIU/mg in germinated). The variations among these studies could be due to the variability in the type of seed or legume used and due to different germination conditions used in these studies.

2.5.2 Macronutrients

2.5.2.1 Starch digestibility

Starch is a polymeric carbohydrate consisting of D-glucopyranose as the building block being connected by glycosidic bonds (Tharanathan and Mahadevamma, 2003; Hizukuri 1986; Hoover et al., 2010). It is composed of two types of molecules, namely amylose (linear, 1→4 linkages) and amylopectin (highly branched, 1→4 and 1→6 linkages). Most of the starch in human diets can be readily converted into D-glucose by the action of amylolytic enzymes; however a portion (known as "resistant starch" [RS]) remains undigested in the small intestine, and then becomes fermented by the gut microbiota in the large intestine. Factors leading to the reduced enzymatic hydrolysis of starch are: 1) tissue/cell structures enclosing the starch (which hinder its swelling and solubility); 2) presence of highly viscous and soluble dietary fibre; 3) high amylose/amylopectin ratio; and 4) presence of anti-nutrients (Ma et al., 2017). These factors can leave a significant amount of un-hydrolyzed starch by alpha amylase, contributing to 34% of RS in raw yellow field pea. Ma et al. (2017), however, showed that germination (30^{o}C for 72 h in dark) of yellow pea could decrease the RS content from 34% to 21%. The authors also showed a corresponding increase in total starch from 43% to 49%, and digestible starch from 9-28%. The increased starch digestibility could be attributed to the partial hydrolysis of starch granules by endogenous enzymes during germination, and the partial removal/decrease in the activity of amylase inhibitors (Ghavidel and Prakash, 2007; Xu et al., 2017; Ma et al., 2017).

2.5.2.2 Protein digestibility

Proteins are highly complexed polymers forming a polypeptide chain which is composed of amino acid residues, linked together in a definite sequence (Richardson, 1981; Ozdal et al., 2013). The amino acids consist of a α-carbon atom which is covalently linked to an amino group, a hydrogen atom, a carboxyl group and a side chain involving R group. The R group may be acidic, basic, neutral, or hydrophobic in nature. This protein structure is arranged in the form of α-helices and β-strands. These α-helices and β-strands determines the stability and organisation of protein structure. Proteins are held together by various covalent and non-covalent forces (*e.g.*, hydrogen bonding, hydrophobic interactions, disulphide bonds and salt bridges). Pulses, such as pea, chickpea, lentil and bean, usually have 17-30% of protein in them with different amounts and sequence of amino acid residues present in them (Tosh and Yada, 2010; Boye et al., 2010). The major portion of storage proteins in pulses comprises globulins (salt soluble) and albumins (water soluble). Albumins usually include enzymatic proteins as well as amylase inhibitors, protease inhibitor and lectins. The molecular mass of albumin proteins range between 5-80 kDa. By contrast, globulin proteins comprise of legumin (11S fraction) and vicilin (7S fraction). Legumin possess a hexameric quaternary structure with a molecular mass of 350 – 400 kDa, which contains basic and acidic subunits with molecular mass of 20 – 40 kDa, respectively. However, vicilin (7S fraction) has a trimeric structure with the molecular mass ranging between 175 – 180 kDa (Boye et al., 2010). Legumin is held together by covalent disulphide bonds while vicilin is held together by hydrophobic interactions (Lam et al., 2016). Another storage protein isolated from pea is convicilin with molecular mass of 290 kDa. It also possesses sulphur-containing amino acids in its residues (Boye et al., 2010). Other minor proteins present in pulses are prolamins (alcohol soluble) and glutelins (soluble in dilute acid or alkali in the presence of reducing agents, also contains methionine and cysteine).

The composition of essential amino acids in proteins and their digestibility determines the nutritional importance of food proteins. It has been reported previously that protein digestibility of pulses is inversely related to the levels of anti-nutrients (tannins and trypsin inhibitors) (Ma et al., 2017). Tannins can bind with proteins through hydrogen bonding and hydrophobic interactions to form insoluble complexes. They are also known to bind irreversibly with the proteins via crosslinking and lead to the formation of covalent linkages, thus reducing their digestibility and nutritional quality (Ozdal et al., 2013). This function of tannins also provide astringency in tannin-

rich foods (Champ, 2002; Ma et al., 2017). Trypsin inhibitors bind irreversibly to the endopeptidase trypsin (protein hydrolysing enzyme) by forming inactive protein complex, which inhibits the activity of trypsin and thus leads to decreased protein digestibility. Frias et al. (1995) reported trypsin inhibitory activity in lentil (*Lens culinaris* var Vulgaris) to be reduced from 5.05 TIU/mg for control to 4.12 TIU/mg after 6 d of germination (20^{o}C in dark, rinsed daily). Singh et al. (2015) reported that the phytic acid content in chickpeas was reduced by 95.74% after germinating them at 25^{o}C for 72 h. The same study also observed the decrease in total phenolic contents by 77.10% in germinated chickpeas. This may be due to the increased enzymatic hydrolysis by phytase and polyphenol oxidase, thus reducing anti-nutrient content and improving digestibility and nutritional quality (Singh et al., 2015). Germination can improve the protein digestibility by inactivating tannins and trypsin inhibitors to enhance protease activity, which leads to increased hydrolysis of the proteins (Ma et al., 2017). Hydrolysis by endogenous enzymes during germination results in degradation of larger protein bodies into simpler forms, which also renders it more susceptible to digestion.

2.5.2.3 Dietary fibre

Dietary fibre (DF) is obtained from the edible parts of plants or analogous carbohydrate cell wall material of plants which are not degraded by hydrolytic enzymes in the human small intestine but is fermented partially or completely in the large intestine by gut microflora (AACC, 2000). Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances. It can be categorized into insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) based on the solubility in water. Martin-Cabrejas et al. (2008) observed a general trend of increases in the level of total dietary fibre (TDF), IDF and SDF after germination of non-conventional legumes (cowpea, jackbean, dolichos, mucuna and soybean) at 25^{o}C for 96 h in dark condition. The TDF content in cowpea increased from 312.2 g/kg in un-germinated cowpea flour to 355.9 g/kg in germinated cowpea flours. Also, the IDF and SDF fractions increased from 303.2 g/kg and 9 g/kg in control cowpea flour to 338.8 g/kg and 17.1 g/kg in germinated counterparts, respectively (Martin-Cabrejas et al., 2008). Another work by Martin-Cabrejas et al. (2003) reported an increase in IDF, SDF and TDF values (IDF: 9.7 g/kg to 13.2 g/kg; SDF: 5.6 g/kg to 13.8 g/kg and TDF: 15.3 g/kg to 27.0 g/kg in control and germinated peas, respectively) after germinating peas at 20^{o}C for 6 d in dark. The increase in IDF could be because of higher gravimetric residues present in

germinated pea and due to its lower content of protein associated with the fibre matrix. In contrast to these researches, Chitra et al. (1996) showed decreased TDF content (from 161.2 g/kg in control to 68.3 g/kg in germinated chickpea) while germinating chickpeas at 25^{o}C for 48 h which could be due to increased alpha-galactosidase activity during germination, leading to decreased oligosaccharide content and thus reducing total dietary fibre.

2.5.3 Bioactive compounds

Germinated pulses contain enhanced levels of numerous bioactive compounds such as minerals, vitamins, GABA, flavonoids and polyphenols which have the potential to benefit human health. These bioactive compounds can either be synthesized or transformed during the germination process. The mineral bioavailability is improved on germination as the content of anti-nutrients bound with the minerals is reduced (Bains et al., 2014). El-Adawy et al. (2003) reported the increases in mineral contents on germination (25^{o}C for 120 h) of peas and lentils (calcium: 86 to 112 mg/100g pea flour and 76 to 94 mg/100g lentil flour; phosphorus: 395 to 426 mg/100g pea flour and 372 to 392 mg/100g lentil flour in control and germinated flour, respectively). Vitamins are organic compounds which are beneficial for human health and are required in limited amounts by our body (Gan et al., 2017). Vitamins are not synthesised in sufficient quantities in humans and must be obtained through diets. Shohag et al. (2012) found that folate content was significantly increased from 0.169 mg/100g fresh weight in raw mung bean seeds to 0.691 mg/100g fresh weight (showed 3.9 folds increase) in mung bean sprouts (germinated at 25^{o}C for 10 d) on 4th day. Masood et al. (2014) showed that the content of vitamin- C reached 9.94 mg/100g on dry weight basis in germinated chickpea (25^{o}C for 120 h), whereas vitamin C content was negligible in raw counterpart.

GABA is a non-protein amino acid that can function as a neurotransmitter to regulate heart rate and blood pressure in our body (Nikmaram et al., 2017; Gan et al., 2017). Germination increases the activity of glutamate decarboxylase enzyme which synthesizes GABA. Kuo et al. (2004) observed that GABA was absent in the raw seeds of beans, lentils and peas, but its content increased dramatically after germination (at 20^{o}C for 6 d in 99% relative humidity), especially after 6 d (44, 32 and 104 mg/100g of the germinated seeds of the three varieties, respectively). Flavonoids and polyphenols are bioactive components with anti-oxidant activities that can be found in pulses in relatively high contents (Pal et al., 2017; Mamilla and Mishra, 2017).

Germination can further enhance the flavonoid content and improve the polyphenol content and antioxidant activity of pulses due to increased activity of polyphenol oxidase enzyme during germination (Pal et al., 2017; Mamilla and Mishra, 2017).

2.6 Effects of germination on functional properties of pulse flours

2.6.1 Protein solubility

During germination, the proteins are hydrolyzed into short peptides and amino acids as a result of increased activities of proteases (Khattak et al., 2008; El-Adawy et al., 2003; Ghavidel and Prakash, 2006). Solubility is related to the balance between protein-solvent and protein-protein interactions, where higher solubility favors the former. Because of the breakdown of the larger and more complex polymers (starch and proteins) into simpler substances, their solubility increases. El-Adawy et al. (2003) observed that the protein solubility of pea (47% in raw and 49% in germinated) and lentil (22% in raw and 29% in germinated) increased with germination at 25ºC for 120 h. Another study by Khattak et al. (2008) also showed that protein solubility of chickpea increased from 23% to 52% after 48 h of germination at 28ºC.

2.6.2 Water holding capacity

Water holding capacity of an ingredient measures the amount of water that can be retained under the test conditions (Menon et al., 2015). Flours with high water holding capacity commonly contain more hydrophilic components of large molecular sizes, such as polar proteins, starch and cellulose. This can also be attributed to increased dietary fibre content during germination, which can lead to improved water holding capacity (Benitez et al., 2013). Water holding capacity is an important functional property because it can influence the texture and mouthfeel of food products (Ai et al., 2017). Ghavidel and Prakash (2006) found that lentils germinated at ambient temperatures (20-25ºC) for 24 h showed an increase in the water holding capacity (974 g/kg in control and 1448 g/kg in germinated), with the largest value observed when the germinated lentils were stored at 60°C (1105 g/kg in control and 1553 g/kg in germinated). El-Adawy et al. (2003) also observed an increase in the water holding capacity of pea (215% in raw to 240% in germinated) and lentil (203% in raw to 219% in germinated) on germination at 25°C for 120 h. Another study by Benitez et al. (2013) observed increased water holding capacity (2000 g/kg in raw and 2600 g/kg in germinated) of cowpea when germinated at 25ºC for 96 h.

2.6.3 Oil holding capacity

Oil holding capacity is primarily attributed to physical entrapment or binding of oil to the apolar moieties of protein and other components. A higher oil holding capacity is usually associated with a more desirable mouthfeel and greater flavor retention in food products (Menon et al., 2015). The efficacy of lipid binding in pulse flours primarily depends upon the surface hydrophobicity of protein, which can be enhanced by germination because this process increases the exposure of non-polar moiety from the interior of protein molecules (Menon et al., 2015; Ghavidel and Prakash, 2006). Germination of pulse seeds at a higher temperature can increase the oil holding capacity of the resultant pulse flour due to improved solubilization and dissociation of the proteins into subunits and subsequent increase in the number of hydrophobic binding sites exposed. Germination also increases the dietary fibre content (along with the non-polar side chains of dietary fibre), which further increases the oil holding capacity (Benitez et al., 2013). Ghavidel and Prakash (2006) showed that the germination of pulses (*e.g.*, green gram, cowpea, lentil and bengal gram) at ambient temperatures (20-25ºC) for 24 h increased the oil holding capacity of their flours (857g/kg for raw and 920g/kg for germinated lentil flour). In addition, the same authors showed that when germinated at a higher storage temperature, a larger increase in their oil holding capacity (964 g/kg for raw and 1040 g/kg for germinated lentils stored at 60°C) was observed. Benitez et al. (2013) also observed enhanced oil holding capacities (800 g/kg in raw and 1200 g/kg in germinated) of cowpea flours after germination at 25°C for 96 h. In contrast, El-Adawy et al. (2003) found a significant decrease while germinating peas (100% in raw to 86% in germinated) and lentils (81% in raw to 75% in germinated) at 25°C for 120 h.

2.6.4 Emulsifying properties

An emulsion is defined as a mixture of two or more immiscible liquids (usually oil and water), where one of the liquids (the dispersed phase) is mixed in to the other (the continuous phase) in the form of small spherical droplets (Singhal et al., 2016). There are generally two types of emulsions: oil-in-water (O/W; such as in milk, mayonnaise and cream) and water-in-oil (W/O; such as in butter and margarine) emulsions, in which the former is a dispersed phase present in the latter continuous phase (Singhal et al., 2016). Emulsions are thermodynamically unstable and tend to separate into individual oil and water layers after some time. These can be stabilized by using

various emulsifiers (*e.g.*, proteins) which adsorb onto the oil-water interface to form a viscoelastic film around the oil droplets and acts as a separating membrane to reduce interfacial tension. Emulsifying capacity refers to the ability of amphiphilic molecules to intermix and retain oil in the solution of two immiscible liquids (Aijie et al, 2014; Xu et al., 2017; Boye et al., 2010). Simply, emulsifying capacity is the amount of oil homogenized per gram of protein material and expressed as mL oil/g protein. In pulse flours, protein is the primary component contributing to the emulsifying capacity. Proteins act to stabilize the oil-water interface to stabilize the emulsion through electrostatic repulsion (depending on the pH and salt level) and through steric hindrance and act to increase the viscosity of the continuous phase that inhibits gravitational sedimentation (Chang et al., 2015). If the interface is insufficiently covered, flocculation and/or coalescence can lead to the formation of larger droplets. Because of density differences between the discontinuous/continuous phases, phase separation can occur. The same study suggests that increased charge repulsion between droplets, high solubility, greater conformational flexibility, and high hydrophobicity (depending upon the application) also contribute to a stronger emulsifying capacity of proteins. Germination can cause partial unfolding and dissociation of proteins that tend to make proteins more surface active, especially as it relates to surface hydrophobicity leading to enhanced emulsification capacity (Ghavidel and Prakash, 2006). The process of germination also involves the synthesis of new proteins which can further enhance the hydrophobicity and their emulsifying ability. The emulsification capacity of lentils increased to 74 mL/g (as compared to 58 mL/g for raw un-germinated lentils) when subjected to germination at 25°C for 24 h (Ghavidel and Prakash, 2006). El-Adawy et al. (2003) also observed an increase in emulsifying capacity of peas (106 mL/g in raw to 113mL/g in germinated) and lentils (92.5 mL/g in raw to 99 mL/g in germinated) on germination at 25°C for 120 h. In contrast to previous research, Benitez et al. (2013) observed decrease in emulsifying activity (45% in raw and 39% in germinated) of cowpea after germination at 25°C for 96 h.

2.6.5 Foaming properties

Similar to emulsions, foams are a dispersion of two immiscible materials where the non-polar gaseous phase becomes dispersed with a continuous liquid or solid phase (Menon et al., 2015); they require intense mechanical energy to form (*e.g.*, whipping or sparging); and are stabilized by an emulsifier (*e.g.*, protein), which unfolds and realigns to form a thick viscoelastic

film at the gas/water interface of the bubbles' lamella (Boye et al, 2010). Foaming capacity refers to the total amount of foam generated using a specific amount of protein (Kohajdova et al., 2013; Boye et al., 2010). Foaming capacity of flours depends upon the structures of the proteins: proteins having good structural flexibility tend to possess strong foaming capacity, whereas those having poor structural flexibility (globular in nature) tend to show weak foaming capacity (Menon et al., 2015). It has been demonstrated that flour with a poor foaming capacity usually results in a low loaf volume when it is used for bread making (Sreerama et al., 2012; Kohajdova et al., 2013). Germination tends to increase the foaming capacity of pulse flours as it leads to improved protein solubility and synthesis of new proteins (Ghavidel and Prakash, 2006). Also, germination can cause surface denaturation of proteins and reduce surface tension of the molecules which results in good foam formation. El-Adawy et al. (2003) also observed an increase in foaming capacity of peas (56% in raw to 63% in germinated) and lentils (64% in raw to 70% in germinated) on germination at 25°C for 120 h. Contrary to this finding, Benitez et al. (2013) found a significant decrease in foaming capacities (64% in raw and 8% in germinated) of germinated cowpeas (25°C for 96 h).

2.6.6 Thermal properties

The thermal properties of a pulse flour are commonly measured using differential scanning calorimetery (DSC). When heated in the presence of a sufficient amount of water, DSC thermogram of a pulse flour will display thermal transitions indicating starch gelatinization, protein denaturation, and dissociation of an amylose-lipid complex (Ai et al., 2017; Chung et al., 2008). DSC thermograms can be used to measure onset temperature, peak temperature, conclusion temperature, and enthalpy change of those thermal transitions. According to previous research, germination did not display a significant effect on the thermal properties of legume starches or flours (Frias et al., 1998). As examples, Frias et al (1998) showed that there were no considerable changes found in the gelatinization properties of starch in lentil seeds before and after germination at 20°C for 6 days.

2.6.7 Pasting properties

Pasting occurs when the starch or flour is heated beyond its gelatinisation temperature, which leads to swelling of starch granules and subsequent leaching out of starch molecules (mainly

amylose and some amylopectin) for the viscosity development (Kaur et al., 2015; Xu et al., 2017; Wani et al., 2012). Pasting properties of starch and flour can be measured using Rapid Visco-Analyzer (RVA) or Brabender Visco-amylograph, which can provide programmed heating temperature profiles and controlled shearing conditions (Wani et al., 2012; Kaur et al., 2015). The instrument can heat starch or flour suspended in water gradually and thus starch granules start to swell. At the pasting temperature, the sample begins to develop viscosity. The swelling of starch granules continues until the peak temperature is reached, where the sample possesses the maximum viscosity (peak viscosity). After the peak temperature, starch granules start to disintegrate and break into fragments, resulting in breakdown in the viscosity until it reaches the lowest viscosity, known as trough viscosity. When the paste of starch or flour begins to cool down, the molecules start to re-associate, which leads to the rise in the viscosity to give the final viscosity at the end point. The difference between final viscosity and trough viscosity is defined as setback viscosity. Figure 1 shows a typical pasting curve of starch or flour with the indications of the aforementioned parameters as measured by RVA or Visco-amylograph. Germination can lead to decreased peak, breakdown, setback and final viscosities as a result of increased activities of hydrolytic enzymes (*e.g.*, alpha-amylase), which hydrolyze large starch molecules into simpler forms (Xu et al, 2017; Sattar et al, 2017; Morad et al, 1980). The decrease in starch content after germination also contributes to the reduced pasting viscosities.

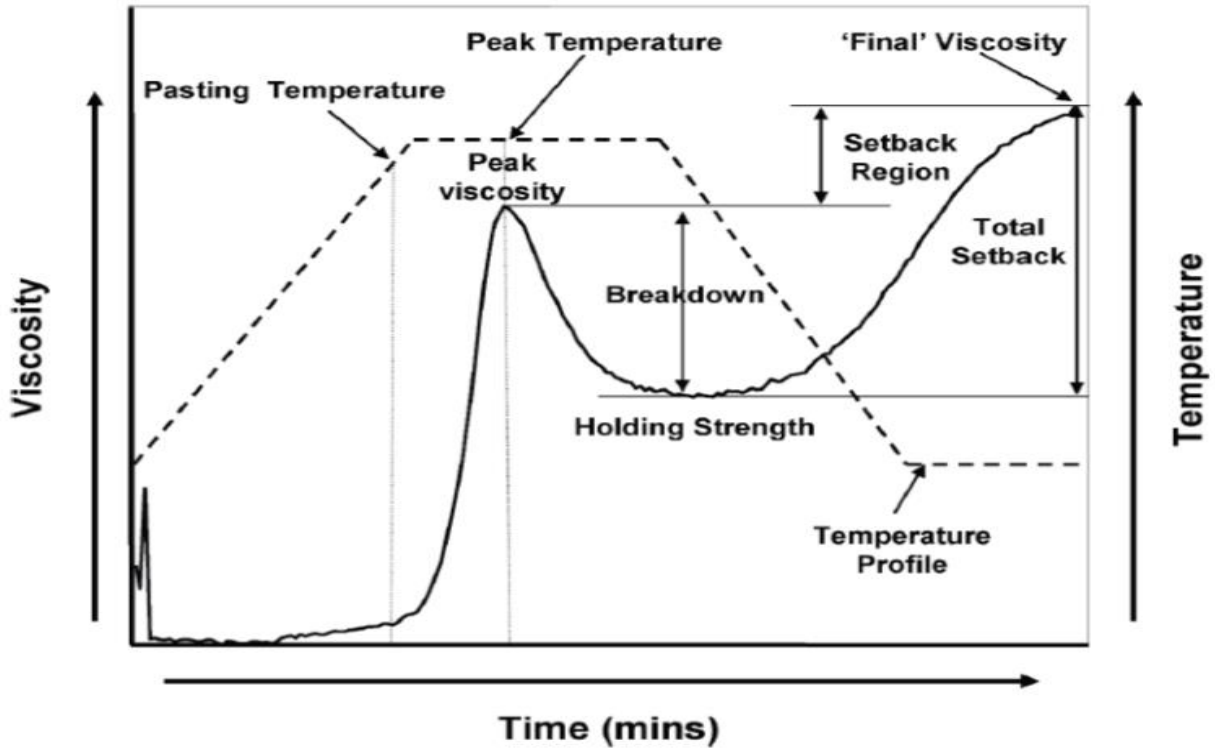

Figure 2.1 A typical pasting curve showing pasting parameters measured with RVA or Visco-amylograph (Wani et al., 2012).

2.7 Pulses and Bread Making

2.7.1 Role of proteins and starch in bread making

The baking of bread usually involves three stages: dough formation, fermentation and baking. Dough formation involves the mixing of ingredients, such as wheat flour, water, yeast, salt (NaCl), shortening/fat, sugars, dairy products (*e.g.,* skim milk powder), enzymes, yeast, emulsifying agents and preservatives. However, the main components of wheat flour, affecting the quality of the baked bread are proteins and starch present in them. They play a critical role in the dough formation and the crumb structure of the baked bread (Avramenko et al., 2018; Hui, 2006). The major proteins which determine the quality of bread making are the gluten proteins (~80% of the total wheat proteins). They are composed of monomeric gliadins (molecular mass between 30-80 kDa) and polymeric glutenins (molecular mass ranging from 80 kDa to several millions) (Hui, 2006; Avramenko et al., 2018; Goesaert et al., 2005). Gluten proteins develop a network structure upon hydration and kneading. The visco-elastic protein network formed largely depends on the gliadin/glutenin ratio. As the glutenins are larger in size, they form a continuous network which is responsible for dough strength, its resistance to deformation and provides dough elasticity (Goesaert et al., 2005; Avramenko et al., 2018). Gliadin aids in providing plasticity to the glutenin polymeric network. The formed gluten network can retain more carbon dioxide during fermentation within the dough. A stronger gluten network is critical to support the crumb structure during baking. Therefore, final volume of baked bread is found to be positively correlated with the dough strength (Goesaert et al., 2005; Hui, 2006). However, the volume of bread can be decreased if the dough shows an extremely high strength. Thus, glutenins and gliadins interact with one another to provide a balance between dough elasticity and dough viscosity, which determines the quality of the resultant bread.

During baking, the gluten proteins undergo various changes, such as denaturation, changes in surface hydrophobicity, disulphide formation, and cross-linking (Hui, 2006; Goesaert et al., 2005; Avramenko et al., 2018). Hence, the quality of gluten (glutenins and gliadins) plays a major role in bread quality. Another component that plays an important role for the bread structure formation is starch. As discussed in the aforementioned sections, starch is composed of amylose (essentially linear) and amylopectin (highly branched) and is semi-crystalline in nature. During baking, when the starch is heated above its gelatinization temperature, starch granules lose birefringence and crystallinity and become gelatinized (Hui, 2006; Goesaert et al., 2005).

Additionally, some amylose molecules leach out (solubilization of amylose double helices) during this process. This leads to the formation of a gel network in which amylopectin acts as the dispersed phase. After baking, the bread will be cooled down. Upon cooling, the generated network is vital to support the structure of the loaf (Goesaert et al., 2005). Therefore, starch, particularly amylose molecules, in wheat flour acts as an essential component to provide order and structure in baked bread and also contributes to loaf firmness (Avramenko et al., 2018).

2.7.2 Pulse flour as an ingredient in bread making

There is ongoing interest in incorporating pulse flours into the formula of pan bread, to improve the quality and nutritional value. Mohammed et al. (2012) prepared a low glycemic and palatable bread by replacing wheat flour with 10%, 20% and 30% chickpea flour. The new formula produced bread with improved or comparable quality when compared to 100% wheat flour control bread in terms of weight, volume, crumb structure, texture, taste, and colour. The composite flour also exhibited an increased percentage of water absorption in Farinograph test. The inclusion of chickpea flour enhanced the dough stability and increased the dough development time and its resistance to deformation (which provides good dough handling properties and tolerance to fermentation) while reducing the extensibility of dough (*i.e.* dough became less viscoelastic). Zafar et al. (2015) prepared bread using a flour blend at a ratio of chickpea: wheat as 35: 65, and the obtained bread showed acceptable taste and texture and, more importantly, a lower glycemic response after being consumed by human subjects. Tshalibe et al. (2013) baked a composite bread by replacing wheat flour with cowpea flour at 10%, 20% and 40% levels. The authors reported that increasing concentrations of cowpea decreased the loaf volume, improved the crust colour, but did not noticeably change the taste, texture, palatability and other sensory attributes in comparison to 100% wheat bread. Jeffers et al. (1978) also prepared bread with acceptable sensory quality by substituting yellow pea flour for wheat flour at a level of 10-15%. Kamaljit et al. (2010) replaced 5% and 10% of wheat flour with pea flour and observed that the resulting bread exhibited similar sensory profiles (including appearance, crust colour, crumb colour, aroma, taste and overall acceptability) to the control wheat bread. Morad et al. (1980) incorporated pea, faba bean and lentil flours at 5%, 10%, 15% and 20% substitution levels for wheat flour and found that the baking absorption increased at all replacement levels, loaf volume increased while using 5% pea flour but decreased for all the other formulations. No undesirable flavour or colour was observed and the

crumb grain appeared to be improved for pea and lentil at 5% and 10% replacement levels. Previous studies on the bread making performance of pulse flours mainly used native pulse flours. The impact of germination on the baking properties of pulse flours, however, has not been well elucidated. Thus, in addition to the abovementioned functional properties, the current study also will examine how the replacement of wheat flour with 10% and 20% germinated pulse flours will influence the dough properties and the quality of the resultant bread. The research will generate useful information for the incorporation of germinated pulse flours in bread formulation to improve its quality attributes and nutritional properties.

3. IMPACTS OF SHORT-TERM GERMINATION ON THE CHEMICAL COMPOSITION, TECHNOLOGICAL CHARACTERISTICS AND NUTRITIONAL QUALITY OF YELLOW PEA AND FABA BEAN FLOURS[1]

ABSTRACT

In the present study, yellow pea (CDC Amarillo) and faba bean (CDC Snowdrop) seed was soaked overnight and then germinated in the dark at ambient temperature for 24, 48 and 72 h. During the short-term germination, germination percentages higher than 96.6% were achieved and progressive growth of radicles was observed for both pea and faba bean. The soaked and germinated seed was dried at 55°C and then milled into flour, and their chemical composition, physicochemical properties and *in vitro* starch and protein digestibility were examined. Overall, soaking and germination did not noticeably alter the chemical compositions of the flours. The most obvious changes in the physicochemical properties were found in the pasting, emulsifying and foaming properties of the pulse flours. Soaking and 24-h germination greatly enhanced the pasting viscosities of the flours; as germination proceeded, their viscosities gradually decreased, resulting from the degradation of starch by endogenous amylase(s) during pasting. Germination progressively improved the emulsion activity and stability, foaming capacity and foam stability of the pulse flours. In addition, germination enhanced the *in vitro* digestibility of starch and protein of the flours; however, germination did not improve their *in vitro* protein digestibility corrected amino acid scores (IV-PDCAAS). Short-term germination of 24-72 h was demonstrated to be an effective approach for generating pulse flours possessing diverse functional properties and enhanced digestibility of macronutrients.

Keywords: yellow pea; faba bean; germination; flour; physicochemical properties; digestibility

[1]Setia, R., Dai, Z.X., Nickerson, M.T., Sopiwnyk, R. Malcolmson, L and Ai, Y. (2019). Impacts of short-term germination on the chemical compositions, technological charatcteristics and nutritional quality of yellow pea and faba bean flours. *Food Research International*, 122, 263-272.

3.1 Introduction

Pulses are the dry edible seeds of crops in the *Fabaceae* family, which include pea, faba bean, bean, lentil, chickpea *etc.* (Asif et al., 2013). Globally, they are popular crops possessing high nutritional value – rich in protein, resistant starch and other dietary fibre, and micronutrients (*e.g.*, folate and other B-vitamins, minerals) (Lopez-Martinez et al., 2017; Tosh and Yada, 2010). Pulses show a relatively low glycemic effect and typically have a low level of fat (except for chickpea) (Boye et al., 2010). The consumption of pulses can offer various health benefits, such as improving glycemic control, enhancing insulin sensitivity, and reducing the risks of chronic diseases (*e.g.*, obesity, cancers) (Boye et al., 2010; Padhi and Ramdath, 2017). Therefore, in recent years pulses have attracted tremendous attention from the food industry, which aims to increase the use of pulses in a broader range of food products to benefit the health of consumers.

Despite the nutritional importance, the wide use of pulses in human diets has been hindered by their cooking difficulties (Leterme and Munoz, 2002), strong flavor profiles (Boye et al., 2010), and presence of anti-nutritional compounds (*e.g.*, inhibitors of digestive enzymes, saponins, lectins and phytic acid) (Boye et al., 2010; Lopez-Martinez et al., 2017). To eliminate or at least alleviate the negative effects of these factors, the food industry has used treatments such as germination, extrusion, roasting, infrared heating, microwave heating, fermentation and enzymatic modification to process whole pulse seeds and their derived ingredients (Alonso et al., 2000; Patterson et al., 2017). Germination, for instance, has been shown to soften cotyledons, reduce cooking time, lower the levels of anti-nutritional factors, and improve the overall nutritional value of pulses (Vidal-Valverde et al., 2002). Moreover, germination can promote the development of desirable aroma and flavor, thus enhancing the organoleptic qualities (Roland et al., 2017).

Research efforts have also been made to investigate the effects of seed germination on the functional properties and nutritional value of the resultant pulse flours. In the case of the former, germination at 20°C for 144 h did not noticeably alter the gelatinization properties of starch in lentil seeds (Frias et al., 1998). Peak, breakdown, setback and final viscosities of flours from various pulses were diminished by germination for 24-96 h, resulting from the breakdown of starch molecules by amylolytic enzymes (Morad et al., 1980; Sattar et al., 2017). Protein solubility of pea and lentil flours was increased from to 47% to 49% and from 22% to 27%, respectively, by germination in the dark at 25°C for 120 h (El-Adawy et al., 2003). Ghavidel and Prakash (2006) found that the emulsifying capacity of resultant lentil flour was increased from 58 mL/g to 74 mL/g

when the seeds were subjected to germination at 25°C for 24 h. The study carried out by El-Adawy et al. (2003) reported an increase in the foaming capacity of pea flour (56% in raw to 63% in germinated) and lentil flour (64% in raw to 70% in germinated) after germinating their respective seeds at 25°C for 120 h. Nonetheless, discrepancies were also found in the literature as germination was reported to lower the emulsifying and foaming capacities of cowpea flours (Benitez et al., 2013). With regard to nutritional value, germination of pulse seeds tended to improve the digestibility of starch and protein, two main macronutrients in pulse flours (Ghavidel and Prakash, 2007; Ma et al., 2017). Germination has been demonstrated to be an effective method that can be utilized to diversify the technological characteristics and enhance the nutritional quality of pulse flours for wider food applications.

According to the literature, previous studies of using germination to modify pulses seeds and flours were typically carried out for long periods (72-168 h), which are not very practical for industrial processing, particularly considering the challenges in microbial control and the low throughput. There is growing interest from the food industry in utilizing short-term germination (*i.e.*, 24-72 h) to modify pulse seeds and their resultant flours. However, there is a lack of understanding of the biological and physicochemical changes occurring in pulse seeds during short-term germination. Therefore, the overarching goal of this project was to examine the effects of germinating yellow pea and faba bean seeds for 24-72 h on the chemical compositions, functional properties and nutritional attributes of the resultant flours. The present study provided useful information about germinated pulse flours that will promote the utilization of pulses for the development of high-quality and nutritious food products.

3.2 Materials and Methods

3.2.1 Materials

Certified seeds of yellow pea (CDC Amarillo variety) and faba bean (CDC Snowdrop variety) were kindly donated by Reisner Farm Ltd. (Limerick, SK, Canada). Potato amylose and maize amylopectin standards used for amylose content determination and enzymes, including protease, α-amylase, amyloglucosidase, trypsin, chymotrypsin, porcine pancreatin and invertase, were purchased from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). The enzymes were used without further purification. Total Starch Assay Kit, Starch Damage Assay Kit, and α-Amylase

SD Assay Kit were purchased from Megazyme International Ltd. (Co. Wicklow, Ireland). All the other chemicals used in the study were of reagent grade or higher purity.

3.2.2 Soaking and germination of seeds

Yellow pea and faba bean seeds were germinated following the method of Frias et al. (2005) with some modifications. Pulse seeds (~350 g each batch) were firstly washed with tap water for cleaning and then soaked in 1 L of 0.07% (w/v) sodium hypochlorite aqueous solution for 30 min for sterilization (Vidal-Valverde et al., 2002). After this step, the sterilization solution was drained off and the seeds were washed 4-5 times with distilled water, followed by soaking in 1 L of distilled water overnight at room temperature (~22°C). On the next day, the soaking water was drained off and the obtained seeds were designated as "soaked (0-h) seeds" in this study. Other batches of soaked seeds were placed on a tray to form an even, single layer of the seeds, which were covered with a wet cheese cloth for germination at room temperature in a dark condition for a duration of 24, 48 or 72 h. The seeds were evenly sprayed with distilled water (~40-50 g) once a day (around 10 am) to maintain adequate hydration. For each germination period, the percentage of germination was calculated by dividing the number of germinated seeds over the total number of seeds in the batch. Radicle length was measured on 10 randomly selected seeds from the same batch using a Vernier caliper. The resultant soaked and germinated seeds were dried in a convection oven at 55 ± 2°C for 24-36 h to reach moisture levels below 10%, and the dried seeds were stored in a freezer prior to milling. The soaking and 24-h, 48-h and 72-h germination of yellow pea and faba bean seeds were performed in triplicate (*i.e.*, three independent batches for each treatment).

3.2.3 Morphology of seed cotyledons

Morphology of the cotyledons of raw (control), soaked and germinated (72-h) seeds in a dry state was examined using a scanning electron microscope (SEM, SU8010, Hitachi High Technologies Canada Inc., Rexdale, ON, Canada). After the seed coat was carefully removed manually, the seed was split into two cotyledons and each cotyledon was gently cut into halves along the long side using a razor blade. The cross section of the seed cotyledon was observed under the SEM after coating the seed surface with 10 nm of gold using Q150T ES coater (Quorum

Technologies Inc., Puslinch, ON, Canada). Representative images were captured at three different magnifications: 30×, 500× and 1,000×.

3.2.4 Milling of seeds

The raw, soaked and germinated pulse seeds were milled into flours using a two-step process: (1) a "pre-break" step: the seeds were milled into coarse grits using a laboratory disc mill (Model 3310, Perten Instruments Canada, Winnipeg, MB, Canada) equipped with a type-1 medium grinding disc at setting 5; and (2) a "pin-mill" step: the coarse grits were further milled using a pin mill (Model 100 UPZ, Hosokawa Alpine, Augsburg, Germany) to prepare fine flours. The obtained fine pulse flours were used for the subsequent analyses.

3.2.5 Alpha-amylase activity and chemical compositions of pulse flours

Alpha-amylase activity of the raw, soaked and germinated pulse flour samples was determined using Megazyme α-Amylase SD Assay Kit. Moisture, ash and crude fat contents of the flours were analyzed in accordance with AOAC Method 925.10, 923.03 and 920.85, respectively (AOAC, 2012). Nitrogen content was quantitated according to the Dumas combustion method using a Nitrogen/Protein Analyzer (CN628, LECO Corp., St. Joseph, MI, U.S.A.), which was converted to protein content (%N × 6.25) following AACC Method 46-30.01 (AACC, 2000). Total starch content was measured using Megazyme Total Starch Assay Kit following AACC Method 76-13.01 (AACC, 2000). Damaged-starch content was determined using Megazyme Starch Damage Assay Kit following AACC Method 76-31.01 (AACC, 2000). Amylose content was quantitated according to the method reported by Chrastil (1987), with potato amylose and maize amylopectin being used as the standards for the construction of a standard curve. Total dietary fibre content was determined using AOAC Method 991.43 (AOAC, 2012). The data from all the analyses were reported on a dry basis (db) of the flours.

3.2.6 Functional properties of pulse flours

3.2.6.1 Thermal properties

Thermal properties of the raw, soaked and germinated pulse flours were determined using a differential scanning calorimeter (DSC Q2000, TA Instruments, New Castle, DE, U.S.A.) according to the method of Song and Jane (2000) with some modifications. Pulse flour (2-3 mg)

was accurately weighed into a Tzero Alodined pan (TA Instruments, New Castle, DE, U.S.A.), which was thoroughly mixed with distilled water (~3-4 volumes, v/w) prior to sealing. After being equilibrated at ambient temperature for more than 2 h, the sample was heated from 10°C to 110°C at a rate of 10°C/min. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures and enthalpy change (ΔH) of the observed endothermic peak in the DSC thermogram were analyzed using Universal Analysis 2000 Software (TA Instruments, New Castle, DE, U.S.A.).

3.2.6.2 Pasting properties

Pasting properties of the raw, soaked and germinated pulse flours were measured using a Rapid Visco-Analyzer (RVA Super 3, Newport Scientific, Sydney, Australia) in accordance with the method of Ai et al. (2017). After suspending the pulse flour in distilled water to reach a dry solids content of 10.6% (w/w), the flour slurry (28.0 g in total) was loaded to the RVA instrument and analyzed using Standard Method 2 in the Thermocline Software provided by the company. The pulse flour from each batch of processing was analyzed at least in duplicate to obtain two well-overlapped pasting curves, the average of six measurements was used to draw the RVA profiles.

3.2.6.3 Protein solubility

Protein solubility of the raw, soaked and germinated flours was determined using a method of Liu et al. (2010) with minor modifications. Deionized water (17.8 g) was added to 0.2 g of pulse flour to prepare a suspension. The pH of the suspension was adjusted to 7.0 with 0.1 M HCl or NaOH, followed by stirring for 24 h to facilitate the dispersion of protein. The pH of the resultant suspension was readjusted back to 7.0, and its total weight was elevated to 20.0 g (*i.e.*, 1% w/w suspension) by adding more deionized water. The mixture was left to stand for 10 min for the precipitation of undissolved particles. An aliquot (10.0 mL) from the top of the mixture was transferred into a 15-mL centrifuge tube and centrifuged at 4,430 *g* for 10 min at room temperature. After the centrifugation, 5.0 g of the supernatant was transferred into a micro-Kjeldahl digestion flask for nitrogen and protein (N% × 6.25) determination. Protein solubility was determined from the ratio of protein within the supernatant relative to that in the flour, multiplied by 100%.

3.2.6.4 Water-holding and oil-binding capacity

Water-holding capacity (WHC) of the raw, soaked and germinated pulse flours was measured in accordance with AACC Method 56-20.01 (AACC, 2000). Pulse flour (5.0 g) was suspended in 25.0 mL of distilled water in a 50-mL centrifuge tube and the tube was kept vertically for a total period of 20 min, with vortexing for 10 s at time intervals of 5 min. The suspension was then subjected to centrifugation at 1,000 *g* for 15 min at room temperature. After the centrifugation, the supernatant was carefully decanted, and the weight of the sediment was recorded. WHC was calculated by dividing the weight of retained water over the dry weight of flour.

Oil-binding capacity (OBC) of the raw, soaked and germinated pulse flours was determined using the method of Nidhina and Muthukumar (2015) with some modifications. Pulse flour (1.0 g) was mixed with 10.0 g canola oil in a 50-mL centrifuge tube and the tube was kept vertically for a total period of 30 min, with vortexing for 10 s at time intervals of 5 min. The flour-oil mixture was then centrifuged at 1,000 *g* for 15 min at room temperature. After the centrifugation, the supernatant was carefully discarded and the weight of the pellet was recorded. OBC was calculated by dividing the weight of bound oil over the dry weight of flour.

3.2.6.5 Emulsifying properties

Emulsion activity (EA) and stability (ES) of the raw, soaked and germinated pulse flours were determined according to the method of Yasumatsu et al. (1972). For EA, pulse flour (4.25 g) was suspended in 75.0 mL of distilled water in a beaker, followed by pH adjustment to 7.0 using 0.1 M HCl or NaOH and stirring overnight. On the following day, pH of the suspension was readjusted back to 7.0 and canola oil (75.0 mL) was then added to this aqueous suspension. The mixture was homogenized at level-4 speed for 1 min using an Omni Macro Homogenizer (Omni International, Marietta, GA, U.S.A.). A portion (60 mL) of the prepared emulsion was then divided evenly into two 50-mL centrifuge tubes (*i.e.*, 30 mL each), followed by centrifugation at 1,300 *g* for 5 min. The heights of the emulsified layer and the entire emulsion in the tube were measured. EA was calculated using the equation as follows:

$$\text{EA}\ (\%) = \frac{\text{Height of emulsified layer}}{\text{Height of entire layer in tube}} \times 100 \qquad \text{[Eq. 3.1]}$$

The remaining portion of the emulsion in the beaker was heated at 80°C in a water bath for 30 min and then cooled to room temperature in another water bath for 15 min. The obtained emulsion was then transferred into two 50-mL centrifuge tubes (30 mL each), followed by centrifugation at 1,300 *g* for 5 min. The heights of emulsified layer and the entire emulsion were recorded, and ES was calculated below:

$$\text{ES}\ (\%) = \frac{\text{Height of emulsified layer}}{\text{Height of entire layer in tube}} \times 100 \qquad \text{[Eq. 3.2]}$$

3.2.6.6 Foaming properties

Foaming capacity (FC) and stability (FS) of the raw, soaked and germinated pulse flours were determined using a previous method (Hall, 1996). Flour suspension was prepared by mixing 0.5 g flour with 49.5 g distilled water (*i.e.*, 1%, w/w), followed by pH adjustment to 7.0 using 0.1 M HCl or NaOH prior to overnight stirring to aid protein dispersion. On the next day, the pH of the suspension was readjusted back to 7.0, and 15.0 mL of the prepared suspension was transferred into a 400-mL beaker for homogenization using Omni Macro Homogenizer (Omni International, Marietta, GA, U.S.A.) equipped with a saw-tooth probe (Part Number: 2989) at level-3 speed for 1 min and level-4 speed for 4 min. To achieve maximum foam formation, the fixture blade was placed slightly below the air-water interface. The foam generated in the beaker was immediately poured into a 50-mL graduated cylinder and the initial foam volume was recorded as V_1. After 30 min, the volume of the remaining foam was recorded as V_2. FC and FS were calculated using the following equations:

$$\text{FC}\ (\%) = \frac{\text{V1}}{\text{15 mL initial volume}} \times 100 \qquad \text{[Eq. 3.3]}$$

$$\text{FS}\ (\%) = \frac{\text{V1} - \text{V2}}{\text{V1}} \times 100 \qquad \text{[Eq. 3.4]}$$

3.2.7 *In vitro* digestibility of starch and protein of pulse flours

3.2.7.1 *In vitro* starch digestibility of uncooked and cooked flours

Starch digestibility of the raw, soaked and germinated pulse flours, with and without cooking, was determined using an *in vitro* method of Englyst et al. (1992) with minor modifications (Ai et al., 2013). Flour sample containing ~600 mg starch (db) was suspended in 15.0 mL of distilled water. The cooking of flour was completed by incubating the centrifuge tube in a boiling water bath for 10 min with vigorous stirring. Sodium acetate buffer (5.0 mL, pH 5.2, 0.4 M, containing 0.08% sodium azide) and guar gum (50.0 mg) were added to the uncooked and cooked samples. After being equilibrated at 37°C in a water bath with shaking (160 rpm) for 15 min, a multi-enzyme solution (5.0 mL) of porcine pancreatin extract and amyloglucosidase was added to hydrolyze the starch. Amounts of glucose released at time intervals of 20 min and 120 min were quantitated using Megazyme D-Glucose Assay Kit. Contents of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were calculated according to the method described by Englyst et al. (1992).

3.2.7.2 *In vitro* protein digestibility and quality

In vitro protein digestibility (IVPD) of the raw, soaked and germinated pulse flours was determined by measuring the pH drop of the flour suspension after being digested by a multi-enzyme solution (Nosworthy et al., 2017). In brief, 31.0 mg chymotrypsin, 16.0 mg trypsin, and 13.0 mg protease were mixed in 10.0 mL deionized water and maintained at 37°C prior to use. The pH of the enzyme solution was adjusted to 8.0 ± 0.05 by adding 0.1 M NaOH or HCl solution dropwise at 37°C. This solution was freshly prepared and used on the same day. Flour containing ~52.5 mg protein was mixed with 10.0 mL deionized water and stirred at 37°C for 1 h. The pH of the flour suspension was adjusted to 8.0 ± 0.05 by adding 0.1 M NaOH or HCl dropwise, and 1.0 mL of the prepared multi-enzyme cocktail was then added to the flour suspension to hydrolyze the protein. The pH of the flour suspension was recorded every 30 s for a total period of 10 min, and IVPD was calculated according to the following equation:

$$\text{IVPD}\ (\%) = 65.66 + 18.10 \times \Delta\text{pH10min} \qquad \text{[Eq. 3.5]}$$

where $\Delta pH_{10\ min}$ refers to the change in pH from the initial value of 8.0 to the value at the end of

10-min hydrolysis.

Amino acid compositions (including all the 18 amino acids) of the raw, soaked and germinated (72-h) pulse flours were determined at POS Bio-Sciences Corp. (Saskatoon, SK, Canada) using a high-performance liquid chromatography (HPLC) and a Pico-tag™ amino acid analysis system (Waters Corporation, Milford, MA, U.S.A.) as described previously by Bai et al. (2018). The essential amino acid score (AAS) was determined as the ratio of essential amino acid content of the target protein to that of the reference protein set by the FAO/WHO using the amino acid requirement for children 2 to 5 years of age (FAO, 1991). The lowest AAS represented the limiting essential amino acid. The aforementioned IVPD of pulse flour was converted to *in vitro* protein digestibility corrected amino acid score (IV-PDCAAS) using the following equation:

$$\text{IV} - \text{PDCAAS}\ (\%) = \text{IVPD}\ (\%) \times \text{limiting amino acid score} \qquad [\text{Eq. } 3.6]$$

3.2.8 Statistical analysis

The soaking and 24-h, 48-h and 72-h germination of yellow pea and faba bean seeds were performed in triplicate (*i.e.*, three independent batches for each treatment). For each batch of the soaked and germinated pulse flours, the analyses were performed in duplicate; the corresponding raw flour samples were also analyzed in six replicates to obtain the same number of measurements for valid statistical analysis. Statistical differences among the values of the raw, soaked and germinated pulse flours of the same type were evaluated using one-way ANOVA, Tukey's multiple comparison test at a significance level of 0.05. Evaluation of the correlations between the different variables was performed using the Pearson correlation test. The statistical analysis was carried out using IBM SPSS Statistics (Version 24.0, IBM Corporation, Armonk, NY, U.S.A.).

3.3 Results and Discussion

3.3.1 Germination percentages and radicle lengths of pulse seeds, and α-amylase activity of pulse flours

The germination percentages of yellow pea ranged from 96.6% to 99.5%, whereas those of faba bean ranged from 97.7% to 98.7% during the 72-h germination (Table 3.1). The large germination percentages indicated the high viability of the used seeds. The radicle length of yellow

Table 3.1 Germination percentage and radicle length of yellow pea and faba bean seeds, and α-amylase activity of the flours[a]

	Germination percentage[b] (%)	Radicle length[c] (mm)	α-Amylase activity (U/g)[d]
Yellow pea			
Raw	-	-	0.5 ± 0.0 a
Soaked	-	-	0.5 ± 0.1 a
24-h germinated	96.6 ± 2.5 a	13.1 ± 2.0 a	0.6 ± 0.0 a
48-h germinated	98.4 ± 2.4 a	30.1 ± 3.3 b	1.4 ± 0.2 b
72-h germinated	99.5 ± 0.3 a	50.7 ± 8.0 c	4.6 ± 0.2 c
Faba bean			
Raw	-	-	0.4 ± 0.1 a
Soaked	-	-	0.4 ± 0.0 a
24-h germinated	97.7 ± 0.4 a	7.2 ± 1.0 a	0.5 ± 0.0 a
48-h germinated	98.7 ± 0.3 b	18.4 ± 1.7 b	0.8 ± 0.1 b
72-h germinated	98.3 ± 0.3 ab	27.3 ± 2.8 c	1.1 ± 0.0 c

[a] Values are presented as average ± standard deviation (n = 3 for germination percentage and radical length measurements; n = 6 for α-amylase activity measurement, on a dry basis); in the same column of one pulse type, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] Calculated by dividing the number of germinated seeds over the total number of seeds of one batch.

[c] Determined for 10 randomly selected seeds in one batch; measurement carried out using a Vernier caliper.

[d] Amylase SD unit as defined in Megazyme α-Amylase SD Assay Kit. 'U' is the amylase SD unit as defined in the kit.

pea increased from 13.1 mm of 24 h to 50.7 mm of 72 h, which was noticeably greater than that of faba bean at the same germination period (7.2-27.3 mm). The data suggested the greater germination rate of yellow pea than faba bean, which is consistent with others (Hsu et al., 1980; Rowland and Gusta, 1977). The α-amylase activity of raw yellow pea and faba bean was quantitated as 0.5 U/g and 0.4 U/g, respectively. These values remained unchanged after soaking but increased progressively during germination, and reached 4.6 U/g and 1.1 U/g for yellow pea and faba bean at 72-h germination. The faster increase in the α-amylase activity of germinated yellow pea is in accordance with the emergence of longer radicles.

3.3.2 Morphology of seed cotyledons

Morphology of the cross sections of seed cotyledons of raw, soaked and germinated (72-h) yellow pea and faba bean is shown in Figure 3.1 and 3.2, respectively. The SEM images of raw yellow pea and faba bean revealed that the starch granules were compactly embedded in the protein and fibre matrix (YP-R and FB-R). The entrapment of starch granules in such a matrix was more obvious in raw faba bean cotyledon as no starch granules were exposed to the surface, which could be because of the higher protein and fibre contents of faba bean (Table 3.2). After soaking, the structure of the protein and fibre matrix became loose and some starch granules were liberated from the matrix (YP-S and FB-S). This phenomenon was more obvious for faba bean as some granules became visible in the cotyledon cross section. During soaking, the pulse seeds imbibed water (reached ~57% moisture content eventually) and swelled, physically causing disruption of the matrix structure (Sefadedeh et al., 1978). After drying at 55°C, the dense structure of the matrix could not be restored. Germination for 72 h further loosened the matrix of both yellow pea and faba bean and thus more starch granules were released from the structure (YP-G and FB-G). The additional rupture of the matrix structure might be attributed to the breakdown of protein and fibre (including cellulose, hemicellulose and pectin) by their respective hydrolytic enzymes (Nnanna and Phillips, 1988; Uriyo, 2001). However, the hydrolysis of granular starch did not seem to take place at 72 h because there was no obvious change in the surface structure of starch granules.

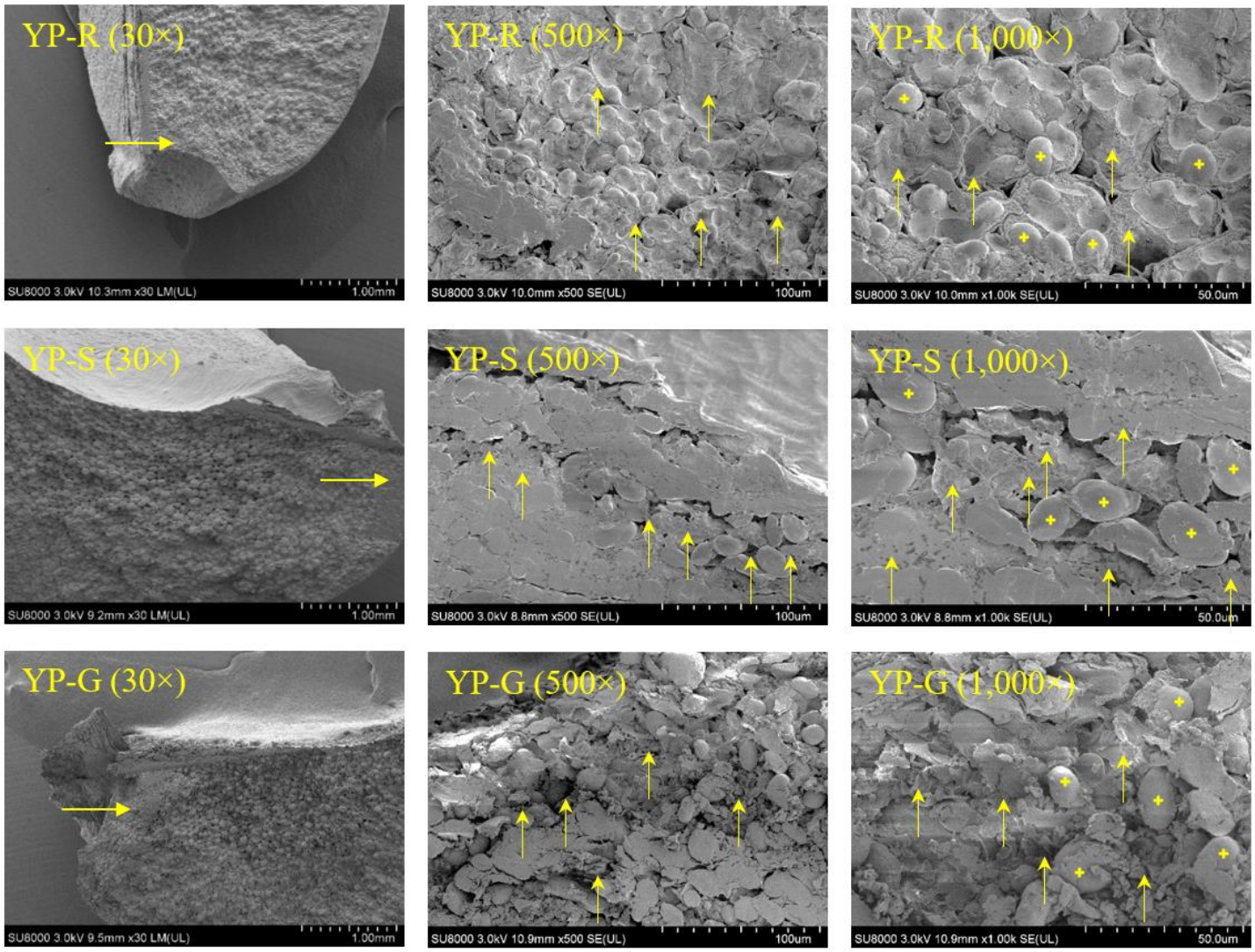

Figure 3.1 Scanning electron microscopy (SEM) images of the cross section of cut cotyledons of yellow pea (YP). R: raw; S: soaked; and G: 72-h germinated. Magnification at which the image was captured is given in parentheses. Right arrows indicate embryo region; plus symbols indicate starch granules; and up arrows indicate protein and fibre matrix.

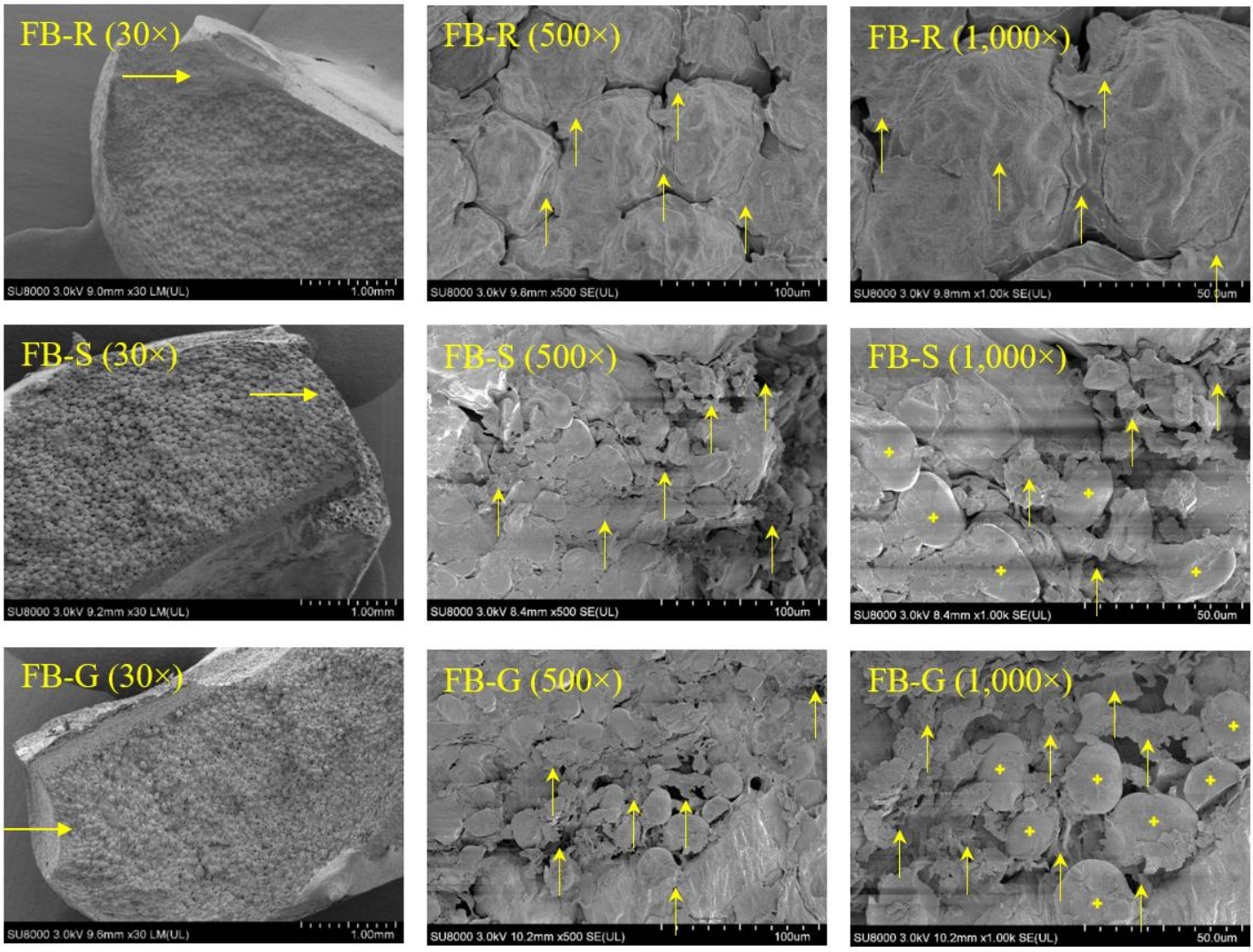

Figure 3.2 Scanning electron microscopy (SEM) images of the cross section of cut cotyledons of faba bean (FB). R: raw; S: soaked; and G: 72-h germinated. Magnification at which the image was captured is given in parentheses. Right arrows indicate embryo region; plus symbols indicate starch granules; and up arrows indicate protein and fibre matrix.

3.3.3 Chemical compositions of pulse flours

Raw yellow pea flour consisted of less ash, protein and total dietary fibre than raw faba bean flour, with the largest difference being found in protein content (22.7% versus 27.5%; Table 3.2). The contents of crude fat, starch, damaged starch and amylose of raw yellow pea flour were slightly higher than those of raw faba bean flour, with the largest difference being found in starch content (46.0% versus 43.3%). Overall, the germination for up to 72 h did not appear to considerably alter the chemical compositions of the flours from both pulses, despite the occurrence of germination and the enhancement of α-amylase activity in the seeds as shown in Table 3.1. The absence of changes in starch, damaged-starch and amylose contents after germination is in good accordance with the observation of no enzymatic hydrolysis of starch granules as shown in SEM images (Figure 3.1 and 3.2). The lack of starch hydrolysis by endogenous enzymes could be explained by the short period of germination and the relatively high resistance of raw pulse starch to enzymatic hydrolysis (Li et al., 2019).

3.3.4 Functional properties of pulse flours

3.3.4.1 Thermal properties

The onset (T_o = 66.4°C), peak (T_p = 72.4°C) and conclusion (T_c = 85.5°C) temperatures of raw yellow pea flour were very close to those of raw faba bean flour (T_o = 66.2°C, T_p = 72.9°C, and T_c = 85.2°C, respectively), while the enthalpy change of the former (ΔH = 6.5 J/g) was substantially higher than that of the latter (5.0 J/g; Table 3.3). The majority of this thermal transition peak corresponded to starch gelatinization. The T_c of raw yellow pea and faba bean flours (85.2-85.5°C) were markedly higher than those of corresponding isolated pulse starches (72.1-77.7°C) (Li et al., 2019). The extension of this endothermic peak of pulse flours to a higher temperature range could be attributed to the occurrence of protein denaturation during heating, which took place at a relatively higher temperature and partly overlapped with the starch gelatinization process (Chung et al., 2008). In general, soaking did not lead to considerable changes in the thermal properties of the pulse flours. Germination did not display a noticeable effect on the T_o of both pulse flours and T_p of yellow pea flour, but reduced T_p of faba bean flour (from 72.9°C to 70.9°C) and T_c of both pulse flours (from 85.5°C to 83.4°C and from 85.2°C to 81.9°C after 72-h germination, respectively). The 72-h germination lowered the ΔH of yellow pea flour from 6.5 J/g to 4.9 J/g and that of faba bean from 5.0 J/g to 4.1 J/g. These changes in the

Table 3.2 Chemical compositions of raw, soaked and germinated yellow pea and faba bean flours[a]

Flour	Ash (%)	Crude fat (%)	Protein (%)	Starch (%)	Damaged starch (%)	Amylose (%)	Total dietary fibre (%)
Yellow pea							
Raw	2.7 ± 0.0 c	1.2 ± 0.0 a	22.7 ± 0.1 a	46.0 ± 1.0 a	1.5 ± 0.1 a	19.1 ± 0.0 a	16.5 ± 0.3 a
Soaked	2.4 ± 0.0 a	1.8 ± 0.1 a	23.6 ± 0.2 ab	47.3 ± 0.3 a	1.3 ± 0.1 a	22.0 ± 0.9 b	14.4 ± 0.6 a
24-h germinated	2.4 ± 0.0 ab	1.5 ± 0.3 a	23.0 ± 1.0 ab	47.5 ± 0.9 a	1.2 ± 0.0 a	21.8 ± 0.5 b	15.3 ± 0.9 a
48-h germinated	2.5 ± 0.0 ab	1.7 ± 0.5 a	23.7 ± 0.3 ab	46.9 ± 0.7 a	1.3 ± 0.0 a	19.7 ± 0.6 ac	14.9 ± 1.0 a
72-h germinated	2.5 ± 0.0 b	1.7 ± 0.5 a	24.1 ± 0.4 b	45.4 ± 1.3 a	1.3 ± 0.1 a	21.1 ± 0.5 bc	14.8 ± 1.0 a
Faba bean							
Raw	3.5 ± 0.0 b	0.9 ± 0.3 a	27.5 ± 0.3 a	43.3 ± 0.5 a	0.9 ± 0.1 b	18.6 ± 0.0 ab	17.4 ± 0.5 a
Soaked	3.2 ± 0.1 a	1.3 ± 0.1 ab	28.4 ± 1.2 a	44.0 ± 1.4 a	0.9 ± 0.0 b	17.1 ± 0.8 a	18.3 ± 1.1 a
24-h germinated	3.2 ± 0.1 a	1.3 ± 0.1 ab	27.4 ± 0.6 a	44.8 ± 1.1 a	0.7 ± 0.1 a	19.8 ± 0.9 b	16.9 ± 0.7 a
48-h germinated	3.3 ± 0.1 a	1.3 ± 0.2 ab	27.6 ± 0.9 a	42.9 ± 0.6 a	0.7 ± 0.0 a	17.6 ± 0.3 a	18.8 ± 0.7 a
72-h germinated	3.3 ± 0.0 a	1.4 ± 0.0 b	28.0 ± 0.8 a	42.9 ± 0.6 a	0.7 ± 0.0 a	19.4 ± 0.2 b	18.6 ± 2.2 a

[a] Values are presented as average ± standard deviation (n = 6; duplicate measurements of three batches) on a dry basis; in the same column of one pulse type, the numbers with the same letter are not significantly different at $p < 0.05$.

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Table 3.3 Thermal properties of raw, soaked and germinated yellow pea and faba bean flours[a,b]

Flour	Thermal transition peak[c]			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Yellow pea				
Raw	66.4 ± 0.1 a	72.4 ± 0.9 a	85.5 ± 0.2 b	6.5 ± 0.4 c
Soaked	66.8 ± 0.3 ab	71.2 ± 0.5 a	84.4 ± 0.4 ab	6.5 ± 0.2 c
24-h germinated	66.7 ± 0.2 ab	71.2 ± 0.3 a	83.8 ± 0.7 ab	5.9 ± 0.2 bc
48-h germinated	67.1 ± 0.1 bc	71.9 ± 1.0 a	84.0 ± 0.5 ab	5.4 ± 0.4 ab
72-h germinated	67.5 ± 0.2 c	72.1 ± 0.6 a	83.4 ± 1.1 a	4.9 ± 0.1 a
Faba bean				
Raw	66.2 ± 0.7 a	72.9 ± 0.3 b	85.2 ± 0.3 b	5.0 ± 0.2 b
Soaked	65.9 ± 0.1 a	70.8 ± 0.3 a	84.0 ± 0.6 ab	5.0 ± 0.2 b
24-h germinated	65.9 ± 0.2 a	71.1 ± 0.2 a	82.3 ± 1.4 ab	4.5 ± 0.2 ab
48-h germinated	66.1 ± 0.2 a	71.2 ± 0.7 a	82.6 ± 1.7 ab	4.4 ± 0.3 a
72-h germinated	66.4 ± 0.1 a	70.9 ± 0.5 a	81.9 ± 0.9 a	4.1 ± 0.1 a

[a] Values are presented as average ± standard deviation (n = 6; duplicate measurements of three batches); in the same column of one pulse type, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] Measured using a differential scanning calorimeter.

[c] T_o = onset temperature; T_p = peak temperature; T_c = conclusion temperature; and ΔH = enthalpy change.

thermal properties of pulse flours could be primarily ascribed to the modification of protein by the seed germination. The germination for up to 72 h exhibited negligible effects on the structures and compositions of starch in both yellow pea and faba bean flours as revealed in Table 3.2 and Figure 3.1 and 3.2. By contrast, the germination obviously disrupted the protein and fibre matrix (Figure 3.1 and 3.2), probably due to enzymatic breakdown. These changes rendered pulse protein more susceptible to thermal denaturation, the process of which occurred at a lower temperature range and required less energy. Further research is required to examine the changes in protein structure at a molecular level.

3.3.4.2 Pasting properties

When pulse flour is heated in excess water with stirring, the mixture starts developing viscosity beyond the gelatinization temperature of starch. After being gelatinized, starch granules swell and some starch molecules (*e.g.,* amylose and small amylopectin molecules) leach out from the swollen granules, both contributing to a rise in viscosity (Debet and Gidley, 2006). Pasting temperatures and peak and breakdown viscosities of raw yellow pea and faba bean flours were comparable (Figure 3.3 and Table 3.4). After the cooked flour pastes were cooled, faba bean flour displayed a substantially larger final viscosity (1,085.2 cP) than yellow pea (845.1 cP). It is interesting to observe that soaking showed an increasing effect on the pasting viscosities of the pulse flours, particularly for yellow pea. Soaking was demonstrated to loosen the protein and fibre matrix of yellow pea and faba bean cotyledons (Figure 3.1 and 3.2). Protein and fibre, components known to restrict the swelling of starch granules in pulse flours to reduce the viscosity (Brummer et al., 2015; Chung et al., 2008), could be more easily separated from starch granules during milling. Consequently, starch granules in soaked pulse flours were able to swell to a greater extent during pasting to provide a higher viscosity when compared with the raw counterparts. After germination for 24 h, the viscosities of both pulse flours were further enhanced, which could be attributed to that the abovementioned matrix structure was destroyed progressively. At 48 h and 72 h, the viscosities of both pulse flours, however, were diminished, which could be due to their increased activity of α-amylase (Table 3.1) and possibly other enzymes, such as β-amylase and α-glucosidase (Stanley et al., 2011). The endogenous amylase(s) in the flours could efficiently degrade starch molecules to promptly lower their pasting viscosities in the early stage of heating

Table 3.4 Pasting properties of raw, soaked and germinated yellow pea and faba bean flours[a,b]

Flour	Pasting temperature (°C)	Peak viscosity (cP)	Breakdown viscosity (cP)	Setback viscosity (cP)	Final viscosity (cP)
Yellow pea					
Raw	79.0 ± 0.8 b	552.8 ± 35.5 a	1.6 ± 1.0 a	293.9 ± 4.8 b	845.1 ± 39.0 b
Soaked	77.6 ± 0.8 ab	798.7 ± 30.2 bc	7.0 ± 8.7 a	408.7 ± 6.0 c	1200.3 ± 18.4 c
24-h germinated	76.4 ± 0.1 a	910.7 ± 48.1 c	11.3 ± 10.3 a	293.7 ± 28.2 b	1193.0 ± 72.7 c
48-h germinated	76.5 ± 0.3 a	754.7 ± 68.0 b	5.7 ± 6.3 a	170.2 ± 4.4 a	919.2 ± 57.6 b
72-h germinated	76.6 ± 0.5 a	603.2 ± 28.4 a	24.8 ± 24.0 a	130.3 ± 32.7 a	708.7 ± 36.1 a
Faba bean					
Raw	80.8 ± 0.2 c	564.7 ± 39.6 b	0.7 ± 0.6 a	521.2 ± 9.4 cd	1085.2 ± 44.2 c
Soaked	80.5 ± 0.9 c	578.0 ± 31.8 b	0.7 ± 0.3 a	541.5 ± 40.9 d	1118.8 ± 72.9 c
24-h germinated	78.6 ± 0.3 b	709.2 ± 6.8 c	7.3 ± 4.0 a	476.7 ± 5.1 c	1178.5 ± 4.0 c
48-h germinated	77.1 ± 0.2 a	508.3 ± 11.4 b	43.3 ± 13.5 b	168.0 ± 7.0 b	633.0 ± 30.4 b
72-h germinated	77.1 ± 0.2 a	387.3 ± 43.5 a	87.0 ± 11.7 c	105.5 ± 9.1 a	405.8 ± 42.2 a

[a] Values are presented as average ± standard deviation (n = 6; duplicate measurements of three batches).

[b] Pasting properties of raw, soaked and germinated yellow pea and faba bean flours determined using a Rapid Visco-Analyzer with 10.6% (w/w, dry solids) flour suspension of 28.0 g total weight.

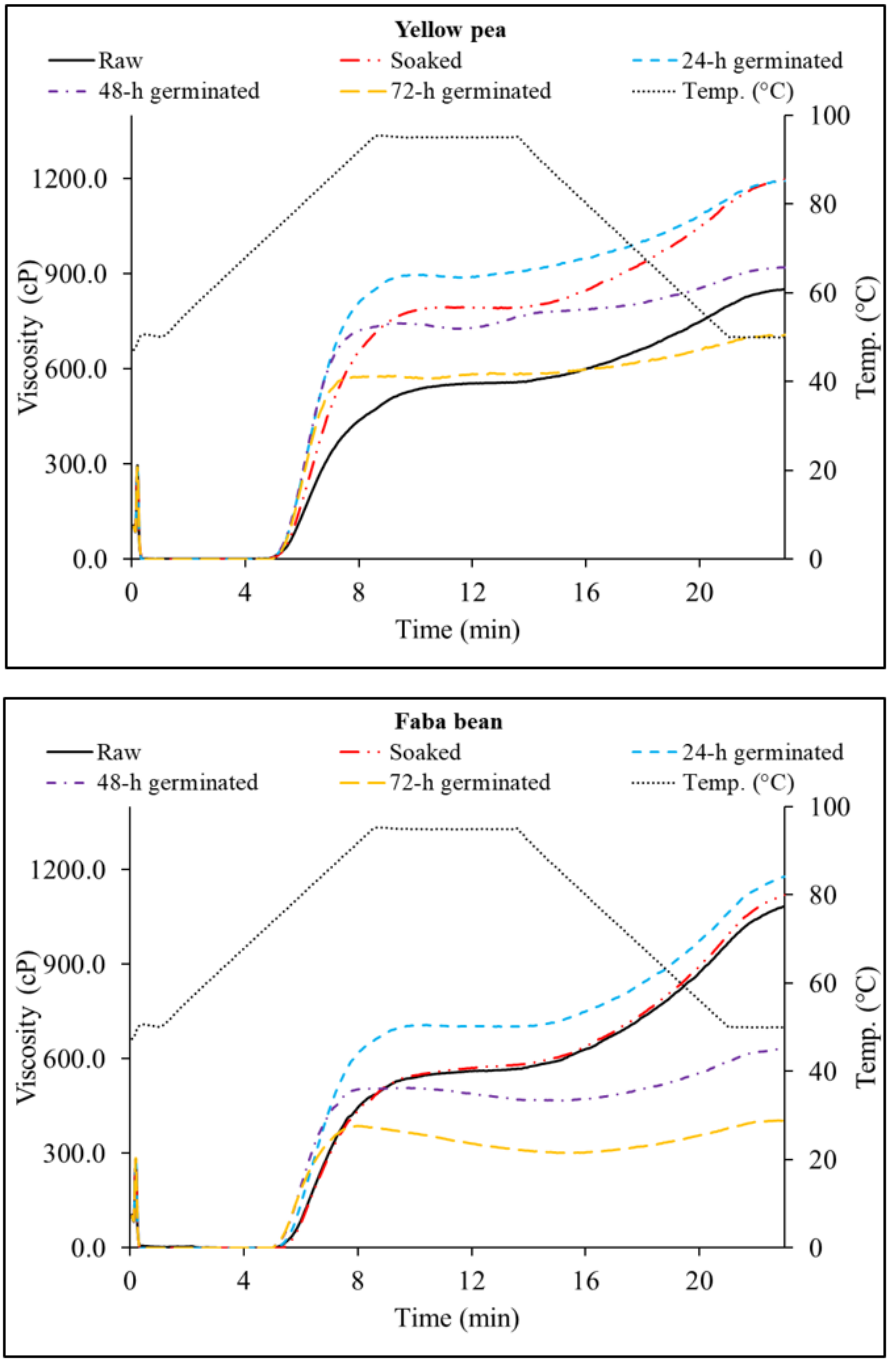

Figure 3.3 Pasting profiles of raw, soaked and germinated yellow pea and faba bean flours measured using a Rapid Visco-Analyzer with 10.6% (w/w, dry solids) flour suspension of 28.0 g total weight.

in RVA (i.e., before they were inactivated by heat at a temperature above 60°C) (Sun et al., 2015; Tian et al., 2016).

3.3.4.3 Other functional properties

Protein solubility (PS) of raw yellow pea and faba bean flours was 92.3% and 78.1%, respectively (Table 3.5). Soaking and germination, in general, enhanced the PS of both pulse flours to a small degree, which could be attributed to the aforementioned breakdown of protein by proteolytic enzymes. Water-holding capacity (WHC) values of raw yellow pea and faba bean flours were comparable, 1.2 g/g and 1.1 g/g, respectively (Table 3.5). Soaking and germination slightly improved WHC, which could be explained by the weakened association between starch and protein/fibre as discussed above. This physical change could expose more hydrophilic moieties in those constituents to bind with water. Oil-binding capacity (OBC) values of the two pulse flours were very close (1.5 g/g), and soaking and germination did not appear to effectively influence their OBC.

Emulsion activity (EA) and stability (ES) of raw yellow pea flour (44.0% and 52.0%, respectively) were slightly lower than those of raw faba bean (47.7% and 53.8%, respectively; Table 3.5). Soaking did not lead to noticeable changes in the EA and ES of both pulse flours. Germination enhanced the EA and ES of the pulse flours to some extent, which could be attributed to partial unfolding and dissociation of protein for promoted surface activity, especially when it was related to surface hydrophobicity responsible for enhanced emulsion activity (Chang et al., 2015; Ghavidel and Prakash, 2006). Foaming capacity (FC) of raw yellow pea flour (114.8%) was stronger than that of raw faba bean flour (107.8%), which could be related to the greater solubility of protein in the former. Their foam stability (FS) was comparable. Soaking did not appear to impact the FC and FS of the two pulse flours. Germination elevated the FC and enhanced the stability of the generated foam (smaller FS meant less decrease in foam volume after 30-min storage according to Eq. 3.4), which could be ascribed to the alteration in protein conformation as described above. EA and ES of the ten pulse flours listed in Table 3.5 were strongly correlated with their FC ($r = 0.767$, $p < 0.01$ and $r = 0.788$, $p < 0.01$, respectively) and FS ($r = -0.782$, $p < 0.01$ and $r = -0.689$, $p < 0.05$, respectively), suggesting the relevance of emulsifying and foaming properties to the changes in the structures and physicochemical properties of protein in the pulse flours.

Table 3.5 Functional properties of raw, soaked and germinated yellow pea and faba bean flours[a,b]

Flour	PS (%)	WHC (g/g)	OBC (g/g)	EA (%)	ES (%)	FC (%)	FS (%)
Yellow pea							
Raw	92.3 ± 0.4 a	1.2 ± 0.0 a	1.5 ± 0.1 a	44.0 ± 0.4 a	52.0 ± 0.3 a	114.8 ± 5.5 a	21.6 ± 0.4 e
Soaked	93.8 ± 0.6 bc	1.3 ± 0.0 ab	1.3 ± 0.1 a	45.6 ± 0.8 a	54.3 ± 0.8 b	114.5 ± 1.9 a	19.4 ± 0.1 d
24-h germinated	93.0 ± 0.5 ab	1.3 ± 0.0 cd	1.4 ± 0.2 a	47.5 ± 1.0 b	60.2 ± 1.1 c	123.9 ± 6.7 a	18.7 ± 0.2 c
48-h germinated	93.8 ± 0.5 bc	1.3 ± 0.0 bc	1.3 ± 0.1 a	50.3 ± 0.1 c	60.8 ± 0.4 c	137.8 ± 6.3 b	18.1 ± 0.0 b
72-h germinated	94.9 ± 0.4 c	1.4 ± 0.0 d	1.4 ± 0.1 a	50.7 ± 0.3 c	61.6 ± 0.5 c	150.6 ± 2.6 b	17.1 ± 0.2 a
Faba bean							
Raw	78.1 ± 0.5 a	1.1 ± 0.0 a	1.5 ± 0.0 a	47.7 ± 0.9 ab	53.8 ± 0.3 a	107.8 ± 2.0 a	21.1 ± 1.0 c
Soaked	78.3 ± 0.5 a	1.2 ± 0.1 b	1.5 ± 0.0 a	47.2 ± 1.0 a	54.2 ± 0.7 a	110.1 ± 0.4 a	20.4 ± 0.4 c
24-h germinated	78.2 ± 0.6 a	1.3 ± 0.3 b	1.5 ± 0.0 a	49.8 ± 0.9 bc	55.7 ± 0.7 b	122.8 ± 4.2 b	19.4 ± 1.0 bc
48-h germinated	78.9 ± 0.3 ab	1.4 ± 0.0 c	1.5 ± 0.0 a	49.4 ± 0.9 bc	55.9 ± 0.3 b	132.7 ± 4.4 c	17.5 ± 0.3 ab
72-h germinated	79.7 ± 0.5 b	1.4 ± 0.0 c	1.5 ± 0.0 a	50.2 ± 0.1 c	56.3 ± 0.1 b	137.8 ± 1.9 c	16.4 ± 0.6 a

[a] Values are presented as average ± standard deviation (n = 6; duplicate measurements of three batches); in the same column of one pulse type, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] PS: protein solubility; WHC: water-holding capacity; OBC: oil-binding capacity; EA: emulsion activity; ES: emulsion stability; FC: foaming capacity; and FS: foam stability.

3.3.5 *In vitro* digestibility of starch and protein of pulse flours

3.3.5.1 *In vitro* starch digestibility of uncooked and cooked pulse flours

Starch can be hydrolyzed by amylolytic enzymes into glucose after ingestion, which is then absorbed to supply energy in human body. Therefore, starch digestibility is an important nutritional attribute of starchy food ingredients and final products. Uncooked, raw yellow pea flour contained markedly more rapidly digestible starch (RDS = 9.7%) and slowly digestible starch (SDS = 23.2%) but less resistant starch (RS = 14.2%) compared with the corresponding faba bean flour (RDS = 4.7%, SDS = 12.4%, and RS = 25.7%, respectively; Table 3.6), suggesting the greater enzymatic resistance of starch in the latter. It has been demonstrated in previous research that the densely packed protein and fibre matrix in pulse cotyledons and milled flours can protect starch from enzymatic hydrolysis, thus substantially reducing its digestibility (Dhital et al., 2016; Wursch et al., 1986). Therefore, the considerably higher enzymatic resistance of starch in the uncooked, raw faba bean flour could be because of the stronger association between starch and protein/fibre as exhibited in Figure 3.1 and 3.2. Soaking decreased the RS contents of both uncooked, raw pulse flours to some degree. Germination gradually increased their RDS contents but lowered the RS contents, suggesting that the treatment enhanced the starch digestibility. The results could be explained by the following three changes: (1) germination loosened the protein and fibre matrix (Figure 3.1 and 3.2), making the starch granules more easily accessible to the hydrolyzing enzymes; (2) germination promoted the activity of endogenous α-amylase (Table 3.1; possibly other amylolytic enzymes as well) and the synthesized amylase(s) could also hydrolyze starch granules during the incubation at 37°C in the Englyst assay; and (3) germination removed some of the amylase inhibitors (Savelkoul et al., 1992).

After being cooked, the RDS contents of yellow pea and faba bean flours were substantially increased, while their SDS and RS contents were markedly decreased. The cooking process gelatinized starch granules and the starch molecules became readily hydrolyzable by the added enzymes (Li et al., 2019). Germination showed an increasing effect on the starch digestibility of cooked yellow pea and faba bean flours, which could be attributed to the three factors as described above. However, it is critical to point out that the hydrolysis by the endogenous amylase(s) probably occurred during the early cooking period (*i.e.*, flour suspension temperature ≤ 60°C; Fig. 3.3 and Table 3.4) in the boiling water bath instead of during the incubation step in the Englyst

assay, because cooking effectively inactivated endogenous enzymes in the pulse flours (Tian et al., 2016).

3.3.5.2 *In vitro* protein digestibility and quality

Impacts of soaking and germination on the *in vitro* digestibility and quality of protein in yellow pea and faba bean were investigated in the current study. Soaking did not noticeably influence the digestibility of protein (Table 3.6). Germination gradually enhanced the digestibility of protein, which could be attributed to the weaker association between protein and starch (Figure 3.1 and 3.2), the existence of endogenous proteases (Nnanna and Phillips, 1988), and the removal of protease inhibitors as a result of this treatment (Savelkoul et al., 1992). Generally, the improvement in protein digestibility by germination was of a smaller degree when compared with the increases in starch digestibility (Table 3.6).

With respect to amino acid compositions, 72-h germination exhibited a more noticeable effect than soaking overall (Table 3.7). In addition, the effect appeared to be dependent upon pulse type: for example, the largest increase was found to be in phenylalanine (from 0.77 to 1.07 g/100 g flour) and aspartic acid (from 2.62 to 3.45 g/100 g flour) for yellow pea and faba bean, respectively; and the most noticeable decrease was found to be in arginine (from 1.85 to 1.55 g/100 g flour) and lysine (from 1.66 to 1.34 g/100 g flour), respectively. The influence of 72-h germination on the amino acid compositions could result from the metabolism of storage protein to support the biosynthesis of enzymes, energy supply, and other biological activities during the germination of pulse seeds (Hsu et al., 1980).

In regard to the nutritional quality of amino acids, both raw yellow pea and faba bean flours had tryptophan as the primary limiting amino acid (Table 3.8), consistent with previous findings (Boye et al., 2010). Soaking did not alter the primary limiting amino acid of faba bean flour, but made threonine as the primary limiting amino acid in yellow pea flour, despite the amino acid score of tryptophan being also low (0.78). After 72-h germination, threonine became the primary limiting amino acid in both yellow pea and faba bean flours, with the essential amino acid score being reduced from 0.79 to 0.67 and from 0.72 to 0.70, respectively. Consequently, although 72-h germination improved the IVPD of both flours (Table 3.6), the treatment diminished the *in vitro* protein digestibility corrected amino acid score (IV-PDCAAS) of yellow pea flour from 62.1% to 53.5% and did not significantly change that of faba bean flour (Table 3.9). The data from this

Table 3.6 *In vitro* starch and protein digestibility of raw, soaked and germinated yellow pea and faba bean flours[a]

Flour	Starch						Protein
	Uncooked[b]			Cooked[b, c]			IVPD (%)[d]
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)	
Yellow pea							
Raw	9.7 ± 0.0 a	23.2 ± 0.0 a	14.2 ± 0.0 b	35.0 ± 0.7 a	4.9 ± 0.3 ab	7.0 ± 0.4 d	78.6 ± 0.1 a
Soaked	10.5 ± 0.6 a	23.3 ± 1.2 a	13.5 ± 1.2 b	35.9 ± 1.7 a	7.5 ± 0.9 b	3.9 ± 0.5 c	78.7 ± 0.1 a
24-h germinated	14.8 ± 0.7 b	24.2 ± 1.4 a	8.4 ± 2.2 a	40.6 ± 1.7 b	4.2 ± 2.5 a	2.7 ± 0.8 bc	79.6 ± 0.1 b
48-h germinated	16.6 ± 0.8 c	23.5 ± 2.1 a	6.7 ± 1.2 a	42.0 ± 0.6 b	3.1 ± 0.6 a	1.7 ± 0.2 ab	79.8 ± 0.1 bc
72-h germinated	17.1 ± 0.6 c	22.9 ± 1.4 a	5.4 ± 1.5 a	42.7 ± 0.8 b	2.1 ± 0.4 a	0.7 ± 0.6 a	79.9 ± 0.1 c
Faba bean							
Raw	4.7 ± 0.2 a	12.4 ± 0.4 a	25.7 ± 0.4 c	37.2 ± 0.1 a	2.6 ± 0.3 ab	3.0 ± 0.3 bc	78.0 ± 0.2 ab
Soaked	5.0 ± 0.1 a	17.5 ± 2.4 ab	21.6 ± 0.9 c	37.1 ± 0.3 a	3.0 ± 0.7 ab	3.9 ± 0.8 c	77.8 ± 0.3 a
24-h germinated	8.3 ± 0.9 b	21.5 ± 3.0 bc	15.0 ± 2.7 b	38.0 ± 0.5 ab	3.6 ± 1.2 b	3.2 ± 0.4 bc	78.4 ± 0.2 ab
48-h germinated	9.3 ± 0.9 b	22.0 ± 1.7 bc	11.6 ± 2.1 ab	39.0 ± 0.9 bc	1.5 ± 0.3 a	2.3 ± 0.3 ab	78.6 ± 0.5 b
72-h germinated	9.9 ± 0.6 b	23.7 ± 1.8 c	9.3 ± 1.8 a	39.9 ± 0.7 c	1.6 ± 0.3 a	1.4 ± 0.4 a	80.4 ± 0.1 c

[a] Values are presented as average ± standard deviation (n = 6; duplicate measurements of three batches); in the same column of one pulse type, the numbers with the same letter are not significantly different at $p < 0.05$.
[b] RDS: rapidly digestible starch, SDS: slowly digestible starch, and RS: resistant starch; values were calculated on a dry starch basis.
[c] Flour was cooked in a boiling water bath for 10 min.
[d] IVPD: *in vitro* protein digestibility; the flours were not cooked for this analysis.

Table 3.7 Amino acid compositions (g per 100 g of flour, on an as-is basis) of raw, soaked and germinated (72-h) yellow pea and faba bean flours

Amino acid	Yellow pea			Faba bean		
	Raw	**Soaked**	**72-h germinated**	**Raw**	**Soaked**	**72-h germinated**
Aspartic Acid	2.58	2.67	2.52	2.62	2.58	3.45
Glutamic Acid	3.84	4.03	3.67	4.05	4.11	4.24
Serine	1.15	1.22	1.21	1.30	1.30	1.49
Glycine	0.77	0.82	0.77	0.95	0.95	0.95
Histidine‡	0.58	0.60	0.52	0.46	0.57	0.63
Arginine	1.85	1.84	1.55	2.17	2.09	2.18
Threonine‡	0.59	0.56	0.51	0.63	0.65	0.64
Alanine	0.81	0.78	0.93	0.94	0.98	1.10
Proline	0.78	0.56	0.96	0.98	1.00	1.12
Tyrosine	0.63	0.67	0.63	0.78	0.70	0.82
Valine‡	0.80	0.85	0.95	1.05	1.05	1.07
Methionine*‡	0.20	0.24	0.17	0.18	0.19	0.18
Cysteine*	0.52	0.58	0.44	0.52	0.50	0.37
Isoleucine‡	0.69	0.75	0.82	0.98	0.98	0.96
Leucine‡	1.21	1.31	1.43	1.77	1.79	1.75
Phenylalanine‡	0.77	0.84	1.07	1.03	1.03	1.04
Lysine‡	1.26	1.27	1.27	1.66	1.65	1.34
Tryptophan‡	0.18	0.19	0.21	0.20	0.20	0.23

* Sulfur-containing amino acid; ‡ essential amino acids.

Table 3.8 Essential amino acid compositions and scores of raw, soaked and germinated (72-h) yellow pea and faba bean flours

Flour	Essential amino acid[a]								
	THR	**VAL**	**MET + CYS**	**ILE**	**LEU**	**PHE + TYR**	**HIS**	**LYS**	**TRP**
a) Content (mg/g protein)									
Yellow pea									
Raw	29	39	35	33	59	68	28	61	9
Soaked	25	38	37	34	59	68	27	57	9
72-h germinated	23	42	27	37	64	76	23	57	9
Faba bean									
Raw	25	42	28	39	70	72	18	66	8
Soaked	24	39	26	36	67	64	21	61	7
72-h germinated	24	40	21	36	65	69	24	50	9
FAO reference	34	35	25	28	66	63	19	58	11
b) Score[b]									
Yellow pea									
Raw	0.84	1.11	1.40	1.20	0.89	1.08	1.48	1.05	0.79*
Soaked	0.74*	1.09	1.48	1.21	0.89	1.08	1.42	0.99	0.78
72-h germinated	0.67*	1.21	1.09	1.31	0.97	1.20	1.22	0.98	0.85
Faba bean									
Raw	0.73	1.19	1.11	1.38	1.06	1.14	0.96	1.13	0.72*
Soaked	0.71	1.12	1.03	1.30	1.01	1.02	1.12	1.06	0.68*
72-h germinated	0.70*	1.14	0.82	1.28	0.99	1.10	1.24	0.86	0.78

[a] THR: threonine; VAL: valine; MET: methionine; CYS: cysteine; ILE: isoleucine; LEU: leucine; PHE: phenylalanine; TYR: tyrosine; HIS: histidine; LYS: lysine; and TRP: tryptophan.

[b] Essential amino acid score = Essential amino acid content / FAO reference value.

* Limiting amino acid.

Table 3.9 Limiting amino acid scores and protein quality data of raw, soaked and germinated (72-h) yellow pea and faba bean flours[a, b]

Flour	Limiting amino acid	Limiting amino acid score	IVPD (%)	IV-PDCAAS[c] (%)
Yellow pea				
Raw	Tryptophan	0.79	78.6 ± 0.1 a	62.1 ± 0.1 c
Soaked	Threonine	0.74	78.7 ± 0.1 a	58.4 ± 0.1 b
72-h germinated	Threonine	0.67	79.9 ± 0.1 b	53.5 ± 0.1 a
Faba bean				
Raw	Tryptophan	0.72	78.0 ± 0.2 a	56.2 ± 0.1 b
Soaked	Tryptophan	0.68	77.8 ± 0.3 a	52.9 ± 0.2 a
72-h germinated	Threonine	0.70	80.4 ± 0.1 b	56.5 ± 0.1 b

[a] Values are presented as average ± standard deviation (n = 6; duplicate measurements of three batches); in the same column of one pulse type, the numbers with the same letter are not significantly different at $p < 0.05$.
[b] IVPD: *in vitro* protein digestibility; and IV-PDCAAS: *in vitro* protein digestibility corrected amino acid score.
[c] IV-PDCAAS (%) = IVPD (%) × limiting amino acid score.

research suggested that soaking and germination did not enhance the nutritional quality of protein in yellow pea and faba bean flours.

3.4 Conclusions

In this research, germination for short durations (24-72 h) was utilized as an effective method to prepare yellow pea and faba bean flours with diverse functional properties and enhanced nutritional value. In comparison with the corresponding control raw flours, soaking (pre-treatment prior to germination) and germination for 24-72 h showed negligible effects on the chemical compositions of the pulse flours. Germination gradually lowered the conclusion temperature (T_c) and enthalpy change (ΔH) of the thermal transition peak of both flours. Soaking and germination for 24 h markedly elevated the pasting viscosities of the pulse flours, which could be due to the disruption of the protein and fibre matrix surrounding starch granules, allowing them to swell to a higher degree during pasting. A longer germination period (48 or 72 h) reduced the pasting viscosities of the pulse flours, resulting from the extensive hydrolysis of starch molecules by endogenous amylase(s) during heating in the RVA. Germination progressively enhanced the emulsifying and foaming properties of the pulse flours. With respect to nutritional attributes, germination increased the *in vitro* digestibility of starch and protein of the pulse flours and altered their amino acid compositions. However, this modification did not improve the *in vitro* protein digestibility corrected amino acid scores (IV-PDCAAS) of the flours. The current study greatly advanced our understanding of the impacts of soaking and short-term germination on the chemical composition, functional properties and nutritional quality of yellow pea and faba bean flours. The acquired new knowledge will be meaningful for the value-added processing industry to employ germination as a useful technology to generate pulse flours with functional and nutritional properties suitable for broader food applications.

3.5 Linkage to Chapter 4

Because 72-h germination induced the most noticeable changes in the chemical composition and functional properties of pulse flours, as demonstrated in Chapter 3, the subsequent study focused on the use of composite flours prepared from 72-h germinated pulse flours and wheat flour and determined their impacts on flour and dough properties as compared to the 100% wheat flour control. Findings from this study will help provide more meaningful information about the

partial substitution (10 or 20%) of wheat flour with 72-h germinated pulse flours in bread making. The results from this research will be valuable to the food industry to include raw and germinated pulse flours into the formulae of breads and other bakery products to manufacture final products with improved quality and enhanced nutritional value for consumers.

4. PHYSICOCHEMICAL PROPERTIES AND BAKING PERFORMANCE OF WHEAT FLOUR COMPOSITED WITH GERMINATED AND UNGERMINATED PULSE FLOURS

ABSTRACT

In this study, raw and 72-h germinated yellow pea (CDC Amarillo) and faba bean (CDC Snowdrop) flours were used to replace 10% or 20% of hard wheat flour to obtain composite flours, and their flour quality, dough properties and bread making performance were evaluated in comparison with a 100% hard wheat flour control. The incorporation of both raw and germinated pulse flours reduced the starch level but increased that of protein. The inclusion of the pulse flours progressively decreased the pasting viscosities of the composite flours, and this phenomenon was more obvious with the germinated samples, primarily due to the presence of endogenous amylase(s). These results corresponded well with decreases in their falling numbers. The incorporation of the raw and germinated pulse flours diminished the solvent retention capacity (SRC) with 5% lactic acid and gluten performance index (GPI), and thus the doughs generated from the composite flours showed shortened dough development time (DDT), poorer dough stability and less elastic structure when compared with the control. As a result of the weakened dough structure, the breads baked from the wheat-pulse flour blends – despite the addition of 2% wheat gluten – exhibited decreased loaf volumes and smaller numbers of cells per slice but greater crumb firmness, with the most significant changes being detected in the samples containing 20% germinated pulse flour. Yellow pea and faba bean flours – in both raw and germinated forms – largely showed similar impacts on the chemical composition and technological characteristics of the composite flours. This research provided insights into the utilization of raw and germinated pulse flours in bread and potentially other bakery products.

Keywords: yellow pea; faba bean; germination; composite flour; flour properties; dough properties; bread making performance

4.1 Introduction

Pulses, including pea, lentil, bean, chickpea and faba bean, are important crops for the agri-food sector in Canada (Lopez-Martinez et al., 2017; Tosh and Yada, 2010). Recently, they are attracting growing interest because of their nutritional value as they are abundant in protein, resistant starch and other dietary fibre, as well as being low in fat (except for chickpeas) and glycemic impact (Boye et al., 2010). Pulses are also a good source of vitamins (*e.g.*, folate and other B vitamins), minerals, and antioxidant compounds, such as phenolic acids, flavonoids and polyphenols. As such, the consumption of pulses can impart various health benefits and reduce the risks of chronic diseases (*e.g.*, obesity, diabetes, cancers and cardiovascular diseases) (Boye et al., 2010; Padhi and Ramdath, 2017).

Pulses are milled to produce pulse flours that are commonly used in food products to deliver the aforementioned nutritional benefits. One good example is to partly replace hard wheat flour with pulse flours in the baking of bread, which is one of the most popular staple foods worldwide. Bread baked from such composite flours contains more protein and fibre when compared with the products prepared from wheat flour alone (Ficco et al., 2018; Portman et al., 2018). Additionally, the use of pulse flours in bread can improve its amino acid quality because the amino acid profiles of pulse flours and wheat flour are complementary: pulse flours are commonly rich in lysine but low in sulphur-containing amino acids (*e.g.*, methionine and cysteine) and tryptophan, while the latter is high in methionine and cysteine but low in lysine (Boye et al., 2010). More interestingly, Zafar et al. (2015) highlighted the significantly reduced glycemic response of whole wheat bread when the whole wheat flour was replaced by chickpea flour at a level of 35%.

Research efforts have also been undertaken to investigate the influence of partial replacement of hard wheat flour with pulses flours on the flour properties, dough quality and bread making performance. Mohammed et al. (2014) reported decreases in the peak viscosities of flour blends with the incorporation of chickpea flour at 10%, 20% and 30% levels, with a larger degree of reduction being observed at a higher level of chickpea flour use. In addition, the composite flours with more chickpea flour substitution showed larger farinograph water absorption values and longer dough development times (DDT) as compared to 100% wheat flour control. Similar results have been demonstrated by other researchers when pulse flours were used to substitute wheat flour at different proportions (Hallén et al., 2004; Kohajdová et al., 2013). However,

discrepancies have also been shown in the literature: Portman et al. (2018) found decreased farinograph water absorption and DDT when using 5%, 10%, 15%, 20% and 40% lentil flour to replace wheat flour in bread formula; Ficco et al. (2018) observed no significant changes in farinograph water absorption and DDT (except an increase in DDT at 30% level) after replacing wheat flour with 10%, 15%, 20% and 30% yellow pea flour. Regarding the quality of the resultant breads, Mohammed et al. (2014) reported that the replacement of wheat flour with chickpea flour at 10-30% lowered the loaf volume, caused darker color of the crust, and resulted in a harder texture of the bread. Similar baking results have been demonstrated for flours composited with various pulse flours at different levels in other studies (Ficco et al., 2018; Portman et al., 2018).

Despite the aforementioned functional and nutritional advantages of pulse flours, the occurrence of antinutrients (*e.g.*, saponins, phytates, phenolics, lectins and enzyme inhibitors) and the undesirable taste and flavor profiles hinder their applications in many food products (Boye et al., 2010; Lopez-Martinez et al., 2017). The food industry has employed different methods to treat pulse seeds and/or flours, such as germination, roasting, extrusion microwave heating, infrared heating, fermentation and enzyme treatments, to diversify the functional properties and enhance the nutritional attributes of the derived flours (Albarracín et al., 2015; Khanam and Platel, 2016; Roland et al., 2017). Among these treatments, germination of seeds has been illustrated to be very effective in improving the overall quality of pulse flours. As examples, short-term germination (24 h) has been reported to remarkably increase the pasting viscosities of yellow pea and faba bean flours, but a longer germination duration (48 h and beyond) reduced their pasting viscosities (Setia et al., 2019). Germination tends to enhance the emulsifying properties and foaming capacity of pulse flours (El-Adawy et al., 2003; Ghavidel and Prakash, 2006; Setia et al., 2019). This treatment also appears to be promising in increasing the digestibility of starch and protein, two major macronutrients in pulse flours (Ghavidel and Prakash, 2007; Setia et al., 2019; Xu et al., 2017). Furthermore, germination can noticeably reduce the strong and pungent flavor of pulse flours (Bellaio et al., 2013; Roland et al., 2017).

Despite the obvious changes in the technological characteristics and nutritional value of pulse flours after germination treatment, there is a lack of knowledge on the performance of germinated pulse flours in real food products, especially when they are used to replace hard wheat flour in bread making. Therefore, the two main objectives of this research were: (1) To investigate the flour quality and dough properties of the composite flours prepared by blending raw or

germinated pulse flours and hard wheat flour at 10:90 and 20:80 ratios; and (2) To bake breads from such flour blends and evaluate their quality attributes in comparison to the 100% wheat bread control. According to our previous work on the influence of germination on the functional attributes of pulse flours (Setia et al., 2019), raw and 72-h germinated yellow pea and faba bean flours were selected in the present study. The findings from this research will be valuable to the food industry to include raw and germinated pulse flours into the product formulas to prepare bread and other bakery goods having acceptable quality and enhanced nutritional value. The research and development efforts will promote the utilization of pulses and pulse-based ingredients in the agri-food sector in Canada and globally.

4.2 Materials and Methods

4.2.1 Materials

Certified yellow pea (CDC Amarillo variety) and faba bean (CDC Snowdrop variety) seeds were kindly provided as gifts by Reisner Farm Ltd. (Limerick, SK, Canada). Total Starch Assay Kit was purchased from Megazyme International Ltd. (Co. Wicklow, Ireland). Commercial hard wheat flour milled from Canada Western Red Spring wheat was supplied by Warburton Foods Ltd. (Saint Francois Xavier, MB, Canada). The other ingredients used for bread making were purchased from various sources: yeast and dough conditioner from AB Mauri Ltd. (Calgary, AB, Canada), table salt from Compass Minerals Canada Corporation (Saskatoon, SK, Canada), fine granulated sugar from Lantic Inc. (Taber, AB, Canada), all-purpose shortening from Richardson Oilseed Ltd. (Winnipeg, MB, Canada), whey powder from Agropur Ingredients (Winnipeg, MB, Canada), and gluten from Manildra Milling Corporation (Hamburg, IA, U.S.A.). All the other chemicals used in the study were of reagent grade, and they were procured from Fisher Scientific Company (Ottawa, ON, Canada).

4.2.2 Germination of pulse seeds

Germination of yellow pea and faba bean seeds was carried out according to our previous method (Setia et al., 2019). After a germination period of 72 h, the germinated seeds were dried in a convection oven at 55 ± 2°C for 24-36 h to have moisture levels below 10%. The dried seeds were stored in sealed Ziploc bags in a freezer before milling. The germination was performed in triplicate (*i.e.*, three independent batches) for both yellow pea and faba bean.

4.2.3 Milling of pulse seeds and blending of flours

Milling of raw and germinated yellow pea and faba bean seeds was completed through two steps. In the first step, the seeds were ground into coarse grits using a laboratory disc mill (Model 3310, Perten Instruments Canada, Winnipeg, MB, Canada) equipped with a type-1 medium grinding disc at setting 5. In the following step, the resultant coarse grits were milled into fine flours using a pin mill (Model 100 UPZ, Hosokawa Alpine, Augsburg, Germany). The obtained fine flours from both raw and germinated pulse seeds were blended with the hard wheat flour at two ratios (*i.e.*, pulse:wheat = 10:90 and 20:80) on a dry basis. The composite flours were used for the subsequent experiments.

4.2.4 Flour properties

4.2.4.1 Chemical composition

Proximate analyses of the composite flours and the wheat flour control were conducted in accordance with official methods. Total starch content was measured using Megazyme Total Starch Assay Kit following AACC Method 76-13.01 (AACC, 2000). Nitrogen content (%N) was quantified using the Dumas combustion method by a Nitrogen/Protein Analyzer (CN628, LECO Corp., St. Joseph, MI, U.S.A.), which was converted to protein content (%N × 6.25 for pulses and %N × 5.7 for wheat) following AACC Method 46-30.01 (AACC, 2000). For the composite flours, the %protein was calculated as: %N × 6.25 × 0.1 + %N × 5.7 × 0.9 for 10:90 ratio and %N × 6.25 × 0.2 + %N × 5.7 × 0.8 for 20:80 ratio, respectively (Krul, 2019). The moisture, crude fat and ash contents were performed following AOAC Method 925.10, 920.85 and 923.03, respectively (AOAC, 2012). The chemical compositions of the flour samples were reported on a dry basis.

4.2.4.2 Pasting properties

Pasting properties of the flour samples were determined using a Rapid Visco-Analyzer (RVA Super 3, Newport Scientific, Sydney, Australia) following the method of Ai et al. (2017). A flour-water suspension (28.0 g in total) was prepared to reach a dry solids content of 10.6% (w/w), which was loaded onto the RVA instrument for the analysis using Standard Method 2 in the Thermocline software. The germinated pulse-wheat flour blends from each germination batch were run at least in duplicate to obtain two well-overlapped pasting curves, and their average was used as one measurement. The average of three measurements (corresponding to three independent

germination batches) was used to draw the RVA profile of each flour. The respective ungerminated pulse-wheat flour blends and the wheat flour control were analyzed in triplicate, and the average of the triplicate measurements was used to prepare the pasting curve of each sample.

4.2.4.3 Falling number

Falling numbers (FN) of the flour samples were determined following AACC Method 56-81.03 using a Falling Number® 1000 instrument (Perten Instruments Canada, Winnipeg, MB, Canada) (AACC, 2000). At the end of the experiment, the recorded FN (as is) was corrected to 14% moisture basis of flour using the following equation:

$$\text{FN (14\% moisture basis)} = \text{FN (as is)} \times (100 - 14) / (100 - \%\text{moisture of flour, in \%)} \quad \text{[Eq. 4.1]}$$

4.2.4.4 Solvent retention capacity

Solvent retention capacity (SRC) of the flour samples was determined according to AACC Method 56-11.02 (AACC, 2000). Four solvents were prepared for the test: deionized water, 5% (w/v) sodium carbonate, 50% (w/v) sucrose and 5% (v/v) lactic acid. Each flour (5 g) was accurately weighed and added into a 50-mL polypropylene centrifuge tube. The solvent (25 g) was transferred into the tube, and the mixture was vortexed for 5 s every 5 min for a total period of 20 min. The flour slurry was then centrifuged at 1,000 *g* for 15 min. After centrifugation, the supernatant was carefully decanted and the tube was drained at 90° angle for 10 min on a paper towel. The weight of gel in the tube was used to calculate SRC:

$$\text{SRC (g/g)} = [(\text{gel weight} / \text{flour weight}) - 1] \times [86 / (100 - \%\ \text{flour moisture})] \quad \text{[Eq. 4.2]}$$

The gluten performance index (GPI), which is a good indicator of the overall quality and performance of gluten in wheat flour, was calculated using the SRC values of 5% lactic acid, 5% sodium carbonate, and 50% sucrose in accordance with the following equation (Arp, Correa and Ferraro, 2018):

$$\text{GPI} = \text{SRC (5\% lactic acid)} / [\text{SRC (5\% sodium carbonate)} + \text{SRC (50\% sucrose)}] \quad \text{[Eq. 4.3]}$$

4.2.5 Dough properties

4.2.5.1 Farinograph

Farinograph analysis was performed on the flour samples using the Farinograph®-AT equipped with a 50-g bowl and WinFarino software (Version 2.6.2) (C.W. Brabender Instruments Inc., South Hackensack, NJ, U.S.A.) according to AACC Method 54-21.02 (AACC, 2000). The farinograph was run for a total duration of 20 min, and the obtained curve was used to calculate farinograph water absorption, dough development time (DDT), dough stability, and dough mixing tolerance index (MTI) according to the procedures described in the official method.

4.2.5.2 Rheology

Dough rheology test on the flour samples was conducted according to the method of Beck et al. (2012) using an AR-1000 rheometer with a 40-mm parallel plate fixture (TA Instruments, New Castle, DE, U.S.A.). The dough formulation consisted of 10.0 g flour (corrected to 14% moisture basis), water (calculated according to farinograph water absorption), and 1.2% sodium chloride (on a 10.0 g flour weight basis). The dough was prepared using a 10-g mixograph (National Manufacturing Division, TMCO, Lincoln, NE, U.S.A.) in accordance with AACC Method 54-40.02 (AACC, 2000). The mixing time was a few seconds beyond the peak time, and the obtained dough was placed on the parallel plate. The temperature was maintained at 22 ± 1°C on a peltier plate temperature system and the gap was set to 2.0 mm. Once the fixture was lowered to the target gap setting, excess dough that spread beyond the rim of the fixture was trimmed away and paraffin oil was applied onto the dough surface exposed to air to prevent drying. The following tests were carried out on the loaded dough sample:

Oscillatory frequency sweep: The frequency was varied from 0.1 to 100.0 Hz at a constant amplitude strain of 0.1% within the linear viscoelastic range. The dynamic storage modulus (G'), loss modulus (G'') and complex modulus (|G*|) of the dough at 1 Hz and 10 Hz were measured. The loss tangent was calculated using the following equation:

$$\text{Tan } \delta = G''/ G' \qquad \text{[Eq. 4.4]}$$

Creep recovery: A constant shear stress (τ_0) of 250 Pa was applied to the dough for 180 s and then removed (τ_0 = 0 Pa). The dough relaxation behavior was monitored for the following 360

s. The strain value was collected as a function of time, and the final result was reported as compliance:

$$J(t) = \gamma(t) / \tau_0 \quad \text{[Eq. 4.5]}$$

where J is the compliance, γ is the strain, and τ_0 is the constant stress (250 Pa) applied during the creep phase. The time- and stress-dependent recoverable shear deformation and the creep compliance J_{max} (at t = 180 s of the creep phase) were measured. The creep recovery compliance J_r (at t = 360 s of the recovery phase), a measure of the mechanical energy stored in the sample during the creep phase, reflected the dough elasticity. The relative elasticity (J_{el}) was calculated as:

$$J_{el} = J_r / J_{max} \quad \text{[Eq. 4.6]}$$

4.2.6 Baking performance

The flour samples were utilized to bake bread following a straight-dough bread baking method – AACC Method 10-10.03 (AACC 2000) with minor modifications in the baking laboratory of Canadian International Grains Institute (Cigi, Winnipeg, MB, Canada). The bread formulation included: 200.0 g of the composite flours or the wheat flour control (corrected to 14% moisture basis) and other ingredients on a 200.0 g flour weight basis – 4% yeast, 4% sugar, 1.2% salt, 4% whey, 3% shortening, 1% dough conditioner, and 2% wheat gluten (only used in the pulse-wheat flour blends to overcome the stickiness of their doughs). The amount of water added to achieve optimal dough development was based on the water absorption data from the farinograph analysis. All the ingredients were mixed using a pin-type mixer (National Manufacturing Division, TMCO) at 118 rpm to prepare a fully developed dough, which was determined by Power to Mixer software (RAR Software Systems, Winnipeg, MB, Canada). After mixing, the dough was covered and allowed to rest for 10 min at room temperature, divided into two 165-g pieces, rounded by hand, and then rested again at room temperature for 10 min in a fermentation bowl before sheeting and molding. The sheeting was completed by passing the dough three times through a sheeter (National Manufacturing Division, TMCO) and the dough sheet was manually rolled and then molded using a three-roll type molder (National Manufacturing Division, TMCO). The molded dough was placed in a baking pan (14.5 cm L × 8.0 cm W × 5.7 cm H), proofed for 60 min in a

fermentation cabinet (85% relative humidity and 37°C; National Manufacturing Division, TMCO) until the height reached 90 mm, and then baked in an electric reel oven (National Manufacturing Division, TMCO) at 190°C for 20 min. The quality characteristics of the baked bread were evaluated using the methods described as follows:

Bread volume: The volume of the bread was determined using a volume meter (Model TexVol BVM–L370, Perten Instruments Canada, Winnipeg, MB, Canada) in accordance with AACC Method 10-14.01 (AACC 2000). The loaf weight, loaf volume and specific volume of the bread were also recorded.

Crumb structure: The bread was sliced using a commercial bread slicer (Oliver Packaging and Equipment Co., Grand Rapids, MI, U.S.A.), and the three slices from the center of the bread loaf were used to evaluate the crumb structure using C-Cell monochrome system CC.200 equipped with Version 2.0 software (Calibre Control International Ltd., Warrington, U.K.). The slice area and number of cells per slice were recorded.

Textural properties of bread crumb: On the following day after baking, the firmness of bread slices was determined in accordance with AACC Method 74-09.01 (AACC 2000) using a TA.HDplus Texture Analyzer (Stable Micro Systems Ltd., Godalming, U.K.) equipped with a 30-kg load cell. The force applied through a cylindrical probe (TA-4) at a constant speed of 1.7 mm/s to compress the centre of two stacked slices to 40% of their original height was reported as the firmness. Measurements were performed in triplicate using six slices taken from the middle of the bread loaf.

4.2.7 Statistical analysis

The germination treatment was performed in triplicate on each pulse type, and the obtained pulse flours of three independent batches were used to prepare the composite germinated pulse-wheat flours at 10:90 and 20:80 ratios. The analyses on each of the composite germinated pulse-wheat flours were performed in duplicate, the average of which was used as one measurement for the calculation of average and standard deviation and for statistical significance analysis (n = 3). The corresponding un-germinated pulse-wheat flour blends and the wheat flour control were analyzed in triplicate. Farinograph analysis was conducted with one replicate on each flour sample due to the limited amounts of the composite flours (Bourré et al., 2019). Statistical differences between the data of composite flours and those of the wheat flour control were assessed using one-

way ANOVA, Tukey's multiple comparison test at a significance level of 0.05 for individual pulse type. Evaluation of the correlations between the different variables was carried out using the Pearson correlation test. The statistical analysis was performed using IBM SPSS Statistics (Version 24.0, IBM Corporation, Armonk, NY, U.S.A.).

4.3 Results and Discussion

4.3.1 Flour properties

4.3.1.1 Chemical compositions

The proximate compositions of the wheat flour control and the raw and germinated pulse-wheat flour blends at 10% and 20% incorporation levels are presented in Table 4.1. The pulse-wheat flour blends consisted of less starch (67.5-73.0% *versus* 75.3%), more protein (15.8-17.9% *versus* 15.3%) and ash (0.8-1.2% *versus* 0.6%), and similar level of crude fat (1.7-2.9% *versus* 2.2%) in comparison with the wheat control. The decrease in starch content and the increases in protein and ash contents after the blending were results of the lower starch content and higher protein and ash contents of the pulse flours (Setia et al., 2019). Also, a greater extent of change was observed with 20% inclusion of pulse flours when compared with the 10% counterparts. Overall, the composite flours with germinated pulse flours showed chemical compositions similar to those with raw counterparts at the same blending level, consistent with our previous findings that germination up to 72 h did not substantially alter the chemical compositions of pulse flours (Setia et al., 2019).

4.3.1.2 Pasting properties

Pasting profiles of the flour samples are displayed in Figure 4.1, and the results are summarized in Table 4.2. In general, the inclusion of raw yellow pea and faba bean flours reduced the pasting temperature, peak time, and pasting viscosities of the composite flours, with more noticeable changes being observed with 20% level. The results could be explained by the fact that these two raw pulse flours had lower pasting temperatures and peak times and less pasting viscosities than the wheat flour control (Setia et al., 2019). The lower pasting viscosities of the flour blends could be partly explained by their smaller starch contents than the wheat flour control (Table 4.1): starch is known to be the primary component contributing to the viscosity development of flours during pasting (Kaur et al., 2015; Wani et al., 2012; Xu et al., 2017).

The use of germinated yellow pea and faba bean flours further diminished the pasting viscosities of the composite flours, which could be mainly attributed to the increased activities of endogenous amylases, such as α-amylase, β-amylase and α-glucosidase, in the germinated flours

Table 4.1 Chemical compositions of wheat flour control and those blended with raw and germinated pulse flours at two ratios[1]

Flour	Starch[2] (%)	Protein[3] (%)	Crude fat[4] (%)	Ash[5] (%)
Wheat control	$75.3 \pm 0.3^{c(C)}$	$15.3 \pm 0.0^{a(A)}$	$2.2 \pm 0.5^{ab(A)}$	$0.6 \pm 0.0^{a(A)}$
Yellow pea (YP)				
R:W (10:90)	72.8 ± 0.8^{b}	15.8 ± 0.0^{b}	2.5 ± 0.4^{b}	0.8 ± 0.0^{b}
G:W (10:90)	73.0 ± 0.7^{b}	16.0 ± 0.2^{b}	1.7 ± 0.3^{a}	0.8 ± 0.0^{b}
R:W (20:80)	69.1 ± 0.6^{a}	16.7 ± 0.1^{c}	2.6 ± 0.2^{b}	1.1 ± 0.1^{d}
G:W (20:80)	70.1 ± 1.1^{a}	17.1 ± 0.2^{d}	2.3 ± 0.1^{ab}	1.0 ± 0.0^{c}
Faba bean (FB)				
R:W (10:90)	$71.4 \pm 0.4^{(B)}$	$16.3 \pm 0.0^{(B)}$	$2.3 \pm 0.1^{(A)}$	$1.0 \pm 0.0^{(B)}$
G:W (10:90)	$70.9 \pm 1.1^{(B)}$	$16.5 \pm 0.1^{(B)}$	$2.6 \pm 0.4^{(A)}$	$0.9 \pm 0.1^{(B)}$
R:W (20:80)	$67.5 \pm 0.2^{(A)}$	$17.4 \pm 0.0^{(C)}$	$2.9 \pm 0.6^{(A)}$	$1.2 \pm 0.1^{(C)}$
G:W (20:80)	$68.1 \pm 0.6^{(A)}$	$17.9 \pm 0.2^{(D)}$	$2.1 \pm 0.4^{(A)}$	$1.2 \pm 0.1^{(C)}$

[1] Values are presented as average ± standard deviation (n = 3, on a dry basis); R: raw, G: germinated, W: wheat; lowercase and uppercase letters represent statistical differences for YP and FB, respectively; the numbers with the same letter are not significantly different at $p < 0.05$.

[2] Determined using Megazyme Total Starch Assay Kit following AACC Method 76−13.01.

[3] Determined using a Nitrogen/Protein Analyzer following AACC Method 46−30.01.

[4] Determined using Soxhlet method following AOAC Method 920.85.

[5] Determined using an electric muffle furnace following AACC Method 08−01.01.

Table 4.2 Pasting properties and falling numbers of wheat flour control and those blended with raw and germinated pulse flours at two ratios[1, 2]

Flour	Pasting temperature (°C)	Peak time (cP)	Peak viscosity (cP)	Breakdown viscosity (cP)	Setback viscosity (cP)	Final viscosity (cP)	Falling number (s)
Wheat control	84.2 ± 0.3$^{c(C)}$	9.2 ± 0.0$^{d(D)}$	2357.7 ± 75.4$^{e(E)}$	1357.3 ± 57.3$^{d(C)}$	1074.0 ± 39.9$^{d(E)}$	2074.3 ± 58.4$^{d(E)}$	421 ± 4$^{d(C)}$
Yellow pea (YP)							
R:W (10:90)	84.1 ± 0.6^{c}	9.1 ± 0.0^{c}	2000.7 ± 103.0^{d}	1124.5 ± 44.5^{c}	976.7 ± 25.1^{c}	1853.3 ± 82.6^{c}	443 ± 3^{e}
G:W (10:90)	81.2 ± 0.7^{b}	8.8 ± 0.1^{b}	1425.3 ± 99.7^{b}	990.0 ± 58.3^{b}	603.2 ± 61.8^{b}	1038.5 ± 102.8^{b}	347 ± 17^{b}
R:W (20:80)	83.0 ± 0.3^{bc}	8.9 ± 0.1^{b}	1817.3 ± 63.3^{c}	989.3 ± 28.1^{b}	914.0 ± 12.0^{c}	1742.0 ± 47.7^{c}	412 ± 1^{c}
G:W (20:80)	77.2 ± 1.1^{a}	8.4 ± 0.1^{a}	1085.8 ± 87.6^{a}	814.8 ± 35.0^{a}	371.2 ± 53.4^{a}	642.2 ± 110.6^{a}	284 ± 16^{a}
Faba bean (FB)							
R:W (10:90)	84.0 ± 0.7$^{(C)}$	9.2 ± 0.0$^{(D)}$	2045.3 ±131.9$^{(D)}$	1146.3 ± 65.6$^{(B)}$	992.0 ± 35.6$^{(D)}$	1891.0 ±102.1$^{(D)}$	442 ± 8$^{(D)}$
G:W (10:90)	82.8 ± 0.2$^{(B)}$	9.0 ± 0.0$^{(C)}$	1595.0 ± 64.0$^{(B)}$	1051.0 ± 38.0$^{(B)}$	715.7 ± 28.2$^{(B)}$	1259.7 ± 71.8$^{(B)}$	362 ± 7$^{(B)}$
R:W (20:80)	82.7 ± 0.0$^{(B)}$	8.9 ± 0.0$^{(B)}$	1741.0 ± 54.4$^{(B)}$	937.3 ± 23.5$^{(A)}$	901.3 ± 7.6$^{(C)}$	1705.0 ± 40.7$^{(C)}$	431 ± 0$^{(D)}$
G:W (20:80)	79.6 ± 0.8$^{(A)}$	8.7 ± 0.1$^{(A)}$	1290.0 ± 29.3$^{(A)}$	908.7 ± 17.3$^{(A)}$	560.2 ± 26.6$^{(A)}$	941.5 ± 49.5$^{(A)}$	325 ± 16$^{(A)}$

[1] Values are presented as average ± standard deviation (n = 3); R: raw, G: germinated, W: wheat; lowercase and uppercase letters represent statistical differences for YP and FB, respectively; the numbers with the same letter are not significantly different at $p < 0.05$.

[2] Determined using a Rapid Visco Analyzer (RVA) with 10.6% (w/w, dry solids) flour suspensions.

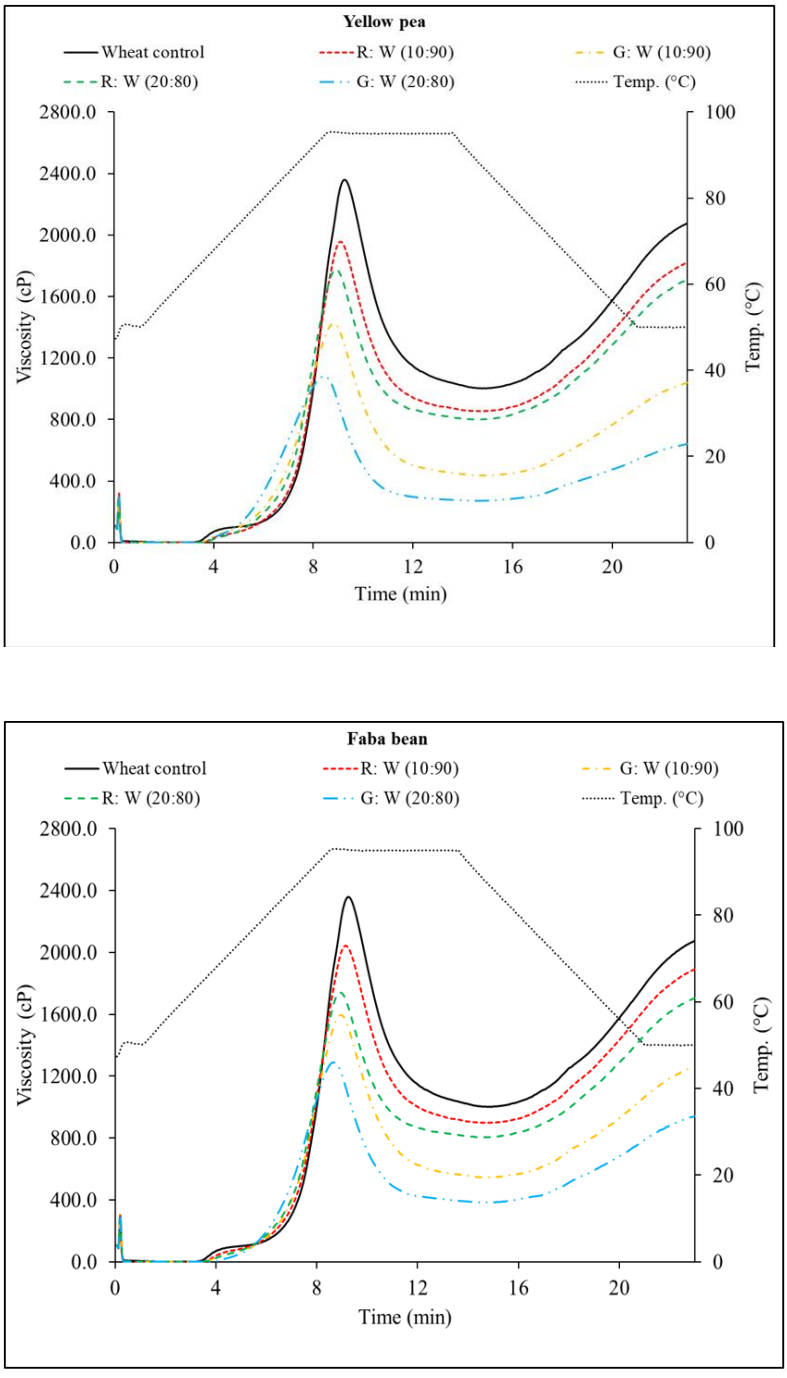

Figure 4.1 Pasting profiles of wheat flour control and those blended with raw and germinated pulse flours at two ratios measured using a Rapid Visco-Analyzer with 10.6% (w/w, dry solids) flour suspension.

(Setia et al., 2019; Stanley et al., 2011). The existing endogenous amylases were able to readily hydrolyze starch in the flour blends during the initial heating stage of RVA analysis (temperature < 60°C), leading to a lower degree of viscosity development. At the same use level, the blends with germinated yellow pea flour exhibited even lower viscosities than those with germinated faba bean flour in the RVA measurement, which corresponded well with the higher activity of α-amylase (possibly other amylases too) in the former (Setia et al., 2019).

4.3.1.3 Falling number

The wheat flour control had a falling number (FN) of 421 s (Table 2), indicating the flour was milled from sound wheat kernels. . The incorporation of raw yellow pea and faba bean flours did not markedly influence the FN of the flour blends, which ranged from 412 to 443 s. The FN of the composite flours gradually decreased with the use of a larger proportion of germinated flours, which could be directly related to the enhanced activities of endogenous amylases in the germinated pulse flours (Setia et al., 2019). It is known that the presence of endogenous amylases, such as in wheat flour with severe pre-harvest field sprouting, can promptly degrade starch molecules during cooking in the FN test (Marti et al.,2018), thus substantially reducing the FN of wheat flour. At the same level of inclusion, the flours composited with the germinated yellow pea flour exhibited a smaller FN when compared with those blended with germinated faba bean flour (347 *versus* 361 s and 284 *versus* 324 s, respectively). The results are in good accordance with the lower pasting viscosities of the flour blends with the incorporation of germinated yellow pea flour as shown in Figure 4.1 and Table 4.2, resulting from the higher activities of amylases in this germinated pulse flour. The FN of all the flour samples were positively correlated their peak viscosities ($r = 0.894$, $p < 0.01$) and final viscosities ($r = 0.964$, $p < 0.01$). With the use of 20% germinated yellow pea flour, the FN of the composite flour dropped to 284 s.

4.3.1.4 Solvent retention capacity

The composite flours showed solvent retention capacity (SRC) with water and 5% sodium carbonate similar to that of the wheat flour control (Table 4.3), suggesting the incorporation of raw and germinated pulse flours did not considerably alter the contents of components that had stronger association with those two solvents (*i.e.*, constituents with hydrophilic moieties and damaged

Table 4.3 Solvent retention capacity of wheat flour control and those blended with raw and germinated pulse flours at two ratios[1, 2]

	Solvent retention capacity				
	Water (g/g)	**5% Sodium carbonate (g/g)**	**50% Sucrose (g/g)**	**5% Lactic acid (g/g)**	**Gluten performance index**
Wheat control	$0.8 \pm 0.0^{b(A)}$	$0.9 \pm 0.0^{b(A)}$	$1.2 \pm 0.0^{c(C)}$	$1.4 \pm 0.0^{e(C)}$	$0.7 \pm 0.0^{c(C)}$
Yellow pea (YP)					
R:W (10:90)	0.7 ± 0.0^{a}	0.9 ± 0.0^{b}	1.1 ± 0.0^{b}	1.2 ± 0.0^{c}	0.6 ± 0.0^{b}
G:W (10:90)	0.8 ± 0.0^{b}	0.9 ± 0.0^{b}	1.1 ± 0.0^{b}	1.3 ± 0.0^{d}	0.6 ± 0.0^{b}
R:W (20:80)	0.7 ± 0.0^{b}	0.9 ± 0.0^{a}	1.1 ± 0.0^{a}	1.0 ± 0.0^{a}	0.5 ± 0.0^{a}
G:W (20:80)	0.8 ± 0.0^{b}	0.9 ± 0.0^{b}	1.1 ± 0.0^{a}	1.1 ± 0.0^{b}	0.5 ± 0.0^{a}
Faba bean (FB)					
R:W (10:90)	$0.8 \pm 0.0^{(A)}$	$1.0 \pm 0.0^{(A)}$	$1.1 \pm 0.0^{(B)}$	$1.2 \pm 0.0^{(B)}$	$0.6 \pm 0.0^{(B)}$
G:W (10:90)	$0.8 \pm 0.0^{(A)}$	$0.9 \pm 0.0^{(A)}$	$1.1 \pm 0.0^{(B)}$	$1.2 \pm 0.0^{(B)}$	$0.6 \pm 0.0^{(B)}$
R:W (20:80)	$0.8 \pm 0.0^{(A)}$	$0.9 \pm 0.0^{(A)}$	$1.0 \pm 0.0^{(A)}$	$1.0 \pm 0.0^{(A)}$	$0.5 \pm 0.0^{(A)}$
G:W (20:80)	$0.8 \pm 0.0^{(A)}$	$0.9 \pm 0.0^{(A)}$	$1.0 \pm 0.0^{(A)}$	$1.1 \pm 0.0^{(A)}$	$0.5 \pm 0.0^{(A)}$

[1] Values are presented as average ± standard deviation (n = 3); R: raw, G: germinated, W: wheat; lowercase and uppercase letters represent statistical differences for YP and FB, respectively; the numbers with the same letter are not significantly different at $p < 0.05$.

[2] Determined in accordance with AACC method 56-11.

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starch, respectively). The inclusion of pulse flours – both raw and germinated yellow pea and faba bean flours – displayed a decreasing effect on the 50% sucrose and 5% lactic acid SRC, which could be due to the lower content of pentosans and the absence of gluten in the pulse flours. Consequently, the gluten performance index (GPI) of the composite flours gradually decreased with a higher level of pulse flours, suggesting a weaker gluten strength. Generally, no obvious difference was found between the blends with raw and germinated pulse flours of the same type at the same blending ratio, which is in good agreement with that the 72-h germination did not considerably change the chemical compositions of the pulse flours as discussed before in section 4.3.1.1

4.3.2 Dough properties

4.3.2.1 Farinograph

The farinograph test revealed the wheat flour control had a water absorption of 65.0% for optimal dough development (Table 4.4), and the value fits well within the range of Canada Western Red Spring hard wheat flour (63.1-68.4%) (Bourre et al., 2019; Portman et al., 2018). The incorporation of raw and germinated yellow pea flours progressively reduced the water absorption of the flour blends, whereas the use of raw and germinated faba bean flours did not follow the same trend. The dough development time (DDT) and stability of the generated doughs gradually decreased and the mixing tolerance index (MTI) gradually increased with the use of a higher level of raw and germinated pulse flours, indicating the formation of a weaker dough structure due to the dilution of gluten protein.

Table 4.4 Farinograph water absorption, DDT, stability, MTI of wheat flour control and those blended with raw and germinated pulse flours at two ratios[1]

Flour	Water absorption (%)	Dough development time (min)	Stability (min)	Mixing tolerance index (Brabender unit)[2]
Wheat control	65.0	7.3	10.8	27
Yellow pea (YP)				
R:W (10:90)	65.3	6.1	4.7	57
G:W (10:90)	63.6	5.3	5.5	61
R:W (20:80)	64.1	4.9	2.9	89
G:W (20:80)	62.7	4.7	3.6	92
Faba bean (FB)				
R:W (10:90)	65.6	5.7	5.8	50
G:W (10:90)	65.6	6.2	6.3	50
R:W (20:80)	65.2	5.0	3.3	73
G:W (20:80)	65.5	5.2	3.3	91

[1] R: raw, G: germinated, W: wheat.

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4.3.2.2 Rheology

Oscillatory frequency sweep. Between the two reported frequencies, the G', G", tan δ and G* of bread doughs at 10 Hz were greater than those of the counterparts at 1 Hz (Table 4.5), which are in good agreement with the behaviors of other viscoelastic materials in oscillatory frequency sweep tests (Rosell et al., 2011). At both frequencies, the incorporation of raw and germinated yellow pea and faba bean flours did not show a clear trend on the G', G", and G* of the doughs when compared with the control from wheat flour. However, tan δ values of the doughs developed from the composite flours were significantly higher than the control wheat dough, and slightly larger tan δ values were observed for the doughs containing raw/germinated faba bean flours than those containing raw/germinated yellow pea flours at both test frequencies.

Creep recovery test. During the creep recovery test, the deformation of the obtained doughs (J_{max}) gradually increased with the incorporation of pulse flours, with the highest value being observed with 20% raw yellow pea and faba bean flours (25.7 and 4.8 1/kPa, respectively; Table 4.6). Changes of a similar trend were found with creep recovery compliance (J_r) of the doughs from composite flours, despite the extents of changes being considerably smaller than those of J_{max}. As a result of these changes, relative elasticity (J_{el}) of the doughs decreased with a higher use level of pulse flours, with the lowest value being observed with 20% raw yellow pea and faba bean flours. The obtained data indicated that the blending of hard wheat flour with pulse flours diluted the gluten protein and led to the formation of less elastic doughs, which exhibited lower resistance to the applied stress force.

Overall, physicochemical properties of the doughs as characterized by farinograph and rheometer revealed that the inclusion of raw and germinated yellow pea and faba bean flours at 10% and 20% levels weakened the dough structure, despite some minor discrepancies were identified between the data from the two analytical methods. The development of weaker doughs from the pulse and wheat flour blends is in good accordance with their smaller 5% lactic acid SRC and GPI than the wheat flour control (Table 4.3). Although the composite flours consisted of more protein than the wheat flour control (Table 4.1), the molecular structures of pulse proteins were distinctly different from the gluten protein in the hard wheat flour. Therefore, the incorporation of raw and germinated yellow pea and faba bean flours was unfavorable for the formation of a strong gluten network. Also, a greater decreasing effect was observed with a higher use level of pulse flours.

Table 4.5 Frequency sweep data of wheat flour control and those blended with raw and germinated pulse flours at two ratios[1]

Flour	1 Hz				10 Hz			
	G' (kPa)	G" (kPa)	tan δ	G* (kPa)	G' (kPa)	G" (kPa)	tan δ	G* (kPa)
Wheat control	$12.46 \pm 0.59^{a(AB)}$	$4.51 \pm 0.20^{a(A)}$	$0.36 \pm 0.00^{a(A)}$	$13.25 \pm 0.63^{a(AB)}$	$19.97 \pm 0.81^{a(A)}$	$8.38 \pm 0.32^{a(A)}$	$0.42 \pm 0.00^{a(A)}$	$21.66 \pm 0.87^{a(AB)}$
Yellow pea (YP)								
R:W (10:90)	11.30 ± 0.73^{a}	4.25 ± 0.30^{a}	0.38 ± 0.00^{b}	12.07 ± 0.79^{a}	18.22 ± 1.22^{a}	7.88 ± 0.56^{a}	0.43 ± 0.00^{b}	19.85 ± 1.34^{a}
G:W (10:90)	12.49 ± 0.79^{a}	4.71 ± 0.25^{a}	0.38 ± 0.00^{b}	13.35 ± 0.83^{a}	20.20 ± 1.13^{a}	8.73 ± 0.44^{a}	0.43 ± 0.00^{b}	22.00 ± 1.21^{a}
R:W (20:80)	11.52 ± 1.02^{a}	4.60 ± 0.50^{a}	0.40 ± 0.01^{c}	12.40 ± 1.13^{a}	19.06 ± 1.83^{a}	8.60 ± 1.01^{a}	0.45 ± 0.01^{c}	20.91 ± 2.09^{a}
G:W (20:80)	15.78 ± 1.16^{b}	6.02 ± 0.42^{b}	0.38 ± 0.00^{b}	16.89 ± 1.23^{b}	25.46 ± 1.93^{b}	11.01 ± 0.80^{b}	0.43 ± 0.00^{b}	27.74 ± 2.09^{b}
Faba bean (FB)								
R:W (10:90)	$11.65 \pm 0.72^{(AB)}$	$4.75 \pm 0.30^{(AB)}$	$0.41 \pm 0.01^{(CD)}$	$12.58 \pm 0.77^{(AB)}$	$20.01 \pm 1.10^{(AB)}$	$9.28 \pm 0.59^{(AB)}$	$0.46 \pm 0.01^{(BC)}$	$22.05 \pm 1.25^{(AB)}$
G:W (10:90)	$10.57 \pm 0.94^{(A)}$	$4.16 \pm 0.39^{(A)}$	$0.39 \pm 0.00^{(B)}$	$11.36 \pm 1.02^{(A)}$	$17.51 \pm 1.60^{(A)}$	$7.93 \pm 0.80^{(A)}$	$0.45 \pm 0.01^{(B)}$	$19.23 \pm 1.78^{(A)}$
R:W (20:80)	$10.33 \pm 0.97^{(A)}$	$4.29 \pm 0.39^{(A)}$	$0.42 \pm 0.00^{(D)}$	$11.19 \pm 1.05^{(A)}$	$17.68 \pm 1.66^{(A)}$	$8.33 \pm 0.75^{(AB)}$	$0.47 \pm 0.00^{(C)}$	$19.55 \pm 1.82^{(A)}$
G:W (20:80)	$13.37 \pm 0.94^{(B)}$	$5.34 \pm 0.40^{(B)}$	$0.40 \pm 0.00^{(C)}$	$14.39 \pm 1.02^{(B)}$	$22.52 \pm 1.86^{(B)}$	$10.28 \pm 0.86^{(B)}$	$0.46 \pm 0.00^{(B)}$	$24.76 \pm 2.05^{(A)}$

[1] Values are presented as average ± standard deviation (n = 3); R: raw, G: germinated, W: wheat; lowercase and uppercase letters represent statistical differences for YP and FB, respectively; the numbers with the same letter are not significantly different at $p < 0.05$.

Table 4.6 Creep recovery data of wheat flour control and those blended with raw and germinated pulse flours at two ratios[1]

Flour	J_{max} (1/Pa × 10^{-3})	J_r (1/Pa × 10^{-3})	J_{el} (× 10^{-3})
Wheat control	$1.0 \pm 0.1^{a(A)}$	$0.6 \pm 0.0^{a(A)}$	$643.5 \pm 10.6^{c(D)}$
Yellow pea (YP)			
R:W (10:90)	1.7 ± 0.4^{b}	1.0 ± 0.2^{a}	550.7 ± 24.1^{b}
G:W (10:90)	1.3 ± 0.2^{b}	0.7 ± 0.2^{a}	551.1 ± 80.3^{b}
R:W (20:80)	25.7 ± 6.2^{d}	1.1 ± 0.1^{a}	43.0 ± 10.1^{a}
G:W (20:80)	13.7 ± 5.5^{c}	0.8 ± 0.1^{a}	60.9 ± 20.7^{a}
Faba bean (FB)			
R:W (10:90)	$1.7 \pm 0.2^{(B)}$	$0.9 \pm 0.0^{(B)}$	$521.6 \pm 24.8^{(BC)}$
G:W (10:90)	$1.8 \pm 0.4^{(B)}$	$1.0 \pm 0.2^{(BC)}$	$558.7 \pm 6.4^{(C)}$
R:W (20:80)	$4.8 \pm 0.9^{(C)}$	$1.2 \pm 0.1^{(C)}$	$219.3 \pm 1.4^{(A)}$
G:W (20:80)	$2.0 \pm 0.5^{(B)}$	$0.9 \pm 0.2^{(BC)}$	$466.2 \pm 49.3^{(B)}$

[1] Values are presented as average ± standard deviation (n = 3); R: raw, G: germinated, W: wheat; lowercase and uppercase letters represent statistical differences for YP and FB, respectively; the numbers with the same letter are not significantly different at $p < 0.05$.

4.3.3 Baking performance

Bread volume: With the inclusion of the raw/germinated pulse flours, weaker bread doughs were formed (Table 4.6) and the resultant doughs possessed very poor handling properties. Thus, 2% wheat gluten (on a 200.0 g flour weight basis) was added to the doughs obtained from the raw/germinated pulse-wheat blends in order to bake breads having acceptable quality. Despite the use of 2% extra wheat gluten, all the breads prepared from the composite flours showed smaller loaf volumes (846.2-954.8 cm^3 with yellow pea flour and 852.7-942.9 cm^3 with faba bean flour; Table 4.7 and Figure 4.2) and slightly higher loaf weights (138.6-142.0 g with yellow pea flour and 137.7-140.1 g with faba bean flour) than the 100% wheat bread control (992.5 cm^3 and 137.3 g, respectively). Consequently, the specific volumes of the breads from the flour blends (6.0-6.9 cm^3/g with yellow pea flour and 6.1-6.8 cm^3/g with faba bean flour) were significantly lower than that of the control bread (7.3 cm^3/g). In general, the largest loaf weight and smallest loaf volume were identified with the composite flours containing 20% germinated yellow pea and faba bean flours.

Crumb structure: The slice area was found to be the highest for 100% wheat bread control (7527.3 mm^2), and the inclusion of raw/germinated pulse flours in the wheat flour bread formulation reduced the slice area (6364.6-7093.0 mm^2 with yellow pea flour and 6291.6-6902.7 mm^2 with faba bean flour). The use of raw/germinated yellow pea and faba bean flours at 10% level did not significantly alter the number of cells per slice, but the use at 20% level decreased this value. Overall, the smallest slice area and number of cells per slice were observed with the composite flours containing 20% germinated yellow pea and faba bean flours.

Textural properties of the crumb: In accordance with the observed differences in the bread volume and crumb structure, the breads generated from the flour blends exhibited firmer crumb texture (240.3-345.6 g with yellow pea flour and 232.0-295.8 g with faba bean flour) than that of the 100% wheat bread control (198.8 g). The bread crumb firmness increased with a greater use level of pulse flours. The largest crumb firmness was identified with the breads prepared with 20% germinated yellow pea and faba bean flours.

Compared with the control bread, the incorporation of pulse flours progressively increased the loaf weight but reduced the loaf volume, specific volume, and number of cells per slice. The results suggested that the bread samples baked from the composite flours – despite the addition of 2% wheat gluten – did not achieve the same volume as the control bread baked from 100% hard

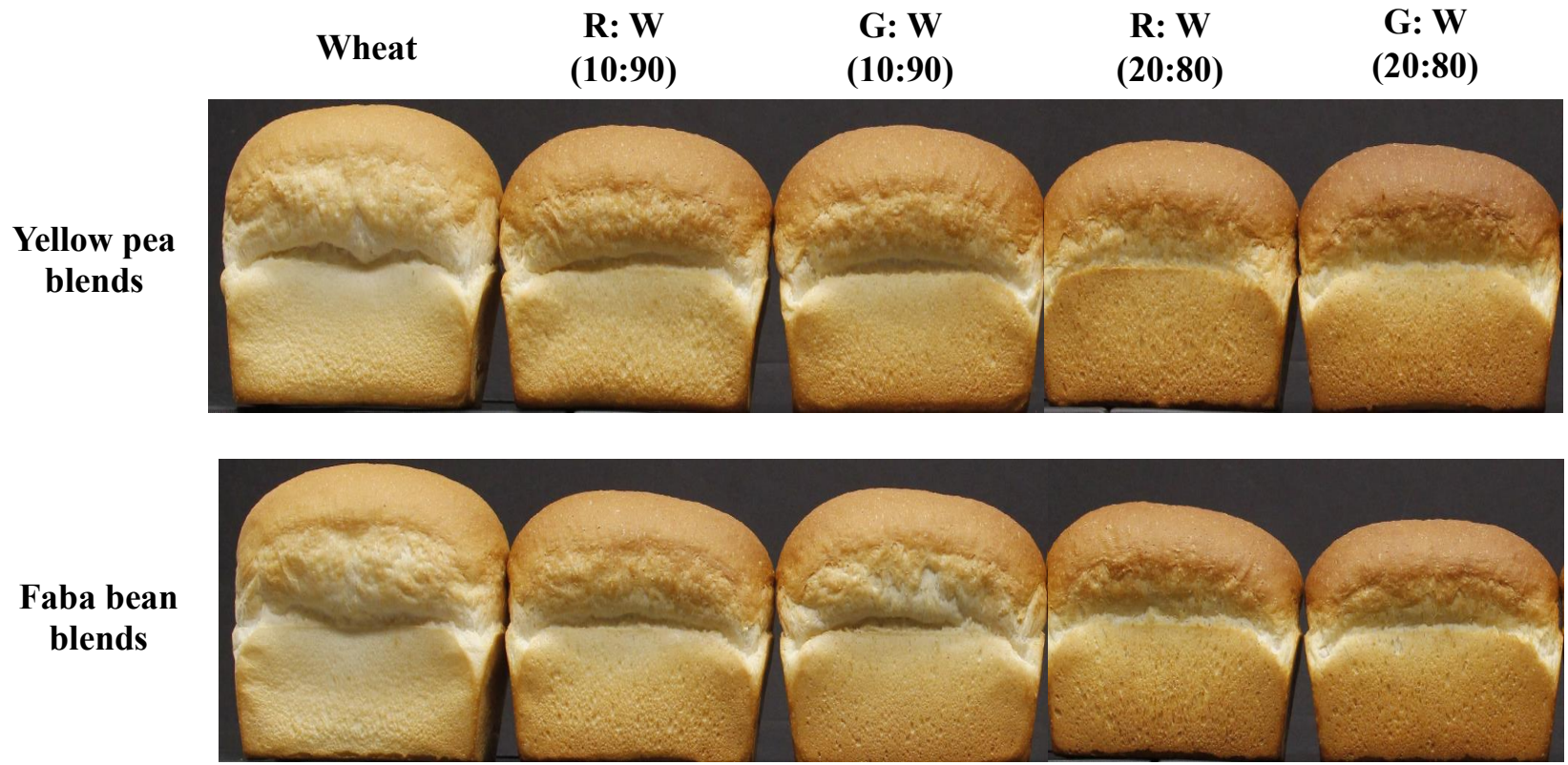

Figure 4.2. Baked bread images of 100% wheat control, raw and 72-h germinated yellow pea and faba bean blends at 10% and 20% incorporation level, respectively; R: raw, W: wheat and G: germinated.

Table 4.7 Characteristics of bread baked from wheat flour control and those blended with raw and germinated pulse flours at two ratios[1]

Flour	Loaf weight (g)	Loaf volume (cm^3)	Specific volume (cm^3/g)	Slice area (mm^2)	Number of cells per slice	Crumb firmness (g)
Wheat control	$137.3 \pm 0.3^{a(A)}$	$992.5 \pm 23.5^{c(C)}$	$7.3 \pm 0.2^{d(C)}$	$7527.3 \pm 190.0^{d(C)}$	$3667.8 \pm 173.8^{b(B)}$	$198.8 \pm 19.8^{a(A)}$
Yellow pea (YP)						
R:W (10:90)	138.6 ± 0.2^{b}	954.8 ± 30.5^{c}	6.9 ± 0.2^{c}	7093.0 ± 218.8^{c}	3708.9 ± 99.3^{b}	240.3 ± 24.9^{b}
G:W (10:90)	139.9 ± 0.4^{c}	939.9 ± 9.2^{bc}	6.7 ± 0.1^{bc}	6961.5 ± 97.0^{bc}	3744.9 ± 72.3^{b}	261.8 ± 8.0^{b}
R:W (20:80)	140.4 ± 0.3^{c}	895.2 ± 13.0^{ab}	6.4 ± 0.1^{b}	6672.4 ± 89.7^{ab}	3417.6 ± 81.5^{a}	285.9 ± 21.8^{bc}
G:W (20:80)	142.0 ± 0.3^{d}	846.2 ± 16.7^{a}	6.0 ± 0.1^{a}	6364.6 ± 101.0^{a}	3259.6 ± 99.4^{a}	345.6 ± 31.2^{c}
Faba bean (FB)						
R:W (10:90)	$137.7 \pm 0.4^{(A)}$	$933.8 \pm 9.9^{(B)}$	$6.8 \pm 0.1^{(B)}$	$6902.7 \pm 43.7^{(B)}$	$3452.0 \pm 123.3^{(B)}$	$232.0 \pm 15.1^{(B)}$
G:W (10:90)	$138.1 \pm 0.8^{(A)}$	$942.0 \pm 7.7^{(B)}$	$6.8 \pm 0.1^{(B)}$	$6931.2 \pm 115.4^{(B)}$	$3320.0 \pm 104.9^{(AB)}$	$239.2 \pm 19.5^{(B)}$
R:W (20:80)	$138.8 \pm 0.8^{(AB)}$	$903.8 \pm 22.8^{(B)}$	$6.5 \pm 0.2^{(B)}$	$6544.9 \pm 178.1^{(A)}$	$3162.8 \pm 166.0^{(AB)}$	$252.7 \pm 38.3^{(B)}$
G:W (20:80)	$140.1 \pm 0.2^{(B)}$	$852.7 \pm 18.0^{(A)}$	$6.1 \pm 0.1^{(A)}$	$6291.6 \pm 103.9^{(A)}$	$3030.6 \pm 144.4^{(A)}$	$295.8 \pm 25.6^{(B)}$

[1] Values are presented as average ± standard deviation (n = 3); R: raw, G: germinated, W: wheat; lowercase and uppercase letters represent statistical differences for YP and FB, respectively; the numbers with the same letter are not significantly different at $p < 0.05$.

wheat flour, which could be attributed to the weaker dough structure with the inclusion of pulse flours as illustrated by farinograph and dough rheology tests. Consequently, the firmness of the breads baked from the flour blends gradually increased. It is important to note that the use of 20% germinated yellow pea and faba bean flours demonstrated the most significant effects on the aforementioned quality attributes of the obtained breads. The data could be ascribed to the presence of endogenous amylases and proteases in the germinated yellow pea and faba bean flours (Ghavidel and Davoodi, 2011; Setia et al., 2019). These endogenous enzymes could efficiently hydrolyze both starch and gluten in the flour during the dough preparation and bread making, and the degraded starch and gluten molecules failed to support the expansion of bread dough for a larger volume and lighter texture. This explanation agrees well with the slightly darker crust color of the breads baked from the germinated pulse-wheat flour blends than the counterparts with raw pulse flours (Figure 4.2). The darker crust color of the former was clearly a sign of a greater extent of Maillard reaction between increased proportions of carbohydrates with a reducing end and proteins with a free amino group as a result of the abovementioned endogenous enzyme hydrolysis of starch and protein (Martins et al., 2000).

4.4 Conclusions

The effects of replacing hard wheat flour with raw and 72-h germinated yellow pea and faba bean flours at 10% and 20% levels on the flour properties, dough quality and bread making performance of the resultant flour blends were investigated in the current study. The pulse-wheat flour blends showed decreased starch contents but increased protein and ash contents due to the compositional differences between the pulse and wheat flours. The composite flours with both yellow pea and faba bean displayed pasting viscosities lower than that of the 100% wheat flour control, and a greater extent of reduction was observed with the use of germinated pulse flours due to the presence of endogenous amylases. Consequently, the germinated pulse-wheat flours also possessed noticeably lower falling numbers. Because of the absence of gluten protein in the raw and germinated yellow pea and faba bean flours, the composite flours showed lower 5% lactic acid solvent retention capacity and smaller gluten performance index, which corresponded well with the weaker network structure of their resultant bread doughs as revealed by farinograph and dough rheology tests. Consistent with the changes in flour

properties and dough quality, the breads baked from the composite flours largely exhibited reduced loaf volumes and decreased numbers of cells per slice, but increased crumb firmness, with more obvious changes being found with the germinated pulse-wheat flour blends. The findings from this research greatly advanced our understanding of the roles that both raw and germinated pulse flours played in flour and dough systems after being composited with hard wheat flour, as well as their influence on the quality attributes of the derived bread products. The presented data also suggested that the inclusion of germinated pulse flours could be more desirable for bakery products that do not require a strong gluten structure, such as cookies and cakes (Tacer-Caba et al., 2015).

5. GENERAL DISCUSSION

The focus of the present research was to investigate the impacts of germination on the functional properties and nutritional quality of yellow pea and faba bean flours and to explore the potential of raw and germinated pulse flours in food products, particularly their performance in bread making. For Study 1, samples of two different pulses, yellow pea (CDC Amarillo) and faba bean (CDC Snowdrop) were obtained and then subjected to germination for 24, 48 and 72 h in the dark at room temperature. The resultant seeds were then dried and milled into flours before further analyses of their functional and nutritional properties. During this short period of germination, germination percentages higher than 97% were achieved with progressive growth of radicles for both yellow pea and faba bean (Table 3.1). The α-amylase activity of raw and germinated yellow pea and faba bean flours did not change significantly after soaking but increased remarkably with the increase in germination duration, especially for yellow pea (Table 3.1). The SEM images in Figure 3.1 and 3.2 showed that the starch was compactly embedded within the protein and fibre matrix. This matrix was disrupted and became loose after soaking, allowing some of the starch granules to be liberated and became more visible. Germination resulted in the breakdown of protein and fibre by their respective hydrolytic enzymes, causing the release of more starch granules from the disrupted matrix. Germination did not noticeably alter the chemical composition of the pulse flours (Table 3.2), but resulted in significant changes in their physicochemical properties. It decreased the conclusion temperature and enthalpy change of the thermal transition peak (Table 3.3), which could be attributed mainly to changes in protein, making it more susceptible to thermal denaturation. Moreover, starch granules in soaked pulse flours swelled to a greater extent and provided higher viscosity as compared to their raw counterparts (Table 3.4 and Figure 3.3). Germination for 24-h further elevated the viscosity due to more disruption of the matrix structure. Interestingly, due to enhanced α-amylase activity, a substantial decrease was observed in the viscosity for the 48-h and 72-h germinated pulse flours. Furthermore, germination tended to enhance protein solubility and water-holding capacity of germinated pulse flours (Table 3.5). It might also lead to partial unfolding of proteins and their dissociation to enhance their

surface activity, which improved the emulsification and foaming properties of the germinated pulse flours as compared to their raw counterparts. In terms of nutritional quality, germination enhanced the digestibility of starch and protein of the pulse flours (Table 3.6), which could be due to the increased hydrolysis of these macromolecules by endogenous enzymes, rendering them more susceptible to enzymatic digestion. However, germination did not improve the *in vitro* protein digestibility corrected amino acid scores (IV-PDCAAS) of the pulse flours. The essential amino acid score was lower for the germinated pulse flours than the corresponding raw flours, indicating that germination did not necessarily improve the nutritional quality of protein in the present research.

Study 2 of this thesis involved the use of raw and 72-h germinated yellow pea and faba bean flours from Study 1 in bread making. These pulse flours were used to replace 10 or 20% of the hard wheat flour in the formula of a pan bread. The flour properties, dough properties, and baking performance of the composite flours were then determined in comparison to the 100% wheat flour formula, which served as a control. In general, the substitution of wheat flour at 10 and 20% with the raw and germinated pulse flours increased the protein and ash contents but diminished the starch contents of the pulse-wheat composite flours (Table 4.1). The pasting viscosities of the pulse-wheat flours blends decreased as compared to the wheat flour control (Table 4.2 and Figure 4.1). The addition of 72-h germinated pulses further decreased the viscosity development, consistent with the increased activity of α-amylase as reported in Study 1. This also led to a reduction in falling number values for germinated pulse-wheat composite flours (Table 4.2). The inclusion of raw and germinated pulse flours had a negative impact on 5% lactic acid SRC and GPI values (Table 4.3). This could be due to the dilution of gluten by pulse proteins that caused a weakened dough structure. The weakening of the gluten network and the inclusion of pulse protein into gluten also lowered the farinograph water absorption, dough development time and dough stability, but increased the mixing tolerance index (Table 4.4). The reduction in the loss tangent, complex modulus and relative elasticity values (Table 4.5 and 4.6) from rheology results confirmed the decreased dough strength, weakened gluten network and stickier dough with the incorporation of pulse flours. Overall, the use of 72-h germinated pulse flours showed more obvious negative effects on these functional attributes. In terms of baking performance, the addition of pulse flours, especially germinated pulse flours, diminished the quality attributes of bread in comparison with the control prepared from 100% wheat flour. The breads prepared from

the flour blends were found to have lower loaf volumes, resulting from lower gas entrapment in the weaker gluten protein network. In addition, the breads prepared from pulse-wheat composite flours showed a denser structure by having a decreased slice area with a lower number of cells (Table 4.7). Moreover, the bread crust became darker and the crumb became yellowish in colour due to a greater extent of Maillard reaction as a result of increased proportions of carbohydrates with a reducing end and proteins with a free amino group from endogenous enzyme hydrolysis available for the browning reaction. Overall, the inclusion of germinated pulse flours did not contribute to improvement in the quality of baked bread. However, they could be used for some other baking applications – such as cookies and cakes – that do not require a strong gluten structure.

6. OVERALL CONCLUSIONS

The overarching goal of this project was to determine the impacts of germination for different times (24, 48 and 72 h) on the chemical composition, functional properties and nutritional value of yellow pea and faba bean flours, as well as to examine the effects of replacing 10 and 20% of wheat flour with 72-h germinated pulse flours on flour properties, dough quality and bread making performance. In the first study, pulses were germinated for different durations (24, 48 and 72 h), and then dried and milled into flours for further analysis. Overall, germination was found to be an effective approach to prepare pulse flours with varied functional properties and improved nutritional attributes. Little change was observed in chemical composition, whereas the conclusion temperature (T_c) and enthalpy change (ΔH) values in the thermal transition peaks for flours from both pulses were found to be lowered significantly by the germination treatment. The disruption of the protein-fibre matrix surrounding the starch granules during soaking and the earlier stage of germination (first 24-h time period) was responsible for a substantial increase in pasting viscosities of flours from both pulses as the starch granules could swell to a greater extent. The prolonged germination periods (48 and 72 h) markedly decreased the pasting viscosities due to the breakdown of starch molecules by endogenous alpha-amylase during heating in the RVA. Other functional properties, such as water-holding capacity and emulsification and foaming properties, were improved with the increase in germination period for flours from both pulses. In addition to the improvement in the functional properties, germination significantly enhanced the nutritional attributes of the pulse flours, which included improving the *in vitro* starch and protein digestibilities. Nevertheless, germination did not improve the *in vitro* protein digestibility corrected amino acid scores (IV-PDCAAS) of the resultant pulse flours. Study 1 significantly advanced our understanding of the influence of soaking and short-term germination (24-72 h) on the chemical composition, functional properties and nutritional value of yellow pea and faba bean flours.

In the second study, 72-h germinated pulse flours were blended with wheat flour at 10 and 20% incorporation levels to prepare composite flours for subsequent analyses. In general, the inclusion of raw and germinated pulse flours decreased the contents of starch and increased the

contents of ash and protein in comparison with the wheat flour control. Pasting viscosities of the control wheat flour were higher than that of the raw pulse-wheat composite flours, owing to the higher starch content of wheat flour. The use of germinated pulse flours further reduced the pasting viscosities due to the increased hydrolysis of starch by endogenous alpha-amylase during heating in the RVA. A similar decrease in falling number due to increased alpha-amylase activity was observed for germinated pulse-wheat composite flours in comparison to their raw counterparts and the wheat flour control. No significant differences were observed in the SRC of water and 50% sodium carbonate, whereas considerable decreases were observed in 5% lactic acid SRC and 50% sucrose SRC and GPI after the incorporation of raw/germinated pulse flours. The results could be due to the weakening of the gluten network with the addition of pulse flours as compared to the 100% wheat flour control. Dough rheology data indicated that the inclusion of raw or germinated yellow pea and faba bean flours made the doughs less elastic with weakened gluten strength. The use of raw/germinated pulse flours in general adversely affected the baking performance of the composite flours. The inclusion of pulse flours (raw or germinated) gave harder texture to the breads with reduced loaf volumes, and the breads were found to be more compact with lower numbers of finer cells present in the crumb as compared with the 100% wheat flour control bread. This part of the research advanced our knowledge on the flour properties, dough properties and baking performance of hard wheat flour with the replacement of 10 and 20% raw or germinated yellow pea and faba bean flours. The results suggested that the germinated pulse flours might not improve the aforementioned properties, but could improve the nutritional value of the finished products. Probably, it would be a better idea to use germinated pulse flours in cookies, cakes and other products that do not require a strong wheat flour dough.

The acquired new knowledge from this research will be meaningful for the value-added processing industry to employ germination as a useful technology to generate pulse flours with suitable functional and nutritional properties for broader food applications. In addition, the results from this research will be valuable to the food industry to include raw and germinated pulse flours into the formulae of bread and other bakery products to manufacture final products with acceptable quality and enhanced nutritional value to the consumers.

7. FUTURE STUDIES

Pulses are a good source of plant proteins, which are consumed widely across the globe. They have the potential to be used to combat malnutrition issues in underdeveloped and developing countries, and are the meatless and gluten-free alternative for vegetarian and no-gluten diets. However, pulse proteins and starch are comparatively harder to digest and have lower vitamin and mineral bioavailability due to the presence of anti-nutrients. They also have harder cell walls, which makes them difficult to cook. Furthermore, pulses have strong and pungent flavour profiles that render them less palatable, thus facing challenges when it comes to consumer acceptance. To address the aforementioned drawbacks, the present study involved the use of germination as a promising approach to enhancing the nutritional, functional and baking performance of pulse flours after being subjected to germination.

Although Study 1 of this research covered most important effects of germination on the functional properties and nutritional value of starch and protein in the pulse flours, more should be investigated in terms of how germination for different periods alters the structure of proteins, which could provide a better idea about how this treatment can affect functional properties. Moreover, the particle size distributions of the raw, soaked and germinated pulse flours also should be investigated as the particle size of flour can significantly influence its functional attributes. Future studies should also look into the isolation of starch and protein from germinated pulse seeds/flours and the characterization of their respective changes in quality and functionality. Furthermore, the analyses of anti-oxidant activity, anti-nutrients, and phenolic and other bioactive compounds should be taken into consideration to provide additional insights into the effect of germination on the nutritional quality of pulse flours. Additionally, flavour and sensory tests of geminated pulse flours or the products derived from them should be carried out to generate more information about their palatability and overall consumer acceptance. Finally, future studies must

include some microbiological tests to determine the quality and safety of the germinated pulse flours and seeds to be used in food products which are meant for human consumption.

Study 2 of this research concentrated on the application and performance of the germinated pulse flours in food products, with a particular focus on a model dough/bread system. The addition of germinated pulse flours was proved to be detrimental for the bread making application. Future studies can look into the application value of raw and germinated pulse-wheat flour blends in other food products, such as cookies, cakes, noodles, pasta and extruded foods. In addition, nutritional studies should be carried out to evaluate the health benefits of various food products with the incorporation of raw/germinated pulse flours, which would provide meaningful fundamental knowledge for broader application of pulse-based ingredients to promote consumer health.

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