EFFECTS OF SHORT-TERM GERMINATION AND AUTOCLAVING ON SELECTED COMPOUNDS IN FABA BEAN AND FABA BEAN APPLICATIONS IN LOW-FAT PORK BOLOGNA

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ABSTRACT

The overall goal of this project was to investigate the levels of selected chemical components of faba bean (*Vicia faba*) seeds produced in North America, and the performance of ingredients derived from faba bean in low-fat pork bologna. This project was divided into two studies. In study I, protein, fat, ash, total starch, and total dietary fibre (TDF) were determined in five faba bean cultivars: Snowdrop, Snowbird, Taboar, Fabelle, and Malik, that were produced in North America from three lots representing biological replicates. The effect of short-term germination and autoclaving on the level of undesired compounds, including phytic acid, vicine/convicine, oligosaccharides, and total phenolics, were also evaluated.

Faba bean varieties had 30-33% protein, 3-4% ash, ~1% fat, 35-40% starch, and 25-27% total dietary fibre. Phytic acid in faba bean was 1.6-1.8% with no statistically significant difference between varieties. Seeds of Fabelle variety had the lowest (p<0.05) vicine and convicine content. No significant difference was found among cultivars for the levels of oligosaccharides. The contents of verbascose (37.5-64.0 mg/g) and stachyose (20.4-25.5 mg/g) were higher than raffinose (8.7-8.9 mg/g) content for all faba bean cultivars. Content of total phenolics among faba bean varieties was significantly (p<0.05) different. Snowdrop and Snowbird had the highest content of total phenolics, while Fabelle had the lowest values for total phenolic content. Germination had no significant effect on phytic acid, vicine (except Snowbird), and convicine levels in faba bean seeds. The oligosaccharides showed significant (p<0.05) reduction upon germination: raffinose by 100%, stachyose by 60%, verbascose by 80% at 72-hour of germination. Upon germination, total phenolic content in Snowdrop and Snowbird showed significant reduction but not Taboar, Fabelle, and Malik varieties. Autoclaving did not present a significant (p<0.05) effect on phytic acid, vicine /convicine, and oligosaccharides while the total phenolic content showed a significant reduction (15-23%) after autoclaving, especially for the varieties of Taboar, Snowbird, and Malik.

In study II, six binders, including wheat flour, pea starch, cotyledon flour (Malik), cotyledon flour (Fabelle), faba bean starch fraction (commercial), and faba bean protein fraction (commercial), were used in production of low-fat pork bologna with two (1.5 and 3%) incorporation levels. Further their chemical composition and functional properties were

investigated, and the physicochemical, textural, and sensory properties of bologna were evaluated. All binders significantly (p<0.05) increased viscosity of the raw meat batters. The cook loss of all bologna products with binders was low (0.5%, w/w) and no significant difference on purge loss among all binders was observed. Products with 3% wheat flour and faba bean protein fraction had significantly lower expressible moisture values when compared to the control. The colour parameters of cooked bologna surface were in a narrow range of L* (69-70), a* (16-18), or b* value (14-16). The texture profile analysis (TPA) showed binders did not change the hardness, cohesiveness, springiness, and chewiness of bologna compared to the control. Bologna with 3% of wheat flour significantly (p<0.05) increased the torsional true stress at failure compared to the control product without a binder while only a limited effect on shear strain was observed. Sensory evaluation of these products by a 12-member trained panel indicated that that the addition of 3% wheat flour resulted in significantly higher sensory firmness scores than the control. Except the products containing Malik cotyledon flour, addition of other binders at 3% significantly (p<0.05) reduced the perception of juiciness of bologna. Products containing binders at 1.5 or 3% showed no effect on graininess and overall flavour intensity scores except the bologna with 3% of Fabelle cotyledon flour which had significantly (p<0.05) increased intensity of foreign flavour. No significant difference was found in overall acceptability among bologna with any of the binders at either level. Faba bean ingredients showed similar effect on textural and sensory properties of bologna products as compared to reference binder ingredients, showing their potential in low-fat meat products.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

*a**	Redness
AACC	American Association of Cereal Chemists
AAFC	Agri-Food Agriculture Canada
AGT	Alliance Grain Traders
AOCS	American Oil Chemist's Society
*b**	Yellowness
CDC	Crop Development Centre
CFIA	Canadian Food Inspection Agency
CIE	International Commission on Illumination
cP	Centipoise
FB	Faba bean
g	Gravitational Force
IDF	Insoluble Dietary Fibre
*L**	Lightness
N	Newton
OAC	Oil Absorption Capacity
p	Probability
RFO	Raffinose Family Oligosaccharide
RVA	Rapid Visco Analyzer
SAS	Statistical Analysis System
SD	Standard Deviation
SDF	Soluble Dietary Fibre
STPP	Sodium Tripolyphosphate
TDF	Total Dietary Fibre
TPA	Textural Profile Analysis
TSW	Thousand Seed Weight (g)
v	Volume
WHC	Water Holding Capacity

1 INTRODUCTION

1.1 Overview

Faba bean (FB), also known as broad bean, fava bean, field bean or horse bean, offers a valuable cover crop and seeds for food and feed. This annual plant requires cool temperatures for growth and can be sustained with a low water requirement (Turco et al., 2016). Canadian prairies provide ideal growing conditions for FB plants. Canada is one of the emerging FB producers in the world by producing 160,000 tones in 2015 while the estimated world production was 4.3 million tons (Oomah et al., 2015; McGill et al., 2017). FB seed provides adequate amount of macronutrients and particularly, higher protein content compared to other *Leguminosae* family plants (Duranti, 2006). According to Bhatty (1974), the early FB cultivars (12) grown in Canada had protein content ranging from 26 to 35% while Boye et al. (2010) and Hood-Niefer et al. (2010) reported values of ~30% for the recent varieties.

Presence of various undesirable chemical compounds such as phytic acid, vicine/convicine, oligosaccharides, and phenolics have limited the use of FB in animal feed and human food. In recent years, the Canadian FB breeding programs have considered reducing the contents of tannin, vicine and convicine in developing new varieties. By mutation in the *vc*-gene that controls vicine and convicine expression in FB, a 10 to 20-fold reduction has been obtained (Duc et al., 1989) without modifying the macronutrient content. Additionally, *zt1* and *zt2* genes were identified in controlling the accumulation of tannins in FB seeds (Duc et al., 1999). The low tannin varieties such as Snowdrop and Snowbird have cream and light seed coat and tannin levels as low as 1%. Low-tannin varieties are suitable for feed due to increased protein digestibility compared to high tannin varieties (Duc et al., 1999). Besides the effort through long-term breeding, different post-harvest processing treatments to reduce or eliminate these undesired compounds have been studied. Germination of seeds is a natural process and may be effective in reducing undesired compounds due to activation or degradation by various *de novo* synthesised enzymes associated with biochemical processes (Haileslassie et al., 2016). As a biomaterial conversion process, seed germination has minimum equipment requirement. Heat

processing has also been studied to reduce the level of undesired compounds in FB seeds but the effects on reduction were not conclusive (Hefni et al., 2015; Mozzoni et al., 2009). Lactic acid bacteria fermentation was a successful process to improve the quality of pulse-based food products. Tannin content could be reduced significantly under the effect of fermentation (Coda et al., 2015).

Reduction of fat intake by consuming low-fat products is a popular consumer trend that exists today. In formulating low-fat processed meat products, direct substitution of fat with water leads to softer texture of the final product, increased cook loss during heat processing, and purge loss during the long-term storage (Claus & Hunt, 1991; Shand, 2000). Use of plant-based binders to improve the texture of the meat products is commonly practiced. Faba bean ingredients such as flours contain high amount of protein and starch (Multari et al., 2015) making them potential candidates to be incorporated into low-fat meat product formulations.

The goal of this project was to investigate selected chemical compounds in cultivars of FB seed grown in North America. Moreover, the effect of short-term germination (up to 72 h) and autoclaving on the levels of undesired compounds in FB was also evaluated. In the second part of the study, ingredients derived from FB and pea starch fraction and wheat flour (reference ingredients as plant binders) were incorporated in a low-fat pork bologna product. The effect of binders on physicochemical, textural, and sensory properties of the pork bologna was determined.

1.2 Hypotheses

1. Different cultivars of FB had similar proximate and carbohydrate content.
2. The cultivar has an effect on the content of undesired compounds of FB.
3. Short-term germination and autoclaving could reduce the level of undesired compounds of FB.
4. Incorporation of FB ingredients could improve the physicochemical, textural, and sensory properties of low-fat pork bologna.

1.3 Objectives

1. Provide composition data of five cultivars of FB produced in North America; Fabelle, Malik, Snowbird, Snowdrop and Taboar.
2. Evaluate the effect of short-term germination and autoclaving on the levels of undesired compounds in FB.

3. Evaluate the feasibility and potential of utilizing FB ingredients as binders in bologna products.
4. Compare sensory properties of FB ingredient-containing low-fat pork bologna with similar products prepared with commercially available plant binders.

2 LITERATURE REVIEW

2.1 Faba bean as a crop

Faba bean (*Vicia faba* L.) is a legume and a vetch but not a true bean. The plant is an annual crop, generally requires cool temperatures for growth and grows under minimum water requirement (Multari et al., 2015). Faba bean (FB) is also referred to as broad bean, fava bean, horse-bean and feed bean and it is a cool season, commercially cultivated grain legume and a cover crop. The center of origin of FB is not conclusive but considered to be the Near East. The subspecies *V. faba paucijuga* which is found in the geographical region from Afghanistan to India at present is probably the primitive form of the cultivated species (Duc, 1999). Faba bean grows well in the Mediterranean region where it is a common human food. In Europe, FB is primarily produced for livestock feed because of the high seed protein content. Among European countries, Britain is the largest producer of FB (Multari et al., 2015). Canada is one of the emerging producers of FB for the global market and most of production occurred in the prairie provinces (Government of Saskatchewan, 2016).

In 2016, the worldwide production of FB dry grains was 4.6 million tons over the cultivated areas of 2.4 million hectors (FAOSTAT, 2016). The main world producer of FB was China with a production of 1.6 million tons from 806,900 hectors of cultivated area (FAOSTAT, 2016). Commercial production in western Canada started in 1972 and since then the area under production has fluctuated (Government of Saskatchewan, 2018). In Canada, FB is mainly grown in the provinces of Alberta, Saskatchewan and Manitoba. In 2016 the total FB cultivated area in the three provinces was 114,500 acres (46,340 hectares) (Phelps, 2017). Canadian faba bean exports decreased in 2016 and 2017 compared to 2014 and 2015. The total production of faba bean in 2016 to 2017 was around 14,000 tons, compared to 24,000-25,000 tons in the previous two years. Mainly this is because of renewed competition from Australia, along with the recent emergence of Baltic countries as competitors in the key Egyptian market (Penner, 2017). The

reduced Canadian export means the domestic consumption of FB has increased in 2016, mostly in animal feed.

The FB plant grows upright, ranging from 1.0 to 1.5 meters tall. It has strong, hollow, erect stems, a strong tap root, compound leaves, large white flowers with dark purple markings and produces pods containing 3-8 seeds. FB plants flower in 45-60 days and seeds requires 110-130 days to mature after planting (Government of Saskatchewan, 2016). The green coloured pods are large (approximately 10 cm long and 2 cm wide) and turn from brown to black at maturity. Seeds can be yellow, green, brown, black, or violet in colour. Faba bean has a distinctive environmental advantage of fixing atmospheric nitrogen more efficiently than other legume species under the same soil and weather conditions (Government of Saskatchewan, 2016).

The Canadian prairies provide ideal growing conditions for the FB crop. It is well adapted to the cooler and wetter regions of prairies which provide adequate soil moisture to reach full yield potential of FB crop. In warmer areas, usually winter planting is done and in northern hemisphere spring is the favored season. As a matter of fact, faba bean is highly sensitive to drought stress (Khan et al., 2010).

Environmental factors could influence chemical component accumulation in FB seeds, such as proline in free form and membrane-bound unsaturated fatty acids which enhance the frost tolerance of the plant (Bhatty, 1974). Multari et al. (2015) showed that in responding to adverse weather conditions, in faba bean leaves and stems, oleic acid was lowered by 3.24% and 1.77% but linolenic acid was increased by 6.28% and 9.06%, respectively. Faba bean protein content is also influenced by environmental factors. Bhatty (1974) reported that the protein content of twelve cultivars of FB grown in the experimental plots at University of Saskatchewan ranged from 26 to 35% but lipid, fibre, and ash content remained constant.

In Canada, small seed FB is preferred for feed uses while large seeds are preferred in the traditional food markets for exporting. Smaller seed varieties such as Snowdrop (335 TSW; grams of thousand seed weight), and Snowbird (495 TSW) consisted 70% Canadian production during 2015 (McGill et al., 2017). For the producer, small and round shape enables the use of existing equipment such as pea seeders and that go through the air seeder easily (Phelps, 2016). Malik (680 TSW) is the only large seed variety produced in a sizable volume in Canada, which took 10% of production in 2015 (McGill et al., 2017).

From a longer-term perspective, Canadian FB production and consumption will continue and expand. If FB supply stays relatively stable, animal feed users will be more comfortable making FB a regular part of their rations, and the higher protein levels can add value to FB. Beyond the feed market, the rapid expansion of pulse protein consumption in human and pet food products could also provide more opportunities but the industry will need to boost processing capacity to build that market.

2.2 Major nutrients in FB seed

2.2.1 Protein

Faba bean seed has one of the highest protein contents among pulses. Faba beans have a long history to be human food and are one of the world's most widely consumed pulses (Mudryj, 2012). The review by Boye et al. (2010) summarized proximate composition of faba bean, protein ~ 30% that is comparable with the protein content of 12 FB cultivars (26 to 35%) reported by Bhatty (1974). Hood-Niefer et al. (2010) also concluded that genotype had a significant effect on protein concentration. In their study, the genotype Gloria had the highest (32.4%) and Florent had the lowest (28.2%) protein content with an average value of 30% among the cultivars.

The two major protein classes found in FB seeds are albumins and globulins. Albumins usually account for most of the regulatory proteins, and globulins account for storage proteins (Makri et al., 2005). Compared to albumins, globulins are salt soluble. Legumin and vicilin are the major globulins found in FB. Faba bean vicilins have several polypeptide fragments (15-66 kDa) which are glycosylated and different from pea vicilins (Lampart-Szczapa, 2002). In general, globulins are built of polymorphic subunits which are encoded by multigene families (Boye et al., 2010). Vicilins have a trimeric structure with 175-180 kDa molecular mass, and legumins have hexametric quaternary structures with 20 kDa alkaline subunits and 40 kDa acidic subunits. The molecular masses of legumins could range from 5 to 80 kDa. Albumins include lectins, amylase inhibitors, protease inhibitors, and enzymatic proteins (Boye et al., 2010).

The nutritional quality of food proteins is particularly determined by the composition of essential amino acids. According to Boye et al. (2010), normally, the pulse proteins including FB proteins are low in methionine, cysteine, and tryptophan but relatively high in lysine, leucine, arginine, glutamic acid, and aspartic acid. The ratio of albumin to globulin proteins of FB may

vary significantly between cultivars. Growing season and geographic location could contribute to the variations in amino acid composition of FB (Boye et al., 2010).

Faba bean can be a more economical high-protein source for food product development. The flour of FB can be a substitute for semolina flour in pasta. The rate of protein enrichment was found to reach 2.25% for every 10% (w/w) of FB flour added in spaghetti (Multari et al., 2015). The FB flour fortified product had higher water absorption and lower dough development ability, compared to the wheat-based product. The sensory evaluation with consumers showed no difference in acceptance between fortified spaghetti and traditional spaghetti. However, the high-water absorption of faba bean flour produced a sticky dough, which is hard to convert to extruded products (Multari et al., 2015). Considering the prevalence of inadequate protein intake among the Canadian population, especially among female seniors (Health Canada, 2016), FB can be a new and economic plant protein source that can be incorporated in developing meat-based products with plant ingredients.

2.2.2 Starch

Starch is the most abundant carbohydrate source in FB seeds. Only a few papers focused on the content and properties of FB starch. Hood-Niefer et al. (2010) reported that starch content in 11 FB cultivars that were produced in Saskatchewan (crop year: 2009) ranged from 41.1 to 47.5%. Amylose contents of these starches ranged from 28.8 to 30.0%. Genotype had significant effect on starch concentration, but not on the amylose concentration in the starch (Hood-Niefer et al., 2010). It is difficult to interpret the variation in properties among pulse starches described currently in the literature. Very little information is available on FB starch properties with respect to different cultivars. Ambigaipalan et al. (2011) had reported the morphology, composition, structure, and physicochemical properties of FB starch isolated from four different cultivars (Fatima, FB 9-4, F 18-20, and SSNS) and the mean apparent amylose content of these four cultivars was 31.78% without any significant difference among cultivars. According to the same study, the confocal laser scanning microscopy examination showed numerous cracks in granules of faba bean starch granules which is related to low granule integrity, so the strain may increase as the granule grows. The gelatinization transition temperature of FB starch (all four cultivars) was low compared to other kinds of starch such as pea starch. Ambigaipalan et al. (2011) have reported mean peak temperature of these four FB cultivars as 69.54 °C; however, higher values of peak temperature (71.9 to 73.3 °C) of same FB cultivars were reported by Hood-

Niefer et al. (2010). Pasting is the phenomenon after the starch is gelatinized. The shear disintegration would occur in starch granules, and the paste obtained on gelatinization was a viscous mass consisting of a continuous phase of amylose and amylopectin (Ambigaipalan et al., 2011). Generally, a lower transition temperature could be associated with presence of shorter amylopectin chains. Faba bean starch paste presented lower viscosity values when compared with other pulse starches (Hood-Niefer et al., 2010). Phosphorous content is another important factor that influence pasting properties of the starch granules. Some plant starches normally have higher content of phosphorous, such as potato starch, therefore the repulsion of ions could weaken the bonding forces between amylopectin clusters, which resulted in a higher starch paste viscosity (Ambigaipalan et al., 2011). The low phosphorous content of FB starch could explain its lower starch paste viscosity.

Ambigaipalan et al. (2011) suggested that FB starch is not suitable for incorporation into food products destined for thermal processing, repeated freezing/thawing, and high shear, since the starch granules show lower integrity. Studying the morphology of starch molecules such as microstructure of amylopectin, chain length of double helices, and number of helices per amylopectin clusters of starch could form the basis for further improvement of FB starch functional properties for food product use (Ambigaipalan et al., 2011). Faba bean starch may be considered as a new ingredient without chemical modifications in food products to generate gels with low-viscosity.

2.2.3 Fibre

Faba bean is rich in fibre that is contributed from seed coat and cotyledon cell walls. Bhatty (1974) analyzed whole seeds of twelve FB cultivars grown in Canada and reported that the total fibre content was in the range of 6.4 to 8.4%. The term dietary fibre was first developed in 1953 by Eben Hipsley to identify the remnant of edible parts of plants that cannot be digested and absorbed in small intestine (Mehta et al., 2015). Dietary fibre can be further classified into insoluble and soluble fibre fractions. This classification is based on solubility in an enzyme solution controlled by various pH during extraction and isolation process. These various pH values in the dietary fibre assay simulate alimentary enzymes in human body (Tosh & Yada, 2010). There is no information specifically on the ratio of soluble/insoluble dietary fibre levels of FB seeds. Only total fibre contents of FB was presented in previous studies.

Soluble and insoluble fibres affect the blood glucose level and insulin sensitivity of individuals by different mechanisms. Primarily, insoluble fibre assists the movement of material through the digestive tract. Soluble fibre helps to regulate blood glucose level and lower blood cholesterol level (Mehta et al., 2015). Chemically, insoluble fibres include cellulose, hemicellulose, lignin and oligosaccharides; pectins, β-glucans, and galactomannan gums are considered soluble fibres (Tosh & Yada, 2010). Soluble fibres are viscous and can form gels in the stomach and small intestine, so that the postprandial glycemic response could be modulated. Three possible mechanisms are described for this modulatory effect by soluble fibre: soluble fibres delay the gastric emptying, in turn a drop in peak glucose concentration occurs (a 35% drop in blood glucose level in subject drank glucose after soluble fibre ingestion); soluble fibre could limit glucose molecules transport through the unstirred water layer; and, soluble fibre could delay small bowel transit and modify the gastrointestinal activity (Mehta et al., 2015). On the contrary, insoluble fibre is non-viscous and shows little influence on postprandial glucose content. Insoluble fibre can increase insulin sensitivity and lowers serum insulin concentration to reduce the risk of diabetes. This change could also be associated with the alterations in gut microbiota. An insoluble fibre could be fermented in large intestine to support the growth of probiotic species (Gao & Yue, 2012).

Fibre can increase stool weight and enhance normal laxation. Adequate fibre intake could also normalize defecation frequency and increase gastrointestinal (GI) transit (Guillon & Champ, 2002). Fibre can increase stool weight by holding water (Quartarone, 2013). As a satiety agent, dietary fibre influences insulin secretion. A high fibre diet could also be a contributor in effective weight management (Mehta et al., 2015). Obesity can increase risks of multiple chronic diseases. Dietary fibre could delay gastric emptying, and therefore provide a sensation of fullness. Dietary fibre itself does not significantly contribute to nutrition and calories, but the functional and metabolic effects they provide make it an important component in healthy human diet. Health Canada suggests fibre intake for men is 30-38 grams of fibre per day while women should take 21-25 grams of fibre per day (Health Canada, 2016). Faba bean could be an economic source to increase the fibre consumption among Canadian populations.

2.3 Major undesired compounds in faba bean seed

2.3.1 Phytic acid

Phytic acid is one of the antinutrient compound reported in FB seeds. In general, phytate collectively describes phytic acid and its salts (Sathe et al., 2002). Phytate is largely stored as a complex salt of K^+, Mg^{2+}, and proteins within globoids, and aleurone grains in seeds. Up to 60-80% of phosphorus present in seeds is in the form of $InsP_6$ (*myo*-inositol hexakisphosphate). Other cations such as calcium, zinc, and iron are also present in measurable quantities. Specific $InsP_6$ can be responsible for signal transduction and cellular regulation (Sathe et al., 2002). Also, $InsP_6$ are involved in discrete processes leading to signal-transducing polyphosphate, such as Ins (1, 4, 5) P_3. Phytates accumulate rapidly during ripening and maturation period of plant seeds and exist in combination with other substances such as proteins, lipids, and starch (Loewus, 2002). The accumulation site of phytate in seeds is within the subcellular single membrane particles, or aleurone grains (Loewus, 2002). The aleurone grains are found in the monocotyledonous seeds such as cereal grains. In legumes (dicotyledonous seeds), globoids are located within the matrix of protein bodies, and are mostly located in cotyledons but not in seed coat. The biogenesis of globoids with the protein matrix is strictly controlled by the potassium, calcium, and magnesium content (Loewus, 2002).

Oomah et al. (2011) reported that growing location and genotype may significantly influence the phytic acid level in FB seeds; values of 5.9 to 15.1 mg/g for Imposa and SSNS-1, respectively has been reported. According to the cluster analysis they conducted, genotypes can be segregated into four groups: high >12 mg/g (Fatima, Florent, and SSNS-1), above average 10-12 mg/g (FB25-56, Disco, and NPZ4-7540), average 8-10 mg/g (AO1155, Divine, Melodie, Snowbird, and Taboar), and low <7 g/mg (Imposa and AZ10).

Pulses may become a major source of dietary phytate intake in food products. However, phytates can chelate minerals such as calcium, zinc, and iron, which will form insoluble precipitates. These complexes will result in a decrease in mineral absorption and bioavailability (Sathe et al., 2002). Phytates are normally heat stable, and they are not easily removed by conventional heat processing methods (Loewus, 2002). On the other hand, since iron or copper cations could catalyze oxidative enzymes that form free radicals, these cations can lead to oxidative damage. As a result, cell membranes could be destroyed; so leaky cells may form. Phytates are able to chelate minerals and protect cells from oxidative damages (Sathe et al.,

2002). For these reasons, sometimes the need for the elimination or reduction of phytic acid may become debatable. The decision of a method to eliminate phytates is solely dependent on the type of food and the target product form in which this food will be consumed.

2.3.2 Vicine/convicine

Vicine and convicine are the most commonly considered antinutritional compounds in faba bean and are concentrated in the seed cotyledons (Khamassi et al., 2013). Endogenous β-glucosidase can hydrolyse vicine and convicine and produce aglycones divicine and isouramil, respectively (Figure 2-1). Aglycones can lead the oxidation of glutathione in red blood cells, which can be harmful to those who cannot synthesize glutathione at normal physiological level because of the deficiency of glucose-6-phosphate dehydrogenase (G6PD) (Khamassi et al., 2013).

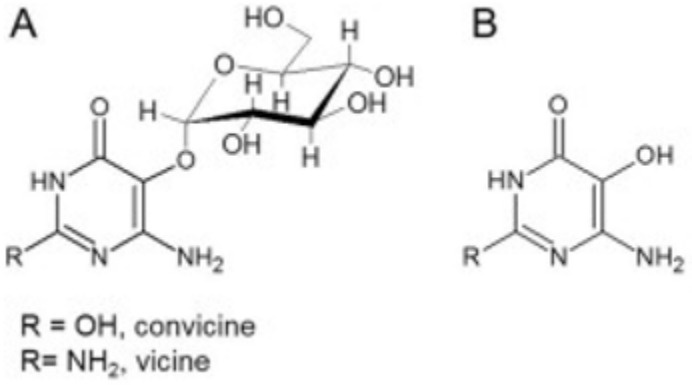

Figure 2-1 Chemical structure of vicine and convicine (A); aglycones of vicine and convicine: divicine and isouramil (B); suggested oxidized aglycones (Pulkkinen et al., 2016)

The health condition related to vicine and convicine of FB seed is called favism (Lattanzio et al., 1982). The symptoms include tiredness, headache, dizziness, nausea, vomiting, shivering, paleness, abdominal pain, and fever in the first 24-hour period after consuming faba bean. In the worst cases, acute favism can generate renal infections that could lead to death. Above 10-20% G6PD deficient individuals in Mediterranean region have reported symptoms similar to favism conditions (Lattanzio et al., 1982). G6PD catalyzes NADP (nicotinamide adenine dinucleotide phosphate) to its reduced form, NADPH, in the pentose phosphate pathway (Figure 2-2). NADPH plays a key role in protecting cells from various oxidative damage. This is

the only way for red blood cells to generate NADPH, so erythrocytes are more vulnerable than other cells to be damaged by oxidative reaction if NADPH is not regenerated (Frank, 2005). A rapid fluorescent spot test for G6PD has been developed which turns the blood to be fluorescent under UV light if present in blood (Frank, 2005).

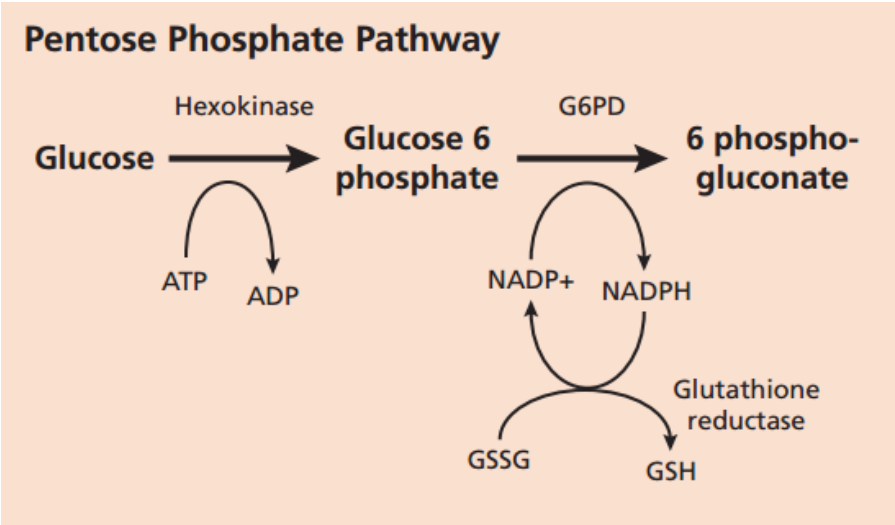

Figure 2-2 The illustration of pentose phosphate pathway (Frank, 2005)

Greene (as cited in Khamassi et al., 2013) mentioned there are about 400 million individuals worldwide are at risk of G6PD deficiency. So far, antioxidants such as vitamin E and selenium have no confirmed effects for the treatment of G6PD deficiency (Frank, 2005).

Animal studies have also exhibited negative health effects of vicine and convicine. Koriem et al. (2008) investigated the effects of convicine on albino rats given oral dose of 2 mg/100g body wt. for 15 and 30 days, respectively. The results indicated that convicine caused a significant reduction ($p<0.05$) in red blood cells with a significant increase ($p<0.05$) in serum and tissue total protein, serum bilirubin, globulin and iron. The results showed that convicine when hydrolyzed to its haemoglobin aglycon isouramil caused signs similar to those observed in the human metabolic disease, favism. Pulkkinen et al. (2015) pointed out that although zero vicine or convicine cultivars have not yet been developed, cultivars with one tenth the level of convicine found in common cultivars have been found among wild-types. The cultivar "Fabelle" is claimed to be low vicine and convicine (Government of Saskatchewan, 2016).

The most widely used method to quantify vicine/convince is high performance liquid chromatography (HPLC). The method was developed in 1981 by Marquardt & Frohlich (1983). Vicine and convicine were extracted into perchloric acid. Using reversed phase chromatography system and ammonium phosphate buffer as the mobile phase, these compounds were detected at 280 nm. In order to improve the consistency of the analytical results, water was used in both the extraction medium and the mobile phase for separation, and the UV detection was at 273 nm (Marquardt & Frohlich, 1983). Pulkkinen et al. (2015) assessed ten regular vicine-convicine-containing faba bean cultivars (Alexia, Aurora, Babylon, Fatima, GLA 1103, Kontu, SSNS-1, Taifun, Tangenta, and Witkiem-Manita). On a dry weight basis, total vicine and convicine concentration of these ten cultivars ranged from 7.25 mg/g (Witkiem-Manita) to 10.42 mg/g (Kontu). In this study the variation of the contents in different crop years was much less than the variation among cultivars. They reported that low-vicine/convicine cultivars (Melodie, Disco and Divine) had one-tenth of the vicine levels in the normal- type cultivars. The concentration of convicine in these low-vicine/convicine cultivars could not be detected (Pulkkinen et al., 2015). Convicine concentrations ranged from 1.0 to 6.0 mg/g, and vicine concentration ranged from 4.0 to 9.0 mg/g.

2.3.3 Oligosaccharides

Raffinose family oligosaccharides (RFOs) are common carbohydrate compounds found in plant seeds, particularly in pulses. Currently, there are few reports in the literature specifically focusing on the composition of RFOs in FB seeds. Rupérez (1998) reported that the total sugar content of FB seeds ranged from 6.69% to 9.99%, and oligosaccharides represented 25–46% of the total sugars; the main oligosaccharides in raw faba beans were verbascose (3.32%), and stachyose (2.21–3.23%).

RFOs contain linear chains of galactosyl residues, which are attached to the glucose moiety of sucrose via the α-1, 6 glycoside linkages. Galactinol is the galactosyl donor, and it is synthesized from UDP-D-galactose and myo-inositol (Peterbauer & Richter, 2001). RFOs could accumulate during seed development and vanish during the germination process (McPhee et al., 2002). Germination has been accepted to reduce the RFOs content by 40 -60% at 20 °C for 48 hours in peas (Guillon & Champ, 2002). During the biosynthesis process, a galactosyl unit is added to sucrose. RFOs in plant seeds are considered as storage carbohydrates, the percentage of

RFOs (on dry weight basis) could be up to 16.2% but are more commonly in the range from 2-10%. Oligosaccharides could provide readily available energy to support growth of plants.

Oligosaccharide can either cause issues or bring benefits to human health. In this study, oligosaccharide is still considered as an antinutrient. Since humans and other monogastric animals are not able to cleave the α-galactosidic linkages, RFOs easily cause flatulence (Peterbauer & Richter, 2001). RFOs could pass to the lower intestine, and the enteric bacteria could use RFOs as substrate to produce hydrogen, carbon dioxide, and methane. These gases will be expelled as flatus (McPhee et al., 2002).

On the other hand, besides its antinutritional properties, the raffinose family oligosaccharides are also considered to be prebiotics. RFOs could be fermented in the large intestine, which can provide multiple health benefits, including increasing bifidobacteria population, promoting mineral absorption, reducing pathogenic flora, and decreasing risks factors associated with obesity and colon cancer (Fan et al., 2014).

2.3.4 Phenolic compounds and tannin

Phenolic compounds are secondary metabolites that have one or more hydroxyl groups attached to an aromatic ring directly. The chemical structure of phenol is shown in Figure 2-3 (Hernandez et al., 2016). Phenolic compounds are a very large group of complex chemical compounds that can be classified into various groups. Flavonoids is one of the major classes of phenolic compounds that has a basic A, B and C-ring structure (Zanotto, 2018). There are 15 carbon atoms arranged in a C6-C3-C6 molecular structure (Vermeris & Nicholson, 2006). Other types of phenolic compounds are: phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), stilbenes, and chalcones. The class of flavonoids consists of the sub classes: flavanones, isoflavones, flavones, dihydroflavonols, flavonols, leucoanthocyanidins, anthocyanidins, anthocyanins, flavans and proanthocyanidins (Zanotto, 2018). Anthocyanins and their aglycones anthocyanidins are plant pigments, and they are key phenolic compounds to develop scarlet, red, mauve, purple and blue colours in plant organs. The pigment development is associated with the protection of inner plant tissues against UV radiations (Cabrera, 1991; Truong et al., 2010). Anthocyanins and their aglycones use the upstream part of the flavonoid pathway and are synthesized through multiple final branches (Vermeris & Nicholson, 2006). Coumaroyl coenzyme A is the substrate of the phenylpropanoid compounds. The flavonoid biosynthetic pathway has been widely investigated and majority of the genes participating in the key passages

of the pathway has been characterized and studied in model species (Zanotto, 2018; He et al., 2008).

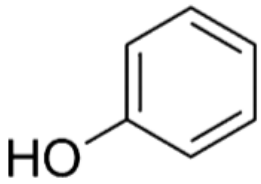

Figure 2-3 Basic structure of a phenolic compound

Condensed tannins belong to proanthocyanidins, and condensed tannins are oligomeric or polymeric flavonoids containing flavans (Zanotto, 2018). Flavan is the basic unit of condensed tannins, which consists of two benzene rings linked together by a three-carbon group (Cabrera, 1991). This three-carbon group is also the basic structure of anthocyanins, the glycosides of anthocyanidins (Cabrera, 1991). Hydrolysable tannins and complex tannins have the ability to bind and precipitate protein molecules (Vermeris & Nicholson, 2006). Condensed tannin is an important anti-nutritional factor reported in FB, which can also generate the bitter and astringent taste response due to their binding ability to protein molecules (Singh et al., 2017; Cabrera, 1991). Faba bean tannins can decrease protein digestibility in poultry because of the formation of tannin-protein complexes, and additionally, starch digestibility can also be influenced by tannin, but the effect on starch is not always obvious (Vilariño et al., 2009).

The chemical effects of tannins are complex and not well understood so far. The condensed tannins in FB have similar biological effects and biochemical properties to those present in cereal grains (Cabrera, 1991). During the seed development stage, the degree of polymerization of condensed tannins starts to change, and these changes could be related to bird repellence. The condensed tannin content of FB has reached the maximum concentration around 30 days after flowering and starts to decline when the degree of polymerization increases (Cabrera, 1991). Phenol related compounds play a key role in plant metabolism and in the interaction of plants and the external environment. Since tannins produce precursors with anthocyanin pigments in the biochemical pathway, there is significant relationship between tannin content and testa colour. The tannin content of grey, green, red, normal, and brown test

colour faba bean seeds are 0.4%, 2.5%, 3.0%, 4.1%, and 4.2%, respectively (Oomah et al., 2011). Dark pigmented varieties normally contain higher tannin content than others.

There is another change in seed pigmentation process related to storage conditions and ripening process (Oomah et al., 2011). During ripening process, the testa colour begins to change due to the oxidation of phenolic compounds. In some cases, seed colour in individual plants is not homogeneous, therefore light or dark colour seed coats may be obtained at the same time. Oomah et al. (2011) also demonstrated that the colour parameters of FB seed coat differed significantly among different genotypes and different locations, upon analyzing 13 faba bean cultivars (A01155, AZ10, Disco, Divine, CDC Fatima, FB2S-56, Florent, Imposa, Melodie, NPZ4-7540, Snowbird, CDC SSNS-1, and Taboar) grown in two different locations in Canada. AO1155, Disco, Divine, and Melodie are low tannin and vicine/convicine genotypes, which were considered as "double zero" with the trademark registration. Divine, Fatima, Florent, Melodie, SSNS-1, and Taboar were coloured genotypes. The mean *L* value (lightness/darkness), *a* value (greenness/redness), and *b* value (yellowness/blueness) of the 13 cultivars of faba bean seeds are 26.1, 0.38, and 0.39 respectively, and the spectrum covered from brown to beige. Not only the genotype had significant effects on faba bean colour variations, but also the location had significant effects on faba bean colour variations. Genotype accounted for 81% of the total variations of FB tannin content. The tannins concentration ranged from 0.5 mg/g to 5.42 mg/g for all 13 cultivars, and only Disco, FB2S-56, and Melodie had low tannin concentrations (less than 1 mg/g). Other 10 cultivars had relatively high concentrations ranged from 4 mg/g to 5 mg/g (Oomah et al., 2011).

2.3.5 Trypsin inhibitor

Trypsin inhibitors can be found in various plants including species in *Leguminoseae*, *Solanaceae*, and *Gramineae* families. FB is an important grain legume in the *Leguminoseae* family, being as a major source of protein in both animal feed and human food. Most legume species such as mung beans, lupins, kidney beans, and FB contain less than 50% of the trypsin inhibitors in soybeans (Savage & Morrison, 2003). However, the existence of trypsin inhibitor has limited the application of FB seeds.

Legume trypsin inhibitors (TI) can be classified into two types based on their molecular size; Kunitz-type (KTI) having molecular mass around 20 KDa, and Bowman-Birk- type (BBTI) at about 8 KDa (Avilés-Gaxiola et al., 2017). In general, soy bean contains both types of TIs.

Other legumes, such as field bean, faba bean, and lentil may contain BBTI only. The molecular structure of KTI consists of three repeating subdomains, and each one is conformed by four consecutive β-strands interconnected by loops (Avilés-Gaxiola et al., 2017). Two di-sulfide bonds are located in KTIs, but none in the active site, and these bonds may stabilize the overall molecular structure. The molecular structure of BBTI was determined in legume species other than soy beans. BBTIs are conformed by two domains; one chymotrypsin inhibiting and the other trypsin inhibiting. These two domains are established by two symmetry-related β-hairpin motifs, and each one has antiparallel β-strands. There are 7 disulfide bonds in the overall structure maintaining the molecular stability of BBTIs (Avilés-Gaxiola et al., 2017).

The main toxicological effect of ingesting of trypsin inhibitors is the hypertrophy of the pancreas due to excessive enzyme secretion. Cholecystokinin which regulates the pancreatic secretion releases and promote secretion of pancreozymins, trpsinogen, and chymotrypsinogen, the precursors to trypsin and chymotrypsin, respectively when levels of these enzymes are lower due to trypsin/chymotrypsin inhibitor-trypsin/chymotrypsin complex formation (Savage & Morrison, 2003). Therefore, when trypsin inhibitors are present, the pancreas may not be functioning to secrete higher enzyme levels to compensate for the loss due to the formation of complexes of enzyme inhibitors. This process will take essential amino acid away from other important body functions, especially pancreatic enzymes are rich in sulfur-containing essential amino acids, which are critical for body growth (Guillamón et al., 2008). The ingestion of trypsin inhibitors may result in adverse effects on animals, including growth depression, pancreatic hypertrophy, and sometimes even death.

The location of trypsin inhibitors may vary, but most of them are found in the plant seed. In mung bean and field bean, high level of trypsin inhibitors has been found in plant leaves. In soy beans, kidney beans, peas, and chickpeas, the majority of trypsin inhibitors were found in the outer part of the cotyledon (Savage & Morrison, 2003). On the contrary, FB seeds has been reported twice as much trypsin inhibiting activity in the seed hull than the cotyledon (Guillamón et al., 2008). Quantitative trypsin inhibitor units (TIU) are used to determine the level of trypsin inhibitors in plants (Gupta et al., 2000). Soybean seeds contained higher TIU (43-84 mg^{-1}) compared to whole lentil seeds (3-8 mg^{-1}) and whole FB seeds (5-10 mg^{-1}) (Guillamón et al., 2008).

2.4 Reduction of undesired compounds in faba bean through breeding

2.4.1 Vicine and convicine

Vicine and convicine are pyrimidine glycosides, which are the major factors limiting the use of faba bean (*Vicia faba* L.) in food and feed (Duc et al., 1989; Crépon et al., 2010). The biosynthetic pathway of vicine and convicine is unidentified. Their aglycones, divicine and isouramil, are effective oxidants and in human beings with glucose-6- phosphate dehydrogenase deficiency, they motive an acute hemolytic anemia called favism, whilst in chickens, they purpose a similar rupture of erythrocytes, particularly in broiler breeds, at the same time as in egg laying hens, productivity is decreased (Crépon et al., 2010). Since there are no known enzymes or enzyme genes that can be sought, a candidate gene approach has been employed in generating FB lines with reduced vicine and convicine levels. Different approaches were used to identify the genes that controlled synthesis of vicine and convicine in faba bean seeds. Wild type faba bean cultivars contain 4–16 g/kg of vicine and convicine (Khamassi et al., 2013) but *vc*-genotypes have 5–10 % of this (Duc et al., 1999) and *vc*- cultivars have ultimately been released (Duc et al., 1989). Since the two compounds are always found together, it appears that they are synthesized in the same pathway. The *vc*- gene has been identified to generate colourless hilum (Duc et al., 1989) but not all colourless-hilum accessions are *vc*-. Gutierrez et al. (2006) mentioned the study of two RAPD (random amplified polymorphic DNA) markers converted to CAP (cleavage amplified polymorphism) is associated with low vicine and convicine level but these markers are further from *vc*- than hilum colour. Cost-effective and highly saturated genetic maps of FB have been developed using single nucleotide polymorphisms (SNPs) because of their low cost, ease of use, and wide distribution throughout the genome (Cottage et al., 2012; Kaur et al., 2014). The simultaneous use of these CAPS allowed accurate fingerprinting of *vc*-allele in FB that facilitate development of cultivars with low levels of vicine and convicine with improved nutritional value however the direct visual assessment of hilum colour of FB would remain as the most cost effective detection method (Gnanasambandam et al., 2012). A 10 to 20-fold reduction in the levels of vicine and convicine has been obtained by mutation in the *vc*-gene that controls vicine and convicine expression in FB, (Duc et al., 1989) but without modifying the levels of macronutrients. Fabelle (medium seeds, 553 TSW) is a low vicine and convicine variety registered in Canada by DL seeds in 2016 (Phelps, 2016; CFIA, 2018).

2.4.2 Tannin

Similar to vicine and convicine, DNA markers were used to determine the genes that controlled the synthesis of tannins. Two complementary genes *zt*1 and *zt*2, which apparently have same function may be just a repetition of each other in the FB genome (Cooper et al., 2017). These two genes control the absence of tannins in FB seeds, generating white flower plants (Torres et al., 2010). Traditional varieties such as Malik and Taboar have brown seed coat, and moderate levels of tannin ranging from 8 to 9% which have been obtained by suppressing *zt1* and *zt2* genes that control the accumulation of tannin compounds (Duc et al., 1999). The low tannin varieties such as Snowdrop (small seeds, 335 TSW) and Snowbird (small to medium seeds, 495 TSW) have cream and light seed coat and tannin levels as low as 1%. Low-tannin varieties are suitable for feed due to increased protein digestibility, compared to high tannin varieties (Duc et al., 1999). Further work may be needed to develop faba bean cultivars low in vicine/convicine and in tannin content.

2.5 Reduction of undesired compounds in faba bean through processing

2.5.1 Germination

Currently consumers are increasingly seeking natural food products that are both healthy and tasty. Germination is one of the natural and an economical method that can improve the nutritional, functional, and sensory properties of pulses. Besides the laboratory germination process, industrial germination process has been developed and enables the development of innovative commercial pulse food products (Luo & Xie, 2013). Pulses are partially germinated under controlled conditions and then dehydrated with hot air to extend the shelf life. The germination process could significantly increase the pleasant sweet taste of the pulse products (Bellaio et al., 2013).

Germination was reported to either reduce or increase level of phytic acid depending on the length of germination occurred. Luo & Xie (2013) reported a significant decrease in the level of phytic acid from 0.84% to 0.53% during 72-h germination and then an increase with longer germination time (up to 0.75% at 120-h germination). Phytic acid serves as a reserve of phosphorus which is generated by the action of phytase during seed germination, resulting in an increase in available phosphorus and, hence, improving the mineral's bioavailability. (Vidal-Valverde et al., 1998).

Anti-nutritional factors such as vicine/convicine could be degraded during germination process. Once the glycosidic bond is cleaved either by enzyme or acids, aglycones will be produced. Since aglycones are confirmed to induce favism, a detection of divicine and isouramil should be done to confirm the effectiveness of the detoxification processes. The degradation pathways of aglycones are still not completely known, but it is confirmed that loss of UV absorptivity could indicate the destruction of aglycone rings (Pulkkinen et al., 2016). To investigate the presence of divicine and isouramil, the same practical HPLC method could be used. The identity of the divicine and isouramil could be proved by LC-MS method. A study completed by Pulkkinen et al. (2016) showed that aglycones could degrade completely at 37°C in 60 – 120 min, when pH level was 3-5. When microbes with β-glucosidase activity (e.g. lactic acid bacteria fermentation process) were employed to hydrolyze vicine and convicine, aglycones released by hydrolysis could decompose if the above temperature and pH combination could be reached (Pulkkinen et al., 2016). Bellaio et al. (2013) found that FB seeds were sterilized by soaking in ethanol solution for 1 min, then the seeds were soaked in distilled water at 25 °C for about 12 hours. After soaking, seeds were placed into thick layers of cotton cloth and allowed to germinate in the dark environment at 25 °C for 72 h. Germinated seeds were rinsed with distilled water. The raw faba bean contained 0.68% of vicine and 0.27% of convicine, and the germinated faba bean contained 0.49% of vicine and 0.19% of convicine. Vicine contents were significantly different at 5% level between raw and germinated FB seeds. Convicine contents were not significantly different at 5% level between raw and germinated faba bean (Bellaio et al., 2013). In Khalil and Mansour's study (1995), vicine was significantly reduced from 6.8 mg/g to 4.9 mg/g after 72-hour of germination, and convicine content remained the same after germination.

Goyoaga et al. (2008) showed that the limited changes of vicine/convicine during germination could be due to translocation of vicine and convicine from the cotyledons to the axis without evidence of use of these compounds in further metabolism. These authors also suggested that a single codominant gene controls the ratio of vicine to convicine in whole mature seeds of *Vicia faba*, and the levels of vicine and convicine in mature seeds were maternally determined. The testa may be the primary site of synthesis and then exported to the cotyledons and seed axis. It was possible that the variation in the levels of vicine and convicine in different tissues of the seed are a result of the interactions between differential synthesis and inter-conversion in the

testa (Goyoaga et al., 2008). Khalil (1995) has reported that the level of vicine and convicine was reduced from 1.45% to 1.03% upon 72 h germination of FB.

Work by Luo and Xie (2013) showed that tannin (0.7% in raw seed) of FB was significantly decreased within 24 h (0.05% of tannin) and 48 h germination (0.03 % of tannin). After 48 h germination, tannin content showed a significant increase up to 0.07% and 1.1% at 96 h and 120 h of germination, respectively. These results indicated that longer germination time may increase the tannin content. The length of germination may be a determining factor to the changes of tannin content. A study by Shetty et al. (2001) further confirmed this by investigating FB germinated up to 8 days. The phenolic content in FB seeds remained same from day 1 to day 5, and started increasing on day 6, and then decreased since day 7 with the highest phenolic content observed on day 6 (3 mg/g). These authors pointed out that the synthesis of free phenolics was regulated via the proline-linked pentose phosphate pathway (PPP), shikimate pathway, and phenylpropanoid pathway. PPP was an alternate route for the breakdown of carbohydrates generating $NADPH_2$ for use in anabolic reactions and provide erythrose-4-phosphate for shikimate pathway. This route was important for the biosynthesis of phenylpropanoid. The guaiacol peroxidase (GPX) enzyme determines the fate of phenolic acids produced. Phenolics were precursors in the peroxidase mediated lignin biosynthetic pathway. Peroxidase, belonging to the oxido-reductive group of enzymes mediated the process of lignification of phenolic acids by utilizing H_2O_2, a photosynthetic by-product. Enhanced peroxidase activity was considered to lead to increased lignin synthesis and subsequent lignification of the cell wall acts as a barrier for fungal infections of the developing seedling (Shetty, 2004).

Limited studies and information are available on the fate of oligosaccharide of FB during the germination process. Goyoaga et al. (2011) reported that the stachyose and verbascose content of FB cultivar Brocal significantly decreased after 48 h of germination and became undetectable after 72 h. Raffinose content also significantly decreased after 96 h of germination. According to this study, at 72 h of germination the sum of RFO (raffinose family oligosaccharides) was only about 10% of initial content. The oligosaccharide content changes with germination were also reported in other pulses. Stachyose content in lima beans, African yam beans and jackbeans, declined progressively until 96 h of germination and reached reductions higher than 70% relative to the original seeds (Oboh et al., 2000). In brown and cream

pigeon peas, verbascose was eliminated at 96 h of germination and stachyose was reduced ~ 50%. Raffinose and verbascose also followed a similar trend during the germination of red lima beans, white lima beans, African yam beans, and jackbeans and became undetectable at 96 h germination (Oboh et al., 2000). The disappearance of oligosaccharides during germination has been attributed to the increase in the level of α-galactosidase enzyme activity which can catalyze hydrolysis of glycosidic linkage in oligosaccharides and result in the increase of smaller carbohydrate molecules such as sucrose (Akinlosotu & Akinyele, 1991).

2.5.2 Heat treatment

Phytate is heat stable, therefore a significant reduction in the level of phytate during cooking cannot be expected unless either the cooking water was discarded, or the food received additional processing treatment such as soaking (soaking water discarded), germination, fermentation, etc. (Sathe & Venkatachalam, 2002). Phytic acid content was reported to reduce after cooking under pressure. In the study by Fernandez et al. (1997), FB seeds were pressure cooked to 120 °C at 1 atm for 15 min and the phytic acid content was reduced by 40%. This may represent an effect of degradation of detectable phytates caused by heat, rather than enzymatic hydrolysis. Soaking in acid solution followed by cooking reduced phytic acid by 30%, and the hard seed cover could prevent the acid solution from reaching the cotyledons and soaking alone had no effect on phytic acid. In contrast, soaking followed by cooking softened the seed cover enough to allow the acid solution to penetrate (Fernandez et al., 1997).

Heat treatments were reported to reduce vicine/convicine of FB seeds in various studies. Khalil (1995) studied boiling of soaked (in distilled water at 25 °C for 12 h, weight:volume 1:20) FB seeds (3 mL water per gram of dry seeds, 45 min until seeds became softer, which can be felt by fingers). The raw FB contained 0.68% vicine and 0.27% convicine (on dry weight basis), and the cooked FB contained 0.44% vicine and 0.18% convicine (on dry weight basis). Vicine contents were significantly (p<5%) different between raw and cooked FB but not the convicine contents. Cardador-Martínez et al. (2012) reported effects of roasting and boiling on vicine and convicine content in ten FB varieties. The whole seeds were roasted at 120 °C for 10 min, and separated cotyledons were milled and assessed. An average vicine content reduction of 6.06%, and 3.2 -30.0% reduction in convicine content has been reported. The boiled FB, separated into cotyledons were assessed. Vicine content reduction value was 19%, and convicine content reduction values ranged from 13-60%. However, none of the studies showed the complete

elimination of phytic acid, vicine/convicine, oligosaccharides, and phytic acid due to boiling or roasting of FB.

Autoclaving has also been reported to reduce vicine and convicine content in FB. Compared to boiling, autoclaving normally requires less processing time but higher temperature (Mozzoni et al., 2009). In the food industry, intensive heat treatments such as autoclaving are required to kill the most heat resistant bacterial microorganisms, which are the spores of *Clostridium* (Mozzoni et al., 2009).

According to the study by Khalil (1995), raw FB seeds contained 0.68% of vicine and 0.27% of convicine, and by cooking the reductions of vicine and convicine were 40% 41%, respectively. Additionally, Khalil (1995) identified autoclaved FB had a higher content of B-group vitamins than cooked FB seeds. They concluded that pressure cooking can increase the levels and retentions of thiamine, riboflavin, and niacin (Mozzoni et al., 2009). In industry, canning or autoclaving is feasible for FB compared to traditional boiling or cooking, the autoclaving process could reduce the processing time significantly (Mozzoni et al., 2009) and could open new markets and create new opportunities for this product.

Similar to the effects of dry heat treatments on vicine/convicine content, tannin content of FB was reduced from 1.45% to 0.65% by boiling and autoclaving, respectively (Khail, 1995). The reduction in phenolic contents of beans during cooking could be due to either destruction of phenolic compounds or other chemical rearrangements of phenolic compounds such as the formation of new insoluble components with other organic substances (Kader, 1995). The variation in the extent of phenolic compounds degradation could be influenced by the seed physicochemical properties, such as seed surface area, coat thickness, coat colours, shapes and hardness, and it was also possible that cooking faba beans led to strong tannin–protein interactions (Giczewska & Borowska, 2003).

So far, limited studies focused on the oligosaccharide contents change in FB upon aotoclaving. Work by Vidal-Valverde et al. (1998) concluded that soaking plus cooking could reduce the content of oligosaccharide in FB from 16-25%. Oboh et al. (2000) reported that in other pulses oligosaccharide level reduces due to boiling in water. Cooking in water (W/C) resulted in a loss of total oligosaccharide by 21% in brown pigeon peas, and 67% in white lima beans, compared to the corresponding raw samples. These reductions may have been due to heat

induced hydrolysis of the oligosaccharides to simple disaccharides and monosaccharides, but oligosaccharides content losses in water were not reported (Oboh et al., 2000).

2.5.3 Fermentation

In recent years, lactic acid bacteria fermentation has been employed as a technology to improve the quality of pulse-based food products. In one study, before the fermentation process, the faba bean dough pH was around 6.7, and after 24-h fermentation, the pH dropped to the range of 4.9 to 5.8 with 27 strains of lacid acid bacteria (Verni et al. 2017). Due to the concern of the microbial control, fermentation may not be used in the current study. Tannin content could be reduced significantly under the effect of the strain L. *plantarum* VTT E-133328 (Coda et al., 2015). But the effects of fermentation on vicine/convicine, phytic acid, and oligosaccharides are still uncertain. The strain of lactic bacteria (L. *plantarum* VTT E-133328) was selected based on its beta-glucosidase activity. L. *plantarum* was cultivated until the late exponential phase of growth was reached. The dough was later mixed with lactic bacteria with cell density of 10^7 cfu/g and fermented at 30°C for 48 hours. The results showed that fermentation could reduce the condensed tannin content by 50% in fermented FB flour, and 42% in protein rich fractions, when compared to non-treated faba bean flour. *L. plantarum* is a ubiquitous species, found in several food ecosystems due to its versatile metabolism, and has the capacity to adapt to different environmental conditions. The reduction of condensed tannin content could also increase protein digestibility.

Another study by Rizzello et al. (2016) also indicated the fermentation process could significantly reduce vicine/convicine in FB flour. Hydrolysis kinetics of vicine and convicine and their derivatives during fermentation with *L. plantarum* DPPMAB24W was studied. A specific HPLC method combined with ESI-MS and MS/MS analysis, including the evaluation procedure of the results, was prepared as the analytical approach to monitor the compounds. The degradation of the pyrimidine glycosides in the fermented flour was complete after 48 h of incubation and the aglycone derivatives could not be detected in any of the samples. The lack of toxicity of the fermented FB was confirmed through ex-*vivo* assays on human blood, proving the experimental results. Findings indicate that cost effective and non-destructive bioprocessing methods can be applied to detoxify FB for industrial applications.

2.6 Generation of faba bean ingredients

2.6.1 Dry processing and fractionation

Dry processing of FB involves conversion of whole seed into flours and fractions rich in fibre, starch or protein on the basis of different densities of various components (Joshi et al., 2017). Fractionation of FB into fractions rich in fibre, protein or starch is done by milling (usually pin milling to achieve suitable particle size distribution) followed by air classification. Seed coat removal is done first as the fibre rich fraction is easily separated and concentrated. In air classification, the seed flour is subjected to an air stream to separate particles depending on the size and density therefore fractions rich in protein and starch could be separated (Swanson, 1990). The smaller, fine protein particles are carried up in the air stream while the larger, coarse starch particles fall by gravitational force (Cloutt et al., 1986). This processing technique has been used to fractionate legumes such as field pea, chickpea, lentil, faba bean, common bean, lima bean, mung bean and defatted lupin and soybean (Joshi et al., 2017). Due to high lipid content, the level of enrichment of the protein fraction was not always efficient in the latter two legumes (Sosulski & Youngs, 1979). Pelgrom et al. (2015) reported an impact mill to generate faba bean protein and starch fraction, which included an internal rotating classifier wheel that allowed the passage of fine particles while coarse particles were returned and milled afterwards. The classifier wheel speed determined the air flow determined the size of the milled flour. After the milling process, the average particle size of protein-rich particles was about 5 μm, and 15 μm for starch granules. Air classification to smaller sizes in the fine fraction could enhance the protein content, but the fine fraction could impair the effect of separation. Decreasing the amount of material build-up during air-classification would be a solution to increase the protein content (Pelgrom et al., 2015).

The major advantage of dry processing method over the wet processing is that the native functionality of proteins and starch could be retained, and it requires less water and energy, since no additional chemical solutions are added in the dry processing (Pelgrom et al., 2015). Protein separation efficiency (PSE) is the percentage of total flour protein recovered in the protein fractions, and normally used to evaluate the performance of air classification of a certain type of pulses (Singhal, 2015). The study by Tyler et al. (1981) reported FB gave a relatively higher PSE value of 84.1% than lima bean (80.2%), cowpea (78.2%), navy bean (80.3%), and field pea (82.8%).

Another study (Gunawardena, 2009) reported that the zero-tannin FB containing 29% crude protein and 46% starch initially, after pin milling and air classification, was able to separate into protein-enriched (up to 63% crude protein) fraction and the starch content was enriched up to 55% in the starch fraction. With air classification the protein recovery of faba bean was higher than that of field pea. Colonna & Mercier (1980) reported this to be 55.2% for FB and 49.8% for field pea; in contrast, starch recovery was higher for field pea (89.8%) than FB (83.5%). Tyler et al. (1981) observed for legumes, including faba bean and field pea, that the proximate content of the air-classified fractions was correlated with the levels of these constituents in the original flour.

2.6.2 Wet processing

Wet processing generally requires the addition of acid or alkaline solution. Pulse protein may be solubilized in the alkaline solution, and multiple physical method may be used to separate solubilized protein from insoluble component and precipitate protein in the solution (Joshi et al., 2017). Flour of FB or dry processed fractions can be wet processed to further increase protein or starch in the final product. Wet processing with food grade additives and processing aids can generate suitable protein fractions for food use. Wet processing can be utilized for preparing both protein concentrates and isolates to achieve 70% and 90% protein, respectively from pulses (Singhal, 2015). The various wet extraction processes include acid/alkaline extraction, isoelectric precipitation, ultrafiltration and salt extraction (Singhal, 2015). Legume flours that can be dispersed in aqueous solutions are normally highly soluble under alkaline or acidic extraction conditions at pH 8-10 and below 4 (Kiosseoglou & Paraskevopoulou, 2011).

Alkaline extraction combined with acid precipitation is the most widely used wet processing method to extract protein components from pulses. For alkaline or acid extraction, proteins are first dissolved under alkaline or acidic conditions, and then the mixture will be clarified. The precipitation process normally takes place at isoelectric point (pI) of the protein molecules at which their solubility is the lowest (Han & Hamaker, 2002). The pI of pulse protein is around pH 4-5 (Joshi et al. 2017). Legume proteins are solubilized at high pH values for alkaline extraction. The solution can then be clarified by removing insoluble material such as insoluble fibre, carbohydrates and insoluble proteins through centrifugation. Acid extraction needs the preliminary extraction of proteins when pH is below 4 (Singhal, 2015). This process

could cause more proteins to be solubilized before the recovery process, since proteins will become more soluble under acidic conditions (pH <4) (Boye et al., 2010). Kaur et al. (2007) concluded that yield of lentil protein isolate ranged from 82 to 84% for Indian cultivars when the protein isolation was done by isoelectric precipitation approach, at pH around 4.5. Vose (1980) prepared faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) protein isolates, and these two legume species after pin milling process were acidified by adding 2 N HCl to the isoelectric point of 4.4-4.6, which produced pea and faba bean protein isolates with a protein content of 92% and 91%, respectively. The advantages of wet fractionation methods over dry fractionation are: aqueous fractionation yields pure fractions and causes less damage to the starch granular structure. In contrast, dry fractionation is fast and economical, with no handling of effluent (Colonna & Mercier., 1980).

2.7 Applications of faba bean or other pulse ingredients in food products

2.7.1 Processed meat products and plant-based binders

Compared to fresh meat, processed meat products generally have improved texture, flavor, appearance and longer shelf-life (Forrest et al., 1975). Manufacturing of processed meat products involves several procedures such as grinding, chopping, the mixing of non-meat ingredients such as seasoning, salt and nitrite to meat batters, and cooking (Xu, 2017). During the first grind, meat and salt may be ground, and water with other ingredients will be ground in the second grind to achieve the maximum protein extraction (Brewer, 2012). Processed meat products consist of a wide range of products, and normally represent sausages, meatballs, burger patties, hams, and bolognas. (Weiss, et al., 2010; Santarelli et al., 2008). Bologna is a typical processed meat product and formulated based on an emulsion system (biphasic). Salt is conventionally used as a preservative to avoid the spoilage of perishable meat products (Weiss et al., 2010). In the emulsion system, protein molecules are extracted and solubilized by salt, which function as continuous phase. Fat particles are dispersed phase, which are surrounded by solubilized myofibrillar protein molecules. Due to the larger fat droplet size and the existence of insoluble proteins, connective tissues (collagen), and other non-meat substances in the continuous phase, meat emulsion systems are not considered as true emulsions (Xu, 2017; Ugalde-Benítez, 2012)

Bologna was usually manufactured using the following major ingredients: lean meat, animal fat, and water. Other minor ingredients include: salt, sodium tripolyphosphate, sodium nitrite, and seasonings (Xu, 2017). Meat protein (mainly in lean meat muscle), fat, and water are the

basics for bologna products, and these ingredients form the emulsion system. Salt can extract and solubilize myofibrillar proteins and promote the formation of the emulsion system. The stable emulsion system subsequently can also entrap adequate amount of water (Xu, 2017; Sofos, 1983). Sodium tripolyphosphate (STTP) is an excellent substance to bind large amount of water via changing the net charge of meat protein molecules. (Forrest et al., 1975). Nitrite are essential to control microbial growth in the meat products and maintaining the appealing pinkish color during the curing process. (Weiss, et al., 2010; Bedale et al., 2016). Seasoning will develop the flavor profile of the meat products (Xu, 2017).

Recently, consumers are more aware of the reduction of fat content in their diets. Leaner fresh meat cuts are preferred, but processed meat products still present higher fat content (for up to 50%), and high-fat content may also contain excessive cholesterol and saturated fat, which will increase the risk for multiple health concerns such as obesity and cardiovascular diseases (Jiménez-Colmenero, 2001; Grasso et al., 2014). The World Health Organization (2015) recommended the total fat in a healthy diet should not exceed 30% of total energy intake to avoid excessive weight gain (Xu, 2017). Additionally, the saturated fat should be consumed less, and unsaturated fat should be consumed more (Word Health Organization, 2015). Cholesterol daily intake should be controlled no more than 300 mg, and the total fat content needs to contribute to 15-30% of the energy in the diet. Saturated fat content should provide less than 10% of the total calories. (Serdaroğlu et al., 2005). Using leaner meat cuts to manufacture final products can directly increase moisture content and reduce fat, but the addition of leaner meat cuts may also induce the cost of production and lead to drier product with a rubbery texture and bland taste (Su & Zayas, 2000). Also, directly replacing fat with water results in the softer texture (Claus & Hunt, 1991), increased cook loss and purge loss during the long-term storage (Shand, 2000). The successful reformulations of reduced-fat meat products is based on various factors (meat protein content, water content, fat replacers or binders, and processing conditions), and maintaining the binding properties of the ingredients is the major challenge during manufacturing low-fat meat products (Carballo, et al., 1995).

Binder is a term used for plant or animal ingredients with a high level of protein which can absorb water and bind fat. In contrast, extenders are non-meat substances with substantial protein content, primarily legume or cereal products such as wheat flour, lentils, beans, and peas (Unatrakarn, 2014; Heinz & Hautzinger, 2007). Plant proteins are more cost-effective and can

replace the meat proteins in the product and maintain the palatability of the product (Heinz & Hautzinger, 2007). Protein-based plant ingredients (soy protein and other legume protein isolates), carbohydrate ingredients (starch and fiber), and gums (xanthan, guar gum, carrageenans, and locust bean gums) have been incorporated as binders in meat products, due to the good functional properties, and lower cost compared to lean meat (Brewer, 2012; Selani et al., 2016). When water percentage increases in the formulation, carbohydrate with good water absorbent should be incorporated. Cereal or pulse starch is widely used as binders due to its binding ability to water and enhancement on textural properties of meat products (Pietrasik et al., 2010). Carballo et al. (1995) found adding waxy corn starch at 5-10% into pork bologna will favor the formation of a more compact and stronger heat-induced protein matrix and cook loss will be reduced.

Although there is limited information on the effect of faba bean binders on meat products, multiple studies discussed the effect of other plant-based binders on meat products. Serdaroğlu et al. (2005) concluded that meatballs incorporated with chickpea flours (protein content of 21%) presented better water holding capacity than the same level of bread crumbs (protein content of 12%), which indicated the emulsion system based on chickpea flour is more stable than that on wheat flour. In the study by Unatrakarn (2014), chick pea flour derived from tempered seed was incorporated into bologna product (5% addition level). No significant (p<0.05) differences of cook loss (0.3-0.4%) and expressible moisture (14.1-15.3%) were found for bologna samples with the addition of chickpea flour, and bologna with the inclusion of wheat flour (5% addition level) presented significantly (p<0.05) lower cook loss (0.3%) and expressible moisture (10.0%) than with the addition of chickpea flour from non-micronized seed. Micronization is a short time infrared treatment used on legumes. Tempering of legume seeds in water prior to micronization resulting in increase in seed size/volume due to water absorption. Tempering is usually used as a pre-treatment prior to micronization to control moisture levels of seeds and to avoid roasting (Fasina et al., 2001). The bologna with the addition 5% wheat flour presented significantly (p<0.05) lower purge losses (3.2%) than the control bologna without any binder (5.4% purge loss), and all samples with chickpea flour from micronized seed (4.0-4.8% purge loss), and the incorporation of chickpea flour from non-micronized seed showed the lowest purge loss (3.9%) among all chickpea ingredients. Adding chickpea flour (micronized seed) increased yellowness of the bologna products compared to the control bologna treatments and bologna with the

addition of wheat flour. Texture profile analysis (TPA) indicated that bologna with chickpea flour from micronized seed (15% and 22%) did not show significantly different (p<0.05) hardness and chewiness, compared to bologna with untampered chickpea flour.

On the other side, using plant-based binders in meat products can also negatively affect textural and sensory properties of the product. Two red pigments myoglobin and hemoglobin contribute to the natural meat color with the interaction with other components such as fat, iron, and connective issue (King & Whyte, 2001). Compounds in the plant binders may interact with these two pigments and affect the natural color. Henning et al. (2016) found that only the addition of 1% dietary fibre derived from pineapple into beef sausage, the surface lightness significantly (p<0.05) increased with more purge loss. In addition, Prinyawiwatkul et al. (1997) mentioned the addition of cowpea flour and peanut flour to chicken nuggets can significantly (p<0.05) enhance the brownish orange color. As for pulse-based binder ingredients, chickpea flour could bring brown color to the meat batter during mixing process (Verma et al., 1984).

Due to the variations of particle size and beany flavor of legume flour, the addition of legume flour may lead to graininess for texture and foreign flavour of meat products (Sanjeewa et al., 2010; Der, 2010; Xu, 2017). Adding 2.5% of pea bran into low-fat pork bologna brought strong foreign beany flavor to the product, and the foreign flavor may be due to lipid oxidation caused by lipoxygenase activity in the binders (Xu, 2017). For the textural properties, Sanjeewa et al. (2010) found that bologna with Kabuli and Desi chickpea flour at 5.0% presented the highest shear forces among all the treatments. The shear forces were positively related to the level of chickpea flour, which showed that the chickpea flour can form a stable gel network with meat constituents. Meanwhile, based on the texture profile analysis (TPA), bologna with chickpea flour had significantly higher (p<0.05) firmness and cohesiveness than with wheat flour, and the difference may be to the protein content in binder ingredients. These texture results were close to that reported by Der (2010) with addition of lentil flour (6 and 12% levels) into beef burgers. These results all indicated the potential of using FB ingredients as binders at various levels in low-fat meat products.

2.7.2 Faba bean ingredients in food products

Besides the excellent nutritional profile, FB is gluten-free and could be utilized to replace wheat flour in other food products, such as gluten-free pasta. Rosa-Sibakov et al. (2015) reported the effects of using flour, starch-rich fraction, and fermented flour of FB to replace semolina

flour in pasta. Pasta prepared with FB flour and fermented FB flour had higher cook loss (10.8-11.5%) and lower water absorption (130-160%) than semolina pasta (6 and 193%) but pasta made with starch fraction showed similar water absorption as semolina pasta. Texture and flavour of FB flour containing pasta was not affected however, fermented FB flour containing pasta showed increased hardness, chewiness, sourness, and flavour intensity. Faba bean ingredients did not change the starch hydrolysis index of the pasta (46-50). The sensory assessment of pasta texture was in line with instrumental evaluation. Giménez et al. (2012) also concluded that the optimum addition of FB flour for the best protein enrichment in wheat-based pasta was at 30% and this level was acceptable in technological terms. The higher protein content brought by FB ingredients played an important role in the stabilization of the protein-polysaccharide matrix. The mixture of FB and wheat flour not only increased the protein content, but also the fibre content of pasta products.

Faba bean ingredients were also studied in baking food products however, traditionally FB ingredients have not been used in baking, as it contains compounds that are problematic to production and sensory characteristics. Coda et al. (2017) prepared bread by replacing wheat flour with 30% of FB flour, native or after sourdough fermentation. Addition of FB flour influenced structure of the breads, causing a slight decrease of volume and higher hardness compared to wheat bread. However, addition of fermented FB flour did not change breadcrumb porosity. The addition of 30% FB flour increased wheat bread protein content from 11.6 up to 16.5% of dry matter. The addition of native FB flour did not affect *in vitro* protein digestibility resulting in similar values to wheat bread (64%). On the contrary, FB sourdough bread showed higher protein digestibility (73%). Generally, the addition of native FB flour caused an improvement of nutritional indexes of the composite bread, which were further enhanced when fermented FB ingredients was used. The free amino acid profile, chemical score, and biological value index of protein were at the highest values in faba bean sourdough bread. In addition, the predicted glycemic index was the lowest in FB sourdough bread.

Commercially, VTT (a research and development institute in Finland) developed a gluten-free pasta product from 100% FB flour or FB fractions with or without fermentation treatment (Nesli, 2014). The products were in line with pasta manufactured from durum wheat semolina. VTT has been able to solve sensory characteristics of FB ingredients by combining mechanical fractionation with bioprocessing, thus improving nutritional, technological and

sensory characteristics. The method is chemical-free as it does not use any of organic solvents and cost effective. Development of ingredients indicated the potential of using FB as new food ingredients in various bakery products such as gluten-free bread.

3 STUDY I: DETERMINATION OF SELECTED COMPOUNDS IN FABA BEAN (*VICIA FABA* L.) AND THE EFFECT OF GERMINATION AND AUTOCLAVING TREATMENT ON THEIR CONTENT

3.1 Abstract

In this study, selected chemical compounds were determined in five faba bean cultivars; Snowdrop, Snowbird, Taboar, Fabelle, and Malik that were produced in North America. Seed samples received from three different production years were used in the study. The effect of short-term germination and autoclaving on the level of undesired compounds, phytic acid, vicine/convicine, oligosaccharides, and total phenolics were evaluated.

There was a slight variation (less than 1%) in moisture, protein, fat, and ash contents of faba bean seeds collected from different years. Faba bean contained 35-40% starch, 30-33% protein, 25-27% total dietary fibre, 3-4% ash, and 1% fat. On average, faba bean contained 2% phytic acid and there was no difference between cultivars. For both vicine and convicine content, Fabelle presented the lowest level of 1 and 0.4 mg/g, respectively while Snowdrop and Snowbird had the highest vicine content at 9.9 and 10.8 mg/g, respectively. Vicine content of Malik and Taboar was at similar levels of 7.3 mg/g. The content of convicine of all cultivars ranged from 3.5 to 4.0 mg/g, except in Fabelle. No significant difference was found among cultivars for oligosaccharides content. All faba bean cultivars showed the contents of verbascose and stachyose were higher than raffinose. The range of oligosaccharide levels in the five cultivars was 8.7 to 8.9 mg/g of raffinose, 20.4 to 25.5 mg/g of stachyose, and 37.5 to 64.0 mg/g of verbascose. Content of total phenolics in faba bean differed significantly (p<0.05) between cultivars. Snowdrop and Snowbird had the higher total phenolic content of 48.4 and 48.3 mg/g, respectively, while Fabelle had the lowest level of 10.8 mg/g. Taboar and Malik had total phenolic content of 38.8 and 37.8 mg, respectively.

Germination had no significant effect on phytic acid, vicine (except Snowbird), and convicine levels in faba bean seeds. Germination significantly (p<0.05) reduced the contents of

raffinose by 100%, stachyose by 60%, verbascose at 80% at 72 h of germination. The germination process significantly (p<0.05) reduced total phenolic content in Snowdrop and Snowbird by 17-18%. However, there was no significant effect on total phenolic content of Taboar, Fabelle, and Malik due to germination. For both Snowdrop and Snowbird, the total phenolic content decreased after 48 h germination. Total phenolic content dropped from 48.4 to 40.0 mg/g, and 48.3 to 40.3 mg/g for Snowdrop and Snowbird after 48 h germination, respectively.

Autoclaving did not provide a significant effect on phytic acid, vicine/convicine, and oligosaccharides. However, autoclaving significantly (p<0.05) reduced total phenolic content in Taboar, Snowbird, and Malik, from 38.8 to 32.9, 48.3 to 39.4, and 37.8 to 30.2 mg/g, respectively. The percentage of total phenolic reduction ranged from 15 to 23%. Autoclaving did not significantly change the total phenolic content of Snowdrop and Fabelle seeds.

3.2 Introduction

Faba bean (FB) is a valuable crop for food and feed. FB seed provides adequate amount of macro-nutrients and particularly higher protein content compared to other plants in the *Leguminosae* family (Duranti, 2006). According to Bhatty (1974), the early cultivars (12 cultivars in the study) of FB produced in Canada had protein content ranging from 26 to 35%. Boye et al. (2010) and Hood-Niefer et al. (2010) also reported values of FB seed protein at about 30%. The total fibre content of FB ranged from 6.4 to 8.4% (Bhatty, 1974), and Guillon & Champ (2002) reported the dietary fibre content of broad bean cotyledons in the range of 6.9 to 9.3%. Starch is the most abundant carbohydrate of FB seeds, ranging from 41.1% to 47.5% in 11 FB cultivars grown in Saskatchewan (Hood-Niefer et al., 2010). FB seed derived ingredients such as flours and fractions offer a useful option to increase protein and starch content of products for the current market demand for pulse-based and plant protein-based foods.

Use of FB as a food or food ingredient is limited partly due to the presence of antinutritional and undesired compounds. Phytic acid, vicine/convicine, oligosaccharides, and phenolic compounds are listed as undesired compounds in FB (Sathe & Venkatachalam, 2002; Khamassi et al., 2013; Peterbauer & Richter, 2001; Crofts, Evans, & McVetty, 1980). Phytic acid can chelate minerals such as calcium, zinc, and iron by forming insoluble precipitates and decreases mineral absorption and bioavailability (Sathe & Venkatachalam, 2002). Vicine and convicine are two pyrimidine glycosides found in FB cotyledons. These can be hydrolyzed by a

native β-glucosidase producing their aglycone counterpart, divicine and isouramil, respectively. Aglycone divicine and isouramil can oxidize glutathione in red blood cells and can cause harmful effects to the individuals deficient in glucose-6-phosphate dehydrogenase (G6PD) who cannot synthesize glutathione at the normal metabolic level (Khamassi et al., 2013). Among the oligosaccharides present in FB, raffinose family oligosaccharides (RFOs) are dominant (Rupérez, 1998). Since humans and other monogastric animals are not able to cleave the α-galactosidic linkages, undigested RFOs could be a fermentable substrate for the enteric bacteria in the lower intestine producing hydrogen, carbon dioxide, and methane (McPhee et al., 2002) causing flatulence (Peterbauer & Richter, 2001). Oligosaccharides have shown similar effects as soluble fibre and can lower the total blood cholesterol levels by lowering low-density lipoprotein (McPhee et al., 2002). In the current study, oligosaccharide is still considered as an antinutrient due to their fermentation in intestine. Phenolics are mainly synthesized by the shikimic acid, phenylpropanoid, and pentose phosphate pathways of the plant (Balasundram et al., 2006). Tannins are one of the major phenolic compounds present in FB. They are water-soluble and have high molecular weight with several phenolic hydroxyl groups which enable them to bind with protein and lower the digestibility (Crofts et al., 1980). Phenolic compounds can negatively affect the sensory properties of foods, and tannins mainly contribute to astringency of foods, due to the binding to proteins (Singh et al., 2017) and are considered as undesired compounds in the current study.

Throughout the years, the Canadian FB breeding program has considered reducing tannin content, vicine and convicine level and the seed size in developing new varieties. Due to the mutation in the *vc*-gene that controls vicine and convicine expression in FB, a 10 to 20-fold reduction in vicine and convicine accumulation has been obtained (Duc et al., 1989) without modifying the macronutrient content. Fabelle (medium size, 553 TSW, thousand seed weight) is a low vicine/convicine variety registered in Canada by DL seeds in 2016 (Phelps, 2016; CFIA, 2018). Traditional varieties of FB have large or medium size seeds and brown seed coat with tannin ranging from 8 to 9%. Low tannin varieties as low as 1% have been developed by suppressing *zt1* and *zt2* genes that control accumulation of tannin and these seeds have cream and light seed coat (Duc et al., 1999). Small seed FB is preferred for feed uses while large seeds are preferred in the food market. The traditional food applications in the Mediterranean regions and Egypt (e.g. falafel) prefer large-seeded, bitter taste, high tannin varieties (McGill et al.,

2017), therefore low tannin varieties may be suitable for the regions where uses and applications of FB are being developed and newly introduced.

Currently, the production of smaller FB seed varieties outweighed the larger seed varieties in Canada. Smaller seed varieties such as Snowdrop, and Snowbird accounted for about 70% of production in Canada during the 2015 crop year (McGill et al., 2017). For the producer, small and round shape FB seeds enable the use of existing pea seeders due to their similar shapes (Phelps, 2016). Malik (680 TSW) is the only large seed variety produced in a sizable volume in Canada, which was about 10% of production in 2015 (McGill et al., 2017).

The breeding process has limitations in eliminating the unwanted compounds in FB that affect their use and nutritional value. Moreover, the cost and time associated with breeding out these can be comparatively higher than using effective post-harvest treatments. Germination is a natural process with minimum equipment requirement and can be effective in reducing undesired compounds due to activation or *de novo* synthesis of various enzymes in germination associated biochemical processes (Haileslassie et al., 2016). Multiple authors have reported the effect of germination of FB on the levels of phytic acid, vicine/convicine, and phenolics, but very limited research was done on oligosaccharides in germinated FB (Luo & Xie, 2013; Bellaio et al., 2013; Khalil & Mansour, 1995; Shetty et al., 2011).

Besides natural process, such as germination, heat processing has also been studied to reduce the level of undesired compounds in FB seeds. Canning is a common heat treatment of FB at commercial level. Canned FB is a convenient food and has an extended shelf-life of the cooked product by killing spores of *Clostridium botulinum* (Güzel & Sayar, 2011; Hefni, et al., 2015; Mozzoni et al., 2009). Autoclaving at 121°C, which has similar heat and pressure effect as canning was less effective in reducing phytic acid in FB compared to germination (Khalil & Mansour, 1995). Reports on other beans on heat treatments and phytic acid levels are not conclusive; Greiner & Konietzny (1998) reported no significant change in phytate content but Khattab & Arntfield (2009) reported that autoclaving significantly reduced 63% of phytate in red kidney bean from Manitoba, Canada. Very little is known about the change of vicine/convicine, oligosaccharides, and phenolics in FB during the autoclaving or canning process. However, heating can induce hydrolysis and break down of large compounds, e.g. oligosaccharides (Oboh et al., 2000).

The purpose of this study is to provide and compare the chemical composition of faba bean cultivars produced in North America with an emphasis on the effect of short-term germination and autoclaving on the levels of selected undesired compounds, phytic acid, vicine/convicine, oligosaccharides, and total phenolics. Based on the results, it could be possible to fully explore the potential of using FB seeds as food ingredient in the current market.

3.3 Materials and methods

3.3.1 Seed collection

Certified FB seeds of five cultivars (Snowdrop, Fabelle, Malik, Taboar, and Snowbird) produced in Saskatchewan, Canada and North Dakota, USA and in three different crop years (2013, 2015, and 2016) were provided by Alliance Grain Traders Inc. Saskatoon and Crop Development Centre (CDC) of University of Saskatchewan, Saskatoon. Three seed lots received for each production year 2016, 2015, and 2013 are referred as seed lot 1, 2 and 3, respectively throughout this study. These cultivars represent various quality traits; Snowdrop and Snowbird are small/medium-seeded and low-tannin, Malik is large-seeded and regular tannin, Taboar is medium-seeded and regular tannin, and Fabelle is medium-seeded, regular tannin and low vicine/ convicine. Each seed sample (12 kg) was received and stored at ambient temperature until sorted for size by screening.

The seed sorting assembly consisted of 24/64-inch round-hole sieve (Can-seed-equip, SK, Canada), 26/64-inch round-hole sieve, and 12/64-inch slot sieves. One kg of faba bean seeds of each cultivar was placed on the 12/64 slot sieve first and the shaking was continued for about 2 min to get rid of impurities (leaves, and branches). For the smaller seed cultivar (Snowdrop, 335 TSW), 1 kg of the seeds were separated on the 24/64-inch round-hole sieve in a similar manner and seeds on the sieve were collected and assumed to have same seed size. Smaller undersized seeds collected under the sieve were discarded. For larger seed cultivars (Snowbird, 495 TSW; Fabelle, 553 TSW; Malik, 680 TSW; and Taboar, 480 TSW), 1 kg of the seeds were separated using 26/64-inch round-hole sieve using the similar method to above. Clean seeds without impurities and separated according to size were sealed in freezer bags (26.8 cm × 27.3 cm) and stored at room temperature for further use.

3.3.2 Soaking and germination of whole faba bean seeds

Dry FB seeds (120 g) were placed in a 1 L glass beaker, and 600 mL (1:5 w/v ratio) of 6% hydrogen peroxide solution was added and the contents were stirred with a clean glass rod for 1 min, followed by soaking for 10 min at room temperature. This disinfecting solution was drained out and the seeds were thoroughly washed with 600 mL of autoclaved distilled water (1:5 w/v ratio, based on dry seed weight) for 1 min. The washing step was repeated 5 times and the disinfected and washed seeds were soaked in 600 mL of autoclaved distilled water (1:5 w/v ratio, based on dry seed weight) at room temperature for 12 h in the dark. Soaked seeds were drained, 40 g of soaked seeds were transferred to freezer bags (16.5 cm × 14.9 cm) and stored at -18 °C until the further analysis. This process will be referred to as the soaking treatment. Soaked seeds were labeled as 0 h of germination. The remaining soaked seeds were used in the germination process.

Seed germination was carried out as follows. The incubator (Autoelex Co. Ltd. South Korea) was sanitized with 70% (v/v) ethanol and set at 25°C. Humidity was maintained at 70% or higher. About 500 mL of autoclaved distilled water was added into the water chamber and replenished every 24 hours. A piece of wet cheese cloth previously soaked in autoclaved distilled water was placed to cover the bottom surface of the incubator, and soaked FB seeds collected after soaking treatment of each cultivar was separately (separated with a polymer splint that came with the incubator) spread on the cheese cloth to have a single or two- seed layer, covered with another piece of soaked cheese cloth, and the incubator lid was closed. Every 12 h, 100 mL of autoclaved distilled water at room temperature was sprayed onto faba bean seeds to maintain a high relative humidity. Gloves sanitized with 70% (v/v) ethanol were used during seed transferring and handling. Germinated seeds of each cultivar were collected at 24 h, 48 h, and 72 h of germination. Approximately 30-40 g of the wet, germinated seeds were randomly selected from each cultivar for each sampling. Germinated seeds were transferred into freezer bags (16.5 cm×14.9 cm) and stored at -18 °C until further analysis. Time of germination was defined as when the seed radicle tip was observed through the ruptured seed coat (Nonogaki et al., 2010). All seeds in the tray were counted to calculate the germination percentage at different germination times.

The germination percentage of each cultivar was calculated based on the following equation:

Germination percentage %= 100 × number of germinated seeds/ total number of seeds in the tray

3.3.3 Soaking and autoclaving of whole faba bean seeds

Dry seeds (50 g) were placed in a 600 mL beaker, 250 mL of autoclaved distilled water were added into the beaker to reach the w/v ratio at 1:5 and the contents stirred with a clean glass rod for 1 min and then wash water was decanted out. This washing step was repeated until 5 washes. Washed seeds were then soaked in autoclaved distilled water (1:5 w/v ratio based on the dry seed weight) at room temperature for 12 h in the dark. Soaked seeds were drained, transferred to a 600 mL beaker, and 100 mL of the autoclaved distilled water (1:2 w/v ratio based on the dry seed weight) was added and the beaker was covered with a piece of aluminum foil. Beakers containing seeds were placed in the autoclave (Amasco 250, Steris, OH, USA) with the settings at 121 °C for 25 min. Autoclaved seeds (with liquid) were allowed to cool down to room temperature, transferred into a 250 mL plastic cup sealed with a lid and stored at -18 °C until further analysis.

3.3.4 Freeze drying and milling of raw and treated (soaked, germinated, and autoclaved) faba bean seeds

Both freezer bags and plastic cup lids were poked with holes using a sterilized stainless steel needle to allow water vapor to release efficiently and placed in the tray freeze dryer (Labconco, MO, USA) chamber until samples reached dry total target moisture content of 1-3 %, w/w. The vacuum set point of the freeze dryer is around 0.01 mBar (-40 to -50 °C). All freeze-dried seeds were milled using an Ultra-Centrifugal Mill (Retsch, PA, USA, Model ZM 200) equipped with a screen (250 µm aperture size) and the rotor speed set at 12,000 rpm. Milled samples were separately packed into freezer bags (16.5 cm×14.9 cm), sealed and stored at -18 °C for further chemical analysis.

3.3.5 Physical properties and chemical composition of faba bean seeds and flours

A) Colour measurement

The HunterLab ColourFlex colourimeter (Hunter Associations Laboratory Inc., Reston, VA, USA) was used to measure the colour of the whole faba bean seed based on *L**, *a** and *b** dimensions with illuminant A and 10 ° observer. For colour measurement of each sample, the transparent polystyrene petri dish (100×15 mm) was filled with the whole faba bean seeds and

covered with the non-transparent lid. Each seed sample was measured twice with 90º rotation between the readings from the bottom. All the samples were measured in triplicate.

B) Proximate analysis and carbohydrate composition

Moisture

The moisture contents were determined by AOAC Method 925.10 (AOAC, 1990). Around 3 to 4 g of faba bean flour samples were dried in the forced air oven (Precision Scientific Thelco, IL, USA, Model 18) at 105 °C overnight. The moisture content was calculated based on the percentage of weight loss after drying.

Crude protein

The protein content was determined by the combustion method based on AACC Method 46-30.01 (AACC, 1999). Around 50 mg of FB flour sample was weighed and analyzed by a total nitrogen analyzer (Thermo Fisher Scientific, Delft, Netherlands, Model FLASH EA 1112). The nitrogen conversion factor of 6.25 was used to calculate protein content.

Crude fat

Crude fat content was determined by the Swedish tube method using hexane solvent based on AOCS method Am 2-93 (AOCS, 1995) with modifications. Around 3 g of FB flour sample was extracted by 25 mL hexane for 20 min in the stainless steel Swedish tubes with vigorous shaking on the vortex mixer. After filtration and solvent evaporation, the oil content was extracted and dried in a forced air oven at 130 °C for 20 min and weighed. Crude fat content was calculated based on the oil weight extracted from the initial FB flour sample weight.

Ash

The ash content was determined by AOAC Method 923.03 (AOAC, 1990). Around 3 g of FB flour sample was charred for 30 min and incinerated in the muffle furnace (Fisher Scientific, Isotemp Muffle Furnace, ON, Canada) at 550 °C overnight. After cooling down, the crucible and remaining ash content was weighed. Ash content was calculated based on the weight of ash and the initial FB sample weight.

Total, insoluble, and soluble dietary fibre

The total, soluble and insoluble dietary fibre were determined by the enzymatic-gravimetric method AACC Method 32-45.01 & 32-50.01 (AACC, 1999) using a Megazyme kit with modifications (Xu, 2017). Exactly 1.000 g of the FB flour sample was digested by 40 mL of pancreatic α-amylase/amyloglucosidase mixture at 37 ºC for 16 h, and pH of the mixture was

titrated to ~8.2 by using 3.0 mL of 0.75 M Tris base solution. Samples were incubated in the water bath at 95-100 ºC for 20 min with occasional shaking. After the water bath, 0.1 mL of protease solution was added, and the mixture was incubated in the water bath at 60 ºC for 30 min. Insoluble dietary fibre was determined by vacuum filtration of the enzyme digest through the glass filter crucible and soluble dietary fibre was obtained by precipitating the filtrate with 4 volumes (filtrate: ethanol = 1: 4) of 95% (v/v) ethanol overnight. Insoluble and soluble dietary fibre were adjusted by ash and protein content in the FB flour sample. The total dietary fibre content is the sum of insoluble and soluble dietary content. The total dietary fibre, insoluble dietary fibre and soluble dietary fibre were calculated by the following equations. Samples were measured in duplicate.

Blank (B) determination (mg) = (BR1 + BR2)/2- PB - PA

Where:

BR1 and BR2 = residue mass (mg) for duplicate blank determinations respectively.

PB and PA = mass (mg) of protein and ash respectively, determined on first and second blank residue.

Total dietary Fibre (TDF), insoluble dietary Fibre (IDF) or soluble dietary Fibre (SDF) (mg/100 g) TDF/IDF/SDF%= ((R1 + R2)/2 - PB - PA – B)/ (M1 + M2/2) × 100

Where:

R1 = Residue mass 1 from M1 in mg

R2 = Residue mass 2 from M2 in mg

M1 = Test portion mass 1 in g; M2 = Test portion mass 2 in g

PA = Ash mass from R1 in mg; PB = Protein mass from R2 in mg

TDF (%) = TDF (mg/100 g)/1000

IDF (%) = IDF (mg/100 g)/1000

SDF (%) = SDF (mg/100g)/1000

Total starch

The total starch content was determined by AACC Method 76-13.01 (AACC, 1999) using a Megazyme kit with modifications (Xu, 2017). About 100 mg of FB flour sample was weighed into a centrifuge tube (16×120 mm, 17 mL) and incubated with 5 mL of aqueous ethanol (80%, v/v) in the water bath at 80-85°C for 5 min. The mixture was mixed thoroughly and additional 5 mL of ethanol (80%, v/v) was added into the centrifuge tube. Tubes were

centrifuged at 1,800×*g* for 10 min. The supernatant was discarded after the centrifugation, and the pellet was resuspended with 10 mL of ethanol. The mixture was stirred and centrifuged again as above. Supernatants were discarded. Then, two mL of dimethyl sulphoxide (DMSO) was added to the tube and mixed by a vortex mixer. The tube was placed in a vigorously boiling water bath for 5 min and removed. Three mL of the thermostable α-amylase was added, and the tube was incubated in the water bath at boiling temperature for 12 min. The tube was stirred vigorously at 4, 8 and 12 min and then immersed into the water bath at 50 °C for 10 min. Then 0.1 mL of amyloglucosidase was added to the tube. The mixture was mixed by the vortex mixer, and the tube was incubated at 50 °C water bath for 30 min. The entire content of the tube was transferred to a 10 mL volumetric flask. The tube was thoroughly rinsed, and volume was adjusted, by distilled water. An aliquot of the solution was centrifuged for 10 min. Duplicate of aliquot (0.1 mL) were transferred to glass test tubes (16×100 mm) and mixed with 3.0 mL of GOPOD (including D-glucose controls and reagent blanks). The reagent blank consisted of 0.1 mL of distilled water and 3.0 mL of GOPOD. D-glucose control consisted of 0.1 mL of D-glucose standard solution (1 mg/mL) and 3.0 mL of GOPOD reagent. The tube was incubated in the water bath at 50 °C for 20 min and absorbance was recorded at 510 nm wavelength against the reagent blank with 60 min. The starch content was calculated by the following equation.

Starch % = ΔA × FW × FV ×0.9

Where:

ΔA = Absorbance value at 510 nm read against the reagent blank

F = 100 (μg of D-glucose)/Absorbance for 100 μg of glucose (conversion from absorbance to μg) FV = Final volume (mL)

W = Sample weight (mg)

0.9 = Adjustment from free D-glucose to anhydrous D-glucose (as occurs in starch).

C) Undesired chemical compounds determination

Phytic acid analysis

Phytic acid level of FB samples were determined according to the method described by Oomah et al. (2010). Faba bean flour (1.0 g) was weighed into a 50 mL centrifuge tube and extracted with 20 mL of 2% (v/v) HCl solution by shaking for 1 h at room temperature. Then the mixture was recovered by centrifugation at 9,000×*g* for 20 min at room temperature. The extract was diluted by mixing 1.0 mL of extract with 24 mL of Super-Q water in another 50 mL

centrifuge tube. The anion exchange column (AG-1-X8, Bio-Rad Laboratories Ltd, Japan) was washed with 6.0 mL of 0.08 % (v/v) HCl solution. The diluted extract eluted through the column, and interfering compounds were removed by washing the column with 10 mL of Super-Q water followed by 15 mL of 0.1 M NaCl. Bound phytate was eluted with 3×10 mL of 0.7 M NaCl. A 150 µL aliquot of the elute was vortexed and mixed with 50 µL of Wade reagent in the well of a microplate. Absorbance of the salicylate-Fe complex was determined at 500 nm using a microplate reader (Bio-Rad Laboratories Ltd, Japan, Model xMark).

Vicine and convicine

Vicine and convicine content was determined according to the method used in the Protein Research Lab, Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, and adopted from Quemener et al. (1982) and Marqurdt et al. (1983) with modifications. Around 50 mg of FB flour samples were mixed with 50 µL of 8 mg/mL of uridine internal standard and 1.45 mL of 0.1 M NaOH in a 2 mL micro-centrifuge tube. The mixture was sonicated at 40 °C for 90 min while mixing the tube content by vortexing for 10 seconds for every 30 min. Then the tube was centrifuged at 23,300×*g* for 10 min at room temperature. The supernatant was collected in another micro-centrifuge tube, and the pH of the mixture was adjusted to 3.5-4.0 with 1 M HCl. Centrifugation was repeated to collect the supernatant. The supernatant was filtered through a 0.45 µm PVDS (polyvinylidene fluoride) syringe filter into an HPLC vial. The extract was analyzed by Waters Alliance C High Performance LC with Waters Symmetry C18, 100 Å, 3.5 micrometer, 2.1×100 mm column (Waters Corporation, MA, USA). Peak identification was based on the commercial vicine standard (Sigma-Aldrich) and mass spectroscopic identification of convicine. Quantitation was based on the external standard curve prepared from vicine considering the recovery of the uridine internal standard.

Oligosaccharides

Around 50 mg of flour sample was weighed and mixed with 1 mL of 1200 µg/mL of D-arabinose water solution, and the mixture was incubated at 60 °C for 1 h (Xu, 2017). Then the mixture was centrifuged at 23,300×*g* for 10 min. The supernatant was obtained. The extraction procedure was repeated by adding 0.5 mL of combined distilled water and the supernatants. A 0.75 mL aliquot of supernatant was mixed with 0.75 mL of 100% acetonitrile and the tube was mixed thoroughly by a vortex for 30 seconds. The solution was incubated at room temperature for 10 min and centrifuged at 23,300×*g* for 10 min. The supernatant was obtained and filtered

through 0.2 µm membrane and analyzed by Acquity UltraPerformance LC (Waters Corporation, MA, USA). The raffinose, stachyose and verbascose contents were calculated based on the external standard curves prepared with the corresponding oligosaccharide (50 to 400 µg/mL), considering the recovery of the D-arabinose internal standard.

Total phenolic content

The phenolic compounds were extracted with ethanol (80%, v/v) (Oomah et al., 2010) and the absorbance was determined by a UV-Visible spectrophotometer. Around 200 mg of the sample was extracted with 6 mL of ethanol (80%, v/v) for 2 h at room temperature. The extraction process was done twice. The supernatant was collected and filtered through the 0.45 µm nylon filter (Canadian Life Science, Edmonton, Canada). A 100 µL of the solution was mixed with 150 µL of HCl (2%, v/v) in ethanol solution (80%, v/v), and the mixture was transferred to a 96-well ultraviolet microplate (flat-bottom). The plate was shaked for 2 min. The absorbance of the mixture was determined by a microplate spectrophotometer (Bio-Rad Laboratories Ltd, Japan, Model xMark) at 280 nm, and catechin was used as the standard.

3.4 Statistical analysis

The overall mean and standard deviation were calculated with three replicates of means for all samples. Observed data were analyzed as a Completely Randomized Design using the Proc Mixed Procedure of SAS 9.4 (SAS, Inst. Inc., Cary, NC). Means were analyzed and separated with the Tukey's method of SAS and a pdmix SAS macro was used to convert mean separation output to letter groupings (Saxton, 1998). Significance was declared at $p<0.05$.

3.5 Results and discussion

3.5.1 Proximate and carbohydrate analysis of whole faba bean seeds

There was a slight variation (less than 1%) of moisture, protein, fat, and ash content of FB seeds collected from different seed lots (Table 3-1). The moisture content and protein of all five FB cultivars were similar. Moisture content of FB ranged from 9.79 % (Fabelle) to 10.57 % (Taboar), and protein ranged from 31.28 % (Snowbird) to 33.30 % (Fabelle). The stable storage conditions provided to FB samples during the study maintained the moisture content at a steady level. There was no significant difference observed in the contents of ash and fat in the whole seed among these five cultivars. The ash content ranged from 3.63 % (Snowbird) to 3.90 % (Malik), and fat from 1.18 % (Snowbird) to 1.26 % (Malik). The data of ash content indicated

very little variation, which was comparable to the ash content of 12 FB cultivars (2.7% to 3.5%) reported in 1974 (Bhatty, 1974).

Table 3-1 Contents of moisture, protein [db], ash [db] and fat [db] (%) of untreated faba bean seeds from three different (seed lot 1 to 3) crop years

Cultivar	Seed lot	Moisture %	Protein %	Ash %	Fat %
Snowdrop	1	10.20±0.54	31.52±1.34	3.61±0.13	1.09±0.01
Taboar	1	10.66±0.32	33.15±1.56	3.77±0.24	1.27±0.02
Snowbird	1	10.29±0.29	31.37±1.23	3.70±0.09	1.26±0.01
Fabelle	1	9.89±0.27	33.42±0.75	3.77±0.08	1.35±0.05
Malik	1	10.06±0.13	33.29±1.02	4.02±0.24	1.24±0.02
Snowdrop	2	9.97±0.08	31.99±0.84	3.66±0.07	1.22±0.04
Taboar	2	10.67±0.28	32.63±0.72	3.85±0.11	1.20±0.07
Snowbird	2	10.18±0.32	31.07±0.51	4.05±0.07	1.24±0.02
Fabelle	2	9.90±0.41	33.18±0.72	3.82±0.05	1.19±0.03
Malik	2	10.19±0.27	33.40±1.01	3.95±0.02	1.17±0.02
Snowdrop	3	9.87±0.17	31.93±4.31	3.63±0.21	1.23±0.06
Taboar	3	10.39±0.38	33.12±0.32	3.80±0.09	1.18±0.05
Snowbird	3	10.48±0.37	31.40±0.83	3.50±0.11	1.29±0.02
Fabelle	3	9.59±0.45	33.31±1.23	3.59±0.13	1.21±0.03
Malik	3	9.75±0.56	33.07±0.64	3.73±0.04	1.16±0.01
Overall mean values*		**Moisture %**	**Protein %**	**Ash[ns] %**	**Fat[ns] %**
Snowdrop		10.01 ± 0.17^{bc}	31.82 ± 0.26^{b}	3.63±0.03	1.18±0.08
Taboar		10.57 ± 0.16^{a}	32.97 ± 0.29^{a}	3.81±0.04	1.25±0.09
Snowbird		10.32 ± 0.15^{ab}	31.28 ± 0.18^{b}	3.75±0.28	1.19±0.04
Fabelle		9.79 ± 0.18^{c}	33.30 ± 0.12^{a}	3.73±0.12	1.21±0.05
Malik		10.00 ± 0.23^{bc}	33.25 ± 0.17^{a}	3.90±0.15	1.26±0.02

[db] Dry basis. [ns] Not significant ($p>0.05$). Means with the different letter in the same column are significantly different ($p<0.05$). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation. *Mean values were calculated by averaging seed lots.

The protein and fat content (Table 3-1) results of this study were close to the results reported in other studies. Boye et al. (2010) reported that the crude protein in FB was around 30%, which agreed with the protein content of 12 cultivars of FB, ranging from 26 to 35%, reported by Bhatty (1974). In another study, Hood-Niefer et al. (2010) also concluded that the protein concentration of FB ranged from 28 to 32%, which was higher than the protein content (25%) of pea. Duc et al. (1999) reported that FB with high tannin content contained 31.0% of protein and 1.9% of fat, and low tannin cultivars had 31.9% of protein and 2.0 % of fat. Sauvant

et al. (2004) also stated the protein content of high-tannin and low-tannin varieties of FB was around 29.4% and 31.1%, respectively. The fat content of high-tannin and low-tannin variety of their study was 1.5% and 1.3%, respectively and in the low side for legumes seeds.

Different seed lots analysed from different crop years only showed a slight difference (≤ 2%) in the contents of total starch or total fibre of each FB cultivar (Table 3-2). Carbohydrate content of FB was around 60% (w/w) of the seed dry matter (Sauvant et al., 2004). There was no significant difference in total starch (Table 3-2) and total fibre contents in the whole seed of five cultivars. The starch content of whole seed FB was around 35 %, and total dietary fibre was 25-26%. Insoluble fibre was dominant in the fibre content. The ratio of insoluble fibre to soluble fibre was roughly 10:1. Overall all cultivars contained 23-24 % of insoluble fibre, and 2.3-2.5 % of soluble fibre. High insoluble fibre content could be due to the incorporation of seed coat in the flour making and no seed coat removal was carried out.

The two major groups of carbohydrates in FB were starch and fibre. Faba bean carbohydrates consisted of various components, such as dextrin, starch, free sugar, lignin, cellulose, hemi-celluloses, and fibre (Bhatty, 1974). Starch was the most abundant carbohydrate, and the results of starch content of the present study was lower than the results (41.1 to 47.5%) reported by Hood-Niefer et al. (2010) for 11 faba bean cultivars grown in Saskatchewan, Canada. Duc et al. (1999) reported a mean starch content of 42% in FB whole seed, which was also higher than the starch content determined in the current study. The difference between the current study and previous studies could be due to the crop year, location, and genotypes of FB. Genotype was reported to have significant effects on starch concentration, but not on the amylose concentration in faba starch (Hood-Niefer et al., 2010).

The utilization of faba bean starch as new ingredients in different food products had drawn attention of the food industry. Ambigaipalan et al. (2011) reported FB starch has higher gelation temperature, higher elasticity, faster retrogradation, and be more resistant to shear thinning compared to other common starch kinds. Additionally, the low phosphorous content of FB starch could result in a lower starch paste viscosity. Such properties of FB starch would make it as a potential candidate to replace chemically modified starch in the current food market.

Table 3-2 Carbohydrate profiles [db] (%) of untreated faba bean seeds from five cultivars

Cultivar	Seed lot	Starch %	Total fibre %	Insoluble fibre %	Soluble fibre %
Snowdrop	1	35.58±2.21	26.69±1.07	24.20±1.12	2.49±0.05
Taboar	1	34.04±1.56	27.67±1.41	25.18±0.98	2.48±0.04
Snowbird	1	35.52±1.70	27.23±0.90	24.93±1.01	2.30±0.11
Fabelle	1	35.62±0.89	26.30±1.01	23.83±0.93	2.47±0.07
Malik	1	36.95±1.02	24.24±0.62	21.75±0.54	2.49±0.08
Snowdrop	2	34.47±2.01	26.29±0.69	23.57±0.78	2.72±0.09
Taboar	2	36.62±1.43	26.21±1.06	23.82±0.69	2.39±0.08
Snowbird	2	34.48±0.80	25.44±0.41	23.08±0.57	2.36±0.16
Fabelle	2	35.75±1.12	24.45±1.26	22.33±1.02	2.12±0.23
Malik	2	35.73±0.75	26.29±0.59	23.94±0.89	2.35±0.30
Snowdrop	3	35.49±0.56	26.03±0.46	23.61±0.75	2.42±0.07
Taboar	3	34.49±1.09	25.95±1.05	23.76±1.03	2.19±0.03
Snowbird	3	36.01±0.45	25.77±1.50	23.20±0.89	2.56±0.15
Fabelle	3	34.11±0.50	25.94±1.21	23.66±0.99	2.28±0.09
Malik	3	34.21±0.72	24.36±1.55	22.07±1.04	2.29±0.15
Overall mean values*		**Starch [ns] %**	**Total fibre[ns] %**	**Insoluble fibre[ns] %**	**Soluble fibre[ns] %**
Snowdrop		35.18±0.62	26.34±0.33	23.80±0.35	2.54±0.16
Taboar		35.05±1.38	26.61±0.92	24.25±0.80	2.35±0.15
Snowbird		35.33±0.78	26.15±0.95	23.74±1.03	2.41±0.14
Fabelle		35.16±0.91	25.56±0.98	23.27±0.82	2.29±0.18
Malik		35.63±1.37	24.96±1.15	22.59±1.18	2.38±0.10

[db] Dry basis. [ns] Not significant (p>0.05). Values are presented as mean ± standard deviation. *Mean values were calculated by averaging seed lots.

Total, insoluble, and soluble fibre content of whole FB seed flour were not significantly different among cultivars. Limited information on fibre content was retrieved for FB cultivars currently grown and available in the market. Dietary fibre or cell wall material content in the testa was normally greater than that of cotyledon. In a study on broad bean, seed coat is rich in dietary fibre and the cotyledon fibre content was in the range of 6.9 to 9.3% (Guillon & Champ, 2002). In 1974, Bhatty reported that the total fibre content was in the range of 6.4 to 8.4% for twelve cultivars of FB whole seed grown in Canada (Bhatty, 1974). Duc et al. (1999) reported the mean crude fibre content of high-tannin and low-tannin FB varieties were 9.9% and 8.8, respectively. Lower values have been reported for the fibre content of FB seed, reflecting in part,

differences in the methodologies used for dietary fibre determination, i.e. AACC 1999 in the current study *versus* AOAC 1970 in early studies. Additionally, the resistant starch content was included in the total dietary fiber content in the current study. The different fibre analysis method employed by previous researchers may attribute to the differences in results of the present study.

3.5.2 Colour parameters and germination percentage and of whole faba bean seed

The *L**, *a**, and *b** values for seed colour (with seed coat on) are in Table 3-3. Among FB cultivars there was no significant difference found in *L**, *a**, and *b** values of FB among cultivars. For all five cultivars, *L* (lightness) ranged from 37.5 to 49.0, *a* (greenness) ranged from 4.7 to 12.6, and *b* (yellowness) ranged from 19.2 to 21.2. Snowbird and Snowdrop had similar brown-green colour. Fabelle, Malik, and Taboar had tan-brown colour.

Table 3-3 The colour parameters of whole faba bean seeds for five cultivars

Cultivar	*L** value [ns]	*a* *value [ns]	*b* *value [ns]
Snowdrop	43.2±1.85	4.7±0.17	19.5±0.79
Taboar	37.5±6.02	10.7±3.24	19.2±1.89
Snowbird	49.0±4.33	12.6±8.14	21.2±1.40
Fabelle	39.2±5.28	9.6±2.67	19.3±1.08
Malik	39.7±9.27	9.6±3.92	19.7±4.09

[ns] Not significant ($p>0.05$). Means with the different letter in the same column are significantly different ($p<0.05$). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation.

Due to the variation of seeds received from different crop years for this study, seed colour variation from year to year was apparent. For the second-year samples received, all five cultivars showed paler colour. The variation in seed coat colour may be due to the amount of the precipitation received in different crop years because more precipitation may cause the paler colour of the seed coat (Government of Saskatchewan, 2016). Therefore, the seeds collected from different crop years may create larger standard deviation values of the mean (e.g. *a* value of Snowbird had a large standard deviation, and the coefficient of variation was more than 50%). A previous study by Oomah et al. (2011) proved that the genotype and growing locations had significant effects on FB seed coat colour. The mean *L* value, *a* value, and *b* value of 13 cultivars of FB grown in Canada were 26.10, 0.38, and 0.39, and the spectrum covered from brown to beige. The difference of the seed colour between the current study and the previous study could be due to the FB genotype, crop year, and growing locations.

3.5.3 Undesired compounds in whole faba bean seed

There was no significant difference found in the levels of phytic acid, raffinose, stachyose, and verbascose among ground seeds of all cultivars (Table 3-4). Due to the variation of seeds received from different seed lots, the content of phytic acid, vicine, and convicine varied among seed lots, which may lead to larger standard deviation values of the mean in some cultivars. Snowdrop presented a higher standard deviation of phytic acid (0.43) and vicine (0.85) than other cultivars. Fabelle had the highest standard deviation (0.47) of the mean convicine level.

Table 3-4 The levels of phytic acid[db] (%), vicine[db] (mg/g) and convicine[db] (mg/g) in untreated faba bean seeds

Cultivar	Seed lot	Phytic acid	Vicine	Convicine
Snowdrop	1	2.08±0.03	10.89±1.01	3.79±0.10
Taboar	1	1.77±0.07	7.59±0.56	3.9±0.23
Snowbird	1	1.83±0.09	10.52±0.98	3.82±0.31
Fabelle	1	1.91±0.01	0.53±0.02	0.14±0.02
Malik	1	1.99±0.12	7.86±0.65	3.68±0.15
Snowdrop	2	1.25±0.11	9.67±0.95	3.37±0.05
Taboar	2	1.59±0.10	7.15±0.41	3.93±0.29
Snowbird	2	1.91±0.08	11.04±0.95	3.59±0.34
Fabelle	2	1.78±0.07	0.57±0.08	0.12±0.02
Malik	2	1.60±0.14	7.02±0.09	3.67±0.76
Snowdrop	3	1.46±0.18	9.25±0.95	3.44±0.13
Taboar	3	1.58±0.09	7.19±0.31	3.97±0.45
Snowbird	3	1.51±0.08	10.89±0.73	3.70±0.06
Fabelle	3	1.61±0.10	1.97±0.98	0.95±0.04
Malik	3	1.60±0.07	6.92±0.52	3.56±0.87
Overall mean values*		**Phytic acid [ns]**	**Vicine**	**Convicine**
Snowdrop		1.60±0.43	9.94±0.85[a]	3.53±0.23[a]
Taboar		1.65±0.11	7.31±0.24[b]	3.93±0.04[a]
Snowbird		1.75±0.21	10.82±0.27[a]	3.70±0.12[a]
Fabelle		1.77±0.15	1.02±0.82[c]	0.40±0.47[b]
Malik		1.73±0.23	7.27±0.52[b]	3.64±0.07[a]

[db] Dry basis. [ns] Not significant (p>0.05). Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation. *Mean values were calculated by averaging seed lots.

There was no significant difference for the phytic acid content in the five FB cultivars (Table 3-4) and the values were in the range of 1.60-1.77%. The phytic acid content in FB was reported to range from 0.39% to 0.84% (Khalil & Mansour, 1995; Luo & Xie, 2013), which was lower than the phytic acid level observed in the current study which may be due to differences in genotypes. Work by Oomah et al. (2011) showed that the location and genotype significantly influenced the phytic acid content in FB seeds. Phytic acid levels ranged from 5.9 to 15.1 mg/g for Imposa and SSNS-1, respectively. Cluster analysis based on phytic acid segregated the genotypes into four groups; high, Fatima, Florent, and SSNS-1 (>12 mg/g); above average, FB25-56, Disco, and NPZ4-7540 (10-12 mg/g); average, AO1155, Divine, Melodie, Snowbird, and Taboar (8-10 mg/g); low, Imposa, and AZ10 (<7 g/mg). Based on levels of phytic acid content, all FB cultivars in the current study can be classified into the high group (>12 mg/g).

In the current study, the vicine content of each cultivar was approximately twice the convicine content (Table 3-4). For both vicine and convicine content, Fabelle presented the lowest level of 1.02 and 0.40 mg/g, respectively. Snowdrop and Snowbird had the highest vicine content at 9.94 and 10.82 mg/g, respectively. Malik and Taboar had the second highest vicine content at 7.27 and 7.31 mg/g, respectively. Except for Fabelle, all other cultivars contained similar level of convicine, ranging from 3.53 mg/g (Snowdrop) to 3.93 mg/g (Taboar). The vicine and convince are synthesized by the seed during the beginning stage of the seed growth, therefore vicine and convicine levels should be high in immature fresh seeds (Cardador-Martínez et al., 2012). Vicine content was reported in the range from 2.88 mg/g to 6.10 mg/g, and convicine from 0.60 to 1.68 mg/g (Cardador-Martínez et al., 2012). Different values of vicine (6.8 mg/g) and convicine (2.7 mg/g) were also reported by Khalil & Mansour (1995). Duc et al. (1999) provided the sum of vicine and vicine content as 8.3 mg/g for high-tannin and 7.6 mg/g for low-tannin FB varieties. Pulkkinen et al. (2015) reported the mean value of vicine of high vicine/convicine faba bean variety was 7.43, and mean value of convicine was 2.50 mg/g by using perchloric acid to extract. According to their study, the mean value of convicine content of low vicine/convicine genotypes was not detectable. In addition, Purves et al. (2017) reported the vicine content of Fabelle was 0.348 mg/g, and convicine content was 0.033 mg/g by using acetone as the extraction solvent. The difference of vicine and convicine content between the current and previous studies could be due to combination of factors; genotypic differences, environmental factors, extraction solvent used, and the analytical method employed.

The levels of raffinose, stachyose, and verbascose (Table 3-5) did not show a significant difference between FB cultivars. Overall, the range of oligosaccharide levels in the five FB cultivars was 8.67 to 8.89 mg/g of raffinose, 20.38 to 25.53 mg/g of stachyose, and 37.54 to 64.00 mg/g of verbascose. Due to the seed receipt from different seed lots, the standard deviation values of mean stachyose and verbascose level were relatively higher than raffinose. FB seeds collected in seed lot 1 presented lower raffinose, stachyose, and verbascose content compared to seed lot 2 and seed lot 3. The variation in the values were quite large between replicates of same variety indicating a possible effect of environment oligosaccharides content. In a previous study by Rupérez (1998), the main oligosaccharides in raw FB were reported as verbascose (33.2 mg/g), and stachyose (22.1–32.3 mg/g). The current study found that stachyose and verbascose as the major ones of the raffinose family oligosaccharides (RFOs) in FB. In the study by Goyoaga et al. (2011), FB cultivars Alameda and Brocal had 5.03 and 4.03 mg/g raffinose, 9.22 and 9.43 mg/g stachyose 29.60 and 28.69 mg/g verbascose, respectively confirming the order of abundance of these oligosaccharides was similar to that is found in the present study: raffinose < stachyose < verbascose.

The content of total phenolic in FB (Table 3-5) showed a significant difference between cultivars. Fabelle had a high standard deviation of 3.45, which resulted the high coefficient of variation (>30). FB seeds collected in seed lot 2 had lower total phenolic content compared to seed lot 1 and seed lot 3. Snowdrop and Snowbird showed higher total phenolic content of 48.35 and 48.28 mg/g, respectively, while Fabelle had the lowest level total phenolic content (10.82 mg/g). Taboar and Malik had total phenolic content of 38.76 and 37.76 mg/g, respectively. The results of total phenolic content in the current study were close to the findings of Oomah et al. (2010). They observed that the total phenolic content ranged from 5.5-41.8 mg/g for13 FB cultivars grown in Canada.

Table 3-5 Oligosaccharides[db] and total phenolics[db] levels (mg/g) in untreated faba bean seeds

Cultivar	Seed lot	Raffinose	Stachyose	Verbascose	Total phenolic
Snowdrop	1	8.16±0.42	18.27±1.01	27.43±1.35	51.65±3.02
Taboar	1	8.19±0.62	14.85±0.90	25.54±1.45	44.29±2.79
Snowbird	1	8.33±0.73	18.40±1.12	34.55±0.98	49.09±3.17
Fabelle	1	8.57±0.85	17.74±0.73	21.93±1.65	12.89±1.34
Malik	1	8.40±0.20	17.88±0.84	32.40±1.89	42.43±2.05
Snowdrop	2	9.47±0.34	24.22±0.69	53.03±3.54	42.82±2.89
Taboar	2	9.60±0.37	23.13±1.01	57.62±3.01	37.46±3.01
Snowbird	2	9.12±0.65	27.72±1.87	89.69±2.98	47.05±2.14
Fabelle	2	9.20±0.72	25.26±1.89	39.91±1.78	6.83±0.98
Malik	2	9.10±0.78	24.04±2.01	61.46±2.45	35.15±2.30
Snowdrop	3	8.75±0.38	34.10±1.99	69.69±0.98	50.58±2.56
Taboar	3	8.74±0.29	23.17±0.92	50.27±0.88	34.52±2.81
Snowbird	3	8.56±0.62	23.77±0.87	67.77±4.02	48.69±2.45
Fabelle	3	8.90±0.59	31.71±1.95	50.78±2.32	12.73±3.65
Malik	3	8.94±0.64	28.88±1.77	76.12±4.34	35.70±2.88
Overall Mean values*		**Raffinose [ns]**	**Stachyose [ns]**	**Verbascose [ns]**	**Total phenolic**
Snowdrop		8.79±0.66	25.53±7.99	50.05±21.29	48.35±4.82[a]
Taboar		8.84±0.71	20.38±4.79	44.48±16.80	38.76±5.01[b]
Snowbird		8.67±0.41	23.30±4.68	64.00±27.76	48.28±1.08[a]
Fabelle		8.89±0.31	24.90±6.99	37.54±14.57	10.82±3.45[c]
Malik		8.81±0.37	23.60±5.51	56.66±22.25	37.76±4.05[b]

[db] Dry basis. [ns] Not significant (p>0.05). Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation. *Mean values were calculated by averaging seed lots.

3.5.4 Germination percentage and of whole faba bean seed

The Canadian Food Inspection Agency (CFIA, 2016) has suggested that the disinfection process of sprouted seed is necessary to control the microbial growth. During the antimicrobial treatment process, germination and packaging areas should be separate to avoid contamination of seed sprouts by non-disinfected seeds or chemical disinfectants. The volume ratio of dry seeds and disinfection agent should be least 1: 5. Seeds should be rinsed by 6% hydrogen peroxide for about 10 min at room temperature. The seeds must be carefully washed with potable water after

the antimicrobial treatment. Rinsing should be repeated sufficiently with potable water to eliminate any residue disinfectant (CFIA, 2016).

Table 3-6 presents the germination percentage observed for each FB cultivar at different stages (Figure 3-1). Figure 3-1 demonstrates the germination process of all five FB cultivars from the beginning to 72 h. No radicles were observed for seeds just after soaking at 0 h. After 24 h, radicles started to rupture the seed coat and kept growing. After 72 h of germination, the length of the radicles of all five cultivars ranged from 1.5-2.5 cm. At 24 h of germination, the germination percentage of cultivars was significantly different; Snowbird and Fabelle had the highest germination percentage of 75% and 76%, respectively (Table 3-6). Taboar had the lowest germination percentage (32%) at 24 h. In general, all cultivars reached the germination percentage of nearly 90% at 72-hour, and there was no significant difference of germination percentage at 48 h and 72 h among cultivars. The germination percentage in the current study reached the goal described by others. According to Vidal-Valverde et al. (1998), a successful germination treatment was defined as a total of 90–100% of the seeds germinated after 6 days of germination.

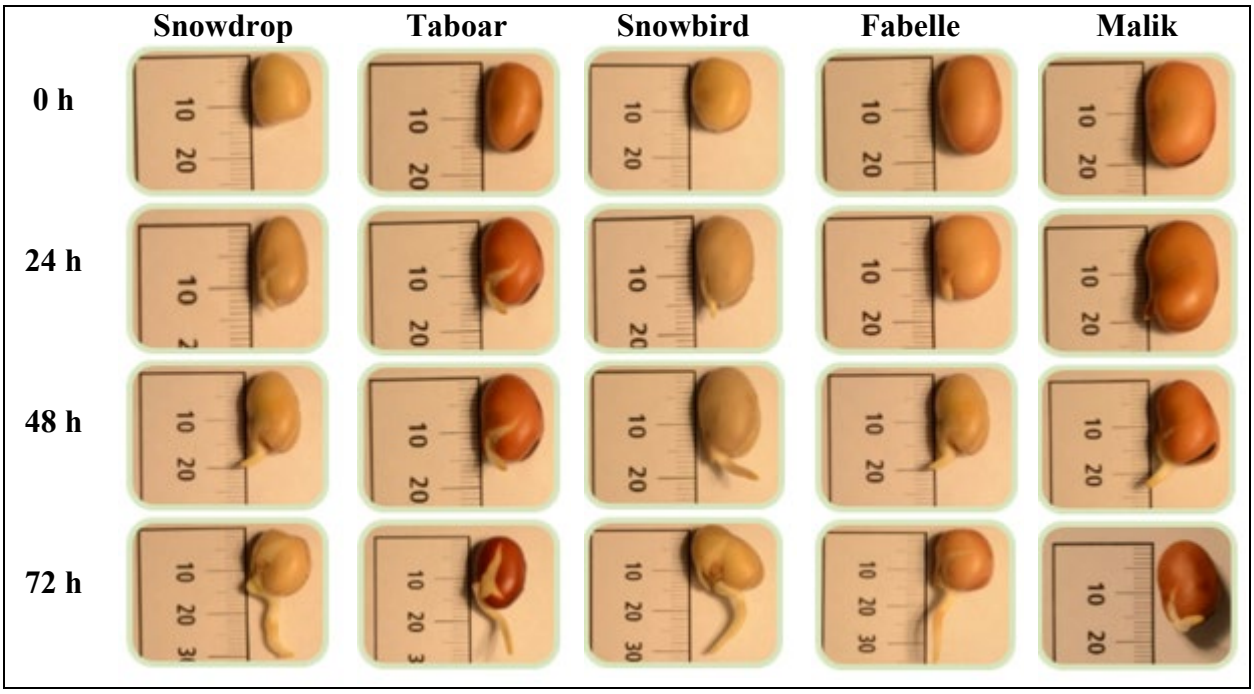

Figure 3-1 Pictures of ungerminated seeds at 0 h (just after soaking in water for 12 h), and germinated seed at 24 h, 48 h, and 72 h

Table 3-6 Germination percentages (%) in 72 h period of faba bean seeds

Cultivar	24 h	48 h[ns]	72 h[ns]
Snowdrop	51±1.9[bc]	76±6.2	88±3.3
Taboar	32±7.3[c]	73±9.3	88±2.1
Snowbird	75±6.8[a]	89±2.0	93±1.7
Fabelle	76±5.7[a]	89±6.1	95±2.1
Malik	59±12.8[ab]	86±3.3	92±4.2

[ns] Not significant (p>0.05). Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation.

3.5.5 Effect of germination on levels of undesired compounds of whole faba bean seed

Germination of FB seeds for 72 h had no significant effect on phytic acid level of these five cultivars (Table 3-7). Because of the difference of the seeds collected from different seed lots (Table 3-3), the standard deviation of the phytic acid content was large, which resulted in a large variance in the phytic acid content in germinated seed. The study by Luo & Xie (2013) reported that 72 h germination at room temperature reduced phytic acid content of FB seeds from 0.84% to 0.53% and a significant increase occurred with longer germination time (increased from 0.53 to 0.75% at 120 h germination). This difference in the phytate level change between the current study and the previous study could be due to the presence of available free minerals such as calcium in FB seeds. The decrease and increase of phytic acid may be related to the hydrolysis and synthesis of InsP6 (the major component of phytic acid, myo-inositol hexakisphosphate), which occurred during germination in cotyledons of beans. Rapid InsP6 degradation occurred in embryonic axes of bean seedlings during root emergence, but after two to three days, InsP6 synthesis was detected (Loewus, 2002). Germinated faba beans showed the largest calcium content, which could be related to the decrease in phytic acid (Vidal-Valverde et al., 1998). Phytic acid served as an important reserve of phosphorus which is generated by the action of endogenous phytase during seed germination, resulting in an increase in available phosphorus and, hence, improving the mineral's bioavailability (Vidal-Valverde et al., 1998). In further studies, the germination effect on phytase activity may need to be measured to better understand enzymatic changes during the germination process.

Table 3-7 Phytic acid[db] content (mg/g) in faba bean seeds during the germination process

Faba bean cultivar	Untreated	Germinated seeds			
		0 h	24 h	48 h	72 h
Snowdrop [ns]	1.60 ± 0.43	1.39 ± 0.44	1.32 ± 0.06	1.39 ± 0.62	1.60 ± 0.72
Taboar [ns]	1.65 ± 0.11	1.73 ± 0.24	1.32 ± 0.25	1.75 ± 0.47	1.69 ± 0.40
Snowbird [ns]	1.75 ± 0.21	1.60 ± 0.33	1.58 ± 0.21	1.86 ± 0.22	1.71 ± 0.09
Fabelle [ns]	1.77 ± 0.15	1.71 ± 0.20	1.52 ± 0.11	1.55 ± 0.66	1.63 ± 0.68
Malik [ns]	1.73 ± 0.23	1.66 ± 0.13	1.54 ± 0.28	1.88 ± 0.53	1.57 ± 0.56

[db] Dry basis. [ns] Not significant (p>0.05). Means with the different letter in the same row are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation.

Table 3-8 Vicine[db] content (mg/g) in faba bean seeds during the germination process

Faba bean cultivar	Untreated	Germinated seeds			
		0 h	24 h	48 h	72 h
Snowdrop	9.94 ± 0.85[a]	9.84 ± 0.79[a]	9.22 ± 0.74[ab]	8.36 ± 0.51[b]	9.18 ± 0.48[ab]
Taboar [ns]	7.31 ± 0.24	7.03 ± 0.48	6.81 ± 0.44	6.44 ± 0.29	6.65 ± 0.41
Snowbird [ns]	10.82 ± 0.27	11.02 ± 1.11	10.57 ± 1.23	9.89 ± 0.85	9.86 ± 0.88
Fabelle [ns]	1.02 ± 0.82	1.20± 1.02	1.19 ± 1.01	1.12 ± 0.67	1.07 ± 0.87
Malik [ns]	7.27 ± 0.52	7.29 ± 0.25	6.68 ± 0.70	6.46 ± 0.21	6.76 ± 0.37

[db] Dry basis. [ns] Not significant (p>0.05). Means with the different letter in the same row are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation.

Germination had no effect on vicine (except one cultivar) and convicine levels in FB seeds. For vicine level (Table 3-8 & Table 3-9), only the cultivar Snowbird showed a significant change during the germination process. At 48 h of germination, the vicine level (8.36 mg/g) was significantly lower (about 19%) than the untreated seed (9.94 mg/g). However, in Khalil and Mansour's study (1995), vicine was significantly reduced from 6.8 mg/g to 4.9 mg/g after 72 h of germination, and convicine content remained the same after germination. In the current study, there was almost no change during the germination in the pyrimidine glucosides content. The ratio between vicine to convicine was 2:1 from the beginning to the end of the germination period observed.

Table 3-9 Convicine[db] content (mg/g) in faba bean seeds during the germination process

Faba bean cultivar	Untreated	Germinated seeds			
		0 h	24 h	48 h	72 h
Snowdrop [ns]	3.53 ± 0.23	3.50± 0.33	3.38 ± 0.33	3.04 ± 0.17	3.36 ± 0.25
Taboar [ns]	3.93 ± 0.04	3.81 ± 0.41	3.74 ± 0.37	3.49 ± 0.02	3.58 ± 0.33
Snowbird [ns]	3.70 ± 0.12	3.80 ± 0.32	3.71 ± 0.48	3.54 ± 0.41	3.55 ± 0.32
Fabelle [ns]	0.40± 0.47	0.45 ± 0.59	0.47 ± 0.55	0.48 ± 0.44	0.44 ± 0.51
Malik [ns]	3.64 ± 0.07	3.49 ± 0.17	3.34 ± 0.49	3.33 ± 0.33	3.41 ± 0.22

[db] Dry basis. [ns] Not significant (p>0.05). Means with the different letter in the same row are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation.

The study by Goyoaga et al. (2008) showed that the vicine concentration at the beginning of germination in the seed axis was nearly 60 mg/g for two FB cultivars which accounted for 1% of the total seed dry weight. The limited changes of vicine/convicine could be due to translocation of vicine and convicine from the cotyledons to the axis and there was no evidence of the use of these compounds in further metabolism. Goyoaga et al. (2008) also suggested that a single co-dominant gene control vicine to convicine ratio in whole mature seeds of *Vicia faba*, and genetic studies showed that the levels of vicine and convicine in mature seeds were maternally determined and the testa may be the primary site of synthesis and then exported to the cotyledons and seed axis. It was possible that the variation in the levels of vicine and convicine in different tissues of the seed are a result of the interactions between differential synthesis or inter-conversion in the testa (Goyoaga et al., 2008). The ratio of the FB seed coat to seed cotyledon may lead to the differences in the effect of germination on vicine/convicine level as observed in the current and previous studies.

Germination can significantly reduce raffinose, stachyose, and verbascose in FB seeds (Table 3-10). Raffinose content (Table 3-10) started to decrease after soaking (at 0 h) in Snowdrop (8.79 untreated to 7.91 mg/g), Snowbird (8.67 to 7.91 mg/g), Fabelle (8.89 to 8.00 mg/g), and Malik (8.81 to 7.91 mg/g) seeds. At 24 h, 48 h and 72 h of germination, raffinose could not be detected in the seeds of all cultivars and can be considered as 100% reduction. Although soaking (0 h) did not change the stachyose content similar to raffinose, the stachyose content (Table 3-10) started to significantly decrease (p<0.5%) after 24 h of germination for Snowdrop and Snowbird Stachyose level of Taboar, Fabelle, and Malik began to drop after 48 h germination. For all five cultivars, the reduction of stachyose was around 60% by end of the

germination period. Verbascose content (Table 3-10) dropped after 24 h of germination in Snowdrop and Malik. For Taboar, Snowbird, and Fabelle, verbascose level began to decrease at 48 h of germination. For all five cultivars, the reduction of stachyose level was around 80% at the 72 h germination.

Previous studies on FB also reported the reduction effect of germination on oligosaccharides. Goyoaga et al. (2011) reported the stachyose and verbascose content significantly decreased after 48 h of germination and became undetectable after 72 h of germination in the FB cultivar Brocal. Raffinose content also significantly decreased after 96 h of germination. According to their study, at 72 h of germination, the sum of RFO (raffinose family oligosaccharides) was only about 10% of initial content in FB. The oligosaccharide content changes were also reported in other pulses during the germination treatment. Stachyose declined progressively until 96 h of germination in lima beans, African yam beans and jack beans, and achieved reductions higher than 70% relative to the original seeds. In brown and cream pigeon peas, verbascose was eliminated at 96 h of germination and stachyose level was reduced by ~50%. Raffinose and verbascose presented a similar trend during the germination of red lima beans, white lima beans, African yam beans, and jack beans, being undetectable at 96 h of germination (Oboh et al., 2000). The germination treatment can be considered as a bio-process to induce the alpha-galactosidase activity, which could promote the enzymatic hydrolysis of RFOs (Vidal-Valverde et al., 1998).

Table 3-10 Oligosaccharide[db] content (mg/g) in faba bean seeds during the germination process

Faba bean cultivar	Untreated	Germinated seeds			
		0 h	24 h	48 h	72 h
Raffinose					
Snowdrop	8.79±0.66^{a}	7.91±0.31^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
Taboar	8.84±0.71^{a}	7.94±0.29^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Snowbird	8.67±0.41^{a}	7.91±0.26^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
Fabelle	8.89±0.31^{a}	8.00±0.32^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
Malik	8.81±0.37^{a}	7.91±0.26^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
Stachyose					
Snowdrop	25.53±7.99^{a}	20.83±4.95^{ab}	11.64±0.80^{bc}	8.54±0.44^{c}	9.04±1.06^{c}
Taboar	20.38±4.79^{a}	18.41±4.44^{a}	13.40±1.46^{ab}	9.38±0.76^{b}	8.73±0.47^{b}
Snowbird	23.30±4.68^{a}	22.95±5.76^{a}	14.24±1.55^{b}	9.28±0.62^{b}	9.03±0.13^{b}
Fabelle	24.90±6.99^{a}	23.05±6.89^{a}	15.27±1.71^{ab}	9.06±0.26^{b}	9.02±0.61^{b}
Malik	23.60±5.51^{a}	21.36±6.18^{a}	12.56±1.20^{ab}	9.21±0.26^{b}	6.31±4.72^{b}
Verbascose					
Snowdrop	50.05±21.29^{a}	40.46±16.26^{ab}	14.20±2.19^{b}	9.32±0.69^{b}	9.64±0.89^{b}
Taboar	44.48±16.80^{a}	45.15±22.50^{a}	19.71±3.30^{ab}	10.73±0.73^{b}	9.57±0.36^{b}
Snowbird	64.00±27.76^{a}	64.50±31.64^{a}	20.87±6.55^{ab}	10.35±0.77^{b}	9.75±0.12^{b}
Fabelle	37.54±14.57^{a}	42.16±21.00^{a}	20.10±3.53^{ab}	9.78±0.84^{b}	10.02±1.13^{b}
Malik	56.66±22.25^{a}	53.82±25.43^{a}	15.67±2.16^{b}	10.02±0.67^{b}	9.62±0.69^{b}

[db] Dry basis. Means with the different letter in the same row are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation.

The germination process significantly reduced total phenolic content (Table 3-11) in Snowdrop and Snowbird. However, there was no significant effect of germination on total phenolic content of Taboar, Fabelle, and Malik. For both Snowdrop and Snowbird, the total phenolic content decreased after 48 h germination. Total phenolic content dropped from 48.35 to 39.97 mg/g, and 48.28 to 40.34 mg/g for Snowdrop and Snowbird, respectively. The reduction of total phenolic was about 17-18%, which was lower than the values reported previously. Luo et al. (2013) found that the 4-day germination process had 96-98% reduction of polyphenol contents. The authors concluded that the loss of polyphenol during sprouting can be attributed to solubilization by enzymes because the prime objective of sprouting was to promote the

development of hydrolytic enzymes that were not active in raw seeds. During the soaking and germination process, some of the phenolic compounds can leach out.

Table 3-11 Total phenolic[db] content (mg/g) in faba bean seeds during the germination process

Faba bean cultivar	Untreated	Germinated seeds			
		0 h	24 h	48 h	72 h
Snowdrop	48.35±4.82[a]	42.48±3.06[ab]	42.04±3.90[ab]	39.97±2.10[b]	44.50±4.42[ab]
Taboar [ns]	38.76±5.01	36.76±1.25	36.01±2.22	34.27±1.73	34.42±1.30
Snowbird	48.28±1.08[a]	44.49±4.00[ab]	41.67±5.05[ab]	40.34±3.60[b]	43.79±2.40[ab]
Fabelle [ns]	10.82±3.45	10.58±3.09	10.69±3.49	11.15±2.63	11.33±3.44
Malik [ns]	37.76±4.05	37.26±2.24	34.96±2.59	34.30±0.90	33.54±0.56

[db] Dry basis. [ns] Not significant (p>0.05). Means with the different letter in the same row are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method. Values are presented as mean ± standard deviation.

Troszyńska et al. (2011) recently demonstrated that the germination process could positively modify the phenolic composition of lentil seeds. The sensory quality of sprouted lentil seed could be enhanced, since strong correlations were found between the negative sensory attributes and the presence of phenolic compounds. Troszyńska et al. (2006) also found that the different length of germination time also had a significant effect on the sensory profile of samples. The 3-day old mung bean sprouts were essentially less bitter than that of the other samples (1 day to 7 days).

In contrast, another study found that the phenolic content increased after germination. Shetty et al. (2001) reported that in the 8-day germination process, the phenolic content in faba bean remained same from day 1 to day 5, and started increasing till day 6, and decreased after day 7. Highest phenolic content was observed on day 6 which was 3 mg/g. These authors pointed out that the synthesis of free phenolics was regulated via the proline-linked pentose phosphate pathway (PPP), shikimate pathway, and phenylpropanoid pathway. PPP was an alternate route for the breakdown of carbohydrates generating $NADPH_2$ for use in anabolic reactions and provide erythrose-4-phosphate for shikimate pathway. This route was important for the biosynthesis of phenylpropanoid. The guaiacol peroxidase (GPX) enzyme determines the fate of phenolic acids produced. Phenolics were precursors in the peroxidase mediated lignin biosynthetic pathway. Peroxidase, belonging to the oxido-reductive group of enzymes mediated

the process of lignification of phenolic acids by utilizing H_2O_2, a photosynthetic by-product. Enhanced peroxidase activity was considered to lead to increased lignin synthesis and subsequent lignification of the cell wall acts as a barrier for fungal infections of the developing seedling.

In the future, longer germination time may be required to fully understand the change of phenolic content during the seed growth. The different reduction in the percentage of the total phenolic content between the current study and literature data could be due to the differences in the extraction solvent used, i.e. ethanol in the current study vs. methanol in previous studies, and the renewed synthesis of polyphenols or degradation of high molecular weight insoluble polymers into smaller molecules that reacted with the reagent (Satwadhar et al., 1981).

3.5.6 Effect of autoclaving on the levels of undesired compounds of whole faba bean seed

Autoclaving involves high temperature and pressure and can breakdown these compounds to some extent from their linkages and also to constituting units. No significant effect on phytic acid (Figure 3-2) content of all five cultivars was found due to autoclaving. Phytates are normally heat stable and cannot easily be removed by convectional heat processing methods (Loewus, 2002). The current study demonstrated that autoclaving was not able to reduce phytic acid level in FB seeds. In preliminary studies, faba bean whole seed were dry heated under pressure, but the samples were burned. Therefore, whole faba bean samples were soaked first, and soaking water was disposed. Additional water was added with faba bean seeds during the autoclaving process. In the study by Fernandez et al. (1997), raw ground dry FB whole seeds were pressure cooked to 120 °C at 1 atm for 15 min and the phytic acid content was reduced by 40%. This may represent an effect of degradation of detectable phytates caused by heat, rather than enzymatic hydrolysis. Soaking in acid solution (liquid was drained after soaking) followed by cooking reduced phytic acid by 30%, and the hard seed cover could prevent the acid solution from reaching the cotyledons. In contrast, soaking followed by cooking process could soften the seed cover, which may allow the acid solution to penetrate through the seed coat (Fernandez et al., 1997). This in turn may have made an acidic environment for endogenous phytase to react. The activity of phytase was optimal at 50 °C, but the enzyme was destroyed at 100 °C. This may explain the relatively lower reduction (30%) in phytic acid content; the phytase enzymes may have been active at the beginning of cooking, before the

enzymes were destroyed by high temperatures. The presence of acids and alkalis during cooking may also contribute to phytate hydrolysis (Sathe & Venkatachalam, 2002).

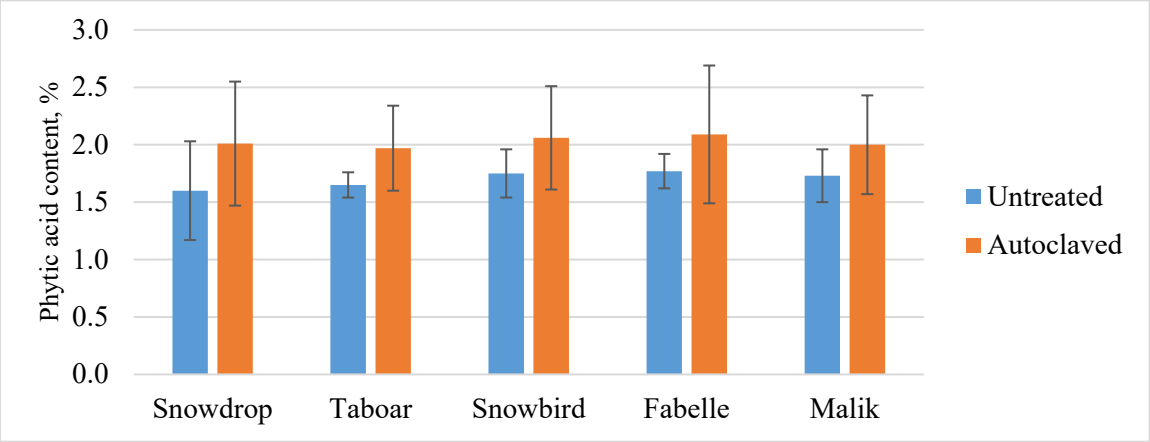

Figure 3-2 Effect of autoclaving on phytic acid content (%) of faba bean seeds
Error bars represent the standard deviations of means

Phytate is heat stable, therefore a significant reduction in the level of phytate during cooking cannot be expected unless either the cooking water was discarded, or the food received additional processing treatment such as soaking (soaking water discarded), germination, fermentation, etc. (Sathe & Venkatachalam, 2002). In the study by Fernandez et al. (1997), soaking water was discarded, and whole faba bean seeds were strained after heat treatment. In the present study, soaking water was discarded but FB seeds were not strained after autoclaving, which could account for the difference of the reduction effect of autoclaving on phytic acid.

The contents of vicine and convicine content also did not change due to the autoclaving (Figure 3-3 and 3-4). Autoclaving could not reduce the level of thermo-stable vicine and convicine. The results of the current study contrast with the published work by Khalil and Mansour (1995), where a reduction in vicine content from 6.8 mg/g to 0.41 mg/g and convicine from 0.27 to 0.16 mg/g was reported. Similarly, Cardador-Martínez et al. (2012) reported that the content of pyrimidine glycosides in FB cotyledons was significantly decreased upon roasting or boiling. The general trend reported in their study was a 6% average decrease in the content of vicine by the roasting treatment, whereas the average decrease of 10% due to boiling. By roasting, the convicine content was reduced by 3–30%. The boiling process produced about 13–60% reduction in the content of convicine. These results showed that boiling was a more effective treatment to reduce glycosides than roasting, and this could be due to the slight water

solubility of glycosides in water, making them more available to heat treatment. The current study did not find autoclaving as an effective treatment to reduce vicine/convicine level of FB. This may be due to the genotypes of the FB seed, and sample preparation (whole seed in the current study vs. seed cotyledon in other studies) used for determination. The seed coat in the current study may reduce the effect of autoclaving to degrade vicine/convicine in the cotyledons.

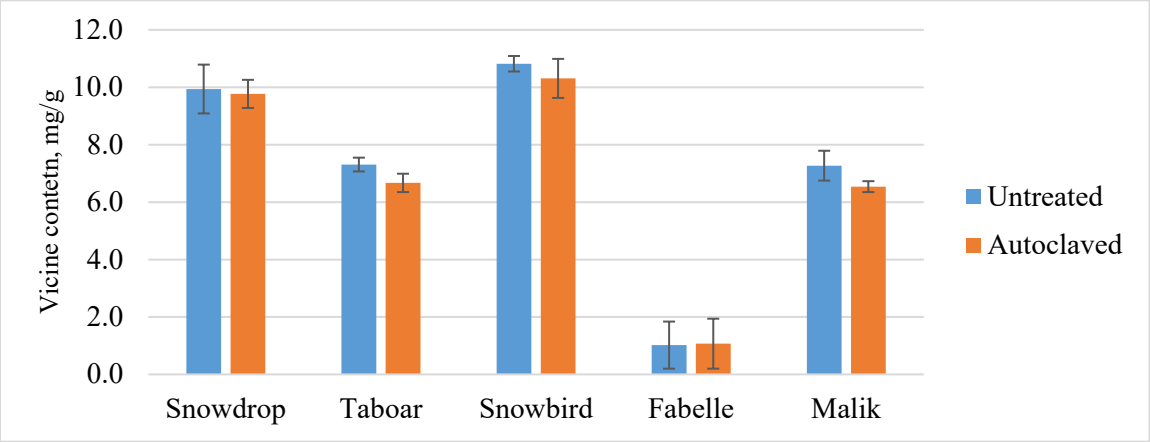

Figure 3-3 Effect of autoclaving on vicine content (mg/g) in faba bean seeds
Error bars represent the standard deviations of means

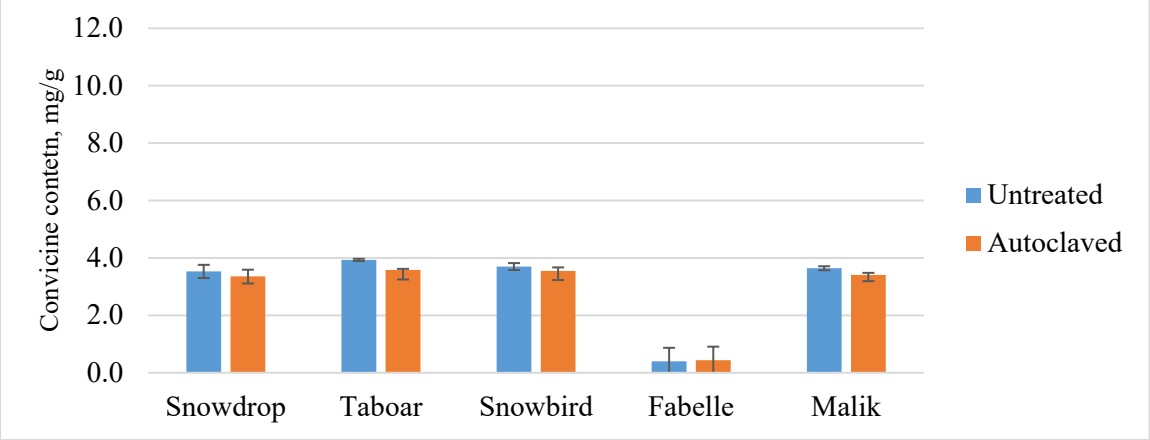

Figure 3-4 Effect of autoclaving on convicine content (mg/g) in faba bean seeds
Error bars represent the standard deviations of means

The vicine and convicine analysis also showed that vicine is the main pyrimidinic glycoside content in all ten cultivars, the vicine: convicine ratio of untreated FB seeds ranged from 3 to 7. Roasted faba bean seeds were in the interval of 3 and 7, and boiled FB seeds were in the interval of 3 and 12. Therefore this study (Cardador-Martínez et al., 2012) concluded that

vicine was the main compound in the seed varieties of *Vicia faba*, however convince was more sensitive to thermal treatment. Convicine was much easier to break down during the heat treatment.

Autoclaving did not show a significant effect ($p<0.05$) on the levels of raffinose, stachyose, and verbascose (Figure 3-5), even though stachyose and verbascose values were lower numerically after autoclaving. Previously, Vidal-Valverde et al. (1998) concluded that soaking plus cooking could reduce the content of oligosaccharide by 16 to 25%. However, the results in the current study did not agree with these previous findings. The reason might be due to the large variation observed in the oligosaccharide content of FB seeds which were collected from different seed lots. As Figures 3-5 showed, the length of error bars (standard deviation of the mean value) for stachyose and verbascose content of each cultivar indicated the uncertainty of the specific value.

The reduction of oligosaccharide level by boiling was reported for other pulses (Oboh et al., 2000). Cooking in water resulted in a loss of total oligosaccharide content by 21% in brown pigeon peas, and 67% in white lima beans, compared to the corresponding raw uncooked samples. These reductions may have been due to heat induced hydrolysis of the oligosaccharides to simple disaccharides and monosaccharides, but oligosaccharides content losses in cooking water were not reported (Oboh et al., 2000).

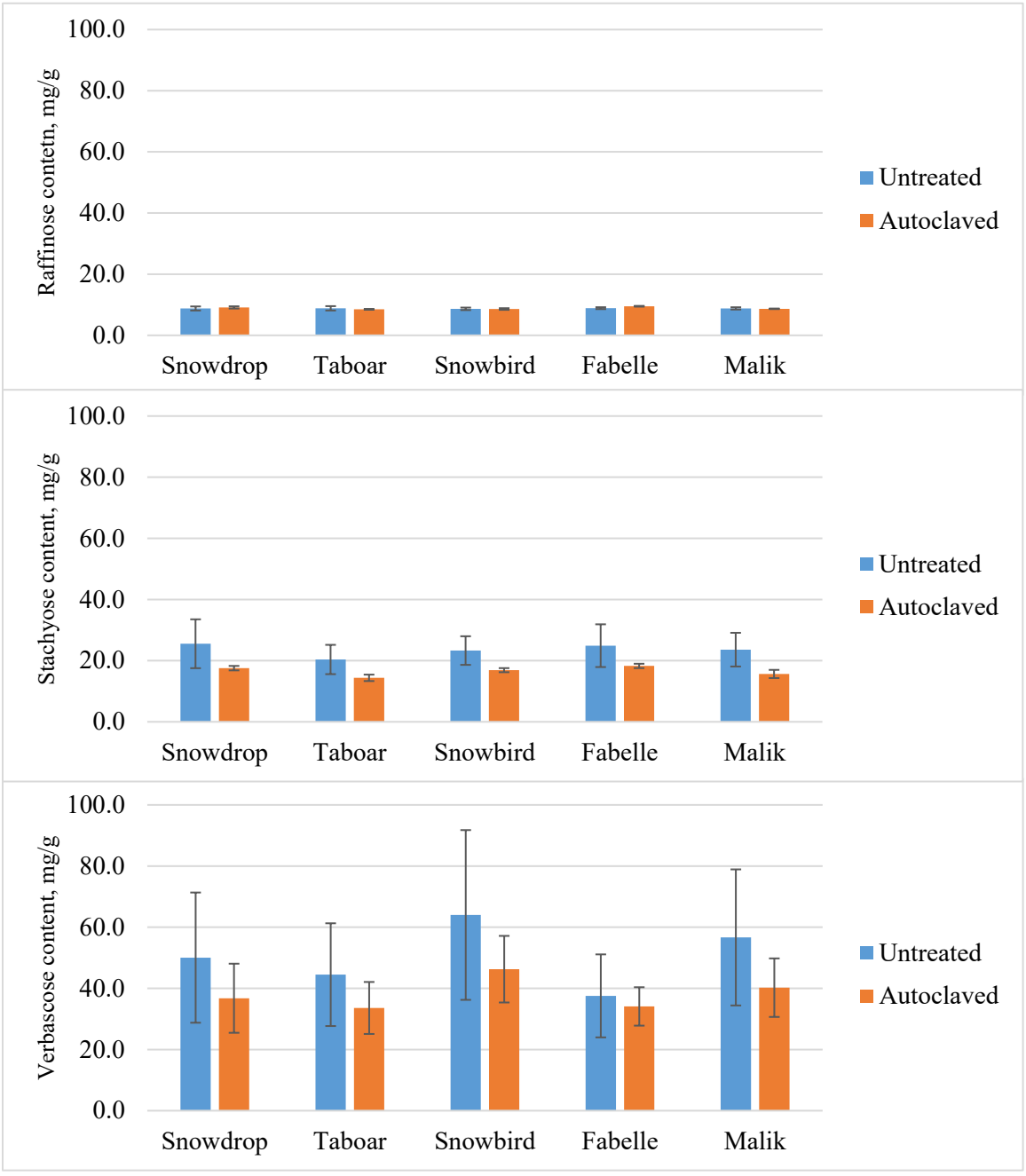

Figure 3-5 Effect of autoclaving on oligosaccharide content (mg/g) in faba bean seeds
Error bars represent the standard deviations of means

The total phenolic content of some faba bean cultivars (Figure 3-6) showed a significant reduction after autoclaving treatment. Autoclaving significantly reduced total phenolic content in Taboar, Snowbird, and Malik, from 38.76 to 32.94, 48.28 to 39.44, and 37.76 to 30.17 mg/g, respectively. The percentage of total phenolic content reduction was in the range of 15 to 23%. Autoclaving did not significantly change the total phenolic content of Snowdrop and Fabelle seeds.

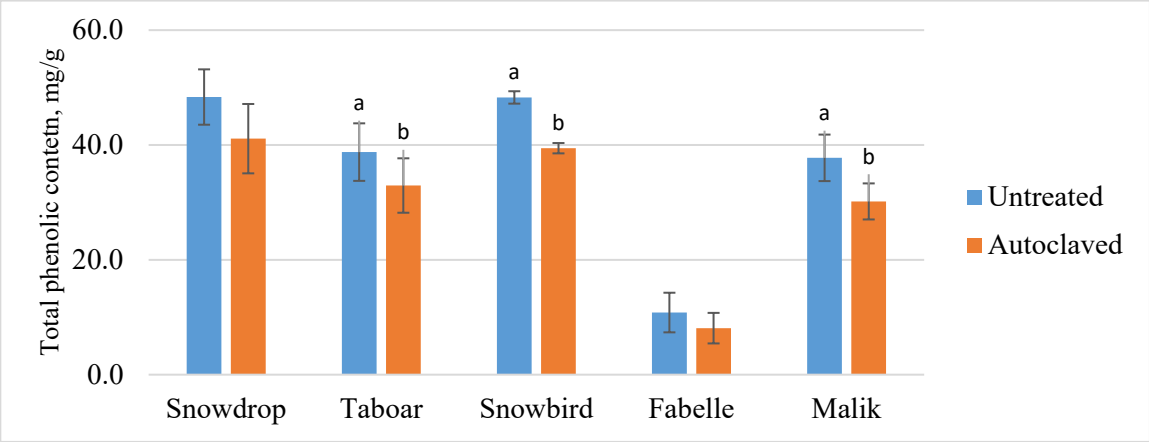

Figure 3-6 Effect of autoclaving on total phenolic content (mg/g) in faba bean seeds
Error bars represent the standard deviations of means. Means with the different letters are significantly different (p<0.05).

The reduction in phenolic contents of beans during cooking could be due to either destruction of phenolic compounds or other chemical rearrangements of phenolic compounds such as the formation of new insoluble components with other organic substances (Kader, 1995). The variation in the extent of phenolic compounds degradation could be influenced by the seed physicochemical properties, such as seed surface area, coat thickness, coat colour, shapes and hardness, and it was also possible that cooking faba beans led to strong tannin–protein interactions (Giczewska & Borowska, 2003).

3.6 Conclusion

Faba bean is a good source of protein, fibre, and starch. Among the five cultivars of FB similar level of macronutrients were found. Of the undesirable components investigated, the level of phytic acid was not affected by cultivar, germination, and autoclaving treatment. Germination up to three days and autoclaving had very limited effect on vicine and convicine

content. Cultivars had a significant effect on vicine and convicine level, and Fabelle had the lowest level of both vicine and convicine. Germination of seeds showed a significant effect on lowering oligosaccharide levels but not autoclaving. Raffinose (100%), stachyose (60%), and verbascose (80%) were all significantly reduced upon 24 h of germination. Autoclaving and type of cultivar both had significant effects on total phenolic content. Fabelle had the lowest phenolic content among all five cultivars. Autoclaving significantly reduced total phenolic content in Taboar, Snowbird, and Malik. Results of this study provided valuable information on undesirable components of FB and short-term germination and autoclaving as suitable processing means for reducing the levels of some undesirable factors and subsequently generating FB suitable for food ingredients and product development.

3.7 Connection to the next study

The overall objective of the current study was to evaluate the effect of short-term germination and autoclaving on the levels of selected undesired compounds in FB seeds. Based on the results, germination and autoclaving treatments had minor effect on most of the undesired compounds, but cultivars were more likely to be the determining factor on the levels of undesired compounds. Therefore, the cotyledon flour of selected cultivars without further treatment such as germination or autoclaving were evaluated to predict the performance of FB flour as a binder/extender in meat systems. In the following study, cotyledon flour of Malik (large seed, regular tannin, vicine and convicine) and Fabelle (low vicine and convicine and tannin) were used as binders in producing low-fat pork bologna. Commercially available FB fractions (starch and protein) were also selected to explore their potential in meat products. In comparison, commercial wheat flour and pea starch fractions were used as reference binders due to the similar chemical composition to FB ingredients. The effect of various binders on physicochemical, textural and sensory properties of finished processed meat products were evaluated in the next study.

4 STUDY II: EFFECT OF FABA BEAN COTYLEDON FLOUR AND FRACTIONS ON PROCESSING, TEXTURAL, AND SENSORY PROPERTIES OF LOW-FAT PORK BOLOGNA

4.1 Abstract

In this study, six binders: wheat flour, pea starch, Malik cotyledon flour, Fabelle cotyledon flour, faba bean starch fraction, and faba bean protein fraction were incorporated into low-fat pork bologna at two levels (1.5 and 3%). The contents of major chemical components and functional properties that are significant in processed meat products as binders and the physicochemical, textural, and sensory properties of the finished low-fat bologna products containing these binders were investigated. The control low-fat bologna formulation included 70.7% pork picnic, 26.8% water, and 2.5% of other ingredients (salt, sodium tripolyphosphate, sodium erythorbate, sodium nitrite and seasoning) with targeted fat content at 10-11%. The effectiveness of binders in the formulation was evaluated as cook loss, expressible moisture (centrifugation), purge loss (2-week storage), surface colour (Hunter L^*, a^*, b^*), texture (TPA and torsional gelometry) of final low-fat product. The products prepared in three different process runs were evaluated by twelve trained panelists for the intensity of firmness, cohesiveness, juiciness, graininess, overall flavour, and foreign flavour and rated the overall acceptability of each sample.

Binders varied in proximate composition, FB ingredients were rich in protein (38% in cotyledon flours and 67% in protein fraction) compared to other plant binders used in the study. Compared to the reference ingredients (wheat flour and pea starch), FB ingredients also had higher water holding capacity and oil absorption capacity. Based on RVA results, FB cotyledon flour showed low peak and final viscosity values compared to pea starch and wheat flour.

All binders had significant ($p<0.05$) effect on increasing the viscosity of the raw meat batters. When heat processed, the cook loss of binder- containing bologna products was low (0.5%, w/w), and there was no significant difference between the purge loss resulted in due to binders. Bologna with 3% added wheat flour or FB protein fraction showed a significant

(p<0.05) decrease in the expressible moisture content compared to the control without a binder. A narrow range of surface colour parameters; L^* (69-70), a^* (16-18), or b^* value (14-16) was observed for the final products. The TPA results showed binders did not change the hardness, cohesiveness, springiness, and chewiness of bologna compared to the control without a binder. Bologna with 3% added wheat flour had significantly (p<0.05) increased torsional true stress value at failure compared to the control. Binders had minor effects on shear strain. Sensory evaluation results indicated that addition of 3% wheat flour to bologna gave significantly (p<0.05) higher sensory firmness scores than the control and in agreement with true shear at failure. Addition of binders at 3% significantly (p<0.05) reduced the perception of juiciness of bologna except those with addition of Malik cotyledon flour. Addition of plant binders at 1.5 or 3% level had no effect on graininess and overall flavour intensity of the final product, however 3% Fabelle cotyledon flour caused a significant (p<0.05) increase in the intensity of foreign flavour, compared to control bologna. Presence of foreign flavour in the all other bologna with plant binders was towards the lower end of the score. No significant difference was found in overall liking among bologna with any of the binders at either level of addition.

The addition of FB ingredients at 1.5 or 3% would not negatively affect the colour, textural, and sensory properties of low-fat bologna products, and functioned similarly to other plant binders current in use. Faba bean may offer a useful option to increase protein content and replace traditional gluten-containing binders in the current market.

4.2 Introduction

Consumers are considering low-fat diets nowadays, due to the increased risks of cardiovascular diseases with the consumption of saturated fat and cholesterol (Astrup et al., 2011). High-fat meat-based products are not a healthy dietary choice. However, in the meat industry, fat content is a crucial ingredient associated with desired flavour and textural properties (Girouard et al., 1997). There could be up to 30% of fat (w/w) in processed meat products, such as frankfurters and bolognas (Choi et al., 2014). The fat level can be reduced by direct water substitution, but it could also alter the texture and water holding abilities of the finished meat products (Claus et al., 1989).

Through the years, multiple ingredients, such as dairy protein, starch, and polysaccharides were developed to offset the taste and texture of low-fat meat products (Colmenero, 1996). Faba bean (FB, *Vicia faba*) is particularly high in protein and starch, but low

in fat content (Multari et al., 2015). Faba beans are predominantly consumed in Asia, the Middle East, Mexico, India, and South America, and its consumption in Western nations including North American regions is still in its infancy (Chandra-Hioe et al., 2016). Increasing the utilization of faba bean in a formulated food product may promote the consumption of low-fat meat products. Therefore, application of faba bean ingredients as binders such as using faba bean starch fraction, protein fraction or dehulled faba bean flour in low-fat meat products may have potential in the current food market.

Few studies have focused on the application of faba bean ingredients in meat products. Faba bean flour presented higher water absorption index than wheat flour, mainly due to the physical-chemical makeup of each crop, and the differences in morphology of their starches (Pietrasik & Janz, 2010). Faba bean application in pasta did not negatively affect the texture, flavour or physical-chemical properties (Giménez et al., 2012). The effect of other legume ingredients on meat products had been reported. Pea fibre was used as a functional ingredient to increase cooking yield and enhance textural properties in low-fat meat products (Pietrasik &Janz, 2010). Chickpea flour was a potential source of high protein ingredient as an extender in emulsified meat products, and its application improved the instrumental texture and sensory properties of bologna products (Sanjeewa et al., 2010).

In this study, cotyledon flour of two faba bean varieties, commercial-grade faba bean starch and protein fractions produced by air classification, commercial pea starch and, commercial wheat flour were evaluated for their functional properties required in processed meat products as an initial step to explore technological feasibilities. In addition, all the above-mentioned plant ingredients were used as binders in low-fat pork bologna formulation, and the physico-chemical, textural, and sensory properties of finished products were also evaluated to understand and compare the effect of faba bean ingredients in the meat system.

4.3 Materials and methods

4.3.1 Preparation of low-fat bolognas from various binders

Fresh pork picnic (boneless) was obtained from a commercial meat processing plant (Maple Leaf Foods Inc. MB, Canada) and stored at -30 °C before further processing. The pork picnic was thawed in the 1 °C cooler for 72 hours before processing. The pork picnic was ground using a grinder (The Biro MFG. Co. Marblehead, OH, USA, Model AFMG-24), first through a

plate with 6.5 mm holes and then to pass through a plate with 3.9 mm holes. The ground meat was kept at 1 °C until further processing.

Six binders were used (Table 4-1), which included Malik cotyledon flour, Fabelle cotyledon flour, faba bean starch fraction (Alliance Grain Traders, SK, Canada), faba bean protein fraction (Alliance Grain Traders, SK, Canada), pea starch fraction (Parrheim Inc. SK, Canada), and wheat flour (Robin hood Inc. ON, Canada). Cotyledon flour were prepared from the whole faba bean seed of Fabelle and Malik varieties. Whole seeds were dehulled using a bench top mill (Satake, Japan) and the cotyledons were picked and milled using an Ultra - Centrifugal Mill (Retsch, PA, USA, Model ZM 200) that is equipped with a screen (250-micron aperture size) and the rotor speed set at 12,000 rpm.

Table 4-1 Low-fat pork bologna formulations with two addition levels of binders

[1]Binder type	Binder level%	Pork picnic%	Ice water%	[2]Others%
Control	0.0	70.70	26.84	2.46
Wheat flour	1.5	70.70	25.34	2.46
	3.0	70.70	23.84	2.46
Malik cotyledon	1.5	70.70	25.34	2.46
	3.0	70.70	23.84	2.46
Fabelle cotyledon	1.5	70.70	25.34	2.46
	3.0	70.70	23.84	2.46
Faba starch	1.5	70.70	25.34	2.46
	3.0	70.70	23.84	2.46
Faba protein	1.5	70.70	25.34	2.46
	3.0	70.70	23.84	2.46
Pea starch	1.5	70.70	25.34	2.46
	3.0	70.70	23.84	2.46

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
[2]Others included 1.32% of salt, 0.29% of Prague powder, 0.35% of sodium tripolyphosphate, 0.05 % of sodium erythorbate, and 0.45% of Wiener German style seasoning.

Each of the binders was used to formulate the pork bologna by replacing the water at two levels: 1.5% and 3% (w/w) (Table 4-1). Other ingredients included up to 26.84% of ice water, 1.32% of salt, 0.29% of Prague powder (6.4% sodium nitrite, 93.6% sodium chloride, Griffith Laboratories, ON, Canada), 0.35% of sodium tripolyphosphate (Unipac Packaging Products Ltd. AB, Canada), 0.05 % of sodium erythorbate (Unipac Packaging Products Ltd. AB, Canada) and 0.45% of Wiener German style seasoning (Hela Spice Canada Inc. ON, Canada). Dry ingredients were weighed and premixed and stored at 1 °C until further processing. The formulations are showed in Table 4-1.

4.3.2 Functional properties of binders

A) Water holding capacity

The water holding capacity (WHC) was estimated using the modified AACC Method 56 -20.01 (AACC, 1999) with modifications (Xu, 2017). Around 1 g sample was weighed into the pre-weighed 50 mL centrifuge tube and mixed thoroughly with 20 mL of distilled water by the vortex mixer for 1 min. The suspension was placed at room temperature and was revortexed for 15 seconds at 5 min and 10 min. After centrifugation at 2,000×*g* for 25 min, the supernatant was carefully discarded, and the tube with sediment was weighed again. The weight of both tube and sediment was recorded and the water holding capacity was determined by the following equation.

WHC (g/g) = ((tube weight +sediment weight) - (tube weight + sample weight))/sample weight

B) Oil absorption capacity

The oil absorption capacity (OAC) was determined by the method of Lin et al. (1974) with modifications (Xu, 2017). Around 1 g sample was weighed into the pre-weighed 50 mL centrifuge tube and mixed thoroughly with 5 mL of corn oil. The mixture was mixed by the vortex mixer for 1 min. After staying for 30 min at room temperature, the tube was centrifuged at 2,000×*g* for 25 min. The supernatant was carefully discarded, and the tube with mixture was weighed again. The weight of both tube and sediment was recorded and the oil absorption capacity was determined by the following equation.

OAC (g/g) = ((tube weight + sediment weight) - (tube weight + sample weight))/sample weight

C) Pasting properties

The pasting properties of binder samples were analyzed by AACC method 76-21.01 (AACC, 1999) using a Rapid Visco Analyzer (Newport Scientific Pty Limited, Australia). Around 3.50 grams of samples (on a 14% moisture basis) were weighed and put into the test canister. Around 25.0 ± 0.1 mL of water were dispensed into the canister with pre-weighed sample. The stirrer was placed into the canister and the blade was vigorously jogged through the sample up and down 10 times. The stirrer was properly centered and left in the canister, and canister was assembled firmly into the paddle coupling. The measurement cycle was initiated until no lumps remained in the mixture. All samples were done in triplicate.

4.3.3 Processing procedures of low-fat pork bologna

Three replications of the pork bologna were processed (Figure 4-1). For each replicate, the bologna treatments were processed in a random order. The ground meat, water, and dry ingredients were mixed and chopped in a bowl chopper (RMF, Missouri, USA) for 1 min at speed #1. The mixture was then chopped again for 2 min at speed #2 to form the meat batter. the meat batter was passed through an emulsion mill (Alexanderwerk, Remscheid, Germany, Type 1E-75F) twice and then transferred into the tumbler (Model VSM-150H, Glass, Frankfurt, DE) and. In the tumbler vacuum was applied to remove the air in the meat batter. The vacuum process was completed when the pressure reached 27 mm in Hg (approximately 5 min). The meat batter so prepared was stuffed into waterproof casings (63 mm diameter) with the sausage filler (Tre Spade Inc. Italy). Cotton strings were used to tie each end of bologna chubs. The processing procedure was carried out with pilot-scale equipment in the meat pilot plant (temperature was maintained at 4 °C) at the University of Saskatchewan (Saskatoon, SK, Canada).

Figure 4-1 The processing and cooking procedures of low-fat pork bologna

4.3.4 Cooking procedures

The finished bologna chubs were kept in the 1 °C cooler overnight and cooked the next day in water using a commercial retort (DIXIE RDTI-3 Canner Co. GA, USA) (Figure 4-1). The bolognas were immersed into a hot water bath warmed up to 50 °C and stayed for 30 min. Then the water temperature increased to 60 °C and 70 °C and maintained at each temperature for 30 min. At the end, the water bath temperature increased to 75 °C and kept until the internal temperature of the bologna reached 72 °C. The total cooking time was approximately 2 h. Product internal temperature was monitored using both a hand-held Fluka digital thermometer (Type T Fluke 51 II thermometer, Fluke Corp., Everett, WA, USA) with a chromel-alumel thermocouple probe and an 8-channel data logger (Model 692-0000 Barnant scanning Thermocouple Thermometer, Barnant Co., Barrington, IL, USA) with copper constantan thermocouples, which were inserted in the geometric center of the bologna chubs. The cooking log was recorded and stored in the computer. After the cooking procedure, bologna chubs were cooled in an ice water bath for 1 hour and stored in the 1 °C cooler until further analysis.

4.3.5 Physical and textural properties of low-fat pork bologna

A) pH of raw meat batter

A pH meter (Fisher Scientific Accumet, ON, Canada, Model AR15) was used to determine the pH values of raw meat batters (Xu, 2017). Around 20 g of sample was put into a filter bag (Nasco Whirl-Pak, WI, USA). Eighty mL of deionized water was added into the bag and the mixture was homogenized by a Stomacher Lab-Blender (Seward Ltd, Bury St. Edmunds,

UK, Model BA6021) for 3 min. After homogenizing, the mixture was screened through the filter. A homogenized solution was obtained. The pH electrode was immersed into the filtered homogenized solution until stable readings were obtained. All the measurements were done in duplicate.

B) Viscosity of raw meat batter

One cup (~250 mL) of the raw meat batter from each treatment was obtained before the stuffing process to determine the viscosity by a programmable rheometer (Brookfield Engineering Laboratories Inc. MA, USA, Model DV-III+) loaded with #7 spindle at the speed of 10 rpm. The viscosity of the raw meat batter and the temperature were recorded at 30 seconds.

C) Cooking loss of cooked bologna chubs

The cooking loss of bologna chubs with each treatment was measured based on the weights before and after cooking. Two chubs of each treatment were used to determine cook loss after cooking and cooling. The chubs were opened, and gently wiped with a clean paper towel and weighed. Cooking loss was calculated according to the following equation.

Cooking loss % = (sample weight before cooking - sample weight after cooking)/sample weight before cooking × 100

Sample weight = Weight of bologna chub –weight of casing –weight of cotton strings

D) Slicing plan of cooked bologna

Two bologna chubs from each treatment was opened and sliced for measuring the physical and textural properties. The portions sliced of each chub for further analysis included: 20 mm × 1 for proximate analysis, 30 mm × 2 for texture profile analysis (TPA), 8 mm ×1 for expressible moisture analysis, 3 mm × 12 for purge loss analysis, 400 mm × 2 for torsion analysis.

E) Proximate analysis

Moisture

Refer to the method in 3.3.5

Protein

Refer to the method in 3.3.5

Fat

The crude fat content of each cooked sample was analyzed using AOAC method 960.39a (1990) with modifications (Xu, 2017). Around 3-4 gram of ground bologna sample was weighed

into a cotton thimble with 1 gram of purified and acid-washed sand. The sample and sand were mixed thoroughly by a glass rod and the mixture was dried at 105 °C overnight. Fat extraction was determined by Soxtherm rapid extraction system (C. Gerhardt GMBH & CO. Germany) using petroleum ether as the solvent with hot extraction at 135 °C for 2 h.

Ash

Refer to the method in 3.3.5

F) Expressible moisture

The expressible moisture was measured using a method by Jaurequi et al. (1998) with modifications (Xu, 2017). A cored sample (~1.0 g) was put on top of the two thimble-shaped filter papers, Whatman # 3 and # 5 filter papers, in a 50 mL centrifuge tube. After centrifugation for 15 min at 750×*g* at 4 °C, the expressible moisture content was calculated as the percentage of weight loss from the original sample. Each sample was done in triplicate.

G) Purge loss

The purge loss was determined based on the drip loss in the vacuum package for 14 days to mimic the retailed storage of processed meat products (Bloukas & Paneras, 1993). Two stacks of six slices (12 slices in total and 3 mm thickness for each slice) of bologna were vacuum packaged in the pre-weighed vacuum bags using a vacuum packaging machine (Sipromac, Quebec, Canada, Model 550A). The weight was recorded, and the bags were stored upright at 4 °C. The package was opened after 14 days of storage and the weight of slices was recorded, respectively. Each sample was done in duplicate. The purge loss was calculated as the percentage of weight loss (drip loss) compared to the initial sample weight.

H) Textural profile analysis (TPA)

The texture profile analysis was determined by the method of Unatrakarn (2014) with modifications. Four sliced and cored bologna samples (25 mm in height and 35 mm in diameters) from each chub were prepared and stayed at room temperature for 1 h. For each treatment, at least total numbers of 8 cores from two bologna chubs were analyzed. The texture profile was measured by compressing the sample twice to 50% of the original sample height (12.5 mm) at a crosshead speed of 100 mm per min by the TMS-Pro Texture Press (Food Technology Corp. Rockville, MD, USA) loaded with a 250 N capacity compressing cell. The texture profile was recorded as a force-time curve and analyzed by Texture Lab pro software to calculate hardness,

cohesiveness, adhesiveness, springiness and chewiness. Each treatment was done in 8-10 replicates.

I) Torsional gelometry

From each treatment, 8-10 cores of bologna samples (28.7 mm length, 19.0 mm diameter) were used (Meullenet et al., 1994). Cores were glued to two slotted plastic styrene chips using cyanoacrylate glue (Loctite 404 instant adhesive, Loctite Corp. CT, USA). Sample cores were then reduced to a dumbbell shape by rotation against a sharp rotating grinding wheel (Model KCI-24A2, Bodline Electric Co. IL, USA) to a minimum 12.5 mm diameter at the midsection. The dumbbell shaped sample core was then put into a bottom torsion fixture attached to a Brookfield digital viscometer (Model DV-I, Brookfield Engineering Laboratories, Inc. MA, USA). The bottom disk was kept stable while allowing the upper disk to rotate at 2.5 rpm. The core was twisted until failure. Shear stress and shear strain at failure were recorded.

J). Colour measurement

The surface colour of the sliced cooked bologna was measured through the transparent vacuum bag using a HunterLab Miniscan XE colourimeter (Hunter Associations Laboratory Inc. VA, USA, Model 45/0L) based on L^* (lightness), a^* (redness) and b^* (yellowness) dimensions with illuminant A and 10 ° observer. The colour of bologna was evaluated in duplicate per treatment from two bologna chubs.

4.3.6 Sensory evaluations of pork bologna samples

The sensory study involving human subjects was reviewed and approved by the University of Saskatchewan Research Ethics Board (BEH 17-354). Eighteen panelists were selected to begin the screening process. Panelists were recruited from the College of Agriculture and Bioresources at the University of Saskatchewan and Agriculture and Agri-Food Canada Saskatoon Research and Development Centre. Formulated bologna samples were used to train and screen the panelists for evaluating different parameters in this sensory study. The training followed the procedures suggested by the American Meat Science Association (2015). Additionally, training references were consistent with those described by Mckillip et al. (2017). (2016). The screening test was done in triplicate with seven formulated bologna samples served at every tasting session (Figure 4-2). The panelists were screened based on their capability to distinguish the sensory attributes in different bologna samples and the repeatability of their

results. The final trained panel included 7 females and 5 males. Panelists ranged in age from 18-55 years old.

Bologna from each treatment were prepared as cubes (1.25 cm × 1.25 cm) and kept in the refrigerator at 4 °C until serving. Four cubes of each treatment were served in a small plastic cup with lid to each panelist. Without revealing any information of the sample, a random three-digit code was used to represent the sample. Panelists were served with samples in a random order. For each replication of the tasting, a total of 13 bologna samples were served on two days within one week. At each day of the tasting, one no-binder control sample plus six samples with binders were evaluated. The sensory study was completed in the sensory evaluation lab located at the College of Agriculture and Bioresources building at University of Saskatchewan. This room was equipped with individual booths. A cup of water, soda crackers, toothpick, paper towel, pencil and spit cup were provided along with the bologna samples on the food tray. Panelists were required to evaluate the attributes of bologna samples in a given evaluation card using eight-point intensity scales, except that graininess was a six-point scale. When the eight-point intensity scale was used for firmness, cohesiveness, juiciness, overall flavour, and foreign flavour, 1 is equal to extremely soft, brittle, dry, bland, or no foreign flavour, and 8 is equal to extremely firm, cohesive, juicy, intense, or intense foreign flavour. When the six-point intensity scale was used for graininess, 1is equal to not detected and 6 is equal to extremely grainy.

Figure 4-2 Training and tasting sessions of the sensory study

4.3.7 Statistical analysis

The overall mean and standard deviation were calculated with three replicates of means for all samples. Observed data were analyzed as a Completely Randomized Design using the Proc Mixed Procedure of SAS 9.4 (SAS, Inst. Inc., Cary, NC). Means were analyzed and

separated with the Tukey's method of SAS and a pdmix SAS macro was used to convert mean separation output to letter groupings (Saxton, 1998). Significance was declared at $p<0.05$.

4.4 Results and discussion

4.4.1 Proximate composition and functional properties of binders

The plant-based binders used in the study varied in the values for proximate composition (Table 4-2). Malik and Fabelle cotyledon flours had 10-11% moisture while the reference binders, wheat flour had the highest (12.81%) and pea starch had the lowest (6.49%) values for moisture. Faba bean starch and protein fractions had the moisture content at 8-9%. Faba bean protein fraction had the highest protein content (67.16%, dry weight basis), and the pea starch fraction had the lowest value at 5.90%. Wheat flour and FB starch fraction had similar protein content at around 13%. Faba bean cotyledon flour of two cultivars had similar protein content at 38%. Boye et al. (2010) reported that the protein content of whole faba bean was around 30 %, and Bhatty (1974) concluded that protein content of 12 cultivars (whole FB seed) was between 26 and 35%. The weight of FB seed coat was around 10% (w/w). The results of the current study were close to previous results.

Table 4-2 Chemical composition of plant binders used in bologna formulations

[1]Binder type	[2]Moisture%	Protein%[db]	Fat% [db]	Ash% [db]	Starch% [db]	Fibre% [db]
Wheat flour	12.81±0.31	13.28±0.32	0.77±0.04	0.72±0.06	69.15±0.34	3.50±0.20
Pea starch	6.49±0.27	5.90±0.10	0.43±0.02	1.35±0.10	82.32±0.38	5.60±0.25
Malik cotyledon	10.60±0.62	38.47±0.14	1.23±0.03	2.94±0.07	32.98±1.53	17.43±0.35
Fabelle cotyledon	10.33±0.10	38.22±0.24	1.20±0.02	3.13±0.08	33.14±0.58	18.79±0.73
Faba starch	8.19±0.19	13.52±0.22	0.53±0.03	2.06±0.05	60.82±1.21	3.15±0.26
Faba protein	9.61±0.58	67.16±1.04	1.66±0.06	5.16±0.32	6.19±0.23	13.77±0.38

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
[2]Only one rep of each sample received. No statistical analysis.
[db]Dry weight basis

The fat content of all binders ranged from 0.43-1.66% (Table 4-2). The pea starch fraction and faba bean starch fraction had relatively lower fat content at 0.43% and 0.53%, respectively. Therefore, addition of these binders in the bologna formulation may not cause a significant effect on the final fat level of low-fat bologna. Other studies also reported the low-fat

content in faba bean. Sauvant et al., (2004) reported the fat content in faba bean was ranging from 1.3 to 1.5%, which also agreed with Duc et al. (1999), which the fat content of faba bean was less than 2%.

The FB protein fraction had the highest ash content at 5.16% (Table 4-2). The high ash content in FB protein fraction could also be due to the contact with metal parts during the air-classifying process (Multari et al., 2015). The reference ingredients, wheat flour and pea starch had similar ash content around 1%. Cotyledon flour from the two faba cultivars presented similar values in ash content (3%).

The starch content varied from 6.19% (faba protein fraction) to 82.32% (pea starch fraction). Cotyledon flour from the two faba bean cultivars had similar starch content at 33%. Wheat flour had the second highest starch content at 69.15%, and faba bean starch (60.82%) was slightly lower than that of wheat flour.

The range of fibre content was from 3.15% (faba starch) to Fabelle cotyledon flour (18.79%). Wheat flour and pea starch also had relatively low fibre content at 3.50 and 5.60%, respectively. Malik cotyledon flour, Fabelle cotyledon flour and faba protein fraction had fibre content at 17.43, 18.79, and 13.77%, respectively. Starch was the most abundant carbohydrate in FB seed, and the values resulted in for cotyledon flours of Malik and Fabelle cultivars (33%) was lower than the results reported by Duc et al. (1999) for whole seed (42%), which may be due to only cotyledon flour was evaluated in the current study. Lower values for the fibre content of whole faba bean seed (6-9 %, have been reported and reflect, in part, differences in the methodologies used for dietary fibre determination, i.e. AACC 1999 in the current study *versus* AOAC 1970 in early studies.

Chemical composition of the ingredients derived from different crops varied and depends on the crop varieties as well as the level of processing received. Faba bean flour generally had lower moisture content, higher protein, higher fat, higher ash, and higher fibre than wheat flour (Giménez et al., 2012). In the current study, pea starch and FB starch fractions were not pure according to the existence of protein content in each fraction respectively (Table 4-2). Also, the proximate composition reflects the seed components captured in the ingredient derived from them i.e. those with the seed coat contained more ash, and those with more cotyledon contained more protein (Bhatty, 1974). During dehulling process a small amount of seed coat may remain

on the surface of the FB cotyledon which can contribute an increase in the fibre content of the cotyledon flour.

Water holding capacity (WHC) and oil absorption capacity (OAC) are important functional properties of plant ingredients that will be incorporated in meat products. The functional properties these ingredients impart may influence the texture, cook yield, and flavour of the finished product. The binders employed in this study varied in water holding capacity (Table 4-3). Pea starch showed the lowest WHC (0.81 g/g), and faba protein fraction had the highest value (1.18 g/g), as a dry powder with no heat treatment. WHC was reported to be positively related to the protein content of dry legume flours (Chau & Cheung. 1998). Thus, it is reasonable that FB protein fraction (61% protein) presented the highest WHC. Additionally, fibre-rich binders are also reported to have high WHC (Wang & Toews, 2011). Therefore Malik (16% fibre) and Fabelle (17% fibre) cotyledon flours displayed the second highest WHC.

Table 4-3 The results of water holding capacity (WHC) and oil absorption capacity (OAC) of plant binders employed in low-fat bologna formulation

[1]Binder type	Water holding capacity (g/g)	Oil absorption capacity (g/g)
Wheat flour	0.90±0.02^{c}	1.22±0.03^{b}
Pea starch	0.81±0.02^{d}	0.90±0.03^{c}
Malik cotyledon	1.04±0.04^{b}	1.28±0.04^{ab}
Fabelle cotyledon	1.09±0.03^{b}	1.30±0.04^{ab}
Faba starch	0.94±0.01^{c}	0.91±0.03^{c}
Faba protein	1.18±0.03^{a}	1.32±0.02^{a}

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
Note: Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method.

Besides the chemical components such as protein, starch, and dietary fibre, the structural characteristics of the chemical constituents would also influence the WHC, including non-polar side chains of protein molecules, extent of gelatinization of starch granules, and ratio of soluble fibre in the dietary fibre fraction. Polysaccharides contribute significantly to the dietary fibre fraction of seed flours. The chains of biological polymers such as polysaccharides that are cross-linked via covalent or non-covalent bonds form a three- dimensional polymer network involving hydrogen bonding, ionic bonding, hydrophobic interactions and van der Waals attraction as

intermolecular interactions and contribute to gel formation in aqueous solutions (Cui et al., 2013).

Faba protein, Malik cotyledon flour, Fabelle cotyledon flour, and wheat flour all had the highest oil absorption capacity (ranging from 1.22 to 1.32 g/g, Table 4-3), and pea starch and faba starch presented comparatively low values (ranging from 0.90 to 0.91 g/g, Table 4-3). Similar to WHC, OAC was also reported to be associated with protein content. The protein molecules associate with lipid molecules via hydrophobic interaction than carbohydrate molecules (Chau & Cheung, 1998). This may explain why FB cotyledon flour of both cultivars, FB protein fraction, and wheat flour showed relatively higher OAC values than starch fractions. Increased OAC has been attributed to the ability of proteins to associate via hydrophobic interactions via non-polar amino acid side chains with the aliphatic chain of lipids (Ma et al., 2011). Additionally, lignin (insoluble fibre content) content in dietary fibre were reported to increase OAC for plant materials (Ma & Mu, 2016). According to Table 4-3, FB cotyledon flour and FB protein fraction had relatively higher insoluble fibre content (14-19%) than pea starch (5.6%) and FB starch fraction (3.2%). Binders with higher fibre content may result in higher OAC.

The investigation of pasting properties of binders would form the basis for understanding and predicting the functionalities expected in the meat products with the addition of these binder. Pasting is the phenomenon following the gelatinization process of the starch. The shear disintegration would occur in starch granules, and the paste obtained on gelatinization is a viscous mass consisting of a continuous phase of amylose and amylopectin (Ambigaipalan et al., 2011). In addition, proteins in the flour matrix may also go through heat induced gelation in the flour pastes and would contribute to viscosity properties (Crosbie et al., 2007).

During the heating process of Rapid Viscometer Analysis (RVA), binders gave significantly different values for peak and final viscosity (Table 4-4 & Figure 4-3). Peak viscosity means the maximum viscosity recorded during the heating process. Pasting time is the time spent to reach the peak viscosity. Pasting temperature refers to the temperature at which the viscosity displays the first rise above the baseline during the heating process. Final viscosity is the viscosity at the end of the cooling process (Crosbie et al., 2007). Pea starch presented the highest peak viscosity (3698 cp) and final viscosity (6584 cp); faba protein fraction which is low in starch had the lowest peak viscosity (44 cp) and the final viscosity (110 cp). Binders with

higher starch content (pea starch fraction and faba bean starch fraction) had the highest peak and final viscosity, and faba protein fraction with the low starch content had the lowest peak and final viscosity.

Table 4-4 Pasting properties of binders

[1]Binder type	Peak viscosity (cP)	Final viscosity (cP)	Pasting time (min)	Pasting T (°C)
Wheat flour	2290±36.1^{b}	2274.33±41.1^{c}	6.10±0.03^{bc}	87.77±0.45^{a}
Pea starch	3698±44.2^{a}	6584.33±58.4^{a}	4.58±0.19^{d}	73.12±0.43^{d}
Malik cotyledon	854±4.5^{c}	1562.33±35.08^{e}	5.70±0.11^{c}	75.94±0.80^{bc}
Fabelle cotyledon	957±13.0^{c}	1704.00±17.06^{d}	5.71±0.14^{c}	76.53±0.51^{b}
Faba starch	2277±71.8^{b}	4511.3±39.31^{b}	7.01±0.02^{a}	74.68±0.40^{c}
Faba protein	44±3.1^{d}	109.67±8.02^{f}	6.37±0.31^{b}	n.d.

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
Note: Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method.
n.d.: not detected

Binders varied significantly in pasting time and pasting temperature (Table 4-4 & Figure 4-3). Faba starch fraction had the longest pasting time of 7.01 min, and pea starch had the shortest pasting time of 4.58 min. Wheat flour had the highest pasting temperature at 87.77°C, and the pasting temperature of faba bean protein fraction was not detected due to the relatively low peak viscosity. Ambigaipalan et al. (2011) previously reported that the mean peak temperature of FB flour pasting was 69.54 °C, however Hood-Niefer et al. (2010) reported higher values of peak temperature (71.9 to 73.3 °C) of FB starch, which were close to the peak temperature of FB binders used in the current study (73-77 °C). The higher pasting temperature of wheat flour was a result of the formation of amylose-lipid complex which restricted the swelling of starch granules during the pasting process (Ai et al., 2013; Nebesny et al., 2005), therefore the paste presented the lower peak viscosity compared to pea starch and FB starch.

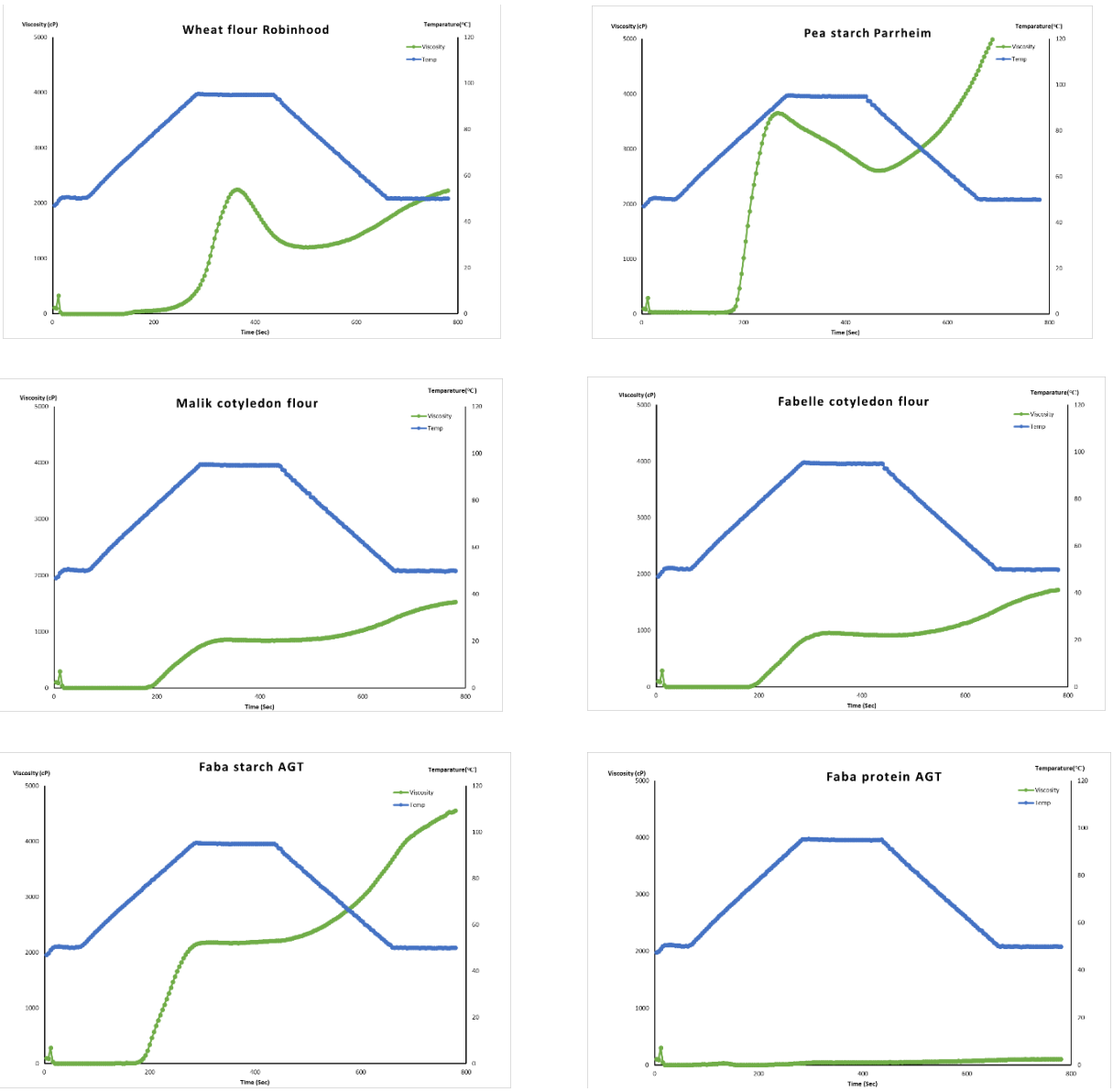

Figure 4-3 Pasting curves of all six binders the blue curve represented the temperature change over heating time, and the green curve represented the viscosity change time. Left Y axis was viscosity of the mixture, and right Y axis was the heating temperature.

The structure of starch granules and amylose/amylopectin content present in various ingredients could affect the pasting properties. Investigation by Ambigaipalan and group (2011) using Confocal laser scanning microscopy showed presence of numerous cracks in granules of faba bean starch granules. High amylose and high amylopectin starches with more cracks may be related to low granule integrity, so the strain may increase as the granule grows and the lower gelatinization transition temperature of FB starches compared to other kinds of cereal and pulse

starches may be due to the cracks in starch granules. As a biological polymer, starch is well known for its high swelling power in heated water because its amorphous region could absorb water in great extent during gelatinization (Agboola et al., 2010). The gelatinization temperature range for pea and lentil starch has been reported between 59.5 and 75.0 °C (Wani et al., 2016). Pea starch can swell more rapidly beyond 65 °C, and the swelling may have slowly increased from 55 to 65 °C (Wang et al., 2014). Generally, a lower transition temperature could reflect the presence of short amylopectin chains (Hood-Niefer et al., 2010). Additionally, based on x-ray diffraction results, Ambigaipalan et al. (2010) reported that FB starch had less well-ordered amylopectin double helices and lower extent of molecular order near the granule surface, compared to pinto bean and black bean starch, which decreased the viscosity during heating.

Amylose contents of the starch were negatively correlated with the peak viscosity (Li et al., 2019). During the initial heating process, amylose could restrict the swelling of starch granules to reduce the peak viscosity, and amylopectin was the primary constituent that contributes to the development of viscosity (Tester & Morrison, 1990). FB starch was reported to have the amylose content between 35 and 40% (Li et al., 2019). Within this range of amylose content in starch, peak and final viscosity were observed to decrease with increasing amylose content (Blazek & Copeland, 2008). The higher amylose content of starch could decrease the melting temperature of granules by disrupting crystallinity in the granular structure (Yuryev et al., 2004). In the current study, the FB starch may contain higher amylose content, compared to the pea starch, which lowered the peak and final viscosity.

Phosphorous content was another important factor that influence the pasting property of the starch granules. Some plant starches normally have higher content of phosphorous, therefore the repulsion of ions could weaken the bonding forces between amylopectin clusters, which resulted in a higher starch paste viscosity and increased granule susceptibility toward shear (Ambigaipalan et al., 2011). In a future study, the knowledge of phosphorous content of binder ingredients could also assist in understanding the pasting properties of the ingredients.

The protein content also had an impact on the peak and final viscosity (Chen et al., 2010). Starch granule-associated proteins, mainly the granule bound starch synthase showed reductions in viscosity of starch pastes measured under large deformation steady shear conditions (Han, 2002). Considering this observation, the inability to obtain values for pasting properties of FB

protein can be explained by the high protein level may have interfered with the development of viscose polymers by starch.

Denaturation temperature of FB protein is also related to the pasting properties of faba protein fraction. Ricci et al. (2018) reported that the denaturation temperature of FB protein isolate (88%, w/w) was 182.5 °C. This temperature value is higher than the maximum heating temperature (95 °C) during the RVA process. The lower heating temperature could not completely denature faba protein fraction, so no gel-like structures can form.

4.4.2 Effect of binder type on pH and viscosity of raw batters

Binder type had no significant effect on raw batter pH and the values ranged from 6.1 to 6.3 (Table 4-5). The control treatment showed the lowest viscosity (107.58 cp). The addition of 3% wheat flour, 1.5 and 3% pea starch, 3% Malik cotyledon flour, 3% Fabelle cotyledon flour, and 1.5% & 3% FB starch and protein fractions significantly (p<0.05) increased the viscosity of raw meat batter compared to the control. Reduced-fat content and more water in the formulation can result in decreased batter viscosity of the comminuted meat products in without any plant-based extender (Claus et al., 1989). Addition of wheat (13.4% protein and 71.2% starch), normal barley (11.8% protein and 66.3% starch) and waxy barley flours (13.0% protein and 61.2% starch) increased batter viscosity by 31%, 35%, and 37%, respectively, compared to the control (Shand, 2000). The increased viscosity of raw meat batters would assist sausage stuffing due to easier handling (Shand, 2000).

Depending on the type of binder the temperature of the raw meat batter changed slightly during processing (Table 4-5). All the binders were at same temperatures before mixing with ground meat. Among different binders, faba bean protein added at 1.5% and 3% significantly (p< 0.05) increased the batter temperature from 8.43 °C (control) to 11.32 and 11.48°C, respectively. Sanjeewa et al. (2010) concluded the temperature increase during processing may be due to the following reasons: temperature of the binders and seasoning which were at the room temperature may cause a temperature rise; less water in the meat system because of direct substitution of water; the addition of flour increased the friction of the system, which may cause a temperature rise. In the current study, binders stored at room temperature might slightly increase the temperature of the meat batter.

Table 4-5 Effect of binders on the viscosity and pH of raw meat batters (n=3)

[1]Binder type	Binder level %	pH[ns]	Temperature (°C)	Viscosity (cp)x10^{-3}
Control	0.0	6.13±0.02	8.43±1.79[b]	107.58±3.33[b]
Wheat flour	1.5	6.16±0.08	9.73±0.85[ab]	129.42±12.55[ab]
	3.0	6.16±0.10	9.93±1.52[ab]	147.00±3.97[a]
Pea starch	1.5	6.22±0.08	10.60±1.05[ab]	139.25±13.27[a]
	3.0	6.25±0.13	10.97±0.94[ab]	148.17±19.14[a]
Malik cotyledon	1.5	6.19±0.15	10.00±0.56[ab]	130.50±10.35[ab]
	3.0	6.23±0.08	10.63±0.55[ab]	137.75±6.41[a]
Fabelle cotyledon	1.5	6.16±0.08	10.77±0.61[ab]	123.92±2.90[ab]
	3.0	6.15±0.08	10.30±0.56[ab]	142.25±3.28[a]
Faba starch	1.5	6.21±0.09	10.60±0.22[ab]	134.42±4.88[a]
	3.0	6.21±0.11	10.88±0.43[ab]	147.50±8.23[a]
Faba protein	1.5	6.15±0.08	11.32±1.11[a]	137.58±8.41[a]
	3.0	6.16±0.06	11.48±1.05[a]	148.92±0.29[a]

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
[ns] Not significant. Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method.

4.4.3 Effect of binders on proximate composition and pH of cooked bologna

Addition of binders significantly changed the moisture, protein, and ash content of the cooked bologna compared to the control (Table 4-6). Due to the direct substitution of water, moisture content was significantly reduced in most formulations, except the 1.5% addition of FB protein fraction. The control bologna had the highest moisture content of 73.41%. The 1.5% difference in binder level resulted in a 1-2% difference in moisture content of the bologna. The protein content of the control bologna formulations was 13.14%, only the addition of Fabelle cotyledon flour at 3% (13.99%), FB protein fraction at 1.5% (14.28%), and 3% (15.56%) significantly increased the protein content with a maximum value about 2.4%. The variation in ash content was small, only the addition of FB protein fraction increased the ash content from 2.55 % (control) to 2.66 % due to the higher ash content of faba bean protein fraction (Table 4-2). As expected due to the low-fat content (Table 4-2), these plant binders had no significant

effect on fat content and the pH values of cooked bologna (Table 4-7). The pH value of bologna ranged from 6.2 to 6.3 and the fat content ranged from 10.1 to 11, with a less than 1% in difference.

Table 4-6 Proximate composition and pH of cooked bologna (n=3)

[1]Binder type	Binder level%	Moisture%	Protein%	Fat%[ns]	Ash%	pH[ns]
Control	0.0	73.41 ± 0.12^{a}	13.14 ± 0.44 ^{d}	10.35 ± 0.24	2.55±0.07^{c}	6.29±0.06
Wheat flour	1.5	72.36 ± 0.15^{b}	13.65 ± 0.67^{bcd}	10.66 ± 0.20	2.57 ±0.01^{c}	6.21±0.02
	3.0	71.50 ± 0.14^{c}	13.83 ± 0.10^{bcd}	10.53 ± 0.47	2.54 ±0.01^{c}	6.19±0.06
Pea starch	1.5	72.39 ± 0.23^{b}	13.42 ± 0.22^{cd}	10.14 ± 0.27	2.54 ±0.03^{c}	6.20±0.04
	3.0	70.78 ± 0.03^{de}	13.62 ± 0.10^{bcd}	10.96 ± 0.14	2.58 ±0.02^{bc}	6.17±0.07
Malik cotyledon	1.5	72.54 ± 0.04^{b}	13.59 ± 0.19^{bcd}	10.98 ± 0.43	2.56 ±0.02^{c}	6.23±0.02
	3.0	70.47 ± 0.02^{e}	13.85 ± 0.10^{bcd}	10.57 ± 0.20	2.61 ±0.02^{abc}	6.24±0.04
Fabelle cotyledon	1.5	72.31 ± 0.05^{b}	13.77 ± 0.19^{bcd}	10.54 ± 0.20	2.58 ±0.02^{bc}	6.19±0.05
	3.0	70.96 ± 0.21^{cde}	13.99 ± 0.15^{bc}	10.94 ± 0.19	2.66 ±0.03^{ab}	6.24±0.06
Faba starch	1.5	72.47 ± 0.13^{b}	13.45 ± 0.19^{cd}	10.61 ± 0.50	2.58 ±0.02^{c}	6.25±0.09
	3.0	71.05 ± 0.11^{cde}	13.75 ± 0.09^{bcd}	10.51 ± 0.23	2.59 ±0.03^{abc}	6.27±0.06
Faba protein	1.5	72.84 ± 0.56^{ab}	14.28 ± 0.13^{b}	10.44 ± 0.37	2.66 ±0.01^{ab}	6.25±0.05
	3.0	71.31 ± 0.43^{cd}	15.56 ± 0.35^{a}	10.87 ± 0.27	2.66 ±0.05^{a}	6.26±0.06

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
[ns] Not significant. Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method.

4.4.4 Effect of binders on cook loss

Binders had no significant effect on purge loss or cook loss of bologna (Table 4-7). Purge loss ranged from 4 to 5% and cook loss ranged from 0.4 to 0.6%. Similarly, the addition of 4% wheat and barley flour in ultra-low-fat bologna (<1% fat) did not affect cook yield (Shand, 2000). However, Pietrasik & Janz (2010) reported that adding 4% of wheat flour to low-fat bologna significantly reduced purge losses to 1.7% and 2.1% from 3.1% and 3.7% after 2 and 4 weeks, respectively. The differences in the results of these studies may be due to the variation in the contents of protein, fat, and water of the formulations.

Table 4-7 Effect of binder on expressible moisture, purge loss and cook loss of cooked bologna (n=3)

[1]Binder type	Binder level%	Cook loss%[ns]	Expressible moisture%	Purge loss%[ns]
Control	0.0	0.57±0.18	12.16±1.45[a]	4.69±0.91
Wheat flour	1.5	0.47±0.02	10.74±0.31[abc]	3.76±1.38
	3.0	0.51±0.03	8.66±0.41[c]	3.74±0.46
Pea starch	1.5	0.43±0.19	10.60±0.10[abc]	4.86±0.12
	3.0	0.43±0.15	11.27±0.16[ab]	3.65±0.44
Malik cotyledon	1.5	0.35±0.17	11.40±1.51[ab]	4.57±0.26
	3.0	0.46±0.16	10.82±0.28[abc]	3.40±0.80
Fabelle cotyledon	1.5	0.44±0.14	12.01±0.46[ab]	5.20±0.73
	3.0	0.34±0.18	10.82±0.23[abc]	3.95±0.98
Faba starch	1.5	0.52±0.06	10.88±0.91[abc]	4.26±1.21
	3.0	0.48±0.02	10.80±0.95[abc]	3.87±0.80
Faba protein	1.5	0.51±0.09	11.23±0.91[ab]	4.56±1.36
	3.0	0.44±0.09	9.77±0.33[bc]	3.67±0.13

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
[ns] Not significant. Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method.

Binders caused a significant effect on expressible moisture of bologna (Table 4-7). Bologna with 3% wheat flour had the lowest expressible moisture (8.66 %), which was significantly different from the control treatment (12.16 %). Similarly, bologna with 3% FB protein fraction also decreased expressible moisture compared to the control (from 12.16 % to 9.77 %). Other binders did not show any significant effect on expressible moisture of bologna. Flours from legumes or cereals contain mainly protein and starch which were biological macromolecules that can imbibe water and form gel matrices upon heating. In the presence of meat protein, they could either form a complex 3D gel network involving various forces such as van der Waals, electrostatic and hydrogen bonding, which trapped fine particles of emulsified meat or the meat matrix simply entrapped the starch and non-meat proteins as fillers (Sanjeewa et al. 2010).

4.4.5 Effect of binders on colour parameters

Binders had significant effect on *L** (lightness), *a** (redness), and *b** (yellowness) value of bologna (Table 4-8). The *L** values ranged from 68.78 (3% wheat flour) to 70.44 (3% Fabelle). Only the addition of 3% pea starch and 3% Fabelle cotyledon flour significantly increased the *L** value to 70.40 and 70.44, respectively.

Table 4-8 Effect of binder on colour parameters of cooked bologna (n=3)

[1]Binder type	Binder level %	*L**	*a**	*b**
Control	0.0	69.2 ± 0.50^{bcd}	17.7 ± 0.25^{ab}	14.2 ± 0.10^{f}
Wheat flour	1.5	69.0 ± 0.96^{cd}	17.7 ± 0.32^{ab}	14.8 ± 0.18^{bcdef}
	3.0	68.8 ± 1.03^{d}	17.8 ± 0.32^{a}	15.3 ± 0.08^{bc}
Pea starch	1.5	69.7 ± 0.82^{abcd}	16.9 ± 0.65^{bc}	14.5 ± 0.03^{def}
	3.0	70.4 ± 0.26^{a}	16.4 ± 0.62^{cd}	15.0 ± 0.16^{bcde}
Malik cotyledon	1.5	69.7 ± 0.10^{abcd}	16.6 ± 0.40^{cd}	14.7 ± 0.28^{cdef}
	3.0	70.1 ± 0.48^{abc}	16.5 ± 0.79^{cd}	15.4 ± 0.32^{b}
Fabelle cotyledon	1.5	69.7 ± 0.98^{abcd}	16.5 ± 0.74^{cd}	14.5 ± 0.14^{ef}
	3.0	70.4 ± 0.77^{a}	16.0 ± 0.44^{d}	15.1 ± 0.3^{bcde}
Faba starch	1.5	70.2 ± 0.43^{ab}	16.7 ± 0.58^{cd}	14.6 ± 0.31^{def}
	3.0	70.1 ± 0.69^{ab}	16.6 ± 0.72^{cd}	15.2 ± 0.09^{bcd}
Faba protein	1.5	69.7 ± 0.77^{abcd}	16.9 ± 0.38^{bc}	15.4 ± 0.27^{b}
	3.0	69.6 ± 0.80^{abcd}	17.0 ± 0.28^{bc}	16.4 ± 0.47^{a}

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
[ns] Not significant. Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different ($p<0.05$). The multi-treatment comparisons were using the Tukey's method.

In terms of the *a** value (redness) of the final product, the observed values ranged from 16.01 (Fabelle 3%) to 17.82 (wheat flour 3%). The addition of 3% pea starch, 1.5% & 3% Malik cotyledon flour, 1.5% & 3% Fabelle cotyledon flour, and 1.5% & 3% faba decreased the *a** value, compared to the control group but with a limited effect.

The *b** value (yellowness) of bologna also varied among binders. The addition of 3% wheat flour, 3% pea starch fraction, 3% Malik cotyledon flour, 3% Fabelle cotyledon flour, 3%

faba starch fraction, and 1.5 and 3% faba protein fraction significantly increased the *b** value, compared to the control. The *b* value ranged from 14.18 (control) to 16.35 (3% faba protein).

The effect of binders from cereals and legumes on colour parameters were discussed in other studies. One study (Shand, 2000) reported similar findings for the colour changes after the addition of cereal and pulse binders. Wheat and barley flour binders (4%, w/w) had marginal effect on colour of low-fat (<1%) bologna. Addition of 7.5–10% chickpea flour to beef sausages had a significant negative effect on the colour of the final product, which were more yellowish than products without adding chickpea flour (Dzudie et al., 2002). An increased *a** value was reported for the low-fat bologna with 4% pea starch (Pietrasik & Janz, 2010). Akesowan (2010) also pointed out decreased *L** and *a** values, but increased *b** values when the addition level of soy protein isolated increased in the pork bologna. The author explained that the pale soy protein isolate color may change the color of pork burgers, and meat myoglobin may be diluted. On the contrary, another study (Claus et al., 1989) found that adding 2% isolated soy protein did not affect the color of beef burgers. This may be due to the paler color of pork than beef.
The different results could be due to the different characteristics of plant-based binder and different myoglobin contents in meat products (Akesowan, 2010). Also, in the current study, the levels of the addition of FB binders (1.5 and 3%) were lower than levels of binders in previous studies (more than 5%), due to the concern of foreign flavour brought by FB ingredients.

4.4.6 Effect of binders on textural properties

Binders had no significant effect on TPA hardness, adhesiveness, cohesiveness, and springiness of bologna compared to the control (Table 4-9 & Table 4-10). Only bologna with 3% Fabelle cotyledon flour had significantly (p<0.05) higher hardness (163 vs. 129 N) and more chewiness (912 vs 801 N*mm) than bologna with 1.5% faba starch fraction.

Previous studies have reported that legume and cereal binders show significant effect on TPA parameters. The study by Sanjeewa et al. (2010) reported that low-fat bologna formulated with chickpea or pea flour (at 2.5 and 5%) had significantly (p<0.05) higher hardness and cohesiveness values than the control without any binder and the wheat flour (2.5 and 5%) containing bologna. In general, the higher protein content in legume flours than the control and bologna with the addition of wheat at 2.5 and 5%, may increase the hardness of bologna samples. Shand (2000) comparing the TPA parameters of ultra-low-fat pork bologna with added carrageenan (2.5%), soy protein concentrate (1%), potato starch (4%), wheat flour (4%) and

barley flour (4%) found that addition of carrageenan and soy protein concentrate had minor effects on texture, while the application of wheat flour and barley flour significantly increased the hardness of the bologna as compared to the control. Addition of common bean flour (21.2% protein and 69.7% carbohydrates) to beef sausages at 2.5%, 5.0%, and 7.5% level resulted in lower hardness values than the control without binder (Dzudie et al., 2002).

Table 4-9 Effect of binders on TPA textural properties (hardness, adhesiveness, and cohesiveness) of cooked bologna (n=3)

[1]Binder type	Binder level%	Hardness (N)	Adhesiveness (N)[ns]	Cohesiveness[ns]
Control	0.0	146.97±10.75[ab]	-0.70±0.10	0.54±0.04
Wheat flour	1.5	147.33±4.72[ab]	-0.70±0.10	0.55±0.02
	3.0	144.60±12.3[ab]	-0.83±0.12	0.58±0.02
Pea starch P	1.5	146.57±7.85[ab]	-0.90±0.17	0.56±0.01
	3.0	144.9±12.86[ab]	-0.97±0.06	0.56±0.02
Malik cotyledon	1.5	141.77±13.30[ab]	-0.97±0.38	0.57±0.01
	3.0	160.77±21.57[a]	-0.87±0.25	0.55±0.02
Fabelle cotyledon	1.5	151.10±4.79[ab]	-0.83±0.12	0.55±0.01
	3.0	163.00±15.96[a]	-1.07±0.40	0.55±0.02
Faba starch	1.5	129.13±6.37[b]	-0.77±0.06	0.57±0.02
	3.0	150.77±6.63[ab]	-0.83±0.23	0.57±0.02
Faba protein	1.5	139.1±12.71[ab]	-0.87±0.15	0.56±0.02
	3.0	136.17±8.75[ab]	-0.92±0.33	0.56±0.01

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
[ns] Not significant. Data did not show significant difference at $p<0.05$. Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different ($p<0.05$). The multi-treatment comparisons were using the Tukey's method.

The differences in the results of previous studies and the present study may be due to the differences in the protein, fat, and water content of the formulations. Due to the strong meat matrix presented in the control treatment of the current study (12-13% of meat protein), the two levels (1.5 & 3%) of binder applications may have very limited effect on texture profile of the bologna. In the previous study (Sanjeewa et al., 2010), the meat protein content in the bologna

formulation was about 9.5%, which indicated the binder applications may show more effect on texture profile due to the weaker meat matrix.

Table 4-10 Effect of binders on TPA textural properties (springiness and chewiness) of cooked bologna (n=3)

[1]Binder type	Binder level%	Springiness %[ns]	Chewiness (N*mm)
Control	0.0	79.75±2.48	866.44±13.31[ab]
Wheat flour	1.5	80.07±0.85	905.07±38.37[ab]
	3.0	83.11±1.74	911.21±83.73[ab]
Pea starch	1.5	81.27±0.91	877.39±25.57[ab]
	3.0	80.49±1.42	857.60±68.62[ab]
Malik cotyledon	1.5	81.01±2.88	839.77±83.81[ab]
	3.0	77.98±4.00	849.2±110.52[ab]
Fabelle cotyledon	1.5	79.93±2.44	911.94±39.36[ab]
	3.0	79.85±0.49	958.90±41.33[a]
Faba starch	1.5	79.95±2.03	801.32±44.48[b]
	3.0	79.65±2.96	904.64±45.09[ab]
Faba protein	1.5	79.97±3.17	830.27±74.18[ab]
	3.0	79.04±1.04	816.97±39.93[ab]

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
[ns] Not significant. Data did not show significant difference at $p<0.05$. Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different ($p<0.05$). The multi-treatment comparisons were using the Tukey's method.

The two levels of addition (1.5% and 3%) of binders had very limited effect on shear stress and strain of the bologna products (Table 4-11). Only bologna with 3% wheat flour showed a significant increase in the shear stress (39.60 kPa), compared to the control treatment (29.29 kPa). The shear stress ranged from 29.29 kPa to 39.60 kPa. Binders had no effect on shear strain, compared to the control. However, shear strain of bologna with 3% wheat flour (1.63) was significantly different from that of bologna with 3 % FB protein (1.42). The shear strain ranged from 1.42 to 1.63.

Table 4-11 Effect of binders on torsional properties of cooked bologna (n=3)

[1]Binder type	Binder level%	Shear stress (kPa)	Shear strain
Control	0.0	29.29±1.66^{b}	1.55±0.06^{ab}
Wheat flour	1.5	30.26±3.18^{b}	1.56±0.08^{ab}
	3.0	39.60±0.95^{a}	1.63±0.04^{a}
Pea starch	1.5	29.09±2.80^{b}	1.52±0.10^{ab}
	3.0	29.73±1.38^{b}	1.50±0.05^{ab}
Malik cotyledon	1.5	29.29±1.28^{b}	1.50±0.10^{ab}
	3.0	29.51±2.78^{b}	1.49±0.09^{ab}
Fabelle cotyledon	1.5	31.63±4.47^{b}	1.51±0.05^{ab}
	3.0	31.68±2.68^{b}	1.53±0.05^{ab}
Faba starch	1.5	32.35±1.41^{b}	1.47±0.05^{ab}
	3.0	29.87±3.17^{b}	1.54±0.06^{ab}
Faba protein	1.5	28.80±2.73^{b}	1.49±0.04^{ab}
	3.0	27.28±2.10^{b}	1.42±0.06^{b}

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
[ns] Not significant. Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method.

Significant differences ($p < 0.05$) were found in torsion shear stress and shear strain values of low-fat pork bologna due to pulse flour levels and type of added flour in previous research (Sanjeewa et al., 2010). The highest shear stress (43.6 kPa) was observed for bologna with 5% Desi chickpea flour, whereas the lowest values were noted for the control (30.5 kPa) and 2.5% pea flour-added bologna (31.5 kPa). The addition of 5% flour in bologna resulted in significantly ($p < 0.05$) higher shear stress values than that with 2.5% flour addition except for the pea flour containing bologna. Shear strain represents the elasticity behaviour of gelled meat products. Low-fat pork bologna formulated with any seed flour had lower (p<0.05) shear strain values than of the control (Sanjeewa et al., 2010). Differences (p<0.05) were found in torsion rigidity (stress/strain) values within the two levels of flour addition and different source of flours. Flour addition increased the torsion rigidity (increased shear stress and decreased shear strain) ($p < 0.05$) compared with the control. The author concluded that when flour addition was increased from 2.5% to 5% in the formulation, changes in torsion rigidity of wheat and Kabuli chick pea

flour added bologna were determined by shear stress, while that of pea flour added bologna were due to changes in shear strain. Shear strain and shear stress made equal contributions to rigidity for the Desi chickpea flour added bologna. In the current study, only addition of 3% wheat flour could significantly increase the shear stress of bologna, which may be due to the lower addition levels of binders compared to the previous study discussed above.

In general, it is known that shear stress is strongly influenced by protein types and concentrations, processing condition, and ingredients while shear strain is affected by protein quality and denotes protein functionality in the food matrix (Hamann, 1988). In the current study, the heating process could promote hydrophobic association of wheat gluten when gluten protein was disrupted due to the increased kinetic energy of the system. Disulphide–sulphydryl interchange reactions were also implicated in heated gluten protein together with electrostatic linkages (Comfort & Howell, 2003). Although the current heat temperature at 72 °C may not be sufficient to form a strong gel network compared to heating to 90°C (Comfort & Howell, 2003), it was possible that the increased kinetic energy of the system during heating caused as much disruption of the structure as it enhanced cross-link formation. The protein gel formed by wheat flour at 3% and meat protein increased the shear stress by 34% of the control. Wheat flour contained gluten, which could lead to more elastic texture than gluten free faba bean ingredients (Multari et al., 2015).

4.4.7 Effect of binders on sensory properties

The low-fat bologna prepared with and out plant binders were evaluated by 12 trained panelists under white light. Addition of binders showed significant (p<0.05) effect on firmness, juiciness, and foreign flavour of the low-fat bologna (Table 4-12 & Table 4-13).

In terms of firmness (Table 4-12), bologna with 3% wheat flour had significantly increased firmness (5.4) compared to the control (4.6). In comparison, the addition of FB protein at 3% in bologna significantly (p<0.05) lowered perception of firmness (4.5) compared to bologna with 3% added wheat flour. The score of firmness was in the middle of 4 and 5, which indicated the intensity of the firmness was between slightly soft and slightly firm. This result was similar with the results obtained from the texture profile analysis even though not exactly the same (i.e. 3% wheat flour could increase the bologna hardness according to the TPA results, which may be due to the presence of gluten content in wheat flour). Additionally, according to the RVA results of binders, faba protein fraction cannot form gels due to high denaturation

temperature (182.5 °C) of FB protein (Ricci et al., 2018). The final internal temperature (around 72-74 °C) of bologna cannot reach the protein denaturation temperature, so bologna added with 3% faba protein fraction may present less firmer texture.

Table 4-12 Effect of binders on the sensory properties (firmness, cohesiveness, juiciness, and graininess) of cooked bologna evaluated by trained panelists (n=12)

[1]Binder type	Binder level%	Firmness	Cohesiveness[ns]	Juiciness	Graininess[ns]
Control	0.00	4.6±0.30[b1]	4.4±0.23	4.9±0.29[a]	2.2±0.32
Wheat flour	1.5	4.7±0.14[ab]	4.5±0.08	4.4±0.17[abc]	2.1±0.27
	3.0	5.4±0.13[a]	4.7±0.19	4.22±0.10[bc]	2.2±0.08
Pea starch	1.5	4.6±0.39[ab]	4.6±0.13	4.5±0.38[abc]	2.4±0.46
	3.0	4.8±0.22[ab]	4.9±0.25	4.0±0.13[bc]	2.8±0.29
Malik cotyledon	1.5	4.8±0.34[ab]	4.7±0.17	4.5±0.25[abc]	2.3±0.25
	3.0	4.8±0.38[ab]	4.9±0.25	4.4±0.29[abc]	2.5±0.35
Fabelle cotyledon	1.5	4.9±0.20[ab]	4.3±0.19	4.7±0.17[abc]	2.2±0.19
	3.0	5.3±0.17[ab]	4.6±0.27c	4.0±0.19[bc]	2.6±0.41
Faba starch	1.5	5.0±0.30[ab]	4.8±0.39	4.4±0.13[abc]	2.4±0.25
	3.0	4.9±0.43[ab]	4.8±0.05	4.1±0.05[bc]	2.7±0.29
Faba protein	1.5	4.7±0.64[ab]	4.6±0.22	4.6±0.21[ab]	2.3±0.29
	3.0	4.5±0.29[b]	4.4±0.32	4.3±0.25[bc]	2.6±0.05

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
Firmness: 1 = extremely soft and 8 = extremely firm
Cohesiveness: 1 = extremely brittle and 8 = extremely cohesive
Juiciness: 1 = dry and 8 = extremely juicy
Graininess: 1 = not detectable and 8 = extremely grainy
[ns] Not significant. Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method.

There was no difference found in cohesiveness (Table 4-12) between types of binder added. The average score of perception of cohesiveness ranged from 4-5 (slightly brittle to slightly cohesive, see Appendix A), which indicated the intensity of the cohesiveness was in the middle point of the scale. These results also corresponded with the results obtained by the instrumental texture profile analysis

For juiciness (Table 4-12), the no binder control bologna showed the highest juiciness score (4.9). Adding wheat flour, pea starch fraction, Fabelle cotyledon flour, faba bean starch fraction, and faba bean protein fraction significantly (p<0.05) decreased the score of juiciness. Application of Malik cotyledon flour at either level (1.5% and 3%) did not affect the juiciness. Refer to the discussion about expressible moisture in this study, adding binders except wheat flour and faba protein fraction did not have significant effect on expressible moisture of bologna. The score of juiciness ranged from 4-5 (slightly dry to slightly juicy, see Appendix A). Xu (2017) reported when including 2.5% pea fibre product, the score for juiciness of low-fat pork bologna was significantly lower than the control group. Malik cotyledon flours did not lower the perception of juiciness, so additional water will not be required to offset the loss of juiciness.

Binders had no effect on graininess (Table 4-12). Graininess was defined as the presence of small particles in bologna after chewing (See Appendix B). The score of graininess ranged from 2-3 (very slightly grainy to slightly grainy, see Appendix A), which indicated that panelists had low perceived grainy mouthfeel. Binders did not significantly bring undesired graininess to the bologna samples as previously expected. Some other studies showed that using fibre-rich pulse ingredients as binders could significantly increase the perception of graininess of bologna products even at 1.25-2.5% addition levels when particle size of legumes was less than 250 μm (Xu, 2017).

Binders did not significantly change the flavour intensity of the bologna (Table 4-13). In general, the score of flavour intensity was around 4 (slightly bland, see Appendix A). However, as noted in Table 4-13, the addition of Fabelle cotyledon flour at 3% significantly increased the intensity of foreign flavour in bologna (3.3), compared to the no-binder control treatment (2.1) and bologna with 3% wheat flour (1.9). The higher value of standard deviation (1.1) for the bologna with 3% Fabelle cotyledon flour may also indicate the variation among panelists. Overall, the score of foreign flavour ranged from 2-3, which indicated that the intensity of graininess was low.

Binders did not have the significant effect on the overall acceptability of the various bologna formulations (Table 4-13). In general, the scores of overall acceptability ranged from 4-5, which indicated that the liking was in the middle of the scale, meaning between dislike slightly and like slightly. Even with lower perception of juiciness, binders did not alter the liking of the bologna products. In addition, due to the small number of the consumer panel (12 panelists), it

may not be sufficient to make a strong conclusion about overall acceptability of the bologna products.

Table 4-13 Effect of binders on the sensory properties (flavour intensity, foreign flavour, and acceptability) of cooked bologna evaluated by trained panelists (n=12)

[1]Binder type	Binder level %	Flavour intensity [ns]	Foreign flavour	Overall acceptability [ns]
Control	0.0	4.5±0.52	2.1±0.39[b]	5.0±0.28
Wheat flour	1.5	3.7±0.41	2.3±0.52[ab]	4.3±0.08
	3.0	3.8±0.35	1.9±0.22[b]	4.4±0.27
Pea starch	1.5	4.3±0.22	2.3±0.38[ab]	4.9±0.21
	3.0	4.4±0.30	2.9±0.63[ab]	4.5±0.21
Malik cotyledon	1.5	4.2±0.19	2.1±0.13[ab]	4.9±0.13
	3.0	4.2±0.10	2.7±0.17[ab]	4.8±0.17
Fabelle cotyledon	1.5	4.3±0.21	2.0±0.14[ab]	5.1±0.08
	3.0	4.1±0.34	3.3±1.09[a]	4.4±0.68
Faba starch	1.5	4.4±0.13	2.2±0.53[ab]	5.1±0.21
	3.0	4.2±0.22	2.5±0.30[ab]	4.6±0.10
Faba protein	1.5	4.5±0.36	2.1±0.08[ab]	5.0±0.22
	3.0	4.5±0.38	2.6±0.29[ab]	4.5±0.21

[1] Wheat flour was of Robinhood brand, Faba bean starch and protein fractions were from AGT Foods, pea starch was from Parrheim and Fabelle and Malik cotyledon flours were prepared in house.
Flavor intensity: 1 = extremely bland and 8 = extremely intense
Foreign flavor: 1 = extremely intense foreign flavor and 8 = no foreign flavor
Overall acceptability: 1 = dislike extremely and 8 = like extremely
[ns] Not significant. Values are presented as mean ± standard deviation. Means with the different letter in the same column are significantly different (p<0.05). The multi-treatment comparisons were using the Tukey's method.

Overall, the sensory results showed that the different addition levels of binders had very limited effect on sensory attributes of the bologna. The sensory studies of bologna with other pulse ingredients were also reported. Claus and Hunt (1991) concluded the addition of 2% pea fibre did not increase the hardness and cohesiveness of low-fat beef bologna but increased the intensity of graininess and juiciness in comparison to the low-fat control treatment. The incorporation of chick pea flour at two levels (2.5% and 5%) increased the instrumental and sensory firmness of the low-fat pork bologna without a biotype difference. For most flavour properties, bologna with 5% Kabuli and Desi chickpea flour performed similarly to control,

whereas panelists noted more foreign-flavours with addition of wheat and pea flour at 5% (Sanjeewa et al., 2010).

4.5 Conclusion

Different binder ingredients varied in functional properties, such as water holding capacity, oil absorption capacity, and pasting properties. FB ingredients had higher water holding capacity and oil absorption capacity, compared to pea starch fraction and wheat flour. No cultivar difference was observed for the pasting properties of FB cotyledon flour. Faba starch fraction can form thicker paste than other FB ingredients. Faba bean protein fraction had poor ability to form paste during the heating process. Faba bean ingredients can form thinner paste without additional chemical modification.

The addition of faba bean ingredients at 1.5 or 3% did not have a major effect on bologna colour. There were significant changes in *L*, *a*, *b*, but the typical cured pink colour was maintained after adding binders. Binders also did not significantly change cook loss and purge loss. Binders had limited effect on textural properties of bologna. Addition of 3% Fabelle cotyledon flour significantly increased the hardness and chewiness of bologna, according to TPA results (but was not noticed by the panelists, only bologna with 3% wheat flour had significantly increased firmness compared to the control). Bologna with 3% wheat flour had significantly increased shear stress. Binders did not have an effect on shear strain.

Adding wheat flour, pea starch fraction, Fabelle cotyledon flour, faba bean starch fraction, and faba bean protein fraction significantly decreased the perception of juiciness. The addition of Fabelle cotyledon flour at 3% significantly increased the intensity of foreign flavour in bologna, compared to the control treatment and bologna with 3% wheat flour. At the higher level of inclusion (3%), faba protein fraction would contribute 2% additional protein to the bologna. Faba bean may offer a useful option to increase protein content and replace traditional plant binders which may contain gluten without significantly changing textural and sensory properties of low-fat meat products in the current market.

5 GENERAL DISCUSSION

The overall objective of this project was to understand the varietal difference on the levels of important macronutrients and undesired compounds of FB with the ability of selected processing on reducing these undesired compounds and the possibility of developing low-fat processed meat products with FB-based ingredients. The studies that were included to encompass this objective were: determination of the level of selected chemical compounds in five different varieties of whole FB seeds, the effect of short-term germination and autoclaving on levels of selected undesired compounds, and the effect of ingredients derived from FB on physicochemical, textural and sensory properties of low-fat pork bologna when used as a binder in comparison with wheat flour and pea starch fraction. The prairie provinces lead Canadian FB production (Oomah et al., 2015). Faba bean is an excellent source of macronutrients, such as protein, starch, and fibre (Multari et al., 2015). Ingredients derived from FB include dehulled flour (cotyledon flour), protein-rich fraction, and starch-rich fraction, however, so far not widely used in food products. In widening the binder market for processed meat products, FB provides a suitable pulse- derived and plant-based binder option that has great potential to be utilized to improve the textural properties in low-fat meat products. The comparative study carried out with other plant-based binder ingredients with similar chemical composition i.e. commercial wheat flour and pea flour provides key information needed in positioning FB ingredients as a binder for low-fat processed meat products.

The study I that investigated five cultivars (Snowdrop, Taboar, Snowbird, Fabelle, and Malik) of FB grown in North America collected from three different crop years provided whether the agronomic and some of the key seed quality traits are represented in the quality parameters that matters in the nutrition and functional performance of FB flour in food systems. The noted quality traits of the seeds were: Snowdrop and Snowbird are small/medium-seeded and low-tannin, Malik is large-seeded and regular tannin, Taboar is medium-seeded and regular tannin, and Fabelle is medium-seeded, regular tannin and low vicine/ convicine. Using seed coat

colour as an identification of varieties was somewhat difficult because of the yearly variation. Snowbird and Snowdrop had similar ocher green colour. Fabelle, Malik, and Taboar presented the similar tanned brown colour. Although no significant difference found in L^*, a^*, and b^* values of FB among cultivars (no difference in seed coat colour), seeds received from the second-year harvest were paler than other years and this colour change was suggested due to the higher than average precipitation received by the plant during growing (Government of Saskatchewan, 2016).

The proximate and carbohydrate analysis showed that cultivar had little influence on macro-composition and very similar moisture, protein, fat, ash, total starch, and total dietary fibre content were found among the five cultivars studied. When these major constituents of faba bean are considered, the lot (production year) had a slight impact (≤ 2%) on total starch and total fibre content of each FB cultivar. Carbohydrate content of FB was around 60% (w/w) of the seed dry matter (Sauvant et al., 2004), which was in line with the results for carbohydrate content obtained in the current study (60-61%). There was no significant difference (p<0.05%) in the contents of total starch (~35%) or total dietary fibre (25 to 26% in the whole seed of five cultivars. In addition, the total dietary fibre content in FB was partitioned as insoluble to soluble in a ratio of ~ 10:1. The proximate and carbohydrate analysis proved that FB seed was rich in protein, starch, and total dietary fibre, and the potential of developing new and alternative food ingredients rich in starch, protein or fibre should be explored.

Undesired compounds such as phytic acid, raffinose, stachyose, verbascose, and total phenolics are common to many legumes and ingredients derived from seeds. Faba bean cultivars had no significant effect on the levels of phytic acid, raffinose, stachyose, and verbascose in whole seeds. The compounds vicine, convicine which are specific to FB only, showed a cultivar difference because Fabelle which is the only registered low vicine/convicine cultivar in Canada was included in the study. According to Duc et al. (1989), mutation in the *vc*-gene that controls vicine and convicine expression has allowed the development of this cultivar that has a 10 to 20-fold reduction without modifying the macronutrient content. Among the cultivars, the level of vicine content was approximately twice of the convicine content. Fabelle, which had the lowest level of vicine and convicine is a cultivar developed to have very low or zero levels of these two pyrimidine glycosides. All other cultivars had regular levels of vicine and convicine found in FB with the highest recorded for Snowdrop and Snowbird followed by Malik and Taboar. The

breeding program to grow low vicine/convicine cultivars was successful, the reduction percentage was found above 90% compared to the other varieties in the current study. The reduced vicine/convicine content allows expanding uses of FB in food products that is currently limited to the individuals has no G6PD deficiency (Lattanzio et al., 1982). A cultivar variation was also observed for the total phenolic content among faba bean cultivars used in the present study. The variety Fabelle showed the lowest level of total phenolic content also and Snowdrop and Snowbird showed higher total phenolic content than others. The cultivars that are low in the contents of vicine/convicine and tannin, such as Fabelle has a tremendous potential to be used as gluten-free ingredients in food products especially, to replace starch and protein.

Germination was one of the treatments evaluated in Study I to reduce the undesired compounds in FB seeds, and it was successful in reducing oligosaccharide content. The washed and soaked whole FB seed showed successful germination when temperature was kept at 25 °C. All cultivars reached the germination percentage of nearly 90% at 72 h. Germination of FB seeds for 72 hours did not have a significant effect on phytic acid level of these five cultivars, which may be related to the hydrolysis and synthesis of InsP6 (the major component of phytic acid, myo-inositol hexakisphosphate), which occurred during germination in cotyledons of beans (Loewus, 2002). The study by Luo & Xie (2013) reported that 72 h germination at room temperature reduced phytic acid content of FB seeds by 37% and a longer germination treatment (120 h) could increase the phytic acid content of FB seeds by 12%. No variation was observed during the germination in the pyrimidine glucosides content. The ratio between vicine to convicine was 2:1 from the beginning to the end of the germination period observed. There was no significant effect on levels of germination on vicine (except Snowbird), and convicine. The limited changes of vicine/convicine during the germination process could be due to translocation of vicine and convicine from the cotyledons to the axis with no evidence of the use of these compounds in further metabolism (Goyoaga et al., 2008). Germination had a strong effect in reducing raffinose, stachyose, and verbascose in all the cultivars of FB seeds. For all five cultivars, a complete disappearance of raffinose and about 60% reduction in stachyose was observed by end of the germination period. However, there was a slight delay in degradation of verbascose in Taboar, Snowbird and Fabelle (48 h) than that observed in Snowdrop and Malik (24 h). For all five cultivars, the reduction of stachyose level was around 80% at 72 h of germination. The disappearance of oligosaccharides was likely accompanied by increases in the

levels of sucrose and other monosaccharides (Vidal-Valverde et al., 1998), although these were not measured in the present study. The disappearance of oligosaccharides and increase in di- and mono-saccharides during germination has been attributed to the increase in the level of α-galactosidase enzyme activity that catalyses hydrolysis of the glycosidic linkage in oligosaccharides and result in the increase of smaller carbohydrate molecules such as sucrose (Akinlosotu & Akinyele, 1991). The germination process significantly reduced total phenolic content in Snowdrop and Snowbird by about 17-18%. However, there was no significant effect of germination on total phenolic content of Taboar, Fabelle, and Malik seeds. The loss of polyphenol during sprouting can be attributed to solubilization by enzymes because the prime objective of sprouting was to promote the development of hydrolytic enzymes that were not active in raw seeds (Satwadhar et al., 1981). During soaking and germination of seeds, some of the phenolic compounds can leach out (Luo et al., 2013).

Autoclaving had a limited effect on the levels of undesired compounds, and only total phenolic content was reduced (by 15 to 23%) after autoclaving. Faba bean seeds were soaked then autoclaved. Autoclaving did not present significant effect on phytic acid, vicine/convicine, raffinose, stachyose, and verbascose. Phytic acid is heat stable, therefore a significant reduction in the level of phytate during cooking cannot be expected unless either the cooking water was discarded, or the food received additional processing treatment such as soaking (soaking water discarded), germination, fermentation, etc. (Sathe & Venkatachalam, 2002). The limited effect of autoclaving on the level of vicine and convicine could be due to the sample preparation (whole seed in the current study vs. seed cotyledon in other studies) used for determination. Cardador-Martínez et al. (2012) reported that the content of vicine/convicine in faba bean cotyledons was significantly decreased upon roasting or boiling. The seed coat may reduce the effect of high heat and pressure penetration into cotyledons during autoclaving. Since vicine/convicine are primarily located in the cotyledons, the effect of autoclaving as a treatment was not that effective in lowering the contents. During the autoclaving process, the reduction of oligosaccharides may be due to heat induced hydrolysis of the oligosaccharides to simple disaccharides and monosaccharides (Oboh et al. 2000). In the present study, oligosaccharides content in cooking water was not separated but dried together for further analysis, therefore any leached-out sugars were included in the analysed samples. This may have been the reason to observe only minor changes in the levels of oligosaccharides of FB samples due to autoclaving. Autoclaving did not

significantly affect the total phenolic content of Snowdrop and Fabelle seeds, however in Taboar, Snowbird, and Malik a 15 to 23% reduction was observed. The reduction in phenolic contents of beans during cooking could be due to either destruction of phenolic compounds or other chemical rearrangements of phenolic compounds such as the formation of new insoluble components with other organic substances (Kader, 1995). Due to the high protein content in FB seeds, heat treatment could also promote stronger tannin–protein interactions (Giczewska & Borowska, 2003) that can interfere with the determination of phenolic content. For further work of autoclaving effect, drained FB seeds (without any liquid) can be analyzed alone, which is commonly done when canned seeds when using canned seeds.

Based on the results, FB cultivars that has contrasting composition differences was selected to use in the application study to incorporate as binders in low-fat pork bologna for study II. Cotyledon flour of Malik (large seed, regular tannin, vicine and convicine) and Fabelle (low vicine and convicine) were used as binder ingredients in comparison with commercially available starch and protein fractions of FB along with commercial wheat flour and pea starch fractions. These binder ingredients differed in starch, protein and fibre contents, therefore the addition level of 1.5 and 3.0 % may pose somewhat different effect on the flow (viscous) properties of uncooked meat batter and the structural properties of the meat gels formed in the cooked product. For chemical composition of binders, the highest protein content was in the faba bean protein fraction and the pea starch had the lowest. Wheat flour and faba bean starch fraction had similar protein content. Faba bean cotyledon flour of two cultivars had the similar protein content. The fat content of all binders ranged from 0.43-1.66%. The starch content varied from 6% (faba bean protein fraction) to 82% (pea starch). Cotyledon flour from Malik and Fabelle had the similar starch content at 33%. Wheat flour had the second highest starch content at 69%, and faba bean starch (61%) was slightly lower than that of wheat flour. The range of fibre content was from 3% (faba starch) to Fabelle cotyledon flour (19%). Wheat flour and pea starch had also relatively low fibre content at 4 and 6%, respectively. Malik cotyledon flour, Fabelle cotyledon flour and faba protein fraction had higher fibre content at 17, 19, and 14%, respectively.

The physico-chemical properties of the binder ingredients that affect the properties of the meat batter and cooked product are water holding capacity, oil absorption capacity, and pasting properties. Pea starch showed the lowest water holding capacity, and faba protein fractions had the highest water holding capacity. Water holding capacity was reported to be positively related

to the protein content (Chau & Cheung. 1998). Thus, it is reasonable that faba bean protein fraction (61% protein) had the highest water holding capacity. Additionally, high fibre content binders were also reported to have higher water holding capacity (Wang & Toews, 2011). Therefore Malik (16% fibre) and Fabelle (17% fibre) cotyledon displayed the second highest water holding capacity. Similar to water holding capacity, oil absorption capacity was also reported to be associated with protein content. The protein molecules generally generated more hydrophobic interaction with lipid molecules than carbohydrate (Chau & Cheung, 1998). This may explain why faba bean cotyledon flour of both cultivars, faba bean protein fraction, and wheat flour showed relatively higher oil absorption capacity. The pasting properties of binders would form the basis for product properties that include meat and binders. Pea starch fraction presented the highest peak viscosity and final viscosity; faba protein fraction presented the lowest peak viscosity and the final viscosity. Binders with higher starch content (pea starch fraction and faba bean starch fraction) had the highest peak and final viscosity, and faba protein fraction with the lowest starch content had the lowest peak and final viscosity. Faba bean starch paste normally presented lower viscosity when compared to other pulse starch (Hood-Niefer et al., 2010). The results indicated that FB cotyledon flour could be used as a minimally processed ingredient without any chemical or physical modification to generate a thinner paste upon cooking.

In the bologna products prepared in study II, incorporation of plant-based binders in the formulation had no significant effect on pH of raw meat batter. The pH values of control raw meat batters were higher than the raw pork picnic (5.8-5.9) because of the addition of sodium tripolyphosphate (pH=9-10). Xu's study (2017) also concluded that the addition of pulse fiber at 1.25 and 2.5% did not change the pH of raw meat batter. The addition of 3% wheat flour, 1.5 and 3% pea starch, 3% Malik cotyledon flour, 3% Fabelle cotyledon flour, and 1.5% and 3% faba bean starch and protein fractions significantly increased the viscosity of raw meat batter. The increased viscosity of raw meat batters would assist sausage stuffing due to easier handling (Shand, 2000). Binders significantly changed the moisture, protein, and ash content of the cooked bologna, compared to the control. Due to the direct substitution of water, moisture content was significantly reduced in most formulations, except the 1.5% addition of FB protein fraction. Due to the high protein content in FB ingredients, the maximum increase in protein content was about 2% when faba protein fraction was added at 3%. Faba protein fraction is an

ideal gluten-free ingredient to increase the overall protein content of the meat products. Due to the limited fat content in binders, no significant effect on the fat content of final cooked product was observed. It is clear that the ingredients derived from FB seeds are promising gluten-free binders to increase the total protein content in processed meat products.

Binders had no significant effect on purge loss and cook loss of the cooked bologna. The expressible moisture content of finished meat products was significantly affected by the binders. Bologna with wheat flour at 3% had the lowest expressible moisture, which was significantly different from the control treatment without a binder. The components such as starch and fibre in pulse flour might help to retain water and fat during the cooking process hence reduced cook loss in meat products (Sanjeewa et al., 2010). Binders had limited effect on the colour of the finished product as observed by L^* (lightness), a^* (redness), and b^* (yellowness) value of cooked bologna. The ranges of L^* (69-70), a^* (16-18), or b^* value (14-16) of the cooked bologna surfaces were narrow for all bologna samples. The original pink colour of cooked bologna was preserved after the addition of binders. For texture profile analysis (TPA), binders had no significant effect on hardness, adhesiveness, cohesiveness, and springiness of cooked bologna, compared to the control without binders. Only bologna with 3% Fabelle cotyledon flour was significantly higher in hardness from bologna with 1.5% faba starch fraction. Dzudie et al. (2002) concluded that the addition of common bean flour to beef sausages at 2.5%, 5.0%, and 7.5% level resulted in lower hardness values than the control without binder. Due to the strong meat matrix presented in the control treatment of the current study (12-13% of meat protein), the low levels (up to 3% w/w) of binder addition provided a limited effect on texture profile of the bologna. For torsional gelometry analysis, only bologna with 3% wheat flour showed a significant increase in the shear stress, compared to the control group. Binders had no effect on shear strain, compared to the control. Shear stress is strongly influenced by the type and concentration of protein and ingredients, and the processing conditions they are subjected to while shear strain is affected by the protein quality that is denoted by protein functionality in the food matrix (Hamann, 1988). In the current study, the stronger protein gel formed by 3% wheat flour and meat protein increased the shear stress by 34% of the control. Since wheat flour contained gluten, the gluten developed may have led to more elastic texture than gluten-free FB ingredients (Multari et al., 2015). The results of the effect of binders on physicochemical and textural properties of low-fat pork bologna indicated that FB ingredients can increases the

protein content and did not significantly change the textural properties of bologna products at the levels used. Bologna samples with binders did not present softer and mushier texture unlike adding other pulse ingredients such as pea bran and pea fibre at 1.25 and 2.5% (Xu, 2017).

The sensory analysis of finished bologna products with the plant binders by twelve trained panelists showed that the binders had a significant effect on firmness, juiciness, and foreign flavour but not on cohesiveness, flavour intensity, graininess, and overall acceptability. Bologna with 3% wheat flour significantly increased firmness compared to the control. In comparison, the addition of FB protein at 3% in bologna had significantly lower perception of firmness than wheat flour at 3%. No binder containing control bologna showed the highest juiciness score. Adding wheat flour, pea starch fraction, Fabelle cotyledon flour, faba bean starch fraction, and faba bean protein fraction significantly decreased the score of juiciness. Application of Malik cotyledon flour at either level (1.5% and 3%) did not affect the juiciness. The addition of Fabelle cotyledon flour at 3% significantly increased the intensity of foreign flavour, compared to the control group. The increase of the foreign flavour could be due to the inclusion of faba bean seed coat during the milling process. Based on the results of overall acceptability, even with lower perception of juiciness, binders did not alter the overall liking of the bologna products. All the bologna products with binders were acceptable to panelists.

In summary, FB is a very good source of protein, starch and fibre and the cultivars Fabelle, Malik, Snowbird, Snowdrop and Taboar consistently demonstrated the stability of the levels of these macronutrients/components. These cultivars may differ in the levels of vicine/convicine and total phenolic content. Treatments such as short-term germination and autoclaving showed very limited effect on phytic acid, vicine, and convicine. Only germination had significant effect on the reduction of oligosaccharides, and autoclaving can reduce phenolic content in some faba bean cultivars. Application of ingredients derived from FB (cotyledon flour, starch fraction and protein fraction) can increase the total protein in low-fat meat products. Two levels of addition of FB ingredients (1.5 and 3%) as binders did not negatively affect the colour, textural, and sensory properties of cooked low-fat pork bologna. Both studies proved that seed coat-free FB-derived ingredients have the potential to be utilized as a plant binder in processed meat products, especially, in developing low-fat emulsion type. These ingredients can further be used in other food products.

6 OVERALL CONCLUSION

The goal of this project was to evaluate the effect of short-term germination and autoclaving on levels of undesired compounds in five faba bean cultivars, and the application of selected faba bean ingredients in low-fat pork bologna. The proximate and carbohydrate analysis of whole faba bean seed indicated that similar protein, fat, ash, total starch, and total dietary fibre levels can be expected from Fabelle, Malik, Snowbird, Snowdrop and Taboar cultivars. Based on the analysis of undesired compounds, there was no difference between cultivars for the levels of phytic acid, raffinose, stachyose, and verbascose. The chemical analysis also confirmed that Fabelle was the only cultivar low in vicine/convicine. Faba bean cultivars were also significantly different in total phenolic content (10.8 to 48.4 mg/g), and Fabelle had the lowest phenolic content. Three-day germination had limited effect on phytic acid, vicine/convicine, and total phenolics. However, germination had a significant (p<0.05) effect on reduction of oligosaccharide levels; raffinose (100%), stachyose (60%), and verbascose (80%) were all significantly reduced upon 24 h of germination. Autoclaving had limited effect on undesired compounds in faba bean and did not reduce the levels of phytic acid, vicine/convicine, and oligosaccharides. Autoclaving only significantly (p<0.05) reduced total phenolic content in Taboar, Snowbird, and Malik (15-23% of reduction). Based on the results, germination and autoclaving treatments had minor effect on most of the undesired compounds, but cultivars were more likely be the determining factor on the levels of undesired compounds investigated in the present study.

In study II, the binder ingredients derived from FB and with varied proximate composition showed differences in water holding capacity and oil absorption capacity. Faba bean cotyledon flour generates pastes with low peak and final viscosity compared to pea starch and wheat flour while the protein fraction of FB showed limited ability to form a paste during heating. Faba bean ingredients can form thinner paste without additional chemical or physical modification. Binders including FB ingredients at 1.5 and 3.0 % level addition had limited effect

in changing colour parameters, cook loss, purge loss and textural propertiesas interpreted by hardness, cohesiveness, adhesiveness, springiness, and chewiness of the bologna, compared to the no binder control. There were no perceived differences in the cohesiveness, graininess, overall flavour intensity, and overall acceptability of low-fat bologna except the products with 3% wheat flour showing increased firmness by the trained panelists. The product juiciness could be decreased due to addition of wheat flour, pea starch, Fabelle cotyledon flour, faba bean starch or faba bean protein. The addition of Fabelle cotyledon flour at 3% can increase the perceived intensity of foreign flavour. Faba bean ingredients can be used in formulating various low-fat meat products while providing extra plant protein to the product without altering overall acceptability. Because of the bland flavour and minor effect to the meat system, faba bean is a candidate gluten-free binder in developing low-fat meat products. Further studies on sensory acceptability of large consumer groups may be useful in confirming acceptability of the faba bean incorporated low-fat meat products.

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APPENDIX A. SCORECARD FOR SENSORY EVALUATION OF BOLOGNA

Panelist
Booth #:
Date:
Sample Code:

Please evaluate the samples and circle the score for each attribute. You could use water and soda crackers to cleanse your palate between samples.

Firmness
Overall, what is your opinion of the **firmness** of this sample?

Extremely soft	Very soft	Moderately soft	Slightly soft	Slightly firm	Moderately firm	Very firm	Extremely firm

Any comments?

Cohesiveness
Overall, what is your opinion of the **cohesiveness** of this sample?

Extremely brittle	Very brittle	Moderately brittle	Slightly brittle	Slightly cohesive	Moderately cohesive	Very cohesive	Extremely cohesive

Any comments?

Juiciness
Overall, what is your opinion of the **juiciness** of this sample?

Extremely dry	Very dry	Moderately dry	Slightly dry	Slightly juicy	Moderately juicy	Very juicy	Extremely juicy

Any comments?

Graininess
Overall, what is your opinion of the **graininess** of this sample?

Not detectable	Very slightly grainy	Slightly grainy	Moderately grainy	Very grainy	Extremely grainy

Any comments?

Flavour intensity
Overall, what is your opinion of the **flavour intensity** of this sample?

Extremely bland	Very bland	Moderately bland	Slightly bland	Slightly intense	Moderately intense	Very intense	Extremely intense

Any comments?

Foreign flavour

Overall, what is your opinion of the **foreign flavour** of this sample?

Not detected	Very weak	Moderately weak	Slightly weak	Slightly intense	Moderately intense	Very intense	Extremely intense

Any comments?

Product acceptability

Overall, what is your opinion of the **product acceptability** of this sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Like slightly	Like moderately	Like very much	Like extremely

Any comments?

APPENDIX B. TERMINOLOGY USED FOR SENSORY EVALUATION

Firmness: the force required to bite through the sample with the incisors/front teeth;

Cohesiveness: the extent to which the sample was deformed between the teeth before it ruptures/breaks down; Degree to which sample deforms (rather than ruptures)

Juiciness: the amount of fluid in the mouth during the first several chews; amount of juice released as teeth apply pressure

Graininess: the presence of small particles after chewing

Overall Flavour Intensity: the amount of typical bologna seasoning and meat flavour present in the mouth after complete mastication

Foreign Flavour: the amount of any atypical or off-flavours present in the mouth after complete mastication (if any present, please describe or identify the flavour in comments section)

Overall Acceptability: degree of acceptability of product