

**Effects of Geographic Region, Seed Type and Processing (Soaking and Infrared Heating) on Basic Nutrients, Anti-Nutritional Factors, Isoflavones and Fatty Acids in Soybean (*Glycine max*) Collected From Malawi**

**By**

**Ce Zhou**

**A Thesis Submitted to the Faculty of Graduate Studies of**

**The University of Manitoba**

**In Partial Fulfillment of the Requirements of the Degree of**

**MASTER OF SCIENCE**

**Department of Food Science**

**University of Manitoba**

**Winnipeg, Manitoba**

**Copyright © 2017 by Ce Zhou**

I hereby declare that I am the sole author of this thesis.

I authorize the University of Manitoba to lend this thesis into other institutions or individuals for the purpose of scholarly research.

Ce Zhou

I further authorize the University of Manitoba to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Ce Zhou

## **Acknowledgements**

I would like to express my immense gratitude to my advisor Dr. Trust Beta, who always gave me helpful instructions and encouragements during this project. Apart from research, she also inspired me the positive and optimistic attitude towards life.

Special thanks go to my committee members Dr. Susan Arntfield and Dr. Rotimi Aluko, who offered me valuable suggestions during my research.

I want to express my sincerest thankfulness to the technical supports from Alison Ser, Pat Heritage, Yang Qiu, Denise Aminot-Gilchrist and the administrative supports from Carola Lange and Jennifer McLaren. My gratefulness also goes to my lab group members, especially to Dr. Lovemore Malunga, who taught me lots of useful experiment techniques since I was a summer student.

I also want to thank the financial support from International trade and Development of Canada through Malawi Farmer-to-Farmer Agroecological (MAFFA) Project. I am grateful for the financial assistance from International Graduate Student Entrance Scholarship (IGSES).

Lastly but not least, I want to express my deepest gratitude to my parents. Their endless love supported me in every progress of my life. I also want to thank a special girl in my life, Jie Zeng, whose love and company give me the courage to face challenges.

## **Abstract**

This study investigated the effects of geographic region, seed type and processing (soaking and infrared heating) on basic nutrients, anti-nutritional factors, isoflavones and fatty acids in soybean collected in Malawi. Compared with local seed, hybrid seed was significantly ( $p < 0.05$ ) higher in crude lipids, carbohydrate and sucrose contents but lower in crude protein and stachyose contents. Except for moisture and raffinose contents, soybeans collected from central and north Malawi were not significantly different in contents of all detected components. Infrared heating significantly affected levels of trypsin inhibitors, lectins and isoflavones. Soaking showed significant effects on contents of oligosaccharides, genistin, malonyldaidzin and malonylgenistin. In conclusion, our findings indicated that within central and north Malawi, seed type can lead to more differences among seed components than geographic region. Soaking and infrared heating served as practical approaches to control some anti-nutritional factors while altering levels of the functional components in soybean at the same time.

## Table of Contents

Acknowledgements.....	ii
Abstract.....	iii
Chapter 1 : Introduction.....	1
Chapter 2 : Literature Review.....	5
2.1 General Review on Soybean and its composition.....	5
2.2 Review on properties of soybean trypsin inhibitors, lectins, flatulence-causing oligosaccharides, isoflavones and fatty acids.....	6
2.2.1 Properties of trypsin inhibitors.....	6
2.2.2 Properties of soybean lectins.....	8
2.2.3 Properties of flatulence-causing oligosaccharides.....	9
2.2.4 Properties of soybean isoflavones.....	11
2.2.5 Properties of soybean fatty acids.....	12
2.3 Review on effects of geographic location and seed type on basic nutrients content and some anti-nutritional factors.....	13
2.3.1 Review on effects of geographic location and seed type on basic nutrients ....	13
2.3.2 Review on effects of geographic location and seed type on some anti-nutritional factors.....	14
2.3.3 Summary.....	15
2.4 Review on effects of some commonly used elimination methods and infrared heating on soybean anti-nutritional factors.....	16
2.4.1 Rational of infrared heating and its advantages.....	16
2.4.2 Effects of some commonly used elimination methods and infrared heating on soybean trypsin inhibitors.....	17
2.4.3 Effects of some commonly used elimination methods and infrared heating on soybean lectins.....	19

2.4.4 Effects of some commonly used elimination methods and infrared heating on soybean flatulence-causing oligosaccharides .....	21
2.4.5 Summary and comparison between anti-nutritional elimination methods .....	22
2.5 Effects of infrared heating in soybean isoflavones and fatty acids .....	23
2.5.1 Effect of infrared heating on soybean isoflavones .....	23
2.5.2 Effect of infrared heating on soybean unsaturated fatty acid .....	24
Chapter 3 : Effects of geographic location and seed type on basic nutrients and anti-nutritional factors in soybeans .....	26
3.1 Abstract .....	26
3.2 Introduction .....	27
3.3 Materials and Methods .....	29
3.3.1 Soybean samples.....	29
3.3.2 Determination of proximate composition (basic nutrients) .....	30
3.3.3 Determination of the content of trypsin inhibitors .....	31
3.3.4 Determination of hemagglutinating activity of lectins .....	32
3.3.5 Determination of oligosaccharide content .....	33
3.3.6 Statistical analysis.....	34
3.4 Results and Discussion.....	34
3.4.1 Effect of growing location and seed type on content of basic nutrients in soybeans.....	34
3.4.2 Effects of growing location and seed type on content of trypsin inhibitors in soybean .....	41
3.4.3 Effects of growing location and seed type on activity of lectins in soybean....	44
3.4.4 Effects of growing location and seed type on content of oligosaccharides in soybeans.....	47
3.4.5 Correlation between detected components .....	52

3.5 Conclusion.....	55
Connecting Statement .....	56
Chapter 4 : Effects of soaking and infrared heating on anti-nutritional factors, isoflavones and fatty acids in soybean .....	57
4.1 Abstract .....	57
4.2 Introduction .....	58
4.3 Materials and Methods .....	60
4.3.1 Chemicals and reagents .....	60
4.3.2 Sample preparation .....	60
4.3.3 Determination of soybean water absorption curve .....	61
4.3.4 Determination of the trypsin inhibitors .....	62
4.3.5 Determination of hemagglutinating activity of lectins .....	63
4.3.6 Extraction and determination of oligosaccharides.....	64
4.3.7 Extraction and determination of isoflavones .....	65
4.3.8 Extraction and determination of fatty acids.....	67
4.3.9 Data analysis.....	68
4.4 Results and Discussion.....	68
4.4.1 Soybean water absorption curve.....	68
4.4.2 Effects of processing on contents of soybean trypsin inhibitors .....	69
4.4.3 Effects of processing on soybean lectins activity .....	71
4.4.4 Effects of processing on contents of soybean oligosaccharides .....	74
4.4.5 Effects of processing on contents of free soybean isoflavones .....	77
4.4.6 Effects of processing on contents of bound soybean isoflavones .....	82
4.4.6 Effects of processing on soybean fatty acids profile .....	83
4.5 Conclusion.....	85

Overall Conclusion .....	86
References.....	88

## List of Figures

Figure 1.1 World soybean production from 1961 to 2014.....	1
Figure 2.1 Structures of Bowman-Birk inhibitor and Kunitz trypsin inhibitor, including the location of binding sites and disulfide bounds.....	7
Figure 2.2 Biosynthesis process of raffinose, stachyose and verbascose. GST: galactosyl transferase. RS: raffinose synthase. STS: stachyose synthase.....	10
Figure 2.3 Chemical structure of isoflavones. ....	12
Figure 4.1 Soybean water absorption curve.....	69
Figure 4.2 Effects of soaking and infrared heating on contents of soybean trypsin inhibitors (dry basis). ....	70
Figure 4.3 Representative results from hemagglutinating assay, A is the rabbit red blood cell (1% in 0.9% NaCl), B is one sample that gave positive results (dilution factor of the sample was 64, some of the rabbit red blood cell are binding together), C is one sample that gave negative results (same sample as in photograph B, where dilution factor was 8192, and rabbit red blood cells were not binding together). The length of the scale labelled in the pictures is 100 $\mu\text{m}$ . Photographs were taken by a microscope camera (Motic®, Moticam 1080 HDMI & USB, BC, Canada).....	72
Figure 4.4 Effects of soaking and infrared heating on soybean lectins activity (dry basis, results of soaking for 3 h and soaking for 16 h are overlapping). ....	73
Figure 4.5 Typical HPLC chromatograph of soybean free isoflavones (wavelength 254 nm), peak 1 is daidzin, peak 2 is glycitin, peak 3 is genistin, peak 4 is for malonyldaidzin, peak 5 is malonylglycitin, peak 6 is malonylgenistin, peak 7 is daidzein and peak 8 is genistein. ....	77
Figure 4.6 Typical HPLC chromatograph of soybean bound isoflavones (wavelength 254 nm).....	83

## List of Tables

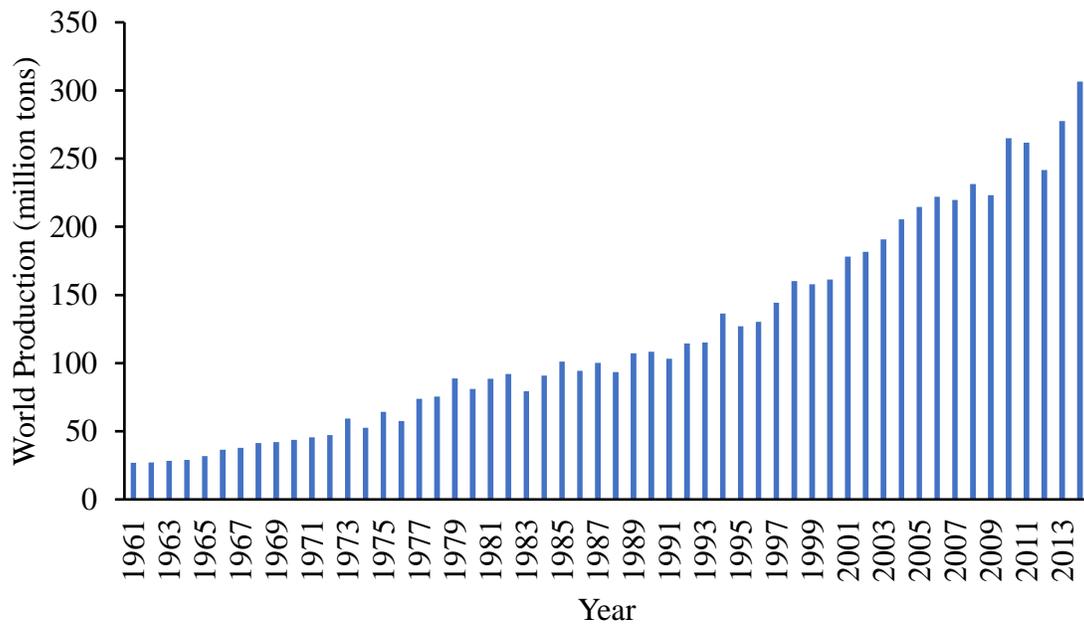
Table 2.1 Effects of several methods on activity or content of trypsin inhibitors in soybean. ....	18
Table 2.2 Effects of infrared heating on trypsin inhibitor activity in soybean and other legumes. ....	19
Table 2.3 Effects of several treatments on activity or content of soybean lectins. ....	20
Table 2.4 Effects of germination and fermentation on soybean oligosaccharides content. ....	21
Table 2.5 Changes in the omega-6/omega-3 ratio in both Kabuli chickpea and green lentil under different infrared heating temperature. ....	25
Table 3.1 Information of soybean samples collected from north and central Malawi. ....	30
Table 3.2 Proximate nutrient (moisture, ash, protein, lipids and carbohydrate) content of two types of soybeans collected from different zones in central and north Malawi. ....	36
Table 3.3 P values from ANOVA indicating the significance of effects from area and seed type on content of basic nutrients (moisture, ash, protein, lipids and carbohydrate) in soybeans. ....	38
Table 3.4 Average contents of basic nutrients in two types of soybeans seeds and two growing regions (central and north Malawi). ....	40
Table 3.5 Trypsin inhibitor (mg/g) of two types of soybeans collected from different zones in central and north Malawi. ....	42
Table 3.6 P values from ANOVA indicating the significance of effects from area and seed type on trypsin inhibitors content in soybeans. ....	43
Table 3.7 Comparisons of average contents of trypsin inhibitors in two types of soybeans grown in central and north Malawi. ....	44
Table 3.8 Lectin activity of two types of soybeans collected from different zones in central and north Malawi. ....	45

Table 3.9 P values from ANOVA indicating the significance of effects from area and seed type on lectin activity in soybeans.....	46
Table 3.10 Comparisons of average lectin activity in two types of soybeans grown in central and north Malawi. ....	46
Table 3.11 Oligosaccharides (mg/g) of two types of soybeans collected from different zones in central and north Malawi.....	48
Table 3.12 P value from ANOVA indicating the significance of effects from area and seed type on oligosaccharides contents in soybeans.....	50
Table 3.13 Comparisons of average contents of oligosaccharides in two soybean types grown in central and north Malawi.....	51
Table 3.14 Correlations between soybean moisture, ash, crude protein, crude lipids, carbohydrate, trypsin inhibitors, lectins, sucrose, raffinose, stachyose and total RFOs...	53
Table 4.1 P value from two-way ANOVA indicating the significance of effects from soaking and infrared heating on trypsin inhibitors contents in soybean.....	71
Table 4.2 P values from two-way ANOVA indicating the significance of effects from soaking and infrared heating on lectins activity in soybean. ....	74
Table 4.3 Average sucrose, raffinose and stachyose contents (mg/g, dry basis) of processed (soaked and infrared heated) soybeans as affected by different soaking time.	75
Table 4.4 P values from two-way ANOVA indicating the significance of effects from soaking and infrared heating on oligosaccharides content in soybean. ....	76
Table 4.5 Types of oligosaccharides found in soybean soaking water.....	76
Table 4.6 Average free isoflavones contents (dry basis) of processed (soaked and infrared heated) soybean treated with the same soaking or infrared heating level.....	79
Table 4.7 P values from two-way ANOVA indicating the significance of effects from soaking and infrared heating on free isoflavone contents in soybean. ....	82
Table 4.8 P value from two-way ANOVA indicating the significance of effects from soaking and infrared heating on bound isoflavones in soybean. ....	83

Table 4.9 P values from two-way ANOVA indicating the significance of effects from soaking and infrared heating on fatty acid profiles in soybean. .... 84

## Chapter 1 : Introduction

Soybeans [*Glycine max.* (L.) Merr.] play an important role in human diets. The history of using domesticated soybean is as early as 2500 – 2300 B.C. (Hartman, West, & Herman, 2011). Nowadays, foods made from soybean, such as, soybean oil, tofu, soybean milk, soy sauce and soybean seed sprouts are well recognized around the world. Figure 1.1 shows the world soybean production from 1961 to 2014. In Africa, Malawi is one of the countries that produce substantial quantities of soybean (Njira, Nalivata, Kanyama-Phiri, & Lowole, 2013). In Malawi, soybean is grown in almost all districts for food, income, livestock feed, export and soil fertility enhancement (Nzima & Dzanja, 2015).



**Figure 1.1** World soybean production from 1961 to 2014.

*Source:* Food and Agricultural Organization of the United Nations (2017)

Soybeans are special in terms of their nutrition. In general, dry soybean seed contains around 5% ash, 20% lipids, 35% carbohydrate and 40% protein (Liu, 2004). Compared with other food legumes, the protein content of soybean is the highest and the oil content is the second-highest (Liu, 2004). In terms of soybean oil, according to Dhakal, et al. (2014), it contains around 12% palmitic acid, 4% stearic acid, 24% oleic acid, 52% linoleic acid and 8%  $\alpha$ -linolenic acid, in which the majority are unsaturated fatty acids.

Besides the nutritional value, what makes soybean more unique is its potential health promoting effects. Soybeans are a great source of isoflavones, which are a bioactive phytochemicals that may help to prevent cardiovascular disease, cancer (colon, breast and prostate), osteoporosis and relief menopausal symptoms (Messina, 1999). Soybean seeds also contain 2 – 3% lecithin, which may be beneficial in lowering cholesterol in human plasma (Bau, Villaume, & Méjean, 2000). Furthermore, consuming soy food often may prevent diabetes and obesity (Friedman & Brandon, 2001).

Apart from nutritional value and possible health promoting effects, soybeans are also well known for their anti-nutritional factors. Some common ones include trypsin inhibitors, flatulence-causing oligosaccharides (Dia, et al., 2012) and lectins (Bajpai, Sharma, & Nath Gupta, 2005). These compounds can cause digestion and absorption problems in humans and animals. In terms of the trypsin inhibitors, they can decrease trypsin activity during digestion, and can be categorized into Bowman-Birk inhibitors and Kunitz trypsin inhibitors (Dia, et al., 2012). It has been reported that the trypsin inhibitor can cause inhibition to the growth of rats and chicks (Becker-Ritt, Mulinari, Vasconcelos, & Carlini, 2004). For flatulence-causing oligosaccharides in soybean, raffinose and

stachyose are the major responsible components (Kumar, et al., 2010). The significant structures of these oligosaccharides are one or more galactose unit (s) attached to one sucrose unit via  $\alpha$  1→6 glycosidic linkages (Peterbauer & Richter, 2001). Humans are lacking the  $\alpha$  1→6 galactosidase enzyme, these oligosaccharides become non-digestible; however, they can be fermented by microflora in large intestine causing flatulence (Kumar, et al., 2010). In the case of lectins, they can attach to the surface of intestinal epithelium and cause absorption problems (Becker-Ritt, Mulinari, Vasconcelos, & Carlini, 2004).

When considering nutritional value, health promoting effects and anti-nutritional factors together, it is ideal that the benefits are maximized and side effects are reduced. Therefore, to make soybeans more suitable for consumption, eliminating anti-nutritional factors while keeping nutritional and functional compounds is desired. There are some possible pre-harvest factors that may contribute to the variation of soybean composition, including seed type (Kumar, et al., 2010; Mohamed & Rangappa, 1992; Sharma, Kaur, Goyal, & Gill, 2014) and growing location (Kumar, et al., 2010; Wang & Murphy, 1994). For post-harvest, many methods including soaking, boiling, heating, germination and fermentation have been developed to remove the anti-nutritional compounds in soybeans. A relatively new method infrared heating, may also be used to affect soybean nutritional value and anti-nutritional factors. However, the effects of infrared heating on soybean isoflavones and fatty acids are still not clear. In this study, a combination of infrared heating and soaking will be used. The two main objectives of present study are:

1. Investigate the effects of geographic location and seed type on basic nutrients, trypsin inhibitors, lectins and oligosaccharides content of soybean collected from central Malawi and north Malawi.
2. Investigate the effects of soaking and infrared heating on trypsin inhibitors, lectins, oligosaccharides, isoflavones and fatty acids content of soybean.

## Chapter 2 : Literature Review

### 2.1 General Review on Soybean and its composition

Soybean is a member of the Leguminosae family and it was originated in northern China (Liu, 2004). Nowadays, over 92% of the soybeans in the world are produced in countries including United States, Brazil, Argentina, China and India (Masuda & Goldsmith, 2009). Except for human consumption, most of the soybeans in the world are crushed into soybean oil and soybean meal, with the latter can be used in livestock and aquaculture feeds (Hartman, West, & Herman, 2011). In Africa, soybean is becoming a major role in agriculture, not only due to its ability to fix nitrogen, but also the protein and oil in the seed (Sinclair, et al., 2014).

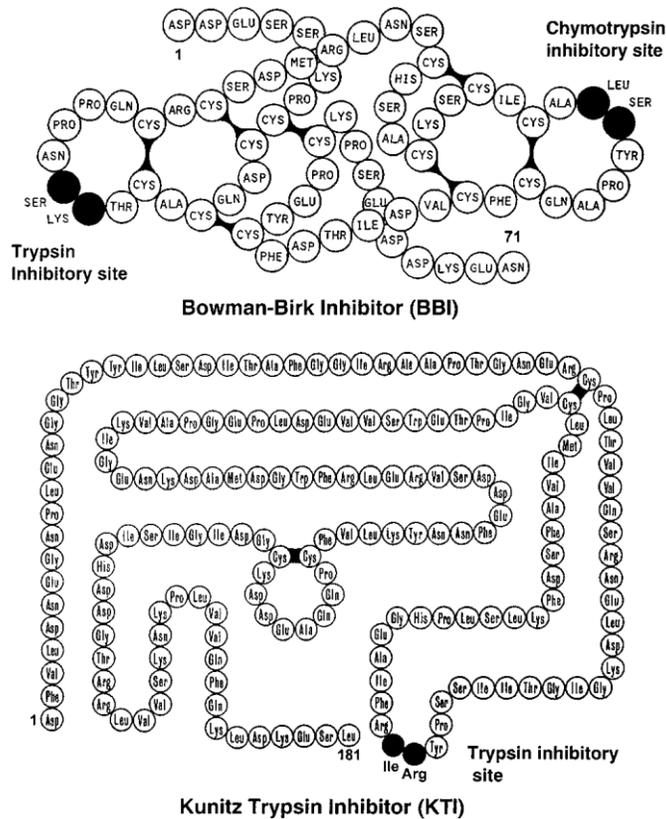
Generally, dry soybean is made up of 40% protein, 35% carbohydrate, 20% oil and 5% ash (Liu, 2004). According to Medic, Atkinson, and Hurburgh Jr (2014), soybean protein can be classified into four groups based on their role, which are metabolic enzymes, structural (including ribosomal and chromosomal) proteins, storage proteins and membrane proteins. In terms of soybean carbohydrates, according to Karr-Lilienthal, Kadzere, Grieshop, and Fahey (2005), around half of it can be classified into structural polysaccharides (including pectic polysaccharides), while the other half is nonstructural (including low weight sugars, oligosaccharides, and small amounts of starch). Soybean oil is mainly made up of linoleic acid (52%), oleic acid (24%), palmitic acid (12%),  $\alpha$ -linolenic acid (8%) and stearic acid (4%) (Dhakal, et al., 2014). Major soybean minerals are potassium, phosphorus, magnesium, sulphur, calcium, chloride and sodium, while the minor minerals are silicon, iron, zinc, manganese, copper, molybdenum, fluoride, chromium, selenium, cobalt, cadmium, lead, arsenic, mercury and iodine (Lokuruka,

2010). These minerals are mainly exist in the forms of sulphates, phosphates and carbonates (Lokuruka, 2010).

## **2.2 Review on properties of soybean trypsin inhibitors, lectins, flatulence-causing oligosaccharides, isoflavones and fatty acids**

### **2.2.1 Properties of trypsin inhibitors**

Soybean trypsin inhibitors are natural proteins existing in soybean seeds. They are mainly located in the protein bodies in the cotyledon (Anderson & Wolf, 1995). Trypsin inhibitors in soybean are believed to be part of the built-in defense mechanism against insects (Kobayashi, Suzuki, Kanayama, & Terao, 2004). They can be categorized into Kunitz trypsin inhibitor (molecular weight ranges from 20 to 25 kD) and Bowman-Birk inhibitor (molecular weight around 8 kD) (Liu, 2004). The Kunitz trypsin inhibitor is a globulin type protein, and it can irreversibly combine with trypsin in a very short time to decrease trypsin activity (Kunitz, 1947). The Bowman-Birk inhibitor is made up of 71 amino acids cross linked by 7 disulfide bonds and can form a complex with trypsin at the ratio of 1:1 (Birk, 1985). The structures of Bowman-Birk inhibitor and Kunitz trypsin inhibitor are shown in Figure 2.1. Due to their ability to combine and inhibit the capacity of trypsin to break down peptide bonds between lysine and arginine, soybean trypsin inhibitors are considered as anti-nutritional factors (Isanga & Zhang, 2008). Armour, Perera, Buchan, and Grant (1998) reported that inclusion of raw soybean in the diet of rat could cause less food intake and lower weight gain.



**Figure 2.1** Structures of Bowman-Birk inhibitor and Kunitz trypsin inhibitor, including the location of binding sites and disulfide bounds.

*Source:* Friedman and Brandon (2001)

Except for the differences in the structures, Bowman-Birk inhibitor and Kunitz trypsin inhibitor also differ in some other properties. According to Friedman and Brandon (2001), Bowman-Birk inhibitor can inhibit both trypsin and chymotrypsin, while Kunitz trypsin inhibitor only have effects on trypsin. The thermal properties of these two inhibitors are also different. As reported by DiPietro and Liener (1989), the purified Bowman-Birk inhibitor was more heat stable than Kunitz trypsin inhibitor. However, Bowman-Birk inhibitor was found to be more heat labile one in the soybean matrix (DiPietro & Liener, 1989).

Besides being considered as anti-nutritional factors, trypsin inhibitors have also been studied in relation to cancer prevention (Messina & Barnes, 1991). Clair, et al. (1990) concluded that Bowman-Birk inhibitors can suppress carcinogenesis in mice liver and gastrointestinal tract. Moreover, as reviewed by Messina and Barnes (1991), Bowman-Birk inhibitors may help to prevent oral and lung cancer. As for Kunitz trypsin inhibitor, Kobayashi, Suzuki, Kanayama, and Terao (2004) first noted that it may block the expression of urokinase-type plasminogen activator, which lead to the inhibition of ovarian cancer cell invasion. Although trypsin inhibitors may be beneficial in prevention of carcinogenesis, only their anti-nutritional properties will be focused in the present study.

### **2.2.2 Properties of soybean lectins**

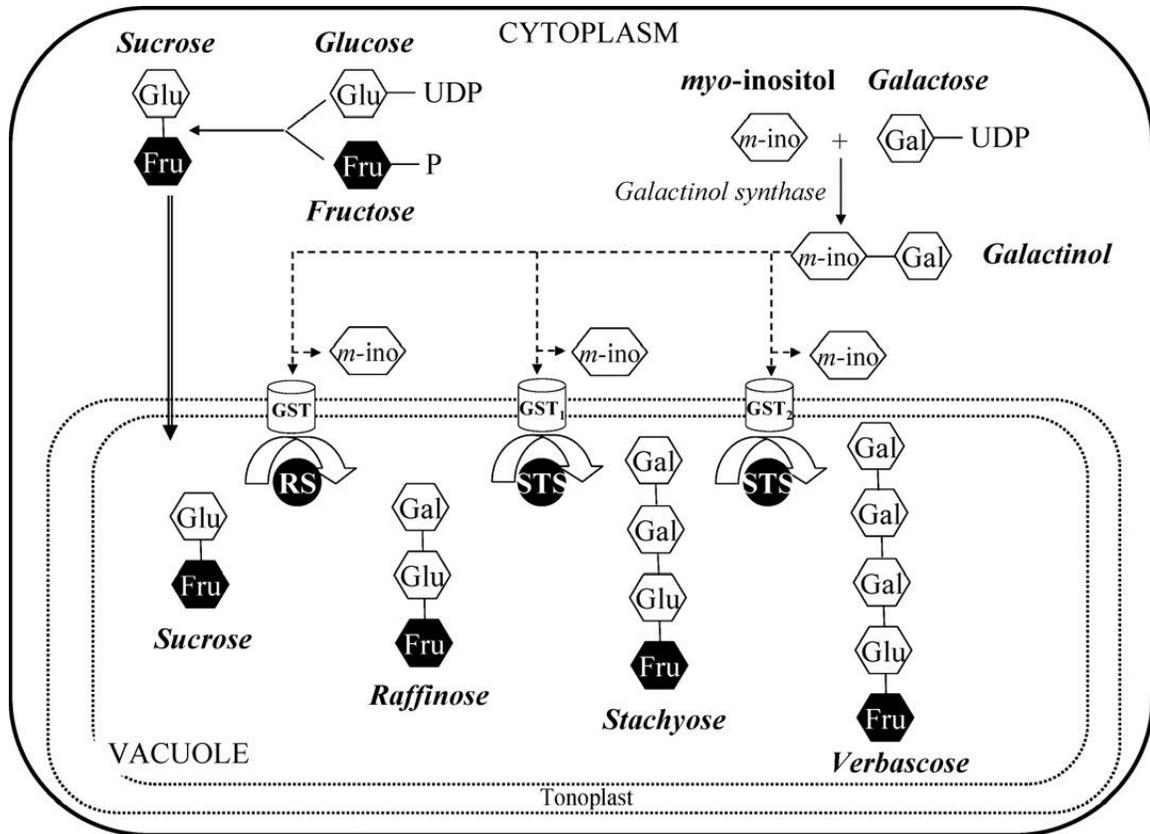
Lectins are important bioactive proteins widely distributed in almost all organisms, which include plants, vertebrates, invertebrates, bacteria and viruses (De Mejía & Prisecaru, 2005). They are able to reversibly bind with free sugars, glycoproteins, glycolipids and sugar residues of polysaccharides (De Mejía & Prisecaru, 2005). In plants, lectins may be part of the defense system, since they have deleterious effects against plant-eating organisms (Peumans & Van Damme, 1995). As for soybean lectins (molecular weight around 120 kD), they are made up of four subunits and mainly located in the protein body of cotyledon (Liu, 2004). Once bound with molecules containing carbohydrates on the epithelial cells, lectins can cause health problems (Palacios, et al., 2004; Rådberg, et al., 2001). It has been reported that lectins in soybean can reduce the growth performance of pigs and chicks (Palacios, et al., 2004). Based on these properties, soybean lectin is considered as one of the anti-nutritional factors.

Although the anti-nutritional effects are not desirable, plant lectins are also thought to possess chemo-preventive activity against cancer and have been employed as therapeutic agents in cancer treatment studies (De Mejía & Prisecaru, 2005). Since lectins can bind to the specific glycans in cell surfaces, they can be used to target drug molecules to certain cells, for example, cancer cells (Bies, Lehr, & Woodley, 2004). Moreover, lectins may be able to prevent invasion of human immunodeficiency virus (HIV) and Ebola virus via binding with glycoproteins on their envelope (Ziółkowska & Wlodawer, 2006). Even though lectins have exciting potentials in medicinal applications, only anti-nutritional properties will be focused in the present study.

### **2.2.3 Properties of flatulence-causing oligosaccharides**

The main flatulence-causing oligosaccharides in soybeans are raffinose and stachyose (Kumar, et al., 2010). Both raffinose and stachyose are linear and derived from sucrose (Karner, et al., 2004). Raffinose is made up of 1 sucrose unit and 1 galactose unit (linked via one  $\alpha 1 \rightarrow 6$  glycosidic linkage), stachyose is made up of 1 sucrose unit and 2 galactose units (Martínez-Villaluenga, Frias, & Vidal-Valverde, 2008). Their structures and biosynthesis processes are shown in Figure 2.2. These oligosaccharides naturally exist in plants and may serve as energy-storing compounds (ElSayed, Rafudeen, & Gollmack, 2014; Frias, et al., 1999; Karner, et al., 2004). Furthermore, accumulation of these oligosaccharides is considered to help matured seed gain tolerance and storability (Karner, et al., 2004; Peterbauer & Richter, 2001). The typical contents of raffinose and stachyose in soybean are 0.9% and 3.5%, respectively (Liu, 2004). Humans cannot digest raffinose and stachyose since they lack the enzyme to break down  $\alpha 1 \rightarrow 6$  glycosidic linkages, however, these two oligosaccharides can be fermented by the microorganisms

in the colon and cause flatulence (Kumar, et al., 2010). Thus, raffinose and stachyose are considered as anti-nutritional factors.



**Figure 2.2** Biosynthesis process of raffinose, stachyose and verbascose. GST: galactosyl transferase. RS: raffinose synthase. STS: stachyose synthase.

Source: Martínez-Villaluenga, Frias, and Vidal-Valverde (2008)

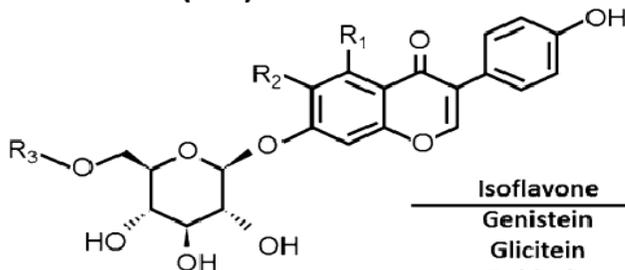
Recently, some of the research has focused on the potential prebiotic effects of raffinose and stachyose (Li, Lu, & Yang, 2013; Van den Ende, 2013). According to Roberfroid, et al. (2010), the prebiotic effects can be defined as the selective stimulation of growth of one or a limited number of microorganisms in the gut microbiota that has health benefits to the host. Raffinose and stachyose can be fermented by the

microorganisms in the small intestine. In other words, it can promote the growth of those microorganisms. More importantly, they can selectively stimulate the growth of beneficial bacteria and inhibit the adherence of pathogens to the epithelial surface (Shoaf, Mulvey, Armstrong, & Hutkins, 2006). Li, Lu, and Yang (2013) reported that feeding mice with raffinose and stachyose may promote the growth of beneficial bacteria in the intestine, including bifidobacteria and lactobacilli. Gulewicz, et al. (2002) found similar results in both *in vitro* and *in vivo* test. Furthermore, raffinose and stachyose can also help to relief constipation in mice (Li, Lu, & Yang, 2013). Using chickens as studying material, Bednarczyk, et al. (2011) found that raffinose and stachyose have dose-dependent effect on the numbers of *Bifidobacterium bifidum*. Despite the potential prebiotic function of raffinose and stachyose, only anti-nutritional effects will be focused in the present study.

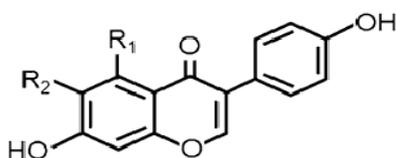
#### **2.2.4 Properties of soybean isoflavones**

Isoflavones are phenolic compounds existing in soybean, and they have some health-promoting effects (Xu & Chang, 2008). Soybean contains a large amount of isoflavones (Luthria, Biswas, & Natarajan, 2007). There are 12 isoflavones isomers, 9 of them are glucosides (daidzin, glycitin, genistin, acetyl daidzin, acetyl glycitin, acetyl genistin, malonyl daidzin, malonyl glycitin and malonyl genistin), 3 of them are aglycones (daidzein, glycitein and genistein) (Song, Barua, Buseman, & Murphy, 1998). Their chemical structures are shown in Figure 2.3. Among all those isoflavones isomers, daidzein, glycitein and genistein are found to have higher bioactivity (Vitale, et al., 2013).

### Glucosides (IFG)



### Aglycones (IFA)



Isoflavone	-R1	-R2	-R3
Genistein	H	OH	-
Glicitein	OCH <sup>3</sup>	H	-
Daidzein	H	H	-
Genistin	H	OH	H
glicitin	OCH <sup>3</sup>	H	H
daidzin	H	H	H
malonyl-genistin	H	OH	COCH <sub>2</sub> COOH
malonyl-glicitin	OCH <sup>3</sup>	H	COCH <sub>2</sub> COOH
malonyl-daidzin	H	H	COCOCH <sub>2</sub> COOH
acetyl-genistin	H	OH	COCH <sub>3</sub>
acetyl-glicitin	OCH <sup>3</sup>	H	COCH <sub>3</sub>
acetyl-daidzin	H	H	COCH <sub>3</sub>

**Figure 2.3** Chemical structure of isoflavones.

Source: Vitale, et al. (2013)

### 2.2.5 Properties of soybean fatty acids

Dry soybean has around 20% oil content (Liu, 2004). The compositions of soybean oil are mainly of linoleic acid, oleic acid, palmitic acid,  $\alpha$ -linolenic acid and stearic acid, with proportions of 52%, 24%, 12%, 8% and 4%, respectively (Dhakal, et al., 2014). Oleic acid, linoleic acid and linolenic acid are unsaturated among the five major soybean fatty acids, with one, two and three double bounds, respectively (Medic, Atkinson, & Hurburgh Jr, 2014). Therefore, unsaturated fatty acids are the major components of soybean oil.

## **2.3 Review on effects of geographic location and seed type on basic nutrients content and some anti-nutritional factors**

### **2.3.1 Review on effects of geographic location and seed type on basic nutrients**

Soybean is a well-known source of protein and lipids. These two components are directly related to nutrition value and quality of soybean. Some pre-harvest factors, including growing location and genotype may affect the basic nutritional components in soybean. Poysa and Woodrow (2002) found that both genotype and growing location have significant effects on soybean protein and oil content. With limited data, Breene, Lin, Hardman, and Orf (1988) drew the conclusion that soybean grown at higher latitude tended to have lower protein content. Moreover, they summarized that the effect of location on oil content was not clear. In another study carried out by Kumar, Rani, Solanki, and Hussain (2006), genotype was found to have significant effects on both protein and oil content. Furthermore, they also concluded that latitude showed a significant negative correlation with soybean protein content and a significant positive correlation with soybean oil content. With soybean collected during 1986, 1987 and 1988 in United States as study material, Hurburgh, Brumm, Guinn, and Hartwig (1990) concluded that the soybean grown in north states (North Dakota, South Dakota, Minnesota, Iowa and Wisconsin) has more oil and less protein than soybean grown in south states (Texas, Arkansas, Louisiana, Mississippi, Tennessee, Kentucky, Alabama, Georgia, South Carolina, North Carolina).

Unlike protein and lipids, the geographic and seed type effects on soybean total carbohydrates and ash are less investigated. The properties of soybean carbohydrates and minerals could contribute to low research interest in this area. Generally, soybean

contains 30-35% carbohydrates (Middelbos & Fahey, 2008). Around half of these carbohydrates are structural polysaccharides, and the other half are nonstructural, which includes low molecular weight sugars, oligosaccharides and some starch (Karr-Lilienthal, Kadzere, Grieshop, & Fahey, 2005). Factors including growing conditions, and cultivar can contribute to the diversity of soybean carbohydrates (Karr-Lilienthal, Kadzere, Grieshop, & Fahey, 2005). Due to the difficulties in digestion, soybean polysaccharides are thought unavailable to humans and may depress the digestion of some other nutrients, including minerals (Lokuruka, 2010). As reviewed by Lokuruka (2010), the majority of minerals in soybean exist in the form of sulphates, phosphates and carbonates. Moreover, soybean is considered as a good source of potassium, phosphorus, calcium, magnesium, sulfur, zinc, manganese, iron, copper, and boron (Cremasco, et al., 2016). Marioli Nobile, et al. (2016) reported that both environment and genotype had influences on soybean mineral profile. Furthermore, Bellaloui, Smith, Gillen, and Ray (2011) concluded that genotype has greater effects on soybean minerals than temperature effects.

### **2.3.2 Review on effects of geographic location and seed type on some anti-nutritional factors**

Trypsin inhibitors, lectins and raffinose family oligosaccharides are common anti-nutritional factors in soybean. Attempts have been made to control the content of these compounds in soybean via agronomy practices. There is still not enough literature indicating how geographic location and seed type affect the content of these anti-nutritional factors. Within that limited information, genotype and genotype  $\times$  location interactions were found to have significant effects on activity of soybean trypsin inhibitors, while the effect of location alone was non-significant (Kumar, Rani, Tindwani,

& Jain, 2003). However, Krishnan, Jang, Baxter, and Wiebold (2012) reported that location demonstrated profound effects on the accumulation of soybean Bowman-Birk inhibitors. According to Gu, Pan, Sun, and Qin (2010), soybean variety can significantly affect lectins content. Using common beans (*Phaseolus Vulgaris* L.), De Mejía, et al. (2003) concluded that growing site, cultivar and their interaction showed significant effects on trypsin inhibitors and lectins, except that the effect of cultivar on lectins was non-significant. As for raffinose family oligosaccharides, Kumar, et al. (2010) discovered that genotype  $\times$  location can significantly affect soybean raffinose and stachyose content, while location alone did not.

### **2.3.3 Summary**

Protein and lipids are the two outstanding nutrients that soybean can provide to us. Some of the research has focused on how growing location and seed type influence these two major nutrients in soybean. At least two studies have concluded that both geographic location and genotype can significantly affect soybean protein and lipid content (Kumar, Rani, Solanki, & Hussain, 2006; Poysa & Woodrow, 2002). More importantly, three different studies pointed out that soybean grown at higher latitude tend to have lower protein content (Breene, Lin, Hardman, & Orf, 1988; Hurburgh, Brumm, Guinn, & Hartwig, 1990; Kumar, Rani, Solanki, & Hussain, 2006). Among those studies, two of them also indicated that higher latitude can result in higher oil content (Hurburgh, Brumm, Guinn, & Hartwig, 1990; Kumar, Rani, Solanki, & Hussain, 2006). Based on available information, growing condition and cultivar were reported to have influence on soybean carbohydrates (Karr-Lilienthal, Kadzere, Grieshop, & Fahey, 2005). Besides, soybean mineral profile can be affected by growing condition and genotype (Marioli

Nobile, et al., 2016); furthermore, genotype may play the major role (Bellaloui, Smith, Gillen, & Ray, 2011).

Even though trypsin inhibitors, lectins, raffinose and stachyose are well-known anti-nutritional factors in soybean, literature showing how their contents are related to geographic location or genotype is negligible. In terms of effects of location on content of soybean trypsin inhibitors, there are conflicts existing between the studies by Kumar, Rani, Tindwani, and Jain (2003) and Krishnan, Jang, Baxter, and Wiebold (2012). Soybean cultivar is reported to have significant influence on lectins (Gu, Pan, Sun, & Qin, 2010). As for content of raffinose and stachyose, effect of location alone is non-significant, while genotype  $\times$  location showed significant effects (Kumar, et al., 2010). Therefore, further research is needed.

## **2.4 Review on effects of some commonly used elimination methods and infrared heating on soybean anti-nutritional factors**

### **2.4.1 Rational of infrared heating and its advantages**

When exposed to infrared radiation, heat will be generated inside a food material (Krishnamurthy, et al., 2008). One source of heat that is generated within the food material is the water molecules' vibration (60 000 – 150 000 MHz), which is caused by the infrared radiation (Fasina, et al., 2001). Based on wavelength, infrared radiation can be divided into far-infrared radiation (3 – 1000  $\mu\text{m}$ ), mid-infrared radiation (1.4 – 3  $\mu\text{m}$ ) and near-infrared radiation (0.78 – 1.4  $\mu\text{m}$ ) (Sakai & Hanzawa, 1994). In food industry, far-infrared radiation is used more often since food materials have high spectral transmissivity when the radiation wavelength is less than 2.5  $\mu\text{m}$  (Krishnamurthy, et al.,

2008; Sandu, 1986). When compared to conventional heating, infrared heating has significantly less heating time, more uniform heating, less loss of nutrients and increased energy efficiency (Krishnamurthy, et al., 2008; Sumnu & Ozkoc, 2010). With these advantages, infrared heating has lots of applications in food industries, for example, drying, roasting, baking, pasteurization and thawing (Sakai & Hanzawa, 1994).

#### **2.4.2 Effects of some commonly used elimination methods and infrared heating on soybean trypsin inhibitors**

Since both Kunitz trypsin inhibitor and Bowman-Birk inhibitor are proteins, their bioactivity will change once their molecular structures are transformed. Protein structure can be changed by physical factors such as heating, or biological activities such as germination and microbial fermentation. Based on that, heating, fermentation and germination are the common methods used and studied to decrease content or activity of trypsin inhibitors in soybeans. However, the effectiveness of those methods varies. Table 2.1 summarizes the effects of several methods on soybean trypsin inhibitors.

**Table 2.1** Effects of several methods on activity or content of trypsin inhibitors in soybean.

Method	Effect on trypsin inhibitors	Reference
Soaking (12 h), Dehulling and boiling (30min)	Level decreased by 82.2%	Egounlety and Aworh (2003)
Boiling at 100 °C for 15 min	Content decreased by 66.5 to 88%	Shivakumar, et al. (2015)
<i>Aspergillus oryzae</i> fermentation (36 h)	Activity decreased from 10.7 TIU/mg to nondetectable level	Chen, Madl, and Vadlani (2013)
<i>Lactobacillus plantarum</i> fermentation (5 days)	Content reduced by 99.2%	Adeyemo and Onilude (2013)
Germination for 144 h at 25 °C	Activity decreased by 45.7%	Kumar, Rani, Pandey, and Chauhan (2006)
Germination for 72 h at 22 – 25 °C	Activity decreased by 57%	Kumari, Krishnan, and Sachdev (2015)

Based on Table 2.1, the effects of different elimination methods on trypsin inhibitors vary from each other. Reduction levels of these methods ranged from 45.7% to around 100%. The time required for these methods is quite different. Germination or fermentation usually need several days, while boiling only take less than 1 hour, which suggest that boiling is more efficient than gemination and fermentation.

Since infrared heating was applied in the food industry, more and more studies have been done to investigate its effects on anti-nutritional factors. However, studies investigating the effects of infrared heating on soybean trypsin inhibitors are few. It is valuable to look to the effects of infrared heating on trypsin inhibitors in some other

legumes, since they widely exist in legumes (Fasina, et al., 2001). Some of the results are summarized in Table 2.2. It can be seen that infrared heating is effective in reducing the activity of trypsin inhibitors.

**Table 2.2** Effects of infrared heating on trypsin inhibitor activity in soybean and other legumes.

Legume type	Effect of infrared heating on trypsin inhibitor activity	References
Soybean	Decreased by 80% (heating at 135 °C)	Chen (2015)
Soybean	Decreased by 93.4% (1342 W for 10 min)	Yalcin and Basman (2015)
Black bean	Decreased by 52.3% (heating at 140 °C)	Fasina, et al. (2001)
Canadian cowpea	Decreased by 92.6% (90 °C for 2.5 min)	Khattab and Arntfield (2009)
Canadian kidney bean	Decreased by 88.8% (90 °C for 3.0 min)	Khattab and Arntfield (2009)
Canadian pea	Decreased by 94.3% (90 °C for 2.5 min)	Khattab and Arntfield (2009)

### 2.4.3 Effects of some commonly used elimination methods and infrared heating on soybean lectins

As reviewed by De Mejía and Prisecaru (2005), some of the lectins are heat labile, however, gentle cooking methods including dry heat and shorting cooking time will not be effective in deactivating them. Armour, Perera, Buchan, and Grant (1998) concluded that soybean lectins were relatively heat-resistant. In terms of deactivating lectins in soybean, there are some methods available, including heating, fermentation and

germination. Lots of studies have investigated the effects of those three treatments on the activity or content of soybean lectins. The effects of several methods on the soybean lectins are summarized in Table 2.3. When considering the efficiency of eliminating soybean lectins, based on the information from Table 2.3, it is not hard to find out that moist heat at higher temperature is a better choice.

**Table 2.3** Effects of several treatments on activity or content of soybean lectins.

Method	Effect on soybean lectin	Reference
Germination for 4 days	Activity reduced by 96%	Bau, Villaume, Nicolas, and Méjean (1997)
Germination for 72 hours	No effect on lectin content	Dia, et al. (2012)
Dry heat at 177 °C for 20 min	Content decreased by 15.5%	Sitren, Ahmed, and George (1985)
Moist heat at 121 °C and 15 p.s.i. for 20 min	Content decreased by 100%	Sitren, Ahmed, and George (1985)
Heating 121 °C and 1.7 atm for 25 min	Activity reduced to not observed after process	Machado, et al. (2008)
Heating fully hydrated soybean in distilled water at 60 °C for 90 min	No effects on lectin activity	Armour, Perera, Buchan, and Grant (1998)
Heating fully hydrated soybean in distilled water at 100 °C for 10 min	Completely deactivated	Armour, Perera, Buchan, and Grant (1998)

In terms of the effects of infrared heating on soybean lectins, the number of research investigations is too little. Therefore, more studies are needed in this area.

#### 2.4.4 Effects of some commonly used elimination methods and infrared heating on soybean flatulence-causing oligosaccharides

To prevent flatulence caused by consuming soybean or soybean products, it is necessary to remove the raffinose and stachyose, or specifically, the  $\alpha 1 \rightarrow 6$  glycosidic linkages in these two oligosaccharides. Unlike proteins, raffinose and stachyose in soybean are not heat labile (Dixit, et al., 2011). The  $\alpha 1 \rightarrow 6$  glycosidic linkages are not easily broken down by heat. However, they may be broken by enzymes produced during fermentation or germination. The effects of germination and fermentation on the soybean oligosaccharides content have been studied for a long time. Besides germination and fermentation, soaking is another method studied often. Table 2.4 summarizes some of those effects.

**Table 2.4** Effects of germination and fermentation on soybean oligosaccharides content.

Method	Effect on oligosaccharides content	Reference
Soaking for 12-14 hours	Raffinose, stachyose decreased by 25.41% and 20.23%	Egounlety and Aworh (2003)
Fermentation with <i>Rhizopus oligosporus</i> for 48 h	Raffinose content remains constant and stachyose almost disappeared	Egounlety and Aworh (2003)
Fermentation with <i>Aspergillus oryzae</i> for 36 h	Both raffinose and stachyose are totally degraded	Chen, Madl, and Vadlani (2013)
Germinated in dark at 27 °C for 5 days	Raffinose and stachyose in both embryonic axes and cotyledon have decreased by 87.6% and 91.7%	Kuo, VanMiddlesworth, and Wolf (1988)
Germinated at 27 °C for 120 h	Content of raffinose and stachyose almost disappear	Guimarães, et al. (2001)

As mentioned by Dixit, et al. (2011), raffinose and stachyose are heat stable. Therefore, infrared heating may have no effects on soybean flatulence-causing oligosaccharides.

#### **2.4.5 Summary and comparison between anti-nutritional elimination methods**

Germination is an effective method to decrease the activity of trypsin inhibitors, lectins and decrease the content of flatulence-causing oligosaccharides. The effect of germination is time dependent, which means longer germination can lead to a greater reduction. However, germination conditions must be carefully controlled since factors such as light, temperature, time and moisture can greatly affect the results (Bau, Villaume, Nicolas, & Méjean, 1997).

Fermentation is an extremely effective method for removing trypsin inhibitors and flatulence-causing oligosaccharides. Based on the cases mentioned, fermentation can lead to 99% or higher reduction in trypsin inhibitors, raffinose and stachyose. Despite using different microorganisms, all the results show that fermentation is a reliable method. Although effective, it is necessary to mention that the whole process must avoid the contamination by unwanted microorganisms.

Infrared heating can generate energy by causing vibration of water molecules in food material (Fasina, et al., 2001). It has the advantages of time-saving, more uniform heating, less loss of nutrients and higher energy efficiency over conventional heating (Krishnamurthy, et al., 2008). Nowadays, some attentions are focused on their effects on anti-nutritional in soybean or other legumes. Based on previous studies, infrared heating

revealed great ability in terms of deactivating trypsin inhibitors in soybean and other legumes (Chen, 2015; Fasina, et al., 2001; Khattab & Arntfield, 2009; Yalcin & Basman, 2015). As for lectins, there is still lack of studies in which the effects of infrared heating are included.

In terms of removing trypsin inhibitors and lectins, high-temperature moist heat is efficient. Germination and fermentation can also be as effective as high temperature moist heat. However, the time efficiency of these three methods are quite different. To reach a desirable reduction in trypsin inhibitors and lectins, heating normally needs to be less than an hour while germination and fermentation usually take several hours or days. The efficiency of heating in removing trypsin inhibitors and lectins are much higher than that of germination and fermentation.

Since flatulence-causing oligosaccharides are heat stable, it is hard to remove them via thermal processes (Dixit, et al., 2011). Soaking could be a solution, since it has been reported able to decrease the content of raffinose and stachyose in soybean (Egounlety & Aworh, 2003; Kumari, Krishnan, & Sachdev, 2015). Therefore, a combination of soaking and infrared heating can have better reduction in anti-nutritional factors. However, the soaking time needs to be controlled, since it can greatly affect the time efficiency of the whole process.

## **2.5 Effects of infrared heating in soybean isoflavones and fatty acids**

### **2.5.1 Effect of infrared heating on soybean isoflavones**

Isoflavones are important functional components in soybean, it is necessary to study their changes in soybean during infrared heating. It has been reported that heat

treatment can change the distribution of isoflavones; significant decreases were observed in daidzein, genistein and glycitein in dehulled soybean after boiling for 20 minutes (Wang & Murphy, 1996). After boiling for 120 minutes or steaming for 100 minutes, the contents of daidzein, genistein and glycitein were increased while the total isoflavones content decreased (Xu & Chang, 2008). As for the effects of infrared heating, it can cause temperature-dependent degradation and conversions of soybean isoflavones (Niamnuy, Nachaisin, Poomsa-ad, & Devahastin, 2012).

### **2.5.2 Effect of infrared heating on soybean unsaturated fatty acid**

As one of the main oils consumed, soybean oil is a good source of unsaturated fatty acids (Stewart, Raghavan, Orsat, & Golden, 2003). As to the effect of infrared heating, limited data are available when using soybean as material. Therefore, it is necessary to look at its effects on unsaturated fatty acids in other types of legumes. It has been reported that infrared heating can significantly increase the content of saturated fatty acids and decrease content of both monounsaturated fatty acids and polyunsaturated fatty acids in mung bean seeds (Padmashree, et al., 2016). Based on their results, it is reasonable to hypothesis that omega-6/omega-3 ratio can be affected by infrared heating. Changes in the omega-6/omega-3 ratios in both Kabuli chickpea and green lentil are summarized in Table 2.5 (Shariati-Ievari, et al., 2016). In terms of health benefits, a lower omega-6/omega-3 ratio is better in the prevention of chronic disease (Simopoulos, 2002). Based on table 2.5, when treatment and experiment materials vary, the changes in omega-6/omega-3 ratios are different. Therefore, in terms of soybean, studies are needed to find out how infrared heating changes the omega-6/omega-3 ratio.

**Table 2.5** Changes in the omega-6/omega-3 ratio in both Kabuli chickpea and green lentil under different infrared heating temperature.

	Kabuli chickpea			Green lentil		
	Raw	130 °C	150 °C	Raw	130 °C	150 °C
$\omega$ -6/ $\omega$ -3ratio	22.35	22.6	21.5	3.78	3.83	3.85

\* *Source:* Shariati-Ievari, et al. (2016).

## **Chapter 3 : Effects of geographic location and seed type on basic nutrients and anti-nutritional factors in soybeans**

### **3.1 Abstract**

Soybean [*Glycine max.* (L.) Merr.] is a good source of protein and lipids. It also contains some anti-nutritional factors, which may affect the utilization of its nutrients. In this study, two types of soybean seeds (hybrid and local) collected from central and north Malawi were examined for content of basic nutrients, trypsin inhibitors, oligosaccharides and lectin activity. Contents of these components were compared to investigate the effects of growing location and seed type. Results indicated that the typical protein, lipid and carbohydrate content of soybean were around 36, 20 and 31 g/100g, respectively. The trypsin inhibitors of all samples ranged from 18.86 to 44.06 mg/g. Most of the samples had lectin activity of about 409 HU/mg and a few had about 819 HU/mg. Sucrose, and the raffinose family oligosaccharides (RFOs) stachyose and raffinose were the major oligosaccharides in soybean, with typical contents of 57, 33 and 4 mg/g, respectively. Hybrid seed had higher lipid and carbohydrate content but lower protein content than local seed. However, neither growing location nor seed type significantly affected the content of trypsin inhibitors and lectin activity, but their interaction effects were significant. For oligosaccharides, seeds from central Malawi had significantly higher raffinose content than that of the soybean grown in north Malawi. Moreover, local seed had significantly higher stachyose and total RFOs content but lower sucrose content than hybrid seed. Trypsin inhibitors were negatively correlated with protein content, while there was no significant correlation between total RFOs and soybean carbohydrates.

### 3.2 Introduction

Soybean [*Glycine max.* (L.) Merr.] is one of the most important food materials in the world. Food products, including tofu, soy sauce, soybean oil, soybean milk and soybean sprouts are favored worldwide. Around 6% of the world arable land is occupied for growing soybean (Hartman, West, & Herman, 2011). According to Masuda and Goldsmith (2009) the average annual world soybean production reached 217.6 million tons in 2005 to 2007, and it is predicted to reach 371.3 million tons by 2030.

Soybean is a great source of protein and lipids. Generally, soybean contains 40% proteins and 20% lipids (Liu, 2004). Besides protein and lipids, another major compound in soybean is carbohydrates, which can make up to 35% of the seed content (Karr-Lilienthal, Kadzere, Grieshop, & Fahey, 2005). Around half of these carbohydrates can be classified as nonstructural and another half are structural polysaccharides (Karr-Lilienthal, Kadzere, Grieshop, & Fahey, 2005). Soybean is also reported to be a source of potassium, phosphorus, calcium, magnesium and iron (Dixit, Antony, Sharma, & Tiwari, 2011; Lokuruka, 2010).

It is also commonly known that soybean contains some anti-nutritional factors, such as trypsin inhibitors, lectins and flatulence-causing oligosaccharides. There are two types of trypsin inhibitors in soybean, the Kunitz trypsin inhibitor, and the Bowman-Birk inhibitor (Liu, 2004). The molecular weight of the Kunitz trypsin inhibitor is 20.1 kD comprising 4 cysteine residues, which form 2 disulfide bridges (Duranti, et al., 2003). The Bowman-Birk inhibitor has a molecular weight of 8 kD (Liu, 2004). It is made up of 71 amino acids which are crosslinked by 7 disulfide bonds (Birk, 1985). Saxena, Jensen, and McGinnis (1963) stated that trypsin inhibitors in soybean can cause growth inhibition

in rats and chicks. Moreover, soybean trypsin inhibitors can also cause pancreatic hypertrophy in some animals (Isanga & Zhang, 2008; Krishnan, 2001).

Soybean lectins are proteins of molecular weight around 120 kD and made up of four identical subunits (Liu, 2004). This group of proteins can recognize and bind with carbohydrate complexes, which are protruding from glycoproteins and glycolipids (Ghazarian, Idoni, & Oppenheimer, 2011). Lectins may cause problems after binding with intestinal epithelium cells (Palacios, et al., 2004; Rådberg, et al., 2001). As noted by Kelsall, et al. (2002), most plants based lectins are resistant to digestion. Long-term intake of low dose lectins can lead to enlargement of the pancreas in rats, which may lead to pancreatic cancer (Kelsall, et al., 2002).

The major flatulence-causing oligosaccharides in soybean are raffinose and stachyose, which belong to raffinose family oligosaccharides (RFOs) and are galactosyl derivatives of sucrose (Kumar, et al., 2010). Raffinose is made up of one sucrose unit and one galactose unit, while stachyose is made up of one sucrose unit and two galactose units (Martínez-Villaluenga, Frias, & Vidal-Valverde, 2008). Due to the  $\alpha 1 \rightarrow 6$  glycosidic linkages that exist in the molecules, raffinose and stachyose are non-digestible to humans (Martínez-Villaluenga, Frias, & Vidal-Valverde, 2008). However, once they enter the large intestine, they can be fermented by the microorganisms producing CO<sub>2</sub>, CH<sub>4</sub> and H<sub>2</sub>S, causing abdominal discomfort or even diarrhea (Kumar, et al., 2010).

Soybean which is high in basic nutrients while low in anti-nutritional factors is always desirable. The contents of these components can be affected by factors, such as growing location and genotype. Some studies have concluded that both genotype and growing location can influence the protein and lipid content of soybean (Kumar, Rani,

Solanki, & Hussain, 2006; Poysa & Woodrow, 2002). Soybean carbohydrates and minerals are also reported to be affected by growing location and genotype (Karr-Lilienthal, Kadzere, Grieshop, & Fahey, 2005; Marioli Nobile, et al., 2016). As for the anti-nutritional factors, literature reports showing the effects of growing location and genotype are too few. There are even conflicts existing between some of the studies.

The objective of this study is to compare the difference in contents of basic nutrients and anti-nutritional factors between the two types of soybean collected from north and central Malawi.

### **3.3 Materials and Methods**

#### **3.3.1 Soybean samples**

The soybeans used for this study was collected from central Malawi (Lobi area) and north Malawi (Ekwendeni area). Four zones were selected to represent in Lobi area while eight zones were selected in Ekwendeni area. Within every zone, soybean samples were collected from different villages. Soybeans from villages under each zone were well mixed to produce one representative sample. There were two types of soybean seed, hybrid and local. Soybean samples were ground to pass through 500 µm sieve. The residue which could not be further ground was well mixed with the part which passed through the sieve. After that, they were stored at -20 °C for further analysis. The soybean sample information is summarized in Table 3.1.

**Table 3.1** Information of soybean samples collected from north and central Malawi.

Area	Zone Code	Zone Name	Hybrid	Local
Central Malawi (Lobi, 14°S, 34°E)	A	Makowe	0 <sup>a</sup>	1 <sup>b</sup>
	B	Mphathi	1	1
	C	Kamenya	1	0
	D	Khulungira	1	1
North Malawi (Ekwendeni, 11°S, 34°E)	EA	Kabanda	1	1
	EB	Edundu	1	1
	EC	Kabwanda	1	1
	ED	Chilinda	1	1
	EE	Kafulufulu	1	0
	EF	Chimbongondo	1	0
	EG	Emityani	1	0
	EH	Mlimo	1	0

<sup>a</sup> 0: there is no sample representing this area.

<sup>b</sup> 1: there is one sample representing this area.

### 3.3.2 Determination of proximate composition (basic nutrients)

Soybean moisture and ash were determined with approved methods of American Association of Cereal Chemists (AACC International, 1999a, 1999b). Crude lipids content was determined with Soxhlet method. Crude protein content was determined by nitrogen combustion method, and the conversion factor used was 6.25. Total carbohydrate was calculated as 100% - (moisture% + ash% + crude lipids % + crude protein%).

### 3.3.3 Determination of the content of trypsin inhibitors

Content of trypsin inhibitors was determined with the method by Hamerstrand, Black, and Glover (1981). Briefly, 1 g ground soybean was added into 50 ml of 0.01 N NaOH and the pH was adjusted to around 8.4 with HCl. The mixture was stirred for 3 h at room temperature with a magnetic stirrer. The extract was appropriately diluted so that 2 ml of it could inhibit 40 – 60% of the trypsin in the following test. There were five test tubes needed for each test, representing one blank, one standard tube and three sample test tubes. Two ml of diluted extract was added into the blank and sample test tubes, while 2 ml of distilled water was added to the standard test tube. After that, 2 ml of trypsin solution was added into sample test tubes and standard test tube. All test tubes were put into a 37 °C water bath for 10 min. Five ml of pre-warmed (37°C) BAPA (N- $\alpha$ -benzoyl-L-arginine 4-nitroanilide hydrochloride, Sigma-Aldrich, Oakville, ON, Canada) solution was added to all test tubes and allowed to react for 10 min in the same water bath. The reaction was stopped by adding 1 ml of 30% acetic acid into all test tubes. Then, 2 ml of trypsin solution was added into the blank test tube. A spectrophotometer (Thermo Scientific, Genesys 10S UV-Vis spectrophotometer, Columbia, MD, USA) was used to read absorbance of all solutions at 410 nm; solution from the blank test tube was used as blank. The content of trypsin inhibitors was calculated as follow:

$$\text{TI, mg/g of sample} = \frac{\text{Standard tube absorbance} - \text{Sample tube absorbance}}{0.019 \times 1000} \times \text{dilution factor}$$

The trypsin solution used was prepared by dissolving 0.0040 g trypsin (Sigma-Aldrich, Oakville, ON, Canada) in 200 ml 0.001 N HCl. The BAPA solution used in the experiment was prepared by dissolving 0.080 g N- $\alpha$ -benzoyl-L-arginine 4-nitroanilide

hydrochloride in 2 ml dimethyl sulfoxide (Sigma-Aldrich, Oakville, ON, Canada) and diluting into 200 ml with prewarmed tris buffer (37°C). Tris buffer was prepared by dissolving 1.21 g tris(hydroxymethyl) aminomethane and 0.59 g CaCl<sub>2</sub>·2H<sub>2</sub>O in 180 ml distilled water, adjusting pH to 8.2 with HCl, and then making up to 200 ml with distilled water.

### **3.3.4 Determination of hemagglutinating activity of lectins**

Hemagglutinating activity of lectins was determined with the method reported by Shi (2015). Briefly, 1 g ground soybean was added into 10 ml of 0.9 % NaCl followed by vortexing well for a homogenous mixture. The mixture was then shaken for 1 h with a wrist action shaker (Burrell Scientific Pittsburgh, PA, USA). Then, the extraction was accomplished by placing the mixture in refrigerator (2 - 4°C) overnight. After that, the mixture was centrifuged (Thermo Scientific, Sorvall RC 6+ Centrifuge, Columbia, MD, USA) at 16770 × g for 10 min. Supernatant was serially diluted in a 96-well microplate with dilution factors ranging from 8 (column 1) to 16384 (column 12). Each diluted supernatant was mixed with equal volume (0.05 ml) of 2% rabbit red blood cell (Cedarlane®, ON, Canada) diluted with 0.9% NaCl. The mixture was then placed onto a Belly Dancer (Stovall Life Science Incorporated, The Belly Dancer®, Greensboro, NC, USA) and shaken for 2 h. The mixture in each well was mixed thoroughly with a pipette, and then one drop of it was transferred onto a glass slide before covering with a slide cover. The coagulation condition of red blood cell was examined by a microscope. The blank used was 1% rabbit red blood cell diluted with 0.9% NaCl. The result of a well was considered positive if at least five red blood cells were observed to be binding to each other. The most diluted extract which could give a positive result was considered to

contain 1 hemagglutinating unit, and the dilution factor of that extract was then recorded.

The following equation was used to calculate hemagglutinating activity:

$$\text{HU/mg} = \frac{\text{Highest dilution factor with positive results} \times S}{V}$$

where

S = Volume of 0.9% NaCl used in original extraction (ml) / weight of ground soybean (mg)

V = Volume of extract mixed with 2% red blood cell, in this study it was 0.05 ml.

### **3.3.5 Determination of oligosaccharide content**

Oligosaccharides in soybean were extracted with the method reported by Landry, Fuchs, and Hu (2016). Briefly, 40 mg of ground soybean was added to 1.8 ml of 50% (v/v) ethanol, and 0.1 ml of adonitol (0.01g/ml) was added as internal standard. The mixture was then mixed with a rotary mixer (Fisher Scientific, Whitby, ON, Canada) at the speed of 30 at room temperature. Then, the mixture was centrifuged at  $9600 \times g$  for 10 min. One ml of the supernatant was mixed with an equal volume of 95% ice cold ethanol and the mixture was stored at  $-20^{\circ}\text{C}$  for 1 h to precipitate protein. After that, the mixture was centrifuged at  $9600 \times g$  for 10 min and the supernatant was dried with nitrogen at  $60^{\circ}\text{C}$ . The residue was dissolved in 1 ml ultrapure water and the solution was filtered with  $0.45 \mu\text{m}$  nylon filter into an HPLC vial.

A Waters AcQuity<sup>®</sup> Arc<sup>™</sup> HPLC equipped with a Waters 2424 ELS detector was used. The column used was Luna<sup>®</sup> 5  $\mu\text{m}$  NH<sub>2</sub> 100 Å, LC Column 250  $\times$  4.6 mm Ea. Column temperature was  $40^{\circ}\text{C}$  and sample was kept at room temperature during analysis.

Solvent A was acetonitrile and solvent B was pure water. The flow rate was 3 mL/min with 80% solvent A and 20% solvent B. Injection volume was 10 µl and total running time was 15 min for each sample.

### **3.3.6 Statistical analysis**

Content of moisture, crude lipids and ash were measured in triplicate. Crude protein content was measured in duplicate. Contents of trypsin inhibitors, lectin activity and oligosaccharides were measured in triplicate. Data were analyzed using both one way and two-way analysis of variance (ANOVA) with proc ANOVA and proc GLM in SAS University Edition. Duncan's multiple range test was used in the comparison of means. Significant level was set at  $p < 0.05$ .

## **3.4 Results and Discussion**

### **3.4.1 Effect of growing location and seed type on content of basic nutrients in soybeans**

The contents of moisture, carbohydrate, ash, crude protein, crude lipids in soybean are summarized in Table 3.2. The major components of soybean are protein, lipids and carbohydrates. The average protein, lipid and carbohydrate content of soybean were around 36, 20 and 31 g/100g. Moisture content of hybrid seed ranged from 6.75 to 7.56 g/100g, while for local seed it ranged from 6.49 to 7.51 g/100g. The lowest ash content of hybrid seed was 4.22 g/100g and the highest content was 4.66 g/100g, while for local seed the lowest and highest values were 4.31 and 4.96 g/100g, respectively. For crude protein content, hybrid seed ranged from 32.43 to 39.04 g/100g, while local seed ranged from 36.37 to 39.64 g/100g. Hybrid seed had crude lipid content ranging from

19.76 to 22.76 g/100g, while that of local seed ranged from 18.67 to 21.14 g/100g. Carbohydrate content of hybrid seed ranged from 29.41 to 33.28 g/100g, while for local seed it ranged from 28.00 g/100g to 30.83 g/100g.

**Table 3.2** Proximate nutrient (moisture, ash, protein, lipids and carbohydrate) content of two types of soybeans collected from different zones in central and north Malawi.

Zone	Moisture (g/100g)		Ash (g/100g)		Crude Protein (g/100g)		Crude Lipids (g/100g)		Carbohydrates (g/100g)	
	Hybrid	Local	Hybrid	Local	Hybrid	Local	Hybrid	Local	Hybrid	Local
A	-	7.18 ± 0.16 <sup>bc</sup>	-	4.62 ± 0.04 <sup>b</sup>	-	36.76 ± 0.24 <sup>f</sup>	-	20.82 ± 0.15 <sup>ab</sup>	-	30.63 ± 0.36 <sup>ab</sup>
B	6.90 ± 0.12 <sup>c</sup>	7.27 ± 0.05 <sup>b</sup>	4.22 ± 0.05 <sup>d</sup>	4.35 ± 0.01 <sup>e</sup>	36.28 ± 0.23 <sup>d</sup>	39.05 ± 0.06 <sup>b</sup>	21.66 ± 0.11 <sup>abc</sup>	19.85 ± 0.40 <sup>b</sup>	30.93 ± 0.01 <sup>de</sup>	29.32 ± 0.44 <sup>b</sup>
C	7.08 ± 0.12 <sup>b</sup>	-	4.50 ± 0.05 <sup>bc</sup>	-	35.14 ± 0.49 <sup>e</sup>	-	21.57 ± 0.18 <sup>bc</sup>	-	31.70 ± 0.20 <sup>bcd</sup>	-
D	7.56 ± 0.08 <sup>a</sup>	7.04 ± 0.05 <sup>c</sup>	4.55 ± 0.02 <sup>b</sup>	4.96 ± 0.02 <sup>a</sup>	32.43 ± 0.04 <sup>f</sup>	38.42 ± 0.19 <sup>c</sup>	22.76 ± 1.01 <sup>a</sup>	18.67 ± 0.21 <sup>c</sup>	33.28 ± 0.11 <sup>a</sup>	30.83 ± 0.44 <sup>a</sup>
EA	6.89 ± 0.13 <sup>c</sup>	7.22 ± 0.09 <sup>bc</sup>	4.56 ± 0.03 <sup>b</sup>	4.47 ± 0.01 <sup>d</sup>	37.14 ± 0.01 <sup>c</sup>	37.85 ± 0.12 <sup>e</sup>	20.59 ± 0.59 <sup>bcd</sup>	19.83 ± 0.68 <sup>b</sup>	30.61 ± 0.57 <sup>def</sup>	30.41 ± 0.60 <sup>ab</sup>
EB	7.22 ± 0.16 <sup>b</sup>	7.51 ± 0.11 <sup>a</sup>	4.56 ± 0.10 <sup>b</sup>	4.47 ± 0.02 <sup>d</sup>	35.24 ± 0.21 <sup>e</sup>	37.95 ± 0.19 <sup>de</sup>	20.69 ± 0.35 <sup>bcd</sup>	20.19 ± 0.34 <sup>ab</sup>	32.43 ± 0.00 <sup>abc</sup>	29.67 ± 0.42 <sup>ab</sup>
EC	6.86 ± 0.05 <sup>c</sup>	6.49 ± 0.10 <sup>d</sup>	4.50 ± 0.04 <sup>bc</sup>	4.31 ± 0.02 <sup>e</sup>	39.04 ± 0.81 <sup>a</sup>	39.64 ± 0.30 <sup>a</sup>	19.76 ± 0.37 <sup>d</sup>	21.14 ± 0.79 <sup>a</sup>	29.81 ± 0.22 <sup>ef</sup>	28.00 ± 0.03 <sup>c</sup>
ED	6.83 ± 0.04 <sup>c</sup>	7.18 ± 0.05 <sup>bc</sup>	4.66 ± 0.05 <sup>a</sup>	4.54 ± 0.04 <sup>c</sup>	36.38 ± 0.04 <sup>d</sup>	38.37 ± 0.05 <sup>cd</sup>	21.10 ± 0.70 <sup>bc</sup>	20.39 ± 0.85 <sup>ab</sup>	31.07 ± 0.63 <sup>cde</sup>	29.50 ± 0.90 <sup>b</sup>
EE	7.13 ± 0.05 <sup>b</sup>	-	4.51 ± 0.01 <sup>bc</sup>	-	37.56 ± 0.05 <sup>c</sup>	-	20.45 ± 0.13 <sup>cd</sup>	-	30.28 ± 0.04 <sup>ef</sup>	-
EF	7.23 ± 0.10 <sup>b</sup>	-	4.42 ± 0.06 <sup>c</sup>	-	38.28 ± 0.26 <sup>b</sup>	-	20.77 ± 0.56 <sup>bcd</sup>	-	29.41 ± 1.05 <sup>f</sup>	-
EG	6.78 ± 0.01 <sup>c</sup>	-	4.54 ± 0.02 <sup>b</sup>	-	34.55 ± 0.16 <sup>e</sup>	-	21.83 ± 0.64 <sup>ab</sup>	-	32.67 ± 0.21 <sup>ab</sup>	-
EH	6.75 ± 0.03 <sup>c</sup>	-	4.53 ± 0.02 <sup>b</sup>	-	35.02 ± 0.14 <sup>e</sup>	-	20.57 ± 0.88 <sup>cd</sup>	-	33.16 ± 1.37 <sup>a</sup>	-

\* Carbohydrate content is calculated as 100% – (moisture % + ash % + protein % + lipids %).

<sup>a</sup> Data in the same column with different letters are significant different ( $p < 0.05$ ).

To investigate whether growing location (north area or central area) and seed type (hybrid or local) had significant effects on soybean components, data were analyzed with two-way ANOVA. Significance of the effects of area and seed type are summarized in Table 3.3. The effects of location were nonsignificant ( $p > 0.05$ ) for all basic nutrients except for crude protein. Seed type showed significant ( $p < 0.05$ ) effects for crude protein, crude lipids and carbohydrates, while it had nonsignificant ( $p > 0.05$ ) effects on moisture and ash content. In terms of interactions between area and seed type, there were significant ( $p < 0.05$ ) effects only on the content of ash and crude lipids. When looking at the basic nutrients individually, area, seed type and their interactions, all had nonsignificant ( $p > 0.05$ ) effects on moisture content. It meant that there was no statistical difference in moisture content between two types of seeds or seeds grown in different areas. Only the interactions of area and seed type showed significant ( $p < 0.05$ ) effects on ash content. It meant that the effect of area (or seed type) on soybean ash content is dependent on seed type (or area). Both area and seed type significantly ( $p < 0.05$ ) affected the crude protein content. However, the effects of their interactions were nonsignificant ( $p > 0.05$ ). These findings suggested that the crude protein content of two types of soybeans or soybean grown in two areas were statistically different. Seed type and its interactions with area showed significant ( $p < 0.05$ ) effects on soybean crude protein content. It meant that the effects of these two factors on crude protein content were dependent on each other. For soybean carbohydrates, only effect from seed type was significant ( $p < 0.05$ ). It meant that the carbohydrate contents of two types of soybean grown in the same area were statistically different. Besides, the carbohydrate contents of each type of soybean grown in different areas were not statistically different.

**Table 3.3** P values from ANOVA indicating the significance of effects from area and seed type on content of basic nutrients (moisture, ash, protein, lipids and carbohydrate) in soybeans.

Proximate	Factor		
	Area	Seed Type	Area × Seed Type
Moisture	0.0826	0.4293	0.3402
Ash	0.2995	0.1106	0.0004*
Crude Protein	0.0241*	<0.0001*	0.1146
Crude Lipids	0.1172	<0.0001*	0.0002*
Carbohydrates	0.0701	0.0004*	0.9318

\* The effect was significant ( $p < 0.05$ )

For the components which were significantly affected by the interaction of the two main effects, further analysis was applied. In the case of ash content, results showed that local seed (average 4.65 g/100g) had significantly ( $p < 0.05$ ) higher values than hybrid seed (4.42 g/100g) in central Malawi. However, in north Malawi, there was no significant difference between the ash contents of local (average 4.44 g/100g) and hybrid seed (average 4.54 g/100g). In terms of crude lipid content, hybrid seed (average 22.11 g/100g) had significantly ( $p < 0.05$ ) higher values than local seed (average 19.78 g/100g) in central Malawi. In north Malawi, however, crude lipid content of hybrid seed (average 20.70 g/100g) was not significantly different from local seed (average 20.39 g/100g). In terms of hybrid seed, the average ash content in central Malawi (average 4.42 g/100g) was significantly lower than that in north Malawi (average 4.54 g/100g). However, for local seed, the average ash content in central Malawi (average 4.65 g/100g) was significantly higher than that in north Malawi (average 4.44 g/100g). Hybrid seed in

central Malawi (average 22.11 g/100g) showed significantly higher crude lipid content than that in north Malawi (average 20.70 g/100g). The crude lipid content of local seed was not significantly different between central (average 19.78 g/100g) and north (average 20.39 g/100g) Malawi.

For the components which were not significantly affected by the interaction effects, the means were presented in Table 3.4. ANOVA procedure indicated that area had nonsignificant ( $p > 0.05$ ) effects for soybean moisture content. Duncan's test showed that such effects were significant ( $p < 0.05$ ). Similar variance was also observed in crude protein content, in which the result from ANOVA was significant while nonsignificant from Duncan's test. Based on the results given by Duncan's test, local seed had significantly ( $p < 0.05$ ) higher crude protein content than hybrid seed. The carbohydrate content of hybrid seed was significantly ( $p < 0.05$ ) higher than found for local seed. Based on these results, the hybrid seed is a better choice for production of soybean oil, while the local seed is more suitable for provision of more proteins. Moreover, with higher carbohydrate content than found in local, hybrid seed may also be a better source of dietary fiber.

**Table 3.4** Average contents of basic nutrients in two types of soybeans seeds and two growing regions (central and north Malawi).

	Average Content (g/100g)		
	Moisture	Crude Protein	Carbohydrate
Central	7.17 ± 0.23 <sup>A</sup>	36.35 ± 2.29 <sup>A</sup>	31.11 ± 1.27 <sup>A</sup>
North	7.01 ± 0.29 <sup>B</sup>	37.25 ± 1.61 <sup>A</sup>	30.58 ± 1.56 <sup>A</sup>
Hybrid	7.02 ± 0.25 <sup>a</sup>	36.10 ± 1.85 <sup>b</sup>	31.40 ± 1.38 <sup>a</sup>
Local	7.13 ± 0.31 <sup>a</sup>	38.29 ± 0.89 <sup>a</sup>	29.76 ± 1.01 <sup>b</sup>

<sup>A, a</sup> Data are expressed as mean ± standard deviation. Value in the same column with different letters are significant different. Values labelled with different sizes of letters are not comparable.

\* Results are generated from Duncan's multiple range test.

Some literature concluded that soybean grown at higher latitude can result in higher oil content and lower protein content (Hurburgh, Brumm, Guinn, & Hartwig, 1990; Kumar, Rani, Solanki, & Hussain, 2006). Such phenomenon was not observed in current study. Thus, there are conflicts existing between current study and literature. There are some possible reasons, which may lead to the variances between current study and literature. Firstly, the selection of growing locations varies. In some studies, the differences between environmental conditions (eg. rainfall and temperature) of growing locations are considerable while not so in others. The distance between growing locations may also contribute to the variance. If the distance is small, it may not cause significant differences in the components as the environments will likely bear resemblance. Secondly, there are genotypic differences in soybean seeds used in the experiments. Some studies used soybean seeds that have similar composition of each component,

which may lead to nonsignificant results. Finally, the difference in agronomic practices may lead to variations. For example, some studies may apply fertilizers during growing of soybean while some studies not.

### **3.4.2 Effects of growing location and seed type on content of trypsin inhibitors in soybean**

The contents of trypsin inhibitors of soybeans are presented in Table 3.5. Hybrid seeds had trypsin inhibitors ranging from 18.86 to 44.06 mg/g, while the contents for local seeds were from 19.98 to 37.96 mg/g. There was about  $2 \times$  difference in the content of trypsin inhibitors between the highest and lowest sample. When comparing two types of seeds, their lowest values were close, while the differences between their highest values were relatively big.

**Table 3.5** Trypsin inhibitor (mg/g) of two types of soybeans collected from different zones in central and north Malawi.

Zone	Trypsin inhibitors (mg/g)	
	Hybrid	Local
A	-	20.92 ± 1.11 <sup>d</sup>
B	28.89 ± 0.29 <sup>d</sup>	33.35 ± 0.37 <sup>b</sup>
C	24.36 ± 1.04 <sup>f</sup>	-
D	44.06 ± 0.03 <sup>a</sup>	20.76 ± 0.67 <sup>d</sup>
EA	28.65 ± 0.33 <sup>d</sup>	28.25 ± 0.26 <sup>c</sup>
EB	26.61 ± 0.45 <sup>e</sup>	19.98 ± 1.10 <sup>d</sup>
EC	18.86 ± 0.57 <sup>g</sup>	28.40 ± 0.67 <sup>c</sup>
ED	23.68 ± 1.13 <sup>f</sup>	37.96 ± 1.95 <sup>a</sup>
EE	19.29 ± 0.88 <sup>g</sup>	-
EF	23.13 ± 0.95 <sup>f</sup>	-
EG	35.11 ± 0.09 <sup>b</sup>	-
EH	33.11 ± 1.19 <sup>c</sup>	-

<sup>a</sup> Data are expressed as Mean ± Standard Deviation. Data in the same column with different letters are significant different ( $p < 0.05$ ).

The significances of effects from growing location and seed type are summarized in Table 3.6. Both area and seed type showed nonsignificant ( $p > 0.05$ ) effects on content of soybean trypsin inhibitors. However, their interactions had significant ( $p < 0.05$ ) effects. It meant that the effect of growing location (or seed type) was dependent on the effect of seed type (or growing location). According to Kumar, Rani, Tindwani, and Jain (2003), location had nonsignificant effects on activity of soybean trypsin inhibitors, while genotype and genotype × location interactions showed significant effects. The results

from current study are partially in agreement with their findings. Krishnan, Jang, Baxter, and Wiebold (2012) drew the conclusion that growing site had profound effects on the accumulation of soybean Bowman-Birk inhibitor. Since the two types of trypsin inhibitors were not determined individually in current study, it is hard to compare with their work. Even though the effect of growing location on Kunitz trypsin inhibitor is not included, their results suggested that growing location can affect content of soybean trypsin inhibitors.

**Table 3.6** P values from ANOVA indicating the significance of effects from area and seed type on trypsin inhibitors content in soybeans.

Compound	Factor		
	Area	Seed Type	Area × Seed Type
Trypsin inhibitors	0.7226	0.2145	0.0304*

\* The effect is significant ( $p < 0.05$ )

To investigate the significant interaction effects, Table 3.7 summaries the average content of trypsin inhibitors of each type of soybean and each area. Based on the table, in central Malawi, hybrid seed had significantly ( $p < 0.05$ ) higher content of trypsin inhibitors than local seed. However, in north Malawi, there were no significant difference between the content of trypsin inhibitors in the two types of seeds. For both hybrid and local seed, the trypsin inhibitors contents were not significantly different between central and north Malawi.

**Table 3.7** Comparisons of average contents of trypsin inhibitors in two types of soybeans grown in central and north Malawi.

	Average Trypsin Inhibitors Content (mg/g)	
	Central Malawi	North Malawi
Hybrid seed	30.99 ± 8.36 <sup>A,a</sup>	25.78 ± 5.64 <sup>A,a</sup>
Local Seed	23.97 ± 5.84 <sup>B,a</sup>	27.74 ± 6.65 <sup>A,a</sup>

<sup>A, a</sup> Value in the same column or row with different letters are significant different. Values labelled with different sizes of letters are not comparable.

### 3.4.3 Effects of growing location and seed type on activity of lectins in soybean

Table 3.8 summarized the lectin activity of soybean seed. Results showed that except for hybrid seed from zone B and local seed from zone ED, the lectin activity of all seeds was about 409 HU/mg. For both two types of seeds, the highest lectin activity was about 819 HU/mg. The highest lectin activity in both central and north area are also about 819 HU/mg.

**Table 3.8** Lectin activity of two types of soybeans collected from different zones in central and north Malawi.

Zone	Hybrid (HU/mg)	Local (HU/mg)
A	-	409 ± 0 <sup>b</sup>
B	819.0 ± 0 <sup>a</sup>	409 ± 0 <sup>b</sup>
C	409 ± 0 <sup>b</sup>	-
D	409 ± 0 <sup>b</sup>	409 ± 0 <sup>b</sup>
EA	409 ± 0 <sup>b</sup>	409 ± 0 <sup>b</sup>
EB	410 ± 0 <sup>b</sup>	409 ± 0 <sup>b</sup>
EC	409 ± 0 <sup>b</sup>	409 ± 0 <sup>b</sup>
ED	409 ± 0 <sup>b</sup>	819 ± 0 <sup>a</sup>
EE	410 ± 0 <sup>b</sup>	-
EF	409 ± 0 <sup>b</sup>	-
EG	409 ± 0 <sup>b</sup>	-
EH	409 ± 0 <sup>b</sup>	-

<sup>a</sup> Data are expressed as Mean ± Standard Deviation. Data in the same column with different letters are significant different ( $p < 0.05$ ).

The p values of the growing location and seed type are summarized in Table 3.9. Interaction effects of area and seed type significantly ( $p < 0.05$ ) affected the lectin activity, while both area and seed type separately showed nonsignificant ( $p > 0.05$ ) effects. The results suggested that the effect of growing location (or seed type) on soybean lectin activity was dependent on the seed type (or growing location). According to De Mejía, et al. (2003) cultivar had no significant effects on activity of lectins in common beans (*Phaseolus Vulgaris* L.), while growing location showed significant ( $p < 0.05$ ) effects. Their results are partially in agreement with the findings of the current

study. One major reason could be the difference in experimental materials (common bean vs. soybean).

**Table 3.9** P values from ANOVA indicating the significance of effects from area and seed type on lectin activity in soybeans.

Compound	Factor		
	Area	Seed Type	Area × Seed Type
Lectins	0.4362	0.4347	0.0029*

\* The effect is significant ( $p < 0.05$ )

To investigate the significant interaction effects, average lectin activities of soybean are summarized in Table 3.10. Based on the table, in central Malawi, hybrid seed had significantly ( $p < 0.05$ ) higher lectins activity than local seed. However, in north Malawi, the differences in lectins activity between the two seeds were not significantly different. In terms of hybrid seed, lectins activity in central Malawi was significantly higher than that in north Malawi. For local seed, there was no significant difference between lectins activity in central and north Malawi.

**Table 3.10** Comparisons of average lectin activity in two types of soybeans grown in central and north Malawi.

	Average Lectins Activity (HU/mg)	
	Central Malawi	North Malawi
Hybrid seed	546 ± 205 <sup>A,a</sup>	409 ± 0 <sup>A,b</sup>
Local Seed	409 ± 0 <sup>B,a</sup>	491 ± 173 <sup>A,a</sup>

<sup>A, a</sup> Value in the same column or row with different letters are significant different. Value labelled with different sizes of letters are not comparable.

#### **3.4.4 Effects of growing location and seed type on content of oligosaccharides in soybeans**

The contents of soybean oligosaccharides are presented in Table 3.11. Sucrose content for hybrid seed ranged from 49.79 to 73.35 mg/g, while that range for local seed was from 49.69 to 60.00 mg/g. Raffinose content of hybrid seed was from 3.74 to 5.97 mg/g. Local seed has raffinose content ranging from 3.75 to 4.64 mg/g. The stachyose content in hybrid seed was from 29.85 to 36.91 mg/g, while in local seed it was from 31.05 to 36.75 mg/g. The total flatulence-causing oligosaccharides in hybrid soybean seed is ranged from 33.69 to 41.75 mg/g. That range for local soybean seed was from 34.80 to 41.22 mg/g. These results confirmed that the major oligosaccharides in soybean are sucrose followed by stachyose and raffinose. Furthermore, stachyose is the major flatulence-causing oligosaccharides in soybean rather than raffinose.

**Table 3.11** Oligosaccharides (mg/g) of two types of soybeans collected from different zones in central and north Malawi.

Zone	Sucrose (mg/g)		Raffinose (mg/g)		Stachyose (mg/g)		Total RFOs *	
	Hybrid	Local	Hybrid	Local	Hybrid	Local	Hybrid	Local
A	-	57.04 ± 3.76 <sup>ab</sup>	-	4.62 ± 0.32 <sup>a</sup>	-	36.60 ± 1.97 <sup>a</sup>	-	41.22 ± 2.24 <sup>a</sup>
B	51.95 ± 1.12 <sup>fg</sup>	49.69 ± 2.43 <sup>c</sup>	4.18 ± 0.16 <sup>a</sup>	4.64 ± 0.01 <sup>a</sup>	30.62 ± 1.33 <sup>b</sup>	35.01 ± 1.70 <sup>abc</sup>	34.22 ± 1.05 <sup>b</sup>	39.07 ± 1.93 <sup>abc</sup>
C	49.79 ± 1.48 <sup>g</sup>	-	4.69 ± 0.91 <sup>a</sup>	-	30.18 ± 4.79 <sup>b</sup>	-	35.33 ± 7.58 <sup>b</sup>	-
D	73.35 ± 2.98 <sup>a</sup>	54.24 ± 2.93 <sup>bc</sup>	5.97 ± 0.54 <sup>a</sup>	4.04 ± 0.05 <sup>ab</sup>	32.28 ± 0.36 <sup>b</sup>	36.75 ± 3.54 <sup>a</sup>	38.43 ± 0.27 <sup>ab</sup>	40.58 ± 4.93 <sup>ab</sup>
EA	58.44 ± 3.28 <sup>de</sup>	60.00 ± 2.98 <sup>a</sup>	4.02 ± 0.24 <sup>a</sup>	3.84 ± 0.07 <sup>b</sup>	33.00 ± 2.27 <sup>ab</sup>	35.28 ± 2.04 <sup>ab</sup>	37.02 ± 2.20 <sup>ab</sup>	38.73 ± 2.64 <sup>abc</sup>
EB	61.75 ± 5.68 <sup>bcd</sup>	54.82 ± 1.32 <sup>abc</sup>	3.83 ± 0.19 <sup>a</sup>	3.75 ± 0.20 <sup>b</sup>	29.85 ± 2.28 <sup>b</sup>	31.05 ± 0.95 <sup>c</sup>	33.69 ± 2.43 <sup>b</sup>	34.80 ± 0.97 <sup>c</sup>
EC	54.46 ± 2.60 <sup>efg</sup>	51.73 ± 4.69 <sup>bc</sup>	4.15 ± 0.26 <sup>a</sup>	3.78 ± 0.33 <sup>b</sup>	31.79 ± 1.95 <sup>b</sup>	31.82 ± 1.77 <sup>bc</sup>	35.94 ± 2.01 <sup>b</sup>	35.59 ± 1.54 <sup>bc</sup>
ED	59.26 ± 2.92 <sup>cde</sup>	53.65 ± 1.16 <sup>bc</sup>	4.85 ± 0.47 <sup>a</sup>	4.33 ± 0.37 <sup>ab</sup>	36.91 ± 3.88 <sup>a</sup>	34.31 ± 1.95 <sup>abc</sup>	41.75 ± 4.22 <sup>a</sup>	38.64 ± 2.15 <sup>abc</sup>
EE	56.06 ± 2.24 <sup>ef</sup>	-	3.74 ± 0.28 <sup>a</sup>	-	31.94 ± 0.59 <sup>b</sup>	-	35.40 ± 0.23 <sup>b</sup>	-
EF	58.66 ± 0.51 <sup>cde</sup>	-	4.21 ± 0.24 <sup>a</sup>	-	32.96 ± 0.51 <sup>ab</sup>	-	37.17 ± 0.75 <sup>ab</sup>	-
EG	64.83 ± 0.83 <sup>b</sup>	-	3.82 ± 0.12 <sup>a</sup>	-	30.50 ± 1.49 <sup>b</sup>	-	34.33 ± 1.60 <sup>b</sup>	-
EH	63.71 ± 1.95 <sup>bc</sup>	-	4.45 ± 0.27 <sup>a</sup>	-	31.86 ± 1.51 <sup>b</sup>	-	36.32 ± 1.52 <sup>ab</sup>	-

\* Total RFOs = raffinose content + stachyose content

<sup>a</sup> Data are expressed as Mean ± Standard Deviation. Data in the same column with different letters are significant different ( $p < 0.05$ ).

The significances of the effects of growing location and seed type on soybean oligosaccharides are summarized in Table 3.12. Area showed significant ( $p < 0.05$ ) effects on raffinose content but no significant ( $p < 0.05$ ) effects on sucrose, stachyose and total RFOs contents. Seed type showed significant ( $p < 0.05$ ) effects on all oligosaccharides. With exception of stachyose and total RFO contents, interactions of area and seed type showed no significant ( $p < 0.05$ ) effects to all oligosaccharides. For sucrose content, only seed type showed significant effect ( $p < 0.05$ ), which means that, no matter where the growing location was, the sucrose contents of two soybean types were statistically different. Both growing location and seed type showed significant ( $p < 0.05$ ) effects on raffinose content. For the two soybean types and soybean grown in two areas, the raffinose content was statistically different. Both seed type and its interaction with area showed significant effects on soybean stachyose content. This result suggested that the effect of growing location (or seed type) on soybean stachyose content was dependent on the seed type (or growing location). When looking at total RFOs content, both seed type and its interaction with area showed significant ( $p < 0.05$ ) effects. It meant that the effects of area and seed type on total RFOs were dependent on each other.

**Table 3.12** P value from ANOVA indicating the significance of effects from area and seed type on oligosaccharides contents in soybeans.

Compound	Factor		
	Area	Seed Type	Area × Seed Type
Sucrose	0.6519	0.0049*	0.7388
Raffinose	0.0002*	0.0371*	0.4179
Stachyose	0.2679	0.0003*	0.0058*
Total RFOs <sup>a</sup>	0.1204	0.0220*	0.0422*

\* Significant at  $p < 0.05$

<sup>a</sup> Total RFOs = raffinose content + stachyose content

To interpret the significant interaction effects on soybean stachyose and total RFOs content, further analysis was applied on the data. In central Malawi, the average stachyose contents of hybrid seed (31.03 mg/g) was significantly ( $p < 0.05$ ) lower than local seed (36.12 mg/g). However, in north Malawi, the average stachyose contents of hybrid and local seed were 32.35 and 33.11 mg/g, values of which were not significantly different. In terms of total RFOs, in central Malawi, hybrid seed (35.99 mg/g) had a significantly ( $p < 0.05$ ) lower average value than local seed (40.42 mg/g). However, in north Malawi, the average total RFOs of hybrid seed (36.50 mg/g) and local seed (36.78 mg/g) were not significantly different. In terms of hybrid seed, the stachyose contents were not significantly different between central (31.03 mg/g) and north (32.35 mg/g) Malawi. However, for local seed, the stachyose content in central (36.12 mg/g) Malawi was significantly higher than that in north (33.11 mg/g) Malawi. The total RFOs content of hybrid seed in central Malawi (35.99 mg/g) was not significant different from that in

north Malawi (36.50 mg/g). Local seed in central Malawi (40.42 mg/g) showed significantly higher total RFOs content than that in north Malawi (36.78 mg/g).

For the oligosaccharides which were not significantly affected by the interaction of the main effects, their means are shown in Table 3.13. Hybrid seed had significantly ( $p < 0.05$ ) higher sucrose content than local seed. Furthermore, the raffinose content of soybean grown in central Malawi was significantly ( $p < 0.05$ ) higher than that of the soybean grown in north Malawi. Even though AVONA results indicated that seed type had significant ( $p < 0.05$ ) effects on raffinose content, the results from Duncan's test showed that differences between two seed types were nonsignificant ( $p > 0.05$ ). As for stachyose, its content in local seed was significantly ( $p > 0.05$ ) higher than that of the hybrid seed. When comparing total flatulence causing oligosaccharides, local soybean seed has significantly ( $p < 0.05$ ) higher levels of RFOs.

**Table 3.13** Comparisons of average contents of oligosaccharides in two soybean types grown in central and north Malawi.

	Average Content (mg/g)	
	Sucrose	Raffinose
Central	56.38 ± 8.08 <sup>A</sup>	4.69 ± 0.71 <sup>A</sup>
North	58.11 ± 4.65 <sup>A</sup>	4.08 ± 0.41 <sup>B</sup>
Hybrid	59.59 ± 6.63 <sup>a</sup>	4.32 ± 0.64 <sup>a</sup>
Local	54.45 ± 4.06 <sup>b</sup>	4.14 ± 0.43 <sup>a</sup>

<sup>A, a</sup> Value in the same column with different letters are significant different. Values labelled with different sizes of letters are not comparable.

\* Results are generated from Duncan's multiple range test. Total RFOs = raffinose content + stachyose content

According to Kumar, et al. (2010), growing location had no significant effects on soybean raffinose and stachyose contents, while its interaction with genotype showed significant effects on these two components. However, in the current study, growing location significantly ( $p < 0.05$ ) affected soybean raffinose content and its interaction with seed type showed no significant effects. As mentioned earlier, such variations could be caused by the actual differences among growing sites, soybean seeds and agronomic practices.

### **3.4.5 Correlation between detected components**

The correlations among detected components are summarized in Table 3.14. Based on the table, soybean ash content was significant ( $p < .05$ ) positively correlated with stachyose and total RFOs. Crude protein content was significant negatively correlated with contents of crude lipids, carbohydrate, trypsin inhibitors, sucrose and raffinose. Crude lipids content was significant positively correlated with contents of trypsin inhibitors and raffinose. Sucrose content was significantly and positively correlated with contents of carbohydrate. Trypsin inhibitors content was significantly positively correlated with contents of sucrose and raffinose. Content of stachyose was significantly and positively correlated with total RFOs.

Protein, lipids and carbohydrates were the major basic nutrients in soybean. In the current study, the average soybean crude protein, crude lipids and carbohydrate contents were 36, 20 and 31 g/100g. Within the soybean seed, higher portion of protein will lead to lower portion of lipids and carbohydrates. Therefore, protein content was negatively correlated with lipids and carbohydrate contents. Interestingly, in terms of crude lipids and carbohydrate, their contents were found not significantly correlated.

**Table 3.14** Correlations between soybean moisture, ash, crude protein, crude lipids, carbohydrate, trypsin inhibitors, lectins, sucrose, raffinose, stachyose and total RFOs.

	Moisture	Ash	Crude protein	Crude lipids	CHO <sup>a</sup>	Trypsin inhibitors	Lectins	Sucrose	Raffinose	Stachyose	RFOs <sup>b</sup>
Moisture	1	0.0901 (0.7223)	-0.1842 (0.4644)	-0.0144 (0.9547)	0.0988 (0.6964)	0.0966 (0.7030)	-0.0283 (0.9111)	0.2129 (0.3964)	0.3381 (0.1700)	0.0821 (0.7462)	0.1528 (0.5449)
Ash	-	1	-0.1325 (0.6003)	-0.3786 (0.1214)	0.3248 (0.1884)	-0.196 (0.4356)	-0.3233 (0.1907)	0.2543 (0.3086)	0.104 (0.6812)	0.4995 <b>(0.0348)</b>	0.5227 <b>(0.0261)</b>
Protein	-	-	1	-0.7244 <b>(0.0007)</b>	-0.9293 <b>(&lt;.0001)</b>	-0.4845 <b>(0.0416)</b>	0.0721 (0.7761)	-0.7294 <b>(0.0006)</b>	-0.5433 <b>(0.0198)</b>	0.3444 (0.1617)	0.1604 (0.5250)
Lipids	-	-	-	1	0.4496 (0.0612)	0.5153 <b>(0.0286)</b>	0.1271 (0.6152)	0.4595 (0.0551)	0.5013 <b>(0.0341)</b>	-0.4554 (0.0576)	-0.2768 (0.2661)
CHO	-	-	-	-	1	0.3851 (0.1145)	-0.1384 (0.5839)	0.7148 <b>(0.0009)</b>	0.4106 (0.0906)	-0.2618 (0.2939)	-0.1223 (0.6289)
Trypsin inhibitors	-	-	-	-	-	1	0.3049 (0.2186)	0.4900 <b>(0.0390)</b>	0.4980 <b>(0.0355)</b>	-0.1172 (0.6431)	0.004 (0.9873)
Lectins	-	-	-	-	-	-	1	-0.2819 (0.2570)	-0.0117 (0.9634)	-0.0724 (0.7752)	-0.102 (0.6872)
Sucrose	-	-	-	-	-	-	-	1	0.4039 (0.0965)	-0.0857 (0.7354)	0.0386 (0.8793)
Raffinose	-	-	-	-	-	-	-	-	1	0.2242 (0.3712)	0.4641 (0.0524)
Stachyose	-	-	-	-	-	-	-	-	-	1	0.9628 <b>(&lt;.0001)</b>
RFOs	-	-	-	-	-	-	-	-	-	-	1

\* Value in the bracket stands for the p value of the corresponding correlations, p value in bold is significant (p < 0.05).

<sup>a</sup> CHO: Carbohydrate <sup>b</sup> RFOs: Raffinose family oligosaccharides (stachyose + raffinose)

Trypsin inhibitors are proteins naturally existing in soybean. The significant and negative correlation between crude protein and trypsin inhibitors suggested that a higher soybean protein content could lead to a lower level of trypsin inhibitors. According to Sakla, Ghali, El-Farra, and Rizk (1988), the correlation coefficient of soybean trypsin inhibitors versus protein percentage was -0.6397, which was in agreement with the current study. However, Zdunczyk, Godycka, and Amarowicz (1997) concluded that there was no significant correlation between trypsin inhibitor activity and protein content in peas, which is in conflict with the findings of current study. Such variation could be caused by use of different study materials.

According to Karr-Lilienthal, Kadzere, Grieshop, and Fahey (2005), about half of the carbohydrates in soybean are nonstructural, which includes low molecular weight sugars, oligosaccharides and a small amount of starch. Based on the results from current study, the major oligosaccharides in soybean were sucrose, stachyose and raffinose. Analysis showed that sucrose but not raffinose and stachyose was significantly and positively correlated with soybean carbohydrate. Possible reason could be the relatively higher content of sucrose among the three oligosaccharides found in soybean. The results of the current study also indicated that there was no significant correlation between soybean carbohydrate and total RFOs content. However, Zdunczyk, Godycka, and Amarowicz (1997) reported that there was significant positive correlation between pea dietary fiber content and total RFOs. There are two possible reasons for the disagreements. One of them is that the seed used in the current study is soybean not peas. Another is that the RFOs content was compared with total carbohydrates not dietary fiber in current study.

### 3.5 Conclusion

The contents of ash, crude protein, crude lipids and carbohydrates were not significantly affected by growing location (central Malawi and north Malawi). However, except for ash, their contents were statistically different from seed type to seed type. Compared with local seed, hybrid seed had higher lipid and carbohydrate content but lower protein content. Both growing location and seed type did not significantly affect the contents of trypsin inhibitors and lectins. The main oligosaccharides in soybean were sucrose, stachyose and raffinose. Stachyose is the major flatulence-causing oligosaccharides in these soybean samples. Soybean grown in central Malawi had significantly higher raffinose content than that of the soybean grown in north Malawi. However, the total RFOs content was not significantly affected by growing region. Local seed had significantly higher stachyose and total RFOs content but lower sucrose content than hybrid seed. Correlation analysis showed that trypsin inhibitors were negatively correlated with protein content. In conclusion, local seed could be a better choice if the soybean is grown for its protein content. Hybrid seed is more suitable for oil extraction in central Malawi. Among the two types of seeds, the higher total RFOs content of local seed may put barriers in its utilization. Growing soybean in north or central Malawi did not lead to significant differences in major nutrients including, ash, protein, lipids and carbohydrates. Except for raffinose content, the two growing regions cannot lead to significant differences in soybean anti-nutritional factors.

### **Connecting Statement**

Chapter 3 focused on the effects of pre-harvest (geographic region and seed type) factors on basic nutrients and anti-nutritional compounds in soybeans collected from Malawi. The results indicated the differences between two types of seeds and seeds collected from different regions. The findings can serve as a good reference when deciding which type of soybean seed should be used if the target is to get more protein or lipids. However, in terms of anti-nutritional factors, the findings showed that geographic location can only significantly affect the raffinose content, while seed type only had significant effects on sucrose and stachyose content. Both geographic location and seed type did not significantly affect the trypsin inhibitors content and lectins activity. Therefore, apart from pre-harvest factors, post-harvest processing is necessary in order to remove these anti-nutritional components.

Chapter 4 will focus on the effects of post-harvest processing (soaking and infrared heating) on soybean anti-nutritional factors. Furthermore, how such processing affects the soybean isoflavones and fatty acids will also be investigated.

## **Chapter 4 : Effects of soaking and infrared heating on anti-nutritional factors, isoflavones and fatty acids in soybean**

### **4.1 Abstract**

Soybean is a good source of protein, lipids and isoflavones. However, it also contains some anti-nutritional factors, including trypsin inhibitors, lectins and flatulence causing oligosaccharides. Elimination of anti-nutritional factors is necessary to make soybean more suitable for consumption. The findings from the current study indicated that infrared heating was effective in deactivating trypsin inhibitors and lectins, and better results could be achieved when soybean moisture content was lower. Soaking significantly ( $p < 0.05$ ) decreased the content of sucrose and raffinose in soybean. The major soybean isoflavones existed in free form while small portion (daidzin and genistin) were in bound form. Infrared heating significantly affected all quantified free soybean isoflavones, while soaking only had significant ( $p < 0.05$ ) effects on genistin, malonyldaidzin and malonylgenistin. The major fatty acids from highest to lowest levels were C18:2, C:18:1, C16:0, C18:3 and C18:0. Neither soaking nor infrared heating significantly ( $p < 0.05$ ) affected the total unsaturated fatty acids in soybean. In general, infrared heating was effective in removing soybean trypsin inhibitors and lectins, while soaking decreased the content of oligosaccharides. The combination of infrared heating and soaking altered the contents of different soybean isoflavones, but showed less effect on fatty acid profiles.

## 4.2 Introduction

Soybean [*Glycine max.* (L.) Merr.] is one of the most important legumes in the world. It can serve as a significant source of proteins and lipids for humans. Foods made of soybean, including tofu, soybean sprouts, soy sauce and soy milk are well recognized worldwide. World soybean production has increased from less than 50 million tons in 1961 to over 300 million tons in 2014 (Food and Agricultural Organization of the United Nations, 2017).

Soybean's role as a functional food is being studied more frequently. It contains a considerable amount of isoflavones, which may help in prevention of cardiovascular disease and some types of cancers (colon, breast and prostate) (Messina, 1999). There are 12 different types of isoflavones in soybean, which includes 3 aglycones (daidzein, glycitein and genistein) and 9 glucosides (daidzin, glycitin, genistin, acetyldaidzin, acetylglycitin, acetylgenistin, malonyldaidzin, malonylglycitin and manonylgenistin) (Song, Barua, Buseman, & Murphy, 1998). Moreover, soybean is also a good source of unsaturated fatty acids (Stewart, Raghavan, Orsat, & Golden, 2003). Soybean oil is reported to have a good ratio of linoleic acid to  $\alpha$ -linolenic acid (7:1) (Connor, 2000).

Soybean is also well known to contain some antinutritional factors (ANFs), which include trypsin inhibitors, lectins and flatulence-causing oligosaccharides. Trypsin inhibitors in soybean can be classified into two types, the Kunitz trypsin inhibitor and the Bowman-Birk inhibitor (Liu, 2004). Both types can bind with trypsin and form complexes (Birk, 1985; Kunitz, 1947). Lectins are natural proteins existing in soybean, which can bind with free sugars, glycoproteins, glycolipids and sugar residues of polysaccharides (De Mejía & Prisecaru, 2005). This property allows lectins to attach to

the epithelial cell surfaces potentially causing problems (Palacios, et al., 2004; Rådberg, et al., 2001). The major flatulence-causing oligosaccharides in soybean are raffinose and stachyose, which belong to the raffinose family oligosaccharides (RFOs) (Kumar, et al., 2010). These two oligosaccharides cannot be digested by humans because of the presence of  $\alpha$  1 $\rightarrow$ 6 glycosidic linkages in their structures (Martínez-Villaluenga, Frias, & Vidal-Valverde, 2008). However, raffinose and stachyose can be fermented by the microorganisms in the large intestine and causing flatulence, abdominal discomfort or diarrhea (Kumar, et al., 2010).

To make soybean more suitable for consumption, the ANFs need to be removed. There are several methods developed to reduce these compounds, which include soaking (Egounlety & Aworh, 2003), fermentation (Adeyemo & Onilude, 2013), germination (Bau, Villaume, Nicolas, & Méjean, 1997), dry heat (Sitren, Ahmed, & George, 1985) and moist heat (Sitren, Ahmed, & George, 1985). Both trypsin inhibitors and lectins are proteins. Heating could change their structures and cause loss of their bioactivity. Therefore, heating is usually effective in removing trypsin inhibitors and lectins. Compared with these compounds, raffinose and stachyose are much more heat stable (Dixit, et al., 2011). However, these two oligosaccharides are water soluble. It has been reported that soaking can decrease their contents in soybean (Egounlety & Aworh, 2003).

Infrared heating is a relatively new technology with growing applications in the food industry. Compared with conventional heating, it has the advantages of shorter heating time, more uniform heating, less loss of nutrients, and higher energy efficiency (Krishnamurthy, et al., 2008; Sumnu & Ozkoc, 2010). It heats up food materials by causing vibration (60 000 – 150 000 MHz) of water molecules (Fasina, et al., 2001).

When studying its effects on the soybean ANFs, it is also worthy to look at the changes in the contents of functional compounds.

The objective of this study was to investigate the effects of soaking and infrared heating on ANFs (trypsin inhibitors, lectins and RFOs), isoflavones and fatty acid profiles in soybean.

### **4.3 Materials and Methods**

#### **4.3.1 Chemicals and reagents**

All solvents or acids used in this study were either HPLC grade or analytical grade. Methanol, acetic acid and HCl were purchased from Fisher Scientific (Whitby, ON, Canada). Dimethyl sulfoxide, toluene and acetyl chloride, bovine trypsin, N- $\alpha$ -benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPA) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Standard compounds including, raffinose, stachyose, daidzin, genistin, glycitin, daidzein, genistein, glycitein and heptadecanoic acid (C17:0) were purchased from Sigma-Aldrich (Oakville, ON, Canada). The rabbit red blood cell was purchased from Cedarlane®. The mixture of fatty acid methyl ester standards was purchased from Nu-Chek Prep Inc. (Elysian, MN, USA).

#### **4.3.2 Sample preparation**

All soybean samples collected from northern (Ekwendeni area) and central (Lobi area) Malawi were well mixed and equally divided prior processing. Soaking was accomplished by adding 60 g of soybean into 200 ml of water (Millipore, Direct-Q). The same experimental bench-top infrared heater (115 volts tubular quartz infrared heating lamp, tungsten wire filament, enclosed in a ceramic casing, Research Inc., USA) used by

Khattab and Arntfield (2009) was applied for infrared heating. The distance between the infrared lamp and heating surface was adjusted to 10 cm. The soaking times were 0.5, 1, 3, 16 h and the infrared heating times were 0, 1.5, 2.5, 3.5 min. Therefore, there were  $4 \times 4 = 16$  processing combinations in total.

After treating with soaking and infrared heating, all samples were freeze dried and ground. A 500  $\mu\text{m}$  sieve was used to sieve the ground soybean. The residue which could not be further ground was well mixed with the part which passed through the sieve. The blank sample was prepared by the same procedures without soaking and infrared heating. Samples were stored at  $-20\text{ }^{\circ}\text{C}$  before analysis.

#### **4.3.3 Determination of soybean water absorption curve**

The water absorption curve was determined by the method reported by Xu and Chang (2008) with modifications. Briefly, 15 g of soybean were soaked in 50 ml water (Millipore, Direct-Q). The soybean seeds were taken out every hour for weighing. Excess water on the soybean seeds was removed with tissue paper. After weighing, soybean was placed in another 50 ml of water (Millipore, Direct-Q). The weight changes were recorded for the first 12 h after soaking commenced. The average moisture content of these samples before soaking was 7.06%. The moisture content of soaked soybean was calculated as follow:

$$\text{Moisture Content (\%)} = \frac{(\text{Soaked weight} - \text{Original weight}) + (\text{Original weight} \times \text{Original moisture content})}{\text{Soaked weight}} \times 100$$

#### 4.3.4 Determination of the trypsin inhibitors

Trypsin inhibitors were determined by the method reported by Hamerstrand, Black, and Glover (1981) with modifications. Briefly, 0.5 g ground soybean was extracted with 25 ml of 0.01 N NaOH. The pH of the mixture was adjusted to around 8.4 with HCl. The tubes containing the mixture were then put on to a wrist action shaker (Burrell Scientific Pittsburgh, PA, USA) for 3h at room temperature. After extraction, the extracts were properly diluted so that they could inhibit 40% - 60% of the trypsin in the following test.

The trypsin solution was prepared by dissolving 0.0004 g trypsin in 200 ml of 0.001 N HCl. The tris-buffer was prepared by dissolving 1.21 g tris(hydroxymethyl) aminomethane and 0.59 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  in 180 ml of distilled water, then adjusting pH to 8.2 with HCl and making up to 200 ml with distilled water. Tris-buffer was pre-warmed to 37 °C prior to use. The BAPA solution was prepared by dissolving 0.080 g BAPA in 2 ml dimethyl sulfoxide and diluting to 200 ml with pre-warmed (37 °C) tris buffer

Five test tubes were required for each determination, one blank, one standard, and three samples test tubes. At the beginning of the test, 2 ml of diluted sample were added to the blank and sample test tubes, while 2 ml of distilled water were added each into the standard test tube. Then, 2 ml of trypsin solution were added into the sample and standard test tubes. The mixtures were then placed into a 37 °C water bath for 10 min. After that, 5 ml of pre-warmed (37 °C) BAPA solution were added to all test tubes. The mixtures were allowed to react for 10 min in the water bath set at 37 °C. The reaction was stopped by adding 1 ml of 30% acetic acid to all test tubes. Then, 2 ml of trypsin solution were added

to the blank. The absorbance of the sample and standard tubes was read against the blank at 410 nm using a spectrophotometer (Thermo Scientific, Genesys 10S UV-Vis spectrophotometer, Columbia, MD, USA). The trypsin inhibitor content was calculated as follow:

$$\text{TI, mg/g of sample} = \frac{\text{Standard tube absorbance} - \text{Sample tube absorbance}}{0.019 \times 1000} \times \text{dilution factor}$$

#### **4.3.5 Determination of hemagglutinating activity of lectins**

The hemagglutinating activity of soybean lectins was determined as reported by Shi (2015) with modifications. Briefly, 1 ml of 0.9% NaCl was added into a micro centrifuge tube containing 0.1 g ground soybean. Then the mixture was placed on to a rotary mixer (Fisher Scientific, Whitby, ON, Canada) for 1 h and the speed was set at 30 rpm. The extraction was accomplished by placing the mixture in the refrigerator (2 – 4 °C) overnight. After that, the mixture was centrifuged (Thermo Scientific, Sorvall Legend Micro 21 Centrifuge, Columbia, MD, USA) at 9600 × g for 10 min and the supernatant was diluted for further analysis. In the first column of a 96 well microplate, 200 µl of diluted supernatant were added (dilution factor 8). Serial dilution was performed with the dilution factor in column 12 reaching 16384. Then, each diluted supernatant was mixed with equal volume (50 µl) of 2% (in 0.9% NaCl) rabbit red blood cell. The mixture was reacted for 2 h on a Belly Dancer (Stovall Life Science Incorporated, The Belly Dancer®, Greensboro, NC, USA) at room temperature. The hemagglutinating condition of each well was checked under a microscope (Fisher Scientific, Whitby, ON, Canada). The content of each well was well mixed and transferred onto a microscope slide. If at least five red blood cells were observed to be binding to each other, the result was considered

as positive. The most diluted extract which could give a positive result was considered to contain 1 hemagglutinating unit. The hemagglutinating activity was then calculated as follow:

$$\text{HU/mg} = \frac{\text{Highest dilution factor with positive results} \times S}{V}$$

where

S = Volume of 0.9% NaCl used in original extraction (ml) / weight of ground soybean (mg)

V = Volume of extract mixed with 2% red blood cell, in this study it was 0.05 ml.

#### **4.3.6 Extraction and determination of oligosaccharides**

Oligosaccharides were extracted using the method reported by Landry, Fuchs, and Hu (2016) with modifications. Briefly, 1.8 ml 50% (v/v) ethanol was added into a micro centrifuge tube containing 40 mg ground soybean. Then, 0.1 ml of adonitol (10 mg/ml) was added as internal standard. The extraction was accomplished by mixing the sample on a rotary mixer (Fisher Scientific, Whitby, ON, Canada) for 1 h at the speed of 30 rpm. After extraction, the sample was centrifuged (Thermo Scientific, Sorvall Legend Micro 21 Centrifuge, Columbia, MD, USA) at  $9600 \times g$  for 10 min. The supernatant was collected and mixed with an equal volume (1 ml) of 95% (v/v) ice cold ethanol. After that, the sample was stored at  $-20\text{ }^{\circ}\text{C}$  for 1 h to precipitate proteins. Then, the sample was centrifuged at  $9600 \times g$  for 10 min. The supernatant was collected and dried with nitrogen at  $60\text{ }^{\circ}\text{C}$  (Thermo Scientific, Reacti-Therm I #TS-18822 Heating Module, Columbia, MD,

USA). The residue was then dissolved in 1 ml of water (Millipore, Direct-Q) and filtered with 0.45  $\mu\text{m}$  nylon filter (Pall Life Sciences, Nylon Acrodisc® 4) into vials.

To identify the oligosaccharides in the soaking water, the same extraction method was used as described before. The only difference was that 40 mg of ground sample was replaced by 40  $\mu\text{l}$  of soaking water.

The quantification of oligosaccharides was achieved by using a high-performance liquid chromatography (HPLC, Waters AcQuity® Arc™ HPLC) equipped with an evaporative light scattering (ELS) detector (Waters 2424 ELS detector). The sample was kept at room temperature during the analysis. The injection volume was 10  $\mu\text{l}$ . The column (Luna® 5  $\mu\text{m}$  NH<sub>2</sub> 100 Å, LC Column 250  $\times$  4.6 mm Ea) temperature was set at 40°C. As for mobile phase, solvent A was acetonitrile and solvent B was pure water. The flow rate was 3 mL/min with 80% solvent A and 20% solvent B. The total running time for each sample was 15 min.

#### **4.3.7 Extraction and determination of isoflavones**

Free isoflavones were extracted as reported by Achouri, Boye, and Belanger (2005) with modifications. Briefly, 0.2 g of ground soybean was well mixed with 1.5 ml hexane for defatting. Then, the mixture was placed onto a rotary mixer (Fisher Scientific, Whitby, ON, Canada) for 15 min at the speed of 30 rpm. Following mixing, the mixture was centrifuged at 9600  $\times$  g for 10 min and the supernatant was discarded. Then, 1 ml of 80% (v/v) methanol was added to the residue and mixed well. The mixture was then sonicated (Branson, 5510) for 15 min and centrifuged at 9600  $\times$  g for 10 min. The residues from the centrifuge were extracted again with the same procedure. The

supernatants were combined and dried at 38 °C with nitrogen. Then, the residue was dissolved in 500 µl of 50% (v/v) methanol and filtered with a 0.45 µm nylon filter (Pall Life Sciences, Nylon Acrodisc® 4) into a vial.

Bound isoflavones were extracted with the method reported by Chen, et al. (2016) with modifications. Briefly, 0.2 g of ground soybean were thoroughly mixed with 4 ml of 2 M NaOH and placed in refrigerator (2 - 4°C) overnight. Then, the pH was adjusted to 1 - 2 with HCl. The mixture was centrifuged (Thermo Scientific, Sorvall RC6+ Centrifuge, Columbia, MD, USA) at  $16770 \times g$  for 10 min. The supernatant was collected and defatted twice with 5 ml hexane. After that, the sample was extracted three times with 5 ml ethyl acetate. All extracts were combined and dried at 38 °C with nitrogen (Thermo Scientific, Reacti-Therm I #TS-18822 Heating Module, Columbia, MD, USA). The residue was dissolved in 0.5 ml of 50% methanol and filtered with a 0.45 µm nylon filter (Pall Life Sciences, Nylon Acrodisc® 4) into a vial.

Isoflavone extracts were analyzed with an HPLC (Waters 2695) equipped with a photodiode array detector (PAD, Waters 2996). A column of 100 × 3 mm with particle size of 26 µm was used (Thermo Scientific, Accucore aQ, Columbia, MD, USA). The sample temperature was set at 15 °C and column temperature was 35 °C. The injection volume was 3 µL. Solvent A was 0.1% acetic acid in water and solvent B was 0.1% acetic acid in methanol. The flowrate was 0.5 ml/min and total run time was 25 min. The gradient was as follows: 0-3.81 min, 9-14% B; 3.81-4.85 min, 14-15% B; 4.85-5.89 min, 15% B; 5.89-8.32 min, 15-17% B; 8.32-9.71 min, 17-19% B; 9.71-10.40 min, 19% B; 10.40-12.48 min, 19-26% B; 12.48-13.17, 26-28% B; 13.17-14.21 min, 28-35% B;

14.21-15.95 min, 35-40% B; 15.95-16.64 min, 40-48% B; 16.64-18.37 min, 48-53% B; 18.37-22.53 min, 53-70% B; 22.53-22.88 min, 70-9% B; 22.88- 25.00 min, 9% B.

Identification of each isoflavone was accomplished by comparing with external standards. For the isoflavones whose external standards were not available, they were identified by their mass spectra. The mass spectrometer (Waters, Micromass Q-ToF micro) was operated in positive mode.

#### **4.3.8 Extraction and determination of fatty acids**

Fatty acid methyl esters (FAMES) were extracted with the one-step method as reported by Lepage and Roy (1986) with modifications. Briefly, 0.2 g ground soybean were thoroughly mixed with 2 ml of methanol/toluene mixture (methanol: toluene = 4:1, v/v) in a glass test tube. 0.1 ml of heptadecanoic acid (C17:0, 10 mg/ml in chloroform) was added as internal standard. Then 0.2 ml of acetyl chloride was slowly added with continuous vortexing. The test tube was flashed with nitrogen to prevent oxidation of fatty acids. After that, the test tube was put into a dry bath (80 °C) for 1 h and cooled down to room temperature. Five ml of 6% K<sub>2</sub>CO<sub>3</sub> solution were added into the test tube and well vortexed. The mixture was separated by letting it sit for 15 min. The top layer was transferred into a micro centrifuge tube and centrifuged (Thermo Scientific, Sorvall Legend Micro 21 Centrifuge, Columbia, MD, USA) at 9600 × g for 10 min. After that, the supernatant was transferred into a 2 ml vial with 500 µl insert.

Soybean FAMES were analyzed with a gas chromatograph (Agilent Technologies, Varian 450, Santa Clara, CA, USA) equipped with a flame ionization detector (FID). The column used was Agilent DB225-MS column (30 m × 0.25 mm diameter and 0.25 µm

film thickness). The injection volume was 1  $\mu$ l. Total running time for each sample was 46.4 min. The sample split ratio was 20:1. The column temperature was set as follows: started at 70°C, held for 1 min; raised to 180°C at 25°C/min, held for 1 min; raised to 200°C at 10°C/min, held for 1 min; raised to 220°C at 2°C/min, held for 6 min; raised to 240°C at 20°C/min, and finally held for 20 min. Hydrogen was the carrier gas and the flow rate was 1.3 ml/min. Fatty acids were identified by comparing with known standards and quantified using an internal standard.

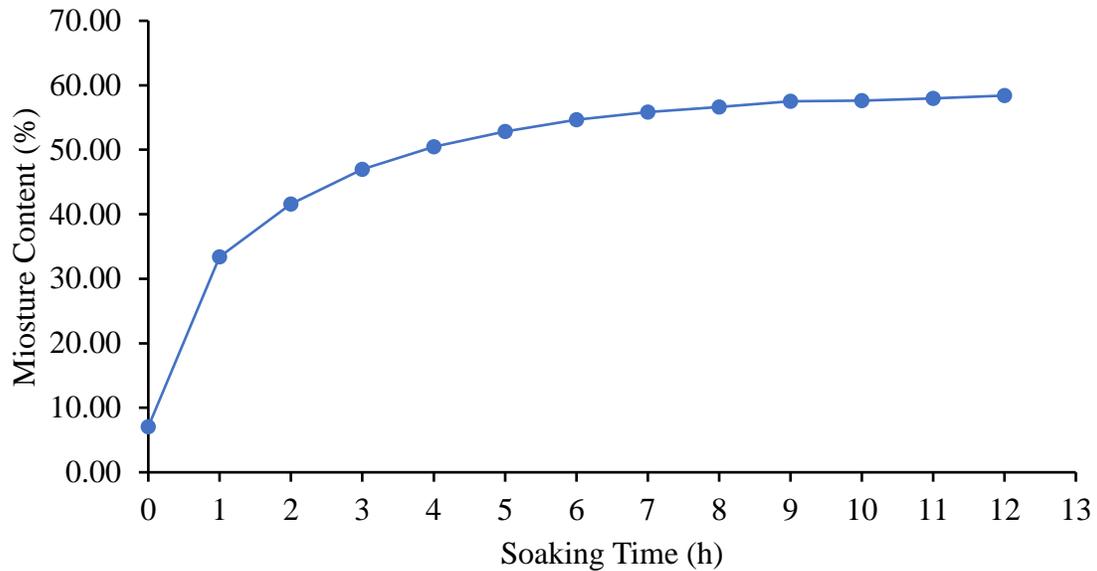
#### **4.3.9 Data analysis**

The treatments were duplicated and all components were measured in triplicate. SAS University Edition was used in data analysis. Two-way analysis of variance (ANOVA) was applied to investigate the effects of soaking and infrared heating. Means were compared with Tukey's test in one-way ANOVA.

### **4.4 Results and Discussion**

#### **4.4.1 Soybean water absorption curve**

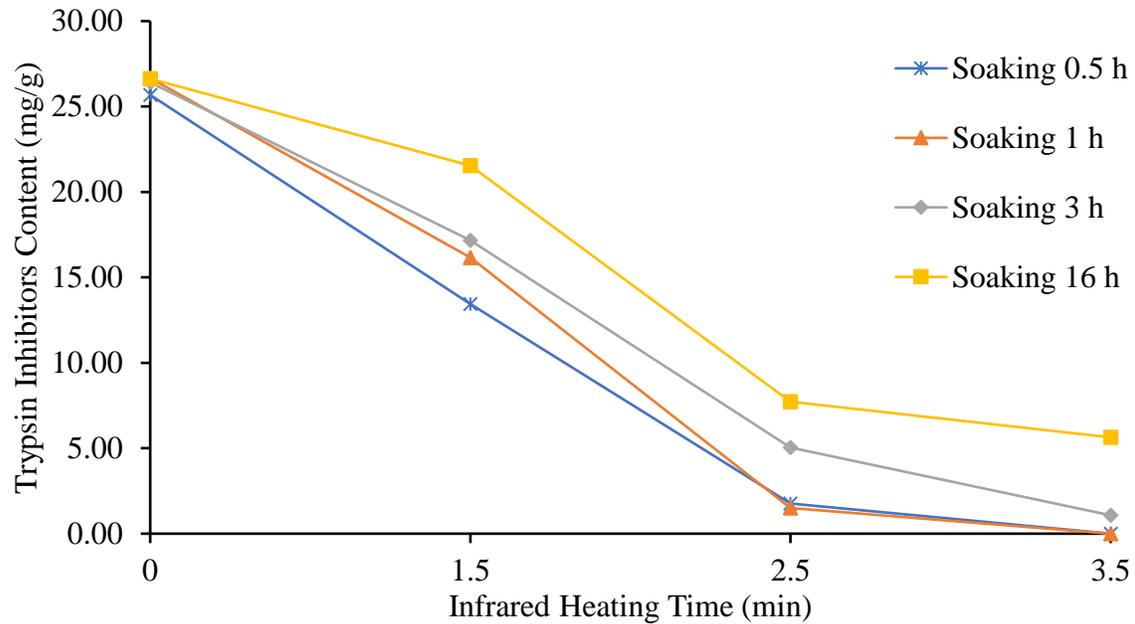
The soybean water absorption curve is presented in Figure 4.1. It can be seen that soybean seeds were almost fully hydrated after soaking for 12 h. The moisture contents of soybean after soaking for 0.5, 1 and 3h were around 20%, 33% and 47%. Soaking for 16 h was considered as fully hydrated in this study. Therefore, the soaking time in this study was chosen to be 0.5, 1, 3 and 16 h.



**Figure 4.1** Soybean water absorption curve.

#### **4.4.2 Effects of processing on contents of soybean trypsin inhibitors**

The effects of soaking and infrared heating on soybean trypsin inhibitors are shown in Figure 4.2. Based on the figure, for each soaking level, the trypsin inhibitors were shown to decrease concomitantly with an increase of infrared heating time. It was evident that following soaking soybean samples for less than 1 h, infrared heating for 3.5 min caused complete elimination of trypsin inhibitors. If the soaking time was longer than 1 h, part of the trypsin inhibitors remained in the sample after infrared heating for 3.5 min. When infrared heating time was similar, longer soaking time caused less reduction in contents of trypsin inhibitors. This was due to the differences in the moisture contents. When infrared heating was the same, higher moisture content caused less increase in the sample temperature, which led to less loss of trypsin inhibitors.



**Figure 4.2** Effects of soaking and infrared heating on contents of soybean trypsin inhibitors (dry basis).

The significance of the effects is summarized in Table 4.1. Soaking, infrared heating and their interactions showed significant ( $p < 0.05$ ) effects on contents of soybean trypsin inhibitors. A significant interaction means that the effects of these two factors were dependent on the levels of each other. For 1.5 min of infrared heat, sample soaked for 1 h had higher levels of trypsin inhibitors than sample soaked for 0.5 h. However, with longer heating times, the 0.5 and 1 h soaking time were equally effective. The 3 and 16 h soaking times were less effective at all heating times.

**Table 4.1** P value from two-way ANOVA indicating the significance of effects from soaking and infrared heating on trypsin inhibitors contents in soybean.

Compound	Factor		
	Soaking	Infrared heating	Soaking × Infrared heating
Trypsin inhibitors	< 0.0001*	< 0.0001*	< 0.0008*

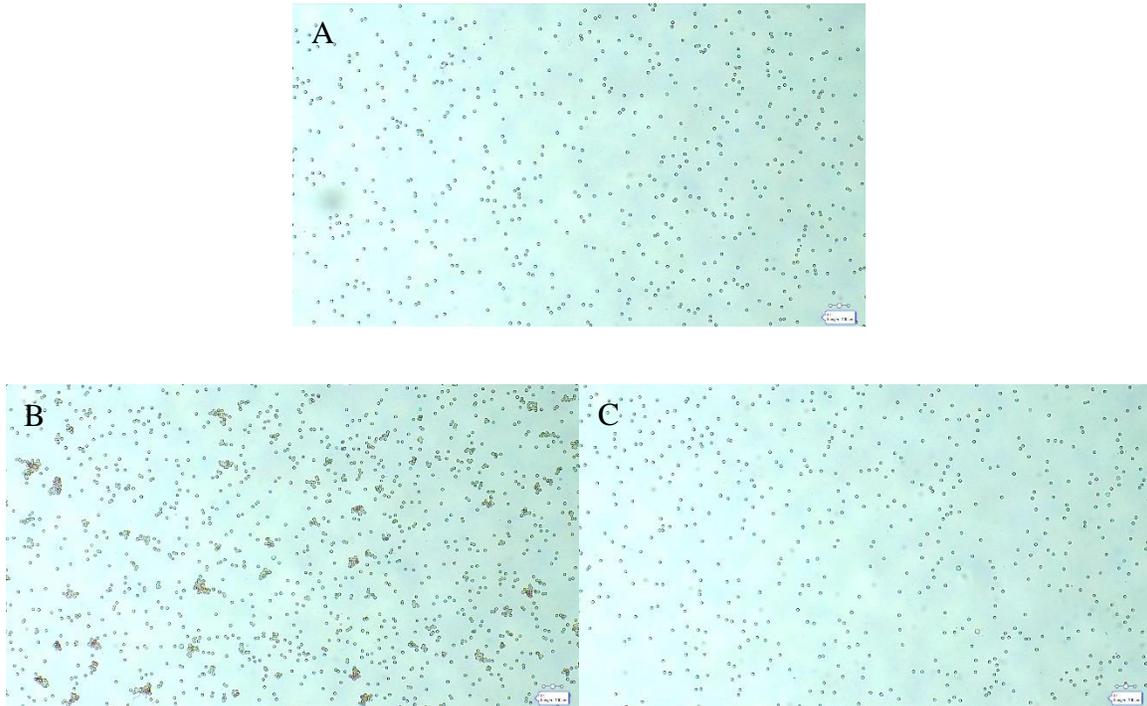
\* The effect was significant ( $p < 0.05$ )

Chen (2015) reported that treating soybean with higher infrared temperature can cause lower activity of trypsin inhibitors, which is in agreement with the results of the current study. According to Yalcin and Basman (2015), both longer infrared heating and soaking time can lead to more reduction in activity of soybean trypsin inhibitors. Their results partially agree with the findings of the current study, where longer soaking time had opposite effects. Possible reasons for such variation could be the difference in sample treatments and detection methods. In the study by Khattab and Arntfield (2009), where exactly the same infrared heater was used, 92.6% (2.5 min), 88.8% (3.0 min) and 94.3% (2.5 min) trypsin inhibitors were deactivated in Canadian cowpea, kidney bean and pea. Results from the current study were quite similar to their findings.

#### **4.4.3 Effects of processing on soybean lectins activity**

The blank, negative and positive results from hemagglutinating assays are shown in Figure 4.3. In both blank and negative samples, rabbit red blood cells were observed to exist individually. However, for the positive sample, some of the cells were binding together and forming clusters, which were evenly distributed as could be viewed in the photographs. The reason for such phenomenon was that lectins can bind to the

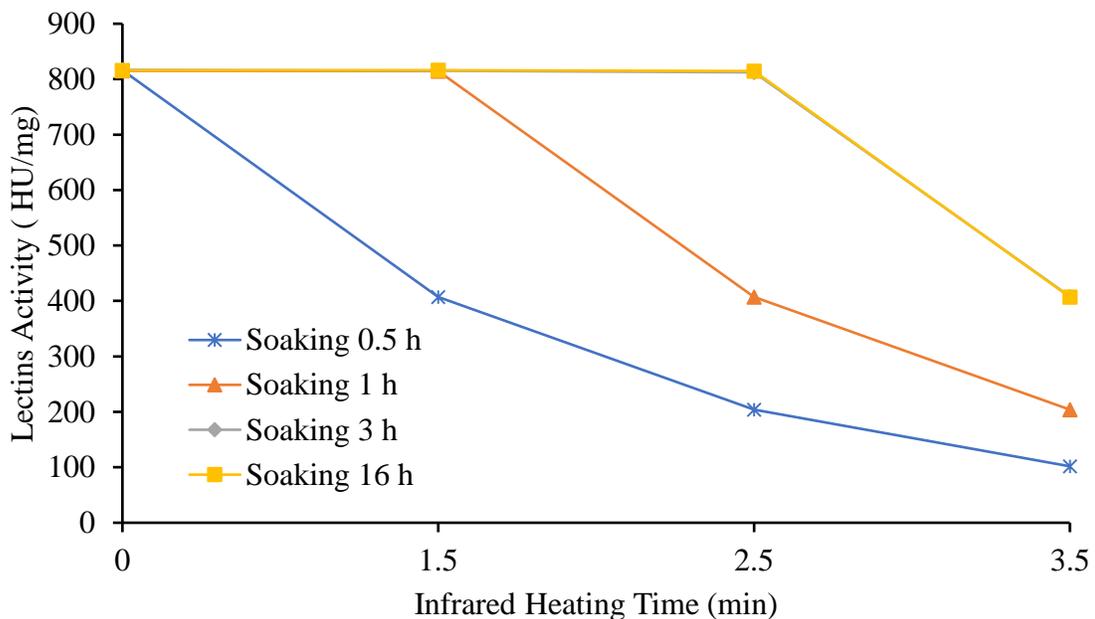
carbohydrate moiety (eg. glycoproteins or glycolipids) on the cell surfaces (De Mejía & Prisecaru, 2005).



**Figure 4.3** Representative results from hemagglutinating assay, A is the rabbit red blood cell (1% in 0.9% NaCl), B is one sample that gave positive results (dilution factor of the sample was 64, some of the rabbit red blood cell are binding together), C is one sample that gave negative results (same sample as in photograph B, where dilution factor was 8192, and rabbit red blood cells were not binding together). The length of the scale labelled in the pictures is 100  $\mu\text{m}$ . Photographs were taken by a microscope camera (Motic®, Moticam 1080 HDMI & USB, BC, Canada).

The lectin activities of processed soybeans are presented in Figure 4.4. For soybean soaked for 0.5 h, infrared heating caused the deactivation of lectins. For soybean

soaked for 1 h, the loss of lectins activity showed up after infrared heating for longer than 1.5 min. However, for soybean soaked for 3 h and 16 h, the deactivation was found when infrared heating time was longer than 2.5 min. When soaking time was 0.5 h, the lectin activity tended to decrease with longer infrared heating time. However, longer soaking time led to reduction of such effects, with the exception that the changes in lectin activity of soybean soaked for 3 h and 16 h were close to each other. For all soaking levels, lectins were not completely deactivated even with the highest infrared heating level. According to De Mejía and Prisecaru (2005), even though lectins are not heat stable, they may not be destroyed by gentle cooking (eg. low temperature or short cooking time). This could be the reason why lectins were not completely deactivated in current study. If the target was to eliminate lectins in soybean completely, infrared heating more than 3.5 min would become necessary.



**Figure 4.4** Effects of soaking and infrared heating on soybean lectins activity (dry basis, results of soaking for 3 h and soaking for 16 h are overlapping).

ANOVA results suggested that effects of soaking, infrared heating and their interactions were highly significant ( $p < 0.0001$ ) (Table 4.2). Significant interactions mean that the effects of these two factors were dependent on each other. Lower activity levels were seen for the 0.5 h soaking time at all heating times; however, the 1 h heating time was only more effective at reducing lectin activity at heating times of 2.5 min and longer and similar samples soaked for 3 and 16 h behaved the same and only showed reduction in lectin activity if the 3.5 min heating time was used.

**Table 4.2** P values from two-way ANOVA indicating the significance of effects from soaking and infrared heating on lectins activity in soybean.

Compound	Factor		
	Soaking	Infrared heating	Soaking $\times$ Infrared heating
Lectins	$< 0.0001^*$	$< 0.0001^*$	$< 0.0001^*$

\* The effect was significant ( $p < 0.05$ )

#### 4.4.4 Effects of processing on contents of soybean oligosaccharides

Average contents of oligosaccharides in processed soybean are summarized in Table 4.3. The major oligosaccharides in soybean are sucrose, followed by stachyose and raffinose. For sucrose contents, there were significant ( $p < 0.05$ ) reductions in the samples soaked for 16 h. Such phenomenon was also observed in terms of raffinose. Even though the stachyose contents of soybean were lower after soaking, the values were not significantly ( $p > 0.05$ ) different. This could be partially explained by the relatively higher content of sucrose and its smaller molecule size.

**Table 4.3** Average sucrose, raffinose and stachyose contents (mg/g, dry basis) of processed (soaked and infrared heated) soybeans as affected by different soaking time.

Soaking Time (h)	Sucrose (mg/g)	Raffinose (mg/g)	Stachyose (mg/g)
0	62.50 ± 3.10 <sup>a</sup>	4.60 ± 0.11 <sup>a</sup>	36.91 ± 1.55 <sup>a</sup>
0.5	57.48 ± 1.09 <sup>a</sup>	3.85 ± 0.53 <sup>bc</sup>	35.00 ± 1.70 <sup>a</sup>
1	58.40 ± 0.59 <sup>a</sup>	4.03 ± 0.17 <sup>ac</sup>	34.76 ± 1.42 <sup>a</sup>
3	57.50 ± 1.31 <sup>a</sup>	3.93 ± 0.31 <sup>ac</sup>	34.54 ± 1.23 <sup>a</sup>
16	50.41 ± 0.77 <sup>b</sup>	3.81 ± 0.25 <sup>bc</sup>	31.82 ± 0.52 <sup>a</sup>

<sup>a</sup> Value are calculated in dry basis and expressed as mean ± standard deviation. Data in each column with different letters are significant different ( $p < 0.05$ ).

The significances of each effects are presented in Table 4.4. Soaking significantly ( $p < 0.05$ ) affected the contents of sucrose and stachyose. Infrared heating showed no significant effects on all oligosaccharides, which was expected since oligosaccharides are relatively heat stable. The interactions of soaking and infrared heating only significantly affected the content of raffinose. It is necessary to mention that soaking showed significant effects on the stachyose content in two-way ANOVA, but the means were not significantly different from one-way ANOVA.

**Table 4.4** P values from two-way ANOVA indicating the significance of effects from soaking and infrared heating on oligosaccharides content in soybean.

Compound	Factor		
	Soaking	Infrared heating	Soaking × Infrared heating
Sucrose	< 0.0001*	0.5912	0.9908
Raffinose	0.0921	0.2902	0.0142*
Stachyose	0.0447*	0.2358	0.9970

\* The effect was significant ( $p < 0.05$ )

According to Egounlety and Aworh (2003), soaking soybean for 12-14 h can lead to 26.68%, 25.41% and 20.23% reduction in contents of sucrose, raffinose and stachyose. Similar but less reductions were observed in the current study. However, only the decrease in sucrose and raffinose were significant ( $p < 0.05$ ).

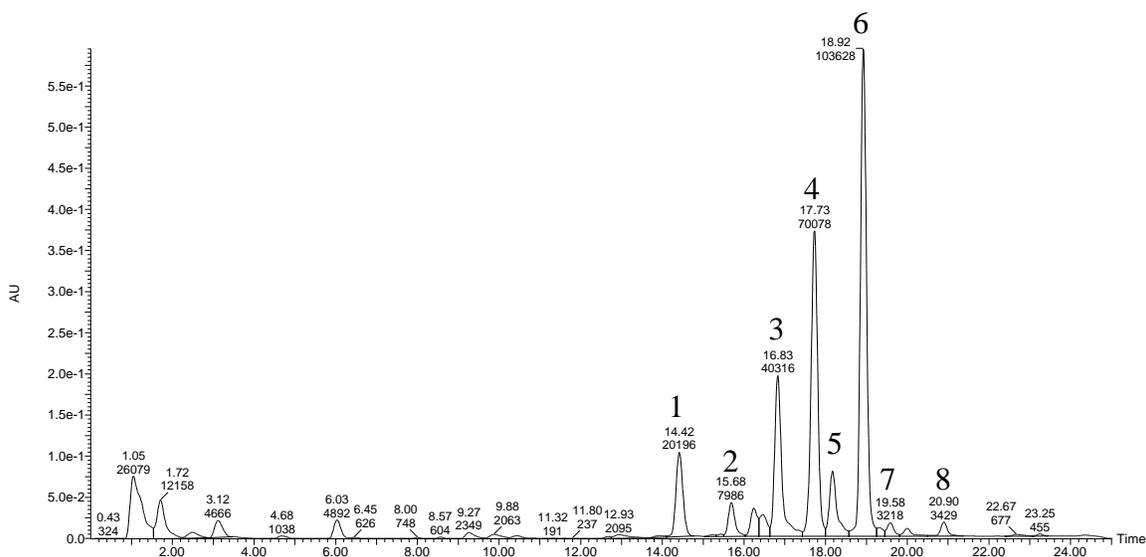
The soaking water (0.5h, 1h, 3h and 16h) was also examined for oligosaccharides (Table 4.5). Interestingly, only fructose was detected in the 0.5h- and 1h soaking water. Besides, only fructose and galactose were found in 3h- and 16h soaking water, with the former being found as predominant oligosaccharide.

**Table 4.5** Types of oligosaccharides found in soybean soaking water.

Soaking Time (h)	Oligosaccharides Detected
0.5	Fructose
1	Fructose
3	Fructose, Galactose
16	Fructose, Galactose

#### 4.4.5 Effects of processing on contents of free soybean isoflavones

A representative chromatographs of free soybean isoflavones is presented in Figure 4.5. In total, there were 8 types of isoflavones found in the soybean samples, which included daidzin, glycitin, genistin, malonyldaidzin, malonylglycitin, malonylgenistin, daidzein and genistein. Among those isoflavones, negative numbers were obtained after calculating contents of glycitin, daidzein and genistein. For other which were quantified, the highest to lowest contents were observed in malonylgenistin, malonyldaidzin, genistin, daidzin and malonylglycitin.



**Figure 4.5** Typical HPLC chromatograph of soybean free isoflavones (wavelength 254 nm), peak 1 is daidzin, peak 2 is glycitin, peak 3 is genistin, peak 4 is for malonyldaidzin, peak 5 is malonylglycitin, peak 6 is malonylgenistin, peak 7 is daidzein and peak 8 is genistein.

The contents of free soybean isoflavones of processed soybean with the same soaking or infrared heating level are summarized in Table 4.6. For raw dried soybean, the contents of malonylgenistin, malonyldaidzin, genistin, daidzin and malonylglycitin were 0.85, 0.66, 0.32, 0.17, 0.09 mg/g, respectively. Thus, malonylgenistin, malonyldaidzin and genistin were the predominant isoflavones in soybean, with their contents making up over 87% of the total isoflavones. For daidzin, both soaking and infrared heating led to significant ( $p < 0.05$ ) increase. For genistin, 3 h (or less) of soaking and infrared heating can lead to significant increase. In terms of malonyldaidzin and malonylglycitin, soaking had no significant effect on their contents, while infrared heating led to a significant decrease. In the case of malonylgenistin content, soaking for 0.5 h and infrared heating for 2.5 min (or more) caused a significant decrease.

**Table 4.6** Average free isoflavones contents (dry basis) of processed (soaked and infrared heated) soybean treated with the same soaking or infrared heating level.

		Daidzin (mg/g)	Glycitin (mg/g)	Genistin (mg/g)	Malonyldaidzin (mg/g)	Malonylglycitin (mg/g)	Malonylgenistin (mg/g)	Daidzein (mg/g)	Genistein (mg/g)
Soybean with same soaking level	0 h	0.17 ± 0.00 <sup>b</sup>	N/A*	0.32 ± 0.00 <sup>c</sup>	0.66 ± 0.00 <sup>a</sup>	0.09 ± 0.00 <sup>a</sup>	0.85 ± 0.00 <sup>ab</sup>	N/A	N/A
	0.5 h	0.26 ± 0.06 <sup>a</sup>	N/A	0.42 ± 0.08 <sup>ab</sup>	0.64 ± 0.10 <sup>a</sup>	0.04 ± 0.04 <sup>a</sup>	0.83 ± 0.07 <sup>b</sup>	N/A	N/A
	1 h	0.26 ± 0.05 <sup>a</sup>	N/A	0.44 ± 0.07 <sup>a</sup>	0.67 ± 0.10 <sup>a</sup>	0.06 ± 0.04 <sup>a</sup>	0.88 ± 0.09 <sup>ab</sup>	N/A	N/A
	3 h	0.26 ± 0.05 <sup>a</sup>	N/A	0.42 ± 0.06 <sup>ab</sup>	0.71 ± 0.04 <sup>a</sup>	0.06 ± 0.03 <sup>a</sup>	0.90 ± 0.04 <sup>ab</sup>	N/A	N/A
	16 h	0.24 ± 0.03 <sup>a</sup>	N/A	0.38 ± 0.04 <sup>bc</sup>	0.71 ± 0.07 <sup>a</sup>	0.05 ± 0.03 <sup>a</sup>	0.92 ± 0.08 <sup>a</sup>	N/A	N/A
Soybean with same infrared heating level	0 min	0.19 ± 0.02 <sup>C</sup>	N/A	0.34 ± 0.02 <sup>C</sup>	0.73 ± 0.05 <sup>A</sup>	0.08 ± 0.04 <sup>A</sup>	0.92 ± 0.06 <sup>A</sup>	N/A	N/A
	1.5 min	0.24 ± 0.04 <sup>B</sup>	N/A	0.40 ± 0.04 <sup>B</sup>	0.72 ± 0.05 <sup>AB</sup>	0.05 ± 0.02 <sup>AB</sup>	0.91 ± 0.05 <sup>AB</sup>	N/A	N/A
	2.5 min	0.28 ± 0.01 <sup>AB</sup>	N/A	0.44 ± 0.04 <sup>A</sup>	0.64 ± 0.06 <sup>BC</sup>	0.04 ± 0.03 <sup>AB</sup>	0.84 ± 0.06 <sup>BC</sup>	N/A	N/A
	3.5 min	0.30 ± 0.04 <sup>A</sup>	N/A	0.47 ± 0.06 <sup>A</sup>	0.62 ± 0.10 <sup>C</sup>	0.03 ± 0.03 <sup>B</sup>	0.83 ± 0.09 <sup>C</sup>	N/A	N/A

\* N/A: Negative numbers were get after calculation.

<sup>a</sup> Value are calculated in dry basis and expressed as mean ± standard deviation. Data in each column with different letters are significant different (p < 0.05).

According to Kudou, et al. (1991), the major isoflavones in soybean exist in malonyl forms, which are heat unstable and can convert to their corresponding glycosides. Furthermore, Niamnuy, Nachaisin, Poomsa-ad, and Devahastin (2012) found that higher infrared temperature and longer heating time can promote conversion of malonylgenistin and acetylgenistin to genistin and genistein. After boiling dehulled soybean in water for 20 min, Wang and Murphy (1996) observed a decrease in contents of daidzin, genistin, glycitin, malonyldaidzin, malonylgenistin and malonylglycitin. As for the effect of soaking, Toshiya, Sakamoto, Takayanagi, and Yokotsuka (2000) concluded that both contents of malonyl and acetyl types isoflavones were decreased while contents of aglycones were increased. Such conversions were reported to be caused by  $\beta$ -glucosidases, since conversions were not happen when inhibitors of  $\beta$ -glucosidases are added during soaking process (Matsuura, Obata, & Fukushima, 1989).

In the current study, both soaking and infrared heating led to significant increase in daidzin and genistin. Besides, infrared heating caused a significant decrease in the contents of malonyldaidzin, malonylglycitin and malonylgenistin. Therefore, based on previous studies, such increase in the content of daidzin and genistin could result following conversions from malonyldaidzin and malonylgenistin. (Kudou, et al., 1991; Matsuura, Obata, & Fukushima, 1989; Niamnuy, Nachaisin, Poomsa-ad, & Devahastin, 2012; Toshiya, Sakamoto, Takayanagi, & Yokotsuka, 2000).

Though heating or soaking may cause the interconversions between soybean isoflavones, it is illogical to only look at the change in their contents without considering other components in the food matrix. As one of the major components in soybean, proteins can interact with other components. According to Siebert, Troukhanova, and

Lynn (1996), proteins can combine with polyphenols and form soluble complexes. Nufer, Ismail, and Hayes (2009) reported that protein may protect isoflavones from degradation, where its content and level of denaturation can affect the extractability of isoflavones. Furthermore, they also explained that denaturation of proteins may promote the extraction efficiency of isoflavones, since the protein bound isoflavones are exposed. Thus, in the current study, the changes in content of individual isoflavones may not only come from interconversions, but may also be caused by the enhancement in extraction efficiency after proteins were denatured.

The p values of the effects of soaking and infrared heating on soybean free isoflavones are presented in Table 4.7. Soaking significantly ( $p < 0.05$ ) affected the contents of genistin, malonyldaidzin and malonylgenistin. Infrared heating showed significant ( $p < 0.05$ ) effects on all quantified isoflavones, however, the p value in the case of malonylglycitin was very close to 0.05. The interactions of the two factors had no significant effects on all quantified isoflavones.

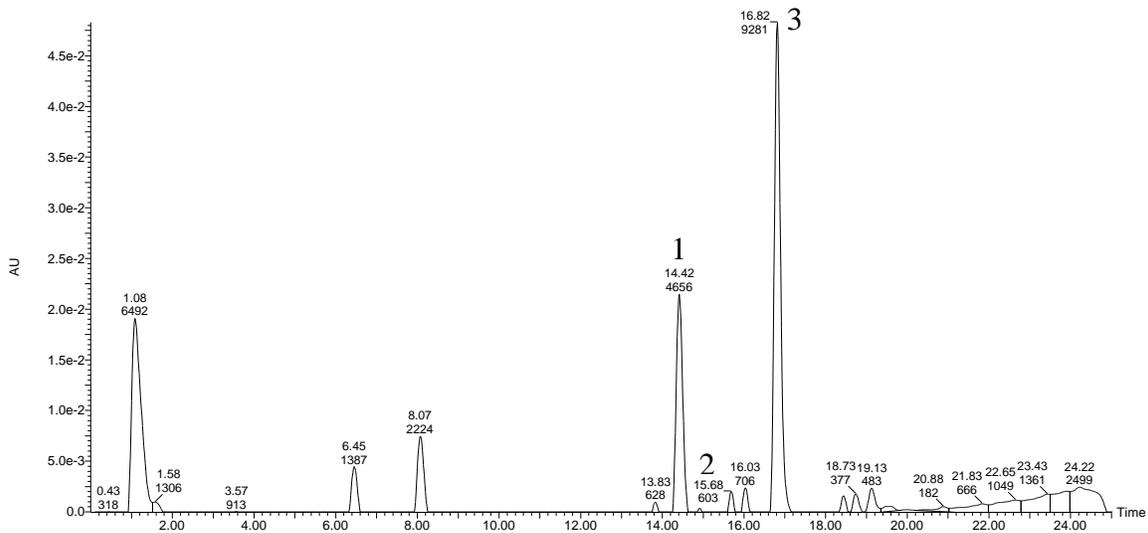
**Table 4.7** P values from two-way ANOVA indicating the significance of effects from soaking and infrared heating on free isoflavone contents in soybean.

Compound	Factor		
	Soaking	Infrared heating	Soaking × Infrared heating
Daidzin	0.2400	< 0.0001*	0.1817
Genistin	0.0116*	< 0.0001*	0.1014
Malonyldaidzin	0.0307*	0.0007*	0.1176
Malonylglycitin	0.7024	0.0496*	0.9501
Malonylgenistin	0.0177*	0.0015*	0.3644

\* The effect was significant ( $p < 0.05$ )

#### 4.4.6 Effects of processing on contents of bound soybean isoflavones

Soybean bound isoflavones were also examined and the typical chromatograph is presented in Figure 4.6. The isoflavones found in bound form included daidzin, glycitin and genistin. Daidzin and genistin could be quantified, while a negative value was obtained when calculating glycitin content. Genistin was the predominant bound isoflavone. Compared with total free isoflavones (average for unprocessed soybean was 2.09 mg/g), the content of total bound isoflavones (average for unprocessed soybean was 0.19 mg/g) was much less. Thus, the majority of the isoflavones in soybean exist in free form (Kim, et al., 2016). In raw dried soybean, the contents of bound genistin and daidzin were 0.14 and 0.05 mg/g. The bound genistin content of processed soybean ranged from 0.32 to 0.58 mg/g, while that range for bound daidzin was from 0.12 mg/g to 0.25 mg/g.



**Figure 4.6** Typical HPLC chromatograph of soybean bound isoflavones (wavelength 254 nm), peak 1 is daidzin, peak 2 is glycitin, peak 3 is genistin.

Results from two-way ANOVA are presented in Table 4.8. Soaking, infrared heating and their interaction had nonsignificant ( $p > 0.05$ ) effects on quantified bound isoflavones. There was agreement with the results from one-way ANOVA.

**Table 4.8** P value from two-way ANOVA indicating the significance of effects from soaking and infrared heating on bound isoflavones in soybean.

Compound	Factor		
	Soaking	Infrared heating	Soaking $\times$ Infrared heating
Bound daidzin	0.4667	0.0521	0.9909
Bound genistin	0.4312	0.0932	0.9982

\* The effect was significant ( $p < 0.05$ )

#### 4.4.6 Effects of processing on soybean fatty acids profile

The major fatty acids in soybean were C16:0, C18:0, C18:1, C18:2 and C18:3, with percentage of 11.08, 3.21, 19.20, 57.43 and 8.59 in total fatty acids. These five acids

made up over 98% of the total fatty acids. The results were in close agreement to the findings by Slavin, et al. (2009). Among the soybeans that were only treated with soaking in the current study, similar situation was observed, except for the soybean soaked for 1 h

The significant effects of soaking and infrared heating on soybean fatty acid profiles are summarized in Table 4.9. Both soaking and infrared heating did not significantly ( $p < 0.05$ ) affect soybean total saturated/unsaturated fatty acids. Neither soaking nor infrared heating significantly ( $p < 0.05$ ) affected any major individual fatty acids. Prinyawiwatkul, Beuchat, McWatters, and Phillips (1996) found a nonsignificant drop in total unsaturated fatty acids after soaking cowpea. However, according to Padmashree, et al. (2016), infrared heating can significantly decrease the unsaturated fatty acids content in mung bean. Such variation could be caused by differences in strength of infrared heating applied and type of study materials.

**Table 4.9** P values from two-way ANOVA indicating the significance of effects from soaking and infrared heating on fatty acid profiles in soybean.

Compound	Factors		
	Soaking	Infrared Heating	Soaking $\times$ Infrared Heating
Saturated Fatty Acids	0.4117	0.2979	0.4028
Unsaturated Fatty Acids	0.4117	0.2979	0.4028
C16:0	0.7222	0.6135	0.5529
C18:0	0.4317	0.0877	0.6156
C18:1	0.1270	0.5717	0.2311
C18:2	0.4755	0.4945	0.4664
C18:3 n3	0.7113	0.2901	0.8005

## 4.5 Conclusion

In the current study, the effects of soaking and infrared heating on soybean trypsin inhibitors, lectins, oligosaccharides, isoflavones and fatty acid profiles were investigated. Infrared heating significantly ( $p < 0.05$ ) decreased the contents of soybean trypsin inhibitors and lectins. Longer infrared heating time led to better reduction of trypsin inhibitors and lectins, while soaking reduced the effects of infrared heating. Sucrose, stachyose and raffinose were the major oligosaccharides in soybean. Soaking for 16 h can led to significant decrease in contents of sucrose and raffinose, but not stachyose. Soaking was not able to remove all the soybean flatulence-causing oligosaccharides, and the amounts of these oligosaccharides were still considerable after processing. The major free isoflavones quantified in soybean were malonylgenistin, malonyldaidzin, genistin, daidzin and malonylglycitin. Daidzin and genistin were also found in bound form. Soaking led to a significant increase in contents of daidzin and genistin. Infrared heating led to a significant increase in contents of daidzin and genistin, while a significant decrease in malonyldaidzin, malonylglycitin and malonylgenistin was observed. Therefore, soaking and infrared heating led to the conversions from malonylgenistin and malonyldaidzin to genistin and daidzin. The major fatty acids include, C16:0, C18:0, C18:1, C18:2 and C18:3, which make up over 98% of the total fatty acids. Neither soaking nor infrared heating can significantly affect the soybean fatty acid profile. In conclusion, infrared heating could be applied to eliminate soybean trypsin inhibitors and lectins. Soaking was not able to remove all soybean flatulence-causing oligosaccharides. Soaking and infrared heating led to the conversions between soybean isoflavones, but they had no effects on fatty acids.

## **Overall Conclusion**

The first part of this thesis mainly focused on how geographic region and seed type affect basic nutrients, trypsin inhibitors, lectins and flatulence-causing oligosaccharides in soybean collected from Malawi. The results pointed out that hybrid seed is more suitable for oil extraction in central Malawi. Local seed could be a better choice for higher protein yield. However, the higher total RFOs content may put barriers in the utilization of local seed. Growing soybean in north or central Malawi will not lead to significant differences in major nutrients including ash, protein, lipids and carbohydrates. Soybeans grown in north and central Malawi were not significantly different in contents of anti-nutritional factors, except for raffinose. These findings can serve as references for the farmers in Malawi when deciding the type of soybean seed to use and region to grow.

The second part of this thesis focused on how soaking and infrared heating change the contents of trypsin inhibitors, lectins, oligosaccharides, isoflavones and fatty acids in soybean. Results from this part suggested that infrared heating was effective in deactivating soybean trypsin inhibitors and lectins, especially with longer treating time and lower moisture content. Soaking was not able to remove flatulence-causing oligosaccharides completely from soybean. Soaking and infrared heating can lead to the conversion of malonylgenistin and malonyldaidzin to genistin and daidzin. Neither soaking nor infrared heating showed significant effects on soybean fatty acids. These findings can serve as references when designing soybean processing procedures.

Even though this thesis bridged some of the research gap, it also created some new questions. For example, since the flatulence-causing oligosaccharides are water soluble, what was the reason that most of these oligosaccharides remained in the soybean

after 16 h of soaking? Soaking and infrared heating can lead to the conversions between isoflavones, but how will such conversions change the antioxidant properties of soybean?

## References

- AACC International. (1999a). Ash-Basic Method. In *Approved Methods of Analysis, 11th Ed Method 08-01.01*). St. Paul, MN, U.S.A.: AACC International.
- AACC International. (1999b). Moisture—Air-Oven Methods In *Approved Methods of Analysis, 11th Ed Method 44-15.02*). St. Paul, MN, U.S.A.: AACC International.
- Achouri, A., Boye, J. I., & Belanger, D. (2005). Soybean isoflavones: Efficacy of extraction conditions and effect of food type on extractability. *Food Research International*, 38(10), 1199-1204.
- Adeyemo, S. M., & Onilude, A. A. (2013). Enzymatic reduction of anti-nutritional factors in fermenting soybeans by *Lactobacillus plantarum* isolates from fermenting cereals. *Nigerian Food Journal*, 31(2), 84-90.
- Anderson, R. L., & Wolf, W. J. (1995). Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. *The Journal of Nutrition*.
- Armour, J. C., Perera, R. L., Buchan, W. C., & Grant, G. (1998). Protease inhibitors and lectins in soya beans and effects of aqueous heat-treatment. *Journal of the Science of Food and Agriculture*, 78(2), 225-231.
- Bajpai, S., Sharma, A., & Nath Gupta, M. (2005). Removal and recovery of antinutritional factors from soybean flour. *Food Chemistry*, 89(4), 497-501.
- Bau, H. M., Villaume, C., & Méjean, L. (2000). Effects of soybean (*Glycine max*) germination on biologically active components, nutritional values of seeds, and biological characteristics in rats. *Die Nahrung*., 44(1), 2-6.

- Bau, H. M., Villaume, C., Nicolas, J. p., & Méjean, L. (1997). Effect of Germination on Chemical Composition, Biochemical Constituents and Antinutritional Factors of Soya Bean ( *Glycine max* ) Seeds. *Journal of the Science of Food and Agriculture*, 73, 1-9.
- Becker-Ritt, A. B., Mulinari, F., Vasconcelos, I. M., & Carlini, C. R. (2004). Antinutritional and/or toxic factors in soybean (*Glycine max* (L) Merrill) seeds: comparison of different cultivars adapted to the southern region of Brazil. *Journal of the Science of Food and Agriculture*, 84(3), 263-270.
- Bednarczyk, M., Urbanowski, M., Gulewicz, P., Kasperczyk, K., Maiorano, G., & Szwaczkowski, T. (2011). Field and in vitro study on prebiotic effect of raffinose family oligosaccharides in chickens. *Bulletin of the Veterinary Institute in Pulawy*, 55, 465-469.
- Bellaloui, N., Smith, J. R., Gillen, A. M., & Ray, J. D. (2011). Effects of maturity, genotypic background, and temperature on seed mineral composition in near-isogenic soybean lines in the early soybean production system. *Crop Science*, 51(3), 1161-1171.
- Bies, C., Lehr, C.-M., & Woodley, J. F. (2004). Lectin-mediated drug targeting: history and applications. *Advanced Drug Delivery Reviews*, 56(4), 425-435.
- Birk, Y. (1985). The Bowman-Birk inhibitor. Trypsin- and chymotrypsin-inhibitor from soybeans. *International Journal of Peptide and Protein Research*, 25(2), 113-131.
- Breene, W., Lin, S., Hardman, L., & Orf, J. (1988). Protein and oil content of soybeans from different geographic locations. *Journal of the American Oil Chemists' Society*, 65(12), 1927-1931.

- Chen, L. Y., Madl, R. L., & Vadlani, P. V. (2013). Nutritional enhancement of soy meal via *Aspergillus oryzae* solid-state fermentation. *Cereal Chemistry*, 90(6), 529-534.
- Chen, Y. (2015). *Effects of micronization, ethanol washing, and enzymatic hydrolysis processing alone or in combination on trypsin inhibitors, lipoxygenase activities and selected "beany" flavour related compounds in soybean flour*. University of Manitoba, Winnipeg, Manitoba.
- Chen, Z., Yu, L., Wang, X., Gu, Z., & Beta, T. (2016). Changes of phenolic profiles and antioxidant activity in canaryseed (*Phalaris canariensis* L.) during germination. *Food Chemistry*, 194, 608-618.
- Clair, W. H. S., Billings, P. C., Carew, J. A., Keller-McGandy, C., Newberne, P., & Kennedy, A. R. (1990). Suppression of dimethylhydrazine-induced carcinogenesis in mice by dietary addition of the Bowman-Birk protease inhibitor. *Cancer Research*, 50(3), 580-586.
- Connor, W. E. (2000). Importance of n-3 fatty acids in health and disease. *The American Journal of Clinical Nutrition*, 71(1), 171S-175S.
- Cremasco, H., Borsato, D., Angilelli, K. G., Galão, O. F., Bona, E., & Valle, M. E. (2016). Application of self-organising maps towards segmentation of soybean samples by determination of inorganic compounds content. *Journal of the Science of Food and Agriculture*, 96(1), 306-310.
- De Mejía, E. G., Guzmán-Maldonado, S. H., Acosta-Gallegos, J. A., Reynoso-Camacho, R., Ramírez-Rodríguez, E., Pons-Hernández, J. L., González-Chavira, M. M., Castellanos, J. Z., & Kelly, J. D. (2003). Effect of cultivar and growing location on the trypsin inhibitors, tannins, and lectins of common beans (*Phaseolus*

- vulgaris* L.) grown in the semiarid highlands of Mexico. *Journal of Agricultural and Food Chemistry*, 51(20), 5962-5966.
- De Mejía, E. G., & Prisecaru, V. I. (2005). Lectins as bioactive plant proteins: a potential in cancer treatment. *Critical Reviews in Food Science and Nutrition*, 45(6), 425-445.
- Dhakal, K. H., Jung, K. H., Chae, J. H., Shannon, J. G., & Lee, J. D. (2014). Variation of unsaturated fatty acids in soybean sprout of high oleic acid accessions. *Food Chemistry*, 164, 70-73.
- Dia, V. P., Gomez, T., Vernaza, G., Berhow, M., Chang, Y. K., & De Mejia, E. G. (2012). Bowman-Birk and Kunitz protease inhibitors among antinutrients and bioactives modified by germination and hydrolysis in Brazilian soybean cultivar BRS 133. *Journal of Agricultural and Food Chemistry*, 60(32), 7886-7894.
- DiPietro, C. M., & Liener, I. E. (1989). Heat inactivation of the Kunitz and Bowman-Birk soybean protease inhibitors. *Journal of Agricultural and Food Chemistry*, 37(1), 39-44.
- Dixit, A. K., Antony, J., Sharma, N. K., & Tiwari, R. K. (2011). Soybean constituents and their functional benefits. In V. K. Tiwari & B. B. Mishra (Eds.), *Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry*, (pp. 367-383). Kerala, India: Research Signpost.
- Dixit, A. K., Kumar, V., Rani, A., Manjaya, J., & Bhatnagar, D. (2011). Effect of gamma irradiation on lipoxygenases, trypsin inhibitor, raffinose family oligosaccharides and nutritional factors of different seed coat colored soybean (*Glycine max* L.). *Radiation Physics and Chemistry*, 80(4), 597-603.

- Duranti, M., Barbiroli, A., Scarafoni, A., Tedeschi, G., & Morazzoni, P. (2003). One-step purification of Kunitz soybean trypsin inhibitor. *Protein Expression and Purification*, 30(2), 167-170.
- Egounlety, M., & Aworh, O. (2003). Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms). *Journal of Food Engineering*, 56(2), 249-254.
- ElSayed, A., Rafudeen, M., & Golldack, D. (2014). Physiological aspects of raffinose family oligosaccharides in plants: protection against abiotic stress. *Plant Biology*, 16(1), 1-8.
- Fasina, O., Tyler, B., Pickard, M., Zheng, G. H., & Wang, N. (2001). Effect of infrared heating on the properties of legume seeds. *International Journal of Food Science & Technology*, 36(1), 79-90.
- Food and Agricultural Organization of the United Nations. (2017). FAOSTAT statistical database. In).
- Frias, J., Bakhsh, A., Jones, D., Arthur, A., Vidal-Valverde, C., Rhodes, M., & Hedley, C. (1999). Genetic analysis of the raffinose oligosaccharide pathway in lentil seeds. *Journal of Experimental Botany*, 50(333), 469-476.
- Friedman, M., & Brandon, D. L. (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry*, 49(3), 1069-1086.

- Ghazarian, H., Idoni, B., & Oppenheimer, S. B. (2011). A glycobiology review: carbohydrates, lectins and implications in cancer therapeutics. *Acta Histochemica*, *113*(3), 236-247.
- Gu, C., Pan, H., Sun, Z., & Qin, G. (2010). Effect of soybean variety on anti-nutritional factors content, and growth performance and nutrients metabolism in rat. *International Journal of Molecular Sciences*, *11*(3), 1048-1056.
- Guimarães, V. M., de Rezende, S. T., Moreira, M. A., de Barros, E. G., & Felix, C. R. (2001). Characterization of  $\alpha$ -galactosidases from germinating soybean seed and their use for hydrolysis of oligosaccharides. *Phytochemistry*, *58*(1), 67-73.
- Gulewicz, P., Szymaniec, S., Bubak, B., Frias, J., Vidal-Valverde, C., Trojanowska, K., & Gulewicz, K. (2002). Biological activity of  $\alpha$ -galactoside preparations from *Lupinus angustifolius* L. and *Pisum sativum* L. seeds. *Journal of Agricultural and Food Chemistry*, *50*(2), 384-389.
- Hamerstrand, G., Black, L., & Glover, J. (1981). Trypsin inhibitors in soy products: modification of the standard analytical procedure. *Cereal Chemistry*, *58*(1), 42-45.
- Hartman, G. L., West, E. D., & Herman, T. K. (2011). Crops that feed the world 2. soybean—worldwide production, use, and constraints caused by pathogens and pests. *Food Security*, *3*(1), 5-17.
- Hurburgh, C. R., Brumm, T. J., Guinn, J. M., & Hartwig, R. A. (1990). Protein and oil patterns in US and world soybean markets. *Journal of the American Oil Chemists' Society*, *67*(12), 966-973.
- Isanga, J., & Zhang, G.-N. (2008). Soybean bioactive components and their implications to health—a review. *Food Reviews International*, *24*(2), 252-276.

- Karner, U., Peterbauer, T., Raboy, V., Jones, D. A., Hedley, C. L., & Richter, A. (2004). myo-Inositol and sucrose concentrations affect the accumulation of raffinose family oligosaccharides in seeds. *Journal of Experimental Botany*, 55(405), 1981-1987.
- Karr-Lilienthal, L., Kadzere, C., Grieshop, C., & Fahey, G. (2005). Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: A review. *Livestock Production Science*, 97(1), 1-12.
- Kelsall, A., FitzGerald, A., Howard, C., Evans, R., Singh, R., Rhodes, J., & Goodlad, R. (2002). Dietary lectins can stimulate pancreatic growth in the rat. *International Journal of Experimental Pathology*, 83(4), 203-208.
- Khattab, R., & Arntfield, S. (2009). Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. *LWT-Food Science and Technology*, 42(6), 1113-1118.
- Kim, M. Y., Jang, G. Y., Lee, Y., Li, M., Ji, Y. M., Yoon, N., Lee, S. H., Kim, K. M., Lee, J., & Jeong, H. S. (2016). Free and bound form bioactive compound profiles in germinated black soybean (*Glycine max* L.). *Food Science and Biotechnology*, 25(6), 1551-1559.
- Kobayashi, H., Suzuki, M., Kanayama, N., & Terao, T. (2004). A soybean Kunitz trypsin inhibitor suppresses ovarian cancer cell invasion by blocking urokinase upregulation. *Clinical and Experimental Metastasis*, 21(2), 159-166.
- Krishnamurthy, K., Khurana, H. K., Soojin, J., Irudayaraj, J., & Demirci, A. (2008). Infrared heating in food processing: an overview. *Comprehensive Reviews in Food Science and Food Safety*, 7(1), 2-13.

- Krishnan, H. B. (2001). Biochemistry and molecular biology of soybean seed storage proteins. *Journal of New Seeds*, 2(3), 1-25.
- Krishnan, H. B., Jang, S., Baxter, I., & Wiebold, W. J. (2012). Growing location has a pronounced effect on the accumulation of cancer chemopreventive agent Bowman-Birk inhibitor in soybean seeds. *Crop Science*, 52(4), 1786-1794.
- Kudou, S., Fleury, Y., Welti, D., Magnolato, D., Uchida, T., Kitamura, K., & Okubo, K. (1991). Malonyl isoflavone glycosides in soybean seeds (*Glycine max* Merrill). *Agricultural and Biological Chemistry*, 55(9), 2227-2233.
- Kumar, V., Rani, A., Goyal, L., Dixit, A. K., Manjaya, J., Dev, J., & Swamy, M. (2010). Sucrose and raffinose family oligosaccharides (RFOs) in soybean seeds as influenced by genotype and growing location. *Journal of Agricultural and Food Chemistry*, 58(8), 5081-5085.
- Kumar, V., Rani, A., Pandey, V., & Chauhan, G. (2006). Changes in lipoxygenase isozymes and trypsin inhibitor activity in soybean during germination at different temperatures. *Food Chemistry*, 99(3), 563-568.
- Kumar, V., Rani, A., Solanki, S., & Hussain, S. (2006). Influence of growing environment on the biochemical composition and physical characteristics of soybean seed. *Journal of Food Composition and Analysis*, 19(2), 188-195.
- Kumar, V., Rani, A., Tindwani, C., & Jain, M. (2003). Lipoxygenase isozymes and trypsin inhibitor activities in soybean as influenced by growing location. *Food Chemistry*, 83(1), 79-83.
- Kumari, S., Krishnan, V., & Sachdev, A. (2015). Impact of soaking and germination durations on antioxidants and anti-nutrients of black and yellow soybean (*Glycine*

- max. L) varieties. *Journal of Plant Biochemistry and Biotechnology*, 24(3), 355-358.
- Kunitz, M. (1947). Crystalline soybean trypsin inhibitor II. General properties. *The Journal of General Physiology*, 30(4), 291-310.
- Kuo, T. M., VanMiddlesworth, J. F., & Wolf, W. J. (1988). Content of raffinose oligosaccharides and sucrose in various plant seeds. *Journal of Agricultural and Food Chemistry*, 36(1), 32-36.
- Landry, E. J., Fuchs, S. J., & Hu, J. (2016). Carbohydrate composition of mature and immature faba bean seeds. *Journal of Food Composition and Analysis*.
- Lepage, G., & Roy, C. C. (1986). Direct transesterification of all classes of lipids in a one-step reaction. *Journal of Lipid Research*, 27(1), 114-120.
- Li, T., Lu, X., & Yang, X. (2013). Stachyose-enriched  $\alpha$ -galacto-oligosaccharides regulate gut microbiota and relieve constipation in mice. *Journal of Agricultural and Food Chemistry*, 61(48), 11825-11831.
- Liu, K. (2004). Soybeans as a Powerhouse of Nutrients and Phytochemicals. In *Soybeans as Functional Foods and Ingredients*: AOCS Publishing.
- Lokuruka, M. (2010). Soybean nutritional properties: The good and the bad about soy foods consumption-A review. *African Journal of Food, Agriculture, Nutrition and Development*, 10(4).
- Luthria, D. L., Biswas, R., & Natarajan, S. (2007). Comparison of extraction solvents and techniques used for the assay of isoflavones from soybean. *Food Chemistry*, 105(1), 325-333.

- Machado, F. P. P., Queiróz, J. H., Oliveira, M. G. A., Piovesan, N. D., Peluzio, M. C. G., Costa, N. M. B., & Moreira, M. A. (2008). Effects of heating on protein quality of soybean flour devoid of Kunitz inhibitor and lectin. *Food Chemistry*, *107*(2), 649-655.
- Marioli Nobile, C. G., Balzarini, M., Aguata, F. M., Grosso, N. R., Soldini, D. O., Zeng, H., Cheng, W.-H., & Martínez, M. J. (2016). Climatic Thresholds for Concentrations of Minerals and Heavy Metals in Argentinean Soybean. *Agronomy Journal*, *108*(2), 532-539.
- Martínez-Villaluenga, C., Frias, J., & Vidal-Valverde, C. (2008). Alpha-galactosides: antinutritional factors or functional ingredients? *Critical Reviews in Food Science and Nutrition*, *48*(4), 301-316.
- Masuda, T., & Goldsmith, P. D. (2009). World soybean production: area harvested, yield, and long-term projections. *International Food and Agribusiness Management Review*, *12*(4), 143-162.
- Matsuura, M., Obata, A., & Fukushima, D. (1989). Objectionable flavor of soy milk developed during the soaking of soybeans and its control. *Journal of Food Science*, *54*(3), 602-605.
- Medic, J., Atkinson, C., & Hurburgh Jr, C. R. (2014). Current knowledge in soybean composition. *Journal of the American Oil Chemists' Society*, *91*(3), 363-384.
- Messina, M., & Barnes, S. (1991). The role of soy products in reducing risk of cancer. *Journal of the National Cancer Institute*, *83*(8), 541-546.
- Messina, M. J. (1999). Legumes and soybeans: overview of their nutritional profiles and health effects. *The American Journal of Clinical Nutrition*, *70*(3), 439s-450s.

- Middelbos, I., & Fahey, G. (2008). Soybean carbohydrates. In L. A. Johnson, White, P. J. and Galloway, R. (Ed.), *Soybeans: Chemistry, Production, Processing and Utilization*, (pp. 269-273).
- Mohamed, A. I., & Rangappa, M. (1992). Screening soybean (grain and vegetable) genotypes for nutrients and anti-nutritional factors. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*, 42(1), 87-96.
- Niamnuy, C., Nachaisin, M., Poomsa-ad, N., & Devahastin, S. (2012). Kinetic modelling of drying and conversion/degradation of isoflavones during infrared drying of soybean. *Food Chemistry*, 133(3), 946-952.
- Njira, K., Nalivata, P., Kanyama-Phiri, G., & Lowole, M. (2013). An assessment for the need of soybean inoculation with *Bradyrhizobium japonicum* in some sites of Kasungu district, Central Malawi. *International Journal of Current Microbiology and Applied Sciences*, 8, 60-72.
- Nufer, K. R., Ismail, B., & Hayes, K. D. (2009). The effects of processing and extraction conditions on content, profile, and stability of isoflavones in a soymilk system. *Journal of Agricultural and Food Chemistry*, 57(4), 1213-1218.
- Nzima, W. M., & Dzanja, J. (2015). Efficiency of soybean markets in Malawi: structure, conduct and performance approach. *International Journal of Business and Social Science*, 6(4).
- Padmashree, A., Semwal, A., Khan, M., Govindaraj, T., & Sharma, G. (2016). Effect of infrared processing on functional, nutritional, antinutritional and rheological properties of mung bean (*Phaseolus aereus*) seeds. *International Journal of Advanced Research*, 4(1), 606-613.

- Palacios, M. F., Easter, R. A., Soltwedel, K. T., Parsons, C. M., Douglas, M. W., Hymowitz, T., & Pettigrew, J. E. (2004). Effect of soybean variety and processing on growth performance of young chicks and pigs. *Journal of Animal Science*, 82(4), 1108-1114.
- Peterbauer, T., & Richter, A. (2001). Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. *Seed Science Research*, 11(03), 185-197.
- Peumans, W. J., & Van Damme, E. (1995). Lectins as plant defense proteins. *Plant Physiology*, 109(2), 347.
- Poysa, V., & Woodrow, L. (2002). Stability of soybean seed composition and its effect on soymilk and tofu yield and quality. *Food Research International*, 35(4), 337-345.
- Prinyawiwatkul, W., Beuchat, L., McWatters, K., & Phillips, R. (1996). Changes in fatty acid, simple sugar, and oligosaccharide content of cowpea (*Vigna unguiculata*) flour as a result of soaking, boiling, and fermentation with *Rhizopus microsporus* var. *oligosporus*. *Food Chemistry*, 57(3), 405-413.
- Rådberg, K., Biernat, M., Linderöth, A., Zabielski, R., Pierzynowski, S., & Weström, B. (2001). Enteral exposure to crude red kidney bean lectin induces maturation of the gut in suckling pigs. *Journal of Animal Science*, 79(10), 2669-2678.
- Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., Wolvers, D., Watzl, B., Szajewska, H., & Stahl, B. (2010). Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*, 104(S2), S1-S63.

- Sakai, N., & Hanzawa, T. (1994). Applications and advances in far-infrared heating in Japan. *Trends in Food Science & Technology*, 5(11), 357-362.
- Sakla, A., Ghali, Y., El-Farra, A., & Rizk, L. F. (1988). The effect of environmental conditions on the chemical composition of soybean seeds: Relationship between the protein, oil, carbohydrate and trypsin inhibitor content. *Food Chemistry*, 29(3), 221-231.
- Sandu, C. (1986). Infrared radiative drying in food engineering: a process analysis. *Biotechnology Progress*, 2(3), 109-119.
- Saxena, H., Jensen, L. S., & McGinnis, J. (1963). Pancreatic Hypertrophy and Chick Growth Inhibition by Soybean Fractions Devoid of Trypsin Inhibitor. *Proceedings of the Society for Experimental Biology and Medicine*, 112(1), 101-105.
- Shariati-Ievari, S., Ryland, D., Edel, A., Nicholson, T., Suh, M., & Aliani, M. (2016). Sensory and Physicochemical Studies of Thermally Micronized Chickpea (*Cicer arietinum*) and Green Lentil (*Lens culinaris*) Flours as Binders in Low-Fat Beef Burgers. *Journal of Food Science*, 81(5), S1230-S1242.
- Sharma, S., Kaur, M., Goyal, R., & Gill, B. (2014). Physical characteristics and nutritional composition of some new soybean (*Glycine max* (L.) Merrill) genotypes. *Journal of Food Science and Technology*, 51(3), 551-557.
- Shi, L. (2015). *A Quantitative Assessment of the Anti-Nutritional Properties of Canadian Pulses*. University of Manitoba, Winnipeg.
- Shivakumar, M., Verma, K., Talukdar, A., Srivastava, N., Lal, S. K., Sapra, R. L., & Singh, K. P. (2015). Genetic variability and effect of heat treatment on trypsin

- inhibitor content in soybean [*Glycine max* (L.) Merrill.]. *Legume Research-An International Journal*, 38(1), 60-65.
- Shoaf, K., Mulvey, G. L., Armstrong, G. D., & Hutkins, R. W. (2006). Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells. *Infection and Immunity*, 74(12), 6920-6928.
- Siebert, K. J., Troukhanova, N. V., & Lynn, P. Y. (1996). Nature of polyphenol– protein interactions. *Journal of Agricultural and Food Chemistry*, 44(1), 80-85.
- Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & pharmacotherapy*, 56(8), 365-379.
- Sinclair, T. R., Marrou, H., Soltani, A., Vadez, V., & Chandolu, K. C. (2014). Soybean production potential in Africa. *Global Food Security*, 3(1), 31-40.
- Sitren, H. S., Ahmed, E. M., & George, D. E. (1985). In vivo and in vitro assessment of antinutritional factors in peanut and soy. *Journal of Food Science*, 50, 418-423.
- Slavin, M., Cheng, Z., Luther, M., Kenworthy, W., & Yu, L. L. (2009). Antioxidant properties and phenolic, isoflavone, tocopherol and carotenoid composition of Maryland-grown soybean lines with altered fatty acid profiles. *Food Chemistry*, 114(1), 20-27.
- Song, T., Barua, K., Buseman, G., & Murphy, P. A. (1998). Soy isoflavone analysis: quality control and a new internal standard. *The American Journal of Clinical Nutrition*, 68(6), 1474S-1479S.

- Stewart, O., Raghavan, G., Orsat, V., & Golden, K. (2003). The effect of drying on unsaturated fatty acids and trypsin inhibitor activity in soybean. *Process Biochemistry*, 39(4), 483-489.
- Sumnu, S. G., & Ozkoc, S. O. (2010). Infrared Baking and Roasting. In *Infrared Heating for Food and Agricultural Processing*, (pp. 203-223): CRC Press.
- Toshiya, T., Sakamoto, A., Takayanagi, T., & Yokotsuka, K. (2000). Changes in isoflavone compositions of soybean foods during cooking process. *Food Science and Technology Research*, 6(4), 314-319.
- Van den Ende, W. (2013). Multifunctional fructans and raffinose family oligosaccharides. *Frontiers in Plant Science*, 4, 247.
- Vitale, D. C., Piazza, C., Melilli, B., Drago, F., & Salomone, S. (2013). Isoflavones: estrogenic activity, biological effect and bioavailability. *European Journal of Drug Metabolism and Pharmacokinetics*, 38(1), 15-25.
- Wang, H.-J., & Murphy, P. A. (1994). Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. *Journal of Agricultural and Food Chemistry*, 42(8), 1674-1677.
- Wang, H.-J., & Murphy, P. A. (1996). Mass balance study of isoflavones during soybean processing. *Journal of Agricultural and Food Chemistry*, 44(8), 2377-2383.
- Xu, B., & Chang, S. K. (2008). Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. *Journal of Agricultural and Food Chemistry*, 56(16), 7165-7175.

Yalcin, S., & Basman, A. (2015). Effects of infrared treatment on urease, trypsin inhibitor and lipoxygenase activities of soybean samples. *Food Chemistry*, *169*, 203-210.

Zdunczyk, Z., Godycka, I., & Amarowicz, R. (1997). Chemical composition and content of antinutritional factors in Polish cultivars of peas. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*, *50*(1), 37-45.

Ziółkowska, N. E., & Włodawer, A. (2006). Structural studies of algal lectins with anti-HIV activity. *Acta Biochim Pol*, *53*, 617-626.