Study of extraction and processing effect on bioactive compounds and nutritional value of soybean for utilization of soybean meal as an natural additive in functional food products

Ekkarat Tangkhawanit

A Thesis Submitted in Partial Fulfillment of Requirements for
degree of Doctor of Philosophy in Food Technology (International Program)
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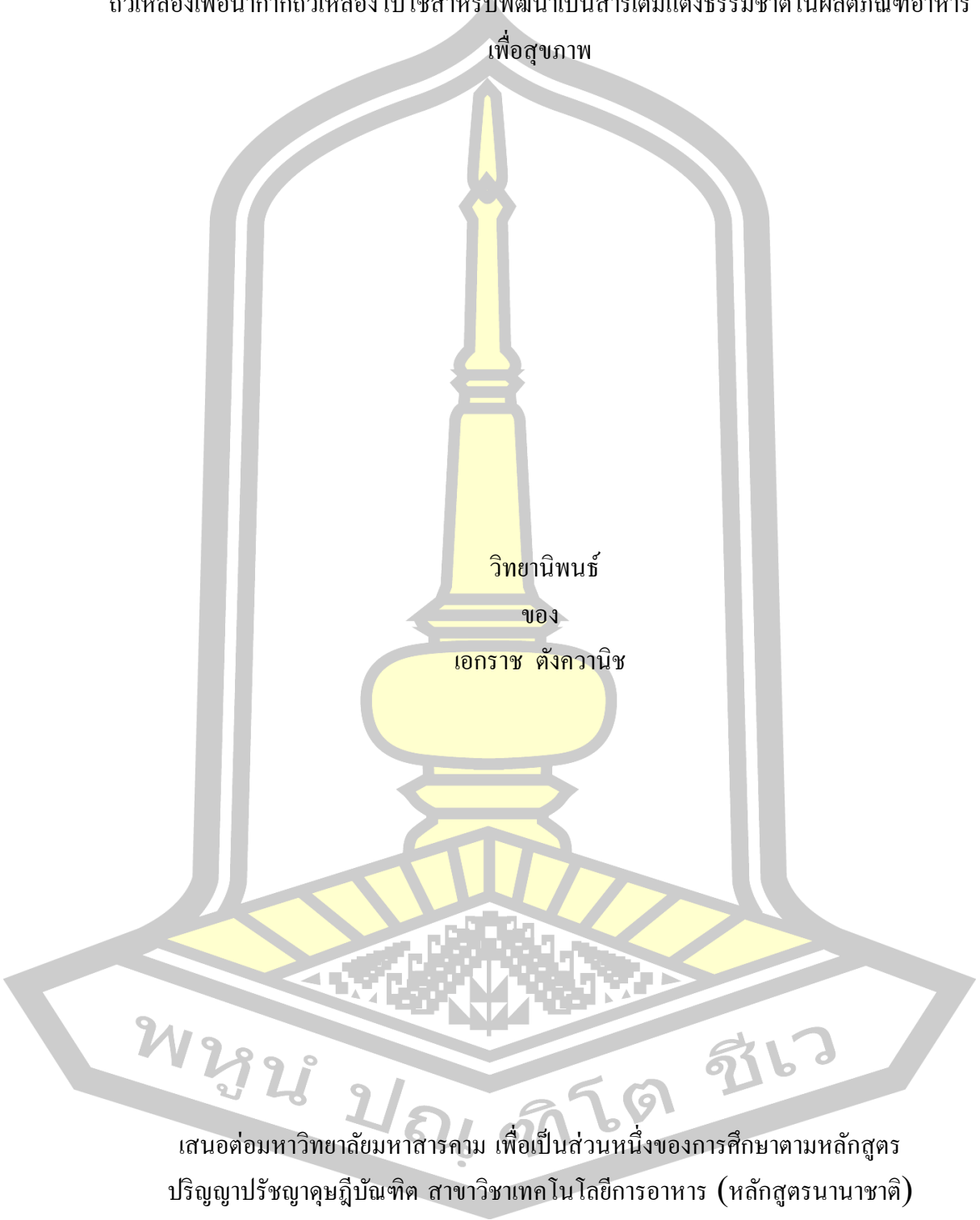
การศึกษาผลของการสกัดและการแปรรูปต่อสารออกฤทธิ์ทางชีวภาพและคุณค่าทางโภชนาการของถั่วเหลืองเพื่อนำกากถั่วเหลืองไปใช้สำหรับพัฒนาเป็นสารเติมแต่งธรรมชาติในผลิตภัณฑ์อาหารเพื่อสุขภาพ



วิทยานิพนธ์

ของ

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เสนอต่อมหาวิทยาลัยมหาสารคาม เพื่อเป็นส่วนหนึ่งของการศึกษาตามหลักสูตร
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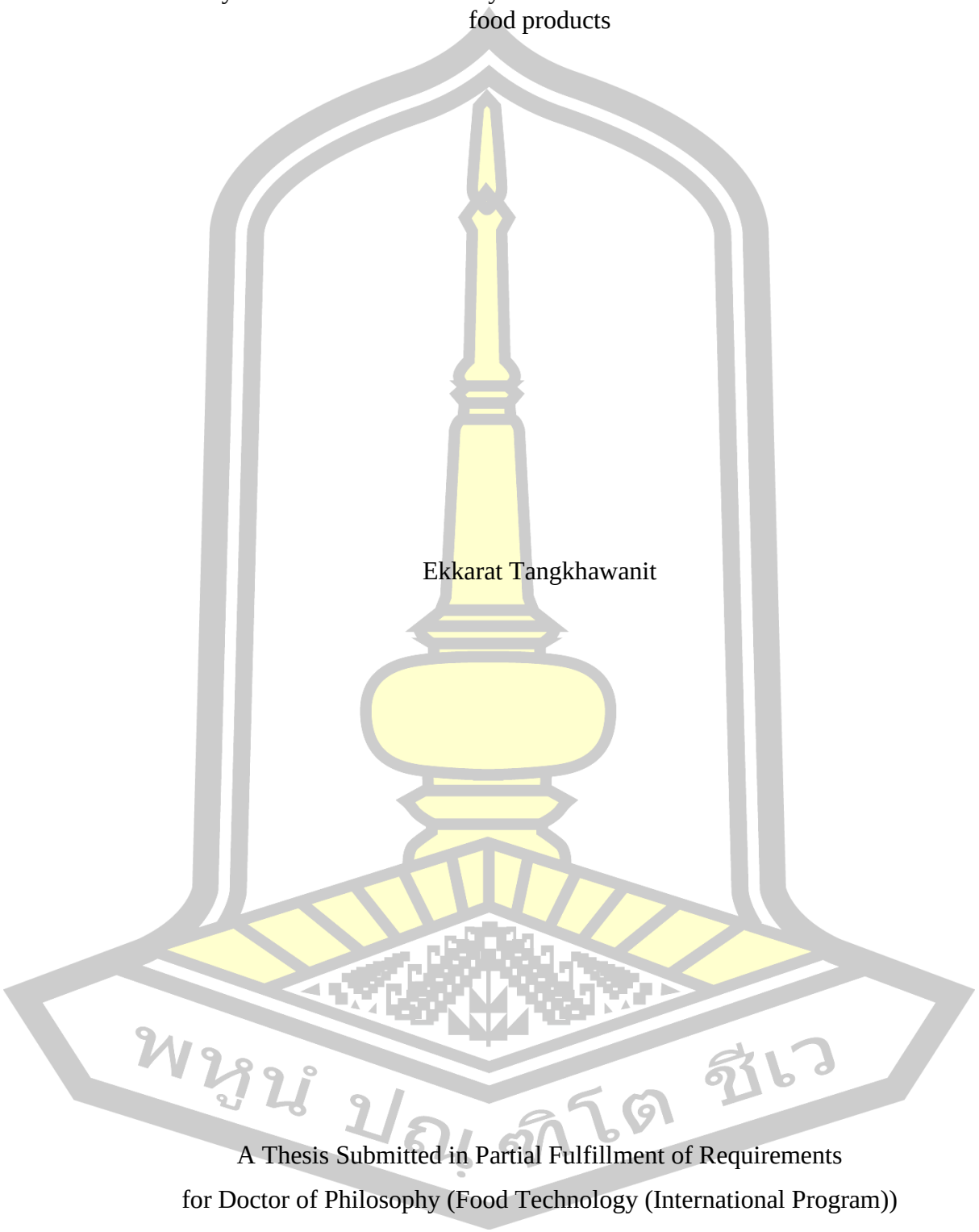

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The examining committee has unanimously approved this Thesis, submitted by Mr. Ekkarat Tangkhawanit , as a partial fulfillment of the requirements for the Doctor of Philosophy Food Technology (International Program) at Mahasarakham University

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TITLE Study of extraction and processing effect on bioactive compounds and nutritional value of soybean for utilization of soybean meal as an natural additive in functional food products

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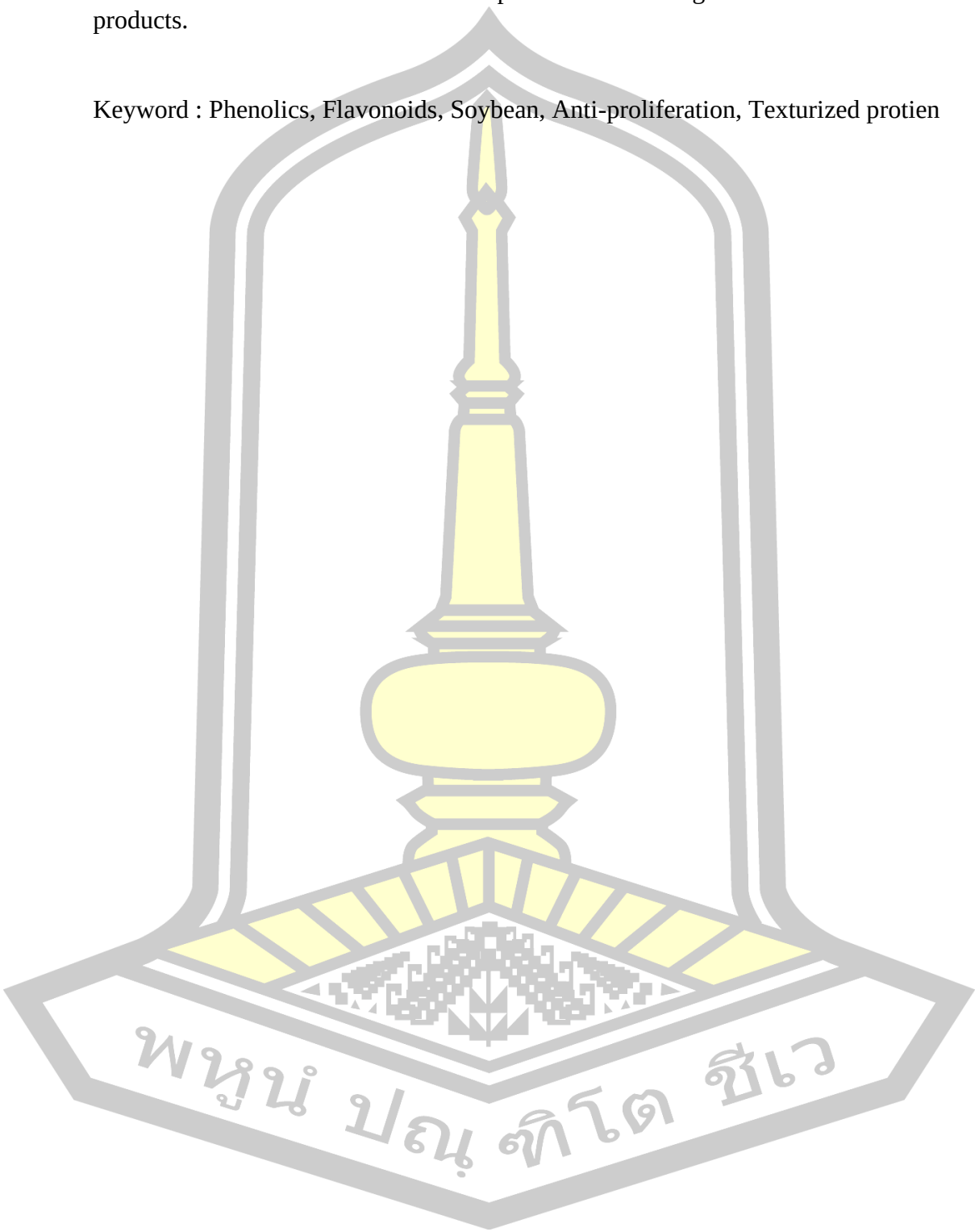
ABSTRACT

The present study investigated the extraction and processing effect on bioactive compounds and nutritional value of soybean for utilization soybean meal as a natural additive in functional food product. The present study investigated the extraction and processing effect on bioactive compounds and nutritional value of soybean for utilization soybean meal as a natural additive in functional food product. Our findings have demonstrated that bioactive compounds and antioxidant activities of oil and soymilk residues are suitable for the food ingredient or food additive add in food to functional food.The results of soybean and soybean residues were indicated that soybean residues from oil and soymilk industries are a rich source of bioactive compounds, namely phenolic acid (protocatechuic, chlorogenic, ferulic, gallic and sinapic acids), flavonoids (rutin, daidzein and genistein) of free and bound form, phytosterols (beta-sitosterol, campesterol and squalene) and essential amino acids (leucine, phenylalanine, tryptophane and methionine) with retained the antioxidant capacity. For drying treatments including hot air oven (HA) and far-irradiation combined with a hot air oven (FIR-HA) with different temperature levels (60, 70 and 80 ^{o}C). The results showed that total phenolic contents of oil and soymilk residues were significantly (p<0.05) different higher when compared with non-treatment samples (NTS). Flavonoid content, the highest values of flavonoids were observed in free flvonoid form. Drying method can be increased daidzein contents by HA treatment and genistein content by FIR-HA treatment, respectively. In overall of antioxidant activities were evaluated by DPPH and FRAP assays; the results found that bound phenolic extracted from oil and soymilk residues were exhibited in DPPH radical scavenging. In contrast, the greatest FRAP values were observed in free phenolic extracted of both residues. Additionally, anti-proliferation capacity of selected soybean samples was shown in % viability in ranged from 5 -10%. However, soybean residues were effective in free radical scavenging and metal chelation. For product development of soy residue from SOI, texturized protein (TP) was made. Physical quality including color, texture was determined. The sensory evaluation of the product indicated that TP was highly accepted (6.2/7 hedonic scale) from the consumers. In addition, the bioactive compounds and its antioxidant activity were comparable to the unprocessed soy residues. In conclusion, this study has provided

valuable in formation of bioactive compounds and biological activity in soy residues. It is recommended to further develop of functional ingredient or functional food products.

Keyword : Phenolics, Flavonoids, Soybean, Anti-proliferation, Texturized protien



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Ekkarat Tangkhawanit

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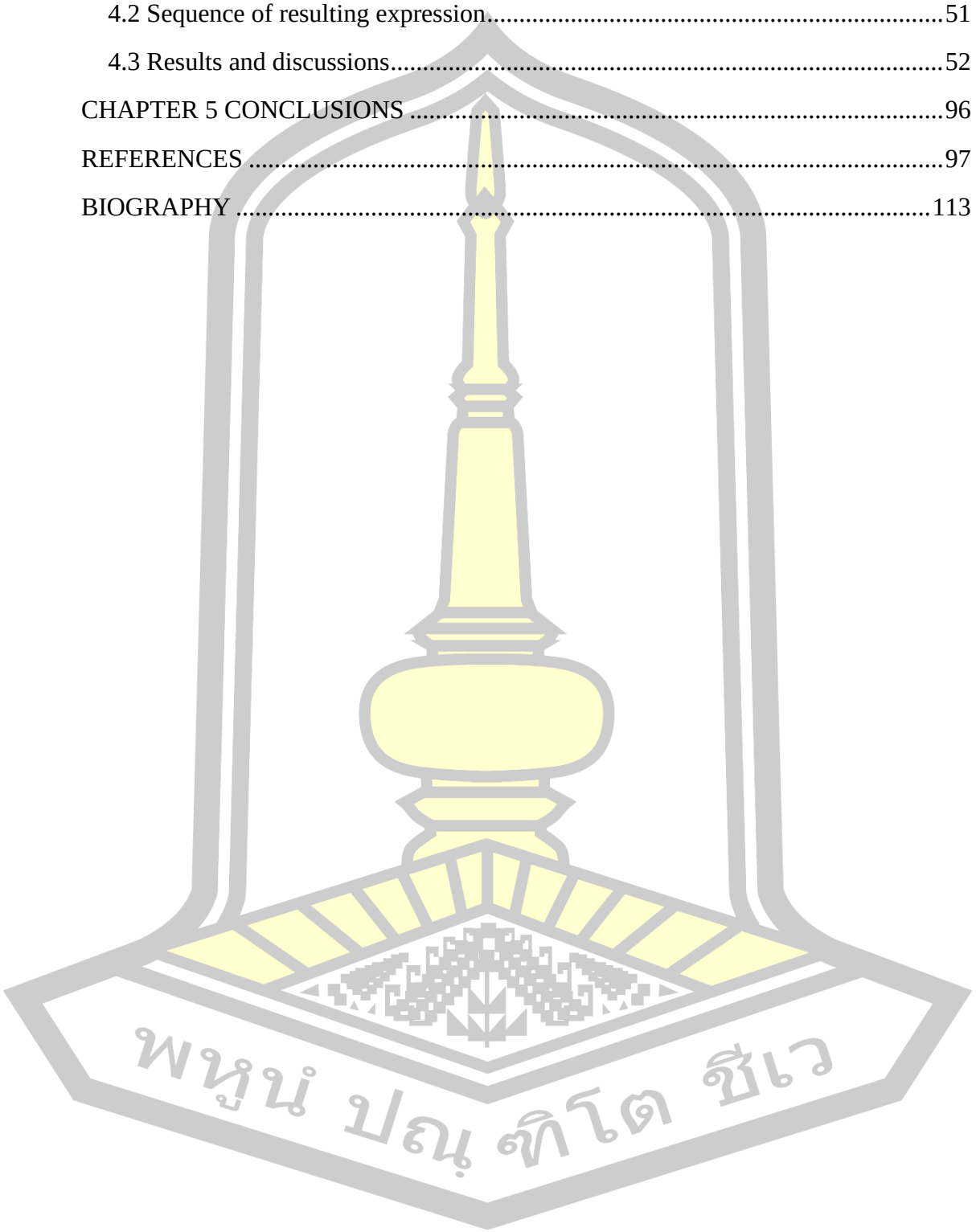

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CHAPTER 1
INTRODUCTION

1.1 Background

Soybean (*Glycine max* (L.) Merrill) is an important crop and popular for many consumers in Asia (Messina, 1999). Soya has cultivated in Thailand and soybean imports are expected to grow every year (Office of Agricultural Economics, 2014) for produced to soybean products such as soybean milk, soybean oil, soy sauce, soy flour, tofu and textured vegetable protein (Devi et al., 2009). In addition, dry soybean seed is a high value of nutrients (protein, lipid, carbohydrate, fiber, vitamins and minerals (Lin, Harnly, Pastor-Corrales, & Luthria, 2008) but also abundant of phytochemicals or bioactive compounds such as phenolic compounds, saponins, phytates, isoflavone and phytoestrogen (Boateng, Verghese, Walker, & Ogutu, 2008; Devi et al., 2009; Díaz-Batalla, Widholm, Fahey, Castaño-Tostado, & Paredes-López, 2006)

However, processes in soybean industry caused a lot of waste or by-product from soybean, to call “soybean meal” such as soybean meal from oil extraction and soybean milk products. Accordingly, soybean meal must be studied nutritive values and bioactive compounds after processed for using too high benefit that caused from the extraction or compression with other processes may affect to change of nutrients and phytochemicals. In addition, previously reported of processing may be increased or decreased on their values such as heat processed may be decreased some bioactive compounds but may be increased some phytochemicals in rice (Wanyo, Meeso, & Siriamornpun, 2014), Gac (Kubola, Meeso, & Siriamornpun, 2013) and flowers (Siriamornpun, Kaisoon, & Meeso, 2012) while information of soybean and soybean meal are rarely studied, especially in Thailand.

Therefore, the aim of the present study will be the investigation of extraction and processing effect on bioactive compounds and nutritional value of soybean for utilization of soybean meal as a natural additive in functional food products. This research expects to obtain high quality and quantity information of important compounds in processed soybean meal, which using for development to a natural

additive in functional food from soybean meal with increasing value of by-product from processing and high potential for consumers.

1.2 Research Objectives

The objectives of study are:

1.2.1 To study the extraction effect of soybean oil and soy milk on content of nutrients and bioactive compounds in soybean meal.

1.2.2 To study the extraction effect of soybean oil and soy milk on biological activities of bioactive compounds in soybean meal.

1.2.3 To determine effects of drying process on nutrients and bioactive compounds content as well as antioxidant properties of soybean meal.

1.2.4 Application of potent soybean meal for development of function food.

1.3 Outcomes

1.3.1 Obtain the knowledge of extraction effect of soybean oil and soymilk on content of nutrients and bioactive compounds in soybean meal.

1.3.2 Obtain the knowledge of extraction effect of soybean oil and soymilk on biological activities of bioactive compounds in soybean meal.

1.3.3 Obtain the knowledge of drying process on nutrients and bioactive compounds content as well as antioxidant properties of soybean meal**.**

1.3.4 Obtain the new functional product from potent soybean meal.

1.4 Hypothesis

1.4.1 Soybean seeds and soybean meal have different bioactive compounds and antioxidant activity from the different source of raw material and processing.

1.4.2 Different drying methods have different effects on bioactive compounds and antioxidant activity.

1.4.3 The levels of bioactive compounds and antioxidant activity increasing when adding in the product.

1.5 Scope of research

1.5.1 Analysis of antioxidant activity and bioactive compounds of soybean seeds and soybean meal from a different source of raw material and processing.

1.5.2 Analysis of phenolic acids and flavonoid composition of soybean seeds and soybean meal, by using HPLC-DAD

1.5.3 Analysis of phytosterols and fatty acid composition of soybean seeds and soybean meal, by using GC-MS

1.5.4 Evaluation the effect of the drying process on antioxidant activity and bioactive compounds in soybean seeds and soybean meal.

1.5.5 Analysis of the cytotoxic activity of a potent soybean meal.

1.5.6 Product development of potent soybean meal powder and evaluates the stability of bioactive compounds and antioxidant activity in the product.

1.6 Defined words

1. Soybean: as soybean seed (*Glycine max* (L.) Merill) and other scientific names such as *Glycine soja*, *Soja max*, *Phaseolus max* and *Dolichos soja*. Soya is an important source of protein, carbohydrate, lipid, fiber and vitamins but also rich of phytochemicals such as phenolics and flavonoids.

2. Soybean meal: a waste or by-product from oil industry and soymilk industry. The residues mainly used as animal feed and turn used as ingredients in other products such as meat and bakery products.

3. Phenolic compounds: chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. Examples in this study are phenolic acids and flavonoids.

4. Phenolic acids: types of aromatic acid compound were included in the class on substances containing a phenolic ring and an organic carboxylic acid function (C6-C1 skeleton). Two important naturally occurring types of phenolic acids are hydroxybenzoic acids and hydroxycinnamic acids, which are derive from non-

phenolic molecules of benzoic and cinnamic acid, respectively. Examples in this study are gallic acid, ferulic acid, *p*-coumaric acid, cafeic acid.

5. Flavonoids: a class of plant and fungus secondary metabolites that is abundant in fruits, vegetables, tea and dry legume seeds. Chemically, flavonoids have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C). This carbon structure can be abbreviated C6-C3-C6. Examples in this study are isoflavone (daidzein and genestein), rutin, myricetin, quercetin, apiginin and kaempferol.

6. Antioxidant activities: a molecule that inhibits the oxidation of other molecules from free radical and can act as scavenge free radical, bind metal ions.

CHAPTER 2
LITERATURE REVIEW

2.1 Soybean

Soybean (*Glycine max* (L.) Merrill) commonly known as soya bean in North America and called “Shu” in Chinese. Soybean originated in China and has been cultivated for a long time (about 5000 years) (Qiu & Chang, 2010). Soya was first cultivated in Southeast Asia, Europe and America. Since the 1940s, soybean has become one of the most important economic crops in America. Currently, America is the largest soybean producer and exporter in the world, namely, about 35% of world production in the 2011/2012 season (United States Department of Agriculture, 2012). United States, Brazil, Argentina and China are the current leaders of soybean production and export in the world, with a combined total harvest of 205.27 tons in the 2011/2012 season (about 86% from worldwide production) (United States Department of Agriculture, 2012). The biggest commercial interests in soy are its oil and hydrolyzed soy protein. In Thailand, soybean imports are expected to grow every year (Office of Agricultural Economics, 2014) (Table 1) for producing soybean products such as soybean milk, soybean oil, soy sauce, soy flour, tofu and textured vegetable protein (Devi et al., 2009). Dry raw soybean has high value nutrients, containing protein, lipid, carbohydrates, fibers, vitamins and minerals (Lin et al., 2008); nutritional values are presented in Table 2.

Before the recent surge in interest in the protein of soybean, soybean oil has been the main product of soybean processing. According to a USDA report, soybean oil is the second largest vegetable oil produced in the world with a total world production of 43 million t. Soybean oil contains 57.7% total polyunsaturated fatty acids, 22.8% total monounsaturated fatty acids and 15.6% total saturated fatty acids (National Nutrient Database for Standard Reference, 2013). In this publication, the panel recommends consuming not over 10% of saturated fat in the diet, and replacing it with monounsaturated or polyunsaturated fat, because these latter fats are associated with lowering the risk of cardiovascular disease (U.S. & Services, 2010).

Figure 1 Soybean seeds.

Recently, the focus has shifted to its other components, especially the protein from soybean. Because of soy protein's PDCAAS (The Protein Digestibility Corrected Amino Acid Score) score (1.0) is ranked the highest among vegetable proteins and is equal to that of milk proteins (casein, whey protein) and egg protein, indicating that soy protein provides complete amino acids to human nutritional value when compared with wheat gluten and other vegetable proteins (Hoffman & Falvo, 2004).

Accordingly, other countries have also produced soybean to manufacture many soybean products, such as soybean milk, soybean oil, soy sauce, soy flour, tofu, and textured vegetable protein (Devi et al., 2009). As a result, there is a great accumulation of soy waste or by-product from soybean processing. This 'soy residue' is approximately 90% from soybean seed and 10% from the soybean seed coat. These residues, coming especially from the soybean milk and soybean oil industries, are called "soybean meal". The soybean information in Table 1 comes from the Thai Office of Agricultural Economics (2014). These data indicate that the demand of soybean in domestic usage will be increasing every year. Most Thai soybean is imported (approximately 95%), of which 82% is used in the oil extraction industry and 18% is used in other products (Fig 2). In addition, by-product from domestic soybean industry was higher, approximately 78%. Previous studies reported that soybean by-products were a good source of total phenolic content, isoflavone and antioxidant activity in soy husk powder (Tyug, Prasad, & Ismail, 2010). Soybean meal (the residue after protein is isolate) retained the bioactive compounds and antioxidant capacity (Alu'datt et al., 2016). Moreover, previous reports indicated that soybean meal is a high-protein meal and mainly used as animal feed but also used for

the other products such as soy protein isolate, meat, bakery products, beverages, soups and infant formulas (Aguiar et al., 2012). However, Thailand has not used soybean meal for human diet products; almost all soy residue in Thailand is used in animal feed products.

Table 1 Soybean information from Office of Agricultural Economics.

Item	2012	2013	2014	2015	2016
1. Cultivated area (million plantation)	0.247	0.196	0.237	0.217	0.212
2. Product / plantation (kg)	257	270	243	262	264
3. Total product (million ton)	0.064	0.053	0.058	0.057	0.056
4. Imported (million ton)	2.12	1.679	1.898	2.557	2.6
5. Exported (million ton)	0.002	0.002	0.012	0.009	0.01
6. Demand in domestic (million ton)	2.21	1.741	1.944	2.605	2.657

From: Office of Agricultural Economics, 2016.

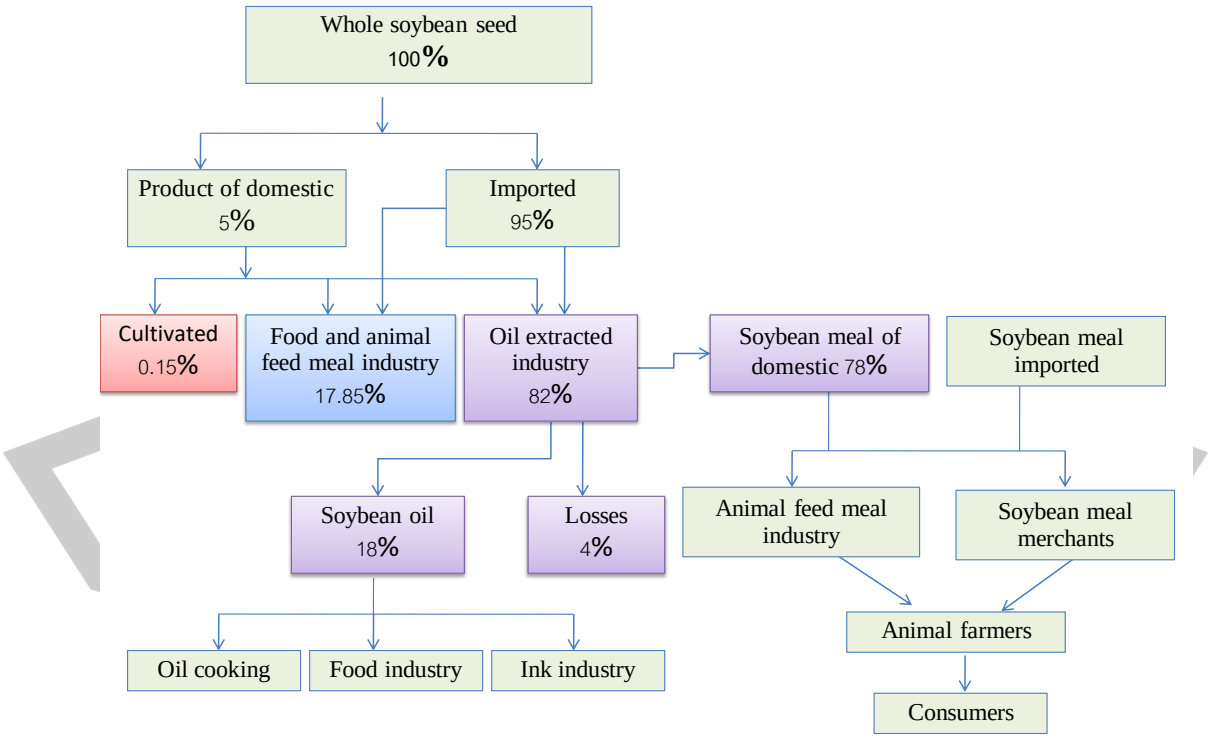


Figure 2 Diagram of soybean and soybean meal with demand in Thailand. From: Office of Agricultural Economics, 2016.

2.1.1 Physical and Chemical compositions of soybean

Soybean has important physical components such as seed coat, cotyledon and embryo.

1. Seed coat: the hull of the mature bean is hard, water-resistant, and protects the cotyledon and hypocotyl from damage. The scar, visible on the seed coat, is called the hilum (colors include black, brown, buff, gray and yellow) and at one end of the hilum is the micropyle, or small opening in the seed coat which can allow the absorption of water for sprouting (Purcell, L. C., Salmeron, M., & Ashlock, 2014).

2. Cotyledon: The first photosynthetic structures, the cotyledons, develop from the hypocotyl, the first plant structure to emerge from the soil. These cotyledons both act as leaves and as a source of nutrients for the immature plant, providing the seedling nutrition for its first 7 to 10 days (Fig. 3) (Purcell, L. C., Salmeron, M., & Ashlock, 2014).

3. Embryo: the part of soybean seed that will develop as a soybean plant, including epicotyls, hypocotyls and radical (contain of lipid and insoluble carbohydrate) (Purcell, L. C., Salmeron, M., & Ashlock, 2014).

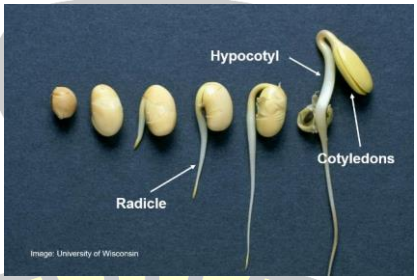


Figure 3 Germination of soybean seeds.
From: http://www.fssystem.com/Agronomy/News/Pages/The-Soybean-Hypocotyl-and-Hypocotyl-Arch.aspx, 2017.

Chemical compositions of soybean seed as below

1. Protein: the protein content of the soybean seed is approximately 36%. The proteins contain several essential amino acids such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (shown on Table 2). Additionally, soy protein is a relatively heat-stable storage protein. This heat stability enables soy food products requiring high-temperature cooking, such as tofu, soymilk and textured vegetable protein (soy flour) to be made.

The Protein Digestibility Corrected Amino Acid Score (PDCAAS) of soy protein is the nutritional equivalent of meat, eggs, and casein for human growth and health. Soybean protein isolate has a biological value of 74, whole soybeans 96, soybeans milk 91, and eggs 97 (FAO/WHO Expert Consulation. Joint, 1989). Soy protein is essentially identical to the protein of other legume seeds and pulses. Moreover, soybeans produced at least twice as much protein per acre than any other major vegetable or grain crop besides hemp. 5 to 10 times more protein per acre than land set aside for grazing animals to make milk, and up to 15 times more protein per acre than land set aside for meat production (Derbyshire, Wright, & Boulter, 1976; Soy benefit, 2012).

2. Fat: whole soybean seeds contain approximately 20% fat and have a very high level of unsaturated fatty acids (about 15%) and saturated fatty acid (5%) (presented on Table 2), which are considered as part of the healthy fat group (USDA Nutrient Database, 2017; Zambiazi, Przybylski, Zambiazi, & Mendonça, 2014). Mono and poly unsaturated in soybean are an essential fatty acid, especially omega-3 and -6 fatty acids for human nutrition. These essential fatty acids are so named because they cannot be synthesized by the human body. In general, consumption of foods rich in omega-3, 6 and 9 fatty acids can help to reduce the risk of cardiovascular disease (Kumar, M. S., & Mishra, 2014).

Table 2 Nutritional value of soybean (mature seeds, raw).

Nutritional value per 100 g					
Energy	1,866 kJ (446kcal)	Cystine	0.66 g	Pantothenic aicd (B_5)	0.79 mg (16%)
Carbohydrates	30.16 g	Phynelalanine	2.12 g	Vitamin B_6	0.38 mg (29%)
Sugars	7.33 g	Tyrosine	1.54 g	Folate B_9	375 µg (94%)
Dietary fiber	9.3 g	Valine	2.03 g	Vitamin B_{12}	0 µg (0%)
Fat	19.94 g	Arginine	3.15 g	Choline	115.9 mg (24%)
Saturated	2.88 g	Histidine	1.10 g	Vitamin C	6.0 mg (7%)
Monounsaturated	4.40 g	Alanine	1.92 g	Vitamin E	0.85 mg (6%)
Polyunsaturated	11.25g	Aspartic acid	5.11 g	Vitamin K	47 µg (45%)
omega-3	1.33 g	Glutamic acid	7.84 g	**Minerals**	
omega-6	9.92 g	Glysine	1.88 g	Calcium	277 mg (28%)
Protein	36.49 g	Proline	2.38 g	Iron	15.7 mg (121%)
Trytophan	0.59 g	Serine	2.36 g	Magnesium	280 mg (79%)
Threonine	1.77 g	**Vitamins**		Manganese	2.52 mg (120%)
Isoleucine	1.97 g	Vitamin A equiv.	1 µg (0%)	Phosphorus	704 mg (101%)
Leucine	3.31 g	Thiamine (B_1)	0.87 mg (78%)	Potassium	1797 mg (38%)
Lysine	2.71 g	Riboflavin (B_2)	0.87 mg (73%)	Sodium	2 mg (0%)
Methionine	0.55 g	Niacin (B_3)	1.62 mg (11%)	Zinc	4.89 mg (51%)
Other constituents					
Water	8.54 g	Cholesterol	0 mg		

From: https://USDA Nutrient Database.com, 2017.

3. Carbohydrate: whole soybean contains 30% to 40% carbohydrate (defatted soybean flour), including polysaccharides 15-18%, oligosaccharide 15% (sucrose, stachyose, raffinose and verbascose) and acidic polysaccharides 8-10% (Karr-Lilienthal, Kadzere, Grieshop, & Fahey, 2005). However, the studies have shown that their consumption is related to promoting health benefits, such as lowering blood cholesterol, reducing blood pressure and protecting against some types of cancer (Kumar, M. S., & Mishra, 2014).

4. Vitamins and minerals: soybean is a good source of vitamins and minerals (see on above Table 2). Soybeans offer a range of nutritional benefits that

contribute folate. And theyare rich in minerals like calcium, zinc, potassium, iron and magnesium. Our nutrients found in beans are called “Phytonutrients”, which are non-essential compounds in food that can provide health benefits and some phytonutrients found in soybean and beans have been reported to reduce risk factors related with cardiovascular disease, reduce blood glucose, insulin and cholesterol concentrations (Kumar, M. S., & Mishra, 2014).

2.2 Phenolic compounds

Phenolic compounds in food originated from one of the main classes of secondary metabolism in plants (Shahidi & Ambigaipalan, 2015). Phenolics can be describe as substances possessing an aromatic ring and contain one or more hydroxyl group on the carbon chain, including their functional derivatives. Phenolics can be found in animal tissues and non-plant materials. They are especially due to ingestion of plant foods, of which the most ubiquitous phenols are polymeric and insoluble lignins that are found in all vascular plants. Many of the food phenolics are soluble in water or organic solvents and phenolics found in foods generally belong to phenolic acids, flavonoids, lignan, stilbenes, coumarins and tannins (Shahidi & Ambigaipalan, 2015).

Phenolics can be used in pharmaceutical, biological, nutraceutical and therapeutic functions via their antioxidant, anti-inflammatory, antiviral, hypolipiddemic, anticancer and hypoglycemic properties (Bravo, 2009; Hollman et al., 1996). The hydroxyl group and carboxylic acids of the aromatic ring in phenolics increase their partial conjugate with food components such as proteins, carbohydrates, lipids and minerals (Alu’datt et al., 2016; Alu’datt, Rababah, Ereifej, & Alli, 2013; Bravo, 2009; Escarpa & González, 2001).

Polyphenols, particularly tannins have been considered as antinutrients because tannins form complexes with proteins thus resulting in reduction of protein digestibility. However, more recently these compounds are considered as important dietary antioxidant (Bravo, 2009; Siddhuraju, 2006). Phenolic antioxidants can separate in classification of phenolic antioxidants on Fig 4 (Shahidi & Ambigaipalan, 2015).

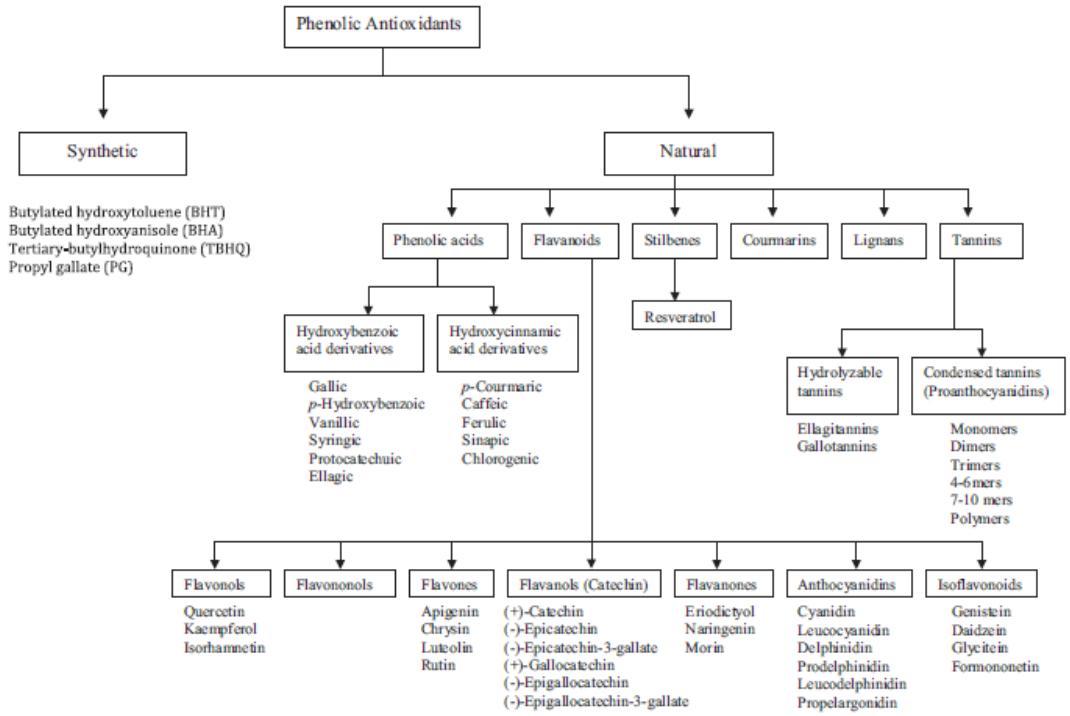


Figure 4 Classification of phenolic antioxidants.
From: Shahidi & Ambigaipalan, 2015.

The polyphenols are find in foods such as vegetables, fruits, legume seeds, beverages, spices, herbs and residue from the food industry (de Oliveira Silva & Perrone, 2015; Shahidi & Ambigaipalan, 2015). They can act as reducing agents (free radical terminators), metal ion chelation (Rice-Evans, C; Miller, NJ; Paganga, 1996) and thus to protect oxidative damage to biomolecules, such as DNA, lipids and proteins. Many researchers have documented antioxidant potential of polyphenol in fruits, vegetables, legumes and residual products (de Oliveira Silva & Perrone, 2015; Kubola & Siriamornpun, 2008; Marathe, Rajalakshmi, Jamdar, & Sharma, 2011; Shahidi & Ambigaipalan, 2015).

Phenolics are classified as primary antioxidants, for example mainly free radical scavenger that delay or inhibit the initiation step or interrupt the propagation step of lipid oxidation, thus decreasing the product of volatile decomposition such as aldehydes and ketones, that cause rancidity (Alamed, Chaiyasit, McClements, & Decker, 2009; Shahidi, 2011; Shahidi & Ambigaipalan, 2015). Capacity of antioxidant in phenolic compounds depends on the number and arrangement of the

hydroxyl groups in molecule structures (Cao, Sofic, & Prior, 1997; Sang et al., 2002). The reaction of phenolic antioxidants are showed in reaction 1.1, 1.2 and 1.3, phenolics can donate hydrogen atom to lipid radicals and produce lipid derivatives and antioxidant radicals, which are more stable and less readily available to promote autoxidation (Shahidi & Ambigaipalan, 2015).

$$R•/RO•/ROO• + AH \rightarrow A• + RH/ROH/ROOH \quad (1.1)$$

$$RO•/ROO• + A• \rightarrow ROA/ROOA \quad (1.2)$$

$$ROO• + RH \rightarrow ROOH + R• \quad (1.3)$$

The effect of antioxidant capacity on autoxidation rates depends on many factors, including the structure of the antioxidant, oxidation conditions and the nature of the sample being oxidized (Shahidi & Ambigaipalan, 2015). The loss of antioxidant activity in phenolic compounds at high concentrations and behave as prooxidant (Gordon, 1990).

2.2.1 Synthetic food phenolics

Synthetic phenolic antioxidants (Fig. 5) currently in use in food are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary-butylhydroquinone (TBHQ). Additionally, octyl gallate (OG) and dodecyl gallate (DG) used as a synthetic antioxidant (Makahleh, A., Saad, B., & Bari, 2015). These synthetic phenolic antioxidants used in the food industry for more than 60 years and added to products in order to protect or extend the onset of lipid oxidation during processing and storage (Saad et al., 2007).

2.2.1.1 Butylated hydroxyanisol (BHA)

BHA is a synthetic monophenolic antioxidant and available as white waxy flakes, which are mixtures of two type isomers (3-tertiary-butyl-4-hydroxyanisole (90%) and 2-tetiary-butyl-4-hydroxyanisole (10%) (Fig 5). BHA can be soluble in fats and oil, and is not soluble in water, which is a more effective antioxidant in preventing deterioration of flavor and color of essential oils (Hettiarachchy, N. S., & Kalapathy, 2000). The effect of BHA and other synergistic antioxidants like BHT, TBHQ or PG provide the most antioxidant activity by mixing

synthetic antioxidant than used to each individual antioxidant. Others reported that BHA is possibly carcinogenic to humans (IRAC, 1987).

The level of BHA and other synthetic antioxidants (BHT and propyl gallate) should not be exceed 1 mg/kg body weight/day and on averages is less than 0.4 mg/kg body weight/day (Kirkpatrick & Lauer, 1986). Moreover, at concentrations as low as 125 ppm, which is acceptable for use in food additive levels, BHA exhibits anti-carcinogenic properties (Williams, Iatropoulos, & Whysner, 1999). BHA is used in bakery and fried products as well as in cereal, potato products, dessert mixes and beverages (Dolatabadi & Kashanian, 2010). Levels for use synthetic antioxidants are present in Fig 6

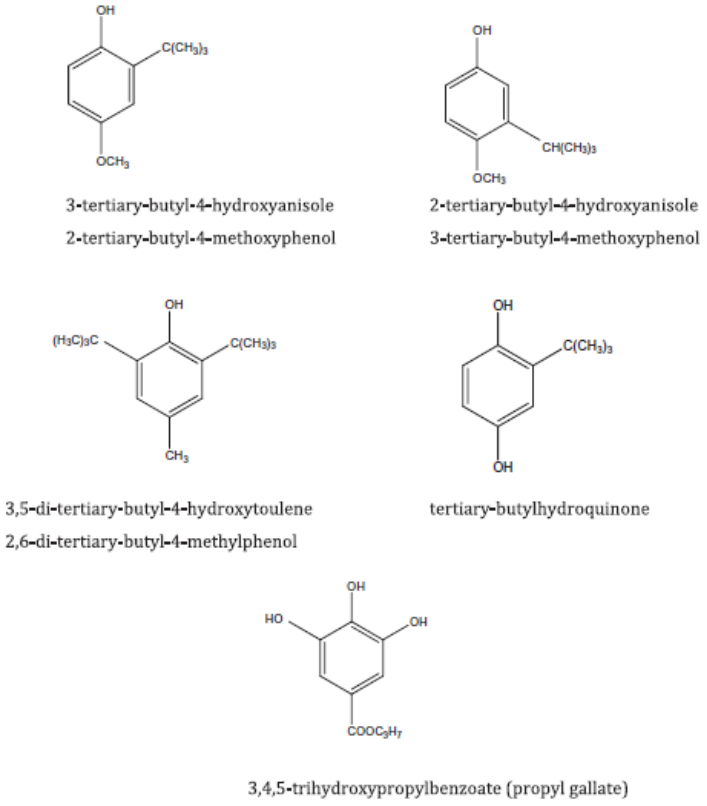


Figure 5 Chemical structures of synthetic antioxidants.
From: Shahidi & Ambigaipalan, 2015.

Food category	Maximum usage level (mg/kg)			
	BHA	BHT	PG	TBHQ
Beverage whiteners	100	100	–	100
Milk powder and cream powder (plain)	100	200	200	–
Butter oil, anhydrous milkfat, ghee	175	75	100	–
Vegetable oils and fats	200	200	200	200
Lard, tallow, fish oil, and other animal fats	200	200	200	200
Fat spreads, dairy fat spreads and blended spreads	200	200	200	200
Fat emulsions mainly of type oil-in-water, including mixed and/or flavoured products based on fat emulsions	200	200	200	200
Fat-based desserts excluding dairy-based dessert products	200	200	200	200
Edible ices, including sherbet and sorbet	200	100	–	200
Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	200	200	50	–
Cocoa and chocolate products	200	200	200	200
Imitation chocolate, chocolate substitute products	–	200	200	–
Confectionery including hard and soft candy, nougats, etc.	200	200	200	200
Chewing gum	400	400	1000	400
Decorations (e.g., for fine bakery wares), toppings (nonfruit) and sweet sauces	200	200	200	200
Whole, broken, or flaked grain, including rice	–	–	100	–
Breakfast cereals, including rolled oats	200	100	200	–
Pre-cooked pastas and noodles and like products	200	200	100	200
Bakery wares	200	200	200	–
Processed meat, poultry, and game products in whole pieces or cuts	200	100	200	100
Processed comminuted meat, poultry, and game products	200	100	200	100
Frozen fish, fish fillets, and fish products, including molluscs, crustaceans, and echinoderms	200	200	–	–
Frozen battered fish, fish fillets, and fish products, including molluscs, crustaceans, and echinoderms	200	200	–	–
Smoked, dried, fermented, and/or salted fish and fish products, including molluscs, crustaceans, and echinoderms	200	200	100	–
Semi-preserved fish and fish products, including molluscs, crustaceans, and echinoderms	200	200	–	–
Fully preserved, including canned or fermented fish and fish products, including molluscs, crustaceans, and echinoderms	200	200	–	–
Herbs, spices, seasonings and condiments (e.g., seasoning for instant noodles)	200	200	200	200
Soups and broths	200	100	200	200
Sauces and like products	200	100	200	200
Yeast and like products	200	–	–	–
Food supplements	400	400	400	–
Snacks – potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	200	–	200	–
Processed nuts, including coated nuts and nut mixtures (with e.g., dried fruit)	200	–	200	–
Ready-to-eat savouries	–	200		200
Water-based flavoured drinks, including "sport", "energy", or "electrolyte" drinks and particular drinks	–	–	1000	–
Desserts – dairy based/fruit based/cereal and starch based/egg based	–	–	90	–
Mustards	–	–	–	200

Figure 6 the usage levels of synthetic antioxidants followed by Codex Alimentarius Commission.

From: Shahidi & Ambigaipalan, 2015.

2.2.1.2 Butylated hydroxytoluene (BHT)

BHT is a monophenol and commercially available as a white powder. However, BHT is not as effective as BHA because of the structure of two tert-butyl groups, which offer better steric hindrance than BHA to the molecule (Hettiarachchy, N. S., & Kalapathy, 2000). In addition, previous studied had reported about BHT may cause internal and external haemorrhaging at high dose that is severe enough to cause death in some strains of mice and guina pigs by effect is due to the ability of BHT to reduce vitamin K dependent blood clotting factor (Ito et al., 1986). Williams, Iatropoulos, & Whysner, (1999) reported that BHT is not genotoxic or reproducibly carcinogenic at high doses of 250 mg/kg/day, but has some tumor-promoting activity.

The acceptable daily intake (ADI) for BHT is 0-0.3 mg/kg body weight (Leclercq, Arcella, & Turrini, 2000).

2.2.1.3 Teriary-butylhydroquinone (TBHQ)

TBHQ is a greater effective preservative for unsaturated vegetable oils, animal fats and meat products. The property of BHT, it does not cause discoloration and not change the flavor or odor of the material (Kashanian & Dolatabadi, 2009). Khan & Shahidi (2001) reported that TBHQ was stronger antioxidant than BHA and BHT because of the two *para*-hydroxyl groups in TBHQ are responsible for the antioxidant activity. In addition, TBHQ could prevent biodiesel and extend the storage time (De Araujo, Barbosa, Viana, & Ferreira, 2011; Goulart, Teixeira, Ramalho, Terezo, & Castilho, 2014). A number of studies have shown that TBHQ has some negative health effect when using high doses, such as precursor to stomach tumours and damage to DNA (Kashanian & Dolatabadi, 2009). Moreover, when TBHQ was hydrolyzed in vitro, its gave 2-tert-butyl-1,4-benzoquinone (TBQ) that TBQ being more strongly cytotoxic (Okubo, Yokoyama, Kano, & Kano, 2003).

2.2.1.4 Propyl Gallate (PG)

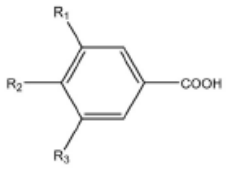
Propyl gallate used in many products such as cosmetic, food-packaging materials, foods containing fats and as an additive in edible fats, oils, mayonnaise, shortening, bakery products, pressure-sensitive adhesives, lubricating oil additives and transforming oils (Zurita et al., 2007). PG is prepared by using esterifacation of gallic acid with propyl alcohol followed by distillation to remove the excess alcohol and is available as a white powder, can solutes in water and has melting point of 148 ^{o}C with loses its effectiveness during heat processing and not stable in frying application that involve on high temperature more than 190 ^{o}C (Shahidi & Ambigaipalan, 2015). A number of studies showed that PG and its metabolite to exhibit liver toxicity and increase carcinogenesis (Eler, Peralta, & Bracht, 2009; Kim, Kang, Lee, Lee, & Lee, 2008). In previous studied reported have indicated that the antioxidative and cytoprotetive properties of propyl gallate may change to prooxidative, cytotoxic and genotoxic in the presence of Cu (II) (H. Jacobi, Hinrichsen, Wess, & Witte, 1999; Heike Jacobi, Eicke, & Witte, 1998).

2.2.2 Natural phenolic antioxidants

Presently, several consumers considered on the toxicity of synthetic antioxidant. Thus, the natural antioxidant of plants products to promote as potential antioxidants to prevent against various diseases from free radicals has been explored (Hou et al., 2003). In addition, the natural antioxidant in foods has multiple activities of antioxidants include inactivating metal catalysts by chelation, reduce hydroperoxide radicals to stable hydroxyl derivatives and interacting with other reducing compounds (Frankel, E. N., & Finley, 2008). Recent years, the natural antioxidants from plant products promoted as food additives in food products to replace synthetic antioxidant. Natural antioxidant compounds are phenolic acids, flavonoid, lignans, terpenes, tocopherols, phospholipids and polyfunctional organic acids and others. The sources of natural phenolic compound found in fruits, vegetables, legumes, nuts, spices and herbs with all parts of plants such as seeds, leaves, roots, bark and flours (Wanasundara & Shahidi, 1996). There have been many reported that the biological activities of phenolic compounds, which are effective antioxidant, metal chelation and free radical scavenge (Shahidi & Ambigaipalan, 2015; Tyug et al., 2010).

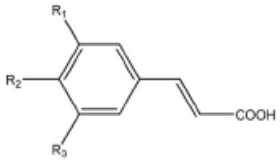
2.2.2.1 Phenolic acids

Phenolic acids are abundant and spread throughout the plant kingdom, interacting with proteins, carbohydrates or lipids in all parts of plants. Furthermore, there are an integral part of the human diet and are consumed as medicinal preparations (Shahidi & Ambigaipalan, 2015). The predominant phenolic acids are hydroxybenzoic and hydroxycinnamic derivatives that found in plants with hydroxycinnamic acids being the more common (Fig 7)



Acid	R_1	R_2	R_3
p-Hydroxybenzoic	H	OH	H
Protocatechuic	OH	OH	H
Vanillic	OCH_3	OH	H
Syringic	OCH_3	OH	OCH_3
Gallic	OH	OH	H

Hydroxybenzoic acid



Acid	R_1	R_2	R_3
p-Courmaric	H	OH	H
Caffeic	OH	OH	H
Ferulic	OCH_3	OH	H
Sinapic	OCH_3	OH	OCH_3

Hydroxycinnamic acid

Figure 7 Chemical structures of phenolic acids.
From: Shahidi & Ambigaipalan, 2015.

These derivatives differ in the group of hydroxylation and methoxylation in their aromatic rings (Mattila & Hellström, 2007; Shahidi & Ambigaipalan, 2015). The basic pathway for synthesis of phenolic acids in plants (Fig 8) begins from sugar through to aromatic amino acids- phenylalanine and in some rare case- tyrosine. That the formation of *trans*-cinnamic acid from phenylalanine and *p*-hydroxycinnamic acid from tyrosine is catalyzed by phenylalanine ammonia lyase and tyrosine ammonie lyase, respectively (Amarowicz, Carle, et al., 2009; Amarowicz, Zegarska, et al., 2009). The common hydroxycinnamic acids are caffeic, *p*-courmaric and ferulic, which found in foods as simple ester form with quinic acid or glucose while the bound hydroxycinnamic acids is well-known and call that chlorogenic acid. In addition, the hydroxybenzoic acid derivatives are the most present in foods in the glucoside forms such as p-hydroxybenzoic, vanillic and protocatechuic acids are the most common forms (Mattila & Hellström, 2007; Shahidi & Chandrasekara, 2010; Shahidi, McDonald, Chandrasekara, & Zhong, 2008; Yeo & Shahidi, 2015). Many studies on phenolic compounds showed that the important role on health protective like antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antimicrobial and other biological properties (Crozier, Lean, McDonald, & Black, 1997; Manach, Mazur, & Scalbert, 2005; Xu, Ye, Liu, Ma, & Chen, 2008). Phenolic acids behave as

antioxidants from the reactivity of their phenol moiety (hydroxyl substituent on the aromatic ring). Thus, there are several mechanisms with the capacity of antioxidant is believed to be radical scavenging via hydrogen atom donation. (Shahidi & Ambigaipalan, 2015). Different of substituents on the aromatic ring affect the stabilization and therefore affect the radical scavenger ability of these phenolic acids (Rice-Evans, C; Miller, NJ; Paganga, 1996). The antioxidant capacity of the free, esterified and glycosylated phenolics with promoting health benefits related with these simple phenolic acids has been reported (Olthof, Hollman, & Katan, 2018; Robbins, 2003). The estimated number of polyphenols in our daily diet ranged between 2590 to 3016 mg/day. Of the total polyphenol intake in the daily diet, 48% gets absorbed in the small intestine, 42% is accessible in the large intestine and 10% remains unabsorbed (Anal, A. K., Kumaree, K. K., & Thapa, 2014).

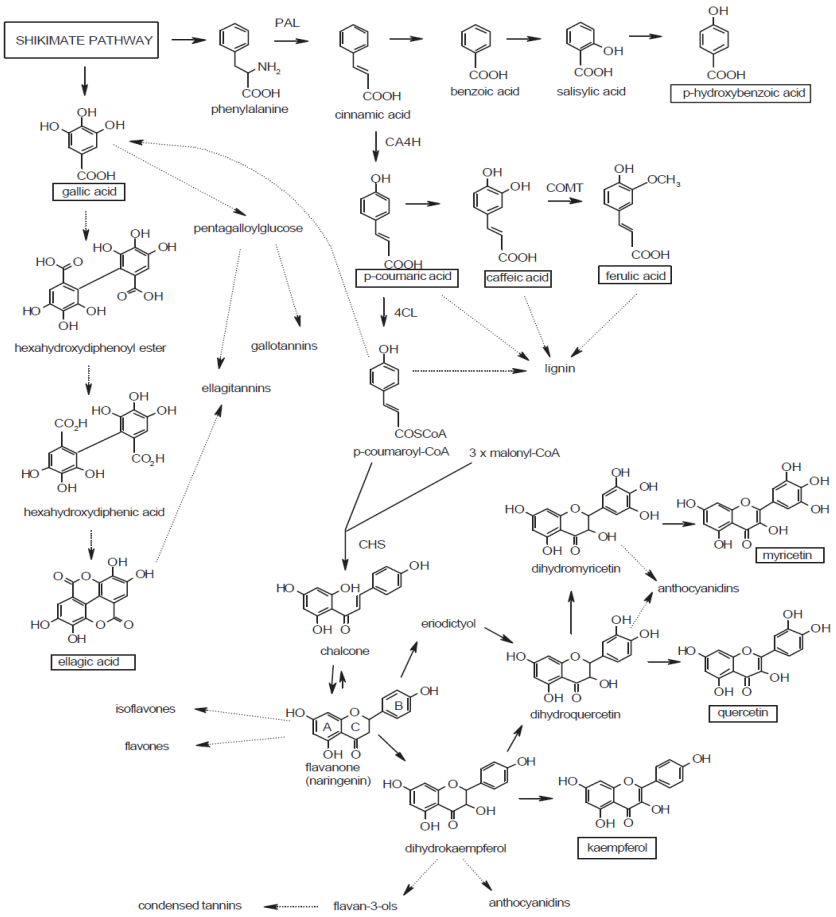


Figure 8 Biosynthetic pathway of phenolic compounds.
From: Shahidi & Ambigaipalan, 2015.

2.2.2.2 Flavonoids

Presently, when referencing phenolics in plant foods, flavonoids are the rich class described. Flavonoids are cyclized diphenylpropanes that commonly occur in plants and particularly plant foods (Cao et al., 1997). Flavonoids include flavones, flavonol, flavanones, isoflavones, flavanonol, flavanol and anthocyanidin. The structure of flavonoids has the characteristic C_6-C_3-C_6 carbon skeleton but different on hydroxyl and methoxy group on the aromatic ring position (Fig 9)

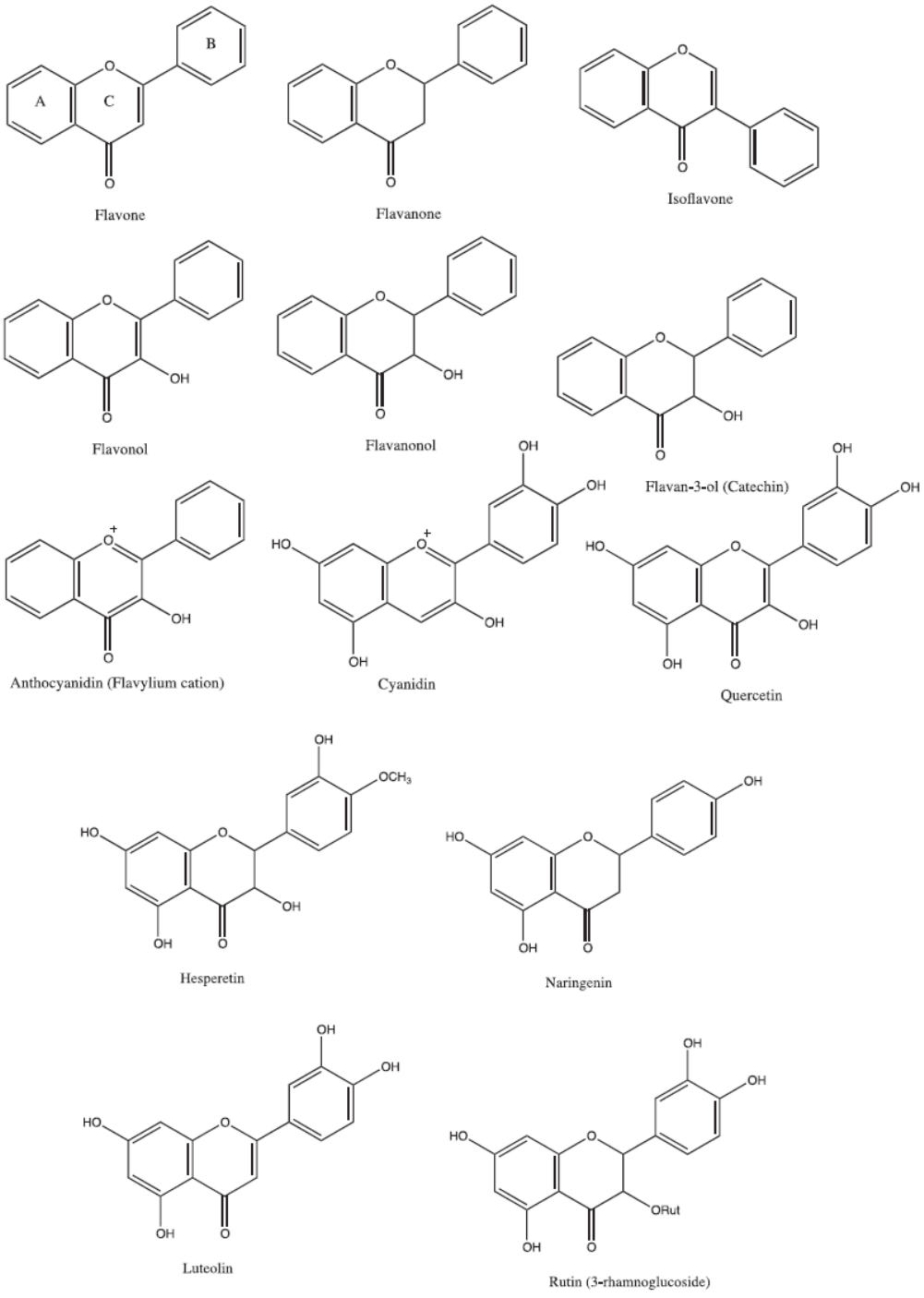


Figure 9 Chemical structures of flavonoids and some selected flavonoid compounds. From: Shahidi & Ambigaipalan, 2015.

The biological activity of flavonoids depends on their chemical structures and the associations of various moieties in the molecules. Han et al, (2009) found that some isoflavonoids are more active as antioxidant than their corresponding

flavonoids. Flavones and flavonols found in aglycone form in foods (approximately 200 flavonols and some 100 flavones have identified in plants). These compounds possess a double bond between C-2 and C-3 while flavonols are different from flavones in that they possess a hydroxyl group in the 3-position and can be regarded as 3-deoxyflavonols (Fig 9) (Shahidi & Ambigaipalan, 2015). In addition, flavonones and flavononols have presented by a saturated C2-C3 bond and an oxygen atom (carbonyl group) in the 4-position. Flavonones may be referred to as dihydroflavones and flavononols differ from flavonones by having a hydroxyl group in the 3-position on the aromatic ring, and are called to as 3-hydroxyflavonones or dihydroflavonols (Shahidi & Ambigaipalan, 2015). Flavonoids found in natural plant foods and are presented some flavonoids in the Table 3

Table 3 Dietary sources of flavonoids.

Class	Name	Dietary sources
Flavone	Chrysin	Fruits skins
	Apigenin	Parsley, celery
Flavonone	Naringin	Citrus, grapefruit
	Naringenin	Citrus
	Taxifolin	Citrus
	Eriodictyol	Lemons
	Hesperridin	Oranges
	Isosakuranetin	Citrus
Flavonol	Kaempferol	Leek, broccoli, endives, grapefruit, black tea
	Quercetin	Onion, lettuce, broccoli, tomato, tea, berries, apples, olive oil, cranberry

Table 3 Dietary sources of flavonoids (continues).

Class	Name	Dietary sources
Flavonol	Rutin	Buckwheat, citrus, red pepper, red wine, tomato skin
Flavononol	Engeletin	White grapeskin
	Astilbin	White grapeskin
Isoflavone	Genistin	Soybean
	Genistein	Soybean
	Daidzin	Soybean
	Daidzein	Soybean
Flavanol	(+)-Catechin, (+)-Gallocatechin, (-)-Epicatechin, (-)-Epigallocatechin, (-)-Epicatechin gallate, (-)-Epigallocatechin gallate	Tea
Anthocyanidin	Epigenidin	Stored fruits
	Cyanidin	Cherry, raspberry, strawberry, grapes
	Delphinium, Pelargonidin	Dark fruits

From: Shahidi & Ambigaipalan, 2015.

Generally, the flavonoid capacities are effective antioxidants depends on three factors: firstly, the metal-chelating potential that is highly chelating dependent on hydroxyls and carbonyl group around the aromatic ring. Secondly, the ability of hydrogen or electron donating substituents able to reduce free radicals, and lastly, the ability of flavonoids to delocalize the unpaired electron leading to the formation of a stable phenoxyl radical. It is ordinarily accepted that the excellent antioxidant properties of flavonoids are occur to presence of catechol hydroxyl groups in the B ring (Musialik, Kuzmicz, Pawcowski, & Litwinienko, 2009; Rice-Evans, C;

Miller, NJ; Paganga, 1996; Zhou, Miao, Yang, & Liu, 2005). Additionally, flavonoids with high capacity of antioxidant have the following characteristic: 1) a 3′,4′-dihydroxyl group in B ring, 2) the 3-OH moiety in the C ring and 3) the C2-C3 double bond in the C ring conjugated with a 4-keto group causing electron delocalization from the B ring, and 4). Both 3-OH group in C ring and 5-OH group in A ring combined with a 4-carbonyl group and C2-C3 double bond (Sun & Powers, 2009).

In addition, soybean and soybean meal not only source of protein and lipid but also contains bioactive compounds such as phenolics and flavonoids. The major bioactive compounds found in soybean were isoflavones, including three aglycones (genistin, daidzein and glycitein), in 7-*O*-β-D-glucosides (genistin, daidzein and glycitein), 6″ *O*-malonyl-7-*O*- β-D-glucosides (malonylgenistin, malonyldaidzein and malonylglycitein) and 6″ *O*-acetyl-7-*O*- β-D-glucosides (acetylgenistin, acetyldaidzein and acetylglycitein) (Udgata & Naik, 2007). Devi et al. (2009) reported that the content of isoflavones and ability of antioxidant in soybean products found that the amount of isoflavones in products after processed remained with antioxidant activity. Marathe et al. (2011) who reported that the antioxidant activity of different common bean varieties in India and found that soybean had total phenolic content 2.17 mg GAE/g and capacity of antioxidant at high level (DPPH, ABTS, FRAP and Chelation). Alu'datt et al. (2016) reported that the free and bound phenolic content with antioxidant activity in by-product from soybean, who found that free and bound phenolic content in soybean residue in the ranged from 0.37-0.92 mg/g and 0.18-1.24 mg/g, respectively. Furthermore, de Oliveira Silva & Perrone (2015) studied on the characterization and stability of bioactive compounds in soybean meal and found that TPC, TFC were remained and in the ranged from 4.4-12.9 mg GAE/g and 6.7-13.6 mg GE/g, respectively.

2.3 Measurement of total phenolic content and antioxidant activity

2.3.1 Total phenolic content (TPC)

Total phenolic content (TPC) is important parameter of total antioxidant capacity (TAC), and widely used for evaluation of antioxidant extracts, including extracts from fruits, herbs, spices, cereals, legumes and other plant foods. The Folin-Ciocalteu assay is originally and well-known method for determination of TPC but the Folin-Ciocalteu assay developed to analyze of protein, which takes advantage of phenolic amino acid tyrosine in proteins (Magalhães, Segundo, Reis, & Lima, 2008). Later, Singleton, Orthofer, & Lamuela-Raventós (1998) adapted the assay to analyses phenolic compounds in wine, and then it became a method analysis for antioxidant assessment of food and plant extracts.

The Folin-Ciocalteu assay based on the reduction of the Folin-Ciocalteu reagent by phenolic compouns, changed color from yellow to blue and determined at 765 nm (Magalhães et al., 2008). The molybdenum center in the complexes were generally accepted as the reduction site, where the Mo^{6+} ion is reduced to Mo^{5+} by receiving an electron donation by the phenolic antioxidant. Thus, the Folin-Ciocalteu method is as an ET-based assay and related with reducing power of phenolic antioxidant. The mostly used gallic acid to reference standard and results of TPC usually expressed as gallic acid equivalents. In addition, some TPC results are also use catechin, caffeic acid or ferulic acid equivalents that suitable standardization of the reported results (Karadag, Ozcelik, & Saner, 2009).

2.3.2 DPPH radical scavenging assay

DPPH radical scavenging assay is preferred to use the method as the first approach for evaluating antioxidant activity. The method is an ET-based method with HAT mechanism being only a marginal reaction pathway in the assay (Prior, Wu, & Schaich, 2005). The DPPH radical is stable free radical with a deep purple color, which is commercially available and does not need to generate prior to the assay**.** The assay is based on electron donation of antioxidants to neutralize DPPH radical by looking on color change of the DPPH measured at 517 nm**.** The discoloration acts as an indicator capacity of antioxidant.

2.3.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is an ET-based method that measures the reduction of ferric ion (Fe^{3+}) to the blue color ferrous (Fe^{2+}) complex by antioxidants in acidic media. Antioxidant activity is determined as increase of absorbance at 593 nm and results are expressed as mmol or micromolar Fe^{2+} equivalents or relative with antioxidant standard (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002)**.** The FRAP assay uses tripyridyltriazine (TPTZ) as the iron-binding ligand and is the method under acidic pH conditions (pH 3.6) in order to maintain iron solubility and more importantly drive electron transfer, which will increase the redox potential and causing a shift in dominant reaction mechanism (Hagerman et al., 1998; Shahidi & Zhong, 2015).

2.4 Stability of bioactive compound during food processing

Difference food processing conditions such as extraction, boiling, drying and others processes to effect on bioactive compounds degradation like isomerization and oxidation, which influence its bioactivity and reduce the functionality for health benefits. The degradation reactions of bioactive compounds are influenced by factors such as reaction medium, temperature, physical state, type of bioactive compound, structure form in natural such as a glycones or binding with glucose, ability of solution, and environmental conditions, which is the most important factors during processing are heat, light and oxygen (Alu'datt et al., 2013; Boateng et al., 2008; de Oliveira Silva & Perrone, 2015; Devi et al., 2009; Marathe et al., 2011; Siah, Wood, Agboola, Konczak, & Blanchard, 2014; Xu et al., 2008).

2.4.1 Extraction and effect of extraction on bioactive compounds with antioxidant activity

Presently, the term "extraction" conveys the idea of pulling something out of something else. It is used to indicate a wide variety of actions, from the surgical removal of a tooth to the retrieval of an item from a database. The extraction will be defined as a separation process, based on differences in solubility. A solvent is used to

solubilize and separate a solute from other materials with lower solubility in the solvent.

It is customary to distinguish between two classes of extraction processes:

1. Solid-liquid extraction: this is where a solute is extracted from a solid phase with the help of a solvent. Examples include extraction of edible oils from oilseeds with organic solvents, extraction of protein from soybeans in the production of isolated soybean protein, etc. Solid-liquid extraction is also termed “leaching”. The mechanism of solid-liquid extraction involves wetting the solid surface with solvent, penetration of the solvent into the solid, dissolution of the extractable, transport of the solutes from the interior of the bulk of the solvent surrounding the solid particles by diffusion and agitation. In some case, the solubilization step may include chemical change promoted by the solvent. The process of extraction with a super-critical fluid (SCF) will be included in this category, it being mainly (but not exclusively) applied to solids.

2. Liquid-liquid extraction: this is a method for extracting a solute from a solution in a certain solvent, by using another solvent. Examples include extraction of penicillin from aqueous fermentation broth by butanol, extraction of oxygenated terpenoids from citrus essential oils using ethanol as a solvent, etc. Liquid-liquid extraction, also known as partitioning, is common in the chemical and pharmaceutical industries and in biotechnology, but much less so in food processing (Fellows, 2009).
The extraction is a separation process based on molecular transport, in which molecules pass from one phase to another under the effect of a difference in chemical potential. The analysis and design of separation operations based on molecular transport require knowledge in three main areas:

1. Equilibrium: net molecular transport stops when equilibrium is reached between the phases- i.e., when the chemical potential of the substance in question is the same in all phases in contact with each other. Although true equilibrium can never be reached within a finite duration, the concept of equilibrium is basic to the calculations. In practice, many of such separation processes consist of a number of consecutive stages through which the phases move, following various flow patterns. Countercurrent movement is the most frequent. The common approach is to simulate the real process as a series of theoretical equilibrium stages and to correct the

deviation from theoretical behavior with the help of empirical or semi-empirical efficiency factors. Equilibrium data may be given in the form of equations, tables or charts. Each stage of the extraction process comprises two operations. First, the two phases are brought into contact for a certain length of time, during which mass transfer between the phases occurs. Subsequently, the two phases are separated from each other by a number of possible methods, such as filtration, decantation, centrifugation, squeezing, draining, etc. Physically, the two operations may take place in the same piece of equipment (e.g., an extraction column) or may require two separate devices. The conditions for optimizing the two operations may be mutually contradictory. Thus, division of one of the phases into fine particles improves the rate of inter-phase mass transfer but makes subsequent separation more difficult.

2. Material and energy balance: each stage of the process must satisfy the laws of conservation of matter and energy, expressed as: In = Out + Accumulation. In steady-state continuous processes there is no accumulation. In the graphical representation of the processes, the equations resulting from material balance are represented as lines of operation.

3. Kinetics: the rate of inter-phase molecular transport depends on diffusion coefficients and turbulence. Transport kinetics determines the rate at which equilibrium is approached. Kinetic effects are often accounted for with the help of the efficiency factors mentioned above.

1.1 Solid-liquid extraction (leaching)

Solid-liquid extraction is a separation process based on the preferential dissolution of one or more of the components of a solid mixture in a liquid solvent. In this context, the term “solid mixture” is used in its practical meaning. In reality, the physical state of the component to be extracted from the raw material is not always solid. In the case of solvent extraction of oil from oilseeds, for example, the oil is already in the form liquid droplets in the raw material.

The multi-stage extraction, this objective of solid-liquid extraction is to extract as much as possible of the solute, using a limited quantity of solvent, so as to obtain a concentrated extract. Obviously, all these conditions cannot be met by single-state extraction, hence the need for multi-stage processes. Multi-stage extraction can

be continuous or semi-continuous. A number of options exist regarding the movement of the solid and the liquid streams with respect to each other.

Mostly, the large-scale solid-liquid extraction processes in the food industry are continuous or quasi-continuous. Batch extraction is used in certain cases, such as the extraction of pigment from plant, isolation of protein from oilseeds, or the production of meat and yeast extracts. In its simplest form, a batch extraction system consists of an agitated mixing vessel where the solid are mixed with the solvent, followed by a solid-liquid separation device. Decanter centrifuges are advantageously used as separators. In continuous multi-stage extraction, the main technical problem is that of moving the solids from one stage to another continuously, particularly if the process is carried out under pressure. The different solid-liquid extraction systems in use differ mainly in the method applied for conveying the solid stream from stage to stage.

1. Fix bed extractors: for fixed bed extractors, the solid bed is stationary. The countercurrent effect is achieved by moving the position where the fresh solvent is introduced. For example, of fixed bed extractor is the quasi-continuous, countercurrent, high-pressure process used for the extraction of coffee in the manufacture of instant (soluble) coffee.

2. Belt extractors: belt extractors are used extensively for extracting edible oil from oilseeds and sugar from crushed sugar cane. The material to be extracted is continuously fed by means of a feeding hopper, so as to form a thick mat on a slowly moving perforated belt. The bed height is kept constant by regulating the feed rate. The mat is continuous, but distinct extraction stages are delimited by the way in which the liquid stream is introduced. Fresh solvent is sprayed to the solid at the tail “section” of the extractor. The first extract is collected at the bottom of the section and pumping to the next section is repeated in the direction opposite to the movement of the belt. The most concentrated extract, known as “full micella” is collected at the bottom of the first (head) section. In their passage from one section to another, the extract may be reheated with the help of heat exchangers.

3. Carousel extractors: also, most commonly used for the extraction of edible oil from oilseeds. This extractor consists of a vertical cylindrical vessel inside which a slowly revolving concentric rotor is installed. The rotor is divided into

segment by radical partition walls, and rotates over a slotted bottom. The segments contain the solid to be extracted. The liquid extractant is introduced at the top and percolates through the solid bed. The extract exits through the slotted bottom and is collected in chambers separated by weirs, to be pumped back onto the solid bed at the next section. The sequence of liquid collection and pumping is in the direction opposite to that of rotation. At the end of one rotation, the segment passes over a hole in the bottom plate through which the spent solids are discharged. At the next station, the chamber is filled with fresh material and the cycle continues.

4. Auger extractors: the solids are conveyed vertically by a large screw conveyor rotating inside a cylindrical enclosure, against a descending stream of extractant liquid. Inclined versions of this class of extractors are also available. Variants of the auger extractor are extensively used for the extraction of sugar from cut sugar-beet chips (cossettes) with hot water.

5. Basket extractors: the material to be extracted is filled into baskets with perforated bottoms. The baskets are moved vertically or horizontally. In the case of vertical basket extractors, also known as bucket elevator extractors, the solvent flows down by gravity through the buckets and is collected at the bottom of the chain. Vertical basket extractors are among the first large-scale continuous solid-liquid extractors (Fellows, 2009).

6. Cold press extractors: there are several ways to extract oil from plants and trees. For example, there is distillation and solvent extraction in which the plant is infused in other substances to extract the aromatic particles. But when extracting oil from the seed, cold pressing is preferred. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant. The cold pressing process does not need an external substance as with other methods. The seeds are crushed and pressed in order to force the oil out. Though the friction caused by the pressure does increase the temperature of the product, this is not high. Manufacturers must keep it within a certain degree range to be able to claim that the oil is cold pressed. According to the Codex Alimentarius Standard 210-1999 and Amendments (2003/2005), cold press oil are obtained, without altering the oil, by mechanical procedures only, e.g., expelling or pressing, without application of heat. They may have been purified by washing

with water, settling, filtering and centrifuging only. The temperature during cold pressing should not exceed 50 ºC (Moreau, R. A., & Kamal-Eldin, 2009). For oil seeds, temperature inevitably increases during pressing due to friction. Only in the case of olive oil, the pressing can proceed successfully at room temperature (below 30 ºC) while cold pressed pumpkin seed oil used the temperature of the seeds during processing should not exceed 60 ºC (Goranovic, 2009). However, the unique of roasted flavor requires heating above 90 ºC. In the case of berry seed oils, cold press is carried out at temperatures between 40 ºC and 60 ºC depending on the composition of the different seeds (Van Hoed et al., 2009).

Processing condition strongly affect the rate and yield of extraction, as well as the quality of the extract product. The influence of processing parameters on performance is essential for the optimal design and operation of extraction processes and systems.

- Temperature: where the thermal damage is not an issue, high temperature is preferred for their positive effect on yields and rate. At high temperature the solubility of the extractable in the solvent is higher and solvent viscosity is lower, resulting in enhanced wetting and penetration capability and higher diffusion coefficients. In the case of volatile, inflammable solvents such as hexane, ethanol or acetone, safety consideration determines the highest application temperature. In the certain case, lower temperatures may be preferred if the selectivity of the solvent towards the desired extractable is improved by lowering the temperature.

- Pressure: solid-liquid extraction at very high temperature implies pressurization to maintain the solvent in liquid state. Ho, Cacace, & Mazza (2007) applied pressurized liquid water at very high temperature (pressurized low-polarity water) for the extraction of biologically active substances from plant tissue. Corrales, García, Butz, & Tauscher (2009) reported that the extract of anthocyanin pigments from grape skins by using high (600-MPa) pressure.

- Particle size: the rate of extraction is improved by reducing the size of the solid particles. For examples, sugar beet is sliced into thin strips (cossettes) and soybeans are ground and flaked prior to extraction. Size reduction facilitates both the internal transport (by increasing the contact area with solvent).

- Agitation: the agitation accelerates external transport to and from the particle surface, but does not affect extraction rate if the rate-controlling factor is internal diffusion (Cogan & Kuwabara, 1967).

- Ultrasound-assisted extraction: intense sonication of the fluid facilitates release of intercellular materials from suspended solids by disrupting cell walls.

In addition, the effect of extraction on bioactive compounds and capacity of antioxidant were reported by others studied; Siger et al. (2017), who reported about physicochemical characteristic of the cold-pressed oil obtained from seeds of *Fagus sylvatica* L. found that the oil extracted from seeds by using cold-pressed extraction also remained bioactive compounds such as tocopherols, phytosterols and essential fatty acids. Zheng, Ren, Su, Yang, & Zhao (2013) found that hot-pressed extraction in peanut meal increased capacity of antioxidant than cold-pressed extraction while decreased on the total free amino acids. Teh & Birch (2013) who showed the physicochemical and quality characteristics of cold-pressed hemp flax and canola seed oils found that the oil extracted also included of bioactive compounds such as tocopherols, beta-carotene, chlorophyll TPC and TFC. Thanonkaew, Wongyai, McClements, & Decker (2012) showed that the stabilization of rice bran by using domestic heating on extraction yield, quality and antioxidant properties of cold-pressed rice bran oil and found that after passed heating with undergone cold-press processing, the rice bran oil remained amount of bioactive compounds and antioxidant activity when compared with raw rice bran (unstabilized). Delfan-Hosseini, Nayebzadeh, Mirmoghtadaie, Kavosi, & Hosseini (2017) reported on their studied of the effect of extraction (solvent, cold-press and MW-cold press) on oxidative stability and rheological properties of purslane seed oil and found that MW-cold press extracted was the highest of TPC, antioxidant activity and oxidative stability and followed by solvent extracted and cold-press extracted, respectively. Vergara-Salinas, Vergara, Altamirano, Gonzalez, & Pérez-Correa (2015) studied of characterization of pressurized hot water extracts of grape pomace: chemical and biological antioxidant activity, found that pressurized hot water extracted on 100 ^{o}C had higher of tannins, TPC than that of pressurized hot water extracted on 200 ^{o}C but FRAP, total antioxidants and MRPs was lower than that of pressurized hot water extracted on 200 ^{o}C.

2.4.2 Hot air oven, far-infrared with hot air (FIR) and effect on quality of drying foods and bioactive compounds with antioxidant activity

Drying is one of the most ancient methods of food preservation known to mankind. Preservation of meat, fish and food plants by drying in the sun or in the naturally dry air of the deserts and mountains has been practiced since prehistoric times, and is still a vital operation in the life of many rural communities. Drying or dehydration is, by definition, the removal of water by evaporation, from a solid or liquid food, with the purpose of obtaining a solid product sufficiently low in water content.

The main technology objectives of food dehydration are:

1. Preservation because of depression of water activity and extend the shelf life of food.

2. Reduction in weight and volume.

3. Transformation of a food to a form more convenient to store, package, transport and use.

4. Imparting to a food product, a particular desirable feature, such as a different flavor, crispiness and chewiness.

The most important on this technological and engineering in food dehydration are the following:

1. The kinetic of drying: with some notable exceptions such as spray drying, drying is a relatively slow process. Knowledge of the factors that affect the rate of drying is essential for the optimal design and operation of drying systems.

2. Product quality: removal of water is not the only consequence of most drying operations. Other important quality-related changes in test, flavor, appearance, texture, structure and nutritive value may occur in the course of drying. The extent of such changes depends on the process conditions.

3. Energy consumption: Most common drying processes use extensive quantities of energy at relatively low efficiency. Energy-wise, drying is a wasteful water removal process, compared to other water removal operations such as evaporation or membrane separation

The mechanism of water removal by drying involves two simultaneous processes, namely transfer of heat for the evaporation of water to the food and transport of the water vapors formed away from the food.

Depending on the mode of heat and mass transfer, industrial drying processes can be grouped in two categories: convective drying and conductive (boiling) drying.

1. Convective drying: hot and dry gas (usually air) is used both to supply the heat necessary for evaporation and to remove the water vapor from the surface of the food. Both heat and mass exchanges between the gas and the particle are essentially convective transfers, although conduction and radiation may also be involved to some extent. This widespread mode of drying is also known as air drying.

2. Conductive (boiling) drying: the moist food is brought into contact with a hot surface. The water in the food is boiled off. In essence, boiling drying is tantamount to evaporation to dryness. Vacuum drying, drum drying and drying in superheated steam are cases of this mode of drying (Fellows, 2009).

2.4.2.1 Hot air oven (cabinet or tray dryers)

Cabinet or tray dryers are used for batch drying of solid foods at a small to moderate scale (approximately 2000-20000 kg per day). They are low cost, semi-continuous mechanism and simple to construct. Cabinet dryers consist of a closed compartment in which trays containing the food to be dried are placed.

The trays rest on shelves with adequate spacing between them. Heated dry air circulates between the shelves. Very often, tray bottom are slatted or perforated in order to provide some air flow through the tray, the drying rate, and hence the moisture content of the raw material, depends on its position on the tray. The material located closest to the entrance of dry air has the lowest moisture content. In order to secure more uniform drying, the direction of air flow may be reversed or the trays may be rotated periodically. The cabinet is usually equipped with movable baffles, adjusted so as to have uniform distribution of the drying air throughout the cabinet. Cabinet driers are frequently found in rural installations, where they are used for drying fruits (grapes, apples), vegetable (onion, cabbage) and herbs (parsley, basil, mint, dill). Air inlet temperatures are usually in the range of 60-80 ^{o}C. Air velocity is a few meters per second, and must be adjusted according to the size, shape and

density of the food particles so as to avoid entrainment of dry particles with the wind. Depending on the product and conditions, the duration of a batch is typically 2-10 hours. Most cabinet or tray dryers feature means for adjustable recirculation of the air. The rate of recirculation is increased as drying progresses, when the air exiting the cabinet is warmer and less humid. Recirculation results in considerable saving in energy costs (Fellows, 2009).

Heat and mass transport phenomena may have profound effects on the quality of dehydrated food (Patel and Chen, 2005). Texture, structure, appearance, color, flavor, taste and nutrition value are all subject to change as a result of the drying process. In physical change from drying process, the foods are shrinkage while the shape of the particle is therefore not affected. During drying, solutes are transported along with water. The distribution of components in the dried product may be different from that of the starting material. The concentration of solutes such as sugars and proteins may be higher at the surface of the dehydrated product. Similarly, flow of liquid fat result in higher fat content at the surface of fat-containing powders obtained by spray drying (Kim et al., 2008). The most common thermal effects are non-enzymatic browning, denaturation of proteins, and thermal destruction of heat-sensitive vitamins and pigments.

In addition, the effect of drying process on bioactive compounds in agricultural products has recently been carried out by several investigators; Chen, Tai, & Chen (2007) reported that the effect of hot-air drying and freeze-drying on the stability of carotenoids in Taiwanese mango and found that freeze-drying method can be remained amount of carotenoids than that of hot-air drying. Katsube, Tsurunaga, Sugiyama, Furuno, & Yamasaki (2009) showed the result of air-drying temperature on antioxidant and stability of phenolic compounds in mulberry leaves and found that when increasing temperature from 40 to 110 ^{o}C, capacity of antioxidant to decrease as well as the content of polyphenolic compounds. Kubola et al. (2013) found that hot air could increase the content of lycopene and beta-carotene. Raksakantong, Siriamornpun, Ratseewo, & Meeso (2011) reported that total flavonoid content of kaprow deang and kaprow khao was the lowest in hot air drying when compared with LRH and FIR. Siriamornpun et al. (2012) found that TPC, TFC and FRAP value of fresh and dried marigold flower by different drying method, the lowest of their values

were found in HA when compared to other drying method (Freeze dry and FIR-hot air)**.**

2.4.2.2 Far-infrared radiation (FIR) Drying

The main commercial application of IR energy is drying low-moisture foods (breadcrumbs, cocoa, flours, grains, malt, pasta product and tea) and in baking application (pizzas, biscuits) or roasting oven for products such as coffee, cocoa and cereals. The technology has also been used to fry or thaw foods, and for surface pasteurization of bread and packaging materials.

Infrared refers to that part of the electromagnetic spectrum between the visible and microwave regions. Electromagnetic spectrum refers to the seemingly diverse collection of radiant energy, from cosmic rays to X-rays to visible light to microwaves, each of which can be considered as a wave or particle traveling at the speed of light. These waves differ from each other in the length and frequency, as illustrated in figure 10

Frequency, ν (nu), is the number of wave cycles that pass through a point in one second. It is measured in Hz, where 1 Hz = 1 cycle/sec. Wavelength, λ (lambda), is the length of one complete wave cycle. It is often measured in cm (centimeters). Wavelength and frequency are inversely related:

$$\nu = \frac{c}{\lambda} \text{ and } \lambda = \frac{c}{\nu}$$

where c is the speed of light, 3 x 10^{10} cm/ sec

Energy is related to wavelength and frequency by the following formulas:

$$E = h\nu = \frac{hc}{\lambda}$$

where h = Planck's constant, 6.6 x 10^{-34} joules-sec

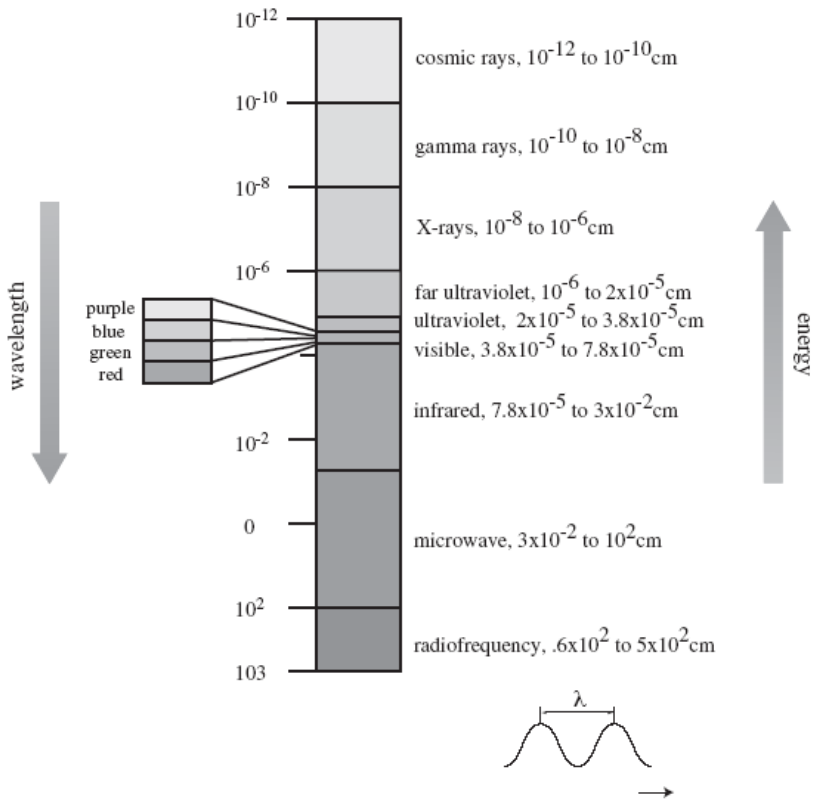


Figure 10 the electromagnetic spectrum.
From: http://www.geo.mtu.edu/rs/back/spectrum, 2017.

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Infrared rays are the part of the light spectrum below the color red in the visible light range. Far-infrared radiation (FIR) has been reported to be successfully applied in drying of foods (Sandu, 1986) and agricultural products since the main components of the agricultural products (i.e. proteins, starches and water) have the bands of far-infrared radiation absorption (Meeso, 2008). FIR creates internal heating via molecular vibration of the material, i.e., molecules absorb the radiation of certain wavelengths and energy, causing excited vibration. Thus, the mechanism of far-infrared drying is different from that of hot air drying (Sandu, 1986), so that the electromagnetic wave energy is absorbed directly by the dried food with reduced energy loss. FIR is thought to liberate and activate low molecular weight natural antioxidant compounds, because it heats material without degrading the constitutive molecules on the sample surface and it transfers heat evenly to the center of the material (Sandu, 1986). The utilization of far-infrared radiation is a novel process that could increase the drying efficiency, save working space, and result in a clean

working environment, etc. (Ratti & Mujumdar, 1995). Previous studies showed that capacity of antioxidant and TPC increased after exposure of rice hulls to FIR radiation (S. C. Lee et al., 2003), peanut hull (S. C. Lee et al., 2006), rice bran and rice husk (Wanyo et al., 2014) and mulberry tea and mulberry leaves drying has determined that TPC increases following combined FIR-HA treatment of mulberry leaves with compared to fresh leaves (Wanyo, Siriamornpun, & Meeso, 2011). FIR rays are biologically active (INOUÉ & KABAYA, 1989) and previous study (S. C. Lee et al., 2003) showed that simple heat treatments could not cleave covalently bound phenolic compounds from rice hulls but FIR treatments could.

The effect of physical on food could be explained that the rapid surface heating changes the flavor and color of foods due to Maillard reaction and protein denaturation, and also seals moisture and flavor or aroma compounds in the interior of the food.

CHAPTER 3

Material and Methods

This research is an experiment research that will be determined and analyzed phenolic compounds, including total phenolic content, total flavonoid content, phenolic acids, flavonoids and isoflavones in soybean meal samples as well as evaluate their antioxidant activities with determination of protein and lipid. In addition, determination of phenolic compounds, phytosterol content, fatty acids composition, cytotoxic activity and antioxidant activity of potent soybean meal extracts from different drying method are investigated. Finally, application of potent soybean meal samples for development of function food product and evaluates the stability of bioactive compounds and antioxidant activity in the product. Therefore, experimental design is followed by:

- Experimental plan
- Instruments and equipment
- Materials
- Chemicals
- Methods
- Statistical analysis

3.1 Experimental plan

This research will be divided into three experiments including (1) study the bioactive compounds and antioxidant activity with proximate analyses (protein and lipid content) in soybean and soybean meal samples from different sources. (2) determination of antioxidant activity and anti-proliferation with bioactive compounds extract samples from different drying methods and (3) to develop product from potent soybean meal as natural food additive for functional food and evaluates the stability of bioactive compounds and antioxidant activity in the product. All experiments will be performed in triplicate.

For experimental plan will be used completely randomized design (CRD) in this research. Analysis of variance will determine to test any difference in result from these methods. Duncan method will use to determine significant differences at $p < 0.05$.

3.2 Instruments and equipment

3.2.1 High performance liquid chromatography system with diode array detector (LC 20A, Shimadzu)

3.2.2 Gas chromatography mass spectrum (GC-MS) system (GCMS-QP2010, Shimadzu)

3.2.3 Ultraviolet-Visible spectrophotometer (Lambda 12, Perkin Elmer, USA)

3.2.4 Centrifuge (Rotina 48 R)

3.2.5 Rotary evaporator (Buchi)

3.2.6 Column Inetsil ODS-3, C18 (4.6 mm x 250 mm, 5 µm)

3.2.7 Column Rtx-Wax (crossbond, carbowax polyethylene glycol) (30 meter, 0.25 mm ID and 0.25 µm df)

3.2.8 Far infrared radiation dryers

3.2.9 Hot air oven (Memmert)

3.2.10 Incubator shaker

3.2.11 Beaker

3.2.12 Erlenmenyer flask

3.2.13 Volumetric flask

3.2.14 Pipette

3.2.15 Vial

3.2.16 Micro pipette

3.2.17 Test tubes

3.2.18 Water bath

3.2.19 Whatman filter paper no. 1 (Whatman, England)

3.3 Materials

Soybean meal samples were obtained from different industry (oil industry and soymilk industry), and from laboratory scale by the same process of two industries. Preparation of oil and soymilk residue samples;

For oil residue sample, seeds were cracked and hulls separated and then seeds were ground to fine powder and dried at 60 ^{o}C for 5 h (moisture content 7-10%). 20 g of sample with added hexane 200 ml and was extracted in Soxhlet for 7 h and then the residue was left in oven 60 ^{o}C until dry and stored at -18 ^{o}C.

For soymilk residue samples, soybean seeds without hulls and were washed 3 times and drained. The seeds were soaked in water ratio 1: 2.2 at 4 ^{o}C, 16 – 18 h and then seeds were drained, ground with water ratio 1: 2.5. The meal was left in oven 40 ^{o}C until dry and stored at -18 ^{o}C.

Total four of soybean residue samples will used for analyses in this research. All samples will be treated by hot air-drying machine at 60, 70 and 80 ^{o}C for 4 hours using hot-air oven (UFE 600, Memmert, Memmert Company, Germany) until moisture content came down to 7-10% (d.b.). In FIR treatment, the sample was FIR-irradiated in the FIR dryer at FIR intensity of 2, 3 and 4kW/m^2 (FIR energy irradiated per FIR heater surface area). Drying temperature will be set at 40 ^{o}C and drying time of 4 hours or samples will be then subjected to shade drying until moisture content came down to 7–10% (dry basis). Control sample (Raw soybean seed) will be shade dried only and stored at 4 °C prior to analyse.

3.4 Chemicals

3.4.1 2,2-Diphenyl-1-picrylhydrazyl , DPPH (Fluka)

3.4.2 2,4,6-Tripiridyl-s-triazine, TBTZ (Fluka)

3.4.3 Folin-Ciocalteu’s reagent (Fluka)

3.4.4 Ferrous sulphate (Carlo)

3.4.5 Sodium sulphate (Merck)

3.4.6 Acetonitrile (Merck)

3.4.7 Standard isoflavones (daidzein and genistein, (Sigma))

3.4.8 Standard phenolic acids and flavonoids (gallic, ferulic, *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, caffeic, syringic, sinapic, chlorogenic and vanillic acids, rutin, kaempferol, myricetin, apigenin and quercetin (Sigma))

3.4.9 Methanol (Merck)

3.4.10 Acetic acid (Fisher Scientific)

3.4.11 Dimethyl sulfoxide (DMSO) (Sigma)

3.4.12 Ethanol (Merck)

3.4.13 Sodium chloride (Fluka)

3.4.14 Potassium hydroxide (Fluka)

3.4.15 Hexane (Merck)

3.4.16 Ethyl acetate (Merck)

3.4.17 Pyrogallol (Fluka)

3.4.18 Pyridine (Sigma)

3.5 Methods

The present study will be divided into three experiments following:

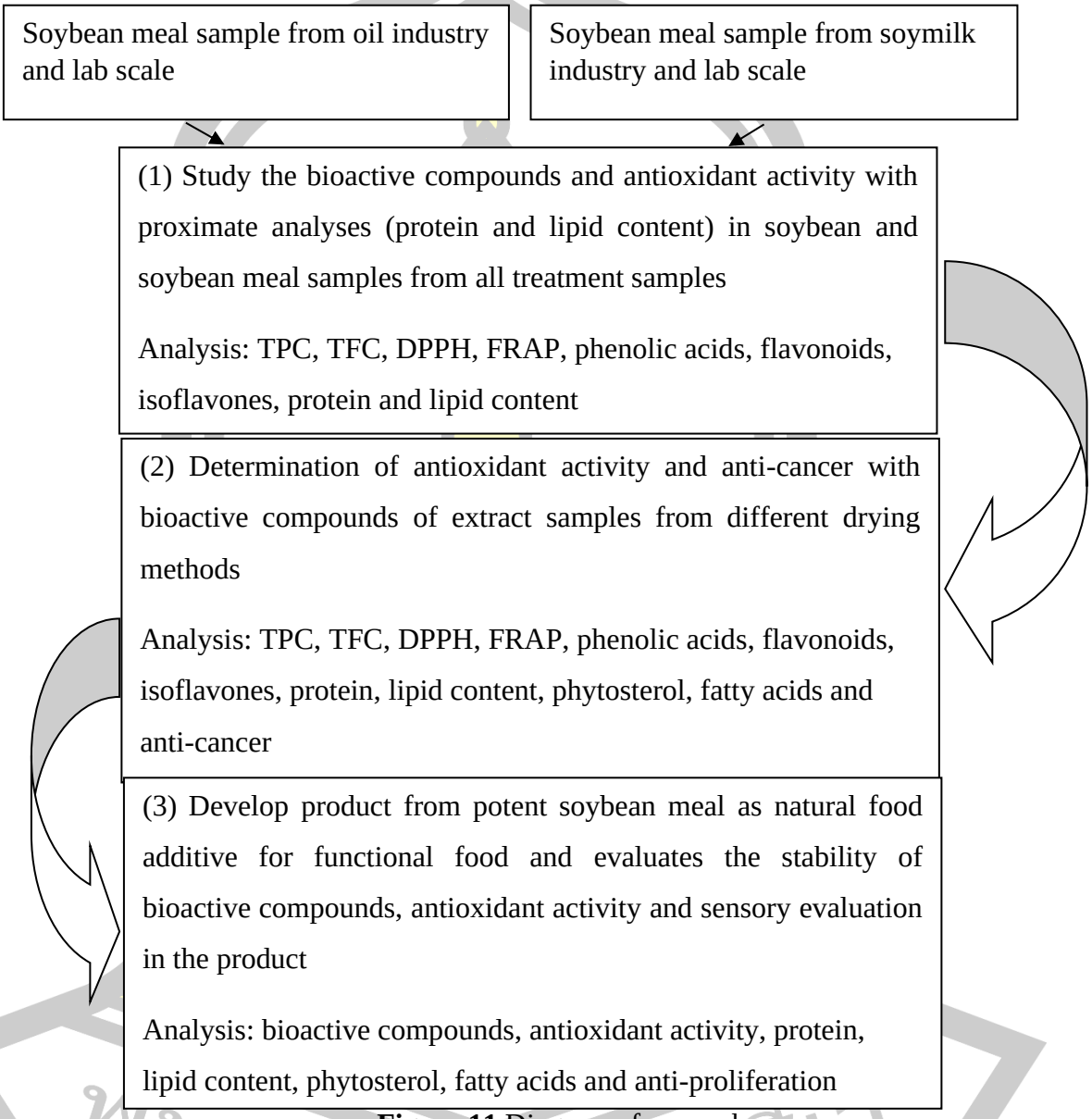


Figure 11 Diagram of research.

3.5.1 Study the bioactive compounds and antioxidant activity with proximate analyses (protein and lipid content) in soybean and soybean meal samples from different samples.

Determination of bioactive compounds and antioxidant activity as below:

3.5.1.1 Sample extractions for free phenolic compounds

Soybean meal samples (oil industry, soymilk industry and lab scale) extracts were prepared based on the method described by Kubola & Siriamornpun (2008) with slight modification. Briefly, 2 g of samples were extracted with 20 ml of 80% ethanol on a shaker set at 150 rpm for 12 h at room temperature. The extract samples were filtered through Whatman filter paper No. 1, and then the extract solutions were used to determine the total phenolics, total flavonoids, phenolic acids, flavonoids, isoflavones and antioxidant activity. Samples were analyzed in three replications.

3.5.1.2 Sample extractions for bound phenolic compounds

The extraction of bound phenolics was prepared based on the method described by Kaisoon, Siriamornpun, Weerapreeyakul, & Meeso (2011) with slight modification. Briefly, the residue samples from free phenolic extracts were extracted with 20 ml of 2 M NaOH on a shaker set at 150 rpm for 12 h at room temperature. The extract samples were filtered through Whatman filter paper No. 1, and then adjusted pH 2.2 by 4 M HCl. The extract solutions were used to determine the total phenolics, total flavonoids, phenolic acids, flavonoids, isoflavones and antioxidant activity. Samples were analyzed in three replications.

3.5.1.3 Total phenolic content (TPC)

Total phenolics in soybean meal samples were analyzed following the Folin-Ciocalteau method (Kubola & Siriamornpun, 2008). Briefly, 0.3 ml of extract samples were mixed with 2.25 ml of 10% Folin-Ciocalteu reagent dissolved in distilled water. After 5 min incubation, 2.25 ml of 6% sodium carbonate (Na_2CO_3) solution was added and the mixtures were left to stand for 90 min at room temperature. The absorbance of the solution samples was measured at 725 nm using a spectrophotometer. The TPC in beans were expressed as mg gallic acid equivalents (GAE) per g dry weight (mg GAE/g DW).

3.5.1.4 Total flavonoid content (TFC)

Total flavonoid content in beans were determined using a modified method, as described previously by Kubola & Siriamornpun (2008). Briefly, 0.5 ml of each sample solution was mixed with 2.25 ml of distilled water and 0.15 ml of 5% $NaNO_2$ solution (w/v). The solution was allowed to stand for 6 min and then 0.3 ml of 10% $AlCl_3$ (w/v) was added to the solution. After 5 min, 0.1 ml of 1 M NaOH (w/v)

solution was added and then the absorbance was measured at 510 nm using a spectrophotometer. Results were expressed as mg rutin equivalents (RE) per g dry weight (mg RE/g DW).

3.5.1.5 DPPH radical scavenging activity

DPPH• scavenging activity was determined using the method of (Brand-Williams, Cuvelier, & Berset, 1995), with slight modifications. Each extract sample (0.1 ml) was added to 3 ml of 0.04 mM DPPH mixture dissolved in 80% ethanol (ethanol: water, 80: 20 v/v). The mixture solution was shaken and allowed to stand in the dark for 30 min at room temperature, and then the absorbance was detected at 517 nm using a spectrophotometer. The DPPH radical scavenging activities of the samples were calculated as % inhibition of DPPH• in the following equation:

$$\text{DPPH}^{\bullet} \text{ scavenging activity (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

$A_{control}$ is the absorbance of the DPPH• solution without test sample and A_{sample} is absorbance of the test sample (DPPH• solution with extract sample).

3.5.1.6 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was measured by the method of Benzie & Strain (1996) with slight modification. Briefly, the fresh FRAP reagent was prepared by mixing 100 ml of acetate buffer (0.3 M, pH 3.6), 10 ml of TPTZ solution dissolved in 40 mM HCl (10 mM) and 10 ml of FeCl3 (20 mM) in a ratio of 10:1:1 and 12 ml of distilled water at 37 °C. The sample extract (60 µl) and 180 µl of deionized water were added to 1.8 ml of FRAP reagent. The mixture solution was shaken and incubated for 4 min at 37 °C in a water bath and then the absorbance was measured at 593 nm against a control, the absorbance of samples was compared with the standard mixture of ferrous sulphate ($FeSO_4.7H_2O$) (0.2 mM –1.0 mM). FRAP values will be expressed as mmol $FeSO_4$ per g dry weight (mmol $FeSO_4$/ g DW).

Identification and quatification of phenolic acids, flavonoids and isoflavones as below:

3.5.1.7 HPLC–DAD system for analysis of phenolic compounds (phenoilc acids and flavonoids)

HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A with diode array detector and chromatographic separations were performed on

a LUNA C-18 column (4.6 x 250 nm i.d., 5 µm). The solvents and used gradient elution condition were described previously by Kubola & Siriamornpun (2011) with some modification. The solvent of mobile phase A was contained a mixture of 1% acetic acid in water; mobile phase B was a pure acetonitrile. The following gradient was used: 0-40 min, from 100% A to 30% A, 70% B with a flow rate 1 ml/min; 40-45 min, from 30% A, 70% B to 20% A, 80% B with flow rate 1 ml/min; 45-55 min, from 20% A, 80% B to 15% A, 85% B with flow rate 1.2 ml/min; 55-57 min from 15% A, 85% B to 10% A, 90% B with flow rate 1.2 ml/min. Operating condition was as followed: column temperature, 40 ^{o}C; injection volume, 20 µl; UV-diode array detection at 280 and 320 for phenolic acids, and 370 nm for flavonoids, respectively. The samples were analyzed in triplicate.

3.5.1.8 HPLC–DAD system for analysis of isoflavones

HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A with diode array detector and chromatographic separations were performed on a LUNA C-18 column (4.6 x 250 nm i.d., 5 µm). The solvents and used gradient elution condition were described previously by Devi et al. (2009) with some modification. The solvent of mobile phase A was DI water (50%), mobile phase B was acetonitrile (10%) and mobile phase D contained a mixture of 1% acetic acid in water (40%). The following gradient was used typically isocratic and flow rate of 1 ml/min for 30 min. Injection volume was 20 µl, UV-diode array detection at 277 nm by comparing with the retention time of standards (genistein and daidzein were using for standard).

3.5.1.9 Proximate analysis

Proximate analysis of the soybean meal samples will be determined according to AOAC (AOAC, 1996). Protein and lipid content were determined in triplicate.

3.5.2 Determination of antioxidant activity and anti-cancer with bioactive compounds of extract samples from different drying methods

Determination of bioactive compounds, antioxidant activity and proximate as same as above (3.4.1)

3.5.2.1 Soybean meal sample from different drying methods

All samples will be treated by hot air-drying machine at 60, 70 and 80 ^{o}C for 6-24 hour using hot-air oven (UFE 600, Memmert, Memmert Company, Germany) until moisture content came down to 7-10% (d.b.). In FIR treatment, the sample was FIR-irradiated in the FIR dryer at FIR intensity of 2, 3 and 4kW/m^2 (FIR energy irradiated per FIR heater surface area). Drying temperature will be set at 60, 70 and 80 ^{o}C and drying time of 2-2.5 h or samples will be then subjected to shade drying until moisture content came down to 7–10% (d.b.). Control sample (Raw soybean seed) will be shade dried only and stored at 4 °C prior to analyses.

3.5.2.2 Phytosterol content by GC-MS

Phytosterol composition and content was analyzed by GC-MS according to the method of Panfili, Fratianni, & Irano (2003), with some modifications. The samples (1.0 g) with an additional 1 mg alpha cholestane as an internal standard were saponified with 2 ml of KOH (600 g/l), 2 ml of ethanol (95%), 2 ml of NaCl (10 g/l) in screw capped tube, and 5 ml of ethanolic pyrogallol (60 g/l) was added as antioxidants. The tubes will be incubated at 70 °C in a water bath and mixed by a vortex mixer every 10 min for 45 min digestion period. The tubes were cooled and then 10 ml of NaCl (10 g/l) was added. The suspension was then extracted twice with 10 ml n-hexane/ethyl acetate (9:1 v/v). The solvent layer was collected and evaporated to dryness in a rotary evaporator at 40 °C under vacuum. Then the dry residue was derivatized with 100 µl of BSTFA: TMCS (99:1) and 1 ml of 99% pyridine, at 60 °C for 30 min. The residue will be dissolved in 2 ml of hexane, and then the clear solution (1 µl) was injected to the GC-MS system (QP2010, Shimadzu, Japan) equipped with a HP5 column (30m, 0.25 mm i.d., 0.25 µm film thicknesses). Helium will be used as a carrier gas at a constant flow rate of 1.0 ml/min. The initial column temperature of 60 °C was held for 1 min, then programmed at a rate of 30 °C /min, held at 150 °C for 10 min, then from 250 °C to 280 °C at 1°C /min and held at 280 °C for 13 min and scan mass range from m/z 40-500. The quantitative calculation is based on the corrected peak area ratios relative to the peak area of the internal standard.

3.5.2.3 Amino acid analysis by using LCMS-MS

Amino acids of soybean meal sample were extracted according to Nimbalkar, Pai, Pawar, Oulkar, & Dixit, (2012) with slightly modifications. The

LCMS/MS was performed using a LCMS-8030 triple-quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) operated in the ESI mode and a Shimadzu LC-20AC series HPLC system (Shimadzu, Kyoto, Japan). The HPLC analysis was operated follow by these conditions; the flow rate was set 0.2 mL/min, the temperature of column oven was set at 38 ºC and autosampler was determined at 4 ºC. The mobile phases were prepared by (A) the water was mixed with formic acid 0.1% (v/v) and (B) the methanol was diluted with the water ratio 1:1 then the mixer was added with formic acid 0.1% (v/v). The autosampler needle was purged with methanol before and after aspiration of the sample. For the operation in MS/MS mode, runs: Multiple Reaction Monitoring (MRM) mode; capillary voltage at 4.5 kV (positive mode; ESI (+)); cone voltage at 1.72k V; ion source temperature of 400 ℃. The amino acid was identified by their m/z values and by comparison with the retention time of standards. All other settings were analyte-specific and were auto-optimized by flow injection of 2 µL of a 1 ppm. solution in methanol containing one analyte.

3.5.2.4 Cytotoxic activity of soybean meal samples

The ethanolic extracts (50%, v/v) of soybean meal samples were dissolved in dimethyl sulfoxide (DMSO) at 20 mg/ml as stock solution which were then diluted with Dulbecco's Modified Eagle Medium (DMEM) to desired concentrations ranging from 10 to 500 µg/ml. The final concentration of DMSO in each sample did not exceed 1% v/v, to keep the cytotoxicity of DMSO at less than 10%. The human cancer cell lines were used as cell models. Anti-cancer activity was performed with neutral red (NR) method (MacHana, Weerapreeyakul, Barusrux, Thumanu, & Tanthanuch, 2012). Briefly, the cancer cell lines were seeded in 96-well plates and treated with various concentrations of the compounds for 24 hours. Then, cells were washed twice with 1x PBS and supernatant was discarded. A total of 100 µl NR solution (50 µg/ml) will be added to each well and incubated at 37 ºC for another hour. NR will be then dissolved by 100 µl of 0.33% HCl. Absorbance of NR dye will be detected by a dual wavelength UV spectrometer at 520 nm and 650 nm. The percentage of cytotoxicity compared to the untreated cells were determined with the equation given below. A plot of % cytotoxicity versus sample concentrations were used to calculate the concentration which showed 50% cytotoxicity (IC_{50})

Cytotoxicity (%) = [100x (Absorbance of untreated group – Absorbance of treated group] / Absorbance of untreated group

The selectivity, which indicates the cytotoxic selectivity (or safety) of the sample extracts against cancer cells versus normal cells, were determined from the IC_{50} of sample extracts in normal cells versus cancer cells (Prayong, Barusrux, & Weerapreeyakul, 2008).

3.5.3 Develop product from potent soybean meal as natural food additive for functional food and evaluates the stability of bioactive compounds, antioxidant activity and sensory evaluation in the product.

After choose the potent soybean meal powder as natural food additive to develop functional food product by following step as below:

Step for develop product from soybean meal powder

Idea for generation and screening

↓

Marketing research

↓

Product information and feasibility study for industry process

↓

Development product of prototype

↓

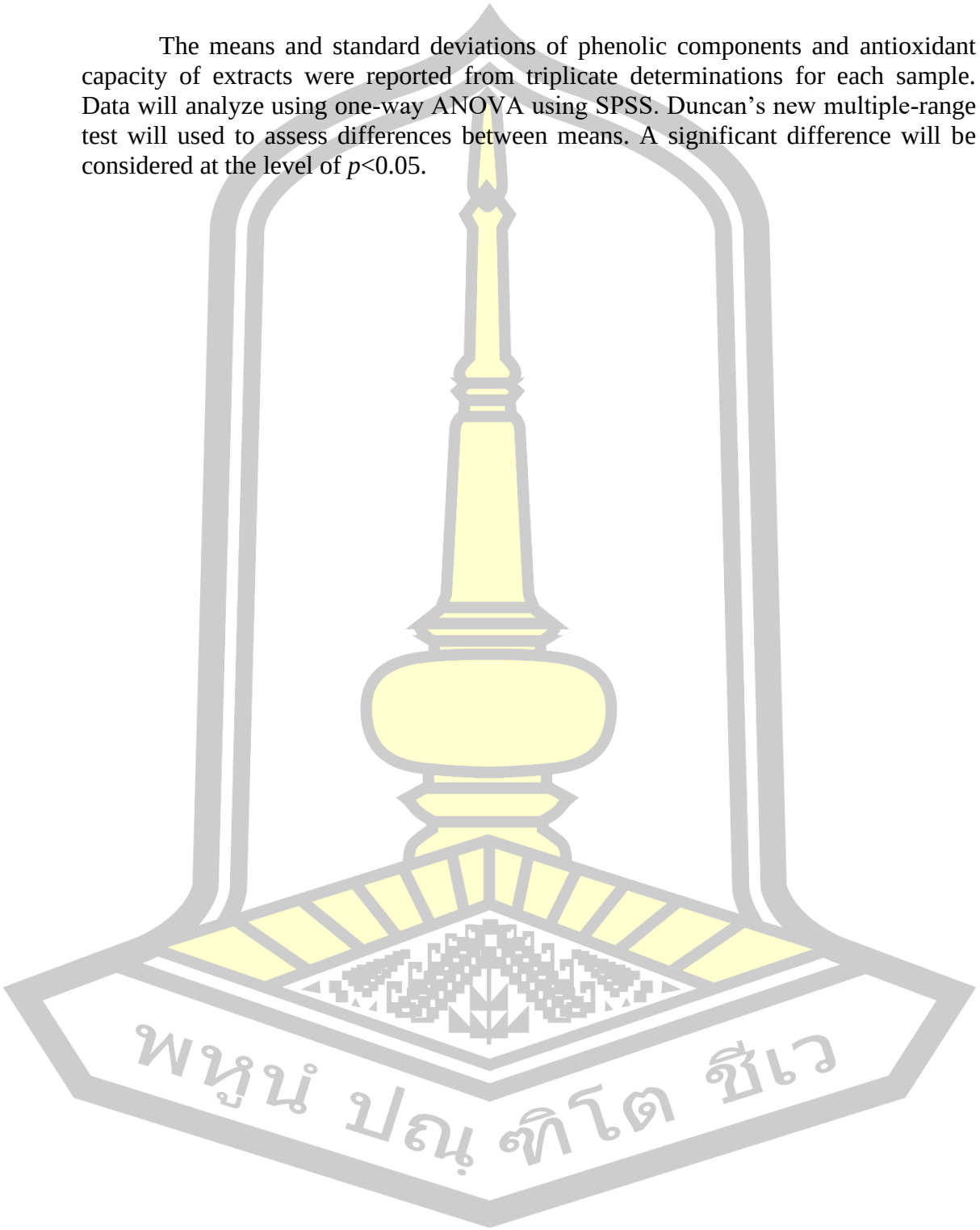
Testing of product (sensory evaluation and chemicals)

3.5.3.1 Sensory evaluation

Sensory evaluations of rice product prototype will be conducted by 30 panelists using a nine-point hedonic scale where nine is like extremely and one dislike extremely. Three coded samples were served and water is provided for rinsing between samples. Control will used to compare with the product prototype for sensory test.

3.6 Statistical analysis

The means and standard deviations of phenolic components and antioxidant capacity of extracts were reported from triplicate determinations for each sample. Data will analyze using one-way ANOVA using SPSS. Duncan's new multiple-range test will used to assess differences between means. A significant difference will be considered at the level of $p<0.05$.



CHAPTER 4

RESULTS AND DISCUSSIONS

The results of data analysis and describing were sequent expressed followed by

4.1 Symbols used for data resulting expression

4.2 Sequence of resulting expression

4.3 Results and discussions

4.1 Symbols used for resulting data expression

In this study, expression of data analysis results was conducted as various symbols.

SD = Standard Deviation

$\overline{X}$ = Means

df = Degrees of freedom

F = F- distribution

P = Probability

4.2 Sequence of resulting expression

The results were sequence expressed following as

Experiment 1: the bioactive compounds and antioxidant activity in soybean and soybean meal samples from all treatment samples.

Experiment 2: determination of antioxidant activity and anti-cancer with bioactive compounds of extract samples from different drying methods.

Experiment 3: develop product from potent soybean meal as natural food additive for functional food and evaluates the stability of bioactive compounds, antioxidant activity and sensory evaluation in the product.

4.3 Results and discussions

Experiment 1: the bioactive compounds and antioxidant activity in soybean and soybean meal samples from all treatment samples.

4.3.1 Total phenolic content

A previous study demonstrated that the consumption of free or bound phenolic compounds from vegetables, fruits and legume seeds have been associated with the prevention of cancer, cardiovascular disease by antioxidants as decrease oxidized of LDL and inhibitors of lipase (Chandrasekara & Shahidi, 2011). The contents of free and bound phenolics of soybean and soybean meal extracts from different food industries are presented in Table 4.

Table 4 Total phenolic content of free and bound phenolic extraction from oil and soymilk samples.

Samples	TPC (free) (mg GAE/g DW)	TPC (bound) (mg GAE/g DW)	Total TPC (mg GAE/g DW)
Oil industry			
DSO*	$77.15 \pm 3.67^{b,A}$	$65.80 \pm 2.^{75b,B}$	142.95 ± 8.02^{b}
SOI	$90.48 \pm 2.61^{a,B}$	$98.39 \pm 2.67^{a,A}$	188.88 ± 5.59^{a}
SOL	93.26 ± 4.24^{a}	99.23 ± 3.88^{a}	192.49 ± 4.22^{a}
Soy milk industry			
DSM	$81.29 \pm 6.65^{a,B}$	$118.75 \pm 13.11^{a,A}$	200.04 ± 8.02^{a}
SMI	79.31 ± 8.77^{a}	93.77 ± 8.13^{b}	173.09 ± 10.21^{b}
SML	88.44 ± 4.55^{a}	87.93 ± 6.50^{b}	176.37 ± 0.36^{b}

*DSO: dry soybean from oil, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale, DSM: dry soybean from soy milk industry, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The different letter in same column were significantly ($p < 0.05$) by compared among treatments, the different capital letter in same row were significantly different ($p < 0.05$) by compared between free and bound, n = 3.

The free phenolic content of oil and soymilk samples ranged from 77.15 - 93.26 mg GAE/g DW and 79.31 – 88.44 mg GAE/g DW, respectively. The highest values of free phenolic content were found in SOL (dry soybean from the oil industry) and SML (soybean meal of soymilk extraction from lab scale). Insoluble-bound phenolic content ranged from 65.80 – 99.23 mg GAE/g DW of oil samples, 87.93 – 118.75 mg GAE/ g DW of soymilk samples, respectively, which the lowest values of bound phenolic content were observed in DSO (dry soybean from the oil industry) and SML samples. Total phenolic content ranged from 142.95 – 200.04 mg GAE/g DW of both samples. DSM (dry soybean from soymilk industry) contained the highest TPC value while DSO contained the lowest of TPC value. Additionally, the total phenolic content of SOI and SOL was significantly (p<0.05) higher when compared to DSO sample while TPC values of SMI and SML were significantly lower than that of DSM sample. The contribution of bound phenolics from oil industry samples to TPC was 46-52% and from soymilk industry samples were 50-59% while free phenolics contributed 47-54%, 41-50% from oil and soymilk industry samples, respectively. The results showed that the majority of phenolic compounds in the soybean and soybean meal retaining after oil extraction and soymilk extraction (water phase extraction) were exhibited in the bound form which according to Wang et al. (2016) reported that the contribution of insoluble-bound phenolics of soybean was higher than that of free phenolics. Adom & Liu (2002) were reported that the content of bound phenolic was significantly higher than free phenolic when compared among all cereal analyzed (corn, wheat and rice). The high bound phenolic content also could be found in potato, pumpkin hull-less seed, grape seed meals and barley varieties (Abdel-Aal & Rabalski, 2013; Ayoub, De Camargo, & Shahidi, 2016; Chu, Sun, Wu, & Liu, 2002; Peričin, Krimer, Trivić, & Radulović, 2009). However, Alu'datt et al. (2016) reported that the content of free phenolic of full fat and defatted of soybean and soybean residue was significantly higher than those of bound phenolic. The high content of free phenolic was found in Brazil nut skin, peanut skin and swallow root (de Camargo, Regitano-d'Arce, Gallo, & Shahidi, 2015; Harish Nayaka, Sathisha, & Dharmesh, 2010; John & Shahidi, 2010). In addition, total phenolic content of soybean residue from industry was significantly higher when compared with other raw beans such as kidney beans, lentils, mung bean and faba bean (Alshikh, de Camargo, & Shahidi, 2015; Chaieb, González, López-Mesas, Bouslama, & Valiente, 2011; Kan et al., 2017; Zhao, Du, Wang, & Cai, 2014). These results indicated that the residue from industry was abundant of soluble phenolic as well as highly insoluble-bound phenolic which a good and cheaper source of bioactive compounds. However, the different of TPC from soybean and soybean meal extracted can be depended on varieties of sample nature, season, harvest, keep storage, solvent to sample ratio, solvent type, time of extraction and different type of processing (Alu'datt et al., 2016).

4.3.2 Total flavonoid content

Table 5 Total flavonoid content of free and bound extraction from oil and soymilk samples.

Samples	TFC (free) (mg RE/g DW)	TFC (bound) (mg RE/g DW)	Total TFC (mg RE/g DW)
Oil industry			
DSO*	$2.79 \pm 0.13^{d,A}$	$0.35 \pm 0.03^{cd,B}$	3.14 ± 1.72
SOI	$1.99 \pm 0.01^{e,A}$	$0.29 \pm 0.01^{cd,B}$	2.28 ± 1.21
SOL	$1.71 \pm 0.03^{f,A}$	$0.10 \pm 0.00^{d,B}$	1.81 ± 1.13
Soy milk industry			
DSM	$3.19 \pm 0.15^{c,A}$	$0.55 \pm 0.13^{bc,B}$	3.75 ± 1.86^{c}
SMI	$6.18 \pm 0.16^{b,A}$	$0.66 \pm 0.05^{b,B}$	6.84 ± 3.90^{b}
SML	$6.45 \pm 0.16^{a,A}$	$5.40 \pm 0.36^{a,B}$	11.86 ± 0.74^{a}

*DSO: dry soybean from oil, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale, DSM: dry soybean from soy milk industry, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The different letter in same column were significantly ($p < 0.05$) by compared among treatments, the different capital letter in same row were significantly different ($p < 0.05$) by compared between free and bound, $n = 3$.

Flavonoids are the phenolic compounds of secondary metabolites from a plant and found in several vegetables, fruits and especially legume seeds such as flavonols and isoflavones. Presently, more than 10,000 flavonoids have been identified which these bioactivities might protect against chronic diseases such as cancer, cardiovascular, obesity and diabetic (Shahidi & Ambigaipalan, 2015). The TFC of free and bound fractions of soybean and soybean meal are shown in Table 5. The total flavonoid content in free flavonoid extract of soybean and soybean meal of both industries in a range of 1.71 to 6.45 mg RE/g DW while bound flavonoid extract of oil and soymilk samples in a range of 0.1 to 5.40 mg RE/g DW. The free TFC values of all samples were significantly ($p<0.05$) higher than that of bound TFC values of all samples. In oil industry samples, the highest value of free TFC was found in DSO, whereas the highest value of free TFC from soymilk industry was observed in SML and SMI. The total flavonoid content of oil and soymilk samples ranged from 1.81-11.86 mg RE/g DW which the highest value of TFC was found in SML while SOL was the lowest of TFC. The information about free and bound TFC content in soybean and soybean meal is rarely reported. Kotásková, Sumczynski, Mlček, & Valášek (2016) reported the free and bound flavonoid contents in *Eragrostis tef* and found that the free flavonoid content was significantly ($p<0.05$) higher when compared with bound flavonoid content. The free and bound flavonoid content of black and red rice were reported by Sumczynski, Kotásková, Družbíková, & Mlček (2016) found that the highest of TFC fraction was observed in free TFC fraction of all

rice samples. Additionally, Bhat & Riar (2017) reported total flavonoid content of whole pigment rice varieties showed that bound flavonoid was significantly lower than that of free flavonoid. Kaisoon et al. (2011) showed that the soluble extracts in edible flowers had higher TFC than bound extracts. In addition, our results were similarly trend with previous study and indicated that the total flavonoid content was presented in free form more than in bound form.

4.3.3 DPPH radical scavenging activity

DPPH radical scavenging assay is widely used methods and investigates the first approach for testing antioxidant properties which is a stable radical with a deep purple color. This assay is based on electron donation of antioxidants to neutralize DPPH radical by observed the discoloration acts as an indicator of the antioxidant capacity (Shahidi & Zhong, 2015). The DPPH radical scavenging activities of free and bound phenolic compound fraction from soybean and soybean residue of both industries are presented in Table 6. The activity of DPPH radical scavenging of free phenolic extract from oil and soymilk industry ranged from 27 to 44% and 30 to 44% with the highest % inhibition of DPPH radical scavenging was observed in DSO and SML sample, respectively. In contrast, the bound phenolic extracts of both samples from oil and soymilk industry were showed strongly on DPPH radical scavenging activity when compared with free phenolic extracts, ranged from 38 to 84% and 57 to 92 % with the highest efficacy rendered by SOL and SML, respectively. Wang et al. (2016) reported insoluble-bound phenolic fraction from soybean was higher DPPH radical scavenging than their corresponding free phenolic fraction. As same as previous reported from Madhujith & Shahidi (2009) found that the capacity of DPPH radical scavenging of bound phenolic extracts of 6 barley varieties was higher compared with free phenolic extracts. Other previous studied also have investigated that bound phenolic had higher antioxidant capacity than free phenolic (Shahidi & Chandrasekara, 2010). Whilst, Kotásková et al. (2016) reported that the DPPH radical scavenging activity of free phenolic extract from brown and white Teff was higher when compared with bound phenolic. Another studies also have indicated that the soluble phenolic extract from edible flower, pigmented rice and soybean residue had higher antioxidant activity of DPPH radical scavenging than bound phenolic (Alu'datt et al., 2013; Kaisoon et al., 2011; Sumczynski et al., 2016). The results of DPPH radical scavenging activity assay indicated that soybean and soybean meal with contain free and bound phenolic and flavonoid contents had effectively electron donation, especially bound phenolic extracts. This may be due to the different of chemical structure attributing to radical scavenging property (Albishi, John, Al-Khalifa, & Shahidi, 2013). Many researchers suggested that capacity of antioxidant of phenolic compounds would be depended on the content, structure, location and number of hydroxyl groups which the antioxidant capacity is enhanced by amount of hydroxyl groups was increased (Shahidi & Zhong, 2015; Xie, Huang, Zhang, & Zhang, 2015).

Table 6 DPPH values of free and bound phenolic extraction from oil and soymilk samples.

Samples	DPPH (free) (% inhibition)	DPPH (bound) (% inhibition)
Oil industry		
DSO*	$44.06 \pm 1.23^{a,B}$	$74.38 \pm 3.21^{b,A}$
SOI	$29.29 \pm 3.09^{b,B}$	$38.18 \pm 4.04^{c,A}$
SOL	$27.28 \pm 2.10^{b,B}$	$84.09 \pm 1.78^{a,A}$
Soy milk industry		
DSM	$34.48 \pm 4.54^{b,B}$	$57.89 \pm 1.38^{c,A}$
SMI	$30.83 \pm 1.89^{b,B}$	$68.81 \pm 3.10^{b,A}$
SML	$44.11 \pm 3.23^{a,B}$	$92.36 \pm 0.05^{a,A}$

*DSO: dry soybean from oil, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale, DSM: dry soybean from soy milk industry, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The different letter in same column were significantly ($p < 0.05$) by compared among treatments, the different capital letter in same row were significantly different ($p < 0.05$) by compared between free and bound, n = 3.

4.3.4 Ferric reducing antioxidant power (FRAP) assay

Generally, the properties of antioxidant activity are two mainly in scavenging of free radicals – hydrogen atom transfer and single electron transfer which the FRAP assay is typically ET-based method that usually measures the ability of antioxidant activity to reduction of ferric ion (Fe^{3+}) complex to ferrous (Fe^{2+}) complex by changed the color of iron to deep blue color in acidic media (Shahidi & Zhong, 2015). The antioxidant activity of the soybean and soybean meal from different industry as determined by reducing power is given in Table 7. The FRAP values of free and bound phenolic extracts from oil and soymilk samples ranged from 0.62 to 0.79, 0.47 to 0.66, 0.73 to 1.18 and 0.17 to 0.66 mmol $FeSO_4$/g DW, respectively. The FRAP values of free phenolic fraction of all oil samples were significantly higher than those of bound phenolic fraction as well as the values of FRAP in all soymilk samples. The highest of FRAP values of free phenolic fraction was observed in SOI and SML samples, which was in agreement with the previous study on pigmented rice, seed coat and cotyledon of black soybean and peanut skin (Bhat & Riar, 2017; de Camargo et al., 2015; Peng, Li, Li, Deng, & Zhang, 2017). However, Wang et al. (2016) found that the FRAP values of insoluble-bound phenolics were significantly higher than that of free phenolics in soybean. Kaisoon et al. (2011) also found the FRAP values of bound phenolic were higher than free phenolic in some edible flower. The efficient of antioxidant activity from phenolic compounds as extracted from plant foods, frequently varies but this does not depend on their quantities, may be dictated by the

chemical structures of their phenolic components (Albishi et al., 2013). Although, our results have been shown to be contain high number of phenolic compounds and exhibited strong antioxidant activities from soybean and soybean residue, especially soybean residue from oil and soymilk industry samples.

Table 7 FRAP values of free and bound phenolic extraction from oil and soymilk samples.

Samples	FRAP (free)	FRAP (bound)	Total FRAP (mmol FeSO4/ g DW)
Oil industry			
DSO*	$0.62 \pm 0.01^{c,A}$	$0.47 \pm 0.01^{b,B}$	1.09 ± 0.10^{b}
SOI	$0.79 \pm 0.00^{a,A}$	$0.66 \pm 0.03^{a,B}$	1.45 ± 0.10^{a}
SOL	$0.71 \pm 0.05^{b,A}$	$0.49 \pm 0.01^{b,B}$	1.20 ± 0.16^{b}
Soy milk industry			
DSM	$0.87 \pm 0.01^{b,A}$	$0.66 \pm 0.01^{a,B}$	1.53 ± 0.14^{a}
SMI	$0.73 \pm 0.02^{c,A}$	$0.43 \pm 0.00^{b,B}$	1.15 ± 0.21^{c}
SML	$1.18 \pm 0.04^{a,A}$	$0.17 \pm 0.00^{c,B}$	1.35 ± 0.72^{abc}

*DSO: dry soybean from oil, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale, DSM: dry soybean from soy milk industry, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The different letter in same column were significantly ($p < 0.05$) by compared among treatments, the different capital letter in same row were significantly different ($p < 0.05$) by compared between free and bound, $n = 3$.

4.3.5 Identification and quantification of phenolic compounds

Phenolic acids are presented as hydroxylated derivatives of hydrobenzoic and hydrocinnamic, which generally found in plants as free, soluble conjugate and insoluble-bound phenolics (Shahidi & Ambigaipalan, 2015). The phenolic compounds and their amount in free and bound extracts of soybean and soybean meal from oil and soymilk industry were determined using HPLC. Results are displayed in Table 8 and 9, respectively. In the oil extracted samples from oil industry and lab-scale (Table 8.), the highest contents of total free phenolic acids were found in SOL (514.68 µg/g DW) followed by SOI (454.21 µg/g DW) and DSO (382.13 µg/g DW), while total bound phenolic acids of SOI were higher but not significantly different at $p<0.05$ when compared with other samples. The results also found that the contents of bound phenolic extracts in all samples were significantly ($p<0.05$) higher than that of free phenolic extracts expect for SOL sample. The predominant free phenolic fractions of all oil extracted samples were protocatechuic acid, chlorogenic acid and ferulic acid, respectively. On the other hand, gallic acid, sinapic acid and *p*-coumaric acid were found in bound form than free form in all oil extracted samples expect for SOI

sample. The high contents of protocatechuic acid in free phenolic extracts were observed in SOL while the high amounts of chlorogenic acid and ferulic acid were found in SOI. In addition, the highest contents of rutin in free phenoilc fractions were found in SOL followed by DSO and SOI, respectively. Quercetin occurred in small quantities in all samples. Isoflavones were used daidzein and genistein to determine, and found that the greatly contents of isoflavone extracts were found in bound daidzein (326.43 to 500.80 µg/g DW), which were significantly (p<0.05) higher when compared with free daidzein and bound genistein. In contrast, the contents of free and bound genistein ranged from 21.05 to 35.08 and 27.61 to 37.35 µg/g DW, respectively, which occurred in small quantities in all samples. The contents of total free and bound phenolic compounds ranged from 996.76 to1148.46 µg/g DW.

The content of phenolic compounds in soymilk industry is presented in Table 9. The results of all soymilk samples showed that free protocatechuic acid was the highest and followed by free ferulic acid and free chlorogenic acid, respectively. The high values of free protocatechuic acid, free chlorogenic acid and free ferulic acid were observed in DSM while the higher contents in all soymilk samples of bound phenolic fractions were gallic acid expect for SML (not detected). Sinapic acid was found in all extracts of all tested samples and the high contents of free and bound sinapic acid were found in DSM as well as the amounts of free and bound *p*-coumaric acid. Rutin was found in all fractions of DSM, SMI and SML which the content of rutin in the free fraction of DSM was significantly higher than that of other samples. Whilst, the high contents of rutin in the bound fraction were also found in SML and SMI, respectively. The content of myricetin ranged from 15.21-15.32 µg/g DW and was found in the free form of all samples which in small quantities. Additionally, isoflavones were found in all sample extracts and the contents of bound daidzein (227.89-318.90 µg/g DW) were significantly higher than those of free daidzein. The contents of genistein in free and bound fractions ranged from 16.98 to 24.56 and 23.89 to 32.97 µg/g DW, respectively. The contents of bound phenolic compounds of SMI and SML were higher when compared with free phenolic compounds of SMI and SML while DSM not different between free and bound fraction. Total free and bound contents of DSM, SMI and SML were 903.37, 860.93 and 585.85 µg/g DW, respectively.

Others previous study, Alu'datt et al. (2016) reported that the components of free and bound phenolic compounds in soybean residue were gallic acid, sinapic acid, ferulic acid and *p*-coumaric acid. For raw soybean, Wang et al (2016) reported protocatechuic acid as the major free and bound phenolics followed by p-coumaric acid and ferulic acid expect for gallic acid was found in soluble conjugate and insoluble-bound phenolics while rutin and quercetin were found in free and conjugate phenolics. In addition, the total isoflavones of soybean meal from the oil industry and experimental ranged from 5.3 to 11.0 mg/g and found in β-glucosides form than other forms (de Oliveira Silva & Perrone, 2015). Kotásková et al. (2016) reported that free and bound phenolics profiles of brown and white teff were *trans-p*-coumaric, ferulic, protocatechuic and gallic acid with rutin catechin and quercetin. The phenolic acid

and flavonoid contents of berry seed meal were found insoluble-bound form than free forms such as protocatechuic, gallic and caffeic acids, and quercetin, quercetin derivatives and catechin (Ayoub et al., 2016). Moreover, many studies showing the correlation between TPC and TFC with antioxidant activity, it is well known that phenolic compounds are highly effective free radical scavengers and metal chelating (Ayoub et al., 2016; Peng et al., 2017; Sumczynski et al., 2016). Wang et al. (2016) showed the correlation of phenolic compounds and found that free, soluble conjugate and insoluble-bound of protocatechuic acid were significantly correlated with FRAP and DPPH but rutin not significant. In addition, Tyug et al. (2010) reported that the high contents of isoflavone extracts were daidzein in soymilk powder and soy husk powder, which the correlation of daidzein and genistein from soymilk powder extracts was significantly correlated with FRAP and TEAC assay. Our results were agreement with previous studies and indicated that the phenolic compounds extract of soybean meal from oil and soymilk industry and lab-scale were retained the greatest of antioxidant activity in free and bound phenolic forms as well as raw soybean.

Table 8 Identifications of free and bound phenolic acids in oil soybean samples.

Contents (µg/g DW)	Oil industry					
	DSO		SOI		SOL	
	Free	Bound	Free	Bound	Free	Bound
Phenolic acids						
Gallic acid	$10.75 \pm 0.40^{c,B}$	$137.73 \pm 9.60^{a,A}$	$10.90 \pm 0.17^{c,A}$	nd	$11.63 \pm 0.15^{c,B}$	$28.99 \pm 2.83^{b,A}$
Procatechuic acid	$92.86 \pm 0.01^{b,A}$	$23.97 \pm 1.01^{c,B}$	$129.07 \pm 12.54^{a,A}$	$31.30 \pm 6.89^{c,B}$	$146.49 \pm 18.00^{a,A}$	$12.81 \pm 3.83^{d,B}$
Chlorogenic acid	$34.54 \pm 2.54^{c,A}$	$8.49 \pm 0.33^{d,B}$	$57.37 \pm 5.04^{b,A}$	$10.07 \pm 0.49^{d,B}$	$80.41 \pm 8.95^{a,A}$	$11.80 \pm 0.06^{d,B}$
p-Coumaric acid	$9.59 \pm 0.09^{d,B}$	$11.08 \pm 0.08^{c,A}$	$9.91 \pm 0.39^{d,B}$	$13.02 \pm 0.28^{b,A}$	$10.04 \pm 0.21^{d,B}$	$14.49 \pm 0.06^{a,A}$
Ferrulic acid	$21.40 \pm 2.23^{c,A}$	$15.82 \pm 1.20^{d,B}$	$35.15 \pm 0.21^{a,A}$	$27.35 \pm 1.68^{b,B}$	27.11 ± 4.82^{b}	26.82 ± 0.18^{b}
Sinapic acid	11.63 ± 0.21^{b}	11.59 ± 0.28^{b}	$13.01 \pm 0.25^{a,B}$	$18.26 \pm 0.66^{a,A}$	$11.73 \pm 0.47^{b,B}$	$17.38 \pm 0.06^{a,A}$
Flavonoids						
Rutin	$106.29 \pm 12.84^{a,A}$	$39.72 \pm 3.51^{d,B}$	$77.12 \pm 2.41^{b,A}$	$45.68 \pm 5.02^{c,B}$	$120.88 \pm 8.15^{a,A}$	$50.25 \pm 4.13^{c,B}$
Myricetin	nd	nd	nd	nd	nd	nd
Quercetin	10.61 ± 0.49	10.61 ± 0.49	10.42 ± 0.00	10.42 ± 0.00	10.86 ± 0.21	10.86 ± 0.21
Isoflavones						
Daidzein	$63.41 \pm 6.71^{d,B}$	$326.43 \pm 15.27^{c,A}$	$76.18 \pm 1.01^{d,B}$	$500.80 \pm 45.28^{a,A}$	$73.76 \pm 0.67^{d,B}$	$420.12 \pm 20.51^{b,A}$
Genestein	$21.05 \pm 2.49^{c,B}$	$29.19 \pm 1.63^{b,A}$	35.08 ± 1.53^{a}	37.35 ± 5.13^{a}	$21.77 \pm 3.11^{c,B}$	$27.61 \pm 0.30^{b,A}$
Total	$382.13 \pm 36.34^{b,B}$	614.63 ± 100.83^{A}	$454.21 \pm 39.48^{ab,B}$	$694.25 \pm 159.36^{a,A}$	514.68 ± 50.76^{a}	$621.13 \pm 126..36^{a}$
Total Free and Bound contents	996.76 ± 164.10		1148.46 ± 169.73		1135.81 ± 75.27	

*DSO: dry soybean from oil, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale, DSM: dry soybean from soy milk industry, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The different letter in same row were significantly ($p < 0.05$) by compared among treatments, the different capital letter in same row were significantly different ($p < 0.05$) by compared between free and bound, n = 3.

Table 9 Identifications of free and bound phenolic acids in soymilk samples.

Contents (µg/g DW)	Soymilk industry					
	DSM		SMI		SML	
	Free	Bound	Free	Bound	Free	Bound
Phenolic acids						
Gallic acid	$12.77 \pm 0.40^{c,B}$	$27.55 \pm 0.39^{a,A}$	$18.83 \pm 0.95^{b,B}$	$22.80 \pm 1.89^{b,A}$	nd	nd
Procatechuic acid	$182.17 \pm 16.39^{a,A}$	$49.44 \pm 3.25^{c,B}$	$100.59 \pm 3.08^{b,A}$	$18.54 \pm 0.99^{d,B}$	$47.61 \pm 1.00^{c,A}$	$12.69 \pm 0.23^{e,B}$
Chlorogenic acid	$27.45 \pm 2.16^{a,B}$	$9.84 \pm 0.05^{c,B}$	10.26 ± 0.39^{b}	nd	11.89 ± 0.17^{b}	nd
p-Coumaric acid	$11.19 \pm 0.39^{a,A}$	$10.76 \pm 0.08^{a,B}$	9.09 ± 0.00^{c}	9.19 ± 0.01^{b}	9.59 ± 0.01^{b}	9.24 ± 0.02^{b}
Ferrulic acid	27.43 ± 5.78^{a}	22.20 ± 0.42^{a}	9.48 ± 0.16^{b}	11.45 ± 1.16^{b}	$26.15 \pm 0.26^{a,A}$	$9.31 \pm 0.02^{b,B}$
Sinapic acid	20.46 ± 1.54^{a}	22.28 ± 0.47^{a}	11.09 ± 0.16^{c}	12.06 ± 0.71^{b}	$13.17 \pm 0.61^{b,A}$	$10.33 \pm 0.01^{c,B}$
Flavonoids						
Rutin	$71.63 \pm 4.73^{a,A}$	$50.41 \pm 3.13^{b,B}$	$35.14 \pm 0.91^{d,B}$	$47.36 \pm 1.78^{c,A}$	$41.20 \pm 7.67^{c,B}$	$51.16 \pm 1.78^{b,A}$
Myricetin	15.21 ± 0.01^{b}	nd	15.25 ± 0.04^{b}	nd	15.32 ± 0.02^{a}	nd
Isoflavones						
Daidzein	$58.13 \pm 0.87^{d,B}$	$227.89 \pm 10.20^{b,A}$	$153.98 \pm 8.79^{c,B}$	$318.90 \pm 14.87^{a,A}$	$47.95 \pm 0.49^{e,B}$	$239.39 \pm 9.82^{b,A}$
Genestein	24.56 ± 4.90^{a}	32.00 ± 2.23^{a}	$23.95 \pm 5.72^{ab,B}$	$32.97 \pm 0.22^{a,A}$	$16.98 \pm 0.39^{b,B}$	$23.87 \pm 0.23^{ab,A}$
Total	451.00 ± 52.07^{a}	452.37 ± 68.16^{a}	387.66 ± 48.99^{a}	473.27 ± 105.73^{a}	229.86 ± 15.84^{b}	355.99 ± 84.49^{a}
Total Free and Bound contents	903.37 ± 0.97^{a}		860.93 ± 60.54^{a}		585.85 ± 89.19^{b}	

*DSO: dry soybean from oil, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale, DSM: dry soybean from soy milk industry, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The different letter in same row were significantly ($p < 0.05$) by compared among treatments, the different capital letter in same row were significantly different ($p < 0.05$) by compared between free and bound, n = 3.

Experiment 2: determination of antioxidant activity and anti-cancer with bioactive compounds of extract samples from different drying methods.

4.3.6 Total phenolic and flavonoid content of soybean residues by using hot air oven (HA) method

The levels of free, bound and total phenolic and flavonoid contents of soybean residues from oil and soymilk industries are presented in Tables 10 and 11, respectively. The highest values of total phenolic content were observed in 70 ^{o}C, followed by 80 ^{o}C and 60 ^{o}C drying of SOI samples, respectively (Table 10). In SOL, free TPC was not significantly different among different drying temperature. However, bound TPC of 80 ^{o}C dried SOL was significantly different (p<0.05), being higher when compared with bound TPC of 60 ^{o}C dried SOL but not significantly different with 70 ^{o}C dried SOL. Total phenolic contents of SOI samples were significantly different (higher) when compared to SOL samples. Hot air oven treatment provided the highest values of free and bound TPC among samples studied including dried samples (Table 10), which showed almost 2 folds phenolics compared to NTS samples. For total flavonoid contents, TFC values were more often found in free form than bound form of SOI and SOL samples. The highest values of unbound and bound TFC were observed in 80 ^{o}C dried SOL (Table 10). This agrees with the report that thermal processing methods alter the total phenolic content or phenolic composition of the insoluble-bound fraction of legumes and grains, with increases or decreases affect the content of phenolic content which possibly will influence their bioactive properties and health promoting advantages (Abdel-Aal & Rabalski, 2013). After heating at the three different temperatures, free and bound phenolics, increased in all samples. Heat or thermal processes can degrade the links of polyphenols with proteins, carbohydrates or lipids, thus releasing soluble phenolics (Dewanto, Wu, & Liu, 2002) as the results show. ……

Table 10 Total phenolic and flavonoid contents from oil residues with HA treatments.

Conditions	Oil industry					
	SOI		Total	SOL		Total
TPC	Free	Bound	mg GAE/g DW	Free	Bound	mg GAE/g DW
NTS	90.48 ± 2.61^{b,C}	98.39 ±2.67^{c,B}	188.88 ± 5.59^{c,A}	93.26 ± 4.24^{b,C}	99.23 ± 3.88^{c,B}	192.49 ± 4.22^{d,A}
60 °C	213.31 ± 1.16^{a,C}	184.00 ± 14.88^{b,DE}	397.31 ± 20.72^{b,A}	186.22 ± 6.71^{a,D}	173.54 ± 6.83^{b,E}	359.77 ± 8.96^{c,B}
70 °C	212.65 ± 6.87^{a,D}	244.73 ± 2.10^{a,C}	457.38 ± 22.68^{a,A}	191.47 ± 2.37^{a,F}	196.38 ± 2.15^{a,E}	387.86 ± 3.46^{a,B}
80 °C	208.76 ± 6.29^{a,C}	198.60 ± 4.17^{b,C}	407.36 ± 7.18^{b,A}	182.13 ± 4.12^{a,E}	198.45 ± 3.16^{a,CD}	380.58 ± 2.16^{b,B}
TFC			mg RE/g DW			mg RE/g DW
NTS	1.99 ± 0.01^{b,A}	0.29 ± 0.01^{c,B}	2.28 ± 1.21^{A}	1.71 ± 0.03^{d,A}	0.10 ± 0.00^{b,C}	1.81 ± 1.13^{b,A}
60 °C	2.46 ± 0.06^{a,A}	0.15 ± 0.01^{d,B}	2.61 ± 1.63^{A}	2.99 ± 0.05^{c,A}	0.29 ± 0.00^{b,B}	3.28 ± 1.91^{A}
70 °C	2.61 ± 0.09^{a,A}	0.52 ± 0.02^{a,B}	3.13 ± 1.48^{A}	3.78 ± 0.11^{b,A}	0.38 ± 0.02^{a,B}	4.15 ± 2.40^{A}
80 °C	2.58 ± 0.05^{a,A}	0.37 ± 0.03^{b,B}	2.95 ± 1.57^{A}	3.94 ± 0.13^{a,A}	0.38 ± 0.02^{a,B}	4.32 ± 2.52^{A}

*NTS; non-treated soybean residue, SOI: soybean residue from oil industry, SOL: soybean residue of oil extraction from lab scale. The values are expressed as means ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in the same row indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

Table 11 Total phenolic and flavonoid contents from soymilk residues with HA treatments.

Conditions	Soymilk industry					
	SMI		Total	SML		Total
TPC	Free	Bound	mg GAE/g DW	Free	Bound	mg GAE/g DW
NTS	79.31 ± 8.77^{c,B}	93.77 ± 8.13^{c,B}	173.09 ± 10.21^{b,A}	88.44 ± 4.55^{c,B}	87.93 ± 6.50^{c,B}	176.37 ± 0.36^{b,A}
60 °C	101.39 ± 6.76^{a,F}	134.94 ± 2.15^{b,D}	236.33 ± 23.72^{a,B}	127.88 ± 4.07^{a,E}	173.74 ± 7.53^{b,C}	301.61 ± 32.44^{a,A}
70 °C	91.53 ± 1.30^{b,E}	137.01 ± 3.52^{ab,C}	228.54 ± 32.15^{a,A}	107.96 ± 4.41^{b,D}	176.32 ± 1.77^{a,B}	284.28 ± 48.34^{a,A}
80 °C	87.14 ± 7.36^{bc,E}	143.27 ± 3.24^{a,C}	230.42 ± 39.69^{a,A}	112.20 ± 4.73^{b,D}	178.99 ± 3.55^{a,B}	291.20 ± 47.23^{a,A}
TFC			mg RE/g DW			mg RE/g DW
NTS	6.18 ± 0.16^{d,B}	0.66 ± 0.05^{a,D}	6.84 ± 3.90^{a,B}	6.45 ± 0.16^{c,B}	5.40 ± 0.36^{a,C}	11.86 ± 0.74^{a,A}
60 °C	9.00 ± 0.14^{a,A}	0.36 ± 0.01^{c,B}	9.36 ± 6.11^{a,A}	8.38 ± 0.35^{b,A}	0.44 ± 0.00^{b,B}	8.81 ± 5.61^{ab,A}
70 °C	7.98 ± 0.17^{c,B}	0.39 ± 0.00^{b,C}	8.38 ± 5.36^{a,AB}	9.76 ± 0.03^{a,A}	0.36 ± 0.00^{c,D}	10.13 ± 6.64^{ab,AB}
80 °C	8.27 ± 0.19^{b,A}	0.17 ± 0.00^{d,D}	8.45 ± 5.73^{a,A}	5.60 ± 0.11^{d,B}	0.33 ± 3.16^{d,C}	5.94 ± 3.73^{b,AB}

* NTS; non-treated soybean residue, SMI: soybean residue from soymilk industry and SML: soybean residue of soymilk extraction at lab scale. The values are expressed as means ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in the same row indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

For SMI, the highest values of free TPC and TFC were found in 60 ^{o}C dried SMI, while 70 ^{o}C dried SMI provided the highest values of bound TPC and TFC (Table 11). For SML, the highest value of free TPC was observed in 60 ^{o}C dried SML. On the other hand, high values of bound TPC were found in 80 and 70 ^{o}C dried SML. Total phenolic contents of all SML samples were significantly (p<0.05) different (higher) than that of all SMI samples. Flavonoids, in 60 ^{o}C dried SMI showed highly values as free TFC, while 70 ^{o}C dried SML provided the highest values of free TFC followed by 60 ^{o}C and 80 ^{o}C dried samples, respectively. Additionally, the bound TFC value of 60 ^{o}C dried SML was significantly higher than that of other dried samples (Table 1B). The results indicated that flavonoid compounds were susceptible to high temperature treatment together with water-soluble extraction. Polyphenols primarily exist in bound structures, forming bridges between polymer chains in their structure. Free and bound forms, would be affected in their behavior during processing, due to their bioavailability for absorption and subsequent physiological effects (Abdel-Aal & Rabalski, 2013). Increases in phenolic compounds after oven treatment were also observed in the previous research of Albishi et al. (2013). This result may be because drying is considered to be adverse due to the possibility of inducing oxidative decomposition either enzymatically by polyphenol oxidase and glycosidase or by thermal degradation of phenolic compounds. However, hot air oven treatment creates a high temperature outside and inside plant tissue, resulting in the disruption of plant cell wall polymers (Abdel-Aal & Rabalski, 2013). Hence, cell wall insoluble phenolics can be released, thus causing extractable phenolic or flavonoid content to increase in the samples. Moreover, the differences in TPC, TFC extracted from soybean residue can depend on the varieties grown, the season, the harvest, the storage conditions, the solvent to sample ratio, the solvent type, the time of extraction, and the type of processing (Alu'datt et al., 2016).

4.3.7 The antioxidant activities of soybean residues by using hot air oven (HA) method

Changes in antioxidant activity of soybean residues from oil and soymilk as affected by different temperatures of HA are presented in Tables 12 and 13, respectively. In oil samples, DPPH values of bound phenolic extracts were significantly (p<0.05) different in NTS and dried soybean residue samples. Bound DPPH values of dried SOI increased from 38% to 90%. However, bound DPPH values of dried SOL did not affect change on percent inhibition of radical scavenging when compared to NTS sample except for 60 ^{o}C dried SOL (Table 12). On the other hand, DPPH values of free phenolic extracts in both samples were not significantly (p<0.05) different when compared to non-treated samples. Bound DPPH values had higher radical scavenging activity than free DPPH values of dried SOI and SOL samples. For FRAP values, the results showed that free FRAP values of dried SOI and SOL were increased from 0.79 to 2.08 and 0.71 to 1.59 mg $FeSO_4$/g DW, respectively. Total FRAP values ranged from 2.39-2.50 and 1.84-2.28 in dried SOI and SOL samples, respectively. These results indicated that the dried soybean residues in both samples had significantly (p<0.05) higher antioxidant capacity than

NTS samples. In soymilk samples, the bound DPPH values of dried SMI and SML ranged from 68-80% and 78-92%, respectively, and had highly radical scavenging activity than the free DPPH values. The highest FRAP values were observed in free phenolic extracts of dried SMI (0.85-0.94 mg $FeSO_4$/g DW) and SML (1.75-1.94 mg $FeSO_4$/g DW). Overall, we found that the bound DPPH and free FRAP values were similarly trending as soybean residues from oil samples (Tables 12 and 13).

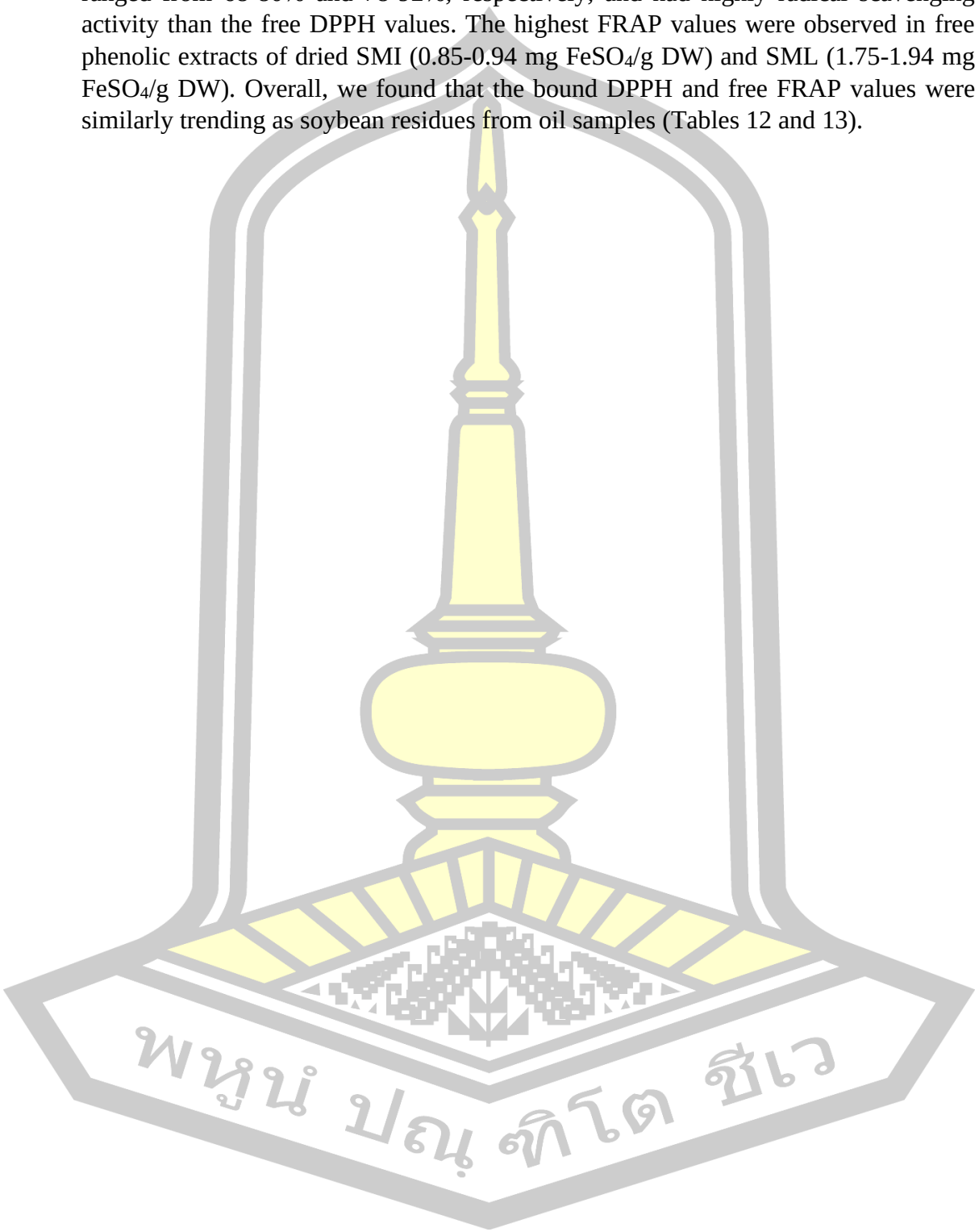


Table 12 the antioxidant activities of the oil residues with HA treatments.

Conditions	Oil industry					
	SOI			SOL		
DPPH	Free *(% inhibition)*	Bound *(% inhibition)*		Free *(% inhibition)*	Bound *(% inhibition)*	
NTS	29.29 ± 3.09^{ab,C}	38.18 ± 4.04^{c,B}		27.28 ± 2.10^{a,C}	84.09 ± 1.78^{a,A}	
60 °C	26.46 ± 1.55^{b,C}	90.74 ± 0.53^{a,A}		22.86 ± 0.55^{b,D}	77.09 ± 0.63^{c,B}	
70 °C	29.32 ± 0.66^{a,C}	82.85 ± 0.89^{b,B}		27.81 ± 0.56^{a,D}	84.92 ± 0.39^{a,A}	
80 °C	29.87 ± 0.84^{a,C}	82.38 ± 0.34^{b,B}		22.52 ± 1.07^{b,D}	86.12 ± 0.26^{a,A}	
FRAP	Free	Bound	Total (mg $FeSO_4$/g DW)	Free	Bound	Total (mg $FeSO_4$/g DW)
NTS	0.79 ± 0.00^{d,B}	0.66 ± 0.03^{a,C}	1.45 ± 0.10^{b,A}	0.71 ± 0.05^{c,B}	0.49 ± 0.01^{b,D}	1.20 ± 0.16^{b,B}
60 °C	2.08 ± 0.04^{a,AB}	0.31 ± 0.00^{c,D}	2.39 ± 1.25^{ab,A}	1.53 ± 0.06^{ab,AB}	0.76 ± 0.02^{a,C}	2.28 ± 0.55^{a,A}
70 °C	1.91 ± 0.11^{b,A}	0.59 ± 0.01^{b,C}	2.50 ± 0.93^{a,A}	1.46 ± 0.02^{b,B}	0.40 ± 0.01^{b,D}	1.86 ± 0.75^{ab,AB}
80 °C	1.75 ± 0.07^{c,AB}	0.64 ± 0.00^{a,C}	2.39 ± 0.78^{a,A}	1.59 ± 0.09^{a,B}	0.34 ± 0.01^{c,D}	1.94 ± 0.89^{ab,AB}

* NTS; non-treated soybean residue, SOI: soybean residue from oil industry, SOL: soybean residue of oil extraction from lab scale. The values are expressed as means ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in the same row indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

Table 13 the antioxidant activities of soymilk residues with HA treatments.

	Soymilk industry					
Conditions	SMI			SML		
DPPH	Free *(% inhibition)*	Bound *(% inhibition)*		Free *(% inhibition)*	Bound *(% inhibition)*	
NTS	$30.83 \pm 1.89^{a,D}$	$68.81 \pm 3.10^{b,B}$		$44.11 \pm 3.23^{a,C}$	$92.36 \pm 0.05^{a,A}$	
60 °C	$16.48 \pm 0.07^{b,D}$	$79.56 \pm 0.28^{a,B}$		$20.59 \pm 0.81^{b,C}$	$81.10 \pm 1.06^{b,A}$	
70 °C	$19.05 \pm 0.42^{b,B}$	$80.38 \pm 0.21^{a,A}$		$20.29 \pm 0.63^{b,B}$	$79.82 \pm 0.64^{c,A}$	
80 °C	$18.51 \pm 0.41^{b,C}$	$79.90 \pm 0.16^{a,A}$		$21.01 \pm 0.46^{b,B}$	$78.88 \pm 0.41^{c,A}$	
FRAP	Free	Bound	Total (mg $FeSO_4$/g DW)	Free	Bound	Total (mg $FeSO_4$/g DW)
NTS	$0.73 \pm 0.02^{b,B}$	$0.43 \pm 0.00^{a,C}$	$1.15 \pm 0.21^{a,A}$	$1.18 \pm 0.04^{c,A}$	$0.17 \pm 0.00^{c,D}$	$1.35 \pm 0.72^{a,A}$
60 °C	$0.94 \pm 0.08^{a,B}$	$0.29 \pm 0.00^{b,D}$	$1.22 \pm 0.46^{a,AB}$	$1.85 \pm 0.06^{ab,A}$	$0.41 \pm 0.05^{b,C}$	$2.26 \pm 1.01^{a,A}$
70 °C	$0.84 \pm 0.07^{a,B}$	$0.22 \pm 0.01^{c,D}$	$1.06 \pm 0.42^{a,B}$	$1.75 \pm 0.03^{b,A}$	$0.46 \pm 0.02^{b,C}$	$2.20 \pm 0.91^{a,AB}$
80 °C	$0.85 \pm 0.00^{a,B}$	$0.25 \pm 0.01^{bc,D}$	$1.10 \pm 0.42^{a,B}$	$1.94 \pm 0.04^{a,A}$	$0.51 \pm 0.01^{a,C}$	$2.45 \pm 1.01^{a,AB}$

* NTS; non-treated soybean residue, SMI: soybean residue from soymilk industry and SML: soybean residue of soymilk extraction from lab scale. The values are expressed as means ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in the same row indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

These results suggest that the natural bioactive compounds could perform differently during processing, depending on the type of processing (e.g. baking, drying, roasting, etc), food matrix structure. Thus, insoluble phenolic compounds could be released from the food matrix. Such changes may be caused by polymerization, Maillard reaction and the oxidation (from heat, oxygen, and enzymes) of phenolics (Abdel-Aal & Rabalski, 2013). The DPPH results are in agreement with previous studies by Wang et al. (2016), who reported that an insoluble-bound phenolic fraction from soybean was higher in DPPH radical scavenging than their corresponding free phenolic fractions. Similarly, Madhujith & Shahidi (2009) found that the capacity of DPPH radical scavenging of bound phenolic extracts of six barley varieties was higher compared with free phenolic extracts. On the other hand, previous studies have also indicated that the soluble phenolic extract from pigmented rice and soybean residues had the higher antioxidant activity of DPPH radical scavenging than bound phenolics (Alu'datt et al., 2016; Sumczynski et al., 2016). The highest values of the FRAP were observed in free phenolic fractions, which were in agreement with the previous studies on pigmented rice, seed coat and the cotyledon of black soybean and peanut skin (Bhat & Riar, 2017; de Camargo et al., 2015; Peng et al., 2017). However, Wang et al. (2016) found that the FRAP values of insoluble-bound phenolics were significantly higher than that of free phenolics in soybean. Kaisoon et al. (2011) also found the FRAP values of bound phenolics were higher than free phenolics in some edible flowers. The results of the antioxidant activities indicated that soybean and soybean residues, containing free and bound phenolic and flavonoid contents, had effective hydrogen atom donation and electron transfer, especially bound and free phenolic extracts, respectively. Many researchers have suggested that the capacity for antioxidant activity of phenolic compounds would depend on the content, structure, location and the number of hydroxyl groups, for which the antioxidant capacity is enhanced if the number of hydroxyl groups is increased (Shahidi & Zhong, 2015; Xie et al., 2015).

4.3.8 Identification and quantification of isoflavone contents of soybean residues by using hot air oven (HA) method

Total isoflavone contents of oil and soymilk samples, treated under different drying temperatures are listed in Table 14. Total isoflavone contents of all samples increased and were significantly (p<0.05) different when compared to NTS samples after hot air drying at three levels of drying temperature. The highly increasing values were observed in free daidzein of all samples as temperatures increased. On the other hand, bound daidzein values of all samples decreased with higher temperatures, except for SML samples. For genistein, the results showed that free genistein values of all samples were slightly changed or decreased when compared to NTS samples, except for SMI samples, while bound genistein values of all samples increased except for SML samples. These results suggest that thermal processing, including drying with air, enhanced the extractability of polyphenol, releasing them from food matrix structures to provide their free form (T. Wang, He, & Chen, 2014). The effects of these changes could be to improve antioxidant properties with increased bioactive

compound, and inhibition of anti-nutrients in soybean such as trypsin, and phytase (Boateng et al., 2008).

In addition, the total isoflavones of soybean meal from the oil industry and experimental samples ranged from 5.3 to 11.0 mg/g; they were found in the β-glucoside form, rather than in any other form (de Oliveira Silva & Perrone, 2015). Additionally, Tyug et al. (2010) reported that the high contents of isoflavone extracts are daidzein in soymilk powder and soy husk powder. Moreover, there were significant correlations of daidzein and genistein from soymilk powder extracts with FRAP and TEAC assay results. The phenolic compound extracts of soybean residues from oil and soymilk samples retained the greatest antioxidant activity in free and bound phenolic forms. Moreover, many studies show a correlation between TPC and TFC with antioxidant activity, indicating the phenolic compounds are highly effective free radical scavengers and metal chelating agents (Ayoub et al., 2016; Peng et al., 2017; Sumczynski et al., 2016). However, there is no literature data to compare the effect of drying method on the bioactive compound of soybean residues from oil and soymilk industries. The data of this study can contribute knowledge concerning the stability of phenolic compounds with respect to the drying conditions of soybean residues.

Table 14 Identification of isoflavones from oil and soymilk residues with HA treatments.

Oil samples	Isoflavone content (µg/g DW)				
SOI	Daidzein		Genistein		
Condition	Free	Boound	Free	Bound	Total
NTS	76.18 ± 1.01^{b,D}	500.80 ± 45.28^{a,A}	35.08 ± 1.53^{c,B}	37.35 ± 5.13^{c,C}	649.41 ± 226.42^{a,AB}
60 °C	310.65 ± 16.05^{c,B}	401.70 ± 20.50^{b,B}	13.35 ± 1.69^{e,E}	29.68 ± 1.95^{d,D}	755.38 ± 197.07^{a,A}
70 °C	451.55 ± 9.22^{c,A}	510.92 ± 19.62^{b,A}	25.38 ± 1.24^{e,C}	57.99 ± 1.19^{d,A}	1045.84 ± 255.52^{a,A}
80 °C	463.14 ± 37.96^{b,A}	494.09 ± 14.07^{b,A}	17.37 ± 4.65^{d,D}	49.53 ± 12.46^{c,AB}	1024.13 ± 257.58^{a,A}
SOL	Daidzein		Genistein		
Condition	Free	Bound	Free	Bound	Total
NTS	73.76 ± 0.67^{c,D}	420.12 ± 20.51^{b,B}	21.77 ± 3.11^{e,C}	27.61 ± 0.30^{d,D}	543.26 ± 190.95^{a,B}
60 °C	213.70 ± 14.84^{c,C}	494.40 ± 24.32^{b,A}	74.30 ± 19.56^{d,A}	53.27 ± 0.65^{d,A}	835.67 ± 203.14^{a,A}
70 °C	231.86 ± 20.50^{c,C}	427.01 ± 18.45^{b,B}	84.27 ± 0.47^{d,A}	45.57 ± 0.29^{e,B}	788.71 ± 172.84^{a,A}
80 °C	301.21 ± 24.37^{c,B}	381.95 ± 14.65^{b,C}	12.90 ± 0.25^{e,E}	42.55 ± 0.84^{d,B}	738.61 ± 184.40^{a,A}

Soymilk samples	Isoflavone content (µg/g DW)				
SMI	Daidzein		Genistein		
Condition	Free	Boound	Free	Bound	Total
NTS	153.98 ± 8.79^{c,F}	318.90 ± 14.87^{b,B}	23.95 ± 5.72^{d,C}	32.97 ± 0.22^{d,B}	529.80 ± 137.71^{a,AB}
60 ºC	567.32 ± 20.50^{b,A}	154.09 ± 15.19^{c,E}	101.51 ± 3.02^{d,A}	32.41 ± 3.06^{e,B}	855.33 ± 240.74^{a,A}
70 ºC	343.07 ± 10.25^{b,C}	241.98 ± 20.50^{c,C}	95.51 ± 0.82^{d,AB}	41.02 ± 0.84^{e,A}	721.58 ± 137.58^{a,A}
80 ºC	372.15 ± 7.08^{b,B}	272.42 ± 10.21^{c,C}	83.50 ± 11.10^{d,B}	42.34 ± 2.92^{e,A}	770.41 ± 155.95^{a,A}
SML	Daidzein		Genistein		
Condition	Free	Bound	Free	Bound	Total
NTS	47.95 ± 0.49^{c,G}	239.39 ± 9.82^{b,D}	16.98 ± 0.39^{e,D}	23.87 ± 0.23^{d,D}	328.19 ± 105.73^{a,B}
60 ºC	233.71 ± 15.95^{c,E}	369.07 ± 1.76^{b,A}	11.87 ± 0.46^{e,E}	45.48 ± 4.09^{d,A}	660.13 ± 167.51^{a,A}
70 ºC	213.85 ± 1.54^{c,E}	366.87 ± 20.50^{b,A}	12.13 ± 0.29^{e,E}	22.61 ± 0.16^{d,D}	615.46 ± 169.60^{a,A}
80 ºC	251.75 ± 0.64^{c,D}	364.31 ± 15.05^{b,A}	11.68 ± 0.00^{e,E}	27.68 ± 0.81^{d,C}	655.42 ± 172.57^{a,A}

* NTS; non-treated, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The values are expressed as mean ± standard deviation. A different small letter in the same raw indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in same column indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

4.3.9 Total phenolic and flavonoid content of soybean residues by using FIR-HA treatment

Total phenolic and total flavonoid contents of soybean residues from oil and soymilk are presented in Table 15 and 16, respectively. For SOI, free and bound TPC of FIR-HA dried samples were significantly (p<0.05) higher when compared with NTS. 80 ^{o}C FIR-HA dried SOI provided the highest values of free TPC. Free TFC values of all FIR-HA dried SOI and SOL decreased while bound TFC values of all FIR-HA dried SOI and SOL increased (Table 15). Similar results were found in free and bound TPC and free TFC of SMI and SML samples except for bound TFC of SMI and SML (Table 16). These results showed that the increases of total phenolic content were convinced by heat combined with far-irradiation (FIR-HA) treatment, thereby verifying a previous report that FIR radiation increases the phenolic compounds of mulberry leaves (Wanyo et al., 2011). FIR-HA can split strong bonds or breaking a particular chemical bond in food matrixes such as covalent bonds and liberate bound phenolic compounds to unbound phenolic compounds (S. C. Lee et al., 2003). On the other hand, flavonoids are the common plant phenolic compounds, that are contained a benzo-y-pyrone structure, which is can be soluble in the water phase and sensitivity on heat, light, oxidation from oxygen or enzyme (Shahidi & Ambigaipalan, 2015). The results indicated that the decrease of flavonoid contents was induced by strong FIR energy, which agrees with previously researched by Wanyo et al. (2014) reported that total flavonoid content in rice bran and rice husk were decreased by FIR-HA treatment.

Table 15 Total phenolic and flavonoid contents of oil residues with FIR-HA treatments.

Conditions	Oil industry					
	SOI		Total	SOL		Total
TPC	Free	Bound	mg GAE/g DW	Free	Bound	mg GAE/g DW
NTS	$90.48 \pm 2.61^{c,C}$	$98.39 \pm 2.67^{c,B}$	$188.88 \pm 5.59^{b,A}$	$93.26 \pm 4.24^{c,C}$	$99.23 \pm 3.88^{d,B}$	$192.49 \pm 4.22^{c,A}$
60 °C	$160.20 \pm 6.62^{b,F}$	$259.18 \pm 7.27^{a,C}$	$419.38 \pm 69.99^{a,A}$	$142.97 \pm 3.15^{b,E}$	$196.83 \pm 4.24^{c,D}$	$339.80 \pm 38.08^{b,B}$
70 °C	$161.01 \pm 4.82^{b,E}$	$257.26 \pm 2.83^{a,C}$	$418.27 \pm 68.06^{a,A}$	$164.14 \pm 4.90^{a,E}$	$227.20 \pm 2.92^{b,D}$	$391.34 \pm 44.58^{a,B}$
80 °C	$172.58 \pm 6.17^{a,D}$	$241.50 \pm 3.91^{b,C}$	$414.08 \pm 48.73^{a,A}$	$165.05 \pm 4.95^{a,E}$	$255.64 \pm 3.88^{a,B}$	$420.70 \pm 64.05^{a,A}$
TFC			mg RE/g DW			mg RE/g DW
NTS	$1.99 \pm 0.01^{a,A}$	$0.29 \pm 0.01^{d,B}$	$2.28 \pm 1.21^{a,A}$	$1.71 \pm 0.03^{a,A}$	$0.10 \pm 0.00^{d,C}$	$1.81 \pm 1.13^{a,A}$
60 °C	$0.13 \pm 0.01^{b,D}$	$0.53 \pm 0.01^{a,A}$	$0.67 \pm 0.28^{b,A}$	$0.11 \pm 0.00^{c,D}$	$0.25 \pm 0.00^{c,C}$	$0.36 \pm 0.10^{b,B}$
70 °C	$0.13 \pm 0.00^{b,C}$	$0.49 \pm 0.00^{b,AB}$	$0.61 \pm 0.25^{b,A}$	$0.13 \pm 0.00^{b,C}$	$0.38 \pm 0.00^{b,B}$	$0.51 \pm 0.17^{b,A}$
80 °C	$0.14 \pm 0.00^{b,C}$	$0.44 \pm 0.01^{c,B}$	$0.59 \pm 0.21^{b,A}$	$0.14 \pm 0.01^{b,C}$	$0.44 \pm 0.01^{a,B}$	$0.58 \pm 0.21^{b,A}$

*NTS; non-treated soybean meal, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale. The values are expressed as mean ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in same row indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

Table 16 Total phenolic and flavonoid contents of soymilk residues with FIR-HA treatments.

Conditions	Soymilk industry					
	SMI		Total	SML		Total
TPC	Free	Bound	mg GAE/g DW	Free	Bound	mg GAE/g DW
NTS	79.31 ± 8.77^{c,C}	93.77 ± 8.13^{d,B}	173.09 ± 10.21^{c,A}	88.44 ± 4.55^{c,BC}	87.93 ± 6.50^{c,BC}	176.37 ± 0.36^{b,A}
60 °C	106.19 ± 3.43^{b,D}	102.04 ± 2.46^{c,D}	208.23 ± 2.92^{b,B}	132.86 ± 3.77^{b,C}	191.52 ± 4.91^{b,B}	324.39 ± 41.47^{a,A}
70 °C	168.69 ± 6.22^{a,C}	143.37 ± 2.89^{a,E}	312.06 ± 17.89^{a,A}	158.99 ± 18.20^{a,D}	193.24 ± 5.07^{b,B}	352.23 ± 24.22^{a,A}
80 °C	166.56 ± 5.52^{a,D}	123.01 ± 1.98^{b,E}	289.58 ± 30.79^{a,B}	165.25 ± 12.95^{a,D}	217.45 ± 4.17^{a,C}	382.70 ± 36.90^{a,A}
TFC			mg RE/g DW			mg RE/g DW
NTS	6.18 ± 0.16^{a,B}	0.66 ± 0.05^{a,D}	6.84 ± 3.90^{a,B}	6.45 ± 0.16^{a,B}	5.40 ± 0.36^{a,C}	11.86 ± 0.74^{a,A}
60 °C	0.98 ± 0.03^{d,AB}	0.19 ± 0.00^{bc,E}	1.17 ± 0.56^{b,A}	0.57 ± 0.08^{d,C}	0.32 ± 0.00^{b,D}	0.89 ± 0.18^{b,B}
70 °C	2.57 ± 0.07^{b,A}	0.26 ± 0.00^{b,D}	2.84 ± 1.63^{b,A}	0.73 ± 0.05^{c,B}	0.38 ± 0.01^{b,C}	1.12 ± 0.24^{b,AB}
80 °C	1.91 ± 0.17^{c,A}	0.13 ± 0.00^{c,D}	2.05 ± 1.25^{b,A}	0.93 ± 0.08^{b,B}	0.41 ± 0.00^{b,C}	1.34 ± 0.36^{b,AB}

* NTS; non-treated soybean meal, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The values are expressed as mean ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in same row indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

4.3.10 Antioxidant activities of soybean residues by using FIR-HA treatment

DPPH radical scavenging and FRAP values of two soybean residues are shown in Tables 17 and 18, respectively. FIR-HA drying samples of free and bound DPPH and FRAP values of SOI and SOL increased when compared with NTS samples except for bound FRAP value SOI and bound DPPH value SOL samples (Table 17). For soymilk residue antioxidant capacity, free and bound DPPH and free FRAP values of FIR-HA dried SMI increased when the temperature increased which significantly (p<0.05) different when compared to NTS samples. Free and bound by DPPH values of FIR-HA dried SML decreased when compared with NTS samples, while bound FRAP value of FIR-HA dried SML was increased. These results indicated that the different processing, raw materials had been affected to the bioactivity of natural bioactive compounds in plant foods (Shahidi & Ambigaipalan, 2015). It is possible explanation for these results by correlation coefficient, the high positive correlation between phenolic contents and free and bound antioxidant activities including DPPH and FRAP values of oil and soymilk residues; r = 0.987 (free DPPH), 0.997 (free FRAP), 0.997 (bound DPPH); 0.717 (free DPPH), 0.958 (free FRAP), 0.899 (bound DPPH), respectively, (data not shown). Our result was also in agreement with Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne (2006) reported that the highest correlation coefficient values were 0.68 < r < 0.97. Additionally, (Kubola & Siriamornpun, 2008) found correlations between TPC and DPPH and FRAP values in bitter gourd samples. However, the negative correlations were found between TFC and antioxidant activities in oil and soymilk residues (r = -0.926 (free DPPH), -0.960 (free FRAP); -0.972 (bound DPPH), -0.944 (bound FRAP), respectively, data not shown), which indicated that if total flavonoid content was decreased which due to antioxidant capacity is decreasing. Constant with this, the present study presented that far-infrared radiation has been improved to enhance the soluble and insoluble phenolics with antioxidant capacity in soybean residue extracts.

Table 17 DPPH and FRAP values of oil residues with FIR-HA treatments.

Conditions	Oil industry					
	SOI			SOL		
DPPH	Free *(% inhibition)*	Bound *(% inhibition)*		Free *(% inhibition)*	Bound *(% inhibition)*	
NTS	29.29 ± 3.09^{c,C}	38.18 ± 4.04^{c,B}		27.28 ± 2.10^{a,C}	84.09 ± 1.78^{ab,A}	
60 °C	36.86 ± 0.45^{b,C}	74.78 ± 0.39^{a,A}		37.27 ± 0.22^{b,C}	46.37 ± 0.28^{c,B}	
70 °C	36.39 ± 0.35^{b,D}	73.49 ± 0.39^{ab,A}		39.68 ± 1.22^{b,C}	64.26 ± 1.32^{a,B}	
80 °C	38.97 ± 0.61^{a,D}	72.32 ± 1.01^{b,A}		41.12 ± 0.51^{a,C}	58.89 ± 1.17^{b,B}	
FRAP	Free	Bound	Total (mg $FeSO_4$/g DW)	Free	Bound	Total (mg $FeSO_4$/g DW)
NTS	0.79 ± 0.00^{c,C}	0.66 ± 0.03^{D}	1.45 ± 0.10^{A}	0.71 ± 0.05^{c,C}	0.49 ± 0.01^{c,E}	1.20 ± 0.16^{b,B}
60 °C	1.35 ± 0.02^{b,B}	0.74 ± 0.02^{C}	2.09 ± 0.43^{A}	1.23 ± 0.07^{b,B}	0.78 ± 0.03^{a,C}	2.01 ± 0.31^{a,A}
70 °C	1.34 ± 0.03^{b,B}	0.65 ± 0.00^{D}	1.99 ± 0.48^{A}	1.47 ± 0.07^{a,B}	0.72 ± 0.06^{b,C}	2.19 ± 0.53^{a,A}
80 °C	1.47 ± 0.13^{a,B}	0.69 ± 0.01^{D}	2.16 ± 0.55^{AB}	1.38 ± 0.08^{a,B}	0.88 ± 0.03^{a,C}	2.27 ± 0.35^{a,A}

* NTS; non-treated soybean meal, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale. The values are expressed as mean ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in same row indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

Table 18 DPPH and FRAP values of soymilk residues with FIR-HA treatments.

	Soymilk industry					
Conditions	SMI			SML		
DPPH	Free *(% inhibition)*	Bound *(% inhibition)*		Free *(% inhibition)*	Bound *(% inhibition)*	
NTS	30.83 ± 1.89^{c,D}	68.81 ± 3.10^{c,B}		44.11 ± 3.23^{a,C}	92.36 ± 0.05^{a,A}	
60 °C	25.86 ± 0.84^{d,C}	82.17 ± 0.67^{b,A}		21.69 ± 0.30^{b,D}	79.53 ± 0.28^{b,B}	
70 °C	39.15 ± 0.84^{b,C}	83.02 ± 0.08^{b,A}		25.74 ± 0.23^{b,D}	78.97 ± 0.89^{c,B}	
80 °C	42.91 ± 0.71^{a,C}	85.28 ± 0.30^{a,A}		29.36 ± 1.41^{b,D}	80.79 ± 1.02^{c,B}	
FRAP	Free	Bound	Total (mg $FeSO_4$/g DW)	Free	Bound	Total (mg $FeSO_4$/g DW)
NTS	0.73 ± 0.02^{d,B}	0.43 ± 0.00^{a,C}	1.15 ± 0.21^{A}	1.18 ± 0.04^{b,A}	0.17 ± 0.00^{b,D}	1.35 ± 0.72^{A}
60 °C	0.99 ± 0.00^{c,B}	0.24 ± 0.02^{b,D}	1.23 ± 0.53^{A}	1.11 ± 0.05^{b,AB}	0.48 ± 0.01^{a,C}	1.59 ± 0.44^{A}
70 °C	1.49 ± 0.06^{b,AB}	0.29 ± 0.01^{b,D}	1.78 ± 0.84^{A}	1.20 ± 0.13^{b,B}	0.53 ± 0.01^{a,C}	1.73 ± 0.47^{A}
80 °C	1.69 ± 0.01^{a,AB}	0.20 ± 0.00^{b,D}	1.89 ± 1.04^{A}	1.39 ± 0.11^{a,B}	0.58 ± 0.00^{a,C}	1.97 ± 0.57^{A}

* NTS; non-treated soybean meal, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The values are expressed as mean ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in same row indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

4.3.11 Identification and quantification of isoflavone contents of soybean residues by using FIR-HA treatment

The predominant isoflavones in soybean seed are a daidzein and genistein, which components of aglycones (daidzein, genistein) and its bound forms, namely, β-glucoside (daidzin, genistin), acetyl-β-glucoside (6$^{//}$-*O*-acetyldaidzin, 6$^{//}$-*O*-acetylgenistin) and malonyl-β-glucoside (6$^{//}$-*O*-malo-nyldaidzin, 6$^{//}$-*O*-malo-nylgenistin). Isoflavone forms in soybean and soybean residues can be changed during various processing steps such as roasting, boiling, and drying (S. W. Lee & Lee, 2009). FIR generates heat inside with molecular vibration of raw materials when high molecules of compounds absorb the radiation containing wavelengths and high energy, and cause vibration due to the high molecular weights of isoflavones can be degraded into low molecular weights (S. W. Lee & Lee, 2009; Sandu, 1986). For examples, malonyl-β-glucoside can be released to acetyl-β-glucoside by decarboxylation reaction. Malonyl- and acetyl-β-glucoside can be converted to β-glucoside by deesterification reaction. All glucoside isoflavone forms can be transformed to aglycones by hydrolysis reaction. In addition, previously studied reported that the agylcone forms display greater bioactive than other glucoside forms (Kwon et al., 2007). In this present study are present isoflavones as free and bound forms, which total isoflavone contents of oil and soymilk residues under different FIR-HA drying temperatures are listed in Table 19. The results found that the highest increases in free and bound genistein values was observed in all soybean residues, while free and bound daidzein values were decreased except for free daidzein SMI sample. However, total isoflavone contents of all FIR-HA dried residues were significantly (p<0.05) different higher than those of NTS samples. These results indicated that the increases genistein in both soybean residues was induced by the FIR-HA treatment, which thereby sustaining a previous study that FIR-HA increases the phenolic compound in mulberry leaves (Wanyo et al., 2011), rice hull samples (S. C. Lee et al., 2003). Moreover, the results showed that the genistein contents were found in the bound form which may be in malonyl-, acetyl- or glucosides and can be converted to free forms by heating from FIR-HA treatment. Yue, Abdallah, & Xu (2010) reported that the most generous isoflavone forms were found in malonyl-β-glucoside and followed by β-glucoside, agylcones and acetyl-β-glucoside. Additionally, Niamnuy, Nachaisin, Poomsa-Ad, & Devahastin (2012) reported that gas-fired infrared combined with hot air vibrating drying showed the highest values of β-glucoside, agylcones, and total isoflavones while malonyl- and acetyl-β-glucoside were lower values in drying soybean.

Table 19 Identification of isoflavones from oil and soymilk residues with FIR-HA treatments.

Oil samples	Isoflavone content (µg/g DW)				
SOI	Daidzein		Genistein		
Condition	Free	Boound	Free	Bound	Total
NTS	76.18 ± 1.01^{b,A}	500.80 ± 45.28^{a,A}	35.08 ± 1.53^{c,D}	37.35 ± 5.13^{c,C}	649.41 ± 226.41^{a,B}
60 ºC	67.65 ± 2.05^{c,B}	26.15 ± 0.35^{d,C}	91.56 ± 24.22^{b,B}	1878.96 ± 36.26^{a,B}	2064.32 ± 908.98^{a,A}
70 ºC	36.01 ± 6.21^{c,DE}	26.73 ± 1.14^{d,C}	124.70 ± 20.42^{b,A}	2267.09 ± 310.34^{a,A}	2454.53 ± 1103.18^{a,A}
80 ºC	34.03 ± 1.43^{c,E}	26.45 ± 0.03^{d,C}	53.63 ± 0.41^{b,C}	1784.30 ± 167.04^{a,B}	1898.41 ± 873.20^{a,A}
SOL	Daidzein		Genistein		
Condition	Free	Bound	Free	Bound	Total
NTS	73.76 ± 0.67^{b,A}	420.12 ± 20.51^{a,B}	21.77 ± 3.11^{c,E}	27.61 ± 0.30^{c,D}	543.26 ± 190.96^{a,B}
60 ºC	38.20 ± 1.69^{c,D}	27.40 ± 0.56^{d,C}	62.28 ± 19.90^{b,C}	1760.77 ± 111.41^{a,B}	1888.65 ± 859.19^{a,A}
70 ºC	69.41 ± 16.34^{c,AB}	28.18 ± 3.23^{d,C}	105.42 ± 8.21^{b,AB}	2198.09 ± 74.96^{a,A}	2401.10 ± 1065.67^{a,A}
80 ºC	49.96 ± 1.70^{b,C}	27.18 ± 1.59^{c,C}	51.97 ± 7.96^{b,C}	1698.33 ± 53.25^{a,B}	1827.44 ± 827.71^{a,A}

* NTS; non-treated, SOI: soybean meal from oil industry, SOL: soybean meal of oil extraction from lab scale. The values are expressed as mean ± standard deviation. A different small letter in the same raw indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in same column indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

Soymilk samples	Isoflavone content (µg/g DW)				
SMI	Daidzein		Genistein		
Condition	Free	Boound	Free	Bound	Total
NTS	153.98 ± 8.79^{c,D}	318.90 ± 14.87^{b,A}	23.95 ± 5.72^{e,F}	32.97 ± 0.22^{d,D}	529.80 ± 137.71^{a,C}
60 ºC	209.92 ± 14.02^{d,C}	27.49 ± 1.54^{e,C}	297.15 ± 10.10^{c,A}	608.31 ± 139.49^{b,C}	1142.87 ± 242.64^{a,B}
70 ºC	372.44 ± 31.40^{c,B}	26.96 ± 1.30^{e,C}	311.30 ± 2.05^{d,A}	737.64 ± 76.11^{b,C}	1448.34 ± 292.13^{a,B}
80 ºC	644.22 ± 3.59^{b,A}	26.18 ± 0.27^{d,C}	53.63 ± 0.41^{c,D}	1784.30 ± 167.04^{a,A}	2508.33 ± 822.47^{a,A}
SML	Daidzein		Genistein		
Condition	Free	Bound	Free	Bound	Total
NTS	47.95 ± 0.49^{b,F}	239.39 ± 9.82^{a,B}	16.98 ± 0.39^{c,G}	23.87 ± 0.23^{c,F}	328.19 ± 105.73^{a,C}
60 ºC	99.22 ± 14.00^{c,E}	27.21 ± 1.20^{d,C}	235.64 ± 68.04^{b,B}	1209.70 ± 71.00^{a,B}	1571.77 ± 551.32^{a,B}
70 ºC	96.19 ± 19.94^{c,E}	27.15 ± 0.96^{d,C}	216.32 ± 0.20^{b,C}	1075.35 ± 70.01^{a,B}	1415.01 ± 487.37^{a,B}
80 ºC	106.69 ± 17.79^{b,E}	26.64 ± 1.35^{d,C}	51.97 ± 7.96^{c,D}	1698.33 ± 53.25^{a,A}	1883.63 ± 818.96^{a,AB}

* NTS; non-treated, SMI: soybean meal from soymilk industry and SML: soybean meal of soymilk extraction from lab scale. The values are expressed as mean ± standard deviation. A different small letter in the same raw indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments. A different capital letter in same column indicates significant difference ($p < 0.05$) compared among treatments, n = 3.

4.3.12 Identification and quantification of phytosterol contents of soybean residues by using drying treatment

The results of phytosterol contents in raw and soybean residues from the oil industry and lab-scale are presented in Table 20. Total phytosterol content of DSO was 1422.4 µg/g DW. The predominant phytosterols in DSO, SOI and SOL were beta-sitosterol, campesterol and squalene. In SOI, the phytosterol contents of all HA and FIR-HA dried samples were not detected except for 60 ºC HA dried sample. For SOL, campesterol and beta-sitosterol content of 80 ºC HA dried samples were significantly (p<0.05) higher when compared to 60 and 70 ºC HA dried samples, while beta-sitosterol content of 70 ºC FIR-HA dried sample was the highest value and followed by 60 and 80 ºC samples, respectively. Campesterol value of 80 ºC FIR-HA dried sample was significantly (p<0.05) lower when compared to 60 and 70 ºC FIR-HA dried samples. On the other hand, squalene contents of all HA dried samples were not significantly among different drying temperature. The highest value of total phytosterol was found in 80 ºC HA dried SOL while the lowest value of total phytosterol was observed in 80 ºC FIR-HA dried SOL sample. Phytosterol contents of raw and soybean residues from soy milk industry and lab-scale are presented in Table 21. For soy milk industry, Total phytosterol content of DSM was 535.17 µg/g DW. The highest value of total phytosterol was found in 60 ºC HA dried SML while the lowest value of total phytosterol was observed in 70 ºC HA dried SMI sample. Beta-sitosterol contents of all temperature FIR-HA dried SMI and SML samples were significantly (p<0.05) higher than that of other HA dried SMI and SML samples except for 60 ºC HA dried SML sample. Octacosanol contents of DSM, SMI and SML in ranging from 39.42 – 59.05 µg/g DW. In addition, the highest value of squalene was observed in 60 ºC HA SML dried sample, while the lowest value of squalene was found in 60 and 70 ºC HA SMI dried samples. The major phytosterols of DSM, SMI and SML were beta-sitosterol, campesterol, octacosanol and squalene, respectively. Our findings were in agreement with previously reported that soybean seed was rich phytosterols, namely beta-sitosterol, campesterol and stigmasterol (Yamaya, Endo, Fujimoto, & Kitamura, 2007). Moreover, health promoting of phytosterols has been studied in anti-cancer, cholesterol-lowering activity (Ostlund, 2004)

Table 20 Phytosterol contents in dry soybean and soybean residues from oil samples with drying treatments.

Samples	Oil industry				Total (µg/g DW)
	Octacosanol	Squalene	Campesterol	Beta-sitosterol	
DSO*	nd	52.86 ± 0.06^{a}	554.51 ± 37.24^{a}	840.31 ± 82.61^{a}	1422.47 ± 140.58^{a}
SOI					
HA					
60 ºC	nd	46.91 ± 0.03^{b}	nd	nd	46.91 ± 0.03^{g}
70 ºC	nd	nd	nd	nd	nd
80 ºC	nd	nd	nd	nd	nd
FIR					
60 ºC	nd	nd	nd	nd	nd
70 ºC	nd	nd	nd	nd	nd
80 ºC	nd	nd	nd	nd	nd
SOL					
HA					
60 ºC	nd	46.84 ± 0.00^{b}	35.12 ± 0.35^{d}	25.38 ± 2.34^{e}	107.36 ± 1.99^{de}
70 ºC	nd	46.86 ± 0.02^{b}	31.53 ± 0.17^{d}	35.06 ± 3.26^{d}	113.46 ± 3.40^{d}
80 ºC	nd	47.10 ± 0.00^{b}	105.15 ± 3.89^{b}	62.97 ± 1.90^{c}	215.22 ± 1.99^{b}
FIR					
60 ºC	nd	47.12 ± 0.05^{b}	50.25 ± 1.52^{c}	6.94 ± 0.46^{f}	104.33 ± 1.11^{e}
70 ºC	nd	47.18 ± 0.07^{b}	47.81 ± 3.85^{c}	94.39 ± 0.02^{b}	189.39 ± 10.16^{c}
80 ºC	nd	47.25 ± 0.09^{b}	31.86 ± 0.29^{d}	2.77 ± 0.74^{g}	81.90 ± 0.94^{f}

*DSO; Dry soybean from oil industry, SOI: soybean residue from oil industry, SOL: soybean residue of oil extraction from lab scale. The values are expressed as mean ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and treatments, n = 3. nd: not detected.

Table 21 Phytosterol contents in dry soybean and soybean residues from soymilk samples with drying treatments.

Samples	Soy milk industry				Total (µg/g DW)
	Octacosanol	Squalene	Campesterol	Beta-sitosterol	
DSM*	39.42 ± 0.02^{f}	48.81 ± 0.45^{fg}	120.16 ± 8.85^{d}	326.76 ± 24.02^{f}	535.17 ± 14.75^{ef}
SMI					
HA					
60 °C	59.05 ± 1.36^{a}	47.47 ± 0.20^{h}	27.93 ± 0.05^{g}	93.39 ± 2.45^{g}	227.85 ± 3.62^{i}
70 °C	53.03 ± 7.97^{bcd}	47.09 ± 0.02^{h}	27.93 ± 0.02^{g}	48.67 ± 3.68^{h}	176.73 ± 4.30^{j}
80 °C	55.59 ± 0.78^{abc}	49.02 ± 0.16^{fg}	62.04 ± 8.14^{f}	216.61 ± 15.92^{c}	383.26 ± 25.02^{h}
FIR					
60 °C	45.06 ± 1.49^{e}	49.86 ± 0.16^{de}	131.21 ± 1.29^{d}	509.64 ± 37.64^{c}	735.78 ± 40.28^{d}
70 °C	52.11 ± 1.19^{bcd}	49.59 ± 0.29^{ef}	60.74 ± 3.72^{f}	430.30 ± 25.44^{e}	592.75 ± 30.06^{e}
80 °C	49.99 ± 6.25^{d}	48.52 ± 0.00^{g}	58.37 ± 3.45^{f}	440.59 ± 20.02^{de}	597.48 ± 10.31^{e}
SML					
HA					
60 °C	55.80 ± 2.24^{abc}	56.94 ± 1.67^{a}	352.85 ± 14.69^{a}	831.11 ± 32.38^{a}	1296.71 ± 21.60^{a}
70 °C	51.08 ± 0.07^{cd}	50.31 ± 0.17^{de}	255.33 ± 3.70^{c}	88.50 ± 4.58^{c}	445.23 ± 8.54^{gh}
80 °C	50.58 ± 0.24^{d}	50.22 ± 0.84^{de}	91.49 ± 3.33^{e}	299.96 ± 11.42^{f}	492.26 ± 14.15^{fg}
FIR					
60 °C	56.47 ± 0.17^{ab}	50.53 ± 0.08^{d}	59.47 ± 0.54^{f}	293.24 ± 6.11^{f}	459.71 ± 6.91^{g}
70 °C	54.59 ± 0.14^{abcd}	54.89 ± 0.16^{b}	275.88 ± 3.63^{b}	780.07 ± 21.48^{b}	1165.43 ± 25.42^{b}
80 °C	58.72 ± 2.56^{a}	53.41 ± 0.05^{c}	280.66 ± 10.39^{b}	483.66 ± 5.73^{cd}	876.49 ± 18.74^{c}

*DSM; Dry soybean from soy milk industry, SMI: soybean residue from soymilk industry and SML: soybean residue of soymilk extraction from lab scale. The values are expressed as mean ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different temperature and treatments, n = 3.

4.3.13 Identification and quantification of amino acid contents of soybean residues by using drying treatment

Amino acid contents of raw and soybean residues obtained from the oil industry and lab-scale are shown in Table 22. The amino acid contents, the predominant amino acids from DSO and SOI were tryptophan, phenylalanine, leucine and methionine. The lowest values of total amino acid content were discovered in DSO, while 60 ^{o}C HA dried SOI provided the highest values of total amino acid content. The highest value of tryptophan and leucine content was found in 60 ^{o}C HA SOI dried sample. In contrast, 70 ^{o}C HA SOI dried sample was provided the highest value of methionine. Phenylalanine, the results showed that all samples of HA dried samples were significantly (p<0.05) higher than that of DSO sample. The contents of amino acid in raw and soybean residues received from soy milk industry and lab-scale are presented in Table 23. The major amino acids from DSM and SMI were leucine, phenylalanine, valine, methionine and arginine. The highest contents of total amino acid were found in 60 ^{o}C and followed by 80 ^{o}C,70 ^{o}C and DSO, respectively. Leucine, phenylalanine, threonine, isoleucine, valine, lysine and methionine contents, the results showed that all samples of HA dried samples were significantly (p<0.05) higher when compared to DSM sample. On the other hands, histidine and tryptophan contents of DSM were significantly (p<0.05) higher than that of all HA dried samples. Our results indicated that soybean residues from oil and soymilk industries were retained the source of essential amino acids. These were used to functional food for human health as according previously reported Song, Liu, Li, & Gu (2013)

Table 22 Identification of amino acid contents in dry and residues of soybean from oil industry with HA treatments by using LCMS-MS.

Amino acid contents (µg/100 g)	Oil industry			
	DSO*	SOI (HA)		
		60 °C	70 °C	80 °C
Lysine	5.92 ± 0.65^{b}	11.13 ± 1.01^{a}	10.48 ± 0.58^{a}	10.01 ± 0.32^{a}
Histidine	1.36 ± 0.72^{c}	5.22 ± 0.40^{b}	5.24 ± 0.26^{b}	6.85 ± 0.19^{a}
Leucine	45.87 ± 1.32^{c}	104.02 ± 6.82^{a}	104.04 ± 2.76^{a}	82.51 ± 6.03^{b}
Phenylalanine	77.84 ± 0.63^{b}	102.44 ± 1.41^{a}	98.25 ± 1.63^{a}	97.21 ± 0.26^{a}
Valine	15.51 ± 4.65^{c}	39.68 ± 3.17^{a}	20.62 ± 8.59^{c}	34.15 ± 1.67^{b}
Tryptophan	223.45 ± 3.10^{c}	270.04 ± 0.49^{a}	241.73 ± 6.16^{b}	242.99 ± 0.09^{b}
Arginine	43.24 ± 2.16^{c}	81.57 ± 5.46^{a}	21.24 ± 3.07^{d}	70.51 ± 1.09^{b}
Isoleucine	9.63 ± 0.62^{b}	15.42 ± 0.14^{a}	14.68 ± 0.11^{a}	13.95 ± 0.13^{a}
Methionine	39.16 ± 4.10^{d}	75.93 ± 7.20^{b}	97.33 ± 3.87^{a}	49.95 ± 4.11^{c}
Threonine	6.69 ± 1.23^{b}	13.13 ± 0.26^{a}	10.05 ± 4.65^{ab}	12.95 ± 0.09^{a}
Total	468.70 ± 10.31^{c}	718.64 ± 25.52^{a}	623.70 ± 19.51^{b}	621.12 ± 29.98^{b}

*DSO; Dry soybean from oil industry, SOI: soybean residue from oil industry, HA: hot air oven method. The values are expressed as mean ± standard deviation. A different small letter in the same row indicates significant difference ($p < 0.05$) compared with different temperature and the same treatments, n = 3.

Table 23 Identification of amino acid contents in dry and residues of soybean from soymilk industry with HA treatments by using LCMS-MS.

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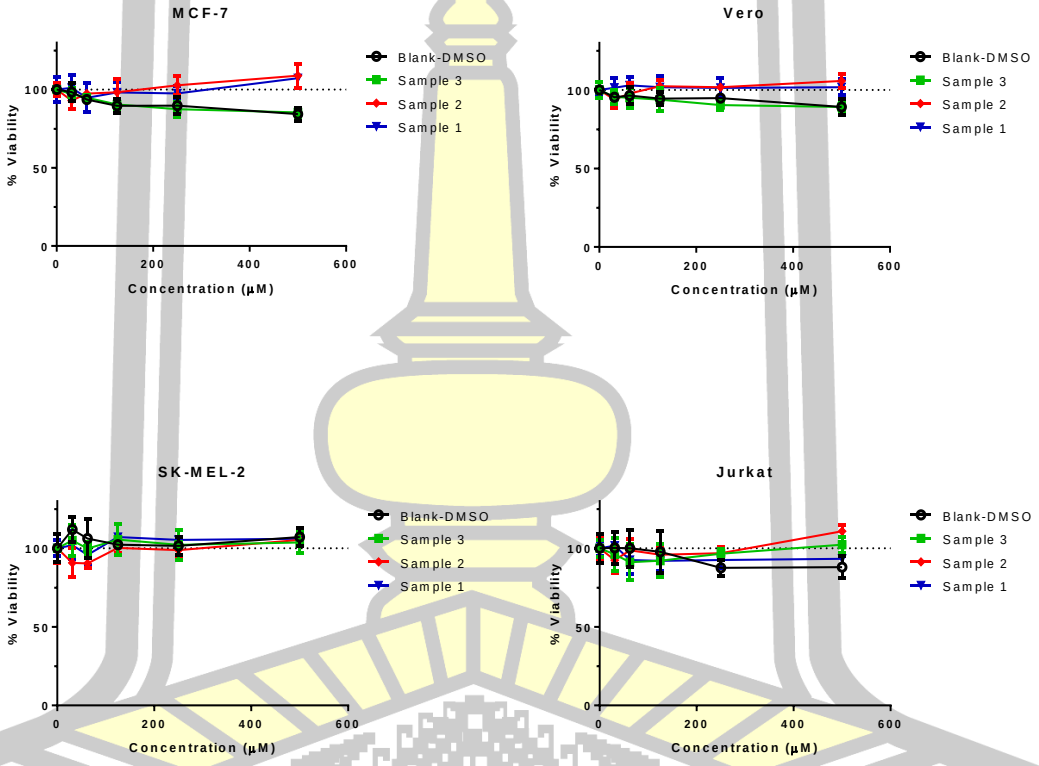
Amino acid contents (µg/100 g)	Soy milk industry			
	DSM*	SMI (HA)		
		60 °C	70 °C	80 °C
Lysine	28.25 ± 0.65^{c}	86.76 ± 2.53^{a}	79.07 ± 1.40^{b}	86.94 ± 0.04^{a}
Histidine	25.34 ± 0.40^{a}	trace	trace	trace
Leucine	207.99 ± 11.08^{c}	1026.79 ± 10.04^{a}	802.12 ± 11.70^{b}	1002.81 ± 39.86^{a}
Phenylalanine	201.38 ± 1.13^{d}	523.09 ± 3.02^{a}	402.96 ± 0.86^{c}	506.20 ± 10.31^{b}
Valine	84.45 ± 8.35^{d}	446.73 ± 4.19^{a}	287.65 ± 23.61^{c}	401.11 ± 10.25^{b}
Tryptophan	310.57 ± 0.17^{a}	34.61 ± 1.34^{b}	24.46 ± 0.26^{d}	29.38 ± 0.63^{c}
Arginine	140.65 ± 4.58^{c}	172.05 ± 0.56^{a}	123.96 ± 1.64^{d}	156.27 ± 0.44^{b}
Isoleucine	32.33 ± 0.06^{c}	69.55 ± 0.51^{a}	54.26 ± 0.28^{b}	70.37 ± 3.02^{a}
Methionine	172.13 ± 10.93^{d}	345.57 ± 1.13^{a}	243.79 ± 11.45^{c}	292.95 ± 10.35^{b}
Threonine	22.99 ± 0.17^{c}	137.24 ± 0.88^{a}	124.00 ± 3.70^{b}	131.11 ± 11.88^{ab}
Total	1226.12 ± 18.33^{d}	2842.55 ± 12.36^{a}	2142.31 ± 26.48^{c}	2677.18 ± 107.43^{b}

*DSM; Dry soybean from soymilk industry, SMI: soybean residue from soy milk industry, HA: hot air oven method. The values are expressed as mean ± standard deviation. A different small letter in the same row indicates significant difference ($p < 0.05$) by compared with different temperature and the same treatments, n = 3.

4.3.13 Soybean extracts cytotoxicity

The results of soybean extract cytotoxicity are presented in Fig 12. The anti-cancer property from soybean residues (raw, 70 ºC HA and FIR-HA of oil industry) and test by four cell lines; MCF-7 (human breast adenocarcinoma), SK-MEL-2 (human melanoma), Vero (African green monkey kidney) and Jurkat (human leukemic T-cell lymphoblast). The results showed that the cytotoxicity activities of all soybean samples by calculating on % viability in ranged from 5 -10% which indicated that the extracts from soybean residues did not inhibit on cancer cell line. However, previously studied reported that the soybean residues can be used for peptide fraction material, which was inhibited colon, liver and lung cancers (Rayaprolu, Hettiarachchy, Chen, Kannan, & Mauromostakos, 2013; Singh, Vij, & Hati, 2014)



MCF-7: Human breast adenocarcinoma, SK-MEL-2: Human melanoman, Jurkat: Human leukemic T-cell lymphoblast, Vero: African green monkey kidney, Sample 1: Raw soybean, Sample 2: 70 ºC HA dried SOI, Sample 3: 70 ºC FIR-HA dried SOI, n = 3.

Figure 12 the anti-cancer capacity of selected soybean samples.

Experiment 3 Develop products from potent soybean meal as natural food additive for functional food and evaluates the stability of bioactive compounds, antioxidant activity and sensory evaluation in the product

4.3.13 Physical property of soy products

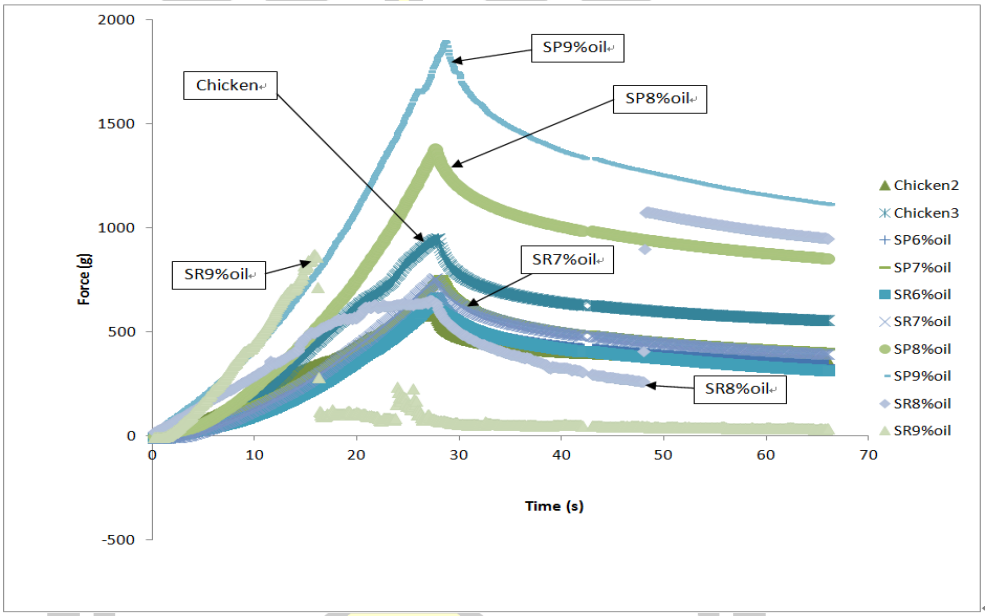


Figure 13 Texture analysis graph of cooked chicken breast and soy products. SP = soy powder, SR = soy residue.

The purpose of soy product development from soybean residue as a natural food additive for functional food and increase soybean residue value from the oil soy industry. Because researcher chose soy residue from oil industry to make soy product that it is easy to management, transporting and operating cost than soy residue from soymilk industry. Moreover, the solvent was using on oil extraction that was not detected referred from certificate of analysis. In addition, the soy product is also containing bioactive compounds with antioxidant activity, which is suitable for future food and human diets.

The results of texture analysis used for comparison of physical or rheological property, the product development from soy powder and soy residue want to nearly meat texture. In this case, using cooked chicken breast to a standard or control sample. For soy products, products have two samples, made from soy powder (SP) and soy residue (SR) which different from % oil (6, 7, 8 and 9%) (Fig 13) with containing alginate powder, used for emulsifier and stabilizer. The result showed that force values (hardness) of cooked chicken breast ranged from 550 – 946 g which depended on part of chicken breast, while sample force values ranged from 641 – 1897 g when % oil was increased. Force values of SP samples (8 and 9% oil) were significantly (p<0.05) higher when compared to SR samples, which were indicated that the texture from SP was hard than SR. However, soy force value samples were nearly cooked chicken breast force value which was possible to accept. Additionally, other texture values such as cohesiveness, springiness, gumminess, and chewiness are

presented in Table 24. The results showed that springiness, gumminess, and chewiness values of SP sample was not significantly (p<0.05) different when compared to SR sample except for cohesiveness value.

For the color of soy products are shown in Table 25. L* value indicates that dark or white color; if L* value is nearly zero score; the sample color is nearly dark color too. For a* value, it is present to green or red color: if a* value is a negative score, the sample color is nearly green while a* value is a positive score. The sample color is nearly red color too. For b* value, if b* value is a negative score which is a blue color, and b* value is a positive color which is a yellow color. The results indicated that the SP sample is more white color than that of SR sample.

Table 24 Texture analysis values of soy product samples.

Samples	Cohesiveness (N)	Springiness (mm)	Gumminess (N)	Chewiness (mj)
SP	0.34 ± 0.04^{B}	1.00 ± 0.00^{A}	20.13 ± 3.72^{A}	20.13 ± 3.72^{A}
SR	0.39 ± 0.02^{A}	1.00 ± 0.00^{A}	21.65 ± 1.17^{A}	21.65 ± 1.17^{A}

The values are expressed as mean ± standard deviation. A different capital letter in same column indicates significant difference (p < 0.05) compared with different samples, n = 3.

Table 25 Color measuring values of soy product samples.

Samples	L*	a*	b*
SP	76.04 ± 0.50^{A}	-3.00 ± 0.03^{A}	18.84 ± 0.16^{B}
SR	70.25 ± 0.63^{B}	-0.96 ± 0.07^{B}	21.43 ± 0.23^{A}

The values are expressed as mean ± standard deviation. A different capital letter in same column indicates significant difference (p < 0.05) compared with different samples, n = 10.

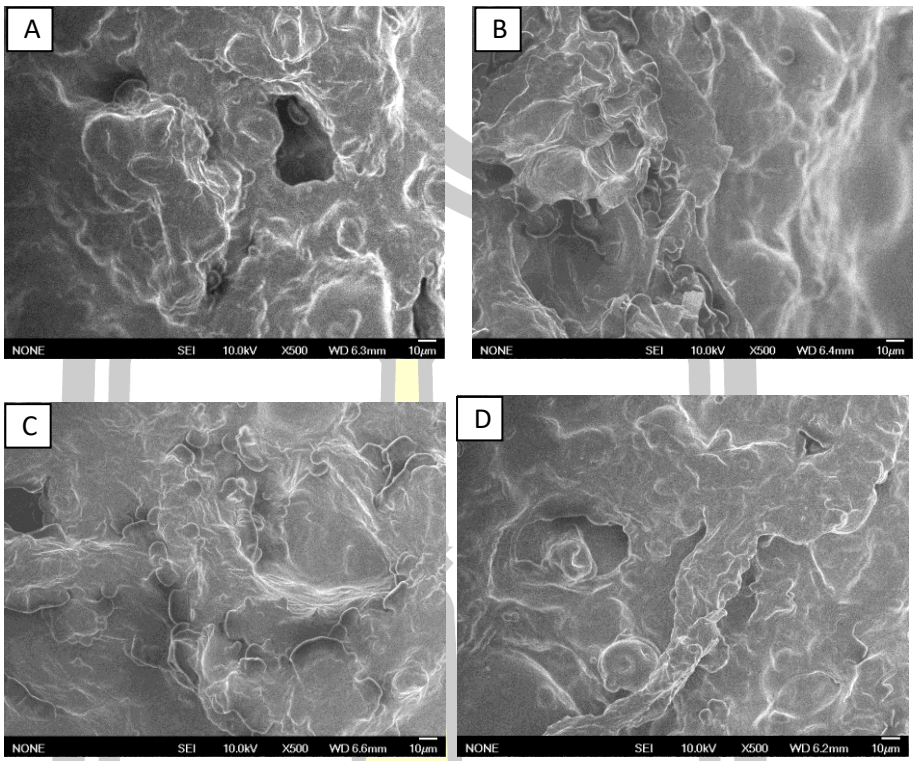


Figure 14 Micrographs illustrating microstructures formed by protein-polysaccharide interaction in the mixture or complex systems of soy products. A: SEM image of soybean residue with 8% oil, scale bar 10 µm, B: SEM image of soybean residue with 9% oil, scale bar 10 µm, C: SEM image of soybean protein with 8% oil, scale bar 10 µm, D: SEM image of soybean residue with 9% oil, scale bar 10 µm.

The images of microstructures formed of soy products (Fig 14) indicated that proteins and polysaccharides interact with oil-water interfaces improving the physical stability of emulsion by gel setting from alginate and heat-treated protein (Wijaya, Patel, Setiowati, & Meeren, 2017). The results showed that microstructures of soy product made from soybean residue is careless structure while the microstructures from soybean protein look too dense. The different microstructures of soy products are different on raw materials; soy protein was planted extract protein from soybean which was highly purified protein while soybean residue was defatted only. Soy protein isolate is often used in many food ingredients for improving; texture, emulsion, foam setting, gel setting and water binding capacity (Wijaya et al., 2017).

4.3.14 Sensory evaluation in soy products

Sensory values are presented in Table 26. Sensory score between SP and SR samples found that appearance and color scores were not significantly (p<0.05) different. In contrast, odor, flavor, texture and overall scores of SR sample were significantly higher when compared with the SP sample. These scores were indicated that soy product made from soy residue was good and acceptable from consumers which could be developed to human diets for future food.

Table 26 Sensory values of soy products by using 7-hedonic scales.

Samples	Appearance	Color	Odor	Flavor	Texture	Overall
SP	5.07 ± 0.52^{A}	5.00 ± 0.53^{A}	3.87 ± 0.43^{B}	4.67 ± 0.71^{B}	$4.23 \pm 0.43^{a,B}$	$4.50 \pm 0.51^{a,B}$
SR	5.20 ± 0.41^{A}	5.47 ± 0.51^{A}	5.83 ± 0.59^{A}	5.83 ± 0.53^{A}	$6.40 \pm 0.50^{a,B}$	$6.23 \pm 0.43^{a,B}$

1 = very dislike, 2 = dislike, 3 = little dislike, 4 = quietly, 5 = little like, 6 = like, 7 = very like
The values are expressed as mean ± standard deviation. A different capital letter in same column indicates significant difference ($p < 0.05$) compared with different samples, n = 30.

4.3.15 Chemical property of soy products

Table 27 Total phenolic and flavonoid contents of soy products.

Samples	TPC (free) (mg GAE/g DW)	TPC (bound) (mg GAE/g DW)	Total TPC (mg GAE/g DW)
SP	$14.98 \pm 0.46^{b,B}$	$170.81 \pm 4.63^{a,A}$	185.80 ± 5.09^{a}
SR	$19.08 \pm 0.32^{a,B}$	$123.47 \pm 5.55^{b,A}$	142.55 ± 5.85^{b}
	TFC (free) (mg RE/g DW)	TFC (bound) (mg RE/g DW)	Total TFC (mg RE/g DW)
SP	$0.37 \pm 0.01^{b,A}$	$0.23 \pm 0.01^{a,B}$	0.61 ± 0.09^{b}
SR	$0.87 \pm 0.01^{a,A}$	$0.21 \pm 0.01^{a,B}$	1.08 ± 0.46^{a}

The values are expressed as mean ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different samples. A different capital letter in same row indicates significant difference ($p < 0.05$) compared between free and bound, n = 3.

Total phenolic and flavonoid content of soy product is presented in Table 27. Total phenolic content of SP sample was significantly ($p<0.05$) higher than that of SR sample. The highest phenolic fraction was observed in bound form of both soy product samples. On the other hand, the highest total flavonoid value was found in SR sample.

Table 28 Antioxidant activities of soy products.

Samples	DPPH (free) (% inhibition)	DPPH (bound) (% inhibition)	
SP	$14.49 \pm 0.37^{a,B}$	$85.41 \pm 0.20^{a,A}$	
SR	$14.66 \pm 0.05^{a,B}$	$79.92 \pm 0.05^{b,A}$	
	FRAP (free) (mmol $FeSO_4$/g DW)	FRAP (bound) (mmol $FeSO_4$/g DW)	Total FRAP (mmol $FeSO_4$/g DW)
SP	$0.22 \pm 0.01^{b,A}$	$0.21 \pm 0.01^{b,A}$	0.43 ± 0.01^{b}
SR	$0.29 \pm 0.01^{a,B}$	$0.39 \pm 0.02^{a,A}$	0.68 ± 0.02^{a}

The values are expressed as mean ± standard deviation. A different small letter in the same column indicates significant difference ($p < 0.05$) compared with different samples. A different capital letter in same row indicates significant difference ($p < 0.05$) compared between free and bound, n = 3.

The antioxidant activities of soy products are shown in Table 28. The results indicated that soy products were effectively on hydrogen donation property which was observed in the bound fraction of both soy product samples. The highest % inhibition DPPH value was observed in bound SP sample, while the highest FRAP value was found in SR sample. These results showed that soy product from soy residue can be utilized as a food additive or food ingredient for functional food in future trend.

Isoflavone contents of soy product samples are presented in Table 29. The results found that total isoflavone content of SR sample was significantly ($p < 0.05$) higher than that of the SP sample. In addition, soy product by using soy residue and soy powder which contained free and bound isoflavones, especially genistein.

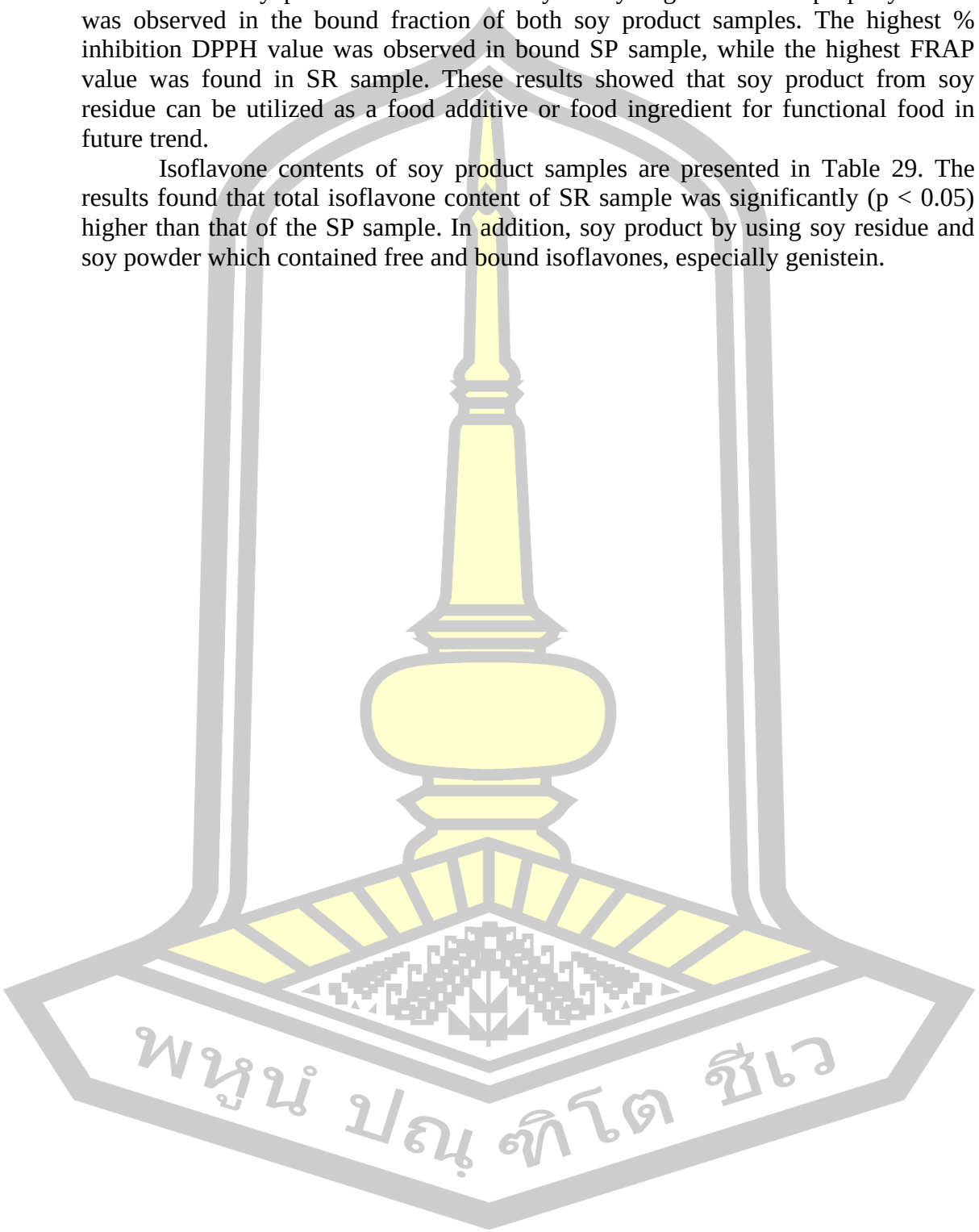


Table 29 Isoflavone contents of soy product samples.

Soy products	Isoflavone contents (µg/g DW)				
	Daidzein		Genistein		
	Free	Bound	Free	Bound	Total
SP	nd	$25.74 \pm 0.01^{a,C}$	$20.69 \pm 0.42^{a,D}$	$40.25 \pm 0.21^{b,B}$	$86.65 \pm 0.64^{b,A}$
SR	nd	$25.84 \pm 0.01^{a,C}$	$18.36 \pm 0.36^{b,D}$	$50.54 \pm 0.33^{a,B}$	$90.31 \pm 0.80^{a,A}$

The values are expressed as mean ± standard deviation. A different capital letter in same row indicates significant difference ($p < 0.05$) compared with different samples. A different small letter in same column indicates significant difference ($p < 0.05$) compared with different samples, n = 3, nd = not detected.

CHAPTER 5

CONCLUSIONS

The results of soybean and soybean residues are indicated that soybean residues from oil and soymilk industries are a rich source of bioactive compounds, namely phenolic acid, flavonoids (rutin, daidzein and genistein) of free and bound form, phytosterols and essential amino acids with retained the antioxidant capacity.

For drying treatments including hot air oven (HA) and far-irradiation combined with a hot air oven (FIR-HA) with different temperature levels. The results showed that total phenolic contents of oil and soymilk residues were significantly (p<0.05) different higher when compared with non-treatment samples (NTS). Flavonoid content, drying method can be increased daidzein contents by HA and genistein content by FIR-HA, respectively.

In overall of antioxidant activities were evaluated by DPPH and FRAP assays; the results found that bound phenolic extracted from oil and soymilk residues were exhibited in DPPH radical scavenging. In contrast, the greatest FRAP values were observed in free phenolic extracted of both residues. Additionally, anti-proliferation capacity of selected soybean samples was shown in % viability in ranged from 5 - 10%. However, soybean residues were effective in free radical scavenging and metal chelation.

For product development of soy residue from SOI, texturized protein (TP) was made. Physical quality including color, texture was determined. The sensory evaluation of the product indicated that TP was highly accepted (6.2/7 hedonic scale) from the consumers. In addition, the bioactive compounds and its antioxidant activity were comparable to the unprocessed soy residues.

Our findings have demonstrated that bioactive compounds and antioxidant activities of oil and soymilk residues are suitable for the food ingredient or food additive add in food to functional food.

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