CHAPTER 2

Literature reviews

2.1 Soy isoflavones

2.1.1 Soybean

The soybean (U.S.A.) or soya bean (U.K.) (*Glycine max* (L.) Merrill) is a species of legume native to East Asia. Soybean is an annual plant that has been used in Asia as a food. Soybean contains essential amino acids for humans so it is a good source of protein. Moreover, soybean also contains significant amounts of fatty acid and the isoflavones. The structure of soybean seed (Figure 2.1) consists of hull (seed coat) and embryo. The seed coat is marked with a hilum and it protects embryo from bacteria and fungi infection. The embryo contains two pieces of cotyledons, which is food reserve structures, and three parts of hypocotyl, radicle and epicotyl. The hypocotyl and radicle, that known as embryonic axis or germ, are located under seed coat at end of hilum. The epicotyl is very small and tucked between cotyledons (Liu, 2012).

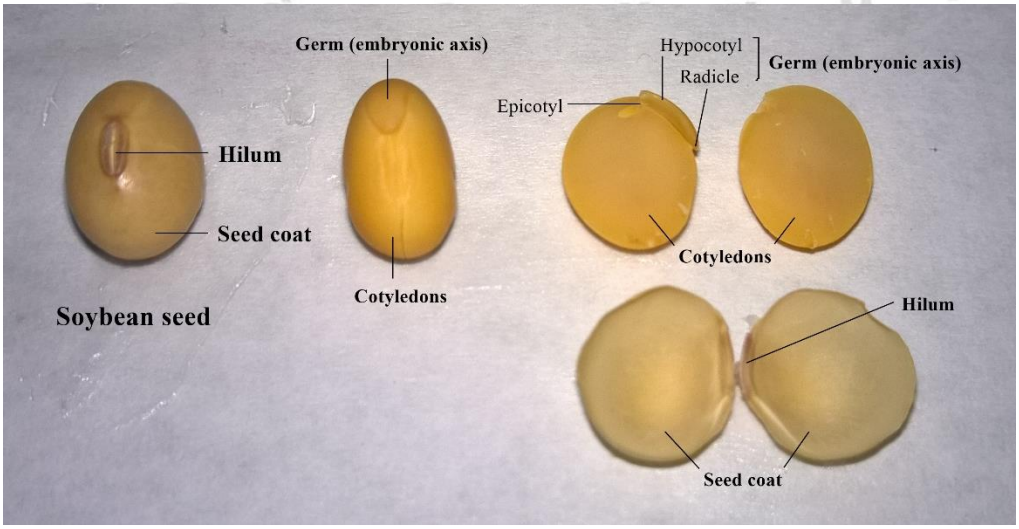


Figure 2.1 Structure of soybean seed.

2.1.2 Isoflavones

Isoflavones are classified as flavonoids which can be found in soybean and soybean products (Table 2.1). Their structure and function are similar to women hormone namely estrogen which will normally decline due to aging. Isoflavones have twelve isomers and can be classified into two main forms including glucosides and aglycones. The glucoside form has nine isomers which are acetyldaidzin, acetylgenistin, acetylglycitin, daidzin, genistin, glycitin, malonyldaidzin, malonylgenistin and malonylglycitin. The aglycone form has three isomers which are daidzein, genistein and glycitein (Liu, 2004; Shimoni, 2004). The chemical structures of isoflavone and estrogen hormone are shown in Figure 2.2. The quantity and the structure of isoflavones in soybeans and soybean products depend on soy's variety (Lee *et al.*, 2003a; Lee *et al.*, 2003b; Lee *et al.*, 2007; Wang and Murphy, 1994), cultivation (Hoeck *et al.*, 2000; Lee *et al.*, 2003a; Wang and Murphy, 1994), processing (Jackson *et al.*, 2002; Kao *et al.*, 2004; Lee *et al.*, 2007) and storage products (Lee *et al.*, 2003a).

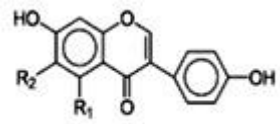
From the study, glucoside form is less estrogenic activity and poorly absorbed in the small intestine comparing to aglycone form (Choi *et al.*, 2002; Setchell, 2000). The glucoside form can be hydrolyzed to aglycones by β-glucosidase from colon microflora (Izumi *et al.*, 2000). However, the metabolism of isoflavones is limited depending on the individual differences, function of diet, medicine intake and time of food digested in the intestine (Setchell, 1998). However, consuming the product containing aglycone forms will help the body absorb aglycone in the small intestine and the body can immediately use the aglycone. This makes the isoflavone aglycones widely studied so as to be applied in medicals, pharmaceuticals and supplement foods.

Table 2.1 Isoflavone in food products

Product	Isoflavone (mg/100g of food)
Soybean	177.89
Roasted soybean	162.50
Soy protein concentrate, aqueous wash	102.07
Soy protein concentrate produced by alcohol extraction	12.47
Soy protein isolate	97.43
Texture vegetable protein	138.20
Dried soybean flour sheet	193.88
Boiled soybean flour sheet	50.70
Green soybean	135.40
Miso soup	42.55
Soy cheddar	7.15
Soy drink	7.01
Soy milk	0.56

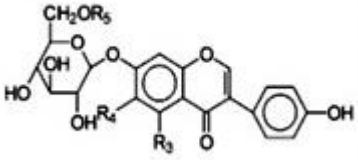
Sources: U.S. Government (2011); Pongsatha (2011)

Aglycone forms



R_1	R_2	Compound
H	H	Daidzein
OH	H	Genistein
H	OCH_3	Glycitein

Glucoside forms



R_3	R_4	R_5	Compound
H	H	H	Daidzin
OH	H	H	Genistin
H	OCH_3	H	Glycitin
H	H	$COCH_3$	Acetyldaidzin
OH	H	$COCH_3$	Acetylgenistin
H	OCH_3	$COCH_3$	Acetylglycitin
H	H	$COCH_2COOH$	Malonyldaidzin
OH	H	$COCH_2COOH$	Malonylgenistin
H	OCH_3	$COCH_2COOH$	Malonylglycitin

Estrogen (Estradiol)

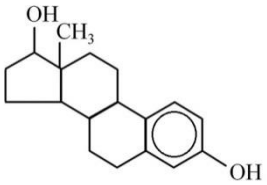


Figure 2.2 Chemical structure of 12 isoflavone isomer and estrogen hormone (Shimoni, 2004).

Generally, isoflavones in soybean seed appear in the form of glucosides whereas the amount of aglycone forms is less. When soybean is fermented, aglycone forms increases because the mircroorganism produce β-glucosidase to hydrolize β-glycosidic linkage in glucoside form. As a result of this process fermented soybean products contains more aglycone forms than nonfermented soybean (Table 2.2). In addition, there are many reports of the deglycosylation of isoflavone glycosides during soybean fermentation by bacterium such as *Bacillus subtilis*, *B. pumilus*, *B. licheniformis*, *B. circulans*, *B. firmus* and *B. megaterium* (Table 2.3).

Table 2.2 Isoflavone aglycones and isoflavone glucosides contents in soybean products and fermented soybean products

Product	Isoflavone aglycones (µg/g)			Isoflavone glucosides (µg/g)						Reference
	DEN	GEN	GLEN	DIN	GIN	GLIN	MDN	MGN	MGLN	
Soybean	ND	ND	ND	1294	966	206	2,510	2,755	200	Kudou *et al.* (1991)
Soy milk	18	19	10	410	710	65	690	871	39	Song *et al.* (1998)
Tofu	46	52	8	25	84	12	159	108	ND	Wang and Murphy (1994)
Miso	135.8	210	19	81	162	6	ND	ND	ND	Klump *et al.* (2001)
Tempeh	137	193	24	2	65	14	255	164	ND	Wang and Murphy (1994)
Thua nao	195	70	32	68	84	191	NT	NT	NT	Dajanta *et al.* (2009)
Chungkukjang	38	30	113	791	866	103	ND	219	ND	Lee *et al.* (2007)

Note: DEN = Daidzein DIN = Daidzin MDN = Malonyldaidzin
GEN = Genistein GIN = Genistin MGN = Malonylgenistin
GLEN = Glycitein GLIN = Glycitin MGLIN = Malonylglycitin
ND = Not detected NT = No data

Table 2.3 Predominant bacteria in fermented soybeans

Fermented soybeans	Bacteria	References
Thua nao	*B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. megaterium*, *B. cereus*	Sundhagul *et al.* (1972); Leejeerajumnean (2003); Chukeatirote *et al.* (2006)
Natto	*B. subtilis* (natto)	Kiuchi and Watanabe (2004)
Kinema	*B. subtilis*, *B. licheniformis*, *B. cereus*, *B. circulans*, *B. thruingensis*, *B. sphaericus*	Tamang (1993); Sarkar *et al.* (1994); Nout *et al.* (1998); Sarkar *et al.* (2002)
Soy daddawa or dawadawa	*B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. megaterium*, *B. cereus*, *B.firmus*	Jideani and Okeke (1991); Omafuvbe *et al.* (2000); Dike and Odunfa (2003); Dakwa *et al.* (2005)
Chungkukjang	*B. subtilis*	Kwak *et al.* (2007)
Doenjang	*B. siamensis*, *B. licheniformis*	Jeong *et al.* (2014)

Source: Modified from Dajanta (2010).

2.1.3 Health impact of isoflavones

According to the study, isoflavones can reduce hot flash during menopause period in woman (Albertazzi *et al.*, 1998; Eden, 1998), it can also prevent breast cancer and prostate cancer (Jung *et al.*, 2006; Nishio *et al.*, 2007). Moreover, it has antimutagenic effect (Park *et al.*, 2003), antihypertensive effect (Okamoto *et al.*, 1995) and antidiabetic effect (Liu *et al.*, 2006). It can reduce the risk of cardiovascular diseases (Bingham *et al.*, 1998; Park *et al.*, 2003; Potter *et al.*, 1998) and decrease the risk of osteoporosis. Other benefit of isoflavones is to improve bone health (Anderson and Garner, 1997; Ikeda *et al.*, 2006; Ishimi *et al.*, 2002).

2.1.3.1 Estrogenicity of isoflavones

During the menopause period in woman, the level of estrogen hormone in blood is changing which may lead to not only heart disease but also osteoporosis in women (American Heart Association, 2007). Other symptoms of the menopause period are shown as hot flash, night sweat, sleepless, vaginal dryness or headache. The changing of estrogen hormone level affects the total parts of the body. Since the body organs occupy two types of estrogen receptors known as alpha (ER-A) and beta (ER-B). ER-A is most found in kidney, uterus and pituitary gland while ER-B is found in the same amount or even more in ovary and brain. Estrogen receptors are also found in blood vascular system and bones, it is then important. It plays important role on women health and body tissue. Therefore the reduction of estrogen hormone level in menopause period can lead to the risk of cardiovascular disease and osteoporosis (Kuiper *et al.*, 1997).

Soy isoflavone has weak estrogenicity toward animal and human (Knight *et al.*, 1996). It is thus interesting for researchers who seek for other choices to reduce health problems in women during the menopause period. According to the comparison study, it is claimed that women menopause period, the consumption isoflavone can enhance weak estrogenicity. However the amount of estrogenicity depends on the number of estrogen receptors.

The women during the menopause period hot flash symptom are divided into two groups: The former group consume soygerm while the latter (controlled group) do not have soygerm. It was found that among the former group who consumed soygerm, the hot flash decrease (44%) while the controlled group who do not have soygerm the hot flash decrease only a little (10%) (Dalais *et al.*, 1998). This result is relevant to Han *et al.* (2002) claiming that women who take isoflavone 100 mg/capsule during the menopause period, the hot flash reduce within 4 months.

2.1.3.2 Prevention of breast cancer and prostate cancer

Based on the study of the Asian population who normally consume more soybean products than the Westerners; it was found that Asian women have 5 times less risk of breast cancer than the Western women. Similarly, the Asian men have 20 times less risk of prostate cancer than Western men. Thus, researchers believe that isoflavone in soybean might reduce prostate cancer by intervening testosterone activity. It might inhibit the growth of cancer cells by decreasing the production of Testosterone hormone (Hargreaves *et al.*, 1999).

2.1.3.3 Reduction of cardiovascular diseases

Women in reproductive age have normally lower risk for coronary artery disease than men. Therefore after menopause period, women or men have equal risk ratio for this disease (American Heart Association, 2007). Hormone replacement therapy (HRT) help control the LDL cholesterol level in the elderly women and also reduce the risk of cardiovascular diseases. There is a study supporting that consuming soy protein which contains genistein can reduce the cholesterol in blood vessels and can be advantageous for heart health (Anderson *et al.*, 1995; Anthony *et al.*, 1996).

2.1.3.4 Prevention of heart diseases

Isoflavone is an antioxidant substance, it is thus prevent oxidation reaction of LDL cholesterol and then it lowers the level of cholesterol. On the contrary, it raises the healthy cholesterol known as HDL (High Density Lipo protein). Phytoestrogen in soybean can stop phytooxidation reaction in LDL cholesterol and can prevent blood clotting in artery wall which causes heart disease. Moreover, phytoestrogen in soybean can increase elasticity of the artery as well (American Heart Association, 2007).

2.1.3.5 Reduction the risk of osteoporosis

Osteoporosis mostly occurs in menopause period of women. This period starts at the age of around 50 up. This age is considered the risk of osteoporosis due to the lack of estrogen hormone. The bone density decreases rapidly and becomes lower as the age increases. Some women who face the menopause period at the earlier

age may lose the bone density more quickly about 1-3% a year especially in their first year of menopause period. When getting older, the loss of bone density is about 0.7 – 1.0% a year. Applying estrogen hormone is one of the best ways to not only prevent the loss of bone density which is the cause of osteoporosis but also prevent back bone fracture. This application includes the menopause period women; the risk of osteoporosis reduces up to 50%. Daidzein is similar to the medicine used to treat osteoporosis in menopause period women; the medicine is known as ipriflavones which is metabolized as daidzein in the human body. Therefore daidzein can inhibit the loss of bone density.

However, researchers still concern about the amount of fermented soybean product consumption. Overdose consumption means the body gains too much isoflavones which might cause side effects on the body. There are many studies on the safety dose of isoflavones. According to the experiment on 28 women during reproductive age receiving isoflavone aglycones 45 mg capsule per day and 14 days continually. The result shows that there are no significant side effects (Hargreaves *et al.*, 1999). Based on another study, 177 recovered from breast cancer patients, who were given soy isoflavones 150 mg. tablets per day plus 4 weeks continually, it was found that there are no significant side effects on endocrine system and mammary gland tissue (Quella *et al.*, 2000).

Based on the above study, the amount of fermented soybean product consumption provides advantages and safety to all consumers at all ages. Nevertheless, there are still no dietary reference intakes (DRI) for isoflavones consumption. Researchers confirm the recommended intake of isoflavones is 20-50 mg/day (Setchell and Cassidy, 1999).

2.2 The *Bacillus* species

The genus *Bacillus* was created in 1872 by Ferdinand Cohn, who renamed *Vibrio subtilil* to *Bacillus subtilis* (Gordon, 1981). The organism was a charter member of a large and diverse genus initiated by Cohn and is a part of the family *Bacillaceae*. Members of the genus *Bacillus* are characterized as Gram-positive, rod-shaped and aerobic or facultative bacteria. This family's distinguishing feature is a production of endospores, which are round, oval, or cylindrical and highly refractile structure formed within the bacterial cells (Slepecky and Hemphill, 2006). The *Bacillus* species are not difficult to isolate. Natural habitats of some *Bacillus* species are shown in Table 2.4. To identify the strain of *Bacillus* species, a sequence of tests according to Table 2.5 can be used.

Table 2.4 Origins of isolates of *Bacillus* species

Name of Bacillus species	Habitats from which isolated
B. subtilis	Soil, water
B. acidiproducens	Vineyard soil
B. alcalophilus	pH 10 enrichment from soil
B. aminovorans	Soil
B. anthracis	Anthrax-diseased animals
B. azotoformans	Soil
B. badius	Feces, foods, marine sources
B. bataviensis	Soil
B. cereus	Soil, foods
B. cibi	Fermented seafood
B. circulans	Soil
B. coagulans	Acid foods
B. cirroflagellosus	Marin mud
B. drentensis	Soil
B. endophyticus	Cotton plants tissue
B. fastidiosus	Soil, poultry litter
B. firmus	Soil, salt marshes
B. galactosidilyticus	Raw milk, infant bile
B. gelatini	Gelatin production plants
B. halmapalus	Soil
B. halodurans	Soil
B. hwajinpoensis	Sea water
B. indicus	Sand
B. isabeliae	Sea salt evaporation pond
B. jeotgali	Fermented seafood
B. korlensis	Sand, soil
B. krulwichiae	Soil
B. laterosporus	Soil, water
B. lentus	Soil, foods
B. licheniformis	Soil
B. marinus	Marine sediment
B. megaterium	Soil
B. mycoides	Soil
B. niabensis	Cotton-waste composts
B. novalis	Soil
B. oshimensis	Soil
B. pumilus	Soil
B. ruris	Raw milk
B. schlegelii	Lake sediment, sugar sludge
B. sphaericus	Soil, water sediments, foods
B. thuringiensis	Soil, foods
B. vietnamensis	Fish sauce
B. weihenstephanesis	Pasteurized milk

Sources: Based on Claus and Berkeley (1986); Slepecky and Hemphill (2006); Logan and Vos (2009)

Table 2.5 Simplified key for the tentative identification of typical strains of *Bacillus* species

1. Catalase:	positive 2	
	negative..... 17	
2. Voges-Proskauer:	positive 3	
	negative 10	
3. Growth in anaerobic agar:	positive..... 4	
	negative 9	
4. Growth at 50 °C:	positive 5	
	negative 6	
5. Growth in 7% NaCl:	positive	*B. licheniformis*
	negative	*B. coagulans*
6. Acid and gas from glucose (inorganic N):	positive.....	*B. polymyxa*
	negative..... 7	
7. Reduction of NO_3 to NO_2:	positive..... 8	
	negative	*B. alvei*
8. Parasporal body in sporangium:	positive	*B. thuringiensis*
	negative	*B. cereus*
9. Hydrolysis of starch:	positive	*B. subtilis*
	negative.....	*B. pumilus*
10. Growth at 65 °C:	positive	*B. stearothermophilus*
	negative 11	
11. Hydrolysis of starch:	positive 12	
	negative..... 15	
12. Acid and gas from glucose (inorganic N):	positive.....	*B. macerans*
	negative 13	
13. Width of rod 1.0 µm or greater:	positive	*B. megaterium*
	negative..... 14	
14. pH in V-P broth <6.0	positive	*B. circulans*
	negative	*B. firmus*
15. Growth in anaerobic agar:	positive.....	*B. laterosporus*
	negative 16	
16. Acid from glucose (inorganic N):	positive	*B. brevis*
	negative	*B. sphaericus*
17. Growth at 65 °C:	positive	*B. stearothermophilus*
	negative 18	
18. Decomposition of casein:	positive.....	*B. larvae*
	negative..... 19	
19. Parasporal body in sporangium:	positive	*B. popilliae*
	negative	*B. lentimorbus*

Note: Numbers on the right indicate the number (on the left) of the next test to be applied until the right-hand number is replaced by a species name.

Source: Norris *et al.* (1981); Logan and Vos (2009)

2.3 Solid-state fermentation

Fermentation is a biological conversion of a complex substrate into simple compounds by various microorganisms such as bacteria and fungi. In the course of this metabolic breakdown, they also release several additional compounds apart from the usual products of fermentation, such as carbon dioxide and alcohol.

Solid-state fermentation is a fermentation of solid substrate at low moisture level or water activity; however, the substrate must possess enough moisture to support growth and metabolism of the microorganism. The water content of a solid mash in solid-state fermentation often varies between 40% and 80% (Shuler and Kargi, 2002). The low moisture content means that the fermentation can only be carried out by limited number of microorganisms. Although, some bacteria is used in the solid-state fermentation, the process is mainly accomplished by yeasts and fungi (Pandey *et al.*, 2000). Examples of some solid-state fermentation processes for each category of microorganisms are reported in Table 2.6. Solid-state fermentation offers numerous advantages for the production of bulk chemicals, enzymes, flavours, colourants and other substances in food industry (Feron *et al.*, 1996; Hesseltine, 1977; Johns and Stuart, 1991; Pandey *et al.*, 1999; Soccol *et al.*, 1994; Tsuchiya *et al.*, 1994).

Generally, substrate with smaller particles provides a larger surface area for microbial attachment and growth, but too small particles may result in an agglomeration of the substrate as well as poor growth. In contrast, larger particles provide better aeration but limited surface area for microbial attachment. Therefore, a suitable particle size must be selected to provide optimal condition for each particular process (Pandey *et al.*, 1999).

Table 2.6 Main groups of microorganisms involved in solid-state fermentation processes

Microflora	**Solid-state fermentation process**
Bacteria	
Bacillus sp.	Composting, natto, amylase
Pseudomonas sp.	Composting
Serratia sp.	Composting
Streptococcus sp.	Composting
Lactobacillus sp.	Ensiling, food
Clostridium sp.	Ensiling, food
Yeast	
Endomicopsis burtonii	Tape cassava, rice
Saccharomyces cerevisiae	Food, ethanol
Schwanniomyces castelli	Ethanol, amylase
Fungi	
Altemaria sp.	Composting
Aspergillus sp.	Composting, industrial, food
Fusarium sp.	Composting, gibberellins
Monilia sp.	Composting
Mucor sp.	Composting, food, enzyme
Rhizopus sp.	Composting, food, enzymes, organic acids
Phanerochaete chrysosporium	Composting, lignin degradation
Trichoderma sp.	Composting, biological control, bioinsecticide
Beauveria sp., *Metharizium* sp.	Biological control, bioinsecticide
Amylomyces rouxii	Tape cassava, rice
Aspergillus oryzae	Koji, food, citric acid
Rhizopus oligosporus	Tempeh, soybean, amylase, lipase
Aspergillus niger	Feed, proteins, amylase, citric acid
Pleurotus oestreatus, sajor-caju	Mushroom
Lentinus edodes	Shii-take mushroom
Penicilium notatum, roquefortii	Penicillin, cheese

Sources: Raimbault (1998) and Couto and Sanromán (2006)

2.4 Extraction of isoflavones

The principle of solvent extraction is the use optimum solvent to separate the wanted substances from mixed substances. Each wanted substance has solubility in different solvent. The extraction can be divided into 3 methods as following

1) Solid/Liquid Extraction is the use of optimum solvent to separate the wanted substances from solid substance.

2) Liquid/Liquid Extraction is the use of optimum solvent to separate the wanted substances from liquid substance.

3) Acid/Base Extraction is the use of acid/base reaction to separate organic compound which has property as acid and base.

Solid/Liquid extraction can be suitably use in the extraction of isoflavones from soygerm since isoflavones have twelve isomers (glucoside forms: acetyldaidzin, acetylgenistin, acetylglycitin, daidzin, genistin, glycitin, malonyldaidzin, malonylgenistin and malonylglycitin; aglycone forms: daidzein, genistein and glycitein). Each isomer has different polarity. Aglycone forms have higher polarity than glucoside forms. As a result, extracting with solvent that holds different polarity will affect the extraction each isomer of isoflavone from raw material. There are many different types of solvent used in isoflavone extraction for instance, methanol, ethanol, acetone and acetonitrile. There are many studies reported about isoflavones extraction by solvent extraction such as applying 80% methanol and 60% acetone could extract the highest isoflavones content from soygerm capsule (Micke *et al.*, 2006). Murphy *et al.* (2002) found that acetonitrile was the best solvent used for isoflavones extraction from soybean food product.

Jankowiak *et al.* (2014) conducted a study about isoflavones extraction from soybean using water. The result was compared with other solvents as well. It was found that extracting using water provided lower isoflavone amount than extracting with mixed ethanol and water. The ratio of 70% ethanol and soybean meal at 20:1 (v/w), 20 ^{o}C shown 47% of total isoflavones content and mostly found malonyl glucoside while glucoside

would be efficiently extracted when the solvent and solid ratio at 40:1 (v/w) and aglycone would be highly extracted on condition that more solvent was applied.

Ethanol is considered one of the most popular solvent used for extracting various substances especially isoflavones (Achouri *et al.*, 2005; Chang and Chang, 2007; Rostagno *et al.*, 2009). According to Bae *et al.* (2005) isoflavones extraction from soygerm using 80% ethanol, 30 ^{o}C for 2 h, could extract the most isoflavones.

Besides solvent, other important factors relevant to the efficient extraction of isoflavones are time, temperature, process and solid content. Simple isoflavones extraction applying mixing and shaking solvent with any solid substance, for 2 hours will gain low isoflavones content (Murphy *et al.*, 2002) Thus, different techniques should be used so as to obtain higher amount of isoflavones content. According to Y. Wu *et al.* (2012) a proper condition for extracting isoflavones from soybean meal by employing ultrasound with ethanol claimed that ethanol concentration, ethanol-soybean meal ratio and extraction time would affect the total isoflavones. The study confirmed that 80% ethanol, 12.81 of ethanol-soybean meal ratio and 55 minutes of extraction time is the most effective method for isoflavones extraction. Furthermore, microwave was applied in another extracting method. Isoflavones extraction using microwave extractor was higher that using the simple method (Song *et al.*, 2007). The finding was relevant to Terigar *et al.* (2010) claiming that isoflavones extraction through microwave extractor under temperature and time varied; the most appropriate condition was 73 ^{o}C for 8 minutes. When applying ethanol and soybean flour ratio at 3:1 the result of isoflavones content was 2 times higher comparing with the simple method.

Pananun *et al.* (2012) studied isoflavones extraction from soybean using high-power ultrasonication (HPU). The HPU at 20,000 Hz. and the amplitude varied 18-54 μm for 1 and 3 minutes was employed and acetonitrile and ethanol were also used as solvent. The study claimed that the higher frequency, the less genistein; contrastingly, this technique provided higher total isoflavones from 600 to 5,813 μg/g which is about 10 times.

Nevertheless, there are many more studies relevant to isoflavones extraction. For instance, the isoflavones extraction by classical soxhlet (Nguyenle *et al.*, 1995), magnetic stirring (Murphy *et al.*, 1999), supercritical fluid extraction, pressurized liquid extraction, and solid phase extraction (Rostagno *et al.*, 2004; Rostagno *et al.*, 2002) etc.

2.5 Purification of isoflavones

Normally, there are two methods for isoflavones purification known as liquid-liquid extraction and solid phase extraction in column chromatography. The latter method uses resin as absorber. Resin is a durable polymer. It is either polar or non-polar. It is a good absorber. It is inexpensive and convenient to reuse (Scordino *et al.*, 2004). Resin is thus widely used in separating and absorbing substances especially, secondary metabolite including anthocyanin (Di Mauro *et al.*, 2002) and hesperidin (Scordino *et al.*, 2003). Resin is known in many different types, polystyrene-dinylbenzene, for instance, Amberlite XAD-4 resins, XAD-16HP resins etc. and polylmethacrylate such as Amberlite XAD-7HP resins (Wu and Lai, 2007). Amberlite XAD-2 is popularly used for extracting evaporated substance in the form of glucoside since Amberlite XAD-2 is a hydrophobic substance it absorbed non-polar glucoside (Crouzet and Chassagne, 1999).

Cho *et al.* (2009) studied isoflavones purification from germinated soybean by using Amberlite XAD-2 and diaion HP-20 as isoflavones absorber. It was found that Diaion HP-20 could more absorb isoflavones than Amberlite XAD-2. Based on (Chang *et al.*, 2004), it was reported that isoflavones purification from soybeen applying Amberlite XAD 16-HP resins, 37% of purified isoflavones was obtained. The separation and purification of genistein from soybean by applying AB-8 resin absorption and gradient separation was report by Li *et al.* (2011). At the first step, AB-8 resin was washed by deionized water, 20 % ethanol and 70% ethanol. Then the resin was washed again by 40 % ethanol and 70% ethanol. Eventually, 90% of purified genistein was found.

2.6 Isoflavones powder production

There are two processes related to isoflavones powder production. One is addition binding agent to increase solid proportion and the other is drying.

2.6.1 Addition binding agent

Isoflavones powder production needs binding agent to increase solid proportion. There are many binding agent used in flour industry such as maltodextrin, sodium alginate, fructo oligosaccharide and inulin. Krishnan *et al.* (2005) explained that some binding agent like gelatin and sodium alginate are higher viscosity than maltodextrin. Its viscosity can be better encapsulated. Based on theory, the more binding agent, the more powder can be produced (Fernández-Pérez *et al.*, 2004). However, too much binding agent might cause the deficiency of encapsulation. Kao and Chen (2007) studied the application of maltodextrin 40%, gelatin 10% and sodium alginate 1% as binding agent for isoflavones powder production; it was found that the amount of isoflavones from sodium alginate powder was the highest content equal to 23,847 microgram per gram of isoflavones powder, incontrastingly, the amount of powder found the highest was maltodextrin, but the amount of isoflavones found was the least content equal to 1,887 microgram per gram of isoflavones powder.

2.6.2 Drying

Nowadays, there are so many different ways to dry materials for example, spray drying, vacuum drying and freeze drying etc. Freeze drying can contribute the product with high stability and can preserve some nutritional value. It is a method to keep wet materials or aqueous solution. Through this process, the product will be frozen and then kept in low humidity environment where ice sublimation occurs or changing from solid state into gas. It takes place without dissolution; and physical chemistry cannot be changed by enzyme. This technique is combined with other drying process where dehumidify is made when air is taken out to generate vacuum state.

Freezing drying is suitable for biological substances or some medical product. These products cannot tolerate high temperature which is generally used in drying process. The freeze dried product will have the least quality changing and it has long stability at room temperature. One of its vital advantages is rehydration.

Freeze drying can be achieved in the following 3 stages (Rungsardthong, 2002).

1. Freezing stage is to solidify water in the product by freezing under freezing point of each product solution. During this stage, every composition of this product which is able to freeze will completely freeze.

2. Sublimation stage (Primary drying stage) is defined as the freezing solution sublimates from the product by decreasing pressure around the product till ice sublimates into vapor simultaneously.

3. Evaporation stage (Secondary drying stage) occurs when non-freezing concentrated solution of each product vanish. This state needs a little bit heating.

According to the stages mentioned above, freeze drying can be achieved by freezing the product under the freezing point of the product solution. And then the product is kept in low pressure so that the ice in the product will continuously sublimate until the product becomes dry. This process needs specific heat for sublimation called heat of sublimation. Ice in the product will sublimate without turning into liquid consequently, the motion of solution in the product occurs very slightly. The structure of the product remains unchanged. As the temperature is low, the pressure is low, and there is no liquid, oxygen in the product cause porous which is stable, denaturation caused by heat and chemicals decrease. In addition, the odor, color, taste and nutrition value are well preserved.

2.7 Conceptual framework

The conceptual framework of this thesis was showed in Figure 2.3.

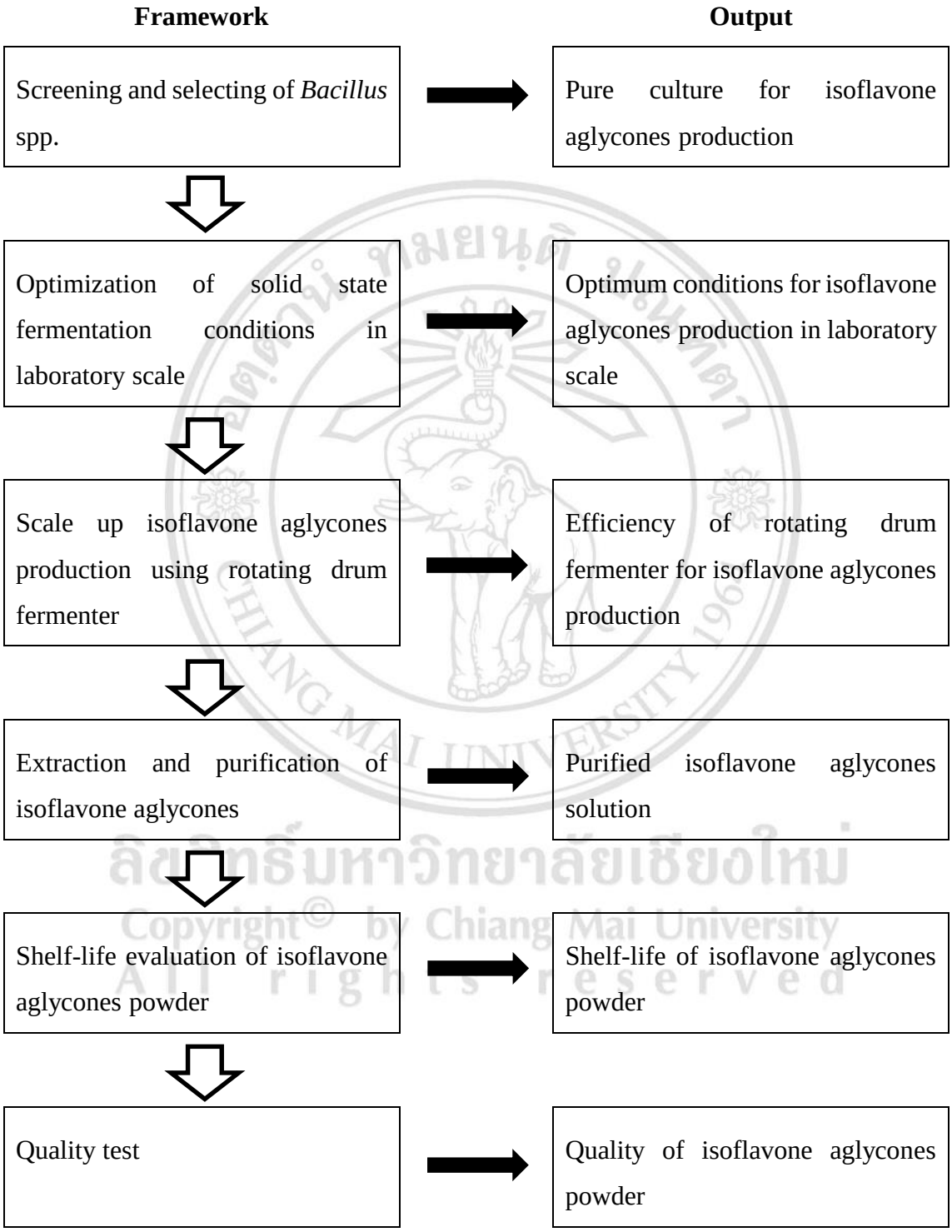


Figure 2.3 Conceptual framework of thesis.