CHAPTER 8

General discussion

Isoflavones are classified as flavonoids which can be found in soybean and soybean products. Their structure and function are similar to women hormone namely estrogen. Isoflavones structure can be classified into two main forms including glucosides and aglycones (Liu, 2004; Shimoni, 2004). Aglycone form has the structure that can be easily absorbed into small intestine. It has high estrogenicity and antioxidant activity. Nevertheless, the majority of isoflavones in soybean are in glucoside form, but the amount of aglycone form can be increased by fermentation process with β-glucosidase from *Bacillus* spp.. Pure culture technology is a method that allows fermentation process to run efficiently because *Bacillus* spp. can grow fully. From the studies, *Bacillus coagulans* PR03, screened from Thai fermented soybean (thua nao), was a suitable pure culture for soybean isoflavone aglycone production. It was able to produce isoflavone aglycones with the maximum amount of 146.21 mg/100 g dry weight (3.81 times higher than non-fermented soybean). This was the first research using *B. coagulans* as predominant bacteria for producing isoflavone aglycones from soybean.

The study of optimum fermentation condition was found that the content of isoflavone glucosides was highest in the soygerm (476.48-1082.89 mg/100 g) and lowest in the hull of the seed (10.20-13.42 mg/100 g); with the cotyledons containing moderate amount between 21.26-37.59 mg/100 g. This result was similar to the report from Wang and Murphy (1994) which showed that isoflavones content in the soygerm was higher than the content in cotyledon. Considering isoflavone glucoside content in germ, it was found that SJ2 variety had the highest amount of glucosides (1,082.89 mg/100g). Because it had highest isoflavone glucosides content, soygerm was used as a substrate for high volume isoflavone aglycones production. Moreover, some components from cotyledon and hull which might affect isoflavone aglycones purification were removed by using only soygerm for the production.

Initial isoflavone glucosides in soygerm depended 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), storage time (Lee *et al.*, 2003b), storage condition (Lee *et al.*, 2003a) and crop year (Wang and Murphy, 1994). From Chapter 4 and 5, it was showed that the initial amounts of isoflavone glucosides in soygerm SJ2 variety were different. In Chapter 4, initial amount of isoflavone glucosides was 1,082.89 mg/100 g dry weight while the amount in Chapter 5 was 966.73 mg/100 g dry weight. Likewise, initial amount of isoflavone aglycones in Chapter 4 was 161.08 mg/100 g dry weight while the amount in Chapter 5 was 335.70 mg/100 g dry weight. However, initial total isoflavones of both Chapter was similar (1,243.97 and 1302.43 mg/100 g dry weight respectively). The different amounts of isoflavones between the 2 chapters might come from a variation in the process of soaking and sterilization of soygerm. The effect of soaking to isoflavone content was explained by the study from Kao *et. al*. (2004), which showed that an increase in soaking temperature would increase the amount of isoflavone aglycones and reduce the amount of isoflavone glucosides. Moreover, Chien *et al.* (2005) indicated that isoflavone glucosides can be transformed to aglycone during moist heating.

The efficiency of isoflavone aglycones production in rotating drum fermenter (5 Kg soygerm/batch) was compared with lab scale production (1 Kg soygerm/batch). It was found that rotating drum fermenter had higher production efficiency than lab scale production because an addition of air injection during fermentation in the rotating drum fermenter enhanced the growth of *B. coagulans* PR03 and the production of β-glucosidase. Furthermore, the rotation in the fermenter could increase the activity between isoflavone glucosides and enzyme.

The air flow rate affected to isoflavone aglycones production. From the study in Chapter 5, it was found that higher air flow rate produced higher isoflavone aglycones (P<0.05). In this study, the highest amount of isoflavone aglycones was achieved using air flow rate of 6 L/min. Based on the results, an increase in air flow rate more than 6 L/min might increase the production rate isoflavone aglycones. However, 6 L/min was the maximum flow rate of this fermenter; thus, further study was not applicable. Nonetheless, higher air flow rate might decrease moisture content in the fermenter

affecting the homogeneity between soygerm and *B. coagualn* PR03, which would result in irregular production of isoflavones aglycones.

Ethanol was used as solvent in extraction process and eluent in purification process. After processes, ethanol can be removed from the extracts by an evaporator. The evaporated ethanol can be reused however further analysis should be performed to ensure the quality and concentration of ethanol.

Nowadays, soy isoflavone had gained more recognition in the field of preventive medicine. Its antimutanenic, antihypertensive and antidiabetic effects can reduce a risk of many diseases such as breast cancer, prostate cancer, cardiovascular diseases and osteoporosis. In addition, it can reduce the severity of some symptoms such as reduce hot flash during the menopause period and relief of menopausal symptom in woman. The majority of isoflavone products are imported in both glucoside and aglycone forms. Therefore, the development of isoflavone production in an enriched aglycones form, which has higher estrogenicity and antioxidant activity, would provide more affordable and better isoflavone products for consumers. From our study, isoflavone was developed into a powder enriched with aglycones content in an amount of 227.78 mg/ 1 g powder. The developed product exhibits a good potential in food supplementary market. Thus, the objective of this study; to produce isoflavone aglycones powder from soygerm, was fulfilled.