

## CHAPTER 9

### Conclusions

The *Bacillus coagulans* PR03, screened from Thai fermented soybean (*thua nao*), was suitable pure culture for soybean isoflavone aglycone production. This was the first research using *B. coagulans* as predominant bacteria for producing isoflavone aglycones from soybean. Moreover, soybean fermentation with *B. coagulans* PR03 revealed high amount of isoflavone aglycones..

Soygerm of SJ2 variety was a suitable source of isoflavone glucosides. The optimum fermentation conditions of lab scale production were 15% (v/w) inoculum concentration of *B. coagulans* PR03 and aerobic fermentation with air flow rate of 1 L/min at 30 °C for 5 days. The kinetic study of isoflavone aglycones production from 1 Kg soygerm/batch had found that specific growth rate of *B. coagulans* PR03 ( $\mu$ ), product yield coefficient ( $Y_{p/s}$ ), specific product usage rate of total glucosides ( $q_{glu}$ ) and specific product formation rate of total aglycones ( $q_{agly}$ ) were 1.04 day<sup>-1</sup>, 0.99 mg/100 g dry weight, 198.38 mg/100 g-day and 185.60 mg/100 g-day, respectively.

The scale up isoflavone aglycones production was using 46 L rotating drum fermenter for 5 Kg soygerm/batch. The optimum air flow rate for isoflavone aglycones production was 6 L/min. The kinetic study of scale up production had found that fermentation time, specific growth rate of *B. coagulans* PR03 ( $\mu$ ), product yield coefficient ( $Y_{p/s}$ ), specific product usage rate of total glucosides ( $q_{glu}$ ) and specific product formation rate of total aglycones ( $q_{agly}$ ) were 4 days, 1.33 day<sup>-1</sup>, 1.01 mg/100 g dry weight, 228.88 mg/100 g-day and 230.67 mg/100 g-day, respectively.

The extraction of isoflavone aglycones by solid-liquid extraction method had found that optimum solvent was 80% ethanol. For the extraction process of isoflavone aglycones with extraction machine, the optimum inverter frequency and extraction time

were 65.90 Hz (equal to 3,986.95 rpm) and 90.31 min, respectively. The resulting crude extract contained total isoflavone aglycones in an amount 293.73 mg/100 g fermented soygerm, respectively.

The purification of isoflavone aglycones by solid-liquid phase column chromatography had found that optimum resin was Amberlite XAD-4. The majority of isoflavone aglycones was found in the portions eluted by 40% and 60% ethanol. The yield and purity of isoflavone aglycones were 75.42% and 71.92%.

The isoflavone aglycones powder production was conducted with 1:3 ratio of purified isoflavone aglycones extract and 1% sodium alginate solution. The color of powder was light yellow with the yield of 3%. The resulting powder contained 22,778.50 mg/ 100 g powder of isoflavone aglycones, which comprised of daidzein, genistein and glycitein at 13,446.51, 3,310.06 and 6,001.92 mg/ 100 g powder, respectively.

For the shelf-life evaluation with accelerate temperature, shelf-life of the isoflavone aglycones powders at refrigerating temperature (4°C), air conditioning (25°C) and room temperature (30, 35 and 40 °C) were 2.09, 2.08, 1.87, 1.63 and 1.38 years, respectively.



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