CHAPTER 5

Scale up isoflavone aglycones production and kinetic study using rotating drum fermenter

5.1 Introduction

From the previous experiment, it was found that soygerm of SJ2 variety was a suitable source of isoflavone glucosides. Moreover, optimum fermentation conditions for isoflavone aglycones by solid state fermentation were also obtained. In this experiment, that information was used to scale up isoflavone aglycones production using a rotating drum fermenter. However, there would be limiting factors; such as rising temperature and oxygen deficiency, when the fermentation process was scaled up (dos Santos *et al.*, 2004; Hölker and Lenz, 2005; Raghavarao *et al.*, 2003). Those factors would affect the microbial growth resulting in lower isoflavone aglycones yield. Therefore, a rotation of fermenter vessel and an influx of oxygen by air pump were used to reduce the effects of rising temperature and oxygen deficiency. Nevertheless, the rotation speed of fermenter vessel was limited to 3.5 rpm, so air flow rate was the only parameter optimized in this chapter. After that, the optimum condition was used to produce isoflavone aglycones and study a kinetic of isoflavone aglycones scaled up production.

5.2 Methods

5.2.1 Instrumental set up

The rotating drum fermenter with 46 L capacity was used in this study. This fermenter was developed and constructed by Traditional Food Research and Development Unit, Science and Technology Research Institute, Chiang Mai University, Chiang Mai, Thailand. Other accessories installed to the fermenter were air pump and air filter (0.2 μm, PTFE-membrane). The configuration of rotating drum fermenter was shown in Figure 5.1.

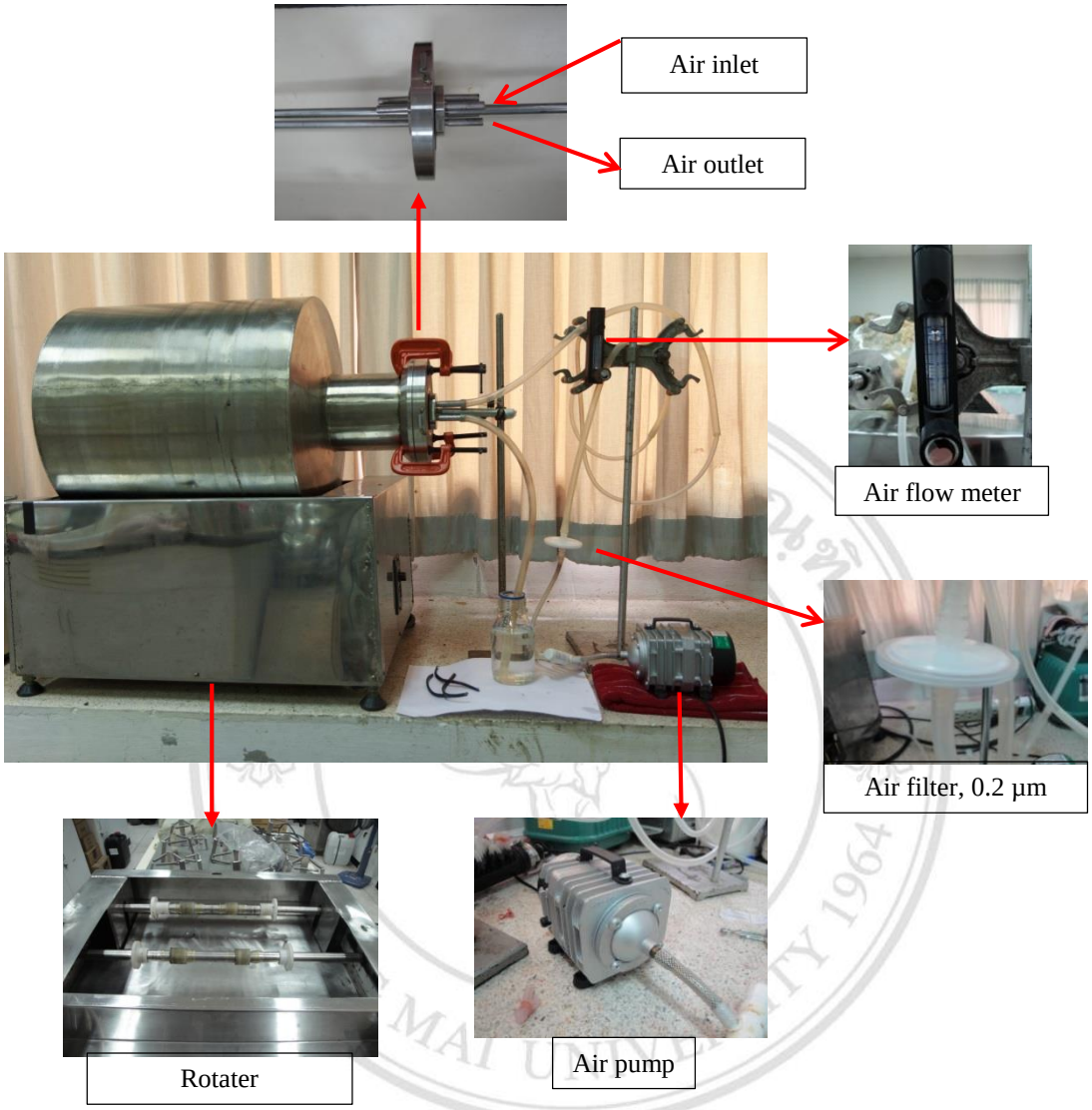

Figure 5.1 The configuration of rotating drum fermenter

For isoflavone aglycones production, 5 kg of soygerm (SJ2 variety) were washed 2 times with clean water and drained for 30 min at ambient temperature (30-35°C). After draining, soygerm was loaded to fermenter vessel, filled with 10 kg of water and sterilized at 121°C for 15 min. After cooling down, the sterilized soygerm was inoculated with 15% (v/w) of *B. coagulans* PR03. The inoculum of *B. coagulans* PR03 was cultured in nutrient broth (NB) and incubated at 37°C and 120 rpm for 18 h before used. During the entire fermentation period, the vessel of fermenter was rotated at 3 rpm. The fermentation was performed at room temperature (30-35 °C) for 5 days.

5.2.2 Study of optimum air flow rate for isoflavone aglycones production in rotating drum fermenter

In this experiment, the air flow rate during fermentation was varied to 1, 3, 5 and 6 Liter/min. After the fermentation, fermented soygerm samples were analyzed for isoflavone aglycones content (see Appendix A), β-glucosidase activity (see section 3.2.3) and total bacterial count (see section 3.2.3). The optimum air flow rate with highest isoflavone aglycones was selected.

5.2.3 Kinetic study of isoflavone aglycones production in rotating drum fermenter

The optimum air flow rate from section 5.2.2 was used to produce isoflavone aglycones. The fermentation time was 7 days. During the fermentation period, fermented soybeans were daily analyzed for isoflavones content (see Appendix A), β- glucosidase activity (see section 3.2.3) and total bacterial count (see section 3.2.3). The results were used to predict the suitable time of isoflavone aglycone harvesting. After the suitable harvesting time was determined, the results during that time were used to study the kinetic of scaled up isoflavone aglycones production including specific growth rate of *B. coagulans* PR03 (μ), product yield coefficient ($Y_{p/s}$), specific product usage rate (q_s) and specific product formation rate (q_p) (see section 4.2.5).

5.3 Results and discussion

5.3.1 Study of optimum air flow rate for isoflavone aglycones production in rotating drum fermenter

According to the study, air flow rate was changed to 1, 3, 4, 5 and 6 L/min. The results of isofalvones content are shown in Table 5.1. It was found that the air flow rate significantly affected the quantities of isoflavone aglycones and isoflavone glucosides (P<0.05). The amounts of daidzein, genistein, glycitein and total aglycones in non-fermented soygerm started at 175.05, 43.28, 117.36 and 335.70 mg/ 100 g, respectively. After fermentation process, it was found that 6 L/min of air flow rate greatly increased total aglycones content to 1,172.83 mg/100 g, which consisted of daidzein, genistein and glycitein in an amount of 538.41, 143.66 and 490.76 mg/100 g, respectively.

For air flow rate of 5, 4 and 3 L/min, the results showed a moderate increase of isoflavone aglycones with the least amount from 1 L/min of air flow rate, which had daidzein, genistein, glycitein and total aglycones at an amount of 319.16, 80.64, 273.14 and 672.93 mg/100 g, respectively. Furthermore, air flow rate at 6 L/min had also showed the highest decrease in isoflavone glucosides especially genistin, which was not detected. It decreased the values of daidzin, glycitin and total glucoside to 5.66, 18.41 and 24.07 mg/100 g, respectively.

The results of β-glucosidase activity and total bacteria count are shown in Table 5.2. It was found that the air flow rate significantly affected to β-glucosidase activity and total bacteria count ($P<0.05$). After fermentation process, it was found that 6 L/min of air flow rate had highest β-glucosidase activity and total bacteria count which were 25.01 mU/g and 7.71 log cfu/g, respectively. 5 L/min of air flow rate secondly affected β-glucosidase activity and total bacteria count, which were 23.03 mU/g and 6.96 log cfu/g, respectively. While, 1 L/min of air flow rate showed smallest β-glucosidase activity (25.01 mU/g) and total bacteria count (7.71 log cfu/g). Correspondingly, the air flow rate of 6 L/min resulted in the highest amount of isoflavone aglycones, β-glucosidase activity and total bacteria count. Therefore, the chosen suitable air flow rate for isoflavone aglycones production in rotating drum fermenter was 6 L/min and was used in the next experiment.

Table 5.1 Isoflavones contents of isoflavone aglycone production at the different air flow rate

Air flow rate (L/min)	Isoflavones content (mg/100 g dry weight)							
	Aglycones				Glucosides			
	Daidzein	Genistein	Glycitein	Total aglycones	Daidzin	Genistin	Glycitin	Total glucosides
1	319.16±7.65 ^{b}	80.64±2.11^{b}	273.14±6.42^{d}	672.93±16.77^{c}	202.26±2.95^{a}	74.45±0.88^{a}	231.82±3.96^{a}	508.53±7.79^{i}
3	505.69±6.72^{bc}	143.43±5.62^{a}	426.40±9.25^{bc}	1,075.53±21.59^{b}	5.16±0.12^{d}	3.33±0.09^{b}	31.43±1.15^{c}	39.92±1.36^{h}
4	513.67±2.14^{bc}	146.82±0.48^{a}	420.79±3.23^{c}	1,081.28±5.85^{b}	5.26±0.16^{d}	ND	28.03±0.17^{d}	33.29±0.01^{g}
5	510.10±4.06^{b}	137.93±2.75^{a}	435.45±7.20^{b}	1,083.49±14.02^{b}	5.14±0.13^{b}	ND	20.45±0.39^{b}	25.59±0.52^{f}
6	538.41±4.47^{a}	143.66±8.38^{a}	490.76±7.15^{a}	1,172.83±19.99^{a}	5.66±0.03^{c}	ND	18.41±0.19^{e}	24.07±0.17^{e}
Non fermented soygerm	175.05±6.74	43.28±2.61	117.36±0.77	335.70±10.12	386.90±2.36	153.85±0.37	425.98±1.97	966.73±3.96

Note: Means within same column with different superscripts are significantly different (P<0.05); ND = not detected.

Table 5.2 β-glucosidase activity and total bacterial count of isoflavone aglycone production at the different air flow rate

Air flow rate (L/min)	β-glucosidase activity (mU/g)	Total bacteria count (log CFU/g)
1	15.08±0.40^{d}	5.88±0.03^{e}
3	16.19±0.18^{c}	6.00±0.10^{d}
4	22.90±0.35^{b}	6.71±0.34^{c}
5	23.03±0.35^{b}	6.96±0.23^{b}
6	25.01±0.86^{a}	7.11±0.05^{a}

Note: Means within same column with different superscripts are significantly different (P<0.05).

5.3.2 Kinetic study of isoflavone aglycones production in rotating drum fermenter

The optimum air flow rate (6 L/min) from section 5.3.1 was applied to produce isoflavone aglycones in rotating drum fermenter. During the fermentation period, fermented soygerm was daily analyzed for isoflavones content, β-glucosidase activity and total bacteria count. The results are shown in Table 5.3. It was found that fermentation time had significant effects on the isoflavone aglycones, isoflavone glucosides, β-glucosidase activity and total bacteria count (P<0.05). The quantity of isoflavone aglycones was increased during the fermentation period. As a result, the quantities of daidzein, genistein, glycitein and total aglycones were increased from 139.49, 45.57, 69.93 and 254.99 mg/100 g to 569.28, 129.86, 487.03 and 1,183.68 mg/100 g, respectively. On the contrary, the quantity of isoflavone glucosides decreased during the fermentation which resulted in the decreasing values of daidzin, genistin, glycitin and total glucosides from 484.88, 122.48, 378.55 and 985.91 mg/100g to 30.21, 5.36, 25.34 and 61.28 mg/100 g, respectively. β-glucosidase activity increased from 8.85 mU/g to the highest value of 27.30 mU/g at the fourth day of fermentation process. After that, the value decreased to 19.47 mU/g at the last day of fermentation. Total bacterial count representing cell number of *B. coagulans* PR03 showed a similar trend as β-glucosidase activity. The value increased from 5.54 to 7.86 log cfu/g at the fourth day of fermentation

and decreased to 5.92 log cfu/g at the last day of fermentation process. The changes of isoflavones, β-glucosidase activity and total bacteria count during soygerm fermentation with rotating drum fermenter are showed in Figure 5.2. The increasing number of *B. coagulans* PR03 and β-glucosidase activity were relevant. For instance, it could be observed that *B. coagulans* PR03 showed logarithmic growth along with β-glucosidase activity until the fourth day of fermentation process. The increasing value of *B. coagulans* PR03 and β-glucosidase activity resulted in hydrolisation of isoflavone glucosides in soygerm transforming it to isoflavones aglycones. As a result, the suitable time for isoflavone aglycones fermentation was 4 days. Therefore, the results during fermentation period of 0-4 days were used to calculate specific growth rate of *B. coagulans* PR03 (μ), product yield coefficient ($Y_{p/s}$), specific product usage rate (q_s) and specific product formation rate (q_p) of scaled up isoflavone aglycones production with rotating drum fermenter.

5.3.2.1 Specific growth rate of *B. coagulans* PR03

From growth curve of *B. coagulans* PR03 in fermented soygerm (Figure 5.2), it was found that the log phase of microbial growth was in the period of 0–4 days. So, the cell numbers in term of cfu/g during that period (Table 5.3) were used to calculate specific growth rate of *B. coagulans* PR03 (μ)

$$\mu = \ln (X_t/X_0) / t$$

$$\mu_{PR03} = \ln (X_5/X_0) / t$$

$$= \ln (10^{7.86}/10^{5.54}) / (4-0)$$

$$= \ln (208.93) / 5$$

$$= 1.33 \text{ day}^{-1}$$

Table 5.3 Isoflavones contents, β-glucosidase activity and total bacterial count of scale up isoflavone aglycone production in 46 L rotating drum fermenter

Time (Day)	Isoflavones content (mg/100 g dry weight)								β-glucosidase activity (mU/g)	Total bacterial count (log CFU/g)
	Aglycones				Glucosides					
	Daidzein	Genistein	Glycitein	Total aglycones	Daidzin	Genistin	Glycitin	Total glucosides		
0	139.49±0.73^{e}	45.57±0.29^{d}	69.93±0.04^{e}	254.99±0.48^{e}	484.88±2.83^{a}	122.48±0.60^{a}	378.55±2.58^{a}	985.91±6.01^{a}	8.85±0.15^{b}	5.54±0.26^{h}
1	267.61±5.21^{d}	52.20±0.74^{d}	182.10±3.67^{d}	501.92±9.61^{d}	315.00±0.24^{b}	104.24±1.08^{b}	312.80±4.68^{b}	732.04±3.84^{b}	17.34±0.73^{a}	5.88±0.32^{g}
2	409.54±2.24^{c}	115.36±0.96^{c}	359.31±1.44^{c}	884.21±4.63^{c}	166.61±0.70^{c}	34.35±1.55^{c}	153.25±1.49^{c}	354.20±3.74^{c}	21.53±0.40^{a}	6.50±0.47^{d}
3	527.67±2.73^{b}	125.37±1.70^{b}	467.99±4.20^{b}	1121.04±3.17^{b}	50.47±0.21^{d}	18.48±1.21^{d}	40.58±1.85^{d}	114.02±2.15^{d}	27.27±0.47^{a}	7.65±0.03^{c}
4	569.28±2.75^{a}	128.98±0.28^{a}	483.24±2.36^{a}	1181.50±4.83^{a}	34.16±0.14^{e}	5.88±0.03^{d}	26.56±0.22^{e}	66.60±0.38^{e}	27.30±0.39^{a}	7.86±0.89^{a}
5	567.64±2.89^{a}	128.75±0.42^{a}	483.55±2.40^{a}	1179.93±4.87^{a}	30.83±1.07^{f}	5.67±0.05^{f}	25.90±1.73^{e}	62.40±2.85^{f}	26.55±0.38^{c}	7.73±0.53^{b}
6	564.87±4.14^{a}	129.86±0.89^{a}	485.06±1.87^{a}	1179.79±6.89^{a}	31.01±0.56^{f}	5.36±0.14^{g}	26.34±2.01^{e}	62.71±1.31^{f}	20.39±0.94^{d}	6.34±0.04^{e}
7	568.00±3.16^{a}	128.65±1.14^{a}	487.03±5.07^{a}	1183.68±9.37^{a}	30.21±0.33^{f}	5.73±0.01^{e}	25.34±0.26^{e}	61.28±0.60^{f}	19.47±0.41^{d}	5.92±0.38^{f}

Note: Means within same column with different superscripts are significantly different (P<0.05).

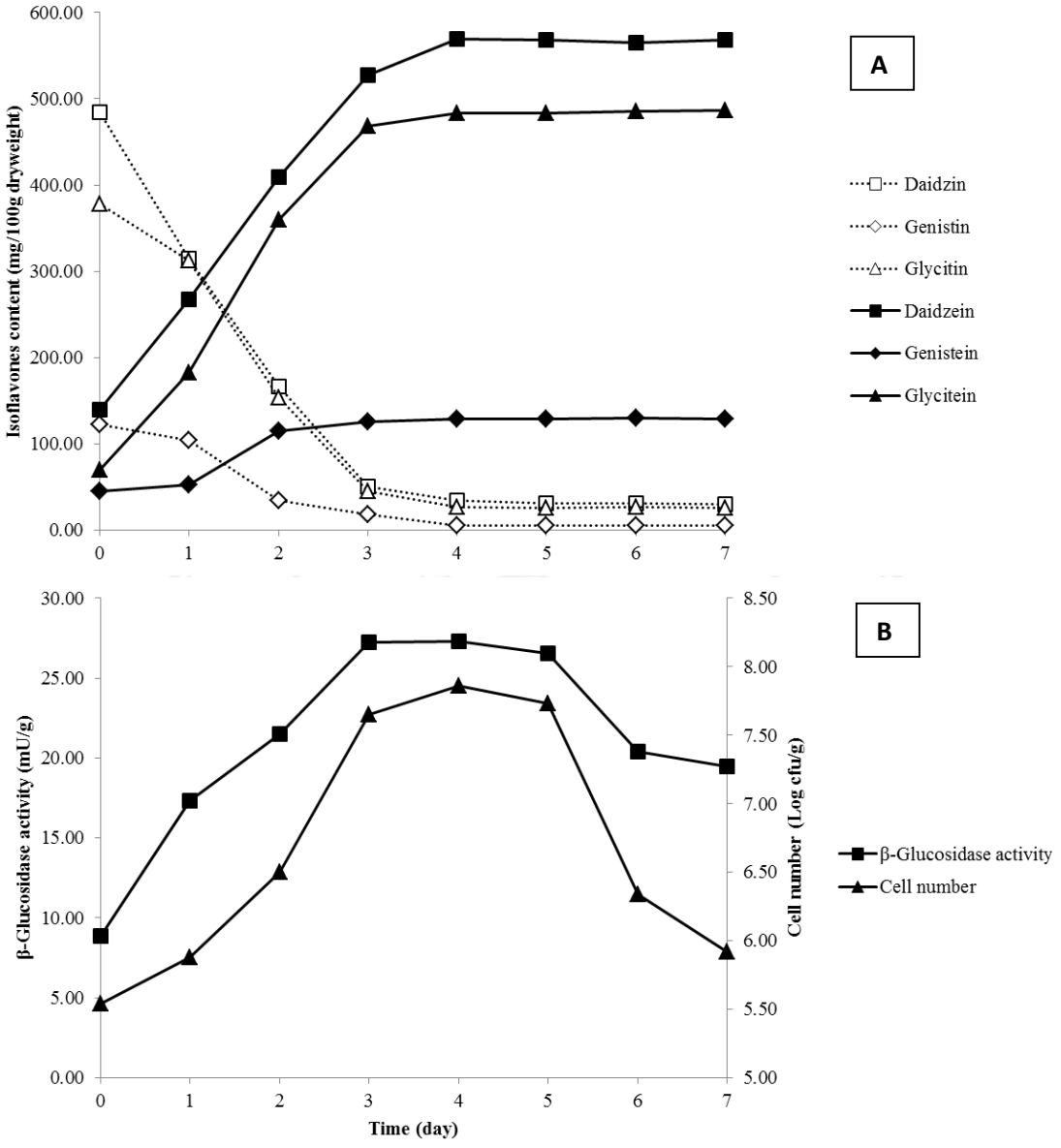

Figure 5.2 Changes of isoflavones contents, β-glucosidase activity and total bacterial count of scale up isoflavone aglycone production in 46 L rotating drum fermenter

5.3.2.2 Product yield coefficient

Similarly, the amounts isoflavone aglycones and isoflavone glucosides during day 0 and day 4th of the fermentation (Table 5.3) were used to calculate product yield coefficient of scaled up isoflavone aglycones production with rotating drum fermenter ($Y_{agy/glu}$).

$$Y_{agy/glu} = (P_{agy}1 - P_{agy}0)/(S_{glu}0 - S_{glu}1)$$

$$= (1{,}181.50-254.99) / (985.91-66.60)$$

$$= 1.01 \text{ mg/100 g dry weight}$$

5.3.2.3 Kinetic of substrate usage

Specific substrate usage rate (q_s) was also calculated using the amounts of isoflavone glucosides from 0-4 day of the fermentation (Table 5.3).

$$q_s = (dS/dX)\,\mu$$

$$q_{daidzin} = [(484.88-34.16) / (\ln 10^{7.86} - \ln 10^{5.54})] \times 1.33$$

$$= 112.22 \text{ mg/100 g-day}$$

$$q_{genistin} = [(122.48-5.88) / (\ln 10^{7.86} - \ln 10^{5.54})] \times 1.33$$

$$= 29.03 \text{ mg/100 g-day}$$

$$q_{glycitin} = [(378.55-26.56) / (\ln 10^{7.86} - \ln 10^{5.54})] \times 1.33$$

$$= 87.64 \text{ mg/100 g-day}$$

$$q_{total\ glucosides} = [(985.91-66.60) / (\ln 10^{7.86} - \ln 10^{5.54})] \times 1.33$$

$$= 228.88 \text{ mg/100 g-day}$$

5.3.2.4 Kinetic of product formation

From Table 5.3, the amounts of isoflavone aglycones in a period of 0-4 days of fermentation were used to determine specific product formation rate (q_p)

$q_p = (dP/dX)\ \mu$

$q_{daidzein} = [(569.28-139.49) / (\ln 10^{7.86} - \ln 10^{5.54})] \times 1.33$

$= 107.00$ mg/100 g-day

$q_{genistein} = [(128.98-45.57) / (\ln 10^{7.86} - \ln 10^{5.54})] \times 1.33$

$= 20.77$ mg/100 g-day

$q_{glycitein} = [(483.24-69.93) / (\ln 10^{7.86} - \ln 10^{5.54})] \times 1.33$

$= 102.90$ mg/100 g-day

$q_{total\ aglycones} = [(1{,}181.50-254.99) / (\ln 10^{7.86} - \ln 10^{5.54})] \times 1.33$

$= 230.67$ mg/100 g-day

5.3.3 Comparison of isoflavone aglycones production between rotating drum fermenter process and lab scale process

The efficiency of isoflavone aglycones production in rotating drum fermenter was compared with lab scale production (section 4.3.5). The results are showed in Table 5.4. For the isoflavone aglycones production, it was found that rotating drum fermenter production had higher specific growth rate (μ), product yield coefficient ($Y_{agy/glu}$), specific daidzin usage rate (q_{din}), specific glycitin usage rate (q_{glin}), specific total glucosides usage rate (q_{glu}), specific daidzein formation rate (q_{dein}), specific glycitein formation rate (q_{glein}) and specific total aglycones formation rate (q_{agy}) than lab scale production. While lab scale production had higher specific genistin usage rate (q_{gin}) and specific genistein formation rate (q_{gein}) than rotating drum fermenter production. However, when considering fermentation time, it was found that isoflavone aglycones production in rotating drum fermenter required only 4 days of fermentation whereas lab scale

production required 5 days. Hence, the rotating drum fermenter was suitable process to use for isoflavone aglycones production from soygerm.

Table 5.4 Comparison of isoflavone aglycones production between rotating drum fermenter process and lab scale production

Efficiency	Rotating drum fermenter process	Lab scale process
Fermentation time (day)	4	5
Specific growth rate: μ_{PR03} (day^{-1})	1.33	1.04
Product yield coefficient: $Y_{agy/glu}$ (mg/100 g)	1.01	0.94
Specific daidzin usage rate: q_{din} (mg/100 g-day)	112.22	91.95
Specific genistin usage rate: q_{gin} (mg/100 g-day)	29.03	32.11
Specific glycitin usage rate: q_{glin} (mg/100 g-day)	87.64	74.33
Specific total glucosides usage rate: q_{glu} (mg/100 g-day)	228.88	198.38
Specific daidzein formation rate: q_{dein} (mg/100 g-day)	107.00	73.46
Specific genistein formation rate: q_{gein} (mg/100 g-day)	20.77	37.42
Specific glycitein formation rate: q_{glein} (mg/100 g-day)	102.90	74.71
Specific total aglycones formation rate: q_{agly} (mg/100 g-day)	230.67	185.60

5.4 Conclusion

The optimum air flow rate for isoflavone aglycones productin was 6 L/min. The kinetic study of isoflavone aglycones production by using 46 L rotating drum fermenter had found that fermentation time, specific growth rate of *B. coagulans* PR03 (μ), 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^{-1}, 1.01 mg/100 g dry weight, 228.88 mg/100 g-day and 230.67 mg/100 g-day, respectively. Moreover, the rotating drum fermenter has higher efficiency than lab scale process and was suitable to use for isoflavone aglycones production.