CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Study on survival rate and moisture content of probiotic bacteria in skim milk solution for spray drying

4.1.1 Spray drying of probiotic in skim milk solution

Evaluation of optimum condition for spray drying of probiotic bacteria, *L. casei* subsp. *paracasei* F-19, *Lactobacillus plantarum* V299 and *L. fermentum* 2311M in 15% (w/v) skim milk solution after incubated at 37°C for log phases. Conditions of spray drying were done with 25 ml/min of feed rate, 15 kg/cm^2 of air atomization and the air outlet temperature at 65°C, 75°C and 85 °C. Spray dry of *L. casei* subsp. *paracasei* F-19 had highest survival rate 72%, 47% and 35% respectively, *L. plantarum* V299 had survival rate at 55%, 49% and 18%, respectively and *L. fermentum* 2311M had lowest survival rate 24%, 20% and 10%, respectively. Moisture content of product was related with air outlet temperature too. At 85°C of air outlet temperature yielded lowest moisture content from 3.2% to 3.8%, at 75°C of air outlet temperature were 5.2% to 5.6%, moisture content is nearly standard of milk powder and the highest moisture content obtained from air outlet temperature at 65°C were 7.9% to 8.1%.

The higher the air outlet temperature decrease the survival rate and also decrease the moisture content of the powder product (see figure 4.1 and 4.2). It was certainly due to dehydration and thermal damage of cell structures and cell components during spray drying (Teixeira *et al*., 1995b). In addition, thermal shock may occur during the introduction of spray droplets into the hotter inlet air, which could have also an effect on cell survival (Kim and Bhowmik, 1990).

Table 4.1 Number of probiotic before and after spray drying in skim milk at various the air outlet temperatures.

probiotic type	AOT (°C)	viable cell before dry (cfu/ml)	viable cell after dry (cfu/ml)	survival rate (%)	moisture content (%)
L. casei F-19	65	6.6×10^{10}	4.8×10^{10}	72	8.1
	75	4.9×10^{10}	2.3×10^{10}	47	5.5
	85	4.6×10^{10}	1.6×10^{10}	35	3.5
L. plantarum V299	65	5.8×10^{9}	3.2×10^{9}	55	8.4
	75	5.3×10^{9}	2.6×10^{9}	49	5.6
	85	6.2×10^{9}	1.1×10^{9}	18	3.8
L. fermentum 2311M	65	7.4×10^{8}	1.8×10^{8}	24	7.9
	75	6.5×10^{8}	1.3×10^{8}	20	5.2
	85	6.1×10^{8}	6.0×10^{7}	10	3.2

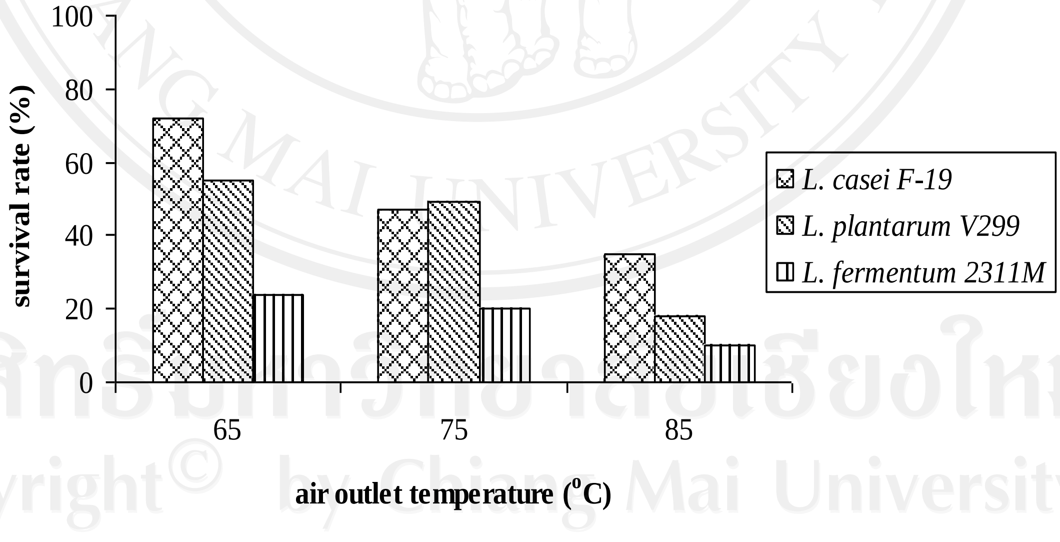

Figure 4.1 Survival of probiotic during spray drying in 15% (w/v) skim milk at different air outlet temperatures.

Many factors were affected the survival of bacteria during spray drying, including inlet and outlet temperatures, composition of carrier medium and choice of strain (Corcoran *et al*., 2006). It was demonstrated that the outlet temperature was inversely linearly related to the survival of probiotic lactobacilli during spray drying. Inlet and outlet temperature of 170^{o}C and 85-90^{o}C, respectively, resulted in good survival of some probiotic lactobacilli, such as *Lactobacillus paracasei* NFBC 338 (Gardiner *et al*., 2000). Strain selection was another important consideration, as different strains were shown to have different survival rates during spray drying (Forster, 1962, Gardiner *et al*., 2000, Lian *et al*., 2002, Corcoran *et al*., 2006 and Picot and Lacroix, 2004). Heat tolerance was linked with survival during spray drying, although other stresses, such as dehydration and osmotic shock were also encountered during the process.

The increasing of the survival rate during spray drying may use encapsulation techniques (Champagne *et al*., 1995) or carriers such as dextrin (Johnson and Etzel, 1993) and increase initial cells in spray drying culture (Morichi, 1974)

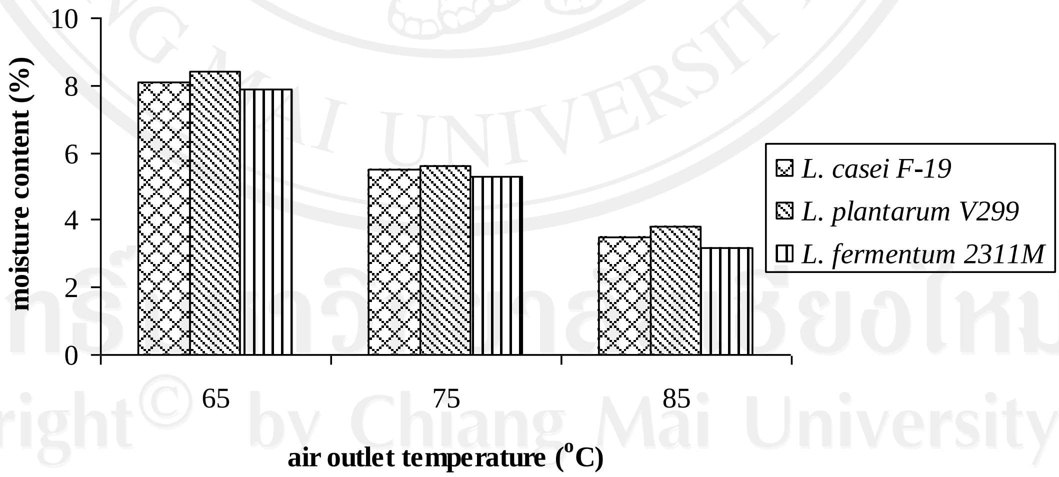

Figure 4.2 Moisture content of probiotic during spray drying in 15% (w/v) skim milk at different air outlet temperatures.

The moisture content of dried product was affected by the air outlet temperature drying. Hight air outlet temperature produces lower moisture content, which was a similar finding to a report by Kim *et al*.(1997).

The difference of probiotic bacteria types, *L. casei* sub.sp. *paracasei* F-19, *L. plantarum* V299 and *L. fermentum* 2311M did not affect the moisture content of dried powder product.

4.1.2 Variation of starter probiotic cells in skim milk solution for spray drying.

4.2.2.1 Cells survival and moisture content.

In addition, when increased the starter cell from 1% to 2% and 3% (w/v), then the survival rate of *L. casei* sub.sp. *paracasei* F-19 increased from 47% to 49% and 55%, *L. plantarum* V299 increased from 49% to 51% and 56% and *L. fermentum* 2311M increased from 20% to 24% and 28%, respectively (see table 4.2 and figure 4.3)

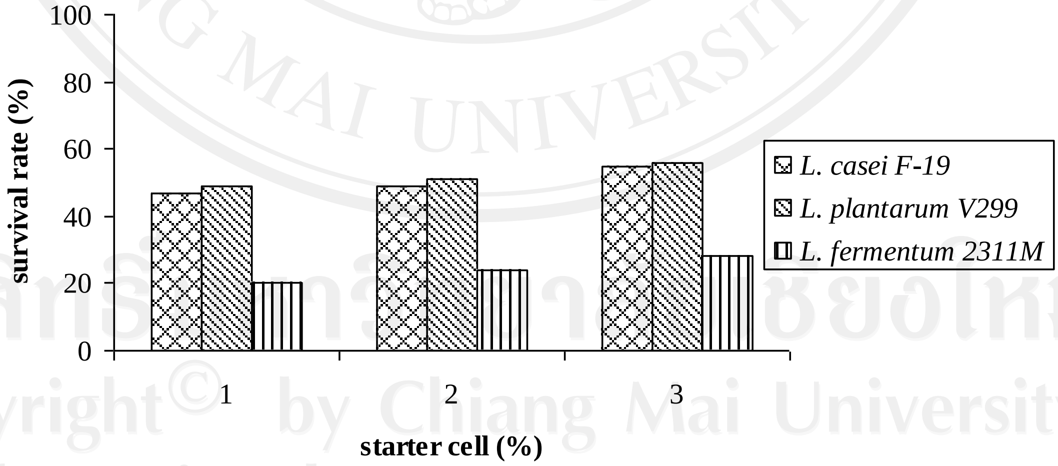

Figure 4.3 Survival of probiotic after spray drying in skim milk at various starter cell at 75°C of air outlet temperature.

Table 4.2 Number of probiotic after spray drying in skim milk at various starter cells at 75°C of air outlet temperature.

probiotic type	starter cell (%)	viable cell before dry (cfu/ml)	viable cell after dry (cfu/ml)	survival rate (%)	moisture content (%)
L. casei F-19	1	4.9×10^{10}	2.3×10^{10}	47	5.5
	2	3.5×10^{11}	1.7×10^{11}	49	5.4
	3	3.8×10^{12}	2.1×10^{12}	55	5.6
L. plantarum V299	1	5.3×10^{9}	2.6×10^{9}	49	5.6
	2	3.7×10^{10}	1.9×10^{10}	51	5.4
	3	2.7×10^{11}	1.5×10^{11}	56	6.1
L. fermentum 2311M	1	6.5×10^{8}	1.3×10^{8}	20	5.2
	2	3.7×10^{9}	9.0×10^{8}	24	5.3
	3	1.8×10^{10}	5.1×10^{9}	28	5.5

4.2.2.2 Viable cells of probiotic bacteria in skim milk spray dried powder during shelf life storage.

4.2.2.2.1 Storage in aluminum seal bag.

Probiotic bacteria in skim milk spray dried powder that were produce by spray drying at 75°C of the air outlet temperature condition and used 2% (w/v) starter probiotic cell were stored at different temperature for 30 days in aluminum seal bag. When stored at 4°C, the viable cells of *L. casei* sub.sp. *paracasei* F-19 decrease from 1.7×10^{11} to 2.2×10^{9} cfu/ml, *L. plantarum* V299 decrease from 1.9×10^{10} to 2.0×10^{9} cfu/ml and *L. fermentum* 2311M decrease from 9.0×10^{8} to 1.3×10^{7} cfu/ml (approximately 2 log cycle). When stored at room temperature, the viable cell of *L. casei* sub.sp. *paracasei* F-19 decrease from 1.7×10^{11} to 3.3×10^{7} cfu/ml, *L. plantarum* V299 decrease from 1.9×10^{10} to 1.2×10^{6} cfu/ml and *L. fermentum* 2311M decrease from 9.0×10^{8} to 1.9×10^{4} cfu/ml (approximately 4 log cycle) and final product texture after storage for 30 days at 4°C and room temperature in aluminum seal bags were not difference, showed very fine and non-caking powder. (showed in table 4.3 and fig. 4.4 - 4.9).

Table 4.3 Viable cells of probiotic bacteria in spray dried skim milk powders during storage at 4°C and room temperature for 30 days at 75°C of the air outlet temperature condition. 2% starter cells in aluminum seal bag.

Storage time (day)	Viable cell in aluminum bag (cfu/ml)					
	L. casei F-19		*L. plantarum* V299		*L. fermentum* 2311M	
	4°C	RT	4°C	RT	4°C	RT
0	1.7×10^{11}	1.7×10^{11}	1.9×10^{10}	1.9×10^{10}	9.0×10^{8}	9.0×10^{8}
15	9.1×10^{10}	1.8×10^{9}	9.8×10^{9}	9.2×10^{8}	8.1×10^{7}	5.9×10^{6}
30	2.2×10^{9}	3.3×10^{7}	2.0×10^{9}	1.2×10^{6}	1.3×10^{7}	1.9×10^{4}

The temperature is critical for microbial survival during storage, and higher survival rates have been obtained at lower storage temperatures (Jonhson and Etzel, 1993).

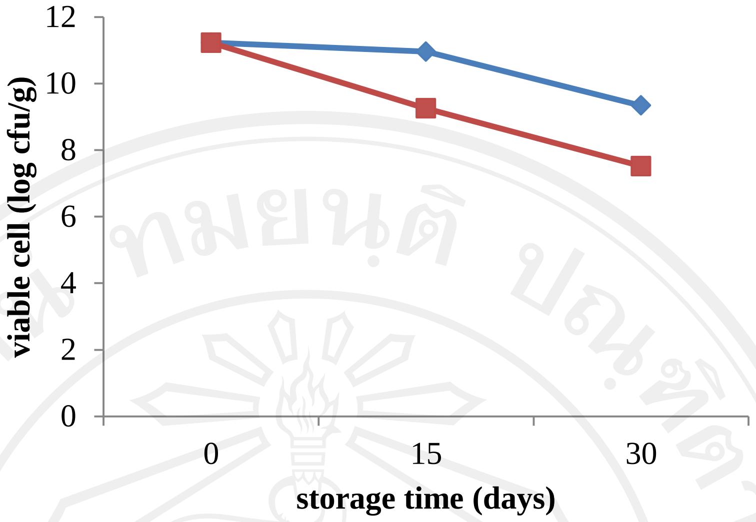

Figure 4.4 Viable cell of *L. casei* sub.sp. *paracasei* F-19 in skim milk spray dried powder during storage at 4°C (◆) and room temperature (■) for 30 days at 75°C of AOT condition.

Figure 4.5 *L. casei* sub.sp. *paracasei* F-19 in skim milk powder product after storage 1 month at 4°C and room temperature in aluminum seal bag.

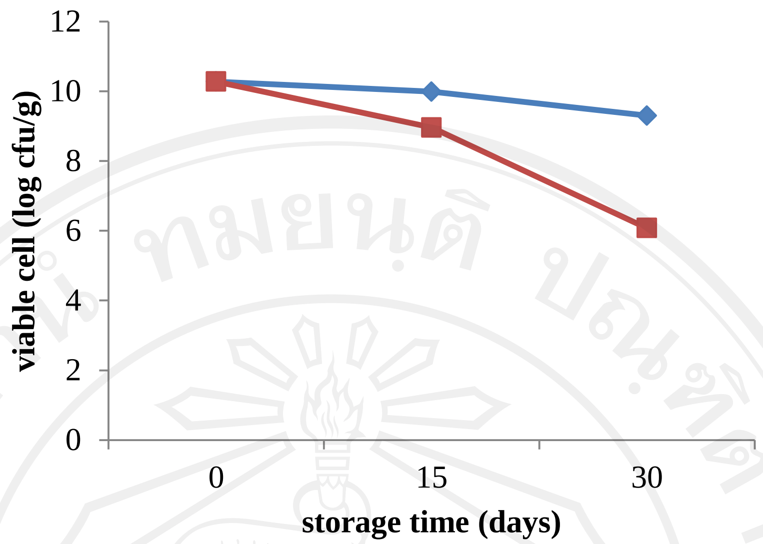

Figure 4.6 Viable cell of of *L. plantarum* V299 in skim milk spray dried powder during storage at 4°C (◆) and room temperature (■) for 30 days at 75°C of AOT condition.

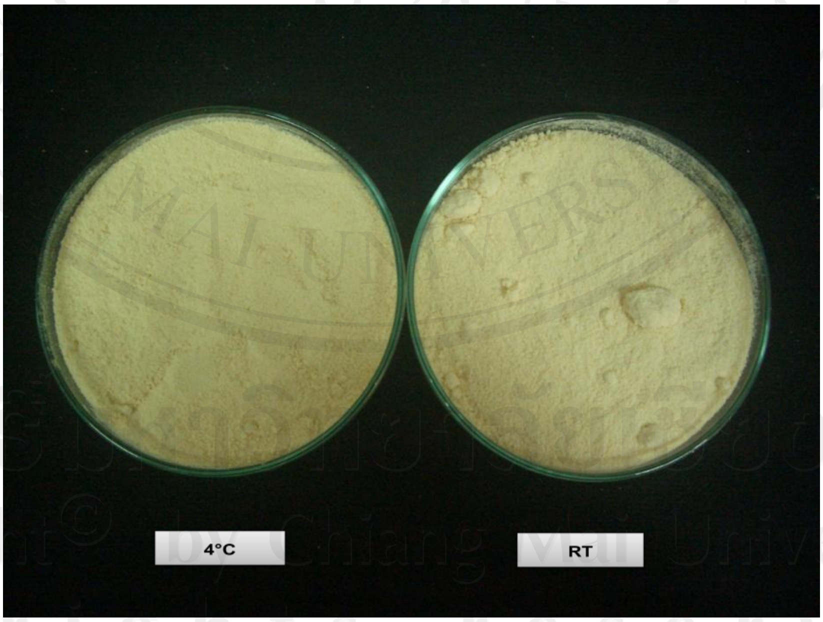

Figure 4.7 *L. plantarum* V299 in skim milk powder product after storage 1 month at 4°C and room temperature in aluminum seal bag.

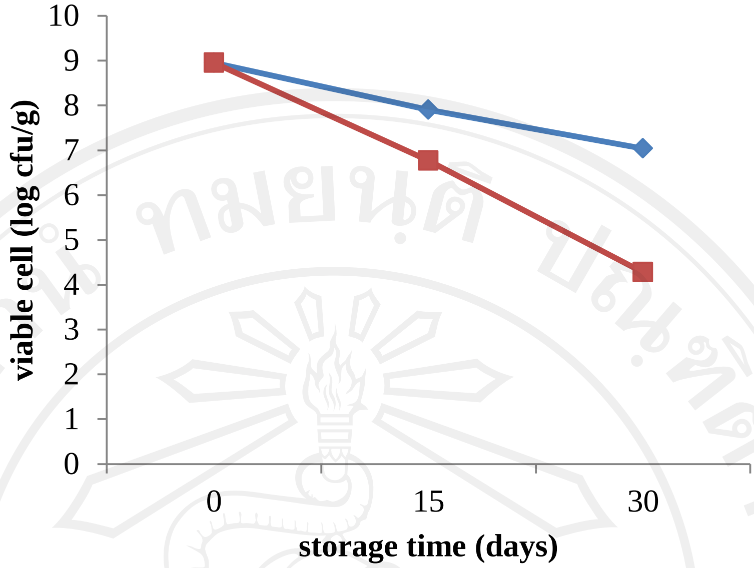

Figure 4.8 Viable cell of of *L. fermentum* 2311M in skim milk spray dried powder during storage at 4°C (◆) and room temperature (■) for 30 days at 75°C of AOT condition.

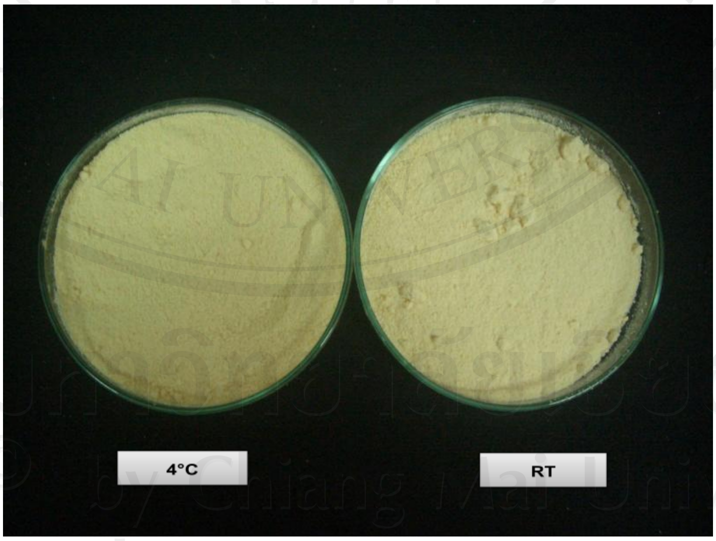

Figure 4.9 *L. fermentum* 2311M in skim milk powder product after storage 1 month at 4°C and room temperature in aluminum seal bag.

4.2.2.2.2 Storage in plastic zip bag.

Probiotic bacteria in skim milk spray dried powder that were produce by spray drying at 75°C of the air outlet temperature condition and used 2% (w/v) starter probiotic cell were stored at different temperature for 30 days in plastic zip bags. It was found that the viable cells when stored at 4°C of *L. casei* sub.sp. *paracasei* F-19 decrease from 1.7×10^{11} to 4.8×10^{6} cfu/ml, *L. plantarum* V299 decrease from 1.9×10^{10} to 3.4×10^{5} cfu/ml and *L. fermentum* 2311M decrease from 9.0×10^{8} to 4.7×10^{4} cfu/ml (approximately 5 log cycle). When stored at room temperature, the viable cell of *L. casei* sub.sp. *paracasei* F-19 decrease from 1.7×10^{11} to 1.6×10^{2} cfu/ml, *L. plantarum* V299 decrease from 1.9×10^{10} to 1.0×10^{2} cfu/ml and *L. fermentum* 2311M decrease from 9.0×10^{8} to 86 cfu/ml (approximately 9 log cycle) and final product texture after storage for 30 days in plastic zip bags at 4°C showed very fine and non-caking powder but stored at room temperature were few caking and dark color (showed in table 4.4 and fig. 4.10 - 4.15).

Table 4.4 Viable cells of probiotic bacteria in spray dried skim milk powders during storage at 4°C and room temperature for 1 month at 75°C of the air outlet temperature condition. 2% starter cells in plastic zip bag.

Storage	Viable cell in Plastic bag (cfu/ml)					
time	*L. casei* F-19		*L. plantarum* V299		*L. fermentum* 2311M	
(day)	4°C	RT	4°C	RT	4°C	RT
0	1.7×10^{11}	1.7×10^{11}	1.9×10^{10}	1.9×10^{10}	9.0×10^{8}	9.0×10^{8}
15	3.3×10^{8}	2.4×10^{4}	6.1×10^{7}	3.6×10^{4}	8.3×10^{6}	2.6×10^{3}
30	4.8×10^{6}	1.6×10^{2}	3.4×10^{5}	1.0×10^{2}	4.7×10^{4}	86

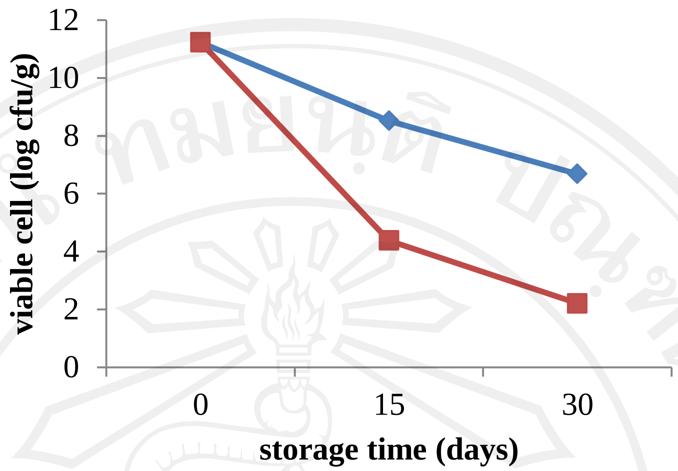

Figure 4.10 Viable cell of of *L. casei* sub.sp. *paracasei* F-19 in skim milk spray dried powder during storage at 4°C (◆) and room temperature (■) for 30 days at 75°C of AOT condition.

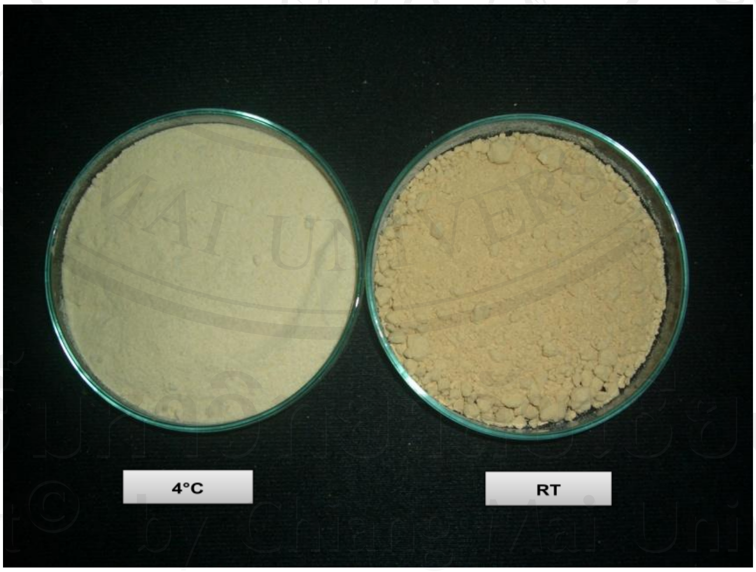

Figure 4.11 *L. casei* sub.sp. *paracasei* F-19 in skim milk powder product after storage 1 month at 4°C and room temperature in plastic zip bag.

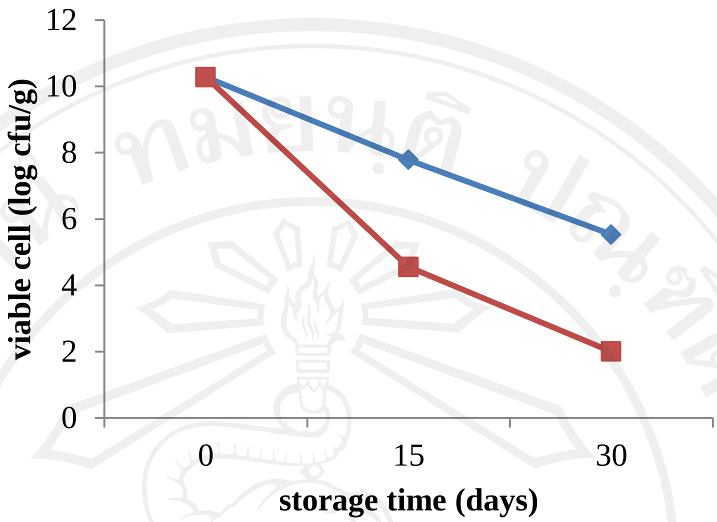

Figure 4.12 Viable cell of of *L. plantarum* V299 in skim milk spray dried powder during storage at 4°C (◆) and room temperature (■) for 30 days at 75°C of AOT condition.

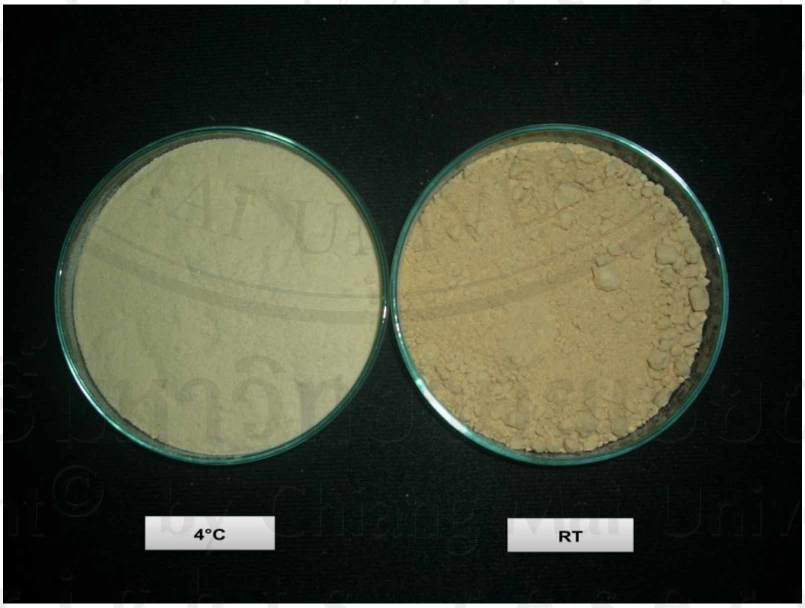

Figure 4.13 *L. plantarum* V299 in skim milk powder product after storage 1 month at 4°C and room temperature in plastic zip bag.

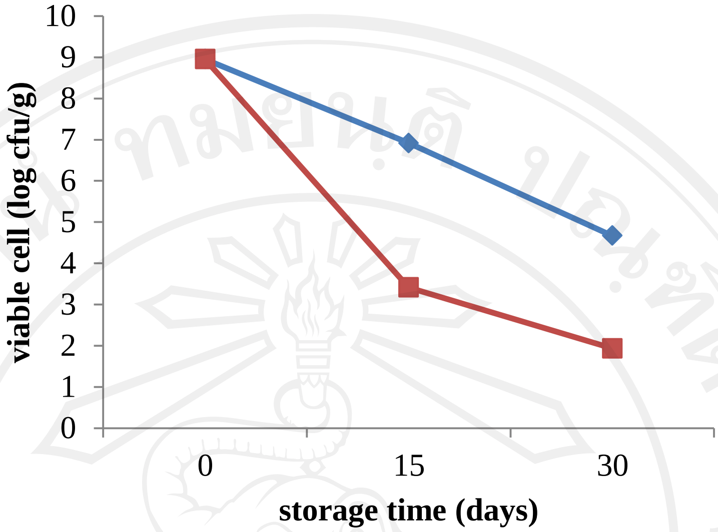

Figure 4.14 Viable cell of of *L. fermentum* 2311M in skim milk spray dried powder during storage at 4°C (◆) and room temperature (■) for 30 days at 75°C of AOT condition.

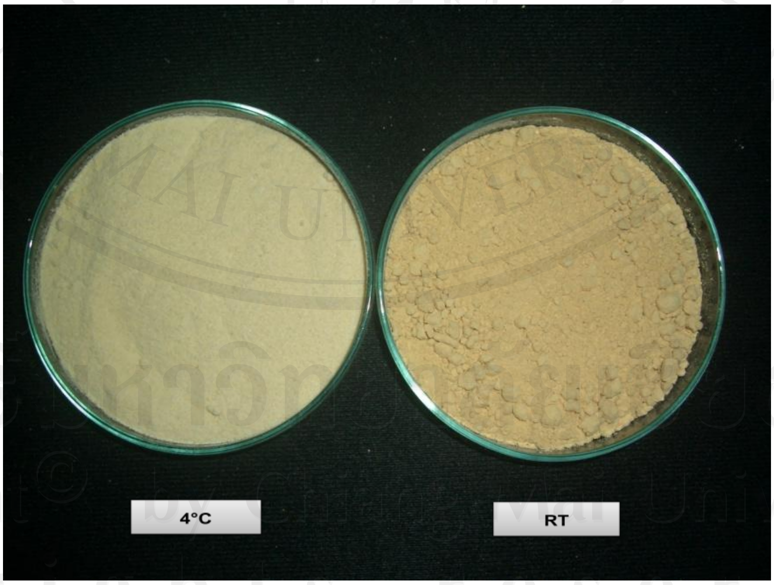

Figure 4.15 *L. fermentum* 2311M in skim milk powder product after storage 1 month at 4°C and room temperature in plastic zip bag.

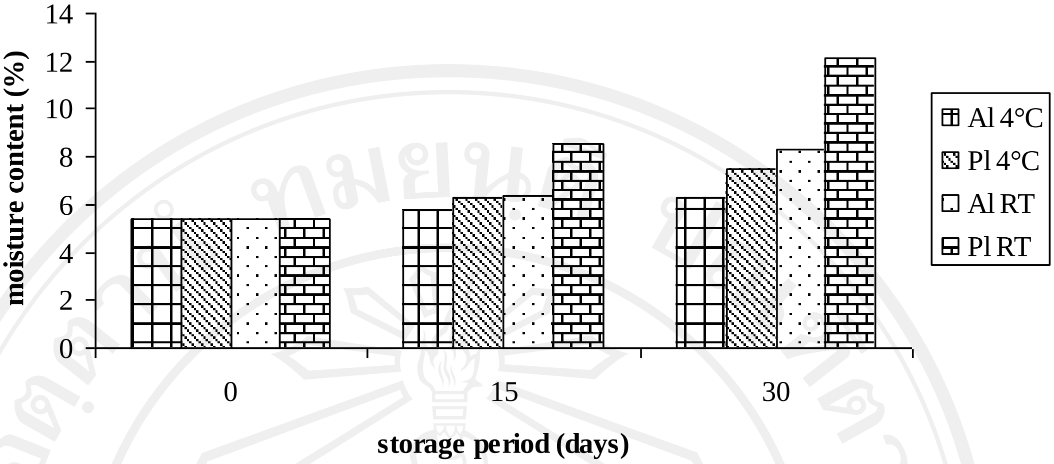

Figure 4.16 Moisture content (%) of *L. casei* subsp. *Paracasei* F-19 containing skim milk powder affected by storage temperatures and packaging materials**.** Al 4 ^{o}C : aluminum bag stored at 4^{o}C, Pl 4^{o}C: plastic bag stored at 4^{o}C, Al RT : aluminum bag stored at room temperature and Pl RT : plastic bag stored at room temperature.

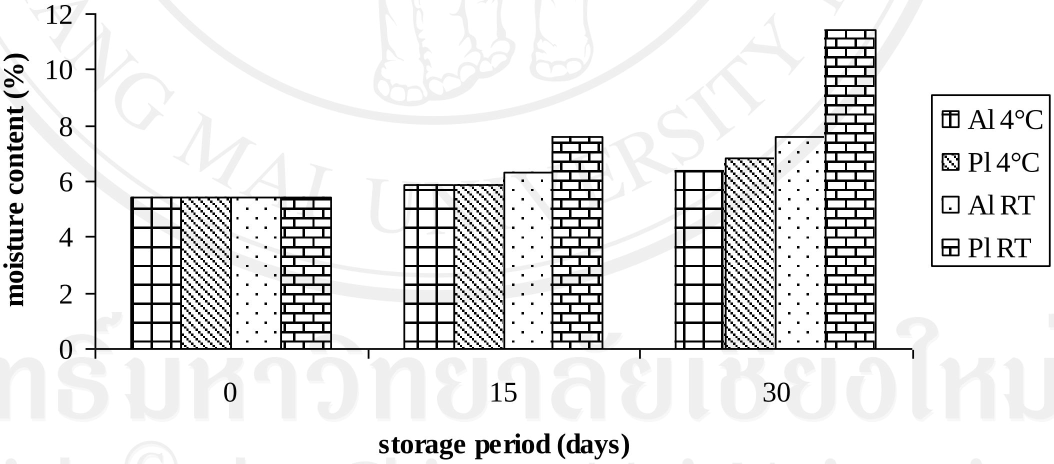

Figure 4.17 Moisture content (%) of *L. plantarum* V299 containing skim milk powder affected by storage temperatures and packaging materials**.** Al 4 ^{o}C : aluminum bag stored at 4^{o}C, Pl 4^{o}C: plastic bag stored at 4^{o}C, Al RT : aluminum bag stored at room temperature and Pl RT : plastic bag stored at room temperature.

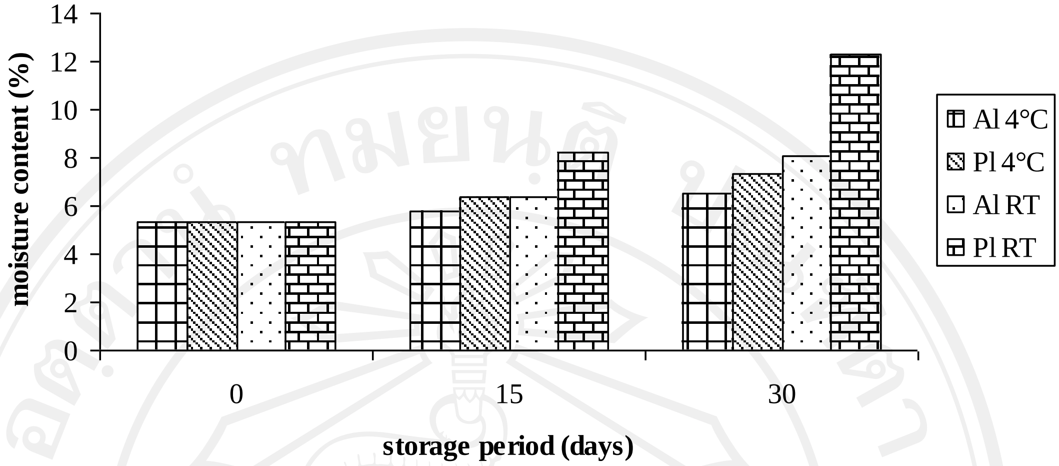

Figure 4.18 Moisture content (%) of *L. fermentum* 2311M containing skim milk powder affected by storage temperatures and packaging materials**.** Al 4 °C : aluminum bag stored at 4°C, Pl 4°C: plastic bag stored at 4°C, Al RT : aluminum bag stored at room temperature and Pl RT : plastic bag stored at room temperature.

Both the storage temperature and the packaging materials contributed in the increase of the powder moisture content. The highest increase of the moisture content was displayed when the powder was packed in the plastic zip bag and kept at room temperature. The powder in this treatment had a moisture content of more than 7% after 15 days storage period indicating the unsuitability of the packaging material to keep the product (Baker, 1997 and Corcoran *et al.,* 2006). The aluminum sealed bag was shown to be better in maintaining the moisture content of the powder, although it could not completely protect the food product. At lower storage temperature, the moisture content of the powder was generally increased at a slower rate and there was not any significantly different between different packaging materials. Results in this experimental section suggested that other packaging materials would need to be used if the powder would be stored for more than one year because the deterioration of the product would be started once the moisture content of the product was more than 4% (Muir and Banks, 2000).

Different storage temperatures and packaging materials significantly affected the increase in the yoghurt powder water activity. The low water activity of the powder during the storage period would limit the chemical reactions that could occur inside the product, such as non-enzymatic browning reaction (Onwulata, 2005).

4.2 Study on survival rate and moisture content of probiotic bacteria in whipping cream solution for spray drying.

4.2.1 Cell survival and moisture content.

Another experiment of spray dry probiotic were cultured in milk and incubated at 37°C for 12 hours. Then mixed with 15% (w/v) whipping cream powder in sample before spray dried at 75°C of air outlet temperature, showed very fine and non-caking powder. The survival rate viable of *L. casei* ssp. *paracasei* F-19, *L. plantarum* V299 and *L. fermentum* 2311M were 21%, 22% and 8%, with moisture content at 5.0%, 5.5% and 4.8%, respectively (table 4.5 and fig.4.19)

Table 4.5 Summary number of probiotic before and after spray drying in whipping cream at 75°C of air outlet temperature.

probiotic type	viable cell before dry (cfu/ml)	viable cell after dry (cfu/ml)	survival rate (%)	moisture content (%)
L. casei F-19	1.1×10^{11}	2.4×10^{10}	21	5.0
L. plantarum V299	1.7×10^{10}	3.7×10^{9}	22	5.5
L. fermentum 2311M	6.6×10^{8}	5.0×10^{7}	8	4.8

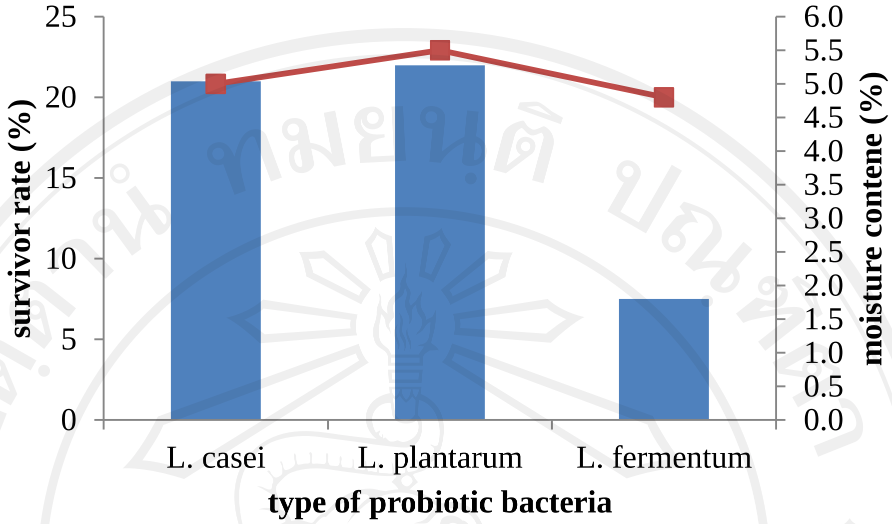

Figure 4.19 Survival of *L. casei sub.sp. paracasei* F-19, *L. plantarum* V299 and *L. fermentum* 2311M during spray drying in 15%(w/v) whipping cream at 75°C of air outlet temperatures (bar graph). The line showed the moisture content of the resulting powders.

4.2.2 Viable cells of probiotic bacteria in skim milk spray dried powder during shelf life storage.

Probiotic bacteria in whipping cream spray dried powder that were produce by spray drying at 75°C of the air outlet temperature condition and used 10% (v/v) starter probiotic were stored at different temperature for 30 days in aluminum seal bag. It was found that the viable cells when stored at 4°C of *L. casei* sub.sp. *paracasei* F-19 decrease from 2.4×10^{10} to 8.5×10^{7} cfu/g, *L. plantarum* V299 decrease from 3.7×10^{9} to 4.0×10^{7} cfu/g and *L. fermentum* 2311M decrease from 5.0×10^{7} to 9.8×10^{5} cfu/g (approximately 2 log cycle). When stored at room temperature viable cell of *L. casei* sub.sp. *paracasei* F-19 decrease from 2.4×10^{10} to 6.9×10^{6} cfu/g, *L. plantarum* V299 decrease from 3.7×10^{9} to 1.4×10^{5} cfu/g and *L. fermentum* 2311M decrease from 5.0×10^{7} to 1.3×10^{3} cfu/g (approximately 4 log cycle) and final product texture after storage for 30 days at 4°C and room temperature in aluminum seal bags were not difference, showed very fine and non-caking powder. (showed in table 4.6 and fig. 4.20 - 4.24).

It may be useful to evaluate the effect of adding protectants, such as dextrin and antioxidants like ascorbic acid and monosodium glutamate, during spray drying. These additives have improved culture viability during powder storage (Teixeira *et al*., 1995a), although other studies have shown that dextrin has little effect (Jonhson and Etzel, 1993) and antioxidants and oxygen absorbers have detrimental effects on culture stability during storage (Champagne *et al*., 1995).

Spray drying was affected to decrease the viable cells and change product properties. Condition of storage had effect to viable cells and characteristics of product. The factors effecting between storage were based on the initial cell in product culture (Morichi. 1974), storage conditions (Gilliland, 1981) and the residual moisture content in powder (De Valdez *et al*., 1985).

Table 4.6 Viable cells of probiotic bacteria in spray dried whipping cream during storage at 4°C and room temperature for 30 days at 75°C of the air outlet temperature condition and 10% (w/w) starter cells in aluminum seal bag.

Storage	Viable cell in aluminum bag (cfu/ml)					
time	*L. casei* F-19		*L. plantarum* V299		*L. fermentum* 2311M	
(day)	4°C	RT	4°C	RT	4°C	RT
0	2.4×10^{10}	2.4×10^{10}	3.7×10^{9}	3.7×10^{9}	5.0×10^{7}	5.0×10^{7}
15	4.7×10^{9}	3.7×10^{8}	2.5×10^{8}	4.6×10^{7}	2.7×10^{6}	5.8×10^{5}
30	8.5×10^{7}	6.9×10^{6}	4.0×10^{7}	1.4×10^{5}	9.8×10^{5}	1.3×10^{3}

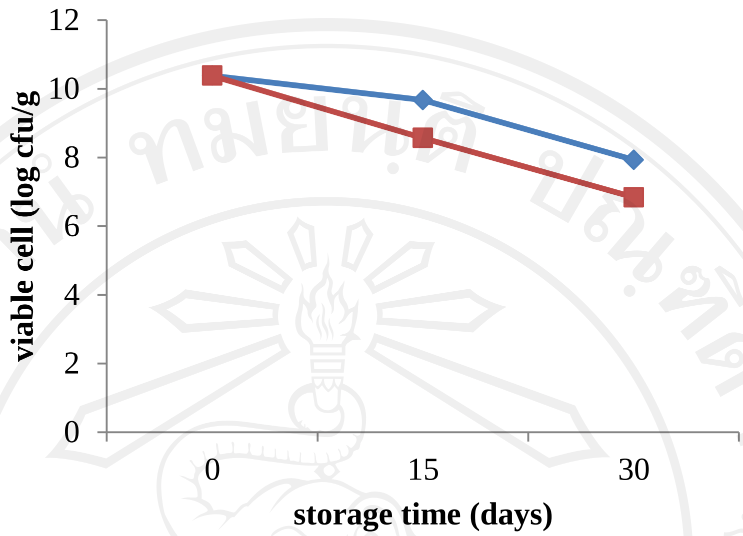

Figure 4.20 Viable cell of *L. casei* sub.sp. *paracasei* F-19 in whipping cream spray dried powder during storage at 4°C (◆) and room temperature (■) for 30 days at 75°C of AOT condition.

Figure 4.21 *L. casei* sub.sp. *paracasei* F-19 in whipping cream powder product of after storage 1 month at 4°C and room temperature in aluminum seal bag.

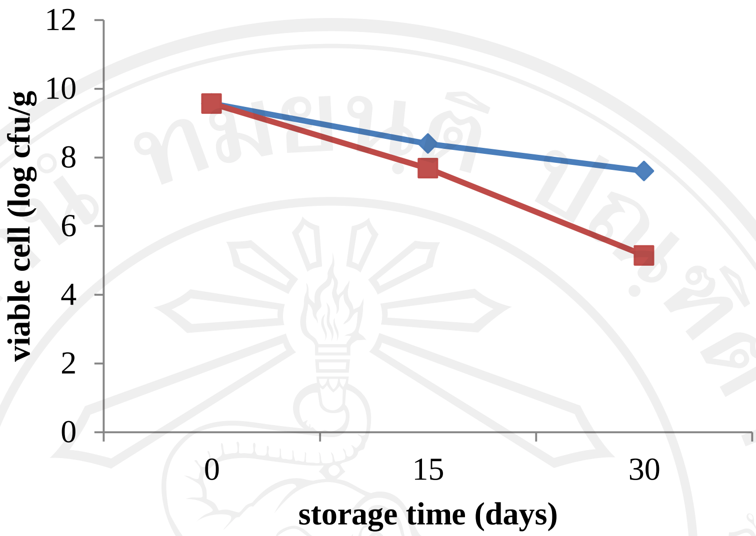

Figure 4.22 Viable cell of *L. plantarum* V299 in whipping cream spray dried powder during storage at 4°C (◆) and room temperature (■) for 30 days at 75°C of AOT condition.

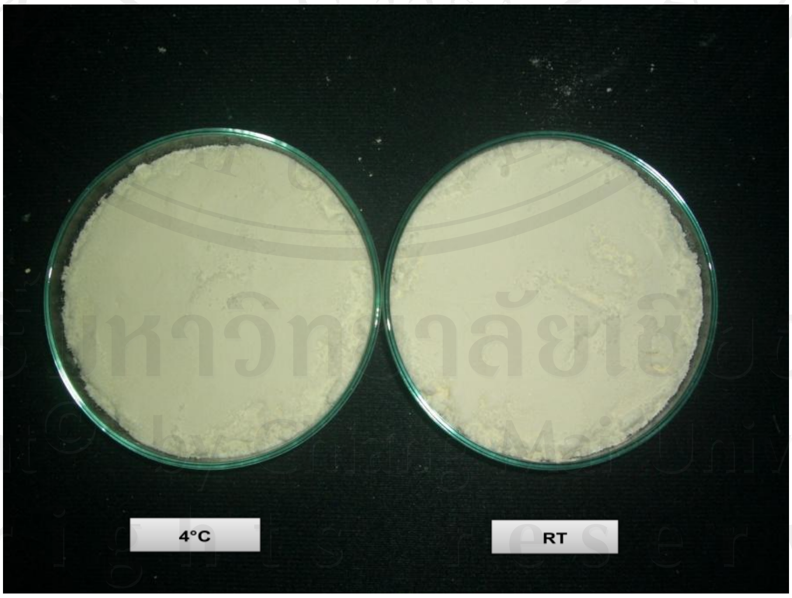

Figure 4.23 *L. plantarum* V299 in whipping cream powder product of after storage 1 month at 4°C and room temperature in aluminum seal bag.

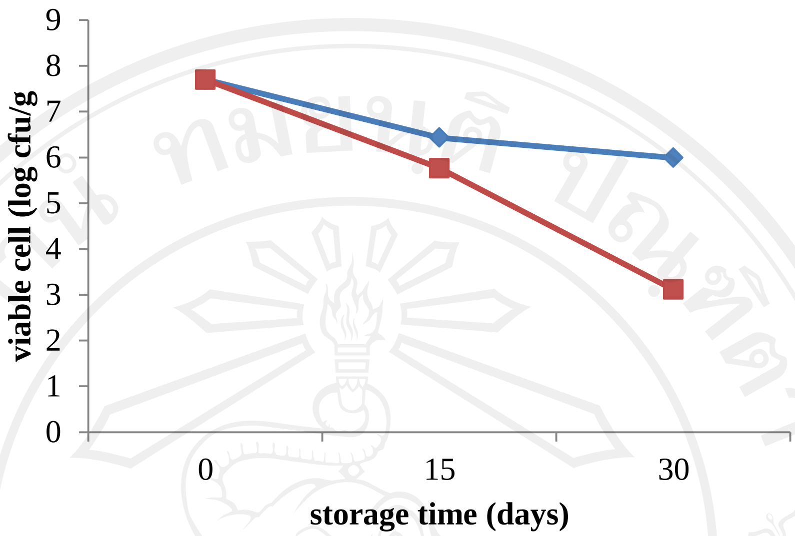

Figure 4.24 Viable cell of *L. fermentum* 2311M in whipping cream spray dried powder during storage at 4°C (◆) and room temperature (■) for 30 days at 75°C of AOT condition.

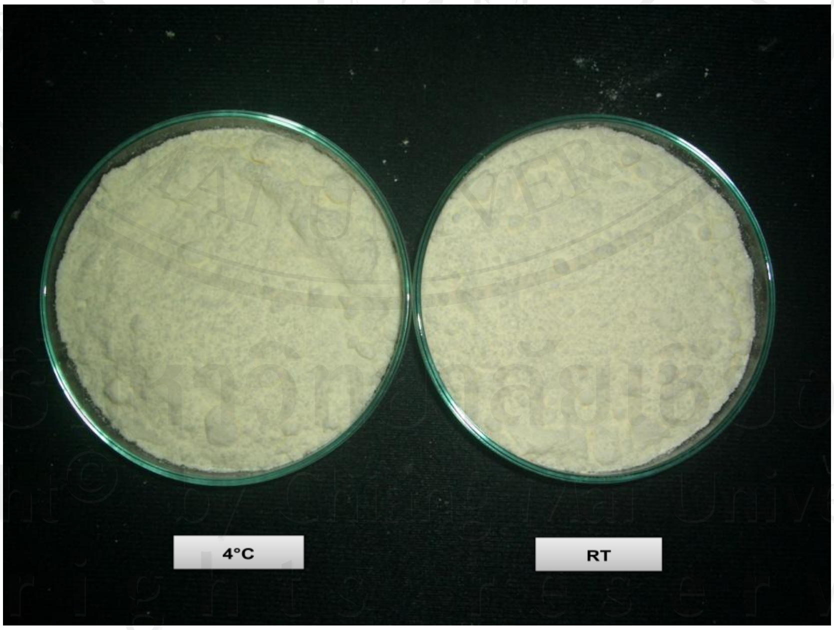

Figure 4.25 *L. fermentum* 2311M in whipping cream powder product of after storage 1 month at 4°C and room temperature in aluminum seal bag.