CHAPTER 4
RESULT AND DISCUSSION

4.1 Finding a suitable concentration of supporting material to immobilized *Lactobacillus acidophilus*

This section was used to investigate the effect of different hi-maize starch concentrations on the physical property of calcium alginate beads and its capability to retain *L. acidophilus* cells. The concentration of sodium alginate and calcium chloride used in this section were 1.8 % (w/v) and 0.1 M, respectively, because of their effective capability to maintain the viability of *L. acidophilus* CSCC 2400 in stimulated gastric conditions (pH 2.0 for 3 h at 37^{o}C) (Chandramouli *et al.*, 2004). The studied hi-maize starch concentration was 0.5 to 2.0% (w/v). These hi-maize concentrations did not significantly affect the bead diameter (Table 10) and the bead volume, which were calculated based on the radius of the beads ($4/3\pi r^3$). The bead volume could mainly be affected by the physical parameter used to prepare the beads, including the syringe size and the distance between the syringe tips with the calcium chloride solutions (Kraseakoopt *et al.,* 2003, Chandramouli *et al.,* 2004 and Anal and Singh, 2007). However, different hi-maize concentrations significantly affected the bead density. The highest bead density was achieved when 1.0% (w/v) hi-maize was added into the alginate solution (Table 10 and Fig. 7). This finding suggested that at this hi-maize concentration, higher levels of hi-maize concentration could be incorporated into the alginate-hi-maize beads, which could lead to higher surface area for probiotic bacteria to attach to the beads. This result was also consistent with the report of Kailasapathy (2005).

When the calcium alginate-hi-maize beads were inoculated with *L. acidophilus*, the immobilized *L. acidophilus* cells in the beads was released in phosphate buffer (0.1 M, pH 7.0) (Sheu and Marshall, 1993) using 2 microbiological procedure, which were gentle shaking for 30 min and a stomacher for 20 min. Between these 2 techniques, it was found that significantly higher numbers of *L. acidophilus* could be enumerated after applying a

Chandramouli, V., Kailasapathy, K., Peiris, P. and Jones, M. (2004) An improved method of microencapsulation and its evaluation to protect Lactobacillus spp. in simulated gastric conditions. . Journal of Microbiology Methods, 56, 27-35.
Kailasapathy, K. (2005) Survival of free and encapsuated probiotic bacteria and their effect on the sensory properties of yoghurt. LWT.

stomacher compared to the gentle shaking (Table 11). At the same time, the stomacher method was applied in the next analysis procedure

Table 10 Physical characteristic of calcium alginate –hi-maize starch beads*

Hi-maize concentrations (% (w/v))	Bead diameter (mm)	Bead volume (mm^3)	Bead density (g/ml)
0.5	$2.14^{a} \pm 0.22^{*}$	$5.1279^{a} \pm 1.47$	$103.82^{b} \pm 14.90$
1.0	$2.11^{a} \pm 0.23$	$5.0184^{a} \pm 1.50$	$119.82^{a} \pm 11.98$
1.5	$2.20^{a} \pm 0.24$	$5.5086^{a} \pm 1.61$	$113.32^{ab} \pm 12.19$
2.0	$2.20^{a} \pm 0.19$	$5.2518^{a} \pm 1.40$	$107.58^{a}b \pm 16.16$

* Each value in the table is the mean± standard deviation of three trials.

Values in the same column with different letters were significantly

Table 11 Different microbiological methods to release immobilized *L. acidophilus* from calcium alginate hi-maize starch beads using phosphate buffer (0.1 M at pH 7.0)

Item	The number of *L. acidophilus*		
	Before immobilization	Gentle shaking for 30 min	A stomacher for 20 min
1. Enumeration (log cfu/g)	7.1816 ± 0.02	6.1344 ± 0.11	6.871± 0.03
2. Cell recovery (%)		(85.42 %)	(95.77 %)

Different hi-maize concentrations were found to significantly affect the amount of *L. acidophilus* cells that were entrapped in the calcium alginate-hi-maize (Table 12). At 1.0% (w/v) hi-maize concentration, a significantly higher number of *L. acidophilus* could be released from the beads compared to those of the bead with higher hi-maize concentrations. This result could be affected by the physical characteristics of the bead that had a higher density value.

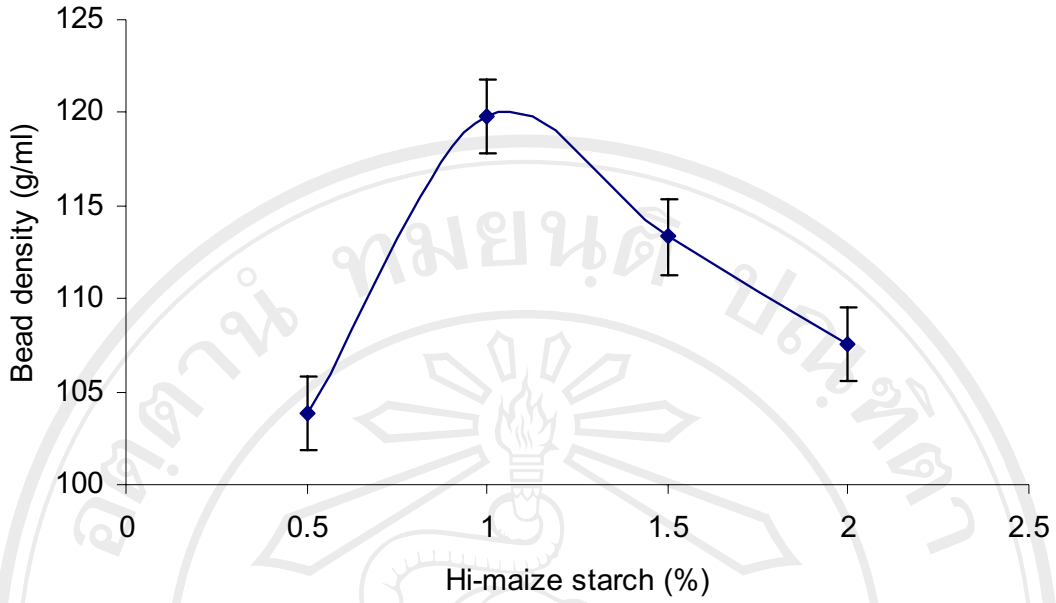

Fig. 7 The bead density of calcium alginate-hi-maize beads affected by hi-maize starch concentrations

Table 12 The cell recovery (%) of *L. acidophilus* released from calcium in alginate-hi-maize beads using 0.1 M phosphate buffer (pH 7.0) and a stomacher for 20 min

Hi-maize concentrations (% (w/v))	*L. acidophilus* before encapsulated (log cfu/ml)	*L. acidophilus* after encapsulated (log cfu/ml)	Cell recovery (%)
0.5	7.40 ±0.03*	7.07±0.13	95.59 ±1.69^{ab}
1.0	7.32 ±0.13	7.11 ±0.07	97.21 ±1.30^{a}
1.5	7.53±0.12	6.89 ±0.29	92.62 ±2.44^{bc}
2.0	7.68 ±0.06	6.95 ±0.25	90.48 ±3.03^{c}

* Each value in the table is the mean± standard deviation of two trials.

Values in the same column with different letters were significantly different by Duncan's multiple range test (p<0.05).

4.2 Finding a best addition time and concentration of immobilized *L. acidophilus* to produce a high recovery of *L. acidophilus* in yoghurt powder

From the previous section, a bead diameter of 2.11 ± 0.23 cm prepared from 1.8% (w/v) alginate, 1.0% (w/v) hi-maize starch and 0.1 M calcium chloride was statistically be chosen to be further studied in the survival of *L. acidophilus* in dried yoghurt. However, this bead diameter was bigger that the spray drier nozzle that would be used to dry the yoghurt. Due to this reason, an investigation to dry immobilized *L. acidophilus* using a vacuum drying method was carried out.

To find a good time and temperature combination to dry immobilized *L. acidophilus* beads by a vacuum drier, 2 preliminary experiments using plain yoghurt were carried out. The first experiment was conducted using a sample volume of 368.5 ml at 40 and 50^{o}C for 24 h drying time (Table 13). The results showed that the dried yoghurts had moisture contents of 9.19 ± 0.03 and 8.42 ± 0.07 after 24 h at 40 and 50^{o}C, respectively. These moisture contents were higher than 5% as was suggested by Corcoran *et al.* (2006) to be good moisture contents of dried milk products. The dried yoghurt processed at 50^{o}C significantly had whiter color and less red and yellow values compared to that of the yoghurt dried at 40^{o}C. This could be due to the higher temperature that produced a higher drying rate more than the lower temperature and gave the lower water activity. In general, the browning reaction of dairy product was due to the reaction between reducing sugar and amino acid called Maillard reaction. The water activity of 0.4-0.7 and a suitable condition would also affect the progress of this reaction (Baker 1997). There was a possibility that the yoghurt dried at 50^{o}C had a brighter appearance than that at 40^{o}C due to a significantly lower a_w of the first dried powder that did not support the browning reaction.

The second preliminary experiment was conducted to find a good condition in the vacuum dryer to dry plain yoghurt to a final moisture content of 5% or lower. This was done by reducing the sample volume from 368.5 ml (or a thickness of 0.50 cm in a 33.5 x 22.0 cm^2 tray) to 110 ml and 70 ml (or a thickness of 0.15 cm and 0.10 cm, respectively). Results of this experiment (Table 13) significantly displayed that reducing the sample volume produced dried yoghurt powders with moisture contents of less than 5%. Lower the initial sample volume was found to be significantly produced lower moisture content

and a_w. At the same time, applying lower initial sample volume significantly produced whiter yoghurt powder.

Table 13 Physical characteristic of dried yoghurt powder produced by a vacuum dryer

Physical characteristics	Initial yoghurt sample	Vacuum drying condition*			
		at 40°C for 24 h	Yoghurt volume (ml) dried at 50°C for 24 h		
			368.5 ml	110 ml	70 ml
1. Color					
L*	88.67 ± 0.33	72.05^{c} ± 1.75	76.90^{b} ± 2.10	78.33^{b} ± 1.52	85.39^{a} ± 0.91
a*	3.48 ± 0.30	3.93^{b} ± 0.38	0.28^{c} ± 0.21	6.15^{a} ± 0.76	3.38^{b} ± 0.42
b*	10.77 ± 1.01	27.82^{b} ± 0.90	26.24^{c} ± 1.51	30.11^{a} ± 1.46	24.25^{d} ± 0.92
2. Moisture content (% (w/v)	76.80 ± 0.04	9.19^{a} ± 0.03	8.42^{b} ± 0.07	4.01^{c} ± 0.04	2.28^{d} ± 0.00
3. a_w	0.89 ± 0.00	0.43^{a} ± 0.00	0.41^{b} ± 0.00	0.33^{c} ± 0.00	0.28^{d} ± 0.00

* Each value in the table is the mean± standard deviation of three trials.
Values in the same column and same row with different letters were significant different by Duncan's multiple range test (p<0.05).

From these two preliminary experiments, it could be concluded that drying yoghurt using a vacuum drier was affected not only by the time and temperature of the drying but also by the initial yoghurt sample volume. Since an initial yoghurt sample of 70 ml (or a thickness 0.10 cm) produced a good moisture content of dried yoghurt, this initial sample volume was chosen to be studied further. In addition, drying temperatures of 40 and 50°C with a maximum drying time of 24 h were considered to be appropriate to dry the yoghurt using a vacuum drier.

When the results of the preliminary experiment were applied into *L. acidophilus* containing yoghurt, drying the yoghurt with an initial free or immobilized *L. acidophilus* cells of 7.11 log cfu/ml at 50°C produced a dried yoghurt powder without any viable *L. acidophilus* cells. This finding indicated that eventhough *L. acidophilus* is a mesophilic bacterium (Wang *et al.,* 2004), drying the microorganism at 50°C for 24 h significantly affected the viability of the bacterium. Similar finding was also reported by Kim *et al.* (1997) that found drying plain yoghurt using a microwave vacuum drying at 50-60°C produced a high lethality rate for *Streptococcus thermophilus* and *Lactobacillus*

bulgaricus. Due to this reason, the main experiment for drying the *L. acidophilus* containing yoghurt was conducted at 40 and 45°C for 16, 20 and 24 h.

Krasaekoopt *et al. (*2006) reported that adding the probiotic before and after yoghurt fermentation was not significantly different and since in this section the focus was to study the survival of *L. acidophilus* after a drying process, therefore, the free or encapsulated cells of *L. acidophilus* were added after the yoghurt fermentation. The yoghurt containing probiotic bacteria at different forms was dried by a vacuum oven at 40 and 45°C for 16, 20 and 24 h. Since this study was interested on the survival rate of *L. acidophilus* during a vacuum drying process and the physical characteristics of the dried yoghurt powder, the survival rate of *S. thermophilus* and *L. bulgaricus* were not enumerated. In general, the survival rate of *L. acidophilus* was not significantly affected by the drying temperature, eventhough lower survival rate was found when *L. acidophilus* cells were dried at 45°C compared to those that dried at 40°C (Fig. 8). Increasing the drying time also did not produce significant reduction in the survival rate of the probiotic bacterium. However, applying 20 h drying time produced a better survival rate of *L. acidophilus* compared to those of the other 2 drying temperatures. Moreover the free cells of *L. acidophilus* was found to have a higher survival rate than those of the immobilized *L. acidophilus* cells, except when the probiotic cells were dried at 45°C for 24 h. This result indicated that an immobilized form of a probiotic bacterium could not protect the microorganism from a drying process. During the vacuum drying, water in food was evaporated at a low temperature under a vacuum condition which caused the pressure to be lower than atmospheric pressure. The absolute pressure of this vacuum condition was 0.1987 bar. From a water phase diagram (appendix D, it showed that at 40 and 45°C of the vacuum conditions, the water in the sample was evaporated at 313 and 318°K, respectively. The *L. acidophilus* cells inside the calcium alginate-hi-maize beads might receive a higher accumulated heat than that of the non-immobilized probiotic cells because the beads had a thicker layer more than the yoghurt structure. In general, a low vacuum time should prevent bacteria from dying but the result revealed that drying for 20 h gave a higher survival rate more than the other drying times. Results in this study suggested that there was an optimum vacuum condition to maintain a high survival rate of *L. acidophilus*. A further investigation using an optimization design technique, such as response surface analysis between vacuum drying time, drying temperature and survival rate of a microorganism might need to be carried out to support the results in this research.

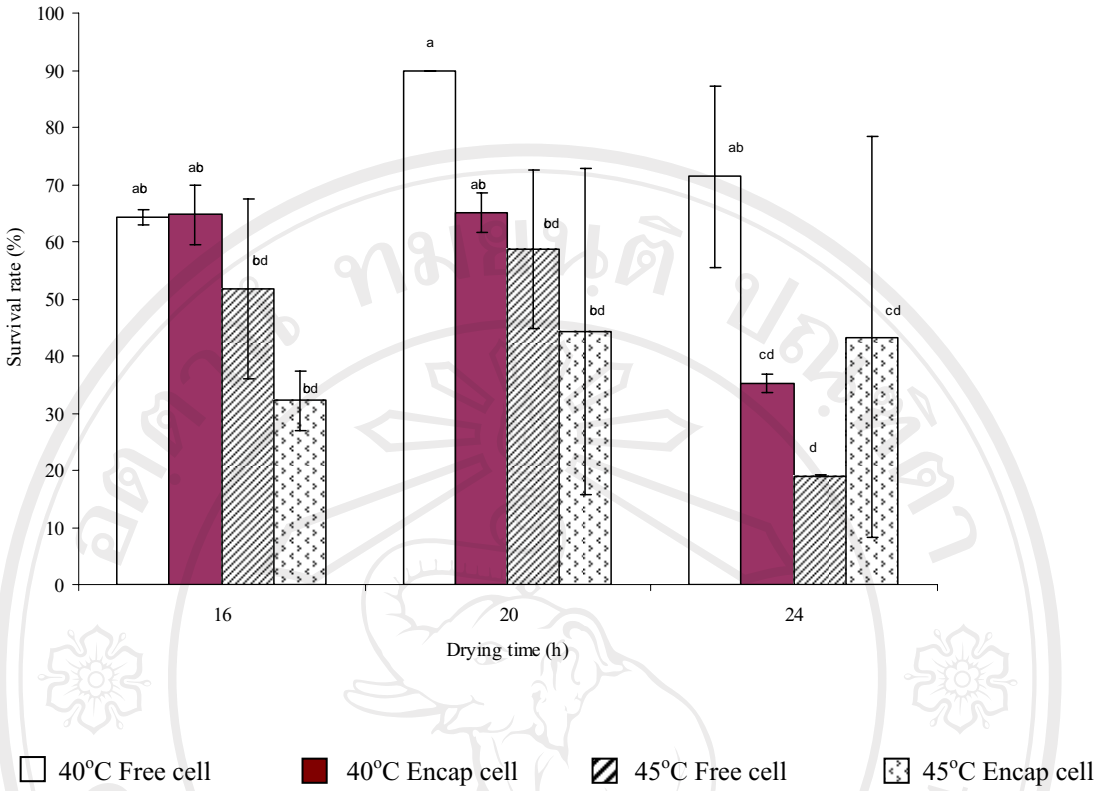

Fig. 8 The survival rate of *L. acidophilus* cells (%) in dried yoghurt affected by the different presentation forms of the probiotic cells, drying temperature and drying times

From the different treatments applied for the *L. acidophilus* cells, it could be concluded that the highest survival rate of *L. acidophilus* was achieved after drying the cells at 40°C for 20 h. Drying the yoghurt was found to significantly affect the yoghurt color, especially the L* and b* values. The initial yoghurt samples before drying had a color values of 88.67 ± 0.33, 10.77 ± 1.01 and 3.48 ± 0.30 for L*, a* and b* values, respectively. After drying at 40 and 45°C for 16-24 h, the yogurt significantly had lower L* value and higher b* value (Fig. 9). Different treatments applied to the yoghurt did not display any significant pattern for the yoghurt color. However, drying for 16 h produced a darker color compared to those samples dried at longer drying times. The highest L* value was found in the dried yoghurt processed at 40°C for 20 h. The a* value gave the highest value when a vacuum temperature at 45°C for 24 h was used while the lowest value was at 40°C for 20 h. The b* value indicated browning reaction because the yoghurt was yellow color when the b* value was high. When the vacuum time was increased the b* value was decreased because the yoghurt powder at 16 h had the water activity more than the others. The high water activity induced browning reaction (Baker, 1997) and the

shorter time was not enough to dry the product. Therefore, the b* value of yoghurt powder at 16 h was higher than the other treatments as well as darker than the other treatments.

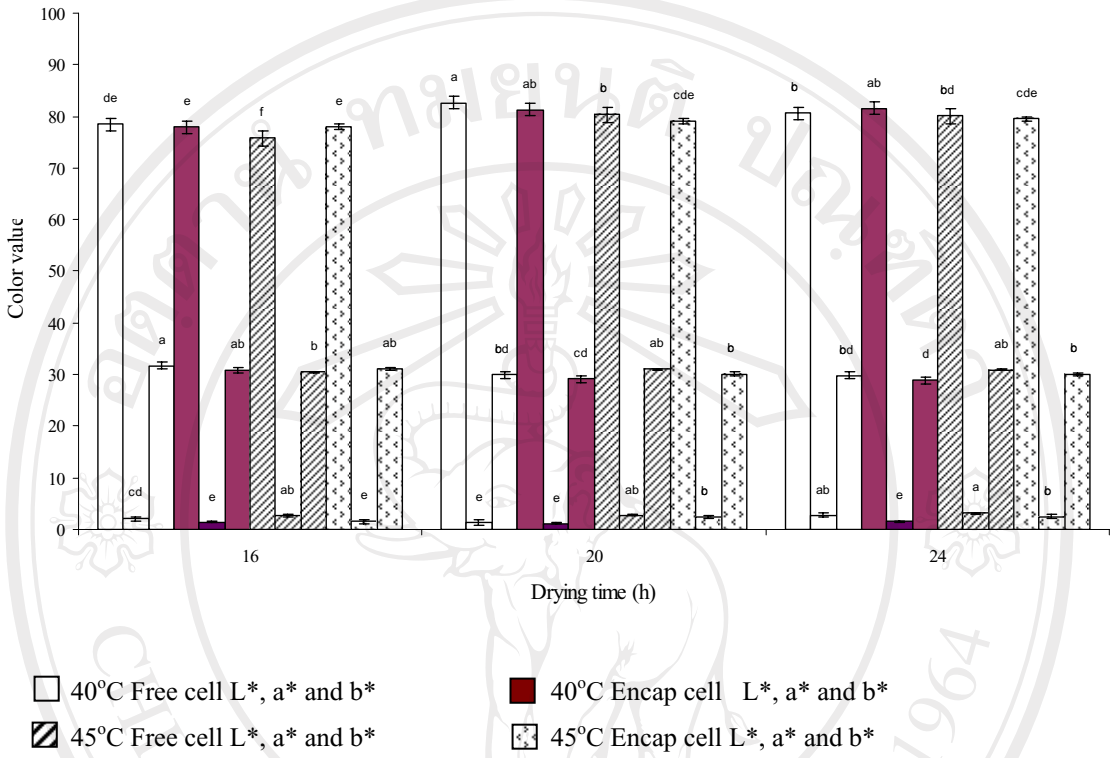

Fig. 9 Color values of *L acidophilus* containing dried yoghurt affected by different presentation forms of the probiotic cells, drying temperatures and drying times

Since water activity of dried yoghurt affects chemical reactions, such as non-enzymatic browning reaction (Tamine and Robinson, 1999 and Onwulata, 2005) and microorganism growth (Baker, 1997), the a_w and moisture contents of different yoghurt treatments were analyzed. The moisture content of dried yoghurt was significantly affected by the drying time (Fig. 10). Longer drying times produced lower moisture contents, which was a similar finding to a report by Kim *et al.* (1997). The different presentation forms of *L. acidophilus* cells and different drying temperatures did not significantly affect the moisture content of the dried yoghurt. However, the different vacuum time was significantly affected to the moisture content.

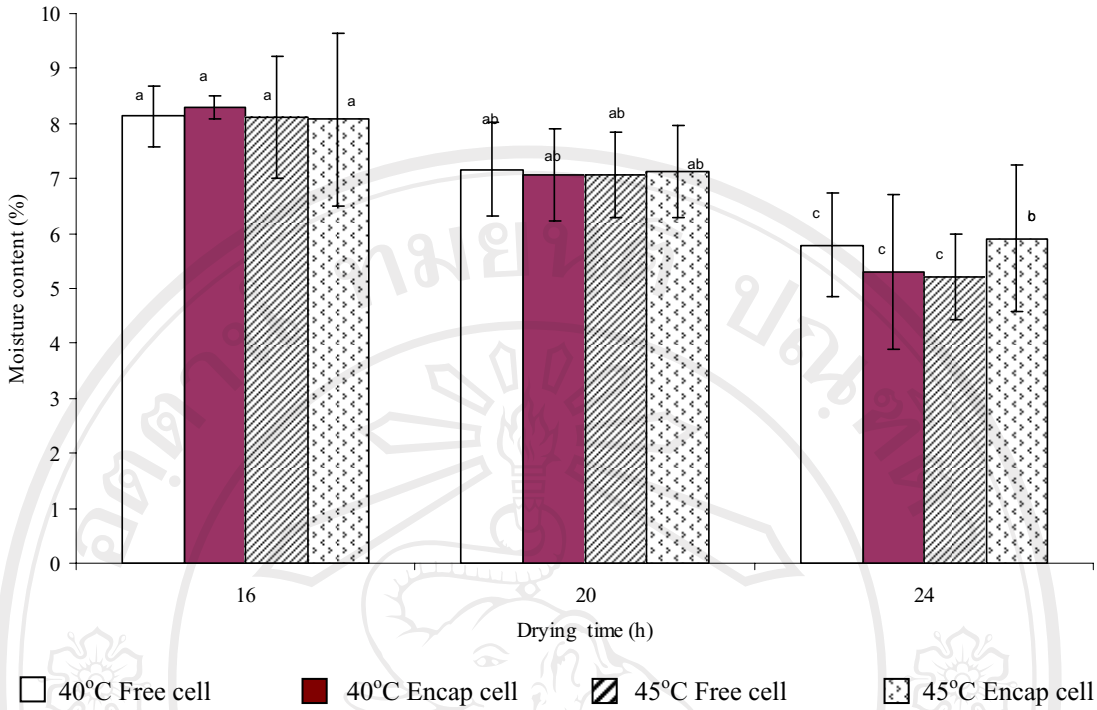

Fig. 10 Moisture content (%) of *L. acidophilus* containing dried yoghurt affected by different presentation forms of the probiotics cells, drying temperature and drying times

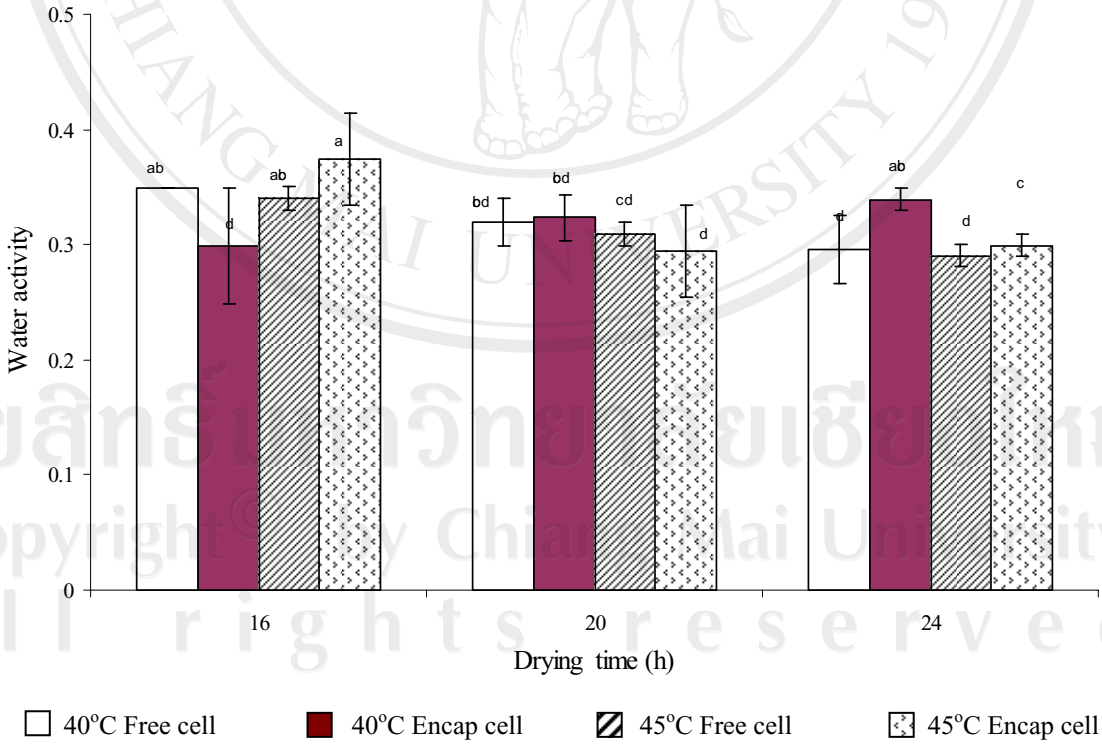

Fig. 11 Water activity of *L. acidophilus* containing dried yoghurt affected by different presentation forms of the probiotics cells, drying temperature and drying times

The rehydration characteristic of the dried yoghurt shown in Fig. 12 displayed that different drying temperatures and drying times did not significantly affect the yoghurt rehydration property. However, since lower rehydration value indicated better water absorption, drying the yogurt for 20 h, especially at 45°C drying temperature, produced lower rehydration values compared to those of the other 2 drying times. The presence of immobilized *L. acidophilus* cells also displayed higher rehydration values compared to those of the free cells. This indicated that the immobilized form of *L. acidophilus* could not absorb water easily and could cause a problem in the rehydrated yoghurt solution.

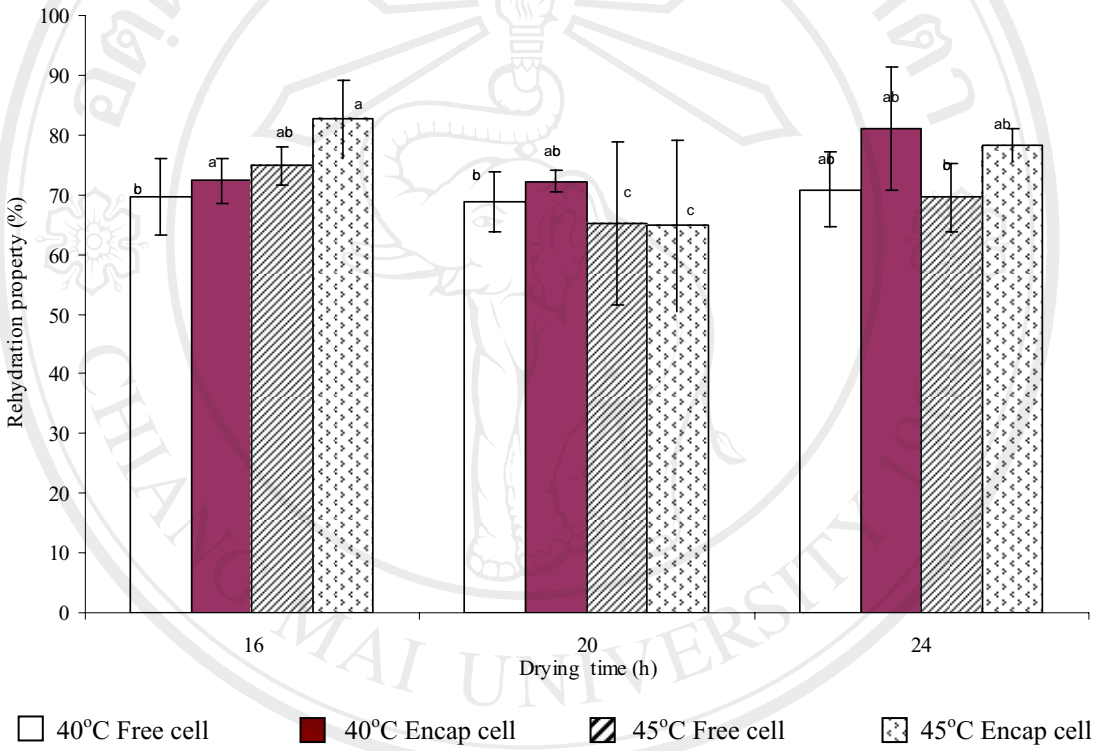

Fig. 12 Rehydration property (%) of *L. acidophilus* containing dried yoghurt affected by different presentation forms of the probiotics cells, drying temperature and drying times

For the dispersibility property of the dried yoghurt (Fig. 13) different presentation forms of *L. acidophilus* cells, different drying times and drying temperature did not significantly affect the yoghurt physical property.

Applying different drying temperature and drying times did not significantly affect the yield of the yoghurt powder (Fig. 14). However, the presence of immobilized *L. acidophilus* cells generally produced higher yield of yoghurt powder compared to those of

the free cells. This could be due to the presence of the beads in the yoghurt that increased the amount of solid in the yoghurt.

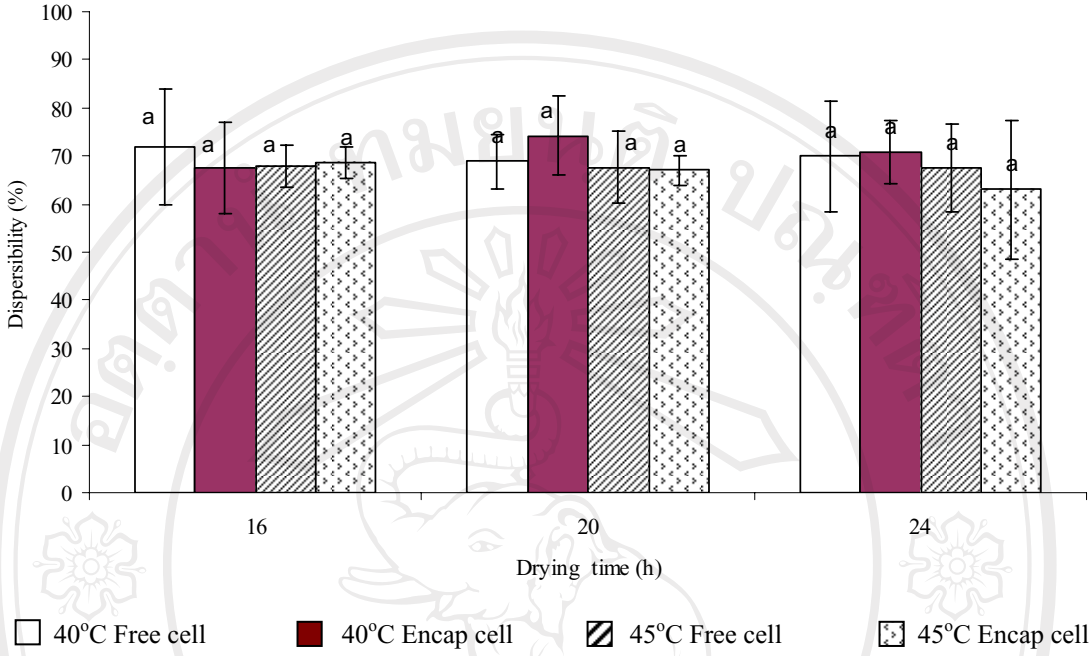

Fig. 13 Dispersibility property (%) of *L. acidophilus* containing dried yoghurt affected by different presentation forms of the probiotics cells, drying temperature and drying times

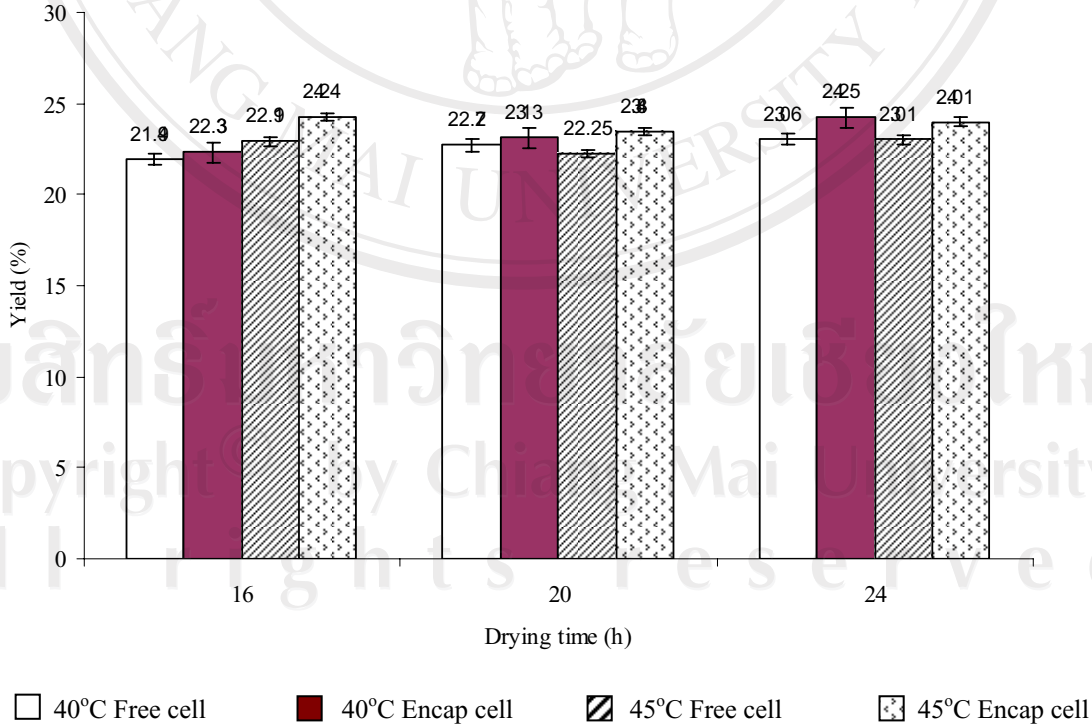

Fig. 14 Yield (%) of *L. acidophilus* containing dried yoghurt affected by different presentation forms of the probiotics cells, drying temperature and drying times

4.3 Production of dried yoghurt powder using different outlet temperature of a spray drier.

Basic yoghurt was prepared by mixing milk powder 15.5% (w/v), skimmed milk powder 10.0% (w/v), carrageenan 0.075% (w/v) and distilled water 74.43% (w/v) following the procedure of Sankhavadhana (2001). The thoroughly mixed solution was then heated to 80-85^{o}C for 5 min, cooled immediately to 45^{o}C and aseptically added with 0.02% (w/v) freeze dried starter culture, that was composed of *S. thermophilus* and *L. bulgaricus* at a ratio of 1:1. The yoghurt fermentation was carried out at 43^{o}C for up to 6 h in which the yoghurt reached a pH value of 4.6. The fermentation process was arrested by immediately cooling the yoghurt to 4^{o}C (Tamine and Robinson, 1999). To confirm that the quality of the basic yoghurt formula was according to the Thai regulation for yoghurt product (Thailand Industrial Standard no. 2146-2546, ประกาศกระทรวงอุตสาหกรรม , 2547) that the yoghurt had a protein content of not less than 3%, the basic yoghurt was subjected to a protein analysis following an AOAC Method no. 991.20 (AOAC, 2000). The result revealed that the basic yoghurt had protein content 5.62 ± 0.05 %, which was higher than the minimum protein content stated in the Thai regulation.

To understand changing inside the reconstituted milk solution during yoghurt fermentation, samples of milk/yoghurt were collected every 2 h and analyzed for its microbial and chemical parameter. The monitoring results clearly displayed that the yoghurt starter cultures rapidly produced lactic acid after 2 h incubation period to reach a pH value of 4.6 ± 0.03 and total titratable acidity of 1.21 ± 0.02% lactic acid at the end of fermentation time (Fig. 15). At the same time, enumeration of the yoghurt starter culture that was conducted by doing 10-fold dilution, pour-plated 1 ml of serial diluted sample in petridishes and thoroughly mixed with M-17 and MRS for *S. thermophilus* and *L. bulgaricus*, respectively, showed that both microorganisms were rapidly multiplied in their numbers during the fermentation period (Fig. 16). The Figure also demonstrated that *S. thermophilus* was grown more rapidly than *L. bulgaricus* at the beginning of the fermentation period because of the production of small peptides and amino acids produced by *L. bulgaricus* (Lourens-Hattingh and Viljoen, 2001 and Walstra *et al.,* 1999). On the other hand, the growth rate of *L. bulgaricus* was higher than those of the *S. thermophilus* after 2 h incubation time due to the presence of formic acid and carbon

dioxide produced by the later microorganism (Walstra *et al.,* 1999). After 4 h incubation time when the pH of the milk had reached 5.19 ± 0.04, the growth of *S. thermophilus* was much slower than those of the *L. bulgaricus* due to the presence of lactic acid (Lourens-Hattingh and Viljoen, 2001 and Walstra *et al.,* 1999). During 6 h fermentation period, the number of *S. thermophilus* was increased from 7.15 ± 0.11 log cfu/ml to 8.58 ± 0.27 log cfu/ml, whereas the number of *L. bulgaricus* was increased from 5.94 ± 0.71 log cfu/ml to 7.82 ± 0.21 log cfu/ml. The final number of both yoghurt starter cultures, which was higher than 6 log cfu/ml confirmed that the yoghurt quality followed the microbial quality for a good yoghurt product recommended by Kraseakoopt *et al.* (2003), Iyer and Kailasapathy (2005) and Ouweland and Salminen (1998). Since the protein content and microbial quality of the basic yoghurt followed the recommendation for chemical and microbial quality of a good yoghurt product, this basic formula was further used in the next experiment.

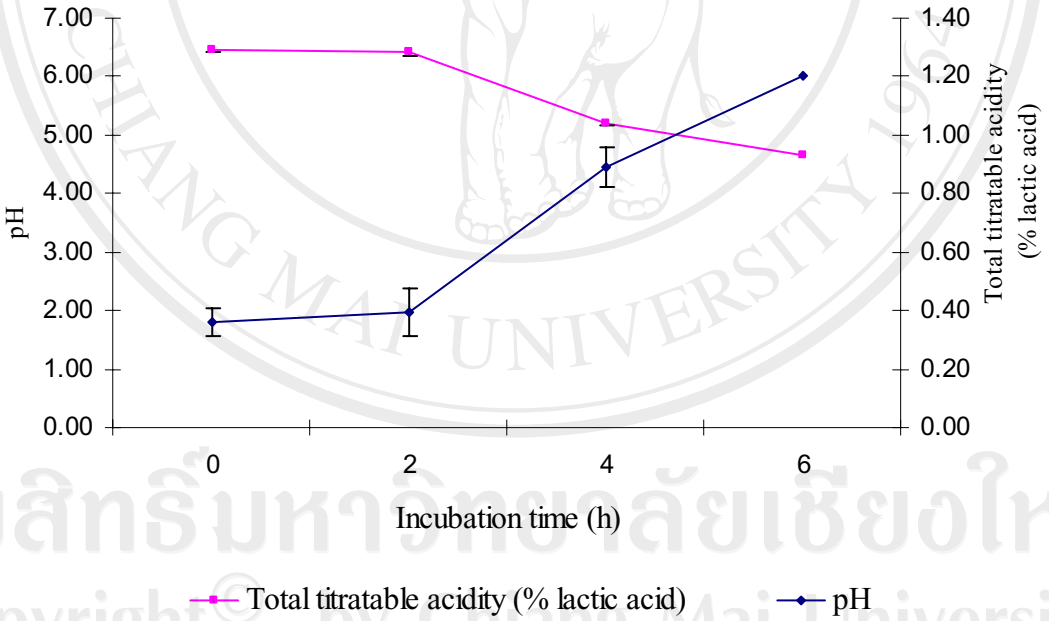

Fig. 15 The acidification profile and the total acidity of yoghurt during fermentation processing

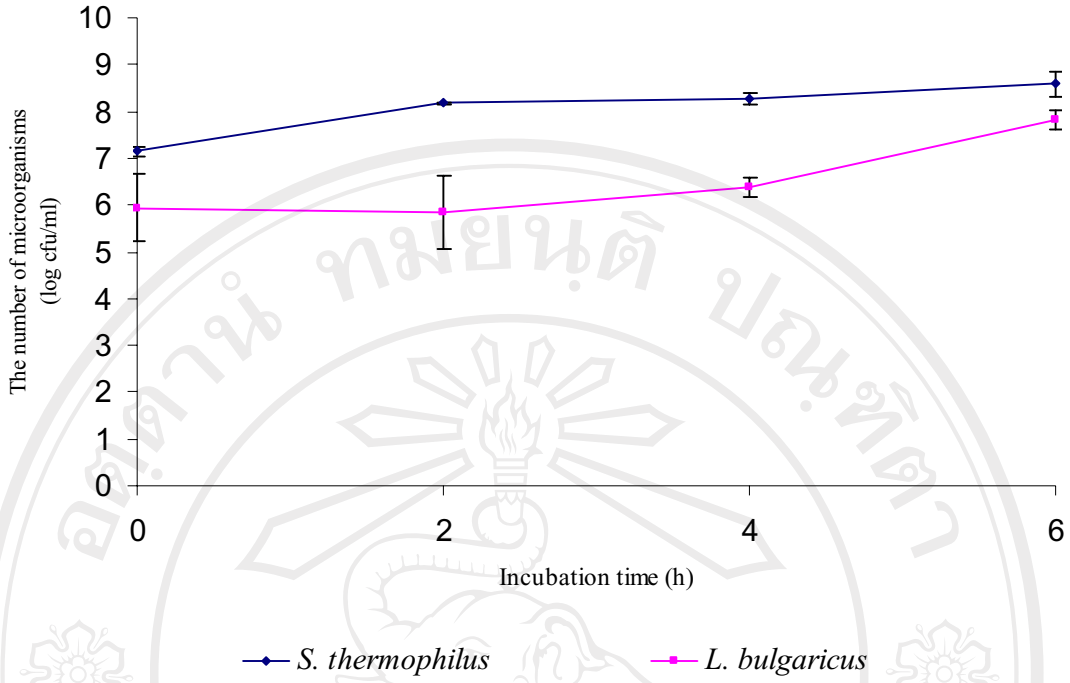

Fig. 16 The number of *S. thermophilus* and *L. bulgaricus* (log cfu/ml) during the yoghurt fermentation

In the main experiment, basic yoghurt was dried using a spray drier model SDE 50 manufactured by J.C. Machinery and civil work Co., Ltd. The type of atomizer used was a nozzle atomizer with a length of 44 cm together with an atomizer pressure at 15 psi, a co-current air flow and an inlet temperature at 180°C. Since the outlet air temperature had a significant effect on the survival rate of yoghurt microorganisms and this outlet temperature should not be higher than 90°C (Kim *et al.,* 1990, Makarukpinyo, 1995 and To and Etzel, 1997) different outlet temperatures of the spray drier of 90 ± 2, 85 ± 2, 80 ± 2 and 75 ± 2°C were studied in this section to understand the survival rate of *S. thermophilus* and *L. bulgaricus* in the final yoghurt powder. At the same time, to get a good drying rate during the spray drying process, the total soluble solid of yoghurt, which was initially 16± 2°Brix, was increased by adding 25% (w/v) of maltodextrin solution to reach a final total soluble solid of 25 ± 2% total soluble solid (Desobry *et al.,* 1997 and Cai and Corke, 2000).

The color of the yoghurt powders based on L*, a* and b* values (Fig. 17) displayed that different outlet temperatures of the spray drier did not significantly affect the L*, a* and b* values of the powder. For the b* value, the yoghurt powder dried at

90°C outlet temperature significantly had more blue color compared to those of the other milk powder.

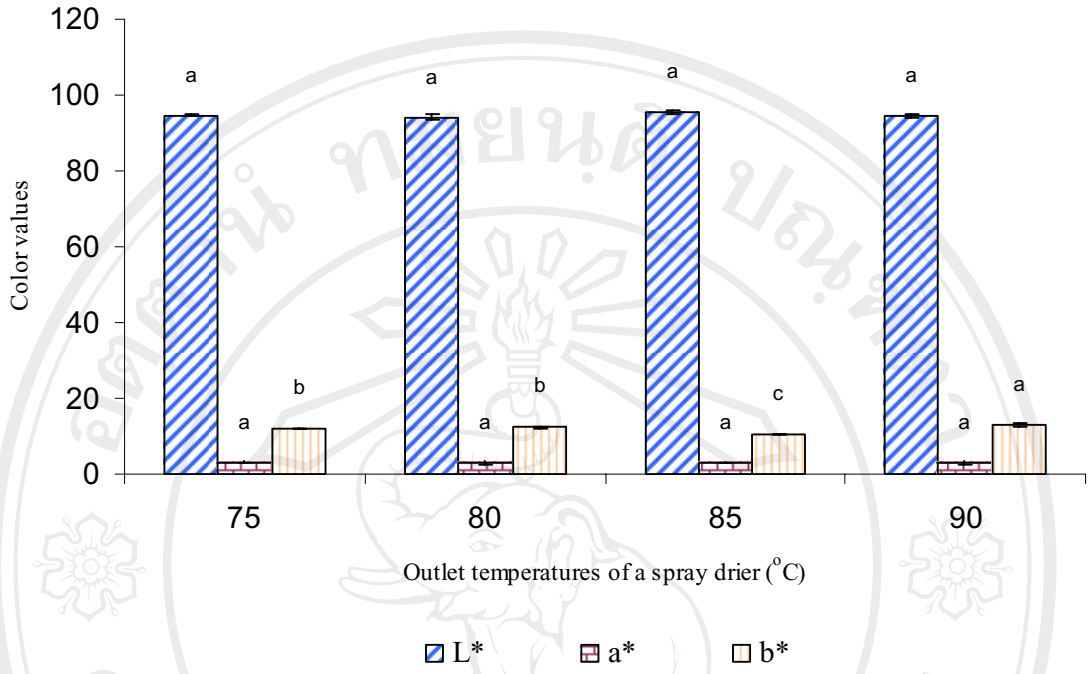

Fig. 17 Color values of yoghurt powder produced by a spray drier using different outlet temperatures

Applying different outlet temperatures of a spray drier significantly affected the moisture content and water activity of the yoghurt powder as were shown in Table 14. Generally, it could be seen that higher outlet temperatures produced lower moisture content and water activity. A significantly lower moisture content and water activity of yoghurt powder dried at 90°C outlet temperature might affect the color values of the powder that was shown to have a higher yellow color. This could be due to the high temperature that could induce the browning reaction. In previous studies, non-enzymatic browning reaction rate was clearly demonstrated to affect water content, water activity, temperature, pH and concentration and the type of reactants. It was also found that coinciding with the glassy transition, physical change of the matrix materials affected the non-enzymatic browning reaction rate (Miao and Roos, 2006). Since the dried food products should have a moisture content lower than 5% (Corcoran *et al.,* 2006) and a water activity between 0.2 to 0.3 (Baker, 1997), drying the basic yoghurt using outlet temperatures of 70°C to 90°C would fulfill these criteria.

Different outlet temperatures of the spray drier were found not to be significantly affected the bulk density of the yoghurt powder. Using a single nozzle atomizer, the yoghurt powders had a bulk density of 0.57 ± 0.10 to 0.63 ± 0.06. The bulk density of a dried product was mainly affected by the material density and the content of included air within the particle. For the last factor, various operating parameters, such as feed pumping, feed agitation and feed atomization could influence the parameter (Master, 1991). Since all of these factors were not varied in this experimental section, the bulk density of the dried powders was not significantly different.

However, the finding of the bulk density affected the physical characteristics of rehydration and water holding capacity of the yoghurt powder. In general, higher bulk density produced higher rehydration values, but lower water holding capacity values. Since the bulk densities of different yoghurt powders were not significantly different, the rehydration and water holding capacity values were also found not to be significantly different. The highest rehydration value and the lowest water holding capacity value were displayed in the yoghurt powder dried at 80°C outlet temperature. For the solubility, at 90°C gave the lowest value because the protein was denatured more than the other temperatures eventhough at this temperature gave the lowest moisture content and water activity.

Table 14 Physical properties of yoghurt powder produced by using different outlet temperature of spray drier

Physical properties	Outlet temperatures of spray drier (°C)*			
	75	80	85	90
1. Moisture content (%)	$1.78^{bc} \pm 0.05$	$1.87^{c} \pm 0.07$	$1.63^{b} \pm 0.16$	$0.72^{a} \pm 0.05$
2. Water activity	$0.161^{c} \pm 0.01$	$0.163^{c} \pm 0.07$	$0.136^{b} \pm 0.01$	$0.098^{a} \pm 0.00$
3. Bulk density (g/ml)	$0.613^{a} \pm 0.05$	$0.667^{a} \pm 0.06$	$0.584^{a} \pm 0.06$	$0.572^{a} \pm 0.10$
4. Reydration (%)	$40.80^{a} \pm 3.13$	$43.88^{a} \pm 5.07$	$39.81^{a} \pm 5.49$	$40.75^{a} \pm 0.97$
5. Water holding capacity	$4.487^{a} \pm 0.25$	$4.412^{a} \pm 0.29$	$4.535^{a} \pm 0.14$	$4.627^{a} \pm 0.26$
6. Solubility (%)	$10.41^{a} \pm 0.99$	$11.05^{a} \pm 0.22$	$11.08^{a} \pm 0.21$	$8.35^{b} \pm 0.28$

* Each value in the table is the mean± standard deviation of three trials.

Values in the same row with different letters were significant different by Duncan's multiple range test ($p<0.05$)

Although drying yoghurt at different outlet temperatures did not affect structure physical characteristics of the yoghurt powder, those outlet temperatures did significantly affect the solubility of yoghurt powders (Table 14). Using an outlet temperature of 90°C, the powder significantly had higher solubility value compared to those of the other powders. This physical parameter could be affected by higher drying rate produced at higher outlet temperatures. Beside solubility, different drying rates at different outlet temperatures also affected the powder yields. Drying the yoghurt at 90 ± 2, 85 ± 2, 80 ± 2 and 75 ± 2°C produced yoghurt powders with yields of 7.91 ± 0.15, 5.41 ± 0.20, 6.25 ± 0.23 and 5.79 ±0.34 g/ml, respectively. This finding indicated that at 90°C outlet temperature, a higher yield of yoghurt powder could be manufactured.

For the chemical properties of yoghurt powder, mainly pH and total titratable acidity, these parameters were not significantly affected by the outlet temperature of the spray drier (Fig. 18). The pH and total titratable acidity of the yoghurt powder would be more affected by the composition of the yoghurt ingredients, time and temperature of the fermentation and the amount of the starter culture (Tamine and Robinson, 1999 and Walstra *et al.,* 1999).

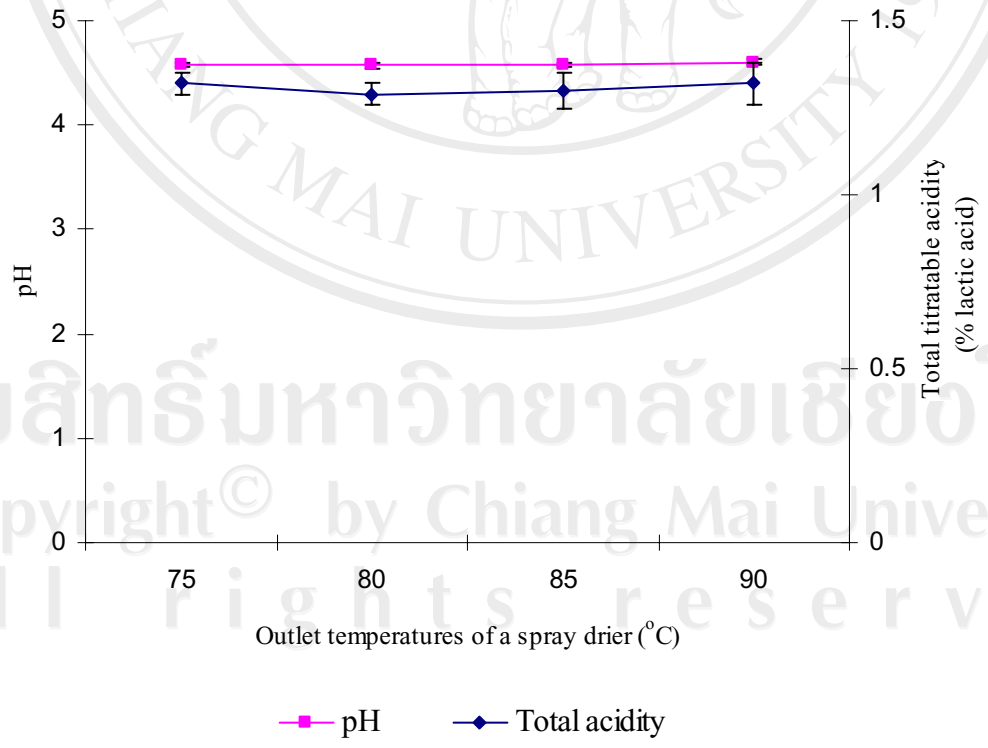

Fig. 18 pH and total titratable acidity (% lactic acid) of yoghurt powder produced by a spray drier using different outlet temperature

In this experimental section, the basic yoghurt sample was not only contained *S. thermophilus* and *L. bulgaricus* at a concentration level of 7.97 ± 0.05 and 5.90 ± 0.32 log cfu/ml, respectively, but also *L. acidophilus* that was aseptically added to the yoghurt at a level of 6.29 ± 0.14 log cfu/ml. The *L. acidophilus* was added together with the yoghurt culture. The results of the lactic acid bacteria enumeration after the drying process clearly displayed that different outlet temperatures significantly affected the survival of the three microorganisms (Fig. 19). Although the literature suggested that the outlet temperature should be lower than 100°C to avoid a complete destruction of yoghurt starter culture (Kim *et al.,* 1990, Makarukpinyo, 1995 and To and Etzel, 1997), the result of this experiment showed that all of the lactic acid bacteria in the yoghurt could not be detected after drying the yoghurt at 90°C outlet temperature. This finding could be affected from the history of the lactic acid bacteria that came from freeze dried cultures, which could affect their heat resistance. The survival of *S. thermophilus* was significantly improved at lower outlet temperatures, whereas the survival of the *Lactobacillus* spp. was significantly higher using an outlet temperature of 80°C compared to those of the other 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°C and 85-90°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. Thermophillic LAB, in particular *S. thermophilus* was reported to survive well during spray drying (Abd-El-Gawad *et al.,* 1989, To and Etzel, 1997 and Bielecka and Majkowska, 2000) which confirmed the finding in this research. Results in this study showed that at lower outlet temperatures, the survival rate of lactic acid bacteria was increased. However a better survival rate of the studied microorganisms at 80°C compared to the outlet drying temperature of 75°C could be due to different drying times of the yoghurt sample to reach a similar water activity in the final product (Table 14). Longer drying times at lower

outlet temperatures of the spray drying could also affect the survival rate of the lactic acid bacteria. Due to a higher r\survival rate of 3 lactic acid bacteria at 80°C, this outlet temperature was applied in the next experimental section.

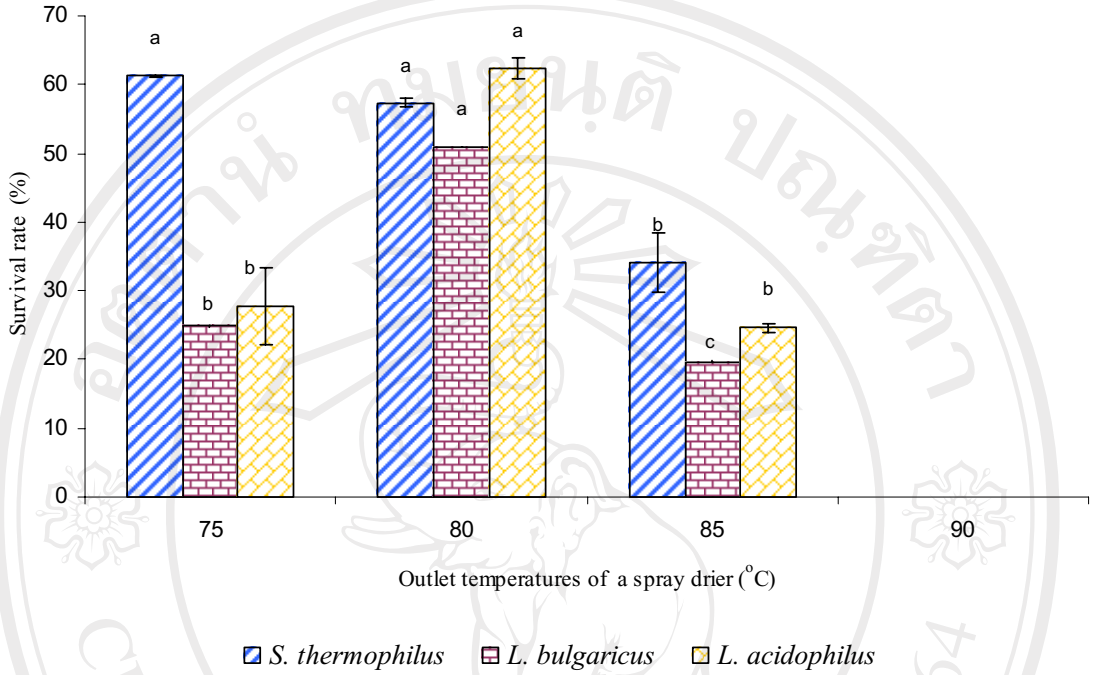

Fig. 19 The lactic acid bacteria enumeration by survival rate (%) in yoghurt powder produced by a spray drier using different outlet temperatures

4.4 Rehydration of *L. acidophilus* containing yoghurt powder using different distilled water temperature

In this section *L. acidophilus* added yoghurt was dried by a spray drier using an outlet temperature of 80 ± 2°C. The yoghurt powder was then rehydrated at a ratio 1: 10 for the powder and distilled water, respectively (Kumar and Mishra, 2004). The temperatures of the distilled water were varied between 30 to 90°C to understand whether the water temperature would affect the physical, chemical and especially microbiological quality of the reconstituted yoghurt solution.

The result clearly displayed that different temperatures of the rehydrated water did significantly affect the survival of the lactic acid bacteria, particularly the yoghurt starter cultures, in the reconstituted yoghurt solution (Fig. 20).

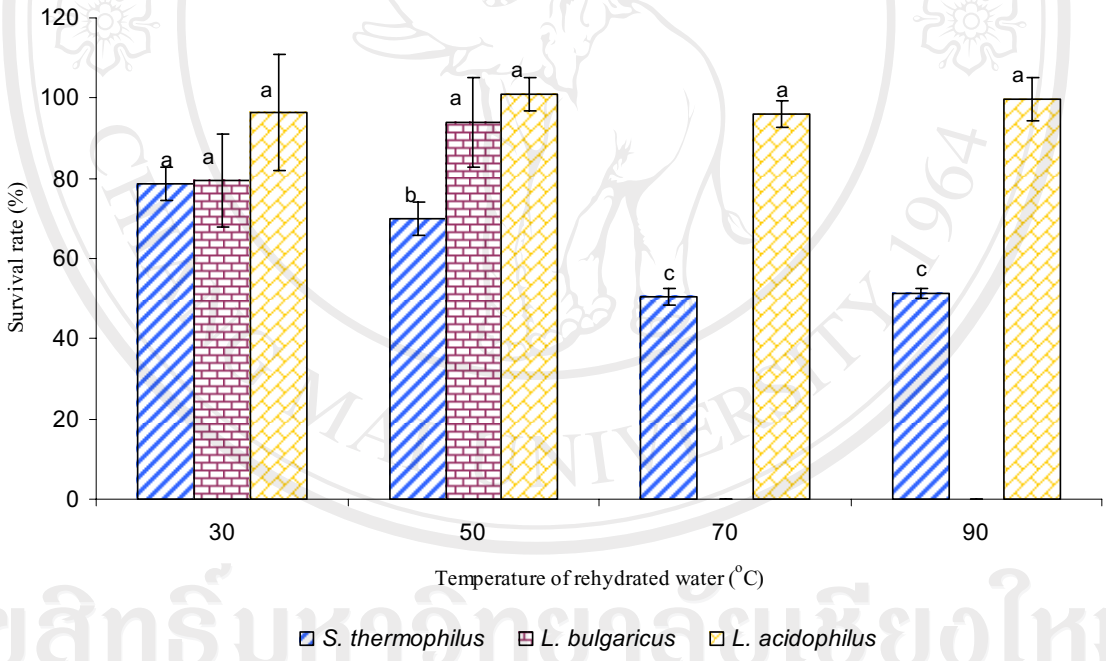

Fig. 20 The survival rate (%) of *S. thermophilus*, *L. bulgaricus* and *L. acidophilus* in rehydrated yoghurt solution affected by different water temperatures of rehydrated water

The survival rate of *L. acidophilus* was high (more than 96% survival) in all the rehydrated water temperatures indicating that any rehydrated water temperature would not affect the survival of the probiotic bacterium. On the other hand, the survival of *S. thermophilus* and *L. bulgaricus* was significantly higher at lower water temperatures. The presence of *L. bulgaricus* could not be detected when the rehydrated water temperatures

were at 70 and 90°C. This finding suggested that eventhough the three lactic acid bacteria used in this research were mesophilic bacteria (Wang *et al.,* 2004), their survival in the reconstituted yoghurt solution would be differed after passing a drying process. It was also interesting to note that although an outlet temperature of a spray drier at 90°C would kill *L. acidophilus*, the microorganism could have a high survival rate when the yoghurt powder was reconstituted with distilled water at 90°C. Different conditions in which the bacterium encountered during a high temperature processing significantly affected the survival rate of the microorganism (Wang *et al.,* 2004). The result in this section also suggested that the application of warm temperature (approximately at 50°C) would be the optimum temperature to rehydrate *L. acidophilus* added yoghurt powder.

For the physical and chemical property of the reconstituted yoghurt solution including rehydration, solubility, water holding capacity, total soluble solid, pH and total titratable acidity, different temperatures of rehydrated water did not significantly affect these parameters (Table 15 and Fig. 21). The rehydration property (%) and the solubility (%) of the reconstituted yoghurt powder at different water temperatures were not significantly different because the yoghurt powder had the same bulk density or the particle size. However, different water temperatures affected the water holding capacity. The result showed that the water holding capacity of the rehydrated yoghurt using water temperature at 30°C was the highest. Increasing the water temperature reduced the water holding capacity of the reconstituted yoghurt. The high value of water holding capacity demonstrated that water was more tightly bound to the protein molecules (Kneifel *et al.,* 1991). The total solid was reduced as the water temperature was increased, but the effect was not found to be statistically different. It was concluded that the protein was denatured during drying process, so the denature protein was difficult to dissolve using rehydrated water at room temperature. In contrast, a high protein product, milk protein concentrated, was dissolved at a high water temperature. An optimum water temperature of 50-60°C would be good to be applied whereas using a rehydrated water temperature of 80°C, it would cause coagulation reactions (Onwulata, 2005).

For the pH and total titratable acidity of rehydrated yoghurt powder, they were not significantly different at different water temperatures. The pH value was around 4.1 and the total titratable acidity was around 1.1% (lactic acid).

Table 15 Physical characteristics of rehydrated *L. acidophilus* containing yoghurt solution affected by different temperature of rehydrated water

Physical characteristics*	Temperatures of rehydrated water (°C)			
	30	50	70	90
1. Rehydration (%)	$31.83^{a} \pm 2.38$	$30.47^{a} \pm 0.17$	$30.76^{a} \pm 0.86$	$30.83^{a} \pm 1.49$
2. Water holding capacity	$2.23^{a} \pm 0.16$	$1.98^{b} \pm 0.08$	$1.51^{c} \pm 0.04$	$0.83^{d} \pm 0.04$
3. Solubility (%)	$10.15^{a} \pm 0.63$	$10.74^{a} \pm 0.66$	$10.70^{a} \pm 0.13$	$10.43^{a} \pm 0.13$
4. Total solid (%)	$8.67^{a} \pm 0.03$	$9.23^{a} \pm 0.77$	$9.45^{a} \pm 0.66$	$9.52^{a} \pm 0.26$

* Each value in the table is the mean± standard deviation of three trials and values in the same row were significant different by Duncan's multiple range test (p<0.05)

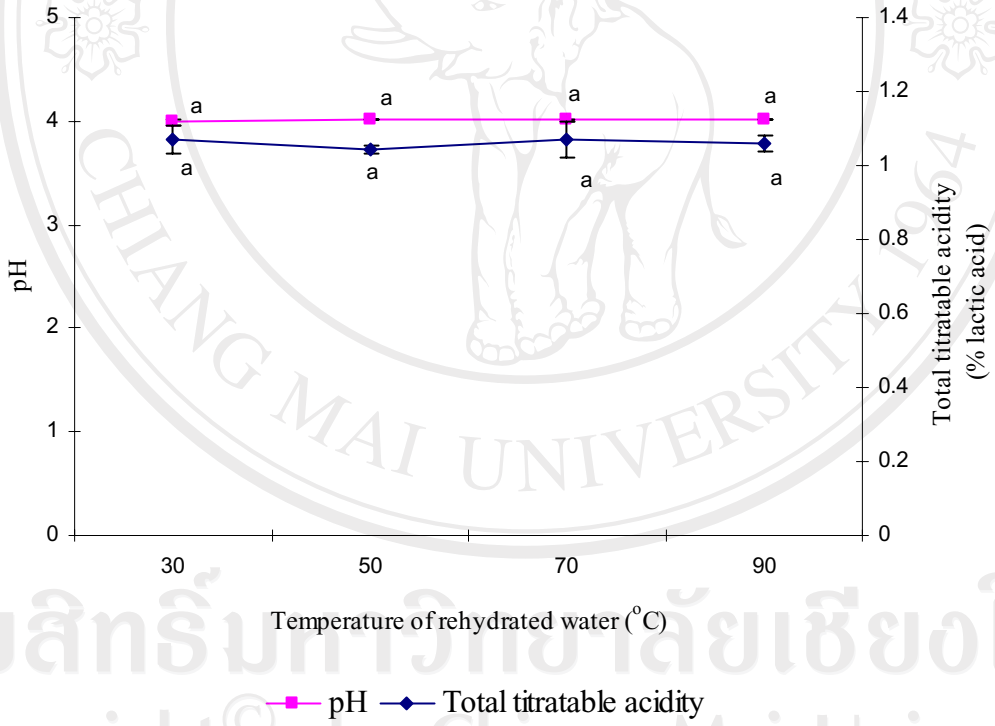

Fig. 21 pH and total titratable acidity of rehydrated *L. acidophilus* containing yoghurt solution affected by different temperature of rehydrated water

The color of *L. acidophilus* containing yoghurt powder was 94.28 ± 1.53, 2.91 ± 0.05 and 12.99 ± 0.28 for L*, a* and b* values, respectively. After adding the rehydrated water at different addition temperatures, the color values of the yoghurt solution was significantly reduced for all the color values (Fig. 22). The color of he rehydrated yoghurt

solution was significantly darker and significantly had higher yellow color when the yoghurt powders was added with higher temperatures of rehydrated water. This could be due to a higher temperature that could induce the browning reaction, so, the rehydrated yoghurt powder at 70°C and 90°C was darker than those of the yoghurt solutions rehydrated using lower rehydrated water temperatures.

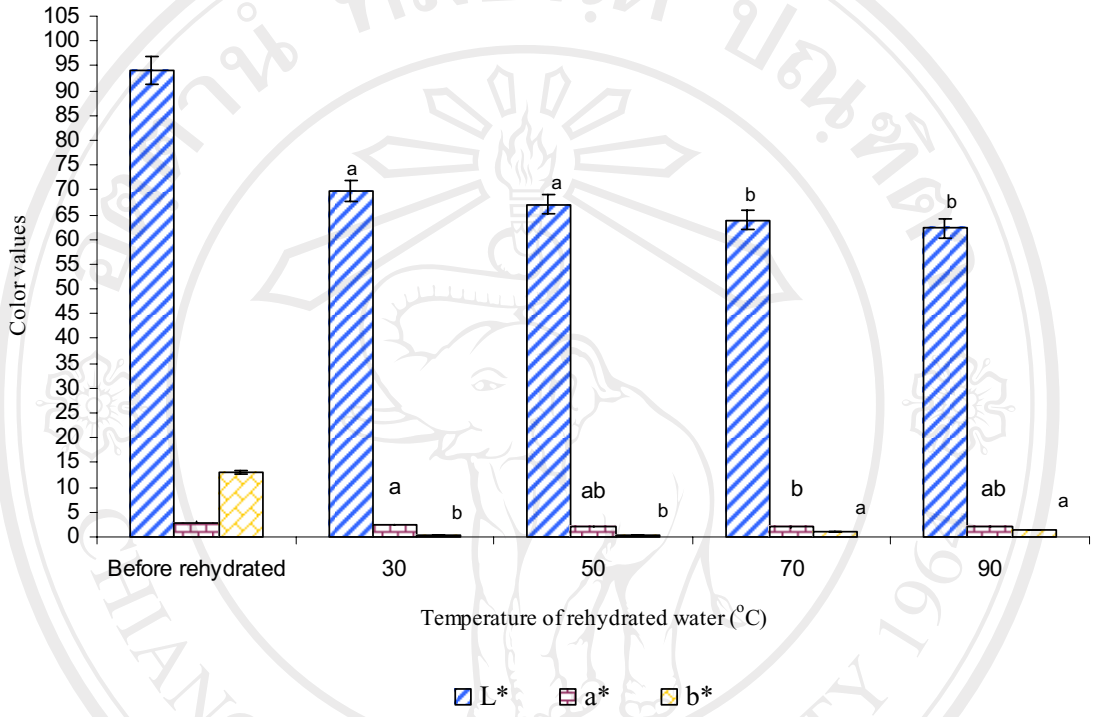

Fig. 22 Color values of rehydrated *L. acidophilus* containing yoghurt solution affected by different temperatures of rehydrated water

Different temperatures of water to rehydrate yoghurt powder were significantly affected the survival rate of the microorganism. Although the survival rate was higher when using the water at room temperature, the total solid of the yoghurt solution was less than the other water temperatures which showed that the yoghurt powder needed a longer time to complete soluble in this water temperature. Therefore, using the water temperature at 50°C should be better because it could make the powder soluble quicker than the water at room temperature eventhough it gave a survival rate of *S. thermophilus* less than that of the water at room temperature.

4.5 The shelf-life of *L. acidophilus* containing yoghurt powder stored at different storage temperature and packed using different packaging materials

Production of *L. acidophilus* containing yoghurt powder was carried out by added 6.29 ± 0.14 log cfu/ml free cells of *L. acidophilus* and drying in an outlet temperature of 80°C. The powder was then packed in 2 different materials of laminated pouches, which were Polyethylene tetraphthalate/ Polypropylene/ Aluminum (PET/PP/Al) and Nylon/ Polyethylene (nylon/PE) and sealed as a vacuum packaging. These packaging materials had an air permeability rate of 7.34×10^{-6} and 1.15×10^{-3} ($cm^3/m^2.d.Pa$) for PET/PP/Al and nylon/PE, respectively. The packed yoghurt powder was stored for 14 weeks either at room temperature, which was around 30 ± 5°C or in a refrigerator at 4 ± 2°C. During the storage period, samples of yoghurt powder were collected every 2 weeks for microbiological analysis and every 4 weeks for physical and chemical analyses.

The monitoring for the viability of *S. thermophilus* in the yoghurt powder clearly displayed that different packaging materials did not significantly affect the survival of the bacterium (Fig. 23).

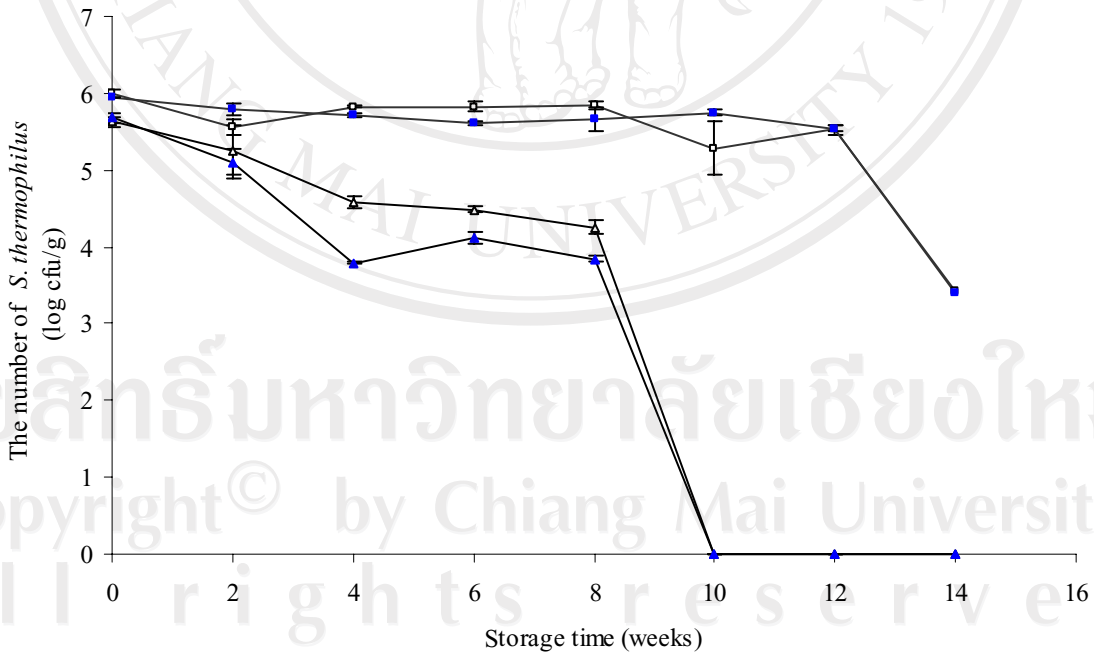

Fig. 23 The number of *S. thermophillus* in *L. acidophilus* containing yoghurt powder affected by storage temperatures of 4°C (□and ■) and room temperature (△ and ▲) and packaging materials, which were PET/PP/Al (white color) and nylon/PE (black color)

However, different storage temperatures did significantly affect the viability of the microorganism. Keeping the yoghurt powder at room temperature reduced stepwisely the *S. thermophilus* viability for up to 1.37 and 1.83 log cfu/g for PET/PP/Al and nylon/PE, respectively, within the first 8 weeks storage followed by a complete destruction of the bacterium viability afterwards. This finding indicated that the storage temperature would not only affect the air permeability of the packaging material during storage (Wang *et al.,* 2004), but it also affected the survival of *S. thermophilus* in dry condition. Larsen and Anon (1990) reported that at lower a_w, the production of lactic acid by *S. thermophilus* and *L. bulgaricus* was reduced. This indicated that the microorganism might adjust to the stress condition of low a_w and reduce their normal metabolism. In this research, the low a_w of yoghurt powder might increase the sensitivity of *S. thermophilus* to the higher storage temperature. At low storage temperature, the viability of *S. thermophilus* was only slightly reduced for up to 0.4836 and 0.3730 log cfu/g for PET/PP/Al and nylon/PE, respectively, within the first 12 weeks of storage. This result suggested that the yoghurt powder would be better stored at low temperature to maintain the viability of *S. thermophilus*.

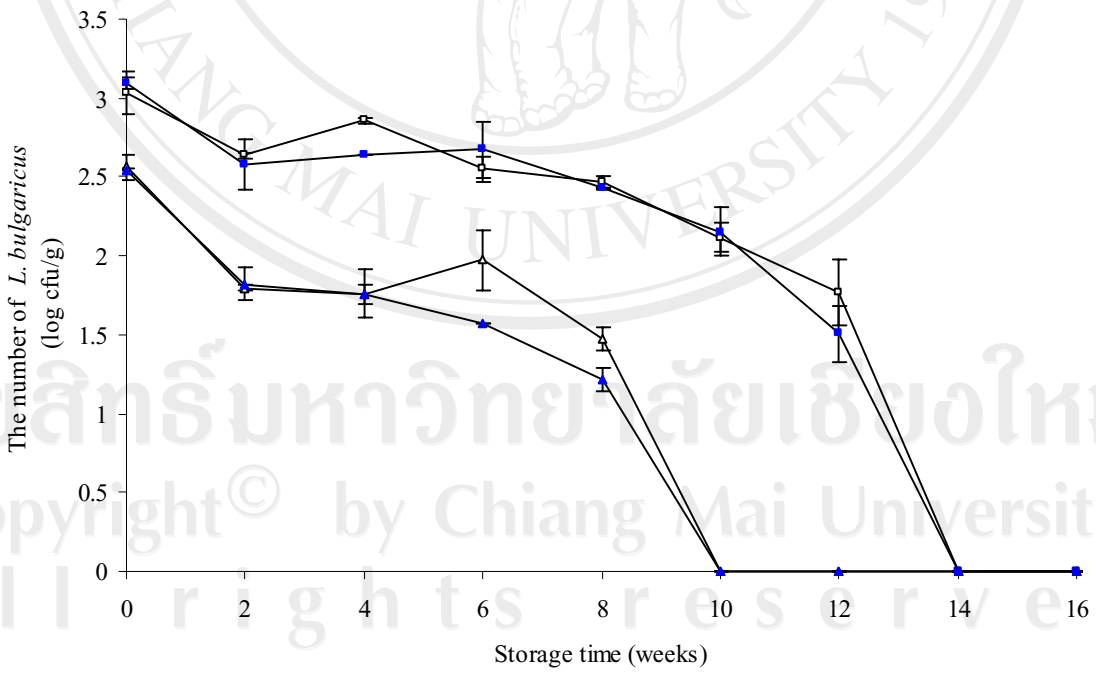

Fig. 24 The number of *L. bulgaricus* in *L. acidophilus* containing yoghurt powder affected by storage temperatures of 4°C (□ and ■) and room temperature (△ and ▲) and packaging materials, which were PET/PP/Al (white color) and nylon/PE (black color)

Although the number of *L. bulgaricus* was 7.82 ± 0.21 log cfu/ml in the yoghurt mixture before a drying process, the higher sensitivity of the bacterium during the drying process caused 50.93 ± 0.86% reduction in its viability after the process. Therefore, the storage of the *L. acidophilus* containing yoghurt powder was started with only 2.54 to 3.03 log cfu/g *L. bulgaricus.* During the storage period, the viability of *L. bulgaricus* was more significantly affected by the storage temperatures compared to the packaging materials (Fig. 24). However, a higher reduction in the *L. bulgaricus* viability numbers was recorded compared to those of the *S. thermophilus* results (Fig. 23). This could be due to the low water activity of the yoghurt powder and also the *L. bulgaricus* characteristic as a microaerophilic bacterium (Bridson, 1993). The higher storage temperature produced a higher lethality rate for *L. bulgaricus* and a complete microorganism destruction was noted after 10 weeks storage at room temperature. Although, the lethality rate of *L. bulgaricus* was lower at 4°C storage temperature, the microorganism could not also be detected at the end of the storage period.

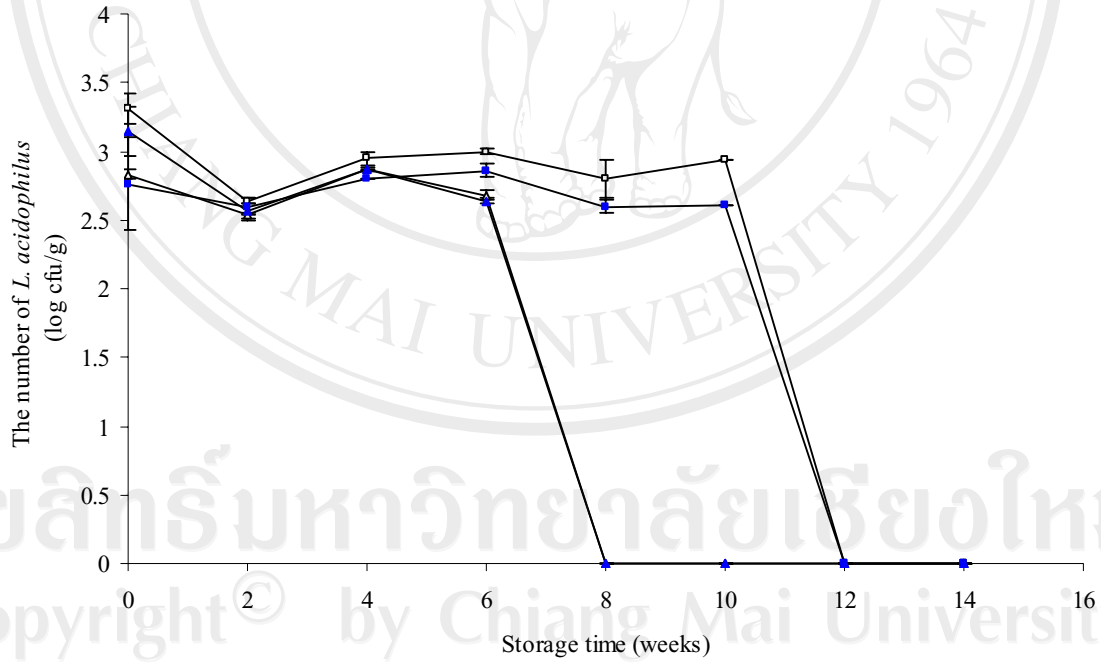

Fig. 25 The number of *L. acidophilus* in yoghurt powder affected by storage temperatures of 4°C (□ and ■) and room temperature (△ and ▲) and packaging materials, which were PET/PP/Al (white color) and nylon/PE (black color)

Interestingly, the survival of *L. acidophilus* was more similar to the *S. thermophilus* compared to the *L. bulgaricus*. The initial number of 2.76 ± 0.34 to 3.31 ± 0.11 log cfu/g of *L. acidophilus* were almost maintained for 10 weeks period at 4°C with a survival rate for more than 79.95 ± 3.16% and for 6 weeks at room temperature with a survival rate for more than 81.61 ± 3.28% (Fig. 25). However, a higher sensitivity of the bacterium to oxygen caused the *L. acidophilus* to be completely lost its viability earlier than the *S. thermophilus*, which was reported to be a facultative anaerobic bacterium (Bridson, 1993).

The acidification profile of *L. acidophilus* containing yoghurt powder showed that in general the pH of the yoghurt powder was significantly reduced in the first month of storage followed by a lower reduction rate of pH for the most of the storage period (Table 16). This could be due to chemical reactions, such as lipid oxidation that could happen at low water activity (Fennema, 1996) and in the presence of low oxygen content inside the packaging. Different storage temperatures and packaging materials did not significantly affect the pH reduction of the yoghurt powder. Although the total titratable acidity results also displayed increasing values, the statistical analysis indicated that the finding was not significantly different (Table 17). Changes in the yoghurt chemical properties could also affect the survival of lactic acid bacteria in the yoghurt powder when the powder was reconstituted.

Table 16 pH of yoghurt powder packed in different packaging materials and storage temperatures

Storage temperature (°C)	Packaging material	Storage period* (weeks)			
		0	4	8	12
4	PET/PP/Al	$4.53^{a} \pm 0.00$	$4.47^{cde} \pm 0.02$	$4.45^{efg} \pm 0.02$	$4.46^{defg} \pm 0.01$
	Nylon/PE	$4.51^{ab} \pm 0.01$	$4.48^{cd} \pm 0.01$	$4.45^{fg} \pm 0.01$	$4.46^{efg} \pm 0.02$
Room temperature	PET/PP/Al	$4.51^{ab} \pm 0.01$	$4.47^{def} \pm 0.00$	$4.46^{efg} \pm 0.02$	$4.45^{fg} \pm 0.02$
	Nylon/PE	$4.49^{bc} \pm 0.02$	$4.45^{fg} \pm 0.03$	$4.45^{fg} \pm 0.00$	$4.49^{g} \pm 0.01$

* Each value in the table is the mean± standard deviation of three trials.

Values in the same column and row with different letters were significantly different by Duncan's multiple range test ($p<0.05$)

Table 17 Total titratable acidity (% lactic acid) of yoghurt powder packed in a different packaging material and storage temperature

Storage temperature (^{o}C)	Packaging material	Storage period *(weeks)			
		0	4	8	12
4	PET/PP/Al	$1.22^{a} \pm 0.07$	$1.34^{abd} \pm 0.01$	$1.13^{bc} \pm 0.02$	$1.27^{abc} \pm 0.05$
	Nylon/PE	$1.27^{abc} \pm 0.03$	$1.38^{a} \pm 0.12$	$1.24^{abc} \pm 0.11$	$1.36^{ab} \pm 0.06$
Room temperature	PET/PP/Al	$1.25^{abc} \pm 0.05$	$1.31^{abc} \pm 0.10$	$1.33^{abc} \pm 0.00$	$1.31^{abc} \pm 0.03$
	Nylon/PE	$1.29^{abc} \pm 0.05$	$1.28^{abc} \pm 0.09$	$1.34^{abc} \pm 0.03$	$1.31^{abc} \pm 0.08$

* Each value in the table is the mean± standard deviation of three trials.
Values in the same column and row with different letters were significantly different by Duncan's multiple range test (p<0.05)

Since the laminated pouches used to pack the *L. acidophilus* containing yoghurt powder were not an air-tight container, the moisture content of the powder was significantly measured during 12 weeks storage at different storage temperatures (Fig. 26). Both the storage temperature and the packaging materials significantly contributed in the increase of the yoghurt powder moisture content. The highest increase of the moisture content was displayed when the yoghurt powder was packed in the nylon/PE laminated pouch and kept at room temperature. The yoghurt powder in this treatment had a moisture content of more than 5% after 8 weeks storage period indicating the unsuitability of the packaging material to keep the product (Baker, 1997 and Corcoran *et al.,* 2006). The PET/PP/Al laminated pouch was shown to be better in maintaining the moisture content of the yoghurt powder, although it could not completely protect the food product. At lower storage temperature, the moisture content of the yoghurt 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 yoghurt 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).

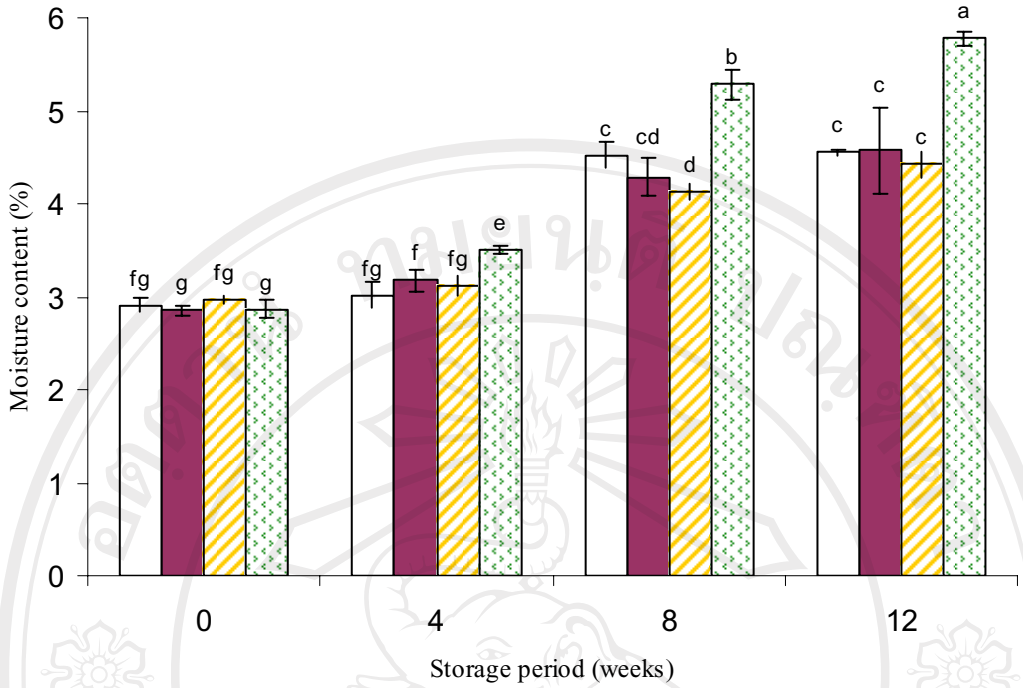

Fig. 26 Moisture content (%) of *L. acidophilus* containing yoghurt powder affected by storage temperatures and packaging materials**.** Symbols: □ PET/PP/Al stored at 4°C; ■ Nylon/PE stored at 4°C; ▨ PET/PP/Al stored at room temperature and ⚃ Nylon/PE stored at room temperature

Confirming the results of the moisture content, the water activity of the *L. acidophilus* containing yoghurt was also significantly increased during the storage period (Fig. 27). Different storage temperatures and packaging materials significantly affected the increase in the yoghurt powder water activity. The increasing rate of the water activity was much slower at lower storage temperature for both packaging materials, while the PET/PP/Al could significantly give a better protection to the yoghurt powder compared to that of the nylon/PE laminated pouch at higher storage temperature. The low water activity of the yoghurt powder during the storage period would limit the chemical reactions that could occur inside the product, such as non-enzymatic browning reaction (Onwulata, 2005). Optimum bacterial stability is encountered in powders with low a_w and Ishibashi *et al.* (1985) recommendend a_w values of 0.2 for maximum stability of probiotic bacteria in powder (Corcoran *et al.,* 2006).

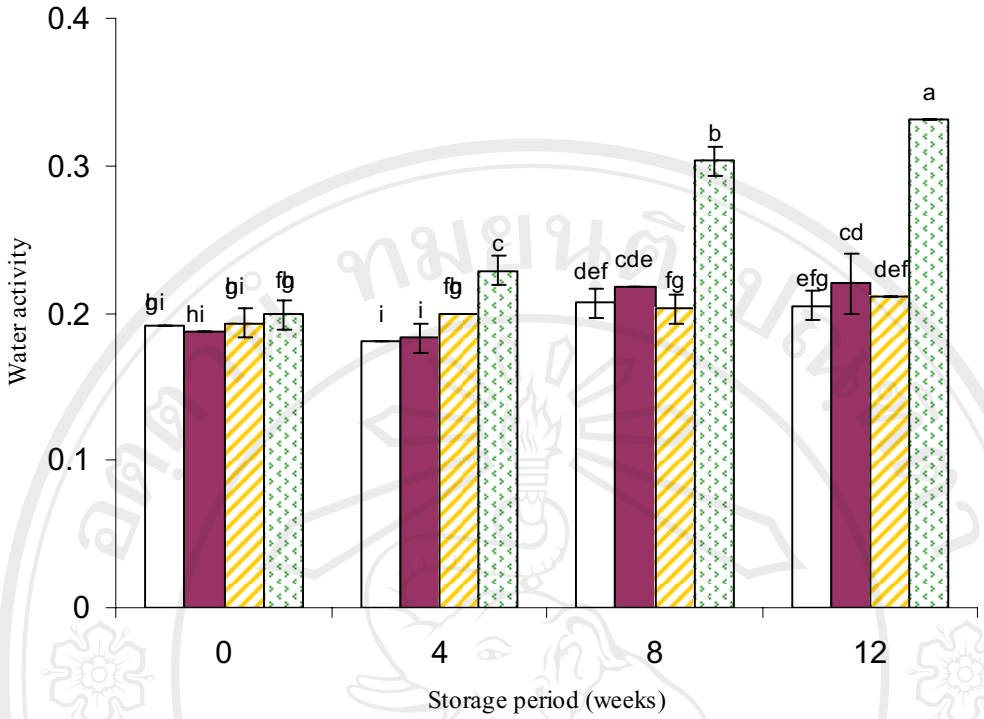

Fig. 27 Water activity (a_w) of *L. acidophilus* containing yoghurt powder affected by storage temperatures and packaging materials. Symbols: □ PET/PP/Al stored at 4°C; ■ Nylon/PE stored at 4°C; ▨ PET/PP/Al stored at room temperature and ▩ Nylon/PE stored at room temperature

Therefore, the color of the *L. acidophilus* containing yoghurt powder, particularly for the L* value, was not significantly changed between at the beginning and at the end of the storage period (Fig. 28). Different packaging material and storage temperature also did not significantly affect this physical property. Increasing the storage time affected the yoghurt powder color. The L* value was decreased whereas a* and b* values were increased. During storage the yoghurt powder at different packaging materials, the oxygen transfer could occur and cause an increase in the oxygen level inside the packaging with a prolong storage period. This could induce discoloration of the product (Kumar and Mishra, 2004).

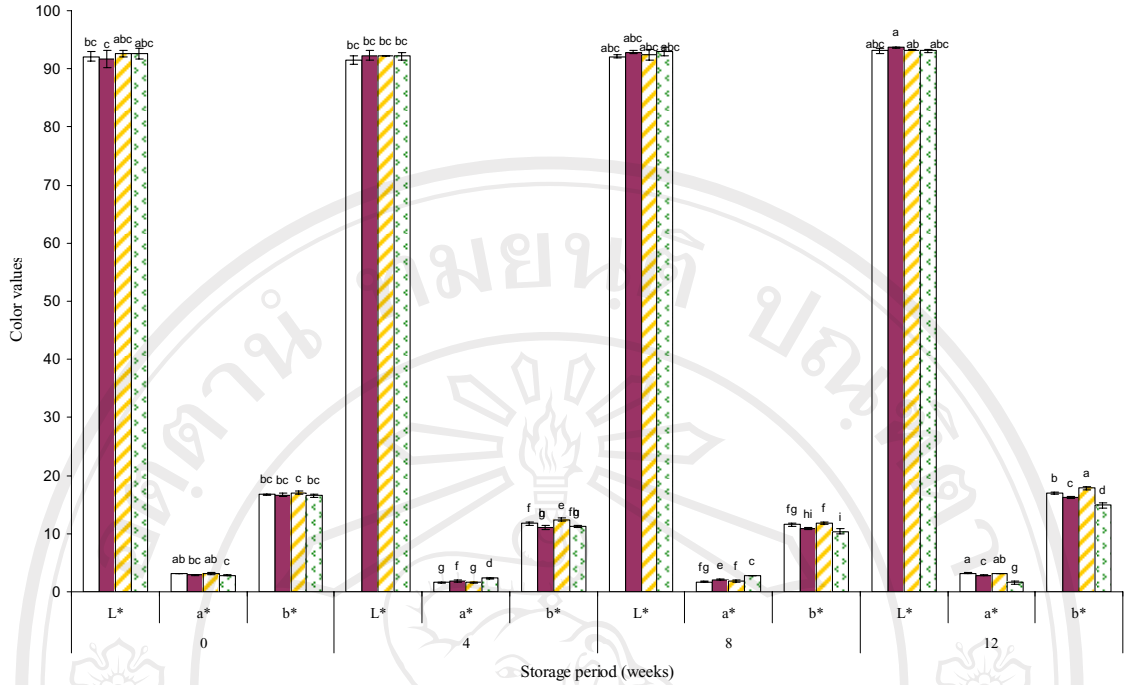

Fig. 28 Color values of *L. acidophilus* containing yoghurt powder affected by storage temperatures and packaging materials**.** Symbols: □ PET/PP/Al stored at 4°C; ■ Nylon/PE stored at 4°C; ◪ PET/PP/Al stored at room temperature and ⊡ Nylon/PE stored at room temperature

The solubility of the *L. acidophilus* containing yoghurt powder was maintained for more than 82.65 ± 0.37% throughout the storage period (Fig. 29). Different packaging materials and storage temperatures did not significantly produce better protection towards the solubility of the yoghurt powder. The highest solubility values were achieved at the beginning of the storage period indicating that the storage period reduced the physical property of the yoghurt powder. Reduction in the solubility of dried skim milk during six months storage at 30°C had also been reported due to the extent of the heat treatment applied to the milk during powder manufacture (Muir and Banks, 2000).

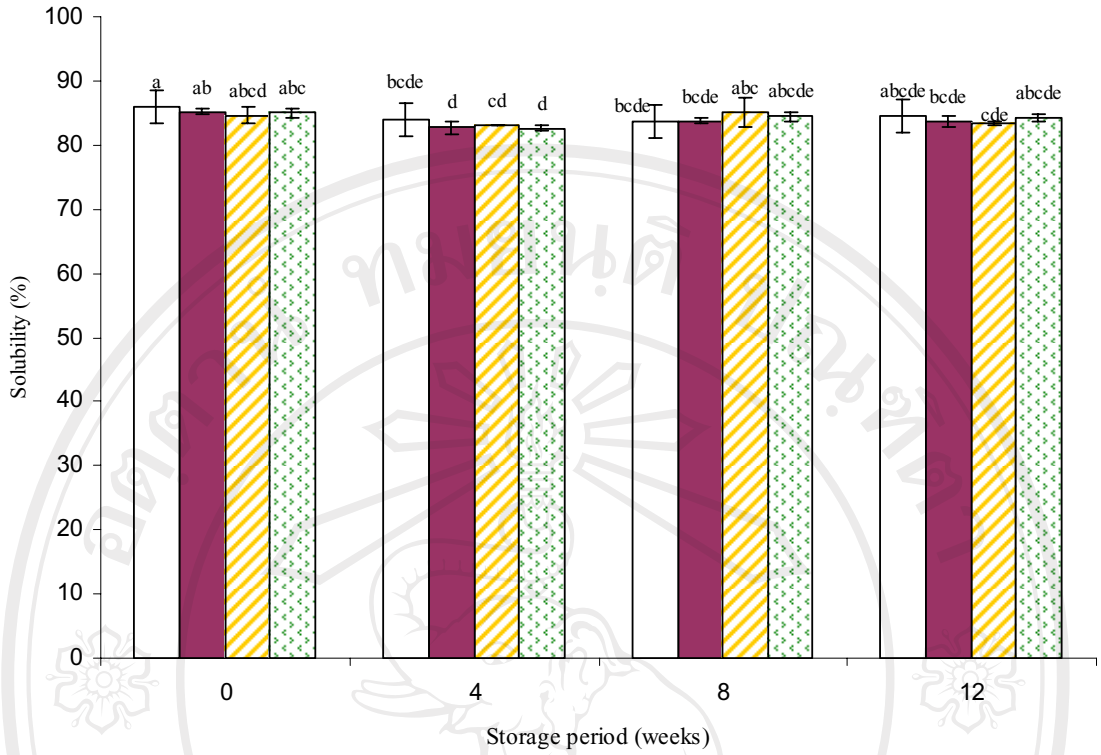

Fig. 29 Solubility (%) of *L. acidophilus* containing yoghurt powder affected by storage temperature and packaging material**.** Symbol: □ (PET/PP/Al) stored at 4°C, ■ (Nylon/PE) stored at 4°C; ▨ (PET/PP/Al) stored at room temperature and ▩ (Nylon/PE) stored at room temperature

The rehydration property of the *L. acidophilus* containing yoghurt powder was not significantly reduced within the first two months storage period (Fig. 30). This physical property was shown to be reduced at the end of the 3 months storage suggesting that the length of the storage period had a significant contribution towards this property. There was not any specific conclusion could be made from different packaging materials and storage temperatures varied in this experimental section.

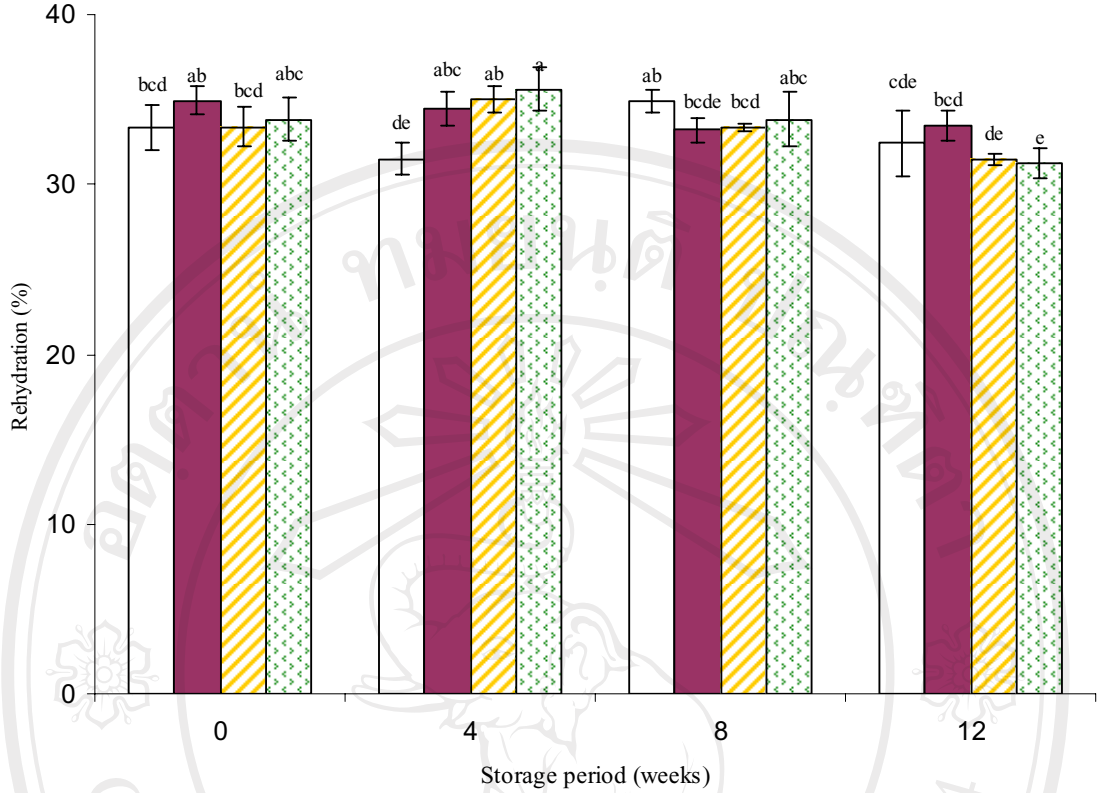

Fig. 30 Rehydration (%) of *L. acidophilus* containing yoghurt powder affected by storage temperature and packaging material**.** Symbol: □ (PET/PP/Al) stored at 4°C, ■ (Nylon/PE) stored at 4°C; ▨ (PET/PP/Al) stored at room temperature and ▩ (Nylon/PE) stored at room temperature

At the beginning of the storage period, the water holding capacity of the *L. acidophilus* containing yoghurt powder was more affected by the packaging materials to keep the product more than the storage temperatures (Fig. 31). Keeping the yoghurt powder in the nylon/PE laminated pouches produced higher values of water holding capacity than those of the PET/PP/Al laminated pouches. However at the end of the storage period, the storage temperatures also affected the yoghurt water holding capacity. At low storage temperature, the water holding capacity of the yoghurt powder was higher, especially for the nylon/PE laminated pouches, compared to those of the higher storage temperature. This result suggesting that both the storage temperatures and the packaging materials affected the yoghurt powder physical property in the long term storage period.

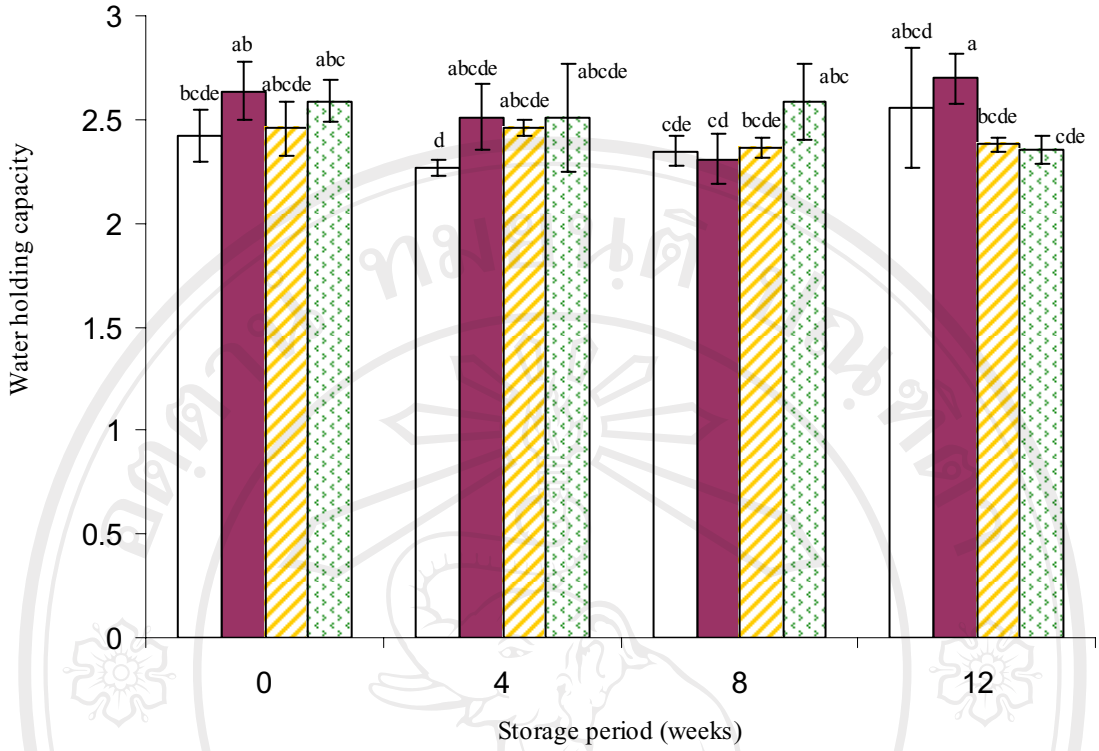

Fig. 31 Water holding capacity of *L. acidophilus* containing yoghurt powder affected by storage temperature and packaging material**.** Symbol: □ (PET/PP/Al) stored at 4°C, ■ (Nylon/PE) stored at 4°C; ▨ (PET/PP/Al) stored at room temperature and ⊡ (Nylon/PE) stored at room temperature

In general, it showed clearly that the bulk density of the *L. acidophilus* containing yoghurt powder was significantly increased during the storage period (Fig. 32). The yoghurt powder stored in the nylon/PE laminated pouches also had higher values of bulk density compared to those of the PET/PP/Al laminated pouches. The result in this experimental section could be affected by the increase in the moisture content of the yoghurt powder (Fig. 26) and the permeability of different packaging materials.

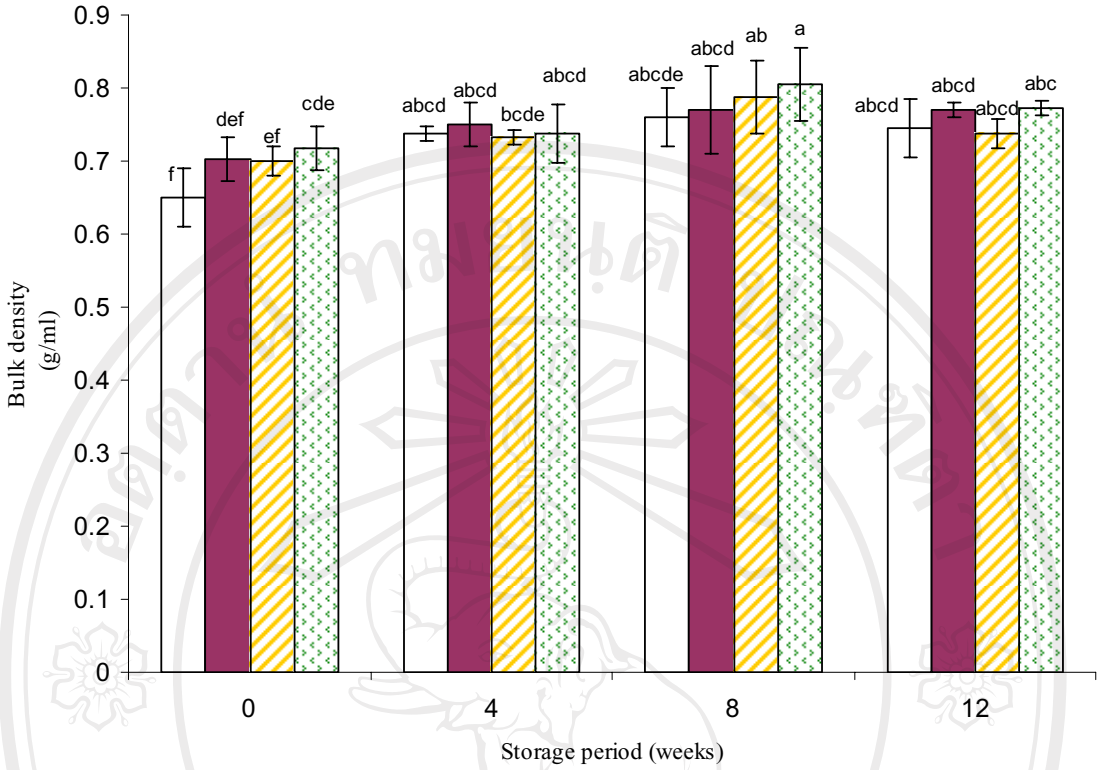

Fig. 32 Bulk density of *L. acidophilus* containing yoghurt powder affected by different storage temperatures and packaging materials. Symbol: □ (PET/PP/Al) stored at 4°C, ■ (Nylon/PE) stored at 4°C; ◪ (PET/PP/Al) stored at room temperature and ◫ (Nylon/PE) stored at room temperature

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