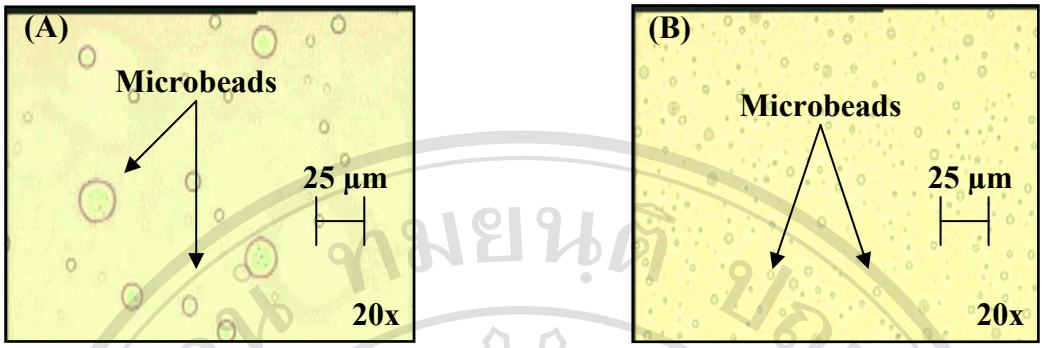
CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Optimization of microbead sizes and microencapsulation of *L. fermentum* 2311M with alginate

The results shown that, agitated for 20-30 minutes were the non-optimum conditions because the various sizes of microbeads were 5-35 µm that show in Figure 4A. The optimum conditions were 50-60 minutes of agitation that obtained the similar size of alginate microbeads. The optimum agitation, 50 minutes was able to produce microbeads sizes 5-10 µm in diameter for alginate microbeads (Figure 4B). While, Krasaekoopt *et al*. (2003) was produced microbeads sizes between 25 µm–2,000 µm by emulsion technique.

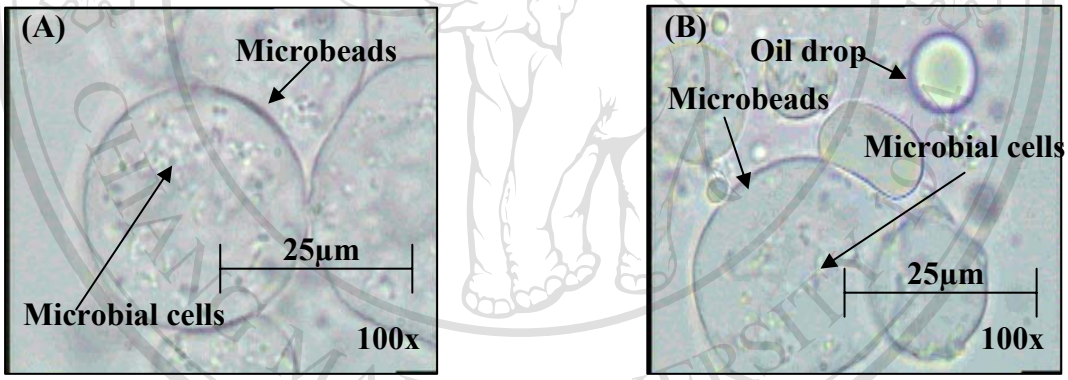
Immobilized *L. fermentum* 2311M were detected in alginate microbeads under the simple light microscope. The optimum agitation time for immobilized bacterial cells in alginate were 50 minutes to produced microbeads sizes 20-80 µm (Figure 5A). The bacterial cells were not detected inside the oil drop sizes 10 µm in conditions for alginate beads production (Figure 5B), when compare with microbeads sizes 25 µm.



Non-optimized condition
20-30 min. agitation
for alginate beads

Optimized condition
50-60 min. agitation
for alginate beads

Figure 4 Optimization of alginate beads production



Alginate microbeads
containing with
Lactobacillus fermentum 2311M

Comparison between
alginate microbeads
and oil drops

Figure 5 Micrograph of the microbeads containing *L. fermentum* 2311M encapsulated in alginate

Sheu *et al*. (1993), produced alginate bead sizes 102, 30 and 15 μm in diameter, respectively. These results that, lactobacilli survived better in bead with mean diameter > 30 μm than in those averaging 15 μm.

Sultana *et al*. (2000), Encapsulated probiotics with an alginate-starch mixture and bead size range of 0.5- 1.0 mm were considerably more viable in yogurt during the storage period.

Ouyang *et al*. (2004) immobilized *Lactobacillus reuteri* in alginate/ poly-L-lysine/pectin/poly-L-lysine/alginate (APPPA) microcapsules for oral delivery and the diameter of microcapsules range 400 ± 25 μm. The APPPA microcapsules can be prepared for bacterial cell encapsulation and are stable in simulated GI condition. No microcapsules damage was reported when exposed to simulated gastric (SGF) and intestinal fluids (SIF) for 12 hours at 250 rpm mechanical shaking at 37.2 ºC. In addition, 93.2 ± 2.3% and 98.9 ± 0.6% of microcapsules were undamaged after 24 hours in SGF and SIF respectively.

4.2 Microencapsulated of *L. fermentum* 2311M in alginate beads

The data of table 2, represent step in encapsulated cell by emulsion technique. Viable cells were calculated as below; (see at Appendix E)

Table 2 Step in encapsulation process on viable counts of alginate beads

Step in encapsulation process	Viable cell count (cfu/ml and cfu/g)
1. Initial cells for encapsulation	2.00×10^{10} cfu/ml
2. NaCl + Cells	2.23×10^{10} cfu/ml
3. Alginate + Cells	2.32×10^{10} cfu/ml
4. Cells of alginate beads/ 1 g of bead	2.31×10^{9} cfu/g
5. Cells leak in $CaCl_2$ solution	4.52×10^{8} cfu/ ml
6.Cells of alginate beads leak in container	7.18×10^{9} cfu/ml
7. Cells of alginate beads/ 1 ml of cell	7.39×10^{9} cfu/ml
8. Cells in alginate beads + Cells in alginate beads leak in container + Cells leak in $CaCl_2$ solution	1.50×10^{10} cfu/ml

From the table 2 shown that initial cell for encapsulated *L. fermentum* 2311M were 2.00×10^{10} cfu/ml and they were encapsulated in alginate beads 2.31×10^{9} cfu/g. Similarly, Mandal *et al*., 2006, reported that encapsulated *L. casei* NCDC-298 in alginate beads. They used initial cell for encapsulated *L. casei* NCDC-298 were 1.00×10^{10} cfu/ml and they were encapsulated in alginate beads 3.7×10^{9} cfu/g.

Table 3 The percentage of efficiency of microencapsulated in alginate

Bacteria	Initial cells	Alginate beads	% of Efficiency
L. casei NCDC-298 (Mandal *et al*.,2006)	$1x10^{10}$ cfu/ml	$3.70x\ 10^{9}$ cfu/g	47.20
L. fermentum 2311M	$2x10^{10}$ cfu/ml	$2.31x\ 10^{9}$ cfu/g	36.95

From the table 3 shown that the percentage of efficiency of encapsulated *L. casei* NCDC-298 were 47.2 %. While, the percentage of efficiency of encapsulated *L. fermentum* 2311M were 36.95 %.

4.3 Survival of alginate beads and free cells at pH 6.5, 1.5

The effect of pH 6.5 on viable count of free and encapsulated cells in alginate beads. The resulted that, no significant reduction in viable count was observed in free cells and encapsulated cells at pH 6.5 on incubation for 3 h (Figure 6). Therefore, we can determined that at pH 6.5 (distilled water) had no effect on survival of free and encapsulated cells.

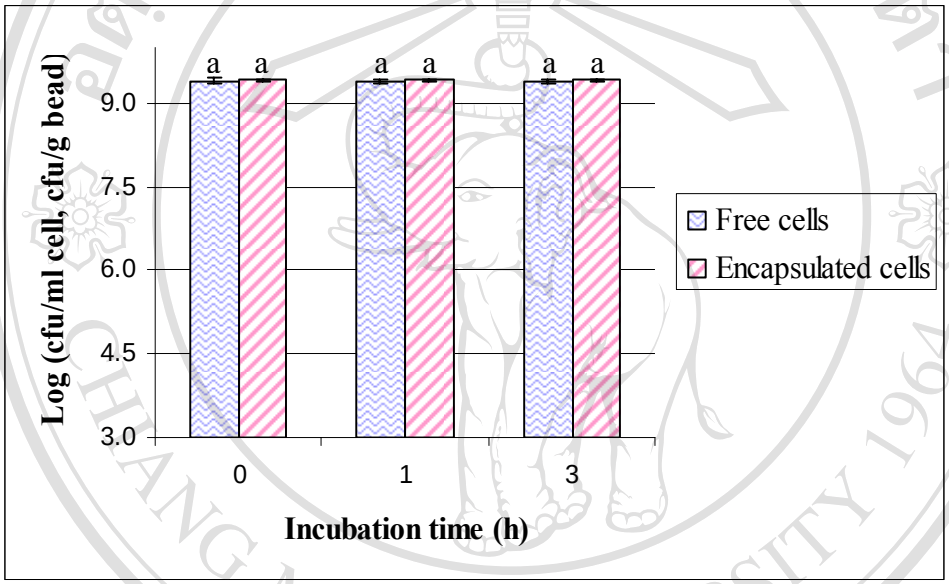


Figure 6 Effect of pH 6.5 on viable counts of free and encapsulated *L. fermentum* 2311M in alginate beads. The error bars represent standard deviation of mean. Mean bars with similar letter (a) at different incubation periods, $P < 0.05$.

Kim *et al*. (2008), reported that at pH 7.0 (control) viability of *L. acidophilus* ATCC 43121 in AGJ remained constant after 3 h incubation at 37 ^{o}C.

Mandal *et al*. (2006), reported the survival of encapsulated *L. casei* in simulated gastric pH. It was found that no significant reduction in viable count was observed in free as well encapsulated cells in distilled water (pH 6.5) on incubation for up to 3 h.

The survival of alginate bead and free cells at pH 1.5. It was found that, there were significant reductions ($P< 0.05$) of free as well alginate encapsulated cells on exposure to pH 1.5. Free cells were decreased from 4.81 to 3.68 log cfu/ml at the end of 1 and 3 h, while encapsulated cells were decreased from 5.59 to 5.21 log cfu/g at the end of 1 and 3 h (Figure 7). So, we can conclude that encapsulated cells can survive at pH 1.5 better than free cells.

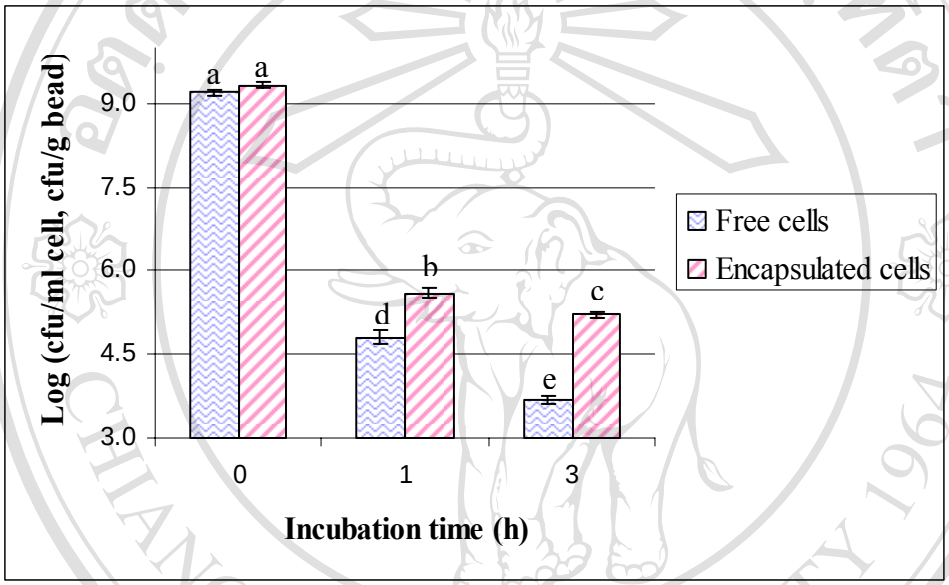


Figure 7 Effect of pH 1.5 on viable counts of free and encapsulated *L. fermentum* 2311M in alginate beads. The error bars represent standard deviation of mean. Mean bars with different letter (a-e) at different incubation periods, $P< 0.05$.

Table 4 Effect of pH 1.5 on viable counts of free and encapsulated cells in alginate of *L. casei* NCDC-298 (Mandal *et al*., 2006) and *L. fermentum* 2311M

Bacteria	Incubation time (h)	FC	% of survival	EC	% of survival
L. casei NCDC-298	0	7.56	100	8.42	100
	1	4.24	56.08	5.96	70.78
	3	3.38	44.70	5.37	63.77
L. fermentum 2311 M	0	9.20	100	9.32	100
	1	4.81	45.43	5.59	60.00
	3	3.68	40.00	5.21	55.90

FC = Free cells (log cfu/ml), EC = Encapsulated cells (log cfu/g)

From the table 4, it was found that the encapsulated cells had the percentage of survival better than free cells on incubation for 3 h. We can determine that encapsulated cells can survive at low pH condition better than free cells. The survival of *L. fermentum* 2311M decreased proportionately with time of exposure to low pH solution.

Favoro-Trindale and Grosso (2002), showed that none of *L. acidophilus* (La-05) survived in the artificial gastric environment of pH 1.0 after 1 h, but microencapsulated *L. acidophilus* (La-05) suffered a reduction of 1 log at pH 1.0 after 2 h incubation.

Hansen *et al*. (2002), who reported that microencapsulation in alginate bead did not effectively protect the microorganism from low pH. So, the death rate of microorganism entrapped in alginate beads decreased proportionately with increased capsule size and alginate concentration (Lee and Heo, 2000).

The viability of encapsulated bacteria in simulated gastric conditions increased with increased capsule size (200-1,000 μm) and also with increased alginate gel concentration from 0.75% to 1.8% (w/v). There was no significant increase (P>0.05) in viable cell number of capsules when the alginate gel concentration was further increased to 2% (w/v) (Chandramouli *et al.*, 2004).

Truelstrup Hansen *et al*. (2002), reported that very large beads (1,000 μm) cause a coarseness of texture in live microbial feed supplements, and small bead of size less than 100 μm did not significantly protect the probiotic bacteria in simulated gastric juice compared to free cell. Therefore, probiotic bacteria should be entrapped within a limited range of bead size.

4.4 Survival of alginate beads and free cells at 0%, 0.15% and 0.3% of bile salt solution

From the Figure 8, we can determine that no significant reduction in viable count was observed in free cells and encapsulated cells at 0% of bile salt on incubation for 3 h. Therefore, we can indicate that at 0% of bile salt (distilled water) had no effect on survival of free and encapsulated cells.

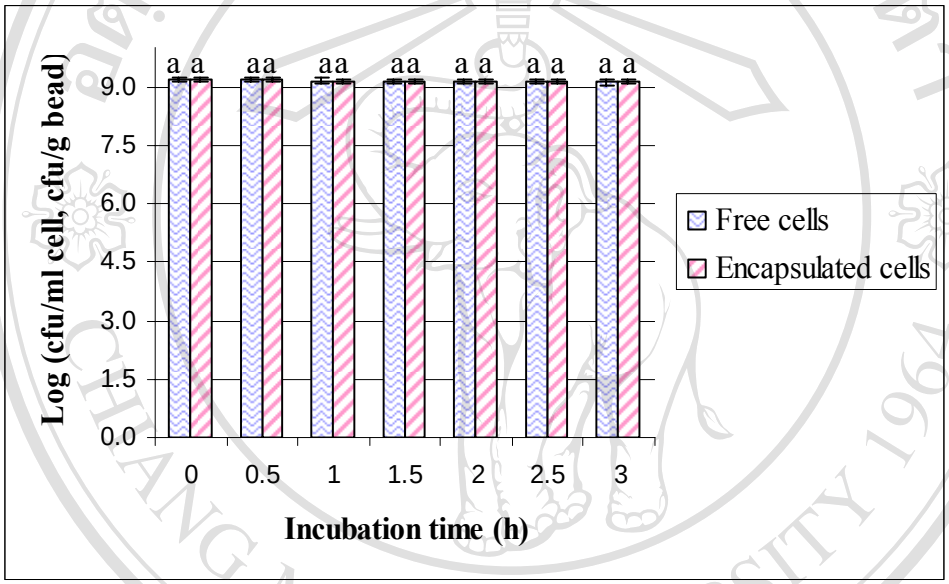


Figure 8 Effect of 0% bile salt on viable counts of free and encapsulated *L. fermentum* 2311M in alginate beads. The error bars represent standard deviation of mean. Mean bars with similar letter (a) at different incubation periods, $P < 0.05$.

Survivals of alginate beads at 0.15% of bile salt solution after incubation for 3 h. The results shown that there were significant reductions ($P < 0.05$) of free as well alginate encapsulated cells on exposure to 0.15% of bile salt solution. Free cells were decreased from 7.65 to 4.35 log cfu/ml at the end of 1 and 3 h, while encapsulated cells were decreased from 8.91 to 8.16 log cfu/g at the end of 1 and 3 h (Figure 9).

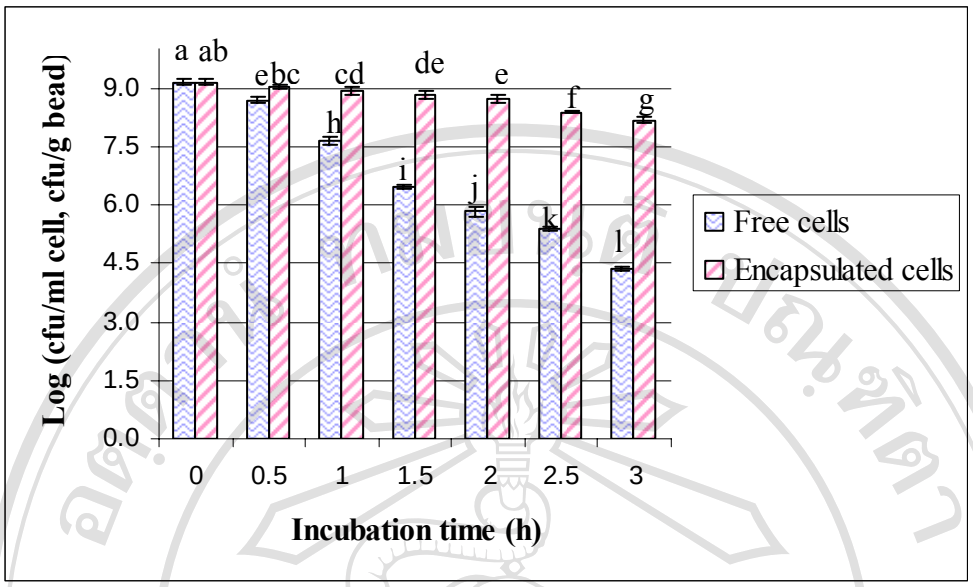


Figure 9 Effect of 0.15% bile salt on viable counts of free and encapsulated *L. fermentum* 2311M in alginate beads. The error bars represent standard deviation of mean. Mean bars with different letter (a-l) at different incubation periods, $P< 0.05$.

Survivals of alginate beads at 0.3% of bile salt solution after incubation for 3 h. The results shown that there were significant reductions ($P< 0.05$) of free as well alginate encapsulated cells on exposure to 0.3% of bile salt solution. Encapsulated cells were decreased from 6.89 to 5.50 log cfu/g at the end of 1 and 3 h, while free cells were destroyed completely after 3 h. (Figure 10).

Therefore, the survivals of encapsulated and free cells in 0.15% and 0.3% of bile salt solution were represented, we can determine that alginate beads can survive at bile salt condition better than free cells.

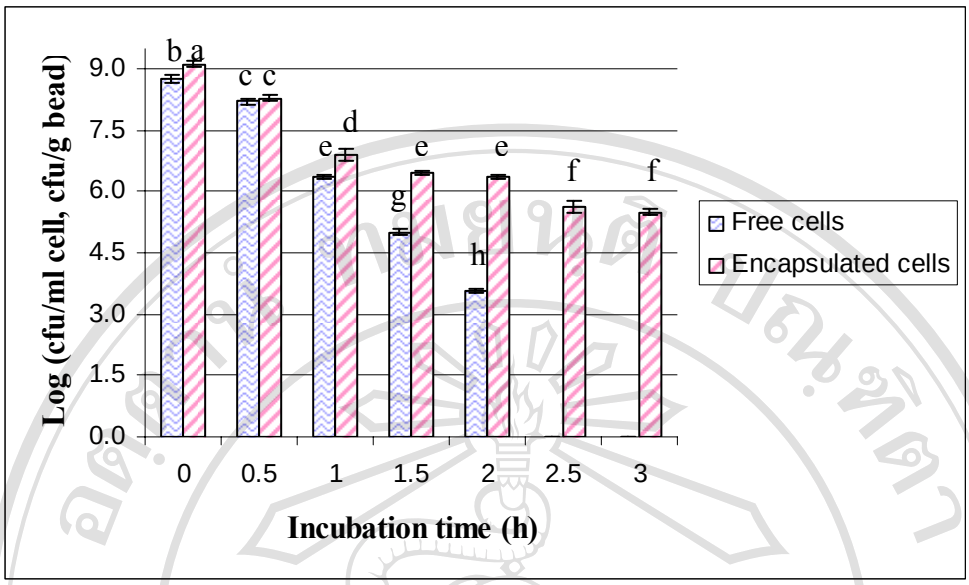


Figure 10 Effect of 0.3% bile salt on viable counts of free and encapsulated *L. fermentum* 2311M in alginate beads. The error bars represent standard deviation of mean. Mean bars with different letter (a-h) at different incubation periods, $P< 0.05$.

Table 5 The survival of free and encapsulated cells in alginate of *L. casei* (Krasaekoopt *et al*., 2004) and *L. fermentum* 2311M after incubation in bile salt solution

Bacteria	Incubation time (h)	FC	% of survival	EC	% of survival
L. casei 0.6% bile salt	0	9.78	100	9.62	100
	1	6.04	61.75	8.80	91.47
	2	2.17	22.18	6.23	64.76
L. fermentum 2311 M 0.3% bile salt	0	8.75	100	9.12	100
	1	6.35	72.57	6.89	75.54
	2	3.55	40.57	6.35	69.62

FC = Free cells (log cfu/ml), EC = Encapsulated cells (log cfu/g)

From the table 5, it was found that the encapsulated cells had the percentage of survival better than free cells on incubation for 2h. We can determine that encapsulated cells can survive at bile salt condition better than free cells. The viability of *L. fermentum* 2311M decreased proportionately with time of exposure to bile salt solution and higher bile salt concentration.

In contrast, Trindade and Grosso (2000), also reported that immobilization of *B. bifidum* and *L. acidophilus* in Ca-alginate beads was not effective in protecting the cells from 2% and 4% bile salt.

While, Mandal (2006), encapsulated *L. casei* NCDC-298 with 2-4% of alginate. It was found that encapsulation with alginate concentration increasing from 2 to 4% improved the viability of cells at similar bile salt concentrations.

4.5 Storage of alginate beads at temperature 4 °C, 8 °C and 20 °C as compared to free cells for 3 months

The alginate beads were stored at 4°C for 3 months to compared with free cells. The results shown that free cells were decreased from 9.37 to 7.57 log cfu/ml while, encapsulated cells were decreased from 9.36 to 7.85 log cfu/g at the end of 3 months (Figure 11).

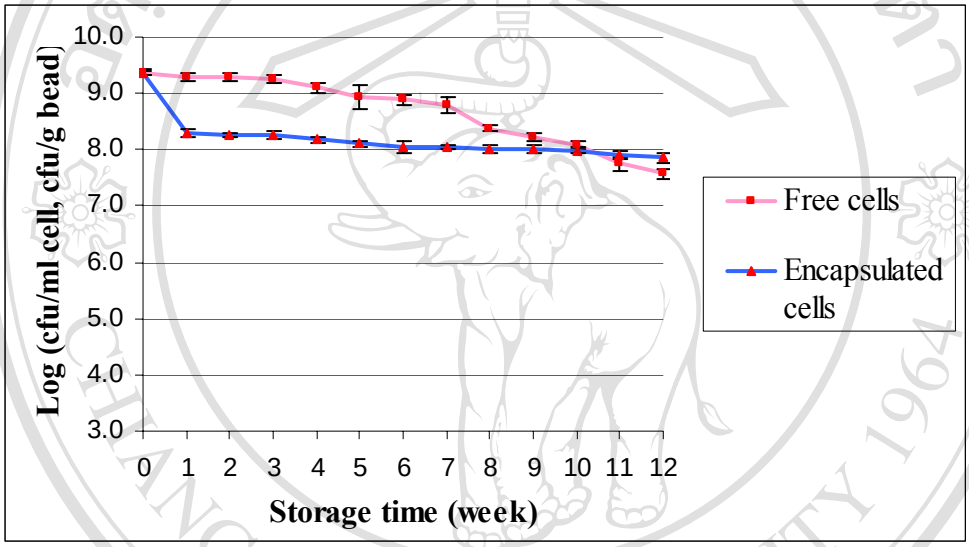


Figure 11 Survival of encapsulated *L. fermentum* in alginate beads, stored in 0.1M $CaCl_2$, at 4°C for 3 months

The alginate beads were stored at 8°C for 3 months to compared with free cells. It was found that free cells were decreased from 9.37 to 6.68 log cfu/ml while, encapsulated cells were decreased from 9.36 to 7.22 log cfu/g at the end of 3 months (Figure 12).

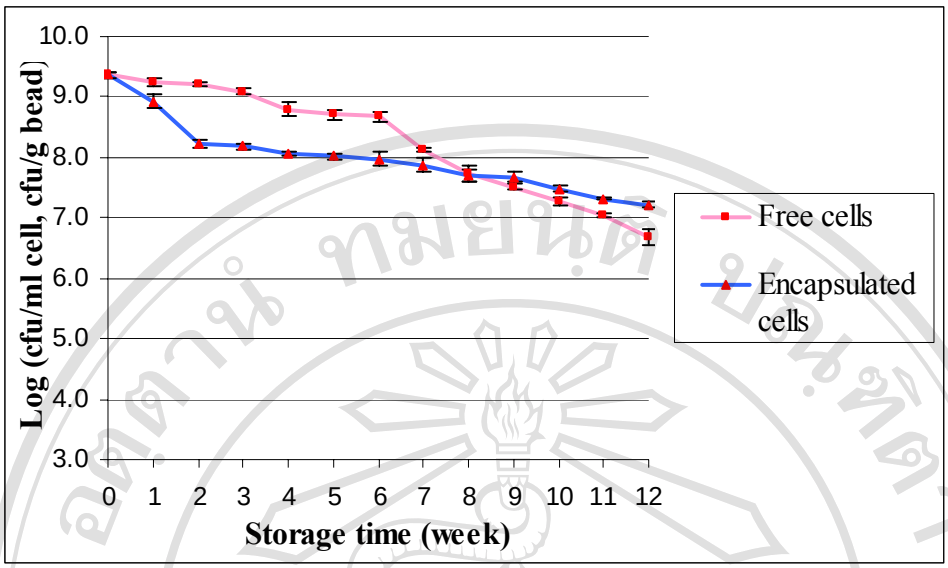


Figure 12 Survival of encapsulated *L. fermentum* in alginate beads, stored in 0.1M $CaCl_2$, at 8°C for 3 months

The alginate beads were stored at 20°C for 3 months to compared with free cells. It was found that free cells were decreased from 9.37 to 3.32 log cfu/ml while, encapsulated cells were decreased from 9.36 to 7.19 log cfu/g at the end of 3 months (Figure 13).

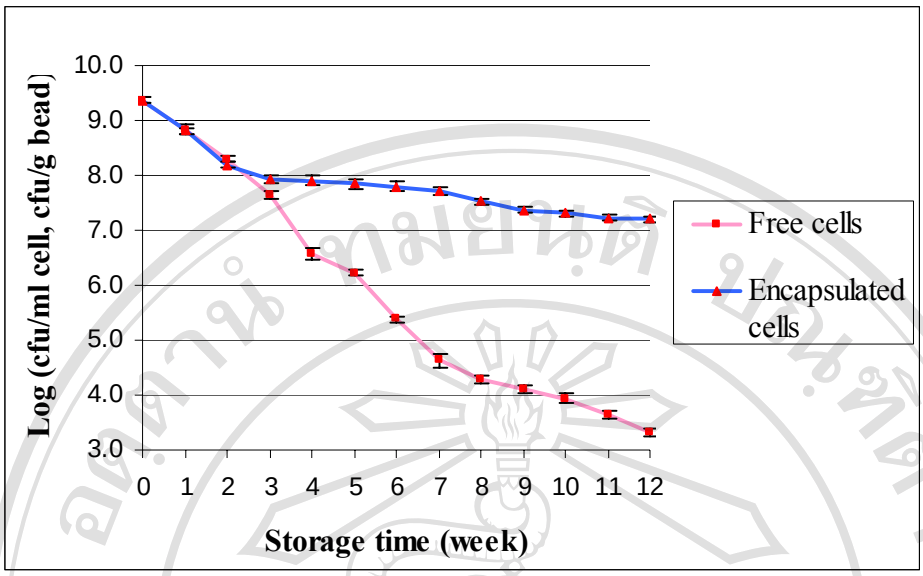


Figure 13 Survival of encapsulated *L. fermentum* in alginate beads, stored in 0.1M $CaCl_2$, at 20 ^{o}C for 3 months

The viable cell numbers of alginate beads at 4 ^{o}C, 8 ^{o}C and 20 ^{o}C for 3 months. It was found that the survival of alginate beads were found to be better than free cells at 4 ^{o}C, 8 ^{o}C and 20 ^{o}C, respectively. Storage at 20°C was found to be the best condition during the storage for 3 months because of free cells at 20°C were drastically reduced more than encapsulated cells at the end of 12 weeks. Therefore, we conclude that stored at 20°C was the best condition for storage.

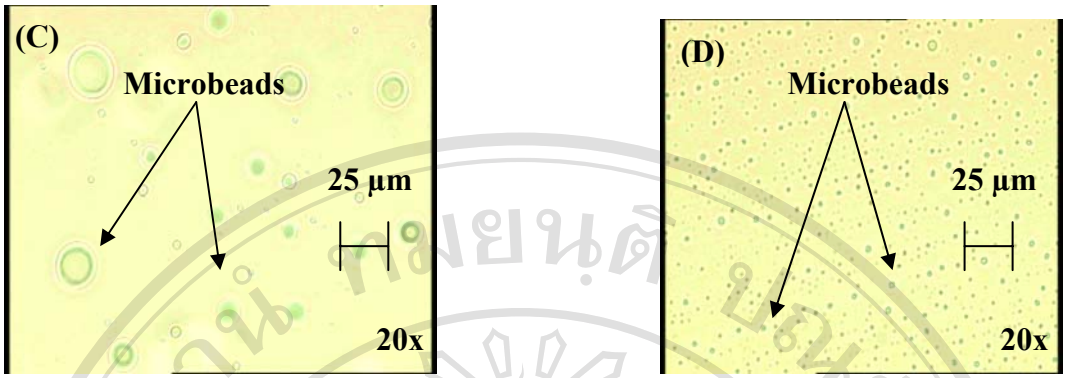
L. acidophilus and *Bifidobacterium* spp. were encapsulated in calcium alginate beads, freeze dried and incorporated into a mix for making frozen fermented dairy desserts prior to freezing. The frozen desserts were stored at -20 ^{o}C for 12 weeks. It was observed that counts of *L. acidophilus* and *Bifidobacterium* spp. in the frozen dessert containing encapsulated bacteria were higher at the end of 12 weeks storage than in those containing non-encapsulated bacteria (Shah and Ravula, 2000).

Viability of microencapsulated *L. acidophilus* and free cells from UHT-and conventionally treated milk during storage at 4°C for 4 weeks. These results implied that the *L. acidophilus* not only survived but also even grew slowly in the first week in the environment inside the alginate beads coated with chitosan. After 1 week, the number of encapsulated cells gradually declined to 8.4 log cfu/g at the end of storage (4 weeks), whereas the number of free cells decreased sharply to 7.2 log cfu/g at week 4, in yoghurt from both UHT-and conventionally treated milk (Krasaekoopt *et al*., 2006).

4.6 Optimization of microbead sizes and microencapsulation of *L. fermentum* 2311M with κ-carrageenan

The results shown that, agitated for 20-30 minutes were the non-optimum conditions because the various sizes of microbeads were 5-35 μm that show in Figure 14A. The optimum conditions were 50-60 minutes of agitation that obtained the similar size of κ-carrageenan microbeads. The optimum agitation, 50 minutes was able to produce microbeads sizes 5-10 μm in diameter for κ-carrageenan microbeads (Figure 14B). While, Krasaekoopt *et al*. (2003) was produced microbeads sizes between 25 μm– 2,000 μm by emulsion technique.

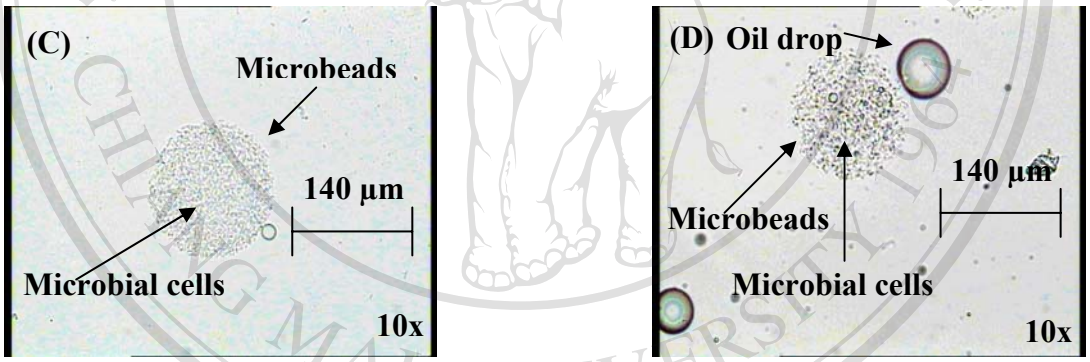
Immobilized *L. fermentum* 2311M were detected in κ-carrageenan microbeads under the simple light microscope. The optimum agitation time for immobilized bacterial cells in κ-carrageenan microbeads were 50 minutes to produced microbeads sizes 120-160 μm (Figure 15A). The bacterial cells were not detected inside the oil drop sizes 50 μm in conditions for κ-carrageenan beads production (Figure 15B), when compare with microbeads sizes 140 μm.



Non-optimized condition
20-30 min. agitation
for κ-carrageenan beads

Optimized condition
50-60 min. agitation
for κ-carrageenan beads

Figure 14 Optimization of κ-carrageenan beads production



κ-carrageenan microbeads containing with *Lactobacillus fermentum* 2311M

Comparison between κ-carrageenan microbeads and oil drops

Figure 15 Micrograph of the microbeads containing *L. fermentum* 2311M encapsulated in κ-carrageenan

Audet *et al*. (1988) immobilized *Streptococcus thermophilus, Lactobacillus bulgaricus* and *S. lactis* in κ-carrageenan-locust bean gum beads (500-1,000 μm and 1,000-2,000 μm diameter) and lactic acid fermentations. The entrapment proceduce was effective for *Streptococcus thermophilus, Lactobacillus bulgaricus* and *S. lactis*, and the viability of those bacteria remained very high throughout entrapment steps and subsequent storage. Bead diameter influenced the fermentation rate: smaller beads (500-1,000 μm) permitted an increase in release rates, lactose utilization and acid production by entrapped cells.

Sun and Griffiths (2000), using *B. infantis*, reported an average gellan: xanthan bead diameter of 3 mm produced by a simple dropping of the mixture though a syringe with a 21 G syringe needle.

Adikhari *et al*. (2003), using co-acervation/emulsion to microencapsulated *B. longum* B6 in κ-carrageenan, reported capsule diameters of 22-350 μm, measured using laser diffractometry.

Muthukumarasamy *et al*. (2006), using *L. reuteri*, reported an average alginate and κ-carrageenan: locust bean gum bead diameter of 38 μm and 90 μm, respectively.

4.7 Microencapsulated of *L. fermentum* 2311M in κ-carrageenan beads

The data of table 3, represent step in encapsulated cell by emulsion technique. Viable cell were calculated as below; (see at Appendix E)

Table 6 Step in encapsulation process on viable counts of κ-carrageenan beads

Step in encapsulation process	Viable cell count (cfu/ml and cfu/g)
1. Initial cells for encapsulation	2.00x 10^{10} cfu/ml
2. NaCl + Cells	2.14 x 10^{10} cfu/ml
3. κ-carrageenan + Cells	2.08 x 10^{10} cfu/ml
4. Cells of κ-carrageenan beads/ 1 g of bead	1.80x 10^{9} cfu/g
5. Cells leak in KCl solution	3.45 x 10^{8} cfu/ ml
6.Cells of κ-carrageenan beads leak in container	6.00 x 10^{9} cfu/ml
7. Cells of κ-carrageenan beads/ 1 ml of cell	5.89 x 10^{9} cfu/ml
8. Cells of κ-carrageenan beads + Cells of κ-carrageenan beads leak in container + Cells leak in KCl solution	1.22 x 10^{10} cfu/ml

From the table 2 shown that initial cell for encapsulated *L. fermentum* 2311M were 2.00 x 10^{10} cfu/ml and they were encapsulated in κ-carrageenan beads 1.8 x 10^{9} cfu/g. Similarly, Adhikari *et al*., 2000, reported that encapsulated *B. longum* B6 in κ-carrageenan beads. They used initial cell for encapsulated *B. longum* B6 were 1.2 x 10^{9} cfu/ml and they were encapsulated in κ-carrageenan beads 1.5 x 10^{9} cfu/g.

Table 7 The percentage of efficiency of microencapsulated in κ-carrageenan

Bacteria	**Initial cells**	**κ-carrageenan beads**	**% of Efficiency**
B. longum B6 (Adhikari *et al*.,2000)	$1.2x10^9$ cfu/ml	$1.5x\ 10^9$ cfu/g	40.83
L. fermentum 2311M	$2x10^{10}$ cfu/ml	$1.8x\ 10^9$ cfu/g	29.45

From the table 3 shown that the percentage of efficiency of encapsulated *B. longum* B6 were 40.83 %. While, the percentage of efficiency of encapsulated *L. fermentum* 2311M were 29.45 %.

4.8 Survival of κ-carrageenan beads and free cells at pH 6.5, 1.5

The effect of pH 6.5 on viable count of free and encapsulated cells in κ-carrageenan beads. The resulted that, no significant reduction in viable count was observed in free cells and encapsulated cells at pH 6.5 on incubation for 3 h (Figure 16). Therefore, we can determined that at pH 6.5 (distilled water) had no effect on survival of free and encapsulated cells.

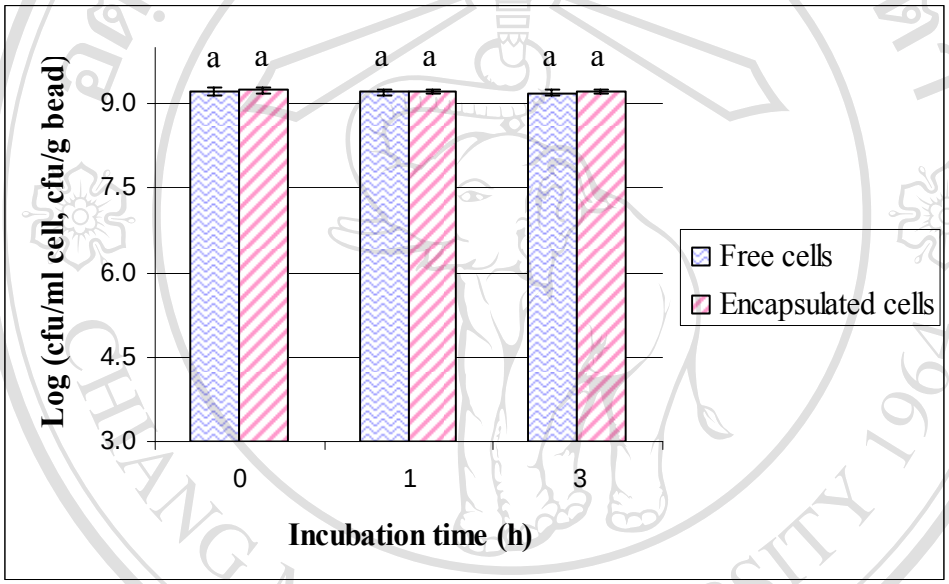


Figure 16 Effect of pH 6.5 on viable counts of free and encapsulated *L. fermentum* 2311M in κ-carrageenan beads. The error bars represent standard deviation of mean. Mean bars with similar letter (a) at different incubation periods, $P< 0.05$.

Kim *et al*. (2008), reported that at pH 7.0 (control) viability of *L. acidophilus* ATCC 43121 in AGJ remained constant after 3 h incubation at 37 ^{o}C.

Effect of pH 1.5 on viable counts of free and encapsulated cells in κ-carrageenan beads. The resulted that, there were significant reductions ($P< 0.05$) of free as well κ-carrageenan encapsulated cells on exposure to pH 1.5. Free cells were decreased from 4.75 to 3.54 log cfu/ml at the end of 1 and 3 h, while encapsulated cells were decreased from 5.54 to 5.14 log cfu/g at the end of 1 and 3 h (Figure 17).

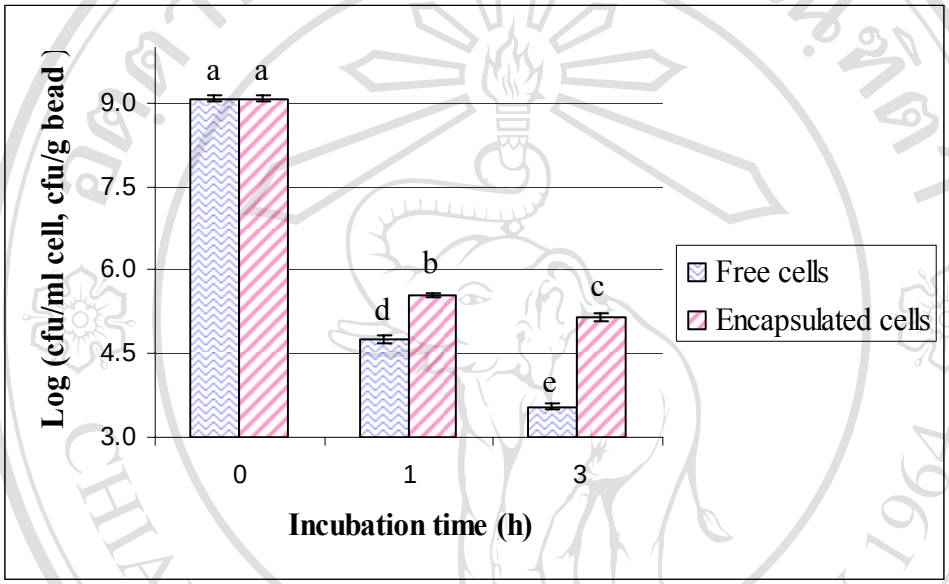


Figure 17 Effect of pH 1.5 on viable counts of free and encapsulated *L. fermentum* 2311M in κ-carrageenan beads. The error bars represent standard deviation of mean. Mean bars with different letter (a-e) at different incubation periods, $P< 0.05$.

Therefore, we can conclude that encapsulated cells can survive at pH 1.5 better than free cells. The survival of *L. fermentum* 2311M decreased proportionately with time of exposure to low pH solution.

4.9 Survival of κ-carrageenan beads and free cells at 0%, 0.15% and 0.3% of bile salt solution

From the Figure 18, we can indicate that no significant reduction in viable count was observed in free cells and encapsulated cells at 0% of bile salt on incubation for 3 h. Therefore, we can determine that at 0% of bile salt (distilled water) had no effect on survival of free and encapsulated cells.

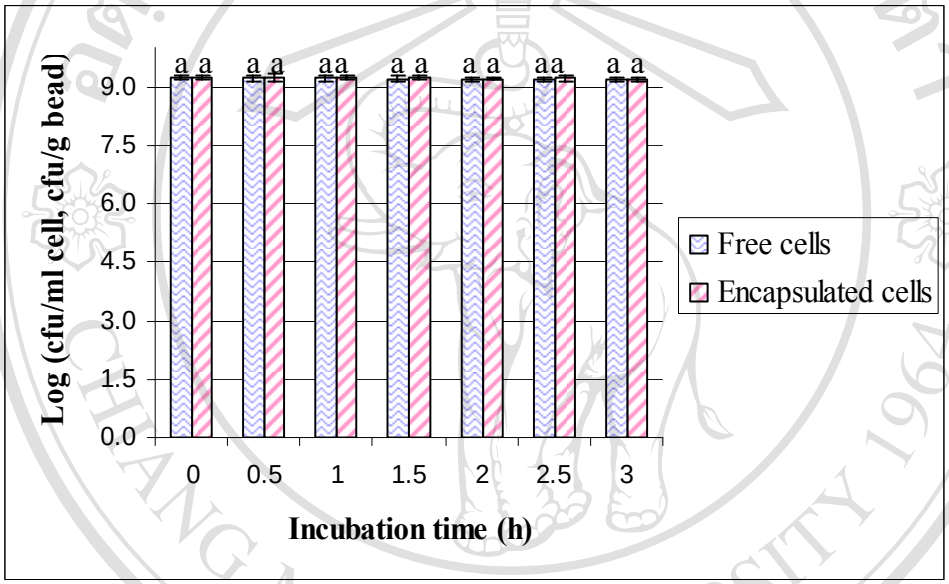


Figure 18 Effect of 0% bile salt on viable counts of free and encapsulated *L. fermentum* 2311M in κ-carrageenan beads. The error bars represent standard deviation of mean. Mean bars with similar letter (a) at different incubation periods, $P< 0.05$.

The κ-carrageenan beads at 0.15% of bile salt solution after incubation for 3 h. The results shown that there were significant reductions ($P< 0.05$) of free as well κ-carrageenan encapsulated cells on exposure to 0.15% of bile salt solution. Free cells were decreased from 7.53 to 3.99 log cfu/ml at the end of 1 and 3 h, while encapsulated cells were decreased from 8.07 to 6.19 log cfu/g at the end of 1 and 3 h (Figure 19).

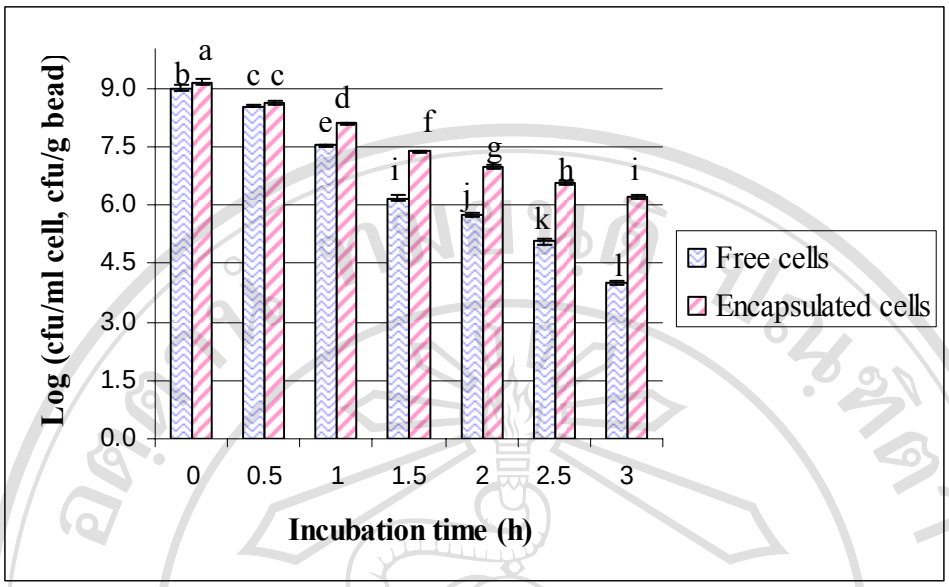


Figure 19 Effect of 0.15% bile salt on viable counts of free and encapsulated *L. fermentum* 2311M in κ-carrageenan beads. The error bars represent standard deviation of mean. Mean bars with different letter (a-l) at different incubation periods, $P< 0.05$.

The κ-carrageenan beads at 0.3% of bile salt solution after incubation for 3 h. The results shown that there were significant reductions ($P< 0.05$) of free as well κ-carrageenan encapsulated cells on exposure to 0.3% of bile salt solution. Encapsulated cells were decreased from 6.91 to 4.45 log cfu/g at the end of 1 and 3 h while, free cells were destroyed completely after 3 h (Figure 20).

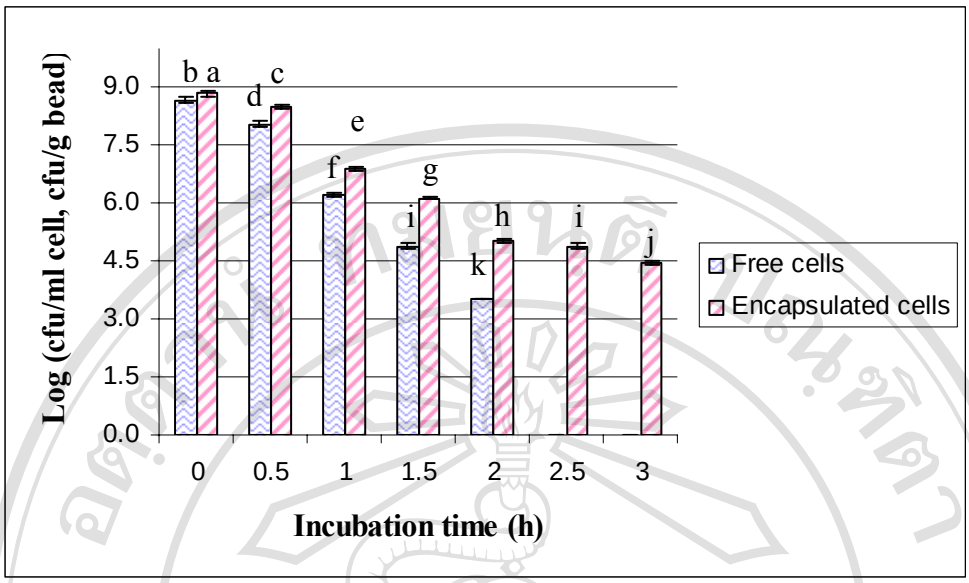


Figure 20 Effect of 0.3% bile salt on viable counts of free and encapsulated *L. fermentum* 2311M in κ-carrageenan beads. The error bars represent standard deviation of mean. Mean bars with different letter (a-k) at different incubation periods, $P< 0.05$.

The survivals of encapsulated and free cells in 0.15% and 0.3% of bile salt solution were represented, we can determine that κ-carrageenan beads can survive at bile salt condition better than free cells. The viability of *L. fermentum* 2311M decreased proportionately with time of exposure to bile salt solution and higher bile salt concentration .

The survivals of non-encapsulated and encapsulated *L. acidophilus* ATCC 43121 were monitored up to 24 h after exposure to 0% (control), 0.3% and 0.5% AIJ. The viability of non-encapsulated *L. acidophilus* ATCC 43121 were decreased from 9.4 x 10^6 to 1.5 x 10^6 and from 7.1 x 10^6 to 9.2 x 10^5 , respectively, at 0.3% and 0.5% bile salt concentration after 24 h incubation at 37°C. While, the viabilities of encapsulated *L. acidophilus* ATCC 43121 were not decreased at 0.3% and 0.5% AIJ after 24 h (Kim *et al*., 2008).

4.10 Storage of κ-carrageenan beads at temperature 4 °C, 8 °C and 20 °C as compared to free cells for 3 months

The κ-carrageenan beads were stored at 4°C for 3 months to compared with free cells. The results shown that free cells were decreased from 9.24 to 6.24 log cfu/ml while, the viability of encapsulated cells decreased proportionately during storage time and they can survive at the end of 9 weeks (Figure 21).

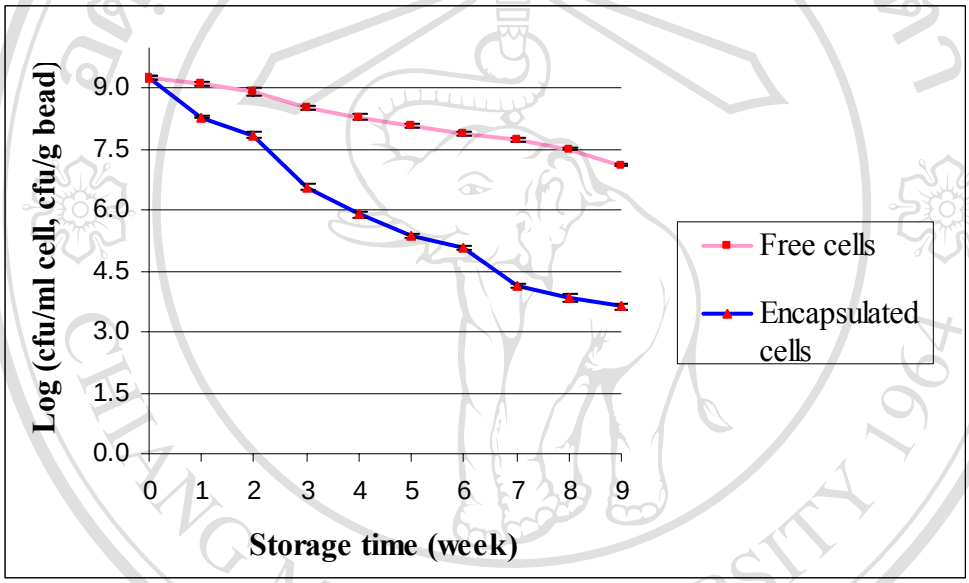


Figure 21 Survival of encapsulated *L. fermentum* in κ-carrageenan beads, stored in 0.05 M KCl, at 4°C for 3 months

The κ-carrageenan beads were stored at 8°C for 3 months to compared with free cells. It was found that free cells were decreased from 9.24 to 4.85 log cfu/ml while, the viability of encapsulated cells decreased proportionately during storage time and they can survive at the end of 8 weeks (Figure 22).

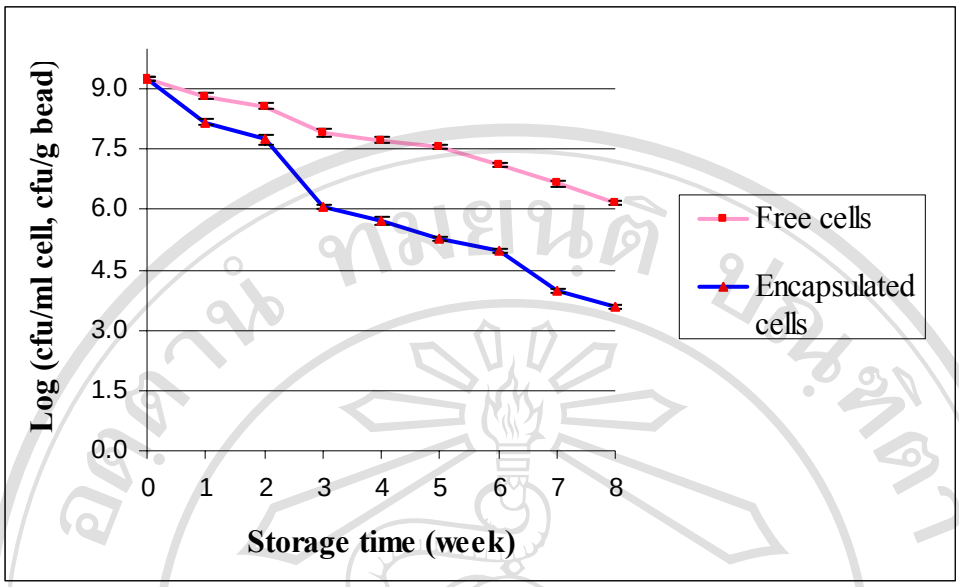


Figure 22 Survival of encapsulated *L. fermentum* in κ-carrageenan beads, stored in 0.05 M KCl, at 8°C for 3 months

The κ-carrageenan beads were stored at 20°C for 3 months to compared with free cells. It was found that free cells were decreased from 9.24 to 2.98 log cfu/ml while, the viability of encapsulated cells decreased proportionately during storage time and they can survive at the end of 7 weeks (Figure 23).

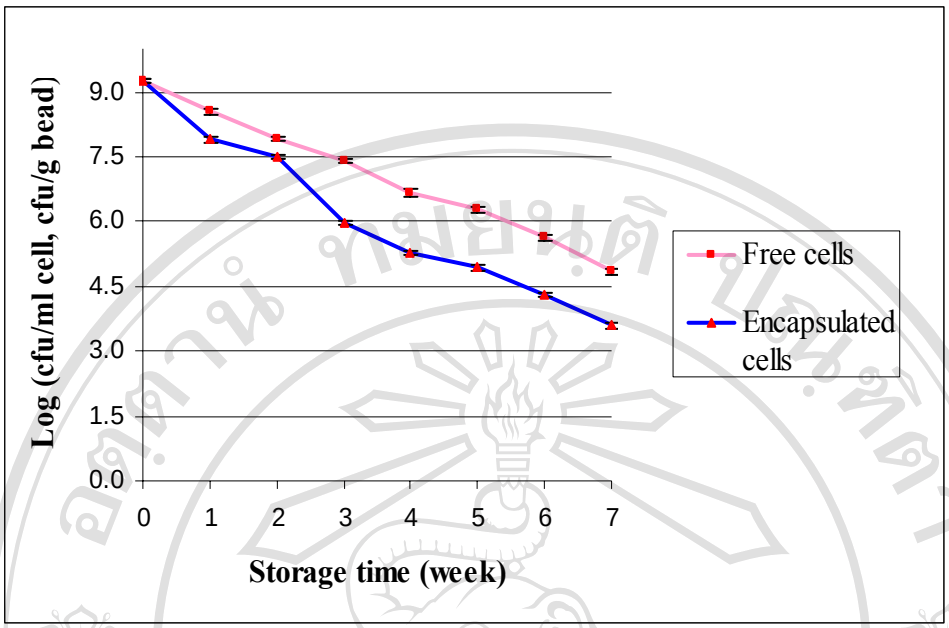


Figure 23 Survival of encapsulated *L. fermentum* in κ-carrageenan beads, stored in 0.05 M KCl, at 20 ^{o}C for 3 months

Immobilization of *S. thermophilus* and *L. bulgaricus* in κ-carrageenan-locust bean gum bead and storage for 2 week. Entrapped cells of *S. thermophilus* stored in 0.05 M KCl or in 0.1% peptonized water for 1 or 2 weeks showed little difference in viability. However, after 2 weeks of storage, small beads exhibited a more pronounced decrease in viability than did larger ones. *L. bulgaricus* cultures showed the same viability during the entrapment process and storage. However, the difference between the two ranges of bead size was evident after 24 h storage in 0.05 M KCl solution. *L. bulgaricus* viability was dramatically affected during storage of the beads in 0.05 M KCl solution.The best results for *L. bulgaricus* were obtained with petonized water: viable cell numbers remained almost constant between 1 and 14 days storage for both bead diameters (Audet *et al.*, 1988).

L. rhamnosus was entrapped by Ca-alginate and κ-carrageenan, and the storage test was conducted after slow freezing and fast freezing treatments of the immobilized cells. The results shown that viable cell variation after fast and slow freezing treatments of immobilized and free *L. rhamnosus*. It was found that the survival of *L. rhamnosus* for immobilized cells was better than for free cells after freezing and Ca-alginate entrapment was better than κ-carrageenan entrapment (De Giulio *et al*., 2005).

Table 8 Effect of encapsulation on population of *B. longum* B6 (Adhikari *et al*., 2000) in set yogurt and *L. fermentum* 2311M in 0.05 M KCl at 4 ^{o}C for 4 weeks

Bacteria	**Storage time (weeks)**	**FC**	**% of survival**	**EC**	**% of survival**
B. longum B6 (2% of κ-carrageenan)	0 day	9.18	100	9.18	100
	week 1	8.94	97.38	9.10	99.12
	week 2	8.69	94.66	9.07	98.80
	week 3	8.66	94.33	8.96	97.60
	week 4	8.64	94.11	9.09	99.01
L. fermentum 2311M (0.5% of κ-carrageenan)	0 day	9.25	100	9.25	100
	week 1	9.10	98.37	8.29	89.62
	week 2	8.92	96.43	8.12	87.78
	week 3	8.51	92.00	6.20	67.02
	week 4	8.30	89.72	5.90	63.78

FC = Free cells (log cfu/ml), EC = Encapsulated cells (log cfu/g)

The viable cell numbers of κ-carrageenan beads was less than free cells at 4°C, 8°C and 20°C during for 3 months. In the table 8, it was found that encapsulated cells in *B. longum* B6 can survive at 4 °C better than free cells. While, encapsulated cells in *L. fermentum* 2311M can survive at 4 °C less than free cells. May be, using low concentration of κ-carrageenan had effect on the survival of microorganisms during storage time. So, increasing κ-carrageenan concentrations also had a positive effect on survival of *L. fermentum* 2311M in harsh conditions.

All of these experiments, we can conclude that the survival of cells in alginate and κ-carrageenan beads can survive better than free cells in low pH and bile salt conditions. While, the storage of cells in alginate beads at 4 °C, 8 °C and 20 °C were better than cells in κ-carrageenan beads during the storage times. This might be use low concentration of κ-carrageenan solution, the difference in gelation mechanism of these two gels that effect of alginate can protect cells better than κ-carrageenan or the gel of κ-carrageenan bead strength can be enhanced using another polymer such as locust bean gum.