CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Growth curves of probiotic bacteria in soymilk yogurt with yogurt starter bacteria

In this section, the growth of a probiotic bacterium of *Bifidobacterium bifidum* or *Lactobacillus acidophilus* in the presence of yogurt starter bacteria, *Streptocoocus thermophilus and Lactobacillus bulgaricus* in soymilk yogurt was monitored for 42 h at 43^{0}C.

Yogurt samples during the fermentation period were separated and analyzed for their chemical and microbiological changes. The result of these analyses was displayed in Tables 4.1 and 4.2 and Figures 4.1 and 4.2

The result from titratable acidity measurement in table 4.1 showed that the amount of lactic acid in soymilk was significantly ($p \leq 0.05$) increased during 42 h fermentation at 43^{0}C. At the beginning of the fermentation time, the soymilk had a titratable acidity of $0.2 \pm 0.00\%$ lactic acid. This amount of lactic acid was increased rapidly within the first 12 h of the incubation period to reach an amount of $0.6 \pm 0.001\%$ in the soymilk yogurt either in the presence of *B. bifidum* or *L. acidophilus.* After this incubation time, the development of lactic acid in the soymilk yogurt was increased in a slower rate. In the last 30 h of the fermentation period, the content of the titratable acidity was only increased up to $0.8 \pm 0.001\%$ lactic acid in the soymilk yogurt with *B. bifidum* and $0.9 \pm 0.00\%$ lactic acid in the soymilk yogurt with *L. acidophilus*. The increase in the amount of lactic acid was mainly due to the use of sugar by microorganisms for their growth and a production of lactic acid as a by-product (Birollo *et al*., 2000). At the same time,

Table 4.1 Chemical properties of soymilk yogurt fermented with *S. thermophilus, L. bulgaricus* and *B. bifidum or L. acidophilus* at 43^0C for 42 h

Incubation time (h)	Soymilk yogurt with *B. bifidum*		Soymilk yogurt with *L. acidophilus*	
	Total titratable acidity (as % lactic acid)	**pH**	**Total titratable acidity (as % lactic acid)**	**pH**
0	0.20 ± 0.00^{a}	6.70 ± 0.01^{k}	0.18 ± 0.00^{a}	6.69 ± 0.00^{l}
2	0.41 ± 0.001^{b}	5.18 ± 0.00^{j}	0.38 ± 0.001^{b}	5.20 ± 0.01^{k}
4	0.45 ± 0.00^{c}	4.50 ± 0.00^{i}	0.45 ± 0.00^{c}	4.50 ± 0.00^{j}
6	0.54 ± 0.00^{d}	4.26 ± 0.00^{f}	0.54 ± 0.00^{d}	4.27 ± 0.01^{i}
8	0.51 ± 0.001^{e}	4.15 ± 0.00^{g}	0.54 ± 0.00^{d}	4.14 ± 0.00^{h}
10	0.63 ± 0.00^{f}	4.14 ± 0.00^{f}	0.63 ± 0.001^{e}	4.09 ± 0.00^{g}
12	0.58 ± 0.001^{g}	4.09 ± 0.01^{c}	0.63 ± 0.002^{e}	4.07 ± 0.01^{f}
15	0.64 ± 0.001^{g}	4.01 ± 0.00^{c}	0.68 ± 0.001^{f}	4.00 ± 0.00^{e}
18	0.66 ± 0.00^{h}	3.99 ± 0.01^{d}	0.68 ± 0.001^{f}	3.98 ± 0.01^{d}
24	0.69 ± 0.001^{i}	3.93 ± 0.02^{c}	0.72 ± 0.001^{g}	3.90 ± 0.01^{c}
36	0.74 ± 0.001^{j}	3.85 ± 0.01^{b}	0.82 ± 0.00^{h}	3.85 ± 0.01^{b}
42	0.80 ± 0.001^{k}	3.80 ± 0.01^{a}	0.86 ± 0.00^{i}	3.76 ± 0.01^{a}

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same column indicated significantly different ($p \leq 0.05$) between the treatments

Table 4.2 Microbiological properties of soymilk yogurt fermented with *S. thermophilus and L. bulgaricus* and *B. bifidum or L. acidophilus* at 43^0C for 42 h

Incubation time (h)	Soymilk yogurt with *B. bifidum*			Soymilk yogurt with *L.acidophilus*		
	B. bifidum (log CFU/ml)	*S. thermophilus* (log CFU/ml)	*L. bulgaricus* (log CFU/ml)	*L. acidophilus* (log CFU/ml)	*S. thermophilus* (log CFU/ml)	*L. bulgaricus* (log CFU/ml)
0	6.89 ± 0.15^{a}	8.25 ± 0.01^{a}	7.67 ± 0.14^{a}	6.92 ± 0.11^{a}	8.06 ± 0.06^{a}	7.83 ± 0.04^{a}
2	6.93 ± 0.06^{a}	8.56 ± 0.01^{b}	7.71 ± 0.08^{a}	6.83 ± 0.08^{a}	8.21 ± 0.09^{a}	7.95 ± 0.01^{ab}
4	7.19 ± 0.05^{b}	8.77 ± 0.09^{c}	8.33 ± 0.03^{b}	7.31 ± 0.00^{ab}	8.86 ± 0.00^{b}	8.12 ± 0.03^{b}
6	7.24 ± 0.01^{b}	9.15 ± 0.08^{d}	8.51 ± 0.48^{b}	7.48 ± 0.36^{b}	9.94 ± 0.33^{c}	8.74 ± 0.04^{c}
8	7.65 ± 0.02^{c}	9.95 ± 0.06^{e}	9.24 ± 0.03^{c}	7.79 ± 0.04^{bc}	10.31 ± 0.23^{cd}	9.20 ± 0.25^{d}
10	8.04 ± 0.13^{d}	10.13 ± 0.08^{f}	10.02 ± 0.18^{d}	8.46 ± 0.54^{def}	10.67 ± 0.11^{d}	9.72 ± 0.21^{e}
12	8.34 ± 0.04^{e}	10.74 ± 0.01^{g}	10.73 ± 0.04^{e}	8.29 ± 0.43^{cde}	11.13 ± 0.19^{e}	10.40 ± 0.01^{f}
15	8.76 ± 0.02^{fg}	11.21 ± 0.06^{h}	10.80 ± 0.01^{e}	8.77 ± 0.14^{ef}	11.69 ± 0.07^{f}	10.90 ± 0.06^{gh}
18	8.70 ± 0.14^{fg}	11.37 ± 0.06^{hi}	10.90 ± 0.09^{e}	8.64 ± 0.21^{def}	11.64 ± 0.16^{f}	10.94 ± 0.08^{gh}
24	8.59 ± 0.14^{f}	11.45 ± 0.03^{i}	10.92 ± 0.06^{e}	8.18 ± 0.08^{cd}	11.82 ± 0.11^{f}	10.99 ± 0.19^{h}
36	8.90 ± 0.05^{g}	11.49 ± 0.04^{i}	10.69 ± 0.02^{e}	8.72 ± 0.03^{def}	11.43 ± 0.30^{ef}	10.95 ± 0.01^{gh}
42	8.80 ± 0.02^{g}	11.66 ± 0.17^{j}	10.87 ± 0.00^{e}	8.90 ± 0.02^{f}	11.57 ± 0.11^{f}	10.70 ± 0.05^{g}

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same column indicated significantly different ($p \leq 0.05$) between the treatments

the result also indicated that *L. acidophlus* could give a better support in the production of lactic acid compared to that of *B. bifidum*.

A measurement of pH values supported the result of total titratable acidity measurement (Table 4.1). During the incubation period, the pH values of soymilk yogurt were significantly decreased (p≤0.05). The initial pH values of soymilk with *B. bifidum* or *L. acidophilus* were 6.70 ± 0.01and 6.69 ± 0.00, respectively. These pH values were significantly (p≤0.05) reduced after 12 h incubation period to reach values of 4.09 ± 0.01 and 4.07 ± 0.01 in the soymilk yogurt with *B. bifidum* or *L. acidophilus*, respectively. At the same time, the texture of the soymilk yogurt had become a curd. When the soymilk yogurts were incubated further up to 42 h, the pH values of the yogurts became 3.80 ± 0.01 and 3.76 ± 0.01 for the soymilk yogurt with *B. bifidum* or *L. acidophilus*, respectively. An explanation for this finding was explaned by Wattananapakasem (2004).

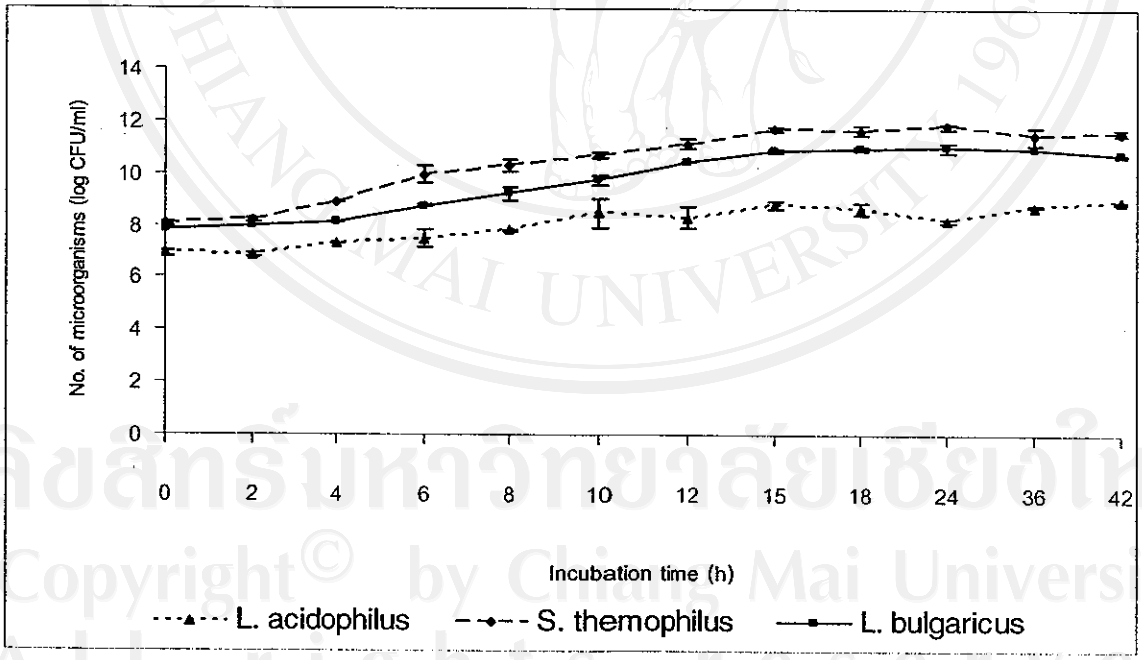

Figure 4.1 The growth curve of *S. thermophilus, L. bulgaricus* and *L. acidophilus* (log CFU/ml) in soymilk yogurt during fermentation at 43^{0}C for 42 h.

The microbiological results in Table 4.2, Figures 4.1 and 4.2 showed clearly that the 2 yogurt starter microorganisms of *S. thermophilus* and *L. bulgaricus* and 2 probiotic bacteria of *B. bifidum* and *L. acidophilus* were significantly increased ($P \leq 0.05$) during the fermentation period at 43^{0}C for 42 h. A rapid growth of these microorganisms were mainly occurred within the first 10 h incubation period. The two probiotic bacteria reached microbial counts of 8.04 ± 0.13 and 8.46 ± 0.54 log CFU/ml for *B. bifidum* and *L. acidophilus*, respectively, after 10 h at 43^{0}C. After 15 h incubation period, the numbers of yogurt and probiotic bacteria were became more constant or slightly reduced (Figure 4.1 and 4.2). This could be due to the presence of lactic acid in the soymilk yogurt that could give a negative effect for both the yogurt and probiotic bacteria. It could be noted that the number of *L. acidophilus* was slightly higher than that of the *B. bifidum* at the end of fermentation time. This could contribute to the presence of a higher lactic acid or a lower pH values of the soymilk yogurt with *L. acidophilus* compared to those of the other soymilk yogurt.

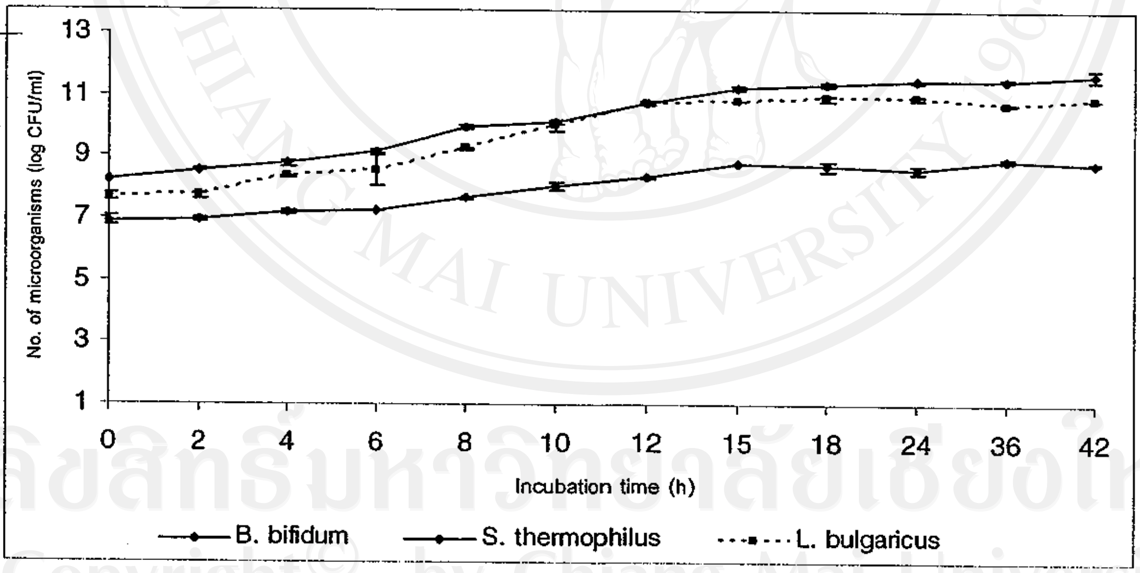

Figure 4.2 The growth curve of *S. thermophilus, L. bulgaricus* and *B. bifidum* (log CFU/ml) in soymilk yogurt during fermentation at 43^{0}C for 42 h.

4.2 Basic formula of soybean yogurt

In this section, a basic formula of soybean yogurt without any addition of probiotic bacteria was prepared and stored at 4^{0}C for 21 days. The basic formula of the yogurt composed of Reconstituted Skimmed milk, Sugar, Soymilk and Commercial Culture (*S. thermophilus* and *L. bulgaricus*). An application of 4^{0}C storage temperature was used because this storage temperature was the most used temperature to keep pasteurized dairy products. During the storage period, yogurt samples were analyzed for their physical and microbiological changes as shown in Tables 4.3 and 4.4.

Table 4.3 Chemical and microbiological properties of soymilk yogurt during storage at 4^{0}C for 21 days.

Chemical and microbiological properties	**Storage time**			
	0 day	**7 days**	**14 days**	**21 days**
Total titratable acidity^{ns} (as % lactic acid) (w/v)	0.50 ± 0.001^a	0.60 ± 0.00^a	0.59 ± 0.01^a	0.62 ± 0.003^a
pH	4.30 ± 0.01^c	4.19 ± 0.01^b	4.14 ± 0.04^a	4.10 ± 0.00^a
S. thermophilus (log CFU/ml)	14.92 ± 0.03^d	13.82 ± 0.09^c	12.97 ± 0.03^b	12.20 ± 0.01^a
L. bulgaricus (log CFU/ml)	13.16 ± 0.05^d	12.47 ± 0.02^c	11.26 ± 0.05^b	10.89 ± 0.04^a

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different ($p \leq 0.05$) between the treatments

Table 4.4 Physical and chemical properties of soymilk yogurt during storage at 4^0C for 21 days.

Physical and chemical properties	0 day	21 day
Consistency values measurement (cm) ^{ns}	24.00 ± 0.00	24.00 ± 0.00
Syneresis values measurement (%) (w/w)	80.95 ± 4.14^a	86.73 ± 3.37^b
Total Solid contents (%) (w/w)	5.33 ± 2.84^a	13.67 ± 0.58^b
Moisture contents (%) (w/w)	94.67 ± 2.84^b	86.33 ± 0.58^a
Total Soluble Solid (^{0}Brix) ^{ns}	7.00 ± 0.00	7.00 ± 0.00
Viscosity values measurement (cp)	1607.7 ± 263.90^b	1457 ± 118.10^a

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different ($p \leq 0.05$) between the treatments

4.2.1 The chemical properties of the basic formula of soybean yogurt during storage at 4^0C for 21 days.

After a fermentation period for 6 h at 43^0C, a basic formula of soybean yogurt had a titratable acidity of 0.5 ± 0.001% lactic acid and a pH value of 4.30 ± 0.01 (Table 4.3) and the yogurt was stored for 21 days at 4^0C. The monitoring of acidities during the storage period showed clearly that the amount of lactic acid was increased during this period causing an increase in the value of titratable acidity to become 0.6% ± 0.003 lactic acid and a significant ($p \leq 0.05$) decrease in the pH value to reach 4.10 ± 0.00 at the end of the storage period. Although at 4^0C yogurt microorganisms would not grow (see section 4.2.3), their metabolism activities still could continue at a slower rate (Dave and Shah, 1996). These activities could be the main reason that caused an increase in acidity at low storage temperature.

For other chemical properties, Table 4.4 showed that the total soluble solid of soybean yogurt was not significantly ($p \leq 0.05$) changed during storage at 4^0C. The total soluble solid of the yogurt was 7.00 ± 0.00^0Brix. However, there was a significant ($p \leq 0.05$) increase in the amount of total solid of the yogurt. With an initial total solid of 5.33 ± 2.84%, the yogurt had 13.67 ± 0.58% total solid at the end of the storage period.

4.2.2 The physical properties of the basic formula of soybean yogurt during storage at 4^0C for 21 days.

For the physical properties, the results were displayed in Table 4.4. For the viscosity, the value was shown to be significantly ($p \leq 0.05$) decreased during the storage period. The change in the viscosity parameter could be due to peolysis reaction that occurred in the storage period (Sankhavadhana, 2001). On the other hand, the syneresis of the soybean yogurt was significantly increased ($p \leq 0.05$) during the storage period. The consistency of the basic formula of soybean yogurt was shown to be not a good value. Since the value reached the maximum value of a Bostwick consistometer that was measured in 30 s. This value meant that the yogurt has a very low consistency characteristic and easy to spread.

4.2.3 The microbiological properties of the basic formula of soybean during storage at 4^0C for 21 days.

The monitoring for the viability of yogurt starter cultures showed that the viable numbers of these microorganisms were significantly ($p \leq 0.05$) reduced during storage at 4^0C. The highest numbers of *S. thermophilus* and *L. bulgaricus* were 14.92 ± 0.03 and 13.16 ± 0.05 log CFU/ml, respectively, at the beginning of the storage period.

Reductions for up to 2.72 and 2.26 log CFU/ml for *S. thermophilus* and *L. bulgaricus*, respectively, were observed after 21 days storage.

4.3 The effect of soymilk yogurt ingredients on the viability of probiotic bacteria in soymilk yogurt during storage at 4^0C for 21 days.

In this section, the study was concentrated to understand the effect of soymilk yogurt ingredients on the viability of probiotic bacteria, which were *B. bifidum* and *L. acidophilus*, during storage at 4^0C for 21 days. The soymilk yogurt was fermented by the normal yogurt bacteria of *S. thermophilus* and *L. bulgaricus* at a level of 2% (w/v) and incubated at 43^0C for 6 h in the absence of the probiotic bacteria. After the soymilk yogurt production, the probiotic bacteria were added aseptically into the yogurt at a concentration of 2% (w/v). There were 7 soymilk ingredients or 7 experimental factors studied in this section, including Reconstituted Skimmed Milk (RSM), sugar, soymilk, carboxymethylcellulose (CMC), kappa-carrageenan, guar gum (GG) and pH levels. Changing in the physical, chemical and microbiological properties of the soymilk yogurt with probiotic bacteria was monitored regularly during storage at 4^0C for 21 days.

4.3.1 Finding the main factors of soymilk yogurt ingredients that affected the viability of probiotic bacteria in soymilk yogurt during storage at 4^0C for 21 days

To understand the effect of 7 soymilk ingredients or 7 experimental factors of soymilk yogurt, this section varied the levels of these experimental factors by applying a Plackett and Burman design. Different levels of each experimental factor and combination of different experimental factors was displayed in Chapter 3 (Tables 3.1 and 3.2). After the production of different treatments, soymilk yogurts were kept at 4^0C for 21 days. During this storage period, yogurt samples were analyzed for their physical, chemical and microbiological changes. Based on these changes, each of the experimental factors was included to be a major or a minor factor. A major factor was defined as an experimental factor that significantly affected more than or equal to half of the analysis attributes, whereas a minor factor was an experimental factor that significantly affected less than half of the analysis attributes.

4.3.1.1 Soymilk yogurt with an addition of *B. bifidum*

A relationship between each experimental factor and soymilk yogurt properties was displayed in Table 4.5. Since one experimental factor could be affected by more than one analysis attribute, the final level of an experimental factor was depended on the total amount of the positive/negative symbol for that particular experimental factor. It the number of the negative symbol was more than that of the positive symbol, then the experimental factor should be chosen at its low level. On the other hand, if the number of the negative symbol was less than that of the positive symbol, then the experimental factor should be selected at its high level. At the same time, if an experimental factor affected more than or equal to half of the analysis attributes, the experimental factor would be categorized as a major factor and it would be further studied in the next section. Whereas, if an experimental factor affected less than half of the analysis attributes, the experimental factor would be included as a minor factor and its level would be selected based on the number of positive and negative symbols.

Analysing the effect of 7 experimental factors on the viability of *B. bifidum* in soymilk yogurt during storage at 4^0C for 21 days showed that RSM could be categorized as a major factor because it affected more than half of the analysis attributes, including total titratable acidity, total solid, total soluble solid, moisture content, consistency, syneresis and the number of *B. bifidum*. Since the number of the negative symbol was more than the positive symbol (Table 4.5), this experimental factor should be selected at its low level of 6% (w/v).

Table 4.5 The effect of experimental factors on the properties of soymilk yogurt added with *B. bifidum* based on a Plackett and Burman design

Analysis attributes	Chemical properties				
	pH measurement	Total titratable acidity (%lactic acid)	Total solid contents (%) (w/w)	Total Soluble Solid (°Brix)	Moisture contents (%) (w/w)
Experimental factor	(+)pH	(-)RSM (+)sugar (-)pH (+)soymilk	(+)RSM (+)sugar	(+)RSM (+)sugar (+)pH	(-)RSM (-)sugar

Table 4.5 (Continue)

Analysis attributes	Physical properties		Microbiological properties		
	Consistency (cm)	Syneresis (%) (w/w)	*B. bifidum* (log CFU/ml)	*S. thermophilus* (log CFU/ml)	*L. bulgaricus* (log CFU/ml)
Experimental factor	(+)RSM (-) k-carrageenan (-)guar gum	(-)RSM (-)sugar (-)k-carrageenan (-) guar gum (-) CMC	(-)RSM (+)pH (-)soymilk (+)guar gum	(+)pH (+)k-carrageenan	(+)k-carrageenan

For an experimental factor of sugar, this factor affected half of the analysis attributes, including total titratable acidity, total solid, total soluble solid, moisture contents and syneresis. Because of this, sugar was categorized as a major factor and it should be used at a high level of 15% (w/v). Similar to the finding of sugar, an experimental factor of pH level was also affected half of the analysis attributes, which were pH measurements, total titratable acidity, total soluble solid and the numbers of *B. bifidum* and *S. thermophilus*. Therefore, this experimental factor was a major factor and it should be applied at a high level of 4.9.

The experimental factor of soymilk was found to affect less than half of the analysis attributes, which were total titratable acidity and the number of *B. bifidum*. Since there were only 2 analysis attributes that was affected by soymilk, this experimental factor was included as a minor factor. Soymilk should also be used at its low level of 15% (w/v), because at this level, the soymilk could give a better support in maintaining the viability of *B. bifidum* in soymilk yogurt during refrigerated storage.

An experimental factor of kappa-carrageenan was categorized as a minor factor because this factor affected less than half of the analysis attributes, including consistency, syneresis and the numbers of *S. thermophilus* and *L. bulgaricus*. This factor should be applied at its high level of 0.3% (w/v) because at this level, the kappa-carrageenan could maintain higher numbers of *S. thermophilus* and *L. bulgaricus* in soymilk yogurt.

Another experimental factor of carboxymethylcellulose was also found to affect less than half of the analysis attributes, which were syneresis, therefore this experimental factor was included as a minor factor. For the suitable level of carboxymethylcellulose, a high level of 0.3% (w/v) should be chosen because the low level of carboxymethylcellulose caused a higher syneresis in soymilk yogurt. Since high syneresis of soymilk yogurt was not desirable, a high level of carboxymethylcellulose should be used to prevent this problem.

For the last experimental factor, guar gum, this factor could affect less than half of the analysis attributes, including consistency, syneresis and the number of *B. bifidum*, therefore this factor was categorized as a minor factor. The suitable level of guar gum to be chosen was a high level of 0.3% (w/v) because at this level, the experimental factor could give a better support in maintaining the number of *B. bifidum* in soymilk yogurt. At the same time, the low level of guar gum increased the syneresis of soymilk yogurt. Because of these reasons, the high level of guar gum was selected to be used in the next experiment.

From the analysis results of soymilk yogurt added with *B. bifidum* based on a Plackett and Burman design, it could be concluded that 7 experimental factors were significantly affected the properties of the yogurt. Three of these experimental factors were included as a major factor and the other four factors were categorized as a minor factor (Table 4.6)

Table 4.6 Major and minor experimental factors that affected the properties of soymilk yogurt added with *B. bifidum* based on a Plackett and Burman design

Major factors		**Minor factor**	
RSM	(-)	Soymilk	(-)
Sugar	(+)	kappa-carrageenan	(+)
pH	(+)	CMC	(+)
		guar gum	(+)

For the three major factors of RSM, sugar and pH levels, they should be applied at 6% (w/v), 15% (w/v) and 4.9, respectively. Whereas for four minor factors, including soymilk, kappa - carragenan, carboxymethylcellulose and guar gum, their suitable levels were 15% (w/v), 0.3% (w/v), 0.3% (w/v) and 0.3% (w/v), respectively.

4.3.1.2 Soymilk yogurt with an addition of *L. acidophilus*

The full analysis results for soymilk yogurt added with *L. acidophilus* were displayed in Appendix B. Similar to the previous section, in this section 7 experimental factors were analyzed for their effects on the viability of *L. acidophilus* in soymilk yogurt during 21 days at refrigerated storage. These experimental factors could be separated as a major or minor factor based on their effects on different analysis attributes. A summary for the relationship between 7 experimental factors with different analysis attributes could be seen in Table 4.7.

Table 4.7 The effect of experimental factors on the properties of soymilk yogurt added with *L. acidophilus* based on a Plackett and Burman design

Analysis attributes	Chemical properties				
	pH measurement	Total titratable acidity (%lactic acid)	Total Solid Contents (%) (w/w)	Total Soluble Solid (°Brix)	Moisture Contents (%) (w/w)
Experimental factor	(+)Sugar (+)pH	(+)Sugar (+)soymilk	(+)Sugar (+)RSM (+)k-carragenan	(+)Sugar (+)RSM (-)guar gum	(-)Sugar (-)RSM (-)k-carragenan

Table 4.7 (Continue)

Analysis attributes	Physical properties		Microbiological properties		
	Consistency (cm)	Syneresis measurement (%) (w/w)	*L. acidophilus* (log CFU/ml)	*S. thermophilus* (log CFU/ml)	*L. bulgaricus* (log CFU/ml)
Experimental factor	(-)soymilk (-)pH (+)RSM (-)k-carragenan (-)guar gum	(-)soymilk (-)guar gum (-)CMC	(+)Sugar (+)pH (+)RSM (+)guar gum	(-)k-carragenan	---

From Table 4.7, it could be observed that RSM affected more than half of the analysis attributes, including total solid, total soluble solid, moisture content, consistency and the number of *L. acidophilus*. Due to these effects, RSM was categorized as a major factor and it should be used at its high level of 12% (w/v) because the number of positive symbol was more than that of the negative symbol.

For an experimental factor of soymilk, the factor affected less than half of the analysis attributes, including total titratable acidity, consistency and syneresis, therefore this factor could be categorized as a minor factor. The suitable level for soymilk to be used was at its high level of 60% (w/v) because at this level, the soymilk could give a better support in maintaining higher total titratable acidity in soymilk yogurt, which was a desirable property of the yogurt. In addition, the low level of soymilk would increase syneresis of soymilk yogurt.

An experimental factor of pH values was found to affect less than half of the analysis attributes, which were pH measurements, consistency and the number of

L. acidophilus, therefore this factor was included as a minor factor. The suitable level of pH value to be used was its high level of 4.9.

The presence of carboxymethylcellulose in soymilk yogurt only affected one of the analysis attributes, syneresis, therefore the experimental factor was categorized as a minor factor. The carboxymethlcellulose should be used at its high level of 0.3% (w/v) because if the factor was applied at its low level, the syneresis of the product would increase.

Another experimental factor of kappa - carragenan affected less than half of the analysis attributes, including total solid, moisture content, consistency and the number of *S. thermophilus*. Due to this finding the factor could be included as a minor factor and it should be applied at its low level of 0% (w/v) because the number of negative symbol was more than that of the positive symbol.

The last experimental factor of guar gum was found to affect less than half of the analysis attributes, which were total soluble solid, consistency, syneresis and the number of *L. acidophilus*, therefore this factor was a minor factor. Although Table 4.7 showed that the number of negative symbol was more than that of the positive symbol, the suitable applied level for guar gum was at its high level of 0.3% (w/v). At this level, guar gum could give a better support in maintaining the number of *L. acidophilus*. Besides that, at the low level of guar gum, the syneresis of soymilk yogurt would increase, which was an undesirable characteristic for yogurt.

Table 4.8 Major and minor experimental factors that affected the properties of soymilk yogurt added with *L. acidophilus* based on a Plackett and Burman design

Major factors		**Minor factor**	
		Soymilk	(+)
Sugar	(+)	CMC	(+)
RSM	(+)	kappa-carragenan	(-)
		guar gum	(+)
		pH	(+)

The 7 experimental factors that affected the properties of soymilk yogurt added with *L. acidophilus* during storage at refrigerated temperature were concluded in Table 4.8. From this Table, it could be seen that sugar and RSM were major factors, whereas soymilk, pH value, carboxymethylcellulose, kappa - carrageeenan and guar gum were minor factors.

The suitable levels for RSM and sugar to be used were 12% (w/v) and 15% (w/v), respectively. For soymilk, kappa - carrageenan, carboxymethylcellulose and guar gum, they should be applied at concentrations of 60% (w/v), 0% (w/v), 0.3% (w/v) and 0.3% (w/v), respectively. At the same time, the pH level of the yogurt after the fermentation process should be adjusted to a pH level of 4.9.

4.3.2 Study the optimum levels for the major factors of soymilk yogurt ingredients that affected the viability of probiotic bacteria in soymilk yogurt during storage at 4^0C for 21 days.

Section 4.3.1 was used to find the main soymilk yogurt ingredients that affect the viability of probiotic bacteria in soymilk yogurt during refrigerated storage by applying a Plackett and Burman design. The optimum levels for these major ingredients were figured out in this section using a factorial experiment in center point (Pongsirikool, 2002).

4.3.2.1 Soymilk yogurt with an addition of *B. bifidum*

The experiment results in the section 4.3.1.1 showed that there were 3 major factors that affected the viability of *B. bifidum* in soymilk yogurt during refrigerated storage. These major factors were RSM, sugar and pH level. To find the suitable levels for these factors, a 2^3 Factorial experiment in center point (Tables 3.3 and 3.4) was used. The complete analysis result for this experiment was displayed in Appendix B and the summary for the statistical analysis of the result could be seen in Table 4.9.

From the statistical result in Table 4.9 and the result from the section 4.3.1.1 for the suitable levels of minor soymilk yogurt ingredients, it could be concluded that the suitable levels of different soymilk yogurt ingredients that would support the viability of *B. bifidum* in soymilk yogurt during refrigerated storage for 21 days were 9% (w/v) RSM, 18% (w/v) sugar, 15% (w/v) soymilk, 0.3% (w/v) carboxymethylcellulose, 0.3% (w/v) kappa - carrageenan, 0.3% (w/v) guar gum and a yogurt pH level of 5.2 (Table 4.10).

Table 4.9 Suitable levels of major soymilk yogurt ingredients for soymilk yogurt added with *B. bifidum* using a 2^3 Factorial experiment in center point

Response variable	Major factors	Level
Total titratable acidity (as % lactic acid) (w/v)	RSM (%) (w/v)	9
	Sugar (%) (w/v)	18
	pH	5.2
Total soluble solid (^{0}Brix)	RSM (%) (w/v)	9
	Sugar (%) (w/v)	18
	pH	5.2
Syneresis (%) (w/w)	RSM (%) (w/v)	9
	Sugar (%) (w/v)	18
	pH	4.6
Moisture Contents (%) (w/w)	RSM (%) (w/v)	9
	Sugar (%) (w/v)	18
	pH	5.2
Total Solid Contents (%) (w/w)	RSM (%) (w/v)	3
	Sugar (%) (w/v)	12
	pH	4.6

Table 4.10 Optimal levels of soymilk yogurt ingredients for soymilk yogurt added with *B. bifidum*

Factors	Optimal level
Reconstituted Skimmed Milk (RSM) (%) (w/v)	9
Sugar (%) (w/v)	18
Soymilk (%) (w/v)	15
Carboxymethylcellulose (CMC) (%) (w/v)	0.3
κ- Carrageenan (%) (w/v)	0.3
Guar gum (%) (w/v)	0.3
pH level before added probiotic	5.2
Distilled water (%) (w/v)	57.1

4.3.2.2 Soymilk yogurt with an addition of *L. acidophilus*

Data in the section 4.3.1.2 displayed that there were 2 major factors that affected the viability of *L. acidophilus* in soymilk yogurt during refrigerated storage. These factors were RSM and sugar. In this section, suitable levels of these factors were studied using a 2^2 Factorial experiment in center point (Table 3.5). Collected data for this experiment could be seen in Appendix B. A conclusion from the statistical analysis for this data was shown in Table 4.11.

Results in Table 4.11 clearly displayed that the suitable levels of RSM and Sugar for soymilk yogurt added with *L. acidophilus* were 15 and 12% (w/v), respectively. Combining the results from Table 4.11 together with the finding from the section 4.3.1.2 for the minor factors of soymilk yogurt ingredients, it could be concluded that the suitable levels of different soymilk yogurt ingredients that would support the viability of *L. acidophilus* in soymilk yogurt during refrigerated storage for 3 weeks were 15% (w/v) RSM, 12% (w/v) sugar, 15% (w/v) soymilk, 0.3% (w/v) carboxymethylcellulose, 0.3% (w/v) guar gum and a yogurt pH level of 4.9 (Table 4.12).

Table 4.11 Suitable levels of major soymilk yogurt ingredients for soymilk yogurt added with *L. acidophilus* using a 2^2 Factorial experiment in center point

Response variable	Major factors	Levels (%)
pH	RSM	15
	Sugar	12
Total Soluble Solid (^{0}Brix)	RSM	15
S. thermophilus (log CFU/ml)	RSM	15

Table 4.12 Optimal levels of soymilk yogurt ingredients for soymilk yogurt added with *L. acidophilus*

Factors	Optimal level
Reconstituted Skimmed Milk (RSM) (%) (w/v)	15
Sugar (%) (w/v)	12
Soymilk (%) (w/v)	60
Carboxymethylcellulose (CMC) (%) (w/v)	0.3
kappa - carrageenan	0
Guar gum (%) (w/v)	0.3
pH level before adding probiotic	4.9
Distilled water (%) (w/v)	12.4

4.4 The effect of nutrient compounds on the viability of probiotic bacteria in soymilk yogurt during storage at 4^0C for 21 days

The experiment result from the section 4.3 was applied in this section. Into these optimal formulations of soymilk yogurt ingredients, different nutrient compounds, including fibersol-2, fructo–oligosaccharides (FOS) and tomato extract, were added to study the effect of these ingredients on the survival of probiotic bacteria in soymilk yogurt during refrigerated storage for 3 weeks.

4.4.1 The effect of Fibersol-2 on the viability of probiotic bacteria in soymilk yogurt during storage at 4^0C for 21 days

In this section, fibersol-2 was added in the soymilk yogurt ingredients together with the normal yogurt starter cultures of *S. thermophilus* and *L. bulgaricus*. After incubation time at 43^0C for 6 h, the yogurt were added with a probiotic bacterium, *B. bifidum* or *L. acidophilus*, and stored at refrigerated temperature for 3 weeks period. During the storage time, yogurt samples were analyzed for their physical, chemical and microbiological properties. The level of fibersol-2 used in this section was 5% (w/v).

4.4.1.1 The effect of fibersol-2 on the viability of *B. bifidum* in soymilk yogurt during storage at 4^0C for 21 days

The optimal formulation of soymilk yogurt ingredients as shown in Table 4.10 was combined with 5% (w/v) fibersol-2 to study the effect of fibersol-2 on the viability of *B. bifidum* in yogurt during refrigerated storage. The analysis results during the storage period were displayed in Tables 4.13 and 4.14 and Figure 4.3.

Table 4.13 Chemical and microbiological properties of soymilk yogurt added with *B. bifidum* and in the presence of 5% (w/v) fibersol-2 during storage at 4^0C for 21 days

Chemical and microbiological properties	Storage time			
	0 day	**7 days**	**14 days**	**21 days**
Total titratable acidity (as % lactic acid) (w/v)	0.40 ± 0.003^a	0.50 ± 0.0006^b	0.50 ± 0.005^b	0.50 ± 0.004^b
pH	5.20 ± 0.00^c	4.90 ± 0.099^b	4.51 ± 0.00^a	4.45 ± 0.00^a
B. bifidum (log CFU/ml)	8.08 ± 0.06^c	7.79 ± 0.03^b	7.73 ± 0.02^b	6.24 ± 0.16^a
S. thermophilus (log CFU/ml)	14.14 ± 0.04^d	13.65 ± 0.05^c	11.74 ± 0.16^b	10.81 ± 0.08^a
L. bulgaricus (log CFU/ml)	12.78 ± 0.08^d	12.17 ± 0.01^c	10.96 ± 0.09^b	10.15 ± 0.08^a

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different (p≤0.05) between the treatments

The chemical measurement results in Table 4.13 showed that the acidity of soymilk yogurt was significantly (p≤0.05) increased during the storage at 4^0C. The value of total titratable acidity was increased from 0.4 ± 0.003% lactic acid (w/v) at the beginning of the storage period to 0.5 ± 0.004% lactic acid (w/v) at the end of the storage time, while the pH values were decreased from 5.20 ± 0.00 to 4.45 ± 0.00 after 21 days at refrigerated storage. This result indicated that the yogurt and/or probiotic bacteria continued to decreased (Table 4.13 and Figure 4.3). For the other chemical analyses, which were total solid, moisture content and total soluble solid (Table 4.14), their values were not significantly (p>0.05) changed during the refrigerated storage.

Table 4.14 Physical and chemical properties of soymilk yogurt added with *B. bifidum* and in the presence of 5% (w/v) fibersol-2 during storage at 4^0C for 21 days

Physical and Chemical properties	0 day	21 day
Consistency values measurement (cm)^{ns}	11.33 ± 0.29	10.50 ± 0.50
Syneresis values measurement (%) (w/w)^{ns}	8.72 ± 0.36	11.47 ± 2.75
Total Solid contents (%) (w/w)^{ns}	19.00 ± 3.00	19.83 ±1.44
Moisture contents (%) (w/w)^{ns}	80.00 ± 3.00	80.17 ± 1.44
Total Soluble Solid (^{0}Brix)^{ns}	20.00 ± 1.00	19.80 ± 1.64
Viscosity values measurement (cp)^{ns}	4495.00 ± 207.35	4159.00 ± 391.00

Note: * Values were mean from 3 replications ± SD

*Different letters that followed numbers within the same row indicated significantly different (p≤0.05) between the treatment

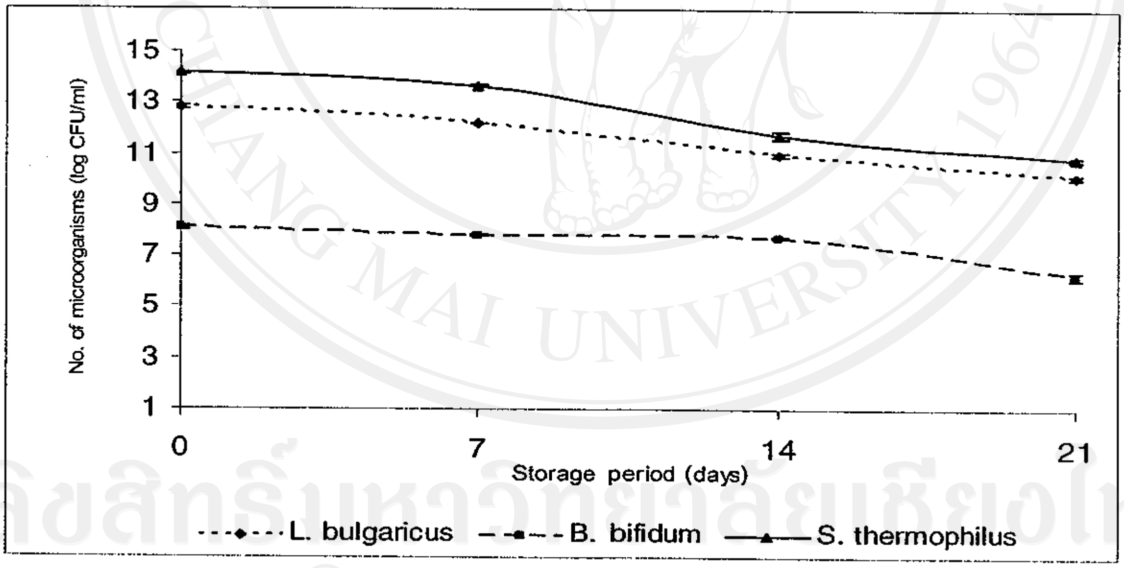

Figure 4.3 The number of *S. thermophilus, L. bulgaricus* and *B. bifidum* (log CFU/ml) in soymilk yogurt added with fibersol-2 during storage at 4^0C for 21 days

The measurement of physical properties of soymilk yogurt added with *B. bifidum*. In the 5% (w/v) fibersol-2 showed that the consistency and viscosity values of the soymilk yogurt were reduced (Table 4.14) during refrigerated storage, while the syneresis of the product was increased (Table 4.14). This result indicated that the physical qualities of soymilk yogurt were changed during storage causing the yogurt to be higher in its viscosity and consistency. There was a possibility due to these changes, the syneresis of the yogurt were affected to be increased too.

For the presence of 3 main microorganisms of S. *thermophilus, L. bulgaricus* and *B. bifidum* in soymilk yogurt, their viabilities were significantly ($p \leq 0.05$) reduced during refrigerated storage as were shown in Table 4.13 and Figure 4.3. The highest reduction in the viable numbers was displayed for *S. thermophilus* that had a reduction for up to 3.33 log CFU/ml after 21 days at 4^0C. At the same time, the reduction in the viable number of *B. bifidum* was shown to be up to 1.84 log CFU/ml during the storage period.

4.4.1.2 The effect of fibersol-2 on the viability of *L. acidophilus* in soymilk yogurt during storage at 4^0C for 21 days

In this section, the effect of adding 5% (w/v) fibersol-2 on the viability of *L. acidophilus* in soymilk yogurt during 4^0C storage was assessed. The yogurt formula used in this section followed the formula result in Table 4.12. For the analysis results based on physical, chemical and microbiological measurements during the storage period, they were shown in Tables 4.15 and 4.16 and Figure 4.4.

Table 4.15 Chemical and microbiological properties of soymilk yogurt added with *L. acidophilus* and in the presence of 5% fibersol-2 (w/v) during storage at 4 ^{0}C for 21 days

Chemical and microbiological properties	Storage time			
	0 day	**7 days**	**14 days**	**21 days**
Total titratable acidity (as % lactic acid) (w/v) ^{ns}	0.80 ± 0.01	0.90 ± 0.002	0.90 ± 0.01	0.90 ± 0.01
pH	4.90 ± 0.00^{c}	4.59 ± 0.02^{b}	4.58 ± 0.06^{b}	4.45 ± 0.02^{a}
L. acidophilus (log CFU/ml)	7.97 ± 0.12^{d}	7.25 ± 0.01^{c}	6.69 ± 0.09^{b}	6.11 ± 0.11^{a}
S. thermophilus (log CFU/ml)	14.44 ± 0.52^{b}	14.20 ± 0.06^{b}	13.19 ± 0.19^{a}	12.77 ± 0.03^{a}
L. bulgaricus (log CFU/ml)	12.68 ± 0.02^{b}	12.33 ± 0.44^{b}	11.54 ± 0.01^{a}	11.10 ± 0.16^{a}

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different (p≤0.05) between the treatments

Similar to the finding in the previous section, the acidity measurement for the soymilk yogurt added with *L. acidophilus* also increased during 21 days at refrigerated storage. The total titratable acidity of the yogurt was increased 0.01% lactic acid after 21 days storage, whereas the pH value was significantly (p≤0.05) reduced for 0.45 (Table 4.15) for the same storage period. This result confirmed the previous finding that the activities of the yogurt and/or probiotic bacteria were continued at 4 ^{0}C storage temperature. Other chemical analyses of total solid, total soluble solid and moisture content were found to be not significantly (p>0.05) changed during the storage period (Table 4.16).

Table 4.16 Physical and chemical properties of soymilk yogurt added with *L. acidophilus* and in the presence of 5% fibersol-2 (w/v) during storage at 4^0C for 21 days

Physical and chemical properties	0 day	21 days
Consistency values measurement (cm)^{ns}	24.00 ± 0.00	24.00 ± 0.00
Syneresis values measurement (%) (w/w)	56.37 ± 5.72^{a}	70.35 ± 2.31^{b}
Total Solid contents (%) (w/w)^{ns}	19.33 ± 2.25	22.33 ± 1.89
Moisture contents (%) (w/w)^{ns}	80.67 ± 2.25	77.67 ± 1.89
Total Soluble Solid (^{0}Brix)^{ns}	18.20 ± 1.06	18.07 ± 0.90
Viscosity values measurement (cp)^{ns}	1498.00 ± 226.00	1130.00 ± 155.12

Note: * Values were mean from 3 replications ± SD

- Different letters that followed numbers within the same column indicated significantly different (p≤0.05) between the treatments

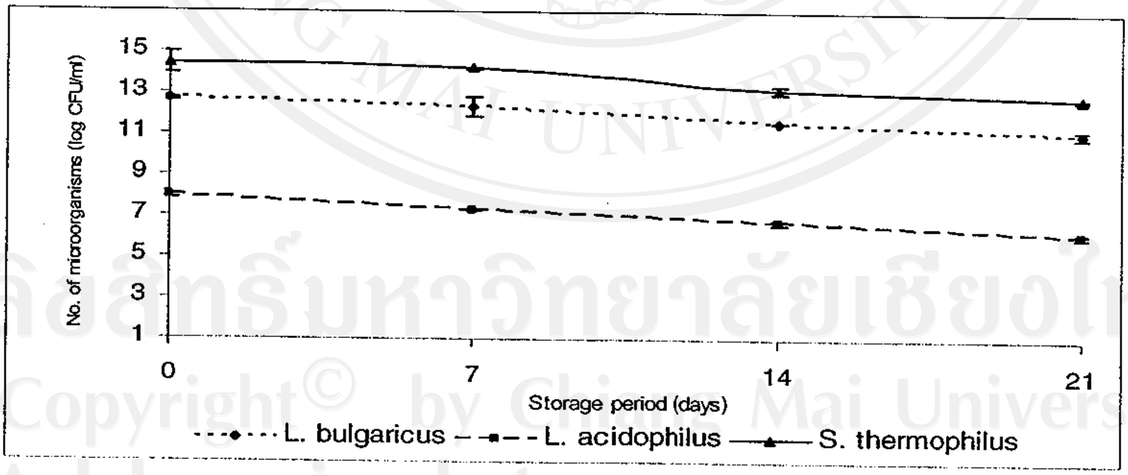

Figure 4.4 The number of *S. thermophilus, L. bulgaricus* and *L. acidophilus* (log CFU/ml) in soymilk yogurt added with fibersol-2 during storage at 4^0C for 21 days

For the physical properties of soymilk yogurt added with *L. acidophilus*, the statistical analysis showed that the syneresis of the soymilk yogurt was significantly ($p \leq 0.05$) increased during refrigerated storage (Table 4.16). Although the viscosity of the soymilk yogurt reduced during 21 days at 4^{0}C, its value was not significantly different ($p \geq 0.05$). No conclusion could be made for the consistency of the soymilk yogurt added with *L. acidophilus*, since the consistency values that were obtained at the beginning and at the end of the storage period were the maximum value of a Bostwick consistency equipment. This data indicated that the consistency of the soymilk yogurt was not good directly after the production process and the physical parameter would not become better during the storage period.

Table 4.15 and Figure 4.4 clearly displayed that the viable count of the 3 main microorganisms in the soymilk yogurt added with *L. acidophilus* were significantly ($p \leq 0.05$) reduced during refrigerated storage for 21 days. During this period, a reduction in viable numbers of 1.58-1.86 log CFU/ml was achieved for these 3 bacteria. The highest reduction in the viable number was shown by *L. acidophilus*, although at the end of the storage period the number of this bacterium was still at a level of 6.11 ± 0.11 log CFU/ml. This finding confirmed the result of the previous section that the number of yogurt and probiotic bacteria were reduced during storage at 4^{0}C.

4.4.2 The effect of fructo-oligosaccharide on the viability of probiotic bacteria in soymilk yogurt during storage at 4^{0}C for 21 days

Another nutrient compound that was added into soymilk yogurt formula was fructo-oligosaccharide. This prebiotic compound was added at a level of 5% (w/v) in the optimal formula of the 2 soymilk yogurt that were displayed in Table 4.10 and 4.12. After producing the soymilk yogurt with an addition of *B. bifidum* or *L. acidophilus*, the yogurts were stored at 4^{0}C for 21 days. During the storage period, yogurt samples were analyzed for their physical, chemical and microbiological properties.

4.4.2.1 The effect of fructo-oligosaccharide on the viability of *B. bifidum* in soymilk yogurt during storage at 4^0C for 21 days

Combining the optimal formula of soymilk yogurt in Table 4.10 with 5% (w/v) fructo-oligosaccharide, this section was used to study the effect of fructo-oligosaccharide on the viability of *B. bifidum* in soymilk yogurt during refrigerated storage. The results of different analyses that were done during the storage time could be seen in Tables 4.17 and 4.18 and Figure 4.5.

Acidity measurement based on total titratable acidity and pH values for soymilk yogurt added with *B. bifidum* showed that the yogurt acidity was significantly ($p \leq 0.05$) increased during 21 days storage at chilled temperature (Table 4.17). The total titratable acidity values were increased from 0.04 ± 0.001% lactic acid (w/w) at the beginning of the storage time to 0.06 ± 0.001% lactic acid (w/w) at the end of the storage period, whereas the pH values were dropped from 5.20 ± 0.00 to 4.44 ± 0.00 after 21 days at refrigerated storage. This finding was similar to the result of the section 4.4.1.1 that demonstrated a continuous activity of yogurt and/or probiotic bacteria in producing lactic acid during the storage period.

Besides the acidity measurement, the measurement of total solid and moisture content also displayed significant ($p \leq 0.05$) changes during the storage period (Table 4.18). The total solid of the soymilk yogurt was found to be increased, while the moisture content was decreased after 21 days storage at 4^0C.

For the last chemical property, total soluble solid, the value of this parameter was slightly reduced at the end of the storage period, but the reduction was not significant ($p > 0.05$) based on the statistical analysis.

Table 4.17 Chemical and microbiological properties of soymilk yogurt products from *B. bifidum* added fructo-oligosaccharide during a storage at 4^0C for 21 day

Chemical and microbiological properties	Storage time			
	0 days	**7 days**	**14 days**	**21 days**
Total titratable acidity (as % lactic acid) (w/v)	0.40 ±0.001^{a}	0.50 ± 0.003^{b}	0.50 ± 0.003^{c}	0.60 ± 0.001^{c}
pH	5.20 ± 0.00^{c}	4.79 ± 0.04^{b}	4.49 ± 0.04^{a}	4.44 ± 0.00^{a}
B. bifidum (log CFU/ml)	8.00 ± 0.06^{c}	7.54 ± 0.06^{b}	7.41 ± 0.26^{b}	6.47 ± 0.06^{a}
S.thermophilus (log CFU/ml)	13.83 ± 0.01^{c}	13.20 ± 0.25^{b}	12.85 ± 0.07^{b}	12.35 ± 0.18^{a}
L. bulgaricus (log CFU/ml)	11.82 ± 0.002^{c}	10.77 ± 0.10^{b}	10.652 ± 0.01^{b}	9.24 ± 0.04^{a}

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different ($p \le 0.05$) between the treatments

Based on the physical properties of consistency and syneresis, the soymilk yogurt added with *B. bifidum* was found to be significantly ($p \le 0.05$) changed during the chilled storage (Table 4.18). The consistency of the soymilk yogurt was significantly ($p \le 0.05$) reduced, while the syneresis was significantly ($p \le 0.05$) increased after 21 storage days.

Another physical characteristic of viscosity was found to be not significantly different ($p > 0.05$) between at the beginning and at the end of the storage period, even though its real values were decreased (Table 4.18).

Table 4.18 Physical and chemical properties of soymilk yogurt added with *B. bifidum* and in the presence of 5% (w/v) fructo-oligosaccharide during storage at 4^0C for 21 days

Physical and chemical properties	0 day	21 days
Consistency values measurement (cm)	10.37 ± 0.32^a	11.40 ± 0.00^b
Syneresis values measurement (%) (w/w)	7.60 ± 0.23^a	15.83 ± 1.21^b
Total Solid contents (%) (w/w)	17.17 ± 2.25^b	22.17 ± 1.04^b
Moisture contents (%) (w/w)	82.83 ± 2.25^b	77.83 ± 1.04^a
Total Soluble Solid (^{0}Brix)^{ns}	20.33 ± 0.29	20.20 ± 0.40
Viscosity values measurement (cp)^{ns}	5855.00 ± 437.52	5489.00 ± 314.93

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different (p≤0.05) between the treatments

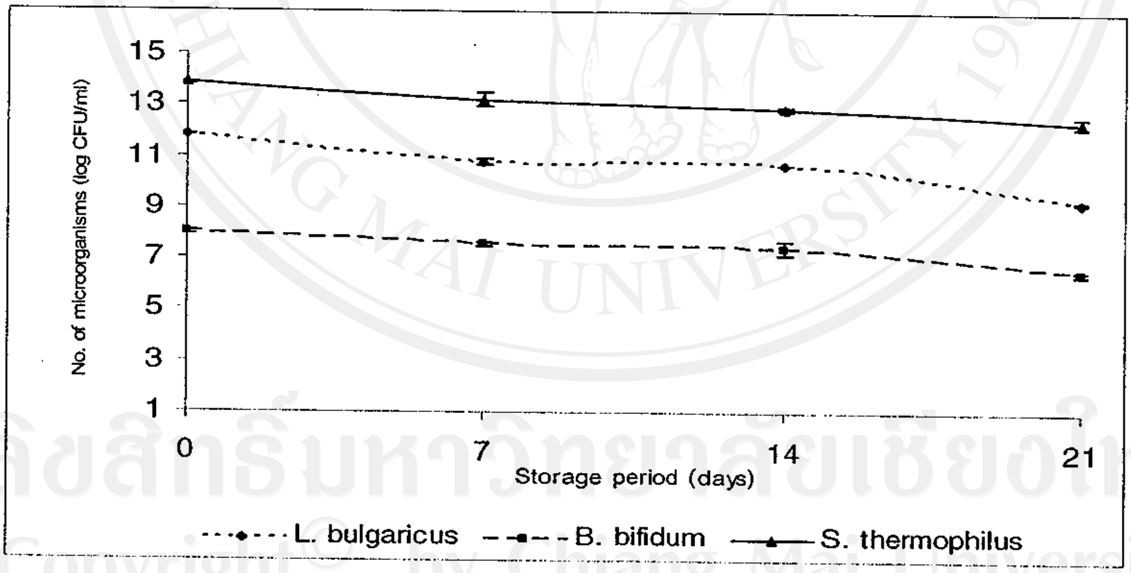

Figure 4.5 The number of *S. thermophilus, L. bulgaricus* and *B. bifidum* (log CFU/ml) soymilk yogurt added with fructo-oligosaccharide during storage at 4^0C for 21 days

The results of microbiological analyses showed that the presence of *S. thermophilus*, *L. bulgaricus* and *B. bifidum* in soymilk yogurt was significantly ($p \leq 0.05$) reduced during 21 days at refrigerated storage (Table 4.17 and Figure 4.5). However, the presence of fructo-oligosaccharide could give a better support towards *S. thermophilus* and *B. bifidum* compared to that of fibersol-2 (Section 4.4.1.1). The reduction in the viable number of *S. thermophilus* and *B. bifidum* was only up to 1.48 and 1.53 log CFU/ml, respectively, which were lower than those in the section 4.4.1.1. On the other hand, the fructo-oligosaccharide did not support the survival of *L. bulgaricus*. A reduction for up to 2.58 log CFU/ml was recorded for this bacterium.

4.4.2.2 The effect of fructo-oligosaccharide on the viability of *L. acidophilus* in soymilk yogurt during storage at 4^0C for 21 days

The effect of fructo-oligosaccharide on the survival of *L. acidophilus* in soymilk yogurt during storage at low temperature was evaluated in this section. The yogurt formulation that was used was according to the previous result in Table 4.12. The final yogurt products were kept at 4^0C and monitored for their physical, chemical and microbiological properties for 21 days. The results for these measurements were exhibited in Tables 4.19 and 4.20 and Figure 4.6.

Between 5 different chemical analyses that were carried out for soymilk yogurt added with *L. acidophilus*, it was only pH value that was found to be significantly ($p \leq 0.05$) reduced during the chilled storage for 21 days (Table 4.19). For total titratable acidity, total solid, moisture content and total soluble solid, the statistical analysis showed that their changes were not significantly different ($p > 0.05$) (Tables 4.19 and 4.20). The reduction of pH values for 0.42 confirmed previous results in the sections 4.4.1.1, 4.4.1.2 and 4.4.2.1 that the activities of yogurt and/or probiotic bacteria to produce lactic acid continued during the storage period.

Table 4.19 Chemical and microbiological properties of soymilk yogurt added with *L. acidophilus* and in the presence of 5% (w/v) fructo-oligosaccharide during storage at 4^{0}C for 21 days

Chemical and microbiological properties	**Storage time**			
	0 day	**7 days**	**14 days**	**21 days**
Total titratable acidity (as % lactic acid) (w/v)^{ns}	0.80 ± 0.00	0.90 ± 0.001	0.90 ± 0.003	0.90 ± 0.004
pH	4.90 ± 0.00^{c}	4.57 ± 0.02^{b}	4.65 ± 0.08^{bc}	4.48 ± 0.02^{a}
L. acidophilus (log CFU/ml)	8.43 ± 0.30^{c}	7.85 ± 0.01^{bc}	7.61 ± 0.08^{b}	6.77 ± 0.30^{a}
S. thermophilus (log CFU/ml)	14.07 ± 0.03^{d}	13.26 ± 0.02^{c}	12.56 ± 0.08^{b}	11.86 ± 0.03^{a}
L. bulgaricus (log CFU/ml)	11.23 ± 0.06^{d}	10.59 ± 0.06^{c}	10.30 ± 0.09^{b}	9.20 ± 0.05^{a}

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different ($p \leq 0.05$) between the treatments

Data in Table 4.19 and Figure 4.6 also clearly demonstrated that the presence of fructo-oligosaccharide in soymilk yogurt could not prevent the reduction in the viable numbers of *S. thermophilus, L. bulgaricus* and *L. acidophilus*. However, comparing this data with the finding in the section 4.4.1.2, where the soymilk yogurt was added with fibersol-2, some conclusions could be made. First, the presence of fructo-oligosaccharide in the soymilk yogurt was better in supporting the survival of *L. acidophilus* compared to that of fibersol-2. During 21 days storage, a reduction of 1.66 log CFU/ml of

L. acidophilus in the presence of fructo-oligosaccharide was recorded, while a reduction of 1.86 log CFU/ml was achieved in the presence of fibersol-2 (Table 4.15).

Table 4.20 Physical and chemical properties of soymilk yogurt added with *L. acidophilus* and in the presence of 5% (w/v) fructo-oligosaccharide during storage at 4^0C for 21 days

Physical and chemical properties	0 day	21 days
Consistency values measurement (cm)^{ns}	24.00 ± 0.00	24.00 ± 0.00
Syneresis values measurement (%) (w/w)	60.07 ± 0.63^a	79.28 ± 0.95^b
Total Solid contents (%) (w/w)^{ns}	28.67 ± 1.89	26.00 ± 2.00
Moisture contents (%) (w/w)^{ns}	71.33 ± 1.89	74.00 ± 2.000
Total Soluble Solid (^{0}Brix)^{ns}	19.00 ± 0.00	18.83 ± 0.29
Viscosity values measurement (cp)^{ns}	942.00 ± 94	785.00 ± 19

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different (p≤0.05) between the treatments

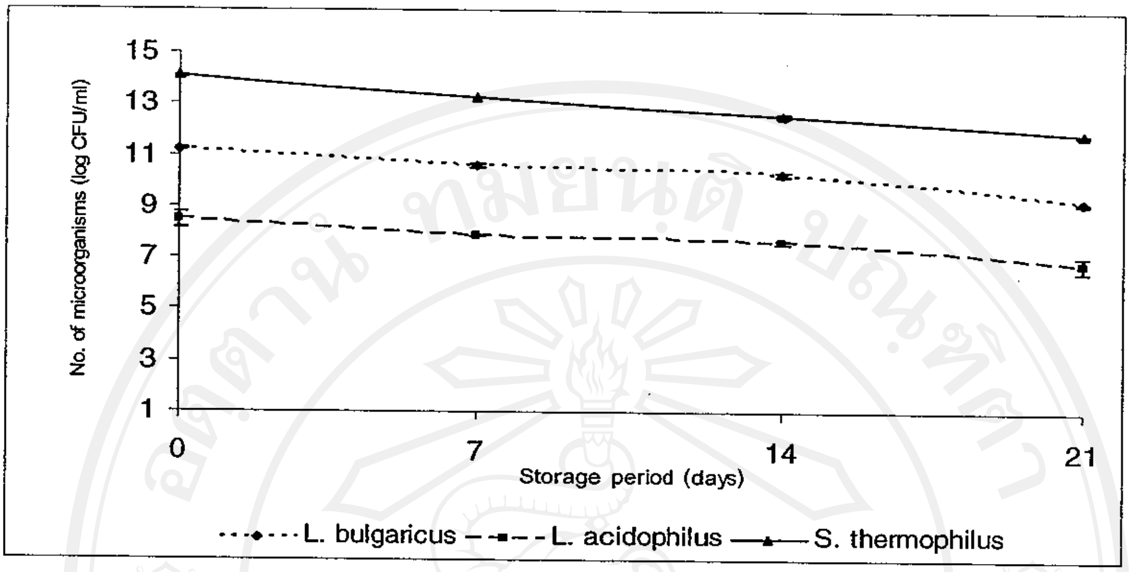

Figure 4.6 The number of *S. thermophilus, L. bulgaricus* and *L. acidophilus* (log CFU/ml) in soymilk yogurt added with fructo-oligosaccharide during storage at 4^0C for 21 days

Another conclusion that could be made was the presence of fructo-oligosaccharide caused more reduction in the viable numbers of yogurt bacteria. Reductions of 2.21 and 2.03 log CFU/ml for *S. thermophilus* and *L. bulgaricus*, respectively, were noted when 5% (w/v) fructo-oligosaccharide was added in soymilk yogurt. The reduction in the viable numbers of yogurt bacteria in this section was higher than those in the section 4.4.1.2, which indicated that fibersol-2 could give a better support in maintaining the viable numbers of yogurt bacteria.

4.4.3 The effect of tomato extract on the viability of probiotic bacteria in soymilk yogurt during storage at 4 ^{0}C for 21 days

The last nutrient compound that was studied in this research was tomato extract. A 5% (w/v) tomato extract (section 3.2.8.1) was added into the optimal formulas of soymilk yogurts (Table 4.10 and 4.12) with an addition of either *B. bifidum* or *L. acidophilus* and kept at 4^0C for 3 weeks. During the chilled storage, yogurt samples were analyzed using several analytical methods to monitor physical, chemical and microbiological changes of the soymilk yogurt.

4.3.1 The effect tomato extract on the viability of *B. bifidum* in soymilk yogurt during storage at 4^0C for 21 days

Applying the optimal formula of soymilk yogurt in Table 4.10 with 5% (w/v) tomato extract, this section was aimed to understand the effect of tomato extract on the viability of *B. bifidum* in soymilk yogurt during low temperature storage. The analysis results during the storage period were displayed in Tables 4.21 and 4.22 and Figure 4.7

In general, changes in the chemical properties of soymilk yogurt added with *B. bifidum* were shown similarity to the finding in the sections 4.4.1.1 and 4.4.2.1. Data in Table 4.21 clearly demonstrated that the acidity of soymilk yogurt was significantly ($p \leq 0.05$) increased during the storage period. After the production of the soymilk yogurt, the yogurt had a total titratable acidity of 0.4 ± 0.002% (w/v) lactic acid with a pH value of 5.20 ± 0.00 (Table 4.21). During the storage period, the total titratable acidity of the soymilk yogurt was gradually increased to reach a value of 0.5 ± 0.00% (w/v) lactic acid at the end of the storage period with a low pH value of 4.38 ± 0.01.

Table 4.21 Chemical and microbiological properties of soymilk yogurt added with *B. bifidum* and in the presence of 5% (w/v) tomato extract during storage at 4^{0}C for 21 days

Chemical and microbiological properties	**Storage time**			
	0 day	**7 days**	**14 days**	**21 days**
Total titratable acidity (as % lactic acid) (w/v)^{ns}	0.40 ± 0.002^{a}	0.50 ± 0.003^{ab}	0.50 ± 0.005^{b}	0.50 ± 0.00^{b}
pH	5.20 ± 0.00^{c}	4.69 ± 0.13^{b}	4.43 ± 0.04^{a}	4.38 ± 0.01^{a}
B.bifidum (log CFU/ml)	7.99 ± 0.12^{c}	7.15 ± 0.07^{b}	6.61 ± 0.20^{a}	6.54 ± 0.12^{a}
S. thermophilus (log CFU/ml)	12.94 ± 0.05^{b}	12.20 ± 0.04^{ab}	11.70 ± 0.01^{a}	11.51 ± 0.62^{a}
L. bulgaricus (log CFU/ml)	11.73 ± 0.05^{d}	10.80 ± 0.03^{c}	10.38 ± 0.001^{b}	9.03 ± 0.09^{a}

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different ($p \leq 0.05$) between the treatments

At the same time, no significant ($p>0.05$) changes could be noted for total solid, moisture content and total soluble solid of the soymilk yogurt during refrigerated storage (Table 4.22). Although the result of this section confirmed the previous finding that the activities of yogurt and/or probiotic bacteria to produce lactic acid continued during chilled storage, a note should be made for a lower pH value of the soymilk yogurt in this section compared to those in the sections 4.4.1.1 and 4.4.2.1 (Tables 4.13 and 4.17). A lower pH value of 0.06 – 0.07 in this section might indicate a better survival of the yogurt and/or probiotic bacteria in the presence of tomato extract during the refrigerated storage that caused more production of lactic acid.

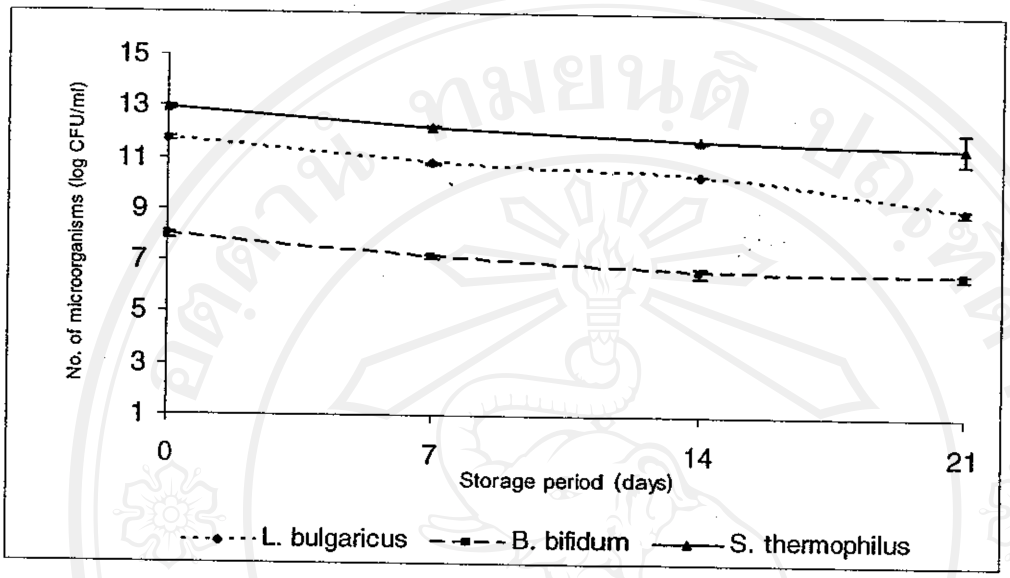

Figure 4.7 The number of *S. thermophilus, L. bulgaricus* and *B. bifidum* (log CFU/ml) in soymilk yogurt added with tomato extract during storage at $4^{0}C$ for 21 days

The three physical measurements, including consistency, syneresis and viscosity, of the soymilk yogurt added with *B. bifidum* were not significantly (p>0.05) changed during the storage period (Table 4.22). However, comparing the physical properties of the soymilk yogurt in this section with the sections 4.4.1.1 and 4.4.2.1, where the soymilk yogurts were added with fibersol-2 and fructo-oligosaccharide (Tables 4.14 and 4.18), it could be seen that the soymilk yogurt in this section had a lower consistency value with higher syneresis and viscosity values than those of the other 2 sections.

Table 4.22 Physical and chemical properties of soymilk yogurt added with *B. bifidum* and in the presence of 5% (w/v) tomato extract during storage at 4^{0}C for 21 days

Physical and chemical properties	0 day	21 days
Consistency values measurement^{ns} (cm)	19.33 ± 1.91	20.73 ± 0.76
Syneresis values measurement (%) (w/w)^{ns}	21.78 ± 4.77	24.17 ± 3.83
Total Solid contents (%) (w/w)^{ns}	20.17 ± 0.29	20.67 ± 3.79
Moisture contents (%) (w/w)^{ns}	79.83 ± 0.29	79.33 ± 3.79
Total Soluble Solid (^{0}Brix)^{ns}	12.67 ± 0.58	13.33 ± 1.89
Viscosity values measurement (cp)^{ns}	6157.00 ± 88.00	5696.00 ± 38.00

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different ($p \leq 0.05$) between the treatments

Since the basic formula of these soymilk yogurts was similar, there was a possibility that the presence of 5% (w/v) tomato extract affected the physical development of the soymilk yogurt in this section during the fermentation period. A further investigation in this area might need to be carried out to give a better explanation for the finding in this section.

The displayed data in Table 4.21 and Figure 4.7 demonstrated a similarity between the finding in this section with other 2 sections of 4.4.1.1 and 4.4.2.1. The viabilities of the 3 main microorganisms, which were *S. thermophilus, L. bulgaricus* and *B. bifidum*, were significantly ($p \leq 0.05$) reduced during the storage period. Reductions in the viable count of 1.43, 2.70 and 1.45 log CFU/ml were observed for *S. thermophilus,* *L. bulgaricus* and *B. bifidum*, respectively.

The viable number reductions of *S. thermophilus* and *B. bifidum* were actually the lowest numbers compared to those in the sections 4.4.1.1 and 4.4.2.1, where the soymilk yogurts were added with fibersol-2 and fructo-oligosaccharide. This result indicated that tomato extract might give a better support in maintaining the viability of *S. thermophilus* and *B. bifidum* in soymilk yogurt during chilled storage compared to those of fibersol-2 and fructo–oligosaccharide (Table 4.23). At the same time, tomato extract was found to be the lowest nutrient compound in maintaining the viability of *L. bulgaricus* (Tables 4.13, 4.17 and 4.21).

Table 4.23 Reductions in the viable numbers of *S. thermophilus*, *L. bulgaricus* and *B. bifidum* in soymilk yogurt added with different nutrient compounds during storage at 4^0C for 21 days

Nutrient compounds (5% (w/v))	Type of microorganisms (log CFU/ml)		
	S. thermophilus	*L. bulgaricus*	*B. bifidum*
Fibersol-2	.3.96 ± 0.13	2.64 ± 0.31	1.78 ± 0.01
Fructo-oligosaccharide	2.58 ± 0.25	1.48 ± 0.02	1.53 ± 0.02
Tomato extract	1.34 ± 0.34	2.70 ± 0.00	1.35 ± 0.04

4.4.3.2 The effect of tomato extract on the viability of *L. acidophilus* in soymilk yogurt during storage at 4⁰C for 21 days

The viability of *L. acidophilus* in soymilk yogurt and in the presence of 5 (w/v) tomato extracts was assessed in this section. A basic formula of soymilk yogurt in the Table 4.12 was added with tomato extract, incubated at 43⁰C for 6 h, cooled down and kept at 4⁰C for 21days. During the storage time, physical, chemical and microbiological properties of the soymilk yogurt were monitored and the results were shown in Tables 4.24 and 4.25 and Figure 4.8.

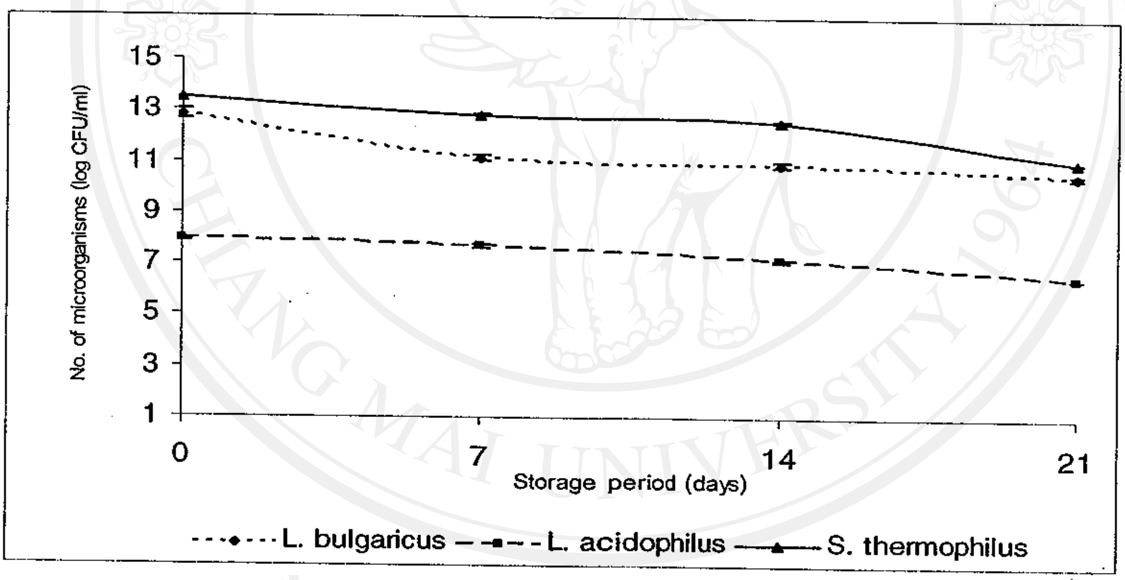

Figure 4.8 The number of *S. thermophilus, L. bulgaricus* and *L. acidophilus* (log CFU/ml) in soymilk yogurt added with tomato extract during storage at 4⁰C for 21 days

The monitoring of the chemical properties of soymilk yogurt added with *L. acidophilus* demonstrated that only the pH value of the yogurt that had a significant ($p \leq 0.05$) reduction during the storage period at 4⁰C (Table 4.24). Other 4 chemical analyses, including total titratable acidity, total solid, moisture content and total soluble solid, of the soymilk yogurt did not change significantly (p>0.05) during the similar storage time (Tables 4.24 and 4.25). A decrease of 0.43 for the pH value that was observed in this section was similar to the findings in the sections 4.4.1.2 and 4.4.2.2 (Tables 4.16 and 4.20) confirming continuous activities of yogurt and/or probiotic bacteria in producing lactic acid during low temperature storage.

Table 4.24 Chemical and microbiological properties of soymilk yogurt added with *L. acidophilus* and in the presence of 5% (w/v) tomato extract during storage at 4^0C for 21 days

Chemical and Microbiological properties	**Storage time**			
	0 day	**7 days**	**14 days**	**21 days**
Total titratable acidity (% as lactic acid) $(w/v)^{ns}$	0.90 ± 0.005	0.90 ± 0.01	1.00 ± 0.02	1.00 ± 0.02
pH	4.90 ± 0.00^b	4.58 ± 0.01^a	4.48 ± 0.02^a	4.47 ± 0.09^a
L.acidophilus (log CFU/ml)	7.89 ± 0.07^d	7.69 ± 0.08^b	7.16 ± 0.04^a	6.48 ± 0.01^a
S. thermophilus (log CFU/ml)	13.47 ± 0.02^d	12.81 ± 0.05^c	12.59 ± 0.04^b	11.06 ± 0.03^a
L. bulgaricus (log CFU/ml)	12.83 ± 0.17^c	11.15 ± 0.12^b	10.93 ± 0.14^b	10.53 ± 0.05^a

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different (p≤0.05) between the treatments

The physical property of soymilk yogurt added with *L. acidophilus* did not change significantly (p>0.05) during storage at 4^0C for 3 weeks (Table 4.25). However, the syneresis and consistency values of the soymilk yogurt in this section were lower and higher, respectively, than those values in the sections 4.4.1.2 and 4.4.2.2, where the soymilk yogurts were added with fibersol–2 and fructo–oligosaccharide (Table 4.16 and 4.20). Since the basic formula of the soymilk yogurt was similar, different physical properties of the soymilk yogurts suggested that different nutrient compounds might give different effects in the development of the soymilk yogurt physical properties during the fermentation time. At the same time, different soymilk yogurt formulas and different compositions of the starter culture that were affected by the presence of different nutrient compounds might also give their influence on the physical characteristics of the yogurt product. This suggestion was concluded from different results that were produced by tomato extract in this section and the section 4.4.3.1.

For the effect of tomato extract in maintaining the viability of *S. thermophilus, L. bulgaricus* and *L. acidophilus* in soymilk yogurt during storage at low temperature, the data in Table 4.23 and Figure 4.8 displayed that the viable counts of the 3 microorganisms were significantly ($p \leq 0.05$) decreased during the storage period. Reductions of 2.41, 2.30 and 1.41 log CFU/ml were observed for *S. thermophilus, L. bulgaricus* and *L. acidophilus*. These results indicated that tomato extract was the lowest nutrient compounds in supporting the viability of yogurt bacteria compared to those of fibersol–2 and fructo–oligosaccharide (Tables 4.15 and 4.19). At the same time, the tomato extract was the highest nutrient compounds in maintaining the viability of *L. acidophilus* compared to those of the other 2 nutrient compounds (Table 4.26).

Table 4.25 Physical and chemical properties of soymilk yogurt added with *L. acidophilus* and in the presence of 5% (w/v) tomato extract during storage at 4^0C for 21 days

Physical and chemical properties	**0 day**	**21 days**
Consistency values measurement (cm)^{ns}	24.00 ± 0.00	24.00 ± 0.00
Syneresis values measurement (%) (w/w)^{ns}	35.78 ± 7.80	35.42 ± 2.93
Total Solid contents (%) (w/w)^{ns}	21.33 ± 2.84	21.00 ± 3.61
Moisture contents (%) (w/w)^{ns}	78.67 ± 2.84	79.00 ± 3.61
Total Soluble Solid (^{0}Brix)^{ns}	16.47 ± 1.37	16.23 ± 2.54
Viscosity values measurement (cp)^{ns}	2058.00 ± 211.00	1964.00 ± 151.00

Note: * Values were mean from 3 replications ± SD

* Different letters that followed numbers within the same row indicated significantly different ($p \leq 0.05$) between the treatments

Table 4.26 Reductions in the viable numbers of *S. thermophilus*, *L. bulgaricus* and *L. acidophilus* in soymilk yogurt added with different nutrient compounds during storage at 4^0C for 21 days

Nutrient compounds (5% (w/v))	Type of microorganisms (log CFU/ml)		
	S. thermophilus	*L. bulgaricus*	*L. acidophilus*
Fibersol-2	1.67 ± 0.04	1.58 ± 0.02	1.85 ± 0.13
Fructo-oligosaccharide	2.21 ± 0.13	2.03 ± 0.24	1.66 ± 0.04
Tomato extract	2.41 ± 0.06	2.30 ± 0.09	1.41 ± 0.03