CHAPTER 4
RESULTS AND DISSCUSSION

4.1 Analysis of green soya bean and green soya bean milk composition.

Physical, chemical and microbiological properties of green soya bean and its milk used in this study were shown in Table 4.1.

Table 4.1 Analyses of green soya bean and green soya bean milk.

Analyses	Green soya bean	Green soya bean milk
1. Physical		
L* value (lightness)	$57.60 \pm 0.94^{a*}$	73.34 ± 0.14^{b}
a* value (red-green)	-20.50 ± 0.72^{b}	-12.61 ± 0.10^{a}
b* value (yellow-blue)	38.77 ± 0.98^{b}	26.02 ± 0.13^{a}
2. Chemical		
Fiber (%) (w/w)	0.062 ± 0.04	0.003 ± 0.00
Carbohydrate (%) (w/w)	11.66 ± 2.27^{b}	1.89 ± 0.40^{a}
Fat (%) (w/w)	16.37 ± 1.56^{b}	6.43 ± 0.4^{a}
Protein (%) (w/w)	5.63 ± 0.21^{b}	1.21 ± 0.24^{a}
Minerals (%) (w/w)	1.66 ± 0.18^{b}	0.42 ± 0.09^{a}
Moisture content (%) (w/w)	64.74 ± 0.86^{a}	90.06 ± 0.05^{b}
Total solid (%) (w/w)	35.26 ± 0.86^{b}	9.94 ± 0.05^{a}
Total soluble solid (°Brix)	8.00 ± 0.00	5.00 ± 0.00
Acidity (as % lactic acid) (w/w)	0.29 ± 0.03^{b}	0.18 ± 0.00^{a}
pH	6.68 ± 0.00	6.71 ± 0.00
3. Microbiological		
Total Plate Count (log CFU/g)	7.67 ± 0.04^{b}	5.69 ± 0.04^{a}

Values are mean from 3 replications ± SD.

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

From Table 4.1, it showed clearly that the green soya bean milk had a different physical, chemical and microbiological property than those of the green soya bean. These differences were mainly due to an addition of warm distilled water (at 60°C) to the green soya bean at a ratio of 1:1 during the production of green soya bean milk. From a color measurement, the green soya bean milk showed significantly ($p \leq 0.05$) whiter, less green and yellow colors compared to those of the green soya bean. At the same time, the green soya bean milk significantly ($p \leq 0.05$) had less carbohydrate, fat, protein, mineral, total solid contents and titratable acidity together with lower values of fiber and total soluble solids compared to those of the green soya bean. On the other hand, the green soya bean milk contained significantly higher moisture contents and pH values compared to those of the green soya bean. The microbiological quality of the green soya bean milk was significantly ($p \leq 0.05$) lower 2.0 log CFU/g than of the green soya bean, which could be due to the temperature of the distilled water added during the extraction process.

Mohameda and Mentreddyb (2004) reported that green soya beans (vegetable soybean) had more lipid and protein, and much higher contents of phosphorus, calcium, potassium, vitamin B_1 and B_2 compared to those in peas or green peas (Section 2.1.3, Table 2.1). Comparing the data from the literature with the results in this research (Table 4.1), It could be seen that the green soya beans in this study had less moisture, protein and fiber contents with higher amounts of fat, carbohydrate and mineral contents. Differences in the nutritional contents could be due to different species of green soya beans, different harvesting periods and places to grow the green soya beans.

4.2 Study the physical, chemical, microbiological and sensory characteristics of a prototype green soya bean yoghurt ice cream.

4.2.1 Evaluation of microorganism in ABT-5 starter cultures freeze-dried.

ABT-5 starter cultures in the form of freeze-dried cultures contained a thermophillic lactic acid bacterium, which was *Streptococcus thermophilus* and probiotic bacteria of *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium bifidum* (BB-12) at a concentration of more than 10^{10} CFU/g (Appendix B-3). These starter cultures had a concentration of 15.85 ± 0.02, 14.78 ± 0.02 and 14.61 ± 0.02 log CFU/g for *S. thermophilus, L. acidophilus* and *B. bifidum*, respectively, in green soya bean yoghurt after the yoghurt was incubated at 43°C for 6 h. The yoghurt also had a final pH value of 4.46 ± 0.01 and a total titratable acidity of 1.59 ± 0.05% (w/w) lactic acid. The numbers of the individual starter cultures in green soya bean milk before fermentation and in the green soya bean yoghurt were shown in Table 4.2.

Table 4.2 The population of ABT-5 starter cultures from a freezed-dried culture in green soya bean milk and green soya bean yoghurt after incubation at 43°C for 6 h.

ABT-5 starter cultures	Microorganism population (log CFU/g)	
	Green soya bean milk	Green soya bean yoghurt
S. thermophilus	13.28 ± 0.01	15.85 ± 0.02
L. acidophilus	12.27 ± 0.01	14.78 ± 0.02
B. bifidum	12.23 ± 0.01	14.61 ± 0.04

Values are mean from 3 replications ± SD.

Table 4.2 showed that the population of the ABT-5 starter cultures in green soya bean yoghurt were higher than in green soya bean milk about 2 log cycles. Similar observation was also made by Shihata and Shah (2002) who reported that the amount of *S. thermophilus, L. acidophilus* and *B. bifidum* were increased about 2 log cycles at the end of fermentation time in cow's milk yoghurt. Vinderola *et al.* (2002) also presented that the numbers of *S. thermophilus, L. acidophilus* and *B. bifidum* at the end of

fermentation time in fermented dairy products were higher than those at the beginning about 2 log cycles. Since the ability of probiotic bacteria in affecting the composition of intestinal microflora is depending on the probiotic level, a suggested level of 10^8-10^9 CFU/g bacteria at the time of consumption has been reported (Lourens-Hattingh and Viljoen, 2001; Mattila-Sandholm *et al.*, 2002). These probiotic cultures must remain viable in the carrier food until the time of consumption. The microbial results in Table 4.2 displayed that the number of probiotic bacteria in the green soya bean yoghurt was higher than the recommended level for probiotic bacteria in healthy food products. This could be due to the growth promoters in green soya bean, such as fiber, raffinose and stachyose in green soya bean, which were prebiotics, that supported bacterial fermentation (Bengmark, 1998).

The microbiological analysis in this study used selective media, such as M17 medium for *S. thermophilus* and Homofermentative Heterofermentative Differential (HHD) medium for *L. acidophilus* and *B. bifidum*. The media could give a better support for microorganism growth. The HHD medium contained ingredients that support bacterial growth, such as fructose, peptone from casein, soytone, casamino acids and yeast extract. Potassium dihydrogen phosphate was a source of phosphate and had a function to be the buffer of medium. The presence of Tween 80 was to decrease the surface tension of bacteria cell walls that could lead to a better condition in bringing food from the medium into the cell (Mc Donald *et al.*, 1987)

Colonies of *S. thermophilus, L. acidophilus* and *B. bifidum* were also characterized based on their colony morphologies and under a microscope examination. The first bacterium was grown on M17 medium for 48 h at 37°C with an aerobically and the last two bacteria were cultivated anaerobically on HHD medium for 72 h at 37°C before they were examined. The examination under a microscope was carried out after the bacteria were staining (Appendix D-5; Harrigan, 1998). The results of this examination could be seen in Table 4.3 and Appendix A-1. This examination also displayed that the ABT-5 culture used in this study was pure and had distinct colony morphologies.

Table 4.3 The colony characteristics, cells shapes and Gram staining of ABT-5 starter cultures for producing green soya bean yoghurt ice cream.

ABT-5 starter cultures	**Colony**	**Gram staining and a microscope examination**
S. thermophilus	Round creamy colonies and shinning surfaces.	Gram positive Ovid cocci.
L. acidophilus	Blue center, surrounded by wide clear green zone colonies.	Gram positive Regularly-long shaped rods.
B. bifidum	Small round white colonies, no surrounding zone and non-shinning surfaces.	Gram positive Club-shaped rods.

Results in the Table 4.3 and Appendix A-1 also showed that *L. acidophilus* and *B. bifidum* could be easily identified and counted on the HHD medium. Mc Donald *et al.* (1987) reported that lactic acid bacteria (LAB) are widely distributed throughout nature. They are important in both production and spoilage of acid food products. LAB can be divided into two physiological groups: the heterofermentative LAB, which produce CO_2, lactic acid, acetic acid, ethanol and mannitol from hexoses and the homofermentative LAB, which produce primarily lactic acid from hexoses. The heterolactics are more important than the homolactics in producing flavour and aroma components such as acetylaldehyde and diacetyl (James, 1927). Homofermentative and heterofermentative LAB can be distinguished by a HHD medium. The cells of homofermentative species were blue to green, while the heterofermentative species would be white colonies when they grew on the medium (Mc Donald *et al.*, 1987).

4.2.2 A production procedure for a prototype product of green soya bean yoghurt ice cream.

A product profile of green soya bean yoghurt ice cream was assessed by an ideal ratio profile test to find important sensory characteristics of the product and to develop the product formula according to consumer demands.

The first sensory evaluation in finding the important sensory characteristics of green soya bean yoghurt ice cream was carried out by 15 panelists. The results of the sensory test were as followed:

1. Appearance — a sensory characteristic: green color (15 panelists).
2. Flavour — sensory characteristics: yoghurt flavour (8 panelists).
   soya bean flavour (11 panelists).
   sweetness (13 panelists).
   sourness (13 panelists).
3. Texture — sensory characteristics: smoothness (12 panelists).
   mouthfeel (11 panelists).
4. Overall — overall opinion about the product (15 panelists).

From the result of the first sensory evaluation, it was summarized that the important characteristics of green soya bean yoghurt ice cream included green color, smoothness, yoghurt flavour, green soya bean flavour, mouthfeel, sweetness, sourness and overall sensory characteristics. This information was then used to measure the acceptance level of a prototype green soya bean yoghurt ice cream based on the ideal figure for the product according to the consumer demand. The data from an ideal ratio profile test was shown in numbers in Table 4.4 and in a cobweb graph in Figure 4.1.

Table 4.4 Sensory characteristics of the prototype and ideal products of green soya bean yoghurt ice cream.

Characteristics	Ratio score of a prototype and ideal products
Green *	0.82 ± 0.18
Smoothness*	0.54 ± 0.17
Yoghurt flavour*	0.63 ± 0.27
Green soya bean flavour *	0.63 ± 0.31
Mouthfeel*	0.46 ± 0.24
Sweetness*	0.65 ± 0.23
Sourness	1.01 ± 0.29
Overalls*	0.45 ± 0.21

Values are mean from 15 replications ± SD.

* Sensory characteristics of the prototype product that were significantly different ($p \leq 0.05$) from the ideal product.

From Table 4.4 and Figure 4.1, it could be seen clearly that the sensory characteristics that need to be improved from the prototype product of green soya bean yoghurt ice cream were green color, smoothness, yoghurt flavour, green soya bean flavour, mouthfeel and sweetness. The reason to do the improvement was because these sensory characteristics significantly ($p \leq 0.05$) received lower scores (< 1.00) compared to those of an ideal product of green soya bean yoghurt ice cream. Improving these sensory characteristics should also improve the overall acceptance of the product. A sensory characteristic of sourness had a value of slightly more than 1.00, therefore this sensory characteristic did not need any improvement.

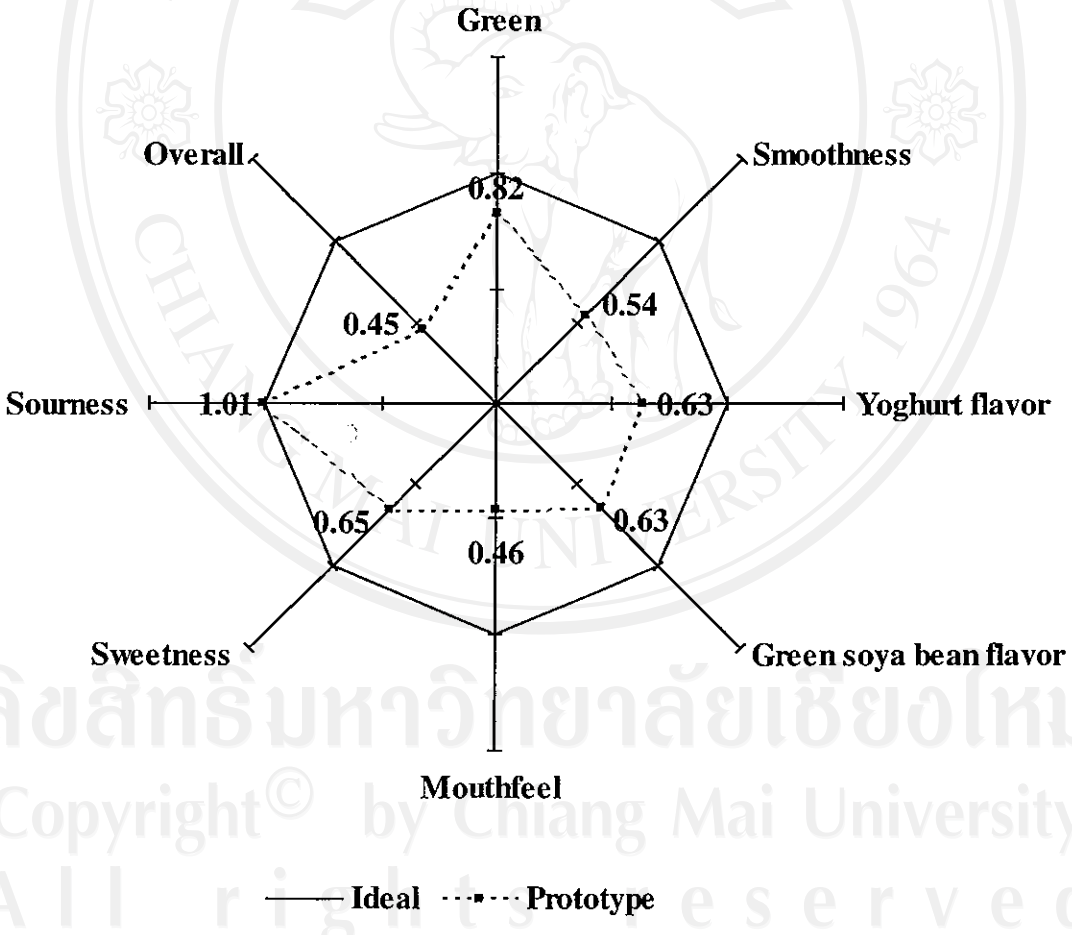

Figure 4.1 The sensory profile of the prototype and ideal product of green soya bean yoghurt ice cream.

The physical, chemical and microbiological characteristics of the prototype product of green soya bean yoghurt ice cream were shown in Table 4.5. From the color measurement of L*, a* and b* values compared with an a* and b* chromaticity diagram, it was indicated that the yoghurt ice cream had an olive green color. This color might be affected by the acidity and the presence of chlorophyll in the product. Since at high acid condition, chlorophyll *a* and *b* are converted to the correspondent pheophytins, in which Mg^{2+} ion is being replaced by protons, to give pheophytins *a* and *b*. Pheophytins had an olive green color and will be familiar as the dominant pigments in 'green' (Coultate, 2002).

Table 4.5 Physical, chemical and microbiological qualities of the prototype product of green soya bean yoghurt ice cream.

Analyses	Results of measurement
1. Physical	
L* value (lightness)	74.71 ± 0.31
a* value (red-green)	-5.33 ± 0.06
b* value (yellow-blue)	20.69 ± 0.28
Overrun (%)	15.91 ± 0.01
Melting rate (g/min)	0.64 ± 0.01
2. Chemical	
Total soluble solid (°Brix)	16.00 ± 0.00
Acidity (as % lactic acid) (w/w)	1.65 ± 0.03
pH	4.45 ± 0.01
Moisture content (%) (w/w)	79.68 ± 0.05
3. Microbiological	
S. thermophilus (log CFU/g)	13.06 ± 0.02
L. acidophilus (log CFU/g)	11.03 ± 0.02
B. bifidum (log CFU/g)	11.01 ± 0.02

Values are mean from 3 replications ± SD.

The physical characteristic of the prototype product of green soya bean yoghurt ice cream was shown that the product had a low overrun value and a high melting rate score. These values contributed to the low smoothness and easy to be melted of the

product. Since the product had a titratable acidity of 1.65 ± 0.03% lactic acid and a pH value of 4.45 ± 0.01, the product had a high sourness (Table 4.4). The numbers of the starter microorganisms found in the green soya bean yoghurt ice cream were less than those in the green soya bean yoghurt (Table 4.2). This could be due to the presence of oxygen that was incorporated during the production of yoghurt ice cream. Probiotic bacteria, especially bifidobacteria, are considered as highly susceptible to oxygen. However, the oxygen tolerance of these organisms has been strain dependent (Talwalker and Kailasapathy, 2003).

4.3 Development of green soya bean yoghurt ice cream formula.

4.3.1 Finding the experimental factors that affect the quality of green soya bean yoghurt ice cream.

Previous section had shown that the prototype of green soya bean yoghurt ice cream had a low sensory acceptance by panellists, therefore a development of the green soya bean yoghurt ice cream formula needed to be carried out. In this section, green soya bean yoghurt ice cream was assessed to find out the yoghurt ice cream ingredients that had a high significant contribution to the final quality of the green soya bean yoghurt ice cream product by applying a Plackett and Burman design experiment. There were 8 green soya bean yoghurt ice cream ingredients that were examined, including skim milk, butter, sugar, ABT-5 starter cultures, carboxymethylcellulose, κ-carrageenan, guar gum, and green soya bean milk. Different combinations and levels of each ingredient could be seen in Chapter 3, section 3.5.3.1. From the statistical design of Plackett and Burman, these ingredients were formulated into 12 treatments. The production of these 12 treatments followed a basic processing of green soya bean yoghurt ice cream in Figure 3.1 and incubated at 43°C for 6 h. The final products were then assessed for their physical, chemical, and microbiological qualities that were displayed in Table 4.6-4.8 together with their sensory acceptance in Table 4.9. For the effects of each green soya bean yoghurt ice cream ingredients on the physical, chemical, microbiological and sensory qualities of the final product, the statistical results could be seen in Table 4.10-4.13.

Table 4.6 The physical quality of green soya bean yoghurt ice cream in the Plackett and Burman design experiment.

Treatment	Color			Overrun (%)	Melting Rate (g/min)
	L* (lightness)	a* (red-green)	b* (yellow-blue)		
1	81.32±0.36	-7.59 ±0.10	24.59±0.80	17.57±0.84	0.15±0.01
2	78.99 ±0.17	-6.61±0.18	22.83±0.47	20.99±0.43	1.10±0.01
3	81.42±0.10	-7.02±0.15	24.88±0.54	27.44±1.05	0.80±0.01
4	84.65±0.25	-5.74±0.09	20.84±0.32	31.27±0.72	0.98±0.01
5	79.69±0.21	-8.66±0.19	25.54±0.75	44.61±0.43	0.27±0.02
6	76.16±0.21	-8.32±0.15	27.62±0.40	36.05±0.56	0.79±0.02
7	79.44±0.41	-6.50±0.26	22.65±0.78	23.56±0.40	0.70±0.01
8	80.00±0.08	-7.72±0.20	25.27±0.53	25.85±0.05	0.81±0.01
9	81.23±0.59	-6.68±0.07	24.85±0.22	21.64±0.71	0.19±0.02
10	75.30±0.58	-6.73±0.17	26.04±0.19	24.61±0.60	0.83±0.02
11	84.75±0.03	-6.27±0.06	19.79±0.15	38.06±0.17	0.84±0.02
12	81.63±0.19	-6.62±0.11	22.58±0.16	10.38±0.26	0.64±0.01

Values are mean from 3 replications ± SD.

Table 4.7 The chemical quality of green soya bean yoghurt ice cream in the Plackett and Burman design experiment.

Treatment	Total Soluble Solid (°Brix)	Total titratable acidity % lactic acid (w/w)	pH	Moisture contents (%) (w/w)
1	18.00±0.00	0.48±0.03	5.80±0.01	76.54±0.17
2	18.00±0.00	0.74±0.03	5.26±0.00	73.58±0.23
3	21.00±0.00	0.92±0.03	4.88±0.01	71.85±0.02
4	21.00±0.00	1.08±0.00	4.66±0.01	72.79±0.07
5	20.00±0.00	0.54±0.00	5.78±0.01	73.66±0.32
6	18.00±0.00	0.66±0.03	5.33±0.01	76.71±0.23
7	13.00±0.00	0.99±0.00	4.77±0.00	83.12±0.10
8	21.00±0.00	0.84±0.03	4.92±0.01	74.10±0.03
9	16.00±0.00	0.95±0.00	4.82±0.01	77.65±0.20
10	24.00±0.00	0.77±0.00	4.99±0.01	71.60±0.23
11	22.00±0.00	0.83±0.03	4.95±0.01	70.50±0.33
12	14.00±0.00	1.13±0.00	4.62±0.01	81.24±0.86

Values are mean from 3 replications ± SD.

Table 4.8 The microbiological quality of green soya bean yoghurt ice cream in the Plackett and Burman design experiment.

Treatment	*S. thermophilus* (log CFU/g)	*L. acidophilus* (log CFU/g)	*B. bifidum* (log CFU/g)
1	15.09±0.04	11.16±0.02	11.26±0.03
2	15.13±0.03	11.99±0.04	12.02±0.04
3	15.09±0.03	11.28±0.04	10.84±0.07
4	15.13±0.01	11.38±0.02	11.19±0.05
5	14.21±0.01	11.03±0.03	10.84±0.07
6	15.12±0.08	12.09±0.00	12.10±0.05
7	15.06±0.04	10.99±0.04	11.06±0.06
8	14.16±0.03	10.93±0.06	10.90±0.08
9	14.26±0.04	12.06±0.01	12.26±0.04
10	14.29±0.05	11.08±0.09	10.79±0.05
11	14.24±0.02	11.06±0.01	11.07±0.03
12	13.21±0.04	11.05±0.04	11.00±0.05

Values are mean from 3 replications ± SD.

Table 4.9 The sensory quality of green soya bean yoghurt ice cream in Plackett and Burman design experiment.

Treatment	Green	Smoothness	Yoghurt flavour	Green soya bean flavour
1	1.05±0.08	0.67±0.37	0.66±0.19	0.98±0.43
2	0.75±0.27	0.74±0.33	0.84±0.29	0.56±0.36
3	0.87±0.25	0.72±0.29	0.78±0.22	0.88±0.37
4	0.81±0.23	0.85±0.18	0.84±0.23	0.74±0.41
5	1.02±0.04	0.84±0.18	0.66±0.39	1.15±0.33
6	1.03±0.11	0.77±0.25	0.89±0.31	1.08±0.33
7	0.80±0.26	0.87±0.15	0.72±0.24	0.83±0.18
8	0.81±0.16	0.99±0.08	0.81±0.12	0.79±0.28
9	0.87±0.16	0.82±0.23	0.76±0.16	0.82±0.29
10	0.72±0.37	0.96±0.10	0.80±0.28	0.85±0.47
11	0.58±0.33	0.96±0.20	0.88±0.27	0.56±0.36
12	0.61±0.15	0.78±0.23	0.83±0.29	0.96±0.21
Prototype product	0.97±0.06	0.65±0.30	0.75±0.23	1.14±0.14

Values are mean ± SD from 13 panelists.

Table 4.9 (Continue)

Treatment	Mouthfeel	Sweetness	Sourness	Overall
1	0.65±0.41	0.64±0.41	0.43±0.28	0.35±0.07
2	0.80±0.36	0.97±0.42	0.65±0.34	0.57±0.19
3	0.65±0.41	0.71±0.38	0.94±0.25	0.46±0.13
4	0.83±0.04	0.77±0.18	0.99±0.10	0.63±0.11
5	0.85±0.32	0.71±0.43	0.62±0.38	0.72±0.21
6	0.74±0.22	0.63±0.20	0.96±0.26	0.46±0.25
7	0.84±0.35	0.69±0.34	0.96±0.47	0.60±0.24
8	0.91±0.28	0.90±0.18	0.86±0.29	0.70±0.09
9	0.74±0.35	0.51±0.32	0.81±0.25	0.67±0.24
10	0.96±0.11	0.91±0.25	0.80±0.41	0.61±0.21
11	0.62±0.35	0.97±0.22	0.91±0.38	0.62±0.22
12	0.53±0.39	0.57±0.24	1.03±0.35	0.45±0.13
Prototype product	0.87±0.17	0.54±0.30	1.23±0.04	0.41±0.28

Values are mean ± SD from 13 panelists.

Table 4.6-4.9 indicated that changing in the green soya bean yoghurt ingredient levels affected the qualities of the final product. These studied ingredients could be divided into 2 categories, which were a main factor and a fixed factor. The main factor was defined as green soya bean yoghurt ice cream ingredients that gave significant effects on the final quality of the product. The ingredients included as the main factors were the ingredients that significantly affected many of physical, chemical, microbiological and sensory quality parameters of the green soya bean yoghurt ice cream. Whereas, the fixed factors were green soya bean yoghurt ice cream ingredients that produced minor effects on the final quality of green soya bean yoghurt ice cream. The fixed factors were the ice cream ingredients that significantly affected less than the main factors in physical, chemical, microbiological and sensory quality parameters of the green soya bean yoghurt ice cream. The significant different levels of the Plackett and Burman design were divided into 4 levels, which were a for $p \leq 0.20$, b for $p \leq 0.15$, c for $p \leq 0.10$ and d for $p \leq 0.05$ (Wiriyajaree, 2002). The ice cream ingredients that were found to significantly affect the final yoghurt ice cream product (the main factors) would then be studied further in the next experiment.

Table 4.10 The effect of individual ingredients on the physical quality of green soya bean yoghurt ice cream based on the Plackett and Burman design experiment.

Dependent variable	Independent variable				
	L* (lightness)	a* (red-green)	b* (yellow-blue)	% Overrun	Melting Rate (g/min)
Skim milk	-4.105[c]	1.803	2.317[b]	0.800	0.255
Butter	7.154[d]	-0.343	-2.024[a]	1.112	-2.908[b]
Sugar	1.873	-2.717[b]	-2.547[b]	0.409	4.620[d]
Starter culture	-3.049[c]	-1.397	1.308	-1.431	-0.972
CMC	0.106	1.219	0.754	-0.048	-0.568
κ-carrageenan	-1.189	1.575	0.087	0.753	-1.569
Guar gum	-0.505	-2.527[b]	-1.214	0.805	0.979
Green soya bean milk	-5.639[d]	3.683[c]	6.514[d]	1.089	-1.267

Different letters that followed numbers within the same column indicated significantly different between the treatments.

a = t-test value significant at P≤0.20 is ±1.886

b = t-test value significant at P≤0.15 is ±2.282

c = t-test value significant at P≤0.10 is ±2.920

d = t-test value significant at P≤0.05 is ±4.303

Table 4.11 The effect of individual ingredients on the chemical quality of green soya bean yoghurt ice cream based on the Plackett and Burman design experiment.

Dependent variable	Independent variable			
	Total Soluble Solid (°Brix)	Total titratable acidity % lactic acid (w/w)	pH	Moisture contents (%) (w/w)
Skim milk	3.638[c]	-3.599[c]	4.074[c]	-2.252[a]
Butter	1.698	-0.854	1.424	-2.881[b]
Sugar	7.519[d]	1.113	-2.079[a]	-5.731[d]
Starter culture	-0.728	-0.596	0.370	0.889
CMC	0.728	-2.408[b]	1.966[a]	-0.861
κ-carrageenan	1.698	-2.667[b]	2.336[b]	-0.715
Guar gum	-0.243	1.631	-2.478[b]	0.232
Green soya bean milk	2.668[b]	-1.476	0.940	-2.024[a]

Different letters that followed numbers within the same column indicated significantly different between the treatments.

a = t-test value significant at P≤0.20 is ±1.886

b = t-test value significant at P≤0.15 is ±2.282

c = t-test value significant at P≤0.10 is ±2.920

d = t-test value significant at P≤0.05 is ±4.303

Table 4.12 The effect of individual ingredients on the microbiological quality of green soya bean yoghurt ice cream based on the Plackett and Burman design experiment.

Dependent variable	Independent variable		
	S. thermophilus (log CFU/g)	*L. acidophilus* (log CFU/g)	*B. bifidum* (log CFU/g)
Skim milk	0.988	2.091[a]	1.253
Butter	0.352	-0.215	-0.480
Sugar	0.365	-1.045	-2.049[a]
Starter culture	0.954	1.568	1.323
CMC	0.338	3.813[c]	4.577[d]
κ-carrageenan	-0.298	-5.565[d]	-4.086[c]
Guar gum	0.405	1.876	1.885
Green soya bean milk	-0.244	1.261	0.129

Different letters that followed numbers within the same column indicated significantly different between the treatments.

a = t-test value significant at P≤0.20 is ±1.886

b = t-test value significant at P≤0.15 is ±2.282

c = t-test value significant at P≤0.10 is ±2.920

d = t-test value significant at P≤0.05 is ±4.303

Table 4.13 The effect of individual ingredients on the sensory quality of green soya bean yoghurt ice cream based on the Plackett and Burman design experiment.

Dependent variable	Independent variable			
	Green	Smoothness	Yoghurt flavour	Green soya bean flavour
Skim milk	1.686	-1.413	-0.671	1.538
Butter	0.963	-1.140	-2.313[a]	0.177
Sugar	-1.686	2.143[a]	3.208[c]	-4.260[d]
Starter culture	0.401	-1.869	-2.611[b]	-1.065
CMC	0.522	-0.319	1.567	-1.834
κ-carrageenan	0.080	2.781[b]	-3.059[c]	0.355
Guar gum	-0.602	2.234[a]	2.313[b]	-1.302
Green soya bean milk	1.445	1.049	-0.522	2.781[b]

Table 4.13 (Continue)

Dependent variable	Independent variable			
	Mouthfeel	Sweetness	Sourness	Overall
Skim milk	1.117	0.972	-4.753[d]	-0.267
Butter	-0.910	-1.250	-2.511[b]	0.100
Sugar	0.869	5.139[d]	1.525	0.567
Starter culture	0.331	-0.417	-3.498[c]	-0.534
CMC	-0.414	0.903	-3.229[c]	-0.167
κ-carrageenan	1.117	2.292[b]	-3.587[c]	0.601
Guar gum	0.703	-0.069	4.036[c]	0.567
Green soya bean milk	1.200	-0.833	0.090	0.667

Different letters that followed numbers within the same column indicated significantly different between the treatments.

a = t-test value significant at P≤0.20 is ±1.886

b = t-test value significant at P≤0.15 is ±2.282

c = t-test value significant at P≤0.10 is ±2.920

d = t-test value significant at P≤0.05 is ±4.303

4.3.1.1 The effect of skim milk on the quality of green soya bean yoghurt ice cream.

Table 4.10 and 4.11 showed that an addition of skim milk in the green soya bean yoghurt ice cream significantly affected the quality of the final product by increasing its pH value ($p \leq 0.10$), total soluble solid ($p \leq 0.10$) and b* value ($p \leq 0.15$) and reducing L* value ($p \leq 0.10$) and titratable acidity ($p \leq 0.10$). In addition, the ingredient supported the growth of starter microorganisms and the effect was found to be significant for *L. acidophius* ($p \leq 0.20$) (Table 4.12). Skim milk supported the microorganisms growth because the ingredient contained high levels of lactose, which was used as a food source by microorganism for growing. Arbuckle (1986) reported that non fat milk solid (NMS) was skim milk that contained protein (37%), lactose (55%) and minerals (8%). The food material has a high food value, is not expensive and can enhance the palatability of ice cream products. The presence of lactose could slightly increase the sweet taste of the product. The protein in NMS helps to make an ice cream product to be more compact and smooth, thus preventing a weak body and coarse texture. Due to these advantages, the presence of NMS is desirable as high levels as possible, except that an excess of NMS may result in saltiness, overcooked or condensed-milk flavour and increase the risk of lactose crystallization during storage. NMS could also increase viscosity and resistance to melting, while at the same time reducing the freezing point. Based on the result of sensory evaluation (Table 4.13), the addition of skim milk in the yoghurt ice cream formula significantly affected the sourness ($p \leq 0.05$) of the final product. Therefore, finding a suitable level of skim milk would be carried out in the next experiment.

4.3.1.2 The effect of sugar on the quality of green soya bean yoghurt ice cream.

The presence of sugar in the green soya bean yoghurt ice cream formula significantly affected the qualities of the final product by increasing melting rate ($p \leq 0.05$) and total soluble solid ($p \leq 0.05$) and decreasing moisture content ($p \leq 0.05$), a* value ($p \leq 0.15$), b* value ($p \leq 0.15$), pH value ($p \leq 0.15$) and the growth of probiotic microorganisms, particularly for *B. bifidum* ($p \leq 0.20$) (Table 4.10-4.12). Sugar gave sweetness to a product, increased total soluble solid content and viscosity of the ingredients, but it also decreased the melting point of an ice cream, so the product would

have a higher rate of melting. In addition, sugar was a source of food for microorganisms to be used in their growth by changing sucrose to glucose and fructose and then turning glucose into lactic acid (Marks, 2004). The total soluble solid and the content of lactic acid in the green soya bean yoghurt ice cream were increased (Table 4.11) and the pH of product decreased (Table 4.11). In the sensory evaluation, the addition of sugar in the yoghurt ice cream formula significantly affected the smoothness ($p \leq 0.20$), yoghurt flavour ($p \leq 0.10$), green soya bean flavour ($p \leq 0.05$) and sweetness ($p \leq 0.05$) of the product (Table 4.13). Therefore, the suitable level sugar in the green soya bean yoghurt ice cream would be investigated further in the next experiment.

4.3.1.3 The effect of butter on the quality of green soya bean yoghurt ice cream.

Fat is of special importance for the flavour and for a solid structure to be formed during freezing, and hence for consistency, appearance and melting resistance. A high fat content leads to a dry, almost grainy texture, a low fat content to a smooth, homogeneous, somewhat slimy texture (Walstra *et al.*, 1999). Arbuckle (1986) reported that milk fat is an important ingredient for ice cream. If the ingredient is applied at a correct level, it will affect the balance of an ice cream mix and also fulfill a legal standard. Milk fat affected the flavour of the final product. Increasing a fat content in an ice cream mix decreases the sizes of ice crystals by interrupting the space in which they have to form. Therefore, the presence of fat restricts ice crystal size and its growth, which are highly important in low-fat ice creams. The presence of butter in the green soya bean yoghurt ice cream formula significantly affected the qualities of the final product by increasing L* value ($p \leq 0.05$) and decreasing b* value ($p \leq 0.20$), melting rate ($p \leq 0.15$) and moisture content ($p \leq 0.15$). At the same time, the ingredient gave a negative effect on the growth of *L. acidophilus* and *B. bifidum*. In the sensory evaluation, the addition of butter in the yoghurt ice cream formula significantly affected the yoghurt flavour ($p \leq 0.20$), and the sourness ($p \leq 0.15$) of the product (Table 4.13). From these results, 2% butter was selected to be used in the next experiment.

4.3.1.4 The effect of ABT-5 starter culture on the quality of green soya bean yoghurt ice cream.

The numbers of starter microorganisms in the green soya bean yoghurt ice cream could be reduced dramatically by acid, freezing injury, high temperature, presence of oxygen or moisture content. Alterations to cell membrane permeability and intracellular dehydration caused by ice crystal formation that may rupture cells could be another mechanism that resulted in microbial inactivation during freezing (Edward, 2003). The presence of ABT-5 starter culture in the green soya bean yoghurt ice cream formula was found to significantly decrease the L* value ($p \leq 0.10$) of the final product. By adding the ABT-5 starter culture, the growth of probiotic microorganisms in the yoghurt ice cream was increased (Table 4.12). The growth of 3 different microorganisms was depending on each other and the growth of these microorganisms together could decrease the fermentation period. The microorganisms also produced a good texture and odour of the yoghurt ice cream. In the sensory evaluation, the addition of ABT-5 starter culture in the yoghurt ice cream formula significantly affected the yoghurt flavour ($p \leq 0.15$) and sourness ($p \leq 0.10$) of the product (Table 4.13). There were many factors that affected the growth of starter cultures, therefore 2% ABT-5 starter culture was chosen to be used in the next experiment.

4.3.1.5 The effect of carboxymethylcellulose on the quality of green soya bean yoghurt ice cream.

The presence of carboxymethylcellulose in the green soya bean yoghurt ice cream formula significantly affected the quality of the yoghurt ice cream by decreasing titratable acidity ($p \leq 0.15$) and increasing the number of *L. acidophilus* ($p \leq 0.10$), *B. bifidum* ($p \leq 0.05$) and pH values ($p \leq 0.20$). In the sensory evaluation, the addition of carboxymethylcellulose in the yoghurt ice cream formula significantly affected the sourness ($p \leq 0.10$) of the product (Table 4.13). Since the presence of the ingredient supported the growth of probiotic microorganisms, a carboxymethylcellulose level of 0.4% was chosen to be applied in the next experiment. A further study might be required to explain the supporting action of carboxymethylcellulose on the microbiological aspect.

4.3.1.6 The effect of κ-carrageenan on the quality of green soya bean yoghurt ice cream.

κ-carrageenan was a heat-stable compound that promotes stabilization of the yoghurt gel by a complex formation with Ca^{2+} and casein (Scott and Hui, 2004). The ingredient also contributed to prevent a separation or uneven distribution of fat and other solids and to prevent or decrease the formation of ice and lactose crystals. In addition, κ-carrageenan can prevent whey-off during storage, before the mix was frozen and later when it passes through a freeze-thaw cycle (Nussinovitch, 1997). An addition of κ-carrageenan into green soya bean yoghurt ice cream formula significantly affected the qualities of the yoghurt ice cream by decreasing the number of *L. acidophilus* ($p \leq 0.15$) and *B. bifidum* ($p \leq 0.15$) and titratable acidity ($p \leq 0.15$) and increasing pH values ($p \leq 0.15$). In the sensory evaluation, the addition of κ-carrageenan in the yoghurt ice cream formula significantly affected the smoothness ($p \leq 0.15$), the yoghurt flavour ($p \leq 0.10$), the sweetness ($p \leq 0.15$) and the sourness ($p \leq 0.10$) of the product (Table 4.13). Since κ-carrageenan was found to reduce the growth of probiotic microorganisms, a level of 0.1% of the ingredient was chosen to be used in the next experiment. A further study might be required to explain the inhibition effect of κ-carrageenan on the microbial growth.

4.3.1.7 The effect of guar gum on the quality of green soya bean yoghurt ice cream.

Guar gum is readily soluble in cold water and is not affected by high temperatures used in the pasteurization of a yoghurt mix (Scott and Hui, 2004). An addition of guar gum in green soya bean yoghurt ice cream formula significantly affected the qualities of the final product by decreasing a* value ($p \leq 0.15$) and pH values ($p \leq 0.15$). In the sensory evaluation, the addition of guar gum in the yoghurt ice cream formula significantly affected the smoothness ($p \leq 0.20$), yoghurt flavour ($p \leq 0.15$), and the sourness ($p \leq 0.10$) of the product (Table 4.13). However the presence of guar gum supported the growth of starter microorganism, therefore a guar gum level of 0.4% was chosen to be applied in the

next experiment. A further study might be required to explain the supporting action of guar gum on the microbiological quality.

4.3.1.8 The effect of green soya bean milk on the quality of green soya bean yoghurt ice cream.

An addition of green soya bean milk in green soya bean yoghurt ice cream formula significantly affected the qualities of the yoghurt ice cream by decreasing L* value ($p \leq 0.05$) and moisture content ($p \leq 0.20$) and increasing b* ($p \leq 0.05$), a* value ($p \leq 0.10$ and total soluble solid ($p \leq 0.15$). The reduction in moisture content or an increase in total solid in the yoghurt ice cream indicated that the amount of water in the yoghurt ice cream was replaced by food compounds when the green soya bean milk was added. Therefore, the milk increased the nutritive value, increased the viscosity and improved the body and texture of the yoghurt ice cream. An increase in total solid improved the whipping ability and shortens the freezing time. Beside that, a higher total solid decreased the percentage of frozen water and permitted a higher overrun (Arbuckle, 1986). In the sensory evaluation, the addition of green soya bean milk in the yoghurt ice cream formula significantly affected the green soya bean flavour ($p \leq 0.15$) of the final product (Table 4.13). The presence of the green soya bean milk also supported the growth of probiotic microorganism of *L. acidophilus* and *B. bifidum*. Therefore a level of 80% green soya bean milk was chosen to be applied in the next experiment.

4.3.1.9 The overall effect of green soya bean yoghurt ice cream ingredients on the quality of the final product.

From the effect of each ingredient on the quality of green soya bean yoghurt ice cream by applying the Plackket and Burman Design with 12 treatments, it could be concluded that the main factors that affected the quality of the yoghurt ice cream were skim milk and sugar because these ingredients were sources of food for microorganisms. Since the physical, chemical and microbiological qualities together with the sensory characteristics of the yoghurt ice cream were significantly affected by these ingredients, optimum levels of these ingredients would be investigated in the next experiment. For the

other ingredients, they were included as fixed factors, including butter, ABT-5 starter culture, κ-carrageenan, carboxymethylcellulose, guar gum and green soya bean milk. For the first 3 ingredients, butter, ABT-5 starter culture and κ-carrageenan, they would be used using their low levels, which were 2, 0.2 and 0.1 % respectively. Whereas, carboxymethylcellulose, guar gum and green soya bean milk were applied in their high levels, which were 0.4, 0.4 and 80%, respectively.

4.3.2 Finding the optimum levels of skim milk and sugar for green soya bean yoghurt ice cream.

In this section, the optimum levels of skim milk and sugar were investigated by applying a 3x3 factorial experiment in Completely Randomized Design (CRD). The skim milk levels that were studied included 8, 10 and 12% (w/v) because the result data from the section 4.3.1.1 showed that an addition of 10% (w/v) skim milk was suitable for a good quality of the final green soya bean yoghurt ice cream. Therefore, the concentration of skim milk would be varied 3 levels including lower and higher level than 10% (w/v) skim milk for finding the optimum level of the skim milk for green soya bean yoghurt ice cream. Whereas, the levels of sugar were 12, 15 and 18% (w/v) because the result data from section 4.3.1.2 showed that an addition of 16% (w/v) sugar was suitable for a good quality of the final green soya bean yoghurt ice cream. Therefore, the concentration of sugar would be varied 3 levels including lower and higher levels than 15% (w/v) skim milk for finding the optimum level of the sugar for green soya bean yoghurt ice cream. The results of this experiment on the qualities of green soya bean yoghurt ice cream were displayed in Table 4.14-4.17.

Walstra *et al.* (1999) wrote that skim milk or NMS contributed to the flavour of ice cream. The ingredient was also responsible for partly reducing the freezing point depression and for an increase in the viscosity of ice cream. The protein of the NMS partly serves to stabilize the foam lamellae during air incorporation and is essential for the formation of fat globule membranes during homogenization. Lactose in the NMS can crystallize at low temperature. The crystals formed should be small in order to prevent

sandiness. In addition, cooling should be quick during freezing and afterward temperature fluctuations should be avoided to prevent big crystals.

Sugar, often sucrose, is essential for the taste and for the freezing point depression. A low level of sucrose may cause too much ice to be formed, whereas a high sugar concentration often makes the ice cream to be too sweet. The sugar also causes a higher viscosity in ice cream, especially when most of the water has been frozen. However, the most important role of the sugar is to reduce the portion of frozen water. If this was happened, the produced ice cream would have a softer consistency and a less cold taste inside the mouth (Walstra *et al.*, 1999).

4.3.2.1 The effect of skim milk and sugar on the physical quality of green soya bean yoghurt ice cream.

Table 4.14 The effect of skim milk and sugar on the physical quality of green soya bean yoghurt ice cream.

Skim milk (%)(w/v)	Sugar (%)(w/v)	Color			Overrun (%)	Melting Rate (g/min)
		L* ns** (lightness)	a* ns (red-green)	b* ns (yellow-blue)		
8	12	75.46±3.42	-5.42±1.14	23.79±5.48	32.17±3.06^{fg*}	0.25±0.02^{b}
8	15	75.41±2.87	-5.49±0.85	24.13±4.79	27.92±2.15^{cde}	0.61±0.03^{d}
8	18	75.89±1.90	-5.38±0.50	22.42±0.36	23.56±0.89^{a}	0.65±0.01^{d}
10	12	75.91±3.1;	-5.56±0.54	22.08±0.76	33.68±1.59^{g}	0.15±0.05^{a}
10	15	75.42±3.38	-5.23±0.54	22.14±0.78	30.17±1.22^{ef}	0.57±0.04^{d}
10	18	75.64±3.20	-5.21±0.81	21.10±1.27	24.80±0.92^{ab}	0.64±0.07^{d}
12	12	76.39±3.67	-5.83±0.48	22.73±2.10	26.06±1.52^{abc}	0.57±0.03^{d}
12	15	77.67±1.31	-6.05±0.99	22.27±1.31	27.23±0.72^{bcd}	0.42±0.12^{c}
12	18	74.83±2.61	-6.28±1.04	22.87±0.87	29.16±0.10^{de}	0.63±0.02^{d}

Values are mean from 3 replications ± SD.

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

** ns are not significant ($p > 0.05$)

From the factorial experiment, the data in Table 4.14 showed clearly that different levels of skim milk and sugar did not significantly affected the color of the yoghurt ice cream, but they significantly affected the overrun and melting rate ($p \leq 0.05$) of the final product. Skim milk and sugar increased the viscosity and the total solids of the mix of yoghurt ice cream. This improves the body and texture of the yoghurt ice cream, increasing the percentage of total solids decreases the percentage of frozen water and frequently permits a higher overrun. Furthermore, increasing sugar tends to increase smoothness of texture and melting rate (Marshall and Arbuckle, 1996). Based on the physical measurement, the optimum levels of skim milk and sugar that produced the highest overrun and the lowest melting rate in the yoghurt ice cream were 10 and 12% (w/v), respectively.

4.3.2.2 The effect of skim milk and sugar on the chemical quality of green soya bean yoghurt ice cream.

Table 4.15 The effect of skim milk and sugar on the chemical quality of green soya bean yoghurt ice cream.

Skim milk (%)(w/v)	Sugar (%)(w/v)	Total Soluble Solid (°Brix)	Total titratable acidity % lactic acid (w/w)	pH	Moisture contents (%)(w/w)
8	12	$20.67 \pm 0.58^{ab*}$	1.32 ± 0.04^{a}	4.95 ± 0.02^{de}	74.04 ± 0.36^{c}
8	15	22.00 ± 1.73^{ab}	1.79 ± 0.05^{bc}	4.83 ± 0.03^{cde}	73.00 ± 0.34^{bc}
8	18	23.67 ± 2.08^{ab}	1.41 ± 0.04^{a}	4.92 ± 0.01^{de}	71.50 ± 0.42^{b}
10	12	21.00 ± 1.00^{ab}	1.55 ± 0.08^{ab}	4.85 ± 0.03^{cde}	74.48 ± 0.83^{c}
10	15	20.33 ± 2.08^{a}	1.93 ± 0.06^{cd}	4.74 ± 0.04^{bcd}	73.82 ± 1.75^{c}
10	18	23.67 ± 0.58^{ab}	2.14 ± 0.05^{de}	4.58 ± 0.06^{ab}	71.09 ± 0.46^{b}
12	12	21.67 ± 2.08^{ab}	2.04 ± 0.20^{cde}	4.64 ± 0.13^{abc}	73.03 ± 0.95^{bc}
12	15	24.00 ± 1.73^{b}	2.25 ± 0.32^{e}	4.52 ± 0.24^{a}	71.54 ± 0.69^{b}
12	18	28.67 ± 2.52^{c}	1.33 ± 0.27^{a}	5.02 ± 0.20^{e}	67.51 ± 2.01^{a}

Values are mean from 3 replications ± SD.

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

Results of chemical analyses in Table 4.15 showed that different levels of skim milk and sugar significantly affected total soluble solid, titratable acidity, pH values and moisture content ($p \leq 0.05$) of the yoghurt ice cream. Applying higher levels of skim milk and sugar would produce yoghurt ice cream with higher total soluble solid and titratable acidity, with lower pH values and moisture content. Skim milk and sugar increased the viscosity and the total solids of the mix of yoghurt ice cream. Increasing the percentage of total solids decreases the percentage of frozen water and an increase in skim milk raises acidity and lowers the pH, including lactic acid that produced by starter microorganisms (Marshall and Arbuckle, 1996).

4.3.2.3 The effect of skim milk and sugar on the microbiological quality of green soya bean yoghurt ice cream.

Table 4.16 The effect of skim milk and sugar on the microbiological quality of green soya bean yoghurt ice cream.

Skim milk (%)(w/v)	Sugar (%)(w/v)	*S. thermophilus* ns** (log CFU/g)	*L. acidophilus* (log CFU/g)	*B. bifidum* (log CFU/g)
8	12	14.27±0.13	10.99±0.13^{a*}	11.53±0.45^{ab}
8	15	14.78±0.52	11.26±0.14^{bc}	11.23±0.18^{ab}
8	18	14.27±0.10	11.09±0.03^{abc}	11.27±0.11^{ab}
10	12	14.85±0.58	11.30±0.16^{c}	11.80±0.53^{b}
10	15	14.54±0.59	11.11±0.13^{abc}	11.30±0.18^{ab}
10	18	14.13±0.08	11.02±0.02^{a}	11.15±0.03^{a}
12	12	14.32±0.11	11.27±0.15^{bc}	11.20±0.28^{a}
12	15	14.23±0.12	11.07±0.05^{ab}	11.13±0.10^{a}
12	18	14.36±0.05	11.26±0.08^{bc}	11.77±0.40^{b}

Values are mean from 3 replications ± SD.

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

** ns are not significant ($p > 0.05$)

Table 4.16 showed that the presences of 3 starter microorganisms were affected differently with different levels of skim milk and sugar. *S. thermophilus* was not significantly affected by different levels of skim milk and sugar and was present at a minimum level of 14.13 ± 0.08 log CFU/g in all the yoghurt ice cream treatments. However, *L. acidophilus* and *B. bifidum* were significantly affected by different concentrations of skim milk and sugar ($p \leq 0.05$). At higher levels of skim milk and sugar, the last two microorganisms were present at higher number in the yoghurt ice cream. It might be due to both of skim milk and sugar supported the growth of these microorganisms.

4.3.2.4 The effect of skim milk and sugar on the sensory characteristic of green soya bean yoghurt ice cream.

Table 4.17 The effect of skim milk and sugar to the sensory quality of green soya bean yoghurt ice cream (a ratio between a product treatment and an ideal product).

Skim milk (%)(w/v)	Sugar (%)(w/v)	Green^{ns*}	Smoothness^{ns}	Yoghurt flavour^{ns}	Green soya bean flavour^{ns}
8	12	0.90±0.11	0.92±0.04	0.80±0.05	1.05±0.15
8	15	0.85±0.14	0.93±0.05	0.90±0.08	1.01±0.10
8	18	0.95±0.09	0.89±0.09	0.87±0.12	1.11±0.13
10	12	0.88±0.10	0.94±0.09	0.81±0.13	1.02±0.13
10	15	0.84±0.15	0.88±0.05	0.84±0.03	1.02±0.15
10	18	0.86±0.09	0.93±0.03	0.84±0.05	0.98±0.09
12	12	0.88±0.12	0.94±0.05	0.85±0.11	0.98±0.12
12	15	0.95±0.14	0.95±0.05	0.75±0.15	0.97±0.10
12	18	0.94±0.11	0.97±0.05	0.87±0.10	1.00±0.03

Values are mean ± SD from 18 panelists.

*ns are not significant ($p > 0.05$)

Table 4.17 (Continue)

Skim milk (%)(w/v)	Sugar (%)(w/v)	Mouthfeel[ns**]	Sweetness	Sourness	Overall[ns]
8	12	0.89±0.06	0.78±0.05[a*]	1.13±0.12[bc]	0.58±0.09
8	15	0.89±0.04	0.79±0.07[a]	1.11±0.06[abc]	0.61±0.08
8	18	0.89±0.06	0.88±0.04[ab]	1.05±0.05[abc]	0.60±0.04
10	12	0.86±0.07	0.78±0.09[a]	1.09±0.08[abc]	0.57±0.12
10	15	0.85±0.04	0.78±0.04[a]	1.20±0.04[c]	0.61±0.14
10	18	0.93±0.04	0.78±0.06[a]	1.15±0.06[bc]	0.63±0.02
12	12	0.95±0.02	0.87±0.08[ab]	1.08±0.18[abc]	0.63±0.12
12	15	0.90±0.09	0.83±0.10[ab]	1.00±0.11[ab]	0.65±0.05
12	18	0.93±0.10	0.94±0.11[b]	0.93±0.07[a]	0.69±0.06

Values are mean ± SD from 18 panelists.

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

** ns are not significant ($p > 0.05$)

From 7 sensory characteristics of green soya bean yoghurt ice cream, there were only the sweetness and sourness of the yoghurt ice cream that were affected by the presence of different levels of skim milk and sugar (Table 4.17). Skim milk and sugar enhance the yoghurt ice cream palatability. Lactose in skim milk added slightly to the sweet taste, largely produced by added sugar and sourness of the yoghurt ice cream depended on the amount of lactic acid. Furthermore, the proteins in skim milk help to make the ice cream more compact and smooth, and thus tend to prevent a weak body and coarse texture (Marshall and Arbuckle, 1996).

Since the level of 12% (w/v) skim milk and 18% (w/v) sugar received the highest value in the overall opinion of sensory panellists and had a high survival number of probiotic bacteria, these levels of skim milk and sugar were chosen to be studied further in the next experiment.

4.4 Study the optimum time and temperature incubation for the production of green soya bean yoghurt ice cream.

The fermentation condition, including time and temperature, could affect the quality of green soya bean yoghurt ice cream because the growth of starter microorganisms and its corresponding product, mainly lactic acid, would be depended on the time and temperature fermentation (de Brabadere and de Baerdemaeker, 1991). Because of this possibility, this section was dedicated to study the optimum time and temperature fermentation to produce a good quality of green soya bean yoghurt ice cream. A 3x3 factorial experiment in CRD was selected to study 3 levels of fermentation temperature, which were 40, 43 and 46°C combined with 3 different fermentation times of 4, 6 and 8 h. The physical, chemical, microbiological and sensory evaluation results were shown in Table 4.18-4.21.

4.4.1 The effect of different incubation times and temperatures on the physical quality of green soya bean yoghurt ice cream.

Table 4.18 The effect of different incubation times and temperatures on the physical quality of green soya bean yoghurt ice cream.

Incubation treatments		Color			Overrun (%)	Melting Rate (g/min)
Temperature (°C)	Time (h)	L*^{ns**} (lightness)	a* (red-green)	b*^{ns} (yellow-blue)		
40	4	75.17±0.31	-7.91±0.21^{b*}	23.93±1.19	28.25±1.00^{a}	0.56±0.05^{bc}
40	6	76.29±1.26	-7.45±0.43a^{b}	24.45±0.54	31.16±2.66^{ab}	0.60±0.02^{c}
40	8	76.87±0.76	-6.54±0.27^{ab}	23.62±0.29	29.23±4.74^{ab}	0.53±0.01^{ab}
43	4	75.94±1.75	-7.12±0.33^{ab}	24.57±0.77	30.91±1.53^{ab}	0.47±0.06^{a}
43	6	76.29±1.70	-6.68±0.66^{ab}	24.26±0.38	31.19±2.21^{ab}	0.51±0.03^{ab}
43	8	75.28±1.63	-6.32±0.67^{a}	24.30±0.64	33.28±0.56^{b}	0.53±0.04^{ab}
46	4	76.94±1.12	-7.56±0.80^{ab}	24.05±0.90	32.61±3.03^{ab}	0.57±0.01^{bc}
46	6	75.35±1.77	-6.80±1.21^{ab}	24.81±0.11	31.91±1.58^{ab}	0.48±0.04^{a}
46	8	75.87±1.88	-6.20±1.39^{a}	24.77±1.14	31.82±1.38^{ab}	0.48±0.05^{a}

Values are mean from 3 replications ± SD.

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

** ns are not significant ($p > 0.05$)

The result displayed on Table 4.18 showed that different incubation time and temperatures did not significantly affected the L* and b* values of green soya bean yoghurt ice cream. However, these different treatments significantly affected ($p \leq 0.05$) the a* value, overrun and melting rate of the yoghurt ice cream. Using an 8 h incubation time was found to decrease the a* value. The product color was changed from a bright green color to an olive green color. This could be due to the lost of Mg^{2+} in chlorophyll and a replacement with two protons that could be easily occured during heat processing or in mild acid. This reaction converted the chlorophyll into pheophytin (Richardson and Finley, 1997). Applying a fermentation temperature at 43°C for 8 h produced the highest overrun value and melting rate for the green soya bean yoghurt ice cream.

4.4.2 The effect of different incubation times and temperatures on the chemical properties of product.

Table 4.19 The effect of different incubation times and temperatures on the chemical quality of green soya bean yoghurt ice cream.

Incubation treatments		Total Soluble Solid (°Brix)	Total titratable acidity % lactic acid (w/w)	pH	Moisture contents^{ns**} (%) (w/w)
Temperature (°C)	Time (h)				
40	4	28.67±0.58^{d*}	0.35±0.02^{a}	6.81±0.11^{e}	69.31±0.79
40	6	27.67±0.58^{c}	0.56±0.03^{b}	6.39±0.26^{d}	69.57±1.06
40	8	26.00±0.00^{ab}	0.83±0.03^{cd}	5.61±0.14^{b}	69.90±2.03
43	4	29.00±0.00^{d}	0.60±0.05^{b}	5.98±0.19^{c}	68.94±1.83
43	6	27.67±0.58^{c}	0.75±0.03^{c}	5.64±0.10^{b}	69.79±1.81
43	8	26.33±0.58^{b}	0.93±0.05^{de}	5.46±0.02^{b}	70.14±1.42
46	4	28.67±0.58^{d}	0.60±0.02^{b}	6.05±0.16^{c}	69.30±0.65
46	6	27.33±0.58^{c}	0.98±0.18^{e}	5.45±0.13^{b}	70.10±1.68
46	8	25.33±0.58^{a}	1.19±0.03^{f}	5.12±0.03^{a}	70.29±1.92

Values are mean from 3 replications ± SD.

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

** ns are not significant ($p > 0.05$)

The chemical properties of green soya bean yoghurt ice cream were significantly affected by different incubation times and temperatures, particularly for total soluble solid, pH values and titratable acidity ($p \leq 0.05$). The total soluble solid and pH values were significantly decreased as longer fermentation time and higher temperature were applied. At the same time, the titratable acidities of the yoghurt ice cream was found to be significantly increased as longer fermentation time and higher temperature were used. The last trend was also applied for the moisture content of the yoghurt ice cream, although the statistical result did not show any significantly different between the treatments. Since starter microorganisms did a conversion of lactose to lactic acid and consequently it would reduce the pH of the yoghurt ice cream, the longer the incubation time and the higher the incubation temperature, the starter microorganisms could have more time to grow and produce lactic acid. A decrease in the total soluble solid of the yoghurt ice cream could be due to a conversion of lactose to lactic acid by the microorganisms.

4.4.3 The effect of different incubation time and temperatures on the microbiological properties of product.

Table 4.20 The effect of different incubation times and temperatures on the microbiological quality of green soya bean yoghurt ice cream.

Incubation treatments		*S. thermophilus* (log CFU/g)	*L. acidophilus* (log CFU/g)	*B. bifidum* (log CFU/g)
Temperature (°C)	Time (h)			
40	4	14.13±0.11^{a*}	11.02±0.02^{a}	11.07±0.03^{a}
40	6	14.28±0.06^{ab}	11.10±0.03^{ab}	11.09±0.02^{ab}
40	8	14.23±0.11^{ab}	11.24±0.03^{c}	11.14±0.03^{ab}
43	4	14.24±0.03^{ab}	11.07±0.02^{ab}	11.25±0.05^{abc}
43	6	14.67±0.38^{bc}	11.20±0.04^{c}	11.28±0.07^{abc}
43	8	15.29±0.15^{d}	11.34±0.06^{d}	11.34±0.09^{abc}
46	4	14.50±0.36^{abc}	11.12±0.04^{b}	11.44±0.33^{abc}
46	6	14.54±0.36^{abc}	11.28±0.06^{cd}	11.56±0.44^{bc}
46	8	14.92±0.49^{cd}	11.35±0.10^{d}	11.65±0.47^{c}

Values are 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 4.20 showed clearly that different incubation conditions significantly affected the growth of 3 starter microorganisms ($p \leq 0.05$) in green soya bean milk. Applying a fermentation condition at 43°C for 8 h produced the highest number of *S. thermophilus* and at 46°C for 8 h produced the highest numbers of *L. acidophilus* and *B. bifidum.* This could be due to the characteristic of the starter bacteria as thermophilic bacteria that had optimum growth temperatures between 40-45°C. At the same time, doing a longer fermentation time would increase the numbers of the starter bacteria (Tamime and Robinson, 1999).

4.4.4 The effect of different incubation time and temperatures on the sensory properties of product.

Table 4.21 The effect of different incubation times and temperatures on the sensory quality of green soya bean yoghurt ice cream (showed in ratio mean score).

Incubation treatments		Green[ns**]	Smoothness[ns]	Yoghurt flavour	Green soya bean flavour
Temperature (°C)	Time (h)				
40	4	1.04±0.05	1.00±0.04	0.75±0.13[a*]	0.86±0.11[a]
40	6	0.96±0.02	0.99±0.06	0.78±0.06[ab]	0.90±0.10[ab]
40	8	0.92±0.07	1.00±0.03	0.85±0.11[ab]	0.95±0.13[ab]
43	4	0.94±0.04	0.98±0.05	0.80±0.03[ab]	1.06±0.11[b]
43	6	0.91±0.07	0.97±0.03	0.88±0.05[ab]	0.98±0.13[ab]
43	8	0.99±0.06	0.97±0.04	0.91±0.04[b]	1.05±0.05[b]
46	4	0.99±0.05	1.01±0.01	0.75±0.04[a]	1.00±0.08[ab]
46	6	0.96±0.02	0.96±0.02	0.81±0.05[ab]	1.07±0.11[b]
46	8	0.97±0.07	0.96±0.05	0.85±0.07[ab]	1.09±0.07[b]

Values are mean ± SD from 18 panelists.

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

** ns are not significant ($p > 0.05$)

Table 4.21 (Continue)

Incubation treatments					
Temperature (°C)	Time (h)	Mouthfeel^{ns**}	Sweetness^{ns}	Sourness^{ns}	Overall
40	4	0.95±0.04	1.05±0.13	0.63±0.06	0.54±0.06^{ab*}
40	6	0.88±0.08	1.05±0.04	0.71±0.09	0.57±0.02^{ab}
40	8	0.83±0.04	0.94±0.25	0.85±0.26	0.57±0.08^{ab}
43	4	0.86±0.11	1.09±0.10	0.72±0.17	0.53±0.02^{a}
43	6	0.89±0.07	0.94±0.03	0.78±0.14	0.62±0.05^{ab}
43	8	0.90±0.06	0.92±0.13	0.91±0.31	0.60±0.00^{ab}
46	4	0.97±0.08	1.03±0.11	0.69±0.02	0.63±0.06^{b}
46	6	0.92±0.08	1.03±0.13	0.76±0.22	0.61±0.08^{ab}
46	8	0.92±0.03	1.05±0.10	0.79±0.20	0.62±0.02^{ab}

Values are mean ± SD from 18 panelists.

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

** ns are not significant ($p > 0.05$)

Conducting a sensory evaluation to green soya bean yoghurt ice cream samples from different incubation conditions showed that panellists could significantly detect differences in the yoghurt and green soya bean flavour ($p \leq 0.05$) between the sample treatments. Differences in the flavour notes of the yoghurt ice cream also significantly affected the overall acceptance of the product ($p \leq 0.05$). Since the panellist gave a high overall score for the sample that was incubated at 43°C for 8 h, and at this condition the yoghurt ice cream had the highest overrun value with high amounts of survival of *S. thermophilus*, *L. acidophilus* and *B. bifidum*, especially for *S. thermophilus*, this fermentation condition was used to be applied in the next experiment.

4.5 Study the shelf life of green soya bean yoghurt ice cream.

4.5.1 Production of green soya bean yoghurt ice cream using the optimum formula and incubation condition.

The optimum formula to produce green soya bean yoghurt ice cream based on the experimental results of the section 4.3 was shown in Table 4.22. This formula was incubated at 43°C for 8 h (from section 4.4) to produce green soya bean yoghurt ice cream. The final product of yoghurt ice cream was then assessed for its physical, chemical, microbiological and sensory characteristics that were discussed in the sections 4.5.1.1 and 4.5.1.2.

Table 4.22 The optimum formula to produce green soya bean yoghurt ice cream.

Ingredients	Quantity (%)(w/v)
Skim milk	9.02
Sugar	13.52
Butter	1.50
ABT-5 starter culture	0.15
κ-carrageenan	0.08
CMC	0.30
Guar gum	0.30
Green soya bean milk	60.10
Distilled water	15.03
Total	100

4.5.1.1 The physical, chemical and microbiological qualities of a developed product of green soya bean yoghurt ice cream.

The physical, chemical and microbiological analysis results for the green soya bean yoghurt ice cream formula in the section 4.5.1 was displayed in Table 4.23.

Table 4.23 The physical, chemical and microbiological characteristics of a developed product of green soya bean yoghurt ice cream.

Analyses	Mean score
1. Physical	
L* value (lightness)	76.31 ± 0.58
a* value (red-green)	-5.73 ± 0.24
b* value (yellow-blue)	28.13 ± 0.75
Overrun (%)	33.29 ± 0.33
Melting rate (g/min)	0.26 ± 0.02
2. Chemical	
Fiber (%) (w/w)	0.02 ± 0.003
Carbohydrate (%) (w/w)	20.90 ± 0.11
Fat (%) (w/w)	2.96 ± 0.09
Protein (%) (w/w)	5.79 ± 0.03
Minerals (%) (w/w)	1.10 ± 0.04
Total soluble solid (°Brix)	27.00 ± 0.00
Total titratable acidity (% lactic acid) (w/w)	1.13 ± 0.00
pH	5.26 ± 0.00
Moisture content (%) (w/w)	69.29 ± 0.04
3. Microbiological	
S. thermophilus (log CFU/g)	14.33 ± 0.05
L. acidophilus (log CFU/g)	11.22 ± 0.03
B. bifidum (log CFU/g)	11.25 ± 0.06
Psychrotroph bacteria (CFU/g)	0 ± 0.00
Yeasts and mould (CFU/g)	< 10
Coliform bacteria (MPN/g)	< 3

Values are mean from 3 replications ± SD.

The result in the Table 4.23 showed that a developed product of green soya bean yoghurt ice cream had a better physical characteristics compared to that of the prototype product of the yoghurt ice cream (section 4.2.2, Table 4.5). The developed yoghurt ice cream product had an olive green color when comparing the result of L*, a* and b* values with an a* and b* chromaticity diagram, because this could be due to the lost of

Mg^{2+} in chlorophyll and a replacement with two protons that could be easily occured during heat processing or in mild acid. This reaction converted the chlorophyll into pheophytin (Richardson and Finley, 1997). This product also had an overrun value that was double than the prototype product (Table 4.5) and had a melting rate that was approximately 40% less than the prototype product (Table 4.5).

The developed product of green soya bean yoghurt ice cream had a pH value of 5.26 ± 0.00 and contained 1.13 ± 0.00% lactic acid. In addition, this product had a nutritional value from the presence of carbohydrate (20.90 ± 0.11%) (w/w), protein (5.79 ± 0.03%) (w/w) and a low fat content (2.96 ± 0.09%) (w/w).

From the microbiological analysis results, Table 4.23 showed clearly that the developed product of green soya bean yoghurt ice cream contained high numbers of *S. thermophilus*, *L. acidophilus* and *B. bifidum.* These microorganisms were presents at levels of 14.33 ± 0.05, 11.22 ± 0.03 and 11.25 ± 0.06 log CFU/g, respectively, which were higher than the recommendation level for probiotic bacteria to give a health benefit effect towards consumers (Wattanasin, 2002). In addition, when the developed product of the yoghurt ice cream was checked for its microbiological quality based on a Thai regulation for ice cream products (The Notification of the Ministry of Public Health No.222 (2001) and No.257 (2002) in the Food Act B.E. 1979), the yoghurt ice cream had low levels of psychrotrophs bacteria, yeast and mould and coliform bacteria. This result indicated that producing the yoghurt ice cream in a clean processing area with a concern for a general hygiene procedure would produce a product that is safe for consumer, particularly from the microbiological point of view.

4.5.1.2 The sensory characteristic of a developed product of green soya bean yoghurt ice cream.

A sensory evaluation for the developed product of green soya bean yoghurt ice cream was carried out by 15 panelists using an ideal ratio profile method. The results of this evaluation were shown in numeric values in Table 4.24 and in a cobwebs graph in Figure 4.2.

Table 4.24 The sensory characteristics of the developed product of green soya bean yoghurt ice cream (showed in ratio mean score).

Characteristics	A ratio between developed and ideal products
Green*	0.94±0.06
Smoothness*	0.95±0.04
Yoghurt flavour*	0.87±0.07
Green soya bean flavour	1.00±0.12
Mouthfeel	0.97±0.11
Sweetness	0.94±0.14
Sourness*	0.92±0.06
Overall*	0.78±0.08

Values are mean ± SD from 15 panelists.

* Sensory characteristics of the developed product of green soya bean yoghurt ice cream that was significantly different than that of the ideal product at $p \leq 0.05$.

It was shown clearly in Table 4.24 that a developed product of green soya bean yoghurt ice cream had sensory characteristic scores that were closer to those of the ideal green soya bean yoghurt ice cream. The sensory characteristics of the developed yoghurt ice cream that were not significantly different with the ideal product included green soya bean flavour, mouthfeel and sweetness. For the sensory characteristics of green color, smoothness, yoghurt flavour and sourness, the panellists gave them values that were significantly lower ($p \leq 0.05$) than those of the ideal product. The low scores of these 4 sensory characteristics could contribute to a lower acceptance of the develop product compare to the ideal one. However, experiments in the previous section did contribute to better values of these 4 sensory characteristics compared to those of a prototype product (Table 4.24 and Figure 4.2). Therefore, the develop product of green soya bean yoghurt ice cream had a higher acceptance value than that of the prototype product of yoghurt ice cream.

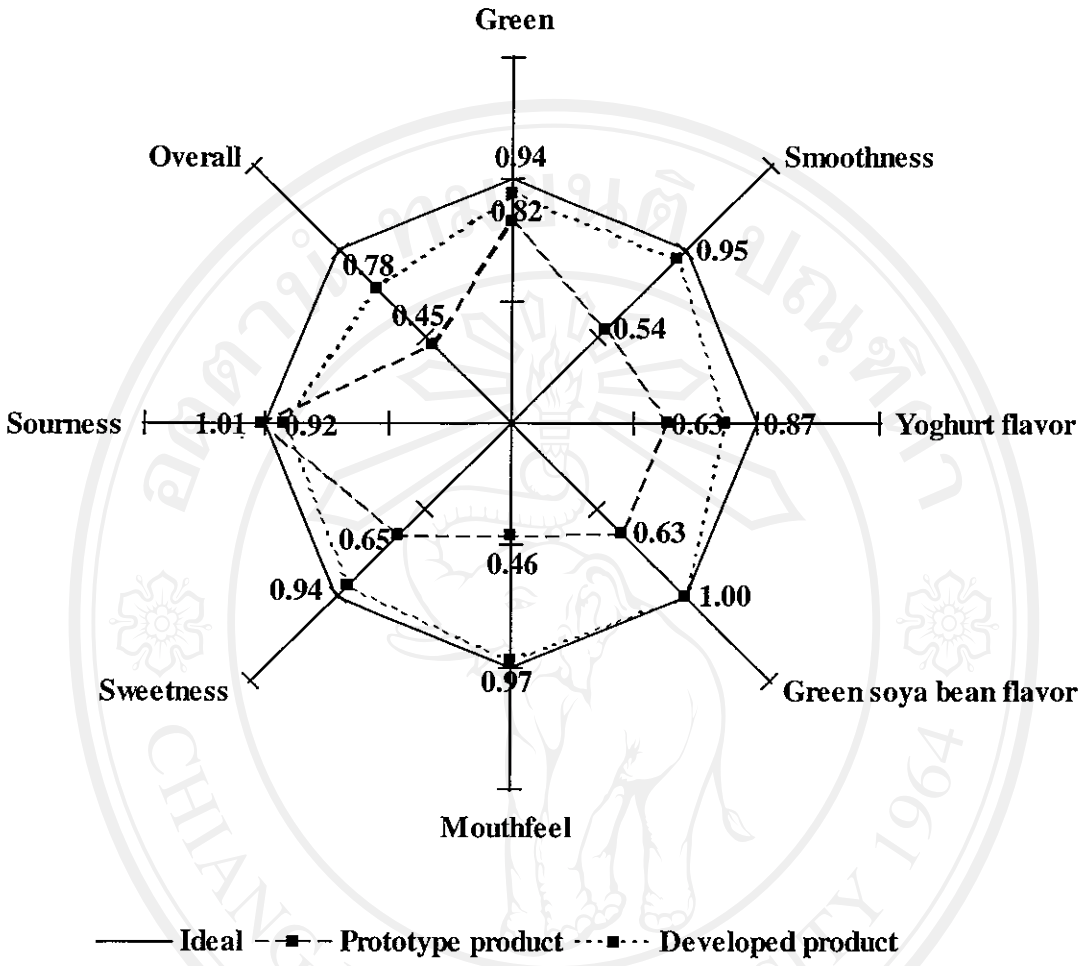

Figure 4.2 Sensory characteristics profile of prototype, developed and ideal products of green soya bean yoghurt ice cream.

The sensory characteristics of prototype, developed and ideal products of green soya bean yoghurt ice cream were compared in a cobweb graph in Figure 4.2. In general, the figure showed that most of the sensory characteristics of the prototype yoghurt ice cream had been improved in the developed product, therefore the developed yoghurt ice cream were closer in the term of sensory characteristics to the ideal one. For the sourness, the developed yoghurt ice cream was significantly valued to have a lower score ($p \leq 0.05$) than that of the prototype product. Since the values of green color, smootness, yoghurt flavour, green soya bean flavour, mouthfeel and sweetness of the developed yoghurt ice cream were better than those of the prototype product, the acceptance of the developed product was also higher than that of the prototype product.

4.5.2 Study the shelf life of green soya bean yoghurt ice cream.

In this last section, green soya bean yoghurt ice cream was prepared based on the experimental results in the sections 4.3 and 4.4, filled in plastic boxes and kept at -18°C for 4 months. During the storage time, samples of the yoghurt ice cream were analysed monthly for physical (Table 4.25), chemical (Table 4.26), microbiological (Table 4.27) and sensory characteristic (Table 4.28) changes, except for starter microorganisms. The 3 starter microorganisms were analysed every 2 weeks.

4.5.2.1 The physical quality of green soya bean yoghurt ice cream during storage at -18°C for 4 months.

Table 4.25 The physical quality of green soya bean yoghurt ice cream during storage at -18°C for 4 months.

Storage time (months)	Color			Overrun^{ns**} (%)	Melting Rate^{ns} (g/min)
	L* (lightness)	a* (red-green)	b* (yellow-blue)		
After processing	76.31±0.58^{a*}	-5.73±0.24^{a}	28.13±0.75^{a}	33.29±0.33	0.26±0.02
1	76.23±0.10^{a}	-5.65±0.12^{ab}	24.71±0.46^{b}	33.55±0.55	0.26±0.01
2	76.16±0.05^{a}	-5.10±0.12^{c}	23.97±0.65^{b}	33.70±0.33	0.26±0.01
3	76.23±0.46^{a}	-5.37±0.28^{bc}	23.86±0.89^{b}	33.27±0.29	0.25±0.01
4	75.28±0.24^{b}	-5.59±0.10^{ab}	24.62±0.13^{b}	33.58±0.43	0.26±0.01

Values are mean from 3 replications ± SD.

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

** ns are not significant ($p > 0.05$)

Changing in the physical quality of green soya bean yoghurt ice cream during 4 months storage at -18°C was shown in Table 4.25. From 3 different measurements, it was only the color property of the green soya bean yoghurt ice cream that had a significant change ($p \leq 0.05$) during the storage period. The overrun and melting rate values were not significantly changed. From Table 4.25, it showed that the L* and b* values of the yoghurt ice cream were significantly decreased during storage at -18°C. This could be

due to a change of chlorophyll in the product into pheophytin that had a color of an olive green color (Richardson and Finley, 1997). Since the changing occurred slowly at low temperature storage. The longer the storage time the higher the color changing would be happened. Therefore, the yoghurt ice cream that had an initial color of green was changed into olive green. This color changing was also affected by the amouth of acid in the green soya bean yoghurt ice cream and the freezing process (Rattanapanon, 2002). For the a* value, the measurement showed that the a* value was slightly changed during storage at -18°C, but the changing was not significantly different between the beginning and the end of the storage time.

4.5.2.2 The chemical quality of green soya bean yoghurt ice cream during storage at -18°C for 4 months.

Table 4.26 The chemical quality of green soya bean yoghurt ice cream during storage at -18°C for 4 months.

Storage time (month)	Fiber^{ns**} (%) (w/w)	Carbohydrate (%) (w/w)	Fat (%) (w/w)	Protein (%) (w/w)	Minerals^{ns} (%) (w/w)
After processing	0.02±0.003	20.90±0.11^{ab*}	2.96±0.09^{bc}	5.79±0.03^{a}	1.09±0.04
1	0.03±0.005	20.41±0.43^{b}	3.64±0.25^{a}	5.30±0.07^{b}	1.05±0.02
2	0.03±0.003	20.38±0.57^{b}	3.40±0.53^{ab}	5.45±0.22^{b}	1.09±0.06
3	0.03±0.005	21.20±0.32^{ab}	3.07±0.07^{bc}	5.47±0.07^{b}	1.05±0.02
4	0.03±0.004	21.50±0.56^{a}	2.71±0.09^{c}	5.40±0.02^{b}	1.06±0.02

Values are mean from 3 replications ± SD.

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

** ns are not significant ($p > 0.05$)

Table 4.26 (Continue)

Storage time (month)	Total Soluble Solid^{ns*} (°Brix)	Total titratable acidity^{ns} % lactic acid (w/w)	pH^{ns}	Moisture contents^{ns} (%) (w/w)
After processing	27.00±0.00	1.13±0.00	5.26±0.00	69.29±0.04
1	26.00±0.00	1.13±0.00	5.26±0.00	69.63±0.17
2	26.00±0.00	1.17±0.00	5.20±0.00	69.71±0.45
3	27.00±0.00	1.13±0.00	5.25±0.00	69.24±0.24
4	26.00±0.00	1.13±0.00	5.28±0.00	69.36±0.36

Values are mean from 3 replications ± SD.

* ns are not significant ($p>0.05$)

Changing in the chemical quality of green soya bean yoghurt ice cream was displayed in Table 4.26. From different measurement, there were only carbohydrate, fat and protein that were changed significantly ($p \leq 0.05$) during storage at -18°C. Most of the time, changing in the chemical properties of frozen food was occurred due to enzyme activities that hydolysed protein and fat in the food affecting pH, redox potential and lipid oxidation. These activities were occurred at a slow rate at low storage temperature (Rattanapanon, 2001). For the other measurement, which were fiber, mineral, total soluble solid, titratable acidity, pH values and moisture contents, they were found to be not significantly different during 4 months storage at -18°C.

4.5.2.3. The microbiological quality of green soya bean yoghurt ice cream during storage at -18°C for 4 months.

Table 4.27 The microbiological quality of green soya bean yoghurt ice cream during storage at -18°C for 4 months.

Storage time (week)	*S. thermophilus*^{ns**} (log CFU/g)	*L. acidophilus*^{ns} (log CFU/g)	*B. bifidum* (log CFU/g)
After processing	14.33±0.05	11.22±0.03	11.25±0.06^{a*}
2	14.31±0.01	11.21±0.04	11.20±0.07^{ab}
4	14.33±0.03	11.22±0.04	11.20±0.03^{ab}
6	14.36±0.06	11.19±0.06	11.16±0.03^{b}
8	14.32±0.03	11.19±0.02	11.17±0.03^{b}
10	14.34±0.06	11.17±0.07	11.16±0.02^{b}
12	14.34±0.06	11.19±0.02	11.17±0.02^{ab}
14	14.34±0.07	11.20±0.04	11.17±0.03^{b}
16	14.35±0.03	11.18±0.05	11.18±0.04^{ab}

Table 4.27 (Continue)

Storage times (month)	Psychrotrophs bacteria^{ns} (CFU/g)	Yeast and moulds^{ns} (CFU/g)	Coliform bacteria^{ns} (MPN/g)
After processing	0±0.00	<10	<3
1	0±0.00	<10	<3
2	0±0.00	<10	<3
3	0±0.00	<10	<3
4	0±0.00	<10	<3

Values are mean from 3 replications ± SD.

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

** ns are not significant ($p > 0.05$)

During 4 months storage at -18°C, the number of psychrotroph bacteria, yeast and mould and coliform bacteria was low in the green soya bean yoghurt ice cream (Table 4.27), which indicated that these microorganisms were not growth at low temperature storage (Adams and Moss, 2002). Beside that, the low numbers of these microorganisms

in the yoghurt ice cream after the processing could give a significant contribution to the good microbiological quality of the yoghurt ice cream during storage. For the 3 starter microorganisms, Table 4.27 showed that *S. thermophilus* and *L. acidophilus* were not significantly changed during the storage period, whereas a significant reduction in the number of *B. bifidum* was observed. However, the numbers of these microorganisms were higher than the recommendation level of 10^8-10^9 CFU/g to give a health benefit effect for consumer (Wattanasin, 2002). In addition, Lopez *et al.* (1998) reported lactic acid bacteria, such as *S. thermophilus*, *L. acidophilus* and *B. bifidum* were survived in frozen yoghurt stored at -23°C for one year. The numbers of these microorganisms were higher than 10^7 CFU/g. Some reports have also suggested additional protections could be added in frozen products to improve the survival of microorganism cells during freezing. The effect of pH and an addition of cryoprotectants, including sucrose and glycerol, had been examined in attempts to maintain the viability of probiotic bacteria during frozen storage (Edward, 2003).

4.5.2.4. The sensory characteristics of green soya bean yoghurt ice cream during storage at -18°C for 4 months.

Table 4.28 The sensory characteristics of green soya bean yoghurt ice cream during storage at -18°C for 4 months (a ratio between developed and ideal products).

Storage time (month)	Green^{ns**}	Smoothness	Yoghurt flavour^{ns}	Green soya bean flavour^{ns}
After processing	0.94±0.06	0.95±0.04^{a*}	0.87±0.07	1.00±0.12
1	0.93±0.09	0.95±0.06^{a}	0.87±0.15	1.02±0.08
2	0.93±0.08	0.95±0.08^{a}	0.86±0.07	1.04±0.11
3	0.90±0.08	0.93±0.08^{ab}	0.79±0.10	1.05±0.18
4	0.88±0.08	0.90±0.07^{b}	0.78±0.16	1.05±0.16

Values are mean ± SD from 15 panelists.

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

** ns are not significant ($p > 0.05$)

Table 4.28 (Continue)

Storage times (month)	Mouthfeel[ns*]	Sweetness[ns]	Sourness[ns]	Overall[ns]
After processing	0.97±0.11	0.94±0.14	0.92±0.06	0.78±0.08
1	0.96±0.11	0.98±0.10	0.92±0.17	0.78±0.10
2	0.95±0.10	0.99±0.12	0.91±0.09	0.77±0.09
3	0.91±0.09	0.99±0.13	0.87±0.12	0.75±0.08
4	0.91±0.12	0.98±0.16	0.86±0.08	0.71±0.13

Values are mean ± SD from 15 panelists.

* ns are not significant ($p>0.05$)

From 7 different sensory characteristics of green soya bean yoghurt ice cream that were monitored during 4 months storage at -18°C, 6 of them were not changed significantly during the storage period. This result could be due to a low storage temperature that was applied. This low temperature might slow down any chemical reactions that affected the sensory attributes of the product (Rattanapanon, 2001). Since most of the sensory characteristics were perceived to be stable during storage, the overall acceptance of the yoghurt ice cream was high and not significantly different throughout the storage time. For the smoothness, the value of this sensory attributes were significantly reduced ($p \leq 0.05$) during the storage period. This could be due to the electricity problem during the storage period that was turned off several times. Temperature fluctuations during on and off of the electricity could induce a formation of ice crystals that significantly affected the smoothness of the yoghurt ice cream.