CHAPTER 1

IMPROVEMENT OF PROBIOTIC-ADDED GOAT AND COW MILK YOGHURT

INTRODUCTION

Goat milk products, especially cheeses and yoghurt are very popular in many countries. Because of the nutritional benefit and values of goat milk, goat milk products are demanded. This demand is also growing because of a greater awareness with traditional medical treatments for such afflictions, especially in developed countries (Haenlein, 2004).

Due to their attributed health benefits, probiotic bacteria (such as *Lactobacillus acidophilus* and/or *Bifidobacterium)* have been increasing included in yoghurts and fermented milk during the past two decades. The addition of probiotic bacteria is made, not only because of certain claimed health-promoting effects in the intestinal tract, but also because of sensory advantages, as well as the expanding variety of products that can be formulated with them (Vinderola *et al.*, 2000). Goat milk can be used for this purpose.

In order to produce therapeutic benefits, a suggested minimum level for probiotic bacteria in fermented milk is from 10^6 to 10^7 cfu/ml (IDF, 1992). However, these organisms often show poor viability in market preparations (Dave and Shah, 1997; Klaver *et al.*, 1993; Ravula and Shah, 1998). Goat's milk has been described as having a higher digestibility and lower allergenic properties than cow's milk. In addition, goat's milk has been attributed with certain therapeutic values in human nutrition (Alferez *et al.*, 2001; Barrionuevo *et al.*, 2002).

In general, the overall properties of yoghurt, such as acidity level, free fatty acid content, the production of aroma compounds (diacetyl, acetaldehyde, acetoin) as well as the sensory profile, and nutritional value, are important traits of the product. These aspects are influenced by the chemical composition of the milk base, processing conditions, the added flavours, and the activity of starter culture during the incubation period (Georgala *et al.*, 1995; Tamime and Robinson, 1999). Mixed strains of starter culture (e.g. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) are normally used for the manufacture of yoghurt; however, mixed strains of *Lactobacillus* species, *Lactobacillus acidophilus, Bifidobacterium* ssp. with one or both of these yoghurt organisms, are used for the production of the so-called probiotic-fermented milks (Tamime and Robinson, 1999). The beneficial health properties of the latter products and their use in the treatment of body ailments are currently well accepted (Oberman and Libudzisz, 1996; Tamime *et al.*, 1995). It is evident that the associative growth that exists between *S. thermophilus* and *L. delbreuckii* ssp. *bulgaricus* can greatly influence the physicochemical properties and the level of aroma compounds in the yoghurt when compared with products made using a single strain of these micro-organisms (Beshkova *et al.*, 1988; Georgala *et al.*, 1995; Tamime and Robinson, 1999).

The objectives of this section were to produce probiotic-added goat and cow milk yoghurt and to determine the effect of the starter cultures, probiotic cultures and other yoghurt ingredients on the overall quality of yoghurt including sensory evaluation properties.

1.1 LITERATURE REVIEW

1.1.1 Goat milk

The importance of goats as the world's provider of essential food in meat and dairy products has been discussed and documented in many recent proceedings of national and international conferences. This importance is also reflected in the largest animal number increase for goats during the last 20 years (Table 1) and the largest increase in goat milk production tonnage compared to other mammalian farm animals. Milk production from goats is probably much greater than in these official statistics, because of the large amounts of unreported home consumption, especially in developing countries. This demand is increasing because of the increase of population. The second aspect of demand for goat milk is the connoisseur interest in goat milk products especially cheeses. This demand is growing because of the increasing levels of incomes. The third aspect of demand for goat milk derives from the affliction of people with gastro-intestinal ailments. This demand is also growing because of a wider awareness of problems with traditional medical treatments to such afflictions, especially in developed countries (Haenlein, 2004).

Despite the much larger volume available of cow milk, a much cheaper production and therefore lower market price for the production and marketing of goat milk and its products is therefore an essential niche in the total dairy industry sector. Especially the third aspect of demand for goat milk, the medical need, deserves in depth discussion and documentation, as it is widely accepted in practice and in anecdotal publications, but sparsely treated with biomedical research. Yet such research is essential for the future of the dairy goat industry, in developing as well as in developed countries (Haenlein, 2004).

Table 1 World numbers of mammalian farm animals (millions) since 1980 and annual milk production

Animal / animal products	**Year**		**Change (%)**
	1980	**1999**	
Animal numbers			
Goat	458	710	+55
Buffaloes	122	159	+30
Pigs	796	913	+15
Cattle	216	1,38	10
Sheep	1,96	1,69	-3
Milk production			
Goat	7,20	12,161	+58
Buffaloes	44,96	60,334	+36
Cattle	42,034	480,659	+14
Sheep	7,870	8,026	+2

Source: Haenlein (2004)

1.1.2 Nutritional studies of goat milk

In Spanish studies with rats, which had 50% of their distal small intestine removed by resection, simulating the pathological condition of malabsorption syndrome, the feeding of goat milk instead of cow milk as part of the diet resulted in significantly higher digestibility and absorption of iron and copper, thus preventing anemia. Also in these studies, the utilization of fat and weight gain were improved with goat milk in the diet, compared to cow milk, and levels of cholesterol were reduced, while triglyceride, high density lipoprotein (HDL), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) values remained normal. It was concluded that the consumption of goat milk reduced total cholesterol levels and the low density lipoprotein (LDL) fraction because of the higher presence of medium chain triglycerides (36% in goat milk versus 21% in cow milk), which decreases the synthesis of endogenous cholesterol. In an Algerian study of 64 infants with malabsorption syndromes, the substitution of cow milk with goat milk caused significantly higher rates of intestinal fat absorption. In a study in Madagascar, 30 hospitalized undernourished children between 1 and 5 years of age were fed either cow or goat milk in addition to their regular diet. Malnutrition is apparently frequent among children in Madagascar and cow milk is not affordable or available in sufficient quantities, while goat milk is cheaper to produce and more readily available. The children on goat milk out-gained the cow milk children in bodyweight by 9% daily over the 2-week trial period and fat absorption tended to be better in the goat milk children (Haenlein, 2004). Nutritional value of cow milk have been previous review by Wal (2004) that cow milk and cow milk products are nutritious food items containing numerous essential nutrients, but in the western societies the consumption of cow milk has decreased partly due to claimed negative health effects. The content of oleic acid, conjugated linoleic acid, omega-3 fatty acids, short- and medium chain fatty acids, vitamins, minerals and bioactive compounds may promote positive health effects. Most milk proteins, even proteins present at low concentrations, are potential allergens. A person may be allergic to casein or whey proteins or to both. Milk allergy may arise in small children (0–3 years) and it is estimated that 2–5% of the children has milk allergy

1.1.3 Yoghurt manufacture

Yoghurt is probably the most popular fermented milk. It is made in a variety of composition of fat and dry matter content, either plain or with added substances such as fruits, sugar or gelling agents. The nutrition values of milk and yoghurt are different as can be seen in Table 2.

Table 2 Comparison between a nutritional value of milk and yoghurt

Constituent units/100g	**Milk**			**Yoghurt**	
	Whole	Skimmed	Full fat	Low Fat	Fruit
Calories	67.5	36	72	64	98
Protein (g)	3.5	3.3	3.9	4.5	5
Fat (g)	4.25	0.13	3.4	1.6	1.25
Carbohydrate(g)	4.75	5.1	4.9	6.5	18.6
Calcium (mg)	119	121	145	150	176
Phosphorus (mg)	94	95	114	118	153
Sodium (mg)	50	52	47	51	-
Potassium (mg)	152	145	186	192	254

Source: Deeth and Tamine (1981)

1.1.4 The yoghurt bacteria

The essential flora of yoghurt consists of thermophilic lactic acid bacteria *S. thermophilus* and *L. bulgaricus*. *S. thermophilus* is a Gram positive facultative anaerobe. It is a cytochrome, oxidase- and catalase-negative organism that is nonmotile, non-spore forming and homofermentative. *S. thermophilus* is an alpha-hemolytic species of the viridans group. It is also classified as a lactic acid bacterium (LAB). *S. thermophilus* is found in fermented milk products. It is a probiotic (it does survive the stomach) and generally used in the production of yogurt. *L. delbrueckii* subspecies *bulgaricus* (until 1984 known as *L. bulgaricus*) is one of several bacteria used for the production of yogurt. It can also be found in other naturally fermented products. Morphologically, it is a Gram positive rod that may appear long and filamentous. It is also non motile, and it does not form spores. It has complex nutritional requirements, including the inability to ferment any sugar except for lactose. This bacterium is also regarded as aciduric or acidphilic, due to the fact that it requires a relatively low pH (around 5.4-4.6) in order to grow effectively. The bacterium feeds on sugars and produces lactic acid which also helps to preserve the product. In milk it breaks down lactose and is often helpful to sufferers of lactose intolerance, whose digestive systems lack the enzymes to break down lactose to simpler sugars. While fermenting milk, *L. bulgaricus* produces acetaldehyde, which is one of the main yogurt aroma components (Walstra *et al.*, 1999).

For a satisfactory flavor to develop, approximately equal numbers of *S. thermophilus* and *L. bulgaricus* should be present. They have a stimulating effect on each other's growth which is known as protocooperation. The proteolytic rods enhance the growth of streptococci by forming small peptides and amino acids, the main amino acid being valine. Milk contains too little of this amino acid and the proteolytic cocci, form the acid slowly. The cocci enhance the growth of the rods by forming formic acid out of pyruvic acid under anaerobic conditions and by a rapid production of CO_2. The stimulatory of formic acid remains unnoticed in intensely-heated milk because in this milk, formic acid has been formed by decomposition of lactose. The production of formic acid by the cocci is, however, essential in industrial practice where more moderate heat treatments of yoghurt milk are applied, e.g., 5-10 min at 85^{o}C. Due to mutual stimulation during combined growth of the yoghurt bacteria in milk, lactic acid is produced much faster than would be expected on the basis of the acid production by the individual pure culture. Some antibiosis also occurs in yoghurt in that the cocci cannot grow after certain acidity has been reached. The properties of bacterial strains used should be matched to each other because not every combination of strains is suitable. Furthermore, both species should be present in large numbers in the product, hence in the starter. The optimum ratio of diplococci to rods depends on the properties of the strains and is often approximately 1:1. This ratio between the yoghurt bacteria is best maintained if the inoculum percentage is 2.5 and the incubation time is 2.5 h at 45 ^{o}C (Walstra *et al.*, 1999).

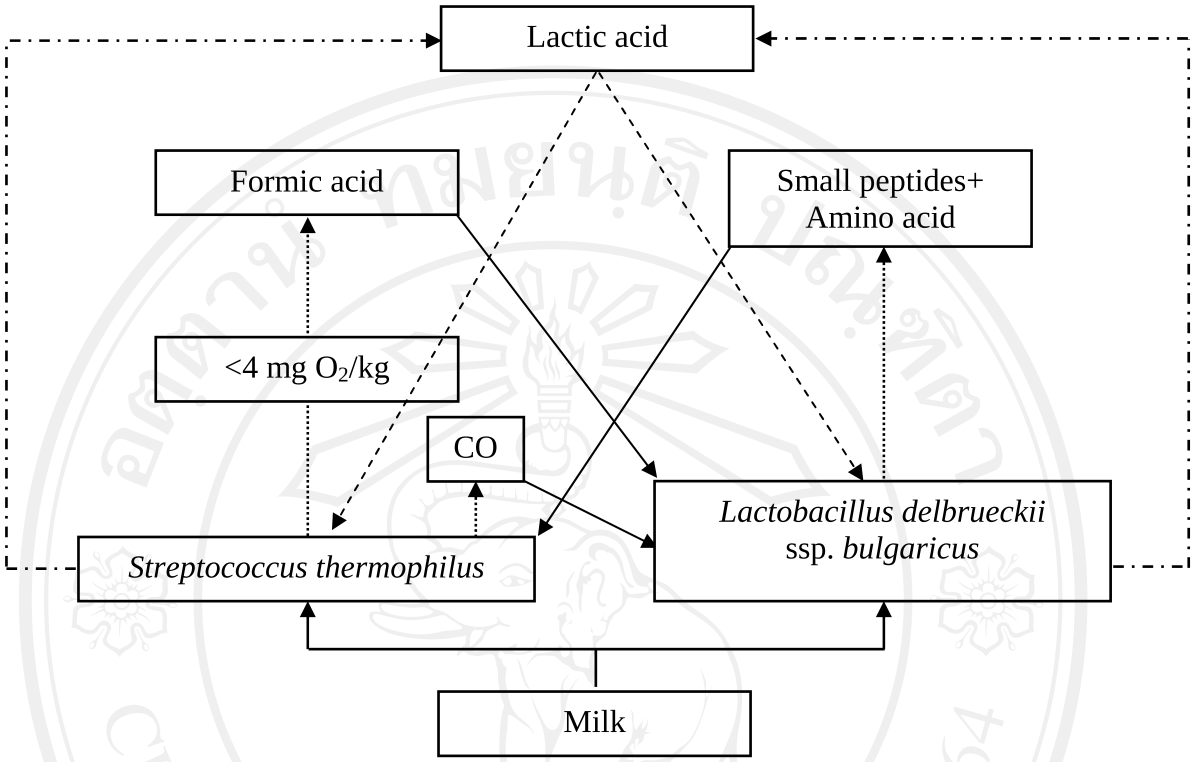

Fig. 1 Outline of the stimulation and the inhibition of the growth of yoghurt bacteria in milk. ______ , formation of lactic acid; ------- , formation of growth factor; -.-.-.-. , stimulation; .... , inhibition (Walstra *et al*., 1999).

1.1.5 Manufacture of set yoghurt

The most common types of set yoghurt are plain varieties made by simple fermentation of milk or milk product using microorganism, without additives, or varieties with stabilizers such as agar gelatin or pectin added, sweetened varieties with added sugar and flavored varieties with fruit juice or flavoring added (Nakazawa and Hosono, 1992). The manufacture of set yoghurt is shown in Figure 2.

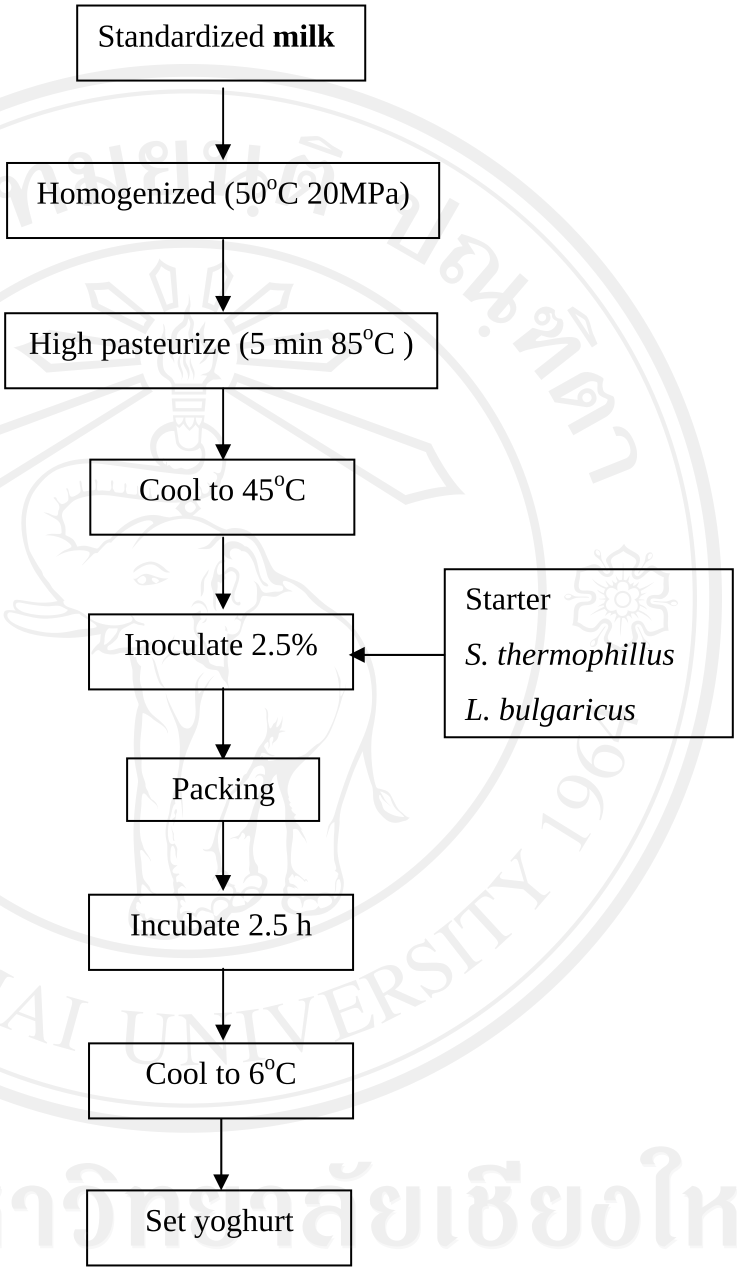

Fig. 2 An example of the manufacture of set yoghurt (Walstra *et al.*, 1999)

1.2 EXPERIMENTAL

1.2.1 Evaluation of physical, chemical and ideal ratio profile of the prototype of probiotic-added goat and cow milk yoghurt

1.2.1.1 The prototype preparation

The yoghurt was prepared based on the basic formula which modified from previous study of rice bran yoghurt containing probiotic (Isara, 2004) as shown in Table 3. All ingredients were mixed together by a homogenizer (Heidolph, Germany). The mixture was then heated to 85^{o}C for 10 min and cooled down to 37^{o}C (Isara, 2004) in a water bath (Memmert, Germany). After that, the starter and probiotic bacteria were inoculated. The product was incubated at 37^{o}C (Isara, 2004) for 12 h or until pH dropped to approximately 4.5 (Krasaekoopt, 2003).

Table 3 Basic formula of probiotic-added goat and cow milk yoghurt

Ingredients	Amount (%) (w/w)
Main ingredients	
Cow milk	42.9
Goat milk	42.9
Other ingredients	
Skimmed milk	7
Sugar	5
Carrageenan	0.2
Yoghurt starter culture	1
Probiotic	1

The basic formula and method above were used to develop a product profile using an ideal ratio profile and 12 panelists.

Microbial cultures in this study were used freeze-dried probiotic cultures of *L. acidophilus* (FD-DVSBB-12^{R}-Probiotic-Tec^{R} Chr. Hansen, Denmark), *B. bifidum* (FD-DVS LA-5^{R}-Probiotic-Tec^{R} Chr. Hansen, Denmark), and freeze-dried yoghurt starter cultures as *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. These cultures were stored at -20^{o}C before used in any experiments.

1.2.1.2 Physical characteristic analysis

The yoghurt sample was analyzed for the following parameters:

- Viscosity using a Brookfield viscometer (Brookfield Rotary viscometer, USA)
- Visual colors using Hunter colorimeter model ColorQuest II (Hunter Laboraory Inc, USA) in terms of L^* (lightness), a^* (redness and greenness) and b^* (yellowness and blueness).

1.2.1.3 Chemical characteristic analysis

The yoghurt sample was analyzed for the following parameters:

- pH by a pH meter (CONSORT C830, CE, Belgium)
- Titratable acidity according to the AOAC method no.947.05 (AOAC, 2000)
- Chemical composition by proximate analysis according to AOAC (2000)
- Total soluble solids by using a hand refractometer (ATAGO, Japan)

1.2.1.4 Microbiological analysis

The population of the following microorganisms was checked according to the following method.

- *S. thermophillus*, M17 (Merck, Germany) agar was used for the enumeration and incubated at 37°C for 72 h aerobically. Colonies of *S. thermophilus* in M 17 Agar

were white-cream, lenticular, with regular sides.

- *L. bulgaricus*, MRS (Merck, Germany) agar was used for the enumeration and incubated at 43°C for 72 h anaerobically. Colonies were circular or trilobate, of white-cream color with an external ring
- *B. bifidum,* MRS agar containing 0.2%/100 g lithium chloride (Sigma, Australia) and 0.3g/100g sodium propionate (Sigma, Australia) was used for enumeration and incubated at 37°C for 72 h under anaerobic conditions. Colonies were of white-cream color lenticular shape with regular sides.
- *L. acidophilus*, HHD agar (Merck, Germany) was used for enumeration and incubated at 37°C for 72 h under anaerobic conditions. Colonies of

L. acidophilus were large, of irregular shape, convex and pyramid shaped surface, light brown with a small central spot of dark green color.

1.2.1.5 Sensory evaluation

The product was subjected to a sensory analysis, using 12 panelists.

1.2.1.6 Statistical analysis

Experimental data were analyzed by SPSS program (SPSS version 11, SPSS Inc., Chicago, USA). Significant differences among means were compared by a Duncan's new multiple range test. The predetermined acceptable level of probability was 5%($p<0.05$) for all the comparison (Montgomery, 2001).

1.2.2 The formula development and influence of the ingredients on the properties of probiotic-added goat and cow milk yoghurt

1.2.2.1 Effect of ingredients on the qualities of goat and cow milk yoghurt

Screening for important ingredients which affected the qualities of mixture goat and cow milk yoghurt based on the formula obtained from 1.2.1.1 was performed. The Plackett and Burman (Plackett and Burman, 1946) experimental design was applied for this purpose. These experimental designs was proved to be able for investigating the dependence of some measured quantity on a number of independent variables (factors) in such a way as to minimize the variance of the estimates of these dependencies using a limited number of experiments (Plackett and Burman, 1994).

1.2.2.2 Effect of important ingredient levels on the qualities of mixture goat and cow milk yoghurt

The level of important ingredients in the section 1.2.2.1 was varied and its effect on the qualities of the mixture goat and cow milk yoghurt was evaluated. Experimental design in this section was based on Central Composite Design (CCD) which was desired for setting up the optimal response (Myers, 1971).

1.2.2.3 Analysis of qualities of goat and cow milk yoghurt.

The qualities of goat and cow milk yoghurt were analyzed in terms of physical, chemical, microbiological and sensory characteristics, based on the method previously mentioned in the Sections 1.2.1.2 to 1.2.1.5.

1.3 RESULT AND DISCUSSION

1.3.1 Evaluation of physical, chemical, microbiological and sensory characteristics of the prototype of the probiotic-added goat and cow milk yoghurt

1.3.1.1 Sensory Evaluation of the prototype of probiotic-added goat and cow milk yoghurt

There were 12 panelists (4 men and 8 women) involving in the sensory evaluation. All panelists were undergraduate and postgraduate students for the degree of Food Science and Technology, Mae Fah Luang University. The panelists evaluated a probiotic-added goat and cow milk yoghurt from the prototype formula (Table 3).

For the 1st sensory evaluation, panelists were given the prototype formula of the probiotic-added goat and cow milk yoghurt and were asked to evaluate the important sensory characteristics of the product in order to develop the product sensory profile. The result of the evaluation showed that most of the panelists agreed with the following properties:

Appearance

- 12 panelists told that the colour of the yoghurt should be white to pale yellow
- 10 panelists told that the consistency of the yoghurt should be homogeneous
- 5 panelists told that the "whey off" is the condition that water in yoghurt was separated from yoghurt cream layer

Texture

- 9 panelists told that yoghurt should have appropriate viscosity

- 9 panelists told that surface area in the cream of yoghurt should be smooth
- 5 panelists told that yoghurt should be slippery on throat and it should be sensible when the yoghurt was swallowed down in the throat

Smell and taste

- 12 panelists told that the smell of yoghurt should have an appropriate smell level of the cow and goat milk
- 9 panelists told that the sour smell should come from the fermentation of yoghurt starter culture
- 8 panelists told that yoghurt should have an appropriate sour taste
- 8 panelists told that yoghurt should have an appropriate sweet taste

Overall acceptance

- 12 panelists told that overall acceptance is the acceptance of overall senses of the product

Result of the test of product outline

From the result of the 1st sensory test, the important sensory characteristics of the probiotic-added goat and cow milk yoghurt based on the opinion of at least half of the panelists were as followed:

1. The color of the yoghurt
2. The consistency of the yoghurt
3. The viscosity of the yoghurt
4. The smoothness of the yoghurt
5. The milk flavour of the yoghurt
6. The sour flavour of the yoghurt
7. The sour taste of the yoghurt
8. The sweet taste of the yoghurt
9. Overall acceptance of the yoghurt

Based on this finding, the prototype yoghurt was further evaluated by 12 panelists in order to find out whether the prototype product had already achieved the ideal sensory characteristic of a good goat and cow milk yoghurt. The result of this sensory panel is shown in Table 4.

Table 4 Sensory evaluation of probiotic-added goat and cow milk yoghurt based on the panelist ideal yoghurt criteria

Sensory Characteristics	Mean ideal ratio profile score
The color of the yoghurt	0.79±0.2*
The consistency of the yoghurt	0.80±0.15*
The viscosity of the yoghurt	0.90±0.28*
The smoothness of the yoghurt	0.85±0.30*
The milk flavor of the yoghurt	0.95±0.31
The sour flavor of the yoghurt	0.76±0.18
The sour taste of the yoghurt	0.91±0.28
The sweet taste of the yoghurt	0.72±0.20*
Overall acceptance	0.62±0.15*

Note: The * shows the difference from ideal score significantly at 95% of confidence level

Data in Table 4 showed that the sensory characteristics of the probiotic-added goat and cow milk yoghurt were lower than the ideal sensory profile of a goat and cow milk yoghurt product. From this information, it was necessary to improve the sensory characteristics of the prototype product by modifying the formula composition to make the product to be the ideal yoghurt.

1.3.1.2 The analysis of physical, chemical and microbiological characteristics of the prototype of probiotic-added goat and cow milk yoghurt

The properties of the prototype of probiotic-added goat and cow milk yoghurt which was analyzed and tested for physical, chemical and microbiological characteristics are displayed in Table 5.

Table 5 Physical, chemical and microbiological characteristics of probiotic-added goat and cow milk yoghurt

Yoghurt Characteristics	Means
L^* Color value (Brightness)	81.40 ±0.18
a^* Color value (Red-Green)	-1.80±0.15
b^* Color value (Yellow - Blue)	8.90±0.18
Viscosity (cp)	4,850±250
pH value	4.25±0.01
Titratable acidity (%, w/w)	1.02±0.06
Protein (%, w/w)	3.25±0.22
Fat (%, w/w)	4.10±0.20
Ash (%, w/w)	0.74±0.04
Moisture (%, w/w)	81.0±0.7
S. thermophilus (log cfu/g)	4.51±0.02
L. bulgaricus (log cfu/g)	3.99±0.02
L. acidophilus (log cfu/g)	8.30±0.02
B. bifidum (log cfu/g)	8.10±0.04

1.3.2 A study for the appropriate ratio between cow milk and goat milk

From the study of the prototype formula of the probiotic-added goat and cow milk yoghurt, it was found that the panelists were divided into 2 groups regarding the milk flavor/smell of the yoghurt. For the first group, panelists could detect the smell of the goat milk which caused both acceptance and rejection. For the other group, testers could not detect the smell of the goat milk. Since the application of goat milk into the product should be increased, an appropriate ratio between cow and goat milks was investigated in this section to find a better ratio of the two raw milk sources that had a high acceptance among the panelists. To do this, the ratio of goat and cow milk were modified into 4 different groups which were 1:1, 1:2, 1:3 and 1:4 for cow milk and goat milk, respectively. The yoghurt characteristics after 12 h fermentation can be seen as followed:

1.3.2.1 Physical, chemical and microbiological properties of probiotic-added goat and cow milk yoghurt at the different ratio levels of goat and cow milks

The results in Table 6 shows the ratio of cow milk and goat milk which affected the physical, chemical and microbiological properties of the yoghurt.

For L^* color value (brightness), the data showed that the brightness of the yoghurt at ratio 1:1 had the highest value, and was not significantly different with the yoghurt at the ratio 1:2 ($p \leq 0.05$). Increasing the amount of the goat milk in the raw material composition significantly reduced the L color value of the yoghurt because the goat milk has more solid substances i.e., turbidity of goat milk is high.

For a^* color value (red, green), it was found that the products had a green color direction which was not significantly different between the treatments. The b^* color value (yellow, blue) indicated that the sample had yellow color direction. Since the b color value was (+) for yellow color direction, the yoghurt sample in this research had low yellow color intensity. The intensity of the yellow color in the yoghurt sample was significantly increased as higher amount of goat milk was added into the basic raw material. Yoghurt samples with the ratio of 1:1 had the lowest yellow intensity and the yellow color will be increased as the ratio of goat milk was higher and therefore, the higher yellow intensity was noted in the ratio of 1:4.

For the viscosity value, it was found that the ratio 1:1 of the cow to goat milk had the lowest viscosity value. Increasing the amount of goat milk in the yoghurt significantly increased the viscosity of the final product as well.

For the pH values, there was not any significant different between the pH value of different yoghurt treatments at 95% of confidence level. All the yoghurt samples had pH values between 4.24-4.26.

The microbial data in Table 6 also showed that the number of yoghurt starter culture and probiotic bacteria were significantly decreased as higher amount of goat milk was added into the basic raw material. The ratio of both yoghurt starter cultures resulted in this study approximately 1:1 which was a good combination of strain in yoghurt product (Walstra *et al*., 1999).

Table 6 The physical, chemical and microbiological properties of probiotic-added goat and cow milk yoghurt as affected by different ratio levels of goat and cow milks

Yoghurt Characteristics	Cow:goat milk 1 : 1	Cow:goat milk 1 : 2	Cow:goat milk 1 : 3	Cow:goat milk 1 : 4
L^* Color value (Brightness)	84.37±0.13^{a}	83.99±0.11^{a}	83.44±0.20^{b}	83.07±0.14^{c}
a^* Color value (Red-Green)	-1.04±0.12^{a}	-1.05±0.02^{a}	-1.09±0.02^{a}	-1.07±0.03^{a}
b^* Color value (Yellow - Blue)	9.37±0.25^{a}	9.52±0.10^{b}	9.57±0.11^{b}	9.76±0.14^{c}
Viscosity(cp)	4,756±265^{a}	4,732 ±136^{a}	5,849 ±179^{b}	5,948 ±145^{b}
pH value	4.25 ±0.02^{a}	4.24 ±0.01^{a}	4.25 ±0.01^{a}	4.26 ±0.01^{a}
Titratable acidity (%, w/w)	0.95 ±0.03^{a}	0.94 ±0.01^{a}	0.91 ±0.03^{a}	0.93 ±0.01^{a}
Protein (%w/w)	3.10 ±0.12^{a}	3.06 ±0.10^{a}	2.87±0.18^{b}	2.65±0.21^{c}
Fat (%w/w)	4.04 ±0.22^{a}	4.00±0.10^{a}	3.91±0.14^{b}	3.70±0.20^{c}
Moisture (%w/w)	81.80±0.80^{a}	80.44±1.12^{c}	80.65±1.10^{b}	80.43±1.00^{c}
S. thermophilus (log cfu/g)	4.90^{a}±0.03	4.87^{a}±0.01	4.65^{b}±0.02	4.65^{b}±0.03
L. bulgaricus (log cfu/g)	4.57^{a}±0.05	4.58^{a}±0.03	4.25^{b}±0.01	4.24^{b}±0.02
L. acidophilus(log cfu/g)	9.24^{a}±0.02	9.30^{a}±0.01	8.66^{b}±0.01	8.61^{b}±0.03
B. bifidum (log cfu/g)	9.00^{a}±0.04	9.02^{a}±0.05	8.59^{b}±0.03	8.44^{c}±0.05

1.3.2.2 The sensory characteristics of probiotic-added goat and cow milk yoghurt affected by different ratio levels of goat and cow milks

The data in Table 7 showed that the overall acceptance of probiotic-added goat and cow milk yoghurt product at the ratio of 1:2 (cow milk : goat milk) had the highest acceptance by the panelists and then the next experiment would use the ratio of 1:2 (cow milk:goat milk).

Table 7 The sensory characteristics of probiotic-added goat and cow milk yoghurt as affected by different ratio levels of goat and cow milks

Sensory characteristics	**Cow:goat milk 1 : 1**	**Cow:goat milk 1 : 2**	**Cow:goat milk 1 : 3**	**Cow:goat milk 1 : 4**
The color of the yoghurt	0.84+0.25	0.85+0.20	0.89+0.21	0.84+0.13
The consistency of the yoghurt	0.71+0.33	0.86+0.15	0.78+0.12	0.79+0.11
The viscosity of the yoghurt	1.31+0.39	1.10+0.20	0.95+0.22	0.90+0.29
The smoothness of the yoghurt	0.74+0.40	0.96+0.20	0.90+0.22	0.88+0.30
The milk flavor of the yoghurt	1.75+0.33	1.70+0.24	1.01+0.21	0.92+0.30
The sour flavor of the yoghurt	1.76+0.27	1.71+0.32	0.88+0.15	0.74+0.27
The sour taste of the yoghurt	0.98+0.36	1.08+0.20	0.84+0.26	0.70+0.31
The sweet taste of the yoghurt	0.94+0.40	0.96+0.25	0.91+0.27	0.91+0.32
Overall acceptance	1.63+0.17	1.69+0.13	1.45+0.18	1.33+0.18

1.3.3 The effect of main yoghurt ingredient levels on different quality parameters of probiotic-added goat and cow milk yoghurt

This section was used to determine the yoghurt ingredients that had significant effects on the quality of the goat and cow milk yoghurt with probiotic. There were 5 factors to be evaluated including skimmed milk (A), sugar (B), carrageenan (C), yoghurt starter culture (*L. bulgaricus, S. thermophilus*) (D) and probiotic cultures (*L. acidophilus* and *B. bifidum*) (E).

The effect of different yoghurt ingredients on the final product quality was assessed by a Plackett and Burman design. The maximum and minimum levels of each yoghurt ingredient were determined based on the panelist ideal yoghurt criteria and can be seen in Table 8. After each of the yoghurt treatment was incubated for 12 hours at 45^{o}C, the yoghurt characteristics was determined as shown in Tables 10 and 11.

Table 8 High and low levels of yoghurt ingredients for a Plackett and Burman design (%)

Factor	Low level (-)	High level (+)
A	10	15
B	5	10
C	0.1	0.2
D	1	2
E	1	2

Source: Isara, 2004

Table 9 Treatments for the Plackett and Burman design

Treatment	A	B	C	D	E	F	G
1	+	+	+	-	+	_	_
2	+	+	_	+	_	_	+
3	+	_	+	_	_	+	+
4	_	+	_	_	+	+	+
5	+	_	_	+	+	+	_
6	_	_	+	+	+	_	+
7	_	+	+	+	_	+	_
8	_	_	_	-	-	-	-

Table 10 Physical, chemical and microbiological properties of probiotic-added goat and cow milk yoghurt based on a Plackett and Burman design

Yoghurt properties	Treatments in the Plackett and Burman design							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
L^* Color value (Brightness)	80.36+0.05	81.95+0.24	82.87+0.01	82.70+0.20	84.45+0.77	85.22+0.12	81.99+0.08	81.78+0.09
a^* Color value (Red-Green)	-1.81+0.02	-2.07+0.05	-2.37+0.05	-2.26+0.02	+2.55+0.02	-2.36+0.01	-2.58+0.04	-2.51+0.03
b^* Color value (Yellow - Blue)	9.29+0.02	9.55+0.02	9.35+0.04	9.76+0.03	11.25+0.03	11.36+0.03	9.74+0.05	9.61+0.05
Viscosity (cp)	2,148+31	2,523+52	4,54+68	4,715+27	3,958+30	3,763+37	1,250+49	1,010+60
pH value	3.89+0.02	3.85+0.01	3.98+0.02	3.87+0.03	3.71+0.02	3.78+0.01	3.96+0.02	4.02+0.02
Titratable acidity (%w/w)	1.31+0.02	1.38+0.04	1.29+0.03	1.15+0.04	1.48+0.05	1.12+0.02	1.20+0.03	1.19+0.02
S. thermophilus(1)	4.88±0.03	4.78±0.01	4.77±0.02	4.89±0.02	5.01±0.03	4.98±0.03	4.95±0.05	4.75±0.01
L. bulgaricus(1)	4.73±0.05	4.92±0.02	4.59±0.03	4.66±0.01	4.95±0.01	4.85±0.02	4.45±0.04	4.56±0.01
L. acidophilus(1)	9.22±0.02	9.55±0.05	9.28±0.05	6.48±0.08	9.73±0.04	9.69±0.06	9.36±0.04	9.10±0.01
B. bifidum(1)	9.30±0.09	9.31±0.07	9.18±0.05	9.44±0.08	9.67±0.07	9.56±0.07	9.43±0.09	8.38±0.08

Note: the data were mean ± S.D. from triplicate analysis, (1) the number of microorganism showed in log cfu/g

Table 11 **Sensory characteristics of probiotic-added goat and cow milk yoghurt based on a Plackett and Burman design**

Sensory characteristics	**Treatments in the Plackett and Burman design**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
The color of the yoghurt	1.15+0.20	1.16+0.15	1.14+0.13	0.89+0.19	0.96+0.20	0.87+0.21	0.89+0.20	0.88+0.22
The consistency of the yoghurt	0.44+0.20	0.76+0.16	0.48+0.15	0.83+0.08	0.98+0.11	0.81+0.20	0.92+0.22	0.90+0.27
The viscosity of the yoghurt	1.36+0.26	1.27+0.44	1.17+0.46	1.06+0.12	1.13+0.17	1.15+0.23	1.01+0.18	1.04+0.21
The smoothness of the yoghurt	0.76+0.23	0.99+0.11	0.70+0.23	1.11+0.18	1.25+0.08	1.01+0.25	0.89+0.16	0.71+0.28
The milk flavor of the yoghurt	0.99+0.26	0.99+0.64	0.95+0.28	0.90+0.23	1.15+0.67	0.85+0.52	0.71+0.20	0.74+0.24

Table 11 Sensory characteristics of probiotic-added goat and cow milk yoghurt based on a Plackett and Burman design (continue)

Sensory characteristics	Treatments in the Plackett and Burman design							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
The sour flavor of the yoghurt	0.88+0.26	0.90+0.49	0.75+0.36	0.97+0.13	1.09+0.17	1.02+0.53	0.93+0.6 8	0.90+0.22
The sour taste of the yoghurt	1.05+0.32	1.01+0.24	1.45+0.36	1.39+0.32	1.48+0.34	1.36+0.46	0.96+0.32	0.98+0.28
The sweet taste	1.02+0.10	1.05+0.11	0.95+0.15	0.98+0.19	0.85+0.21	0.81+0.16	0.90+0.14	0.83+0.10
Overall acceptances	0.65+0.26	0.75+0.13	0.56+0.25	0.73+0.12	0.88+0.12	0.74+0.22	0.73+0.18	0.67+0.21

1.3.4 Different analyses for the main yoghurt ingredients that affected the probiotic-added goat and cow milk yoghurt characteristics

To consider the effect of yoghurt ingredients on the quality of the final yoghurt based on a Plackett and Burman design, the collected data was analyzed by a SPSS program. Each of the ingredients had a t-test score (Table 12). The t-test score used in this research applied a confidence level at 75% ($p \leq 0.25$). In order to decide which ingredients produced major or minor effects on the final yoghurt product, the following criteria were used:

If the t-test score has a significant negative value, it means that the yoghurt ingredient has an adverse effect on the yoghurt quality. When the amount of the ingredient is increased, the correlated of the yoghurt characteristic will be decreased. Therefore, this ingredient will be categorized as a minor factor which gives a minor effect on the yoghurt property.

On the other hand, when the t-test score has a significant positive value, it indicates that the specific yoghurt ingredient has a positive effect on the product quality. The value of a dependent variable will be increased as the quantity of the independent variable is higher. Therefore, this dependent variable will be increased and produces a major effect on the yoghurt characteristic.

Table 12 The t-test score for the physical, chemical, microbiological and sensory properties of the probiotic-added goat and cow milk yoghurt based on a Plackett and Burman design

Dependent variables	Independent variables				
	Skim milk	Sugar	Carageenan	Yoghurt Starter	Probiotic
L^* Color value (Brightness)	-2.926[e]	-6.764[c]	-17.708[e]	-1.710[b]	2.014[c]
a^* Color value (Red-Green)	0.651[e]	-0.868[a]	-3.362[a]	-0.651[c]	0.868[a]
b^* Color value (Yellow - Blue)	97.045[e]	22.212[e]	0.447[a]	4.621[e]	3.130[e]
Viscosity (cp)	4.457[e]	1.082[c]	1.339[e]	-1.294[b]	-1.052[b]
pH value	-3.922[e]	0.784[c]	5.099[e]	-5.491[e]	5.883[e]
Titratable acidity (%w/w)	2.527[d]	0.168[c]	-0.910[a]	-0.101[e]	0.236[e]
Yoghurt starter culture (log cfu/g)	1.897[c]	0.108[c]	-0.44[b]	0.046[e]	-0.139[c]
Probiotic	2.045[c]	2.098[c]	1.023[b]	0.996[e]	2.334[d]
The color of the yoghurt	5.769[e]	1.236	1.099[c]	-1.099[c]	-1.236[c]
The consistency of the yoghurt	-9.200[e]	2.061[c]	-11.606[e]	7.918[e]	-1.193[c]
The viscosity of the yoghurt	4.747[e]	0.421[c]	3.545[e]	-0.661[c]	0.180[b]
The smoothness of the yoghurt	-2.478[d]	2.819[c]	-8.116[e]	0.769[c]	0.598[b]
The milk flavor of the yoghurt	11.767[e]	0.784[e]	-5.099[e]	-0.784[c]	1.961[c]

Table 12 The t-test score for the physical, chemical, microbiological and sensory properties of the probiotic-added goat and cow milk yoghurt based on a Plackett and Burman design (continue)

Dependent variables	Independent variables				
	Skim milk	**Sugar**	**Carageenan**	**Yoghurt Starter**	**Probiotic**
The sour flavor of the yoghurt	-1.252 [a]	-0.364 [e]	-0.121 [a]	1.091 [e]	1.091 [e]
The sour taste of the yoghurt	0.299 [c]	-3.483 [e]	-1.095 [a]	-1.095 [e]	4.279 [e]
The sweet taste	0.282 [b]	5.927 [e]	-1.223 [a]	0.094 [b]	1.035 [b]
Overall acceptances	-3.103 [e]	5.814 [e]	-5.217 [e]	1.640 [b]	1.342 [e]

Note: numbers in the same column with different symbols are significantly different

a = t-test score at the confidence level of 75% ($p \leq 0.25$) is = ±1.301

b = t-test score at the confidence level of 80% ($p \leq 0.20$) is = ±1.476

c = t-test score at the confidence level of 85% ($p \leq 0.15$) is = ±1.699

d = t-test score at the confidence level of 90% ($p \leq 0.10$) is = ±2.015

e = t-test score at the confidence level of 95% ($p \leq 0.05$) is = ±2.571

1.3.4.1 The effect of skimmed milk on the probiotic-added mixture goat and cow milk yoghurt characteristics

From Table 12, it was found that skimmed milk affected the chemical, physical and microbiological properties of the final yoghurt product. With increasing amount of the skimmed milk, the viscosity, b^* color value, the number of microorganisms and total acidity were also increased whereas the pH of the yoghurt decreased. Because milk protein is soluble in water, therefore the viscosity of the yoghurt was affected. Beatriz *et al.* 2006 reported that transglutaminase addition improved the quality of set style and stirred yoghurt and skim milk powder is a better option for increase dry matter. Viscosity of stirred yoghurt with shear rate of 100 (s^{-1}) increased with the addition of skimmed milk.

Skimmed milk promotes the growth of some microbes such as *L. bulgaricus*, *S. thermophilus* and probiotic because skimmed milk is a food for the growth of microbes. This is possibly due to the presence of nutrients in skimmed milk powder (SMP), particularly lactose as a carbon source. This is in agreement with the results of Donkor *et al.* (2005) who found that the viability of the yoghurt starter including *L. bulgaricus* (Lb 11842) and *S. thermophilus* (ST 1342) was significantly enhanced ($p< 0.05$) in soy yogurt supplemented with SMP during fermentation as well as during the 28 d storage.

For the sensory evaluation, the increasing amount of skimmed milk to a high score level caused the consistency, the smoothness and overall acceptances to decrease with a statistical significance ($p\leq0.05$). On the other hand, the viscosity was increased with a statistical significance ($p\leq0.05$). Because skimmed milk has low amount of lipid when skimmed milk was precipitated, there is no fat globules to help spread protein. It causes protein to blend together firmly, so the product tends to be solid clot and has high viscosity but the surface area is unacceptable for testers. Then skimmed milk should be used at low level.

1.3.4.2 The effect of carrageenan on the probiotic-added goat and cow milk yoghurt characteristics

From Table 12, it was found that when the amount of carrageenan increased from low to high level, the brightness (L^* value) was decreased. The product had a darker color, the consistency, the smoothness and the overall acceptance decreased with statistical significances ($p\leq0.05$). On the other hand, the viscosity would be increased if the carrageenan was used in a high level, because carrageenan is a polysaccharide which helps to produce a gel and causes a higher viscosity (Isara, 2004). Therefore, the appropriate level of carrageenan was a low level.

1.3.4.3 The effect of sugar on the probiotic-added goat and cow milk yoghurt characteristics

From Table 12, it was found that sugar affected the physical and microbiological properties of goat and cow milk yoghurt. A high level of sugar raised the yellow (b^* value) color with a statistical significance ($p\leq0.05$). When sugar is heated, the products tend to be more yellow (Isara, 2004). The amount of yoghurt starter culture and probiotic was increased with statistical significances ($p \leq 0.15$) when the sugar was used at a high level. Akın *et al*. (2006) studied about the survival of yogurt and probiotic bacteria in probiotic ice-cream with different sugar concentrations and with or without supplementary inulin during 90 days. Sugar concentrations significantly affected viable bacteria numbers ($p \leq 0.01$). The highest number was in the samples with 18% sugar. At the same time, at high level of sugar the sour taste of the product decreased with a statistical significance ($p \leq 0.05$). In addition, the sweet taste score and overall acceptances increased with statistical significances ($p \leq 0.05$). Sugar was a sweetening agent of the product and was used to be the food for microorganisms for their growth. Normally, the microbe groups such as lactic acid bacteria can change sucrose into glucose and fructose and further change the glucose into lactic acid. The changing of sugar to be lactic acid by using lactic bacteria will decrease the amount of sucrose in the product, and will increase the acid volume in the product. Then the use of sugar in a high level (10%) should affect the product characteristics and the acceptance of goat and cow milk yoghurt with probiotic would be better.

1.3.4.4 The effect of yoghurt starter cultures and probiotic on the probiotic-added goat and cow milk yoghurt characteristics

From Table 12, it was found that the use of *L. bulgaricus* and *S. thermophilus* at a high level (2%) decreased the pH of the product with a statistical significance ($p \leq 0.05$). The pH value decreased because during the fermentation process *L. bulgaricus* and *S. thermophilus* produced acid in the product. Due to mutual stimulation during combined growth of yoghurt bacteria in milk, lactic acid is produced much faster than would be expected on the basis of the acid production by pure culture the individual. The rods are less susceptible to acid and continue to grow. Probiotic can produce less acid in the product than *L. bulgaricus* and *S. thermophilus* (Walstra *et al.*, 1999). The reduced probiotic bacteria count both for lactobacilli and bifidobacteria can be caused by over acidification in fermented products. The over acidification caused by the growth of *L. bulgaricus* is the main factor in the death of bifidobacterial cells in milk products (Walstra *et al.*, 1999).

From the result above, the appropriate formula of yoghurt which would be used in the next experiment was as followed;

1. Ratio of cow milk and goat milk 26.85% : 53.73%
2. Skimmed milk 8.06%
3. Sugar 8.06%
4. Carrageenan 0.08%
5. Yoghurt starter culture 1.61%
6. Probiotic bacteria 1.61%