Effects of Fat Substitute on Survived Probiotic Bacteria in Yoghurt Ice Cream

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Abstract

The objective of this research was to compare amounts of fat substitutes supplied in the industries in order to determine their ability to encapsulate probiotic microbes in the production of yoghurt ice cream to ensure maximum survived probiotic microbes. In this research, two fat substitutes, Purity SM 100 and CRYSTAL texTM 648, were used to substitute skim milk in the standard formulas in order to study numbers of survived probiotic microbes and to test the sensory acceptance. For the sensory acceptance, it was found that the probiotic yoghurt ice cream containing 20% Purity SM100 is acceptable at the maximum significance ($p \leq 0.05$) because its flavor and taste was better than other samples as well as the best overrun with statistical significant differences ($p \leq 0.05$). However, by addition of both fat substitutes at different concentrations, pH values of probiotic yoghurt ice cream products did not have statistical significant differences ($p \geq 0.05$). When Lactobacillus acidophilus, Bifidobacterium, and Streptococcus thermophilus microbial starters were added into the probiotic yoghurt ice cream using both fat substitutes in place of skim milk at ratios of 10%, 15%, and 20%, respectively, under a storage condition of -20oC for 4 weeks, it was found that types and amounts of fat substitutes affected the surviving rate of probiotic microbes in the product with a statistical significance ($p \leq 0.05$). The addition of fat substitutes, Purity SM100 and CRYSTAL texTM 648, at all concentration levels made probiotic microbes survived in the product, which illustrated that both fat substitutes used in above experiments could encapsulate probiotic microbe cells. The survived probiotic microbes were in the range of $10^7 - 10^8$ CFU/ml in comparison to the probiotic yoghurt ice cream with the standard formula without the addition of fat substitutes that has survived probiotic microbes of only 10^6 CFU/ml.

Key words : Fat Substitue, Probiotic Bacteria, Yoghurt Ice Cream

Introduction

Probiotic is the microorganism which exists mostly in the products that processed from the fermented milk. The probiotic in the products contributes in the digestive system of a human, in addition it will help to make the alimentary canal work normally, it help to decrease a chance in infecting with the alimentary canal. But most yogurt ice cream often have a quantity of probiotic lower than 106 CFU/g. Moreover it has saturated fat very much as a kind of lauric acid and myristic acid. The research was to study the processing of products from yogurt ice cream by emphasizing at the quantity of probiotic microorganisms existed in yogurt by taking the fat replacer to use as wrapper on probiotic microorganisms to have most survival quantity, and it was an option for the consumers who took care of health.

Experimental Method

1. The study for a period of time on yogurt preparation for using as the composition in the probiotic yoghurt ice cream

Prepare the yoghurt from ready made microorganism for producing the yogurt (The East Asiatic (Thailand) PLC) by using the quantity of microorganism in the milk in table 1 and the process in Figure 1 Take this yoghurt to measure the pH, the total dissolved solids, and the quantity of lactic acid for every hours until the yoghurt has constant quantity of lactic acid, and applied it as the components of yogurt ice cream formula.

Table 1 Comparison of the ratio of the microorganism and the quantity of milk

Amount of milk to be inoculated	1,250 l / 500 gal	2,500 l / 1,000 gal	5,000 l / 2,000 gal	10,000 l / 4,000 gal
Amount of DVS culture	250 g	500 g	1,000 g	2,000 g

Source : The East Asiatic (Thailand) PLC

2. The study of standard formula of yoghurt ice cream

The study of standard formula of yogurt ice cream by taking both standard formulas in the table 2 to produce the ice cream, and bring them to test the sensory attribute : color , smell , smoothness and overall liking, used 30 examiners, by the method of scoring in 9 levels of preference (9 Point Hedonic scale), and took the results to analyze the variance and the difference of the vary facts.

Table 2 Standard formula of yoghurt ice cream

Ingredient	Quantity of Composition	
	1	2
Sugar	150	110
Whipping Cream	200	200
Whey Powder	30	70
Stabilizer	5	5
Water	300	465
Yoghurt	400	370

Source : 1. Somjit Sukapat, 2541

2. Wannaporn Jitjumrean, 2547

3. Study the type of fat substitute in the Probiotic Yoghurt Ice Cream

Take the yoghurt ice cream formula that can develop to study the type and the type of the fat replacer, for example , Purity SM100 and CRYSTAL tex^{TM}648 by planning the factorial experiment: First factor : they were two types of fat replacer, for example, Purity SM100 and CRYSTAL tex^{TM}648, the second factor was as follows: the quantity of fat replacer in 3 levels, for example , 10, 15 and 20 of the quantity of the whey of the powdered milk, and take the produced ice cream to measure the quality as follows:

3.1 The physical quality analysis

3.1.1 Leavening rate according to the method (Marshall and Arbuckle,1996)

3.1.2 Dissolved rate according to the method (Geilman and Schmidt, 1992)

3.2 The chemical quality analysis

3.2.1 Measure the pH with the pH meter

3.2.2 Analyze the quantity of fat according to the method of AOAC 2000

3.3 Analysis of the microbial quality

3.3.1 Quantitative analysis of S.thermophilus (Dave and Shas, 1996)

3.3.2 Quantitative analysis of L.acidophilus (Dave and Shas, 1996)

3.3.3 Quantitative analysis of B.bifidum (Dave and Shas, 1996)

3.3.4 Sensory quality analysis

4. Quantitative study of lactic acid bacteria during keeping the probiotic yoghurt ice cream

Take the yogurt ice cream to put in the plastic cup, close the lid and kept at -20°C and took a random to test the quantity of lactic acid bacteria in ice cream products every weeks for one month in order to check the amount of microorganisms remaining in the products.

Results of data analysis and discussion

1. A study for a period of time on yoghurt preparation for using as the composition in ice cream products

Probiotic Yoghurt

From producing the yoghurt and adding *S.thermophilus, L.acidophilus, B.bifidum* in the compounds of yoghurt , then the yoghurt was ripen at 40°C, which was the appropriate temperature for the progress of the microbe that used as the yogurt microbe, and measured the pH value , the total dissolved solids, and the percentage of lactic acid of yoghurt for every one hour until the percentage of lactic acid in the yoghurt was equal to 0.90 - 0.97. It was found that, in the first three hours the pH value inclined to be stable and the pH value started to decrease from 6.60 to 3.23 but it has started to decrease quickly since the fourth hour. Because at the first period the microorganism in yoghurt that has just grew up was *S. thermophilus* only which this microorganism could digest the protein and nitrogen from a source of nitrogen that was not the protein.

Besides, *S. thermophilus* was able to build lactic acid, acetic acid and formic as well. It made pH value , in the products decreased (Shihata and Shah, 2000) In the part of *L.acidophilus* and *B. bifidum* were microorganisms that had to use amino acid and peptides which *S. thermophilus* built as food in the growth so it made both kind of these microorganisms built lactic acid with *S. thermophilus*. It made the pH value started to decrease quickly since the fifth hour. When the pH value decreased lower than 5, it made *B. bifidum* which did not resist to acid and died slowly. It made the pH value not decreased quickly, the percentage of lactic when it ended the period of fermentation time at 0.97. The percentage of lactic acid increased quickly since the third hour to the seventh hour. Because the microorganism began to digest the lactose sugar in milk to lactic acid which caused the total dissolved solids in yogurt started to decrease until 6 ºBrix. When it analyzed the protein and fat quantity in the products, it was found that the example of yoghurt had 3.04 protein quantities and 3.20 fat quantities.

2. The study of standard formula of yoghurt ice cream

Choose two amounts of yoghurt ice cream formula for applying to choose as the standard formula in making the probiotic yogurt ice cream. From the sensory test with the consumers, it was found that the consumers accepted the first ice cream formula. From statistical analysis, it was found that the quality of color, taste, flavour, and texture , there was not statistical difference ($p \leq 0.05$) but there was obvious difference at the total preference of the consumer. Therefore, the researcher chose to use the first yoghurt ice cream formula as standard formula in probiotic yoghurt ice cream development. For the first formula, it had more quantity of yoghurt for using as the compositions than the second formula, therefore it made more special flavor of yoghurt, which it caused the first formula received the total preference scores more than the second formula.

Table 3 The average preference scores of choosing the ice cream standard formula

Samples	Sensory Characteristics				
	Taste	Color	Flavour	Texture	Total Preference
First formula	8.03±0.78	8.13±0.68	8.03±0.80	8.03±0.71	8.25±0.63
Second Formula	8.08±0.72	8.20 ±0.66	7.95±0.67	8.05±0.72	7.93±0.55

3. The study of the type of the fat replacer in the probiotic yoghurt ice cream

Prepared seven samples of yoghurt ice cream as follows: ICE01, ICE02, ICE03, ICE04, ICE05, ICE06 , and the control sample, and took them to analyze the physical quality, the chemical quality, the microbiological quality and the sensory quality. The results of the experiment were as follows:

Table 4 The results of statistical analysis in the physical aspect of the probiotic yoghurt ice cream

Type of Fat Replacer	Factor	
	Dissolving Rates	Percentage of Leavening
ICE01	21.50^{a}±0.707	95.37^{bc}±0.077
ICE02	15.50^{bc}±0.707	95.85^{a}±0.070
ICE03	13.00^{c}±1.414	96.04^{a}±0.014
ICE04	18.50^{ab}±0.707	94.52^{d}±0.050

Table 4 The results of statistical analysis in the physical aspect of the probiotic yoghurt ice cream (to be continued)

Type of Fat Replacer	Factor	
	Dissolving Rates	Percentage of Leavening
ICE05	16.00^{ab}±0.000	95.05^{c}±0.028
ICE06	15.00^{bc}±1.414	95.50^{b}±0.028
Control	20.50^{a}±0.707	94.43^{c}±0.156

Note : a - b , the different alphabets in the horizon means the average which there are statistical differences ($p \leq 0.05$) , * Values within column followed by a different letter are significantly.

From the study of the type of the fat replacer in the probiotic yoghurt ice cream, it made ice cream had dissolving rates, and percentage of leavening. There was statistical difference ($p \leq 0.05$) as shown in Table 4. In the aspect of dissolving of the probiotic yoghurt ice cream, which added the Purity SM100 at 10% , there was statistical difference ($p<0.05$) with ICE04. It had shown that adding the Purity SM100 and CRYSTAL tex^{TM} 648 at 10% , it did not help in dissolving of the probiotic yoghurt ice cream, but adding both types of the fat replacer at 15% and 20 % in the yogurt, it would help the samples of probiotic yoghurt ice cream dissolved slowly in the room temperature , including having high percentage of leavening. This experimental results corresponded with the experiment of Pelan(1997) said that dissolving rate would have relations with the percentage of leavening. That was when the percentage of dissolution decreased, the percentage of leavening would rise as shown in Table 4, it has shown that the samples of probiotic yoghurt ice cream that had percentage of dissolution approximately , it would have percentage of leavening approximately too.

From the comparative ratio of the Purity SM100 and CRYSTAL tex^{TM} 648 when it compared with the Control samples, it showed that both kinds of substances made the dissolving rate in yoghurt ice cream better. When it compared with each one, it has shown that CRYSTAL texTM 648 was able to help the ice cream dissolved slowly at room temperature. When 10% CRYSTAL tex^{TM}648 were used, it would make the dissolving rate better equal to using the Purity SM100 at 15% and 20%.

From the analysis of percentage of leavening, it showed that percentage of leavening in the samples in the section of 94-96% from Table 4 when it compared both kinds of fat replacer adding in the ice cream. It would affect the percentage of leavening in the products of both kinds of substance, there was statistical difference ($p \leq 0.05$). It was considered that both substances had properties on

stability alms, and being emulsifier alike, but the Purity SM100 caused the ice cream which was added this fat replacer had higher percentage of leavening. Because this kind of this substance entered, it would make aeration cell in yogurt ice cream had small-sized, it made the air kept in the yoghurt ice cream better than adding CRYSTAL tex™ 648. Although it was added in the same ratio, the results of Goff (2003) said that the substance that had property as emulsifier, and the substance which provided the stability would help the fat grain catch with the air and it could leavened better. If we want to use CRYSTAL tex™ 648 in the products , as a result, we had to add this substance in the increasing quantity for one time on order to get the characteristics of good dissolution and leavening the same as adding Purity SM100.

The type and the quantity of both fat replacers, for example, Purity SM100 and CRYSTAL tex™ 648 , in the quantity of 10, 15 and 20%, indicated that the probiotic yoghurt ice cream had significant difference in pH values ($p \leq 0.05$). The pH values were at 4.6-4.8 which were lesser than the ice cream formula of the control sample which had highest pH value but it was still at pH value that the probiotic microorganism could grow up.

Table 5 The analysis results in sensory testing of the probiotic yoghurt ice cream samples

Samples	Sensory Characteristics				
	Taste	Color	Flavour	Texture	Total Preference
ICE01	7.78^{b}	8.05^{a}	6.83^{cd}	6.38^{b}	6.93^{c}
ICE02	7.73^{b}	8.07^{a}	7.23^{bc}	6.93^{a}	7.52^{b}
ICE03	8.03^{a}	8.02^{a}	8.03^{a}	6.93^{a}	8.03^{a}
ICE04	7.95^{a}	8.13^{a}	6.40^{d}	6.38^{b}	6.93^{c}
ICE05	7.95^{a}	8.13^{a}	7.12^{c}	6.93^{a}	7.50^{b}
ICE06	8.03^{a}	7.98^{a}	7.70^{ab}	6.93^{a}	7.70^{ab}

Note : a - b , the different alphabets in the horizon means the average which there are statistical differences ($p \leq 0.05$) , * Values within column followed by a different letter are significantly.

From the study of yoghurt ice cream by adding two types of fat replacers, they were Purity SM100 and CRYSTAL tex™ 648 at 10, 15 and 20 percentage of a quantity, the results of this study in Table 5 were as follows: the aspect of color factor of the probiotic yoghurt ice cream showed that there were no significant differences ($p \geq 0.05$), in the part of taste, flavour, texture and total preference of yoghurt ice cream that used both types of fat replacers had significant difference ($p \leq 0.05$). It was found that yoghurt ice cream which have added CRYSTAL tex™ 648 gave better flavour. In the part of

taste, it was shown that in every samples had significant difference ($p \leq 0.05$) in the sample ICE03 added 20% Purity SM100, it was most accepted. In the aspect of texture, the examiner accepted the sample added the substances at 15-20% from the table 5 : the samples ICE02, ICE03, ICE05 , and ICE06 had significant difference ($p \leq 0.05$). With the samples ICE01 and ICE04 added the fat replacer at 10%, in the part of total preference the probiotic yoghurt ice cream that was accepted most from the examiner was ICE03 which was added 20% Purity SM100 in the ice cream. Because this example gave the feeling of yogurt taste, including the feeling of smooth on the tongue more than other examples so there was significant difference ($p \leq 0.05$ with other examples. It summarized that the example of probiotic yoghurt ice cream that was accepted in the aspect of sensory from the consumers most was ICE03. From the analysis of fat and protein quantity in the example ICE03, it was found that protein quantity in the products was equal to 13.97 and the fat quantity was equal to 10.13.

4. The study of lactic acid bacteria quantity between keeping the probiotic yogurt ice cream

The study results on the quantity of probiotic microorganism used as the yoghurt microbe in the yoghurt products. When the ice cream has been spun with the ice-cream maker for 20 minutes and the **lactic acid bacteria quantity was analyzed**: *S.thermophilus, L.acidophilus and B.bifidum* were kept at -20°C for 4 weeks in order to check the amount of probiotic microorganisms remaining in the products. When it was compared with the probiotic microorganism, it would analyze the quantity of probiotic microorganism after the ice cream has already spun (Table 6)

From the table, it was indicated that every ice cream samples had initial microorganisms during 10^9 - 10^{10} CFU/ml excepted the ice cream formula of the Control sample. At the beginning it had microorganism quantity , *B.bifidum*, in lesser quantity than other examples: there were microorganism quantity during 10^8 CFU/ml. But when it was analyzed statistics, it was shown that the microorganism quantity in every samples had no significant difference ($p \geq 0.05$). In the part of checking the microorganism, *E.Coli*, which was used as the indicator of cleanness of the processing procedure, the analysis results did not find *E.Coli* microorganism.

Table 6 The quantity of probiotic microorganism in the products in the fourth week

Samples	Quantity of Microorganism			
	Lactic Acid Bacteria	S.*thermophilus*	*L.acidophilus*	*B.bifidum*
Control 1	80×10^{5c}	1×10^{6c}	39×10^{6b}	45×10^{6}
ICE01	40×10^{7c}	15×10^{8a}	49×10^{7b}	50×10^{6}
ICE02	90×10^{7c}	17×10^{8a}	70×10^{7b}	90×10^{7}
ICE03	49×10^{8a}	14×10^{8ab}	90×10^{8a}	65×10^{7}
ICE04	40×10^{7c}	65×10^{7ab}	47×10^{7b}	55×10^{6}
ICE05	30×10^{8b}	75×10^{7ab}	88×10^{8a}	55×10^{7}
ICE06	55×10^{8a}	10×10^{8bc}	88×10^{8a}	40×10^{7}

Note : a - b , the different alphabets in the horizon means the average which there are statistical differences ($p \leq 0.05$) , * Values within column followed by a different letter are significantly

Changing the quantity of yoghurt microbe, and probiotic microorganism, Lactic Acid Bacteria, *S.thermophilus, L.acidophilus, and B.bifidum* when they have been kept at -20°C for four weeks. It was found that the microorganism quantity inclined to decrease during keeping. Making a comparison between the table 7, 8, 9 and 10 which were microorganism quantities in the first, second, third and fourth weeks, respectively with the table 6, which the microorganism quantities were analyzed after the procedure of spinning ice cream.

S.thermophilus in the examples tended to decline since the zero week which *S.thermophilus* would begin to decrease the quantity quickly down in the third to fourth week by decreasing from 10^{10} CFU/ml to 10^{7}- 10^{8} CFU/ml of all samples which added the fat replacer, Purity SM 100 and CRYSTAL tex™ 648 , in the last week, the samples that added Purity SM 100 at 15 percentage of a quantity.

It contributed to cover most microorganism cell, *S.thermophilus*, following with the sample that added Purity SM100 at 20 and 10 percentage of a quantity, respectively. In the part of fat relacer, CRYSTAL tex™ 648, the quantity that helped to cover most cell of *S.thermophilus*, that was fat replacer at 20 percentage of a quantity. But when the amount of cell microorganism, *S.thermophilus*, as taken to analyze statistics, it was found that the remaining microorganism quantity of every examples had no significant difference ($p \geq 0.05$).

L.acidophilus tended to decline between keeping it, but during the first and second weeks the amounts of *L.acidophilus* increased a little, and decreased continuously. At the end of the last week,

it was found that ICE03, which added the fat replacer, Purity SM 100 at 20 percentage of a quantity, was the example that left most amount of *L.acidophilus*. In the part of ICE05 and ICE 06, which added CRYSTAL tex^{TM}648 at 20 and 15 percentage of a quantity, respectively, having the amount of *L.acidophilus* at 10^8 CFU/ml. ICE 04 which added CRYSTAL tex^{TM}648 at 10 percentage of a quantity remaining microorganism quantity at least. When it was analyzed statistics, it was found that ICE03, ICE05 and ICE06 had significant difference ($p \geq 0.05$) with the samples ICE01, ICE02, ICE04 , and the Control.

B.bifidum tended to decrease continuously, but it decreased quickly during the third and fourth weeks, ICE 02 had quantity of *B.bifidum* remaining most. Following with ICE 03 from table 10, It was indicated that adding the fat replacer, Purity SM 100, and CRYSTAL tex^{TM}648 at 10 percentage of a quantity which had an effect on *B.bifidum*, remaining at 10^6 CFU/ml. In the part of adding Purity SM 100 and CRYSTAL tex^{TM}648 at 15 and 20 percentage of a quantity which had an effect on *B.bifidum*, remaining at 10^7 CFU/ml.

Decreasing the amount quickly, it caused from the microorganism cell was pierced by the ice crystal as it was kept for a long time, and the water of ice cream which became ice. So it could not take the nutrient to the microorganism cell (Dave and Shah, 1997). But *S.thermophilus, L.acidophilus* and *B.bifidum* had cell wall as gram positive, which was the characteristics of the microorganism that resisted freezing. Therefore, keeping the probiotic yogurt ice cream at -20°C, it made the quantity reduction of probiotic microorganism occurred very slowly (Adams and Moss, 2000)

From the above experiment, it was indicated that both Purity SM 100 and CRYSTAL tex^{TM}648 used as the fat replacer in the yoghurt ice cream products. They had properties in covering the probiotic microorganism in ice cream products when it compared with the yoghurt ice cream formula in the control sample which did not add any fat replacer in the ice cream. The control formula would have remaining microorganism remains during 10^5- 10^6 CFU/ml. In the part of other ice cream formula that added the fat replacer in the ice cream, the microorganism quantity would be left during 10^7- 10^8 CFU/ml. If making the comparison on the quantity microorganism remaining in the sample that added Purity SM 100 and CRYSTAL tex^{TM}648, it was found that the remaining microorganism was not different. But using Purity SM 100 would be accepted by more consumers , they would find that it was soft and helped to pull the taste of yoghurt ice cream better than the ice cream which added CRYSTAL tex^{TM}648.

Discussion and Conclusion

1. The period of time on fermenting yogurt which was appropriate was 7 hours, by ripening at 40°C. It would make yoghurt had a quantity of lactic acid bacteria at 0.90-0.97 percentage by using the yoghurt microbe and mixed with *Lactobacilluss acidophilus, Bifidobacterium* and *Streptococcus thermophilus.*

2. The fat replacer which was appropriate for using with the formula of making yoghurt ice cream. It was Purity SM 100, by using it in stead of the whey at 20 percentage of a quantity. Purity SM 100 would help to cover the cell of probiotic microorganism in the yoghurt ice cream products in order to have the amount of surviving microorganism more than using CRYSTAL tex^{TM}648. That was making the microorganism quantity, remaining during 10^7- 10^8 CFU/ml and more than the production in the normal formula that did not add any fat replacer in the ice cream, by remaining the probiotic microorganism only 10^7- 10^8 CFU/ml.

3. The formula of probiotic yoghurt ice cream that was accepted in the sensory aspect from most consumers was ICE03, which added Purity SM 100 in the ice cream at 20 percentage of a quantity. As a result of the flavour, taste, texture and total preference which were better, including the ice cream was soft better than using CRYSTAL tex^{TM}648. But CRYSTAL tex^{TM} 648 is one of the good kind of fat replacer because it has a property in replacing the fat and it has the property in helping to cover the microorganism cell in order to have the amount of microorganism remaining survived as using Purity SM100. Although they were left in the a little quantity but they could make the consumers to receive the benefit in the aspect of probiotics better than consuming the yoghurt ice cream that did not add Purity SM 100 and CRYSTAL tex^{TM}648.

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