Original Article

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Probiotic Frozen Yogurt

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Abstract

Recent trends show that consumers have been searching for functional foods to improve bodily functions and overall health. The purpose was to develop new formulations of frozen yogurt, a food product widely accepted among health conscious consumers, by adding probiotics (*Lactobacillus* spp.). The developed frozen yogurt formulae were as follows: Formula 1 with no probiotics, Formula 2 containing 3%w/w of probiotics, and Formula 3 containing 4%w/w of probiotics. The survival rate of probiotics in Formula 2 and Formula 3 at Day 30 was 33.89% and 26.67%, respectively. Sensory evaluation was carried out among 60 panelists, using the 9-point hedonic scale method for food acceptance. According to Analysis of Variance, the mean scores of these three formulae were not significantly different ($p > 0.05$). Formula 1, 2, and 3 obtained the mean score of 7.25 ("like moderately" to "like very much"), 6.97 ("like slightly" to "like moderately"), and 7.27 ("like moderately" to "like very much"), respectively.

Keyword: Frozen yogurt, Probiotics, Survival rate

INTRODUCTION

Frozen yogurt is a frozen dessert that combines the flavors and textures of ice-cream and sherbet. The unique flavor of frozen yogurt was attributed from the compounds produced by the strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. In recent year, there has been considerable interest in probiotics and its significant roles in the intestinal tract of human.

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host[1]. Probiotic food is defined as a food product that contains viable probiotic microorganisms in sufficient populations incorporated in a suitable matrix[2]. The populations of 10^6-10^7 cfu/g in the final products are established as therapeutic quantities of probiotic cultures in processed foods[3]. According to Thai FDA, the present legislation states that the minimum viable quantity of probiotic culture should be "not less than 10^6 colony/g of probiotic foods during the period of storage and use"[4]. Several health benefits are attributed to the ingestion of foods containing probiotic cultures. Probiotics were thought to beneficially affect the host by improving its intestinal microbial balance, thus inhibiting pathogens and toxin- producing bacteria[5]. Some of the health benefits related to probiotics are: anti-microbial activity, prevention and treatment of diarrhea, relief of symptoms caused by lactose intolerance, anti-mutagenic and anti-carcinogenic activities and stimulation of the immune system[6]. However, there is no single probiotic strain capable of providing all the benefits mentioned above[6]. The commonly used probiotic strains in different dairy products are: *Lactobacillus acidophilus, L. casei Shirota, L. casei immunitas, L. crispatus, L. gasserei, L. johnsonii, L. plantarum, L. reuterii, B. lactis, B. longum, Enterococcus faecium, and E.faecalis*[7]. Animal studies showed that *L. paracasei* was a potent strain

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with immune modulation properties and had a significant preventive effect on colitis[8].

Frozen dairy products is an ideal vehicle for the delivery of probiotic organisms in the human diet. The addition of viable probiotic cultures to frozen yogurt provides the product with additional functionality and value. Studies showed that *L. paracasei subsp. paracasei* did not interfere and even improved the sensory preference of the mousse[9,10,11].

The objective of this study was to isolate probiotic bacteria from a commercial fermented dairy product and develop acceptable probiotic frozen yogurts. Acceptability and viability of the probiotic frozen yogurts were evaluated.

MATERIALS AND METHODS

Isolation and Cultivation of probiotics

Probiotic drinking yogurt (Meiji®) was purchased from a retail supermarket and used as probiotic source of bacteria. The probiotic microorganism claimed to be present in the product was *Lactobacillus paracasei.*

The drinking yogurt was diluted with MRS broth and the 10^{-4}, 10^{-5}, and 10^{-6}dilutions were spread onto MRS agar. The plates were then incubated at 37° C, under an anaerobic condition for two days. The morphology was identified using Gram stain method. Single colony of the probiotic bacterium was isolated and inoculated into a tube containing 5 ml of 10% skim milk (Difco,USA), then incubated at 37° C under an anaerobic condition for 48 hours. The number of probiotic bacterium was increased by transferring the cultured skim milk into a flask containing 200 ml 10% skim milk , then incubated at 37° C under an anaerobic condition for 48 hours. The latter cultured skim milk was then centrifuged at 4,000 rpm for 10 minutes. The supernatant was discarded and the bacterial cells was stored at 4°C.

Preparation of yogurt

Plain yogurt (Duchie®) was purchased from a retail supermarket and was used as the yogurt starter culture. Experimental plain yogurt was prepared by heating pasteurized whole milk (Meiji®) at 90-95°C for 5 minutes and subsequently cooled to 43°C. It was then inoculated with 2%w/w starter culture yogurt. The inoculated mix was incubated at 43°C until the acidity of 0.85-0.9 was obtained.

Probiotic frozen yogurt preparation

The frozen yogurt mix was prepared by combining experimental plain yogurt, 66.26 %w/w, whipping cream (Foremost®, 35.5% fat), 11.6 %w/w, sucrose, 12 %w/w, guar gum, 0.3 %w/w and water, 9.84 %w/w.

Weighed guar gum was sprinkled onto hot water (60°C). Other ingredients were weighed and mixed in a container. Hydrated guar gum suspension was poured into the mix and pasteurized at 85°C for 25 seconds. The mix was divided into three separated containers. The prepared probiotic microorganisms were weighed and incorporated into the mixes. All three yogurt mixes were each transferred into the ice-cream maker. The finished products, the frozen yogurt and two formulae of probiotic frozen yogurts, were stored at -18°C for further tests.

Titratable acidity

Acidity determinations were performed using 9 g of sample, diluted with 9 ml of water, and titrated against 0.1 N NaOH, using 0.5 ml phenolphathalein as the indicator. The titratable acidity was calculated as percent lactic acid as follows: % of acidity = ml of 0.1 N NaOH used in titration x 0.009x100/ grams of sample used

Sensory evaluation

The frozen yogurt and two probiotics frozen yogurts formulae were evaluated among sixty panelists, using 9-point Hedonic Scale method. Two columns of attribute evaluation, namely Liking ("dislike extremely" (1) to "like extremely" (9)) and Intensity (Lowest intensity (1) to Highest intensity (9)) were included in the sensory evaluation questionnaires. The attributes evaluated in the test

were aroma, texture, sourness, sweetness, creaminess, color, and melting, and the overall acceptability. The mean scores of Liking and Intensity for each attribute and the overall acceptability were calculated. All data were statistically analyzed by using Analysis of Variance. The means of the treatments that were significant at the 0.05 level of probability were separated by Duncan's Multiple Range Test utilizing Sirichai program. (Dr.Sirichai Unsrisong, Maejo University)

Survival rate

One g of ice-cream samples were prepared in serial dilutions (1:10), using a phosphate buffer. Each dilutions (0.1 ml) were pipetted onto MRS agar and spreaded, then incubated at 37°C under an anaerobic condition for 48 hours. The colony forming units of the probiotic bacteria were determined on Day 1, 2, 3, 4, 5, 6, 7, 10, 14, 21 and 30 of the storage time.

RESULTS AND DISCUSSIONS

Probiotic isolation and cultivation

It was found that Meiji® drinking yogurt contained $6x10^9$ cfu/g of the probiotics. The prepared probiotic coagulum was smooth in texture, with light brown in color and characteristic aroma. The morphology of the organisms and the prepared probiotics were shown in Figure 1 and 2, respectively.

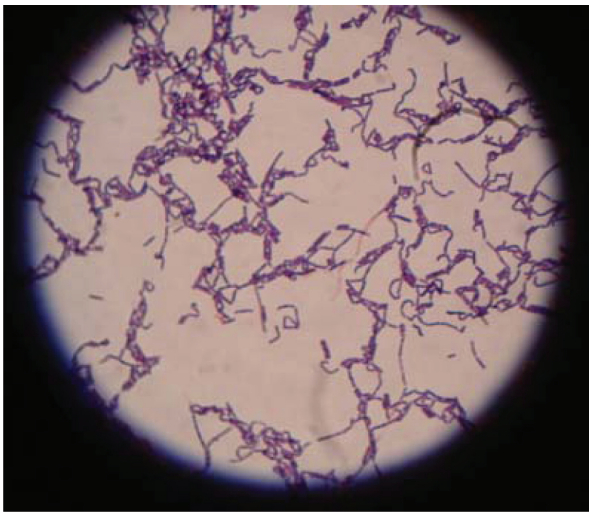

Figure 1. Morphology of *Lactobacillus paracasei*

Figure 2. Prepared probiotic

The probiotics was identified as *L.paracasei*. Meiji® drinking yogurt was selected as the probiotic source for the isolation of the starting probiotic bacteria since the yogurt contained a single strain of probiotic bacteria, thus it was more practical for isolation. Furthermore, the isolation from drinking yogurt was less complicated than from other food sources of probiotic bacteria. The multiplication and the culturing method of *L. paracasei* strain was with good results and less complicated as well.

Preparation of frozen yogurts

Dutchie® yogurt was selected as the source for yogurt starter culture. Two yogurt bacteria used in the commercial yogurt product were *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. In the process of preparing the Experimental plain yogurt, the 2 strains of yogurt bacteria were anticipated to contribute to the desirable attributes of yogurt such as smooth texture, optimal viscosity, optimal flavor, and optimal aroma. The prepared yogurt mixes were incubated for 4, 5, 6, 7, and 8 h and prepared into frozen yogurt form in order to compare the flavour and other attributes. According to Table 1, all 5 frozen yogurts showed similar melting, sweetness, and texture. The frozen yogurts, with 4 and 5 hour incubation periods, showed low level of sourness whereas the frozen yogurts with 7 and 8 h incubation periods showed high level of sourness. On the other hand, the frozen yogurt prepared from the yogurt which had been incubated for 6 h showed smooth texture , quick melting and optimal sourness; only the sweetness needed to be increased. Consequently, 3 concentrations of sucrose, 10%w/w, 12%w/w, and 14%w/w, were added to the yogurt formulae. It was found that the 12% sucrose provided optimal attributes for the yogurt formulations as shown in Table 2.

Table 1. Attributes of frozen yogurts with different incubation times

Formula	Incubation time (h.)	% Acidity	Attributes of frozen yogurts			
			Sourness	Sweetness	Meltability	Texture
1	4	0.85	low	mild	quick	smooth and sticky
2	5	0.88	low	mild	quick	smooth and sticky
3	6	0.92	optimal	mild	quick	smooth and sticky
4	7	0.97	high	mild	quick	smooth and sticky
5	8	1.01	high	mild	quick	smooth and sticky

Table 2. Attributes of three frozen yogurts with different sucrose contents

Sucrose (%w/w)	Attributes of Frozen Yogurts		
	Sweetness	Meltability	Texture
10	low	optimal	smooth and sticky
12	optimal	optimal	smooth and sticky
14	high	optimal	smooth and sticky

Table 3. Attributes of the frozen yogurts

Formula	Attributes of Frozen Yogurts		
	Texture	Colour	Creaminess
1 (no probiotic)	smooth	white	optimal
2 (3% probiotic)	smooth	light brown	optimal
3 (4% probiotic)	very smooth	brown	optimal

Table 4. Sensory evaluation of probiotic frozen yogurts

Attributes	Mean scores of sensory evaluation			Mean scores of intensity		
	491	652	873	491	652	873
Aroma	6.77A	6.40A	6.77A	5.68a	5.18a	5.18a
Color	7.78A	7.05B	6.47C	4.25c	4.93b	5.47a
Texture	6.37B	6.30B	7.02A	6.08b	5.83b	6.63a
Sourness	7.02A	6.48A	6.87A	5.27a	5.22a	5.42a
Sweetness	6.83A	6.60A	6.77A	5.28a	5.63a	5.57a
Creaminess	6.77A	6.20B	5.58C	4.98c	5.53b	6.13a
Meltability	6.87A	6.60A	6.95A	4.97a	5.12a	5.17a
Overall acceptability	7.25A	6.97A	7.27A			

a,b,c and A,B,C. Means not sharing letter in common differ significantly ($P<0.05$) as determined by Duncan's Multiple Range Test.
Scale of values was 1 (dislike extremely) to 9 (like extremely).
491 = Formula 1 (no probitic), 652 = Formula 2 (3%probiotic), 873 = Formula 3 (4% probiotic)

From Table 3, all 3 formulae of frozen yogurts showed smooth texture and good creaminess but the color of Formula 1 (no probiotic) was lighter than the Formula 2 (3% probiotic) and Formula 3 (4% probiotic). Formula 1 was used as control.

Sensory evaluation of probiotic frozen yogurts

Sensory evaluation of the 3 formulae of frozen yogurts were carried out among 60 panelists, using 9-point Hedonic Scale Method. The mean scores of Liking and Intensity of the 3 frozen yogurts were as shown in Table 4.

According to Analysis of Variance for the acceptability of each attribute, there was no difference in the mean scores of Liking for aroma, sourness, melting, and the overall acceptability among the 3 formulae ($P>0.05$). The attributes which showed significant difference were color, creaminess, and texture as follows ($P<0.05$):

Color. The panelists preferred the formula with no probiotics the most; Formula 1 obtained the mean score of Liking of 7.78 ("like moderately" to "like very much").

The mean score was significantly higher than those of Formulae 2 (3% probiotics) and 3 (4% probiotics) which were 7.05 ("like moderately" to "like very much") and 6.47 ("like slightly" to "like moderately"), respectively P<0.05). The mean score of Formula 2 was significantly higher than Formula 3 (P<0.05). The content of probiotics bacteria which were added into the latter two formulae might contribute to the undesirable light brown color of the finished products which was darker in color than the formula with no probiotics. The color intensity mean score of the 3 formulae were significantly different (P<0.05). Formula 3 (4% probiotics) showed the darkest color, then Formula 2 (3% probiotics) and Formula 1 (no probiotics), the lightest. Thus, in order to obtain higher acceptance, lighter colored probiotics coagulum should be developed in the future, so lighter colored frozen probiotics yogurt could be subsequently produced. On the other hand, other ingredients/ colors should be used to mask the darker colors of the frozen yogurts.

Creaminess. The panelists preferred the formula with no probiotics the most. Formula 1 obtained the mean score of Liking of 6.77 ("like slightly" to "like moderately") which was significantly different from Formula 2 and 3 which obtained the mean scores of 6.20 ("like slightly" to "like moderately") and 5.58 ("neither like nor dislike" to "like slightly"), respectively (P<0.05). Considering the mean scores of Intensity, it was found that there was significant difference between each formula and the formula with the highest mean score of Intensity (Formula 3, with the mean score of 6.13) obtained the lowest mean score of Likings (5.58)(P<0.05). The creaminess intensity mean score of the 3 formulae were significantly different. Formula 3 (4% probiotics) showed the highest creaminess, then Formula 2 (3% probiotics) and Formula 1 (no probiotics), the lowest. The ingredients in the media used for the multiplication of the bacteria might contribute to the high creaminess.

Texture. Formula 3 (4% probiotics) obtained the highest mean score of Liking of 7.02 (" like moderately" to "like very much") which was significantly higher than Formula 1 (no probiotics) and Formula 2 (3% probiotics) which obtained the mean scores of Liking of 6.37 and 6.30 ("like slightly" to "like moderately"), respectively. Formula 3 obtained the highest mean score of Intensity (6.63) and the mean score was significantly higher than the other two formulae(P<0.05). Ingredients used on the media in the multiplication process might contribute to the high desirable smoothness of Formula 3.

The overall acceptability mean score. According to Analysis of Variance, Formula 1 (no probiotic) and Formula 3 (4% probiotic) obtained the mean scores of 7.25 and 7.27 ("like moderately" to "like very much"), respectively, and Formula 2 (3% probiotic) obtained the mean score of 6.97 ("like slightly" to "like moderately"). However, the mean scores of the 3 formulae were not significantly different (P> 0.05). In conclusion, the addition of probiotics did not interfere with the overall acceptance of the frozen yogurt products. Various flavoring agents should be included in future studies.

Survival rate

The initial count of probiotic in Formula 2 (3%probiotic) was $126x10^8$ cfu/ml and that of Formula 3 (4% probiotic) was $129x10^8$ cfu/ml. The counts of probiotic in the two formulae decreased gradually after storage (Table 5). At 30 days, the counts of probiotic in Formula 2 (3% probiotic) and Formula (4% probiotic) were $61x10^8$ and $64x10^8$ cfu/ml respectively. However the bacterial contents of the two formulae were still higher than $1.0x10^6$ cfu/ml, the number regulated by Thai FDA for the probiotic products .

The survival rate of the two probiotic frozen yogurts at day 30 were 33.89% and 26.67%, respectively (Figure 3).

Table 5. Probiotic populations in the probiotic frozen yogurts at Day 1, 2, 3, 4, 5, 6, 7, 10, 14, 21 and 30

Day	Formula 2 (3% probiotic)	Formula 3 (4% probiotic)
1	126 x 10^8	129 x 10^8
2	122 x 10^8	127 x 10^8
3	120 x 10^8	123 x 10^8
4	122 x 10^8	122 x 10^8
5	118 x 10^8	120 x 10^8
6	116 x 10^8	119 x 10^8
7	112 x 10^8	115 x 10^8
10	104 x 10^8	114 x 10^8
14	100 x 10^8	103 x 10^8
21	87 x 10^8	92 x 10^8
30	61 x 10^8	64 x 10^8

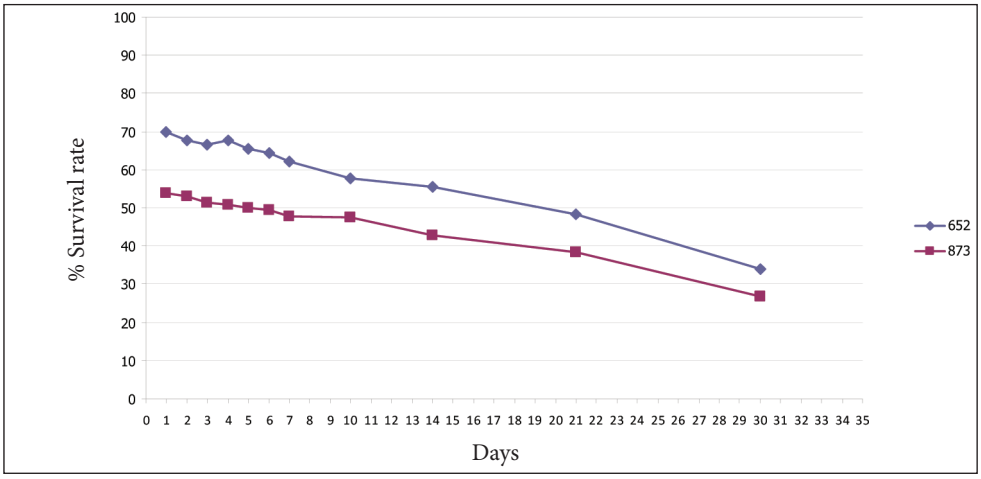

Figure 3. Survival rate of the probiotic cultures of the probiotic frozen yogurts

CONCLUSION

Among the functional foods, the dairy products, especially frozen yogurt is a good vehicle to transfer probiotics to the human intestinal tract. Consumption of probiotic bacteria via dairy food product is an ideal way to re-establish the intestinal micro-flora balance. Our study showed the potential in the development of probiotic frozen yogurt products which provide the advantage of viable probiotics in the products.

More studies are needed to further investigate the probiotic survival in an acidic and alkaline conditions of human intestinal tract as well as therapeutic effects of live probiotic cells on human health.

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