SUCROSE AS A CRYOPROTECTIVE AGENT IN FREEZE-DRIED YOGURT FOR STARTER

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ABSTRACT

The objectives of this study were 1) to observe the growth of lactic acid bacteria on freeze-dried yogurt for mixed-starter with the addition of sucrose as cryoprotective, 2) to determine the optimum sucrose concentration to be used as a cryoprotective agent and 3) to determine the yogurt quality produced using freeze-dried mixed-starter. There were two stages of the trial. On the first stage, freeze-dried mixed-starter was made with the addition of sucrose (5, 8 or 11%) before and after the fermentation. On the second stage, the freeze-dried mixed-starter produced on the first stage was used for making yogurt weekly. Data were analyzed using 2x3x4 factorial design. Time of sucrose and sucrose concentration did not affect the number of lactic acid bacteria, either on liquid or freeze-dried mixed-starter.

Key words: Dry yogurt, Sucrose, Cryoprotective

INTRODUCTION

Yogurt is a fermented milk product which needs an appropriate handling, in which the starter or its products must be preserved under low temperature and this is the problem faced by households who want home-made yogurt. Production of freeze-dried yogurt without sucrose as a cryoprotective showed the decrease of bacteria count from 2,254.55 million down to 4.31 million of *Lactobacillus bulgaricus* and from 1,093.76 million down to 1.99 million of *Streptococcus thermophillus* during the first two week (Rihastuti, 1994). Therefore, it is needed to produce freeze-dried yogurt for mixed-starter with addition of sucrose as a cryoprotective agent.

The use of additives has been studied extensively to protect bacterial cells from freezing damage. Adonitol and glycerol have been used as cryoprotective agents in storage of lactobacilli. Sucrose has also been claimed to be a good cryoprotector (Allaeddinoglu *et al.*, 1989). The cryoprotective effect of glycerol and sucrose might be attributed to their capacity to bind water and reduce intracellular and extracellular ice crystal formation.

The objectives of this trial is 1) to observe the growth of lactic acid bacteria in freeze-dried yogurt as a mixed-starter for yogurt making with the addition of sucrose as a cryoprotective agent, 2) to determine the optimum sucrose concentration to be used as a cryoprotective agent and 3) to determine the quality of yogurt produce using freeze-dried mixed-starter.

MATERIAL AND METHODS

There were two stages of the trial. On the first stage, freeze-dried mixed-starter was made of pasteurized skimmed milk inoculated with 5% of mixed bacteria of *Lactobacillus bulgaricus* and *Streptococcus thermophillus* at the ratio of 1:1, incubated under 42 °C for four hours. Sucrose was added at the rate of 5, 8 and 11%, either before or after fermentation. The products were then freeze-dried, refrigerated and semi-vacuum packed. The lactic acid bacteria plate count test was done at the 1st, 2nd and 3rd week. Besides, observations were made on the lactic acid

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Table 1. Bacteria count at four hours of fermentation (10^8/ml)

Bacteria	Sucrose concentration (%)			
	0	5	8	11
S. thermophillus	59.27	45.00	55.20	50.67
L. bulgaricus	81.33	72.67	70.00	73.33

bacteria development, pH, lactic acid percent and sugar upon four hours of incubation.

On the second stage, yogurt was made from the pasteurized skimmed milk, which was divided, into six parts to be inoculated with the starters produced from the first stage of this trial. Weekly observations were made on lactic acid percent, pH and lactose content using chloramin T method (Sudarmadji *et al.*, 1984). Data were analyzed using 2x3 and 2x3x4 factorial analyses of variance for the first and the second stage trial, respectively.

RESULTS AND DISCUSSION

Bacteria developments of liquid starter

Both *S. thermophillus* and *L. bulgaricus* bacteria counts reached the maximum point at four hours of incubation in all cases. At this point, dry yogurt making process was stopped, at which point bacteria development was on the logarithmic phase. Beyond four hours of incubation, bacteria development would either decrease or on the stationer phase (Stainer *et al.*, 1970). Addition of sucrose of all percentages did not affect the bacteria count of the yogurt at the four hours of incubation (Table 1). The developments of both species of bacteria were affected ($P<.01$) by time length of fermentation.

Sucrose addition after fermentation resulted in bacteria count $59.27x10^8$ of *S. thermophillus* and $81.33x10^8$ of *L. bulgaricus* and these figures were greater than those before fermentation. According to Kosikowski (1978), sugar addition should be done after fermentation to slow down or to stop the bacteria development.

Bacteria count of freeze-dried mixed starter

There were no significant effects of sucrose addition, sucrose concentration and time length of storage on bacteria count of both species (Table 2 and 3). However, all figures showed that lactic acid bacteria counts were greater than the minimum requirement of starter, which are $10x10^8$.

Yogurt quality

pH. There were no significant effects of sucrose addition, sucrose concentration and time length of storage on the pH of yogurt produced using the freeze-dried mixed starter (Table 4). The pH of the yogurt was

Table 2. Average *S. thermophillus* bacteria count of freeze-dried started (10^8/ml)

Sucrose addition	Sucrose concentration (%)	Weeks of storage				Average	
		0	1	2	3		
After fermentation	5	50.00	48.33	51.07	50.00	49.85	
	8	52.00	51.00	55.00	54.33	53.08	
	11	48.33	47.67	47.00	47.33	47.58	50.17
Before fermentation	8	44.67	43.33	45.00	43.83	44.21	
	8	50.33	50.00	54.67	46.00	50.25	
	11	48.33	46.00	49.33	50.40	48.52	47.66
Average		48.94	47.72	50.34	48.65	-	-

Table 3. Average *L. bulgariacus* bacteria count of freeze-dried mixed starter (10^8/ml)

Sucrose addition	Sucrose concentration (%)	Weeks of storage					Average
		0	1	2	3		
After fermentation	5	78.33	80.67	77.90	79.00	78.98	
	8	80.00	79.67	79.67	81.00	80.08	
	11	80.00	81.37	80.00	80.00	80.34	79.80
Before fermentation	8	68.00	67.00	70.00	71.67	69.17	
	8	70.67	74.67	70.00	75.33	72.67	
	11	66.67	70.00	72.00	70.00	69.67	70.50
Average		73.94	75.56	74.93	76.17	-	-

much higher than the standard that was in the range of 4.5 to 4.6 (Lampert, 1975; Harper and Hall, 1976; Kosikowski, 1978). The pH was so high, because the incubation was terminated at five hours. If incubation were continued, there would have been a pH decrease.

Lactic acid %. There were no significant effects of sucrose addition, sucrose concentration and time length of storage on lactic acid % of yogurt (Table 5). In accordance with pH, lactic acid % was much lower than expected. To be a standard yogurt, lactic acid should be .85 to 1.00 (Lampert, 1975; Kosikowski, 1978). Again, it was due to the termination of incubation at five hours. Lactic acid % of yogurt using starter with sucrose addition after fermentation was higher ($P<.01$) than that with sucrose addition before fermentation. Lactic acid bacteria activities were suppressed by sucrose added before fermentation. During freeze-drying process, the bacteria were on the dormant condition and active again when exposed to suitable media. Peppler (1967) stated that the lag phase of freeze-drying process could be lengthen for the dormant condition of bacteria. Metabolism of bacteria will proceed upon rehydration in a normal condition (Handayani, 1988).

Yogurt sugar. Sucrose addition, sucrose concentration and time length of storage did not affect yogurt sugar significantly (Table 6). Comparing yogurt sugar with the sugar in the milk used as raw material, resulted in the highest sugar reduction (15.99%) on yogurt using starter with 8% sucrose added before fermentation. Sugar reduction on yogurt using starter with 8% sucrose added after fermentation was the highest (14.20%).

Dry matter. Sugar addition, sucrose concentration and time length of storage affected ($P<.01$) dry matter content of yogurt.

Table 4. Yogurt pH of freeze-dried mixed starter affected by sucrose addition and concentration and time length of storage

Sucrose addition	Sucrose concentration (%)	Weeks of storage					Average
		0	1	2	3		
After fermentation	5	5.27	5.23	5.17	5.20	5.22	
	8	5.20	5.13	5.23	5.23	5.20	
	11	5.23	5.23	5.20	5.27	5.23	5.22
Before fermentation	8	5.23	5.13	5.27	5.27	5.23	
	8	5.17	5.20	5.27	5.30	5.23	
	11	5.23	5.10	5.27	5.27	5.22	5.23
Average		5.22	5.17	5.23	5.26	-	-

Table 5. Lactic acid % of freeze-dried mixed starter affected by sucrose addition and concentration and time length of storage

Sucrose addition	Sucrose concentration (%)	Weeks of storage					Average
		0	1	2	3		
After fermentation	5	0.63	0.66	0.67	0.65	0.65	
	8	0.65	0.66	0.66	0.64	0.65	
	11	0.59	0.65	0.62	0.62	0.62	0.64[a]
Before fermentation	8	0.70	0.67	0.69	0.62	0.67	
	8	0.68	0.69	0.69	0.69	0.69	
	11	0.67	0.68	0.67	0.72	0.69	0.68[b]
Average		0.65	0.67	0.67	0.66	-	-

[a,b] Means with different superscript, differ (P<.01)

There were an interaction effect (P<.01) between time of sucrose addition (before and after fermentation) and sucrose content on dry matter content (Table 7). When sucrose was added after fermentation, dry matter content was higher (P<.01) than that when sucrose was added before fermentation (15.25 *vs.* 9.06%). This was due to the fact that sugar reduction was higher when sucrose was added after fermentation. Fermentation is a metabolism process involving carbon containing substance such as carbohydrate, protein and fat through enzymatic process of specific microorganism (Hargrove, 1970, cited by Wittier and Webb, 1970).

Dry matter content was increased by the higher rate of fermentation. Besides, sucrose, as a cryoprotector when added before fermentation prohibit bacteria from their metabolism activities, resulting in lower dry matter content of yogurt.

CONCLUSION

Sucrose addition and sucrose concentration had no effect on lactic bacteria count both on liquid and freeze-dried mixed starter. Freeze-drying process resulted in the decrease of lactic acid bacteria count. However, lactic acid bacteria count of freeze-dried mixed starter produced in this trial, met the minimum requirement of standard starter.

Sucrose addition, sucrose concentration and time length of freeze-dried mixed starter storage did not affect yogurt quality using freeze-dried mixed starter, in terms of pH and yogurt sugar. In order to

Table 6. Yogurt sugar of freeze-dried mixed starter affected by sucrose addition and concentration and time length of storage (%)

Sucrose addition	Sucrose concentration (%)	Weeks of storage					Average
		0	1	2	3		
After fermentation	5	7.07	7.05	7.19	7.00	7.08	
	8	6.73	7.39	7.28	7.39	7.19	
	11	7.05	7.44	7.42	7.07	7.24	7.17
Before fermentation	8	7.14	7.23	7.14	7.16	7.17	
	8	6.75	6.96	7.05	7.41	7.04	
	11	7.05	6.73	6.91	7.00	7.92	7.04
Average		6.96	7.13	7.16	7.17	-	-

Table 7. Yogurt dry matter content of freeze-dried mixed starter affected by sucrose addition and concentration and time length of storage (%)

Sucrose addition	Sucrose concentration (%)	Weeks of storage					Average
		0	1	2	3		
After fermentation	5	14.54	5.11	15.06	11.60	14.08[a]	
	8	14.65	14.03	14.15	14.39	14.30[a]	
	11	17.58	7.95	16.77	16.21	17.38[b]	15.25[a]
Before fermentation	8	9.20	9.25	9.19	8.87	9.13[cd]	
	8	9.83	9.18	8.79	7.67	8.87[c]	
	11	9.69	9.69	9.56	7.82	9.19[d]	9.06[a]
Average		12.75[g]	12.53[g]	12.25[g]	11.09[h]	-	-

[a,b,c,d] Different superscripts show different means affected by sucrose concentration (P<.01)

[c,f] Different superscripts show different means affected by sucrose addition (P<.01)

[g,h] Different superscripts show different means affected by time of storage (P<.01)

make a yogurt, which meets the standard requirements, incubation with freeze-dried mixed starter, should be lengthen beyond five hours.

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