USE OF EXTRACELLULAR BIOEMULSIFIER FROM *STREPTOCOCCUS THERMOPHILUS* STRAINS IN PRODUCTION OF FROZEN YOGHURT AS A PROBIOTIC FOOD

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Abstract

Four bacterial strains of *Streptococcus thermophilus* p were cultivated in milk permeate and in M17 broth with lactose or glucose added to select the best strain which can grow on milk permeate or on M17 to give the highest extracellular bioemulsifiers yield which was produced by *S. thermophilus* p. The best strain was *Streptococcus thermophilus* p which gave the highest growth on milk permeate at 37°C for 24 h at pH 7.

Butter oil-in-water emulsions were stabilized over a broad range of conditions, from pH 2 to 11, with up to 5% sodium chloride or sucrose in the aqueous phase. In the presence of a low concentration of various solutes, emulsions were stable to three cycles of freezing and thawing.

The main objective of this study was to manufacture frozen yogurt using different ratios of extracellular bioemulsifiers, which was produced by *S. thermophilus* p. The results showed that using extracellular bioemulsifiers had slight effect on increasing the specific gravity and weight per gallon in the resultant frozen yogurt. In addition, extracellular bioemulsifiers reduced the rate of melting. Generally, using of extracellular bioemulsifiers in the manufacture of frozen yogurt improved whipping quality of the mix and gave smoother body and texture. The preferable concentration of bioemulsifier produced by S. *thermophilus* p was 0.2% which enhanced viscosity, freezing point and overrun, and the product was more acceptable to consumers.

INTRODUCTION

Emulsifiers are surface-active compounds capable of reducing surface and interfacial tension at the interfaces between liquids, solids and gases, thereby allowing them to mix or disperse readily as emulsions in water or other liquids.

The greatest waste disposal problem in cheese industry today is milk permeate. Up to half million ton / year of raw permeate may be discharged into sewage disposal system in Egypt. However, utilization of this waste in fermentation for producing bioingredients is preferable, as permeate contains large amounts of biodegradable compounds such as lactose, vitamins, nitrogenous substances ...etc. Using *Streptococcus thermophilus* to ferment permeate is proposed as an alternative to reduce environmental pollution.

Bioemulsifiers are produced by probiotic strains, *Streptococcus thermophilus* P, using conventional synthetic media and its applications was reported by Rodriguez *et al.* (2006).

A probiotic is defined classically as a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. This definition, however, was initially intended for use with animal feed products. For human nutrition, the following definition has been proposed: "a live microbial food ingredient that is beneficial to health".

Frozen desserts have been increased markedly in Egypt, because of their popularity. The frozen dessert is the frozen yoghurt which has the flavour of yoghurt and rheological properties of ice cream. Yoghurt is the most popular fermented milk in Egypt and all over the world. Consumption of yoghurt in Egypt has nearly double in the past three years. The nutritive value of yoghurt is based on the nutritive value of milk from which it is made. It has increased digestibility, and healthy effects in certain condition.

Frozen yoghurt can be made using different sweeteners, stabilizers, emulsifiers flavours and sources of milk solids not fat according to their availability and price These different ingredients might affect the survival of (probiotic bacteria) bifidobacteria and *lactobacillus acidophilus* and quality of the resultant frozen yoghurt.

Emulsifiers mechanism can be summarized as follows: they lower the fat/water interfacial tension in the mix, resulting in protein displacement from the fat globule surface, which in turn, reduces the stability of the fat globule allowing partial coalescence during the whipping and freezing process. This leads to the formation a structure of fat in the frozen product that contributes greatly the texture and meltdown properties(Groff and Jordan, 1989). The extent of protein was displacement of the memberan and hence the extent of dreness was achived is a function of the emulsifier concentration. (Marshall *et al.*, 2003).

Consumer interest in frozen yoghurt stems from the desirable nutritional properties attributed to the product. In addition to low-fat formulation, frozen yoghurt supplemented with probiotic bacteria, such as *lactobacillus acidophilus* and bifidobacterium sp. provides additional health benefits. Health aspects attributed to the consumption of fermented dairy products supplemented with probiotic bacteria include improved lactose utilization, anticarcinogenic activity, control of intestinal infection, and improved flavor and nutritional quality (Baig and Prasad, 1995). The utilization of lactose during fermentation makes the product more easily digested by lactose intolerant people (Lorora, and Martin, 1990).

The aim of this study was to develop a low-cost alternative medium for bioemulsifier production by *S. thermophilus* P. Milk permeate was evaluated as alternative media and compared with the conventional synthetic medium for S. *thermophilus* P to produce a food grade extracellular bioemulsifiers and to study the behaviour of this new extracellular bioemulsifiers on probiotic frozen yoghurt production.

MATERIALS AND METHODS

Bacteria Strains: Different lactic acid bacteria strains were used in this study. *Streptococcus thermophilus* P was obtained from the Laboratrium wiesby GmbH &Gotteskogslrasse 40-42, D-25899 Niebull, Germany. *Streptococcus thermophilus* EMCC 1043 was obtained from Cairo MIRCEN, Faculty of Agriculture, Ain Shams University.

Streptococcus thermophilus 026802, and *Streptococcus thermophilus* 026809 were obtained from the Chr. Hansen's Laboratories, Copenhagen, Denmark.

Milk permeate: Fresh buffalo milk permeate was obtained from the Unit of Milk Industry, Animal Production Research Institute, Agriculture Research Center, Giza. , Egypt. Milk permeate contained, 4.8% lactose, 0.2 % nitrogenous substances and fat free.

Fresh whole buffalo's milk 6% fat was obtained from the herd of the Dairy cattle of the Faculty of Agriculture, Ain Shams University.

Low heat skim milk powder (SMP) made in Holland was obtained from the local market in Cairo.

Fresh Cream was mechanically separated from the fresh buffalo's milk that obtained from the herd of the Dairy cattle at the Faculty of Agriculture, Ain Shams University

Commercial grade granulated sugar cane was obtained from the local market. Sodium Carboxy methylcellulose (CMC), made by BDH Chemicals LTD. poole, England and Vanilla (Chem. Rein 100%) made by Boehringer Manheim GMB, Germany and emulsifier mono-glyceride (Glycerol monoleate) (GMO) were obtained from the local market.

Microbiological media: The following media were obtained from Oxoid Division of Oxoid LTD, London: Violet Red Bile agar (V.R.B.A.), Trypton soya agar (T.S.A.), yeast extract peptone agar (Y.E.P.A.), S.S. agar and Baird parker's agar.

Media used and growth conditions

Streptococcus thermophilus strains were grown in either M17 broth or milk permeate medium previously sterilized at 121 °C for 15 min. The media were

inoculated with 2% volume of precultured strains grown in M17 broth for 24h, then, incubated at 37°C in a shaking water bath (JULABO LABORTE CHNIK GMBH D.7633 Seelbach, Germany) with agitation rate 150 rpm for 24h. Experiments were carried out in triplicate. Samples were taken every 24h. The samples were analyzed for Biomass dry weight, carbohydrate, lactose and bioemulsifier yield.

Screening of strains

Erlenmeyer flasks (500 ml) containing 100 ml of Modified M17 broth or milk permeate were inoculated with 6% of 24 h active culture and incubated at 37°C for 24 h.

Four bacteria strains of *Streptococcus thermophilus* were tested on one liter Modular fermentor (Mini Bioreactor, A. Gallen Kamp & Co. Ltd.) operated at 37°C for their ability to produce significant amounts of the emulsifying agent.

Production of emulsifier

To test bioemulsifier production using alternative fermentation media, batch fermentations were carried out as shown in Table 1. The conventional synthetic medium was prepared according to the supplier instructions (OXOID, Basingstoke, England). *Streptococcus thermophilus* P was tested on one liter Modular bioreactor (Mini Bioreactor, A. Gallen Kamp & Co. Ltd.) fitted with agitation control, as well as temperature and pH measurement control was used. The temperature was maintained at 37 °C for 24 h, the pH at 6.7 by automatic addition of a potassium hydroxide solution, and the agitation speed was set at 150 rpm. The total working volume was 0.5 L.

In order to compare the amount of cell-bound bioemulsifiers produced by the bacteria grown in standard and optimized medium, the bioemulsifiers were released by the stationary phase cells using the PBS extraction procedure as described below. Briefly, the bacteria were left at room temperature for 2 h in 100 ml potassium buffer pH 7 with gentle stirring for cell-bound bioemulsifiers release. Subsequently, the bacteria were removed by centrifugation and the remaining supernatant liquid was filtered through a 0.22 mm pore-size filter (Millipore). The supernatant was dialyzed against demineralized water at 4 C in a Spectrapor membrane tube (molecular weight cut off 6000–8000, Spectrum Medical Industries Inc., CA) and freeze-dried. The mass produced bioemulsifiers (milligram per gram cell dry weight) was determined.

Table 1. Medium composition used in the fermentation for tested strain (*S. thermophilus* P).

Medium	
A	M17 broth
B	MP (50 g/L Lactose + 3 g/LYE + 5g/L PEP
C	MP (50 g/L Lactose + 3 g/LYE + 10g/L PEP
D	MP (50 g/L Lactose + 22 g/LYE + 15g/L PEP

MP, Milk Premeate, YE, yeast extract, PEP, peptone.

Biomass Dry Weight: At the end of fermentation period (24 h), bacteria cells in fermentation medium were killed by thermal treatment (75 °C for 15 min.) as suggested by (Ming-Lo, 1997).Yeast cells were harvested by centrifugation at 16000 g for 20 min, and then, dried at 105 °C until the change of weight was negligible.

Methods of analysis

The following parameters were determined in bioemulsifiers:

Emulsification activity determination

Emulsification activity was evaluated as described by Cameron *et al.* (1988). Bioemulsifiers was dissolved (0.2 %) in 4 ml of distilled water and 6 ml of kerosene or butter oil .The tube homogeneity is achieved by vortex. After one hour, the emulsified proportion of kerosene or butter oil was compared with the total volume of kerosene or butter oil added. Emulsion generated by vigorous mixing separated completely within one hr, in absence of any emulsifying agents.

Evaluation of emulsification stability

Effect of some parameters, namely, pH range of 2-11, sodium chloride concentrations up to 5% sucrose concentration up to 15%, heat treatment at 63°C / 30 min., freezing at -18°C for 16 hr followed by thawing at 25°C for 8h and refrigeration at 4°C for 30 days was carried out. The stability of purified intracellular emulsifier was evaluated as mentioned by Cameron *et. al.*, (1988).

Preparation of yoghurt

For prepraring yoghurt, buffalo's milk was standardized to 4% fat. milks was exposed for yoghurt manufacture according to Tamime & Riboinson(1985) milk was cooled to 42 °C and starter composed of 3% of normal starter *Steptococcus thermophilus* and *Lactobacillus delbreukii* subsp *bulgaricus* ,at the ratio of 2:1 respectively) and the other milk was composed of 2% *Bifidobacterium befidium* ATCC 15696 , 1% *Lb. acidophilus* TISTR 450 and 2% of normal starter. The yoghurt milk was filled into suitable containers covered and incubated at 42 °C for about 3h . The containers were transferred to the refrigerator at 5 °C, where they were kept for overnight to use in frozen yoghurt manufacture..

Preparation of frozen yoghurt

Frozen yoghurt base mix (the control) was prepared to contain 4% fat.12% MSNF, 15% sucrose, 0.2% stabilizer CMC. 0.05% vanilla and 0.2% commercial emulsifier (Glycerolmonoolate) the other frozen yoghurt base mix was prepared to contain the same contents except the emulsifier which was added at different ratios of extratracellular bioemulsifer from *S. thermophilus* p at the levels of 0.1, 0.2, or nil% respectively as shown in Table (2). The manufacture was carried out as described by Bradly & Winder (1977). Three replicates were done for every treatment.

Table 2. The formulas (kg / 100 kg) of frozen yoghurt mix by replacement of extracellular bioemulsifier with different levels.

Ingredient	Level of replacement				
	% of extracellular bioemulsifiers				
	Nil (Control)	GMO 0.2%	0.05%	0.1%	0.2%
Cream (38% fat, 4.5 % SNF)	27.30	27.30	27.30	27.30	27.30
Liquid milk (4% fat)	52.18	52.18	52.18	52.18	52.18
Dried skim milk (95% DM)	4.22	4.22	4.17	4.12	4.02
Sucrose	16.1	16.1	16.1	16.1	16.1
Stabilizer (CMC)	0.20	0.20	0.20	0.20	0.20
Intracellular bioemulsifier	0.00	0.00	0.05	0.10	0.20
Yoghurt	100.00	100.00	100.00	100.00	100.00

SNF: Solids not fat DM: Dry matter
GMO: glecerol monoolate.
CMC: Sodium carboxy methylcellulose

Chemical analysis of frozen yoghurt

Fresh and stored frozen yoghurt were analyzed for acidity according to Ling (1963) and the pH of the sample was determined by using a glass electrode pH meter (lutron pH-206. UK). Dry matter (DM) content was determined , fat content and protein content (Total nitrogen x 6.38) were determined according to AOAC (1994). Ash content was determined according to Pearson (1973).

Physical properties of frozen yoghurt

Specific gravity

The specific gravity of frozen yoghurt was determined by the method described by Winston, (1958). The weight per gallon of both mixes and frozen ice cream in pounds was directly calculated according to Burke (1947) by multiplying the specific gravity of the mix by the factor 8.34 (the weight per gallon of water in pounds). Overrun percent was according to Arbuckle (1986). Melting resistance: Melting rates were determined as described by Reid and Painter (1933).

Microbiological analysis

Bifidobacterial count was determined using modified MRS agar supplemented with 0.05% L-Cystein and 0.3% lithium chloride according to Dave and Shah (1996). Lactobacilli count was determined using MRS agar according to De Man *et al.* (1960). Total bacterial counts of frozen yoghurt were determined using standard plate count, SPC, method (Marth, 1978).

Sensory evaluation: The organoleptic properties of frozen yoghurt treatments were assessed according to Farag *et al*, (1993). for flavor (50 points), body and texture (40 points), and 10 points for melting quality (100 total score) by 20 panelists of the experienced staff members of the dairy Science Department, Animal Production Research Institute, Agriculture Research Center.

RESULTS AND DISCUSSION

Bioemulsifier production using conventional synthetic medium

Fermentation control runs were carried out using the conventional synthetic medium M17 broth (A as defined in Table 1).

Bioemulsifier production using milk premeate

Fermentations were carried out using milk premeate supplemented with yeast extract and peptone as culture broth for studied strains. Different sets of medium contain in yeast extract and peptone were evaluated. For both strains growing in all the tested milk premeate medium (B, C, D as defined in Table 1), the results indicated lactose consumption, biomass growth and bioemulsifier production, respectively.

The purified emulsifiers were tested for their stabilization under a widerange of chemical and physical conditions which might be encountered in various applications. To facilitate the detection of the possible effects of pH, sodium chloride and sucrose on emulsification, emulsions were prepared with 0.2% (w/v) purified emulsifier.

Data recorded in Table (3) show, the effect of different levels of pH values on the emulsion of *S. thermophilus* P emulsifier (containing 0.2% purified emulsifier).

It was noticed that the maximum relative emulsifier activity was obtained at pH 4, 6 and 8. However, at pH 10 1.9% loss of relative emulsifier activity was noticed. The results showed that the best pH values which gave the highest relative emulsifier activity ranged between 4 - 8.

Data recorded in Table 3 show also the effect of different concentrations of sucrose. It was observed that, in presence of 2 to 6 % (w / v) sucrose, there was no loss in emulsion activity, compared to the estimated loss with 10 % and 15 % sucrose.

Data recorded in Table 3 show the effect of different concentrations of sodium chloride It was observed that in presence of 2 to 6 % (w / v) sodium chloride, there was no loss in emulsion activity , compared to the loss found when NaCl concentration increased above that. These results are in agreement with those results obtained by Roushdy (1997).

Table 3. Stability of emulsion with a purified extracellular bioemulsifier from Streptococcus thermophilus P at different pH values, sucrose and sodium chloride concentrations.

pH value	butter oil phase emulsified (%)	sucrose %	butter oil phase emulsified (%)	Nacl %	butter oil phase emulsified (%)
2	90	0	91.66	0	91.66
4	93	1	91.66	2	91.66
6	93	2.5	83.30	4	91.66
8	93	5	81.66	6	91.66
10	91	10	75.00	8	66.66
11	91	15	75.00	12	66.66

Effect of heat treatments and storage condition on relative emulsifier activity of extracellular emulsifier

Physical treatments known to reduce emulsion stability were tested on emulsion containing 0.2% purified emulsifier. Different heat treatments (60 , 70 , 80 and 90 °C) were used to show the effect of heating on relative emulsifier activity. The results were illustrated in Table (4). It was noticed that the effect of heating times were more pronounced than the temperature used. Thus, the broad range of pH stability, thermo stability and salt tolerance suggested that, the isolate bioemulsifier could be useful in the dairy industry. (Cirigliano and Carman 1984).

The same table indicates that emulsification activity decreased to 85.3% after 3 cycles of freezing and thawing. Similar results have been reported for S. *ceervisiae* bioemulsifier bioemulsifier (Cameron *et al.,* 1988).

Effect of storage condition was also presented in table (4). Obtained results showed that storing at room temperature (25°C) caused more decreasing in emulsification activity of bioemulsifier comparing to storing in refrigerator (4°C).

Table 4. Effect of heat treatments, freezing, thawing and storage condition on stability of emulsions with a purified extracellular bioemulsifier from *Streptococcus thermophilus* P.

Treatment	butter oil phase emulsified (%)	
	Before	After
Heat treatments		
63 °C for 30 min	91.66	58.33
72°C for 15 sec	91.66	77.00
85 °C for 10 min	91.66	76.66
90°C for 10 min	91.66	66.66
Freeze-thaw cycles:		
1st	91.66	91.0
2nd	91.6	89.0
3rd	90.0	85.3
Storage at :		
4 °C for 30 days	91.66	90.2
25+1 °C for 15 days	91.66	87.0

Chemical composition of frozen yoghurt mixes

Chemical composition of frozen yoghurt mixes are shown in Table 5. TS%, Fat% and total protein content did not affect with the type of starter cultures used in all treatments compared with control frozen yoghurt. This may be due to the same chemical composition in the initial milk used in the manufacture of frozen yoghurt.

Total solids content: From the results also presented in Table 5, it could be noticed that the average dry matter of frozen yoghurt mixes with or without the addition of extracellular bioemulsifiers from *S. thermophilus* P by 0.05, 0.1 and 0.2% were 75.6, 79.4 and 72.9%, respectively which are similarl to the control (Table 5).

Fat content: Table 5 show that fat content in all frozen yoghurt mixes including control was in narrow figures and ranged between 12.0 to 12.15% in all mix samples. The slight differences in fat values are due to the experimental error since the fat was adjusted in all mix samples during preparation to be 12%.

Protein content: From the results presented in Table 5, it was noticed that the whole replacement of commercial emulsifier with extracellular bioemulsifier from *S. thermophilus* P had no effect on protein content of frozen yoghurt.

Ash content, titratable acidity and pH of all mixes were nearly similar to the control.

Table 5. Chemical properties of frozen yoghurt mixes as affected by the percentages of extracellular bioemulsifiers from *Streptococcus thermophilus* P.

Property	Nil	% GMO	% of extracellular bioemulsifiers		
	Control	0.2	0.05	0.1	0.2
Total solids %	70.2	79.1	75.6	79.4	72.9
Fat %	12.02	12.0	12.15	12.03	12.05
Protein % (T.N x 6.38)	4.67	4.74	4.68	4.73	4.78
Ash %	1.104	1.101	1.093	1.098	1.108
pH value	5.036	5.6	4.91	5.04	4.84
Titratable acidity %	0.8	0.78	0.9	0.92	0.79

GMO: glecerol monoolate.

Physical properties of frozen yoghurt mixes

Specific gravity and weight per gallon

Physical properties of frozen yoghurt mixes with different levels of extracellular bioemulsifiers produced by *S. thermophilus* P are presented in Table 6. The specific gravity of control mix was 0.977 while it was 1.071, 1.378 and 1.005 for mixes with 0.05, 0.1 and 0.2% of extracellular bioemulsifier. The forementioned values indicated that the added bioemulsifier has no effect on the specific gravity values or weight per gallon of frozen yoghurt mixes. Weight per gallon of frozen yoghurt mixes were, 8.9326, 8.6549, 8.38142and 8.1525 for control and treatments with 0.05, 0.1 and 0.2% of extracellular bioemulsifier respectively.

Freezing point: Freezing point of frozen yoghurt mixes with different levels of exracellular bioemulsifiers are also presented in Table (6).It is clear that freezing point decreased when emulsifiers were used, and decreasing was in positive relation with emulsifier ratio.

Viscosity: Viscosity values of treatments are reported in Table 6. Among treatments with bioemulsifier, the viscosity values increased by increasing the ratio added of bioemulsifier to the mix being highest in the treatments with 0.2% of extracellular bioemulsifier. Viscosity increased proportionally by increasing of new extracellular bioemulsifier ratios.

Table 6. Physical properties of frozen yoghurt mixes asaffected by the percentages of extracellular bioemulsifiers from Streptococcus thermophilus p.

Property	Nil		% of extracellular bioemulsifiers		
Specific gravity	Control	GMO 0.2 %	0.05	0.1	0.2
	0.977	0.945	1.071	1.378	1.005
Weight per gallon	8.153	7.879	8.933	8.655	8.381
Freezing point C	-2.45	-2.75	-2.80	-3.25	-3.15
Viscosity (c.p)	301.9	432.2	434.4	488.3	491.7

GMO: glecerol monoolate.

Physical properties of resultant frozen yoghurt

Specific gravity: Specific gravity of frozen yoghurt with different levels of extracellular bioemulsifiers produced by *S. thermophilus* P are presented in Table 7. Frozen yoghurt with extracellular bioemulsifier had sp. gr. values of 0.950, 0.954 and 0.841 for 0.05, 0.1 and 0.2 % emulsifier respectively. which mean a slight effect on specific gravity values.

Weight per gallon

Weight per gallon of the frozen yoghurt mixes were in the same trend as specific gravity since it was calculated by sp. gr. values. Weight per gallon of frozen yoghurt was, 7.925, 7.955, 7.012and 6.7657 for control and treatments with 0.05, 0.1 and 0.2 % of extracellular bioemulsifier respectively.

Overrun: Overrun values of the frozen yoghurt reported in Table 7 were 60.92, 62.52, 64.53 and 58.60% for control and treatments with 0.05, 0.1 and 0.2 % of extracellular bioemulsifier respectively. From the data presented it can be seen that the control sample had significantly lower overrun value than that with extracellular bioemulsifier. The results also indicated that the samples with extracellular bioemulsifier possessed significantly higher overrun values.

Melting resistance: Table 7 showed the effect of adding extracellular bioemulsifier on the melting resistance of frozen yoghurt. From these data, it could be seen that the melting resistance of frozen yoghurt proportionally increased with increasing of extracellular bioemulsifier added.

Table 7. Physical properties of fresh frozen yoghurt as affected by the level percentages of extracellular bioemulsifiers from Streptococcus thermophilus P.

Property	Nil	% of extracellular bioemulsifiers			
Specific gravity	Control	0.2 % GMO	0.05	0.1	0.2
	0.811	0.988	0.950	0.954	0.841
	6.765	8.240	7.925	7.955	7.012
Weight per gallon					
Overrun %	58.60	54.70	60.92	62.52	64.53
Melting resistance loss %					
After15 min	33.3	26.10	11.20	8.80	11.7
After 30 min	57.10	33.80	31.80	28.80	34.30
After 45 min	66.52	55.79	57.00	62.40	44.00
After 60 min	76.70	79.50	79.50	78.40	77.26
After 75 min	96.00	91.70	86.70	83.43	80.99

GMO: glecerol monoolate.

Effect of storage period on acidity and pH of resultant frozen yoghurt

Data presented in table 8 show changes of titratable acidity and pH values of frozen yoghurt samples during storage period. Obtained values clearly show very slight increase in T.A.% and very slight decrease in pH values during storage period for 6 weeks.

Table 8. Effect of storage period on acidity (%) and pH values of frozen yoghurt made with different level of extracellular bioemulsifiers from Streptococcus thermophilus p.

Treatment % of extracellular bioemulsifiers	Storage period (weeks)				
	0	2	4	6	8
	pH values				
Control	5.3	4.82	4.82	4.82	4.82
GMO (0.2 %)	5.6	5.24	5.24	5.20	5.20
0.05	4.91	4.73	4.71	4.60	4.60
0.1	5.04	4.95	4.93	4.85	4.83
0.2	4.84	4.88	4.88	4.83	4.81
	Titratable acidity %				
Control	0.70	0.70	0.70	0.70	0.70
GMO* (0.2 %)	0.73	0.74	0.74	0.75	0.75
0.05	0.69	0.70	0.71	0.72	0.72
0.1	0.73	0.74	0.75	0.76	0.76
0.2	0.70	0.69	0.69	0.71	0.72

*GMO: (glecerol monoolate).

Effect of storage period on chemical properties of resultant frozen yoghurt

Total solids conten: From the results presented in Table 9, it could be noticed that the average of T.S% with the addition of extracellular bioemulsifiers from *S. thermophilus* P by 0.05, 0.1 and 0.2% were 31. 4, 30.5 and 30.6% respectively. During storage period, no significant changes could be noticed.

Fat content: Table 9, shows that fat content in all resultant frozen yoghurt samples including control were in narrow figures and ranged between 11.9 to 12.08%. During storage period, slight differences in fat values were found due to the experimental error since the fat was adjusted in all mix samples during preparation to be 12%.

Protein content: From the results presented in Table 9, it was clear that the whole replacement of commercial emulsifier with extracellular bioemulsifier from *S. thermophilus* P has slightly effect on protein contents of resultant frozen yoghurt. No remarkable changes were detected with protein content values throughout storage period.

Ash content: The replacement of extracellular bioemulsifiers showed slightly effect on the ash content during storage period of resultant frozen yoghurt.

Table 9. Effect of storage period on chemical composition of frozen yoghurt made with different levels of extracellular bioemulsifiers from Streptococcus thermophilus P.

Treatment % of extracellular bioemulsifiers	Storage period (weeks)				
	0	2	4	6	8
	Total solids (%)				
Control	29.8	29.75	29.80	29.80	29.91
GMO (0.2 %)	30.9	31.12	31.12	31.05	31.20
0.05	31.4	31.49	31.44	31.64	31.55
0.1	30.5	30.50	30.54	30.42	30.70
0.2	30.6	30.66	31.01	30.83	30.86
	Fat %				
Control	12.0	11.9	11.9	12.0	12.0
GMO (0.2 %)	11.9	12.0	12.0	12.1	12.1
0.05	12.0	12.0	12.0	12.0	12.0
0.1	11.9	11.9	11.9	11.9	11.9
0.2	11.9	11.9	12.0	12.0	12.0
	Protein % (T.N x 6.38)				
Control	4.67	4.67	4.69	4.68	4.90
GMO (0.2 %)	4.74	4.77	4.77	4.79	4.82
0.05	4.68	4.71	4.69	4.73	4.75
0.1	4.73	4.76	4.78	4.75	4.82
0.2	4.78	4.79	4.84	4.80	4.83
	Ash %				
Control	1.104	1.100	1.122	1.123	1.157
GMO (0.2 %)	1.049	1.076	1.055	1.077	1.127
0.05	1.039	1.098	1.075	1.141	1.121
0.1	1.047	1.005	1.066	1.094	1.155
0.2	1.048	1.086	1.100	1.120	1.135

GMO: glecerol monoolate.

Bacteriological properties of resultant frozen yoghurt

The microbiological quality of produced frozen yoghurt is illustrated in Table 10. Counts of total bacterial decreased gradually in all treatments up to the end of storage period. This may be attributed to the effect of freezing during storage on bacterial growth (Banwart, 1980.

These result are in agreement with those obtained by Kebary (1996), Baig and Prased (1997) who found that total bacterial counts decreased during preparation and storage of frozen yoghurt.

Lactic acid bacterial counts followed similar trends of the total bacterial count. Lactic acid bacterial counts decreased gradually in all treatments up to the end of storage period (Table 10). These results are in agreement with those obtained by Inoue *et al.* (1998) who stored frozen yoghurt for 6 months at -35 and found that the counts of viable lactic acid bacteria decreased during storage.

Also results indicated that the count of Bifidobacteria decreased gradually as storage period advanced in all treatments. The count of Bifidobacteria in all treatments are still higher than the minimum level (10^6cfu/ml) and that will achieve the beneficial effects of Bifidobacteria (Samona and Robinson 1991, Hunger and Peitersen, 1992).

Table 10. Effect of storage period on microbiological analysis of frozen yoghurt made with different levels of extracellular bioemulsifiers from *Streptococcus thermophilus* p.

Treatment % of extracellular bioemulsifiers	Storage period (weeks)				
	0	2	4	6	8
	Total bacterial count (cfu/ml x 10^6)				
Control	٣١٠	٢٧٥	٢٤٠	١٩٠	١٨٥
GMO (0.2 %)	3..	٢٩٠	٢٧٠	١٥٠	٩٥
0.05	٣٩٠	٢٨٠	31٠	٢٤٠	٢٢٠
0.1	٣٦٠	30٠	٢٩0	٢٦٠	٢٣٠
0.2	30٠	30٥	٢٩٥	٢٨٠	٢٧٠
	Lactic acid bacterial count (cfu/ml x 10^6)				
Control	300	290	280	280	125
GMO (0.2 %)	180	160	135	121	98
0.05	250	220	180	140	90
0.1	300	250	210	190	180
0.2	223	210	205	155	100
	Bifidobacterial count (cfu/ml x 10^6)				
Control	105	95	74	64	60
GMO (0.2 %)	107	100	90	81	75
0.05	130	120	115	95	82
0.1	270	260	132	125	103
0.2	230	218	202	180	115

GMO: glecerol monoolate.

Organoleptic properties of resultant frozen yoghurt

The average sensory score points of different resultant frozen yoghurt treatments manufactured with adding extracellular bioemulsifiers are shown in Table

11. It is obvious that the control sample had total score 91.5 points. However, the resultant frozen yoghurt containing 0.05, 0.1 and 0.2% extracellular bioemulsifiers possessed total score points 96.5, 97.5 and 98.5 respectivly. These data indicated that the addition of extracellular bioemulsifiers to frozen yoghurt mixes, at the concentration of 0.05, 0.1 and 0.2% enhanced the quality of resulting frozen yoghurt. The highest score of melting quality was found in the treatment with 0.2% of extracellular bioemulsifiers. These results are in accordance with those of Marshal *et al.* (2003) who concluded that low levels of emulsifier added as emulsifying agent achieved satisfactory physical and sensory properties in the resulting frozen yoghurt.

Table 11. Organoleptic scores of frozen yoghurt made with different levels of extracellular bioemulsifiers from S. *thermophilus* p.

Treatment % of extracellular bioemulsifiers	Storage period (weeks)				
	0	2	4	6	8
	Flavour (50)				
Control	٤٨	47	46	46	45
GMO (0.2 %)	49	48	47	45	45
0.05	٥٠	49	48	47	46
0.1	٥٠	49	48	47	46
0.2	٥٠	49	48	46	46
	Body and Texture (40)				
Control	35.5	35.5	34	33	31
GMO (0.2 %)	37	37	36	32	31
0.05	37.5	37	37	35	34
0.1	38	38	37	36	35
0.2	39	38	37	36	35
	Melting quality (10)				
Control	9.0	8.0	7.0	6.0	5.0
GMO (0.2 %)	8.0	8.0	7.0	6.0	5.0
0.05	9.0	9.0	8.0	7.0	6.0
0.1	9.5	9.0	8.0	6.5	6.0
0.2	9.5	9.0	8.0	7.5	6.5
	Total score (100)				
Control	92.5	90.5	87	85	81
GMO (0.2 %)	94	93	90	83	81
0.05	96.5	95	93	89	86
0.1	97.5	96.5	93.5	90	87.5
0.2	98.5	96	93	89.5	87.5

GMO: glecerol monoolate.

CONCLUSION

Streptococcus thermophilus p showed a good performance for glucose or lactose to bioemulsifier fermentation using the costly M17, which includes among others yeast extract and peptone. When the conventional synthetic media were replaced by cheaper alternative media, as milk premeate, in all cases fermentations were carried out effectively with high yields and productivities of bioemulsifier. The best results, even higher than those obtained with the conventional synthetic media, were obtained using supplemented milk' premeate , thus it can be used as an alternative economical medium for bioemulsifier production.

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إستخدام مادة الاستحلاب الحيوية لسلالات بكتريا الـ *Streptococcus thermophilus* فى إنتاج اليوجورت المجمد كغذاء داعم للحيوية

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تهدف هذه الدراسة إلى تنمية اربع سلالات من بكتريا *Streptococcus thermophilus* في بيئة راشح اللبن واختبارها لإنتاج مادة الاستحلاب الميكروبية من خارج الخلية لاستخدامها استخداما خاصا فى الأغذية. و لقد تم استخلاص مادة الاستحلاب هذه من كل نوع أو سلالة من البكتريا المختبرة و التى تضم ثلاث أنواع من الأجناس بخلاف *Streptococcus thermophilus* P و من أحد النواتج الأربعة المختبرة وجد أن لسلالة واحدة *Streptococcus thermophilus* P انتجت مادة استحلاب لها خواص استحلاب أفضل من خواص مواد الاستحلاب الشائعة الاستخدام فى مجال الأغذية مثل الجليسرول مونو أوليت. و قد وجد أن السلالة (*Streptococcus thermophilus* P) و التى تم اختبارها للدراسة قد أعطت مواد استحلاب خارجية ذات خواص استحلابية ممتازة.

و قد حدث ثبات لزيت الزبد فى الماء بالمستحلبات على مدى واسع من الاشتراطات فوجد أنه كان ثابتا على درجة pH ٢-١١ و كذلك مع تركيز ٥% كلوريد صوديوم وحتى تركيز ٥٠ % من السكروز فى الطور المائى. و فى وجود تركيزات منخفضة من مادة الاستحلاب الخارجية كانت المستحلبات ثابتة خلال ٣ دورات من التجميد والتسييح.

وتم استخدام مادة الاستحلاب الخارجية بتركيزات مختلفة (٠,٠٥، و٠,١ و ٠,٢%) بهدف تحسين خواص اليوغورت المجمد الناتج بالمقارنة بالكنترول.

وأظهرت النتائج أنه لا يوجد تأثير ملحوظ على كل من الوزن النوعي والوزن للجالون واللزوجة ولكن لوحظ انخفاض في نقطة التجمد بزيادة نسبة المستحلب بينما حدث إنخفاض تدريجي في قيمة الـ pH في المخلوط بزيادة النسبة المضافة من مادة الإستحلاب الميكروبية. وأن هناك زيادة تدريجية في الربع ونقص في سرعة السيولة خاصة خلال النصف ساعة الأولى في اليوغورت المجمد بإضافة مادة الإستحلاب الميكروبية. أما من حيث الخواص الحسية متضمنة النكهة والقوام والتركيب وجودة السيولة، فإن جميع المعاملات كانت مستساغة تماماً و خالية من أي عيوب وأن المعاملة التي تحتوي على مادة إستحلاب ميكروبية بنسبة ٠,٢% حصلت على أعلى درجات التحكيم مقارنة بالمخلوط المحتوي على المستحلب التجاري.

وقد أظهرت النتائج المتحصل عليها أن استخدام مادة الاستحلاب المنتجة بواسطة سلالة البكتريا *Streptococcus thermophilus* P بتركيز ٠,٢ % أعطى نتائج جيدة من حيث اللزوجة ونقطة التجمد كما عمل على زيادة نسبة الربع والقابلية للخفق وانعكس هذا على خواص الانصهار عند التحكيم الحسي مما ادى إلى الحصول على أفضل درجات التحكيم.