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INFLUENCE OF SOME ADDITIVES ON THE PROPERTIES OF FLAVORED WITH FRUIT PROBIOTIC FERMENTED MILK

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ABSTRACT

The effect of Glucon Delta-Lactone (GDL), disrupted *Saccharomyces cerevisiae* cells after freezing (DSCAF) and yeast extract (YE) on the properties of flavored with fruit probiotic fermented milk was investigated. The obtained results revealed that 5% of DSCAF activated the bacterial growth more than other additives. Therefore, it was selected to make fermented milk from UF retentate of cows' milk (14 % T.S.) with 3% ABT – 4 culture. This additive accelerated the fermentation time throughout 2 hours, but GDL (0.4%) and YE (0.4%) reduced the fermentation time an hour. The addition of 5% of mango or strawberries pulp slightly decreased the fermentation time and partly decreased rheological quality. However, the counts of probiotic bacteria increased to more than the minimum therapeutic dose and mold and yeast were affected in the presence of DSCAF (5%). These organisms were detected on the seventh day of cold storage and had limited numbers. This result was confirmed when the growth of *Aspergillus niger* and *Aspergillus flaveus* reduced in the presence of DSCAF (5%), *Aspergillus niger* grew only 2.2cm while *Aspergillus flaveus* scored 2.6 cm growth zone.

Keywords: Fermented milk, probiotic bacteria, *Saccharomyces cerevisiae* Glucono-Delta-Lactone (GDL), yeast extract.

INTRODUCTION

The use of probiotic organisms such as *Lactobacillus acidophilus* and *Bifidobacterium* spp. in fermented milks became popular by the end of 1970s as a result of the increased knowledge about these organisms. New fermented products containing *Lb. acidophilus*, *Bifidobacterium* spp., *Lactobacillus casei* Shirota, *Lactobacillus thamnosus* GG, and Lactobacillus reuteri have been developed in Europe. However, *Lb. acidophilus* and *Bifidobacterium* spp. are most commonly used as probiotics. It is estimated that over 70 products containing *Lb. acidophilus* and *Bifidobacterium* spp. including yogurt, buttermilk, frozen desserts, and milk powder are produced worldwide. Probiotic organisms are also available as powders, capsules, and tablets (Mittal and Garg, 1992). A number of genera of bacteria (and yeast) are used as probiotics. Traditionally, probiotic organisms have been added to yogurt and other fermented foods; however, recently, these organisms are incorporated in drinks and marketed as supplements including tablets, capsules, and freeze dried preparations. Today, there are over 70 bifidus- and acidophilus-containing products produced worldwide. More than 53 different types of milk products that contain probiotic organisms are marketed

in Japan alone. The probiotics in Europe are very popular, but their use is largely restricted to the yogurt sector (Shah, 2000a).

Lb. acidophilus tends to grow slowly in milk, leading to the risk of overgrowth of undesirable microorganisms. Ironically, most strains of *Lb. acidophilus* do not survive well in fermented milk due to the low pH, and it is difficult to maintain large numbers in the product. *Lb. acidophilus* grows poorly in milk even as they show a high level of β-galactosidase activity. This is partly related to low concentration of small peptides and free amino acids in milk, which would be insufficient to support the bacterial growth. Bifidobacteria are fastidious organisms and have special nutritional requirements, thus often these bacteria are difficult to isolate and grow in the laboratory (Shah, 1997; 2002). The leading commercial probiotic lactobacilli and bifidobacteria were shown by Krishnakumar and Gordon, (2001); Holm, (2003); Playne *et al*., (2003).

A number of health benefits are claimed in favor of products containing probiotic organisms. Some of the health benefits are well established, while other benefits have shown promising results in animal models. However, additional studies are required in humans to substantiate these claims. Health benefits imparted by probiotic bacteria are strain specific, and not species- or genus- specific. Health benefits of probiotic bacteria include antimicrobial activity and gastrointestinal infections, improvement in lactose metabolism, antimutagenic properties, anticarcinogenic properties, reduction in serumcholesterol, antidiarrhoeal properties, immune system stimulation, improvement in inflammatory bowel disease, and suppression of *Helicobacter pylori* infection (Kurmann and Rasic, 1991). There is sufficient evidence to support the view that oral administration of Lactobacilli and bifidobacteria is able to restore the normal balance of microbial populations in the intestine (Ouwehand *et al*, 1999). Technological problems have arisen with using the therapeutic bacteria in preparing fermented milks, particularly, the relatively long time needed for obtaining a satisfactory yoghurt coagulum. Accordingly some authors have directed their interest towards enhancing the growth of the therapeutic bacteria in milk to shorten the coagulation period (Mahmoud, 1999).many additives such as organic or inorganic were used to active probiotic bacteria.
Therefore, the present study was carried out to investigate the effect of adding disrupted cells of *Saccharomyces cerevisiae* after freezing (DSCAF), yeast extract and Glucono Delta- Lactone (GDL) on activation of probiotic bacteria and properties of flavored with fruit Probiotic Fermented Milk from UF retentate of cows milk (14 % T.S.)

MATERIALS AND METHODS

UF retentate of cows' milk (14 % T.S.) was obtained from Royal Food Factory, Mansoura-Egypt, using a pilot plant ultrafiltration unit (CARBOSEP 151 SPEC Co. France. Mango and strawberries pulp were purchased from Nile For Agricultural Industry Co, Glucono-Delta-Lactone (GDL) and yeast extract (YE) were obtained from (Pfizer Chemicals, Ireland).

Commercial bread yeast was used as a source for the isolation of *Saccharomyces cerevisiae*. The most appropriate medium for this purpose was the modified Saborou medium (Savova and Nikolova, 2002). The isolated strain was identified as *Sacc*. *cerevisiae* according to the procedures described by Barnett *et al*. (1990) and in consultant with Department of Microbiology, Soils, Water and Environment Research Institute, ARC, Giza, Egypt.

Partial extraction of *Sacc. cerevisiae* (Meyen ex E.C Hansen) was prepared by growing *Sacc. cerevisiae* on plates of malt extract agar medium for 4 days at 28 °C. The resulted cells were scraped gently with Pasteur pipet using 10 mL of sterilized water. The cell suspension was adjusted to 10^{11} cell Ml^{-1} and kept under freezing for 48 h to allow partial disruption of the cell walls. After melting, the obtained disrupted cells were considered as 100% concentration of yeast extract

ABT-4 culture *(Lactobacillus acidophilus; Bifidobacterium bifidium* and *Streptococcus thermophilus)* was obtained from Chr. Hansen-(Denmark). *Aspergillus niger* and *Aspergillus flaveus* were Obtained kindly from Prof. Dr. Mohamed EL-Metwally - Plant Pathology Res. Institute Agric. Res. Center, Giza, Egypt.

The effect of DSCAF (3 or 5%), yeast extract (0.2 or 0.5%) and Glucono Delta- Lactone (GDL) (0.2 or 0.4%) were examined separately, all additives were added to sterilized MRS broth medium which inoculated with ABT-4 culture and incubated at 37±2°C for 24 h, *Lactobacillus acidophilus; Bifidobacterium bifidium* and *Streptococcus thermophilus* were enumerated on selective media at (0.0, 3.0, 6.0, 9.0, 12.0 and 24 h) during that period.

The best concentration of additives(5% of DSCAF, 0.4%GDL and 0.4%YE) were chosen and added to UF retentate of cows' milk (14 % T.S.) to make fermented milk. All samples were incubated at 37±2°. Titratable acidity and pH values were determined and counts of probiotic bacteria were enumerated after each hour until full coagulation. 5% of DSCAF accelerated the fermentation time. Therefore, we used that additive to make fruity probiotic fermented milk.

5% of DSCAF was added to fresh UF retentate of cows' milk (14 % T.S.) and mixed well then was divided into three portions, 5% of mango pulp (15% T.S.) was added to the first portion, 5% of strawberries pulp (15% T.S.) was added to the second portion, the third portion was considered as control without adding fruits. All portions were warmed to 55 °C and homogenized using Rannie Lab-100, 2 stage homogenizer (Rannie, Copenhagen, denmark) at 200 $Kg/cm^{۲}$ for the 1 stage and 50 $Kg/cm^{۲}$ for 2nd stage. All treatments were heated at 85 °C for 5 min, cooled immediately to 40°C and inoculated with 3% (v/v) of actively culture of ABT – 4, then incubated at 37°C. The resultant fermented milks were stored at 6±2°C for 11 days. Samples were analyzed chemically, microbiologically, rheologically and organoleptically when fresh and after 3, 7 and 11 days of storage period. pH value was measured using laboratory pH meter with a glass electrode Model pH-206 Lutron Inst. Co. UK. Titratable acidity expressed as lactic

acid (%) was determined according to the method reported by Ling (1963). Acetaldehyde was determined as given by Lees and Jago (1969), diacetyle was determined as described by Westerfeld (1945). Total nitrogen and non protein nitrogen (NPN) were determined by the semi-micro Kijeldahl method according to Ling (1963). The total volatile fatty acids (TVFA) were determined by the method of Kosikowski (1966)

Total viable bacterial count (Standard plate colony count, SPC) mold and yeast count and coliform group counts, were carried out according to the American Public Health Association (1992). The count of spore-forming bacteria was determined according to Chalmer (1962). Counts of psychrotrophic bacteria count was estimated by using PCS medium (Bridson, 1990). Staphyloeoccus medium No.110 (DIFCO, 1974) was used to count and detect staphyiococci. *Bifidobacterium bifidium* was enumerated according to Dave and Shah (1996) using modified MBS agar supplemented with 0.05% L-cystein and 0.3% lithium chloride. *L. acidophilus* was enumerated according to Gilliland and Walker (1990) using modified MRS agar supplement with 0.2% Oxagal. *St. theromophiius* count was determined using M17 agar (Terzaghi and Sandine, 1975).

All additives were separately were added in sterilized oxitetracycline glucose yeast extract agar before pouring medium in the dishes, a disk of fungal growth (*Asp. niger* or *Asp. flaveus***)** put at the center of the dish the plates were incubated at 25°C for 3 days**.** Fungal growth was measured after 3 days with subtraction disc diameter.

Curd tension was measured by the method of Chandrasekhara *el al*, (1957). The apparatus used consisted of knives of constant weight (5g). H-shaped with needle in the middle ending with a hook, and a wire crossing a freely rotating wheel attached to the knife at one end and a pan (5g) at the other The knife was placed in a 100 ml beaker, yoghurt mixture inoculated with the yoghurt starter without/or with GDL (50ml) were added and incubated at 42 °C until set The curd tension was measured as weight in grams to remove the knife from the yoghurt.

The organoleptic properties of fruity probiotic fermented milk samples were evaluated for flavour (60 points), body and texture (30 points) and appearance (10 points) according to Bodyfelt *et al*. (1988).

RESULTS AND DISCUSSION

Table (1) showed that the effect of 5% of disrupted cells of *Sacch. .cervesia* after freezing(DSCAF) activated the bacterial growth of *L. acidophilus; Bif bifidium* and *Str thermophilus* more than all additives followed by 3% of DSCAF, 0.4 yeast extract (YE), 0.2% YE, and 0.4% GDL then 0.2% GDL. These results might be due to the abundance of necessary compounds for the bacterial growth in solution of DSCAF as vitamin B complex, antioxidants, minerals (iron, zinc, phosphorus and chromium). Regarding the results of adding (YE) consistent with Goh et al. (1983). The bacterial growth also was slightly enhanced in the presence of GDL more than in control, these results agree with El-Etriby, et *al*., 1997

The additives which achieved the best bacterial growth were selected to make fermented milk from UF retentate of cows' milk (14 % T.S.) with 3% ABT – 4 culture. The pH values were characterized by a decreasing trend after adding the additives and culture throughout incubation period (Table 2), titratable acidity gradually increased at the same time. The fermented milk which made with 5% of DSCAF had low pH and high acidity4.63 and 0.69, respectively, as compared with (4.62 and 0.70) (4.71 and 0.68) in the presence of 0.4 % YE and 0.4 % GDL when fully coagulation, respectively. This refers to 5% of DSCAF which stimulated lactic acid bacteria to ferment milk lactose to organic acids. Adding 5% of DSCAF caused a reduction in the time of fermentation 2 hours, followed by 0.4% of YE and 0.4 % of GDL, which accelerated fermentation time an hour compared with control samples. El-Etriby, *et al.,* 1997 found that 0.3 % of GDL decreased the fermentation time of yoghurt to 3.5 hour during manufacturing of yoghurt and agreed with reported by Mohamed (1994)

Table (1): Effect of some additives on growth of *L. acidophilus*, *Bif. bifidum* and *St. theromophilus* (Log CFU/ml).

Treatments	%	Bacteria	Time (hours)					
			0.0	3.0	6.0	9.0	12.0	24.0
control	W a	A	3.11	3.50	4.05	5.07	6.46	7.43
		B	3.09	3.48	4.06	5.00	6.31	7.28
		S	3.05	3.42	3.90	4.81	6.03	6.12
BSCAF	3	A	3.12	3.74	4.35	5.44	7.05	8.24
		B	3.08	3.70	4.33	5.41	7.01	8.17
		S	3.05	3.64	4.11	5.01	6.63	7.81
	5	A	3.11	3.78	4.44	5.61	7.35	8.53
		B	3.07	3.73	4.41	5.52	7.21	8.36
		S	3.06	3.65	4.18	5.10	6.74	7.96
Y E	0.2	A	3.10	3.61	4.18	5.22	6.85	7.77
		B	3.09	3.58	4.13	5.15	6.77	7.58
		S	3.04	3.54	4.08	5.03	6.44	7.33
	0.4	A	3.13	3.64	4.21	5.28	6.59	7.83
		B	3.09	3.62	4.15	5.19	6.48	7.62
		S	3.05	3.59	4.12	5.07	6.34	7.39
G D L	0.2	A	3.11	3.5٣	4.58	5.١١	6.٥٠	7.48
		B	3.57	3.4٩	4.58	5.0٢	6.3٤	7.31
		S	3.05	3.4١	3.9١	4.8٣	6.05	6.14
	0.4	A	3.11	3.55	4.11	5.١٥	6.٥٥	7.54
		B	3.07	3.51	4.0٩	5.0٤	6.3٦	7.35
		S	3.05	3.43	3.93	4.8٥	6.0٥	6.17

A = *L ACIDOPHILUS* B = *BIF. BIFIDUM* S = *ST. THEROMOPHILUS*
W A= WITHOUT ADDING THESE RESULTS ARE AVERAGE OF 3 REPLICATES

Table (2): The changes of pH-values and acidity of probiotic fermented milk during incubation at 37±2°.

Time of incubation (h)	Treatments							
	Control		5% D SCAF		Y E (0.4 %)		G D L (0.4 %)	
	pH	TA%	pH	TA%	pH	TA%	pH	TA%
A. A. A I	6.59	0.19	6.59	0.19	6.59	0.19	6.59	0.19
1	6.38	0.20	6.20	0.23	6.31	0.21	6.17	0.24
2	6..10	0.30	5.93	0.35	6.02	0.31	6.09	0.29
3	5.61	0.38	5.39	0..45	5.49	0.39	5.59	0.37
4	5.30	0.48	4.85	0.58	5.11	0.52	5.29	0.46
5	4.95	0.57	4.63	0.69	4.88	0.63	4.96	0.57
6	4.82	0.62	-	-	4.62	0.70	4.71	0.68
7	4.61	0.71	-	-	-	-	-	-

TA%= titratable acidity% these results are average of 3 replicates
A. A. A I= after adding additives and inoculum

Lactic acid bacteria were enumerated in the control and in all treatments by using selective media every each hour during incubation period. It could be noticed from Table (3) that the counts of *L acidophilus* and *Bifi. bifidum* gradually increased in the control and in the other three treatments during the first two hours. On the contrary, counts of *St. theromophilus* increased rapidly in the same period. After that (*L acidophilus* and *Bif. bifidum*) and *St. theromophilus* took a counter-trend to the above until full coagulation. On the other hand, *L acidophilus* and *Bif. bifidum* almost increased 4 logarithmic cycles logarithmic in the presence of DSCAF(5%) and YE (0.4%) after 5 hours and 6 hours respectively, *St. theromophilus* almost increased 3 logarithmic cycles in the control and all treatments. *L acidophilus* and *Bifidobacterium bifidum* increased 3—3.5 logarithmic cycles in the presence of 0.4 % GDL and the control after 6 and 7 hours respectively, The high growth of the probiotic bacteria might be attributed to the stimulation of the growth St. thermophilus which consumed dissolved oxygen in milk which considered toxic for probiotic bacteria, particularly, bifidobacteria. Results obtained are similar to those reported by Murti et al. (1993), who stated that bifidobacteria stimulated maximal growth of yoghurt starter bacteria and abundance of necessary compounds for the bacterial growth in solution of DSCAF.

Table (3): Growth of lactic acid bacteria in the presence of some additives in UF retentate cows' milk (14%TS) during incubation at 37±2° until coagulation. (Log CFU/ml).

Incubation time (hrs)	Treatments											
	Control			5% DSCA F			0.4 % Y E			0.4 % G D L		
	A	B	S	A	B	S	A	B	S	A	B	S
After inculcation	4.11	4.05	3.60	4.09	4.05	3.62	4.10	4.10	3.61	4.10	4.10	3.60
1	4.41	4.33	3.91	4.51	4.42	4.12	4.48	4.41	4.15	4.43	4.36	4.11
2	4.81	4.77	4.65	5.31	5.11	4.95	5.08	5.01	4.87	5.02	4.98	4.82
3	5.25	5.21	5.32	5.63	5.50	5.59	5.41	5.31	5.39	5.36	5.27	5.29
4	5.62	5.53	5.65	6.79	6.68	5.88	6.34	6.33	5.78	6.24	6.28	5.71
5	6.10	6.02	5.91	7.95	7.81	6.49	7.19	7.11	6.14	7.13	7.05	6.05
6	7.81	7.69	6.21	-	-	-	8.02	7.85	6.45	7.92	7.74	6.39
7	7.23	7.13	6.53	-	-	-	-	-	-	-	-	-

A = *L acidophilus* B = *Bifidobacterium bifidum* S = *St. theromophilus*
These results are average of 3 replicates

DSCAF (5%) was selected as the best additives, fermented milk samples were made using UF cows' milk retentate (14%TS) with addition 5% of mango pulp or 5% of Strawberry pulp with 3% highly activiteABT-4 culture, control and all samples were analyzed chemically, rheologically, microbiologically and organoleptically. when fresh and after 3, 7 and 11 days of cold storage and

It could also be noticed from table (4) that the pH-values decreased and the acidities increased in all treatments in all treatments compared with the control samples during storage. The samples containing (5% mango pulp and 5% B SCAF had the highest titratable acidity, T.N/D.M%, N PN/D.M% and TVFA, Acetaldehyde and diacetyle, followed by samples fortified with (5% strawberries pulp and 5%YFE) then the control samples. On contrary, the samples containing (5% mango pulp and 5% BSCAF) had the lowest pH-values, compared with another treatment and the control. Moreover, acetaldehyde and diacetyle increased until the seventh day of storage period, then decreased till the end of cold storage.

Table (4): Chemical analysis of fruity probiotic fermented milk during cold storage.

Treatments	S. p. days	Items						
		pH	Acidity	T.N/ D.M%	N PN/ D.M%	TVFA	Acetal	diace
Control	0	4.53	0.69	4.86	0.319	6.50	24.00	12.00
	3	4.41	0.75	4.58	0.329	7.90	32.00	15.00
	7	4.21	0.79	4.49	0.375	9.80	39.00	18.00
	11	4.13	0.85	4.15	0.418	10.2	37.00	17.00
mango pulp (5 %)	0	4.32	0.74	5.22	0.328	8.20	35.00	14.00
	3	4.19	0.80	5.02	0.339	9.80	44.00	18.00
	7	4.02	0.87	4.89	0.386	10.5	51.00	23.00
	11	3.97	0.95	4.48	0.431	11.3	55.00	21.00
Strawberries pulp (5%)	0	4.41	0.71	4.92	0.321	6.90	33.00	13.00
	3	4.31	0.76	4.69	0.332	8.20	42.00	16.00
	7	4.09	0.82	4.58	0.378	10.00	50.00	20.00
	11	4.05	0.89	4.35	0.425	10.5	51.00	18.00

TVFA= T.V.F.A ml 0.IN Na OH/100g diace = diacetyle s.p.=store period
Acetal = Acetaldehyde
these results are average of 3 replicates

These results agree with those obtained by El-Sayed and El-Shafei (1996), who found that the addition of *Bif. infantis* filtrate stimulated the acetaldehyde production by yoghurt bacteria. Another noteworthy observation was that decreasing trend of TCC after 7 days presumably due to demonstrated ability of lactic organism to reduce acetaldehyde to ethanol or oxidize it to acetic acid. These results agree with those obtained by Mohamed (1994) The curd- tension of fruity fortified probiotic fermented milk was greatly affected by adding fruit pulp and storage of period (Table 5). The curd tension of samples decreased with adding pulp each of mango and strawberries. When samples were kept in the refrigerator, their curd tensions increased gradually. The control samples recorded the highest curd- tension compared with the other two treatments; it was (26.0 – 42.0 g),

(25.0 – 41.0) and (24 – 38.40) when fresh and after 11 days of cold storage for control and in the presence of 5 % mango pulp and 5 % Strawberries pulp, respectively. This might be due to the weakness of the casein network when adding fruit pulp.

Table (5): Changes in both curd tension and wheying off in fruity fermented milk during cold storage.

Treatments	Items		
	Storage period (days)	Curd tension (g)	Wheying off (rnl /100ml)
Control	00	26.00	3.40
	3	34.50	3.20
	7	39.60	3.00
	11	42.00	2.60
mango pulp (5 %)	00	25.40	3.60
	3	33.50	3.40
	7	38.40	3.40
	11	41.00	3.40
Strawberries pulp (5%)	00	24.00	4.20
	3	31.60	3.50
	7	37.50	3.20
	11	38.40	3.20

These results are average of 3 replicates

The high curd tension of stored yoghurt might be due to complete setting of the curd. On the other hand, wheying off took the opposite trend. these results agree with El-Etriby, *et al.,* (1997), who found that Uf retentate (14% T.S.) was the best concentration to make yoghurt and curd tension was 39.5 g, but wheying off was 3.40 ml/100ml in flavoured yoghurt after 11 days of cold storage

Data in table (6) showed that the viable counts of *Bif. bifidum, L. acidophilus a*nd *St. thermophilus* slightly decreased during the first 7 days of cold storage. After 7 days to the end of storage period all lactic acid bacteria gradually decreased. On the eleventh day of the cold storage, the numbers of lactic acid bacteria sharply decreased. Counts of lactic acid bacteria almost decreased half a logarithmic cycle at the end of cold storage compared with fresh samples. These results agreed with Shimamura *et al*. (1992) However, viability of the probiotic bacteria in all treatments remained above 10^6 cfu /ml or g until the expiration date, which is the recommended minimum dose to receive the health benefits of these organisms (Shin et a/., 2000).

Results as shown in table (7) reveal that the control samples had the lowest total bacterial count, compared with treatments in the presence of mango and Strawberries pulp possibly due to the presence of fruit, which supported bacterial growth, Obviously from the same Table, both of coliform and *Staph. aureus* were not detected in all of the treatments and the control whether in fresh or stored. This indicates that the manufacturing process was conducted under hygienic practices. Therefore, this infectious or/ and undesirable bacteria could be avoided. Counts of sporeforming bacteria indicated in this Table are clearly close to each other in all the treatments. This behaviour continued after 1, 3, 7 and 11 days of storage as the counts

were still close to each other. sporeforming bacteria are unavoidable under the commonplace practices of raw milk production.

Table (6): Viability of lactic acid bacteria during cold storage for 11 day (Log CFU/ml).

Storage period (days)	Treatments								
	control			mango pulp 5%			Strawberries pulp 5%		
	A	B	S	A	B	S	A	B	S
0.0	7.29	7.17	6.54	7.58	7.45	6.55	7.32	7.21	6.22
3	7.21	7.08	6.48	7.36	7.35	6.48	7.25	7.12	6.11
7	7.11	6.91	6.35	7.24	7.16	6.31	7.09	7.02	6.25
11	6.74	6.54	6.02	6.71	6.61	5.95	6.58	6.44	5.89

A = *L acidophilus* B = *Bifidobacterium bifidum* S = *St. theromophilus*
These results are average of 3 replicates

Table (7): Microbiological analysis of fruity probiotic fermented milk during cold storage (Log CFU/ml).

Treatments	Storage period (days)	Microbial tests					
		T.VC	CF	ST	SPF	Psych	Mould &yeast
Control	0.0	8.12	ND	ND	2.20	ND	ND
	3	6.88	ND	ND	2.17	ND	ND
	7	6.11	ND	ND	2.18	ND	1.3
	11	4.52	ND	ND	2.16	1.5	1.5
mango pulp 5 %	0.0	8.34	ND	ND	2.23	ND	ND
	3	7.13	ND	ND	2.25	ND	ND
	7	6.41	ND	ND	2.25	ND	1.6
	11	4.95	ND	ND	2.23	1.8	2.1
Strawberries pulp 5%	0.0	8.32	ND	ND	2.21	ND	ND
	3	7.22	ND	ND	2.22	ND	ND
	7	6.35	ND	ND	2.24	ND	1.8
	11	4.81	ND	ND	2.25	1.7	2.3

These results are average of 3 replicates ND means not detected
T.VC = total bacterial count CF= Coliform group Psych= Psychrotrophic bacteria
ST= *Staph. aureus* SPF= aerobic sporeforminq bacteria

Accordingly, it is expected to encounter them in most of dairy products, as they are not affected by pasteurization. Since fermented milks are characterized by low pH-values, which don't enhance the growth of sporeforming bacteria, it is reasonable not to have significant discrepancies in counts during the storage since the fermented milk environment doesn't favour the growth of the sporeformers.

Since samples of yoghurt were stored in refrigerator, it is necessary to detect the presence of psychrotrophic bacteria. Psychrotrophic bacteria may release heat-resistant proteases and lipases, these enzymes won't be totally inactivated and may give rise to off-flavours (Tamime, 2009). These bacteria were detected in few numbers on eleventh day of storage in control and other treatments. Their numbers were limited and close to each other. Generally, molds and yeasts were absent in all treatments and control until the third day of cold storage However, molds and yeasts could be detected after 7 days in the control and all treatments. This means that such

organisms could not reach detectable levels after 3 days. Control sample scored the lowest number of molds and yeasts, the two other treatments contained higher of these organisms this may be due to Increasing of acidity.
It is noticeable that the addition of DSCAF sharply reduced the level of molds and yeasts in control ant treatments samples; this may be because of what DSCAF contains antioxidants compounds and anti-material compounds produced by therapeutic bacteria. These results were confirmed by another experiment which is summarized in Table (9).

Results given in table (8) show the changes in sensory evaluation of fruity probiotic fermented milk, whether fresh or after cold storage for 1, 3, 7 or 11 days. The total scores gradually increased in all examined treatments in the first week, and then gradually decreased till the end of storage period. On the other hand, fermented milk fortified with 5%mango pulp and 5% B S A F had the highest total scores of 93.0, followed by control then fortified 5 % Strawberries pulp and 5% DSCAF which gained the lowest scores of 91.0 in the first week samples. The flavour was highly affected by adding pulp of fruits and possessed the highest effect in the fresh samples or during storage because it had a moderate favourite and acceptable acidic taste.
It is worth mentioning that the samples appearance were marginal impact in total score on the other hand, negative impact emerged with Adding fruit pulp on Body and texture, this may be due to the weakness of the casein network

Table (8): Organoleptic properties of fruity probiotic fermented milk during cold storage

Cold storage period (day)	Treatments	Flavor (F) (60)	Body and texture (B&T), (30)	Appearance (A), (10)	Total (T), (100)
Control	Fresh	49.0	28.0	9.0	86.0
	3	53.0	28.0	9.0	90.0
	7	53.5	29.0	9.0	92.0
	11	52.5	27.5	9.0	89.0
Mango pulp (5%)	Fresh	51.5	27.5	9.0	87.0
	3	54.0	28.0	8.5	90.5
	7	56.5	28.0	8.5	93.0
	11	53.0	26.0	8.0	87.0
Strawberries pulp (5%)	Fresh	50.5	27.0	8.0	85.5
	3	53.5	27.5	8.0	89.0
	7	55.5	27.5	8.0	91.0
	11	51.0	25.0	8.0	84.0

Table (9): Effect of the additives on the growth of *Aspergillus niger* and *Aspergillus flaveus*

Fungi													
Aspergillus niger							*Aspergillus flaveus*						
Treatments													
C	DSCAF		YE		GDL		C	D SC A F		Y E		GDL	
-	3%	5%	0.2%	0.4%	0.2%	0.4%	-	3%	5%	0.2%	0.4%	0.2%	0.4%
9	3.8	2.2	5.1	4.8	4.5	3.6	9	4.1	2.6	5.5	4.8	5.0	4.1

C= control. The diameter growth disc was thrown
9 means full growth of fungi on the dish

Data presented in table (9) showed that in control both fungi filled the surface of the dish after 3 days, DSCAF was the best on inhibition of both *Aspergillus niger* and *Aspergillus flaveus* followed by GDL then YE. On the other hand, DSCAF (5%) scored less fungal growth dislike YE (0.2%) scored more fungal growth. Another important note, *Aspergillus niger* was more affected than *Aspergillus flaveus* by all additives. These findings reinforced our results in detection fungi during sample storage. With regard GDL these results Consistent with El-Etriby, *et al.,* 1997

Conclusion: This paper recommends addition of DSCAF (5%) during manufacturing probiotic fermented milk and adding (5%) of pulp fruits

REFERENCES

Barnett, J.A., R.W. Payne and D. Yarrow, 1990. Yeasts : characteristics and identification 2nd Edn., Cambridge Univ. Press, Cambridge, UK., pp: 1002.

Bodyfelt, F. W.; Tabias, J. and Trout, G. M. (1988). The sensory evaluation of dairy products. Pp. 227-270. Von Nostrand Reinhold, New York dairy foods. J. Dairy Sci. 83:894–907.

. Bridson, E.Y., 1990. The Oxoid Manual. 6th Edn., Unipath Ltd., Basingstoke, UK. Chalmer, C.H., 1962. Bacteria in Relation to the Milk Supplies. 4th Edn., Edward Arnold Ltd., London

Chalmer, C. H. (1962). Bacteria in relation to milk supplies. 4th Ed., Edward Arnold Ltd, London.

El-Etriby,H.M. El-; R.K. El-Dairouty, and A.H. Zaghloul,(1997) 'hysicochemical and bacteriological studies on mango yoghurt manufactured from ultrafiltered milkre tentate using glucono-delta- lactone(gdl). egyptian j. dairy sci., 25: 349-365.

El-Sayed, I. M. and ElShafei, H. K. (1996). Stimulating effect of Bif. infantis on the growth and biochemical activities of yoghurt starter bacteria. Alex. J. Agric. Res. 41(2): 159-166.

Gilliland, S. E. and Walker, D. K. (1990). Factors consider when selection a culture L. acidophilus as a dietary adjunct to produce hypocholesteromic effect in human. J. Dairy Sci. 73: 905-911.

Goh, J. S.; Kwon, I. K. and Kim, Y. O. (1983). Studies on the growth of *Bif. bifidum* ATCC 11863 in milk. Korean J. Dairy Sci. 8(1): 48-58.

Holm F. 2003. Gut health and diet: The benefits of probiotic and prebiotics on human health. The World of Ingredients. 2:52–55.

Kosikowski. F.V (1966) Cheese and fermented milk food, Cornell Univ. Ithaca NY

Krishnakumar V, Gordon IR. 2001. Probiotics: Challenges and opportunities. Dairy Ind. Inter. 66(2):38–40.

Kurmann, J. A. and Rasic, J. L. J. (1991). The health potential of products containing bifidobacteria pp 117-158. In: R. K. Robinson ed. Therapeutic properties of fermented milks El-Sevier Applied Sci., London.

Lee, G.J. and Jago, G.R. (1969). Methods for" estimation of acetaldchyde in cultured dairy products Australian J.Dairy Tech.. 24:181.

Ling, E. R. (1963). Text Book of Dairy Chemistry Vol. 2, Practical 3rd Ed. Chapman and Hal LTD, London.

Mahmoud, H. M. A. (1999). Production of bifidus fermented milk by stimulating Bif. bifidum ATCC11147. J. Agric. Sci. Mansoura Univ., 24(4): 1863-1870.

Mittal BJ, Garg SK. 1992. Acidophilus milk products: Manufacture and therapeutics. Food Reviews Intern.8(3):347–389.

Mohamed, F. A. F. (1994). Utilization of *L. acidophilus* and *Bif. bifidum* to produce fermented buffaloe's . Ph. D. Thesis, Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

Murti, T. W.; Bouillanne, C.; Landor, M. and Desmazeaud, M. J. (1993). J. Food Sci. 58: 153-157. (C.F. J. Dairy Res., 1995, 62: 151-187).

Ouwehand AC, Kirjavainen PV, Shortt C, Salminen S. 1999. Probiotics: Mechanisms and established effects. Intern. Dairy J. 9:43–52.

Playne MJ, Bennet LE, Smithers GW. 2003. Functional dairy foods and ingredients. Aust. J. Dairy Technol. 58(3):242–264.

Savova, I. and M. Nikolova, 2002. Isolation and taxonomic study of yeast strains from Bulgarian dairy products. J. Collection, 3: 59-65

Shah NP. 1997. Bifidobacteria: Characteristics and potential for application in fermented milk products. Milchwissenschaft 52(1):16–21.

Shah NP. 2000a. Probiotic bacteria: Selective enumeration and survival in

Shah NP. 2002. Bifidobacterium spp.: Applications in fermented milks. Encyclopedia of Dairy Science, Academic Press, London, pp. 147–151.

Shimamura, S.; Ishibashi, A.B.E.F.; Migakawa, N.; Yaeshima, H.;Araya, T. and Tomita, M. (1992). J. Dairy Sci. 75: 3296-3306. (C. F. J. Dairy Res. 1995, 62: 151-187).

Shin-Hanseung; Hwa, L. J.; Pestka, J. J.; Ustonul, Z.; Shin, H. S. and Lee, J. H. (2000). Viability of bifidobacteria in commercial dairy products during refrigerated storage. J. Food Prot. 63(3): 327-331.

Tamime, A.Y., 2009. Milk Processing and Quality Management. Blaekwell Publishing, New York.

Terzaghi, B. E. and Sandine, W. E. (1975). Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. 29: 807.

Difco Manual of Dehydrate Culture Media and Reagents (1974). Pub. Difco Laboratory Incorporated, Detroit, Michigan, 48, 201, U.

Ling, E. R. (1963). Text Book of Dairy Chemistry Vol. 2, Practical 3rd Ed. Chapman and Hal LTD, London.

Dave, R. I. and Shah, N. P. (1996). Evaluation of media for selective enumeration of St. thermophilus, L. bulgaricus, L. acidophilus and bifidobacteria. J. Dairy Sci. 79(9): 1529-1536.

Chandrasekhare. M.R.; Bhagawan, R.K.; Swaminathan, M and Subrahmanyan, V, (1957). The use of mammalian milk and processed milk foods in the feeding of infants Indian J. of Child Health. 6, 701

Westerfeld, W.W., 1945. A colorimetric determination of blood acetoin. J. Biol. Chem., 161: 495-499.

تاثير بعض الإضافات على خواص اللبن المتخمر ببكتريا البروبيوتك والمدعم بالفاكهة

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فى مُحاولةٍ لدراسة تاثير بعض المواد على نشاط بكتريا البروبيوتك اثناء تصنيع لبن مُختمر مُدعم بالفاكهة، وخواص هذا المنتج تم دراسة تاثير كل من GDL بنسبة ٠.٢ و ٠.٤ % و مُستخلص الخميرة بنفس النسب و الخميرة *Sacch. Cerevisiae* المُحطمة بعد التجميد والانصهار بنسبة ٣% و ٥% ، حيث تم دراسة تاثير هذه الإضافات على نمو البكتريا العلاجية فى بيئة MRS السائلة لمدة ٢٤ ساعة ، وُجِد ان (DSCAF 5%) و٠.٤ % YE و٠.٤ % GDL حققت افضل نمو ، فتم دراسة تاثيرها عند تصنيع لبن مُختمر من مَركز لبن بقرى UF (T.S. ١٤ %) .إنخفض وقت التجبن ساعتان عند اضافة (DSCAF(5% وساعة واحدة عند إضافة ٠.٤ % YE او ٠.٤ % GDL فى تلك الاثناء تم تقدير pH والحموضة وعدد بكتريا حامض اللاكتيك ، حققت بكتريا *L. acidophilus* اعلى نمو حوالى (8 (Log CFU/ml) تبعها فى ذلك بكتريا *Bif. bifidum* واخيرا جاءت *St. thermophilus*.

تم تصنيع لبن مُختمر من مَركز لبن بقرى UF (T.S. ١٤ %) مضاف اليه(5% DSCAF) ثم قسم اللبن الى ٣ اقسام الاول بدون فاكهة (كنترول) والثانى مضاف إليه ٥% لب المانجو والثالث مضاف إليه ٥% لب الفراولة ، وتم تتبع خواص العينات كيمائيًا وميكروبيًا وريلوجيًا وحسيًا خلال التخزين البارد ، بإضافة لب الفاكهة قلت الجودة الريولوجية والحسية لكن تحسنت بعض الصفات الكيمائية وحققت العينات اكثر من الحد الادنى لعدد البكتريا العلاجية فى نهاية فترة التخزين ولُوحظ قلة اعداد الفطريات مما حدا بنا تقدير تاثير الاضافات السابقة على نمو فطرى *Aspergillus niger* and *Aspergillus flaveus* وقد حققت DSCAF(5%) اكبر تثبيط لهذه الفطريات.

قام بتحكيم البحث

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