VIABILITY OF *Bifidobacterium* SP. IN ICE MILK PRODUCT ENHANCED BY SOME HERB OILS

Gooda, Effat; T. El Nemr and Malak Abbas.

Department of Dairy Science and Technology, Faculty of Agriculture (El Shatby), Alexandria University, Alexandria, Egypt.

ABSTRACT

Bio-ice milk product (lactose hydrolyzed) enhanced by peppermint oil and caraway oil were manufactured by culturing milk with *L. acidophilus, St. thermophilus* and *Bifidobacterium* sp.. Survivability of *Bifidobacterium* sp. was studied during 30 days of frozen storage at –25°C. Caraway oil bio-ice milk mixture was superior toward the viability of *Bifidobacterium* sp. at level above the minimum therapeutic level 10^5 CFU/g after 22 days of refrigerated storage. Also caraway oil bio-ice milk mixture showed higher acceptance in sensory evaluation comparing with the other treated bio-ice milk containing peppermint and control.

INTRODUCTION

Since Metchnikoff 1908 noted that the probiotic bacteria not only compete with “unhealthy fermentation” in human intestine, but also produce a number of beneficial health effects on the host by improving its intestinal microbial balance, *Lactobacillus* and *Bifidobacterium* sp. have been used commonly in food (Playne, 1991).

Probiotics including lactic acid bacteria and bifidobacteria, are important in the treatment of a wide range of human disorders including lactose intolerance, diarrhea, food allergies, intestinal infection, constipation, gastroenteritis, hepatic, encephalopathy, flatulence, colitis, gastric, acidity, high blood cholesterol and cancer (Godward *et al.*, 2000). These strains produce natural antibiotics and organic acids (such as lactic and acetic acid) that are inhibitory toward gram negative bacteria (Rasic, 1983).

Probiotic strains used have anticarcinogenic effect that can prevent the conversion of procarcinogenic into carcinogenic due to the content of their enzymes such as β-galactosidase, nitroreductase and azoreductase (Goldin *et al.*, 1984: Oda *et al.*, 1983 and Reddy *et al.*, 1983). Probiotic food products are gaining popularity with the increased awarenases of consumers as indicated by statistics showing market growth (Anon, 1997; 1999).

Yoghurt and another dairy fermented products were the famous dairy products in this regard (Aspasia and Robinson, 1994; Kailasapathy and Rybka, 1997; Malak *et al.*, 2000; and El-Nemr *et al.*, 2001). Also probiotics frozen dairy products were the point of many studies (Molder *et al.*, 1990) which were made by culturing milk with *Bifidobacterium* and *Lactobacillus* sp. before freezing.

Enhancement of probiotic strains growing in milk was the issue of several researchers including supplementation of peanut and amino acids (Murad *et al.*, 1997); Soya milk (Tridjoko *et al.*, 1992); Milk hydrolysate (Gomes *et al.*, 1998) and herb oils (Malak *et al.*, 2000 and El Nemr *et al.*,

2001). Herb oils are distinguishably used not only for initiating the growth of *bifidobacteria* and *Lactobacillus* sp. and their therapeutic action, but also might be used as flavouring materials such as peppermint (Arbuckle, 1975) and caraway oil. β-galactosidase was added to stimulate the growth and acid production of the starter strains as mentioned by Khattab *et al.* (1986).

Previous advantages of herb oils and milk lactose hydrolysis made us to believe in the production of the probiotic ice-milk product (low-fat) supplemented with herb oils (peppermint and caraway). Also, to study the viability of starter strains during storage of the ice milk product.

MATERIALS AND METHODS

Microorganisms:

Active thermophilic lactic culture which defined mixed strain cultures containing *Lactobacillus acidophilus; bifidobacteria* and *Streptococcus thermophillus* obtained from Hansen A/S, Denmark.

Starter culturing:

Thermophilic lactic culture directly poured with parameter of 5u/25L, 40°C, 5 hours, pH (4.5 – 4.8) to sterilized antibiotic free reconstituted skim milk 10% (W/V), then incubated at 37°C till coagulation.

Media:

Bifidobacterium sp. was counted on Lithium chloride – galactose – agar according to Lapierra (1992), using double layered plate. Whereas the other microbial content were counted as total bacterial count on standard plate colony (SPC) according to (APHA, 1990).

Herb oils:

Selected therapeutic oils, peppermint oil (*Mentha piperita*) and caraway oil (*Carum carvi*) were used at level of concentration of 0.05% for both oils in the mixture before aging (action through culturing). The previous concentration of herb oils were added before whipping and freezing. Caraway oil was purchased from Pembroek Industrwey 22, 1231Kh 1005 Drecht – Holland; whereas peppermint oil was purchased from Industries GMB SAAD LTDA virgili 124 Barcelona, Spain. All these oils were of pharmaceutical grade.

Manufacture of probiotic ice – milk:

Batches of ice milk mixture were standardized to have 3% milk fat, 12% milk solids not fat (MSNF), 15% sugar and 0.5% stabilizer – emulsifier. The following flow chart is showing the plan of the work:

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a. To the part a, sugar, stabilizer and emulsifier were added and aged overnight at 5^{o}C.

b. To the part b, starter culture and herb oil in treatment 3, 4, 5 were added and incubated at 37^{o}C till complete coagulation, then cooled and refrigerated.

Prior to freezing the mixtures, the two parts a and b, were well mixed together and then the flavouring ingredients (the access amounts of herb oil were added and frozen by batch freezer system at –3°C). The ice milk was filled in 125ml plastic cups and hardened at –20°C.

Therefore the five treatments of bio–ice milk which manufactured were:

1. Control, plain bio–ice milk with non – hydrolyzed milk lactose.
2. Plain bio – ice milk with hydrolyzed milk lactose.
3. As in No. 2 + peppermint oil.
4. As in No. 2 + double concentration of peppermint oil.
5. As in No. 2 + caraway oil.

Lactose hydrolysis:

Lactose of milk was partially hydrolyzed using β-galactosidase extracted (Maxilact, 20.000 ONPG unit/g) obtained from Gist Brocades Delf-Holland.

Sensory evaluation:

The ice milk was organoleptically evaluated in fresh and stored products, in accordance with the hedonic scale from 1 – 5. The ice milk samples were scored for firmness, chewiness, sourness, off flavour, iciness and total impression.

RESULTS AND DISCUSSION

The probiotics cultures which have been used grew and increased in number from 1.2×10^2 at zero time to reach 1.5×10^7 in control (treatment 1) till 2.6×10^6 in treatment 5 of *Bifidobacterium* sp. and from 3.5×10^2 at zero time to 4.2×10^7 (treatment 1) till 1.6×10^8 in treatment 5 of lactic acid culture. The increase in bacterial numbers was in correspondence with increase in acidity and decrease in pH value and affected in the reduction of clotting time. From Table (1) it was clear that the addition of β-galactosidase increased the bacterial activity (treatment 2, 3, 4, 5) and using herb's oil affected in different degrees on bacterial counts, pH, acidity and the reduction in clotting time.

Table (1): **Effect of β-galactosidase and herb oil on some properties of Bio-ice milk mixture and viability of culture bacteria before freezing.**

Treatments	*Bifidobacterium* sp.	T.C	pH	Acidity (%)	Reduction in Clotting time (min)
1	1.5×10^7	4.2×10^7	4.78	0.74	0.00
2	2.3×10^7	6.7×10^7	4.47	0.98	30.0
3	1.7×10^7	7.6×10^7	4.60	0.84	40.0
4	2.2×10^7	8.3×10^7	4.72	0.80	55.0
5	2.7×10^7	1.6×10^8	4.74	0.80	50.0

Viable counts of *Bifidobacterium* sp. and other bacterial counts were revealed in Table (2). Data clearly indicate that using β-galactosidase, peppermint oil and caraway oil greatly enhances the growth of *Bifidobacterium* sp. in bio-ice milk mixture. Pronounced increase in *Bifidobacterium* growth was noticed in the mixture which contained a caraway oil (2.6×10^7 CFU/ml) at the end of coagulation time and still found after 30 days of storage at –20°C in a count of 4.7×10^2 CFU/ml. Although the presence of lactic acid bacteria which might restrict the growth of *Bifidobacterium* (Aspasia and Robinson, 1994), but the supplementation with peppermint and caraway oil, developed the growth of Bifidobacterium and the other microbial counts.

Table (2): Probiotic bacteria counts and the total bacterial counts in the different treatment of ice milk product during refrigerated storage.

	Storage periods (days)	Treatments				
		1	2	3	4	5
Bifidobacterium sp.	1	1.25×10^7	2.2×10^7	2.1×10^7	1.9×10^7	2.3×10^7
	4	0.9×10^7	1.3×10^7	1.38×10^7	1.41×10^7	1.6×10^7
	6	3.3×10^6	6.4×10^6	6.1×10^6	5.5×10^6	6.7×10^6
	9	2.3×10^5	1.7×10^5	1.1×10^6	2.7×10^6	4.1×10^6
	22	1.1×10^3	2.1×10^3	1.1×10^1	2.5×10^1	3.5×10^5
	30	1.5×10^1	2.0×10^1	1.5×10^1	1.8×10^1	4.7×10^2
Total bacterial counts	1	4.1×10^7	6.3×10^7	7.1×10^7	7.6×10^7	1.1×10^8
	4	3.7×10^7	5.1×10^7	6.3×10^7	not detected	0.9×10^8
	6	3.6×10^7	2.3×10^7	4.1×10^7	4.7×10^7	6.8×10^7
	9	3.6×10^6	4.3×10^6	3.7×10^7	1.1×10^7	3.1×10^7
	22	4.5×10^4	1.4×10^5	1.9×10^5	3.5×10^5	4.3×10^5
	24	4.1×10^2	3.5×10^3	1.7×10^3	3.5×10^3	5.5×10^3

Many researchers studied viability of bifidobacterium in frozen dairy products, Holcomb *et al.* (1991) reported that *Bifidobacterium* and *Lactobacillus acidophilus* were able to survive and grow in frozen yoghurt before and after freezing. Modler *et al.* (1990) studied survival of *Bifidobacterium* in ice cream over 70 days of frozen storage and found approximately 90% of these bacteria survival during the storage period. On

the other hand Rodrigues *et al.* (1996) reported that 4% inoculum of 1:1 *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was sufficient to obtain dietetic yoghurt ice cream containing viable count of both bacterial strains at levels above the minimum therapeutic level of 10^5/g.

Table (3) gives the average score for sensory evaluation of bio-ice milk mixture flavoured by peppermint and caraway oil during storage period. Generally the final product was acceptable for all the judgers and the bio-ice milk mixture flavoured with caraway oil gained the highest score and more preferable followed by peppermint as flavouring agent (No. 3).

Table (3): Sensory evaluation of different bio-ice milk treatment during freeze storage.

Treatments	Storage period (days)	Sensory evaluation		
		Flavour (5)	Texture (5)	Total impression
]	1	4.0	4.0	Good and accepted
	7	4.0	4.0	Good and accepted
	14	4.0	3.8	Good and accepted
	21	3.5	3.6	Sour and ice
	30	3.0	3.5	Sour and ice
2	1	4.0	4.0	Good and accepted
	7	4.0	4.0	Good and accepted
	14	4.0	4.0	Good and accepted
	21	3.8	3.5	Slightly sour & cool
	30	3.6	3.0	
3	1	4.2	4.0	Sweety and mind mint flavour, more acceptable
	7	4.2	4.0	Sweety and mind mint flavour, more acceptable
	14	4.0	4.0	Sweety and mind mint flavour, more acceptable
	21	3.9	4.0	Sweety and mind mint flavour, more acceptable
	30	3.9	3.6	Little mint flavour, still acceptable
4	1	3.5	4.0	Strong mint flavour
	7	3.6	4.0	Strong mint flavour
	14	3.6	4.0	Strong mint flavour
	21	3.8	4.0	Accepted flavour
	30	3.8	3.8	Accepted flavour
5	1	4.3	4.0	Good mild accepted caraway flavour
	7	4.2	4.0	Good mild accepted caraway flavour
	14	4.2	4.0	Good mild accepted caraway flavour
	21	4.0	4.0	Good mild accepted caraway flavour
	30	4.0	3.8	Good mild accepted caraway flavour

fig1

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القدرة على النمو والبقاء لسلالة البيفيدوباكتيرم فى المثلوج اللبنى المحسن بإضافة بعض زيوت الأعشاب الطبية

عفت جودة ، طارق النمر ، ملك عباس

قسم علوم وتكنولوجيا الألبان ، كلية الزراعة (الشاطبى) ، جامعة الإسكندرية - الإسكندرية

المثلوج اللبنى الحيوى المحسن بإضافة مستخلص النعناع والكراوية تم تصنيعه بإنماء سلالة مختلطة من البيفيدوباكتيرم خلال عملية التصنيع. ولقد تم دراسة القدرة على النمو والبقاء لتلك السلالة خلال ٣٠ يوم على درجة حرارة –٢٠°م. ولقد أظهر مخلوط الكراوية أعلى معدلات للنمو والبقاء مقارنة بالمخاليط الأخرى حيث أحتفظ بالمعدلات العلاجية لتلك السلالة (٣,٥ × ١٠° خلية لكل جرام) بعد ٢٢ يوم من التخزين. كما أظهر أيضا ذلك المخلوط تفوقه تجاه الخواص الحسية مقارنة بالمخاليط الأخرى).