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CARAMINATIVE BIO-YOGHURT: ENRICHMENT OF BIFIDO-YOGHURT WITH SOME HERB OILS

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ABSTRACT

The ability of supplementing *Bifidobacterium* yoghurt with oils of herbs (Tolue or Anissed or Peppermint or Caraway oil) was investigated. Acceptable concentrations were 0.4, 0.2, 0.05 and 0.05% (v/v), respectively. Clear increases in acetaldehyde content, β-galactosidase activity and total organic acids were detected. On the other hand, the supplementation gives an additional benefits for yoghurt, not only therapeutic and caraminative effects but also enhancing of *Bifidobacterium* in milk. Sensory evaluation showed that fresh or stored bio-yoghurt containing Tolue or Anissed oils were acceptable followed by Peppermint and Caraway against plain bio-yoghurt.

INTRODUCTION

Yoghurt is the most important product of dairy fermented milk using yoghurt starter bacteria containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophillus*. It has been the main interest of numerous investigations (Räsic and Kurmann, 1978; Marshall, 1987; Alisonlevick, 1996 and Kailasapthy and Rybka, 1997).

Bifidobacterium spp. are considered to be “probiotic” which causes beneficial effects on foods fermented, particularly dairy fermented products (Gilliland, 1979 and Klaver, 1989). They provided microbial balance within the digestive tract (Fuller, 1991). Recently, many studies reported the beneficial effects of bifidobacteria on human health, including protection of infants from infections, lowering serum cholesterol, beneficial for lactose intolerant people, anticarcinogenic activity, and provision of B-vitamins group (Loria and Martin, 1991; Gupta, 1992; Dinakar and Mistry, 1994; Blanchette and Roy, 1995 and Damebodi and Gilliland, 1998). On the other hand, Mitsuoka (1990) reported that the bifidobacteria may be effective for the therapy of enteric and hepatic disorders and for stimulation of immune response.

For overcoming the difficulties of poorly growing of bifidobacteria in milk and their requirement of special conditions and media for their propagation with the addition of growth promoting substances, many articles pointed that issues (Gilliland, 1979; Khattab *et al*., 1986; Driessen, 1988; Murad *et al*., 1997; Nagendra and Warnakulsuriya, 1997 and Rajiv and Nagendra, 1997). Oils are distinguishably used for their therapeutic action, for flavouring or as starting materials for the synthesis of other compounds. These oils, such as Peppermint, Tolue, Caraway and Anissed oil, with a high phenol content, have antiseptic properties and are used as caraminatives (Tyler and Robbers, 1999). Oils showing antispasmodic activity and much

used in popular medicine. These caraminative effects express (a) stimulation of the stomach lining, (b) reflexive increase in stomach secretions resulting in improved digestion, (c) antispasmodic or spasmolytic effects on smooth muscle, (d) limiting the development of undesirable microorganisms, and (e) promotion of bile flow, facilitating digestion and absorption of nutrients.

The previous healthy effects lead us vigorously for enriching bio-yoghurt containing *Bifidobacterium* spp. with some herb oils for their previous benefits.

MATERIALS AND METHODS

Microorganisms :

Strains of yoghurt bacteria *Streptococcus salivarius* subsp. *thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were obtained from Hansen's Laboratories, Copenhagen, Denmark.

Bifidobacterium bifidum ATCC 11147 was obtained from American Type Culture Collection (USA).

Propagation of cultures :

Bifidobacterium bifidum was inoculated in selective medium (Lapierra, 1992) and then propagated in 10% (w/v) reconstituted skim milk (antibiotic free) and incubated at 37^{o}C for 48 hrs. Yoghurt starter bacteria were cultured in 10% (w/v) reconstituted skim milk at 42^{o}C for 3 hrs.

Media :

Bifidobacterium bifidum was cultured and counted on Lithium chloride-galactose-agar according to Lapierra (1992) using double layered plate. Whereas, the yoghurt samples were counted for total bacterial count on Standard Plate Colony Count (SPC) according to APHA (1992).

Bifidus milk preparation :

Homogenized pasteurized cows' milk was supplemented with 2% (w/v) skim milk powder, antibiotic free (U.S.A) and inoculated with 2% (v/v) of active grown subculture *Bifidobacterium bifidum* at 37^{o}C for 1 hr before inoculation with yoghurt culture (2% v/v). After coagulation, these products were kept under refrigeration (4$\pm$1^{o}C) for 14 days. The samples were analyzed for pH, acidity, acetaldehyde content, β-galactosidase activity, total organic acids, firmness, synersis, *Bifidobacterium* count, yoghurt starter count and organoleptic properties after 1, 3, 7, 10 and 14 days.

Herb oils:

Selected caraminative therapeutic oils; Tolue balsam oil (*Myroxylon balsamum*), Peppermint oil (*Mentha piperita*), Caraway oil (*Carum carvi*) and Anissed oil (*Pimpinella anisum*) were used, at concentrations of 0.4, 0.05, 0.05 and 0.2% (v/v), respectively. Anissed, Tolue and Caraway oils were purchased from Pembroek Industrieweg 22, 1231 Kh 1005 Drecht, Holland; whereas, Peppermint oil was purchased from Industrias GMB SAAD LTDA

Virgili 124 Barcelona, Spain. Previous oils were added before inoculation of yoghurt starter. All these oils were pharmaceuticle grade.

Methods of analysis:

1. Titratable acidity:

was examined as the percentage equivalent of lactic acid (Desjardins *et al*., 1990).

2. Firmness:

Firmness of yoghurt sample was detected as gram of stress according to Shalabi (1987).

3. Synersis :

Wheying of (synersis) was determined according to Harwalkar and Kalab (1983).

4. Acetaldehyde :

Acetaldehyde content, as ppm, was assessed using basic fuchsin reagent according to Robinson *et al*.(1977).

5. β-galactosidase activity :

Chromogenic substrate O-nitrophenol-β-galactoside (ONBG) (Sigma Chemical Co., St. Louis, Mo, U.S.A) was used for the determination of lactase activity according to Lin *et al*.(1989) at 420 nm. One unit of enzyme activity released 1 μM of O-nitrophenolamine.

6. Organic acids :

Organic acids were determined using Gas Chromatographic Method as described by Kang *et al*.(1988) with the following parameters: Column 80/100 carboback 4% carbowax 20M 2mm x 2m, FID detector; temp. 150°C Isotherm; Carrier gas N_2 20 ml/min; foil gases: H_2 1 m/min and air ½ ml/min; sensitivity 16 x 10^2; shart speed 5 mm/min; detector temp. 270°C. The apparatus used was Shimadzu GC 4CM, Japan.

7. Sensory evaluation :

The organoleptic properties of yoghurt samples were assessed according to El-Etriby et al.(1997) by a taste panel of 10 well trained persons from Dairy Science and Technology Department. The properties assessed were flavour, acid, bitterness, appearance, texture and firmness. The characteristics in each property were given scores out of 10.

RESULTS AND DISCUSSION

Firstly, different concentrations of herb oils were inspected with *Bifidobacterium* yoghurt aiming for the suitable concentration used. These concentrations were 0.05, 0.1, 0.2, 0.3 and 0.4% (v/v) from the oils. The organoleptic properties of yoghurt with various concentrations of oils revealed that the accepted levels were 0.4, 0.2, 0.05 and 0.05% (v/v) for yoghurt containing Tolue, Anissed, Peppermint and Caraway oils, respectively. Chosen concentrations were added to the milk after 1 hr of incubation with *Bifidobacterium*.

Addition of herb oils had no great effect on coagulation time. The averages of coagulation times were 170, 165, 170, 160 and 165 min, for control, Tolue, Anissed, Peppermint and Caraway bio-yoghurt, respectively.

Physico-chemical properties :

The data in Table (1) revealed the changes in pH, acidity and acetaldehyde content of caraminative bio-yoghurt supplemented with herb oils during storage period. pH of control treatment (without oils) and all oils bio-yoghurt treatments slightly decreased during the storage period. On the other hand, similar tendency in the acidity level which increased between 0.80% and 1.24% for the first day and the end of storage period of all treatments. Hild (1979) found a relation between the yoghurt flavour and acetaldehyde content. An increase in acetaldehyde content was noticed for the treatments containing oils (Table 1), which reached the the percentage increases of 93.36, 32.8, 28.71 and 16.26% for Tolue, Anissed, Caraway and Peppermint bio-yoghurt, respectively against control treatment. Acetaldehyde content of all treatments was increased by increasing storage period until 7 days then decreased. This increase is not due to only the oils, but also it could be realized that the *Bifidobacterium bifidum* had the best acetaldehyde production ability. This trend of acetaldehyde development agrees with the results of Akalin (1996) and El-Etriby *et al*.(1997).

Table (1): Changes in pH, acidity and acetaldehyde content of caraminative bio-yoghurt supplemented with herb oils during storage period. (average of 3 replicates)

Treatments	Storage period (days)	pH	Acidity (lactic acid %)	Acetaldehyde content (ppm)
Control	1	4.62	0.85	21.10
	3	4.37	0.99	23.10
	7	4.18	1.09	30.90
	10	4.11	1.20	19.10
	14	4.00	1.24	19.00
Tolue oil	1	4.67	0.80	46.70
	3	4.39	0.98	51.60
	7	4.19	1.15	55.60
	10	4.11	1.20	20.90
	14	4.12	1.22	17.89
Anissed oil	1	4.60	0.86	31.40
	3	4.40	0.90	59.10
	7	4.25	1.07	60.90
	10	4.15	1.12	51.70
	14	4.12	1.18	27.00
Peppermint oil	1	4.70	0.80	25.20
	3	4.41	0.98	31.70
	7	4.24	1.06	49.40
	10	4.11	1.09	39.40
	14	4.09	1.10	21.40

Caraway oil	1	4.64	0.84	29.60
	3	4.49	0.89	35.10
	7	4.24	1.00	51.00
	10	4.09	1.10	49.10
	14	4.05	1.13	32.70

Table (2) demonstrates the highest level of β-galactosidase activity on 3rd and 7th days during storage period. On 3rd day of storage, bio-yoghurt supplemented with Anissed, Caraway and Peppermint oil showed percentages of increase being 8.1, 6.0 and 4.8% against the control treatment in activity of β-galactosidase.

Table (2): Changes in β-galactosidase activity and total organic acids of caraminative bio-yoghurt supplemented with herb oils during storage period (average of 3 replicates)

Treatments	Storage period (days)	β-galactosidase activity (units/g)	Total organic acids (mg/g)
Control	1	74.50	90.90
	3	77.50	*
	7	76.50	127.38
	10	75.50	*
	14	74.50	190.10
Tolue oil	1	72.60	86.67
	3	76.50	*
	7	77.50	160.50
	10	77.50	*
	14	68.40	233.46
Anissed oil	1	77.50	90.82
	3	84.30	*
	7	78.40	102.12
	10	78.40	*
	14	77.50	204.48
Peppermint oil	1	77.50	109.08
	3	81.40	*
	7	78.40	115.56
	10	78.46	*
	14	68.20	137.97
Caraway oil	1	73.50	99.00
	3	82.40	*
	7	79.40	104.63
	10	78.40	*
	14	67.50	145.44

*** Not determined.**

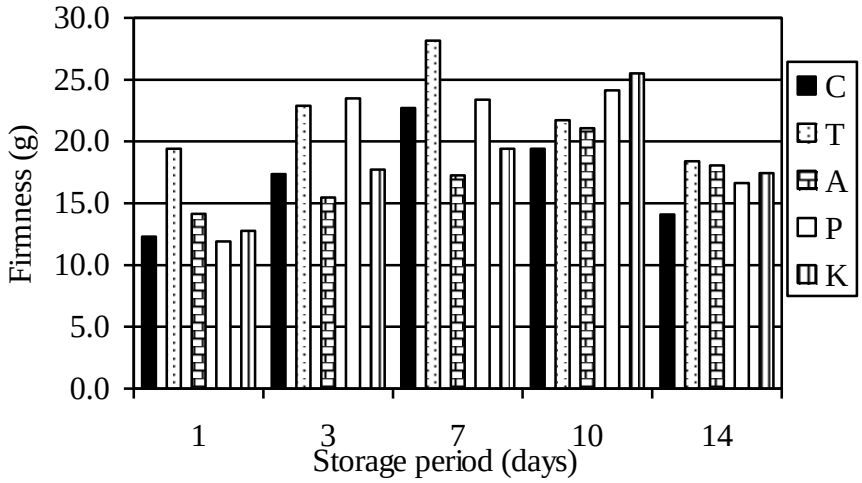
Several characteristics that would be beneficial in a bifidobacteria culture for yoghurt use are high β-galactosidase activity (Kolars *et al*., 1984 and Lin *et al*., 1991). On the other hand, a wide increase of total organic acid was obtained during the storage period (Table 2). Tolue and Anissed bio-yoghurt produced the highest level of total organic acid (233.44 and 204.48

mg/g) through 14 days of storage period comparing with other treatments. Misra and Kuila (1991) reported that the consumption of bifidus milk with high numbers will provide organic acids, antibiotic factor and live bifidobacteria in addition to other nutritional components.

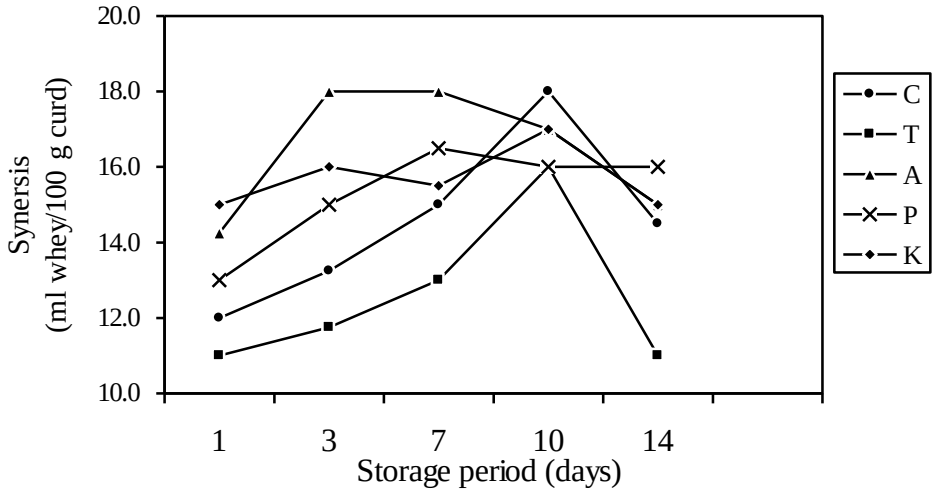
The relationship between synersis (wheying out) and firmness of caraminative bio-yoghurt was studied (Figure 1). Similar tendency of firmness for all treatments comparing with control treatment (Fig. 1A) which slightly increased until 7-10 days. These values were decreased in all treatments after 14^{th} days of storage. Similar observation was noticed in wheying out of all the treatments. The amount of whey per 100 g curd (Fig. 1B) reached 18, 16 and 17 ml for the control, Tolue and Caraway bio-yoghurt on 10^{th} day of storage, comparing with 18.0 and 16.5 ml for Anissed and Peppermint oils bio-yoghurt on 7^{th} day of storage. These results are in agreement with the trend of the data reported by El-Etriby *et al*. (1997).

Microbiological properties:

Viable counts of *Bifidobacterium bif*idum and yoghurt starter bacteria are shown in Table (3). Data clearly indicate that, in each treatment including control, the growth of *Bifidobacterium* was optimized after 3^{rd} day of storage period, then decreased during storage period. It seemed that oils enhanced the growth of *Bifidobacterium*. Distinguished increase of *Bifidobacterium* growth was revealed for the Peppermint oil bio-yoghurt (1.43×10^7 cfu/ml) at 3^{rd} day storage. Although the presence of yoghurt cultures may restrict the growth of *Bifidobacterium* in milk (Aspasia and Robinsonm, 1994), the supplementation of oils developed the growth particularly with the yoghurt starter. It means that the enrichment of bio-yoghurt with herb oils seemed as a two-edged weapon, firstly they play as a therapeutic and caraminative factor (Yaeshima, 1996), and second initiating the growth of *Bifidobacterium*. Many studies reported the ability of activation the growth of *Bifidobacterium* by the supplementation of peanut and amino acids (Murad et al., 1997) or soymilk (Tridjoko *et al*., 1992).



A: Firmness. (average of 5 replicates)



B: Synersis (average of 3 replicates)

Figure 1. Firmness and synersis of caraminative bio-yoghurt supplemented with herb oils during storage period.

C = Control; T = Tolue oil bio-yoghurt; A = Anissed oikl bio-yoghurt; P = Peppermint oil bio-yoghurt; K = Caraway oil bio-yoghurt

Table (3): Viable counts (cfu x 10^{-6}/g) of yoghurt bacteria and Bifidobacterium bifidum during fermentation of caraminative bio-yoghurt supplemented with herb oils. (average of 3 replicates.

	Storage period (days)	Control (plain)	Tolue oil bio-yoghurt	Anissed oil bio-yoghurt	Peppermint oil bio-yoghurt	Caraway oil bio-yoghurt
Bifidobacterium bifidum	0	0.50	0.50	0.50	0.05	0.05
	1	0.73	4.15	5.28	13.58	1.58
	3	1.25	6.00	6.60	14.30	6.08
	7	0.99	0.91	0.70	1.13	2.90
	10	0.98	0.70	0.70	1.85	0.45
	14	0.47	0.50	0.32	0.78	0.31
Yoghurt bacteria	0	0.90	0.90	0.90	0.90	0.90
	1	1.15	3.75	8.03	11.30	0.86
	3	3.00	3.00	13.00	8.00	1.15
	7	1.18	1.74	2.63	2.49	3.64
	10	0.72	1.33	1.09	2.80	1.36
	14	0.55	0.66	0.32	0.80	0.35

Consequently, a relationship between β-galactosidae activity and viable counts of *Bifidobacterium* was noticed (Tables 2 and 3). The highest activity of β-galactosidase for all treatments seems to be the cause of the optimization of *Bifidobacterium* counts at 3rd day of storage. This means that the β-galactosidae develops the growth of *Bifidobacterium*, which agrees with the data previously reported by Khattab *et al*.(1986).

On the other hand, similar trend of yoghurt starter bacteria counts was detected ($3.64x10^6$) for the Caraway oil bio-yoghurt at 7th day of storage. The rest treatments optimized between 1st and 3rd days of storage period.

Organoleptic properties :

Table (4) gives the average score for organoleptic properties of caraminative bio-yoghurt supplemented with oils containing herb oils during storage period. Overall the highest total score was given to Tolue and Anissed oils bio-yoghurt and then Peppermint and Caraway oils bio-yoghurt.

Table (4): Organoleptic scoring of caraminative bio-yoghurt supplemented with herb oils during storage period.

Treatments	Storage period (days)	Organoleptic properties						Average 10
		Flavour 10	Acid 10	Bitterness (no bitterness) 10	Appearance (10)	Texture 10	Firmness 10	

Control bio-yoghurt	1	8.7	9.5	10	10	10	10	9.70
	3	8.9	10	10	10	10	10	9.70
	7	8.1	9.5	10	10	10	10	9.60
	10	8.3	9.5	10	9.0	10	10	9.40
	14	8.4	9.0	10	9.0	9.5	10	9.30
Tolue oil bio-yoghurt	1	9.9	10	10	10	10	10	9.90
	3	9.9	10	10	10	10	10	9.90
	7	9.7	10	10	10	10	10	9.95
	10	9.8	9.9	10	10	10	10	9.95
	14	9.0	9.5	10	10	9.0	10	9.50
Anissed oil bio-yoghurt	1	8.7	9.5	10	10	10	10	9.70
	3	8.3	9.5	10	10	10	10	9.60
	7	8.4	9.0	10	10	9.5	10	9.40
	10	9.0	8.5	9.0	8.5	9.5	10	9.08
	14	8.9	8.7	8.0	8.0	9.0	9.0	8.60
Peppermint oil bio-yoghurt	1	7.5	8.5	10	10	10	10	9.30
	3	7.1	9.1	10	10	9.5	10	9.20
	7	7.9	9.0	7	10	7.5	9	8.40
	10	7.9	8.7	5	7.0	7.1	8	7.20
	14	6.1	8.0	3	7.0	7.0	8	5.60
Caraway oil bio-yoghurt	1	6.1	7.6	10	10	9.5	10	6.50
	3	6.1	6.7	9.0	9.0	9.1	10	8.30
	7	6.3	6.1	5.0	8.5	8.7	9.0	7.20
	10	6.7	6.1	3.0	7.0	5.1	6.5	5.70
	14	5.9	4.7	1.0	6.0	4.0	6.0	4.60

Generally, from the present investigation, it could be concluded that the fortification of bifidus-milk with caraminative herb oils has double effect; increasing the therapeutic and caraminative effects beside increasing *Bifidobacterium* growth in milk.

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الزبادى العلاجى: تدعيم زبادى البيفيدوباكتيريم ببعض زيوت الأعشاب الطبية

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تم دراسة إمكانية إدخال بعض زيوت الأعشاب الطبية العلاجية (التيليو – الينسون – الفليا والكراوية) فى تصنيع اللبن الزبادى المدعم بسلالة البيفيدوباكتيريم بيفيدم بجانب بكتيريا بادىء الزبادى.

ولقد أظهرت الدراسة أن التركيزات المثلى للإستخدام كانت 0.4، 0.2 ، 0.05، 0.05% (حجم/حجم) بالترتيب على التوالى. كما أظهرت الدراسة أن إستخدام تلك التركيزات أعطى معدلات كبيرة فى المحتوى من الأسيتالدهيد والبيتاجالاكتوسيداز وكذلك الأحماض العضوية الكلية. كما أعطى هذا التدعيم فوائد إضافية للبن الزبادى ليس كونه ذو قيمة علاجية وإنما إمتد إلى تنشيط نمو سلالة البيفيدوباكتيريم بيفيدم فى اللبن. وكان تقييم الخواص العضوية الحسية فى صالح اللبن الزبادى المدعم بالتيليو والينسون يليه المدعم بالفليا والكراوية مقارنة باللبن الزبادى الخالى من تلك الزيوت.