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IMPACT OF *Bifidobacterium befidium* ON VITALITY AND QUALITIES OF BIO-YOGHURT MADE WITH DIFFERENT STRAINS OF LACTIC ACID BACTERIA

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ABSTRACT

Four strains of lactic acid bacteria as the following I- *Str. thermophilus* + *Lactobacillus acidophilus* + *B. befidium* (1:2:1) treatment (B). II- *Str. thermophilus* + *Lactobacillus helveticus* + *B. befidium* (1:1:2) treatment (C). *Str. thermophilus* + *Lactobacillus bulgaricus* + *B. befidium* (1:2:2) treatment (D) were evaluated to their abilities at viability and growth rate when grown with *Bifidobacterium befidium* to produce bio-yoghurt as compared to traditional yoghurt made by traditional yoghurt starter IV- *Str. thermophilus* + *Lactobacillus bulgaricus* (1 : 1) treatment (A). Resultant yoghurt chemically, rhiologically, microbiologically and sensory evaluated when it was fresh or during storage period. Results showed that pH values in all treatments were higher than those in control and the acidity was in inverse trend to the pH in all treatments and control. T.V.F.A. values were increased in all treatments and control with the progress in storage periods as well as, treatment (B) gained the highest T.V.F.A. value among other treatments and control. Curd syneresis was in a correlation with the progress of acid content. Also, total bacterial count was increased with progress in storage until 3 days followed by a decrease but treatment (D) gained the highest growth rate among other treatments and control yoghurt. The strains were enhanced when they grown in a combination with *Bifidobacterium befidium* all except *Lactobacillus acidophilus*. No growths on macconkey agar media, no molds and yeast in all treatments and control yoghurt in fresh or stored product. The presence of proteolytic and lipolytic bacteria was increased with the progress in storage periods. Sensory evaluation data showed the consumers acceptance of all treatments except treatment (B) where it was weak in body and texture and more flat in aroma. From these results we can concluded that bio-yoghurt with healthy properties can be made with a combination with *Bifidobacterium befidium* to enhance its viabilities and to achieve the probiotic dose number (10^6 cfu/gm) without any aroma, body and texture defects. Also, these results orientate the future research works to enhance the properties of bio-yoghurt contains *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium befidium* as starter culture.

Keywords: Probiotic bacteria, bio-yoghurt, *Bifidobacterium befidium*.

INTRODUCTION

A new direction in the worldwide scientific programs in the field of milk industry is usage of the health protection abilities of the L.A.B. The fermented milks are the most direct approach to influence of L.A.B. on the human body. The yoghurt and yoghurt milks are defined as "new health-care foods" of the 21st century. During the last years the fermented milks science and technologies are developed rapidly. The restricted number of probiotic bacteria strains, which are used in the milk industry must be increased with LAB strains. These new strains have to produce new antimicrobial active substances and to form new ecological bio-systems, which can produce

fermented milks with guaranteed safety, organoleptic features and health benefits Simova, (2007).

Today, there are over 70 bifidus containing products produced worldwide, including sour cream, butter milk, yoghurt, powder milk and Cottage cheese. Little is known about the survival of *Bifidobacteria* in fresh cheese as well as their isolation on selective media, whereas, *Bifid, Befidium, Bifid. longum with Lb. acidophilus*, *Streptococcus thermophilus* and *Bifido. befidium* with cream starter culture were used for the production of some cheese types (Tamime *et al.,* 1995).

The health promoting effects of probiotic LAB include metabolic stimulation of vitamin synthesis and enzyme production, stabilization of gut microflora and competitive exclusion of enteric pathogens, enhancement of innate host defenses by production of antimicrobial substances, reduction of serum cholesterol by assimilation mechanisms, decreased risk or colon cancer by detoxification of carcinogens and tumor suppression by modulation of cell mediated immunity (Gerritse *et al*., 1990). *Lactobacillus acidophilus, Bifidobacteria* and *Lactobacillus casei* are considered to be probiotic because their consumption in certain numbers may exert various health benefits beyond inherent basic nutrition. They can be used alone or in association with other lactic acid bacteria for organoleptic or technological reasons. The flavour and consistency of milk fermented with this organism are often poor. Therefore, it has been incorporated into mixed starters used for yoghurt manufacture. *Lactobacillus acidophilus* normally metabolizes acetaldehyde to alcohol and also utilizes pyruvate in the presence of glucose and produces diacetyl. *Bifidobacteria* differ from lactic acid bacteria in that they produce not only lactic acid but also acetic acid as major fermentation products. They can ferment a wider range of carbohydrates than most lactobacilli found in fermented milks (Davies and Law, 1984).

This work aims to produce bio-yoghurt with good properties and effective dose of probiotic bacteria from combinations among probiotic lactic acid bacteria and *Bifidobacterium befidium*.

MATERIALS AND METHODS

Source of milk :

Fresh whole buffalo's milk which is standardized to 3 % milk fat and, 3.2 % protein was obtained from Agriculture Research and Experiments Center, Faculty of Agriculture, Mansoura University.

Lactic acid bacteria *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus acidophilus 145* (Lb acid type 145), *Lactobacillus helveticus* and *Bifidobacterium species 420 Befidium ssp*. strains obtained from the Microbiological Resources Center (Cairo MIRCEN) - Faculty of Agriculture, Ain Shams University.

Preparation of starter cultures:

Sterilized reconstituted skim milk powder was inoculated with the given bacterial isolates and incubated at 40 ± 2 ^{o}C, until coagulation. They are usually coagulated through 16 hrs.

Production of bio-yoghurt:

Fresh buffalo's milk used for bio-yoghurt making was standardized by reducing fat through cream separator to 3 % fat, standardized milk was heated to 95 °C for 15 min. and stirred, then subjected to cooling at 40°C, then inoculated with starter culture and incubated until coagulation takes place. Then the resultant yoghurt refrigerated and stored at 5±2 ºC until the end of storage periods.

Chemical analysis:

pH values were measured by using a glass electrode pH meter, type (digital pH Meter) Kinck. The determination was performed according to the British Standard Institution (B.S.I., 1976). The conventional Gerber's method was followed for detecting the fat content using the special butyrometer tubes for yoghurt as described by the British Standard Institutions (B.S.I) method (1955). Titratable acidity and the total nitrogen content of fermented milk were examined by following the method mentioned by Ling (1963). Total volatile fatty acids (T.V.F.A.'s) were determined using a direct distillation method according to Kosikowski (1978).

Rhiological analysis :

Curd synersis was detected after coagulation of bio- yoghurt from different treatments, the volume of separated whey was measured according to the method described by Ghaleb and Rashed (1983). The separated whey was collected in a graduated glass cylinder and measured first after 10 minutes and then regularly after 30, 60, 90 and 120 minutes.

Microbiological analysis:

Total bacterial counts of yoghurt were determined according to the American Public Health Association (1978) by plating the proper dilution in duplicates using nutrient agar medium (Difco manual, 1966). Mackonky agar was prepared as described by Oxoid manual (1962), to detect the presence of enterococci bacteria. Plates were incubated at 37˚C for 16-18 hrs. before counting. For counting the proteolytic bacteria present in the examined samples, the proteolytic agar medium (oxoid) described by Chalmer (1962) was used. The plates were incubated at 35 ºC for 48 hours. As with the lipolytic bacterial count, it was detected according to Berry (1933). The plates were incubated for 4 days at 30 ºC. The count of *Bifidobacteria* was determined according to Dave and Shah (1996) by using modified MRS agar supplemented with 0.05% L-cystein and 0.3% Lithuim chloride. The plates were incubated at 37 ºC for 48 hrs under anaerobic conditions. *Lactobacillus subsp.* counts were determined according to Gilliland and Walker (1990) by using modified MRS agar supplemented with 0.2 % oxagal. The plates were incubated at 37˚ C / 48 hrs before counting. Potato dextrose agar described by the Oxoid Manual (1962) was used for the enumeration of moulds and yeasts. Plates were incubated at 20-25 ºC for 5 days before counting.

Sensory evaluation:

Sensory evaluation was carried out according to Bodyfelt *et al.,* (1988). Treatments of bio- yoghurt scored for flavour out of 60 points, for body and texture out of 30 points and for appearance out of 10 points.

RESULTS AND DISCUSSION

Fresh buffaloe's milk (standardized to 3 % fat) was heated to 90 °C for 15 min. followed by cooling to 40 °C, inoculated with mixed strains under study and probiotic bacteria then incubated until coagulation followed by storage in refrigerator at 5±2 °C and chemically analyzed for pH value, titratable acidity, fat percent and total volatile fatty acids. Proteolytic and lipolytic bacterial counts, total count, mould & yeast count, and coliform bacteria after 0, 3, 7, 10 and 15 days were detected.

Chemical analysis :

Data in table (1) showed that pH values in all treatments were higher than those in control at fresh and during all storage periods and it was above 4.6 for bio-yoghurt in the fresh product. Moreover, the pH values were decreased in all treatments and control during storage periods. In addition, acidity content was in opposite trend with the pH and had the same trend in control and treatments in fresh and at all storage periods. Moreover, the change coefficient in the acidity was higher in control yoghurt when compared with other bio-yoghurt treatments. Bio-yoghurt made with *Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium befidium* 1:2:1) (treatment B) gained the highest pH value among other treatments. On the other hand, the treatment (D) *Str. thermophilus* + *Lactobacillus bulgaricus* + *B. befidium* (1:2:2) was the nearest treatment to the control yoghurt and this might be due to the interaction and cooperation between starter bacteria and the abundance of growth factors in some treatments for others. In addition, treatment (C) (*Str. thermophilus* + *Lactobacillus helveticus* + *B. befidium* 1:1:2) were the highest coefficient change (6.4) among all treatments and control yoghurt and this might be resulted from the increasing in the growth rate of culture strains, this behavior was stable until 10 days of storage periods, where the treatment B (*Str. thermophilus* + *Lactobacillus acidophilus* + *B. befidium* 1:2:1) listing the highest change coefficient and this also might be due to the resistance of culture starter to the bio-yoghurt circumstances. These results were in agreement with Abdel-Baky *et al*., (1987) and Ishibashi and Shimamura (1993), they reported that the pH of the finished bio-yoghurt must be maintained above pH 4.6.

Illustrated data in the same table shows the changes in moisture values. There was a gradual decrease in the moisture content of all treatments during the storage periods. Moreover, there were slight differences among either treatments or control yoghurt. This result might be due to the keeping of samples in refrigerator so, the cooling caused evaporation for some moisture of samples as well as total solids concentrated and increased .the differences in the change coefficient were slight and this might be due to the position of package in the refrigerator during storage. These results go in line with Gamal EL-Dein (1998).

The same data in table (1) showed the values of T.V.F.A.'s determination. These data indicates that the treatment (B) which consists of [*Str. thermophilus* + *L. acidophilus* + *Bifido. befidium* (*1:2:1*)] gained the highest value when compared with control treatment or the other treatments.

These results might be due to the ability of *Bifidobacterium befidium* on the production of some growth factors comes from the degradation of protein and lactose on bio-yoghurt, these growth factors increased the ability of another starter bacteria on the analysis of fat content and realizing the free fatty acids. On the other hand, T (D) had the lower T.V.F.A.'s content when compared with either control or other treatments but the change coefficient in the break down of fat and realizing the free fatty acids was highest in this treatment when compared with other treatments or control yoghurt during all storage periods. These results were in agreement with Eltibe, (2000).

Table (1): Changes of titratable acidity, pH values, fat content, total volatile fatty acids and moisture for yoghurt made from different mixed starters during storage at 5±2 ºC up to 15 days.

Test	Treatment	Storage periods (days)								
		Fresh	3	Coefficient %	7	Coefficient %	10	Coefficient %	15	Coefficient %
Acidity	A	1.10	1.32	20.0	1.55	40.9	1.58	43.6	1.62	47.3
	B	0.83	0.98	18.1	1.26	51.8	1.32	59.0	1.35	62.7
	C	1.08	1.15	6.5	1.39	28.7	1.56	44.4	1.59	47.2
	D	1.12	1.28	14.3	1.47	31.3	1.55	38.4	1.60	42.9
pH	A	4.54	4.37	-3.7	4.26	-6.2	3.97	-12.6	3.90	-14.1
	B	5.09	4.95	-2.8	4.59	-9.8	4.35	-14.5	4.32	-15.1
	C	4.66	4.36	-6.4	4.33	-7.1	4.03	-13.5	4.00	-14.2
	D	4.65	4.43	-4.7	4.28	-8.0	4.45	-4.3	4.08	-12.3
Moisture	A	83.6	82.9	-0.8	82.4	-1.4	81.9	-2.0	81.5	-2.5
	B	84.4	84.1	-0.4	83.7	-0.8	83.4	-1.2	83.1	-1.5
	C	84.9	84.2	-0.8	83.8	-1.3	83.5	-1.6	83.2	-2.0
	D	84.7	84.3	-0.5	83.6	-1.3	83.3	-1.7	83.1	-1.9
T.V.F.A 0.1 ml NaOH/ 100g	A	26.0	31.2	20.0	38.3	47.3	46.8	80.0	51.5	98.1
	B	32.2	36.8	14.3	40.0	24.2	45.6	41.6	53.2	65.2
	C	19.2	20.4	6.3	22.0	14.6	28.4	47.9	36.8	91.7
	D	11.6	14.8	27.6	19.0	63.8	22.1	90.5	31.6	172.4
Fat	A	3.0	----	----	----	----	----	----	2.6	-13.3
	B	3.0	----	----	----	----	----	----	2.1	-30.0
	C	3.0	----	----	----	----	----	----	2.8	-6.7
	D	3.0	----	----	----	----	----	----	2.5	-16.7
T.P	A	3.24	----	----	----	----	----	----	----	----
	B	3.12	----	----	----	----	----	----	----	----
	C	3.19	----	----	----	----	----	----	----	----
	D	3.29	----	----	----	----	----	----	----	----

A (Control) : Yoghurt made with *Str. thermophilus + Lactobacillus bulgaricus* (1 : 1).
B : Bio-yoghurt made with *Str. thermophilus + Lactobacillus acidophilus + B. befidium* (1:2:1).
C: Bio-yoghurt made with *Str. thermophilus + Lactobacillus helveticus + B. befidium* (1:1:2).
D: Bio-yoghurt made with *Str. Thermophilus + Lactobacillus bulgaricus + B. befidium* (1:2:2).

Data in the same table shows the changes in fat content of bio-yoghurt when it was fresh and at the end of storage periods. These data indicates that, there isn't any changes in fat content either among all treatments or control yoghurt, but after 15 days of storage the fat content decreased by different ratios among treatments. The treatment (B) which consists of [*Str. thermophilus* + *L. acidophilus* + *Bifido. befidium* (1:2:1)] gained the highest decrease among all treatments and control. Moreover, the control has the

lowest decrease and this might be resulted from the high lipolytic activity in all treatments when compared with control treatment. These results were in agreement with Eltibe, (2000).

Data in the same table indicates the values of total protein determination of bio- yoghurt and control yoghurt when it was at zero time only. These data indicates that there were slight differences in T.P content among all treatments and control. This result might be because all treatment and control made by the same milk and under the same conditions of manufacture.

Data in table (2) showed the changes of syneresis (ml/100g) for all treatments. These data indicated that the amount of released whey increased with the progress of syneresis time from (10 to 120 min) while it was fresh, it is noticed that treatments (D) & (A) gained the highest values, these results due to the high acid content in these treatments comparing with other treatments.

These results agreed with Amal EL-Saady (2010) who reported that the amount of released whey increased with the progress of syneresis time from (10 to 150 min).

Table (2): Changes of curd syneresis for yoghurt made from different mixed starters related with the acid content.

Treatments	acidity	Synersis (ml/100 g) after				
		10	30	60	90	120 min
A	1.10	0.8	1.4	1.9	2.3	2.50
B	0.83	0.7	0.9	1.7	1.90	2.30
C	1.08	0.8	1.2	1.8	1.92	2.40
D	1.12	0.9	1.3	1.9	2.12	2.60

A (Control) : Yoghurt made with *Str. thermophilus* + *Lactobacillus bulgaricus* (1 : 1).
B: Bio-yoghurt made with *Str. thermophilus* + *Lactobacillus acidophilus* + *B. befidium* (1:2:1).
C: Bio-yoghurt made with *Str. thermophilus* + *Lactobacillus helveticus* + *B. befidium* (1:1:2).
D: Bio-yoghurt made with *Str. Thermophilus* + *Lactobacillus bulgaricus* + *B. befidium* (1:2:2).

Data illustrated in table (3) showed the changes in number of total bacterial count. These data indicates that the numbers of bacteria in bio-yoghurt and control increased gradually with the progress of the storage up to 3 days, but after 3 days the numbers of bacteria for bio-yoghurt and control were decreased until the end of storage periods. It is noticed that control which consists of (*Streptococcus thermophilus* plus *Lactobacillus bulgaricus* 1: 1) and treatment (B) which consists of (*Str. thermophilus*: *L. acidophilus*: *Bifido. befidium* 1: 2: 1) gained the highest counts. These results were in agreement with Amal EL-Saady (2010) who reported that the number of bacteria in the examined samples increased gradually with the progress of the storage to 6 days after which the number of bacteria lowered.

Moreover, treatment (D) which consist of (*Str. thermophilus*: *L. bulgaricus*: *Bifido. befidium* 1:2:2) gained the highest growth rate among another treatments and control.

It is quite clear from the same table that moulds & yeast were absent in all treatments of bio-yoghurt and control treatment after manufacture in both fresh or stored ones. These results might be due to the presence of antimicrobial substances produced by starter culture. These results disagreed

with Ammara (2000) and Amal EL-Saady (2010), they reported that moulds & yeast could not be detected after manufacture (fresh) and after 3 days of cold storage. However, moulds & yeast were detected and counted after 6 days.

Table (3): Total bacterial count and mould & yeasts for yoghurt made from different mixed strains during storage at 5±2 ºC up to 15 days.

Storage periods (days)	Treatment	Total count $x10^7$ cfu/ml	Coefficient %	Moulds and yeast $x10^2$ cfu/ml
Fresh	A	60	---	N.D
	B	40	---	N.D
	C	36	---	N.D
	D	35	---	N.D
3	A	70	16.7	N.D
	B	54	35.0	N.D
	C	48	33.3	N.D
	D	52	48.6	N.D
7	A	40	-33.3	N.D
	B	30	-25.0	N.D
	C	34	-5.6	N.D
	D	36	2.9	N.D
10	A	8	-86.7	N.D
	B	16	-60.0	N.D
	C	2	-94.4	N.D
	D	5	-85.7	N.D
15	A	2	-96.7	N.D
	B	4.5	-88.8	N.D
	C	2	-94.4	N.D
	D	1.2	-96.6	N.D

A (Control) : Yoghurt made with *Str. thermophilus* + *Lactobacillus bulgaricus* (1 : 1).
B: Bio-yoghurt made with *Str. thermophilus* + *Lactobacillus acidophilus* + *B. befidium* (1:2:1).
C: Bio-yoghurt made with *Str. thermophilus* + *Lactobacillus helveticus* + *B. befidium* (1:1:2).
D: Bio-yoghurt made with *Str. Thermophilus* + *Lactobacillus bulgaricus* + *B. befidium* (1:2:2).

Data in table (4) showed the changes in numbers of proteolytic bacteria of bio- yoghurt and control. This data indicates that the proteolytic bacteria were increased gradually with the progress of storage up to 7 days, after 7 days these numbers were decreased. Treatments (A) and (B) gained the highest values and treatment (C) gained the lowest values, in addition treatments (B) & (C) gained the highest increase when compared with the other treatments. These results were in agreement with Rajagopal and Sandine (1990), they mentioned that Lactobacilli were highly proteolytic (61.0 to 144.6 mg of tyrosine/ml of milk) and *Streptococcus thermophilus* were less proteolytic (2.4 to 14.8 mg of tyrosine/ml of milk).

On the other hand, the same data in table (4) indicates that the number of lipolytic bacteria take the same behavior such as proteolytic bacteria. It is found that treatment (B) gained the highest value when compared with control and another treatments or bio-yoghurt. Moreover, treatment (C) gained the highest growth rate in both fresh until the end of storage. These data were in agreement with Abd El-Salam *et al*., (1994) and Amal EL-Saady (2010), they reported that proteolytic bacteria counts was higher in all

treatments in both fresh and after 6 days of storage and low from 9th to 15th days.

Data in table (4) showed that all treatments for bio-yoghurt and control were free from coliform in both fresh and stored ones. These results indicate that the manufacture of bio-yoghurt was carried out using the proper hygienic practices, results in the elimination of the contamination with such undesirable bacteria. These results agreed with Jordano *et al*., (1991); EL-Nagar and Shenana (1998) and Ammara (2000), they reported that coliform bacteria was not detected in all samples whether fresh or after storage.

Table (4): Proteolytic, lipolytic and coliform bacterial counts for yoghurt made from the different mixed starters during storage at 5±2 ºC up to 15 days.

Storage periods (days)	Combination	Proteolytic bacteria x10² cfu/ml	Coefficient %	Lipolytic bacteria x10² cfu/ml	Coefficient %	Coliform x10² cfu/ml
Fresh	A	8	----	4	----	N.D
	B	5	----	9	----	N.D
	C	1	----	2	----	N.D
	D	4	----	2	----	N.D
3	A	9	12.5	7	75.0	N.D
	B	8	60.0	11	22.2	N.D
	C	2	100.0	4	100.0	N.D
	D	5	25.0	3	50.0	N.D
7	A	11	37.5	10	150.0	N.D
	B	10	100.0	12	33.3	N.D
	C	7	600.0	6	200.0	N.D
	D	7	75.0	4	100.0	N.D
10	A	4	-50.0	5	25.0	N.D
	B	2	-60.0	4	-55.6	N.D
	C	1	0.0	1	-50.0	N.D
	D	3	-25.0	3	50.0	N.D
15	A	1	-87.5	4	0.0	N.D
	B	1	-80.0	4	-55.6	N.D
	C	1	0.0	1	-50.0	N.D
	D	2	-50.0	N.D	----	N.D

A (Control) : Yoghurt made with *Str. thermophilus* + *Lactobacillus bulgaricus* (1 : 1).
B: Bio-yoghurt made with *Str. thermophilus* + *Lactobacillus acidophilus* + *B. befidium* (1:2:1).
C: Bio-yoghurt made with *Str. thermophilus* + *Lactobacillus helveticus* + *B. befidium* (1:1:2).
D: Bio-yoghurt made with *Str. Thermophilus* + *Lactobacillus bulgaricus* + *B. befidium* (1:2:2).

Data in table (5) shows the survival of Lactic acid bacteria and probiotic bacteria in bio-yoghurt and control yoghurt. These data apparent that viable count of *Streptococcus thermophilus* increased gradually after manufacture to reach maximum after 3 days for all treatments and control then decreased during the storage periods. It's noticed that treatment (D) gained the highest value among another treatment and control. Moreover, treatment (D) gained the highest growth rate coefficient among another treatments and control. These results might be due to the presence of growth factors which produced from the protocooperation between *Streptococcus thermophilus* and

Lactobacillus bulgaricus which enhanced by the presence of *Bifidobacterium befidium*.

Lactobacillus bulgaricus for control increased gradually to reach maximum after 10 days. In addition, the viable count of *Lactobacillus bulgaricus* when it was in a combination with *Streptococcus thermophilus* and *Bifidobacterium befidium* {treatment (D)} was enhanced and that appeared in the increasing of the growth rate which was high in 7 days when compared with the high growth rate in (control) which was at day 10. These results might be caused by high analysis of protein fractions caused by *Streptococcus thermophilus* and *Bifidobacterium befidium*.

These results were in agreement with Iwana *et al*., (1993); Mihail *et al*., (2009) and Mengjin *et al*., (2009), they reported that *Streptococcus thermophilus* stimulated the growth of *Lactobacillus bulgaricus* by creating the necessary anaerobic conditions in the reactor and *Lactobacillus bulgaricus* produce the necessary amino acids and peptides for the *Streptococcus thermophilus* growth.

In the same table, *Lactobacillus acidophilus* increased gradually to reach maximum after 7 days then decreased until the end of storage.

This result agreed with Iwana *et al*., (1993); Olson and Aryana (2008) and Bari *et al*., (2009), they reported that higher inoculation levels of *Lactobacillus acidophilus* could increase the viability of *Lactobacillus acidophilus* and if the Lactobacilli counts are altered by wide variations in the *Lactobacillus acidophilus* inoculation level.

Data in the same table indicated that, there was an enhancement in the growth rate (68 x 10^7 cfu/ml) of *Lactobacillus helveticus* by the addition of *Bifidobacterium befidium* when compared with its growth with *Streptococcus thermophilus* only (64 x 10^7 cfu/ml). On the other hand, the viable count of *Lactobacillus helveticus* was decreased after 3 days of storage. Our data go in line with Iwana *et al*., (1993).

Bifidobacterium befidium increased gradually for all treatments after manufacture to reach maximum when 7 days except T (B) reached to maximum when reached 10 days then decreased until the end of storage. Moreover, *Bifidobacterium befidium* was able to keep its vitality until 10 days followed by decreasing at the end of storage periods and that might be resulted from the abundance of growth factors produced by other strains which were in a combination with *Bifidobacterium befidium*.

These results were in agreement with Iwana *et al*., (1993); EL-Nagar and Shenana (1998); El-Dieb *et al*., (2009) and Bari *et al*., (2009), they investigated the number of *Lactobacillus acidophilus* and *Bifidobacterium befidium* were found to be higher in the samples with higher levels of added probiotic bacteria. The counts of *Streptococcus thermophilus* increased slowly during storage up to 7 days and decreased later, *Lactobacillus acidophilus* and *Bifidobacterium befidium* decreased during storage periods. Increasing of cysteine improved the viability of *Bifidobacterium befidium* and *Lactobacillus bulgaricus*. Also, they reported that the initial number of *Bifidobacteria* in manufactured bio-yoghurt was 10^7-10^8 cfu/ml and their number was stable during the storage periods.

Table (5): Survival of probiotic and lactic acid bacteria in bio-yoghurt during storage periods for 15 days at 5±2 ºC.

Storage periods (days)	Treatment	*Str. thermophilus* x10^7 cfu/ml	Coeffecient %	*L. bulgaricus* x10^7 cfu/ml	Coeffecient %	*L. acidophilus* x10^7 cfu/ml	Coeffecient %	*L. helveticus* x10^7 cfu/ml	Coeffecient %	*B. befidium* x10^7 cfu/ml	Coeffecient %
Fresh	**A**	10	----	7	----	NE	NE	NE	ND	NE	NE
	B	37	----	NE	NE	44	----	NE	NE	26	----
	C	48	----	NE	NE	NE	NE	68	----	80	----
	D	59	----	70	----	NE	NE	NE	NE	40	----
3	**A**	16	60.0	14	100.0	NE	NE	NE	NE	NE	NE
	B	40	8.1	NE	NE	49	11.4	NE	NE	35	34.6
	C	51	6.3	NE	NE	NE	NE	68	zero	84	5.0
	D	64	8.5	80	14.3	NE	NE	NE	NE	49	22.5
7	**A**	12	20.0	35	400.0	NE	NE	NE	NE	NE	NE
	B	20	-45.9	NE	NE	55	25.0	NE	NE	41	57.7
	C	37	-22.9	NE	NE	NE	NE	45	-33.8	60	-25.0
	D	30	-49.2	50	-28.6	NE	NE	NE	NE	60	50.0
10	**A**	7.0	-30.0	49	600	NE	NE	NE	NE	NE	NE
	B	23	-37.8	NE	NE	38	-13.6	NE	NE	48	84.6
	C	20	-58.3	NE	NE	NE	NE	32	-52.9	58	-27.5
	D	21	-64.4	33	-52.9	NE	NE	NE	NE	56	40.0
15	**A**	2.9	-71.0	35	400.0	NE	NE	NE	NE	NE	NE
	B	6.5	-82.4	NE	NE	24.6	-44.1	NE	NE	43	65.4
	C	9.8	-79.6	NE	NE	NE	NE	11	-83.8	38	-52.5
	D	9.2	-84.4	18	-74.3	NE	NE	NE	NE	40	zero

A (Control) : Yoghurt made with *Str. thermophilus* + *Lactobacillus bulgaricus* (1 : 1).
B : Bio-yoghurt made with *Str. thermophilus* + *Lactobacillus acidophilus* + *B. befidium* (1:2:1).
C: Bio-yoghurt made with *Str. thermophilus* + *Lactobacillus helveticus* + *B. befidium* (1:1:2).
D: Bio-yoghurt made with *Str. Thermophilus* + *Lactobacillus bulgaricus* + *B. befidium* (1:2:2).

Organoleptic properties :

Data in table (6) showed the changes in organoleptic properties in bio- yoghurt and control. Data indicated that the (control) and (C) & (D) gained the highest total score points when fresh and after 3 days of storage periods. Then scoring of all treatments was decreased until the end of storage. These results were in agreement with Badran, Sanaa (2009).

Table (6): Organoleptic properties of probiotic yoghurt made from different mixed starters during storage periods at 5±2 ºC up to 15 days.

Storage periods (days)	Treatment	Appearance (10)	Body texture (30)	Flovour (60)	Total (100)
Fresh	A	9.0	28.0	57.0	94.0
	B	7.0	24.0	55.0	86.0
	C	9.0	28.0	57.0	93.0
	D	9.0	28.0	57.0	94.0
3	A	9.0	28.0	57.0	94.0
	B	7.0	24.0	55.0	86.0
	C	9.0	27.0	57.0	93.0
	D	9.0	28.0	57.0	94.0
7	A	8.0	27.0	56.0	91.0
	B	6.5	23.5	54.0	84.0
	C	7.5	26.0	56.0	90.0
	D	8.0	27.0	56.0	91.0
10	A	7.5	27.0	55.5	90.0
	B	6.0	22.0	53.0	81.0
	C	7.0	25.5	56.0	89.0
	D	7.5	27.0	55.5	90.0
15	A	6.0	24.0	52.0	82.0
	B	4.0	17.0	45.0	66.0
	C	5.0	20.0	48.0	73.0
	D	6.0	24.0	52.0	82.0

A (Control) : Yoghurt made with *Str. thermophilus* + *Lactobacillus bulgaricus* (1 : 1).
B : Bio-yoghurt made with *Str. Thermophilus* + *Lactobacillus acidophilus* + *B. befidium* (1:2:1).
C: Bio-yoghurt made with *Str. thermophilus* + *Lactobacillus helveticus* + *B. befidium* (1:1:2).
D: Bio-yoghurt made with *Str. Thermophilus* + *Lactobacillus bulgaricus* + *B. befidium* (1:2:2).

Conclusion

From previous data we can produce bio-yoghurt containing different lactic acid bacteria strains in a combinations with *Bifidobacterium befidium* and the resultant bio-yoghurt have the same properties of traditional yoghurt and had the probiotic dose number 10^6 cfu/g of yoghurt, but the bio-yoghurt containing *Str. thermophilus* + *L. acidophilus* + *B. befidium* (1:2:1) T (B) not accepted to the consumers, so, we must found means to enhance this product in future researches.

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تـأثير الــ *Bifidobacterium befidium* علـي حيويـة وصـفات الزبـادي الحيوي المصنع بسلالات مختلفة من بكتريا حامض اللاكتيك

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اختبرت أربع سلالات من بكتريا حامض اللاكتيك بنموها مع الـ *Bifidobacterium befidium* كالتالي:

١- (*Streptococcus thermophilus* : *Lactobacillus acidophilus* : *Bifidobacterium befidium* {1:2:1}) حيث أشير إليها بالمعاملة B .

٢- Streptococcus *thermophilus* + *Lactobacillus helveticus* + *B. befidium* (1:1:2) أشير إليها بالمعاملة C .

٣- Streptococcus *thermophilus* + *Lactobacillus bulgaricus* + *B. befidium* (1:2:2) أشير إليها بالمعاملة D

للتغيرات في معدلات نموها وحيويتها وتأثير ذلك علي صفات الزبادي الحيوي الناتج ومدي تقبل المستهلكين له بغرض إنتاج ألبان متخمرة ذات اثر علاجي باحتوائها علي السلالات موضع الدراسة لما لها من صفات صحية وعلاجية وقورنت جميع المعاملات بالزبادي المصنع من الباديء التقليدي *Streptococcus thermophilus* + *Lactobacillus bulgaricus* (1 : 1). تم تقييم جميع

المعاملات وعينة المقارنة كيماوياً وميكروبيولوجياً وريولوجياً وحسياً. أظهرت نتائج التحليل الكيماوي أن قيم الـ pH لجميع المعاملات كانت اعلي من الموجودة في الكنترول وكانت فلورا البادىء المكون من (*Lactobacillus acidophilus* : *Streptococcus thermophilus*) اعلي البادئات المختبرة في تحمل الظروف الموجودة في الـ bio-yoghurt المصنع كما حدث انخفاض في قيم الـpH خلال فترات التخزين و كان سلوك الحموضة معاكسا في جميع المعاملات والكنترول لسلوك الـpH .لقد شجع وجود الـ *Bifidobacterium befidium* جميع السلالات وضاعف من معدلات نموها وزمن التضاعف لهذه السلالات ماعدا المعاملة المحتوية علي المحتوية علي الـ *Lactobacillus acidophilus* حيث لم يحدث تحسن في معدلات النمو الخاصة به. زادت قيم الأحماض الدهنية الطيارة في جميع المعاملات مع التقدم في التخزين وحققت المعاملة المحتوية علي *Lactobacillus acidophilus*: *Streptococcus thermophilus* اعلي هذه المعدلات في حين كانت المعاملة المحتوية علي *Bifidobacterium befidium* (*Lactobacillus bulgaricus* : *Streptococcus thermophilus*) اقلها في تطور الأحماض الدهنية الطيارة. حدثت زيادة مطردة في أعداد البكتريا المحللة للبروتين وكذلك المحللة للدهن مع اختلاف المعاملات فيما بينها حيث كان أعلاها المعاملة B واقلها المعاملة C. لم يكن هناك ظهور للفطريات والخمائر وكذلك ميكروبات القولون في جميع المعاملات حتي نهاية التخزين. حدثت زيادة في التعداد الكلي للميكروبات في جميع المعاملات خلال بداية فترة التخزين وكان أعلاها معاملة الكنترول وحققت المعاملة D اعلي معامل نمو مقارنة بباقي المعاملات والكنترول ولوحظ انخفاض العدد الكلي للميكروبات بجميع المعاملات والكنترول مع التقدم في التخزين.كانت هناك اختلافات بسيطة فيما بين المعاملات والكنترول في نسبة البروتين والدهن منسوبا إلي المادة الجافة حيث زادت نسبة كلا منهما مع التقدم في التخزين وصاحب ذلك انخفاض طفيف في المحتوي الرطوبي لجميع المعاملات والكنترول.

قام بتحكيم البحث

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