Viability of *Lactobacillus plantarum* BfEL 92122 in association with commercial yoghurt starter in probiotic yoghurt

BY

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Abstract

The ability of potentially probiotic strain *Lactobacillus plantarum* BfEL 92122 to milk fermentation, acid & bile tolerance, bile salt hydrolase activity and the possibility to use different levels of *L. plantarum* association with commercial yoghurt starter for the manufacture of probiotic yoghurt were investigated. This strain exhibited ability on acid & bile tolerance and bile salt hydrolase activity. Also, it coagulated milk after ~ 24 hr incubation at 37°C, population reached 10^8 cfu/ml and the pH values ~ 4.6. Yoghurt made with 2% commercial yoghurt starter which contain *L. delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* spp. *thermophilus*, 1:1 as a control (T1), probiotic yoghurt made with 2, 2.5 and 3% bio-yoghurt starter which contain commercial yoghurt starter and *L. plantarum* culture, 1:1 (T2, T3 and T4, respectively). Yoghurt treatments were assessed for coagulation time, rheological properties, chemical analysis, microbiological quality and sensory evaluation when fresh and during storage up to 14 days at ~ 5°C. T1 and T4 nearly the same in coagulation time compared with T2 and T3, while progressive increase in acid production during storage were observed in T1 compared with T2, T3 and T4 especially at the end of storage. *L. plantarum* did not affect the growth of commercial yoghurt starter or chemical composition of yoghurt. Addition of *L. plantarum* in yoghurt production along with commercial yoghurt starter (T3 and T4) allowed to obtain yoghurts with an improve in the rheological, sensory properties and numbers of potentially probiotic bacteria at desired level $10^6 – 10^8$ cfu/g.

Key words: *L. plantarum*, probiotic, viability, acid tolerance, bile tolerance

Introduction

Yoghurt is generally fermented with a mixture of two species, *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus salivarius* spp. *thermophilus***.** In recent years, yoghurt has become popular vehicles for incorporating the probiotic bacteria. Probiotics are defined to be live micro-organisms that beneficially affect the host health (Mattila-Sandholm *et al*., 2002). For probiotic cultures, most commonly, *Lactobacillus* and *Bifidobacterium* strains are used (Holzapfel and Schillinger, 2002) and probiotic products are considered to be safe and have GRAS (Generally Regarded As Safe) status. Some *lactobacilli* are used as probiotics, *e.g*. *Lactobacillus plantarum* (Gomez *et al*., 1996; Francois *et al*., 2004; Maragkoudakis *et al*., 2006; Ismail *et al.,* 2007 and Modzelewska *et al*., (2008).

Probiotic microorganisms must fulfill certain criteria before they can be used in food production. This includes safety and functionality aspects and the technological properties of strains. Functional aspects of probiotic selection include: tolerance to low pH, bile salts and bile salt hydrolase activity or cholesterol removal. Jones *et al*. (2004) and Ismail *et al*. (2007**)** show that, *L. plantarum* 80 (PCBH1) and *L. plantarum* BfEL 92122 cells can efficiently break down and remove bile acids, and establish a basis for their use in lowering blood serum cholesterol. Technological properties, *e.g.* beneficial influence on sensory properties of products, survivability during food processing and stability in the product during storage (Saarela *et al*. 2000). Another important factor, which might be crucial, is its ability to proliferate on a large scale. Moreover, a strain should be appropriately chosen for a product in which it will be applied to facilitate a desired process, *e.g.* acid fermentation. The final product should be characterized by accepted shelf-life and sensory properties (colour, flavour, taste, texture), and desired numbers and activity of the probiotic strain during the whole storage period or even longer, as well as interactions of the probiotics with the starter cultures (Heller, 2001).

The aim of this study was to investigate the ability of potentially probiotic strain *Lactobacillus plantarum* BfEL 92122 to milk acidification, acid & bile tolerance, bile salt hydrolase activity and the possibility to use different levels of *L. plantarum* with commercial yoghurt starter for the manufacture of probiotic yoghurt.

MATERIALS AND METHODS

Materials

- Fresh mixed milk (cows and buffaloes, 1:1) were obtained from the herds of Faculty of Agriculture, Moshtoher, Benha University.
- Skimmed milk powder was obtained from Agri-Best Holland, purchased at Al-Bassyouny and Partners Comp., Meit Ghamr Dakahleia, Egypt.
- Bile salts: Ox-bile salt (Sodium tauroglycocholate) was obtained from BDH Chemicals Ltd Poole England. Sodium salts of taurodeoxycholic acid, TDCA) was obtained from Sigma-Aldrich Chemie GmbH Germany, while sodium desoxycholate from Difco Laboratories, Incorporated, Detroit, Michigan.
-Gas Generating Kit was obtained from Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK)

- Cultures:

Lactobacillus delbrueckii ssp. *bulgaricus* Lb-12 and *Streptococcus salivarius* ssp. *thermophilus* TH-4 were obtained from Chr. Hansen,s A/S. Denmark. While, *Lactobacillus plantarum* BfEL 92122 from Institute of Microbiology, Federal Research center for Nutrition and Food (BfEL), Kiel, Germany (by contact).

- Activation of cultures:

Lactobacillus delbrueckii ssp. *bulgaricus* Lb-12, *Streptococcus salivarius* ssp. *thermophilus* TH-4 and *Lactobacillus plantarum* BfEL 92122 were activated (subcultured) 3 times before use in sterile de Man, Rogosa, Sharpe (MRS) or M17 broth

(according type) using 1% inoculum and incubated for 24 h at 37°C, then reactivated twice ($10^6 – 10^8$ cfu/ml) and conserved in refrigerator (Abd El-Fattah, 1999).

Commercial yoghurt starter (*Lactobacillus delbrueckii* ssp. *bulgaricus* Lb-12 & *Streptococcus salivarius* ssp. *thermophilus* TH-4, 1:1) and *Lactobacillus plantarum* BfEL 92122 were separately and together at ratio of 1:1 (as a bio-yoghurt starter) incubated at 37ºC, during reactivation by three successive transfers in sterile 11% reconstituted skimmed milk powder (10^6-10^8 cfu/ml). The active starter cultures were kept in refrigerator until use (through 24 hr, Badawi *et al*., 2004)

- Yoghurt manufacture:

Some trials were conducted to know the ratio from bio-yoghurt starter which can be inoculated to yoghurt milk to give acceptable yoghurt and the count of *Lactobacillus plantarum* reach to 10^6-10^8 cfu/g in fresh yoghurt. The obtained results clear that the best ratio from bio-yoghurt starter were 2, 2.5 and 3% compared with control yoghurt made with 2% commercial yoghurt starter.

Yoghurt was manufactured according to Tamime, (1978) from fresh mixed milk standardized to ~ 3% milk fat. It was heated to 85°C for 30 min, immediately cooled to 42ºC and divided to four portions (5 Kg each), and then inoculated with 2% commercial yoghurt starter (as a control yoghurt, T1), 2, 2.5 and 3% bio-yoghurt starter T2, T3 and T4, respectively (as a probiotic yoghurt).

All treatments were put into yoghurt plastic cups (100 ml) and incubated at 42ºC until the pH reached ~ 4.7 (coagulation time is recorded). Then, the treatments transferred to refrigerator and maintained at ~5ºC. Yoghurts were analysed for the rheological, chemical, microbiological tests, and they were sensory evaluated when fresh and after 7, and 14 days.

Methods of analysis:

-Milk acidification

The ability of *Lactobacillus plantarum* BfEL 92122 compared with commercial yoghurt starter to grow and acidify milk was analysed by inoculation of 9 ml of sterile skimmed milk with 0.01 ml of bacterial culture. Fermentation proceeded at 37°C for 24 hr (Rönka *et al*., 2003). After inoculation, 2, 4, 6, 8, 12, 16, 20 and 24 hr of fermentation, the bacterial counts and pH were measured.

-Acid tolerance test

Lactobacillus plantarum BfEL 92122 was tested for tolerance to low pH for up to 90 min according to Lan-szu and Bart, (1999). Acid tolerance test was evaluated by growing *L. plantarum* BfEL 92122 in MRS broth adjusted to acidic pH 3.5 and 2 by adding HCl and non-acidified MRS broth pH 6.5 and incubating at 37°C for 90 min in an anaerobic conditions (Gas Generating Kit, Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK). Samples were collected during incubation period intervals 0, 30, 60 and 90 min and plate counts were done using MRS agar and the pour plate technique. Acid tolerance was determined by comparing the final plate count after 90 min with the initial plate count at 0 hr. The experiments were repeated twice.

Lactobacillus plantarum BfEL 92122 was subcultured at least 3 times before experimental use. Also, the inoculation (10% v/v) into the broth and growth monitoring using the plate count method.

-Bile salt tolerance test

Ox-bile salt was used to study bile tolerance of the *Lactobacillus plantarum* BfEL 92122 according to the method of Gilliland and Walker, (1990). Activated (overnight) culture was diluted into 10 ml MRS broth medium containing different concentrations (0, 0.1, 0.3, 0.5, 1 and 3%) of the ox-bile salt. The control comprised MRS broth without bile salt. Samples were incubated at 37°C and bacterial growth was monitored by measuring absorbance with spectrophotometer (Shimadzu, UV-120-02) at O.D 620 nm at hourly

intervals for 7 to 8 hr. The inoculation (10% v/v) into the broth and all experiments were replicated twice.

-Bile salt hydrolase activity assay

Lactobacillus plantarum BfEL 92122 was tested for bile salt hydrolase activity with a plate assay on MRS agar supplemented with 0.5% sodium salts of taurodeoxycholic acid, TDCA, (Scott and Dwayne, 2001). Activated (overnight) *Lactobacillus plantarum* culture was diluted and plated onto MRS agar containing TDCA. The plates were incubated anaerobically at 37°C for 48 hr. Bile salt hydrolase activity was indicated by deoxycholic acid precipitate around the colonies.

- Chemical analysis:

Titratable acidity, total solids, fat, ash and protein contents of yoghurt treatments were determined according to the methodology mentioned by A.O.A.C, (1990). Lactose content was determined as suggested by the phenol-sulphuric method of Barnett and Abdel-Tawab, (1957). Acetaldehyde content was determined according to the method described by Lees and Jago, (1969). pH value of yoghurt samples was determined using a pH meter (JENCO Model 1671, USA)

- Microbiological examinations:

Lactic acid bacteria (LAB); yeasts & moulds and coliforms were counted according to Elliker *et al*. (1956); IDF, (1990) and APHA, (1992) respectively. While, the counts of *Streptococcus salivarius* ssp. *thermophilus* TH-4 was counted as described by Ryan *et al.* (1996).

Counts of *Lactobacillus plantarum* BfEL 92122 in pure cultures was determined on MRS agar, whereas the numbers of *L. plantarum* and *L. delbrueckii* ssp. *bulgaricus* in yoghurts were determined on MRS with maltose and bromocresole purple (Burbianka *et al.*, 1983). Colonies were counted after 72 hr of incubation at 30°C or 42°C (according type) under anaerobic conditions.

- Rheological analysis:

Curd firmness of yoghurt was measured using the Penetrometer Model Koehler Instruments Co., (USA) controller as described by Kammerlehner and Kessler, (1980), the depth of penetration (0.1 mm = penetrometer unit) was measured after 5 sec at ~25°C (using cone weight 30 g and cone angle 45°C. The higher record by the penetrometer reading, refer to less firmness of yoghurt. Curd syneresis (the serum separation) was determined according to the method of Mehanna and Mehanna, (1989)

- Sensory evaluation:

Yoghurt samples were evaluated organoleptically by 10 of the Staff Members of Food Science Department, Faculty of Agriculture, Moshtoher, Benha Univ., scoring was carried out as recommended by Harby and El-Sabie (2001) as follow: flavour (50 points), appearance (10 points), body & texture (40 points) and total scoring (100 points)..

- Statistical analysis:

Statistical analysis for the obtained data was carried out according to the method described by Clarke and Kempson, (1997).

RESULTS AND DISCUSSION

Functional aspects of probiotic selection include: *e.g.* tolerance to low pH, and bile salts. Moreover, a strain should be appropriately chosen for a product in which it will be applied to facilitate a desired process, *e.g.* acid fermentation. The final product should be characterized by accepted shelf-life and sensory properties (colour, flavour, taste, texture), and desired numbers and activity of the probiotic strain during the whole storage period or even longer.

Milk acidification

Bacterial strains used in fermented dairy products should be characterized by good technological properties such as the ability to ferment and acidify milk to the pH

value of 4.4–4.6 after 14–16 hr, to maintain viability in products during storage and to exert a benificial influence on the sensory characteristics of products. Also, the ability of the bacteria to proliferate in milk has a great technological significance (Rönka *et al*., 2003). The above-mentioned requirements do not refer to all probiotic strains, because some of them show a limited ability to proliferate in milk and acidify milk to the isoelectric point.

L. plantarum BfEL 92122 showed slow growth in milk, after ~ 24 hr incubation at 37°C, populations reached $1o^8$ cfu/ml and the pH value was 4.6 (Fig 1a and b), which is a sufficient number of probiotic bacteria in a food product to affect the host. While commercial yoghurt starter (*Lactobacillus delbrueckii* ssp. *bulgaricus* Lb-12 and *Streptococcus salivarius* ssp. *thermophilus* TH-4, 1:1) exhibit the same result after ~ 6 hr incubation at 37°C. This may be due to that the proteolytic activity of *L. plantarum* has a significant influence on its growth rate and acid production (Modzelewska et al., 2008). Thus, it cannot be used separately as starter cultures in fermented products, but their application as adjuncts is possible.

Acid tolerance:

The results as shown in Fig (2) clear the survival of *L. plantarum* BfEL 92122 at different pH values (6.5, 3.5 and 2) during incubation at 37°C for 90 min. Little or no reduction was noticed in the count of *L. plantarum* during incubation at pH 6.5 and 3.5, while it did not survive at pH2 for 90 min. So, *L. plantarum* BfEL 92122 is considered to be acid-tolerant strain. Ismail *et al*. (2007) studied the effect of different pH values on the survival lactobacilli strains at 37°C for 90 min. They considered survival at pH 3.5 as a criterion for acid tolerance, although acid tolerance until up to pH 2 was tested to detect highly acid tolerant strain. Barada *et al.* (1991) mentioned that, the probiotic bacteria are exposed to acid-stress in the stomach with its low pH (pH3.5 or lower). The food transit time through the human stomach is about 90 min.

a **b**

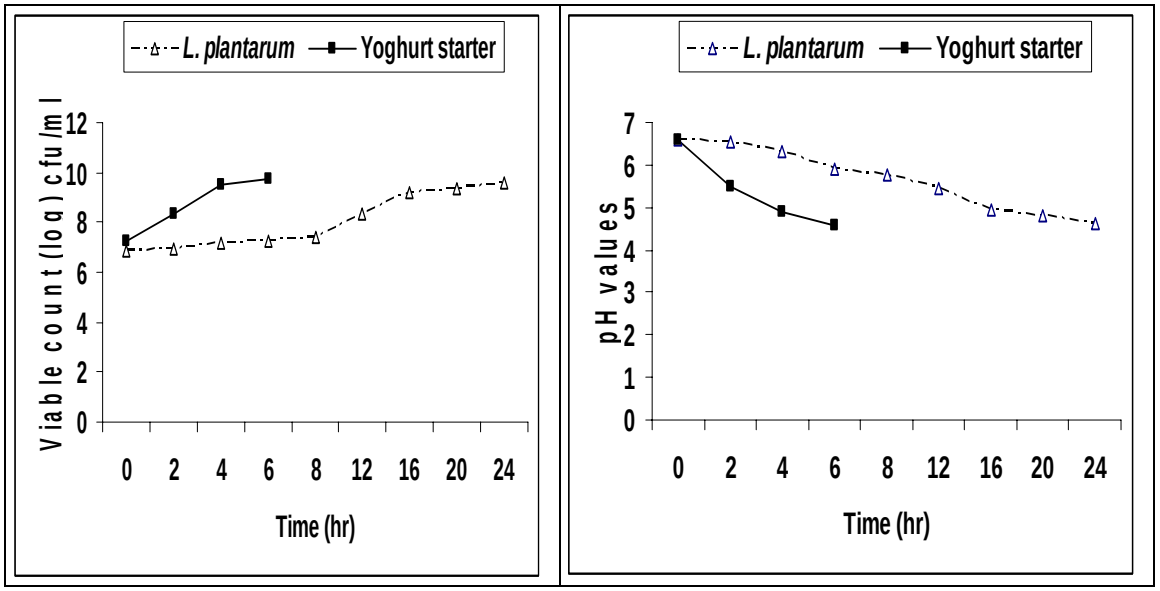


Fig (1a and b): Survival of *L. plantarum* BfEL 92122 & commercial yoghurt starter cultures (log cfu/ml) separately and pH of milk during fermentation at 37ºC.

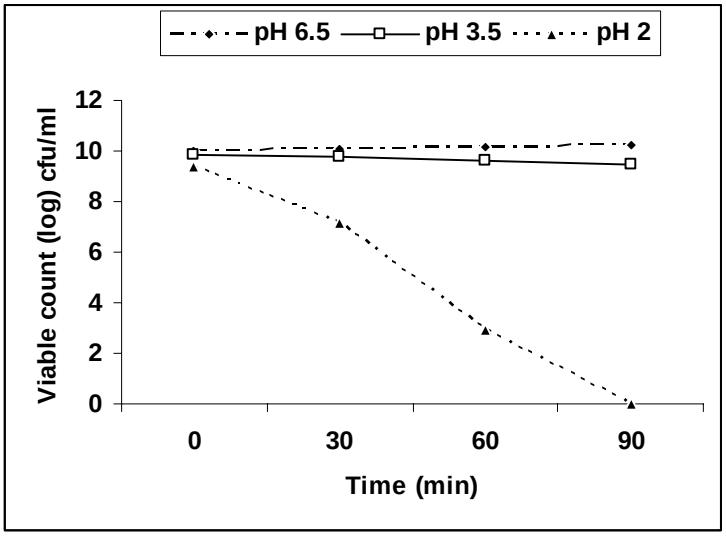


Fig (2): Survival of *L. plantarum* BfEL 92122 (log cfu/ml) in MRS medium at different pH values during incubation at 37ºC for 90 min.

Bile tolerance and hydrolase activity:

Data in Fig (3) show the effect of different concentrations (0, 0.1, 0.3, 0.5; 1, and 3%) of ox-bile salt on the growth of *L. plantarum* BfEL 92122. This strain exhibited highly resistant to bile salt and able to grow in 3% bile.

On the other hand, *L. plantarum* BfEL 92122 tested for bile salt hydrolase activity, expressed bile salt hydrolase and deconjugated taurine-bile acid and produced precipitate around the colonies on agar medium which containing 0.5% TDCA. Bile salt hydrolase activity was indicated by deoxycholic acid precipitate around the colonies (data shown in Fig 4). These results are confirmed with those represented by Ismail *et al*. (2007).

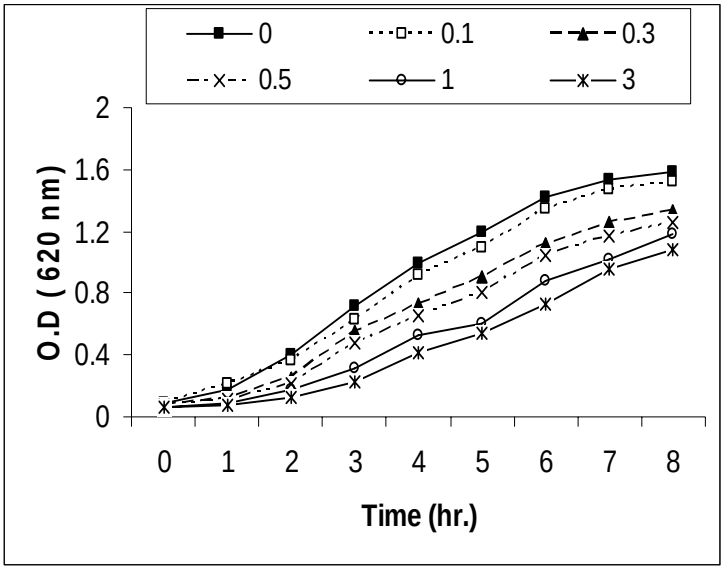
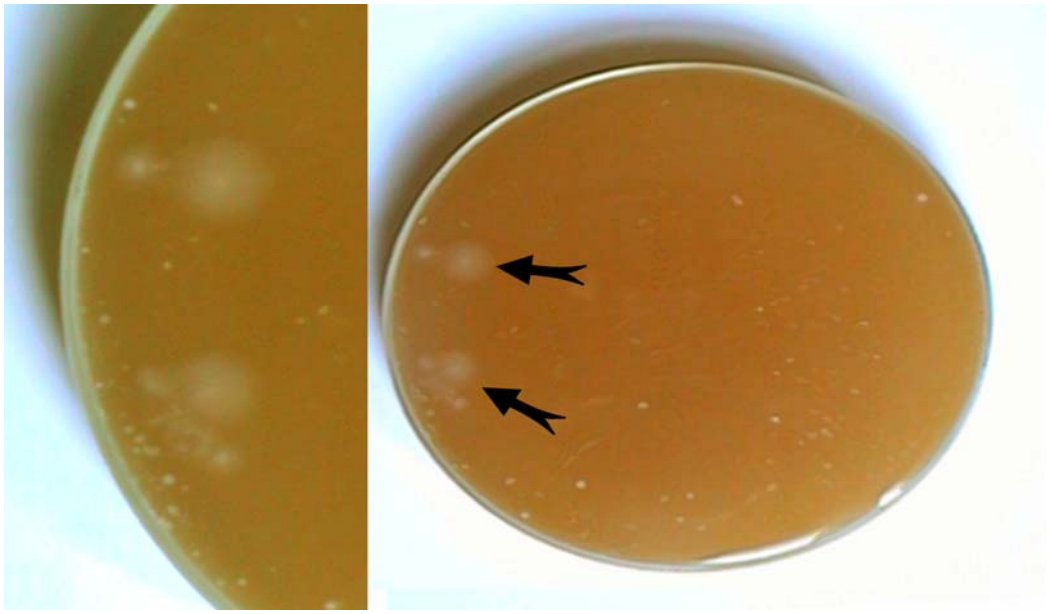


Fig (3): Effect of different concentrations (%) of ox-bile salts on growth of *L. plantarum* BfEL 92122



Fig(4). Bile salt hydrolase activity of *L. plantarum* BfEL 92122 as detected by plate assay method on MRS supplemented with TDCA.

Some properties of yoghurt

Setting time, curd syneresis and curd firmness:

Results presented in Table (1) show the setting time, curd syneresis and curd firmness values of yoghurt made with either 2% commercial yoghurt starter alone as a control T1 or associated with 2, 2.5 and 3% bio-yoghurt starter (T2, T3 and T4, respectively) as a probiotic yoghurt. These results indicate that, yoghurt treatments T1 and T4 presented highly significant decrease ($P<0.01$) in setting time compared with T2 and T3. This may be attributed to different types and levels of starter culture in the treatments as *L. plantarum* has slow acidification property (Francois *et al*., 2004 and Modzelewska *et al*., 2008).

The rheological properties of probiotic yoghurts compared with control yoghurt were assessed by monitoring the rate of serum separation (syneresis) and curd firmness of the product at the intervals storage at ~ 5°C. Curd syneresis of T1 and T4 were slightly

Table (1): Setting time, curd syneresis and curd firmness of probiotic yoghurt when fresh and during storage.

Treatments	Setting time (min)	Curd syneresis (g/15gm curd /10 min)			*Curd firmness (0.1mm)		
		Fresh	7 days	14 days	Fresh	7 days	14 days
T1	224^{C}	2.7^{A}	2.47^{BC}	2.29^{CD}	266^{D}	252^{F}	228^{I}
T2	252^{A}	2.0^{D}	1.95^{EF}	1.81^{F}	280^{A}	266^{D}	245^{G}
T3	240^{B}	2.3^{CD}	2.15^{DE}	1.93^{EF}	275^{B}	257^{E}	236^{H}
T4	217^{C}	2.6^{AB}	2.41^{BC}	2.14^{DE}	268^{C}	245^{G}	220^{J}
LSD	**10.74**		**0.2074**			**1.837**	

*The higher record by the penetrometer reading, refer to less firmness of yoghurt.

T1= yoghurt made with 2% commercial yoghurt starter **T2**= yoghurt made with 2% bio-yoghurt starter

T3= yoghurt made with 2.5% bio-yoghurt starter **T4=** yoghurt made with 3% bio-yoghurt starter

higher ($P<0.05$) than treatment T3 followed by T2 when fresh and through the storage period. These results may be due to the high acidity of T1 and T4 which contain high level of commercial yoghurt starter. Curd syneresis of all treatments slightly decreased ($P<0.05$) during storage period. La Torre *et al.* (2003) Found that, the probiotic fermented milk made with starter culture (*Bifidobacterium bifidum*, *B. lactis* and *B. infantis*) had the minimum value of serum separation, and milks made with commercial yoghurt starter had maximum values. The decrease during the storage period was linear and the rate of decrease was dependent on the type and level of starter culture used. The differences between yoghurt means were significant.

Yoghurt firmness was measured as the penetration distance in 0.1 mm. The high record by the penetrometer reading, refer to less firmness of yoghurt. The variations of firmness measurements in all treatments were different during storage. The highest firmness value was recorded for T1 followed by T4 and then T3, while T2 recorded the lowest firmness when fresh and during storage. Bonczar *et al*. (2002) found that, the hardness of probiotic-fermented milk (contain *S. thermophilus, L. acidophilus and bifidobacterium* ssp.) was a little lower than yoghurts made with commercial yoghurt starter.

The yoghurt firmness gradually increased in all treatments during storage, which may be refer to a slight increase of total solids content and acidity development as well as the complete setting of curd during the storage. These results are coincided with Ibrahim *et al.* (2004). Also, La Torre *et al*. (2003) studied the manufacture of set-type probiotic fermented milks (*Bifidobacterium bifidum*, *B. lactis* and *B. infantis*) and yoghurt made with commercial yoghurt starter and they found that, the firmness of these products increased during the storage period, and the rate of increase was linear and independent of the starter culture. Thus is in harmony with these results. The differences between yoghurt treatments were highly significant ($P<0.01$).

Chemical composition of yoghurt:

The chemical composition of yoghurt made with commercial yoghurt starter either alone (T1) or in association with *Lactobacillus plantarum* BfEL 92122 (T2, T3 and T4) are presented in Table (2). A slight or no effect ($P>0.05$) could be observed on the total solids, fat, protein and ash contents among the different treatments of the same age. These results agree with (Dave and Shah, 1997).

On the other hand, titratable acidity was the highest ($P<0.01$) in T1 followed by T4 compared with T2 and T3 in fresh yoghurt. This may be due to the higher level of commercial yoghurt starter in T1 and T4 than T2 and T3. The differences in the acidity may be also due to that *L. plantarum* grow slower in milk than yoghurt starter (Francois *et al*., 2004 and Modzelewska *et al*., 2008). Whereas, acidity increased ($P<0.01$) gradually during the storage period in all treatments and this may be due to an increase in metabolites and other biochemical changes made by LAB, even at low temperatures (Yadav *et al*., 2007).

The opposite trend of acidity results was observed with respect to pH values. A continuous decrease in pH values ($P<0.01$) of all treatments during storage was noticed. Dave and Shah, (1997) and Modzelewska *et al.* (2008) mentioned that, the pH of yogurts made with *L. plantarum* 14 or *L. fermentum* 4a resembled acidity of the control yoghurt.

Table (2): Chemical composition of probiotic yoghurt when fresh and during storage period.

Treatments	Storage period (days)	T.S. %	Fat %	Protein %	Ash %	Acidity %	pH	Lactose %	Acetald-hyde (µg/100 g)
T1	Fresh	13.49	3.20	3.56	0.828	0.78^{H}	4.57^{D}	4.38^{BC}	117^{B}
T2		13.42	3.18	3.48	0.876	0.66^{I}	4.70^{B}	4.58^{A}	77^{F}
T3		13.57	3.25	3.86	0.907	0.68^{I}	4.72^{A}	4.49^{AB}	90^{D}
T4		13.77	3.25	4.03	0.907	0.75^{H}	4.62^{C}	4.44^{BC}	108^{C}
T1	7	13.61	3.18	3.49	0.861	1.38^{B}	4.15^{I}	4.05^{F}	128^{A}
T2		13.54	3.11	3.39	0.911	1.05^{G}	4.30^{E}	4.33^{CD}	87^{DE}
T3		13.76	3.20	3.75	0.882	1.18^{F}	4.25^{F}	4.24^{DE}	106C
T4		13.81	3.17	3.90	0.915	1.26^{DE}	4.20^{H}	4.14^{EF}	120^{B}
T1	14	13.71	3.17	3.46	0.883	1.50^{A}	4.03^{J}	3.25^{J}	67^{G}
T2		13.67	3.09	3.34	0.916	1.25^{E}	4.23^{G}	3.79^{G}	61^{H}
T3		13.82	3.18	3.68	0.893	1.29^{D}	4.20^{H}	3.61^{H}	75^{F}
T4		13.87	3.14	3.81	0.920	1.33^{C}	4.15^{I}	3.50^{I}	84^{E}
LSD		-	-	-	-	0.030	0.016	0.107	5.071

T1= yoghurt made with 2% commercial yoghurt starter cultures **T2**= yoghurt made with 2% bio-yoghurt starter
T3= yoghurt made with 2.5% bio-yoghurt starter **T4=** yoghurt made with 3% bio-yoghurt starter

After 14 days of storage, pH and titratable acidity of probiotic yoghurts were ~ 4.2 and 1.3, respectively, which were acceptable to the assessors. The final pH of yoghurt manufactured with the combination of commercial yoghurt starter and different concentrations of *L. plantarum* BfEL 92122 were highly significant different

During storage of yoghurt, the lactose content decreased ($P<0.01$) in all treatments. The results are in agreement with those found by Rasic & Kurmann, (1978) and Deeth & Tamime, (1981) who reported that about 20-30% of lactose content is fermented during yoghurt processing. The reduction in lactose during storage reflected its continued fermentation to lactic acid and some aroma components during storage, and mainly due to its utilization by lactic acid bacteria as a main source for energy.

Acetaldehyde is considered as the most prominent compound for the typical yoghurt aroma. The analysis of variance for acetaldehyde between treatments when fresh and during storage was highly significant ($P<0.01$). The maximum content of acetaldehyde was belonged to the control (T1) followed by T4 and then T3 which contain higher level of commercial yoghurt starter, while the minimum amount was belonged to

(T2) which contains low level of commercial yoghurt starter. Beshkova *et al*. (1988) found that *L. delbrueckii* ssp. *bulgaricus* produces higher amounts of aroma metabolites in milk. The milk fermented by *L. acidophilus* or *Bifidobacteria* is often characterized by lack of acetaldehyde, which is quantitatively the principal and the most important constituent of yoghurt aroma. The absence of alcohol dehydrogenase in lactic acid bacteria involved in yoghurt is a desirable feature for starter cultures. However, some *L. acidophilus* strains possess an alcohol dehydrogenase which converts the acetaldehyde to ethanol resulting in lack of flavour in acidophilus milk (Marshall and Cole, 1983).

Acetaldehyde content slightly increased during the first week of storage, then it decreased ($P<0.01$) as prolonging the storage period in all treatments. This decrease may be due to the demonstrated ability of numerous lactic acid bacteria to convert the acetaldehyde to ethanol and/or evaporation from the samples (Tamime and Robinson, 1999)

Microbiological examination

The changes of viable count of *Lactobacillus plantarum* BfEL 92122, lactic acid bacteria (LAB), *Streptococcus salivarius* spp. *thermophilus* and *Lactobacillus delbrekii* ssp. *bulgaricus* during storage of yoghurts are shown in Fig (5).

It can be seen that the population of *L. plantarum*, grown in association with yoghurt bacteria, had initiated count of (10^6 to 10^8 cfu/g), in accordance with international recommendations and guidelines for probiotic and starter cultures in milk products (Maragkoudakis *et al*. 2006) and this number was almost stable in treatments T3 and T4 during the storage period up to 14 days at ~ 5°C, while the number of this organism was $<10^6$ (cfu/g) in treatment T2. This may be due to that *L. plantarum* BfEL 92122 is considered as a tolerant to low pH and it survived very well at pH 3.5 (Fig, 2) and Ismail *et al.* (2007). It was found by Dave and Shah, (1997) that probiotic organisms have weak proteolytic activity and require free amino acids for better multiplication. So, the presence of *L. delbrueckii* ssp. *bulgaricus* in starter cultures, which has been known

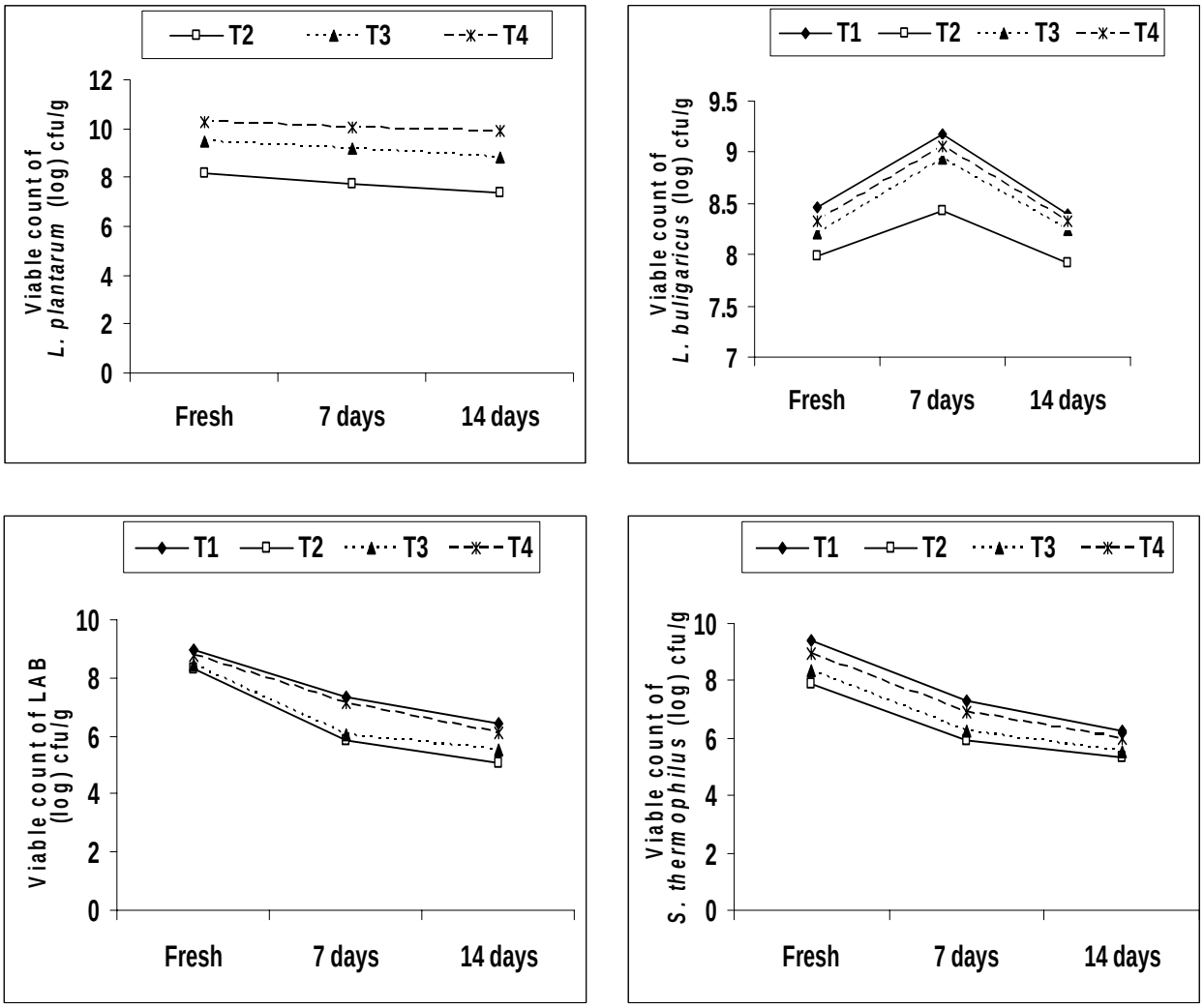


Fig. (5): Growth of *L. plantarum* BfEL 92122 and commercial yoghurt starter cultures of yoghurt when fresh and during storage.

for its symbiosis and proteolytic nature (Shankar and Davies, 1976), produces free amino acids in yoghurt which can be used by other organisms and would have promoted the growth of probiotic bacteria and remain stable (Singh *et al*., 1980). The variation between treatments (T2, T3 and T4) in the numbers of *L. plantarum* may be due to different concentrations of this organism which added in milk yoghurts.

It is obvious that the changes in the counts of LAB and *Streptococcus salivarius* ssp. *thermophilus* of yoghurt from different treatments decreased during storage and the decrease was more obvious at the beginning of storage. *Str. salivarius* ssp. *thermophilus* rapidly lost viability and a reduction in the viable counts of more than 2 to 3 log cycles was observed While, the numbers of *L. delbrueckii* ssp. *bulgaricus* increased during storage, reached the maximum after 7 days then started to decline till 14 days. These differences in the inactivation rate of *Streptococcus salivarius* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus* may be attributed to the increase of acidity which affects streptococci while, lactobacilli tolerate. The obtained results are in agree with Mehanna *et al.* (2003).

From the previous results, it was concluded that the starter culture survival rates were not affected by variations in levels of *L. plantarum* in starter culture. These results are in agreement with Maragkoudakis *et al*.(2006) who examined *Lactobacillus plantarum* ACA-DC 146 and *L. paracasei* ssp. *tolerans* ACA-DC 4037 for their potential application as adjuncts in the production of traditional Greek set-type yoghurt. Both strains displayed low milk acidification activity, while no inhibition was observed towards or from the yoghurt starters used (*L. delbrueckii* ssp. *bulgaricus* ACA-DC 84 and *S. thermophilus* ACA-DC 6). This allows the co-existence of the *L. plantarum* BfEL 92122 in yoghurt as adjuncts.

So, probiotic yoghurt treatments (T3 and T4) can be regarded as probiotic, because the counts of *L. plantarum* during the entire shelf-life were higher than 10^6 cfu /g (Modzelewska *et al*., 2008)

Coliform bacteria and yeasts & moulds were not detected in all treatments either fresh or stored which is due to severe heating of milk and the good sanitary condition during production of yoghurt as well as the role of LAB in preservation of the product which associated with their ability to produce a range of antimicrobial compounds.

Organoleptic properties

The sensory characteristics of fermented milks play an important role in product acceptance by consumers. During the storage period of the probiotic yoghurts up to 14 days the organoleptic properties were done compared with control yoghurt, and the sensory data are shown in Fig (6). The overall acceptability for the products reflects the opinion of the panel, and the acceptability influenced by type and level of starter cultures.

From the sensory results, it was clear that T4 followed by T3 were closed to T1 when fresh, in addition, no differences ($P>0.05$) were noticed in the flavour and body & texture between them (exhibiting a rich, smooth, traditional taste, acceptable acidity). T2 recorded the lowest scores and this might be attributed to that it contains lower level of commercial yoghurt starter and *L. plantarum* than the other treatments (exhibiting less acetaldehyde, weakly body & texture and less acidity). The use of *L. plantarum* in fermented milk gives very slow acidification property (Francois *et al.*, 2004). Yoghurt containing *L. plantarum* ACA-DC 146 had a mild, neutral taste, (Maragkoudakis *et al*., 2006). Appearance of all treatments scored very high during the whole storage period.

On the other hand, the products from T4 followed by T3 were gained higher scores than T1 ($P<0.05$) especially at the end of the storage period. The control yoghurt showed high acidity which affect on the rheological properties. This is in according with Bonczar *et al*. (2002) who found that, the organoleptic scores of control yoghurt (contain *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) received higher scores than probiotic-fermented milk (contain *S. thermophilus, L. acidophilus* and *bifidobacterium* ssp.) when fresh, mainly because of more intensive flavour and better consistency, while after 14 days the scores in probiotic-fermented milk were higher than the traditional yoghurt, mainly because the control yoghurt appeared to be more acid than the probiotic-fermented milk. Also, Modzelewska *et al.* (2008) found that, flavour and appearance of yogurts with potentially probiotic strain (*L. plantarum* 14 or *L. fermentum* 4a) were better

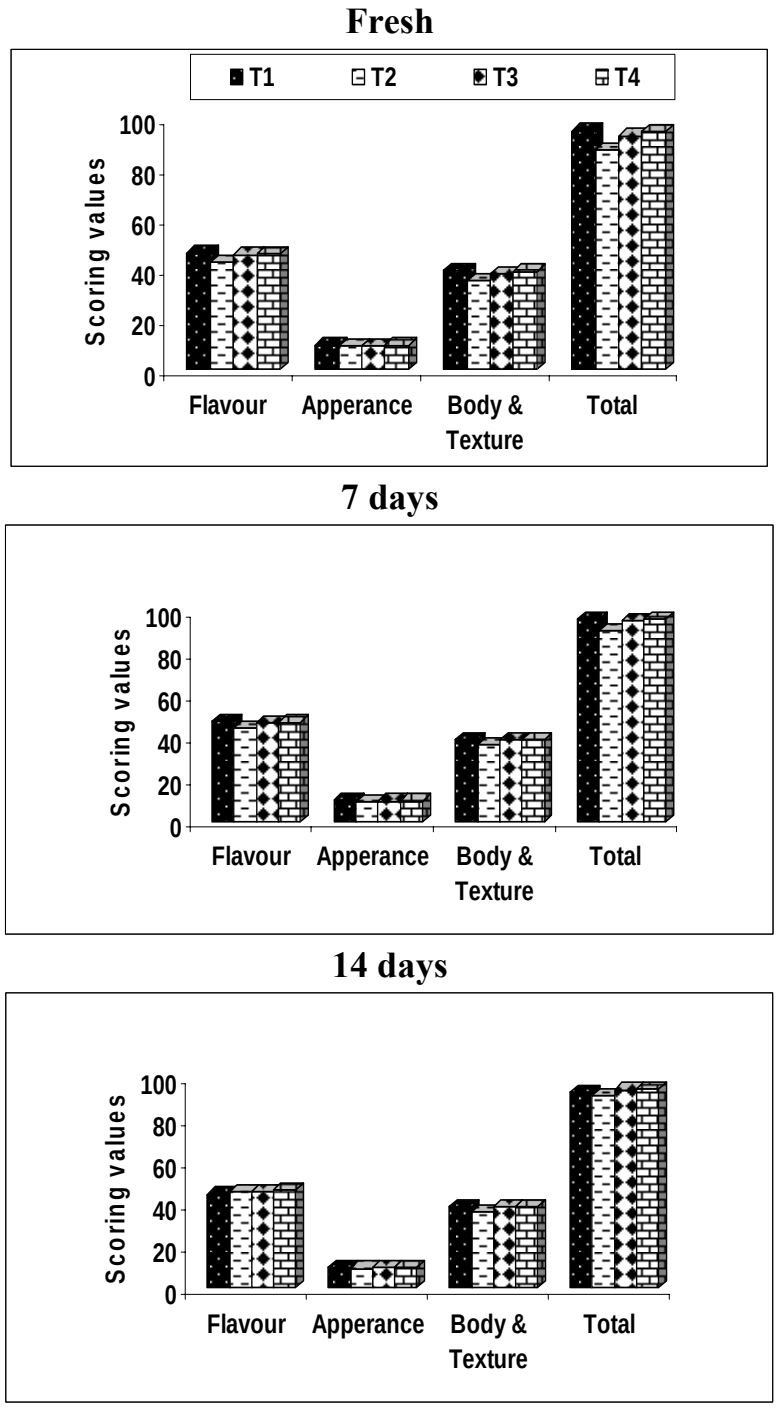


Fig. (6): Organoleptic properties of probiotic yoghurt compared with control yoghurt when fresh and during storage.

than or similar to control yogurt (contain *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) up to 14 days of storage and also the texture of yogurts containing *L. plantarum* 14 gained higher notes than the other treatments.

The total scores comprising all evaluated features indicated slight differences ($P<0.05$) of sensory quality between T3 and T4 and T1, which suggests the possibility of using *L. plantarum* BfEL 92122 association with commercial yoghurt starter as adjuncts to produce probiotic yoghurt. These results agree with Francois *et al*. (2004) and Modzelewska *et al*. (2008).

The statistical analysis of sensory data clear that all factors have the same importance to the analysis and it was clear that the interaction between the treatments and the sensory characteristics (flavour and texture) was significant ($P<0.05$) which may be due to the type and level of starter culture bacteria as it affects these characteristics.

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إنتاج زبادي حيوي بإضافة سلالة *Lactobacillus plantarum BfEL* 92122 مع بادئ الزبادي التجاري

أجريت هذه الدراسة لتقييم تأثير إضافة سلالة *Lactobacillus plantarum* BfEL 92122 مع بادئ الزبادي التجاري (*L. delbrueckii* ssp. *bulgaricus* and *S. salivarius* ssp. *thermophilus*, 1:1) بهدف إنتاج زبادي حيوي. ولتقييم السلالة حيويا فقد تم دراسة قدرتها علي إنتاج الحموضة في اللبن ومدي مقاومتها للـ pH المنخفض وللتركيزات المختلفة من ملح الصفراء وكذا قدرتها علي تحلل ملح الصفراء. وقد أظهرت النتائج قدرة السلالة علي إنتاج الحموضة وتجبن اللبن بعد 24 ساعة /37°م بعدد يصل الي 10^8 (خلية/مل) كما أن حيويتها لم تتأثرعلي pH 3.5 أو 3 ٪ ملح صفراء كما أظهرت قدرتها أيضا علي تحلل ملح الصفراء.

كما أجريت محاولات لمعرفة التركيز المناسب من بادئ الزبادي الحيوي (*L. plantarum* + بادئ الزبادي التجاري 1:1) لتصنيع الزبادي الحيوي وبحيث يصل عدد السلالة الحيوية الي 10^6– 10^8 (خلية/جم) في المنتج. وقد تم تصنيع زبادي المقارنة T1 بإضافة 2 ٪ من بادئ الزبادي التجاري ، بينما صنعت معاملات الزبادي الحيوي T2 ، T3 و T4 بإضافة 2 ، 2.5 و 3 ٪ من بادئ الزبادي الحيوي ، علي الترتيب. سجل وقت التجبن وفحصت الخصائص الريولوجية والتحليلات الكيماوية والجودة الميكروبيولوجية وكذا الخصائص الحسية في عينات الزبادي الطازج وخلال فترة التخزين حتي 14 يوم / ~ 5°م. وجد أن معدل نمو بادئ الزبادي التجاري وكذا التركيب الكيماوي للزبادي لم

يتأثربإضافة *L. plantarum* وأن إضافة *Lactobacillus plantarum* BfEL 92122 مع بادئ الزبادي التجاري في المعاملة (T3 و T4) حسنت الخصائص الريولوجية والحسية مع ثبات المستوي المطلوب لأعداد *L. plantarum* (10^6 – 10^8 خلية/جم) في الزبادي الحيوي.