Survival of some Probiotics in Bio-Yoghurt Production

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Abstract: Viability of *Lactobacillus acidophilus* (*L. acidophilus*) and *Bifidobacterium bifidum* (*B. bifidum*) incorporated with yoghurt culture in three combinations of starter cultures; Treatment 1 (yoghurt culture + *L. acidophilus*), T2 (yoghurt culture + *B. bifidum*) and T3 (yoghurt culture + *L. acidophilus* + *B. bifidum*) were studied. Analyses of pH, titratable acidity (TA), syneresis, acetaldehyde content and sensory evaluation after 1, 3, 7, 14 and 21 days of cold storage of yoghurt were under taken. Counts of *L. acidophilus* was recorded 5.85 $\log_{10}$ cfu g^{-1} in T1 which were higher than counts in T3 (5.79 $\log_{10}$ cfu g^{-1}) at the end of storage period. On the other hand, the viability of *L. acidophilus* and *B. bifidum* was stable until 14 days of storage period. The results showed that the addition of *B. bifidum* to yoghurt culture (T2) increased TA of bio-yoghurt comparing with addition of *L. acidophilus* (T1). Syneresis in all treatments was in the range of 20 to 33% and it was found higher in T3 than control. The highest sensory values were in control, T1 and T2 until the end of 14 days of storage period.

Keywords**:** Yoghurt, Probiotic, Bio-yoghurt.

INTRODUCTION

Probiotics definition as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" by Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO, 2001). An important challenge for industrial producers was viability and stability of probiotic bacteria in their products during either the technology or marketing Caldeano and Perdigon (2004). Probiotic cultures with good technological properties should grow easily in milk, improve sensory characteristics of the product and should be viable during the whole product shelf life (Magarinos *et al*., 2008). Bifidobacteria and lactobacilli were the famous probiotic bacteria incorporated into dairy products (Lourens-Hattingh and Viljoen, 2001).

The viability of *L. acidophilus* in bio-yoghurt improved by incorporated into milk with yoghurt culture before fermentation (Tamime, 2005). This allows propagation of *L. acidophilus* to some extent in milk, which improves the initial number after processing and assists its adaptation to the product environment; this will help their survival during storage. The possible interactions between selected strains should be considered when choosing the best combinations in order to optimize their performance in the process and their viability.

Probiotic bacteria must be viable and available at a high concentration, typically 10^6-10^7 cfu g^{-1} of product to achieve health benefits (Shah *et al*., 2000).

The aim of this work was to determine the viability of probiotic bacteria (*L. acidophilus* and *B. bifidum*) incorporated with yoghurt starter culture in making bio-yoghurt. To achieve this goal, three different mixtures in addition to control were prepared and their technological properties and sensory evaluation during storage at 4°C for 21 days were studied.

MATERIALS AND METHODS

Bacterial strains:

Streptococcus thermophilus 1043 (*S. thermophilus*), *Lactobacillus delbrueckii* ssp. *bulgaricus* 20080 (*L. delbrueckii* ssp. *bulgaricus*) and *L. acidophilus* 20079 were obtained from Ain Shams University, Faculty of Agriculture, Microbiological Resources Center (Cairo MIRCEN). *B. bifidum* 6071 was procured by China Center of Industrial Culture Collection (CICC), Beijing, China.

Preparation of yoghurt:

Fresh cow's milk was obtained from the local market. The average of chemical composition of milk was 4.12-4.14, 4.0-4.20 and 11.45-11.50% for protein, fat and total solids (TS), respectively. Yoghurt was prepared as described by Tamime and Robinson (1985). Briefly, whole cow's milk (3.5% fat and 12.7% TS) was heat treated up to ≈ 85ºC for 10 min then cooled to 42ºC and inoculated with 2% of starter culture. Three starter cultures were prepared as following:

Control: Yoghurt culture (*S. thermophilus* + *L. delbrueckii* ssp. *bulgaricus*).

T1: Yoghurt culture + *L. acidophilus*.

T2: Yoghurt culture + *B. bifidum*.

T3: Yoghurt culture + *L. acidophilus* + *B. bifidum*.

All the treatments were distributed into 120 ml sterilized leaded glass cups and incubated at 42ºC until clotting. Yoghurt was kept at ≈ 5 ± 1ºC for 21 days and then the samples were taken when fresh, 3, 7, 14 and 21 days and tested for viable bacterial count, chemical analyses and sensory evaluation.

Microbiological analysis:

S. thermophilus was enumerated on M17 agar at 37°C/48 h. *L. delbrueckii* ssp. *bulgaricus* was counted on MRS agar with pH adjusted to 5.2 at 42°C/48 h (Dave and Shah, 1997). MRS-sorbitol agar (1.0% D-sorbitol) was used for enumerated of *L. acidophilus* at 37°C/48 h, while, *B. bifidum* was enumerated on LP-MRS (lithium propionate-MRS) agar in anaerobic jar at

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37°C/72 h has described by Vinderola and Reinheimer (1999).

Chemical analysis:

The pH value of the produced bio-yoghurt samples were measured as described by BSI (1985) using Jenco pH meter (model 671P, USA). The titratable acidity was determined according to BSI (2010). Yoghurt was then analyzed for total solids by the method of Kurt *et al*. (1996). Acetaldehyde content and syneresis of yoghurt were estimated as described by Lees and Jago (1969) and Keogh and O'Kennedy (1998), respectively.

Sensory evaluation:

The organoleptic properties of fresh and stored bio-yoghurt were carried out according to the procedures of Kebary and Hussein (1999) by the panels of 8 staff members of the Food and Dairy Science and Technology Department, Faculty of Environmental Agriculture, El-Arish University. The sensory attributes included: flavour (50 points), body and texture (30 points) and appearance (20 points).

Statistical analysis:

Statistical analyses for the obtained data were carried out according to the method described by Clarke and Kempson (1997).

RESULTS AND DISCUSSIONS

Chemical composition of bio- yoghurt:

The total solids contents, pH, titratable acidity, syneresis and acetaldehyde content are shown in Table (1). The total solid of all the produced bio-yoghurt were showed slight increase, which was mainly attributed to slight evaporation during cold storage periods. The total solids of produced bio-yoghurt did not affected by the type of starter cultures used. These results were in agreement with that of Cunha *et al*. (2002).

pH and TA:

Generally, the greatest drop of pH was noticeable after 14 days of the storage in all treatments. There were significant differences ($P < 0.05$) between treatments where the values of pH were decreased and TA increased. The pH value was 4.53 in T1, while it was 4.28 for T3 during the storage period, which was generally considered detrimental to the survival of probiotic bacteria (Dave and Shah, 1997).

Comparing the results of TA according to the added starter cultures, addition of *B. bifidum* to yoghurt culture (T2) increased TA of bio-yoghurt as compared with the addition of *L. acidophilus* (T1). These results agreed with Tamime *et al*. *(*1995) and Kehagias *et al*. (2006) which attributed such results to the formation of both acetic and lactic acids by *B. bifidum*. Post acidification of yoghurt treatments is attributed to the metabolic activity of the starter bacteria during the cold storage periods of the product. Also, Beal *et al*. (1994) reported that post-acidification was greater by using a mixed starter culture *S. thermophilus* with *L. delbrueckii* ssp. *bulgaricus* than with other bacterial strain associations; this explains the associative growth that exists between these selected bacteria.

Whey syneresis: Syneresis is generally defined as separation of aqueous phase from continuous phase or gel network, which is an undesirable property in fermented milk products (Aghajani *et al*., 2012). Syneresis of all treatments decreased during storage period. This is accordance with obtained by Isleten and Karagul-Yuceer (2006). Moreover, the acidity of the yoghurt could be a further contributing factor since the increasing in acidity is known to stimulate syneresis in yoghurt and rearrangement of casein particles in the gel network, and the rate of solubilization of colloidal calcium particles are the driving factor for the syneresis (Tamime *et al*., 1995; Lee and Lucey, 2004).

The high separation of whey was found in T3 followed by control meanwhile, the lowest value was obtained for T1. This could be attributed to the differentiations in metabolic activities of starter cultures. Starter cultures, product type and storage time were effected on syneresis values (Panesar and Shinde, 2012). Some strains of lactic acid bacteria produce exopolysaccharides (EPS) which affect syneresis of fermented products. The EPS have the ability to bind water and reduce syneresis (De Vuyst and Degeest, 1999).

Acetaldehyde content: Acetaldehyde is mainly responsible for the typical aroma of yoghurt. The results showed that there were significant differences ($P < 0.05$) of acetaldehyde content among treatments during storage period. After 7 days of cold storage, the highest acetaldehyde values were observed and it was 57.47 mg g^{-1} for T3, whereas the lowest value was observed for control (54.44 mg g^{-1}). Therefore, the differences in acetaldehyde contents could be attributed to the differences of starter culture.

The high increase of acetaldehyde content, particularly in T3 may be due to the addition of two strains of probiotic bacteria (*L. acidophilus* + *B. bifidum*). These results refer to probiotic bacteria which stimulated the growth of yoghurt starter and the acetaldehyde production, these data were in agreement with Murti *et al*. (1993).

With the progress of storage periods, acetaldehyde content of all treatments started to decrease. The lowest value was 34.03 mg g^{-1} for control after 21 days. The decrease in the acetaldehyde levels could be related to the hydrolysis by microbial enzymes to form other substances such as converting acetaldehyde to ethanol by the enzymes of yoghurt bacteria. Yuguchi *et al*. (1989) mentioned that the higher amount of acetaldehyde might be due to the metabolism activity of bifidobacteria.

Microbiological properties of bio-yoghurt:

In general, there were significant differences ($P < 0.05$), in counts of *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, *L. acidophilus* and *B. bifidum* between treatments when fresh and during storage period (Table 2).

Table (1): Changes of total solid (TS) %, pH, titratable acidity %, acetaldehyde contents (mg g^{-1}) and syneresis (ml100g^{-1}) of bio-yoghurt stored at 4°C for 21 days

Properties	Treatments*	Storage period (day)				
		Fresh	3	7	14	21
TS%	C	14.94^{b}	15.43^{b}	15.53^{c}	15.67^{c}	15.71^{b}
	T1	14.97^{a}	15.45^{a}	15.59^{a}	15.73^{a}	15.75^{a}
	T2	14.96^{a}	15.44^{a}	15.56^{b}	15.69^{b}	15.73^{a}
	T3	14.93^{b}	15.43^{b}	15.52^{d}	15.66^{c}	15.70^{b}
pH	C	4.47^{b}	4.40^{b}	4.35^{b}	4.30^{c}	4.29^{b}
	T1	4.53^{a}	4.44^{a}	4.39^{a}	4.34^{a}	4.31^{a}
	T2	4.48^{b}	4.43^{a}	4.36^{b}	4.32^{b}	4.29^{b}
	T3	4.45^{c}	4.38^{c}	4.34^{c}	4.28^{d}	4.28^{c}
TA%	C	0.85^{a}	0.89^{a}	0.92^{a}	0.96^{a}	1.00^{a}
	T1	0.79^{c}	0.83^{c}	0.86^{b}	0.91^{c}	0.94^{c}
	T2	0.83^{b}	0.85^{b}	0.90^{a}	0.93^{b}	0.96^{c}
	T3	0.82^{b}	0.84^{cb}	0.89^{a}	0.92^{cb}	0.98^{b}
Acetaldehyde contents (mg g^{-1})	C	41.75^{c}	44.96^{d}	54.44^{c}	42.94^{c}	34.03^{d}
	T1	43.61^{b}	52.57^{c}	55.25^{b}	44.47^{b}	35.94^{c}
	T2	43.21^{b}	53.52^{b}	56.03^{b}	48.83^{a}	37.23^{b}
	T3	44.37^{a}	56.08^{a}	57.47^{a}	49.48^{a}	40.62^{a}
Syneresis (ml 100 g^{-1})	C	32.28^{b}	29.64^{b}	26.42^{b}	24.92^{a}	21.73^{b}
	T1	30.15^{d}	26.44^{d}	24.87^{d}	22.35^{c}	20.71^{c}
	T2	31.63^{c}	29.53^{c}	25.67^{c}	22.27^{d}	20.11^{c}
	T3	33.16^{a}	30.39^{a}	27.39^{a}	24.48^{b}	22.39^{a}

*C: (yoghurt starter culture), T1: (yoghurt culture + *L. acidophilus*), T2: (yoghurt culture + *B. bifidum*) and T3: (yoghurt culture + *L. acidophilus* + *B. bifidum*).Values with different letters in the same column are significantly different (P < 0.05).

Table (2): Survival (log_{10} cfu g^{-1}) of *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus, L. acidophilus* and *B. bifidum* in bio-yoghurt during storage at 4°C for 21 days

Starter culture	Treatments*	Storage period (day)					
		Fresh	3	7	14	21	Mean
S. thermophilus	C	9.21	8.89	8.81	8.76	8.65	8.86^{a}
	T1	9.01	8.93	8.80	8.74	8.56	8.80^{a}
	T2	8.83	8.75	8.63	8.57	8.48	8.65^{a}
	T3	8.86	8.82	8.75	8.67	8.53	8.72^{a}
L. delbrueckii ssp. *bulgaricus*	C	8.07	7.94	7.78	7.62	7.43	7.76^{b}
	T1	7.95	7.46	7.52	7.03	6.83	7.35^{b}
	T2	7.85	7.81	7.76	7.66	7.58	7.73^{b}
	T3	7.73	7.58	7.43	7.28	6.92	7.38^{b}
L. acidophilus	T1	7.41	7.30	7.09	6.76	5.85	6.88^{c}
	T3	7.34	7.25	7.08	6.57	5.79	6.80^{c}
B. bifidum	T2	7.48	7.12	6.80	6.65	6.53	6.91^{c}
	T3	6.83	6.81	6.76	6.18	5.73	6.46^{c}

*C: (yoghurt starter culture), T1: (yoghurt culture + *L. acidophilus*), T2: (yoghurt culture + *B. bifidum*) and T3: (yoghurt culture + *L. acidophilus* + *B. bifidum*). Values with different letters in the same column are significantly different (P < 0.05).

Counts of yoghurt culture:

The average of initial microbial count for each of the activated cultures was 10^7 cfu g^{-1}. After 24 h of fermentation, the counts (log values) were 9.21 and 8.07 for *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, respectively. The values of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were decreased by 0.56 and 0.64 cycles, respectively after 21 days of the storage period. The symbiotic nature of yoghurt culture shows that *S. thermophilus* produces lactic acid, pyruvic acid, formic acid and CO_2. Lactic and formic acids stimulate the growth of *L. delbrueckii* ssp. *bulgaricus* and also, *S. thermophilus* create favorable conditions for the growth of *L. delbrueckii* ssp. *bulgaricus* throughout assimilates oxygen in milk which in turn produces peptides and amino acids that stimulate the growth of *S. thermophiles* (Radke-Michell and Sandine, 1984).

S. thermophilus was obviously dominated in yoghurt and bio-yoghurt. Oliveira *et al*. (2002) reported that *S. thermophilus* predominated in all products presenting counts higher than 9 $\log_{10}$ cfu ml^{-1} in yoghurt prepared with mixed culture. However, by the end of the storage period, the counts decreased to 8.65, 8.56, 8.48 and 8.53 $\log_{10}$ cfu ml^{-1} in control, T1, T2 and T3 treatments, respectively. Birollo *et al*. (2000) observed that *S. thermophilus* remained viable (10^9 cfu ml^{-1}) after 40 days of storage period at 6°C. On other hand, *L. delbrueckii* ssp. *bulgaricus* counts reached 10^8 cfu ml^{-1} within the first 10 days, under the same storage conditions, and after 15 to 45 days the counts continued to be 10^7 cfu ml^{-1}.

During the storage course, the viability of *L. delbrueckii* ssp. *bulgaricus* was reduced by 1.19 $\log_{10}$ cfu g^{-1} when grown with *S. thermophilus*. Venir *et al*. (2007) found in fresh yoghurt, *S. thermophilus* counts between 10^4-10^8 cfu ml^{-1} and 10^6-10^7 cfu ml^{-1} for *L. delbrueckii* ssp. *bulgaricus.* In this study, yoghurt culture count was 8.53-9.21 and 6.83-8.07 log cfu g^{-1} for *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, respectively.

***L. acidophilus* of bio-yoghurt:**

L. acidophilus showed a steady decline in counts during the interval storage periods and contained $>10^6$ cfu $g^{-1}$for 14 days of storage period. This result was mainly attributed to low pH values and organic acids accumulation in which they are amongst the important factors contributing to the loss of cell viability of probiotics. This data are in agreement with Nighswonger *et al*. *(*1996) and Donkor *et al*. (2006). The decrease of *L. acidophilus* counts was in agree with Zacarchenco and Massaguer-Roig (2004) who observed a reduction of 2 $\log_{10}$ cycles ml^{-1} in the counts of *L. acidophilus* when grown with *S. thermophilus* and *B. longum* at the end of the storage period. The growth of *L. acidophilus* in T1 (5.85 $\log_{10}$ cfu g^{-1}) was higher than that of T3 (5.79 $\log_{10}$ cfu g^{-1}) at the end of storage period. On the other hand, Gilliland and Speck (1977) reported that hydrogen peroxide, a substance produced by *L. delbrueckii* ssp. *bulgaricus* metabolism, was the main chemical compound responsible for the reduction of *L. acidophilus* viability in yoghurt.

***B. bifidum* of bio-yoghurt:**

Several factors have been claimed to be responsible for the loss of viability of probiotic organisms: acidity of products, acid produced during refrigerated storage (post acidification), level of oxygen in products, oxygen permeation through the package, sensitivity to antimicrobial substances produced by bacteria (Dave and Shah, 1997). Counts of *B. bifidum* remained at 7 $\log_{10}$ cfu g^{-1} from an initial inoculum of 7 $\log_{10}$ cfu g^{-1} during 3 days of cold storage and decreased up to the end of the storage periods to reached 6.53 $\log_{10}$ cfu g^{-1}in T2 and reduced to 5.73 $\log_{10}$ cfu g^{-1} in T3.

However, the addition of *S. thermophilus* may also increase the survival of certain strains of *Bifidobacterium* through the reduction of oxygen pressure as mentioned by Ishibashi and Shimamura (1993) and Nogueira *et al*. (1998).

The counts of viable *L. acidophilus* and *B. bifidum* were more than 10^6 cfu g^{-1} until 14 days of storage period in all treatments. These counts were decreased to less than 10^6 cfu g^{-1} after 21 days of storage period in all treatments except T2.

Sensory evaluation:

The scores of sensory evaluations of bio-yoghurt during storage at ≈5±1°C for 21 days are presented in Table (3). Generally, all samples were acceptable by the sensory evaluation panelests. The storage was the principle factor influencing the sensory properties, this may be attributed to the developed acidity and whey separation, which may participated the pleasant acid flavour of yoghurt. Similar observations were reported by Routray and Mishra (2011) who found that the storage time had a negative impact on the flavour scores of yoghurt, thereby attributed to the changes in the aroma compounds.

Significant differences ($P < 0.05$) were observed among all the treatments. The results showed that addition of *B. bifidum* to yoghurt culture in T2 was most preferable bio-yoghurt from sensory properties than the addition of *L. acidophilus* in T1. The highest score was 97.16 points for T2 containing *B. bifidum* followed by T1 containing *L. acidophilus* with 96.53 points. Bio-yoghurt made with *L. acidophilus* or *B. bifidum* had the highest score for flavour acceptability than other treatments. High flavour acceptability of yoghurt made with probiotic bacteria could be due to their acetaldehyde contents. These data are in agreement with Tawfik *et al*. (2003) who suggested that the combination of yoghurt culture bacteria with bifidobacteria produced fermented dairy products with preferable flavour and presence of acetaldehyde was the important component for good yoghurt flavour, and Ayad *et al*. (2010) reported that using *B. bifidum* with yoghurt culture enhanced body and texture of all treatments. Hassan *et al*. (2003) indicated that the texture of yoghurt results from a complex interaction between milk protein, acid and exocellular polysaccharide produced by the starter culture. As storage progressed the texture score decreased in bio-yoghurt. This could be attributed to that the level of metabolites (mainly acetic acid) produced by the bacterial strains which can influence the organoleptic assessment.

Table (3): Sensory evaluation scores of bio-yoghurt stored at 5 ± 1°C for 21 days

Storage period (day)	Treatments*	Flavour	Body & texture	Appearance	Total score
		50 points	30 points	20 points	100
Fresh	C	48.20	28.00	19.33	95.53[a]
	T1	49.00	29.00	18.33	96.53[a]
	T2	49.00	29.00	19.16	97.16[a]
	T3	47.66	27.50	18.33	93.49[b]
3	C	47.83	28.00	19.33	94.16[b]
	T1	48.66	29.00	18.33	95.99[a]
	T2	48.66	29.00	19.33	96.99[a]
	T3	45.16	27.50	16.66	89.32[c]
7	C	46.33	27.33	18.50	92.16[b]
	T1	47.50	28.00	17.83	93.33[b]
	T2	47.50	28.16	18.50	94.16[b]
	T3	45.33	26.16	17.16	88.65[c]
14	C	44.66	26.00	17.33	87.99[c]
	T1	45.16	26.50	17.00	88.66[c]
	T2	45.00	26.33	17.16	88.49[c]
	T3	43.33	24.50	15.33	83.16[d]
21	C	41.00	25.00	15.83	81.83[d]
	T1	43.66	24.33	15.83	83.82[d]
	T2	44.00	24.83	15.83	84.66[d]
	T3	41.33	22.00	13.66	76.99[d]

*C: (yoghurt starter culture), T1: (yoghurt culture + *L. acidophilus*), T2: (yoghurt culture + *B. bifidum*) and T3: (yoghurt culture + *L. acidophilus* + *B. bifidum*). Values with different letters in the same column are significantly different ($P < 0.05$).

The results indicated that treatment 2 (yoghurt culture and *B. bifidum*) and treatment 1 (yoghurt culture and *L. acidophilus*) were the best combination of starter culture in bio-yoghurt production due to the viability, technological properties and sensory evaluation. At the same time, bio-yoghurt should be consumed until 14 days of storage period to achieve the acceptable standard viable counts of probiotics.

REFERENCES

Aghajani, A. R., R. Pourahmad and H. R. Mahdavi Adeli (2012). Evaluation of physicochemical changes and survival of probiotic bacteria in synbiotic yoghurt. Journal of Food Biosciences and Technology, 2: 13-22.

Ayad E., A. Darwish, S. Darwish and M. El-Soda (2010). Production of novel functional yoghurt-like products. Egyptian Journal of Dairy Science, 38: 183-199.

Beal, C., N. Deschamps, V. Juillard, H. De Roissart, J. Richard and B. Saraux (1994). Cinetiques de croissance et d'acidititification des bacteries lactiques. In *Bacteries lactiques,* Aspects Fondamentaux et Technologiques. De Roissart, H. and F. M. Luquet, (Eds), pp. 367-401. Uriage, FRA: Lorica.

Birollo, G. A., L. A. Reinheimer and C. G. Vinderola (2000). Viability of lactic acid microflora in different types of yoghurt. Food Research International, 33: 799-805.

BSI (British Standards Institution) (1985). Determination of pH value. BS770, Part 5.

BSI (2010). Milk and dried milk: determination of titratable acidity (Reference method) ISO, 6091.

Caldeano, C. M. and G. Perdigon (2004). Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation, Journal Appl. Microbial, 97: 673-681.

Clarke, G. M. and R. E. Kempson (1997). Introduction to the design and analysis of experiments. Arnold, a member of the Holder Headline Group, 1st eds., London, UK.

Cunha, C. R., L. M. Spadoti, P. B. Zacarchenco and W. H. Viotto (2002). Efeito do fator de concentracao do retentado na composicao e proteolise de queijo minas frescal de baixo teor de gordura fabricado por ultrafiltracao. Cienciae Tecnologia de Alimentos, 22: 82-87.

Dave, R. I. and N. P. Shah (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. International Dairy Journal, 7: 31-41.

De Vuyst, L. and B. Degeest (1999). Heteropolysaccharides from lactic acid bacteria. FEMS Microbiology Reviews. 23: 153-177.

Donkor, O. N., A. Heuriksson, T. Vasiljevic and NP. Sha (2006). Effect of acidification on the activity of probiotics in yoghurt during cold storage. International Dairy Journal, 16: 1181-1189.

FAO/WHO (Food and Agriculture Organization of the United Nations and World Health Organization) (2001). Health and nutrition properties of probiotics in food including powder milk with live lactic acid bacteria. pp. 2.

Gilliland, S. E. and L. M. Speck (1977). Instability of *Lactobacillus acidophilus* in yoghurt. Journal of Dairy Science, 60: 1395-1398.

Hassan, A. N., R. Ipsen, T. Janzen and K. B. Qvist (2003). Microstructure and rheology of yoghurt made with cultures differing only in their ability to produce exopolysaccharides. Journal Dairy Science, 86: 1632-1638.

Ishibashi, N. and S. Shimamura (1993). Bifidobacteria: Research and development in Japan. Food Technology, 47: 129-134.

Isleten, M. and Y. Karagul-Yuceer (2006). Effects of dried dairy ingredients on physical and sensory properties of nonfat yoghurt. Journal Dairy Science, 89: 2865-2872.

Kebary, K. M. K. and S. A. Hussein (1999). Manufacture of low fat Zabady using different fat substitutes. Acta Alimentaria Budapest, 28: 1-14.

Kehagias, C., S. Koulouris, J. S. Arkoudelos and A. Samona (2006). Viability and biochemical activity of bifidobacteria in association with yoghurt starter cultures in Bifidus milk and bio-yoghurt during storage at 4°C. Egyptian Journal of Dairy Science, 34: 151-158.

Keogh, M. K. and B. T. O'Kennedy (1998). Rheology of stirred yogurt as affected by added milk fat, protein and hydrocolloids. Journal of Food Science, 63: 108-112.

Kurt, A., S. Cakmakci and A. Caglar (1996). Sutve Mamiilleri Muayeneve Analiz Metotlan Rehberi. Ataturk Univ, Yay. No: 252/d, Erzurum, Turkey.

Lees, G. J. and G. R. Jago (1969). Methods for the estimation of acetaldehyde in cultured dairy products. Australian Journal of Dairy Technology, 24: 181-185.

Lee, W. J. and J. A. Lucey (2004). Structure and physical properties of yoghurt gels: Effect of inoculation rate and incubation temperature. Journal Dairy Science, 87: 3153-3177.

Lourens-Hattingh, A. and B. C. Viljoen (2001). Yoghurt as probiotic carrier food, International Dairy Journal, 11: 1-17.

Magarinos, H., P. Cartes, B. Fraser, S. Selaive, M. Costa, F. Figuerola and O. Pizarro (2008). Viability of probiotic microorganisms (*Lactobacillus casei* Shirota and *Bifidobacterium animalis* Subspp. *lactis*) in a milk-based dessert with cranberry sauce. International Journal of Dairy Technology, 61: 96-101.

Murti, T. W., C. Bouillanne, M. Landon and M. J. Desmazeaud (1993). Bacterial growth and volatile compounds in yoghurt-type products from soymilk containing *Bifidobacterium* ssp. Journal of Food Science, 58:153-157.

Nighswonger, B. D., M. M. Brashears and S. E. Gilliland (1996). Viability of *L. acidophilus* and *L. casei* in fermented milk products during refrigerated storage. Journal of Dairy Science, 79: 212-219.

Nogueira, C., H. Albano, P. Gibbs and P. Teixeira (1998). Microbiological quality of Portuguese yoghurts. Journal of Industrial Microbiology and Biotechnology, 21: 19-21.

Oliveira, M. N., I. Sodini, F. Remeuf, J. P. Tissier and G. Corrieu (2002). Manufacture of fermented lactic beverages containing probiotic cultures. Journal of Food Science, 67: 2336-2341.

Panesar, P. S. and C. Shinde (2012). Effect of storage on syneresis, pH, *Lactobacillus acidophilus* count, *Bifidobacterium bifidum* count of *Aloe vera* fortified probiotic yoghurt. Current Research in Dairy Science, 4: 17-23.

Radke-Michell, L. and W. E. Sandine (1984). Associative growth and differential enumeration of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*: A Review Journal Food Prot., 12: 383-391

Routray, W. and H. N. Mishra (2011). Scientific and technical aspects of yoghurt aroma and taste: A Review. Comprehensive Reviews Food Science and Food Safety, 10: 208-220.

Shah, N. P. (2000). Probiotic bacteria: selective enumeration and survival in dairy foods. Journal Dairy Science, 83: 894-907.

Shah, N. P., J. F. Ali and R. K. Ravula (2000). Populations of *L. acidophilus, Bifidobacterium* spp., and *Lactobacillus casei* in commercial fermented milk products. Biosciences Microflora, 19: 35-39.

Tamime, A. Y. (2005). Probiotic dairy products, Oxford, UK: Blackwell Publishing Ltd, PP 41.

Tamime, A. Y. and R. K. Robinson (1985). Yoghurt: Science and Technology. Oxford, Pergamon Press Ltd.

Tamime A. Y., V. M. Marshal and R. K. Robinson (1995). Microbiological and technology aspects of milks fermented by bifidobacteria. Journal of Dairy Research, 62: 151-187.

Tawfik, N. F., O. M. Sharaf, G. A. Amin, G. M. Khalafalla, S. A. El-Gizawy and B. A. Azzat (2003). Utilization of some microorganisms as dietary adjuncts ш. Production and application. Egyptian Journal Dairy Science, 31: 221-231.

Venir, E., M. D. Torre, M. L. Stecchini, E. Maltini and P. D. Nardo (2007). Preparation of freeze-dried yoghurt as a space food. Journal of Food Engineering, 80: 402-407.

Vinderola, C. G. and J. A. Reinheimer (1999). Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the

presence of yoghurt bacteria. International Dairy Journal, 9: 497-505.

Yuguchi, H., A. Hiramatsu, K. Doi and O. S. Idach (1989). Studies on the flavor of yoghurt fermented with Bifidobacteria: Significance of volatile components and organic acids in the sensory acceptance of yoghurt. Journal Zootech. Science, 60: 734-741.

Zacarchenco, P. B and S. Massaguer-Roig (2004). Differential enumeration of *Bifidobacterium longum* and *Lactobacillus acidophilus* in the presence of *Streptococcus thermophilus*. Milchwissenschaft, 59: 258-261.

حيوية بعض سلالات بكتريا الداعمات الحيوية في الزبادي الحيوي

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تم إنتاج ثلاث معاملات مختلفة من الزبادي الحيوي باستخدام سلالتين من بكتريا البروبيوتك *L. acidophilus* و *B. bifidum* مع بادئ الزبادي واختبار المنتج من خلال تقدير الـ pH والحموضة وانفصال الشرش ومحتوى الاسيتالدهيد والخواص الحسية للزبادي الحيوي وأيضا أعداد البكتريا وحيويتها خلال فترة ٢١ يوم من التخزين البارد على درجة ٥±١°م. وقد أظهرت النتائج أن أعداد بكتريا *L. acidophilus* في المعاملة الأولى (*L. acidophilus* + بادىء الزبادي) وفى المعاملة الثالثة (*B. bifidum* + *L. acidophilus* + بادىء الزبادي) ظلت أعلى من ١٠^٦ وحدة مكونة للمستعمرات لكل جرام حتى اليوم الرابع عشر ثم انخفضت لأقل من هذا العدد في اليوم ٢١ من التخزين البارد. وأوضحت النتائج أيضا أن إضافة *B. bifidum* إلى بادئ الزبادي في المعاملة الثانية (*B. bifidum* + بادىء الزبادي) أدى إلى ارتفاع قيم الحموضة مقارنة ببقية المعاملات. وبتقدير انفصال الشرش كانت القيم تتراوح ما بين ٢٠-٣٣٪ وكانت مرتفعة في المعاملة الثالثة وتلاها الكنترول. وكانت أعلى قيم للتقييم الحسي للمعاملة الثانية (٩٧.١٦) ويليها المعاملة الأولى (٩٦.٥٣) والتي استخدم فيها بكتريا الداعمات الحيوية في صورة مفردة مع بادئ الزبادي.