The Production of Set-Type-Bio-Yoghurt with Commercial Probiotic Culture

L. Yilmaz-Ersan and E. Kurdal

***Abstract*—In this study, the yoghurt and bio-yoghurt were produced using different commercial probiotic combinations of *S. thermophilus, L. bulgaricus, L. acidophilus, Bifidobacterium* ssp., *L. lactis* and *L. casei*. The samples were analyzed for microbiological, physico-chemical and sensorial properties at a 5-day interval during storage. Culture combinations and storage time significantly influenced some properties of the samples. While titratable acidity and lactic acid (%) increased, syneresis, pH, lactose and acetaldehyde decreased during storage. Changes in fat, ash, protein contents of yoghurt samples during storage period were not remarkable. Viable probiotic bacterial counts in all bio-yogurts were above 10^7 cfu g^{-1} at the end of storage except for C sample. Considering sensory properties of bio-yoghurt samples, the highest overall acceptabilities by panelists were obtained for C sample.**

***Index Terms*—Bio-yoghurt, probiotic culture.**

I. Introduction

The beneficial effects of foods with added nutritive value on human health are being highly promoted by health professionals. This awareness has led to an increased market demand particularly within children and other high-risk individuals for functional dairy products containing probiotic bacteria as it's positive effect on health has come to the forefront of the public's attention.

The probiotics, live non-pathogenic microorganisms, are defined as microbial cell supplement which exert positive impact on the health of the host when ingested alive in sufficient amount. The main health benefits include improved the intestinal microbial balance, lactose metabolisation, stimulation of the digestive and immune system, reduction of blood cholesterol level, prevention against urinary infections, cardiovascular diseases, diarrhea, osteoporosis, as well as anti-mutagenic and anti-carcinogenic properties [1]–[7].

The most prominent probiotic bacteria associated with food products worldwide are *Lactobacillus* and *Bifidobacterium* species, which are common but non-dominant members of the indigenous microflora of the human gastro-intestinal tract. The health benefits are not only dependent on the choice of microorganism with specific therapeutic properties, but it is also essential that these live microorganisms are consumed in sufficient quantities to exhibit the desired beneficial health effects. Several authors have suggested that the minimum necessary concentration at the moment of ingestion is $10^6 – 10^9$ viable cells g^{-1} in the final product, and this is named as "the therapeutic minimum" [8]–[13].

Fermented dairy foods are the ideal food matrix for probiotics, which promotes growth and enhances viability of these organisms. Of these foods, yoghurt is the most popular, and provides higher levels of protein, carbohydrate, calcium and certain B vitamins than milk. In general, yoghurt is produced by lactic acid fermentation of pasteurized milk using *Streptococcus thermophilus* and Lactobacillus *delbrueckii* subsp. *bulgaricus*, so called yoghurt starter culture. These bacteria are not bile acid resistant and do not survive in the passage of intestinal tract. Thus, recently, probiotic bacteria such as *L. acidophilus*, *Bifidobacterium* ssp., *L. casei* and *L. rhamnosus* incorporated into yoghurt starter culture due to their bile-resistant properties and beneficial health effects. The resulting product, called as "yoghurt-like products", "probiotic" or "bio-yoghurts", are becoming more popular due to the ability of excellent health effects of probiotic bacteria [14]–[17].

The objective of this work was to determine the effects of the starter cultures on microbiological, physico-chemical and sensorial properties of the set-type bio-yoghurt produced using commercial blends of starter cultures (*S. thermophilus, L. bulgaricus, L. acidophilus, Bifidobacterium* ssp., *L. lactis* and *L. casei*). The bio-yoghurt samples were evaluated during storage of 25 days at refrigerated temperature (4±1 ℃).

TABLE I: Sample Code, Cultures and Incubation Conditions

Code	**Starter Cultures**	**Incubation temperature (°C)***	**Incubation time (h)***
A (Control)	*Streptococcus thermophilus* *Lactobacillus bulgaricus*	42	3
B	*Streptococcus thermophilus* *Lactobacillus acidophilus* *Bifidobacterium* ssp.	42	5.5
C	*Streptococcus thermophilus* *Lactobacillus acidophilus* *Lactobacillus lactis* *Bifidobacterium* ssp.	42	7
D	*Lactobacillus acidophilus* *Bifidobacterium lactis*	40	6.5
E	*Lactobacillus acidophilus* *Bifidobacterium lactis* *Lactobacillus casei*	40	6.5

*Recommendations of Starter Culture Suppliers

II. Materials and Methods

A. Materials and Methods

For manufacturing of yoghurt samples, raw bovine milk was obtained from a local dairy plant (EKER Dairy Co., Bursa, Turkey). Skimmed milk powder was purchased from

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Lutfiye Yılmaz-Ersan is with the Department of Food Engineering, Faculty of Agriculture, Uludag University, Bursa, Turkey (e-mail: lutfiyey@uludag.edu.tr).

Pınar Dairy Co., Izmir, Turkey

Sample code, the microorganisms used in commercial starter cultures and incubation conditions were described in Table I.

B. Manufacture of Experimental Bio-Yoghurt

Control yoghurt and bio-yoghurts were manufactured using five commercial starter cultures. The milk was tempered to 45^{o}C, fortified with 3% (w/v) nonfat dry milk, heated to 90 °C for 10 min and cooled to incubation temperature. The starter cultures were added and incubation was carried out according to the recommendations of the manufacturer until pH 4.6. The yogurts, produced in three replicates, were kept at room temperature (20^{o}C) for 30 min., stored at 4 ±1 °C and assessed 1, 5, 10, 15, 20 and 25 days of storage.

C. Microbiological Analysis

The culture growth and viability were evaluated taking 1 mL of each sample, decimally diluting it and plating on media described in Table II on 1, 5, 10, 15, 20 and 25 days of storage. The suitability of media for the selective growth of *S. thermophilus, L. bulgaricus, L. acidophilus, Bifidobacterium* ssp., *L. lactis* and *L. casei* was evaluated as described in Table II.

TABLE II: CULTURE MEDIA FOR SELECTIVE GROWTH ACCORDING TO THE LITERATURE

Culture	Culture Media	Incubation	Temperature (°C)	Time (h)	References
S. thermophilus	M17-Agar	Aerobic	37	72	Dave and Shah [18]– Donkor *et al.*[19]
L. bulgaricus	MRS agar (pH 5.2)	Anaerobic	43	72	Tharmaraj and Shah [20]
L. acidophilus	Bile-MRS agar	Aerobic	37	72	Vinderola and Reinheimer [21]
Bifidobacterium ssp.	LP-MR agar	Anaerobic	37	72	Vinderola and Reinheimer [21] – Lapierre *et al.* [22]
L. lactis	Elliker Agar	Anaerobic	42	72	Zimbro and Power [23]
L. casei	Vancomycin-MRS agar	Anaerobic	37	72	Tharmaraj and Shah [20]

D. Physico-Chemical Analysis

Different physico-chemical parameters such as moisture, ash, fat, protein and lactose in all prepared bio-yoghurt samples were estimated by the method described in A.O.A.C. [24]. The pH values of the samples were measured using digital pH meter (Analyzer model 315i/SET, WTW, Germany). The titratable acidity (LA %) was determined according to A.O.A.C. [25] during storage. Syneresis was estimated using a drainage test according to Atamer and Sezgin [26]. The quantification of lactic acid [27] and acetaldehyde [28] contents were determined spectrophotometric ally.

E. Sensory Evaluation

Ten panelists, selected depending on their availability and willingness to participate in the study, from staff of Department of Food Engineering, Uludag University, evaluated the sensory properties of the bio-yoghurt samples, after preliminary training sessions. Samples in three digit random number coded plastic cups were provided to panelists. The panelists were asked to note any defects or undesirable characteristics. Sensory evaluations were applied during the storage process using a five-point score system (5 excellent, 1 unacceptable).

F. Statistical Analysis

A factorial experiment was designed with the product type and the storage time as the main factors. Analysis of variance (ANOVA) using 99 % confidence intervals was run on each of the analyzed variables. Analysis of variance with mean separations using the LSD multiple range test as the level of significant difference was used to determine the effect of starter culture differences and storage time on physico-chemical, microbiological and sensorial properties. Different letters were used to label values with statistically significant differences among them. All analyses were performed using the Minitab for Windows (Version 10) Statistical Software Package (Minitab Inc., State College, PA).

III. RESULTS AND DISCUSSION

A. Incubation Period

Incubation of the yoghurt samples was ceased when the pH of the samples reached 4.6. The results obtained showed that the incubation periods of experimental yoghurts differed significantly (P<0.01, data not shown). While the sample A (control yoghurt) reached the target pH within 3 h, the incubation times of the B, C, D and E, containing probiotic bacteria combination, were 4.30, 6.30, 8 and 10 h, respectively. The incubation period of the samples was found to be dependent on the type or concentration of the probiotic cultures used.

B. Microbiological Characteristics of the Experimental Bio-Yoghurts

The changes in the viable counts of yoghurt and probiotic bacteria during storage are presented Table III. *S. thermophilus* counts of control and bio-yoghurt samples were effected significantly by culture combination type (P<0.01). The highest *S. thermophilus* counts were found in sample C (as 8.72 $\log_{10}$ cfu g^{-1}), where the lowest were for control yoghurt (A) with 8.10 $\log_{10}$ cfu g^{-1}. For the reason that, *L. bulgaricus* was used only in control yoghurt, the effect of storage time over *L. bulgaricus* counts, which was found significant (P<0.01). *L. bulgaricus* counts decreased throughout the storage time, the highest counts were on 1st day (as 8.42 $\log_{10}$ cfu g^{-1}) whereas the lowest values were on 25th day (as 7.55 $\log_{10}$ cfu g^{-1}). The highest *L. acidophilus* counts of bio-yoghurts were determined in E (as 7.89 $\log_{10}$ cfu g^{-1}), whilst the lowest values were in C (as 6.10 $\log_{10}$ cfu g^{-1}). Among produced samples, the highest *Bifidobacterium* ssp. counts were in E (as 7.90 $\log_{10}$ cfu g^{-1}), whereas sample B displayed the lowest value (as 6.13 $\log_{10}$ cfu g^{-1}). *L. lactis,* since being found only in probiotic culture combination C, analyzed statistically with respect to storage time, that displayed significant differences on counts (P<0.01). *L. lactis* counts throughout storage time varied from 9.29 $\log_{10}$ cfu g^{-1} to 8.54 $\log_{10}$ cfu g^{-1}. Dave and Shah [29], Vinderola *et al.* [30] and Gustaw *et al.* [31] reported that counts of probiotic strains showed a decrease over storage time. Nevertheless, for all bio-yoghurts the probiotic populations were approximately 10^6 cfu g^{-1} at the end of 25-day-storage. Recommendations for potential to exert the health-promoting effects the therapeutic minimum is quite variable. In general, the food industry applies the recommended level of 10^6 cfu g^{-1} at the time of consumption for probiotic bacteria to have the expected health effects [8]–[10]. According to the results of present study, all probiotic strains grew well and exhibited satisfactory viability levels after storage. The strains selected, acidity, storage temperature, oxygen content, pH and hydrogen peroxide due to bacterial metabolism, inoculation level, fermentation time, post-acidification and food matrix may be the major factors affecting the viability of probiotic microorganisms during manufacture and storage of fermented products [29], [30].

TABLE III: THE CHANGES OF VIABLE BACTERIA COUNTS OF SAMPLES DURING STORAGE (LOG CFU G^{-1})

Bacteria	Days of Storage	A	B	C	D	E
S. thermophilus	1	8.51^{cA}	9.23^{bA}	9.31^{aA}	-	-
	5	8.36^{cB}	8.88^{bB}	9.14^{aB}	-	-
	10	8.23^{cC}	8.70^{bC}	8.81^{aC}	-	-
	15	8.15^{cD}	8.65^{bD}	8.76^{aD}	-	-
	20	7.80^{cE}	8.57^{bE}	8.73^{aE}	-	-
	25	7.81^{cF}	8.39^{aF}	8.21^{bF}	-	-
L. bulgaricus	1	8.42^{A}	-	-	-	-
	5	8.21^{A}	-	-	-	-
	10	8.09^{A}	-	-	-	-
	15	7.71^{A}	-	-	-	-
	20	7.69^{A}	-	-	-	-
	25	7.55^{A}	-	-	-	-
L. acidophilus	1	-	7.50^{cA}	7.17^{dA}	8.31^{bA}	8.43^{aA}
	5	-	7.37^{bB}	6.88^{cB}	8.14^{aB}	8.14^{aB}
	10	-	7.29^{bC}	6.55^{cC}	7.91^{aC}	7.96^{aC}
	15	-	7.20^{cD}	6.04^{dD}	7.84^{bD}	7.93^{aD}
	20	-	7.16^{cE}	5.45^{dE}	7.69^{bE}	7.88^{aE}
	25	-	7.08^{bF}	5.30^{cF}	7.59^{aF}	7.54^{aF}
Bifidobacterium ssp.	1	-	7.01^{dA}	7.07^{cA}	8.50^{aA}	8.48^{bA}
	5	-	6.45^{cB}	6.47^{cB}	7.93^{bB}	8.32^{aB}
	10	-	6.05^{dC}	5.80^{cC}	7.77^{bC}	7.93^{aC}
	15	-	5.96^{cD}	5.70^{dD}	7.60^{bD}	7.95^{aD}
	20	-	5.74^{cE}	5.57^{dE}	7.44^{bE}	7.80^{aE}
	25	-	5.66^{cF}	5.56^{dF}	7.16^{bF}	7.59^{aF}
L. lactis	1	-	-	9.29^{A}	-	-
	5	-	-	9.00^{ABC}	-	-
	10	-	-	8.77^{ABC}	-	-
	15	-	-	8.70^{ABC}	-	-
	20	-	-	8.62^{ABC}	-	-
	25	-	-	8.54^{BC}	-	-
L. casei	1	-	-	-	-	8.07^{A}
	5	-	-	-	-	7.81^{A}
	10	-	-	-	-	7.61^{A}
	15	-	-	-	-	7.55^{A}
	20	-	-	-	-	7.46^{A}
	25	-	-	-	-	7.33^{A}

a,b Different superscript lowercase letters denote significant differences (P<0.01) between different samples
A,B Different superscripts capital letters denote significant differences (P<0.01) between different times of storage

C. Physico-Chemical Properties of the Experimental Bio-Yoghurts

The chemical composition of cow milk used for the production of yogurt and bio-yogurt fell within the following averages: titratable acidity 0.15% (LA), pH 6.62, total solids 12.66%, fat 3.80% and protein 3.17%.

The changes of some physico-chemical properties of yoghurt and bio-yoghurts during storage are presented Table IV. As it shows, the average pH values for all samples ranged from 4.60 to 4.10 during the storage. The highest pH value was found in D (as 4.43), and the lowest pH value was 4.24 for control (A). A gradual and consistent decrease in pH was noted significantly during 25-day-storage (P<0.01), but did not fall below pH 4.0, which is generally considered detrimental to the survival of probiotic bacteria [29].Whilst in control yoghurt only lactic acid is produced, lactic and acetic acids are produced by *L. acidophilus* and *Bifidobacteria* in bio-yoghurt. However, the post acidification, development of acidity during shelf life of yoghurt because of the conversion of lactose to lactic acid, was higher in control yoghurt compared to bio-yoghurts. Singh *et al.* [32], Ozer *et al.*, [33] and Ranathunga and Rathnayaka [34] reported that the enzyme activity of starter bacteria used in bio-yoghurt production resulted in significant decrease in pH during storage.

TABLE IV: CHANGES OF PHYSICO-CHEMICAL PROPERTIES OF SAMPLES DURING STORAGE

Physico-chemical Properties	Days of Storage	A	B	C	D	E
pH	1	4.45^{bA}	4.48^{bA}	4.47^{bA}	4.60^{aA}	4.41^{cA}
	5	4.36^{cB}	4.44^{bB}	4.43^{bB}	4.53^{aB}	4.39^{dA}
	10	4.25^{cC}	4.34^{bC}	4.34^{bC}	4.40^{aC}	4.32^{bB}
	15	4.20^{dD}	4.34^{bC}	4.34^{bC}	4.39^{aC}	4.31^{cB}
	20	4.16^{cE}	4.30^{aD}	4.30^{aD}	4.31^{aD}	4.24^{bC}
	25	4.10^{dF}	4.26^{bE}	4.25^{bE}	4.31^{aD}	4.14^{cD}
Titratable Acidity (LA%)	1	1.58^{aC}	1.25^{cD}	1.41^{bD}	1.19^{dC}	1.19^{dE}
	5	1.68^{aB}	1.43^{bC}	1.47^{bC}	1.28^{dB}	1.38^{cC}
	10	1.74^{aAB}	1.53^{bB}	1.51^{bB}	1.34^{cA}	1.34^{cD}
	15	1.79^{aA}	1.54^{bB}	1.51^{bB}	1.36^{cA}	1.58^{bB}
	20	1.81^{aA}	1.56^{cB}	1.57^{cA}	1.38^{dA}	1.68^{bA}
	25	1.83^{aA}	1.61^{cA}	1.57^{dA}	1.39^{eA}	1.69^{bA}
Syneresis (mL 25 g^{-1})	1	4.95^{bD}	4.65^{dC}	4.65^{dF}	5.66^{aD}	4.90^{cF}
	5	5.06^{dC}	5.13^{cB}	4.87^{eE}	6.13^{aC}	5.18^{bE}
	10	5.10^{dC}	5.17^{cB}	5.11^{dD}	6.24^{aB}	5.39^{bD}
	15	5.11^{eC}	5.70^{bA}	5.38^{dC}	6.25^{aB}	5.57^{cC}
	20	5.92^{cB}	5.76^{dA}	5.45^{eB}	6.33^{aB}	6.08^{bB}
	25	6.20^{cA}	5.80^{cA}	6.23^{cA}	7.86^{aA}	6.92^{bA}
Lactose (%)	1	4.42^{eA}	4.98^{bA}	4.60^{dA}	5.17^{aA}	4.85^{cA}
	5	4.27^{eB}	4.66^{bB}	4.51^{dB}	4.81^{aB}	4.60^{cB}
	10	4.12^{dC}	4.53^{bC}	4.46^{cC}	4.66^{aC}	4.52^{bC}
	15	4.12^{dC}	4.45^{cD}	4.43^{cC}	4.66^{aC}	4.52^{bC}
	20	4.02^{eE}	4.35^{cE}	4.41^{bC}	4.58^{aD}	4.31^{dD}
	25	4.07^{dD}	4.30^{bF}	4.30^{bD}	4.56^{aD}	4.26^{cE}
Lactic Acid (mg 100 g^{-1})	1	1.06^{aD}	0.73^{bD}	0.77^{bE}	0.75^{bE}	0.70^{cD}
	5	1.15^{aC}	0.85^{cC}	0.94^{bD}	0.87^{cD}	0.73^{dD}
	10	1.18^{aC}	0.92^{cB}	0.97^{bC}	0.94^{cC}	0.78^{dC}
	15	1.22^{aAB}	0.96^{bA}	0.99^{bC}	0.99^{bB}	0.80^{cB}
	20	1.23^{aAB}	0.96^{cA}	1.04^{bB}	1.03^{bA}	0.96^{cA}
	25	1.25^{aA}	0.97^{cA}	1.08^{bA}	1.04^{bA}	1.02^{bA}
Acetaldehyde (ppm)	1	20.66^{bA}	14.67^{dA}	27.48^{aA}	20.81^{bA}	18.39^{cA}
	5	16.08^{cB}	14.11^{dA}	21.76^{aB}	16.26^{cB}	17.31^{bA}
	10	14.06^{bC}	13.57^{bAB}	18.30^{aC}	11.17^{cC}	13.19^{bB}
	15	13.58^{bC}	13.29^{bAB}	17.94^{aC}	10.60^{cC}	11.56^{cC}
	20	13.40^{bC}	12.96^{bC}	15.03^{aD}	10.56^{cC}	10.97^{cC}
	25	12.91^{bD}	12.74^{bC}	14.54^{aD}	10.10^{cC}	10.01^{cC}

a,b Different superscript lowercase letters denote significant differences ($P<0.01$) between different bio-yoghurt samples

A,B Different superscripts capital letters denote significant differences ($P<0.01$) between different times of storage

Titratable acidity is a very important factor, which affects the shelf life and the acceptability of fermented dairy products [35]. The titratable acidity rate of samples displayed significant differences due to product type and storage time ($P<0.01$). The titratable acidity of control and bio-yoghurts varied from 1.19 to 1.83% throughout storage time. In contrast to pH, the acidity of samples showed significant increase ($P<0.01$) during storage due to acid formation. Since *L. bulgaricus* is the main bacteria responsible for acid production, control sample had the highest acidity at the beginning and at the end of the storage period. The lowest acidity (1.39) was observed in the sample D at the end of the storage period. The level of acidity in bio-yogurts was found to be lower than control yogurt. These results were in agreement with Singh *et al.* [32], Ranathunga and Rathnayaka [34], Vahicic and Hruskar [36], Guler-Akin and Akin [37], and Ozer *et al.* [38]. It appears that the composition of starter culture, fermentation temperature and storage period could influence the overall level of acidity and pH of stored yoghurt samples [32].

Syneresis is generally defined as separation of aqueous phase from continuous phase or gel network, which is an undesirable property in fermented milk products [17]. The highest syneresis at the end of the storage was determined in sample D, whereas the lowest value was obtained in sample C. Because of differentiations in metabolic activities of starter cultures, product type and storage time were significant on syneresis being in agreement with Panesar and Shinde [39] ($P<0.01$). Besides, the acidity of the yogurts can be a further contributing factor, since higher acidity is known to stimulate syneresis in yogurt [40], [41].

The main carbohydrate in dairy and dairy products is lactose so called “milk sugar”. The use of different culture combinations resulted in significant differences over product type and storage time in terms of lactose values ($P<0.01$). Due to the production yield of lactic acid, the decrease in lactose content of bio-yoghurt samples during storage was determined to be culture dependent. These findings were in accordance with the observations of Sady *et al.* [42] and Bano *et al.* [43].

Lactic acid is suggested to be one of the major compounds of yogurt flavor [44]. The lactic acid values of yoghurt samples displayed significant differences due to product type and storage time (P<0.01). A noticeable increase in lactic acid concentration was observed for all the bio-yoghurts throughout the storage period. The concentrations varied from 0.97 to 1.25 mg 100 g^{-1}. These results were in agreement with that obtained by Dave and Shah [29], who they reported a similar pattern of increase in parallel to titratable acidity.

Acetaldehyde is mainly responsible for the typical aroma of yogurt. In samples, the highest acetaldehyde value was 27.48 ppm in sample C, whereas the lowest was in sample D with 13.81 ppm. Therefore the differences in acetaldehyde contents could be the result of inoculum level and probiotic bacteria used, as in lactobacilli species the activity of alcohol dehydrogenase, which catalyzes the formation of acetaldehyde from threonine, is species-dependent. These results could be attributed to the fact that the counts of the total lactic acid bacteria in sample C were higher than those of other samples. The acetaldehyde content seemed to decrease during storage; the highest value being 27.48 ppm for the 1st day and the lowest being 10.01 ppm on 25th day. The decrease in acetaldehyde levels can be related to the hydrolysis by microbial enzymes to form other substances, such as ethanol. In addition, carbonyl compound production capacity of bifidobacteria and lactobacilli are limited when compared to the yoghurt starter bacteria [38]–[40]. The panelists mentioned a mild flavor in bio-yoghurts. Similar results were also reported by Guler-Akin and Akin [37] and Bonczar *et al.* [45].

No significant differences were observed in fat, ash and protein contents of the yoghurt samples (P>0.05; data not shown).

D. Sensory Evaluations of the Experimental Bio-Yoghurts

The popularity of yogurt depends mainly on its sensory characteristics, of which are characterized as the microbial factors, processing parameters, source of milk and the additives used. The mean scores of the sensorial attributes (appearance and color, consistency, odor, taste and overall acceptability) for samples given by the panelists were presented in Table V. The sensory response to the samples demonstrated that the use of probiotic culture combination positively influenced the overall sensory characteristics. The panel found significant differences (P<0.01) for each sample for consistency, odor, taste and overall acceptability, which reflects the advantages of probiotic culture as effective components on the general sensory properties of yogurt. The overall acceptability of yoghurt samples was determined on the basis of the average of the total score obtained for different sensory attributes. The highest overall acceptabilities were obtained for the sample C, and the lowest acceptability scores were for the sample E. Especially, the overall acceptability scores of samples increased during storage of up to 10 days and thereafter decreased for all criteria. This could be associated with development of acidity and decreases in acetaldehyde contents.

TABLE V: CHANGES OF SENSORY PROPERTIES OF SAMPLES DURING STORAGE

Properties	Days of Storage	A	B	C	D	E
Appearance and Color	1	4.27	3.48	3.35	3.70	3.77
	5	4.63	3.88	3.88	3.88	3.67
	10	4.30	3.95	4.30	4.70	4.10
	15	3.88	3.57	5.00	3.86	4.14
	20	4.69	3.80	4.32	3.56	3.82
	25	3.84	3.84	3.84	4.00	3.92
Consistency	1	4.00^{bAB}	4.13^{bB}	4.38^{aA}	3.75^{cB}	3.63^{cdC}
	5	4.20^{bA}	4.40^{aA}	4.20^{bB}	4.20^{bA}	3.40^{cC}
	10	4.30^{cA}	4.60^{aA}	4.60^{aA}	4.20^{cdA}	4.45^{bA}
	15	4.13^{bAB}	4.19^{abB}	4.32^{aAB}	3.82^{cB}	3.94^{cB}
	20	4.41^{aA}	3.89^{cC}	4.13^{bB}	3.57^{dC}	3.82^{cB}
	25	3.78^{cB}	4.10^{bB}	4.41^{aA}	4.21^{bA}	3.62^{cdC}
Odor	1	4.25^{cD}	4.50^{bA}	4.75^{aA}	4.50^{bC}	4.50^{bB}
	5	4.39^{bC}	4.44^{bB}	4.63^{aB}	3.84^{cD}	3.89^{cE}
	10	4.50^{bB}	4.59^{bA}	4.75^{aA}	4.67^{aB}	4.09^{cCD}
	15	4.90^{abA}	4.55^{cA}	4.75^{bA}	5.00^{aA}	4.90^{abA}
	20	4.48^{aB}	4.25^{cdC}	4.34^{cD}	4.50^{aC}	4.04^{dD}
	25	4.19^{bE}	4.25^{bC}	4.44^{aC}	3.75^{cE}	4.19^{bC}
Taste	1	4.13^{dB}	4.38^{bA}	4.23^{cB}	4.25^{cB}	4.50^{aA}
	5	3.73^{cD}	4.15^{bB}	4.34^{aAB}	3.38^{dD}	3.28^{dD}
	10	4.00^{cC}	4.50^{aA}	4.42^{aA}	4.17^{bB}	3.67^{dC}
	15	4.30^{cA}	4.45^{bA}	4.60^{abA}	4.50^{bA}	4.70^{aA}
	20	4.09^{aB}	3.94^{bC}	4.14^{aB}	4.00^{abC}	3.73^{cC}
	25	3.82^{cD}	3.83^{cC}	4.38^{aAB}	4.00^{bC}	4.00^{bB}
Overall Acceptability	1	4.16^{aC}	4.12^{aB}	4.18^{aC}	4.05^{abB}	4.10^{aB}
	5	4.24^{aB}	4.22^{aB}	4.26^{aC}	3.83^{bC}	3.56^{cD}
	10	4.28^{bB}	4.41^{aA}	4.52^{aB}	4.44^{aA}	4.08^{cB}
	15	4.30^{bcB}	4.19^{cB}	4.67^{aA}	4.30^{bcA}	4.42^{bA}
	20	4.42^{aA}	3.97^{bC}	4.23^{aC}	3.91^{bB}	3.85^{bC}
	25	3.91^{bD}	4.01^{bC}	4.27^{aC}	3.99^{bB}	3.93^{bC}

a,b Different superscript lowercase letters denote significant differences (P<0.01) between different bio-yoghurt samples
A,B Different superscripts capital letters denote significant differences (P<0.01) between different times of storage

IV. Conclusion

Different probiotic culture combination and storage time had significant effects on the pH, titratable acidity, syneresis, lactic acid concentration, acetaldehyde contents, viable bacterial counts and sensory characteristics of bio-yogurts. Based on our results, probiotic bacteria used were found to survive throughout storage period and are suitable to provide sufficient number of viable bacteria counts at the time of product consumption. Sample C received the highest sensory scores from the panelists; however, total sensorial scores for all yoghurts seemed to decrease throughout storage. Further studies are needed to achieve the best physico-chemical and sensory characteristics of the final product to satisfy consumer demands.

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Lutfiye Yilmaz-Ersan is an assistant professor of Department of Food Engineering at the Uludag University, Bursa, Turkey. More recently, she has worked dairy and dairy products. From 2007 to 2008 (14 months) she worked at University of Nebraska-Lincoln USA, Department of Food Science and Technology as a visiting scientist. Her areas of interest include dairy and dairy products, probiotics and prebiotics.