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RESEARCH ARTICLE

Set Type Yoghurt Production by Exopolysaccharide Producing Turkish Origin Domestic Strains of *Streptococcus thermophilus* (W22) and *Lactobacillus delbrueckii ssp. bulgaricus* (B3)

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Summary

Lactobacillus delbrueckii spp. bulgaricus (B3) and *Streptococcus thermophilus* (W22) with high exopolysaccharide (EPS) production were combined with commercial culture in various percentages and used in yoghurt production. Lactic acid and acetaldehyde contents, titratable acidity, tyrosine, viscosity and consistency values, and EPS level were measured throughout storage for twenty one day with 10-day intervals. The yoghurt sample produced with commercial culture only (control) had the highest level of acidity. Tyrosine content of the yoghurt produced by wild strains (sample D) was higher than that of others, whereas acetaldehyde and EPS levels were lower. The domestic strains did not influence the viscosity of yoghurt significantly (P>0.01). On contrary, the gel firmness of yoghurts produced with *Lactobacillus delbrueckii spp. bulgaricus* (B3) was lower than the other samples. The yoghurt sample made by isolated strains only had the highest viscosity values. Yoghurt produced by using isolated strains was found not to be acceptable by the panellists.

Keywords: *Exopolysaccharides, Yoghurt, Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus*

Ekzopolisakkarit Üretimi Yüksek Yerli *Streptococcus thermophilus* (W22) ve *Lactobacillus delbrueckii ssp. bulgaricus* (B3) Suşları ile Set Tip Yoğurt Üretimi

Özet

Bu çalışmada, yoğurt üretiminde ekzopolisakkarit üretimi yüksek olan yerli *Lactobacillus delbrueckii ssp. bulgaricus* (B3) ve *Streptococcus thermophilus* (W22) bakterileri ticari kültürle farklı oranlarda karıştırılarak kullanılmıştır. Üretilen yoğurtlar 21 gün süreyle buzdolabı koşullarında depolanmış ve 10 gün arayla analize tabi tutulmuştur. Deneme örneklerinde laktik asit, asetaldehit, titrasyon asitliği, tirozin değeri ve ekzopolisakkarit miktarı belirlenmiş; ayrıca konsistens ve viskozite ölçümleri de alınmıştır. Sadece ticari kültürün kullanıldığı kontrol yoğurdunun asitliği diğer örneklerden daha yüksek düzeyde bulunmuştur. Sadece izole suşların kullanıldığı örnek ise tirozin içeriği açısından diğerlerinden daha yüksek değer vermiştir. Buna karşın bu örneğin asetaldehit ve ekzopolisakkarit içeriği daha düşük çıkmıştır. Yerli suşlar yoğurdun viskozitesini etkilememiş, *Lactobacillus delbrueckii ssp. bulgaricus* (B3)'ün kullanıldığı yoğurtta pıhtı sıkılığı daha düşük bulunmuştur. Sadece izole suşlar kullanılarak üretilen yoğurdun (D örneği) viskozitesi diğerlerinden bir miktar yüksek bulunmuş ve söz konusu örnek yapılan duyusal değerlendirme sonucunda panelistlerce beğenilmemiştir.

Anahtar sözcükler: *Ekzopolisakkarit, Yoğurt, Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus*

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INTRODUCTION

Lactic acid bacteria (LAB) have been extensively studied in the food industry, not only in view of their ability to acidify and hence preserve food products from spoilage, but as well for their contribution to the organoleptic properties of the final fermented food [1,2]. Among them a combination of *Streptococcus thermophilus* and *Lb. delbrueckii ssp. bulgaricus* which grow synergistically are widely used as starter cultures for the production of yoghurt [3-5]. It is widely known that some LAB like *Streptococcus thermophilus* and *Lb. delbrueckii ssp. bulgaricus* secrete EPS [2,3,6,7]. The use of cultures producing EPS play an important industrial role in the texture development of yoghurts and other fermented milks, low fat cheeses and dairy desserts [3,6,8].

In general, the food industry is particularly interested in natural viscosifiers and texture enhancers, the so-called biothickeners. These are mostly plant carbohydrates (e.g., locust bean gum, starch, alginate, pectin and guar gum), animal hydrocolloids (e.g., gelatin and casein), or bacterial biopolymers (e.g. xanthan and gellan) [1]. However most of the plant carbohydrates used are chemically modified to improve their rheological properties. Their use is hence strongly restricted, especially in the European Union (E numbers) [6]. The use of cultures producing EPS increases resistance of yoghurt coagulum to thermal and physical shocks, and play an important role in achieving satisfactory firmness and apparent viscosity of yoghurt [1,8]. EPS also effective in protecting microbial cells against phagocytosis, phage attacks, antibiotics, toxic compounds, osmotic stress and bacteriocins [2,9-11]. With these considerations in mind, it was aimed to produce set-type yoghurt by using EPS producing Turkish origin domestic strains of *Streptococcus thermophilus* and *Lb. delbrueckii ssp. bulgaricus,* and to evaluate the quality of the resulting products.

MATERIAL and METHODS

Culture and Growth Conditions

The strains of *Lb. delbrueckii ssp. bulgaricus* B3 and *Streptococcus thermophilus* W22 used in this study were isolated from village type yoghurt by Aslim et al.[12]. The strains isolated were identified as *Lb. delbrueckii ssp. bulgaricus* B3 and *Streptococcus thermophilus* W22 on the basis of morphological and biochemical characteristics, and 16S rDNA sequence analysis were done [2]. The criteria for selection of the strains were based on their quantity of EPS production. Each strain was grown in skim milk (10 g/100 ml) and incubated at 40°C for 18 h. In addition, the control yoghurt was produced incorporating a commercial blend of starter culture coded TM-081 (Rhodia-EZAL group).

Production of Yoghurt

Fresh cow milk was obtained from the Ankara University, Faculty of Agriculture, Department of Dairy Technology. The gross composition of raw milk was: 7.38±0.53 titratable acidity (°SH), 6.55±0.09 pH, 3.08±0.06 total fat and 12.66±0.22 total solids. The raw milk was heated up to 40°C and skimmilk powder was added so that total solids content was increased to 15%. After homogenization at 60°C at 200 kg/cm^2, milk was heat treated at 85°C for 20 min and cooled to 42°C, and then divided in four equal portions. Each portion was inoculated with 3% (v/v) commercial starter (A); 1.5% (v/v) commercial starter plus 1.5% (v/v) *Lb. delbrueckii ssp. bulgaricus* B3 (B); 1.5% (v/v) commercial starter plus 1.5% (v/v) *Streptococcus thermophilus* W22 (C); 1.5% (v/v) *Lb. delbrueckii ssp. bulgaricus* B3 and 1.5% (v/v) *Streptococcus thermophilus* W22 (D). Inoculated milk was, then, incubated at 42°C for 4 to 5 h until pH 4.6 was attained. Following incubation, the samples were stored at 4°C for 21 days. Analyses were performed in the experimental yoghurts after 1, 11 and 21 days of storage. The experiment was repeated three times in duplicate.

Chemical Analysis

The dry matter, titratable acidity and total fat contents were determined by the methods given in Turkish national standard for yoghurt [13]. The spectrophotometric methods were followed for the analysis of lactic acid [14] and tyrosine contents [15]. Acetaldehyde content was determined according to Lees and Jago [16]. The firmness of yoghurt curd was determined by a penetrometer (Stanhope-Seta Surrey, England) using the cone-form penetration body with an apical angle of 45°C, and a weight of 72.5 g. The depth of penetration was measured at 5 s at a product temperature of 7±1°C. HAAKE-Viscometer VT 181/VT 24 was employed for determination of viscosity. Sensory properties were evaluated by 10 experienced panellists according to the scoring sheet given in Turkish national standard [13]. Analysis of variance was performed using MINITAB [17], and the results were analyzed as a randomized plot design. Means were compared by the least significant differences method [18,19].

Isolation and Quantification of EPS

The yoghurt samples were boiled at 100°C for 10 min. After cooling, they were treated with 17 ml/100 ml of 85% trichloracetic acid solution and centrifuged [20].

After removal of the cells and protein by centrifugation, the EPS was precipitated with ethanol. The EPS was recovered by centrifugation at 4°C at 14000 rpm for 20 min. Total EPS (expressed as mg l^{-1}) was estimated in each sample by phenol-sulphuric method [21] using glucose as a standard [22].

RESULTS

Total solids of the samples A, B, C and D were as follows respectively (g 100 g^{-1}); 14.60±0.103, 14.51±0.108, 14.51±0.106, 14.48±0.092 at the beginning of storage period. The fat contents of the samples (g 100 g^{-1}) were found to be 3.03±0.033, 2.96±0.088, 3.00±0.000, 3.00±0.000 respectively.

Table 1 describes some chemical properties of yoghurt during the storage. Lactic acid value and the titratable acidity of yoghurt samples increased throughout the storage period. There was no significant difference between the samples with regard to titratable acidity and lactic acid (P>0.01).

There was a significant difference (P<0.01) between yoghurt samples in terms of tyrosine contents of the samples. The highest tyrosine value was obtained from the sample D, where local strains of yoghurt bacteria with high EPS production were used. This sample was followed by sample B (including *Lb. delbrueckii ssp. bulgaricus* B3).

No significant difference was noted between the samples with regard to acetaldehyde levels (P>0.01). In all samples, the highest acetaldehyde content was recorded on the 11th day of storage, and then decreased.

EPS producing starters are used to improve physical properties of yoghurt. In this study, it was observed that there was no statistically significant difference between the samples with regard to EPS concentrations (P>0.01). However, the highest EPS concentrations was recorded on the 11th day of storage from the sample B (256 mg/L). The sample D had the lowest EPS concentrations (175 mg/L). Given the average of EPS values, it was shown that sample B (219 mg/L) and C (220 mg/L) were relatively higher than the sample A (196 mg/L).

Table 1. *Some chemical and physical properties of yoghurt samples*
Tablo 1. *Yoğurt örneklerinin fiziksel ve kimyasal özellikleri*

Properties	**Storage (Day**	**Treatments**			
		A	**B**	**C**	**D**
Titratable Acidity (°SH)	1 11 21	49.25±2.88 55.40±2.85 56.39±3.07	47.47±1.71 52.74±2.52 54.26±2.75	47.02±3.58 50.67±4.87 52.36±5.45	45.87±3.69 48.00±3.73 50.05±4.80
Lactic Acid (g/100g)	1 11 21	0.83±0.34 0.89±0.41 0.98±0.84	0.82±0.39 0.87±0.41 0.90±0.27	0.79±0.61 0.84±0.53 0.86±0.77	0.76±0.49 0.75±0.62 0.79±0.83
Tyrosine Value (mg/g)	1 11 21	0.278±0.017 ^{ab} 0.312±0.027 ^{ab} 0.400±0.011 ^{ab}	0.291±0.017 ^{b} 0.356±0.022 ^{b} 0.379±0.028 ^{b}	0.272±0.013 ^{ab} 0.300±0.030 ^{ab} 0.346±0.015 ^{ab}	0.344±0.024 ^{a} 0.410±0.064 ^{a} 0.471±0.026 ^{a}
Acetaldehyde (ppm)	1 11 21	17.05±4.70 22.68±2.17 18.87±0.72	18.41±4.32 23.55±1.61 16.57±1.47	14.92±4.92 21.60±2.68 14.96±0.17	12.21±1.65 16.78±0.95 15.25±1.41
EPS (mg/l)	1 11 21	206±13.5 200±13.7 181±42.7	215.67±7.67 265.30±17.6 176.30±68.2	243.33±6.17 251.00±31.9 166.70±37.4	188.0±22.01 174.7±30.80 169.7±73.60
Penetrometer Value (x1/10 mm)	1 11 21	306.33± 6.33 280.00±10.40 286.00± 5.69	309.67±7.84 276.00±12.30 279.30±10.10	305.33±07.80 286.70±14.70 285.00±13.70	311.33±9.53 282.70±13.50 286.30±14.30
Viscosity (cP)	1 11 21	1441.7±98.2 1683.0±10.1 1758.0±13.1	1275±94.6 1475±13.9 1575±13.9	1308.3±79.5 1508.0±21.4 1558.0±20.4	1275.0±14.40 1536.7±7.26 1512.7±7.22

A: Commercial culture (3%)
B: Commercial culture (1.5%) plus isolated Lb. delbrueckii ssp. bulgaricus B3 (1.5%)
C: Commercial culture (1.5%) plus isolated Streptococcus thermophilus W22 (1.5%)
D: Isolated Lb. delbrueckii ssp. bulgaricus B3 (1.5%) plus isolated Streptococcus thermophilus W22 (1.5%)
a, b, ab, Means within the same row with different superscripts differ significantly (P<0.01)

The good quality of yoghurt is characterized with high firmness and low whey separation. In the present study, there was no significant difference between the yoghurt samples in terms of gel firmness (P>0.01). The EPS(+) strains seemed to be ineffective on gel firmness.

In the yoghurt samples, the viscosity measurements were in good correlation with the gel firmness readings. It is thought that the slight increase in viscosity was caused by the EPS(+) strains.

Given the results of sensory evaluation *(Table 2)*, we observed that there was no difference between the samples in terms of apperance and odour. On examination of structure and texture, the samples B and C, in which strains of *Lb. delbrueckii ssp. bulgaricus* B3 along with commercial culture and *Streptococcus thermophilus* W22 were used had higher scores than the others. The samples B and C most were given higher scores by the panel group, whereas sample D, was found to be too sweet.

Table 2. *Organoleptic properties of yoghurt samples (Mean ± SE)*
Tablo 2. *Yoğurt örneklerinin duyusal nitelikleri (ortalama ± standart hata)*

Treatments	Storage (Day)	Appearance (Max 5 Points)	Body and Texture (Max 5 Points)	Odour (Max 5 Points)	Taste (Max 5 Points)
A	1	5.000±0.000	4.233±0.122	4.933±0.067	4.000±0.115
	11	4.933±0.067	3.933±0.067	5.000±0.000	3.667±0.167
	21	4.933±0.067	3.767±0.145	4.600±0.231	3.500±0.252
B	1	4.867±0.133	4.300±0.252	5.000±0.000	4.200±0.306
	11	5.000±0.000	4.233±0.033	5.000±0.000	4.167±0.186
	21	4.633±0.186	3.933±0.067	4.700±0.153	3.933±0.296
C	1	4.933±0.067	4.467±0.033	5.000±0.000	4.467±0.333
	11	5.000±0.000	4.300±0.153	5.000±0.000	4.133±0.133
	21	4.400±0.100	3.967±0.203	4.700±0.153	3.933±0.067
D	1	5.000±0.000	4.133±0.133	5.000±0.000	3.833±0.203
	11	4.933±0.067	4.167±1.012	5.000±0.000	3.833±0.441
	21	4.833±0.167	3.900±0.265	4.833±0.167	3.467±0.808

A: Commercial culture (3%)
B: Commercial culture (1.5%) plus isolated Lb. delbrueckii ssp. bulgaricus B3 (1.5%)
C: Commercial culture (1.5%) plus isolated Streptococcus thermophilus W22 (1.5%)
D: Isolated Lb. delbrueckii ssp. bulgaricus B3 (1.5%) plus isolated Streptococcus thermophilus W22 (1.5%)

DISCUSSION

The development of acidity is important not only form milk fermentation, but also necessary for a well-balanced aroma, texture and flavor of yoghurt. Acid development by domestic strains was poor (especially sample D) in comparison with the commercial starter. Özer and Atasoy [23] used viscous and non-viscous commercial strains of *Lb. delbrueckii ssp. bulgaricus* (B3) and *Stretococcus thermophilus* (CH-1) in yoghurt production. The authors determined the levels of titratable acidity and lactic acid in yoghurt produced by viscous culture as 44°SH and 0.62 g 100 g^{-1} respectively. These figures were reported to be 53.4°SH and 0.77 g 100 g^{-1} in yoghurt produced by non-viscous culture.

Although lactic acid bactreria are generaly weakly proteolytic compared with other groups of bacteria such as Bacillus, Proteus, Pseudomonas, and coliforms, some strains of LAB show high proteolytic activity in many fermented dairy products [24]. Proteolytic activity of the starter used contributes to texture development of the final product. Proteolytic ability of lactic acid bacteria is dependent on species and strains. It is known that the proteolytic activity of *Lb. delbrueckii ssp. bulgaricus* is higher than that of *Streptococcus thermophilus.* In our research, the highest tyrosine value was obtained from the sample D. Özer and Atasoy [23], found that yoghurt produced by viscous cultures provided a higher level of tyrosine than that of yoghurt produced by non-viscous cultures. Proteolytic activities of starter bacteria may have some adverse effects on fermented milk. In yoghurt, production of bitter peptides is largely attributed to proteolysis by *Lb .delbrueckii ssp. bulgaricus* during storage.

Yoghurt sample produced with viscous and/or domestic culture had less acetaldehyde level than those manufactured with mixed or only commercial yoghurt bacteria. The acetaldehyde production ability of EPS(-) strains of yoghurt bacteria is high, whereas acetaldehyde production capacity in ropy or viscous strains of the yoghurt bacteria decreases considerably [23,25,26].

It was reported that the amount of EPS produced by the LAB ranged from 150 to 600 mg/L, depending on strains under optimal culture conditions [6,9,27]. In sample D, where only domestic strains were, incorporated it was seen that the amount of EPS was lower than those of the other samples. Similar results were obtained by Marshall and Rawson [28], who produced yoghurt, using strains of *Streptococcus thermophilus* and *Lb. delbrueckii ssp. bulgaricus* with and without exopolysaccharide production ability and showed that the lowest level of EPS was obtained from the sample in which EPS(+) strains were used together. On the other hand, Bouzar et al.[29] showed that mixed cultures of a non-ropy strains of *Streptococcus thermophilus* and different ropy strains of *Lb. delbrueckii ssp. bulgaricus* produced EPS at a faster rate.

The firmness of the gel and the ability to retain the water are influenced by the structure of the gel formed or the type of the culture used [11]. In the present study, the EPS(+) strains seemed to be ineffective on gel firmness. In many studies, it was stated that the higher the EPS level in yoghurt produced by ropy strains, the lower the curd firmness. The EPS(+) could interfere with the association between casein micelles resulting in a less firm coagulum [28,30,31]. The gel firmness of the samples increased during the cold storage. In contras, Güzel-Seydim et al.[32] stated that penetrometer values of all samples decreased significantly during storage.

Several strains which are capable of forming EPS, were isolated and analyzed [33]. In present study, viscosity of samples produced by the domestic strains was found to be higher than that of control sample. Some researcher explained that, there was no clear correlation between the EPS concentration and viscosity, and polysaccharide type was far more important than EPS quantity in terms of viscosity. It was reported that the formation of protein strand and protein-protein bond development is partly prevented by excessive formation of poly-saccharide flaments attached to the protein matrix and thus reducing rigidity of the yoghurt gel [9,28,30,34].

The primary function of lactic cultures used to manufacture of yoghurt is to produce lactic acid required for the formation of the coagulum. Additionally they produce volatile compounds reponsible for the typical flavour and texture of yoghurt. Careful selection of the strains employed and good monitoring throughout the manufacturing process of yoghurt are, therefore, essential to control the metabolic end-products, final pH, flavour and aroma and texture efficiently. According the results obtained, the yoghurt sample made by domestic starter strains only had a negative result, especially in terms of sensory qualities. The use of combined cultures of commercial starter and *Lb. delbrueckii ssp. bulgaricus* B3 and/or *Streptococcus thermophilus* W22 may offer an alternative to yoghurt processors. Our findings are crucial for the dairy industry to be used domestic strains as starter culture and to be taken place in the culture collections.

REFERENCES

1. Vaningelgem F, Zamfir M, Mozzi F, Adriany T, Vancanneyt M, Swings J, De Vuyst L: Biodiversity of Exopolysaccharides produced by *Streptococcus thermophilus* strains is reflected in their production and their molecular and functional characteristics. *Appl Environ Microb,* 70, 900-912, 2004.

2. Durlu-Özkaya F, Aslim B, Ozkaya MT: Effect of exo-poly-saccharides (EPSs) produced by *Lactobacillus delbrueckii ssp. bulgaricus* strains to bacteriophage and nisin sensitivity of the bacteria. *LWT-Food Sci Technol,* 40, 564-568, 2007.

3. De Vuyst L: Technology aspects related to the application of functional starter cultures. *Food Technol Biotech,* 38, 105-112, 2000.

4. Hassan AN, Corredig M, Frank JF: Capsule formation by starter cultures affects the viscoelastic properties of yoghurt during structure formation. *J Dairy Sci,* 85, 716-720, 2002.

5. Tarakçı Z: Influence of kiwi marmalade on the rheology characteristics, color values and sensorial acceptability of fruit yogurt. *Kafkas Univ Vet Fak Derg,* 16, 173-178, 2010.

6. De Vuyst L, Vanderveken F, Van de Ven S, Degeest B: Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. *J Appl Microbiol,* 84, 1059-1068, 1998.

7. Welman AD, Maddox IS: Exopolysaccharides from lactic acid bacteria: Perspectives and challenges. *Trends Biotechnol,* 21, 269-274, 2003.

8. Ngoufack ZF, El-Noda AN, Tchouanguep FM, El-Soda M: Effect of ropy and capsular exopolysaccharides producing strain of *Lactobacillus plantarum* 162RM on characteristics and functionally of fermented milk and soft Kareish type cheese. *Afr J Biotechnol,* 3, 512-518, 2004.

9. De Vuyst L, Degeest B: Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol Rev,* 23, 153-177, 1999.

10. Sutherland IW: Polysaccharides for microbial exopoly-saccharides. *Carbohyde Polym,* 38, 310-328, 1999.

11. Ruas-Madiedo P, Hugenholtz J, Zoon P: An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *Int Dairy J,* 12, 163-171, 2002.

12. Aslim B: Bazı laktik asit bakterilerinin EPS üretimlerinin belirlenmesi. TUBITAK Proje No: TBAG-2090 (IDIT 129), Ankara, 2005.

13. TSE (Türk Standartları Enstitüsü): Yoğurt, (TS 1331), Ankara, Turkey, 1989.

14. Steinsholt K, Calbert HE: A rapid colorimetric method for determination of lactic acid in milk and milk products. *Milchwissenschaft,* 31, 402-408, 1960.

15. Tunail N: A study on some important physiological properties of lactic acid bacteria isolated from White brined cheese used as starter. *Associate Proffesor Ship Thesis,* Ankara, 1978.

16. Lees GL, Jago GR: Methods for the estimation of acetaldehyde in cultured dairy products. *Aust J Dairy Technol,* 24, 181-185, 1969.

17. Ryan BF, Joiner BL, Ryan TA Jr: Minitab Handbook. PWS-KENT Publishing Company, Boston, USA, 1985.

18. Düzgüneş O, Kesici T, Kavuncu O, Gürbüz F: Araştırma ve Deneme Metotları (İstatistik Metotları II). Ankara Üniv Ziraat Fak Yayınları, 1021, Ankara Üniv Basımevi, Ankara, 1987.

19. Ergün G, Aktaş S: ANOVA modellerinde kareler toplamı yönteminin karşılaştırılması. *Kafkas Univ Vet Fak Derg,* 15, 481-484, 2009.

20. Frengova GI, Simova ED, Beshkova DM, Simov ZI: Production and monomer composition of exopolysaccharides by yoghurt starter cultures. *Can J Microbiol,* 46, 1123-1127, 2000.

21. Dubois M, Gilles KA, Hamilton JK, Pebers PA, Smith F: Colorimetric Method for determination of sugars and related substances. *Anal Chem,* 28, 350-356, 1956.

22. Torino MI, Taranto MP, Sesma F, De Valdez GF: Heterofermentative pattern and exopolysaccharide production by *Lactobacillus helveticus* ATCC 15807 in response to environmental pH. *J Appl Microbiol,* 91, 846-852, 2001.

23. Özer B, Atasoy F: Effects of addition of amino acids, treatment with ß-galactosidase and use of of heat-shocked cultures on the acetaldehyde level in yoghurt. *Int J Dairy Technol,* 55, 166-170, 2002.

24. Rajagopal SN, Sandine WE: Associative growth and proteolysis of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in skim milk. *J Dairy Sci,* 73, 894-899, 1990.

25. Robinson RK, Tamime AY, Chubb LW: Acetaldehyde as an indicator of flavour intensity in yoghurt. *The Milk Industry,* 79, 4-6, 1977.

26. Tamime AY, Robinson RK: Yoghurt Science and Technology. 3rd ed., Woodhead Published Ltd., Cambridge, England, 1999.

27. Duboc P, Mollet B: Applications of exopolysacharides in dairy industry. *Int Dairy J,* 11, 759-768,2001.

28. Marshall VM, Rawson HL: Effect of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of the stirred yoghurt. *Int J Food Sci Tech,* 34, 137-143, 1999.

29. Bouzar F, Cerning J, Desmazeaud M: Exopolysaccharide production and texture-promoting properties of mixed-strain starter cultures in yoghurt production. *J Dairy Sci,* 80, 2310-2317, 1997.

30. Hassan AN, Frank JF, Schmidth KA, Shalabi SI: Textural properties of yoghurt made with encapsulated nonropy lactic cultures. *J Dairy Sci,* 79, 2098-2103, 1996.

31. Hess SJ, Roberts RF, Ziegler GR: Rheological properties of nonfat yoghurt stabilized using *Lactobacillus delbrueckii ssp. bulgaricus* producing exopolysaccharide or using commercial stabilizer system. *J Dairy Sci,* 80, 252-263, 1997.

32. Güzel-Seydim ZB, Sezgin E, Seydim AC: Influence of exopolysaccharide producing cultures on the quality of plain set type yoghurt. *Food Control,* 16, 205-209, 2005.

33. Grobben GJ, Chin-Joe I, Kitzen V, Boels IC, Boer F, Sikkema J, Smith MR, De Bont JAM: Enhancement of exopolysaccharide production by *Lactobacillus delbrueckii subsp. bulgaricus* NCFB 2772 with a simplified defined medium. *Appl Environ Microb,* 64, 1333-1337, 1998.

34. Sebastiani H, Zelger G: Texture formation by thermophilic lactic acid bacteria. *Milchwissenschaft,* 53, 15-20, 1998.