African Journal of Microbiology Research Vol. 6(22), pp. 4696-4701, 14 June, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1319
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Effect of protective culture and biopreservatives on strained yoghurt quality

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Accepted 27 December, 2011

In this research, the protective culture (*Lactobacillus rhamnosus* LC705 and *Propionibacterium freudenreichii* JS) and biopreservatives as Nisin (Nisaplin), Delvocid® (natamycin) or Nipasol M (propyl paraben) on the physical, chemical and sensory properties of strained yoghurt were studied in relation to its viability of undesirable yeasts-moulds during storage at 4±1°C for 28 days. Based on chemical and physical characteristics studied, there were no effective or negative effect originated from these preservatives. Moreover control samples had lower scores especially at the end of storage. The preservative supplemented strained yoghurts were protected from yeast and mould growth. The inhibitory action of protective culture against yeast and moulds was depended on type of preservatives. Also, use of protective culture or bio preservatives did not affect sensory properties of strained yoghurt.

Key words: Biopreservatives, fermented milks, protective cultures, strained yoghurt, yoghurt.

INTRODUCTION

Fermented milks with different names and ripened by microorganisms under similar conditions are available in most countries. These fermented products have always constituted a major element in the diet of many populations especially those of Middle East (Guizani et al., 2001). The lactic acid fermentation is one of the oldest methods studied for the preservation of milk whereas these relatively acidic products could still be prone to spoilage (Sayan and Sahan, 2002).

Fermented milks are widely produced in many countries. This type of process is one of the oldest methods used to extend the shelf-life of milk, and has been practiced by human beings for thousands of years. The exact origin(s) of the manufacture of fermented milks is difficult to establish, but it is safe to assume that it could date to more than 10,000 years ago as the way of life of humans changed from food gathering to food producing. This change also included the domestication of certain mammals such as the cow, sheep, goat, buffalo and camel; it is most likely that the transition occurred at different dates in different countries. However, archaeological evidence of certain civilizations (Sumerians, Babylonians, Pharos and Indians) suggests that they were well advanced in agriculture and in the production of fermented milks (Tamime, 2002).

Strained yoghurt is produced by removing yoghurt whey and so water content decreases to about 70%. This product is similar to Labneh in the Middle East, Skry in Ireland, Chakka and Shirkhand in India and Ymer in Denmark. Concentrated yoghurt is made by removing yoghurt whey traditionally in a cloth bag or centrifugation (Seckin and Ozkilinc, 2011). Strained yoghurt has a longer shelf life than the traditional yoghurt due to an increased lactic acid concentration.

Differences in the traditional methods seen during the production of labneh can influence the compositional, nutritional properties and microbiological quality of the product. Therefore, the variation in production of fermented dairy products like labneh is implicitly dependent on the area of production and the environmental conditions during manufacturing process. The condition employed in the manufacturing processes provides microbiologically safe products with

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recognizable sensory and structural characteristics in an efficient and reproducible way.

A lot of research in the field of food science has focused on new preservation technologies but very few of these methods have been implemented by the food industry until now. An increasing number of consumers prefer minimally processed foods, prepared without chemical preservatives. In biopreservation, storage life is extended and/or safety of food products is enhanced by using natural or controlled microflora, mainly LAB and/or their antibacterial products such as lactic acid, bacteriocins and others (Devlieghere et al., 2004).

There are several articles pertaining to the chemical, nutritional and microbiological quality of the products in the literature (Karla et al., 1973; Gupta and Prasad, 1989; Rajmohan and Prasad, 1995). However, there are studies on the use of natural antimicrobial preservatives on chemical and sensory properties of labneh and viability of lactic acid bacteria, yeasts and moulds (Hanlin et al., 1993; Caplice and Fitzgerald, 1999; Sarkar, 2006). Also, use of biopreservatives is an important alternative technology that could be used to extend the shelf life of ready to consume fermented milks and preserve the freshness, flavor, texture and nutrient value of these products (Khurana and Kanawjia, 2007). The objective of this study is to investigate effect of protective culture and some bio preservatives on strained yoghurt quality.

MATERIALS AND METHODS

Fresh raw cow milk was obtained from the dairy herds of Ege University, Faculty of Agriculture. The chemical composition of raw milk was 11.77% total solids, 3.60% fat, 4.26% protein, 4.52% lactose and 6.65 pH. Commercial freeze-dried starter culture containing *Lactobacillus delbrueckii* subsp, *bulgaricus* and *Streptococcus thermophilus* (Texel, France) was used for yoghurt fermentation; skim milk powder used for total solid standardization (Pınar Sut Co, Izmir-Turkey). Mixed culture of *Lactobacillus rhamnosus* LC 705 and *Propionibacterium freudenreichi: Js* (Bioprofit™) (Visby vac®, Niebull, Germany), Delvocid® (natamycin) (Gist Brocades, Netherlands), propyl paraben (Nipasol M) (Nipa Lab, Glamorgan, UK) and nisin (Nisaplin) (Aplin & Barrett Ltd., Trowbridge-Wilts) were used for anti-yeast and anti-fungal preservation.

Manufacture of strained yoghurt

The yoghurts were produced according to standard methodology for manufacture of set type yoghurt that includes pasteurization (95℃ for 5 min), cooling (43 ± 1℃), inoculation (3% yoghurt culture), incubation (until 4.7 pH), pre-cooling (22℃ f or 30 min) and cold storage during overnight. Before inoculation of yoghurt culture bulk milk was divided into 7 different portions for addition of 0.25% Bioprofit (Bio 25), 0.50% Bioprofit (Bio 50), 25 - 50 RU/kg Nisin (N25-N50), 50 mg Delvocid® (DUCT) and 75 mg Nipasol M (NPSL) including control sample. The control yoghurt (C) vats were prepared without protective culture and bio preservatives. The curds were cooled overnight at hanging cloth bags to drain the whey and then strained yoghurt samples were poured into plastic cups (150 g) and held at 4℃ for 28 days.

Methods

The pH was determined with a pH meter (Hanna pH 211 Microprocessor, Portugal). Lactose titratable acidity (SH), protein and fat content were determined according to Oysun (1996). Acetaldehyde (Robinson et al., 1977), tyrosine (Citti et al., 1963), acid degree value (ADV) (Barrantes et al., 1996) of strained yoghurt samples were detected.

Bacterial enumerations were carried out at days 1th, 7th, 14th, 21th, and 28th in triplicates of each batch. Samples (10 g) were diluted with ringer solution (90 mL). Afterwards, serial dilutions were carried out, and bacteria were counted, applying the pour plate method. *L. bulgaricus* counts were enumerated in MRS agar (pH 5.8) as anaerobically at 42℃ for 48 h whereas *S. thermophilus* counted in M17 agar (pH 6.9) as aerobic incubation at 37℃ for 4 8 h (Bracquart, 1981). Yeasts and moulds were enumerated using YGC Agar (pH 6.8) (Merck Kga A, Darmstadt, Germany) and incubated aerobically at 25 ± 1℃ for 5 days (Anonymous, 2005) .

Samples were evaluated for their sensory characteristics (taste-aroma, and consistency) and overall acceptability on a 5-point hedonic scale (5 excellent, 3 indifferent, and 1 unacceptable) performed by a panel of judges selected according to their accomplishment of a general sensory aptitude test after 1 day of storage at 4℃ (Bodyfelt et al., 1998). All sensory analyses were carried out in triplicate.

Statistical analysis of the data was done using the analysis of variance in SPSS© v.9.05 (SPSS Inc., Chicago, USA). Means with a significant difference were compared by Duncan's multiple range tests. Significance of difference was set at $p < 0.05$ in all cases. All analyses were performed in duplicate.

RESULTS AND DISCUSSION

The physical and chemical properties of strained yoghurt throughout storage are given in Table 1. Data that were obtained shows similar changes in acidity and pH values of samples ($p<0.05$) whereas significant differences were observed during the storage period of the strained yoghurt manufactured with yoghurt culture added Bioprofit, Nisin (Nisaplin), Natamycin (Delvocid) and Propyl paraben (Nipasol M). The pH value decreased significantly ($p<0.05$) during storage and generally lower pH was determined in control samples. It was seen that during the storage period natural strained yoghurt samples were more active than strained yoghurt that contains added Bioprofit and other preservatives. Titratable acidity was affected by the addition of different preservatives until 28th day of storage and the acidity gradually increased during storage progressed ($p<0.05$). Similar observations were reported by Abrahamsen and Holmen (1981), Mehaia and Khadragy, (1999), Al.Kadamany et al. (2003), Al.Otaibi and Demerdash, (2008) and Kesenkaş, (2010).

According to our results, some insignificant differences may be sourced in preparation methods used as well as due to differences in compositional characteristics of the milk and yoghurt. Threonine aldolase converts to threonine glycine and acetaldehyde. For optimal flavor development in yoghurt, the level of acetaldehyde content must be 23-41 mg/kg of yoghurt (Guven et al., 2005). As seen from Table 2; all data obtained in this

Table 1. Physical and chemical properties of strained yoghurt samples during storage at 4℃ (n=3 ± sd).

Variable	Days	C	Bio 25	Bio 50	N 25	N 50	DUCT	NPSL
pH	1	4.20 ± 0.05	4.13 ± 0.06	4.39 ± 0.12	4.18 ± 0.06	4 .28 ± 0.10	4.38 ± 0.06	4.34 ± 0.04
	7	4.08 ± 0.07^{a}	4.10 ± 0.10^{a}	4.38 ± 0.14^{b}	4.15 ± 0.05^{a}	4.26 ± 0.08^{a}	4.35 ± 0.03^{b}	4.32 ± 0.06^{b}
	14	3.93 ± 0.04^{a}	4.08 ± 0.04^{b}	4.23 ± 0.03^{c}	4.13 ± 0.04^{b}	4.23 ± 0.05^{c}	4.33 ± 0.02^{bc}	4.30 ± 0.07^{bc}
	21	3.78 ± 0.00^{a}	4.00 ± 0.07^{b}	4.13 ± 0.04^{c}	4.08 ± 0.06^{bc}	4.13 ± 0.06^{bc}	4.25 ± 0.04^{d}	4.25 ± 0.08^{d}
	28	3.75 ± 0.07^{a}	4.03 ± 0.07^{b}	4.10 ± 0.05^{b}	4.08 ± 0.07^{b}	4.15 ± 0.07^{b}	4.18 ± 0.04^{c}	4.20 ± 0.09^{c}
Titratable Acidity (°SH)	1	72.93 ± 2.18^{a}	70.19 ± 2.14^{a}	66.81 ± 2.65^{a}	57.11 ± 1.86a	55.79 ± 1.89^{b}	59.79 ± 2.24^{b}	61.05 ± 3.07^{bc}
	7	74.86 ± 2.10^{a}	71.94 ± 2.22^{a}	68.17 ± 2.66^{a}	57.50 ± 1.90^{b}	56.90 ± 2.07^{b}	61.60 ± 2.80^{b}	62.90 ± 3.08^{b}
	14	77.42 ± 2.27^{a}	73.33 ± 2.26^{ab}	70.47 ± 2.70^{b}	60.05 ± 2.07^{c}	58.61 ± 2.35^{c}	63.59 ± 2.84^{c}	65.12 ± 2.94^{c}
	21	89.68 ± 1.73^{a}	75.67 ± 3.07^{b}	71.58 ± 2.74^{b}	62.05 ± 2.38^{c}	61.12 ± 2.65^{c}	66.18 ± 3.07^{bc}	66.90 ± 2.86^{c}
	28	82.25 ± 1.29^{a}	76.52 ± 2.27^{a}	72.14 ± 2.89^{b}	65.85 ± 2.24^{c}	63.55 ± 2.22^{c}	66.66 ± 3.12^{c}	68.19 ± 2.83^{bc}
Acetaldehyde (ppm)	1	24.70 ± 4.84	24.92 ± 0.89	24.45 ± 0.78	24.84 ± 0.89	24.23 ± 0.93	23.93 ± 0.68	24.09 ± 0.72
	7	25.33 ± 1.89	25.64 ± 0.92	25.48 ± 0.89	25.63 ± 0. 97	25.06 ± 0.58	24.73 ± 0.58	24.34 ± 0.89
	14	25.70 ± 1.11	26.20 ± 0.96	26.83 ± 0.96	26.67 ± 0 .85	25.57 ± 0.68	26.35 ± 0.61	25.33 ± 0.87
	21	26.89 ± 1.68	26.79 ± 0.89	27.00 ± 0.97	26.74 ± 1 .07	26.67 ± 0.72	25.58 ± 0.57	26.05 ± 0.92
	28	27.10 ± 1.68	26.68 ± 0.78	26.59 ± 0.98	26.16 ± 0 .66	26.02 ± 0.76	24.13 ± 0.69	26.07 ± 0.39
Tyrosine (mg/g)	1	0.48 ± 0.06	0.43 ± 0.16	0.40 ± 0.09	0.45 ± 0.03	0 .36 ± 0.08	0.35 ± 0.06	0.34 ± 0.07
	7	0.49 ± 0.66	0.46 ± 0.02	0.42 ± 0.07	0.48 ± 0.03	0 .39 ± 0.13	0.39 ± 0.07	0.35 ± 0.08
	14	0.52 ± 0.12	0.48 ± 0.07	0.44 ± 0.06	0.50 ± 0.06	0.42 ± 0.02	0.43 ± 0.05	0.40 ± 0.02
	21	0.56 ± 0.05	0.54 ± 0.08	0.48 ± 0.05	0.53 ± 0.17	0.44 ± 0.06	0.45 ± 0.17	0.47 ± 0.03
	28	0.69 ± 0.03	0.59 ± 0.03	0.53 ± 0.04	0.58 ± 0.08	0.54 ± 0.11	0.50 ± 0.06	0.50 ± 0.05
ADV (meq KOH/kg)	1	6.55 ± 0.08^{a}	5.94 ± 0.65^{b}	5.65 ± 0.89^{a}	5.86 ± 0.26^{a}	5.46 ± 0.07^{a}	5.55 ± 0.92^{a}	5.54 ± 0.19a
	7	6.86 ± 0.07^{a}	6.23 ± 0.36^{b}	5.87 ± 0.37^{a}	6.02 ± 0.25^{b}	5.60 ± 0.14^{c}	5.65 ± 1.02^{c}	5.65 ± 0.22^{c}
	14	7.09 ± 0.10^{a}	6.43 ± 0.37^{a}	5.94 ± 0.42^{b}	6.20 ± 0.08^{b}	5.88 ± 0.16^{c}	5.90 ± 0.65^{c}	5.90 ± 0.52^{c}
	21	7.38 ± 0.18^{a}	6.62 ± 0.42^{b}	6.10 ± 0.45^{c}	6.33 ± 0.21^{c}	6.02 ± 0.22^{c}	6.10 ± 0.86^{c}	6.20 ± 0.56^{bc}
	28	7.82 ± 0.19^{a}	6.72 ± 0.46^{b}	6.29 ± 0.05^{c}	6.44 ± 0.19^{c}	6.20 ± 0.36^{c}	6.25 ± 0.37^{c}	6.31 ± 0.65^{c}

C = Control, Bio25 = 0.25% Bioprofit^{TM}, Bio50 =0.50% Bioprofit^{TM}, N 25 = 0.25% Nisaplin , N 50 = 0.50% Nisaplin , DUCT =50 mg /kg Natamycin, and NPSL =75 mg/kg Nipasol M, $^{a, b, c, d}$ Means in the same row with different superscripts are different ($p<0.05$).

study are in this level. Although samples that contain delvocid had lower values, addition of different preserving agents such as Bioprofit and Nipasol M were not restricted acetaldehyde contents ($p>0.05$). Thus, it had no antagonistic effect to threonine aldolase sources of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. Moreover acetaldehyde concentration of strained yoghurts

Table 2. Sensory properties of strained yoghurt samples during storage at 4℃ (n=3 ± sd).

Variable	Days	C	B 0.25	B 0.50	N 25	N 50	DUCT	NPSL
	1	4.9 ± 0.01	5.0 ± 0.00	5.0 ± 0.00	4.9 ± 0.02	5.0 ± 0.00	4.9 ± 0.01	4.9 ± 0.02
	7	4.9 ± 0.00	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00	4.9 ± 0.02	4.9 ± 0.05
Appearance	14	4.6 ± 0.05	4.8 ± 0.01	4.9 ± 0.01	4.7 ± 0.01	4.9 ± 0.02	4.7 ± 0.05	4.6 ± 0.14
	21	3.1 ± 0.04[a]	4.3 ± 0.02[b]	4.6 ± 0.05[b]	4.5 ± 0.02[b]	4.7 ± 0.04[b]	4.5 ± 0.06[b]	4.5 ± 0.07[b]
	28	3.0 ± 0.03[a]	3.9 ± 0.02[b]	4.3 ± 0.07[b]	4.1 ± 0.07[b]	4.4 ± 0.06[b]	4.2 ± 0.09[b]	4.5 ± 0.06[b]
	1	4.7 ± 0.02	4.8 ± 0.03	4.7 ± 0.08	4.8 ± 0.09	4.8 ± 0.09	4.7 ± 0.08	4.5 ± 0.07
	7	4.6 ± 0.05	4.7 ± 0.04	4.7 ± 0.10	4.6 ± 0.06	4.8 ± 0.08	4.8 ± 0.10	4.5 ± 0.12
Taste	14	4.6 ± 0.06	4.7 ±0.06	4.6 ± 0.08	4.7 ± 0.12	4.6 ± 0.12	4.5 ± 0.09	4.6 ± 0.15
	21	3.9 ± 0.12	4.2 ± 0.08	4.6 ± 0.12	4.3 ± 0.07	4.5 ± 0.13	4.4 ± 0.11	4.3 ± 0.07
	28	3.1 ± 0.11	3.9 ± 0.09	4.3 ± 0.11	4.3 ± 0.08	4.4 ± 0.15	4.2 ± 0.13	4.3 ± 0.06
	1	5.0 ± 0.00	5.0 ± 0.00	4.9 ± 0.03	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00	4.8 ± 0.08
	7	5.0 ± 0.00	4.9 ± 0.05	4.8 ± 0.05	4.8 ± 0.03	4.9 ± 0.07	4.9 ± 0.05	4.8 ± 0.12
Odour	14	4.7 ± 0.11	4.8 ± 0.06	4.8 ± 0.06	4.8 ± 0.07	4.8 ± 0.04	4.8 ± 0.07	4.5 ± 0.09
	21	4.0 ±0.05	4.1 ± 0.09	4.4 ± 0.14	4.2 ± 0.11	4.3 ± 0.08	4.3 ± 0.12	4.1 ± 0.15
	28	3.6 ± 0.13	3.9 ± 0.08	4.3 ± 0.12	4.3 ± 0.12	4.3 ± 0.10	4.3 ± 0.08	4.0 ± 0.12
	1	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00	4.8 ± 0.12	4.9 ± 0.06	4.9 ± 0.03	4.8 ± 0.07
	7	5.0 ± 0.00	5.0 ± 0.00	4.9 ± 0.11	4.8 ± 0.15	4.8 ± 0.11	4.9 ± 0.02	4.7 ± 0.10
Consistency	14	5.0 ± 0.00	4.8 ± 0.08	4.7 ± 0.14	4.8 ± 0.19	4.7 ±0.08	4.7 ± 0.08	4.7 ± 0.11
	21	4.6 ± 0.08	4.5 ± 0.12	4.5 ± 0.13	4.6 ± 0.15	4.7 ± 0.09	4.5 ± 0.10	4.3 ± 0.17
	28	4.2 ± 0.12	4.3 ± 0.09	4.4 ± 0.08	4.5 ± 0.16	4.6 ± 0.17	4.5 ± 0.12	4.1 ± 0.15

C =Control, Bio25 = 0.25% Bioprofit™, Bio50 =0.50% Bioprofit™, N 25 = 0.25% Nisaplin , N 50 = 0.50% Nisaplin , DUCT =50 mg /kg Natamycin, and NPSL =75 mg/kg Nipasol M.

did not significantly vary during storage period that might be related to low alcohol dehydrogenase activity that resulted in lower hydrolysis of acetaldehyde to ethanol.

Proteolysis that is an indicator of tyrosine content is usually not an important factor in flavour or defects in fermented milks but, it is important in cheese texture and flavour development during ripening. Since, this enzyme activity by the starter cultures may be able to produce peptides and amino acids that could be used as growth factors (Dave and Shah, 1997). In general, tyrosine contents of samples measured as free amino groups did not significantly change at the first day of storage ($p>0.05$) whereas the control, bioprofit 0.25%, nisin (25RU/kg) samples had higher contents ($p<0.05$) and increased gradually during storage period in all samples ($p<0.05$). As stated that starter cultures have smooth proteolytic activity and sometimes it results in bitterness (Guven et al., 2005). The restricted level of bitterness in fermented milk products is 0.5 mg/ml of tyrosine content (Guzel et al., 2005). Therefore, the lower taste scores of all samples at the 28th day of storage might be related to cover typical yoghurt flavor by bitterness.

Total free acids expressed as ADV were in all samples during storage and significant differences were only found at 28th day of storage where the samples had maximum amounts ($p<0.05$). Similarly other researchers were stated to increased total fatty acids in yoghurts and concentrated yoghurts during storage period

Table 3. Microbiological properties of strained yoghurt samples during storage at 4℃ (log cfu/g, n=3 ± sd).

Variable	Days	C	B 0.25	B 0.50	N 25	N 50	DUCT	NPSL
Lactobacillus delbrueckii ssp. *bulgaricus*	1	11.63 ± 0.01	11.63 ± 0.01	11.62 ± 0.00	11.61 ± 0. 02	11.62 ± 0.00	11.63 ± 0.01	11.63 ± 0.02
	7	11.63 ± 0.00	11.63 ± 0.00	11.62 ± 0.00	11.61 ± 0.00	11.62 ± 0.00	11.62 ± 0.02	11.61 ± 0.05
	14	11.62 ± 0.05	11.62 ± 0.05	11.62 ± 0.01	11.62 ± 0 .01	11.61 ± 0.02	11.61 ± 0.05	11.61 ± 0.14
	21	11.61 ± 0.04	11.61 ± 0.04	11.61 ± 0.05	11.61 ± 0 .02	11.60 ± 0.04	11.60 ± 0.06	11.60 ± 0.07
	28	11.59 ± 0.03	11.59 ± 0.03	11.60 ± 0.07	11.60 ± 0 .07	11.60 ± 0.06	11.60 ± 0.09	11.61 ± 0.06
Streptococcus thermophilus	1	11.63 ± 0.02	11.61 ± 0.03	11.60 ± 0.08	11.62 ± 0. 09	11.59 ± 0.09	11.58 ± 0.08	11.60± 0.07
	7	11.64 ± 0.05	11.61 ± 0.04	11.59 ± 0.10	11.63 ± 0. 06	11.59 ± 0.08	11.58 ± 0.10	11.60 ± 0.12
	14	11.65 ± 0.06	11.63 ±0.06	11.60 ± 0.08	11.61 ± 0. 12	11.59 ± 0.12	11.59 ± 0.09	11.60 ± 0.15
	21	11.64 ± 0.12	11.63 ± 0.08	11.59 ± 0.12	11.62 ± 0 .07	11.58 ± 0.13	11.58 ± 0.11	11.59 ± 0.07
	28	11.63 ± 0.11	11.61 ± 0.09	11.58 ± 0.11	11.60 ± 0 .08	11.58 ± 0.15	11.56 ± 0.13	11.56 ± 0.06
Yeast and moulds	1	2.66 ± 0.00^{a}	2.24 ± 0.00^{b}	1.18 ± 0.03^{c}	2.06 ± 0.00^{b}	1.72 ± 0.00^{c}	1.86 ± 0.00^{c}	2.04 ± 0.08^{b}
	7	2.95 ± 0.00^{a}	2.30 ± 0.05^{b}	1.94 ± 0.05^{c}	2.13 ± 0.03^{c}	1.83 ± 0.07^{c}	1.89 ± 0.05^{c}	2.10 ± 0.12^{c}
	14	3.08 ± 0.11^{a}	2.13 ± 0.06^{b}	2.04 ± 0.06^{b}	2.26 ± 0.07^{b}	1.95 ± 0.04^{b}	2.02 ± 0.07^{b}	2.15 ± 0.09^{b}
	21	3.18 ± 0.05^{a}	2.60 ± 0.09^{ab}	2.19 ± 0.14^{b}	2.40 ± 0.11^{b}	2.19 ± 0.08^{b}	2.13 ± 0.12^{b}	2.22 ± 0.15^{b}
	28	3.32 ± 0.13^{a}	2.65 ± 0.08^{b}	2.33 ± 0.12^{c}	2.48 ± 0.12^{c}	2.30 ± 0.10^{c}	2.22 ± 0.08^{c}	2.25 ± 0.12^{c}

C =Control, Bio25 = 0.25% Bioprofit^{TM}, Bio50 =0.50% Bioprofit^{TM}, N 25 = 0.25% Nisaplin , N 50 = 0.50% Nisaplin , DUCT =50 mg /kg Natamycin, and NPSL =75 mg/kg Nipasol M.

(Guven and Karaca, 2003; Guven et al., 2005, Guzel-Seydim et al., 2005). Moreover, higher ADVs in control and Bio 25 samples until the end of storage might be associated with growth of lipolytic yeasts.

Rajmohan and Prasad, (1989) found nisin or nisin producing organisms capable of producing 1000 IU/ g to be effective in controlling the lipolysis during storage in Dahi. Incorporation of 15 RU/g nisin into dahi retained all its desirable characteristics up to 35 days. Sensory evaluation of strained yoghurt made with some preserving agents during storage summarized in Table 2. It is obvious that nisaplin 50 RU/kg gained high scores of the evaluation. This could be attributed to the effect of nisaplin that improves the keeping quality and prevents the growth of yeasts and moulds till the end of storage time. Also nisin imparts no adverse flavour to strained yoghurt. Moreover, the sensory properties of strained yoghurt were made with Delvocid, Nipasol M and Nisaplin 25 RU/kg did not significantly differ from each other. On the other hand, the control samples had lower scores as a result of especially yeasts and moulds growing, that impart off-flavour, bitterness, discoloration. Off flavour of control and bioprofit (0.25% protective culture) yoghurts might be caused by contamination of microorganisms, mainly yeasts.

The off flavours may be characterized as yeasty, fruity, cheesy, bitter and occasionally soapy-rancid. A flavour threshold is generally reached at a count of about 10^4 yeasts and moulds /g (Walstra et al., 1999). These results are in agreement with those reported by Guizani et al. (2001), El-Diasty et al. (2008) and Sayan and Sahan, (2002). Gupta and Prasad, (1989) reported to use nisin at a concentration of 100 IU/g extended the shelf life of yoghurt from 3-7 days without significant change in flavor, body, texture and consistency.

Yeasts and moulds were significantly ($p<0.05$) inhibited in Bioprofit 0.50%, Delvocid and Nipasol M samples during the storage period. Yeasts and moulds were significantly increased in control and Bioprofit 0.25% samples in the storage respectively (Table 3). Moreover, none of tested strained yoghurts exhibited any

coliform contamination. Also the lactic acid bacteria (lactococci and lactobacilli) comprised the major share of bacteria. It is well known that these organisms play major role in the producing of cultured dairy products. This group of bacteria growing in all samples was not significantly affected by antimicrobial agents used. Soumalainen and Mäyrä-Mäkinen, (1999) used as protective culture (10^7 cells per gram) contains *Lactobacillus rhamnosus* LC 705 and *Propionibacterium freudenreichii* JS in dairy products. It is reported that the product inhibited yeasts.

The results indicate that all antimicrobial agents used in this study had usually antifungal properties and inoculation of Nisin 50 RU/kg was an effective factor for that case. However, further studies on the effect of especially Bioprofit cultures and Nisin are needed.

Conclusion

Use of protective culture and biopreservatives is an important technique that could be used to extend the shelf life of ready to consume fermented milks. Strained yoghurts containing protective cultures and some preservatives have not shown any impressive or negative effects on physical, chemical, and sensory properties of yoghurts. However, strained yoghurts preservative supplemented were protected from yeast and mould growth. These effects depend on the concentration and variety of the used preservative.

ACKNOWLEDGEMENT

The authors thank the Ege University Scientific Research Fund (Project No: 2004-ZRF-012) Council for financial support to this study.

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