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Effect of using different probiotic cultures on properties of Torba (strained) yoghurt

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Summary

The viability of *Lactobacillus casei* LAFTI® L26, *Bifidobacterium animalis* subsp. *lactis* LAFTI® B94 and *Lactobacillus acidophilus* LAFTI® L10, their proteolytic activities and effects on chemical, textural and sensory properties of Torba yoghurts were assessed during 14 days of storage at 4 °C. These probiotic cultures were separately added after the fermentation of milk with yoghurt culture but prior to packaging of the product. Probiotic bacteria reached the recommended level of 6 log cfu/g in Torba yoghurt except *B. animalis* subsp. *lactis* B94. The addition of probiotic bacteria resulted in an appreciable proteolytic activity but also textural defects due to the lower total solid content in the final product.

Key words: Torba yoghurt, probiotic bacteria, viability, proteolytic activity, texture

Introduction

Probiotic foods are defined as "foods containing live microorganisms, which actively enhance health of consumers by improving the balance of microflora in the gut when ingested live in sufficient numbers" (Fuller, 1992; Shah, 2004). In terms of the growth and viability of probiotic bacteria in retail products, fermented milks are excellent vehicles for the transfer of selected strains to humans (Itsaranuwat et al., 2003). Dairy products containing probiotic cultures such as *Bifidobacterium* sp. and *Lactobacillus* sp. have been produced around the world for many years and it is estimated that currently more than 80 products containing these bacteria are being produced worldwide (Maity et al., 2008). Among these products the most widely encountered one is yoghurt. The probiotic bacteria can be incorporated into yoghurt by using different methods. They may be inoculated together with the yoghurt culture prior to fermentation process, or alternatively, added to the yoghurt after fermentation is completed (Gilliland et al., 2002; Uysal et al., 2003a). In the later case, probiotic bacteria are separately grown in a batch of milk in order to achieve high viable counts, while another batch of milk is fermented with the traditional starter cultures. After the fermentation stages, the two batches are mixed to obtain a probiotic fermented milk (Tamime et al., 2005).

Several factors must be considered when using probiotic bacteria in fermented products like yoghurt. Primarily, the probiotics must be viable and present in high count at time of consumption to achieve the desired benefits (Gomes et al., 1995). Thus, a probiotic dairy product should contain at least 10^6-10^7 cfu/mL of viable probiotic bacteria at the time of consumption (Boylston et al., 2004) and, should be consumed regularly in a quantity of higher than 100 g per day in other words at least 10^9 cfu per day (Gomes and Malcata, 1999; Medici et al., 2004; Matijević et al., 2009).

Today, several types of concentrated fermented milks are produced in many countries to extend the

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keeping quality and, although these products have different local names, in practice they are similar (Özer, 2006). "Torba" yoghurt is one of the most consumed concentrated fermented milk product in Turkey. It is also known as "Süzme" or "Kese" yoghurt. Similar strained products are manufactured by different methods in many countries like in Balkan and Eastern Mediterranean countries, in Turkmenistan and the Indian subcontinent (Tamime and Robinson, 2007). The traditional method of making Torba yoghurt is achieved by straining cold plain yoghurt using a cloth bag. The basic principle of using the traditional cloth bag method is to remove whey from plain yoghurt until the desired level of total solids has been reached (Özer, 2006). Torba yoghurt has a relatively high content of total solids (20-25%) and has a creamy texture.

Several authors have investigated the chemical, physical and microbiological composition (Cağlar et al., 1997; Kırdar and Gün, 2002; Güler and Şanal, 2009), losses of nutrients (Nergiz and Seçkin, 1998), effects of different concentration methods (Uysal, 1993), effect of fat replacers and whey concentrates (Moharrami, 1992; Uysal et al., 2003b; Yazıcı and Akgün, 2004), effect of using glucono delta lactone (Uysal et al., 2004), determination of critical control points (Karabıyık, 2006) and finally the effect of protective cultures (Parlak, 2002) in Torba yoghurt. Although sensorial and physical properties of Torba yoghurt containing probiotic bacteria were investigated before (Akın, 1999), there is no reported research on the survival and activity of probiotic bacteria in Torba yoghurt.

Therefore, the objectives of this study were: i) to manufacture Torba yoghurt containing *Lactobacillus acidophilus*, *Lactobacillus casei* or *Bifidobacterium animalis* subsp. *lactis* cultures; ii) determine the viability of these bacteria during storage; iii) monitor the effects of their metabolic activity; iv) investigate certain chemical, textural and sensorial characteristics of Torba yoghurts as effected by different probiotics.

Materials and methods

Probiotic cultures

Commercially available probiotic cultures of *L. casei* LAFTI® L26, *B. animalis* subsp. *lactis* LAFTI® B94 and *L. acidophilus* LAFTI® L10 were obtained from DSM Food Specialties (Izmir, Turkey). The probiotic cultures were grown in reconstituted skim milk (12% total solids), which was autoclaved at 115 °C for 10 minutes and incubated at 37 °C for 18 hours.

Production of Torba yoghurt

Torba yoghurt production was carried out in Sakıpağa Dairy and Food Co. Izmir, Turkey. A schema for production of Torba yoghurt is presented in Figure 1. The Torba yoghurt was subsequently divided into four portions (10 kg); one portion was used as control while the other portions were separately mixed (1:10 ratio) with probiotic cultures of LAFTI® L26 (8.27 log cfu/g), LAFTI® B94 (8.11 log cfu/g) and LAFTI® L10 (8.38 log cfu/g) to achieve an approximate concentration of 10^7 viable probiotic cells/g. Finally, four different Torba yoghurts (L26, B94, L10 and C) were produced and all treatments were stored for 1, 7 and 14 days at 4 °C.

Chemical analyses

The raw milk and Torba yoghurt samples were analyzed for fat (Gerber), total solids (oven drying at 103 °C), total protein (Kjeldahl) and ash content after 1st day of storage. The lactose content was determined spectrophotometrically (Kurt et al., 1999) and the pH measured with a pH meter (model 211, Hanna Instruments).

Enumeration of probiotic bacteria and yoghurt starters

The colony counts of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, *L. acidophilus*, *L. casei* and *B. animalis* subsp. *lactis* were enumerated in each sample (10 g) following serial dilution in Ringers' solution. Appropriate dilutions were plated using the following media: MRS Agar for the enumeration of *L. delbrueckii* subsp. *bulgaricus* incubated anaerobically at 42 °C for 48 h; M17 agar for the enumeration of *S. thermophilus* incubated aerobically at 37 °C for 48 h.; MRS-sorbitol agar, MRS-vancomycine agar and MRS-nalidixic acid, neomycin sulphate, lithium chloride and paromomycin sulphate (NNLP) agar incubated anaerobically at 37 °C for 72 h were used for the enumeration of *L. acidophilus*, *L. casei* and *B. animalis* subsp. *lactis* respectively (Tharmaraj and Shah, 2003). Yeasts

Raw cow milk
Svježe kravlje mlijeko
↓
Thermal treatment (70 °C 30 s)
Toplinski tretman
↓
Standardization of fat (3.2 %)
Standardizacija udjela mliječne masti
↓
Evaporation (12 % non-fat solids, 4-4.5% fat)
Evaporacija(12 % suhe tvari bez masti, 4-4,5% masti)
↓
Homogenization (180-200 bar)
Homogenizacija (180-200 bar)
↓
Heat treatment (95 °C, 5 min)
Toplinski tretman (95 °C, 5 min
↓
Cooling (42-44 °C)
Hlađenje (42-44 °C)
↓
Inoculation (with % 2.5 yoghurt culture)
Inokulacija s 2,5% jogurtnom kulturom
↓
Incubation (43 °C until pH 4.7)
Inkubacija (43 °C do pH 4,7)
↓
Cooling of Yoghurt (overnight at 4 °C)
Hlađenje jogurta (na 4 °C preko noći)
↓
Mixing (gently)
Blago miješanje
↓
Straining (in cloth bags at 4 ºC until about ~20 % total solids)
Cijeđenje (u krpama na 4 ºC na oko 20 % suhe tvari)
↓
Packaging and storage (500 g, 4 °C)
Pakiranje i čuvanje (500 g, 4 °C)

Fig. 1. Flow diagram of Torba yoghurt production
Grafikon 1. Dijagram proizvodnje Torba jogurta

and moulds were enumerated with YGC agar incubated at 25 °C aerobically for 3-5 days.

Determination of proteolytic activity

The influence of proteolytic activity of probiotic cultures used in the production of Torba yoghurts were determined in the final product by measuring liberated amino acids and peptides using the *o*-phthaldialdehyde (OPA) method described by Church et al. (1983) and Donkor et al. (2006). Samples (1 g) were mixed with 5 mL of % 0.75 % (w/v) trichloroacetic acid and vacuum-filtered using a Whatman # 1 filter paper (Whatman International Ltd, Kent, England). The filtrate (150 μL) was added to 3 mL of OPA reagent and the absorbance measured at 340 nm in a Spekol 1300 spectrophotometer (Analytic Jena AG, Jena, Germany) after 2 min at room temperature (20 °C).

Texture profile analysis and measurement of viscosity

The texture analysis was performed with a TA-XTPlus Texture Analyzer (Stable Micro Systems, Godalming, UK) with a 35 mm diameter probe using 5 kg load cell. The penetration of 30 mm was determined at following speeds: pre-test 1 mm/s, test 1mm/s and post-test 10 mm/s. The following texture parameters were recorded: firmness; maximum compression force in extrusion thrust into sample (g), consistency; area within curve during extrusion thrust (g.s), cohesiveness; maximum compression force during withdrawal of probe from sample (g) and index of viscosity; area within negative region of curve during probe withdrawal (g.s).

The apparent viscosity of samples was measured with Brookfield DV-II+Pro viscometer (Middleblro, MA USA) with helipath stand and T-bar type spindle (T-F) at 0.6 r.p.m.

Sensory evaluation

Eight panellists experienced with the Torba yoghurt evaluated the samples by using a mixed-point system (rating scale of 1-5) during 14 days storage at 4 °C. The sensory vocabulary comprised attributes describing appearance, consistency, odour, aroma/flavour and overall acceptability.

Statistical analysis

One way analysis of variance was applied and whenever it was adequate, Duncan's Multiple Range Test was applied in order to determine the differences between Torba yoghurt samples. Also, the effect of storage on samples was determined by the same method by using SPSS© 15.0 for Windows. In all cases, the 0.05 probability level was considered. All experiments and analyses were completed in triplicate.

Results and discussion

Chemical properties and composition

The chemical characteristics of raw milk used for production and of Torba yoghurt after 1 day of storage, are shown in Table 1. The total solids con-

Table 1. Chemical composition of raw milk and Torba yoghurt samples (n=3, ± s)
Tablica 1. Kemijski sastav sirovog mlijeka i uzoraka Torba jogurta (n=3, ± s)

	Total solids Ukupna suha tvar (g/100g)	Fat Mast (g/100g)	Protein Protein (g/100g)	Lactose Laktoza (g/100g)	Ash Pepeo (g/100g)
Raw milk Sirovo mlijeko	11.79±0.00	3.50±0.00	2.70±0.00	3.78±0.02	0.70±0.01
L26[1]	19.66±0.30[a]	6.70±0.34[a]	6.82±0.01[a]	4.30±0.17	0.93±0.04
B94[2]	19.38±0.14[a]	6.50±0.17[a]	6.52±0.35[a]	4.42±0.13	0.98±0.02
L10[3]	19.14±0.72[a]	6.90±0.51[a]	6.53±0.20[a]	4.62±0.28	0.95±0.04
C[4]	21.24±0.75[b]	7.90±0.17[b]	7.63±0.74[b]	4.66±0.26	0.96±0.02

[1]Torba yoghurt samples containing *L. casei* L26/Torba jogurt sadržava *L. casei* L26
[2]Torba yoghurt samples containing *B. lactis* B94/ Torba jogurt sadržava *B. lactis* B94
[3]Torba yoghurt samples containing *L. acidophilus* L10/Torba jogurt sadržava *L. acidophilus* L10
[4]Control sample/Kontrolni uzorak
[a, b]Means within the same row without a common superscript are significantly different (P<0.05)/Vrijednosti u redovima označene različitim slovima signifikantno su različite (P<0,05)

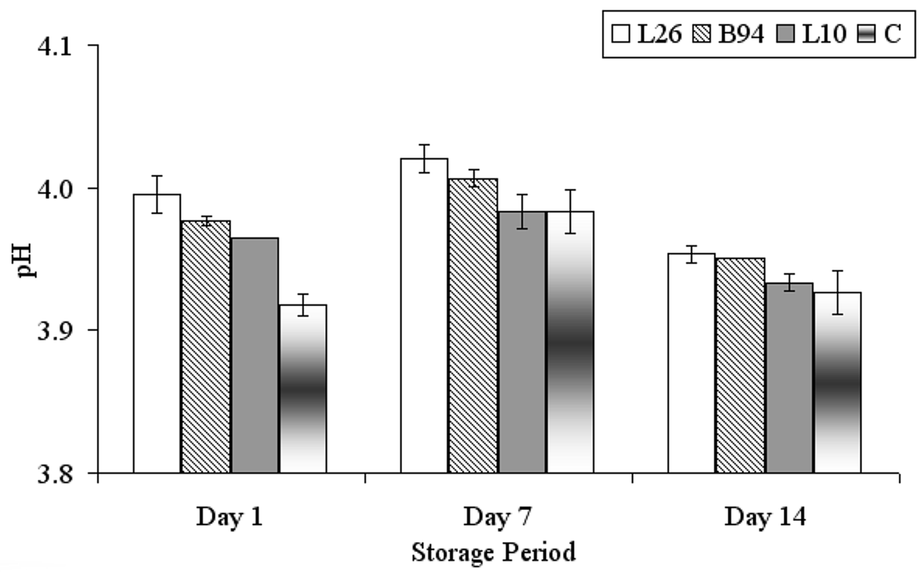

Fig. 2. pH of Torba yoghurts during 14 days of storage (error bars represent the standard deviation of means (n=3))
Grafikon 2. pH Torba jogurta tijekom pohrane od 14 dana (graničnici predstavljaju standardnu devijaciju srednje vrijednosti (n=3))

tent in the control sample was significantly higher (21.24%) compared to the other Torba yoghurt samples (P<0.05). Similar results were found for fat and protein contents (P<0.05). The lactose and ash contents did not significantly differ among the samples. The decreases of the mentioned constituents in probiotic Torba yoghurts can definitely be attributed to mixing Torba yoghurts with probiotic cultures prior to packaging. The gross composition of all Torba yoghurts was quite lower than those reported by Akın (1999), Uysal et al. (2003b; 2004), Güler and Şanal (2009).

The change of pH values in Torba yoghurts during storage are presented in Figure 2. During the storage of 14 days the differences between pH values were significant (P<0.05). The initial pH value of the control (pH 3.92) was lower compared to probiotic Torba yoghurts and the highest pH value was observed in samples containing *L. casei* L26 (pH 4.0). Among the probiotic Torba yoghurt samples the lowest pH values during the entire storage were observed for L10 (3.97 to 3.93).

Generally, pH values slightly increased in the first 7 days of storage but declined to the lowest values at the end of storage in all samples (P<0.05). The increase in pH in the first 7 days might be attributed to the accelerated yeast growth (Table 2) thus assimilation of lactic acid. On the other hand, declines at the end of storage are indicating continued residual fermentation. Similar pH values were obtained by Yazıcı and Akgün (2004), whereas lower values were reported by Uysal et al. (2003b; 2004) and Akkaya et al. (2009) in Torba yoghurts. Contrary, Akın (1999) found higher pH values (4.27-4.64) in Torba yoghurts containing probiotic bacteria. It has to be emphasized that yeasts and mould are undesired contaminants in Torba yoghurt and their presence is the consequence of insufficient hygiene during production.

Viable counts of yoghurt bacteria and yeast-moulds

Viable counts of *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus* and yeast-moulds during storage are presented in Table 2. The viable counts of *L. delbrueckii* subsp. *bulgaricus* generally decreased during storage in all samples (P<0.05), particularly in sample L10. The final concentration of *L. delbrueckii* subsp. *bulgaricus* at day 14 significantly differed between samples (P<0.05) and the control sample had the lowest count (8.07 log cfu/g). It can be assumed that the viability of *L. delbrueckii* subsp. *bulgaricus* was enhanced in the presence of probiotics during this period. The counts of *S. thermophilus* increased in all samples in first 7 days, and except in sample L26 (P<0.05), a slight decrease followed during the subsequent period of storage. Although similar tendency was found for viable counts of *S. thermophilus* (8.52 log cfu/g) in L26 and control samples at day 7, probiotic Torba yoghurts had generally significantly higher *S. thermophilus* counts than control sample during the entire storage period. It is apparent that probiotic cultures had a significant (P<0.05) effect on high viability of *S. thermophilus* throughout the cold storage. Additionally, the ratio

Table 2. Viability of yoghurt bacteria and yeasts-moulds in Torba yoghurt samples during 14 days of storage (log cfu/g, n=3, ± s)

Tablica 2. Aktivnost bakterija jogurta, plijesni i kvasaca u uzorcima Torba jogurta tijekom 14 dana pohrane (log cfu/g, n=3, ± s)

Microorganism Mikroorganizam	Yoghurts Jogurt	Day 1 1. dan	Day 7 7. dan	Day 14 14. dan
Lactobacillus bulgaricus	L26	8.65 ± 0.07^{b}	8.61 ± 0.08^{b}	8.46 ± 0.05^{aY}
	B94	8.63 ± 0.11^{a}	8.21 ± 0.11^{b}	8.32 ± 0.11^{bY}
	L10	8.60 ± 0.15	8.30 ± 0.11	8.22 ± 0.19^{XY}
	C	8.76 ± 0.08^{b}	8.29 ± 0.25^{a}	8.07 ± 0.07^{aX}
Streptococcus thermophilus	L26	8.47 ± 0.05^{aY}	8.52 ± 0.04^{aX}	8.62 ± 0.04^{bXY}
	B94	8.31 ± 0.08^{aXY}	8.83 ± 0.04^{cY}	8.67 ± 0.09^{bY}
	L10	8.46 ± 0.13^{aY}	8.76 ± 0.04^{bY}	8.68 ± 0.10^{bY}
	C	8.26 ± 0.06^{aX}	8.52 ± 0.05^{bX}	8.47 ± 0.08^{bX}
Yeasts - Moulds	L26	2.08 ± 0.26^{a}	2.14 ± 0.14^{aX}	3.39 ± 0.06^{bX}
	B94	1.82 ± 0.36^{a}	2.71 ± 0.06^{bY}	3.54 ± 0.06^{cX}
	L10	1.53 ± 0.47^{a}	3.31 ± 0.19^{bZ}	3.54 ± 0.06^{cX}
	C	2.20 ± 0.17^{a}	2.73 ± 0.18^{bY}	3.71 ± 0.10^{cY}

$^{a, b, c}$Means in the same row with different superscripts significantly differ (P<0.05)/Vrijednosti u redovima označene različitim slovima signifikantno su različite (P<0,05)

$^{X, Y}$Means in the same column with different superscripts among yoghurt samples significantly differ (P<0.05) /Vrijednosti u kolonama označene različitim slovima signifikantno su različite (P<0,05) of *S. thermophilus* to *L. delbrueckii* subsp. *bulgaricus* varied marginally during the entire storage and it was approximately 1:1 for all samples. Similar viable counts of *L. delbrueckii* subsp. *bulgaricus* counts were found in concentrated yoghurts by Özer and Robinson (1999), but they reported higher viable counts of *S. thermophilus*. Akkaya et al. (2009) who studied growth-death kinetics of *Listeria monocytogenes* in Torba yoghurt enumerated lower counts of yoghurt bacteria. Moreover, the viable counts of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in Torba yoghurt samples were in the same magnitude as reported by Salem et al. (2007).

Due to the inherent low pH and low storage temperatures, the major contaminants of Torba yoghurt are yeast and moulds. This is the case when the traditional cloth-bag production method is used and the necessary hygiene practices are not fully observed. Table 2 shows the total yeast-mould counts in Torba yoghurt samples during the storage. The yeast-mould counts increased constantly from day 1 to day 14 ($P<0.05$) in all samples. Other than initial counts, the difference between samples was significant ($P<0.05$) and the control sample had the highest counts (3.71 log cfu/g) at day 14. The lower yeast-mould counts in probiotic samples at this period could be attributed to the competition for nutrients. The yeast-mould counts are in accord with those of Atamer et al. (1988) and Uysal (1993).

Viability of probiotic bacteria

Figure 3 shows the viability of *L. casei* L26, *B. animalis* subsp. *lactis* B94 and *L. acidophilus* L10 in Torba yoghurt samples. The counts of probiotic bacteria at the first day of storage were 7.14, 5.36, 8.67 log cfu/g respectively. Although significant decreases were noted for all probiotic bacteria after 7 days of storage ($P<0.05$), the counts increased slightly and after 14 days the finally counts of *L. casei* L26, *B. animalis* subsp. *lactis* B94 and *L. acidophilus* L10 reached 6.95, 4.12 and 8.51 log cfu/g respectively.

It is clear that *B. animalis* subsp. *lactis* B94 showed in the environment of Torba yoghurt higher sensitivity than *L. acidophilus* L10 or *L. casei* L26. Özer et al. (2005) observed that values of pH 4.5 or lower affect negatively the cell viability of the probiotic organism in yoghurts. Similarly, Akalın et al. (2004) cited that the most important factor in bifidobacterial mortality was the low pH of the yoghurt and any drop below pH 4.3 greatly affected the viability of bifidobacteria. Not surprisingly, pH value around 4.0 in *B. animalis* subsp. *lactis* B94 sample

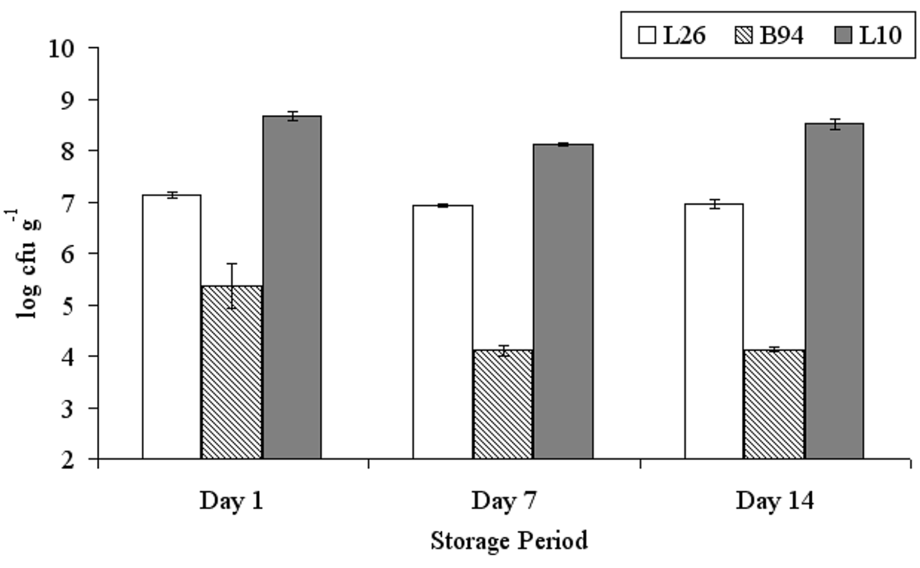

Fig. 3. Changes in the viable counts of probiotic bacteria in Torba yoghurt samples during 14 days of storage (error bars represent the standard deviation of means (n=3))

Grafikon 3. Promjene broja probiotičkih bakterija u uzorcima Torba jogurta tijekom 14 dana pohrane (graničnici predstavljaju vrijednost standardne devijacije srednje vrijednosti)

(Fig. 2) resulted in lower counts of bifidobacteria. *L. casei* L26 maintained more constant counts throughout the storage. In spite of some differences during the storage (P<0.05), low pH values did not effect the survival of *L. casei* L26 as much as *B. animalis* subsp. *lactis* B94. Similar observations were made by Donkor et al. (2006). *L. acidophilus* L10 seems to be more tolerant to acidic condition in Torba yoghurt. Compared to the other two probiotic organisms tested, its counts remained at high level (Fig. 2).

For probiotics tested in the present study, it can be concluded that except for *B. animalis* subsp. *lactis* B94, the minimum therapeutic and/or recommended level of above 6 log cfu/g, was maintained in L26 and L10 Torba yoghurt samples throughout the whole period of storage.

According to literature the stability of probiotic bifidobacteria and lactobacilli in fermented milk products is variable. Dave and Shah (1997) reported a rapid decline in the counts of *Bifidobacterium* subsp. in yoghurt made with commercial starter cultures. Yeganehzad et al. (2007) demonstrated that *L. acidophilus* could survive in concentrated yoghurt at sufficient levels (>6 log cfu/mL) for up to 21 days. Furthermore, Gilliland et al. (2002) observed that viability of *L. casei* in yoghurt-like product was strain dependent and the viability greatly varied.

Proteolytic activity

The production of yoghurt is a complex process involving many physical and chemical changes including proteolysis, which involves the progressive hydrolysis of the caseins to polypeptides, peptides and amino acids. The OPA-based spectrophotometric assay detects released α-amino groups, which result from milk proteins hydrolyses, thus giving a relative measurement of proteolytic activity (Shihata and Shah, 2000). Proteolytic activities during storage in Torba yoghurts containing probiotic bacteria and in control samples are shown in Figure 4. The statistical analysis showed that storage period was significantly effected the amount of liberated amino acids only in Torba yoghurt containing *L. casei* L26 and control sample (P<0.05) and they had the maximum absorbance values of 0.92-0.93, respectively at day 7. Other than initial period, the proteolysis in control sample was significantly (P<0.05) higher than that of Torba yoghurts containing probiotic bacteria. This higher absorbance values determined in control samples could be clearly attributed to higher protein content as indicated in Table 1.

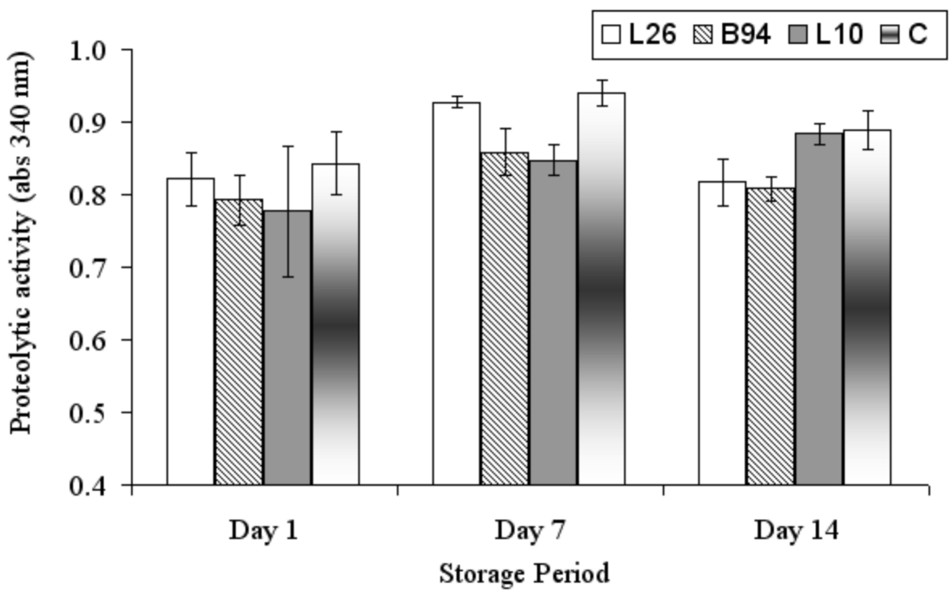

Fig. 4: Proteolytic activity of yoghurt and probiotic cultures in Torba yoghurt samples (error bars represent the standard deviation of means (n=3))

Grafikon 4. Proteolitička aktivnost jogurta i probiotičkih kultura u uzorcima Torba jogurta (graničnici predstavljaju standardu devijaciju srednje vrijednosti (n=3))

Briefly appreciable proteolytic activity was detected in all Torba yoghurt samples but did not follow a consistent trend. Moreover the amino acids released in Torba yoghurts containing *L. casei* L26 and *L. acidophilus* L10 could be effected the survival of these probiotic bacteria. Because the ability of many lactic acid bacteria to grow to high cell densities in milk is dependent on a proteolytic system that can liberate essential amino acids from casein-derived peptides (Donkor et al., 2007).

Texture profile analysis and viscosity

Details of textural properties of Torba yoghurt samples are shown in Table 3. The firmness, consistency, cohesiveness and index of viscosity values showed a significant increase (P<0.05) in all samples during storage. Moreover, the addition of probiotic bacteria cultures prior to packaging showed a significant decrease (P<0.05) in all texture parameters analyzed. The higher values in the control sample are most probably due to the higher total solid as well as protein content (Table 1). Chandan (2006) reported that texture characteristics of yogurts are primarily related to their moisture content and protein level. Additionally Becker and Puhan (1989) found that higher protein content gives a higher firmness value in yogurt.

Similar firmness values were obtained by Yazıcı and Akgün (2004) in strained yoghurts produced with fat replacers. Besides the textural profile, viscosity is another important property of fermented milk products that affects the mouth feel. Contrary to texture parameters, storage period did not have significant effect on viscosity of any of the samples (Table 3). However, control samples again had the

Table 3. Textural properties and viscosity of Torba yoghurt samples during 14 days of storage (n=3, ± s)
Tablica 3. Teksturalna svojstva i viskoznost uzoraka Torba jogurta tijekom 14 dana skladištenja (n=3, ± s)

	Yoghurts Jogurti	Day 1 1. dan	Day 7 7. dan	Day 14 14. dan
Firmness Čvrstoća (g)	L26	179.84 ± 3.59^{aX}	292.66 ± 4.24^{bY}	290.20 ± 9.23^{bX}
	B94	165.54 ± 14.0^{aX}	$263.75\pm5.50b^{XY}$	298.69 ± 11.4^{cX}
	L10	149.23 ± 10.4^{aX}	232.37 ± 2.44^{bX}	259.36 ± 2.25^{cX}
	C	348.17 ± 66.2^{aY}	516.36 ± 32.8^{bZ}	571.62 ± 42.5^{bY}
Consistency Konzistencija (g.s)	L26	3663.4 ± 104^{aX}	5945.4 ± 244^{bY}	6043.0 ± 238^{bX}
	B94	3290.4 ± 274^{aX}	5329.6 ± 239^{bXY}	6337.1 ± 116^{cX}
	L10	2985.3 ± 227^{aX}	4659.9 ± 254^{bX}	5590.2 ± 141^{cX}
	C	6936.2 ± 155^{aY}	10680 ± 908^{bZ}	11936 ± 104^{bY}
Cohesiveness Kohezivnost (g)	L26	137.55 ± 12.3^{bY}	187.39 ± 12.4^{aY}	219.56 ± 29.6^{aY}
	B94	113.32 ± 7.04^{cY}	163.68 ± 13.8^{bY}	197.07 ± 13.7^{aYZ}
	L10	91.32 ± 15.5^{bY}	135.99 ± 5.21^{aZ}	153.25 ± 5.67^{aZ}
	C	290.21 ± 63.8^{bX}	349.51 ± 21.3^{abX}	415.66 ± 47.2^{aX}
Index of viscosity Indeks viskoznosti (g.s)	L26	242.50 ± 19.7^{bY}	297.22 ± 10.0^{aY}	347.28 ± 20.5^{aY}
	B94	201.49 ± 17.2^{cY}	330.25 ± 78.2^{bY}	371.80 ± 103^{aY}
	L10	197.52 ± 24.8^{Y}	283.17 ± 37.0^{Y}	366.24 ± 31.9^{Y}
	C	465.04 ± 75.9^{bX}	729.39 ± 127^{aX}	594.93 ± 62.4^{abX}
Viscosity Viskoznost (Pa.s)	L26	621.66 ± 58.1^{X}	616.76 ± 56.4^{Y}	589.27 ± 20.5^{X}
	B94	529.35 ± 72.3^{X}	575.12 ± 37.1^{XY}	600.61 ± 46.7^{X}
	L10	480.72 ± 61.8^{X}	496.65 ± 28.4^{X}	544.31 ± 44.1^{X}
	C	1001.89 ± 143^{Y}	985.49 ± 69.3^{Z}	900.14 ± 30.5^{Y}

$^{a, b, c}$Means in the same row with different superscripts significantly differ (P<0.05)/Vrijednosti u redovima označene različitim slovima signifikantno su različite (P<0,05)
$^{X, Y, Z}$Means in the same column with different superscripts among yoghurt samples significantly differ (P<0.05)/Vrijednosti u kolonama označene različitim slovima signifikantno su različite (P<0,05)

Table 4. Sensory properties of Torba yoghurt samples during 14 days of storage (n=3, ± s)
Tablica 4. Senzorska svojstva uzoraka Torba jogurta tijekom 14 dana skladištenja (n=3, ± s)

	Yoghurts Jogurti	Day 1 1. dan	Day 7 7. dan	Day 14 14. dan
Appearance Izgled	L26	4.50±0.50	4.58±0.38	3.83±1.60
	B94	4.58±0.52	4.66±0.57	3.66±1.52
	L10	4.58±0.52	4.50±0.50	3.66±1.52
	C	4.57±0.25	4.66±0.28	3.66±2.30
Consistency Konzistencija	L26	3.83±0.57	3.91±0.14^{X}	3.33±0.57^{X}
	B94	3.75±0.66	4.00±0.00^{X}	3.33±0.57^{X}
	L10	3.91±0.62	4.00±0.00^{X}	3.33±0.57^{X}
	C	4.33±0.76	4.66±0.28^{Y}	4.50±0.86^{Y}
Odour Okus	L26	5.00±0.00^{b}	4.66±0.57^{b}	2.00±1.00^{a}
	B94	5.00±0.00^{b}	5.00±0.00^{b}	2.00±1.00^{a}
	L10	5.00±0.00^{b}	5.00±0.00^{b}	2.00±1.00^{a}
	C	5.00±0.00^{b}	4.66±0.57^{b}	2.16±1.04^{a}
Aroma/Flavor Aroma	L26	4.41±0.52^{b}	4.00±0.00^{b}	2.33±0.57^{a}
	B94	4.50±0.50^{b}	4.08±0.14^{b}	2.33±0.57^{a}
	L10	4.50±0.50^{b}	3.50±0.86^{ab}	2.33±0.57^{a}
	C	4.25±0.75^{b}	4.00±0.50^{b}	2.33±0.57^{a}
Total Ukupno	L26	17.75±1.56^{b}	17.16±0.76^{b}	11.50±3.50^{a}
	B94	17.83±1.52^{b}	17.75±0.43^{b}	11.33±3.51^{a}
	L10	18.00±1.32^{b}	17.00±1.00^{b}	11.33±3.51^{a}
	C	18.33±1.75^{b}	18.00±1.50^{b}	12.66±3.51^{a}

$^{a, b, c}$Means in the same row with different superscripts significantly differ (P<0.05)/Vrijednosti u redovima označene različitim slovima signifikantno su različite (P<0,05)
$^{X, Y}$Means in the same column with different superscripts among yoghurt samples significantly differ (P<0.05)/Vrijednosti u kolonama označene različitim slovima signifikantno su različite (P<0,05)

highest viscosity from the beginning and throughout the storage (P<0.05). This also can be attributed to the higher total solids and protein content in the control compared to probiotic Torba yoghurts. As reported by Tamime and Robinson (2007), the higher the levels of solids in the yoghurt the greater the viscosity of the end product. Furthermore Özer et al. (1998; 1999) concluded that rheological properties of the labnehs seemed to be dependent on the level of protein and total solid elevation procedure.

Sensory evaluation

The results of sensory evaluation of Torba yoghurts on the basis of appearance, consistency, odour and aroma/flavour are summarized in Table 4. It appears that addition of probiotic bacteria to Torba yoghurt did not affect the sensory properties.

Panellists could only detect difference in consistency scores after 7 days of storage (P<0.05) and the control samples had the highest scores. Additionally storage time had an adverse impact only on the odour and aroma/flavour scores (P<0.05). These scores decreased sharply at the end of storage, effecting also the total score (P<0.05). During this period the most noted defect by the panellist was yeasty flavour. Similarly Al-Kadamany et al. (2002) pointed out an increased yeasty flavour during storage of concentrated yoghurt produced by in-bag straining.

Conclusion

The results of the present study suggest that probiotic Torba yoghurt could be produced successfully by using *L. casei* L26 and *L. acidophilus* L10 rather than *B. animalis* subsp. *lactis* B94. The survival of *L. acidophilus* L10 was better than the other two bacteria in Torba yoghurt environment. The acidity of Torba yoghurt seemed to be the main factor affecting the viability of probiotic bacteria, particularly *B. animalis* subsp. *lactis* B94. The use of probiotic bacteria with yoghurt culture resulted in lower proteolytic activity but higher *S. thermophilus* counts in comparison with control sample. On the other hand, addition of probiotic cultures to plain yoghurt in the manufacture of Torba yoghurt significantly decreased the total solid and protein content, adversely affecting the textural properties and viscosity. However panellists detected this negative effect after the midpoint of storage e.g. 7 days. This study also showed that shelf-life of probiotic Torba yoghurt should be less than 14 days because of yeasty flavour and so confirmed that major contaminants of Torba yoghurt are yeasts and moulds.

For further studies, the possibility for reaching the recommended minimum level of 10^6 cfu/g in Torba yoghurt by addition of microencapsulated probiotic bacteria, especially strains of Bifidobacteria, should be investigated.

Utjecaj različitih probiotičkih kultura na svojstva Torba (čvrstog) jogurta

Sažetak

Aktivnost *Lactobacillus casei* LAFTI® L26, *Bifidobacterium animalis* subsp. *lactis* LAFTI® B94 i *L. acidophilus* LAFTI® L10, njihova proteolitička aktivnost i utjecaj na kemijska, teksturalna i senzorska svojstva Torba jogurta procjenjivana su tijekom 14 dana skladištenja na 4 °C. Ove probiotičke kulture odvojeno su dodane nakon fermentacije mlijeka s jogurtnom kulturom, ali prije pakiranja proizvoda. Probiotičke bakterije dosegnule su preporučeni stupanj od 6 log cfu/g u Torba jogurtu osim *B. animalis* subsp. *lactis* B94. Dodatak probiotičkih bakterija rezultirao je značajnom proteolitičkom aktivnošću ali i pogreškama u teksturi, što je učinak manjeg udjela ukupne suhe tvari u konačnom proizvodu.

Ključne riječi: Torba jogurt, probiotičke bakterije, proteolitička aktivnost, sastav

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