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ORIGINAL RESEARCH

Use of *Bacillus indicus* HU36 as a probiotic culture in set-type, recombined nonfat yoghurt production and its effects on quality

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Viability of a probiotic and carotenoid-producing bacterium, Bacillus indicus *HU36 in vegetative form, along with the yoghurt cultures in set-type, recombined nonfat yoghurt and its effects on quality were determined during the storage at 4 °C. The number of* B. indicus *HU36 cells in yoghurt remained about 5 log cfu/mL after 14 days, but decreased to 3.5 log after 21 days. The bacterium resulted in increased yellowness, but did not affect the rheological properties of the yoghurt. Sensorial properties of the yoghurt were acceptable compared to a commercial probiotic yoghurt.* B. indicus *HU36 can thus be used as a probiotic culture in yoghurt production.*

Keywords *Bacillus indicus* HU36, Probiotic, Yoghurt, Viability, Quality.

INTRODUCTION

Probiotics are viable micro-organisms used as food supplements to exert health benefits such as protection against diarrhoea, improvement in lactose metabolism, antimutagenic and anticarcinogenic properties, reduction in serum cholesterol and stimulation of immune system in the host (Cremonini *et al.* 2002; Cross 2002; Lei *et al.* 2006; Shah 2010). Development of functional foods containing probiotic cultures has been a subject of interest due to these health benefits. Dairy products are the most common probiotic foods because of the facts that they can contain high amount of viable probiotic bacteria even after processing and that they protect the bacteria against the acid secretions in the stomach due to their high buffering capacity (Champagne *et al.* 2005).

Bifidobacteria and *Lactobacillus* species have been among the most common probiotic bacteria added to yoghurt (Dave and Shah 1997). Low tolerance to acidic pH and gastric juice is the main issue affecting the technological performance of various probiotic cultures. Development of new probiotic cultures that do not negatively affect the organoleptic qualities of the food product as well as being highly viable in both the product and the gastrointestinal tract has been a subject of great interest (Heller 2001).

Bacillus species such as *B. subtilis, B. pumilus, B. coagulans,* and *B. clausii* have been used as probiotics (Sanders *et al.* 2003; Hong *et al.* 2005; Cutting 2011). The safety- and health-promoting properties of the *Bacillus* species as probiotics have been investigated and confirmed by several researchers (Duc *et al.* 2004, 2006; Hong *et al.* 2005, 2008). *B. indicus* HU36 has gained special interest because of its safety as a probiotic supplement and its high capacity to produce carotenoids (Duc *et al.* 2006; Hong *et al.* 2008). A recent study showed that *B. indicus* HU36 remained viable after gastric and duodenal digestion, and the carotenoid content of this bacterium is readily bioavailable (Sy *et al.* 2013). Being a probiotic and having carotenoid production capacity, *B. indicus* HU36 can be used in various food formulations. We recently found that *B. indicus* HU36 can potentially be used in the production of probiotic bitter chocolate with a high survival rate in the chocolate (Erdem *et al.* 2014).

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The main objective of this research was to investigate the use of *B. indicus* HU36 as a probiotic culture in yoghurt. The specific objectives were the following: (i) to determine the effects of *B. indicus* HU36 on the rate of pH drop during fermentation in set-type, recombined nonfat yoghurt production; (ii) to investigate the viability of *B. indicus* HU36 in the yoghurt with commercial yoghurt cultures; (iii) to determine the effects of *B. indicus* HU36 on the postfermentation quality attributes of the yoghurt such as pH, colour, and sensory and rheological properties.

MATERIALS AND METHODS

Materials

DSM (Difco Sporulation Medium) agar, LB (Luria-Bertani) broth and LB agar were obtained from Oxoid (Basingstoke, UK). MRS (acc. to De Man, Rogosa and Sharpe) and M17 (acc. to Terzaghi) agars were obtained from Merck (Darmstadt, Germany). Mixture of freeze-dried DVS (Direct Vat Set) commercial yoghurt cultures was provided by Chr. Hansen (YC-350, 50-U pouches, Horsholm, Denmark). Commercial skimmed milk powder (brand name: 'PINAR', Pinar Süt Mamülleri San. A.Ş., İzmir, Turkey) in a high barrier plastic package laminated with an aluminium layer was purchased from a local market.

Inoculum preparation

A natural isolate of *B. indicus* HU36 was supplied by the coordinator (Prof. Dr. Simon Cutting) of the EU 7th Framework Project 'COLORSPORE' (Project number: 207948) on DSM agar plates. The bacteria were activated with three successive transfers in LB broth before use. In addition, stock cultures were prepared in glycerol solution and stored at −18 °C. *B. indicus* HU36 was grown according to the method described by Nicholson and Setlow (1990) with some modifications. *B. indicus* HU36 on DSM agar was transferred to LB broth and incubated overnight at 30 °C in a water bath with an orbital shaker (New Brunswick Scientific Co, Inc. Classic Series, C2 platform shaker, Edison, NJ, USA) with aeration at 250 rpm. Overnight grown cultures were transferred to a fresh LB broth and incubated at 37 °C in the shaker until the medium reached an optical density of approximately 1.0 at 600 nm. The broth containing the bacteria was diluted [1:5 (v/v)] and used to inoculate LB agar, which was then incubated at 37 °C for 24 h. The bacterial cells were harvested by scraping them from the surface of the agar. The harvested *B. indicus* HU36 cells were suspended in sterile distilled water, and their concentration was adjusted approximately to 8 log cfu/mL.

The freeze-dried DVS commercial yoghurt culture consisting of *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was suspended in heat-treated (95 °C for 15 min) reconstituted nonfat milk (1% w/v) and activated for 15 min at 42 °C before use in the production of yoghurt (Korbekandi *et al.* 2009).

Yoghurt production

The commercial skimmed milk powder with 1.25% (w/w) fat content was reconstituted in sterilised distilled water to obtain a 13% (w/v) total solid content. The reconstituted nonfat milk (400 mL) was then transferred to a sterile flask and heat-treated at 95 °C for 15 min in the water bath followed by cooling to the fermentation temperature (42 °C) in an ice water bath.

Bacillus indicus HU36 yoghurt samples were prepared by two different methods. In the first method (designated as BI-I), the reconstituted nonfat milk was first inoculated with *B. indicus* HU36 (8 log cfu/mL) and incubated at 37 °C for 2 h in the shaker. Then, this pre-inoculated milk was inoculated with the commercial yoghurt cultures from the stock suspension (0.4% v/v). In the second method (designated as BI-II), the reconstituted nonfat milk was simultaneously inoculated with *B. indicus* HU36 (8 log cfu/mL) and the commercial yoghurt cultures (0.4% v/v). The control yoghurt was produced by inoculating the reconstituted nonfat milk only with the commercial yoghurt cultures (0.4% v/v). Each of the inoculated milk samples was shared out to 50-mL sterile tubes, sealed and incubated at 42 °C in the water bath until pH 4.5 was attained. Then, the yoghurt samples were stored at 4 °C for 21 days.

Experimental design

Five different sets of experiments were performed to investigate: (i) rate of pH drop during fermentation, (ii) viability of *B. indicus* HU36 and the yoghurt cultures, and the changes in pH during storage, (iii) rheological properties, (iv) texture profile and colour changes and (v) sensory characteristics. The experiments for sensory evaluations (v) and analysis of the rate of pH drop during fermentation (i) were replicated twice. The other experiments (ii, iii, iv) were repeated three times.

Evaluation of the rate of pH drop during fermentation

Changes in pH of the inoculated milk during fermentation were measured by a pH meter (Hanna pH 211 Model pH meter, Ann Arbor, MI USA) at intervals of 15 min until pH 4.5 was attained. A sample was removed from the water bath to measure the pH at each sampling period and was discarded after the measurement. The following kinetic parameters were evaluated: maximum rate of pH drop (V_{max}): the maximum rate of change in pH by time (dpH/dt) in mUnit pH/min; t_{max}: the time at which the V_{max} was reached in hour; pH_{Vmax}: the pH at which the V_{max} was reached; $t_{pH5.0}$: the time to reach the pH 5.0 in hour; and $t_{pH4.5}$: the time to reach the pH 4.5 in hour (i.e. the total fermentation time). The rate of pH drop (V_{max}) was obtained by dividing the maximum difference in the pH between the two consecutive measurements by the corresponding time interval.

Determination of the viable bacterial counts

Bacterial counts in the yoghurt samples were determined after the fermentation and once a week until the end of the storage period. *B. indicus* HU36 colonies were enumerated on LB agar incubated at 37 °C for 24 h. The number of *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* were determined on M17 and MRS agars, respectively, incubated at 37 °C for 5 days under anaerobic conditions. The anaerobic conditions were created by packaging the petri dishes in bags made of a high gas barrier material (laminated aluminium foil-polyethylene, AL-PE) that were flushed with 100% CO_2 before being sealed using a packaging machine (Multivac C200; Multivac Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany).

Determination of pH change in yoghurt during storage

The pH of the yoghurt samples were measured once a week using the pH meter (Hanna pH 211 Model pH meter) calibrated using buffer solutions. A separate sample was removed at each sampling period for the measurement.

Colour analysis of yoghurt

Colour parameters (L^*-, a^*-, and b^*-values, chroma, hue) of the yoghurt samples were measured each week using a Chroma Meter (Model CR-400; Konica Minolta Sensing Inc., Osaka, Japan) during the 21-day storage. Whiteness Index (WI) for the samples was calculated according to Vargas *et al.* (2008) using the equation: $WI = 100-[(100-L^*)^2 + a^{*2} + b^{*2}]^{1/2}$.

Sensory analysis of yoghurt

Descriptive profiling analysis was carried out by six trained panellists selected among the members of the Food Engineering Department at Istanbul Technical University. All the panellists had previous experience in sensory analysis. A set of references was selected by the panellists from commercially available products to evaluate various quality aspects of the *B. indicus* HU36 yoghurt as follows: set-type natural yoghurt (3% fat), set-type probiotic yoghurt (3% fat), set-type light yoghurt (1.5% fat), stirred-type yoghurt with dried apricot and mayonnaise (for colour evaluation). Evaluations were conducted at room temperature under white illumination. The panel members discussed and agreed upon the physical quality attributes (general appearance, colour, whey separation, lumpiness, firm body and yoghurt-like texture), their definitions and how to quantify the attributes on a scale from 0 to 7 as given in Table 1. Blindly labelled samples were introduced to the panel members at the room temperature in plastic bowl in random order for the evaluation of their attributes listed in Table 1. A commercial probiotic yoghurt obtained from a local store was also included in the test for the purpose of comparison.

Table 1 The glossary of descriptors used for descriptive sensory profiling of yoghurt

Descriptors	*Definition/Reference*
General appearance	Reference: Commercial probiotic yoghurt = 6 (0 to 7 scale)
Colour	Appearance of the product ranging from white to orange (0 to 7 scale) References: Commercial probiotic yoghurt = 0, mayonnaise = 4, yoghurt with dried apricot = 7
Whey separation	The amount of free whey on the surface of the yoghurt cup (0 to 7 scale) References: natural yoghurt = 0, light yoghurt = 1, commercial probiotic yoghurt = 4
Lumpiness	Uneven and nonhomogenous appearance of yoghurt mass (0 to 7 scale) References: commercial probiotic yoghurt = 1, natural yoghurt = 3, light yoghurt = 5
Firm body and yoghurt-like texture	Yoghurt-like firm body showed in the spoon (0 to 7 scale, 7 means firm) References: Yoghurt with dried apricot (stirred type) = 0, commercial probiotic yoghurt = 3, light yoghurt = 5, natural yoghurt = 7

Rheological analysis

Rheological properties of the yoghurt samples were measured at 4 °C using a Rheometer (Haake RheoStress RS 50; Haake Rheometer, Karlsruhe, Germany) with a parallel plate sensor with 35 mm diameter and 1 mm gap. The samples were analysed immediately after being removed from the storage. Before placing yoghurt samples in the rheometer's plate for the analysis, they were stirred gently 10 times with a spatula. The samples were exposed to increasing (upward flow curve) and decreasing (downward flow curve) shear rates ranged from 0 to 290 per second within 120 seconds for each run, and the corresponding shear stress data were obtained. The averaged data from the first 40 seconds of the upward flow curve were fitted to power-law equation: $\tau = K\gamma^n$ where τ is the shear stress (Pa), γ is the shear rate (per second), K is the consistency index (Pa s^n), and n is the flow behaviour index.

The viscoelastic properties of the yoghurt samples were determined using the dynamic oscillation tests (Paseephol *et al.* 2008). The linear viscoelastic range was verified by a stress sweep from 0.1 to 15 Pa at a constant frequency of 1 Hz. The dynamic measurements were carried out at a constant shear stress of 0.5 Pa, and the frequencies ranged from 0.05 to 100 Hz. The dynamic complex viscosity (η^*), the storage modulus (G'), the loss modulus (G'') and the phase angle (tan δ) representing the ratio of G'' to G' were evaluated.

Texture profile analysis (TPA) was conducted using a texture analyser (TPA; Lloyd Instruments-TA Plus, West

Sussex, UK) with 20-mm-diameter cylindrical probe. The sample was penetrated to a depth of the 50% of its height in a double compression cycle, using a cross-head speed 1 mm/second. The following textural attributes were analysed: hardness, cohesiveness, springiness, chewiness, gumminess and adhesiveness.

Statistical analysis

The data were subjected to the analysis of variance (ANOVA) and the Tukey tests to determine significant differences among the sample means using a statistical software program (MINITAB Release 12.2; Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Fermentation kinetics

The changes in average pH during fermentation and the kinetic parameters of the fermentation at 42 °C are shown in Figure 1 and Table 2, respectively. The pH of the milk inoculated with the *B. indicus* HU36 (BI-I and BI-II) showed a more rapid reduction compared to the control and reached the desired final pH (4.5) 30 min earlier during fermentation. Addition of *B. indicus* HU36 at both conditions (BI-I and BI-II) resulted in significantly higher V_{max} and lower t_{Vmax}, $t_{5.0}$ and $t_{4.5}$ values compared to the control yoghurt ($P < 0.05$). This was apparently due to an increased acid production of the yoghurt cultures in the presence of the competing bacterium, *B. indicus* HU36, because inoculation of the milk with *B. indicus* HU36 alone did not cause a significant change in the pH of the milk (data not shown). The differences between the kinetic parameters (V_{max}, t_{Vmax}, $t_{5.0}$, $t_{4.5}$) of BI-I and BI-II yoghurt were not significant ($P > 0.05$). These findings indicated that the addition of *B. indicus* HU36 speeded up the fermentation process and thus shortened the time required for yoghurt production. Similar observations in which presence of a culture caused a more rapid reduction in pH in yoghurt production have been reported in literature (Kristo *et al.* 2003; Oliveira *et al.* 2009; Saccaro *et al.* 2009). Tamime and Robinson (1999) proposed that a synergistic effect may be found between probiotic bacteria and yoghurt cultures: *L. delbrueckii subsp. bulgaricus* partially digests casein to peptides which can be further metabolised to free amino acids by probiotic bacteria and *S. thermophilus*. A similar synergistic effect of *B. indicus* HU36 and the yoghurt culture may be a reason for the higher rate of pH drop observed in the *B. indicus* HU36 yoghurt samples (BI-I and BI-II) in our study.

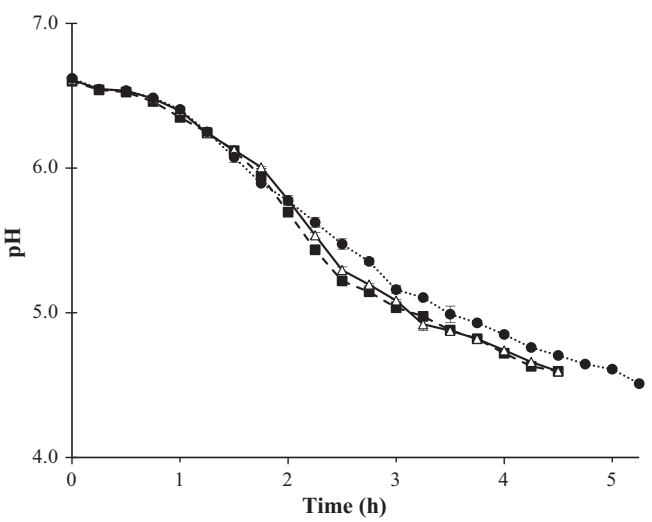

Figure 1 Changes in pH of the milk during fermentation at 42 °C as affected by the addition of *B. indicus* HU36. Added before yoghurt starter culture-I): (-■-); added with yoghurt starter culture (BI-II): (–△–); no addition (control): (-●-). Results expressed as the mean of the two independent measurements with standard deviations.

Viability of *B. indicus* HU36 and the yoghurt cultures

The viable numbers of *B. indicus* HU36, *L. delbrueckii subsp. bulgaricus* and *S. thermophilus* in yoghurt samples during 21 days of storage at 4 °C are shown in Figure 2. The initial number of *B. indicus* HU36 (8.65 log cfu/mL, data not shown) decreased to 6.57 and 5.43 log cfu/mL, respectively, in BI-I and BI-II yoghurt after the fermentation (Figure 2). Higher population of *B. indicus* HU36 was observed in BI-I compared to BI-II in the first 7 days of the storage period ($P < 0.05$). This may be associated with a better adaptation of *B. indicus* HU36 to the environment

Table 2 Kinetic parameters of the rate of pH drop during fermentation at 42 °C as affected by *B. indicus* HU36 addition to milk samples in yoghurt production[a,b]

B. indicus HU36 addition	*Maximum rate of pH drop,* V_{max} (mUnit pH/min)	*Time to reach* V_{max}, t_{Vmax} (h)	*pH at* V_{max}, pH_{Vmax}	*Time to reach pH 5.0,* $t_{5.0}$ (h)	*Time to complete fermentation,* $t_{4.5}$ (h)
Added before yoghurt starter culture (BI-I)	18.00 ± 0.99^{A}	2.13 ± 0.18^{B}	5.70 ± 0.01^{A}	3.00 ± 0.00^{A}	4.75 ± 0.00^{A}
Added with yoghurt starter culture (BI-II)	17.35 ± 0.92^{A}	2.37 ± 0.18^{B}	5.40 ± 0.17^{AB}	3.00 ± 0.00^{A}	4.75 ± 0.00^{A}
No addition (Control)	13.00 ± 0.47^{B}	3.00 ± 0.00^{A}	5.16 ± 0.03^{A}	3.50 ± 0.00^{B}	5.25 ± 0.00^{B}

[a]Results expressed as the mean of the two independent measurements with standard deviations.

[b]Values with different upper case letters at the superscripts in the same column are significantly different ($P < 0.05$).

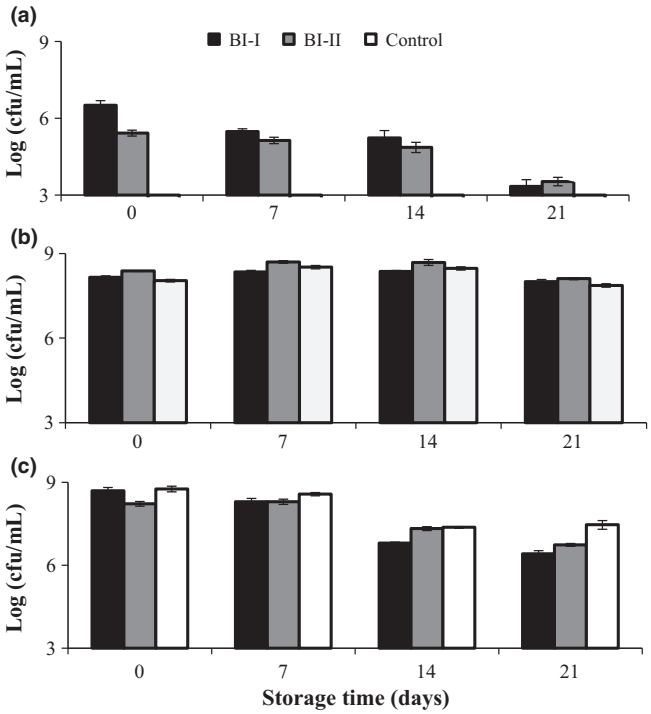

Figure 2 Viability of (a) *B. indicus* HU36, (b) *Lactobacillus delbrueckii* subsp. *bulgaricus* and (c) *Streptococcus thermophilus* in the yoghurt samples during 21 days of storage at 4 °C. Results expressed as the mean of the three independent measurements with standard deviations.

during the 2-h incubation prior to the yoghurt culture addition in the BI-I yoghurt. Pre-inoculation of milk with *B. indicus* HU36 did not change the pH at the end of the 2-h incubation period. On the other hand, the counts of *B. indicus* HU36 decreased to similar levels (5.32 and 5.06 log cfu/mL) in both BI-I and BI-II yoghurt after 14 days of storage, followed by further reduction to 3.5 log cfu/mL after 21 days (Figure 2). While the recommended number for probiotics in a product is accepted as 6 log cfu/mL, it is also accepted that the number of the probiotic bacteria exerting health benefits in food varies as a function of the strain and the targeted health benefits (Schrezenmeir and Vrese 2001). Thus, *in vivo* studies should be performed to determine number of *B. indicus* HU36 needed to show its health benefits in a host. Moreover, higher number of *B. indicus* HU36 in yoghurt at the end of storage time may be achieved by increasing the initial inoculation rate of the organism, which would not increase the overall cost considerably from the industrial standpoint.

The counts of *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* before yoghurt fermentation were both around 8 log cfu/mL (data not shown). The number of *L. delbrueckii subsp. bulgaricus* was slightly higher in BI-II yoghurt than the others and overall remained unchanged during the 21 days of storage in all samples (Figure 2). Viability of *S. thermophilus* in the presence of *B. indicus* HU36 was slightly lower than its viability in the control yoghurt ($P < 0.05$), and there was a small reduction in its count after 14 days (Figure 2). These results indicated that *B. indicus* HU36 may suppress the growth of *S. thermophilus* while promoting the growth of *L. delbrueckii subsp. bulgaricus*.

Changes in pH during storage

The pH of the yoghurt samples was about 4.57 ± 0.10 at the beginning and decreased further during storage (Figure 3). The pH reduction in BI-I and BI-II yoghurt was lower than that in the control yoghurt during storage ($P < 0.05$). Development of acidity during storage arises from the activity of the yoghurt cultures (Saccaro *et al.* 2009). This is undesirable as the yoghurt becomes sourer resulting in lower consumer acceptance. Cultures with reduced 'overacidification' behaviour should be used to prevent pH drop in yoghurt during storage (Lourens-Hattingh and Viljoen 2001). According to Heller (2001), probiotic cultures not promoting the acidity of the product during storage should be selected for developing marketable products. In this standpoint, *B. indicus* HU36 resulted in a favourable effect in maintaining the acidity of the yoghurt during storage.

Colour properties

Changes in L^*-value, WI and b^*-value of yoghurt samples during storage at 4 °C are presented in Figure 4. The BI-I and BI-II yoghurt had lower WI compared to the control yoghurt ($P < 0.05$). The b^*-values (yellowness) of the yoghurt with *B. indicus* HU36 (BI-I and BI-II) were significantly higher than that of the control yoghurt. These differences may be associated with the carotenoid content of the *B. indicus* HU36 cells. However, there was no difference

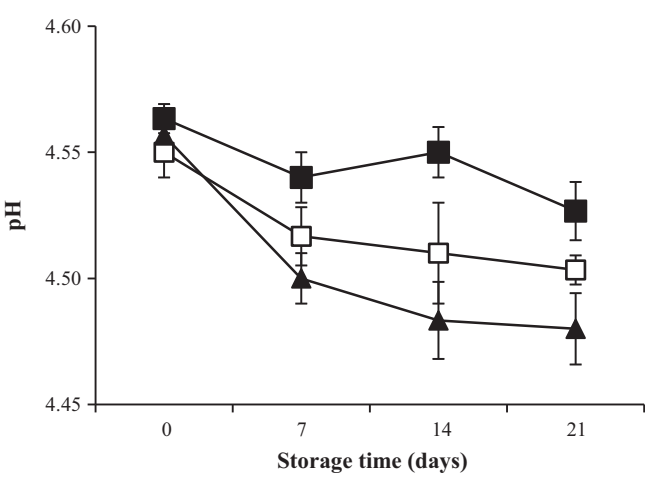

Figure 3 Changes in pH of the yoghurt samples during 21 days of storage at 4 °C. BI-I: (–□–); BI-II: (–■–); control: (–▲–). Results expressed as the mean of the two independent measurements with standard deviations.

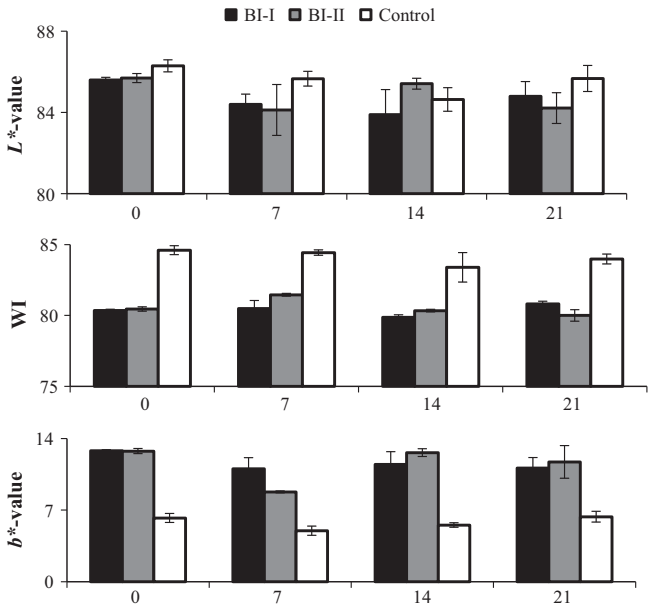

Figure 4 Changes in lightness (L^*-value), whiteness index (WI) and yellowness (b^*-value) of the yoghurt samples during 21 days of storage at 4 °C. Results expressed as the mean of the three independent measurements with standard deviations.

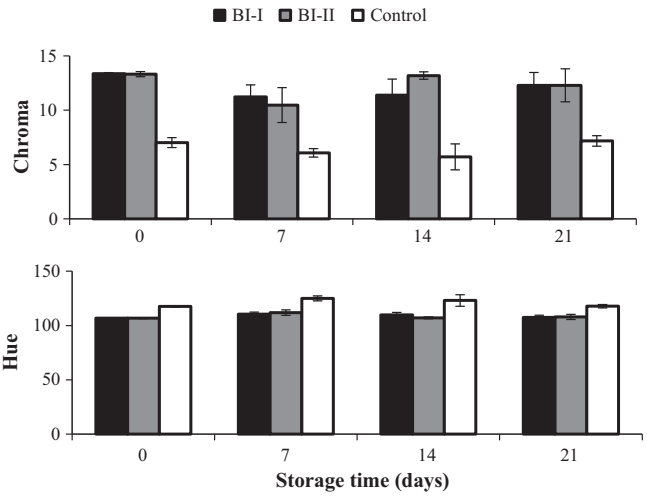

Figure 5 Changes in chroma and hue angles of the yoghurt samples at 4 °C during 21 days of storage. Results expressed as the mean of the three independent measurements with standard deviations.

between the b^*-values of the BI-I and the BI-II yoghurt samples ($P > 0.05$), showing pre-inoculation of *B. indicus* HU36 did not affect yellowness (b^*-values) in yoghurts. *B. indicus* HU36 increased the chroma of the yoghurt compared to the control (Figure 5). This may positively affect the consumer preference for the yoghurt. Hue angles for the *B. indicus* HU36 yoghurt (BI-I and BI-II) were significantly smaller and closer to yellow than that of the control yoghurt (Figure 5).

Sensory analysis

The results of the sensory analysis are shown in Figure 6. Although the yoghurt with *B. indicus* HU36 (BI-I and BI-II) had higher whey separation than the control yoghurt, it was lower than the whey separation in the commercial probiotic yoghurt on day 7. Lumpiness in BI-I and BI-II was higher than the commercial probiotic yoghurt, but the scores was similar to those for the control yoghurt (BI-II) or the light yoghurt reference (BI-I) on day 7. The lumpiness scores of the BI-II and the commercial probiotic yoghurt were similar after fermentation (on day zero). Whey separation and lumpiness in BI yoghurts may be related with the activity of *B. indicus* HU36 cells before the yoghurt organisms become dominant (Driessen and Stadhouders 1980).

The scores for the yoghurt-like texture in BI-I and BI-II yoghurts were between the scores of the control and the commercial probiotic yoghurt and thus can be considered acceptable. The *B. indicus* HU36 yoghurt (BI-I and BI-II) received higher colour scores compared to the control or the commercial probiotic yoghurt. This was associated with the

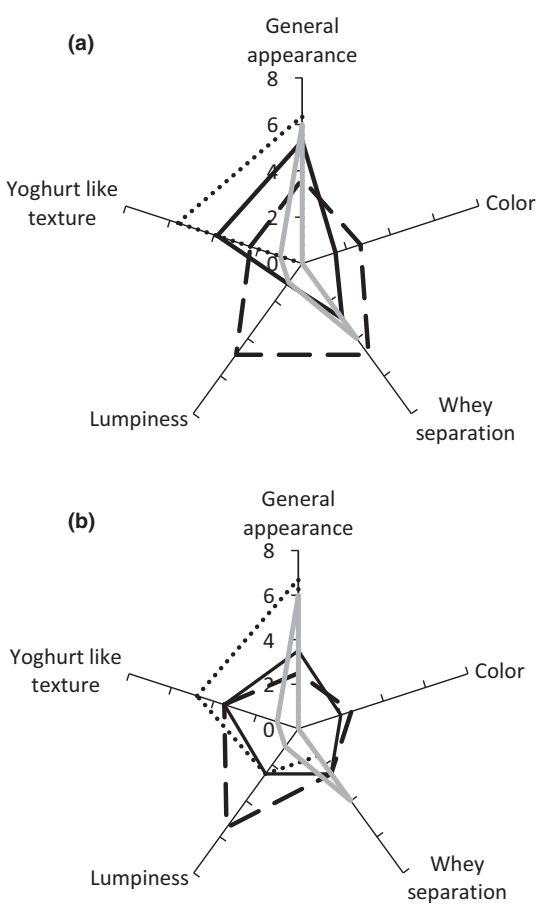

Figure 6 Sensory analysis of the yoghurt samples (a) after fermentation and (b) after 7 days of the storage period. BI-I: (– –); BI-II: (——); control: (·····); commercial probiotic yoghurt: (——). Results expressed as the mean of the two independent measurements with standard deviations.

increased yellowness as assessed by the higher b^*-values. The yoghurt with *B. indicus* HU36 (BI-I and BI-II) received lower scores on the general appearance compared to the control yoghurt ($P < 0.05$). This may be due to the colour difference in the *B. indicus* HU36-containing yoghurt compared to commercial yoghurt accustomed by the panel.

Rheological properties

B. indicus HU36 at both conditions (BI-I and BI-II) did not affect the rheological properties (*n* and K) of the yoghurt samples (data not shown). The average flow behaviour index (*n*) in the power law was 0.41, which agrees with the reported pseudoplastic characteristic of yoghurt (Bourne 2002). The average consistency index (*K*) was 10.2.

Viscoelastic properties of the yoghurt samples are presented in Table 3. As a result of stress sweep test, stress at 0.5 Pa in viscoelastic range of the yoghurt was selected for the dynamic tests. *B. indicus* HU36 did not affect viscoelastic parameters, G', G'', tan δ and η^* of the yoghurt ($P > 0.05$). While G', G'' and η^* increased, tan δ decreased with the storage period ($P < 0.05$, Table 3). Overall, the G' value of the yoghurt samples was higher than G'' values. G' and G'' give information about the solid-like and liquid-like behaviour of the sample. Higher G' value showed more solid-like structure, which is expected for a viscoelastic system such as yoghurt. In addition, the reduction in tan δ during storage was an indication of an increased firmness in the yoghurt samples.

Texture profile analysis indicated that *B. indicus* HU36 and the storage period did not affect hardness, cohesiveness, springiness, gumminess, chewiness and adhesiveness of yoghurt samples ($P > 0.05$) (data not shown). The average values of the hardness, the cohesiveness, the springiness, the gumminess, the chewiness and the adhesiveness of the yoghurt samples were 0.79 *N*, 0.32, 16.66 mm, 0.25 *N*, 4.18 and 2.39 *N*mm, respectively.

Table 3 Viscoelastic properties of yoghurt samples determined at 1 Hz[a,b,c,d,e]

		Storage (days)[d]			
	Yoghurt[c]	*0*	*7*	*14*	*21*
G' (Pa)	BI-I	83.7^{B}	80.0^{B}	122.6^{A}	120.2^{A}
	BI-II	90.4^{A}	101.2^{A}	140.9^{A}	140.5^{A}
	Control	88.5^{B}	108.7^{AB}	134.5^{A}	122.5^{AB}
G'' (Pa)	BI-I	23.3^{B}	21.7^{B}	33.6^{AB}	32.0^{B}
	BI-II	25.5^{A}	27.4^{A}	38.1^{A}	37.7^{A}
	Control	25.1^{A}	28.8^{A}	35.8^{A}	32.6^{A}
tan δ (°)	BI-I	0.278^{A}	0.272^{AB}	0.273^{AB}	0.266^{B}
	BI-II	0.282^{A}	0.271^{A}	0.270^{A}	0.268^{A}
	Control	0.284^{B}	0.265^{A}	0.266^{A}	0.266^{A}
η^* (Pa.s)	BI-I	13.8^{B}	13.2^{B}	20.2^{A}	19.8^{A}
	BI-II	15.0^{A}	16.7^{A}	23.2^{A}	23.2^{A}
	Control	14.6^{B}	17.9^{AB}	22.2^{A}	20.2^{AB}

[a]Results expressed as the mean of the three independent measurements with standard deviations.

[b]G': the storage modulus; G'': the loss modulus; tan δ: the phase angle; η^*: the dynamic complex viscosity.

[c]Different upper case letters in superscripts within the same column show statistical difference ($P < 0.05$).

[d]No statistical difference due to storage period was detected ($P < 0.05$).

[e]BI-I: yoghurt in which *B. indicus* HU36 was added for a 2-h prefermentation followed by the addition of yoghurt starter culture BI-II: yoghurt in which *B. indicus* HU36 and the yoghurt starter culture were added simultaneously; control: yoghurt without *B. indicus* HU36.

CONCLUSIONS

The number of *B. indicus* HU36 in both BI-I was 6.5 log cfu/mL after the fermentation but decreased to 5 log cfu/mL and 3.5 log in both BI-I and BI-II yoghurt at the end of 14th and 21st day of the storage, respectively. These values are lower than the general recommended numbers (6 log cfu/mL) for probiotics. Viability of the bacterium in yoghurt may be maintained at a higher level by increasing the initial inoculation rate, which requires further studies. The use of *B. indicus* HU36 in yoghurt production did not affect the rheological and the textural properties of the yoghurt during storage. Overall, *B. indicus* HU36 resulted in sensory attributes (except colour and general appearance) within the range of the control and the commercial probiotic yoghurt. In conclusion, *B. indicus* HU36 could potentially be successfully used as a probiotic culture with *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* in yoghurt production. Addition of *B. indicus* HU36 to milk before the yoghurt culture (as in BI-I) or simultaneously with the yoghurt culture (BI-II) did not affect their viability and the other quality attributes of the yoghurt. Thus, simultaneous addition of *B. indicus* HU36 and the yoghurt culture to milk (BI-II) can be suggested in the yoghurt production for convenience.

Further clinical studies to investigate specific number of viable *B. indicus* HU36 required to exert the health benefits should be conducted. Moreover, treatments such as encapsulation to improve stability of *B. indicus* HU36 in food and also human gastrointestinal tract should be investigated to expand the potential use of the probiotic organism in food.

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