Dairy Sci. Technol. 89 (2009) 583–600

DOI: 10.1051/dst/2009039

Available online at:
www.dairy-journal.org

Original article

Effect of exopolysaccharides and inulin on the proteolytic, angiotensin-I-converting enzyme- and α-glucosidase-inhibitory activities as well as on textural and rheological properties of low-fat yogurt during refrigerated storage

Lata RAMCHANDRAN, Nagendra P. SHAH*

School of Biomedical and Health Sciences, Faculty of Health, Engineering and Science, Victoria University, Werribee Campus, P.O. Box 14428, Melbourne, Vic. 8001, Australia

Received 4 February 2009 – Revised 10 September 2009 – Accepted 28 September 2009
Published online 28 October 2009

Abstract – Whey separation during storage continues to be one of the major problems in low-fat yogurts. Exopolysaccharides (EPS) produced by lactic acid bacteria have been recognized as a solution to this problem. Inulin is accepted as a fat replacer in products such as low-fat yogurts, in addition to providing physiological benefits. A combination of EPS and inulin could give both health- and texture-promoting properties to low-fat yogurt. Therefore, the aim of this study was to comprehensively study the influence of using an EPS-producing strain of *Streptococcus thermophilus* along with inulin (3%, wt/vol) on the viability of *S. thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, their proteolytic, ACE- and α-glucosidase-inhibitory activities, as well on the textural and rheological properties of low-fat yogurt during storage at 4 °C for 28 days. The time to reach a pH of 4.5 was less in the presence of EPS-producing *S. thermophilus*. However, during storage, EPS and inulin together did not influence the pH and lactic acid, and the effect on ACE-inhibition activity varied with the period of storage. The presence of EPS showed a protective effect on the survival of *Lb. delbrueckii* ssp. *bulgaricus* and partially on the extent of proteolysis. The α-glucosidase-inhibitory activity was more apparent in EPS-containing yogurt. The yield of EPS varied with the period of storage, being maximal (110.77 mg·100 g^{-1}) at d 14. EPS-containing yogurts showed lower firmness, spontaneous whey separation, storage modulus, yield stress, consistency index and hysteresis area than non-EPS producing yogurts. It was concluded that low-fat yogurt with a stable and compact texture having anti-hypertensive and anti-diabetic potential could be obtained using an EPS-producing strain of *S. thermophilus*.

ACE inhibition / inulin / rheology / low-fat yogurt / exopolysaccharide

摘要 – 胞外多糖和菊粉对低温贮藏过程中酸奶生物活性和质构特性的影响。乳清析出是低脂酸奶贮存中出现的主要问题之一。关于乳酸菌产生的胞外多糖可以解决酸奶乳清析出问题已经得到共识。菊粉作为脂肪替代品可以应用在低脂酸奶中，此外还赋予产品的功能性。菊粉与胞外多糖结合应用在低脂酸奶中可以赋予酸奶的保健和质构特性。因此，本文目的是研究产胞外多糖菌株 *Streptococcus thermophilus* 和菊粉 (3% wt/vol) 对菌株

*Corresponding author (通讯作者): Nagendra.Shah@vu.edu.au

S. thermophilus 和 *Lactobacillus delbrueckii* ssp. *bulgaricus* 的存活能力、蛋白质水解、ACE 和 α-葡萄糖苷酶抑制活性、以及对低脂酸奶在 4 °C 贮存 28 天的质构和流变性的影响。 在产胞外多糖菌 *S. thermophilus* 存在下，酸奶达到 pH 4.5 的时间较短。 然而，在贮藏期间， EPS 和菊粉并不影响 pH 和乳酸的生成，但是对 ACE 抑制活性的影响则是随贮藏期间而发生变化。 胞外多糖对 *Lb. delbrueckii* ssp. *bulgaricus* 的存活具有保护效应，并且可以使蛋白质部分水解。 含有胞外多糖的酸奶对 α-葡萄糖苷酶活性具有明显的抑制作用。 在贮藏期内，胞外多糖产量是变化，在第 14 天胞外多糖的产量达到最大值 (110.77 mg·100 g^{-1})。含胞外多糖酸奶的硬度、自然的乳清析出量、储存模量、屈服应力、稠度指数和滞后面积均低于不含胞外多糖的酸奶。 因此，应用产胞外多糖菌株 *S. thermophilus* 可以生产出稳定、质地均匀，并具有潜在抗高血压、抗糖尿病功能的低脂酸奶。

ACE-抑制作用 / 菊粉 / 流变性 / 低脂酸奶 / 胞外多糖

Résumé – Effet des exopolysaccharides et de l'inuline sur l'activité protéolytique, l'activité inhibitrice de l'enzyme de conversion de l'angiotensine I et de l'α-glucosidase, ainsi que sur les propriétés texturales et rhéologiques de yaourt allégé en matière grasse au cours du stockage réfrigéré. L'exsudation de lactosérum au cours du stockage demeure un des problèmes majeurs des yaourts allégés en matière grasse. Les exopolysaccharides (EPS) produits par les bactéries lactiques sont reconnus pour remédier à ce problème. L'inuline est admise comme substitut de matière grasse dans des produits tels que le yaourt allégé en matière grasse, et constitue en plus un apport sur le plan physiologique. La combinaison des EPS et de l'inuline pourrait procurer au yaourt allégé en matière grasse des propriétés favorisant à la fois la santé et la texture. Le but de cette étude était donc d'étudier l'influence de l'utilisation de souches de *Streptococcus thermophilus* productrices d'EPS avec de l'inuline (3 %, p/v) sur la viabilité de *S. thermophilus* et *Lactobacillus delbrueckii* ssp. *bulgaricus*, leurs activités protéolytiques, inhibitrices de l'ACE et de l'α-glucosidase, et également sur les propriétés texturales et rhéologiques de yaourt allégé en matière grasse au cours du stockage à 4 °C pendant 28 jours. Le temps pour atteindre un pH de 4,5 était inférieur en présence de *S. thermophilus* produisant des EPS. Cependant, au cours du stockage, les EPS associés à l'inuline n'influençaient pas le pH et l'acide lactique, et l'effet sur l'activité inhibitrice de l'ACE variait au cours du temps de stockage. La présence d'EPS montrait un effet protecteur sur la survie de *Lb. delbrueckii* ssp. *bulgaricus* et partiellement sur l'étendue de la protéolyse. L'activité inhibitrice de l'α-glucosidase était plus évidente dans le yaourt contenant des EPS. Le rendement en EPS variait au cours de la période de stockage, avec un maximum (110,77 mg·100 g^{-1}) le 14^{e} jour. Les yaourts contenant des EPS présentaient une fermeté, une séparation spontanée de lactosérum, un module de conservation, un seuil de contrainte, un indice de consistance et une aire d'hystérésis plus faibles que les yaourts sans EPS. On peut conclure que du yaourt allégé en matière grasse à texture stable et ferme, ayant des activités anti-hypertensives et anti-diabétiques, peut être obtenu en utilisant une souche de *S. thermophilus* produisant des EPS.

yaourt allégé en matière grasse / exopolysaccharide / inuline / inhibition de l'ACE / rhéologie

1. INTRODUCTION

Interest in the role of prebiotics as functional food ingredients has been increasing rapidly over the past few years. A prebiotic is an ingredient that allows the growth and activity of the selected gastrointestinal microflora and thereby confers benefits upon the well-being and health of the consumer. The prebiotics are now considered to be one of the practical and efficient means of manipulating the gut microflora, provided the health-promoting species are present in the bowel. The most common prebiotics include inulin and oligofructosaccharides which are found in many vegetables, including onion, asparagus, Jerusalem artichoke and chicory root. Yogurts are among the main dairy products in which prebiotics are commonly added. The properties of inulin

as a fat replacer in low-fat/no-fat dairy products are attributed to its capacity to form microcrystals that interact with each other forming small aggregates, which occlude a great amount of water, creating a fine creamy texture that provides a mouth sensation similar to that of fat [2].

Fermented milks such as yogurts have been reported to provide a range of beneficial properties to human beings, including assimilation of cholesterol, anti-tumorigenic effect, prevention of gastrointestinal infection and lowering of blood pressure. The blood pressure-lowering effects in such products are mediated through the inhibition of angiotensin-I-converting enzyme (ACE). Many of the peptides released from milk proteins due to the action of cell wall-associated proteinase activity during fermentation have been found to possess such an inhibitory property. Since these proteolytic enzymes are not very specific, a great variety of peptides are liberated during fermentation [11]. Therefore, the selection of suitable bacteria with optimal proteolytic activity is important for these products to exhibit ACE-inhibitory properties [21]. The content of potent ACE-inhibitory peptides depends on the balance between their formation and further breakdown [14]. Increasing health consciousness among consumers has boosted the demand for low-fat yogurts with additional health benefits, leading to an increase in yogurts-containing prebiotics and probiotics.

However, spontaneous appearance of whey on the surface of set yogurt, especially low-fat yogurt, remains a major concern to the manufacturers. This also affects the consumer acceptability of the product. One approach to solve this problem relies on the in situ use of generally recognized as safe, food grade, exopolysaccharide (EPS)-producing strains of lactic acid bacteria [6]. Several authors have reported the effect of EPS on the rheology and texture of yogurt [1, 9, 10, 24], and some have studied the effect of added inulin on the rheological and sensory properties of yogurt [8, 15, 19]. However, so far no work has been carried out to study the influence of EPS-producing strains of yogurt starters along with inulin on the rheological and physiological properties of low-fat yogurt. We have carried out preliminary work to examine the influence of varying levels of Raftiline HP® (2% and 3%) on the physico-chemical properties of yogurt with specific reference to viability, proteolysis and ACE inhibition and textural properties [27]. However, in this study, the rheological parameters were studied only in the fresh product to know how added Raftiline affected the rheology of the product in order to select the best level of Raftiline that could be beneficially incorporated into yogurt for our future study [27]. So far, there is no reference in the literature that has studied the influence of an EPS producer along with inulin on the textural and biochemical properties of yogurt.

In this study, we have examined the influence of EPS-producing culture against a non-EPS producing culture in the presence of a specific level of Raftiline (3%). In particular, this work aimed at comprehensively examining the influence of EPS along with inulin (Orafti HP®, earlier known as Raftiline HP®) on the viability of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, changes in pH and lactic acid content, proteolysis and ACE- and α-glucosidase (α-glu)-inhibitory activities, as well as on the firmness, spontaneous whey separation and rheological parameters during storage of low-fat yogurt at 4 °C for 28 days. The yield of EPS was also monitored during the period of storage.

2. MATERIALS AND METHODS

2.1. Propagation of yogurt starters

Two strains of *S. thermophilus*, 1275 (EPS producer) and 1342 (non-EPS producer), were obtained from the Starter

Culture Collection of Victoria University (Werribee, Vic., Australia). *Streptococcus thermophilus* 1275 produces capsular as well as ropy EPS [36]. *Lb. delbrueckii* ssp. *bulgaricus* 1368 was obtained from the Australian Starter Culture Research Centre Ltd. (Werribee, Vic., Australia). The organisms were stored in 40% (wt/vol) glycerol at −80 °C. The frozen cultures of *S. thermophilus* were first transferred into M17 broth, and those of *Lb. delbrueckii* ssp. *bulgaricus* into MRS (deMann Rogosa Sharpe) broth for their activation. This was followed by one transfer each to sterile reconstituted skim milk (RSM; 12% wt/vol) supplemented with 1% yeast extract and 2% glucose (wt/vol) and one to sterile RSM before the bulk cultures were prepared. The temperature of each incubation was 37 °C for *S. thermophilus* and 42 °C for *Lb. delbrueckii* ssp. *bulgaricus*. For each transfer, the rate of inoculation was 1% (vol/vol), and the incubation period was 20 h each.

2.2. Yogurt making

Skimmed milk (Skinny Milk, Parmalat Australia Ltd., Brisbane, QLD, Australia) standardized to 12% total solids with skim milk powder was used to prepare the low-fat yogurts. The temperature of the milk was raised to 60 °C for the addition of skim milk powder and then to 70 °C for the addition of inulin (3%, wt/vol, Orafti HP®; BENEO-Orafti, Mandurah Australia Pty. Ltd., Vic., Australia). The heating was then continued to a temperature of 85 °C, and the heated yogurt mix was held at this temperature for 30 min followed by cooling to 45 °C in a water bath maintained at about 4 °C. The mix was then inoculated with *S. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*, each at the rate of 1% (wt/vol) followed by mixing. The inoculated mix was then dispensed in 50 mL polystyrene cups with lids, followed by incubation at 42 °C until the pH dropped to 4.5 ± 0.1. At this stage, the cups were immediately transferred to a walk-in refrigerator maintained at 4 ± 1 °C. Two types of yogurts were prepared – one using the non-EPS producing strain of *S. thermophilus* 1342 (NEPSY) and the other using the EPS-producing strain of *S. thermophilus* 1275 (EPSY).

2.3. Preparation of filtrates for analysis of proteolysis and ACE- and α-glu-inhibitory activities

The filtrates of inoculated samples of yogurt mix (0 h) were prepared by lowering their pH to 4.5 with glacial acetic acid followed by centrifugation (Sorvall R2 7, Thermo Scientific, Waltham, USA) at 4000× *g* for 30 min at 4 °C. Filtrates of low-fat yogurt samples stored at 4 °C (d 1–28) were prepared by centrifuging at 4000× *g* for 30 min at 4 °C. All the supernatants thus obtained were filtered through 0.45-μm membrane syringe filter and stored at −20 °C until assayed.

2.4. Measurement of pH

The changes in pH during preparation of yogurts and at d 1, 7, 14, 21 and 28 of their storage were measured using a pH meter (Model 8417, Hanna Instruments, Singapore).

2.5. Determination of lactic acid

The concentration of lactic acid ($mg \cdot 100\ g^{-1}$) in all samples (d 1–28) of low-fat yogurt stored at 4 °C as well as the 0 h samples of mixes was determined by the high-performance liquid chromatography (HPLC) as described by Ramchandran and Shah [27]. Briefly, 1 g of the sample was vortexed with 40 μL of concentrated nitric acid and with 500 μL of 0.005 $mol \cdot L^{-1}$ sulphuric acid and centrifuged for 30 min at 14 000× *g* in Eppendorf 5415C centrifuge (Crown

Scientific, Melbourne, Vic., Australia). The supernatant thus obtained was filtered into HPLC vials using 0.45-μm membrane syringe filter. An Aminex HPX-87H, 300 × 7.8 mm ion exchange column (BioRad Life Science Group, Hercules, USA) fitted with a guard column maintained at 65 °C and attached to a Varian HPLC (Varian Analytical Instruments, Walnut Creek, USA) fitted with a UV/Vis detector was used to separate and detect the lactic acid in the samples. The sample (20 μL) was injected into the column and eluted using 0.005 mol·L^{-1} sulphuric acid at a flow rate of 0.6 mL·min^{-1}, and lactic acid was detected at 220 nm. The retention times of standard working solutions of L(+) lactic acid (prepared from a stock solution of 5.1990 g·50 mL^{-1}) were used to identify the peaks, and the peak areas were used to calculate the concentration of lactic acid in the samples.

2.6. Viability of yogurt starters

Streptococcus thermophilus 1275 and 1342 and *Lb. delbrueckii* ssp. *bulgaricus* 1368 were enumerated in freshly inoculated mixes (0 h) and at weekly intervals in the stored low-fat yogurts by pour plate technique using M17 and reinforced clostridial agar, respectively [5]. The counts were reported as $\log_{10}$ colony-forming units (CFU) per gram of yogurt. In the results presented, the 0 h counts were subtracted from those of the yogurt samples at d 1, 7, 14, 21 and 28 of storage to represent the actual changes in numbers due to growth and survival of the yogurt starters during yogurt making and storage at 4 °C.

2.7. Determination of crude EPS content

The quantity of crude EPS in EPSY was determined at weekly intervals during the storage period of 28 days at 4 °C by the method described by Purwandari et al. [25] with some modifications. Fifty grams of yogurt sample were centrifuged (J2-HS, Beckman, Fullerton, USA) at 11 000× *g* for 10 min at 4 °C. The EPS in the supernatant was precipitated by mixing it with two volumes of cold ethanol and quiescent storage at 4 °C for about 18–20 h. This was followed by centrifugation at 11 000× *g* for 15 min at 4 °C. The precipitates thus obtained were dissolved in 20 mL of MilliQ water and made protein free by mixing with 500 μL of 80% (wt/vol) trichloroacetic acid and storing at 4 °C for another 18–20 h. This was followed by centrifugation (Sorvall R2 7, Thermo Scientific, Waltham, USA) at 2000× *g* for 15 min at 4 °C. The steps of EPS re-precipitation and protein precipitation were repeated two more times. The collected crude EPS was dried at 40 °C until two consecutive weights did not show a difference of more than 0.001 g. The 0 h results of EPSY were subtracted from all the samples to rule out any differences due to co-precipitation of the added inulin. The results were expressed as milligrams of crude EPS per 100 g of yogurt.

2.8. Extent of proteolysis

The extent of proteolysis was determined by measuring the free amino acid content in the filtrates of yogurt mixes (0 h) as well as of low-fat yogurt samples by the method of Church et al. [3] as described by Ramchandran and Shah [27]. The filtrate (150 μL) was vortexed with 3 mL of OPA (o-phthaldialdehyde) reagent for 5 s, and the resulting absorbance was measured at 340 nm within 2 min using NovaSpec®-II spectrophotometer (Pharmacia, Biotech, Uppsla, Sweden). The readings of the 0 h samples as well as the reagent blank were deducted from the corresponding readings of yogurt filtrates to obtain the amount of free amino acids released as a consequence of the proteolytic activity of the starter cultures during fermentation and storage.

2.9. Determination of ACE-inhibitory activity

The ACE-inhibitory activity was determined in the filtrates of yogurt mixes as well as of the low-fat yogurts by the method of Cushman and Cheung [4] as described by Ramchandran and Shah [27]. The filtrate (30 μL) was mixed with Hip-His-Leu (200 μL, 5 mmol·L^{-1} in 0.1 mol·L^{-1} borate buffer) and borate buffer (60 μL, 0.1 mol·L^{-1} solution containing 0.3 mol·L^{-1} NaCl, pH 8.3) and incubated at 37 °C for 10 min. This was followed by the addition of ACE solution (20 μL, 0.1 unit·mL^{-1}) and incubation at 37 °C for 30 min. The reaction was terminated by the addition of 250 μL of 1 mol·L^{-1} HCl. The hippuric acid formed was extracted in 1.7 mL of ethyl acetate and after quiescent standing for 10 min, 1.2 mL of the separated solvent layer was siphoned out and dried on a boiling water bath. The absorbance of the dried hippuric acid, dissolved in 1 mL of deionized water, was measured at 228 nm using UV/Vis Pharmacia, LKB-UltrospecIII spectrophotometer (Pharmacia, Uppsala, Sweden). The percent inhibition was calculated using the following formula:

$$\text{ACE inhibition } (\%) = \left[1 - \frac{C - D}{A - B}\right] \times 100,$$

where A is the absorbance in the presence of ACE and without the sample, B is the absorbance without both ACE and the sample, C is the absorbance with ACE and the sample and D is the absorbance with the sample but without ACE. The IC_{50} values, defined as the protein concentration in the sample (mg·mL^{-1}) required to inhibit 50% of the ACE activity, were also determined. The protein content of the filtrates was determined by the method of Lowry et al. [22] using bovine serum albumin as a standard.

2.10. α-Glu-inhibitory activity

The method of Zhang et al. [35] as modified by Ramchandran and Shah [26] was followed to measure the α-glu-inhibitory activity of the filtrates of yogurt mixes as well as of the low-fat yogurts. To 300 μL of 0.1 mol·L^{-1} phosphate buffer (pH 6.5), 150 μL of 20 mmol·L^{-1} *p*-nitrophenyl-α-glucoside (Sigma Chemicals, St. Louis, USA) solution and 50 μL of the sample were added, and the mixture was incubated at 45 °C for 10 min. This was followed by the addition of 100 μL of α-glu enzyme solution (0.2 units·mL^{-1}, from yeast, Sigma Chemicals) and incubation for another 10 min at 45 °C. The reaction was terminated by the addition of 2 mL of sodium carbonate solution (0.1 mol·L^{-1}) and the amount of *p*-nitrophenol released was determined by measuring the absorbance at 400 nm using NovaSpec®-II UV spectrophotometer (Pharmacia, Biotech, Uppsla, Sweden). The percent inhibition and IC_{50} were calculated as indicated for ACE inhibition (Sect. 2.9).

2.11. Spontaneous whey separation

Spontaneous whey separation in the stored low-fat yogurt was determined by the siphon method as described by Amatayakul et al. [1]. A cup of yogurt was weighed upon removal from the refrigerator and tilted at an angle of 45° to collect the surface whey. The surface whey was siphoned out using a syringe attached with a needle. The siphoning was performed within 10 s to avoid forced leakage of whey from the curd. Thereafter, the cups were re-weighed, and the whey separation was calculated and expressed as the percentage spontaneous whey separation.

2.12. Firmness of yogurt

The firmness of the low-fat yogurts was measured as the force (gf) required to break

the gel using a texture analyser TA-XT.2 (Stable Micro Systems, Godalming, UK) with a P20 probe (diameter 20 mm) and 25 kg load cell. The speed and the depth of penetration were set at 1 mm·s^{-1} and 10 mm, respectively. The ratio of cup diameter to probe diameter was 3.5:1. The measurements were performed as soon as the samples were removed from the refrigerator. The firmness of the yogurt samples was expressed as gf.

2.13. Rheological measurements

The low-fat yogurts in cups, stored at 4 °C, were gently stirred five times in clockwise direction prior to rheological analysis. The viscoelastic properties were determined by small amplitude oscillatory measurement (SAOM) using a controlled stress/controlled rate rheometer (Physica MCR 301, Anton Paar, GmbH, Germany) equipped with a temperature and moisture regulating hood and cone-plate geometry (CP50-1, 50 mm dia, 1° angle and 0.02 mm gap, Anton Paar). The temperature of the system was regulated controlled at 5 ± 1 °C by a viscotherm VT2 circulating bath and a Peltier system (Anton Paar). The samples were loaded on the inset plate and presheared at a shear rate of 500 s^{-1} for 30 s and equilibrated for 150 s before SAOM was performed. The samples were first subjected to a frequency sweep test (frequency ramp from 0.1 to 10 Hz) at a constant strain of 0.5% (determined from an amplitude sweep performed at 1 Hz) to ascertain the shear rate, storage modulus, loss modulus and damping factors. This was followed by a shear rate sweep (0.1–100 s^{-1} upward and downward sweeps) to generate the flow curves and measure the shear stress and viscosity. The flow behaviour of the samples was determined using the Herschel-Bulkley model which is as follows:

$$\sigma = \sigma_0 + k \cdot \dot{\gamma}^n,$$

where σ_0 is the yield stress, k is the consistency index, $\dot{\gamma}$ is the shear rate and n is a dimensionless number that indicates the closeness to Newtonian flow ($n < 1$ indicates pseudoplastic liquid). The calculation of hysteresis loop area between the upward and downward curves and analysis of the data of the rheological measurements were performed using the supporting software Rheoplus/32 V2.81 (Anton Paar).

2.14. Experimental design and statistical analysis

The experiment was designed with culture (strains of *S. thermophilus*) and replications as the main plot and time as the subplot. This block was replicated three times with two subsamplings. The results of various determinations were analysed by two-way analysis of variance (ANOVA) using the General Linear Model procedure of SAS system [30]. The data of yield of EPS were analysed by one-way ANOVA and Tukey's test for multicomparison of the mean values. The level of significance was set at $P = 0.05$. Where appropriate, correlational analysis was employed using Microsoft Excel Statpro software.

3. RESULTS AND DISCUSSION

3.1. pH

The acidification time to reach the pH of 4.5 in the presence of EPS-producing *S. thermophilus* was 273 min, which was less than in the presence of non-EPS producing strain (282 min). A similar observation was made by Doleyres et al. [9] and Purwandari et al. [25].

The changes in pH during storage of the low-fat yogurts, NEPSY and EPSY, are given in Table I. There was no difference ($P > 0.05$) in the pH between NEPSY and EPSY throughout the storage period.

Table I. Changes in pH and lactic acid content (mg·100 g^{-1}) during storage for 28 days of control (NEPSY) and experimental (EPSY) low-fat yogurts at 4 °C.

Type of yogurt	Period of storage (day)				
	1	7	14	21	28
pH					
NEPSY	4.50^{aA}	4.40^{aA}	4.33^{aA}	4.50^{aA}	4.49^{aA}
EPSY	4.51^{aA}	4.43^{aA}	4.36^{aA}	4.51^{aA}	4.50^{aA}
SEM			0.05		
Lactic acid content					
NEPSY	0.97^{bA}	1.14^{aA}	1.17^{aA}	1.20^{aA}	1.20^{aA}
EPSY	0.76^{bB}	1.12^{aA}	1.20^{aA}	1.21^{aA}	1.22^{aA}
SEM			0.05		

Values are the mean of six observations.
SEM, standard error of mean values; NEPSY, control yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and non-EPS producing strain of *S. thermophilus*; EPSY, yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and EPS-producing strain of *S. thermophilus*.
[ab] Mean values in the same row with different alphabets are significantly different for each type of yogurt.
[AB] Mean values in the same column with different alphabets are significantly different for a particular day of storage for each parameter.

This is similar to the observation of Doleyres et al. [9] who concluded that the low amount of EPS does not have any influence on the post-acidification activity. Both the yogurts showed an increase ($P > 0.05$) in pH on d 21 to values similar to those of d 1. Considering that there was no increase in the lactic acid content of both the yogurts after d 7 (Tab. I), it is possible that some basic metabolites could have been produced at the low temperature (4 °C) that caused the increase in pH at d 21 [34].

3.2. Lactic acid

The lactic acid content of NEPSY and EPSY as observed during the storage at 4 °C for 28 days is also given in Table I. Both the yogurts exhibited a significant ($P < 0.05$) increase in the lactic acid concentration at d 7, and the values did not change during the remaining storage period. There was no difference ($P > 0.05$) in the lactic acid content of NEPSY and EPSY throughout the storage period.

3.3. Viability of *S. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*

The changes in the viability of the yogurt starters during storage at 4 °C are presented in Table II. There were no changes ($P > 0.05$) in the counts of the non-EPS producing strain of *S. thermophilus* throughout the storage period, whereas the EPS-producing strain showed a decrease ($P < 0.05$) at d 28, to numbers similar to those at d 1. Also, there were no differences ($P > 0.05$) between their numbers in NEPSY and EPSY during storage, except during the last week of storage of EPSY. The counts of *S. thermophilus* in both the yogurts were marginally higher in the presence of inulin than those observed in an earlier study in the absence of inulin [28]. In contrast, there were significant decreases in the counts of *Lb. delbrueckii* ssp. *bulgaricus* in NEPSY at d 7 and 14, whereas there were no changes in counts in EPSY throughout the storage period. Moreover, the counts were higher ($P < 0.05$) in EPSY

Table II. Changes in viability of yogurt starters (Δ $\log_{10}$CFU·g^{-1}) and EPS content (mg·100 g^{-1}) during storage for 28 days of control (NEPSY) and experimental (EPSY) low-fat yogurts at 4 °C.

Type of yogurt	Period of storage (day)				
	1	7	14	21	28
S. thermophilus					
NEPSY	2.06^{aA}	2.09^{Aa}	2.19^{aA}	2.15^{aB}	2.11^{aA}
EPSY	2.15^{abA}	2.10^{bA}	2.24^{abA}	2.36^{aA}	2.03^{bA}
SEM			0.05		
Lb. delbrueckii ssp. *bulgaricus*					
NEPSY	1.57^{aB}	0.94^{bB}	0.27^{cB}	0.22^{cB}	0.15^{cB}
EPSY	2.00^{aA}	1.95^{aA}	1.94^{aA}	1.87^{aA}	1.82^{aA}
SEM			0.04		
EPS content					
EPSY	38.15 ± 26.37^{a}	27.23 ± 12.23^{a}	110.77 ± 9.97^{b}	57.84 ± 18.45^{a}	43.79 ± 24.78^{a}

Values are the mean of six observations; values of EPS are Mean ± SD.
SEM, standard error of mean values; NEPSY, control yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and non-EPS producing strain of *S. thermophilus*; EPSY, yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and EPS-producing strain of *S. thermophilus*.
^{abc} Mean values in the same row with different alphabets are significantly different for each type of yogurt.
^{AB} Mean values in the same column with different alphabets are significantly different for a particular day of storage for each parameter.

as compared to those of NEPSY throughout the storage period. This confirms the protective effect of EPS on the survival of *Lb. delbrueckii* ssp. *bulgaricus* during storage which was not affected by the presence of inulin. Amatayakul et al. [1] also observed the protective effect of ropy EPS on *Lb. delbrueckii* ssp. *bulgaricus*.

3.4. Crude EPS content

The yield of crude EPS (mg·100 g^{-1}) in EPSY is shown in Table II. The EPS content was similar ($P > 0.05$) throughout the storage period, except for a sharp (~ four times) increase ($P < 0.05$) at d 14 followed by a decrease ($P < 0.05$) at d 21. The increase at d 14 could be due to the enhanced numbers ($P < 0.05$) of the EPS producer (*S. thermophilus* 1275) observed at d 14 (Tab. II), whereas the decrease in the EPS content could be attributed to the presence of enzymes capable of degrading EPS [7]. Also, the yield of EPS was higher in the presence of inulin, particularly from d 7, than those reported in our previous work in yogurts without added inulin [28]. Doleyres et al. [9] found that the EPS content in yogurt remained stable during the 4-week storage at 5 °C, while Amatayakul et al. [1] reported an increase in the concentration of EPS in yogurt made with ropy starter cultures but not in yogurt made using capsular EPS-producing starter cultures. Purwandari et al. [25] observed a decrease in the EPS content of yogurts during storage. As yet, there are no reports on the changes in EPS content of inulin-containing yogurts.

3.5. Proteolysis

The changes in the extent of proteolysis, as measured by the increase in the level of free amino acids, are shown in Figure 1.

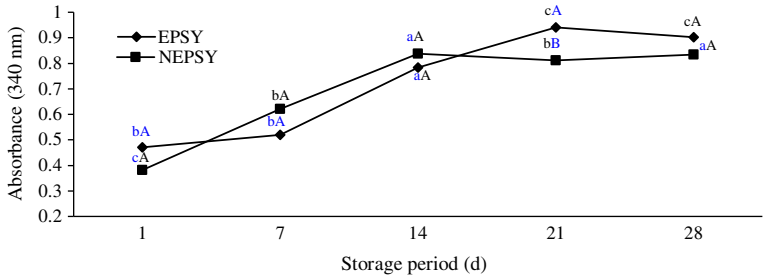

Figure 1. Changes in proteolysis (ΔA_{340}) of control (NEPSY) and experimental (EPSY) low-fat yogurts during storage for 28 days at 4 °C. NEPSY, control yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and non-EPS producing strain of *S. thermophilus*; EPSY, yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and EPS-producing strain of *S. thermophilus*.
[abc] Mean values with different alphabets are significantly different within each type of yogurt.
[AB] Mean values with different alphabets are significantly different between each type of yogurt for a particular day of storage.

While the proteolytic activity in NEPSY increased ($P < 0.05$) until d 14, that in EPSY remained stable until d 7 before showing a continuous increase ($P < 0.05$) until d 21. Proteolytic activity was higher ($P < 0.05$) in EPSY than in NEPSY at d 21 only. The increase in the extent of proteolysis was much higher in NEPSY (0.24 units) than in EPSY (0.05 units) during the first week of storage, which resulted in a higher ($P > 0.05$) extent of proteolysis in NEPSY at d 7. However, during the third week, the extent of proteolysis increased by 0.156 units in EPSY compared to a negligible decrease in NEPSY, which resulted in increased absorbance values of EPSY towards the end of the storage period. So far, no work has been carried out to study the influence of EPS on proteolysis of inulin-containing yogurt.

3.6. ACE inhibition

The ACE-inhibitory activity (%) along with the corresponding IC_{50} ($mg \cdot mL^{-1}$) values of the low-fat yogurts is given in Table III. No ACE-inhibitory activity was observed in the 0 h filtrates of the yogurt mixes. At d 1 and 14, the inhibitory activity was higher ($P < 0.05$) in NEPSY than in EPSY, but at the end of storage (d 21 and 28) it was the reverse. Consequently, the IC_{50} value of EPSY was also lower than that of NEPSY at d 21 and 28. The activity was similar ($P > 0.05$) at d 7. The ACE-inhibitory (%) values were higher in the EPS^+ yogurts in the presence of inulin than those observed in our previous study in the EPS^+ yogurts in the absence of inulin [28]. During storage, the ACE-inhibitory activity increased ($P < 0.05$) in NEPSY at d 7 and dropped ($P < 0.05$) at d 21 before increasing ($P < 0.05$) again at d 28. In the case of EPSY, the activity increased ($P < 0.05$) at d 7 but decreased ($P < 0.05$) at d 14 before increasing ($P < 0.05$) at d 28. Although there was no correlation between the extent of proteolysis and ACE inhibition, the continued proteolytic activity (Fig. 1) could have resulted in changes in the content of peptides showing ACE-inhibitory potential which caused variations in the activity during storage. Gobetti et al. [14] have also made a similar observation. Additionally, Fuglsang et al. [14] have reported that a high OPA index does not necessarily indicate higher ACE inhibition. So far, there have been no reports on the

Table III. Changes in ACE- and α-glu-inhibition (%) and corresponding IC_{50} ($mg \cdot mL^{-1}$) values in low-fat yogurts stored for 28 days at 4 °C.

	Period of storage (day)									
	1		7		14		21		28	
	ACE-I	IC_{50}	ACE-I	IC_{50}	ACE-I	IC_{50}	ACE-I	IC_{50}	ACE-I	IC_{50}
NEPSY	22.20^{bcA}	2.36	38.28^{aA}	1.15	36.35^{aA}	1.32	12.59^{cB}	4.20	25.66^{bB}	1.51
EPSY	15.82^{bB}	3.66	39.50^{aA}	1.09	23.78^{bB}	1.98	17.81^{bA}	2.84	36.30^{aA}	1.28
SEM					2.21					
	α-Glu-I	IC_{50}	α-Glu-I	IC_{50}	α-Glu-I	IC_{50}	α-Glu-I	IC_{50}	α-Glu-I	IC_{50}
NEPSY	ND	–	ND	–	ND	–	6.71^{aB}	0.394	10.79^{aA}	0.180
EPSY	7.63^{b}	0.379	15.68^{a}	0.138	11.16^{ab}	0.211	11.58^{abA}	0.218	14.93^{aA}	0.155
SEM					1.52					

Values are the statistical mean of six observations.
SEM, standard error of mean values; NEPSY, control yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and non-EPS producing strain of *S. thermophilus*; EPSY, yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin; EPS-producing strain of *S. thermophilus* and ND, activity not detected.
[abc] Mean values in the same row with different alphabets are significantly different within a particular treatment.
[AB] Mean values in the same column with different alphabets are significantly different for a particular day of storage.

Table IV. Changes in firmness (gf) and spontaneous whey separation (%) during storage for 28 days of control (NEPSY) and experimental (EPSY) low-fat yogurts at 4 °C.

Type of yogurt	Period of storage (day)				
	1	7	14	21	28
Firmness					
NEPSY	74.60^{aA}	84.01^{aA}	85.72^{aA}	89.43^{aA}	90.10^{aA}
EPSY	68.94^{aA}	70.41^{aA}	70.17^{aB}	74.04^{aB}	72.50^{aB}
SEM			4.26		
Spontaneous whey separation					
NEPSY	3.26^{abA}	2.96^{abA}	2.07^{bA}	3.56^{aA}	4.08^{aA}
EPSY	2.55^{aA}	1.96^{abA}	1.22^{bB}	2.40^{aB}	2.56^{aB}
SEM			0.27		

Values are the mean of six observations.
SEM, standard error of mean values; NEPSY, control yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and non-EPS producing strain of *S. thermophilus*; EPSY, yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and EPS-producing strain of *S. thermophilus*.
^{abcd} Mean values in the same row with different alphabets are significantly different for each type of yogurt.
^{AB} Mean values in the same column with different alphabets are significantly different for a particular day of storage for each parameter.

influence of EPS-producing strains of starters on the ACE-inhibitory potential of yogurt-containing inulin.

3.7. α-Glu-inhibition

The ability of the low-fat yogurts to inhibit the activity of α-glu with the corresponding IC_{50} values ($mg \cdot mL^{-1}$) is shown in Table III. The 0 h filtrates of the yogurts did not show any α-glu-inhibitory activity. Among the two types of yogurts, EPSY showed the inhibitory activity throughout the storage period; the activity increased ($P < 0.05$) at d 7 and then remained stable for the rest of the storage period. On the other hand, the activity was not detected in NEPSY until d 21. In an earlier work, the authors found that *S. thermophilus* 1275 and *Lb. delbrueckii* ssp. *bulgaricus* 1368 showed α-glu-inhibitory activity in the presence of inulin when grown individually for 6 h at 37 °C. We also observed that in the absence of inulin, low-fat yogurts with EPS- and non-EPS producing strains of *S. thermophilus* did not show any such activity [28]. It is thus possible that the presence of inulin could have aided the generation of components showing α-glu-inhibitory activity. The higher ($P < 0.05$) α-glu-inhibitory potential of EPSY as compared to that of NEPSY could be due to the variation in the strain of *S. thermophilus*. Thus, it appears that in the presence of EPS-producing strain of *S. thermophilus* and inulin, the low-fat yogurts showed some anti-diabetic potential due to their ability to inhibit α-glu.

3.8. Spontaneous whey separation

The spontaneous percent whey separation exhibited by NEPSY and EPSY during storage at 4 °C for 28 days is presented in Table IV. The appearance of surface whey in NEPSY and EPSY was similar ($P > 0.05$) initially (until d 7), but for the rest of the storage period it was lower

($P < 0.05$) in EPSY than in NEPSY. The trend of whey separation in NEPSY during storage showed a decrease until d 14 followed by a significant increase at d 21; in EPSY, also a decrease ($P > 0.05$) was observed until d 14 followed by an increase ($P < 0.05$) to the initial values at d 21 and 28. The EPS content of EPSY showed a good inverse correlation ($r = -0.78$) to the spontaneous whey separation during storage indicating that the yogurts had a better and more stable structure in the presence of EPS that prevented syneresis. However, it was noted that the spontaneous whey separation was relatively higher in EPS^+ yogurts-containing inulin in comparison to those observed in EPS^+ yogurts without inulin [28]. Amatayakul et al. [1] did not find any change in syneresis during storage of yogurt. Several researchers have observed a reduction in syneresis of yogurts made with EPS-producing cultures [1, 9, 28]. The shear-induced microstructure in yogurt made with EPS-producing culture has been shown to consist of compartmentalized protein aggregates between channels containing EPS, which hinders syneresis [17]. The improved water-holding capacity of yogurts-containing EPS, due to the high water-binding property of EPS, is another reason for decreased syneresis [33].

3.9. Firmness

Table IV presents the changes in firmness (gf) of the low-fat yogurts during storage at 4 °C. The firmness of NEPSY and EPSY remained stable ($P > 0.05$) throughout the storage period. Also, EPSY had lesser firmness ($P < 0.05$) than NEPSY during storage, except at d 1 and 7 when both the yogurts had similar ($P > 0.05$) firmness. Interestingly, a comparison to our earlier study [28] revealed that the inclusion of inulin did not appear to influence the firmness of EPS^+ yogurt but increased that of EPS^- yogurts. This confirms that fermentation with EPS-producing strains resulted in yogurts with low gel firmness as has been reported by several workers [1, 10]. The presence of EPS could interfere with the association between casein micelles resulting in less firm coagulum [29]. Studies of yogurt microstructure have shown void spaces around EPS-producing bacteria that can affect the integrity of the protein matrix [17] which also explains the lower firmness of EPS-containing yogurts. The changes in firmness showed a good correlation to the extent of proteolysis observed in NEPSY ($r = 0.95$) and EPSY ($r = 0.86$). Gassem and Frank [12] reported an increase in the firmness of yogurts prepared from milk proteolysed with microbial proteases.

3.10. Rheology

The storage and loss moduli (Pa) and damping factor (at 1.5 Hz) of NEPSY and EPSY as obtained from their frequency sweeps are given in Table V. In general, the loss modulus (G″) was lower than the storage modulus (G′) for both the yogurts throughout the storage period indicating that the yogurts exhibited characteristics typical of a weak viscoelastic gel. The values of both G″ and G′ of EPSY were lower ($P < 0.05$) than those of NEPSY during storage. The lower G′ values indicate that EPS-containing yogurt gels had solid-like character, as was also observed by Purwandari et al. [31]. However, the damping factor (tan δ) was similar ($P > 0.05$) for both the types of yogurts throughout the storage period, except at d 7 and 21, when EPSY showed a higher ($P < 0.05$) value than NEPSY. During storage, the tan δ values of both the yogurts decreased with time, being significant ($P < 0.05$) at d 7 and 14 for EPSY while for NEPSY it was at d 7 only. Decreases in tan δ values indicate that rearrangement of yogurt structures occurs during storage to a more solid-like gel [9]. This is consistent with the increase in G′ as shown in Table V. Lucey [23] suggested that extensive particle rearrangement during

Table V. Changes in viscoelastic properties (at 1.5 Hz) during storage for 28 days of control (NEPSY) and experimental (EPSY) low-fat yogurts at 4 °C.

Type of yogurt	Period of storage (day)				
	1	7	14	21	28
Storage modulus (Pa)					
NEPSY	366.67^{bA}	452.00^{bA}	677.33^{bA}	813.67^{bA}	1429.83^{aA}
EPSY	179.33^{bB}	242.17^{abB}	220.50^{abB}	317.67^{abB}	382.17^{aB}
SEM			105.98		
Loss modulus (Pa)					
NEPSY	100.12^{bA}	119.77^{bA}	176.48^{bA}	210.65^{bA}	369.67^{aA}
EPSY	49.45^{bB}	64.63^{abB}	57.72^{abB}	82.70^{abB}	99.23^{aB}
SEM			27.49		
Damping factor (tan δ)					
NEPSY	0.274^{aA}	0.265^{bB}	0.261^{bcA}	0.259^{cB}	0.259^{cA}
EPSY	0.277^{aA}	0.268^{bA}	0.262^{cA}	0.261^{cA}	0.259^{cA}
SEM			0.001		

Values are the mean of six observations.
NEPSY, control yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and non-EPS producing strain of *S. thermophilus*; EPSY, yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and EPS-producing strain of *S. thermophilus*.
^{abcd} Mean values in the same row with different alphabets are significantly different for each type of yogurt.
^{AB} Mean values in the same column with different alphabets are significantly different for a particular day of storage for each parameter.

structure formation results in dense clusters of aggregates and lower G′ values. Similar observations regarding G″, G′ and tan δ have been made by other researchers [9, 18, 25]. The viscosity of the stored yogurt samples decreased with increasing shear rate (data not shown) confirming their non-Newtonian behaviour. The viscosity of EPSY was, in general, lower than that of NEPSY. Studies on the effect of EPS on the viscosity of yogurt have shown controversial results including an improvement in viscosity [6, 16], a decrease in viscosity [18] and no correlation between EPS production and viscosity [32]. Differences in viscosity of various strains may be due to the differences in the intrinsic viscosity of the EPS produced [29] or differences in EPS localization within the gel [33].

The upward flow curves (shear stress) of the two types of low-fat yogurts were fitted to the Herschel-Bulkley model to study the changes in their flow behaviour during storage. The values of yield stress (σ_0), consistency index (k) and flow behaviour index (n) thus obtained are presented in Table VI. The increase in the values of yield stress (Pa) during storage for both NEPSY and EPSY was not significant. Also, the yield stress of EPSY was lower ($P < 0.05$) than that of NEPSY during storage, except at d 1 when the values were similar ($P > 0.05$). This is in concurrence with the firmness of the yogurts (Tab. IV) as indicated by the strong correlation obtained between the firmness and yield stress values of NEPSY ($r = 0.83$) and EPSY ($r = 0.86$). Polysaccharides, by non-specific entanglements, are believed to prevent the interactions of dispersed particles, which explained the lower yield stress and firmness of EPSY. Hassan et al. [17] have reported that EPS^+ samples broke down more easily than EPS^- samples because of

Table VI. Flow behaviour (predicted by the Herschel-Bulkley model) and hysteresis loop area of control (NEPSY) and experimental (EPSY) low-fat yogurts during storage for 28 days at 4 °C.

Period of storage (day)	Rheological parameters									
	σ_0 (Pa)		k (Pa·s)		n		R^2		Hysteresis (Pa·s^{-1})	
	NEPSY	EPSY	NEPSY	EPSY	NEPSY	EPSY	NEPSY	EPSY	NEPSY	EPSY
1	8.00^{bA}	5.25^{bA}	2.06^{aA}	1.22^{aB}	0.62^{aB}	0.73^{bA}	0.990	0.983	260.91^{bA}	134.08^{bB}
7	11.65^{bA}	6.88^{abB}	1.40^{aA}	1.30^{aA}	0.77^{aA}	0.73^{abA}	0.990	0.985	295.41^{bA}	207.36^{abB}
14	15.28^{bA}	6.84^{abB}	2.33^{aA}	1.22^{aA}	0.65^{aA}	0.75^{abA}	0.985	0.986	486.46^{bA}	189.49^{abB}
21	18.06^{abA}	9.31^{abB}	3.18^{aA}	1.07^{aA}	0.74^{aA}	0.81^{abA}	0.983	0.985	515.27^{bA}	253.84^{abB}
28	27.84^{aA}	11.06^{aB}	4.01^{aA}	0.94^{aB}	0.66^{aB}	0.86^{aB}	0.985	0.985	897.06^{aA}	298.08^{aB}
SEM	1.96		0.52		0.05				64.52	

Values are the mean of six observations.
SEM, standard error of mean values; NEPSY, control yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and non-EPS producing strain of *S. thermophilus*; EPSY, yogurt prepared from skim milk standardized to 12% total solids containing 3% inulin and EPS-producing strain of *S. thermophilus*.
[abc] Mean values in the same row with different alphabets are significantly different for each type of yogurt.
[AB] Mean values in the same column with different alphabets are significantly different for a particular day of storage for each parameter.

the presence of fewer protein-protein interactions at the critical sites in the network to overcome. Doleyres et al. [9] also obtained lowest yield stress values for 13% (total solids) milks fermented with EPS-producing yogurt culture. However, we observed an increase in the yield stress values of EPS$^+$ yogurts in the presence of inulin as compared to those reported in the absence of inulin [28]. Thus, it appears that the presence of inulin molecules aided protein-protein interactions. The consistency index (Pa·s) of NEPSY and EPSY did not change ($P > 0.05$) during the storage period. The consistency index of EPSY was also lower than that of NEPSY throughout the storage period, being significant ($P < 0.05$) only at d 1 and 28. This is in concurrence with the lower viscosity of EPSY than NEPSY (data not shown). This, however, is contrary to the observation of higher consistency coefficients in EPS-containing yogurts made by several authors [9]. This may be due to strain variations. Sebastiani and Zelger [31] have reported that EPS from different EPS-producing strains have different viscosifying effects in yogurts. The flow behaviour index of all the yogurt samples was < 1, further confirming their non-Newtonian behaviour. Both EPSY and NEPSY maintained their flow behaviour index throughout the storage period.

The hysteresis loop area (Pa·s^{-1}) between the upward and downward curves (shear rate 0.1–100 s^{-1}), showing the thixotropic behaviour of NEPSY and EPSY (Tab. VI), indicated that EPSY did not show any change in the values throughout the storage period, while NEPSY showed continuous increase in the values, being significant at d 28. The hysteresis loop area of EPSY was lower than that of NEPSY, being significant ($P < 0.05$) throughout the storage period. This is contrary to the published reports that indicate a high degree of hysteresis in EPS-containing yogurts [1, 10]. This could be due to the differences in the types of EPS produced, which in turn is strain dependent. De Vuyst et al. [6] have reported

that EPS having lower-molecular mass showed less-pronounced thixotropic character than EPS having high-molecular mass. Hassan et al. [17] have found that incompatibility between protein particles and EPS produced by bacteria during fermentation could lead to the formation of denser and larger aggregates. Koksoy and Kilic [20] have found that thixotropy in ayran increased with reduction in particle size and higher surface charges. The changes in hysteresis loop area correlated positively with the consistency index of NEPSY ($r = 0.92$) but showed an inverse correlation in EPSY ($r = -0.81$). Variations in rheological and textural properties may arise from the differences in the produced EPS (e.g. molecular size or degree of branching) or in the way EPS is incorporated in the protein network in the yogurt gels [9]. The continuously changing balance of repulsive/attractive interactions between EPS and milk proteins over the fermentation process is believed to be an important issue for the final texture and stability of fermented milk [13].

4. CONCLUSION

The presence of EPS-producing strain of *S. thermophilus* and inulin did not affect pH and lactic acid concentration of the low-fat yogurts, but exhibited a protective effect on the survival of *Lb. delbrueckii* ssp. *bulgaricus* during storage at 4 °C. No change in the EPS content was observed during storage of the inulin containing low-fat yogurts, except for a sharp increase at d 14. The proteolytic activity of yogurt produced with EPS-producing strain of *S. thermophilus* and inulin was higher than that of the control (NEPSY) at d 1, 21 and 28, indicating a time-dependent effect of EPS. The ACE-inhibitory activity varied with the time of storage in both the types of yogurts, being higher in EPS-containing yogurt at d 28. The EPS- and inulin-containing yogurt showed better α-glu-inhibitory activity as compared to the control. Yogurts made with EPS-producing strain of *S. thermophilus* and inulin appeared to have a stable and compact structure as indicated by the reduction in appearance of spontaneous whey separation, lower firmness, G′ values and yield stress. The consistency index and hysteresis loop area were lower in the EPS- and inulin-containing yogurt.

Acknowledgements: The authors acknowledge the help provided by Dr. Todor Vasiljevic in studying rheology of yogurt and statistical analysis of the data.

REFERENCES

[1] Amatayakul T., Halmos A.L., Sherkat F., Shah N.P., Physical characteristics of yogurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios, Int. Dairy J. 16 (2006) 40–51.

[2] Bot A., Erle U., Vreeker R., Agterof W.G.M., Influence of crystallisation conditions on the large deformation rheology of inulin gels, Food Hydrocoll. 18 (2004) 556–574.

[3] Church F.C., Swaisgood H.E., Porter D.H., Catignani G.L., Spectrophotometric assay using o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins, J. Dairy Sci. 66 (1983) 1219–1227.

[4] Cushman D.W., Cheung H.S., Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung, Biochem. Pharmacol. 20 (1971) 1637–1648.

[5] Dave R.I., Shah N.P., Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, and bifidobacteria, J. Dairy Sci. 79 (1996) 1529–1536.

[6] De Vuyst L., Zamfir M., Mozzi F., Adriany T., Marshall V., Degeest B., Vaningelgem F., Exopolysaccharide-producing *Streptococcus thermophilus* strains as functional starter cultures in the production of fermented milks, Int. Dairy J. 13 (2003) 707–717.

[7] Degeest B., Mozzi F., De Vuyst L., Effect of medium composition and temperature and

pH changes on the exopolysaccharide yields and stability during *Streptococcus thermophilus* LY03 fermentation, J. Food Microbiol. 79 (2002) 161–174.

[8] Dello Staffolo M., Bertola N., Martino M., Bevilacqua A., Influence of dietary fiber addition on sensory and rheological properties of yogurt, Int. Dairy J. 14 (2004) 263–268.

[9] Doleyres Y., Schaub L., Lacroix C., Comparison of the functionality of exopolysaccharides produced in situ or added as bioingredients on yogurt properties, J. Dairy Sci. 88 (2005) 4146–4156.

[10] Folkenberg D.M., Dejmek P., Skriver A., Guldager H.S., Ipsen R., Sensory and rheological screening of exopolysaccharide producing strains of bacterial yogurt cultures, Int. Dairy J. 16 (2006) 111–118.

[11] Fuglsang A., Rattray F.P., Nilsson D., Nyborg N.C.B., Lactic acid bacteria: inhibition of angiotensin converting enzyme in vitro and in vivo, Antonie van Leeuwenhoek 83 (2003) 27–34.

[12] Gassem M.A., Frank J.F., Physical properties of yogurt made from milk treated with proteolytic enzymes, J. Dairy Sci. 74 (1991) 1503–1511.

[13] Girard M., Schaffer-Lequart C., Attractive interactions between selected anionic exopolysaccharides and milk proteins, Food Hydrocoll. 22 (2008) 1425–1434.

[14] Gobetti M., Minervini F., Grizzello C., Angiotensin converting enzyme-inhibitory and antimicrobial peptides, Int. Dairy J. 14 (2004) 1075–1080.

[15] Guven M., Yasar K., Karaca O.B., Hayaloglu A.A., The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture, Int. J. Dairy Technol. 58 (2005) 180–184.

[16] Guzel-Seydim Z.B., Sezgin E., Seydim A.C., Influences of exopolysaccharide producing cultures on the quality of plain set type yogurt, Food Control 16 (2005) 205–209.

[17] Hassan A.N., Frank J.F., Schmidt K.A., Shalabi S.I., Formation of yogurt microstructure and three-dimensional visualization as determined by confocal scanning laser microscopy, J. Dairy Sci. 78 (1995) 2629–2636.

[18] Hess S.J., Roberts R.F., Ziegler G.R., Rheological properties of nonfat yogurt stabilized using *Lactobacillus delbrueckii* ssp. *bulgaricus* producing exopolysaccharide or using commercial stabilizer systems, J. Dairy Sci. 80 (1997) 252–263.

[19] Kip P., Meyer D., Jellema R.H., Inulins improve sensoric and textural properties of low-fat yogurts, Int. Dairy J. 16 (2006) 1098–1103.

[20] Koksoy A., Kilic M., Use of hydrocolloids in textural stabilization of a yogurt drink, ayran, Food Hydrocoll. 18 (2004) 593–600.

[21] López-Fandiño R., Otte J., van Camp J., Physiological, chemical and technological aspects of milk-protein derived peptides with antihypertensive and ACE-inhibitory activity, Int. Dairy J. 16 (2006) 1277–1293.

[22] Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., Protein measurement with the Folin-Phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[23] Lucey J.A., Cultured dairy products: an overview of their gelation and texture properties, Int. J. Dairy Technol. 57 (2004) 77–84.

[24] Lucey J.A., Tamehana M., Singh H., Munro P.A., A comparison of the formation, theological properties and microstructure of acid skim milk gels made with bacterial cultures or glucono-δ-lactone, Food Res. Int. 31 (1998) 147–155.

[25] Purwandari U., Shah N.P., Vasiljevic T., Effects of exopolysaccharide-producing strains of *Streptococcus thermophilus* on technological and rheological properties of set-type yogurt, Int. Dairy J. 17 (2007) 1344–1352.

[26] Ramchandran L., Shah N.P., Proteolytic profiles, and angiotensin-I converting enzyme and α-glucosidase inhibitory activities of selected lactic acid bacteria, J. Food Sci. 73 (2008) M75–M81.

[27] Ramchandran L., Shah N.P., Growth, proteolytic and ACE–I activities of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* and rheological properties of low fat yogurt as influenced by the addition of Raftiline HP®, J. Food Sci. 73 (2008) M368–M374.

[28] Ramchandran L., Shah N.P., Effect of EPS on the proteolytic and ACE-inhibitory activities and textural and rheological properties of low-fat yogurt during refrigerated storage, J. Dairy Sci. 92 (2009) 895–906.

[29] Ruas-Madiedo P., Hugenholtz J., Zoon P., An overview of the functionality of exopolysaccharides produced by lactic acid bacteria, Int. Dairy J. 12 (2002) 163–171.

[30] SAS, SAS/STAT Software, Changes and enhancement through release, SAS Institute Inc., Cary, USA, 1996.

[31] Sebastiani H., Zelger G., Texture formation by thermophilic lactic acid bacteria, Milchwissenschaft 53 (1998) 15–20.

[32] Shihata A., Shah N.P., Influence of addition of proteolytic strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* to commercial ABT starter cultures on texture of yogurt, exopolysaccharide production and survival of bacteria, Int. Dairy J. 12 (2002) 765–772.

[33] Sodini I., Remeuf F., Haddad S., Corrieu G., The relative effect of milk base starter, and process on yogurt texture: a review, Crit. Rev. Food Sci. Technol. 44 (2004) 113–137.

[34] Tinson W., Broome M.C., Hillier A.J., Jago G.R., Metabolism of *Streptococcus thermophilus*. 2. Production of CO_2 and NH_3 from urea, Aust. J. Dairy Technol. 37 (1982) 14–16.

[35] Zhang J.-F., Zheng Y.-G., Shen Y.-C., Inhibitory effect of valienamine on the enzymatic activity of honeybee (*Apis cerana* Fabr.) α-glucosidase, Pest. Biochem. Physiol. 87 (2007) 73–77.

[36] Zisu B., Shah N.P., Effects of pH, temperature, supplementation with whey protein concentrate and adjunct cultures on the production of exopolysaccharides by *Streptococcus thermophilus* 1275, J. Dairy Sci. 86 (2003) 3405–3415.