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Screening of lactic acid bacteria for highly producing extra and intracellular folate and their potential use in production of bio-enriched yogurt

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ABSTRACT

In this study, firstly, Microbial Assay (MA) was performed to evaluate the levels of intra and extracellular folate (Quatro folate) produced by 43 lactic acid bacteria strains isolated from traditional Iranian dairy products. The results obtained from strain screening showed that two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus*, two strains of *Streptococcus thermophilus*, two strains of *Lactobacillus delbrueckii* subsp. *lactis* and one strain of *Lactobacillus helveticus* are highly folate producing. However, two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* produced higher amounts of folate (133.23 μg/mL and 102 μg/mL, respectively). In the second phase, eight yogurt samples were produced by using the two highest folate producing strains and compared with the control sample. The folate production in yogurt samples showed that Y8 sample has the highest amount of folate (117.93, 38.81 μg/mL determined by microbial and HPLC assay methods, respectively). It was found that the microbial assay method has the ability to measure different forms of folate derivatives compared to the HPLC method. The results exhibited that the isolates examined in this study showed the ability to produce extracellular folate at higher levels and can be used as bio-enrichment cultures to produce functional foods.

1. Introduction

Folate is one of the essential micronutrients in the metabolism of living cells. This water-soluble vitamin is from the family of B group vitamins, which includes vitamins such as thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), pantothenic acid (B5), biotin (B7 or H), folate (B9- B11 or M) and cobalamin (B12). Each of these vitamins are chemically different and cooperate with other vitamins in order to maintain their homeostasis; because they have decisive functions in metabolic processes such as energy production and red blood cell production (Fishman et al., 2000; Hamzehlou et al., 2018; Le Blanc et al., 2011). The human body is not able to synthesize B group vitamins including folate, so humans must supply it through external sources. Among foods, vegetables are known as the main source of folate supply in the human diet (Malin, 1977). Although this group of vitamins are present in many foods; but it is possible that these vitamins are easily lost due to heating or processing of foods and cause a sharp decrease in the level of this vitamin in the body; this is the main factor for deficiency of this category of vitamins in many parts of the world (Iyer & Tomar, 2009). Man cannot live without folate; because this type of vitamin B plays an important role in the basic functions of cell metabolism, such as: DNA replication, repair and methylation, and synthesis of nucleotides, vitamins and some amino acids. The health of multicellular organisms like human starts at the level of individual cells, so that if our cells are healthy, we are also healthy. Healthy cells, in turn, depend on the continuous and flawless replication of our DNA. DNA can also be seriously damaged by the attack of free radicals; therefore, it is crucial to protect the human genome by antioxidant compounds (Sybesma, 2003; Le Blanc et al., 2007). Due to its antioxidant properties, folate can protect the human genome from the attack of free radicals, and as a result, it plays an important role in DNA repair and, following that, transcription mechanisms (Duthie et al., 2002). Folate deficiency associated with Alzheimer's disease (Luchsinger et al., 2007; Morris, Evans, Bienias et al., 2005), as well as other diseases such as coronary heart disease (Lee J et al., 2015; Danesh & Lewington, 1998; Kelly, Anne, & FCCP, 2010), osteoporosis (Lee Blanc et al., 2007; Baines et al., 2007), increased risk of breast cancer (Charles et al., 2004; Tjønneland et al., 2006) and colorectal cancer (Choi & Mason, 2002; Giovannucci et al., 1998; Gordon, 1999; Leahy et al., 2005; LeBlanc et al., 2007; van Guelpen et al., 2006), poor cognitive performance (Durga et al., 2007), hearing loss (LeBlanc et al., 2011; Dobie, 2007) and Neural tube defects (Sarma et al., 1995; Czeizel et al., 2013, Czeizel and Dudas, 1992; MRC,

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1991). Oral folates mainly folic acid, is currently available as supplemental form. Food folates are in the tetrahydrofolate (THF) form with additional glutamate residues making them polyglutamates (Gregory, 2001). Most naturally occurring folates are pteroyl glutamates that have 2 to 7 glutamates residues each linked by amide bonds through the Y-carboxyl glutamate. Pteroyl polyglutamates with 11 glutamic acid residues exist naturally. The main folates inside the cells are pteroyl pentaglutamates, while the main and significant folates outside the cells are pteroyl monoglutamates (McNulty & Pentieva, 2004). Folic acid is a synthesized form of folate, which is commonly used as food enrichment and nutritional supplements (Hanson et al., 2000). Lactic acid bacteria are a group of microorganisms that usually found in a wide range of environmental habitats. These environments include: the digestive tract, the urinary tract of mammals, and foods, especially milk and its products (Axelsson, 2004). Some lactic acid bacteria that are used as industrial starters; such as *Lactococcus lactis* and *Streptococcus thermophilus*, have been reported that they are able to synthesize folate during the fermentation process (Laino et al., 2013). The food industry must now take the next step to use this information to select folate-producing strains as part of their primary cultures to produce fermented products with high amounts of this essential vitamin. The objective of this study was to evaluate the ability of 43 lactic acid bacteria isolated from traditional Iranian dairy products to produce extra and intracellular folate. The best two of folate producing strains were then tested in experimental yogurt production for the development of folate bio-enriched yogurt.

2. Materials and methods

2.1. Microbial strains

In this research, a total of 43 strains of lactic acid bacteria isolated from traditional yogurt of Khorasan nomads, Khorasan Masske butter and traditional kishk, whose technological characteristics were previously investigated (12 strains of Streptococcus thermophilus, 15 strains of *Lactobacillus delbrueckii* subsp. *bulgaricus*, 13 strains of *Lactobacillus delbrueckii* subsp. lactis, 3 strains of Lactobacillus helveticus) were assayed for folate production. Microorganisms examined in this study were obtained from the microbial bank of Department of Food Science and Technology, Ferdowsi University of Mashhad (Table 1) (Ghiamati et al., 2016; Haji Mohammadi Farimani, 2014; Haji Mohammadi Farimani et al., 2016; Vosough et al., 2022).

2.2. Activation of isolates

The strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus helveticus* were first grown on related culture media (M17 or MRS broth) for enrichment. The enriched bacteria were purified using Streak plate on M17 or MRS agar and incubated at 37 and 42 °C respectively for 24–48 h. Stock culture was then prepared and stored at −20 °C until use.

2.3. Selection of extracellular folate-producing strains

To select folate-producing lactic acid bacteria, all isolates were tested under the same conditions for extracellular and intracellular folate in the medium according to the method of Albano et al. (2020). Forty-three strains examined in this study were tested using microbiological assay method. The examined strains, which were kept at −20 °C, were used to inoculate fresh MRS broth and were then incubated at 37 °C for 24 h. After growth, each strain was centrifuged at 3287×*g* for 5 min, then the supernatant was collected and stored at −20 °C for folate determination. The concentration of folate in the culture supernatant of each isolate was determined in triplicate using the activated reference strain (L. casei subsp. rhamnosus NCIMB 10463) in FACM medium.

Table 1
Lactic acid bacteria isolates (Lactobacillus and Streptococcus strains).

Strains	Isolation number	Source
Lactobacillus delbruckii subsp. *bulgaricus*	55	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	52	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	26	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	56	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	22	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	85	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	29	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	16	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	25	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	27	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	18	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	50	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	54	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	23	Nohur Y
Lactobacillus delbruckii subsp. *bulgaricus*	49	Nohur Y
Lactobacillus delbruckii subsp. *lactis*	63	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	73–1	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	69	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	73	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	66	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	65	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	68	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	71	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	62	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	64	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	67	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	73–2	Tomagh Y
Lactobacillus delbruckii subsp. *lactis*	75	Tomagh Y
Lactobacillus helveticus	82	Hamza-Canelo Y
Lactobacillus helveticus	86	Hamza-Canelo Y
Lactobacillus helveticus	87	Hamza-Canelo Y
Streptococcus thermophilus	79	Hamza-Canelo Y
Streptococcus thermophilus	Y106	Masske B
Streptococcus thermophilus	Y19	Masske B
Streptococcus thermophilus	Y109	Masske B
Streptococcus thermophilus	K72	Traditional K
Streptococcus thermophilus	K55	Traditional K
Streptococcus thermophilus	Y75	Masske B
Streptococcus thermophilus	R57	Traditional K
Streptococcus thermophilus	R34	Traditional K
Streptococcus thermophilus	K73	Traditional K
Streptococcus thermophilus	B170	Masske B
Streptococcus thermophilus	K47	Traditional K

Y: Yogurt; B: Butter; K: Kishk.

2.4. Selection of intracellular folate-producing strains

In order to extract intracellular folate, 1 ml/100 mL of each strain was inoculated in broth culture medium (7.5 g/100 ml) related to the studied strains (MRS or M17) and incubated under optimal conditions. After growth, each strain was centrifuged at 12,000×*g*, for 10 min. The supernatant was then diluted (1:1) with phosphate buffer 0.1 (pH 7.2) containing sodium ascorbate 0.1 g/100 mL and stored at −20 °C to measure the amount of extracellular folate produced by each strain. The cells were then suspended in the same buffer (5 ml) and 100 mg/mL lysozyme was added and incubated for 5 min at 37 °C. The samples (cells and supernatants) were then heated for 5 min at 100 °C in order to release folate from cells and folate-bound proteins. Fully hydrolyzed polyglutamates into monomeric form was achieved by incubation of each specimen with pancreatic enzyme for 2 h at 37 °C, as the microbial assay has sensitivity to longer-chain polyglutamylfolate (Iyer, Tomar, Kapila, Mani, & Singh, 2010). The samples were then heated for 30 min at 95 °C in water bath and cooled to 30 °C. After cooling, aliquot of 1 mL of each sample was transported to sterile tube and centrifuged for 5 min at 8000×*g*, the supernatant was then separated and microbial assay was performed (Nor et al., 2010; Puwastien et al., 2005; Sybesma et al., 2003).

2.5. Measurement of folate (extracellular and intracellular) using microbial assay method

Folate contents were estimated by MA using *L. casei* subsp. *rhamnosus* NCIMB 10463 as reference strain (Iyer, Tomar, Kapila, Mani, & Singh, 2010). Each examined strain, which was stored at −20 °C, was then activated in MRS broth at 37 °C for 24 h. After growth, 1 mL of the culture medium was centrifuged and the pellet was washed 3 times with saline solution. The resulting sediment was re-suspended in the original volume to inoculate fresh FACM medium at 2 ml/100 mL and incubated at 37 °C for 24 h. Next, 1000 μL of fresh FACM primary culture containing 20 μL of chloramphenicol were added to each well of 96-well micro titer plate, 80 μL of *Lactobacillus rhamnosus* grown in FACM medium was then inoculated to the wells and finally 900 μL of the supernatant were added. Bacterial samples grown in FACM medium were added to each well and mixed. After the incubation period (48 h at 37 °C), the cell turbidity of each sample was measured with an ELISA reader (micro plate reader model Elx800, Biotek company), at OD580 nm. The folate concentration of samples was determined by comparing the OD obtained for the examined samples to that of plotted standard curve (Laino et al., 2013; Iyer et al., 2010).

The positive control was prepared according to the steps mentioned in the above section, but instead of using the supernatant of bacteria, Quatro folate (5-methyltetrahydrofolate) (Ashbal Chemical Company., Iran) was added.

2.6. Standard curve for quantitative calculation of extracellular and intracellular folate based on microbial assay method

Based on different concentrations of Quatro folate (5-methyltetrahydrofolate), the growth of folate consuming bacteria (*Lactobacillus rhamnosus* (ATCC7469)) was obtained based on the microbial assay method. The amount of extracellular folate (Quatro folate) in the yogurt samples was calculated based on the resulting equation (Fig. 1).

2.7. Evaluation of exopolysaccharide production by selected strains for yogurt production

It is believed that yogurt texture is a key attribute that determines the good quality yogurt and consumer acceptability (Iranian Standard and Industrial Research Organization, 2024). In order to identify the strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* producing exopolysaccharide, 2 g/100 mL of glucose, fructose, sucrose and lactose were added separately to the yogurt preparation mixture and kept in an incubator for 72 h (Welman & Maddox, 2003). Exopolysaccharide producing strains have a glazed (mucoid) phenotype. Also, in order to identify the strains of *Streptococcus thermophilus* producing exopolysaccharide they were cultured on milk red ruthenium (RRM) containing 0.5% yeast extract, 10% skim milk, 1% sucrose, 1.5% agar and 0.08 g/L ruthenium red. Since ruthenium red stains the bacterial cell wall, therefore, red colonies are related to strains that are unable to produce exopolysaccharide, and transparent colonies are related to strains that produce exopolysaccharide (Ricciardi et al., 2002).

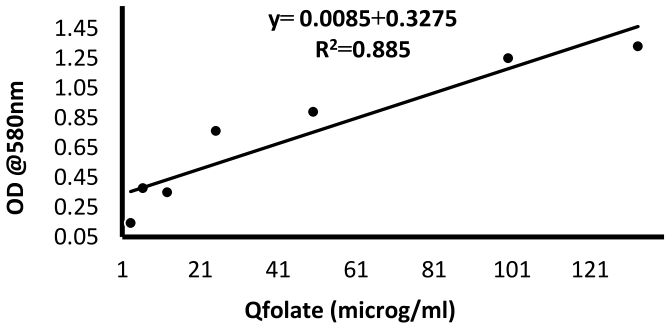

Fig. 1. Standard curve of Qfolate (5- Methyl tetrahydrofolate) by different analytical techniques Microbial assay.

2.8. Physical and chemical experiments of milk used for yogurt production

The physical and chemical characteristics of milk used for yogurt production were determined according to the standard methods.

2.9. Yogurt production using commercial starters and selected isolates

2.9.1. Preparation of commercial starter culture

The whole content of the syringe contained 18 mL of freshly activated starter mixture in milk obtained from Pegah Khorasan Company was used to inoculate 600 mL of heat-treated milk (3ml/100 ml).

2.9.2. Combining selected strains for yogurt production

To prepare yogurt samples, five strains exhibited the highest folate production (two strains of *Lactobacillus bulgaricus*, two strains of *Streptococcus thermophilus* and one strain of *Lactobacillus lactis*) were selected to be used as primary starter cultures for yogurt production. Yogurt samples Y1, Y2, Y3, Y4, Y5, Y6, Y7, Y8 were produced with an inoculation about 3% of starter cultures according to the standard and kept in an incubator at 42 °C until pH = 4.6 (Laino et al., 2013; Iyer, Tomar, Kapila, Mani, & Singh, 2010; LeBlanc, Pía Taranto, Molina, & Sesma, 2010a).

2.10. Folate determination

The determination of folate derivatives in food generally includes three steps: release of folate from the cell matrix, separation of polyglutamate into mono and D-glutamate forms, and detection of biological activity or chemical concentration of the resulting folates (Iyer et al., 2010). Among the common diagnostic methods: microbial assay method, relying on the growth of *Lactobacillus rhamnosus* (Arcot & Shrestha, 2005), and HPLC method (Iyer et al., 2010) were carried out in this study.

2.11. Preparation of yogurt samples

500 μl of yogurt samples were taken under sterile conditions and then mixed with 500 μl of protective buffer (0.1 mol/L phosphate buffer, pH = 6.8) and 1.5 g/100 mL of ascorbic acid to prevent folate oxidation. The resulting mixture (1 ml) was heated for 5 min at 100 °C in order to precipitate the proteins in the yogurt samples and release the folate derivatives bound to the proteins. It was then centrifuged at 10,000×*g* for 6 min at 4 °C, and the resulting supernatant was stored at 20 °C to measure folate (Quatro folate) (Laino et al., 2013).

2.12. Folate measurement using microbial assay method

This method is relying on the growth of *Lactobacillus rhamnosus* (Arcot & Shrestha, 2005). To determine folate concentration of each yogurt sample, the activated reference strain, *L. casei* subsp. *rhamnosus* NCIMB 10463, was used in triplicate in FACM medium. Microbial assay was performed on a 96- well micro titer plate. In each well, 1000 μl of fresh FACM starter culture containing 20 μl of chloramphenicol was inoculated, then 80 μl of *Lactobacillus rhamnosus* grown in FACM was added to the wells and finally mixed with 900 μl of sample supernatant yogurt. After 48 h of incubation at 37 °C, the turbidity of the samples was measured with an ELISA reader (microplate reader model Elx800, Biotek Company) at OD580nm. Standard curve was prepared by plotting % transmittance values against logarithmic values of Qfolate content in relevant dilutions (Keagy, 1985; Tamura et al., 1997; Iyer et al., 2010) and the percent transmittance was read at 580 nm by ELISA reader.

Folate concentration of yogurt samples was determined using the equation obtained from the standard curve (Fig. 1) (Laino et al., 2013; Iyer, Tomar, Kapila, Mani, & Singh, 2010).

2.13. Folate measurement and standard curve using HPLC method

The analytical HPLC systems (1260 Infinity II LC System), SPD-20 A UV detector, Phenomenex Luna C18 column (5 μM particle size, 100 Å pore size, 4·6 × 250 mm) and CBM20A system controller connected to a desktop PC with Chromatography CLASS VP™ software was used. In order to measure folate (Quatro folate) by HPLC method. A stock solution of folate (Quatro folate) (concentration of 1·005 mg/ml) was made in 0.1 M phosphate buffer with pH of 6.5. It was diluted further within the range of 1, 10, 100, 500, 1000 μg/mL to determine the retention time (RT) and the association between peak area and folate concentration. Followed by passing through 0.22 μM filter, 20 μl standard folate (Quatro folate) and extract (supernatant) from yogurt samples was loaded. The UV detector cell and column temperatures were kept at 40 °C. column equilibration and gradient elution were achieved with the mobile-phase consisting of acetate buffer (acetic acid 0.166 mol/l; potassium hydroxide 0.01 mol/l; pH 2·8) and acetonitrile filtered through 0.45 μM nylon filter and degassed by sonication before use. The mobile phase used in chromatography consists of acetate buffer (potassium hydroxide 0.1 mol/L; acetic acid 0.166 mol/L and pH of 2.8) and acetonitrile (Iyer et al., 2010).

To plot the quantitative standard curve of folate in yogurt samples using the HPLC method, the best fitted equation between the concentration of quatrofolate and the area under the curve (mAU*S) with different concentrations of quatrofolate (1, 10, 100, 500, 1000 μg/ml) (5-methyltetrahydrofolate) was obtained. The HPLC system was conditioned with the mobile phase until the triplicate injections of standard solution showed identities of retention time ((RT) and peak area. Detection was performed in the UV region, at 290 nm at a flow rate of 1 mL/min, using 10% acetonitrile plus 90% aqueous buffer initially, changing to 24% acetonitrile plus 76% aqueous buffer phase after 12 min. Based on the equation obtained from the standard curve, the amount of extracellular folate (quatrofolate) in yogurt samples was calculated (Fig. 2).

2.14. Physicochemical properties of yogurt

2.14.1. Titratable acidity (TA)

To measure the titratable acidity of yogurt, briefly, 9 g of the yogurt sample was mixed with 9 g of carbon dioxide-free water, then 0.5 mL of 1% phenolphthalein reagent was added and titrated with NaOH (0.1 mol/L) until a pale pink color appeared. Finally, according to the formula, the acidity of the samples was reported in terms of %lactic acid (James, 1995; Katsiari et al., 2002).

$$\frac{N*0.009*100}{M} = \text{Acidity}(\%)$$

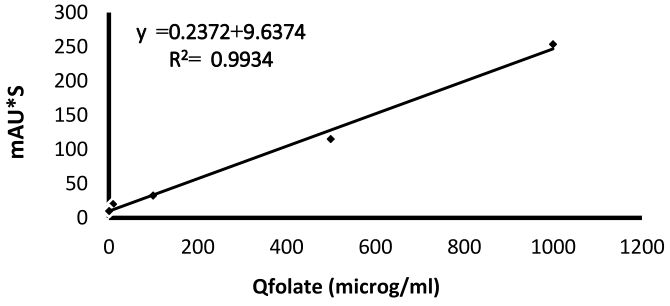

Fig. 2. Standard curve of Qfolate (5- Methyl tetrahydrofolate) by different analytical techniques High performance Liquid Chromatography.

2.14.2. pH

pH measurement was done by directly placing the pH meter electrode (model 744 made by METROHM Switzerland) in the homogenized yogurt. The pH meter was standardized using pH 4.0 and pH 7.0 reference buffer solutions (Kailasapathy, 2006; Roberfroid, 2002; Sahan, N et al., 2008).

2.14.3. Syneresis

To measure the syneresis, 30 g of yogurt sample was poured into a 50 mL falcon propylene tube and centrifuged at 1530×*g* for 20 min at 4 °C. After centrifugation, the supernatant of each sample was weighed and the results were presented using the following formula as grams of water per 100 g of yogurt sample (Nguyen et al., 2017).

$$\text{Syneresis}\ (\%) = \frac{\text{weight of whey}\ (g)}{\text{weight of yogurt}\ (g)}$$

2.15. Texture analysis

A combination of retrograde extrusion and texture profile analysis was used to determine the texture parameters (Kose et al., 2018; Kumar & Mishra, 2003). During the TPA mode test, the P/50 probe was used, the test depth was 1.5 cm, the temperature was 25 °C and the speed was 1 mm/s, for a sample of 100 g of yogurt. Hardness, adhesiveness cohesiveness and gumminess, were determined and the mentioned characteristics are defined according to the TPA standard curve (Salvador & Fiszman, 2004).

2.16. Sensory evaluation

In this experiment, 100 g of each yogurt treatment was given to the panelists. The panellists were advised to rinse their mouth with plain water before evaluating each sample. Using five-point hedonic method (5 excellent, 1 unacceptable) characteristics such as: color, odor, appearance, flavor, texture, and overall acceptance, which are considered as important criteria to verify the general acceptability of yogurt scored by panelists were evaluated (Huang et al., 2022; Pachekrepapol et al., 2021). The sensory characteristics were evaluated by a panel consisting of 10 members (Tekinşen and Keleş,1994. Meilgaard, M.C et al., 1999). Sensory evaluation carried out considering ethical aspects of using human subjects. Personal data were treated and managed based on the European regulation (EU) 2016/679 on the protection of natural persons regarding the processing of personal data and on the free movement of such data (Regulation, 2016).

2.16.1. Statistical analysis

Results were obtained from three independent experiments and each data point was measured in duplicate. Data are presented as mean ± standard deviations (SD). Statistical analyses were performed with Minitab21 v21.4.2 (Proprietary software. Pennsylvania, USA) using ANOVA general linear models followed by Tukey's post hoc test, and $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Results

3.1.1. Intracellular and extracellular folate production by LAB strains

The folate concentrations of the strains incubated at 37 °C are shown in Table 2. The highest level of folate obtained in this study was 133.23 μg/mL (Lb.25). The results showed that among the 15 isolates of *Lactobacillus delbrueckii* subsp. *bulgaricus*, two isolates coded 25 and 26 had the highest folate levels. Furthermore, the screening of 13 *Lactobacillus delbrueckii* subsp. *lactis*, showed that strain No. 73 produced highest folate than other examined isolates. The folate production

Table 2
Intracellular and extracellular folate production by Lactobacillus strains and Streptococcus strains (microbial assay method).

Bacteria	Strain (Code)	Intracellular folate (μg/mL)	Extracellular folate (μg/mL)
Lactobacillus delbrueckii subsp. *bulgaricus*	55	0^{d}	89.99 ± 0.45^{c}
Lactobacillus delbrueckii subsp. *bulgaricus*	52	0^{d}	71.76 ± 2.02^{d}
Lactobacillus delbrueckii subsp. *bulgaricus*	26	35.87 ± 0.87^{b}	102.34 ± 5.53^{b}
Lactobacillus delbrueckii subsp. *bulgaricus*	56	0^{d}	49.70 ± 0^{ef}
Lactobacillus delbrueckii subsp. *bulgaricus*	22	0^{d}	8.52 ± 0^{h}
Lactobacillus delbrueckii subsp. *bulgaricus*	85	0^{d}	68.23 ± 4.10^{d}
Lactobacillus delbrueckii subsp. *bulgaricus*	29	0^{d}	3.23 ± 0.52^{h}
Lactobacillus delbrueckii subsp. *bulgaricus*	16	0^{d}	53.52 ± 0.87^{e}
Lactobacillus delbrueckii subsp. *bulgaricus*	25	0^{d}	133.23 ± 0.74^{a}
Lactobacillus delbrueckii subsp. *bulgaricus*	27	0^{d}	38.81 ± 3.43^{g}
Lactobacillus delbrueckii subsp. *bulgaricus*	18	0^{d}	8.81 ± 0.45^{h}
Lactobacillus delbrueckii subsp. *bulgaricus*	50	46.19 ± 1.32^{a}	91.46 ± 1.57^{c}
Lactobacillus delbrueckii subsp. *bulgaricus*	54	6.76 ± 0.52^{c}	66.76 ± 6.84^{d}
Lactobacillus delbrueckii subsp. *bulgaricus*	23	0^{d}	43.82 ± 0^{fg}
Lactobacillus delbrueckii subsp. *bulgaricus*	49	0^{d}	75.87 ± 3.96^{d}
Lactobacillus delbrueckii subsp. *lactis*	63	0^{d}	33.53 ± 1.56^{ab}
Lactobacillus delbrueckii subsp. *lactis*	73–1	0^{d}	34.40 ± 23.10^{ab}
Lactobacillus delbrueckii subsp. *lactis*	69	0^{d}	37.95 ± 4.31^{ab}
Lactobacillus delbrueckii subsp. *lactis*	73	0^{d}	39.81 ± 0.94^{a}
Lactobacillus delbrueckii subsp. *lactis*	66	58.23 ± 0.87^{b}	10.43 ± 3.59^{ab}
Lactobacillus delbrueckii subsp. *lactis*	65	0^{d}	3.52 ± 2.02^{b}
Lactobacillus delbrueckii subsp. *lactis*	68	64.70 ± 2.39^{a}	25.29 ± 9.09^{ab}
Lactobacillus delbrueckii subsp. *lactis*	71	0^{d}	17.64 ± 7.07^{ab}
Lactobacillus delbrueckii subsp. *lactis*	62	0^{d}	29.70 ± 2.10^{ab}
Lactobacillus delbrueckii subsp. *lactis*	64	43.23 ± 0.53^{c}	12.05 ± 3.24^{ab}
Lactobacillus delbrueckii subsp. *lactis*	67	0^{d}	9.40 ± 2.91^{ab}
Lactobacillus delbrueckii subsp. *lactis*	73–2	0^{d}	32.63 ± 4.25^{ab}
Lactobacillus delbrueckii subsp. *lactis*	75	0^{d}	39.70 ± 6.42^{a}
Lactobacillus helveticus	82	0^{d}	9.99 ± 1.34^{b}
Lactobacillus helveticus	86	66.17 ± 1.05^{b}	2.2 ± 0.68^{c}
Lactobacillus helveticus	87	66.76 ± 0.52^{a}	17.94 ± 3.72^{a}
Positive control			2.67 ± 23.9
Streptococcus thermophilus	79	0^{d}	24.99 ± 2.10^{ef}
Streptococcus thermophilus	Y106	0^{d}	14.40 ± 2.10^{gh}
Streptococcus thermophilus	Y19	0^{d}	8.52 ± 2.10^{hi}
Streptococcus thermophilus	Y109	0^{d}	61.46 ± 4.88^{c}
Streptococcus thermophilus	K72	0^{d}	28.52 ± 0^{e}
Streptococcus thermophilus	K55	0^{d}	24.11 ± 5.01^{ef}
Streptococcus thermophilus	Y75	8.23 ± 0.45^{c}	2.93 ± 0.45^{i}
Streptococcus thermophilus	R57	0^{d}	44.40 ± 1.57^{d}
Streptococcus thermophilus	R34	43.52 ± 0.45^{b}	19.70 ± 2.62^{fg}
Streptococcus thermophilus	K73	66.46 ± 0.87^{a}	90.29 ± 0.52^{a}
Streptococcus thermophilus	B170	0^{d}	6.76 ± 0.52^{hi}
Streptococcus thermophilus	K47	0^{d}	74.11 ± 1.87^{b}
Positive control			61.53 ± 9.53

Different letters indicate significant difference ($p < 0.05$).

ability of 3 isolates of *Lactobacillus helveticus* showed that isolates 87 and 86 had the highest and lowest folate levels, respectively (Table 2). Among the 12 strains of *Streptococcus thermophilus*, strains No. K73 and No. K47 produced the highest folate (Table 2).

3.1.2. Screening of exopolysaccharide producing bacteria

Among the 43 bacterial strains examined in this study, only the strains which had the ability to produce high amount of extracellular folate were chosen for exopolysaccharide production test. According to the results, only strain No.73 of *Lactobacillus delbrueckii* subsp. *lactis* was not able to produce exopolysaccharide (Table 3).

3.1.3. Physical and chemical experiments of milk used for yogurt production

The chemical and physical features of milk are presented in Table 4.

3.1.4. Combining selected strains for yogurt production

Yogurt samples were produced according to (Table 5).

3.1.5. physicochemical properties of produced yogurts

3.1.5.1. Titratable acidity. Among all prepared yogurt samples, Y5, Y6 samples had the highest titratable acidity. The lowest level of acidity was related to samples Y3, Y7, Y8 and commercial yogurt. Based on the results; there was a significant difference between the acidity of most yogurt samples with commercial yogurt ($p < 0.05$) (Table 6).

3.1.5.2. pH. The highest pH was related to sample Y3, Y7 and commercial yogurt, and the lowest pH was related to samples Y5 and Y6 ($p < 0.05$). (Table 6).

3.1.5.3. Syneresis. The Y6 yogurt sample had the highest amount of syneresis, while the lowest amount was related to Y5 and commercial yogurt samples. Based on the results obtained from this test; there was a significant difference between commercial yogurt sample and yogurt

Table 3
Exopolysaccharide production.

Bacteria	**Strain (Code)**	Exopolysaccharide production
Lactobacillus delbrueckii subsp. *bulgaricus*	25	+
	26	+
Streptococcus thermophilus	K47	+
	K73	+
Lactobacillus delbrueckii subsp. *Lactis*	73	–
	75	+

Table 4
Milk analysis.

% (by wet weight)				
protein by wet weight	Fat	Dry matter	pH	Acidity
83.28 ± 0.04	±01.3	008.0 ± 64.11	04.0 ± 82.6	±053.1

Table 5
Yogurts produced based on used bacterial strains.

Yogurt samples	Bacterial strains
Y1	St. k73*lb.25
Y2	St. k73*lb.26
Y3	St. k47*lb.25
Y4	St. k47*lb.26
Y5	St. k73*lb.26*l. lactis.75
Y6	St. k73*lb.25*l. lactis.75
Y7	St. k47*lb.26*l. lactis.75
Y8	St. k47*lb.25*l. lactis.75
Y9	Commercial yogurt

St: *Streptococcus thermophilus*, K: Kishk.

Table 6
Physical and chemical properties of produced yogurts.

Sample	Titratable acidity (% lactic acid)	pH	Syneresis (%)
Y1	1.65 ± 0.008^{bc}	4.39 ± 0^{ab}	14.17 ± 0.01^{e}
Y2	1.60 ± 0.004^{c}	4.39 ± 0.20^{ab}	15.01 ± 0.02^{b}
Y3	1.47 ± 0.008^{d}	4.43 ± 0.004^{a}	15.08 ± 0.008^{b}
Y4	1.65 ± 0.01^{bc}	4.36 ± 0^{bc}	14.84 ± 0.008^{c}
Y5	1.81 ± 0.008^{a}	4.30 ± 0.008^{c}	13.9 ± 0.008^{f}
Y6	1.70 ± 0^{b}	4.31 ± 0.004^{c}	15.19 ± 0.012^{a}
Y7	1.45± 0^{d}	43.4 ± 16.00^{a}	14.57 ± 0.008^{d}
Y8	1.41 ± 0.01^{d}	40.4 ± 0.004^{ab}	14.51 ± 0.008^{d}
Commercial yogurt	1.47 ± 0.008^{d}	4.45 ± 0.004^{a}	13.97 ± 0.008^{f}

samplesY6, Y3, Y2, Y4, Y7, Y8 and Y1 ($p < 0.05$). (Table 6).

3.1.6. Textural characteristics of produced yogurts

3.1.6.1. Hardness. The highest and lowest hardness values were related to Y4 and Y8 samples, respectively. No significant difference was observed between commercial yogurt and other samples (Table 7).

3.1.6.2. Adhesiveness. The highest stickiness was related to sample Y4 and commercial yogurt and the lowest stickiness was related to samples Y3 and Y6. In the stickiness test; there was a significant difference between Y3 and Y6 yogurt samples with commercial yogurt ($p < 0.05$). However, no significant difference was observed between other samples and commercial yogurt (Table 7).

3.1.6.3. Cohesiveness. The highest Cohesiveness was related to samples Y7 and Y8 and the lowest degree of Cohesiveness was related to sample Y2. In the Cohesiveness test; there was a significant difference between commercial yogurt sample and Y7 and Y8 yogurt samples ($p < 0.05$) (Table 7).

3.1.6.4. Gumminess. The highest gumminess corresponds to Y6 sample and the lowest amount was related to samples Y5, Y2 and commercial yogurt. In the Gumminess test; there was a significant difference between commercial yogurt samples and Y6 yogurt samples ($p < 0.05$) (Table 7).

Table 7
Textural characteristics of produced yogurts.

Sample	Hardness (N)	Adhesiveness (Nm)	Cohesiveness	Gumminess (N)
Y1	22.22 ± 0.80^{bc}	0.24 ± 0.02^{ab}	0.18 ± 0.01^{bc}	3.08 ± 0.28^{ab}
Y2	18.19 ± 0.52^{abcd}	0.28 ± 0.01^{ab}	0.12 ± 0.004^{c}	1.21 ± 0.02^{c}
Y3	21.32 ± 1.35^{abc}	0.20 ± 0.009^{b}	0.19 ± 0.004^{bc}	2.43 ± 0.11^{abc}
Y4	24.70 ± 2.44^{a}	0.39 ± 0.004^{a}	0.21 ± 0.008^{bc}	2.17 ± 0.45^{abc}
Y5	14.36 ± 1.40^{cd}	0.33 ± 0.03^{ab}	0.25± 0^{b}	1.15 ± 0.04^{c}
Y6	20.71 ± 0.47^{abc}	0.21 ± 0.004^{b}	0.17 ± 0.01^{bc}	3.35 ± 0.04^{a}
Y7	15.59 ± 0.74^{bcd}	0.25 ± 0.04^{ab}	0.38 ± 0.02^{a}	1.97 ± 0.03^{abc}
Y8	12.84 ± 0.012^{d}	0.30 ± 0.008^{ab}	0.44 ± 0.004^{a}	1.66 ± 0.31^{bc}
Commercial yogurt	18.31 ± 0.35^{abcd}	0.37 ± 0.004^{a}	0.18 ± 0.008^{bc}	1.30 ± 0.07^{c}

3.1.7. Sensory evaluation

The highly accepted samples were Y1, Y2, Y3, Y5 and Y6 and the lowest accepted sample was commercial yogurt. In general acceptance; there was significant difference between yogurt samples Y1, Y2, Y3, Y5 and Y6 with commercial yogurt ($p < 0.05$) (Table 8).

3.1.8. Measurement of folate in yogurt samples

3.1.8.1. Comparison of microbial assay and HPLC methods. Folate concentrations of different yogurt samples are shown in Table 9. Yogurt samples with the highest amount of Quatro folate measured by HPLC method were Y3, Y5 and Y8 samples (Table 9). There was a significant difference (p-value <0.05) among the different treatments of yogurt and commercial yogurt determined by microbial assay method. However, in both evaluation methods, the Y8 yogurt sample had the highest amount of extracellular folate production in comparison with other samples.

4. Discussion

Based on the results obtained in this study, it has been confirmed that certain strains of lactic acid bacteria are capable of producing folate derivatives. Therefore, not only some species of industrially important lactic acid bacteria such as *Lactobacillus lactis* and *Streptococcus thermophilus* which have been proven to produce this type of vitamin B, but also other lactic acid bacteria such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Leuconostoc lactis*, Propionibacterium, and Bifidiobacterium are also potentially capable of producing this vitamin (Crittenden et al., 2003; Gangadharan et al., 2010; Le Blanc

Table 8
General acceptance of yogurt samples.

Sample	overall acceptance
Y1	4.5 ± 0.63^{a}
Y2	4.1 ± 0.79^{a}
Y3	4 ± 0.42^{a}
Y4	3.8 ± 0.83^{ab}
Y5	4.3 ± 0.74^{a}
Y6	4.1 ± 0.66^{a}
Y7	3.8 ± 0.83^{ab}
Y8	3.9 ± 0.89^{ab}
Commercial yogurt	2.8 ± 0.57^{b}

Table 9
The amount of folate (Quatro folate) produced in yogurt samples based on microbial assay (MA) and HPLC method.

Yogurt samples	Microbial assay (μg/mL)	HPLC (μg/mL)
Y1	43.23 ± 0.52^{f}	14.19 ± 0.01^{d}
Y2	98.23 ± 0.45^{d}	32.61 ± 0.01^{b}
Y3	114.40 ± 1.28^{b}	38.30 ± 0.48^{a}
Y4	40.87 ± 1.57^{f}	15.16 ± 0.04^{d}
Y5	109.11 ± 0.52^{c}	36.68 ± 0.44^{a}
Y6	27.93 ± 0.52^{g}	9.09 ± 0.15^{e}
Y7	68.22 ± 0.45^{e}	23.38 ± 0.77^{c}
Y8	117.93 ± 1.05^{a}	38.81 ± 0.05^{a}
Commercial yogurt	17.35 ± 0.52^{h}	5.6 ± 0.28^{f}

et al., 2011; Lin & Young, 2000; Pompei et al., 2007).). In many studies, it has been proven that the ability of microbial starter cultures to produce or utilize folate is significantly different and depends on the bacterial strain (Le Blanc et al., 2010). Therefore, since folate production is a strain dependent feature, it seems necessary to screen strains to find a suitable strain capable of producing sufficient amounts of folate (Crittenden et al., 2003). *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are species of lactic acid bacteria that are used as starter cultures in the preparation of various fermented dairy products such as yogurt, and other fermented milk products. The amount of folate in yogurt depends on the strain of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* utilized as commercial starter. All examined strains of *Streptococcus thermophilus* were able to produce folate, but only two strains (K73 and K47) had the highest amount of folate production. Based on the current study, it has been found that many strains of this species (*Streptococcus thermophilus*) be able to produce folate. Many researches confirmed that some fermented dairy products, such as yogurt, contain more folate than non-fermented dairy products. It is concluded that microbial fermentation of milk, is the main source of folate derivatives production. Therefore, the production of folate derivatives by the streptococcus strains examined in this study seems reasonable, considering that the origin of all the studied bacteria is yogurt or the yogurt derivatives products (local curd and butter process) (Iyer et al., 2010). In this study, among the examined strains, the highest level of extracellular folate production was related to *Lactobacillus delbrueckii* subsp. *bulgaricus*, so that this species produced extracellular folate in greater numbers and with higher amounts than *Streptococcus thermophilus*. Among 15 strains of *Lactobacillus delbrueckii* subsp. *bulgaricus*, 2 strains (25 and 26) were able to produce extracellular folate higher than 100 μg/mL, which is a significant amount of production. Most authors claim that, *Streptococcus thermophilus* normally produce folates (Iyer, Tomar, Kapila, Mani, & Singh, 2010) whereas *Lactobacillus delbrueckii* subsp. *bulgaricus* is a folate consumer, so the selection of adequate combination of strains is essential to develop fermented foods with increased folate concentrations (Le Blanc et al., 2011; Rao et al., 1984; Kneifel et al., 1991). The results of this study are similar to the screening performed on *Lactobacillus delbrueckii* subsp. *bulgaricus* strains on fermented dairy products in Argentina, where strains of *Lactobacillus delbrueckii* subsp. were identified as folate producers (Laino et al., 2012). All 13 *Lactobacillus lactis* strains examined in this study were able to produce extracellular folate, although in low amounts, while the 3 *Lactobacillus helveticus* strains evaluated in this study were able to produce lower amounts of folate than the other strains mentioned in this study. The production of smaller amounts of extracellular folate for this bacterium is consistent with other study on *Lactobacillus helveticus* CD6 isolated from fermented milk with the ability to produce only one derivative of folic acid (Quatro folate (5-MeTHF)) (Ahire et al., 2013). Many studies reported that commercial lactic acid bacteria such as *Lactobacillus lactis* and *Streptococcus thermophilus* have the ability to synthesize folate. Also, the amount of folate in cow's milk varies from 20 to 50 mg/L (Le Blanc et al., 2007), while its concentration in yogurt may increase to values higher than 200 mg/L depending on the starter culture and storage conditions (Waters et al., 2002). Nowadays, it is known that some strains of lactic acid bacteria such as *Lactobacillus lactis* produce intracellular folate., where up to 90% of the total folate produced remains intracellularly as 5,10-methenyl-THF and possibly 10-formyl-THF derivatives (Sybesma et al., 2003). However, *Streptococcus thermophilus* ability to produce a total folate derivative intracellularly is much lower. This difference in the production of intracellular and extracellular folate derivatives can probably be explained in connection with the distribution of the polyglutamyl chain with different length in the folate derivatives produced in those two microorganisms. It is thought that one of the main functions of polyglutamyl tail in folate derivatives is to maintain folate inside the cell. In addition, in *Streptococcus thermophilus*, distribution of folate derivatives inside and outside cells is also affected by the pH. Thusly, cells grown at low pH produce more extracellular folate derivatives than cells grown at high pH. The same trend exists for intracellular folate production, so that at low intracellular pH, a higher concentration of folate derivatives will be protonated, which will increase transport across the cell membrane (Sybesma et al., 2003b). In order to confirm the effect of pH, research has also shown that in yogurts produced with strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, fermentation time (incubation) can be one of the most important factors in the production of extracellular folate derivatives (Laino et al., 2013). Maximum folate production was observed at the beginning of the stationary phase (6 h of incubation). This state refers to the physiological variables of the cell as a factor that can affect the production of metabolites (Laiño et al., 2017). Another reason that may possibly cause the production of extracellular folate derivatives by the strains studied in this research is the existence of a symbiotic relationship in the strains isolated from yogurt (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) (Reddy et al., 1976). It has already been confirmed in similar studies that *Streptococcus thermophilus* contains enzymes necessary for the production of para-amino benzoic acid and folate, so yogurt is naturally considered as a rich source of para-amino benzoic acid. The presence of these derivatives at high levels in yogurt can probably accelerate the growth and coexistence of *Lactobacillus bulgaricus* during co-cultivation with *Streptococcus thermophilus* and as a result the production of more folate derivatives (Igarashi & Kashiwagi, 2000; Van de Guchte et al., 2006). *Streptococcus thermophilus* has also been reported to have a complete pathway for folate biosynthesis from the precursor GTP or PABA (Van de Guchte et al., 2006). It has been shown in various researches that the correct combination of strains in optimal growth conditions for fermentation can provide a product with sufficient and high amount of folate (Laino et al., 2012). This fact can be considered a reason for the high amount of folate produced in Y8 yogurt sample determined by both methods of measuring folate (Quatro folate). However, regarding the pH of manufactured yogurt samples, it cannot be accurately correlated the decrease in pH and acidification of yogurt, with the increase of folate in the samples. Sample Y8 (pH = 4.40), as a sample of yogurt, which has a lower pH than commercial yogurt, produced the highest level of folate than commercial yogurt (pH = 4.45) (Laino et al., 2012). The level of folate production in the mentioned sample is much higher than commercial yogurt (Laino et al., 2012). It can also be stated that the growth of *Streptococcus thermophilus* during the fermentation process in yogurt production can have a direct relationship with folate production; so that the amount of folate increases gradually with the growth of this bacterium. However, based on the results obtained in this study, it has been determined that folate production is a trait dependent on the strain and even the growth of the folate-producing strain, but the production of this metabolite may not always have a direct relationship with the growth of the folate-producing strain. (Laino et al., 2012). Also, the increasing trend of folate production determined by microbial assay method can be seen in samples coded Y6, Y4, Y1, Y7, Y2, Y5, Y3 and Y8 respectively. While such trend is slightly different for folate determined by the HPLC method. The lowest amount of folate production is related the

commercial yogurt (control). The results obtained from this study showed that Y8 yogurt sample produced maximum folate at 42 °C in the first 6 h of fermentation, because based on the previous study, it was determined that the temperature of incubator affects the amount of folate production in the sample (Laino et al., 2012). Regarding the measurement of folate (Quatro folate) with the microbial assay method, it is worth to mention that although this method is sensitive and somewhat time-consuming, it is a repeatable method with high efficiency. In addition, the cost of performing this assay is relatively low and all forms of folate (mono to polyglutamate) are measured even at the nanogram level (Forssen et al., 2000; Pffeiffer et al., 1997; Arcot & Shrestha, 2005; Iyer et al., 2010). The results obtained from this study showed that the microbial assay method is not only capable of measuring extracellular and intracellular folate (total folate), but also has the ability to measure folic acid in samples. The measurement of folate derivatives by the HPLC method showed lower amounts of folate compared to the microbial assay method. The most important reason for determining lower values in this method might be attributed to the limitation of the UV detector to identify all folate derivatives (Horn, 1997; Ginting and Arcot, 2004; Kariluoto et al., 2001; Arcot & Shrestha, 2005; Mehrabi, Shoja Al-Sadati, Mousavi, & Hashemi, 2015). Nutritionists have stated that food can be considered a good source of folate when it provides at least 10–20% of the daily requirement for folate (Ohio State University, 2005). Based on this, commercial or even traditional yogurts cannot be considered as a good source of folate suppliers if folate-producing strains are not utilized in their preparation.

Regarding the evaluation of technological properties of yogurt samples, since *Streptococcus thermophilus* has the ability to produce more lactic acid than *Lactobacillus bulgaricus*, Therefore, yogurt with higher acidity can indicate primary inoculation with a higher proportion of *Streptococcus thermophilus*. (70% of lactic acid produced in yogurt is related to *Streptococcus thermophilus* bacteria (Tamime & Robinso, 1999). Based on the results, it can be stated that Y5, Y6, Y4, Y1 and Y2 yogurt samples with the highest level of acidity among the yogurt samples compared to commercial yogurt, are probably affected by the different growth of starters, especially the growth of *Streptococcus thermophilus* in the production of mentioned samples. (Sah et al., 2016). The higher the growth rate of *Streptococcus thermophilus* strain in the yogurt, the more acidic metabolites are produced, and finally, with the increase in yogurt acidity, its pH also decreases (Tamime & Robinso, 1999). Based on the results, yogurt samples Y6 and Y5 have a lower pH than other samples, especially commercial yogurt. This phenomenon can be explained by the different activity and growth of indigenous starters compared to commercial starters (Sah et al., 2016; Tarakçi & Küçüköner, 2003). The results of this study are inconsistent with previous research conducted which shown commercial yogurt samples compared to yogurt samples produced with native starters and probiotics; had a lower pH, (Kailasapathy, 2006).

Exopolysaccharide production by microorganisms used in yogurt production plays an important role in improving the textural properties and thus reducing the syneresis of the produced yogurts. Since all the strains used in the production of yogurt samples in this study have the ability to produce exopolysaccharide, therefore, the produced yogurts have a relatively good firmness, and the firmness in yogurt is probably directly related to the increase in acidity and the decrease in pH (Mokoonlall et al., 2016). In the occurrence of syneresis phenomenon in yogurt, it can be said that the amount of syneresis in produced yogurt samples is related to microbial growth and acidity change (Mokoonlall et al., 2016). This relationship can be seen in Y5 yogurt sample, which has the lowest amount of syneresis compared to other yogurts produced. This phenomenon is probably due to the low acidity and high pH of Y5 yogurt compared to other samples. However, this relationship was not visible in the case of commercially produced yogurt, despite the fact that it has the lowest % syneresis after yogurt sample Y5. On the other hand, the difference in the growth pattern between the commercial strains used in yogurt production compared to other locally isolated strains can be another reason for the less syneresis of commercial yogurt. Microbial exopolysaccharides as a stabilizer play an essential role in improving the gel structure in yogurt. However, the excessive production of microbial exopolysaccharide can have a positive effect on the stability of yogurt and some of its textural properties such as gumminess, adhesiveness, and cohesiveness (Shihata & Shah, 2002; Kailasapathy, 2006). In this study, samples Y3, Y6 and Y7, Y8 and Y1, Y6; respectively, had a significant difference ($p < 0.05$) in adhesiveness, cohesiveness and gumminess with commercial yogurt. This can probably be caused by the production of more microbial exopolysaccharide in the mentioned yogurt samples. Since the amount of exopolysaccharide production in yogurts produced in this research compared with commercial and locally isolated strains has not been investigated, therefore it is not possible to make a precise conclusion in this case. The results obtained from the sensory test showed that the yogurts produced with locally isolated strains have more general acceptance and favorability than commercial yogurts. Yogurt samples produced with locally isolated strains have more acidity than commercial yogurt samples. According to other studies, the lactic acid produced in yogurt intensifies the aromatic and nutty flavors in the product, as well as the favorable proteolytic and lipolytic activity of yogurt starters play a significant role in creating flavor compounds (Tamime & Robinso, 1999). Therefore, in addition to producing more lactic acid, probably the proteolytic and lipolytic activity of native strains were also higher than commercial strains.

5. Conclusion

In this study, two strains of *Streptococcus thermophilus* (K73 and K47), two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* (25 and 26) and yogurt sample Y8 (St. k47*lb.25*l. lactis.75) showed the highest amount of extracellular folate production. The results of this research showed that the production of extracellular folate in lactic acid bacteria is strain dependent, which can be largely related to the origin of the isolate. In this study, most strains of *Streptococcus thermophilus* and a small number of *Lactobacillus bulgaricus* strains had the ability to produce extracellular folate compared to other investigated strains. It is possible to produce fermented foods, including dairy products with high level of folate using LAB strains utilized in this study. The analysis of the methods used to determine folate in this study showed that the HPLC method has high sensitivity. However, only some specific derivatives of folate can be measured with this method, but the microbial assay method can be used to estimate the total folate. In general, many of the LAB strains examined in this study have the potential to be used in the production of folate enrichment fermented products. This can be an efficient tool to prevent folate absorption deficiency in countries where there are no folate fortification programs.

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CRediT authorship contribution statement

Fatemeh Hosseini: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Mohammad Bagher Habibi Najafi:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Mohammad Reza Edalatian Dovom:** Writing – review & editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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