

Improved quality of traditional East European soured milk produced with wild-type *Lactococcus lactis* and fortified with local dill (*Anethum graveolens*)

Justina Milerienė¹, Kristina Kondrotienė¹, Loreta Šernienė¹, Vaida Andrulevičiūtė²,
Neringa Kašėtienė¹, Dalia Sekmokienė¹, Lina Laučienė¹, Mindaugas Malakauskas¹

¹Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

²Department of Biochemistry, Medicine Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Keywords: antioxidant properties, fermentation, wild-type *Lactococcus lactis*, dill extract, soured milk.

Abstract. Recently, main product development and research in the dairy industry have been targeted to the enrichment of products with local natural preservatives and wild-type starters leading to enhanced product safety and sustainability. The objective of the present study was to enhance the safety, quality, sensory acceptability, and sustainability of traditional East European soured milk by adding wild-type *Lactococcus lactis* bacteria and dill (*Anethum graveolens*) CO₂ extract. All fortified samples showed lower pH (0.05–0.1 points) at Days 1–14, lower D (–) lactate content (8–25%) throughout the whole storage, higher phenolic content (1–2%) and slightly higher overall acceptability at the end of storage compared with unfortified samples. Due to these properties and little impact on the industrial starter and wild *L. lactis*, dill CO₂ extract could be incorporated to soured milk production. Both tested wild-type *Lactococcus lactis* strains were able to reduce the pH of the milk to standard value 4.46–4.64 in 8 h and produced mainly L (+) lactic acid during storage time. Wild-type *L. lactis* maintained LAB counts at 1 log unit higher than the control sample until Day 14. Wild-type strains showed high viability during storage time, and actively acidified milk creating acceptable flavor, so they could be promising starters as a single strain or co-starter cultures.

Introduction

Consumers seek dairy foods for a healthier lifestyle, so synthetic preservatives are now being replaced by natural various plant extracts (Burt, 2004; Khorshidian et al., 2018), and local wild lactic acid bacteria (LAB) cultures are being investigated as possible starters (Sarao and Arora, 2017; Yerlikaya, 2019).

The most important research area in the dairy industry (Sekomkiene, 2018) is the enrichment of dairy products with active plant components with aromatic, anti-oxidizing and antimicrobial properties, but it is still insufficiently investigated (Veiga et al., 2020). Many various biologically active substances (mainly bioflavonoids) are found in plant extracts and they are known to have a huge positive impact on the human body and to change the main characteristics of most dairy products they are used in (Gabriel-Danut et al., 2009).

Soured milk denotes a dairy product produced by the acidification of milk, giving a tart taste. This product is made at home commonly or produced commercially and consumed in Europe, especially in Eastern Europe. Traditionally, fresh milk is left to sour by keeping it in a warm place for a day. Naturally occurring acid causes milk to coagulate in this process

and at the same time inhibits the growth of harmful bacteria and improves the product's shelf life (Pophaly et al., 2018).

Modern commercial soured milk may differ from milk that has become sour naturally. Soured milk produced by fermentation with different strains introduces the consumer to new flavors. In Eastern Europe, it can be widely used in different food recipes, like cold summer soured milk soups, which are often spiced up with local herbs. Dill (*Anethum graveolens*) is the most common among them, due to its pleasant and distinct flavor, availability of the plant throughout the summer period and emotional impact on traditional East European consumer. Recently, dill has found its place in the recipes of ready to use niche commercial soured milk products. The shelf life of these products is shorter than the traditional plain soured milks. Thus, the novelty of this study is in exploring the possibilities of fortifying soured milk with dill extracts. The effect of various aqueous dill extracts (Amirdivani and Baba, 2011; Marhamatizadeh et al., 2012; Abbas et al., 2013) and essential dill oils (Hassanien et al., 2014) has been widely analyzed. The main active bio-components in dill extract are carvone, limonene, dill apiol and α -phellandrene (Jianu et al.; Chahal et al., 2017). The properties of CO₂ dill extract in various dairy matrixes are less explored. *Lactococcus lactis* is a well-known starter culture used in large-scale manufacture of a vast range of fermented dairy products (fermented milk, sour cream, butter, soft and hard cheeses, etc.) due to de-

Correspondence to Justina Milerienė, Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania, Tilžės 18, Kaunas LT-47181, Lithuania. E-mail: justina.mileriene@lsmu.lt

sirable properties such as acid production, flavor development, and bacteriophage resistance (Kelly et al., 2010) including wild-type isolates and dairy starter cultures, were screened on the basis of their phenotype and the macrorestriction patterns produced from pulsed-field gel electrophoresis (PFGE). However, specialized dairy starters have evolved to become essential components of industrial processes and are no longer fit to survive outside the dairy environment (Kandasamy et al., 2018) preservation and sensory qualities. These foods turn out to play a central role in the diet of several cultures because of its enriched health benefits that are known to possess antimicrobial, antidiabetic, anti-atherosclerotic, antioxidant and anti-inflammatory activities. Consequently, fermentable microorganisms, fermentation process and its products draw scientific interest. Currently fermented food production is mainly carried out using starter cultures for a precise and expectable fermentation. Lactic acid bacteria (LAB). Therefore, studies on technological, biochemical and organoleptic properties of newly isolated wild-type LAB, which may have genes that can be used to enhance the metabolism of dairy strains, are emerging (Nuryshev and Stoyanova, 2016; Yerlikaya, 2019; Fusieger et al., 2020) important substances that add buttery flavor notes in dairy products. Twenty-three *L. lactis* subsp. *lactis* isolates were obtained from dairy products (milk and cheese). Consumption of locally sourced strains isolated from food and subsequently used in the production of fermented milk products can significantly improve the condition and adaptation of the intestinal microflora (Cavanagh et al., 2015).

Wild-type LAB are a demanding microorganism and requires various nutrients and special environmental conditions for growth and propagating. Supplementation of carrier foods with nutritious and/or protective/sustaining ingredients such as plant extracts can, therefore, improve its viability having a potential synergistic effect on spoilage bacteria as well (Abdollahzadeh et al., 2018) physicochemical, rheological, and sensory characteristics of probiotic fermented milk was investigated. DE was added to milk at the level of 0–12 g/100 mL; the mixtures were then fermented with *Lactobacillus acidophilus* La-5. The initial probiotic concentrations ranged between 8.16 and 8.77 log₁₀ CFU/g. Although the highest DE concentration led to a significant count reduction (from 8.16 to 6.44 log₁₀ CFU/g. The application of plant extracts and wild LAB is a promising technology, which has been successfully used for bio preservation and functionality of milk products (Sarao and Arora, 2017).

In a recent study, we analyzed technological potential of *Lactococcus lactis* strains naturally present in raw and fermented milk (Kondrotiene et al., 2018). Therefore, to conduct this study, we selected two wild *Lactococcus lactis* subsp. *lactis* strains (LL16 and LL76) possessing most potential properties. Soured milk

produced with newly isolated wild-type strains from Lithuanian raw and naturally fermented milk may be able to introduce the new desirable flavors, and their abilities as a potential starter were tested in this study. The study was undertaken to determine the suitability of locally produced dill CO₂ extract (*Anethum graveolens*) together with wild-type *Lactococcus lactis* strains for formulation of traditional East European soured milk. Fortification of traditional soured milk with locally produced dill CO₂ extract is an innovation that might be more commercially attractive and thus worth being tested.

Materials and methods

Lactic acid bacteria and plant extracts

Two wild *Lactococcus lactis* strains were isolated from raw (LL16) and fermented (LL76) small scale local farm milk samples and stored at –80°C in M17 broth (Merck, Germany) in the presence of 30% glycerol until further analysis (Kondrotiene et al., 2018). Before conducting any experiments, strains were revitalized in MRS broth (Biolife, Milano, Italy) by growing for 18 h at 30°C.

Dill (*Anethum graveolens*) CO₂ extract was purchased from a small-scale organic farm in Kaunas region. The extract demonstrated antimicrobial activity against *Bacillus cereus*, *Listeria monocytogenes*, and *Brochothrix thermosphacta*. The total phenolic count of the dill CO₂ extract was 7.15 ± 0.8 GAE mg/g of extract and antioxidant activity (ABTS) was 53.21 ± 0.12 μmol TEA/g, respectively.

Preparation of experimental soured milk

Soured milk was prepared according to traditional soured milk preparation technology (Walstra et al., 1999). Standardized (2.52% fat, 3.03% protein, 4.54% lactose, 8.12% non-fat solids, pH 6.69 ± 0.02, determined with FoodScan (Foss, Denmark)), homogenized and pasteurized cow's milk was collected from local dairy factory and kept refrigerated (4°C) until analysis. After warming up milk to 30°C, it was distributed into three 400 mL vats and individually inoculated with 2% (approx. 4 log CFU bacteria per vat) of wild *L. lactis* strains (LL16 and LL76, respectively) and a commercial starter as a control (C). The commercial starter (F-DVS) for milk fermentation was purchased from CHR Hansen, Denmark. Deep-frozen bulk granules of mesophilic LD-type culture of mixed *L. lactis*, *L. cremoris*, *Leuconostoc sp.*, and *St. thermophiles* (LAB concentration > 1 × 10¹⁰ CFU/mL) were stored and prepared according to manufacturer's instructions to reach approx. 4 log CFU bacteria per vat. After inoculation, the content of each vat was divided into 2 parts (200 mL each) again, one portion was fortified with dill extract (10 μL/100 mL milk) and the other one remained unfortified (C-D, LL16-D, LL76-D). The samples were incubated for 8 h at 24°C, cooled to 4°C and stored for 28 days.

Analysis of experimental sour milk samples

Soured milks were sampled in triplicate for pH, D/L lactates and microbial examination at the beginning of storage (Day 1) and after 7, 14, 21 and 28 days of storage at 4 °C.

pH of samples was measured directly with a pH-meter (Sartorius Professional meter for pH Measurement, Germany).

The concentrations of L(+) and D(-) lactates (g/100 g) were determined using Megazyme assay Kit (Megazyme International Ireland, Bray, Ireland) and following manufacturer's instructions.

The changes of lactic acid bacteria were determined after 7, 14, 21 and 28 days of storage at 4°C. Soured milk samples (10 g) were aseptically taken from each triplicate, placed into a sterile stomacher bag (VWR Blender bag, US), diluted (1:10, w/v) in sterile Peptone water (Liofilchem) and homogenized with Stomacher 400 Circulator (Seward, UK) for 2 min. Decimal dilutions were prepared according to ISO 6887-5 (2010) with sterile Peptone water (Liofilchem) and plated on corresponding media. Quantification of microbiological counts was carried out using the pour plate technique. LAB counts (\log_{10} CFU/mL) were enumerated on MRS agar (Biolife, Milano, Italy) and incubated under aerobic conditions at 30°C for 72 h.

Sensory analysis of milks was performed on Day 1, 14, and 28 by a panel of tasters comprising 10 to 12 participants, both men and women, with ages ranging from 22 to 50 years old. The panelists were selected and instructed to work according to ISO 8586 (2012) and had practical skills to evaluate milk products. General acceptability of samples (20 g) was analyzed using a 9 mm lineal rating scale ranging from no or very low acceptability (score 0–1) to an excellent one (8–9). The samples were coded with 3-digit randomized numbers and served at room temperature, before the evaluation. Panelists were exposed to each sample in random order and were asked to assess general acceptability. Two evaluation sessions were performed.

Antioxidant activity was determined by the 2,2'-azinobis-(3-ethylbenzthiazoline)-6-sulfonate (ABTS) method as described earlier with minor modification (Re et al., 1999). The ABTS+ solution was prepared mixing ABTS (7 mM) and potassium persulfate (2.45 mM) and stored in the dark for 16 h before use. To determine scavenging activity of different supercritical CO₂ extracts, the ABTS radical solution was diluted with distilled water to an absorbance of 0.800 at 734 nm. The absorbance of 20 µL of extracts with 3 mL of ABTS solution was measured after 1 h storing in the dark. A series of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) solutions (100–500 mg/L) were used for calibration. The Trolox equivalent antioxidant capacity (TEAC) values were calculated from the calibration curve and the radical scavenging capacity (RSC) values were ex-

pressed in µmol Trolox equivalents (TE) per product g (µmol TE/g).

Total phenolic content (TPC) of the samples was determined using the Folin-Ciocalteu method (FC) and expressed in milligrams of the Gallic acid equivalents (GAE) per 100 g of sample (Lotito and Frei, 2004) and flavonoid-rich foods may help protect against chronic diseases by antioxidant mechanisms. In the present study we investigated: (1. Fermented milk (3 g) was extracted with methyl alcohol (3 mL) for 10 min using a vortex. The tubes were centrifuged at 700 g for 10 min and the upper phase layer was collected and analyzed immediately.

Statistical analysis

The data analysis was performed by SPSS statistical package (Chicago, SPSS Inc., SPSS 24). Data were analyzed using descriptive statistics (Explore) and one-way analysis (ANOVA) methods. Means were compared using Bonferroni's multiple range tests, and statistical significance was standardized by ANOVA at $P < 0.05$.

Results

The study revealed significant differences of the acidity parameters of the soured milk samples during storage time (Table 1). Both of our tested wild-type *Lactococcus lactis* strains were able to reduce the pH of milk from 6.6 ± 0.03 to a standard pH for fermented milks (4.46–4.64) after an 8-h fermentation process.

Interestingly, all fortified samples showed significantly lower pH on Day 1 compared with unfortified samples. No such differences among fortified and plain samples were detected later throughout the storage period. pH values significantly increased on Day 7 and decreased to the Day 1 level on Day 14, during the storage. In all extract-free samples, it remained at the Day 14 level, increasing significantly in dill fortified samples at the end of storage.

LAB counts were monitored in soured milks during the whole storage time (Table 1). LAB counts remained stable during the first 2 weeks of storage and then gradually decreased till the end of the storage period (28 days) in all samples. On Day 14, there was a significant drop in LAB counts detected in control sample C and all samples fortified with dill extract. The same drop was spotted in the plain samples with wild-type starters a week later – on Day 21. The lowest viability of LAB was monitored in the sample with wild LL76, and the highest was observed in the sample with wild LL16 ($P < 0.05$) and in control samples.

Reduction of pH is caused by the metabolic process of lactic acid bacteria which produce organic acids. In our samples, LAB produced mainly L (+) lactate during storage time (Table 2). The amounts of undesirable D lactate increased during storage in all samples. Significantly lower concentrations of D lactate were detected in all the samples fortified with dill extract. This tendency was especially prominent in the samples with wild-type strains LL16 and LL76.

Table 1. Counts of lactic acid bacteria (LAB, log₁₀ CFU/mL) and pH in sour milk samples without extract (C, LL16, LL76) and fortified with dill extract (C-D, LL16-D, LL76-D) during storage time at 4°C

Storage	Day 1	Day 7	Day 14	Day 21	Day 28
Samples	Lactic acid bacteria (log ₁₀ CFU/mL)				
C	7.67 ± 0.01a	7.43 ± 0.01a	5.96 ± 0.02bA	6.00 ± 0.03bA	6.26 ± 0.02bA
C-D	7.49 ± 0.02a	7.49 ± 0.02a	5.91 ± 0.01bA	6.23 ± 0.02bA	5.49 ± 0.01bB
LL16	7.18 ± 0.01a	7.45 ± 0.03a	7.04 ± 0.01aB	5.57 ± 0.03bA	4.85 ± 0.01bC
LL16-D	7.22 ± 0.02a	7.11 ± 0.01a	6.28 ± 0.03bA	4.60 ± 0.02bB	4.11 ± 0.03bD
LL76	7.00 ± 0.01a	7.08 ± 0.02a	7.08 ± 0.01aB	5.68 ± 0.02bA	4.74 ± 0.01cC
LL76-D	7.20 ± 0.03a	6.90 ± 0.03a	6.60 ± 0.02bA	4.70 ± 0.03bB	3.86 ± 0.02cD
Samples	pH				
C	4.52 ± 0.03aA	4.69 ± 0.07B	4.54 ± 0.01aA	4.52 ± 0.01aB	4.57 ± 0.01aB
C-D	4.49 ± 0.01 bA	4.64 ± 0.01B	4.50 ± 0.01bA	4.52 ± 0.01aB	4.61 ± 0.01aB
LL16	4.43 ± 0.04 A	4.66 ± 0.12 B	4.42 ± 0.01 bA	4.47 ± 0.01 bB	4.47 ± 0.01aB
LL16-D	4.36 ± 0.12 bA	4.62 ± 0.04 B	4.43 ± 0.03 bA	4.46 ± 0.02 bB	4.47 ± 0.01aB
LL76	4.44 ± 0.05 A	4.69 ± 0.07 B	4.49 ± 0.03 bA	4.50 ± 0.08 aB	4.50 ± 0.01aB
LL76-D	4.40 ± 0.01 bA	4.58 ± 0.01 B	4.52 ± 0.01 bA	4.53 ± 0.01aB	4.51 ± 0.01aB

Values presented are means of three replicates ± standard deviation.

Means in the same row with different lowercase letters indicate significant differences ($P < 0.05$) among storage days.

Means in the same column with different capital letters indicate significant differences ($P < 0.05$) among strains.

Table 2. The concentrations of L(+) and D(-) lactates (g/100 g) in sour milk samples without extract (C, LL16, LL76) and fortified with dill extract (C-D, LL16-D, LL76-D) during storage at 4°C

Storage	Day 1	Day 7	Day 14	Day 21	Day 28
Samples	L (+) lactate (g/100 g)				
C	1.53 ± 0.00Aa	0.87 ± 0.00Ab	1.23 ± 0.00Ac	1.34 ± 0.02Ad	1.13 ± 0.00Ae
C-D	0.91 ± 0.00Ba	0.72 ± 0.00Bb	1.68 ± 0.00Bc	1.94 ± 0.02Bd	1.81 ± 0.02Be
LL16	2.17 ± 0.00Ca	0.83 ± 0.01Cb	1.95 ± 0.02Cc	2.26 ± 0.02Cd	2.94 ± 0.02Ce
LL16-D	1.69 ± 0.01Da	0.65 ± 0.00Db	2.43 ± 0.00Dc	1.54 ± 0.02Dd	3.28 ± 0.01De
LL76	2.76 ± 0.00Ea	0.94 ± 0.00Eb	2.90 ± 0.01Ec	2.60 ± 0.01Ed	2.05 ± 0.04Ee
LL76-D	2.98 ± 0.01Fa	1.31 ± 0.01Fb	3.85 ± 0.00Fc	3.49 ± 0.01Fd	3.48 ± 0.01Fd
Samples	D (-) lactate (g/100 g)				
C	0.19 ± 0.04Aa	0.60 ± 0.03Ab	0.31 ± 0.02Ac	0.59 ± 0.01Ab	0.26 ± 0.02Ad
C-D	0.03 ± 0.03Ba	0.05 ± 0.00Ba	0.06 ± 0.01Ba	0.08 ± 0.02Bb	0.10 ± 0.02Bb
LL16	0.17 ± 0.03Aa	0.54 ± 0.01Cb	0.36 ± 0.04Cc	0.91 ± 0.03Cd	0.81 ± 0.01Ce
LL16-D	0.18 ± 0.02Aa	0.12 ± 0.00Db	0.06 ± 0.03Bc	0.06 ± 0.02Bc	0.05 ± 0.04Dc
LL76	0.22 ± 0.03Ca	0.57 ± 0.00Eb	0.38 ± 0.01Cc	0.37 ± 0.04Dc	0.42 ± 0.05Ed
LL76-D	0.08 ± 0.04Da	0.09 ± 0.03Fa	0.12 ± 0.02Db	0.09 ± 0.00Ba	0.05 ± 0.03Da

Values presented are means of three replicates ± standard deviation.

Means in the same row with different lowercase letters indicate significant differences ($P < 0.05$) among storage days.

Means in the same column with different capital letters indicate significant differences ($P < 0.05$) among strains.

We detected no significant differences in general acceptability among samples (Table 3) during storage. Storage affected the acceptability negatively; all samples demonstrated a slight decrease in acceptability on Day 28. Nevertheless, the samples fortified with dill extract showed the tendency to slightly reduce the sensory acceptability of control and experimental samples on Day 1 and enhance it at the end of the experiment.

Table 3 shows that the process of fermentation and storage (Day 1 versus 28) influenced the total phenolic content (TPC) with a slight decrease over the storage time, with the highest values found in the samples fortified with dill on Day 1 ($P \geq 0.05$). Significantly higher contents of TPC were found in all plain samples on Day 14 and Day 28.

Antioxidant activity also varied depending on the strain (Table 3). Between wild type strains, the high-

Table 3. Total phenolic content (GAE mg/100 g), and antioxidant activity ($\mu\text{mol TE/g}$), and overall acceptability (1-9) of sour milk samples without extract (C, LL16, LL76) and fortified with the dill extract (C-D, LL16-D, LL76-D) during storage at 4°C

Sample	Total phenolic content (GAE mg/100 g)			Antioxidant activity ($\mu\text{mol TE/g}$)		
	Day 1	Day 14	Day 28	Day 1	Day 14	Day 28
C	1.46 \pm 0.01 ^a	1.25 \pm 0.04 ^{bA}	1.26 \pm 0.08 ^{bA}	0.36 \pm 0.08 ^{aA}	1.02 \pm 0.03 ^{bA}	0.06 \pm 0.07 ^c
C-D	1.53 \pm 0.09 ^a	1.61 \pm 0.06 ^{bB}	1.30 \pm 0.08 ^{cA}	0.05 \pm 0.06 ^{aB}	0.59 \pm 0.01 ^{bB}	0.05 \pm 0.02 ^a
LL16	1.39 \pm 0.06 ^a	1.53 \pm 0.06 ^{bB}	1.16 \pm 0.08 ^{cB}	0.55 \pm 0.01 ^{aA}	0.60 \pm 0.03 ^{aB}	0.08 \pm 0.08 ^b
LL16-D	1.41 \pm 0.08 ^a	1.26 \pm 0.06 ^{bA}	1.09 \pm 0.02 ^{cB}	0.08 \pm 0.05 ^{aB}	0.14 \pm 0.07 ^{bC}	0.04 \pm 0.08 ^a
LL76	1.50 \pm 0.02 ^a	1.48 \pm 0.02 ^{bB}	1.49 \pm 0.04 ^{bA}	0.21 \pm 0.02 ^{aA}	0.34 \pm 0.02 ^{bB}	0.01 \pm 0.05 ^a
LL76-D	1.52 \pm 0.06 ^a	1.31 \pm 0.03 ^{bA}	1.20 \pm 0.02 ^{cA}	0.46 \pm 0.01 ^{aA}	0.26 \pm 0.05 ^{aC}	0.02 \pm 0.03 ^b
Sample	Overall sensory acceptability (1-9)					
	Day 1	Day 14	Day 28			
C	6.97 \pm 2.96 ^a	6.12 \pm 2.56	5.67 \pm 1.26 ^b			
C-D	6.41 \pm 2.57 ^a	6.21 \pm 1.75	5.91 \pm 2.27 ^b			
LL16	7.03 \pm 2.49 ^a	6.48 \pm 1.95	5.73 \pm 1.29 ^b			
LL16-D	6.73 \pm 3.24 ^a	6.53 \pm 2.31	5.83 \pm 2.32 ^b			
LL76	7.73 \pm 2.77 ^a	6.62 \pm 2.12	5.73 \pm 1.27 ^b			
LL76-D	7.46 \pm 1.66 ^a	6.72 \pm 1.96	5.96 \pm 2.23 ^b			

Values presented are means of three replicates \pm standard deviation.

Means in the same row with different lowercase letters indicate significant differences ($P < 0.05$) among storage days.

Means in the same column with different capital letters indicate significant differences ($P < 0.05$) among strains.

est antioxidant activity was determined in strain LL16 on Day 1 and the lowest in LL76. The antioxidant capacities obtained in this study increased on Day 14 and decreased on Day 28 in all samples, but they are significantly lower comparing with the samples fortified with dill extract. No differences in antioxidant activity were detected among the samples at the end of the storage.

Discussion and conclusions

One of the most important properties of a starter in dairy fermentation is the ability to produce acid rapidly (Bello et al., 2013). At the beginning of the storage (before Day 14), herbal soured milks had faster rates of pH reduction than the plain ones ($P < 0.05$). Similar pH tendencies in dill fortified fermented products were detected by Amirdivani and Baba (2011). In a study of yoghurts fortified with thyme, grape and green tea extracts, Alwazeer et al. (2020) concludes that addition of herbal extracts into the formulations of fermented milk can affect the metabolism of starters, especially their metabolic flux, acidification rate and reducing activity.

LAB starters must demonstrate multiple benefits in foods such as delaying spoilage and producing bioactive metabolites. Bioactive metabolites that are specifically the result of LAB are increasingly identified in foods. Ensuring the presence of these metabolites in products is contributing to health functionality (Champagne et al., 2018). Lactic acid as a LAB metabolite is important not only as a bio-preservation agent (Corsetti et al., 2015), it also comes in two bio-

active lactic acid forms: L (+) and D (-). High concentrations of D (-) lactic acid are harmful to humans and should be avoided in food, whereas L (+) lactic acid is the preferred isomer in food products (Reddy et al., 2008). D-lactate can cause health complications, and the expected median lethal dose (LD50) per orally poisoned rats is around 4.5 g/kg (Pohanka, 2020). Wild *L. lactis* strains produced mainly L (+) lactic acid in our samples during storage time. Most of it was produced in a LL76 sample fortified with dill extract ($P < 0.05$). Interestingly, dill extract added to the control and experimental products significantly decreased D (-) lactic acid concentration. This is in agreement with other studies (Marhamatizadeh et al., 2012).

One of the criteria for a wild-type LAB to be regarded as acceptable starters is consumer acceptance and survival of microorganisms through the processing and storage of a final product (Sarao and Arora, 2017). In this study, the ability of the wild *L. lactis* strain to maintain viable counts at the same level as an industrial mix of starter cultures shows promising technological potential. Such isolates could be considered for replacement or supplementation of commercial starters used to produce fermented products. However, the fortification of soured milk samples with the dill CO_2 extract had a negative impact on the viability of LAB at the end of the storage. The results of our study revealed the significant decrease of LAB counts in all samples with the dill extract: on Day 14 in the samples with wild-type strains, and on Day 28 in control samples. At the end of the storage, wild-type strains were at 1 log lower counts than

those of the control sample. The same tendency appeared in the fortified samples – all samples with dill expressed 1 log less viable cells than their unfortified counterparts. The decrease in the LAB count was less noticeable in control samples with dill containing a commercial starter. The main component in the dill extract is carvone (Chahal et al., 2017), which can disrupt a pH gradient and membrane potential of cells (Champagne et al., 2018). In 1995, Oosterhaven et al. announced that carvone reduced the growth of *S. thermophilus* and *L. lactis* through interrupting the metabolic energy status of the cells. The use of dill essential oil *in vitro* was not effective on the growth of some lactic acid bacteria: *L. delbrueckii* ssp. *bulgaricus* and *St. thermophilus* (Abbas et al., 2013). Nevertheless, the results regarding its effect on LAB are controversial. It has been reported that addition of some aromatic and essential oils to yoghurt during its manufacture had a stimulatory effect on lactic acid bacteria by enhancing their growth and acid production (Abou Ayana, 2011). It can be stated that the impact of the main antimicrobial agent on non-target organisms highly depends on the method of extraction, dosage, and dairy matrix and starter interactions (Nadal et al., 2010).

Flavor is one of the most important attributes for consumers that can be negatively affected by putting more attention to bio-preservation of dairy produce (Khorshidian et al., 2018), such as choosing strong unpleasant volatile and non-volatile metabolites producing *L. lactis* strains and applying too high concentrations of plant extracts thus increases the off-flavor in the final bouquet. In our study, there were no significant differences among samples regarding sensory evaluation, i.e., the samples with wild-type strains were equally acceptable and dill fortification by the end of the 28 days of storage was able to slightly enhance the sensory acceptance of soured milk samples.

There were significant differences in antioxidant activity among strains in our study, indicating that the antioxidant activity of *L. lactis* can vary by source and strain (Ozdogan et al., 2012) namely its resistance to bile salt, pepsin, pancreatin, acid and antibiotics. Moreover, the ability of *L. lactis* to inhibit the adhesion of *Escherichia coli* ETEC and *Salmonella Typhimurium* SL1344 to Caco-2 cells were examined and also the hydrophobicity, iron-ion chelating ability, and determination of α, α -diphenyl- β -picrylhydrazyl (DPPH). The antioxidant capacities were storage dependent: increasing at the beginning and decreasing at the end of the storage period. Since plain-samples contain no plant extracts, the TPC values in sour milk reflect phenolic compounds related to milk protein breakdown by LAB. Surprisingly, less antioxidant activity was

detected in the fortified samples. Contrary results were found in the similar studies by Amirdivani and Baba (2011). TPC and antioxidant capacity per g of a pure dill extract (7.15 GAE mg /100g, ABTS 53.21 μ mol TE/g, respectively) were quite high, so other factors may have interfered in the colorimetric determination of TPC and ABTS in soured milks.

In conclusion, development of dairy products with local wild-type starters and new flavors has potential benefits thereby increasing sales and consumer's satisfaction. Traditional preparation of soured milk may be beneficial by including plant extracts to enhance the flavor as well as product quality. In view of its organoleptic properties, the dill extract could most readily be incorporated in manufactured East European fermented dairy products that are traditionally associated with herbs (savory dishes such as herbal sour milk or soft white curd cheese) or spices (dried and baked/smoked varieties of curd cheese). Due to the prominent phenolic content, ability to reduce D (-) lactate in fermented milk products and acceptable aroma, the dill extract can be proposed to dairy producers as a natural, safe, biodegradable flavor enhancer. Antagonism between the extract and food ingredients is undesirable and further research is needed, so it could be avoided in practical applications.

Industrial dairy *L. lactis* strains have undergone a significant genome decay and are considered to be unable to survive outside of this niche (Kelly et al., 2010) including wild-type isolates and dairy starter cultures, were screened on the basis of their phenotype and the macrorestriction patterns produced from pulsed-field gel electrophoresis (PFGE). With the emergence of wild-type *L. lactis* with promising sensory and technological properties, such isolates could be used producing traditional and novel fermented products with the potential to sustain the natural diversity of gut microbiota. Combinations of probiotic *L. lactis* and the dill extract can be exploited to maximize the phenolic content in the product and to minimize the concentrations required to achieve a particular D (-) lactate decreasing effect.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

This research was funded by the European Regional Development Fund according to the supported activity 'Research Projects Implemented by World-class Researcher Groups' under Measure No. 01.2.2-LMT-K-718.

References

1. Abbas H. M., Mohamed S. H. S., Zaky, W. M., Kassem J. M., Salem, M. M. E., Said- H. A. H., et al. Impact of Antimicrobial Properties of Some Essential Oils on Cheese Yoghurt Quality. *World Applied Sciences Journal*. 2013. T. 27. P. 497–507. DOI:10.5829/idosi.wasj.2013.27.04.13623.
2. Abdollahzadeh S. M., Zahedani M. R., Rahmdel S., Hemmati F., and Mazloomi S. M. Development of *Lactobacillus acidophilus*-fermented milk fortified with date extract. *LWT - Food Science and Technology*. 2018. T. 98. P. 577–582. DOI: 10.1016/j.lwt.2018.09.042.
3. Abou Ayana I. Improvement of the Properties of Goat's Milk Labneh using some Aromatic and Vegetable Oils. *International Journal of Dairy Science*. 2011. T. 2. P. 112–123. DOI:10.3923/ijds.2011.112.123.
4. Alwazeer D., Bulut M., and Tunçtürk Y. Fortification of milk with plant extracts modifies the acidification and reducing capacities of yoghurt bacteria. *International Journal of Dairy Technology* 2020. T. 73. P. 117–125. DOI:10.1111/1471-0307.12643.
5. Amirdivani S., and Baba A. S. Changes in yogurt fermentation characteristics, and antioxidant potential and in vitro inhibition of angiotensin-1 converting enzyme upon the inclusion of peppermint, dill and basil. *LWT - Food Science and Technology*. 2011. T. 44 P. 1458–1464. DOI:10.1016/j.lwt.2011.01.019.
6. Bello B. D., Zeppa G., Bianchi D. M., Decastelli L., Traversa A., Gallina S., et al. Effect of nisin-producing *Lactococcus lactis* starter cultures on the inhibition of two pathogens in ripened cheeses. *International Journal of Dairy Technology*. 2013 T. 66. P. 468–477. DOI:10.1111/1471-0307.12064.
7. Burt S. Essential oils: Their antibacterial properties and potential applications in foods - A review. *International Journal of Food Microbiology*. 2004. T. 94. P. 223–253. DOI:10.1016/j.ijfoodmicro.2004.03.022.
8. Cavanagh D., Fitzgerald G. F., and McAuliffe O. From field to fermentation: the origins of *Lactococcus lactis* and its domestication to the dairy environment. *Food Microbiology*. 2015. T. 47. P. 45–61.
9. Chahal K., Kaur R., Kumar A., and Bhardwaj U. Chemistry and biological activities of *Anethum graveolens* L. (dill) essential oil: A review. *Journal of Pharmacognosy and Phytochemistry*. 2017. T. 6. P. 295–306.
10. Champagne C. P., Gomes da Cruz A., and Daga M. Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*. 2018. T. 22. P. 160–166. DOI:10.1016/j.cofs.2018.04.008.
11. Corsetti A., Perpetuini G., and Tofalo R., "Biopreservation effects in fermented foods," in *Advances in Fermented Foods and Beverages: Improving Quality, Technologies and Health Benefits* (Elsevier Inc.). 2015. P. 311–332. DOI:10.1016/B978-1-78242-015-6.00013-X.
12. Fusieger A., Martins M. C. F., de Freitas R., Nero, L. A. and de Carvalho, A. F. Technological properties of *Lactococcus lactis* subsp. *lactis* bv. *diacetylactis* obtained from dairy and non-dairy niches. *Brazilian Journal of Microbiology*. 2020. T. 51. P. 313–321. DOI:10.1007/s42770-019-00182-3.
13. Gabriel-Danut M., Gabriela R., Elisabeta B., Aida V., Liliana G., Doina A., et al., Research concerning the production of a probiotic dairy product with added medicinal plant extracts. in *The Annals of the University of Dunarea de Jos of Galati*. 2009. P. 32–37.
14. Hassanien M. F. R., Mahgoub S. A., and El-Zahar K. M. Soft cheese supplemented with black cumin oil: Impact on food borne pathogens and quality during storage. *Saudi Journal of Biological Science* 2014. T. 21. P. 280–288. DOI:10.1016/j.sjbs.2013.10.005.
15. International Organization for Standardization 2010: ISO 6887-5:2010 Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination Specific rules for the preparation of milk and milk products. 13. Available at: <https://www.iso.org/standard/50306.html> [Accessed March 16, 2020].
16. International Organization for Standardization 2012: ISO 8586:2012 Sensory analysis – General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. 28. Available at: <https://www.iso.org/standard/45352.html>.
17. Jianu C., Mișcă C., Stoin D., Bujancă G., and Alexandra Teodora L.-G., Section Advances in Biotechnology chemical composition and antioxidant properties of dill essential oil. 2018. DOI:10.5593/sgem2018/6.4.
18. Kandasamy S., Kavitate D., and Shetty P. H. "Lactic Acid Bacteria and Yeasts as Starter Cultures for Fermented Foods and Their Role in Commercialization of Fermented Foods," in *Innovations in Technologies for Fermented Food and Beverage Industries* (Springer International Publishing). 2018. P. 25–52. DOI:10.1007/978-3-319-74820-7_2.
19. Kelly W. J., Ward L. J. H., and Leahy S. C. "Chromosomal Diversity in *Lactococcus lactis* and the Origin of Dairy Starter Cultures," in *Genome Biology and Evolution*. 2010. P. 729–744. DOI:10.1093/gbe/evq056.
20. Khorshidian N., Yousefi M., Khanniri E., and Mortazavian A. M. Potential application of essential oils as antimicrobial preservatives in cheese. *Innovative Food Science and Emerging Technologies*. 2018. T. 45. P. 62–72. DOI:10.1016/j.ifset.2017.09.020.
21. Kondrotiene K., Kasnauskite N., Sernien, L., Gölz G., Alter T., Kaskoniene V., et al. Characterization and application of newly isolated nisin producing *Lactococcus lactis* strains for control of *Listeria monocytogenes* growth in fresh cheese. *LWT - Food Science and Technology*. 2018. T. 87. P. 507–514. DOI:10.1016/j.lwt.2017.09.021.
22. Lotito S. B., and Frei B. Relevance of apple polyphenols as antioxidants in human plasma: Contrasting in vitro and in vivo effects. *Free Radical Biology and Medicine*. 2004. T. 36. P. 201–211. DOI:10.1016/j.freeradbiomed.2003.10.005.
23. Marhamatizadeh M. H., Jaafari F., Rezazadeh S., Ehsandoost E., and Mohammadi M. Effects of dill extract (*Anethum graveolens* L.) on growth and survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in probiotic milk and yoghurt. *Global Veterinaria*. 2012. T. 9. P. 252–257. DOI:10.5829/idosi.gv.2012.9.3.6543.
24. Nadal E. S., Sayas-Barberá E., Fernández-López J., and Pérez-Alvarez J. A. "Food Formulation to Increase Probiotic Bacteria Action or Population," in *Bioactive Foods in Promoting Health* (Elsevier Inc.). 2010. P. 335–351. DOI:10.1016/B978-0-12-374938-3.00022-0.
25. Nuryshev M. Z., and Stoyanova L. G. New Probiotic Culture of *Lactococcus lactis* ssp. *lactis*: Effective Opportunities and Prospects. *Journal of Microbiology and Biochemical Technology*. 2016. T. 8. DOI:10.4172/1948-5948.1000299.
26. Oosterhaven K., Poolman B., and Smid E. J. S-carvone as a natural potato sprout inhibiting, fungistatic and bacteriostatic compound. *Industrial Crops and Products*. 1995. T. 4. P. 23–31. DOI:10.1016/0926-6690(95)00007-Y.
27. Ozdogan D. K., Akcelik N., Aslim B., Suludere Z., and Akcelik M. Probiotic and antioxidative properties of *L. Lactis* I127 isolated from milk. *Biotechnology and Biotechnological Equipment*. 2012. T. 26, P. 2750–2758. DOI:10.5504/bbeq.2011.0091.
28. Pohanka M. D-Lactic Acid as a Metabolite: Toxicology, Diagnosis, and Detection. *BioMed Research International*. 2020. Volume 2020. P. 1 – 9. DOI: 10.1155/2020/3419034
29. Pophaly S. D., Chauhan M., Lule V., Sarang P., Tarak J., Thakur K., et al. "Functional Starter Cultures for Fermented Dairy Products," in *Microbial Cultures and Enzymes in Dairy Technology* (IGI Global). 2018. P. 54–68. DOI:10.4018/978-1-5225-5363-2.ch003.
30. Re R., Pellegrini N., Proteggente A., Pannala A., Yang, M., and Rice-Evans C., Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 1999. T. 26, P. 1231–1237. DOI:10.1016/S0891-5849(98)00315-3.
31. Reddy G., Altaf M., Naveena B. J., Venkateshwar M., and Kumar E. V. Amyolytic bacterial lactic acid fermentation -

- A review. *Biotechnology Advances*. 2008. T. 26. P. 22–34. DOI:10.1016/j.biotechadv.2007.07.004.
32. Sarao L. K., and Arora M. Probiotics, prebiotics, and micro-encapsulation: A review. *Critical Reviews in Food Science Nutrition*. 2017. T. 57, P. 344–371. DOI:10.1080/10408398.2014.887055.
 33. Sekmokiene, D. The influence of aqueous extracts on curd-type cheese quality. *researchgate.net*. 2018. DOI:10.6001/zemesukiomokslai.v25i2.3754.
 34. Veiga M., Costa E. M., Silva S., and Pintado M., Impact of plant extracts upon human health: A review. *Critical Reviews in Food Science Nutrition*. 2020. T. 60. P. 873–886. DOI:10.1080/10408398.2018.1540969.
 35. Walstra P., Geurts T. J., Noomen A., Jellema A., and Boekel M. A. J. S. van. *Dairy technology: principles of milk properties and processes*. Dairy Technology– Principles of Milk Properties and Processes. 1999
 36. Yerlikaya, O. Probiotic potential and biochemical and technological properties of *Lactococcus lactis* ssp. *lactis* strains isolated from raw milk and kefir grains. *Journal of Dairy Science*. 2019. T. 102. P. 124–134. DOI:10.3168/jds.2018-14983.

Received 5 November 2020

Accepted 28 December 2020