Agar-immobilized basil–lactic acid bacteria bioproducts as goat milk taste-masking agents and natural preservatives for the production of unripened goat cheese

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ABSTRACT

Goat milk cheeses have become popular recently; however, many consumers do not choose these products because they have specific sensory properties that are not acceptable to all consumers and the shelf life of the cheese is short. The concept of this work was to increase overall acceptability and shelf life of unripened goat milk cheese by using *Ocimum basilicum* and lactic acid bacteria (*Lactobacillus plantarum* LUHS135, *Lactobacillus paracasei* LUHS244, *Pediococcus pentosaceus* LUHS100, *Pediococcus acidilactici* LUHS29, and *Lactobacillus brevis* LUHS140) bioproducts (basil-LAB) immobilized in agar. A basil-LAB bioproduct could be a promising multifunctional ingredient for cheese manufacturing because it has a low pH, high LAB count, and high total phenolic compound content (after fermentation pH decreased by 25.4%, LAB count averaged 7.2 log₁₀ cfu/g, and total phenolic compound content increased by 30.9%). Use of different LAB in the preparation of basil-LAB bioproducts had a significant influence on cheese pH and hardness, and compared with cheese samples prepared with nonfermented basil, cheese samples prepared with basil-LAB bioproducts had, on average, higher pH (by 2.6%) and lower hardness (by 36.0%), similar to the control cheese (without basil). Overall acceptability of cheese was significantly influenced by the basil-LAB bioproduct immobilization process; in all cases, cheese samples prepared with fermented and immobilized basil-LAB bioproduct had better acceptability (5 points). After 120 h of storage, cheese samples prepared with basil-LAB bioproducts fermented with LUHS135, LUHS244 and LUHS140, no enterobacteria were found, and we detected strong negative and moderate negative correlations, respectively, of LAB count with enterobacteria count and yeast/mold

count ($r = -0.7939$ and $r = -0.4495$, respectively). Finally, immobilization increased LAB viability in fresh goat milk cheese, which led to a reduction in enterobacteria and mold/yeast contamination during storage and an increase in overall acceptability compared with nonimmobilized basil-LAB. Therefore, basil-LAB bioproducts fermented with LUHS135, LUHS244, and LUHS140 strains can be recommended for preparing fresh goat milk cheese with extended shelf life and high acceptability.

Key words: unripened curd cheese, goat milk, lactic acid bacteria, *Ocimum basilicum* L., immobilization

INTRODUCTION

In recent years, goat milk and its products have received much attention as functional foods for maintaining the nourishment and health of the young and elderly, especially those who have an allergy to cow milk (Yangilar, 2013). Goat milk differs from cow and human milk in having better digestibility, buffer capacity, alkalinity, and therapeutic values, as well as having fat with better physical properties (i.e., surface tension, viscosity, and specific gravity) than cow milk (Park et al., 2007). Goat milk is commonly used for the production of fresh unripened cheese (Janštová et al., 2010), a versatile nutrient-dense dairy product susceptible to physical, chemical, microbiological, and biochemical spoilage that can decrease its shelf life and cause serious risks to consumers' health (Jalilzadeh et al., 2015; Gouvea et al., 2017; Khorshidian et al., 2017). Changes that limit shelf life include creaming of fat, gelation of protein solutions, syneresis of curds and crystallization of minerals, nonenzymatic browning and oxidation of fat, growth of microorganisms, enzymatic degradation, ripening of cheese, and fermentation (Kilcast and Subramaniam, 2011). Increasing demand from consumers to use food products without preservatives or with natural preservatives has compelled food industries to use preservatives of herbal and microbial origin instead of artificial preservatives (Gouvea et al., 2017; Khorshid-

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ian et al., 2017). Basil (*Ocimum basilicum* L.) belongs to the Lamiaceae family of herbs, which, in addition to being used as a spice, is medicinally known as a powerful antibacterial, antimutagenic, and chemopreventive agent (Stanojevic et al., 2017). These plants are considered to be a rich source of essential oils that have biological activity and use in different areas of human activity (Avetisyan et al., 2017). The essential oils from various basil cultivars can contain alcohols (linalool), oxides (1,8-cineole), phenols (eugenol, methyl eugenol, methyl isoeugenol, thymol), esters (methyl cinnamate), aldehydes (citral) and camphor; 1,8-cineole, methyl cinnamate, methyl chavicol, and linalool are the constituents responsible for the distinct aroma of basil plants (Klimankova et al., 2008). Numerous papers have been published on the antimicrobial and antioxidant properties of basil essential oils and their constituents (Soković et al., 2007; Liu et al., 2012; Zabka et al., 2014; Huang et al., 2015).

To increase the preparation of goat milk products and consumer demand, it is necessary to increase their safety, improve their flavor, and to increase their functional value; lactic acid bacteria (LAB) can be used to achieve this (de Souza and Silva Dias, 2017) as they are regarded as health-friendly for consumers (Bulajic et al., 2017). Lactic acid bacteria are the dominant indigenous microorganisms of raw milk cheese, producing pathogen-inhibiting substances and leading to the manufacture of cheese with desirable microbiological qualities (Tamang et al., 2016). Our previous research confirmed that the positive effect of LAB on raw milk fresh cheese can be enhanced by combining LAB and savory plant bioproducts (Mozuriene et al., 2016). Plant bioproducts significantly reduced the counts of enterobacteria, yeast, fungi, and spores of mesophilic bacteria in cheese samples and increased the amount of volatile compounds in cheese. Bioproducts derived from a combination of LAB and savory plants increased the acceptability and shelf life of cow milk curd cheese, and therefore can be recommended for the production of better quality curd cheese (Mozuriene et al., 2016).

The concept of this work was to increase overall acceptability and shelf life of unripened goat milk cheese by using *Ocimum basilicum*-LAB bioproducts (basil-LAB) immobilized in agar.

MATERIALS AND METHODS

Plant Material and Microorganisms Used for Fermentation

Fresh *O. basilicum* was obtained from a local market. Before the experiment, plants were washed under

running water and homogenized by using a Bosch MSM67170 homogenizer (Robert Bosch GmbH, Stuttgart, Germany) to obtain a puree consistency.

Lactobacillus plantarum LUHS135, *Lactobacillus paracasei* LUHS244, *Pediococcus pentosaceus* LUHS100, *Pediococcus acidilactici* LUHS29, and *Lactobacillus brevis* LUHS140 strains were obtained from the culture collection of the Lithuanian University of Health Sciences (Kaunas, Lithuania). The LAB were cultured at 30°C for 24 h in de Man, Rogosa, and Sharpe (MRS) broth (CM0359, Oxoid Ltd., Basingstoke, UK) and used for further experiments.

Preparation of Agar-Immobilized Basil-LAB Bioproducts

The homogenized plant samples (500 mL) were inoculated with pure LAB cultures (3% by volume). A cell suspension of LAB containing about $9.4 \log_{10}$ cfu/mL was added to the homogenized plant sample, followed by fermentation for 24 h at $30 \pm 2^\circ\text{C}$. Prepared basil-LAB bioproducts were immobilized in agar. First, agar powder was soaked in water (70.2 g/L) for 20 min and then melted by heating for 5 min. Basil-LAB bioproducts were incorporated into the agar mass (80:20, basil: agar, % by volume) at the end of the process, when the agar solution was cooled to $30 \pm 2^\circ\text{C}$. The obtained mass was poured into molds and dried at room temperature ($21\text{--}23^\circ\text{C}$) for 24 h to a gel-like texture. The mass was divided, by cutting with a knife, into granules 3 mm in diameter and directly used for the analysis and preparation of unripened goat milk cheese. During this experiment, 5 basil-LAB bioproducts were prepared by using different LAB strains (*L. plantarum* LUHS135, *L. paracasei* LUHS244, *P. pentosaceus* LUHS100, *P. acidilactici* LUHS29, and *L. brevis* LUHS140). Nonfermented homogenized *O. basilicum* was tested as a control. Unripened goat milk cheese was prepared by using 5 immobilized, 5 nonimmobilized, and 1 nonfermented *O. basilicum*, and without *O. basilicum*.

Preparation of Unripened Goat Milk Cheese

Raw goat milk (normal, clean, and white; natural flavor without any foreign matter or adulteration; pH 6.6, milk solids-not-fat 8.25%, total microorganisms $<5 \times 10^4$ cfu/mL, somatic cells $<7 \times 10^5$ cells/mL, protein content 3.8%, fat content 4.0%, total solids 13.1%) was collected from Laurikieciu farm (Pakruojis, Lithuania). The curd cheese was made using 10 L of raw goat milk per treatment. Raw milk intended for unripened (fresh) cheese was pasteurized in a batch pasteurizer at 72 to 73°C for 15 to 20 s. After spontaneous coagulation

(after 24 h at $30 \pm 2^\circ\text{C}$) of the milk, curd was gently mixed, using a spoon, with 5% basil-LAB bioproduct (by weight) and 2% salt; it was then gently cut into cubes of approximately 100 g, drained, placed in nylon containers, and pressed (0.5-kg weight) for 12 h at 4°C . Curd cheese samples for analysis were collected within 24 h of the manufacturing process.

Analysis of Acidity Parameters of Basil-LAB Bioproducts and Curd Cheese

The pH values of basil-LAB bioproducts were measured and recorded with a pH electrode (PP-15, Sartorius, Goettingen, Germany). A cheese slurry was prepared by blending 20 g of grated cheese with 12 mL of water; then, 20 g of sample was mixed with 250 mL of distilled water and filtered through Whatman #1 filter paper to determine the total titratable acidity (TTA, T°), and 25 mL of the filtered sample was titrated with 0.1 mol/L NaOH. Phenolphthalein was used as an indicator.

Analysis of Total Phenolic Compound Content and Determination of Free Radical Scavenging Activity

The total phenolic compounds (TPC) content of basil-LAB bioproducts was determined by using a spectrophotometric method, as reported by Vaher et al. (2010). The absorbance of samples was measured at 765 nm using a V-1100D spectrophotometer (J.P. Selecta S.A., Barcelona, Spain). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (FRSA) of the samples was determined according to the method reported by Zhu et al. (2011).

Evaluation of Color, Texture, and Overall Acceptability

Color characteristics of basil-LAB bioproducts and curd cheese were assessed using a CIELAB system (Chromameter CR-400, Konica Minolta, Tokyo, Japan). The hardness parameter was evaluated by using a Brookfield texture analyzer (Middleborough, MA). Sensory analysis of basil-LAB bioproducts and curd cheese was carried out according to ISO method 6658 (ISO, 2017) for overall acceptability by 10 selected assessors using a 5-point hedonic scale ranging from 1 (extremely dislike) to 5 (extremely like). Ten assessors were recruited internally (Department of Food Safety and Quality, Lithuanian University of Health Sciences, Kaunas, Lithuania; ISO, 2008, 2012): 5 females and 5 males, from 35 to 45 yr old. Individuals who were

familiar with this study were excluded from the panel. The previous training of the assessors was based on descriptive analysis (Gaze et al., 2015; Janiaski et al., 2016; Torres et al., 2017). Selected assessors were non-smokers, interested in sensory analysis, and motivated to participate.

Microbiological Analysis of Basil-LAB Bioproducts and Unripened Goat Milk Cheese

Viability of LAB was evaluated in basil-LAB bioproducts and cheese samples; LAB counts were determined on MRS agar (Liofilchem, Roseto degli Abruzzi, Teramo, Italy) using standard plate count techniques (ISO, 1998). Plates were incubated at 30°C for 72 h under anaerobic conditions (using an AnaeroGen atmosphere generation system; Oxoid).

Curd cheese analysis was performed after 24, 72, and 120 h of storage at 4°C ; LAB counts were determined on MRS agar (Liofilchem) using standard plate count techniques. Enterobacteria were determined on violet red bile glucose agar (VRBA, Liofilchem) after incubation at 37°C for 24 h. Yeasts and fungi were determined on chloramphenicol agar (CM0549, Oxoid) after incubation at 25°C for 5 d. All experiments were carried out in triplicate, and the number of microorganisms was expressed as $\log_{10}$ of colony-forming units per gram ($\log_{10}$ cfu/g).

Statistical Analysis

All analytical determinations were performed at least in triplicate. Data obtained were analyzed using the statistical package SPSS for Windows XP V15.0 (2007; SPSS Inc., Chicago, IL). Significance of differences between treated samples was evaluated using Duncan's multiple range tests at a 5% level.

RESULTS AND DISCUSSION

Acidity Parameters and LAB Count in Basil-LAB Bioproducts

Acidity parameters and LAB count in basil-LAB bioproducts are shown in Figure 1. Comparing the pH of samples before and after fermentation, the greatest decrease in pH was found in basil fermented with strain LUHS244 (25.4% decrease), and the smallest in spontaneously fermented basil and basil fermented with strain LUHS29 (14.6 and 17.5% decreases, respectively). Fermentation with LAB generally increases the organic acid mass fraction and antioxidant activity of the fermentable substrate (Bustos et al., 2017). The

essential oil of basil is characterized by a high degree of antimicrobial activity but it does not inhibit the development of LAB in dairy starter cultures (Kostova et al., 2014). The LAB count in fermented basil ranged from 7.02 ± 0.08 to $7.47 \pm 0.06 \log_{10}$ cfu/g (fermented with LUHS135 and LUHS100, respectively), and in all cases was higher than the LAB count in nonfermented basil ($3.02 \pm 0.07 \log_{10}$ cfu/g). However, acid formation in cheese samples with added basil essential oil can be lower but within acceptable values (Kostova et al., 2016). According to our results, basil-LAB bioproducts could be promising ingredients for cheese manufacturing because LAB were not inhibited and showed good growth and acidification rates in basil substrate.

TPC Content and FRSA Before and After Immobilization

The results for TPC content and DPPH FRSA of basil-LAB bioproducts before and after fermentation and immobilization are given in Figure 2a and 2b, respectively. Fermentation with selected LAB significantly increased ($P \leq 0.05$) the TPC content in basil-LAB bioproducts (by 12.0% in basil fermented with LUHS140 and by 49.8% in basil fermented with LUHS135). However, we did not establish a significant effect of fermentation on the FRSA (by DPPH assay) of basil-LAB bioproducts. The immobilization process did not affect the TPC content in basil-LAB bioproducts, but a significantly lower FRSA was obtained for immobilized basil samples fermented with LUHS29,

LUHS244, and LUHS135 (by 12.4, 10.2, and 11.7%, respectively; $P \leq 0.05$). The use of LAB or lactic acid fermentation is considered one of the most suitable technologies to exploit the functional potential of plant matrices and to enrich them with bioactive compounds (Pellati et al., 2004). Fermentation with selected LAB is largely used to enhance antimicrobial, antioxidant, and immune-modulatory features (Rizzello et al., 2013). Enhancement of antioxidant activity during fermentation is mainly ascribed to the release of bioactive compounds by LAB (Hur et al., 2014), and the addition of basil can increase the functional properties of dairy products (Mosiyan et al., 2017). Basil could be used in the food industry as a safer alternative to synthetic antioxidants (Stanojevic et al., 2017).

Figure 1. pH and lactic acid bacteria (LAB) count ($\log_{10}$ cfu/g) in basil-LAB bioproducts (data are expressed as mean $\pm$ SD; $n = 3$). NSF = fermented spontaneously, F = fermented; 135 = *Lactobacillus plantarum* LUHS135, 244 = *Lactobacillus paracasei* LUHS244, 100 = *Pediococcus pentosaceus* LUHS100, 29 = *Pediococcus acidilactici* LUHS29, and 140 = *Lactobacillus brevis* LUHS140.

Figure 2. (a) Total phenolic compound (TPC) content (mg of gallic acid equivalents, GAE) and (b) 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (FRSA) of basil-lactic acid bacteria (LAB) bioproducts (data are expressed as mean $\pm$ SD; $n = 3$). NSF = fermented spontaneously, F = fermented, I = immobilized; 135 = *Lactobacillus plantarum* LUHS135, 244 = *Lactobacillus paracasei* LUHS244, 100 = *Pediococcus pentosaceus* LUHS100, 29 = *Pediococcus acidilactici* LUHS29, and 140 = *Lactobacillus brevis* LUHS140. Mean values within a column with different letters (a–f) are significantly different ($P \leq 0.05$).

Overall Acceptability and Color Characteristics Before and After Immobilization

Overall acceptability and color characteristics of basil-LAB bioproducts before and after immobilization are shown in Table 1. Spontaneously fermented basil had the poorest overall acceptability (2 points) and was not selected for preparation of goat milk fresh cheese. Immobilization did not affect the overall acceptability of basil-LAB bioproducts; the most acceptable were those fermented with LUHS140 (5 points), followed by samples fermented with LUHS135, LUHS244, and LUHS29 (4 points), and samples fermented with LUHS100 (3 points).

Immobilization had a different influence on the lightness values (L^*) of the bioproducts because the different metabolites formed by the different LAB can influence color. In samples fermented with LUHS135, LUHS100, and LUHS140 after immobilization, L^* increased by 6.3, 8.2, and 7.4%, respectively; in samples fermented with LUHS29, it decreased by 6.8%, and in samples fermented with LUHS244, no significant changes in L^* were observed after immobilization. After immobilization, the redness (a^*) of basil-LAB bioproducts fermented with LUHS135, LUHS244, and LUHS100 increased significantly (by 20.6, 16.8, and 5.8%, respectively; $P \leq 0.05$). In contrast, a^* values for samples fermented with LUHS29 and LUHS140 decreased after immobilization (by 19.5 and 18.6%, respectively; $P \leq 0.05$). Significantly different values of yellowness (b^*) were detected in basil-LAB products after immobilization. In samples fermented with LUHS135, LUHS244, and LUHS140, we detected a significant increase in b^* value; in contrast, a significant decrease in b^* value was detected in samples fermented with LUHS100 and LUHS29 (by 10.7 and 6.8%, respectively; $P \leq 0.05$).

In terms of functional properties, LAB can contribute to the release or production of compounds such as exopolysaccharides, vitamins, bioactive peptides, bioactive lipids, and enzymes in food; generally, fermented foods prepared with autochthonous LAB have better sensory acceptance (de Souza and Silva Dias, 2017). Our previous study showed that the chemical composition of volatile compounds had a less strong odor (lower concentration of essential oils) and was more acceptable to consumers, and some of the compounds are transformed by the appearance of new volatile compounds as a result of fermentation with different LAB (Juodeikiene et al., 2013). However, more research is needed on the transformation of basil volatile compounds during this process. Color is extremely important in food products because of its direct effect on appearance, and color is related to consumer acceptance (IFIC-FDA, 2010). Low L^* values (darkening) are associated with

Table 1. Overall acceptability (5-point hedonic scale ranging from 1 = extremely dislike to 5 = extremely like) and color characteristics of basil-lactic acid bacteria (LAB) bioproducts¹

Parameter ²	Bioproduct ³									
	NSF	F135	F244	F100	F29	F140	F135	F1244	F1100	F1140
Overall acceptability	2 ± 1 ^a	4 ± 1 ^b	4 ± 1 ^b	3 ± 1 ^a	4 ± 1 ^b	5 ± 1 ^c	4 ± 1 ^b	4 ± 1 ^b	3 ± 1 ^a	5 ± 1 ^c
L^*	24.54 ± 0.25 ^b	27.85 ± 0.28 ^c	25.10 ± 0.10 ^c	26.24 ± 0.26 ^d	28.96 ± 0.29 ^f	25.12 ± 0.28 ^c	29.60 ± 0.15 ^d	25.01 ± 0.19 ^c	28.38 ± 0.21 ^f	26.98 ± 0.18 ^d
a^*	5.11 ± 0.15 ^e	3.49 ± 0.23 ^b	3.57 ± 0.15 ^b	3.65 ± 0.14 ^b	4.00 ± 0.10 ^{cd}	4.78 ± 0.07	4.21 ± 0.11 ^d	4.17 ± 0.13 ^d	3.86 ± 0.19 ^c	3.89 ± 0.15 ^c
b^*	17.48 ± 0.17 ^c	16.48 ± 0.16 ^b	17.90 ± 0.13 ^d	18.04 ± 0.17 ^d	18.30 ± 0.18 ^d	18.86 ± 0.23 ^f	20.29 ± 0.35 ^f	20.84 ± 0.25 ^f	16.22 ± 0.13 ^b	20.14 ± 0.31 ^f

^{a-f}Mean values within a column with different letters are significantly different ($P \leq 0.05$).

¹Data are expressed as means ± SD ($n = 3$).

² L^* = lightness; a^* = redness ($-a^*$ of greenness); b^* = yellowness ($-b^*$ of blueness).

³NSF = spontaneously fermented basil bioproduct; F135 = basil fermented with *Lactobacillus plantarum* LUHS135; F244 = basil fermented with *Lactobacillus paracasei* LUHS244; F100 = basil fermented with *Pediococcus pentosaceus* LUHS100; F29 = basil fermented with *Pediococcus acidilactici* LUHS29; F140 = basil fermented with *Lactobacillus brevis* LUHS140; F135 = basil fermented with *Lactobacillus plantarum* LUHS135 and immobilized in agar; F244 = basil fermented with *Lactobacillus paracasei* LUHS244 and immobilized in agar; F100 = basil fermented with *Pediococcus pentosaceus* LUHS100 and immobilized in agar; F29 = basil fermented with *Pediococcus acidilactici* LUHS29 and immobilized in agar; F140 = basil fermented with *Lactobacillus brevis* LUHS140 and immobilized in agar.

oxidation and enzymatic and microbiological degradation and are undesirable because they cause consumer rejection (Dattatreya and Rankin, 2006). Nonetheless, fermentation with selected LAB (because they produce different metabolites and have an influence on the different chemical composition of the fermentable substrate) has a different influence on the color characteristics of basil-LAB bioproducts because color can be influenced by metabolites formed (acids, peroxide, and so on).

Effect of Basil-LAB Bioproducts on Acidity, Texture, Color, and Overall Acceptability of Goat Milk Fresh Cheese

Results for acidity and texture parameters and for overall acceptability of goat milk fresh cheese are presented in Tables 2 and 3. Comparing all cheese samples, a significantly higher pH was found for cheese samples with nonimmobilized basil fermented with LUHS244 (5.82 ± 0.06 ; Table 2), and use of different LAB for preparation of basil-LAB bioproducts had a significant effect on cheese pH ($P = 0.04$; Table 3). Immobilization and the interaction of immobilization and different LAB were not significant for cheese pH (Table 3). A significantly higher TTA ($P \leq 0.05$) was obtained for cheese samples prepared with untreated basil and for samples prepared with basil fermented with LUHS135, LUHS244, LUHS100, and LUHS29 (higher by 33.3, 53.3, 40.0, 53.3, 33.3, and 66.7%, respectively). In contrast to pH, the TTA of cheese samples was significantly affected by the immobilization process ($P = 0.002$), and the interaction of the factors analyzed was significant ($P = 0.004$). The hardest texture was obtained for the cheese samples prepared with untreated basil and for samples prepared with basil fermented with LUHS244 and immobilized basil-LAB bioproducts (0.5 mJ). Use of different LAB for basil fermentation significantly affected cheese hardness ($P = 0.001$), and the interaction of different LAB and the immobilization process was significant ($P = 0.001$). Cheese-making relies on the fermentation of lactose by LAB to produce lactic acid, which lowers the pH and, in turn, assists coagulation, promotes syneresis, and contributes to cheese texture. Lactic acid bacteria also produce lipases and proteases, which are necessary for cheese texture formation (Medjoudj et al., 2018). A very weak positive correlation was found between cheese hardness and TTA ($r = 0.11$). Overall acceptability of cheese was significantly influenced by the immobilization process ($P = 0.011$), and a moderate positive correlation was found between TTA and the overall acceptability of cheese samples ($r = 0.53$). According to Costa et al. (2014), a correlation exists between the exposure sessions and an increase in acceptance of the exposure groups, and it

Table 2. pH, total titratable acidity (TTA), color characteristics, and overall acceptability of curd cheese¹

Parameter ²	Curd cheese ³											
	CC	CNF	CF135	CF244	CF100	CF29	CF140	CF135	CF1244	CF1100	CF129	CF1140
pH	5.42 ± 0.04 ^a	5.31 ± 0.10 ^b	5.40 ± 0.07 ^a	5.82 ± 0.06 ^b	5.44 ± 0.06 ^a	5.33 ± 0.12 ^a	5.42 ± 0.10 ^b	5.46 ± 0.06 ^a	5.51 ± 0.11 ^a	5.40 ± 0.11 ^a	5.41 ± 0.10 ^a	5.31 ± 0.06 ^a
TTA, T ^o	0.15 ± 0.03 ^{ab}	0.20 ± 0.09 ^c	0.12 ± 0.06 ^a	0.11 ± 0.04 ^a	0.11 ± 0.05 ^a	0.13 ± 0.02 ^a	0.23 ± 0.04 ^c	0.21 ± 0.05 ^c	0.23 ± 0.03 ^c	0.20 ± 0.05 ^c	0.25 ± 0.04 ^{cd}	0.13 ± 0.06 ^a
Texture, mJ	0.30 ± 0.05 ^b	0.50 ± 0.07 ^d	0.30 ± 0.09 ^b	0.30 ± 0.07 ^b	0.30 ± 0.07 ^b	0.40 ± 0.04 ^c	0.20 ± 0.06 ^b	0.20 ± 0.02 ^a	0.50 ± 0.06 ^d	0.40 ± 0.06 ^c	0.30 ± 0.05 ^b	0.30 ± 0.06 ^b
L*	90.90 ± 0.96 ^k	69.69 ± 0.48 ^b	64.75 ± 0.51 ^a	73.58 ± 0.29 ^c	74.66 ± 0.25 ^c	71.87 ± 0.45 ^d	70.34 ± 0.76 ^e	80.19 ± 0.73 ^f	83.43 ± 0.69 ^j	81.27 ± 0.63 ^h	83.33 ± 0.52 ⁱ	81.84 ± 0.63 ^h
a*	5.78 ± 0.41 ^b	4.04 ± 0.31 ^a	6.71 ± 0.36 ^d	6.20 ± 0.15 ^c	6.79 ± 0.19 ^d	6.50 ± 0.34 ^d	6.18 ± 0.23 ^c	6.74 ± 0.27 ^d	6.56 ± 0.41 ^d	7.05 ± 0.32 ^e	6.09 ± 0.21 ^c	6.92 ± 0.42 ^e
b*	21.19 ± 0.15 ^b	22.41 ± 0.31 ^c	25.17 ± 0.20 ^f	24.36 ± 0.19 ^e	25.77 ± 0.29 ^f	20.47 ± 0.31 ^a	22.62 ± 0.19 ^e	24.97 ± 0.16 ^e	23.75 ± 0.19 ^d	25.00 ± 0.31 ^f	26.30 ± 0.18 ^g	24.78 ± 0.21 ^e
Overall acceptability	4 ± 1 ^a	4 ± 1 ^a	4 ± 1 ^a	4 ± 1 ^a	4 ± 1 ^a	4 ± 1 ^a	4 ± 1 ^a	5 ± 1 ^b	5 ± 1 ^b	5 ± 1 ^b	5 ± 1 ^b	5 ± 1 ^b

^{a-f}Mean values within a column with different letters are significantly different ($P \leq 0.05$).

¹Data are expressed as mean ± SD ($n = 3$).

²L* = lightness; a* = redness (–a* of greenness); b* = yellowness (–b* of blueness).

³CC = control cheese samples produced without basil or basil bioproducts; C = cheese; basil or basil bioproducts used for cheese preparation: NF = nonfermented; F = fermented; I = immobilized; 135 = *Lactobacillus plantarum* LUHS135, 244 = *Lactobacillus paracasei* LUHS244, 100 = *Pediococcus pentosaceus* LUHS100, 29 = *Pediococcus acidilactici* LUHS29, 140 = *Lactobacillus brevis* LUHS140.

could be that increasing exposure sessions could be a strategy to increase goat milk product acceptance. Finally, further studies, as well as projective methods, are recommended to establish the sensory profiling and consumer perception of cheese sensory properties (Fonseca et al., 2016; Esmerino et al., 2017; Oliveira et al., 2017). In all cases, basil-LAB bioproducts reduced the L^* values of cheese samples, but higher L^* values were established for cheese samples prepared with immobilized compared with immobilized basil-LAB bioproducts (Figure 3). Lightness values of 93.36 have been found for ricotta containing only goat milk (Borba et al., 2014), which is due to the presence of smaller fat globules compared with cow milk and conversion of β -carotene to vitamin A (Park et al., 2007). The factors analyzed in the current study (use of different LAB for basil fermentation and immobilization) and their interaction were significant for L^* values of cheese ($P = 0.0001$). In all cases, basil-LAB bioproducts had higher a^* values than the control cheese and cheese prepared with untreated basil, and use of different LAB for basil fermentation significantly affected a^* values of cheese ($P = 0.0001$). The lowest b^* value was observed for the control cheese samples (21.19 ± 0.15). In cheese samples prepared with fermented nonimmobilized basil-LAB bioproducts, b^* values ranged from 20.47 ± 0.31 to 25.77 ± 0.29 (cheese samples prepared with LUHS29 and LUHS100, respectively); for cheese samples prepared with fermented immobilized basil-LAB bioproducts, b^* values ranged from 23.75 ± 0.19 to 26.30 ± 0.18 (cheese samples prepared with LUHS244

and LUHS29, respectively). All factors analyzed had a significant effect on the b^* values of cheese, and the interaction of factors analyzed was significant ($P = 0.0001$). In all cases, better acceptability was obtained for cheese samples prepared with fermented and immobilized basil-LAB bioproducts (5 points). Control cheese samples and samples prepared with nonimmobilized basil-LAB bioproducts had poorer acceptability (4 points). Many factors influence the flavor, texture, and sensory qualities of goat milk cheeses. The flavor of cheese arises from a series of complex reactions involving microbial metabolism and enzymatic reactions, which include proteolysis of proteins, lipolysis of fats, and fermentation of carbohydrates (Moatsou and Park, 2017). Selected LAB improve nutritional quality, shelf life, and acceptability of soft white goat cheese (Mohammed et al., 2018), and basil enhances the volatile composition and acceptability of dairy products (Gurkan and Hayaloglu, 2017). According to our results, the use of immobilized basil-LAB bioproducts is a suitable method to improve the overall acceptability of fresh goat cheese; immobilization leads to the production of cheese with better acceptability compared with cheese produced with nonimmobilized basil.

Microbiological Parameters of Curd Cheese During Storage

Microbiological parameters of curd cheese during storage are shown in Table 4. After 24 h of storage, no enterobacteria or mold/yeast were found in cheese

Table 3. The influence of different lactic acid bacteria (LAB) and immobilization process on fresh cheese parameters¹

Source	Dependent variable ²	Mean square	F-value	P-value
Different LAB	pH	0.051	4.489	0.04
	TTA, T°	0.002	0.898	0.512
	Texture, mJ	0.035	9.541	0.0001
	L^*	197.497	589.265	0.0001
	a^*	2.767	28.027	0.0001
	b^*	5.901	104.502	0.0001
	Overall acceptability	0.0001	0.0001	1.000
Immobilization	pH	0.015	1.267	0.271
	TTA, T°	0.031	12.370	0.002
	Texture, mJ	0.012	3.258	0.084
	L^*	903.215	2,694.891	0.0001
	a^*	0.288	2.918	0.100
	b^*	12.519	221.725	0.0001
	Overall acceptability	7.500	7.500	0.011
Different LAB $\times$ immobilization	pH	0.22	1.902	0.143
	TTA, T°	0.013	5.213	0.004
	Texture, mJ	0.027	7.330	0.001
	L^*	15.321	45.712	0.0001
	a^*	0.271	2.741	0.052
	b^*	11.786	208.743	0.0001
	Overall acceptability	0.0001	0.0001	1.000

¹Data are expressed as mean $\pm$ SD ($n = 3$).

²TTA = total titratable acidity; L^* = lightness; a^* = redness ($-a^*$ of greenness); b^* = yellowness ($-b^*$ of blueness).

samples, and the LAB count in cheese samples prepared with basil-LAB bioproducts was significantly higher than that of control cheese or cheese samples prepared with nonfermented basil. In all of the cheese samples prepared with basil-LAB bioproducts, the LAB count was $>6.0 \log_{10}$ cfu/g, meeting the requirements for probiotic products. Comparing the LAB count in cheese samples prepared with nonimmobilized and immobilized basil-LAB bioproducts, a significantly higher ($P \leq 0.05$) LAB count was found in cheese samples prepared with immobilized LUHS135, LUHS244, LUHS29, and LUHS140 basil-LAB bioproducts (by 12.3, 10.5, 7.9, and 20.8%, respectively). According to the results obtained, in most cases (except LUHS100 bioproducts), immobilization increased the stability of LAB in

cheese, and a higher viable cell count was obtained in the end product. The average LAB count in fresh goat cheese prepared from nonpasteurized milk can reach 10^8 cfu/g (Randazzo et al., 2002; Janštová et al., 2010). The relatively low level of contamination in the samples of natural fresh cheese can be explained by the fact that the cheese in the current study was made from pasteurized milk (Janštová et al., 2010). After 72 h of storage, enterobacteria were found in control cheese samples (prepared without basil or with untreated basil) and in cheese samples prepared with LUHS100 and LUHS29 basil-LAB bioproducts. A moderate negative correlation was obtained between LAB count and enterobacteria in cheese samples ($r = -0.6365$). *Enterobacteriaceae* counts in milk can reach 10^2 to 10^3 cfu/g under normal

Figure 3. Images of the unripened goat milk curd cheese. CC = control cheese produced without basil bioproducts; C = cheese, NF = nonfermented, F = fermented, I = immobilized; 135 = *Lactobacillus plantarum* LUHS135, 244 = *Lactobacillus paracasei* LUHS244, 100 = *Pediococcus pentosaceus* LUHS100, 29 = *Pediococcus acidilactici* LUHS29, and 140 = *Lactobacillus brevis* LUHS140. Color version available online.

Table 4. Microbiological parameters (counts, log₁₀ cfu/g) of curd cheese during storage¹

Parameter	Sample ²											
	C	NF	F135	F244	F100	F29	F140	F1135	F1244	F1100	F129	F1140
After 24 h												
LAB	2.34 ± 0.12 ^a	3.20 ± 0.14 ^b	6.40 ± 0.15 ^d	6.41 ± 0.10 ^d	6.92 ± 0.12 ^e	6.83 ± 0.10 ^e	6.05 ± 0.13 ^e	7.19 ± 0.10 ^f	7.08 ± 0.13 ^f	6.39 ± 0.10 ^d	7.37 ± 0.08 ^g	7.31 ± 0.12 ^g
Enterobacteria	—	—	—	—	—	—	—	—	—	—	—	—
Mold/yeast	—	—	—	—	—	—	—	—	—	—	—	—
After 72 h												
LAB	3.89 ± 0.10 ^a	5.16 ± 0.12 ^b	6.43 ± 0.15 ^d	6.80 ± 0.12 ^d	6.69 ± 0.13 ^d	6.35 ± 0.13 ^d	6.55 ± 0.10 ^d	7.10 ± 0.11 ^e	7.00 ± 0.10 ^e	5.87 ± 0.13 ^c	6.59 ± 0.09 ^d	6.29 ± 0.10 ^d
Enterobacteria	2.34 ± 0.12 ^d	2.42 ± 0.10 ^d	—	—	2.04 ± 0.09 ^e	2.12 ± 0.10 ^e	—	—	—	1.32 ± 0.07 ^a	1.57 ± 0.10 ^b	—
Mold/yeast	—	—	—	3.05 ± 0.07 ^b	3.26 ± 0.09 ^e	2.46 ± 0.10 ^a	2.34 ± 0.03 ^a	—	—	—	—	—
After 120 h												
LAB	4.52 ± 0.14 ^a	4.47 ± 0.10 ^a	6.4 ± 0.11 ^d	6.77 ± 0.09 ^e	6.13 ± 0.10 ^e	6.76 ± 0.10 ^e	6.50 ± 0.09 ^d	7.10 ± 0.07 ^f	7.08 ± 0.11 ^f	5.45 ± 0.09 ^b	5.40 ± 0.11 ^b	6.10 ± 0.09 ^c
Enterobacteria	5.21 ± 0.10 ^c	5.03 ± 0.09 ^c	—	—	3.34 ± 0.14 ^b	3.29 ± 0.10 ^b	—	—	—	2.34 ± 0.03 ^a	2.34 ± 0.03 ^a	—
Mold/yeast	4.16 ± 0.10 ^f	2.91 ± 0.12 ^c	2.28 ± 0.06 ^b	3.95 ± 0.11 ^e	3.42 ± 0.08 ^d	2.34 ± 0.03 ^b	3.29 ± 0.11 ^d	—	2.10 ± 0.10 ^a	2.39 ± 0.08 ^b	2.45 ± 0.12 ^b	2.30 ± 0.11 ^b

^{a-f}Mean values within a column with different letters are significantly different ($P \leq 0.05$).
¹Data are expressed as mean ± SD (n = 3).
²C = cheese; NF = nonfermented, F = fermented, and I = immobilized basil or basil bioproducts used for cheese preparation; 135 = *Lactobacillus plantarum* LUHS135, 244 = *Lactobacillus paracasei* LUHS244, 100 = *Pediococcus pentosaceus* LUHS100, 29 = *Pediococcus acidilactici* LUHS29, 140 = *Lactobacillus brevis* LUHS140.

conditions, and the counts may increase during the first week to reach 10⁶ to 10⁷ cfu/g in the inner portion of cheese; however, *Enterobacteriaceae* are sensitive to low pH values, and the use of LAB starters can reduce *Enterobacteriaceae* contamination by lowering the pH (Tornadijo et al., 2001). A weak positive correlation was found between LAB count and mold/yeast count in all cheese samples (r = 0.3142). Although a high LAB count in cheese can reduce enterobacteria, yeasts are usually in a symbiotic relation with LAB; thus, increasing LAB has no influence on yeast count. After 120 h of storage, similar tendencies after 72 h were obtained for the enterobacteria; however, in cheese samples prepared with basil-LAB bioproducts, the enterobacteria count was lower by 35.9 and 36.9% in cheese samples prepared with nonimmobilized LUHS100 and LUHS29 bioproducts, respectively, and lower by 55.1% in cheese samples prepared with immobilized LUHS100 and LUHS129 bioproducts, respectively, than that in control cheese samples. In some cases, enterobacteria were not found (in cheese samples prepared with basil-LAB bioproducts fermented with LUHS135, LUHS244, and LUHS140). After 120 h of storage, strong and moderate negative correlations were found between LAB count and enterobacteria count and between LAB count and yeast/mold count (r = −0.7939 and r = −0.4495, respectively). Goat milk is most commonly used for the production of fresh unripened cheese, and bacterial spoilage may occur in fresh cheeses having a sufficiently high pH (Janštová et al., 2010). Basil essential oils are promising antimicrobial and antifungal agents for food formulations; their antibacterial activity is attributed to their high linalool and estragole content, but the antimicrobial spectrum is restricted to specific bacteria (*Staphylococcus* spp., *Enterococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter* spp., *Listeria* spp., *Proteus* spp., *Salmonella* spp., *Serratia marcescens*, and *Yersinia enterocolitica*) and fungi (*Candida* spp., *Rhodotorula* spp., and *Saccharomyces cerevisiae*) (Sakkas and Papadopoulos, 2017). Basil extracts are recommended as natural food preservatives because of their role in the control of food-borne pathogens as biological food contaminants, in addition to their antioxidant and anti-inflammatory activities (Hamad et al., 2017). According to our results, immobilization increases LAB viability in fresh cheese, and basil-LAB bioproducts reduce the contamination of cheese during storage.

CONCLUSIONS

Fermented basil could be a promising multifunctional ingredient for cheese manufacturing because its inclu-

sion resulted in cheese with a lower pH, a higher LAB count, a higher content of total phenolic compounds, and a lower enterobacteria count (in cheese samples prepared with basil-LAB bioproducts fermented with LUHS135, LUHS244, and LUHS140). Immobilization of the fermented basil led to higher sensory acceptability of cheeses. We confirmed the hypothesis of this study: immobilization increased LAB viability in fresh goat milk cheese, which led to a reduction in contamination during storage and an increase in overall acceptability compared with use of nonimmobilized basil-LAB bioproducts. Basil-LAB bioproducts fermented with LUHS135, LUHS244, and LUHS140 strains are recommended for the preparation of extended-shelf-life goat milk fresh cheese that is acceptable to consumers.

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