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ORIGINAL PAPER

The effect of *Lactobacillus rhamnosus* added to milk in the first hour after milking on the protection of cheesemaking suitability traits*

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Abstract

The quality of milk determines its technological usefulness, especially in cheese production. The presence of undesirable microorganisms in milk results in a decrease in its quality and destruction of the micellar structure of casein proteins. Unfavorable changes occurring in milk concern primarily the protein, therefore there is a need to protect this milk component. The aim of the experiment presented in this article was to clarify the influence of the addition of *Lactobacillus rhamnosus* (LR) on the hygienic quality and technological suitability of raw milk. The use of protective additives such as lactic acid bacteria (LR) strains helps to protect milk proteins. In this experiment, the effect of the addition of LR on the hygienic quality and cheesemaking usefulness of raw milk was examined. The changes occurring in milk were determined by analyses performed on milk samples taken 1, 5, 15, 25, 38 and 48 h after milking. In milk with the addition of LR, lower total bacteria count (TBC) variability and higher lactose content were noted, and a significant negative correlation between the lactose content and TBC was found. The amount of κ-casein in milk with LR was on average 0.006 mg mL^{-1} higher than in control milk ($p<0.05$), and the correlation with TBC was negative ($p<0.05$). The experiment also showed that the κ-casein content began to decrease after the 5th hour of storage, but this process was more dynamic in milk without bacterial protection (on average 0.003 mg mL^{-1} less). The study confirms that the addition of LR can effectively improve the quality of milk and its suitability for cheesemaking.

Keywords: cow's milk, casein, *Lactobacillus rhamnosus*

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INTRODUCTION

Milk for food production, including long-ripened cheeses, must be of the highest quality. However, despite its high quality standards, the storage and handling of raw milk often distorts its primary microflora. According to de Oliveira et al. (2015), Pinto et al. (2006) and Mcphee and Griffiths (2011), the bacteria found in milk are mainly psychrotrophic strains of *Pseudomonas*, *Achromobacter*, *Aeromonas*, *Serratia*, *Alcaligenes*, *Chromobacterium* and *Flavobacterium* spp. Less numerous groups include *Corynebacterium*, *Streptococcus*, *Lactobacillus* and *Microbacterium* spp. (Cempirkova 2002, Cempirkova 2007, Qin et al. 2023). The deterioration of milk quality is largely due to the presence of undesirable microorganisms, whose metabolism negatively affects the quality of the components that determine the use of this raw material in cheesemaking process (Nörnberg et al. 2010). These changes mainly affect the protein and fat fractions. There is therefore a need to look for possibilities to protect components such as protein and especially caseins, which determines the quality of the curd and cheese yield. The main aim of such measures is to protect the micellar structure.

Of the microorganisms present in milk, particular attention should be paid to bacteria of the *Clostridium* and *Bacillus* genera, which have the ability to survive heat treatment (De-Jonghe et al. 2010, Velázquez-Ordoñez et al. 2019). It has been shown that lowering the temp. of milk to 4°C is not sufficiently effective in inhibiting the metabolic activity of these two types of bacteria. The proteases they produce hydrolyze milk proteins, leading to changes which deteriorate their properties, consequently reducing their usefulness for obtaining good cheese yield (Fox et al. 2000). In order to mitigate these changes, additives are used at the beginning of the processing to favour the correct orientation of lipo- and proteolysis processes (Cleto et al. 2012, Marchand et al. 2012). One method of milk protein protection is the use of selected cultures of lactic bacteria (starter cultures). Their addition to pasteurized milk promotes protection against the development of pathogens, mainly by inhibiting their biosynthesis or binding in the cell wall (Dalie et al. 2010). Abbes et al. (2013) shows that among the tested bacterial strains, the greatest potential for binding aflatoxin in milk was demonstrated by the *Lactobacillus rhamnosus* strain. *Lactobacillus rhamnosus* is a gram-positive, facultative anaerobic bacterium that belongs to the *Lactobacillus* genus. It is commonly found in dairy products and other fermented foods (Calasso, Gobbetti 2011). The studies carried out so far confirm that *Lactobacillus rhamnosus* has a unique metabolic profile that focuses on carbohydrate metabolism and lactic acid production, with the possibility of utilizing amino acids and producing other metabolites (Makarova et al. 2006, Douillard et al. 2013). Moreover, studies suggest that *L. rhamnosus* has the potential to protect proteins from various types of environmental stress, including heat, acidic conditions, and oxidative stress.

The aim of the present experiment was to clarify the influence of the addition of *Lactobacillus rhamnosus* (LR) on the hygienic quality and technological suitability of raw milk. The experiment was based on the assumption that the applied treatment would not cause the exceedance of the accepted standards for the total number of microorganisms. It was hypothesized that the addition of LR would support the protection of casein fraction proteins of milk, thereby affecting the efficiency of fresh cheese production.

MATERIALS AND METHODS

Sampling

The research was carried out in January and February. The research material consisted of 7 composite samples of bulk milk. Samples were collected in 48-hour cycles (two morning and two evening milkings), simulating the 48-hour storage time of the raw material on the farm. The milk collected was divided into two groups: control (C) – without the addition of bacteria, and experimental (LR) – with the addition of *Lactobacillus rhamnosus* acting as a vaccine. LR bacteria were added to the milk (in a dose of 0.01 mg L^{-1}) after the end of each milking, which lasted an average of 1 hour. The samples were transported to a laboratory accredited by the Polish Accreditation Centre (Quality Certificate ICAR; PN-EN ISO/IEC 17025), where each sample was poured into 2 beakers (duplicate). The prepared milk samples were stored at 4°C. During that time, the milk was continuously stirred with a magnetic stirrer (Thermolyne®MirakTM hotplate, Merck KGaA, Darmstadt, DE). The changes occurring in the material thus obtained were determined by analyses performed in milk samples after 1, 5, 15, 25, 38 and 48 h after milking.

Milk analysis

Overall composition of milk (protein, fat, lactose and dry matter) was determined a Bentley Combi 150 device (Bentley Instruments Inc., Chaska, USA). The total bacterial count (TBC) was determined in raw milk on a Bactoscan 8000s (Bentley Instruments Inc., Chaska, USA). The total casein level was measured after precipitation and purification in acetate buffer (pH 4.6, 20°C). The first stage of the experiment involved clotting and purification of casein, which was performed using buffer with pH of 4.6. In the resulting sediment (clot), the total nitrogen level was measured using the Kjeldahl method (AOAC, 2000), and converting the results to the total protein level by the coefficient of 6.38. For the determination of total protein, a BUCHI kit (BUCHI Labortechnik AG 9230 Flawil, Switzerland) was used, compatible with the TitroLine®5000 titrator (SI Analytiks®, Xylem Analytics Germany Sales GmbH & Co, Germany). Measurements of α-, β- and κ-casein

were performed on an HPLC liquid chromatograph (Varian Inc., Palo Alto, USA). The resulting clot was dissolved in TRIS-HCl buffer (pH 8.0). Protein separation was performed on an Aeris XB-C-18 column, 3.6 u, 250×4.6 mm (Phenomenex Inc., Torrance, USA) at a temp. of 40.0°C (flow: 1.5 mL min^{-1} by 30 min), where eluent A was 0.1% trifluoroacetic acid aqueous solution (TFA), and eluent B was 0.1% TFA solution in acetonitrile. The concentration of proteins was measured on the basis of a calibration curve drawn according to the bovine standard (Sigma-Aldrich, St. Louis, USA). Based on the results, the casein:protein ratio was calculated. Milk acidity was determined by titration (titrimetric method, AOAC, 2000) and expressed in Soxhlet-Henkel degrees (°SH).

Curd making process

Milk samples (50 ml) were pasteurised for 90 s to reach 72°C. During pasteurisation, the samples were stirred, then they were cooled to 30°C and an enzyme preparation (Hymax M; 1:30.000) was added. After 30 min, the curd was cut and reheated in a water bath (50°C for 15 minutes), then cooled in a water bath to 20°C and left for about 15 min (curd settling). Separation of the curd from the whey was performed by gravity. The mass obtained was centrifuged (MPW 260 RH, MPW Med. Instruments, Warsaw, PL) at a speed of 2500×g for 30 min at a temp. of 20°C. The separated curd was stored at -75°C until lyophilization was performed, which was carried out until a constant sample weight was reached (Alpha 1-2LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, D). Total protein and crude fat contents were determined in the freeze-dried product (AOAC, 2000). Based on the difference in weight of the samples before and after freeze-drying, the dry matter content was calculated. The amount of ash was determined after burning the samples in a muffle furnace (FCF 22 S, Czylok, Jastrzebie Zdroj, PL) at a temp. of 450°C. Based on the weights of the milk used and the curd obtained, the cheesemaking yield expressed as a percentage was calculated. The quality of fresh curd structure was assessed on the basis of water holding capacity (WHC), using own modification of the Pohja and Niinivaara (1957) method. The modification involved a load of 0.2 kg of curd placed between two glass plates. The amount of leakage was measured on blotting paper (88 g m^{-2}; Whatman, Inc., Maidstone, GB) and expressed as a percentage. In the whey obtained, the mass of the parts not retained in the curd structure (cheesecake dust) was determined using separation on strainers (84 g m^{-2}; Alchem Group Sp. z o.o., Torun, PL) and subsequent freeze-drying to a constant sample weight (Alpha 1-2LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, D). The cheese dust content was calculated from the difference in sample weight before and after lyophilization.

Statistical analysis

The results were compiled statistically in Statistica 13.0 software (StatSoft Inc., Tulsa, OK, USA). A one-way analysis of variance was used (ANOVA). The data were mathematically calculated using analysis of variance with repeated measures, according to a linear model (GLM). The significance of differences between the averages was estimated using the Duncan test at $P<0.05$. The results expressed as mean values and standard error (SEM) are presented in tables and graphs. The visualization of the results consists of charts showing the average values for the tested features and the standard deviation (SD).

RESULTS AND DISCUSSION

Our analysis of the results for the main components of bulk milk showed that the use of *Lactobacillus rhamnosus* (LR) inhibited the breakdown of lactose during 48 hours of storage (Table 1). On average, LR milk contained 0.07 percentage point (p.p.) more of this component compared to control milk ($p<0.05$). At the same time, a significant negative correlation was found between the lactose content and TBC in milk ($p<0.05$). An effect of the changes associated with the lower lactose content of C milk was the difference in acidity found between the groups ($p<0.05$). This difference, as well as the positive correlation found between acidity and TBC ($p<0.05$), indicates that the application of milk protection in the form of LR reduces milk acidification caused by the titratable acidity of lactose-metabolising bacteria.

Table 1

Composition of control milk and milk with the addition of *Lactobacillus rhamnosus*

Component	Mean value		SEM	Correlation TBCx
	control milk C	milk with addition of LR		
Protein (%)	3.56	3.57	0.003	-0.142
Fat (%)	3.44	3.42	0.001	-0.168
Lactose (%)	4.41^{a}	4.48^{b}	0.005	-0.376*
Dry matter (%)	11.49	11.51	0.006	0.109
Casein:protein (%)	77.2^{a}	77.8^{b}	0.059	-0.763*
Total casein (mg mL^{-1})	2.73^{a}	2.77^{b}	0.002	-0.446*
κ-casein	0.223^{a}	0.229^{b}	0.005	-0.368*
α-casein	0.832	0.834	0.003	-0.219
β-casein	1.659	1.663	0.004	-0.221
Acidity (°SH)	6.79^{a}	6.74^{b}	0.026	0.495*

$^{a, b}$ – means in a row with different letters differ significantly ($p<0.05$), * <0.05

Between the studied groups, there were no significant differences in total protein, fat and dry matter. These components were relatively weakly correlated with TBC. As shown in Table 1, milk with LR protection applied had a higher total casein content and a higher casein:protein ratio ($p<0.05$). Negative and relatively strong correlations of both indices with TBC were also shown ($p<0.05$). With regard to κ-casein, its amount in LR milk was on average 0.006 mg mL^{-1} higher than in C milk ($p<0.05$). Also in this case, a negative correlation with TBC was found ($p<0.05$). The analysis of these relationships presented in Figure 1 shows that the labelled κ-casein values in C milk were subject to greater dispersion compared to LR milk (Figure 2).

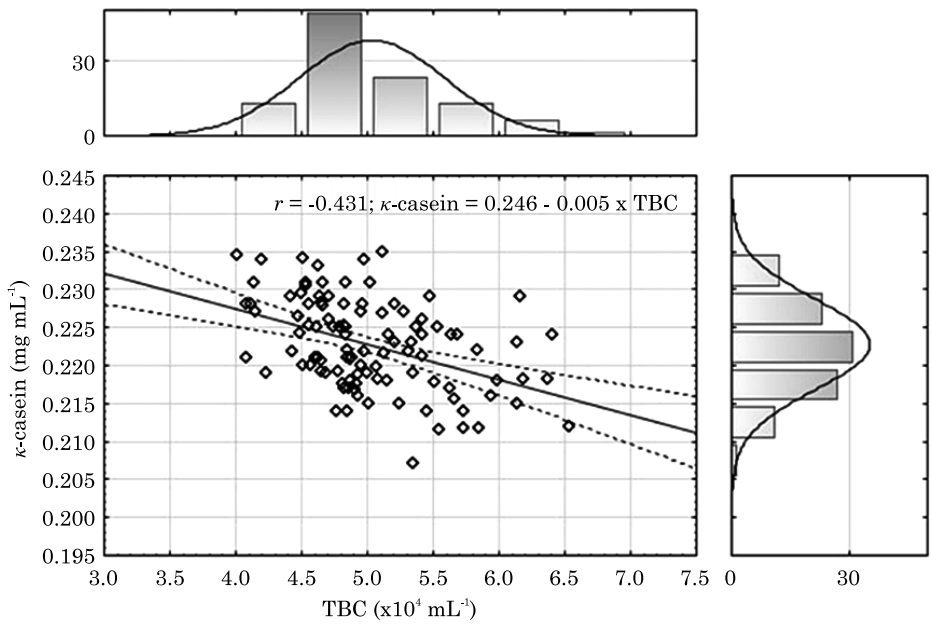

Fig. 1. The correlation coefficient of total bacteria count (TBC) and κ-casein in control milk (C)

The results of the analyzed milk parameters presented in Table 2 showed that the 48-hour storage time reduced the content of lactose and total casein ($p<0.05$). Compared to the first hour of storage, the differences were 0.04 p.p. and 0.02 mg mL^{-1} respectively. The consequence of the changes resulting from the bacterial degradation of lactose was an increase in the acidity of the stored milk, which was confirmed by the difference of 0.08°SH ($p<0.05$). Changes in the casein to protein ratio were also detected ($p<0.05$). It was found that after 25 h of storage, the share of casein in the total protein content decreased. The negative microbial influence on this parameter was also confirmed by the positive correlation between milk acidity and TBC ($p<0.05$, Table 1).

The results presented in Figure 3 show that the addition of *Lactobacillus rhamnosus* to milk did not cause an excessive increase in TBC. Indeed,

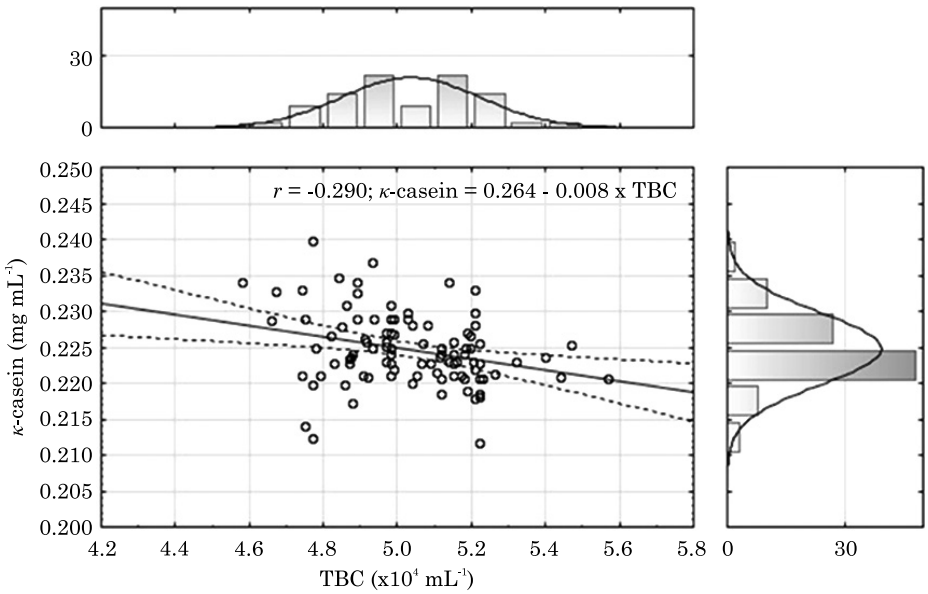

Fig. 2. The correlation coefficient of total bacteria count (TBC) and κ-casein in milk with the addition of Lactobacillus rhamnosus (LR)

Table 2

Comparison of milk composition in different storing time

Component	Storing time (h)						
	1	5	15	25	28	38	48
Lactose (%)	4.49^{a}	4.49^{a}	4.47^{b}	4.46^{b}	4.46^{b}	4.45^{c}	4.45^{c}
Casein:protein (%)	77.5^{a}	77.4^{a}	77.4^{a}	77.2^{c}	77.2^{c}	77.2^{c}	77.2^{c}
Total casein (mg mL^{-1})	2.77^{a}	2.77^{a}	2.78^{a}	2.76^{ab}	2.76^{ab}	2.75^{b}	2.75^{b}
Titratable acidity (°SH)	6.71^{a}	6.70^{a}	6.71^{a}	6.73^{b}	6.74^{b}	6.79^{c}	6.79^{c}

$^{a, b, c}$ – means in a row with different letters differ significantly (p<0.05)

the values of this characteristic in the first hour of milk storage ranged from 4.89 x104 mL^{-1} in C milk to 4.95 x104 mL^{-1} in LR milk. In both types of milk, a similar trend of increase in microbial counts was noted. The TBC in C milk increased by 0.2 x104 mL^{-1} and in LR milk by 0.15 x104 mL^{-1}. The differences between the mean TBC values were not statistically confirmed, but it is noteworthy that significantly smaller standard deviation (SD) values were found in LR milk (0.252 – 0.134 x104 mL^{-1}). In unprotected (C) milk, the SD values were several times higher than the SD values found in LR milk (0.707 – 0.350 x104 mL^{-1}).

The result concerning the maximum TBC values obtained after 48 h of storage also remains important (Figure 3). The values obtained did not exceed the limits for bulk milk, i.e. the EU standard less than 100.000 ml (Regulation EC, 2004). In the case of LR milk, this fact is particularly interes-

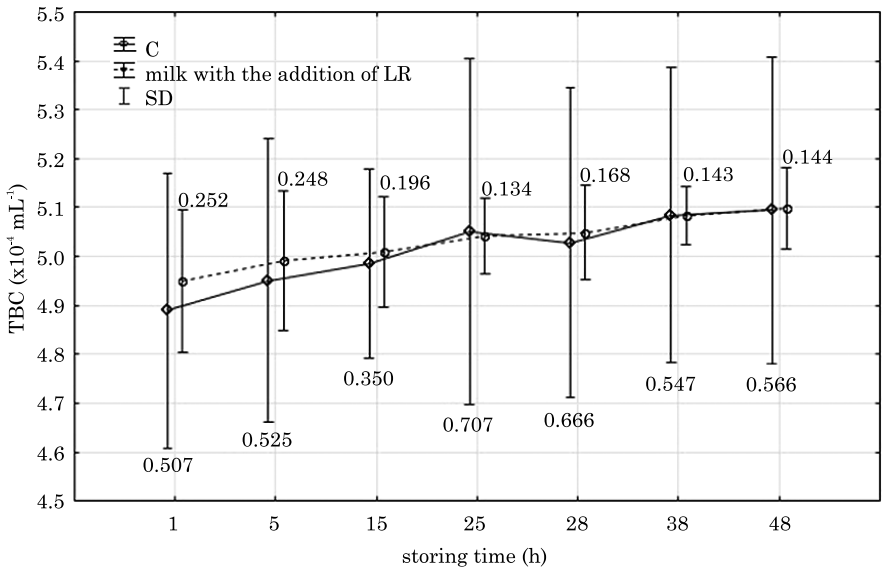

Fig. 3. Average content and standard deviation (SD) of TBC in bulk milk in different storing time

ting, as it also showed less degradation of κ-casein (Figure 4) and a negative correlation of the content of this component with TBC ($p<0.05$, Table 1). The data presented in Figure 4 show that the κ-content started to decrease

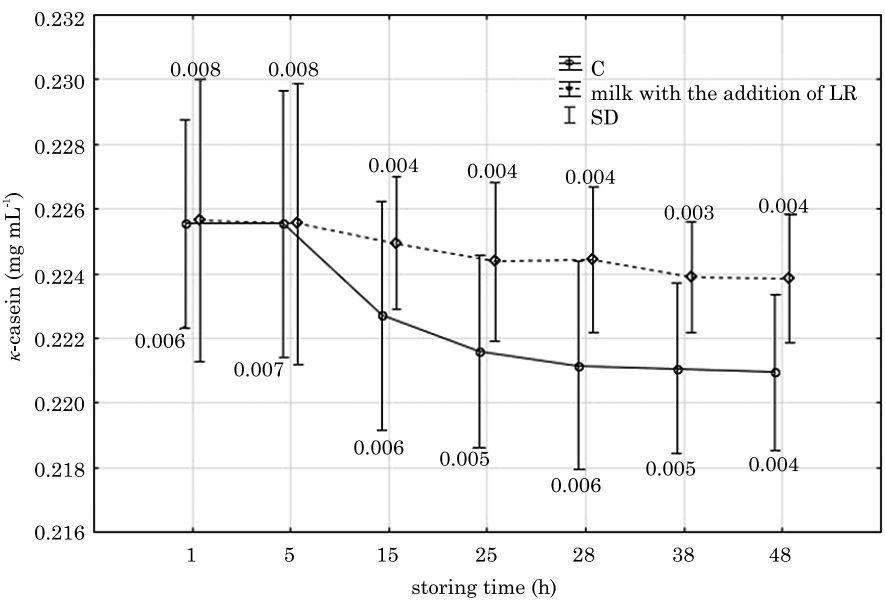

Fig. 4. Average content and standard deviation (SD) of κ-casein in bulk milk at different storing time

after the 5th hour of storage, but in the case of milk C without bacterial protection, this process was more dynamic (on average 0.003 mg mL^{-1} less).

The results presented in Table 3, concerning the efficiency indicators of milk processing for the production of curd, show that milk with the addition of *Lactobacillus rhamnosus* has a much higher yield of curd than the control milk, the difference being 1.26 p.p. ($p<0.05$).

Table 3

Indicators of milk processing efficiency for curd manufacturing

Indicator	Mean value		SEM	Correlation	
	C	milk with addition of LR		κ-casein x	TBCx
Curd yield (%)	19.16^{a}	20.42^{b}	0.035	0.438*	-0.195*
Water holding capacity (%)	30.15	29.42	0.076	0.508*	-0.375*
Curd dry matter (%)	39.02	39.93	0.116	0.660*	-0.504*
Protein (%)	40.47	40.93	0.049	0.601*	-0.601*
Fat (%)	52.48	52.81	0.009	0.517*	-0.428*
Ash (%)	5.05	5.07	0.011	-0.524*	0.427*
Whey:					
Dry matter (%)	66.33	66.26	0.109	ns	ns
Protein (%)	7.33	6.88	0.005	-0.213*	ns
Ash (mg dL^{-1})	2.79	2.51	0.076	-0.328*	0.217*

$^{a, b}$ – means in a row with different letters differ significantly ($p<0.05$), ns – not significantly, * $p<0.05$

Safeguarding the quality of raw milk at the storage stage is an important measure for maintaining its processability. Among other things, it is an important element in improving the quality of maturing cheeses, including their health-promoting qualities, and in ensuring that the maturing process is carried out correctly (Lu et al. 2016, Reale et al. 2016).

The studies by Crow and Curry (2002) and Sulieman (2017) shed some light on our results. This is mainly due to the fact that *Lactobacillus rhamnosus* (LR) belongs to facultative hetero-fermentative lactic acid bacterial strains, and these bacteria are often an indigenous, albeit niche, milk microbiome. They are often used as starter cultures added to pasteurized milk, mainly in the production of maturing cheeses. As mesophiles, they show a fairly high tolerance to temperature, maintaining vital functions above 40°C (Curry, Crow 2004, Tuganbay et al. 2008). Tian et al. (2020) showed that more favorable cheese flavor is achieved owing to LR metabolism. Furthermore, the metabolic profile of LRs is mainly based on glucose, and the pyruvate formed is in effect broken down to form diacetyl, which can enhance the buttery aroma of the final product. During LR fermentation, these bacteria show the ability to produce bioactive peptides derived from

casein (Guo et al. 2016, Solieri et al. 2018). The results given by the cited authors may explain the slight decrease in the amount of κ-casein in milk after the addition of LR. Indeed, the presence of these bacteria may have promoted the formation of bioactive peptides that enhanced the bacteriocidal mechanism of milk. As can be seen in the aforementioned studies, microbiological quality is a key factor in this research, because it affects the quality of milk casein. Our results demonstrate that the protective effect of a *Lactobacillus rhamnosus* strain is evident at the milk storage stage. The metabolism of *Lactobacillus rhamnosus* may enhance the competition with raw milk microflora in raw milk. We indirectly confirmed this effect in our study. Indeed, we showed that when LR cultures were added to the milk, the casein content decreased more slowly compared to uninoculated milk. In this case, the fact that there was no change in the total number of microorganisms, which may indicate that LR competed with the milk microflora, also remains important. Among other things, this characteristic of *Lactobacillus rhamnosus* may enhance the competition with the microflora in raw milk. Given its rather high tolerance to temperature, it is most likely that this influence of LR activity can be transferred at the next stage to the cheese maturation process.

Liu et al. (2016) showed that the addition of *Lactobacillus rhamnosus* cultures could, during ripening of Cheddar cheese, increase the proteolysis activity and further lysis of metabolites formed secondary to the breakdown of casein. This effect was explained by the amount of peptidase originally produced by LR. These results remain consistent with the ones obtained by Ong et al. (2006). Liu et al. (20160 further showed that the higher intensity of proteolytic processes at later maturation is mainly caused by enzymes originally produced by *Lactobacillus rhamnosus* cultures added to milk. They confirmed this with a higher water-soluble nitrogen content.

The fact that the use of LR for the protection of raw milk, as proposed in our study, may also have a positive effect on the processes occurring during cheese maturation remains important. This may be indicated by the study carried out by Ong et al. (2006), who showed that in the case of adding LR, the transfer of LR proteolytic activity to the ripening process is more efficient owing to the relatively high persistence of these bacteria in the structure of the resulting curd. This can be explained to some extent by a study by Lane and Fox (1997), in which it was shown that by plasmin and starter cultures were important factors in suppressing casein lysis. However, unlike plasmin, the amount of which is influenced by the hygienic quality of milk, the addition of *Lactococcus lactis* ssp. *cremoris* promotes the formation of enzymes that initiate the generation of mainly small peptides and free amino acids during maturation. Furthermore, research by Azarnia et al. (2010) has shown that the bacterial enzyme-mediated digestion of β-casein in milk promotes the formation of peptides, the presence of which limits the hydrolysis of casein during the later stages of cheese maturation. This

is confirmed by Bergamini et al. (2009). A similar effect was obtained for the formation of the κ-casein content in raw milk, which remains consistent with our research results. Indeed, in milk with the addition of LR, the qualitative changes associated with its proteolysis were weaker. In light of the study by Tarazanova et al. (2018), in which it was shown that the presence of adhesin can significantly affect the interaction between bacteria and milk components, it can be hypothesized that the addition of LR weakens the adhesion of bacteria to micellar casein.

The results obtained by Liu et al. (2016) also remain important regarding the bioprotection of casein in raw milk, as suggested in our study. The LR cultures added to the milk caused a reduction in the number of *Enterococcus*, with a simultaneous stability in the number of *Lactobacillus* bacteria. This effect is partly explained by the relationship described in the study of Ong et al. (2006), in which the addition of LR also resulted in lower *Lactococcus* numbers. To some extent, the results of Liu et al. (2016) and Ong et al. (2006) explain the result obtained in our experiment. This is because we found no clear changes in the development of the total microbial count during storage, despite the inoculation of raw milk with LR bacteria. In contrast, in milk with the additive, the variation in the total microbial count was lower. It should be noted that a significant result of adding LR to milk was lower variability in TBC, which we confirmed with lower SD values. Ong et al. (2006) explain the effect they obtained mainly by increased autolysis of *Lactococcus*, which was induced by changes caused by the presence of *L. rhamnosus* metabolites. The results of the described studies correspond with the tendency observed in clinical studies (Al-Madhagi, Alramo 2023). Although not directly in milk, they confirm the antagonistic effect of the *L. rhamnosus* GG on the growth of *Clostridium* and *Salmonella* bacteria in the digestive system. It should be noted that both of these bacteria are also present in raw milk and can promote technological defects during the maturation of cheeses. The antagonism described by Al-Madhagi and Alramo (2023) can be explained by a mechanism that inhibits the adhesion of these bacterial cells to the intestinal walls. The quoted results may further explain the tendencies observed in our experiment. To some extent, the TBC formation result we obtained can be explained by the synergistic effects reported in the aforementioned studies, e.g. Liu et al. (2016), Ong et al. (2006). Certainly, this effect can also be attributed in part to the reduced microbial adhesion to casein micelles and the competition between *Lactobacillus rhamnosus* and the other microorganisms in milk, as suggested by Solieri et al. (2015). In our opinion, this synergy enhanced the antimicrobial natural defense mechanism of milk. Thus, the introduction of microbial antagonizing strains into raw milk, described by Lu et al. (2016) and Murphy et al. (2016) as producing proteinases, may in effect enhance the efficiency of cheese maturation and the functional characteristics of the cheese. In existing practice, this is achieved by matching the type of metabolism and growth conditions of the starter culture to the type of

cheese (Martinovic et al. 2013, Desfossés-Foucault et al. 2014, Ganesan et al. 2014). Typically, cultures are used as an additive to pasteurized milk (Coelho et al. 2022). In addition, they can produce bioactive compounds. In the above experiment, *Lactobacillus rhamnosus* was chosen because it is a naturally occurring strain in milk cultures. *Lactobacillus rhamnosus* has a high ability to adhere to human intestinal cells, and it is often used as a probiotic, especially in the treatment of small intestinal bacterial overgrowth (Sun et al. 2019). Although *Lactobacillus rhamnosus*, *Lactobacillus plantarum* and *Lactobacillus casei* are quite often used as probiotics and helper cultures, there is little research published on the potential of these strains being used to protect casein in raw milk. What is more, like other lactic acid bacteria, *Lactobacillus rhamnosus* has the ability to ferment lactose, a naturally occurring sugar in milk. *Lactobacillus rhamnosus* bacteria present in milk consume lactose and other sugars while producing lactic acid, which causes the milk to coagulate and form curd. Ribeiro et al. (2021) and Medveďová et al. (2020) confirmed that other strains such as *Lactococcus lactis*, *Enterococcus faecalis* and *Lactococcus garvieae* inhibit the growth of *Staphylococcus aureus* in milk. The lack of lactose and other sugars in milk also inhibits the development of other unfavorable bacteria, which are deprived of food. This hypothesis is confirmed by the results obtained in our experiment.

CONCLUSIONS

Safeguarding raw milk with LR bacteria can be of great utilitarian importance, especially in regions of the world where maintaining milk hygiene can be hampered by unfavorable environmental conditions, above all in regions with relatively high temperatures and in barns with less strict hygiene standards. Less degradation of casein, which is the main cheese protein, was also observed in LR milk. It is important that the curd yield obtained in the experiment is higher in the case of milk with the addition of *Lactobacillus rhamnosus*. High curd yield is desirable in the cheesemaking industry for several reasons. Higher curd yields mean that more cheese can be produced from the same amount of milk. This can lead to greater efficiency and lower costs in the cheesemaking process. In addition, a higher curd yield means that the amount of cheese produced is more consistent from batch to batch. This is important for maintaining product quality and meeting customer expectations. Making more cheese from the same amount of milk can also benefit the environment by reducing the overall amount of milk needed to make cheese, thus reducing the carbon footprint. The bioprotection of raw milk with *Lactobacillus rhamnosus* bacteria, as proposed by us, may have a utilitarian dimension especially in terms of counteracting the excessive growth of the milk microflora and protecting the casein fraction of milk proteins.

Author contributions

Conceptualization: K.M., B.G.; Data collection and curation: K.M.; Formal analysis: K.M., B.G.; Investigation: K.W.; Methodology: K.M.; Writing – original draft: K.M., K.W.; Writing – review & editing: K.W.

Conflicts of interest

The authors report no conflict of interest.

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