ABSTRACT

Goat milk is a good carrier for probiotic bacteria; however, it is difficult to produce fermented goat milk with a consistency comparable to that of fermented cow milks. It can be improved by the addition of functional stabilizers, such as inulin, or treatment with transglutaminase. The aim of this study was to determine the effect of cold storage of inulin and microbial transglutaminase on the viability of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* ssp. *lactis* Bb-12 in fermented goat milk. Microbiological analysis included the determination of the probiotic bacteria cell count in fermented milk samples, whereas physico-chemical analysis included the analysis of fat content, titratable acidity, and pH of raw, pasteurized, and fermented goat milk samples. No positive influence of inulin or microbial transglutaminase on the viability of probiotics in fermented goat’s milk samples was observed. Nevertheless, the population of probiotics remained above 6 log cfu/g after 8 wk of storage at 5°C.

*Key words: *probiotic, goat milk, transglutaminase, inulin

Short Communication

Consumers are aware of the health benefits of fermented milks containing lactic acid bacteria, especially probiotic bacteria such as *Lactobacillus acidophilus*, *Lactobacillus casei*, and bifidobacteria (Abd El-Gawad et al., 2005; Saxelin et al., 2005; Savard et al., 2011). Milk products containing probiotic bacteria are becoming popular due to their health promoting properties (Ravula and Shah, 1998; Say and Şahan, 2002; Abd El-Gawad et al., 2005). According to Food and Agriculture Organization of the United Nations and World Health Organization requirements, fermented milks should contain at least $10^6$ cfu of labeled (i.e., probiotic) bacteria per milliliter (Ravula and Shah, 1998; Wang et al., 2012; Mituniewicz-Malek et al., 2013). During manufacture and storage, the viability of probiotic bacteria in fermented milks is dependent on several factors, such as acidity, hydrogen peroxide and oxygen content, concentrations of organic acids, temperature, and so on (Lankaputhra and Shah, 1995; Lankaputhra et al. 1996; Shah, 2000).

Globally, goat milk products are becoming increasingly popular as specialty and healthy substitutes for cow milk products (Alférez et al., 2001; Barrioomeño et al., 2002; Slacanac et al., 2010). Farnsworth et al. (2006) suggested that fermented goat milk is an excellent carrier for probiotic bacteria. It is difficult, however, to produce fermented goat milk with a consistency comparable to that of fermented cow milks. Goat milk has naturally lower $\alpha_{s1}$-CN content and higher proportions of $\kappa$-CN than in cow milk. This leads to a softer curd than in cow milk (Li and Guo, 2006; Park, 2007; Uniacke-Lowe et al., 2010). Traditional methods used to improve the texture of fermented milk, such as yogurt, include increasing the total milk solids and adding stabilizers. It has recently been demonstrated that the microstructure of fermented milks can be improved by applying transglutaminase (Lorenzen et al., 2002; Farnsworth et al., 2006; Rodriguez-Nogales, 2006). The influence of transglutaminase on milk proteins has been investigated (Lauber et al., 2000; Schorsch et al., 2000a,b; Bönisch et al., 2007); however, only few data are available from studies concerning its effect on goat milk protein (Farnsworth et al., 2002, 2003; Rodriguez-Nogales, 2006). Transglutaminase catalyzes an acyl transfer reaction between the $\gamma$-carboxyamide groups of peptide-bound glutamine residues and the $\varepsilon$-amino groups of lysine residues, leading to the formation of covalently cross-linked protein polymers (Jaros et al., 2007; Ardelean et al., 2012). Transglutaminase is now widely used in ready-to-eat meals, such as seafood, sushimi, meat products, and so on, to improve their functional properties. Approaches to modifying the texture of cultured dairy products also include functional stabilizers, such as inulin, which has a growth-stimulating
and protective effect on microflora (Gibson, 2004; Aryana and McGrew, 2007; Donkor et al., 2007). These stabilizers may improve the viability of probiotic bacteria cells during both the storage of food products and their passage through the gastrointestinal tract (Kolida et al., 2002; de Wiele et al., 2004; Hernandez-Hernandez et al., 2012; Bedani et al., 2013). Limited data exist on the influence of prebiotics on probiotic lactobacilli or bifidobacteria in dairy products stored at low temperatures (Shah et al., 1995). Protein networks created in fermented goat milk by transglutaminase may affect the growth and viability of the lactic acid bacteria and probiotic cultures (Farnsworth et al., 2006). The aim of the current study was to investigate the effects of microbial transglutaminase and inulin treatment on the viability of probiotics in fermented goat milks during storage at low temperatures.

Raw goat milk was the raw material for the production of experimental fermented goat milk. Raw milk came from the Kozi Gródek private organic farm in Wołczkowo, near Szczecin (Poland). It was heat-treated in a tank at 85°C for 15 min, and then cooled to 42°C. Fermented goat milk was produced using 2 different starters of freeze-dried probiotic monocultures, L. acidophilus La-5 (FD-DVS LA-5 - Probio-Tec) and Bifidobacterium animalis ssp. lactis Bb-12 (FD-DVS BB-12 - Probio-Tec). Both were purchased from Chr. Hansen (Copenhagen, Denmark).

The enzyme, microbial transglutaminase (mTGase), was added as a preparation of Activa MP manufactured by Ajinomoto Co. Inc. (Tokyo, Japan). According to the manufacturer, this enzyme was obtained from Streptovercillum moberansae and the preparation consists of a mixture of 1% Ca²⁺-independent transglutaminase and 99% maltodextrin. This was used for the production of fermented goat milk. During the production of fermented goat milk, this preparation was used in its original form without further purification. The Orafi GR Inulin (Tienen, Belgium) preparation was used for the production of fermented goat milk. This contains 92% inulin and an 8% glucose, fructose, and sucrose mixture. Average degree of polymerization is 10 (ranging from 2 to 60). It has β(2–1) bonds in longer carbon chains consisting of fructose units. The Orafi GR Inulin preparation has a neutral taste and low solubility. It is less sweet than sugar, 10% sweetness in comparison to 100% sugar sweetness.

The research samples were produced on a laboratory scale from pasteurized goat milk. Six types of fermented milk samples were produced: milk sample fermented by Lb. acidophilus (NFLA), milk sample fermented by bifidobacteria (NFBB), NFLA containing inulin (NFLA-IN), NFBB containing inulin (NFBB-IN), NFLA containing microbial transglutaminase (NFLA-TG), and NFBB containing microbial transglutaminase (NFBB-TG). To produce the first 2 (NFLA and NFBB), probiotic monocultures LA-5 and BB-12, respectively, were used. After pasteurization and cooling to 42°C, the goat’s milk was inoculated with LA-5 or BB-12 starters and subjected to fermentation at 42°C for ca. 5 h (to pH 4.7). The probiotic monocultures were activated before milk inoculation and used in the form of a bulk, which was added to the milk samples in the amount of 5%. Two nest samples (NFLA-IN and NFBB-IN) were produced from milk supplemented with the inulin preparation at a dosage of 2% wt/wt. The last 2 samples (NFLA-TG and NFBB-TG) were obtained with identical dosages of probiotic starters and with the addition of mTGase at a dosage of 0.04%. Before the fermentation process, the milk samples were supplemented with mTGase and incubated at 42°C for 2 h; then the TG was inactivated at 80°C for 1 min, the milk was cooled to the fermentation temperature (42°C), and the LA-5 or BB-12 starters were added.

After the fermentation process, all experimental milk samples were cooled to 5°C ± 1 and stored for a period of 21 d under such conditions. A total of 96 samples were stored in refrigerator. Samples for analysis were collected after 1, 7, 14, and 21 d of refrigerated storage.

The microbiological analysis included the determination of the probiotic bacteria cell count in fermented milk samples using the plate method, in 2 parallel replicates and 3 independent replicates for each analyzed sample. Because the cultures used for fermentation of goat milk were monocultures of bacteria, a medium de Man, Rogosa, Sharpe agar was used for the determination of bacteria cell number (Merck, 2007). Preparations of samples for microbiological analysis, as well as dilutions, were performed according to EN ISO 6887-5:2010 (ISO, 2010). Plates of de Man, Rogosa, Sharpe agar (cat. no. 1.10660; Merck, Darmstadt, Germany) inoculated with sample dilutions were incubated at 37°C for 72 h in anaerobic conditions (Anaerocult A, cat. no. 1.13829; Merck). After incubation, the number of bacteria cells was converted into colony-forming units in 1 mL (cfu/mL).

Physical and chemical analyses included the analysis of raw and pasteurized (fat content, titratable acidity, and pH) milk samples and fermented milk samples (pH), in 2 parallel replicates and 3 independent replicates for each analyzed sample. The fat content was determined using the Gerber method according to ISO 2446:2008 (ISO, 2008). The titratable acidity was determined via the titration of a known amount of samples with 0.1 N NaOH, using phenolphthalein as indicator. The pH value of milk samples was measured with a pH-meter.

The mean value and SD were calculated from the data obtained from the experiments. These data were
compared using Tukey HSD’s Multifactor ANOVA in Statgraphics Centurion XV, version 15 (Statpoint Technologies Inc., Warrenton, VA). Statistical significance was set at $P < 0.05$.

The raw goat milk used in the experiments contained 4.37% fat (Table 1), and this parameter did not change as a result of the pasteurization process. The titratable acidity of raw and pasteurized goat milk samples was 8.2 Soxhlet-Henkel degrees (°SH), and the pH values were 6.77 and 6.50 in raw and pasteurized goat milk samples, respectively. A lowering of pH value due to the effect of pasteurization is known in the case of cow and goat milk. The fermentation of goat milk by probiotic bacteria led the pH value of samples to decrease to 4.39 to 4.73. Determination of the basic composition of goat milk used in the experiments, as well as its acidity, was to demonstrate that all samples are prepared on the basis of identical milk. Pasteurization was a step in the preparation of the milk samples, so we checked how some of the characteristics of milk change under its influence.

The storage period ($P < 0.01$) had a statistically significant effect (at the 95.0% confidence level) on the bacteria number in fermented goat milk samples fermented by a culture of *Lactobacillus acidophilus* La-5 (Figure 1). Furthermore, the addition of either inulin or mTGase did not improve the survival rate of probiotic bacteria. The initial population of *L. acidophilus* La-5 ranged from 7.9 to 8.6 log cfu/mL. After 21 d of storage under refrigerated conditions, the population of *L. acidophilus* La-5 decreased to 7.4, 7.2, and 7.6 in the control milk sample, the milk sample with inulin, and the milk sample with transglutaminase, respectively. In general, the lowest number of bacterial cells was observed in samples of goat milk containing inulin (NFLA-IN). This was not a consequence of the addition of inulin, but rather due to the lower dosage of starter than in the other samples. The initial population of *L. acidophilus* La-5 in NFLA-IN samples was about 0.3 to 0.5 of logarithmic cycle lower than in NFLA and NFLA-TG samples. Trend of cell viability of this strain was identical in all the samples, regardless of the initial starter dose used for milk. The low pH value and refrigerated storage conditions of goat milk samples meant that at d 21 of storage the number of bacterial cells had been significantly reduced to below ca. 7.5 log cfu/mL, irrespective of the addition of inulin or mTGase. Both the addition of inulin or mTGase ($P < 0.01$) and the storage period ($P < 0.01$) have a statistically significant effect (at the 95.0% confidence level) on the pH value of fermented goat milk samples fermented by a culture of *L. acidophilus* La-5 (Figure 1). The initial pH of fermented milks ranged from 4.39 to 4.66. In general, the highest final pH values (4.21–4.27) were observed in samples of goat’s milk containing inulin (NFLA-IN) and control milk samples; the lowest final pH values (4.13) were noted in samples of goat milk with the addition of mTGase (NFLA-TG).

Currently, a trend to use yogurt starter cultures containing probiotic bacteria without *Lactobacillus delbrueckii* ssp. *bulgaricus* has been observed. The elimination of *L. delbrueckii* ssp. *bulgaricus* from yogurt starter cultures allows the inhibition of the postacidification of final products (Shihata and Shah, 2000). The third generation of fermented milk drinks is a completely different group of products than yogurt; they are prepared exclusively with the use of probiotic bacteria. Several studies have been concerned with the improvement of the growth and viability of probiotic bacteria in such products containing maize starch or inulin (Shin et al., 2000; Bruno et al., 2002; Akalin et al., 2004). Donkor et al. (2007) observed the positive effect of inulin on a population of *L. acidophilus* LAFTI L10 in yogurts stored at 4°C for 28 d. Although the addition of inulin did not improve the viability of probiotic bacteria during storage, the authors noted that the concentration of probiotics was above the recommended therapeutic level of 6 log cfu/mL (Kurmann and Rasic, 1991) at the end of storage. The decrease in the viability of *L. acidophilus* in cow milk yogurt or other milk-based products with a pH of 4 to 5 has been observed in many studies (Dave and Shah, 1997; Buriti et al., 2007; Zarba et al., 2008; Buriti et al., 2010). In contrast, Hel-land et al. (2004) obtained the viability of *L. acidophi-lus* La-5 at a population 7 to 8 log cfu/g in milk-based puddings stored at 4 to 6°C for 21 d. It is known that the main factors affecting the viability of *Lactobacillus* spp. in food are the probiotic strains, the inoculum concentration, the fermentation time, the decrease in the medium pH value, the presence of oxygen or hydrogen peroxide, the concentration of bacterial metabolites, and the storage temperature (Shah, 2000; Talwalkar and Kailasapathy, 2004; Donkor et al., 2006). However, Bedani et al. (2013) demonstrated that the pH value obtained in their study did not affect the viability of *L. acidophilus* La-5. Those data are consistent with the studies of Bozanic et al. (2001) and Varga et al. (2006), demonstrating that inulin does not influence the viability of *L. acidophilus* La-5 in fermented cow and goat

<table>
<thead>
<tr>
<th>Milk</th>
<th>Fat content, %</th>
<th>Titratable acidity, °SH</th>
<th>pH</th>
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</thead>
<tbody>
<tr>
<td>Raw</td>
<td>4.37</td>
<td>8.2</td>
<td>6.77</td>
</tr>
<tr>
<td>Pasteurized</td>
<td>4.37</td>
<td>8.2</td>
<td>6.50</td>
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°SH = Soxhlet-Henkel degrees.
milks stored at 4 to 5°C for 4 to 6 wk. Sanlidere Aloglu and Öner (2013) demonstrated an increase in the firmness of samples of labneh (a concentrated type of Mediterranean and Middle Eastern yogurt prepared from goat milk) produced with transglutaminase at ranges of 2 to 4 U/g of protein, compared with an untreated sample. These changes were observed in samples without important differences in total solid, protein, and fat contents. Domagała et al. (2013) proved that the modification of goat milk with transglutaminase (at a dosage of 2 U of transglutaminase/g of protein) improved the sensory quality and textural characteristics and reduced the syneresis of set yogurt when compared with the control product. The present study demonstrated that the use of mTGase does not change the viability of the \textit{L. acidophilus} strain compared with an untreated sample of fermented goat milk. The data obtained are in agreement with the results of Şahan et al. (2004) on the viability of aerobic mesophilic bacteria, aerobic spore-forming bacteria, and psychrotrophic bacteria in samples of labneh stored at refrigeration temperature for 60 d. Interesting results were obtained by Neve et al. (2001) on the viability of \textit{L. delbrueckii} \textit{bulgaricus} culture in cow yogurt when transglutaminase was supplied simultaneously with the yogurt starter bacteria without subsequent inactivation. The cited authors observed an increase in bacteria cell viability during cold storage.

In the present study, both the addition of inulin or mTGase ($P < 0.01$) and storage period ($P < 0.01$) have a statistically significant effect on bifidobacteria count in fermented goat milk samples fermented by a culture of \textit{B. animalis} ssp. \textit{lactis} Bb-12 at the 95.0% confidence level (Figure 2). The initial population of \textit{B. animalis} ssp. \textit{lactis} Bb-12 ranged from 7.9 to 9.0 log cfu/mL. In general, the greatest decrease in the population of bifidobacteria was observed at 14 d of refrigerated storage. After 21 d of storage under refrigerated conditions, the population of \textit{B. animalis} ssp. \textit{lactis} Bb-12 decreased to 7.8, 7.2, and 7.8 in control milk samples, milk samples with inulin, and milk samples with transglutaminase, respectively. Samples with the addition of mTGase (NFBB-TG) or inulin (NFBB-IN) had significantly fewer bacterial cells than the control samples (NFBB); this was not the result of the additives used or refrigerated storage conditions, but rather the low dosages of bacterial cells at the beginning of the experiment. Both the addition of inulin or mTGase ($P < 0.01$) and storage period ($P < 0.01$) had a statistically significant effect on pH value in fermented goat milk samples fermented by a culture of \textit{B. animalis} ssp. \textit{lactis} Bb-12 at the 95.0% confidence level (Figure 2). The initial pH of fermented milks ranged from 4.56 to 4.73. The lowest final pH value was observed in samples of goat milk.

Figure 1. Average bacteria count and pH value of fermented goat milk samples stored at 5°C for 21 d (n = 6). (A) Samples of goat milk fermented by \textit{Lactobacillus acidophilus} La-5; (B) samples of goat milk fermented by \textit{L. acidophilus} La-5 containing inulin; (C) samples of goat milk with microbial transglutaminase fermented by \textit{L. acidophilus} La-5.
with mTGase (NFBB-TG, 4.11). The other samples of goat milk (NFBB and NFBB-IN) did not differ significantly from each other in terms of the final pH value (4.19 and 4.21, respectively).

_Bifidobacterium_ spp. remained viable in a probiotic goat milk yogurt produced by Wang et al. (2012). The population of bifidobacteria was above 10^6 cfu/g during storage at 4°C for 4 wk. Additionally, inulin has a positive effect on the viability of bifidobacteria, but it is less effective than the other carbohydrate sources studied (Shin et al., 2000). Compared with the untreated control sample, the pretreatment of the milk with transglutaminase (2 or 4 U/g of protein) did not affect the populations of probiotic cultures in goat milk yogurt samples (Farnsworth et al., 2006). The population of probiotics remained above 6 log cfu/g after 8 wk of storage at 4°C. According to the cited authors, the enzymatic cross-linking of proteins by transglutaminase seems to have a positive role on the viability of probiotics. The use of inulin at 5% has a beneficial effect on the viability of bifidobacteria after 28 d of refrigerated storage (Varga et al., 2006). Kehagias et al. (2008) studied the acidity and growth of _Bifidobacterium longum_ in skimmed cow, ewe, and goat milk samples. The population of bifidobacteria was higher in goat or ewe milk than in cow milk. During the first 8 to 12 h of the 24-h fermentation, the increase in pH value was more intensive in goat or ewe milk than in cow milk. Wang et al. (2012) observed significant differences in pH values and titratable acidity between weeks during storage of a probiotic goat-milk yogurt fermented by _Bifidobacterium_ spp. As previously mentioned, the decrease in pH of yogurt may be responsible for the reduced viability of probiotic bacteria, including bifidobacteria (Hood and Zoitola, 1988; Shah and Jelen, 1990; Lourens-Hattingh and Viljoen, 2001).

In our study, changes in the population of probiotics in fermented beverages produced from goat milk enriched with inulin or produced with the use of microbial transglutaminase are probiotic strain-dependent. The decrease in the pH of fermented goat milk was responsible for the reduction of the population of probiotic bacteria. Bifidobacteria were more sensitive than lactobacilli. Nevertheless, the population of probiotics remained above 6 log cfu/g after 8 wk of storage at 5°C. The positive influence of inulin or transglutaminase on the survival rate of bacterial cells in samples of fermented goat milk was not observed in our experiments.

**REFERENCES**


