





LOW-LACTOSE FERMENTED GOAT MILKS WITH BIFIDOBACTERIUM ANIMALIS SSP. LACTIS BB-12

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ABSTRACT

Goat milk is a good carrier for probiotic bacteria and due to its specific composition is a high-quality material for manufacturing food for certain sectors of the population with needs. Probiotic bacteria in fermented and unfermented milk products can be used to alleviate the clinical symptoms of lactose intolerance. The aim of this study was to develop a low-lactose probiotic fermented beverage prepared from goat's milk and to evaluate the effect of prior lactose hydrolysis on the viability of *Bifidobacterium animalis* ssp. *lactis* Bb-12, pH and total acidity, colour, textural and sensory parameters of these products. Results showed the influence of lactose hydrolysis on the acidity of fermented goat milk, colour, syneresis and the viability of bifidobacteria. The lactose hydrolysis of milk resulted a higher hardness in probiotic fermented goat milk. Moreover, the lactose-free probiotic fermented milk had a more distinct sweet taste than the control one and was characterized by a less sour flavour.

Keywords: probiotics, bifidobacteria, fermented milk, goat milk, lactase, lactose hydrolysis

INTRODUCTION

Nowadays, industries and consumers interest of functional foods has been exponentially increasing (Minervini et al., 2009). Using of milk with nutritional properties in combination with bacterial strains with probiotic properties represents one of the technology options for manufacturing dairy functional beverages (Kumar et al., 2015).

Probiotics are the main bioactive components of fermented dairy foods and numerous economic indicators show that products with probiotics are still in the forefront of innovation in the functional food sector (Ozer and Kirmaci, 2009; Ventura et al., 2004). The minimum levels for probiotic bacteria in fermented milks are 6 log cfu·g-1 at the end of expiry dates ($\bar{F}AO/WHO$, 2002). Nowadays, various types of milk products with Bifidobacteruim ssp. are available at the food markets. Bifidobacteria are one of the first microorganisms to colonize the gastrointestinal tract of the newborn and constitute the largest group of intestinal microflora during breastfeeding (Favier et al., 2003). Despite the changes in intestinal microflora observed with age and diet, bifidobacteria continually occur in adults' intestinal microflora, and constitute from 3% to 6% of the total population (Lay et al., 2005). It is believed that bifidobacteria have a positive impact on health benefits on their host (O'Callaghan and Van Sinderen, 2016). Acidifying the contents of the intestine by metabolites produced by Bifidobacterium spp. inhibits the growth of pathogenic microorganisms, reduce cholesterol levels and stimulates apoptosis of tumor cells (Jędrzejczak-Krzepkowska and Bielecki, 2011). According to Ziarno et al. (2011) there are many factors that may affect the ability of fermentation and survival of microorganisms in food products. Their varied survival may be due to different sensitivity of used strains of probiotic bacteria, the fermentation time, storage conditions, pH of the product, sugar concentration, dry matter content and the access to nutrients (Jedrzejczak-Krzepkowska and Bielecki, 2011; Znamirowska et al., 2018).

Goat milk, due to its specific composition, is a high quality material for manufacturing food for certain sectors of the population with particular needs (Sanz Ceballos et al., 2009). According to Park et al. (2007), goat milk in comparison to cow milk, has better digestibility, alkalinity, buffering capacity and therapeutic values in human nutrition. Moreover, the goat milk is a reach source of elements: calcium, potassium, iron, copper and manganese and the concentration of these components is higher than in cow milk (Barlowska et al., 2013). Joon et al. (2017) and Senaka et al. (2012) report that people with lactose intolerance can drink goat milk due to its better digestibility. Goat milk is easily

absorbed than cow's milk and leaves less undigested residue behind in the colon to literally ferment and could cause the uncomfortable symptoms of lactose intolerance. Lactose is a unique mammalian milks disaccharide sugar composed of glucose and galactose. Moreover, lactose is a fermentable substrate, first being hydrolysed by facultative or anaerobic microorganisms, allowing for anaerobic metabolism of the resultant simple sugars (Solomons, 2002). Lactose intolerance is related to primary or secondary lactase deficiency and depends not only on the expression of lactase, but also on the dose of lactose, intestinal flora, gastrointestinal motility, small intestinal bacterial overgrowth and sensitivity of the gastrointestinal tract to the generation of gas and other fermentation products of lactose digestion. Treatment of lactose intolerance can include lactose-reduced diet and enzyme replacement (Deng et al., 2015). Moreover, probiotic bacteria in fermented and unfermented milk products can be used to alleviate the clinical symptoms of lactose intolerance (Oak and Jha, 2018). There are "low in lactose" or "lactose free" dairy products on the market that have been specially manufactured to reduce their lactose content. As there are currently no harmonised rules at EU level for the use of terms such as 'lactose-free', Member States may maintain or adopt relevant national measures. In UE legislation, the term "lactose-free" has only been defined for infant and follow-on formula as ≤10 mg/100 kcal (**EFSA, 2010**).

The aim of the present study was to develop a new low-lactose probiotic fermented beverage prepared from goat's milk and to evaluate chosen physicochemical, textural and sensory parameters of these product.

MATERIAL AND METHODS

Raw goat's milk analysis

Raw goat's milk for the manufacture of fermented milks was obtained three times (June-July) directly from a farm "Zuza" in village Zabratówka, near Rzeszów (Poland). The cold morning milk (4°C) was transported directly to the laboratory and the physicochemical and microbiological analysis of the milk samples was carried out before the manufacture of fermented milks. The total bacterial count (TBC) and somatic cell count (SCC) were determined in BactoCount IBC M/SCC semi-automatic counter (Bentley Instruments Inc., USA), chemical composition and freezing point were determined in milk composition analyzer Bentley B-150 (Bentley Instruments Inc., USA) and pH was determined with a digital pH meter Toledo FiveEasy TM (Mettler Toledo, Switzerland) (Tab 1).

Table 1 Quality of raw goat milk (n=15)

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Properties	Mean±standard deviation	
TBC·1000 (cfu·mL ⁻¹)	207.33±13.53	
SCC·1000 (per 1 mL)	661.50 ± 19.82	
pН	6.72 ± 0.06	
Freezing point (°C)	-0.569±0.01	
Protein (%)	2.62 ± 0.09	
Fat (%)	2.51±0.16	
Lactose (%)	3.97 ± 0.10	
Total solids (%)	10.16 ± 0.20	

Low-lactose milk production process

Raw goat milk was filtered, pasteurized (85°C, 30 min), then transferred to sterile flasks and cooled to 6°C. After cooling, milk was divided into 2 batches: one with lactase incorporated (MGE) and the other working as the control without the enzyme addition (MGD). The procedure of lactose hydrolysis in the milk was performed in accordance with the manufacturer's guidelines. 0.05% (w/v) of commercial NOLA® Fit (Chr. Hansen, Denmark) was added to the goat milk. The activity of enzyme preparation of the β -galactosidase is 5500 BLU·g¹ and is expressed in Bifido Lactase Units (BLU). According to the enzyme manufacturer, 20h in temperature from 6°C to 10°C is sufficient time to reduce lactose content by almost 100%. Goat milk containing the enzyme (MGE) was stored in the temperature of 6°C for 20h. For the control fermented milk (MGD), the milk was not treated with lactase enzyme.

Fermented probiotic goat milk manufacture

The milk was heated to 37°C and each batch of milk was inoculated with 5% (w/v) probiotic monocultures DVS starter Bb-12 (*Bifidobacterium animalis* ssp. *lactis*) (Chr. Hansen, Denmark). Then the milk was thoroughly mixed, poured into 100 mL sterile plastic containers with lids and incubated at 37°C, until the pH reached 4,8 (~10 hours). Next, the samples of fermented probiotic goat milk were cooled to 6°C and stored at the same temperature for 7 days and tested.

Bacteriological analysis

Bifidobacterium animalis ssp. lactis Bb-12 was evaluated using MRS Agar (Biocorp, Poland) incubated under anaerobic condition at 37°C for 72 h (**Lima** et al., 2009). The vacuum desiccator and GENbox anaer (Biomerieux, Poland) were used to maintain anaerobic conditions. Anaer indicator (Biomerieux, Poland) was used for controlling anaerobic conditions. The viable counts were expressed as the log cfu·g-¹.

Evaluation of pH and total acidity

pH values were determined with a digital pH meter Toledo FiveEasy TM (Mettler Toledo, Switzerland). The total acidity (TA) was estimated according to **Jemaa** *et al.* (2017). Fermented goat milk samples (25 g) where added with 1 mL of phenolphthalein (at 5% w/v) and then titrated with 0,1 M NaOH solution to an end point of stable faint pink colour for 30 sec. TA was expressed as grams of lactic acid per L.

Colour measurement

The colour measurement was carried out with the Chroma Meter CR-400 (Konica Minolta, Japan) and was monitored with the CIE LAB system (in values: $L^*a^*b^*$) (Achanta *et al.*, 2007).

Syneresis

Syneresis of fermented probiotic milk was determined using the centrifugal method (centrifuge LC-04R-N, Zenith Lab Inc., USA) according to **Zhao** et al. (2018).

Texture profile analysis

Texture profile analysis (TPA) according to **Domagala** *et al.* (2013), with modifications, was performed with Brookfield CT3 texture analyzer (Brookfield AMETEK, USA), controlled by a PC computer. Fermented goat milk samples in 100-mL cups (35 mm in diameter), at a temperature 10° C± 1° C, were penetrated by a plastic cylinder type TA3/100 (25.3 mm in diameter) to the depth of 15 mm at a rate of 1 mm·s⁻¹ and press force 0.1 N. Hardness, adhesiveness, stringiness length, cohesiveness, springiness and gumminess were measured.

Sensory evaluation: pre-test

The sensory evaluation pre-test was conducted after seven days of storage at temperature 6°C, using a trained panel of 20 judges (14 females and 6 males, 23–38 years of age, were recruited from the University of Rzeszów). A triangular test was applied to verify the discriminatory ability of each assessor. The sensory panel was served five samples at a time (in three-digit random number coded plastic cups) and asked to rinse their mouths between samples with water. The panelists evaluated the presence of creamy milky flavour, sour flavour, sweet flavour, goaty flavour, abnormal flavour, sour odour, goaty and abnormal odour (Tab 2) on a nine-point rating scale with edge markings (from 1 = not perceptible to 9 = extremely strong) (Barylko-Pikielna and Matuszewska, 2014; PN-ISO, 1998; PN-ISO, 1999).

Table 2 Definitions of the attributes in descriptive organoleptic analysis of probiotic fermented goat milk

Attribute	Definition
Creamy - milky flavour	The taste stimulated by milk powder (none - intensive)
Sour flavour	The taste stimulated by lactic acid (none - intensive)
Sweet flavour	The taste stimulated by sucrose (none - intensive)
Goaty flavour	Animal-like, lingering, associated with a harsh odor and sharp taste; caprylic acid (none – intensive)
Abormal flavour	Unidentified flavour that is not characteristic (none – intensive)
Sour odour	The intensity of odor associated with sour milk, i.e. lactic acid (none - intensive)
Goaty odour	Animal-like, lingering, associated with a harsh odour and sharp taste; caprylic acid (none – intensive)
Abnormal odour	Unidentified odour that is not characteristic (none – intensive)

Statistic analysis

The experiment was performed in three repetition and all of the analyses were performed in quintuplicate. From the obtained results the mean and standard deviation were worked out statistically in the software Statistica v. 13.0 (StatSoft, USA). A one-way analysis of ANOVA variance was performed and significance of differences between the averages (p≤0.05) was estimated with Tukey's test.

RESULTS AND DISCUSSION

Bacteriological evaluation

The viable cell count of probiotic bacteria in fermented milk should be a minimum of 6 log cfu·g⁻¹ (FAO/WHO, 2002). Bifidobacteria in both fermented goat milk samples resulted viable after 7 days of refrigerated storage, at cell counts not lower than the minimum value. A significantly higher (p<0.05) amount of Bb-12 (9.32 log cfu·g⁻¹) was observed in the control sample (MGD), comparing to the low-lactose fermented milk (MGE) (9.08 log cfu·g⁻¹). Slačanac *et al.* (2013) report that due to the specific composition and structure of goat milk (higher content of some mineral compounds, short-chain fatty acids and better

bioavailability of proteins) bifidobacteria are more active in goat milk fermentation compared with cow milk fermentation. Mituniewicz-Małek et al. (2017) stated that the viable cell count of Bifidobacterium animalis ssp. lactis Bb-12 in fermented goat milk stored at 5±1°C for 7 days was 7.4 log cfu·g-1. Some studies show the preference of some Bifidobacterium species for di- or oligosaccharides over monosaccharides (Amaretti et al., 2006; Parche et al., 2006). Moreover, Amaretti et al. (2007) reported that some bifidobacteria species preferentially use lactose over glucose as a carbon source when grown in the presence of both sugars (Amaretti et al. 2007). Observations reported by Schmidt et al. (2016) also clearly point on strain-dependent differences in the preferred carbon sources. In Gonzalez-Rodriguez et al. (2013) study, strain Bb-12 was grown in MRSfc supplemented with 2% (w/vl) lactose, glucose, or galactose to establish the representative growth profiles. Growth was evaluated by measuring the turbidity of the culture by determination of the optical density at 600 nm (OD600). Authors reported that galactose was a very poor substrate for growth. However, in lactose or glucose, maximal OD₆₀₀s of 8.2±0.9 and 8.3±0.9, respectively, were obtained, with the cells growing at maximum specific growth rates of $0.38\pm0.03~h^{-1}$ and $0.40\pm0.03~h^{-1}$, respectively.

Evaluation of pH and total acidity

Acidity is one of the important factors allowing a fermented goat milk to be suitable for human consumption. Moreover, the viable cultures of bacteria are the basic for the manufacture of fermented goat's milk and their activity causes changes in fermented beverages (Mituniewicz-Malek et al., 2017). In the present study, the influence of lactose hydrolysis on acidity of fermented milks was noticeable and had a statistically significant effect (p≤0.05) on pH and total acidity in probiotic goat's milk fermented by a culture of Bifidobacterium animalis ssp. Lactis (Tab 3). The highest pH (4.77) and the lowest total acidity (0.74 g·L⁻¹) were observed in MDG samples (low-lactose variant). Similar conclusions were made by Skryplonek et al. (2017) who reported that lactosefree yoghurts had lower pH (4.38) and higher lactic acid concentration (0.927%) than control voghurts (pH=4.44; % lactic acid=0.914). Authors (Skryplonek et al., 2017) explain that breaking down lactose to monosaccharides facilitates bacterial metabolism and enhances the fermentation process. Mituniewicz-Malek et al. (2017) observed that the fermented goat milk had different pH values depending on the monoculture of Bifidobacterium longum and Bifidobacterium animalis ssp. lactis used in the manufacturing process. In a first day of cold storage, goat milk fermented with Bifidobacterium animalis ssp. lactis BB-12 had the highest pH (5.32) and the lowest pH was observed in samples fermented with Bifidobacterium longum (4.54).

Colour evaluation

The colour attributes of the probiotic goat fermented milk were significantly affected (p<0.05) by the influence of lactose hydrolysis (Tab 3). "L" value represents lightness (100) and blackness (0), "a" value represents red (+) to green (-) hues, whereas "b" represents yellow (+) to blue (-) hues (Zare et al., 2012). The application of the lactase enzyme caused a decrease in luminosity (L*) of fermented goat milks, as well as in chromatic colour parameters (a* and b*). In both types of fermented milks, chromatic colour parameters assumed negative values, which indicates that the colour of beverages was more green than red (a*) and more blue than yellow (b*). A decrease in the L*, a* and b* value in lowlactose fermented milk (MGE), in comparison to MGD sample, could be caused by a higher acidity of this product. According to Skryplonek et al. (2017) lactose-free frozen yoghurt was brighter than the control yoghurt and had a higher value of L* parameter (94,80). The colour coordinates changes can be also connected with a different level of gel opacity, which is related to the casein ratio and their aggregation level in milk. Moreover, the absence of β -carotene in goat milk together with its elevated proportion of small fat globules can explain the increase in whiteness index of goat milk yoghurt samples. Also, the increase in the syneresis index during storage of yoghurt samples may explain the changes in colour (Vargas et al., 2008).

Table 3 Total acidity (g·L¹), pH, syneresis (%), colour (L*a*b*) and *Bifidobacterium* Bb-12 (log cfu·g¹¹)

		Type of ferr	Type of fermented milk	
Parameters	s	MGD (n=15)	MGE (n=15)	
Bifidobacterium Bb-12 (log cfu·g-1)		9.32b ±2.46	$9.08a \pm 2.89$	
pН		$4.77b\pm0.04$	$4.74a\pm0.07$	
Total acidity	y (g·L ⁻¹)	$0.74a\pm0.01$	$0.76b\pm0.03$	
Syneresis (%)		$64.32b\pm1.14$	50.72a±2.20	
	L*	95.41b±0.38	95.05a±0.36	
Colour	a*	$-2.79b\pm0.01$	$-2.74a\pm0.01$	
	b*	10.24b±0.04	10.20a±0.04	

Legend: table shows mean \pm standard deviation; a, b - mean values in rows denoted by different letters differ statistically at p \leq 0.05

Syneresis measurement

According to Domagala et al. (2013) syneresis is a separation of the liquid phase from the gel. This process may be spontaneous or may occur only when the gel is mechanically disrupted while cutting, agitating, or freezing. This visible defect may occur during milk fermented beverages storage and can affect the final product acceptance (Joon et al., 2017). When the total solids concentration and the protein content is increased it results in a rise in gel hardness and wheyholding capacity in yoghurt. Moreover, the milk type and the type of applied starter culture may affect the fermented beverages syneresis (Domagala, 2009; Lucey, 2004). It was found that low-lactose fermented milks (MGE) revealed the lowest syneresis (50.72%), whereas MGD samples were found to be highest (64.32%). Moreover, the influence of lactose hydrolysis on syneresis in fermented probiotic milks showed the significant effect (p≤0.05) (Tab 3). Kárnyáczki and Csanádi (2017) observed difference between the whey leakage of control yoghurt and lactose-free yoghurt samples. Syneresis in control yoghurt was higher (21.47%) while the lower (14.63%) was determined in lactose-free yoghurt. Nagaraj et al. (2009) reported that syneresis of yoghurt from hydrolysed milk was increased. Martins et al. (2012) tested products with hydrolysed more than 97% of lactose during the fermentation with 0.5 g·L⁻¹ and 1.0 g·L⁻¹ lactase and observed that products with less enzyme concentration exhibited lower syneresis. Authors explained this phenomenon by a lower number of exopolysaccharides (EPS) synthesised in case of higher enzyme concentration. In addition, **Schmidt et al.** (2016) reported that in a case of set gels, forced syneresis was not influenced by lactose hydrolysis, whereas significant differences between the starter cultures were observed. Moreover, authors observed that the determination of exopolysaccharides concentration showed that more EPS were synthesised in hydrolysed milk in comparison with the reference substrate.

Texture evaluation

Texture is considered an important quality indicator of fermented goat milk that determines whether such products are attractive components of a basic human diet. Moreover, the quality of curd depends on the texture profile of used milk, the production technology, the additives and particularly the type and activity of cultures (Mituniewicz-Malek et al., 2017; Zare et al., 2011). Goat milk fermented products have softer consistency and show a weaker gel in comparison to products obtained from cow milk (Joon et al., 2017; Vargas et al., 2008). The hardness of control and low-lactose fermented goat milks ranged from 0.36 N to 0.38 N and the highest values were observed in MGE samples and the lowest in MGD products. Furthermore, apparent hardness values were affected ($p \le 0.05$) by the lactose concentration in fermented milks. Texture parameters of the fermented goat milks, such as stringiness length and gumminess, were slightly higher in low-lactose products (Tab 4). The adhesiveness of both type of fermented milks was similar. However, the springiness of MGE fermented milk was lower than those of control products (MGD). Kárnyáczki and Csanádi (2014) observed that the preliminary lactose hydrolysis of milk resulted a firmer texture of yoghurt and the hardness and the adhesion force of the lactose-free yoghurt were higher in comparison to the control product.

Table 4 Texture of low-lactose fermented goat milks

Texture profile analysis	Type of fermented milk	
_	MGD	MGE
	(n=15)	(n=15)
Hardness (N)	$0.36a\pm0.01$	$0.38b\pm0.01$
Adhesiveness (mJ)	$0.18a\pm0.04$	$0.18a\pm0.11$
Stringiness length (mm)	4.22a±0.57	$4.64a\pm0.37$
Cohesiveness	$0.82a\pm0.05$	$0.81a\pm0.03$
Springiness (mm)	$13.64a\pm0.61$	13.36a±0.73
Gumminess (N)	$0.30a\pm0.02$	$0.31a\pm0.02$

Legend: table shows mean \pm standard deviation; a, b - mean values in rows denoted by different letters differ statistically at p \leq 0.05

Sensory evaluation: pre-test

Results from sensory evaluation pre-test of the probiotic fermented goat milk are presented in a Table 5. Significant differences were observed only in sour and sweet flavour between control (MGD) and low-lactose (MGE) fermented goat milk (p \leq 0,05). Regarding flavour attributes, there were no differences (p \leq 0,05) among control and low-lactose product. The scores for creamy-milky flavour, goaty flavour, abnormal flavour, goaty odour and abnormal odour were slightly higher in low-lactose group of fermented milks than in the control group. Moreover, the lactose-free variant of probiotic fermented milk had a more distinct sweet taste than the control and was characterized by a less sour flavour. Lactose hydrolysed products taste sweeter because of the higher sweetness of the individual monosaccharides (Schmidt et al., 2016).

Table 5 Sensory evaluation pre-test of low-lactose fermented goat milks

Sensory attributes	Type of fermented milk	
-	MGD	MGE
	(n=60)	(n=60)
Creamy-milky flavour	3.90a±1.70	5.00a±1.97
Sour flavour	$7.00b\pm1.49$	$5.50a\pm1.47$
Sweet flavour	2.90a±1.52	$4.75b\pm2.27$
Goaty flavour	2.95a±1.93	$3.95a\pm2.04$
Abnormal flavour	$2.63a\pm2.36$	2.85a±2.52
Sour odour	5.32a±2.41	4.40a±2.54
Goaty odour	$2.00a\pm0.58$	$2.15a\pm0.67$
Abnormal odour	$1.79a\pm0.86$	1.90a±0.85

Legend: table shows mean \pm standard deviation; a, b - mean values in rows denoted by different letters differ statistically at p \le 0.05

According to **Nagaraj** *et al.* (2009) yoghurt from milk with 50% and 70% of lactose hydrolysed before fermentation had a creamier texture and a better flavour than yoghurt made from not hydrolysed milk. However, 90% lactose hydrolysis resulted in yoghurts with lower viscosity and a too sweet flavour. **Schmidt** *et al.* (2016) reported that lactose hydrolysis in yoghurt mix showed an improvement in body and texture of 50% and 70% lactose hydrolysed than

unhydrolysed yoghurt which may be due to increased content of monosaccharides that are more soluble and imparted soft body and a creamier texture. Skryplonek et al. (2017) observed that the lactose-free variant of frozen yoghurt was described by a panelist as creamy and smooth and had a more pronounced sweet taste than the control product. Goat milk is characterized by a higher concentration of caproic, caprylic, and capric acid, which are responsible for its distinctive taste and make this product not well accepted by consumers (Mayer and Fiechter, 2012; Park et al., 2007; Schmidt et al., 2016). Therefore, goat milk yoghurt presents a lower overall acceptance compared with cow milk yogurt because of its unpleasant "goaty" taste, even in goat milk yogurt with added fruit pulp (Eissa et al., 2010; Masamba and Ali, 2013; Senaka Ranadheera et al., 2012). It may be considered that the higher sweetness in the low-lactose fermented milk influenced the slightly higher sense of goaty flavour by a panelists.

CONCLUSION

Results showed the influence of lactose hydrolysis on the properties of probiotic fermented goat milk. Bifidobacteria in both fermented goat milk samples resulted viable after 7 days of refrigerated storage and the number of viable cells exceeded 9 log cfu g.-1, indicating these fermented milks meet the therapeutic minimum required for probiotic beverages. There was determined significantly lower amount (about 0.3 log cfu·g-1) of Bifidobacterium Bb-12 in low-lactose fermented milk in comparison to the control sample. The application of the lactase enzyme caused a decrease in L* value (luminosity) of fermented goat milks, as well as in chromatic colour parameters (a* and b*). A decrease in the colour parameters values in low-lactose fermented milk could be caused by a higher acidity of this products. There were also observed difference between the syneresis of control and lactose-free fermented milk samples. Syneresis in control beverage was higher, while the lower was determined in lactose-free product. The lactose hydrolysis of milk resulted a higher hardness in probiotic fermented goat milk. Moreover, the lactose-free variant of probiotic fermented milk had a more distinct sweet and goaty taste than the control and was characterized by a less sour flavour.

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