ผลของจุลินทรีย์ทางการค้าต่อคุณสมบัติทางเคมีกายภาพ จุลชีววิทยา และประสาทสัมผัสของบรีชีสนมแพะ

Effect of Commercial Microorganisms on Physicochemical, Microbiological and Sensory Properties of Goat Brie Cheese

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บทคัดย่อ

บรีชีสนมแพะเป็นผลิตภัณฑ์นมที่เกิดจากการหมักของจุลินทรีย์ที่มีคุณค่าทางโภชนาการสูงเหมาะสำหรับผู้ที่แพ้นมวัว ซึ่งกล้าเชื้อมีความสำคัญอย่างยิ่งในกระบวนการแปรรูปและการเก็บรักษาชีส แบคทีเรียกรดแลคติกจึงเป็นปัจจัยที่สำคัญในการพัฒนาผลิตภัณฑ์ชีสจากนมแพะ งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของกล้าเชื้อแบคทีเรียกรดแลคติกต่อคุณภาพทางกายภาพเคมี จุลชีววิทยา และประสาทสัมผัสของชีสนมแพะ โดยทำการศึกษาบรีชีสนมแพะ 3 กลุ่มที่ใช้กล้าเชื้อแตกต่างกัน ดังนี้ กลุ่มควบคุม (cheese culture and *Penicillium candidum*), กลุ่ม T1 (*Geotrichum candidum*, *P. candidum*, *Lactococcus lactis*, and *Streptococcus thermophilus*), และกลุ่ม T2 (*G. candidum*, *P. candidum*, and *L. lactis*) และวิเคราะห์คุณภาพทางกายภาพเคมี ประสาทสัมผัส และจุลชีววิทยาของบรีชีสนมแพะในวันที่ 1, 12 และ 30 ผลของการศึกษาพบว่า วันที่ 30 ของการเก็บรักษาบรีชีสนมแพะทั้ง 3 กลุ่มมีค่า pH เพิ่มขึ้น และมีปริมาณกรดลดลง บรีชีสนมแพะที่มีอายุการเก็บรักษาได้นานมากขึ้นจะมีปริมาณน้ำลดลง ขณะที่โปรตีนและไขมันเพิ่มขึ้น จึงส่งผลต่อการเปลี่ยนแปลงทั้งเนื้อสัมผัส สี และประสาทสัมผัส โดยบรีชีสนมแพะกลุ่ม T1 มีค่าความแข็ง ความเคี้ยวได้ และค่าสีเหลืองเพิ่มขึ้น รวมทั้งมีคะแนนความชอบด้านสี กลิ่นนมแพะ ความแข็ง รสเค็ม รสหวาน รสเปรี้ยว และความรู้สึกที่ยังกรุ่นอยู่ในปากมากกว่าบรีชีสนมแพะสูตรอื่น ตลอดอายุการเก็บรักษา บรีชีสนมแพะทั้ง 3 สูตรมีปริมาณแบคทีเรียทั้งหมด ยีสต์ และแบคทีเรียกรดแลคติกเพิ่มขึ้นอย่างต่อเนื่อง เมื่อบรีชีสนมแพะมีอายุการเก็บรักษาที่นานขึ้น แบคทีเรียกรดแลคติกจะส่งผลต่อคุณภาพทางด้านเนื้อสัมผัส สี และการทดสอบทางประสาทสัมผัสของผู้บริโภค ดังนั้นการใช้ *Streptococcus thermophilus* เป็นกล้าเชื้อร่วมในการผลิตบรี

ชีสนมแพะสามารถส่งผลให้บรีชีสนมแพะเก็บรักษาไว้ได้นานยิ่งขึ้นและมีคุณสมบัติเป็นที่ยอมรับมากกว่าบรีชีสนมแพะสูตรอื่น

คำสำคัญ: บรีชีส นมแพะ แบคทีเรียกรดแลคติก กล้าเชื้อ

Abstract

Goat Brie cheese is a highly nutritious dairy product suitable for individuals with cow milk allergy. The starter culture plays a crucial role in cheese processing and storage. Lactic acid bacteria are important in goat cheese production. The objective of this research was to investigate the effect of lactic acid bacteria starter culture on the physicochemical, microbiological and sensory properties of goat Brie cheese. Three types of goat Brie cheese were produced from goat milk using different starter cultures as follows: control (cheese culture and *Penicillium candidum*), T1 (*Geotrichum candidum*, *P. candidum*, *Lactococcus lactis*, and *Streptococcus thermophilus*), and T2 (*G. candidum*, *P. candidum*, and *L. lactis*). Analyses of the aforementioned parameters of goat Brie cheese were performed on days 1, 12, and 30. On day 30 of storage, all three goat Brie cheese treatments led to increased pH and reduced acidity. Aged goat cheese contained less water, but more protein and fat leading to change in texture, color, and sensory properties. T1 goat Brie cheeses had increased hardness, chewiness and yellow values. Furthermore, its preference ratings for color, goat milk odor, hardness, salty flavor, sweet taste, sour taste, and aftertaste than those of the other goat Brie cheese formulae. Throughout their shelf life, all three types of goat Brie cheese had an increasing quantity of total bacteria, yeast, and lactic acid bacteria. When goat Brie cheese had a long shelf life, lactic acid bacteria affected cheese texture, color, and consumer sensory ratings. Therefore, use of *S. thermophilus* as a co-starting culture in goat Brie cheese production could make older goat Brie cheese more acceptable than goat Brie cheese formulae.

Keywords: Brie cheese; Goat milk; Lactic acid bacteria; Starter culture

1. Introduction

Goat milk is an important food source with unique composition, containing small and easily digestible fatty acids. Its total basic chemical composition is better than that of cow milk. Furthermore, goat milk has a higher solid content, which is a combination of proteins, carbohydrates, fats, and mineral content, than cow milk [1], [2]. The milk solid content is dependent on the animal species and feed and can be used as a raw material for food processing in various types of dairy products, such as cheese. In 2017, the global dairy goat population was predicted to be 218 million. Globally, the number of dairy goat populations has been steadily increasing, with a major increase in the 1990s. Over the last decade (2007–2017), the global dairy goat population has expanded by approximately 22% [3]. Goat milk production has increased due to the

increasing number of consumers who have lactose intolerance and cannot consume cow milk. Lactose intolerance has been found in up to 65-70% of adults worldwide. The incidence varies according to ethnicity. Lactose intolerance is most common in Asian countries and is rarely found in Scandinavia and Europe [4]. In Thailand, the increased hydrogen gas in the breath after lactose consumption was measured in 45 adults aged 21-31 years in a study conducted in 2004. The results revealed that 51% of the participants were lactose intolerant. The latest studies conducted in 2017 observed lactose intolerance in up to 100% of individuals in China, Japan, and Vietnam, which reflected the significant increase in lactose intolerance associated with cow milk consumption in the Asian continent. Therefore, the actual figure for this condition in Thailand was probably above 51% [5].

Cheese is a dairy product produced via microbial fermentation. Thailand's cheese industry is currently estimated to be worth approximately US$ 87 million. The market is projected to expand at a yearly rate (CAGR 2021-2025) [6]. The cheese production market can be divided into two main groups. Processed cheeses are cheeses that undergo processing of their texture to become more convenient for human consumption and have prolonged shelf life. Unprocessed cheeses are cheeses with a texture that does not need to be improved. These cheeses have held market shares of 54% and 46%, respectively. The microorganisms that produce lactic acid can convert lactose into lactate, and acidity rates have a direct effect on curd texture. The acidity rate directly affects demineralization, such as that of calcium [7]. The soft texture of cheese is a consequence of calcium demineralization, which is a critical structural component of milk in both soluble (including ionic) and colloidal (including casein-bound) forms [8]. These cheeses have a silky white surface and are covered with *Penicillium camemberti* or *Penicillium candidum* mycelium. This mold is responsible for the cheese's unique style, flavor, and aroma. Brie and Camembert cheeses are made using these microorganisms [9]. *Lactobacillus*, *Streptococcus*, and *Lactococcus* are common lactic acid bacteria used as starter cultures [10], [11], [12]. *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* spp. *lactis* are the two strains that are most often utilized in the manufacture of cheddar cheese [13]. They have a strong acid-producing capacity and are beneficial for protein decomposition and autolysis, which are important for cheese production [14]. Therefore, the appropriate selection of microbial strains is crucial in dairy product processing. Expertise is required in the selection of microorganisms to suit the physicochemical properties of cheese. Biochemical reactions may occur during cheese processing and storage. Therefore, researchers select *Streptococcus thermophilus*, which has good growth properties under low oxygen or anaerobic conditions. It is a thermophilic bacterium that is utilized to produce fermented dairy products, such as yogurt and a range of cheeses. It can convert lactose in milk to lactic acid and form formic acid. It is also a microorganism that is relatively cheap and easy to control with good heat resistance. Brie cheese

can be produced from goat milk using *S. thermophilus* as a starter culture. Therefore, it is an alternative dairy product for consumers who have lactose intolerance associated with cow milk consumption. Furthermore, it is helpful in the development of agricultural processing products for farmers. Hence, we have studied the development of Brie cheese products from goat milk derived from goats in Chachoengsao Province to further develop and improve these products and continue to generate income for farmers. In this research, we aim to investigate the effect of lactic acid bacteria starter culture on the physicochemical, sensory and microbiological properties of goat Brie cheese.

2. Materials and methods

2.1. Study area

The study was carried out at the Faculty of Science and Technology, Quality Control Lab, Rajamangala University of Technology Tawan-ok (RMUTTO), Thailand, and a goat milk farm in Chachoengsao Province, Thailand, from December 2020 to June 2021.

2.2. Materials

Goat milk samples were randomly collected from goats on a farm in Chachoengsao Province, Thailand. Animals were fed natural vegetation for approximately 8 h per day and supplementary feeding in the form of concentrate feed mixture to cover the energy requirements after returning from the pasture at night. Water was offered daily in the morning. The milk samples were immediately maintained and stored under refrigerated conditions until transferred to the laboratory for analysis within 24 h.

2.3. Bacterial strains

The commercial freeze-dried mixed microbial starters (Sacco, Italy) used in the different formulae were as follows: i) the control formula comprised cheese culture and *Penicillium candidum*, ii) treatment 1 (T1) comprised *Geotrichum candidum*, *P. candidum, Lactococcus lactis*, and *S. thermophilus*, and iii) treatment 2 (T2) comprised *G. candidum*, *P. candidum*, and *L. lactis*.

2.4. Brie cheese production from goat milk

For cheese production, the milk was pasteurized at 72 °C for 15 min. Then, the milk temperature was reduced to 35 °C, and 0.06% of each starter culture was added to each group, and the mixture was allowed to stand for 40 min. Later, 0.25% renin (Sacco, Italy) was added and the mixture was allowed to stand for 45 min, cut into pieces with a size of 2 × 2 × 2 cm, and slowly stirred well four times every 15 min. The mixture was poured into the sieve to drain out the whey water and flipped to the other side of the curd cubes at 15 min, 30 min, 1 h, 2 h, and 8 h. The cheese cubes were soaked in 20% saline solution for 50 min, kept at 14 °C for 24 h, and incubated at 13°C for 11 days. Subsequently, the samples were stored at 4 °C and analyzed on days 1, 12, and 30.

2.5. Physicochemical analyses

2.5.1. pH and titratable acidity analysis

The pH value of the cheese samples (10 g of cheese pulverized with 10 mL of distilled

water) was measured using a pH meter (Consort C830, Belgium) [15]. The cheese lactic acid content was determined using the titratable acidic method of AOAC [16] by weighing 10 g of sample and titrating with 0.1% NaOH solution using phenolphthalein (Ajax Finechem) as an indicator. Subsequently, the total acid content was titrated with 1 mL of sodium hydroxide standard solution that made an equivalent reaction with 0.009 g of lactic acid (KemAus, Australia).

2.5.2. Color analysis

Cheese color values were determined using a spectrophotometer (Konica Minolta CM-3500d, Japan). Data processing was performed using the SpectraMagic software (version NX). The results were expressed as the CIE L*, a*, and b* color values, where L* is lightness value, a* is redness (+) to greenness (-), and b* is yellowness (+) to blueness (-) [17].

2.5.3. Texture analysis

Cheese texture measurement (hardness, adhesiveness, springiness, cohesiveness and chewiness) was performed using the TA-XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) [18].

2.5.4. Proximate analysis

The AOAC method was used to determine the fiber volume, moisture, ash, protein, fat, and carbohydrate contents of goat Brie cheese [16].

2.6. Sensory analysis

The sensory test participants recruited 112 untrained sensory test subjects to determine the sensory acceptability of goat Brie cheese. The three cheeses were cured for 1, 2 and 30 days, with the cured cheeses being refrigerated throughout their shelf life. Sensory test participants aged 20 to 60 ate cheese on a regular and were not allergic to milk. The tester administered sensory tests to ascertain the impact of various sensory characteristics. color, cream aroma, milk goat aroma, hardness, adhesiveness, salty taste, sweet taste, sour taste, bitter taste, and an aftertaste that lingers in the mouth. A nine-point hedonic scale was implemented (9*:* like it extremely; 8*:* like it very much; 7*:* like it moderately; 6*:* like it slightly; 5*:* neither like nor dislike it; 4*:* dislike it slightly; 3*:* dislike it moderately; 2*:* dislike it very much; 1*:* dislike it extremely) using an adapted form of the method of González and Zárate [19]. This analysis was approved by the ethics committee of our institution (RMUTTO REC No. 002/2021).

2.7. Microbiological analysis

The total bacterial plate count, yeast count, and lactic acid bacteria count were determined using an adapted form of the method of Taboada et al. [20]. First, 25 g of goat Brie cheese and 0.1% peptone solution were mixed well using a Stomacher (400 Circulator, UK) for 2 min. Then, the mixture was diluted using the serial dilution method. A total of 0.1 mL of sample was cultured on agar. Total bacteria were analyzed using plate count agar (PCA), the yeast count was determined using Sabouraud dextrose agar (SDA), the lactic acid bacteria count was determined using De Man, Rogosa, and Sharpe (MRS). The samples were incubated at 37 °C for 24-48 h. Colonies were counted in the culture medium. The results were reported as colony forming units (log CFU g^{-1}).

2.8. Goat Brie cheese changes during storage

The appropriate changes in goat Brie cheese were compared with those of the control formula during storage at 4 ±1 °C. The physicochemical and microbiological properties were analyzed on days 1, 12, and 30.

2.9. Statistical analysis

Statistical analysis was conducted by planning a complete randomized design trial. Results are presented as the mean ± standard deviation (mean ± SD). The mean differences were tested using one-way ANOVA and Duncan's multiple range test were carried out at significance level of 0.05 using SPSS version 11.5 based on data from triplicated tests.

3. Results and discussion

3.1. pH and acidity analysis of goat Brie cheese

By measuring the pH and acidity of goat Brie cheese, we found that the control, T1, and T2 cheese types had initial pH values of 4.74, 4.93, and 4.73, respectively. After incubating the samples at 13 °C for 12 days, the pH of the control and T1 samples slightly decreased ($P>0.05$). T2 cheese showed a significant increase in pH ($P\leq0.05$). After the cheese was stored for 30 days, the control and T1 cheeses had significantly increased pH ($P\leq0.05$), whereas the T2 cheese had slightly increased pH ($P>0.05$) (Figure 1a). The initial acid contents were 14.93%, 10.67%, and 11.73%, respectively. The acid content of the control cheese was significantly higher than that of the other cheese types ($P\leq0.05$). However, on days 12 and 30 of storage, the acid percentage continued to decrease. T1 cheese had a higher acid percentage on day 12, which decreased on day 30. This is consistent with the increase in pH during storage. However, T2 cheese had a stable acid percentage throughout its shelf life (Figure 1b). Therefore, the cheese pH and acidity levels depended on the addition of the specific starter culture. González and Zárate [19] used local starter cultures isolated from goat milk cheese (*Lactococcus lactis* subsp. *lactis* TF53, *Lactobacillus plantarum* TF191, and *Leuconostoc mesenteroides*). During goat cheese production, it was found that the initial pH was 5.27, which slightly increased during the 90-day storage (5.27–5.33). The acid content was 1.24–1.50%. However, goat cheese produced using a commercial starter culture (*Lac. lactis* subsp. *lactis*, *and Lac. lactis* subsp. *cremoris*, and *S. thermophilus*) had an initial pH of 5.18, which, then, slightly increased (5.18 – 5.54), whereas its acid content was 1.21–1.32%.

Lactic acid bacteria play a major role in cheese processing. In the first phase of the fermentation process of lactic acid bacteria from the homofermentative group (Lactococci) and the heterofermentative group (Lactobacilli) acid is released, resulting in a rapid pH decrease [21]. During the prolonged cheese incubation period, the pH was increased and the acid content decreased due to the use of lactic acid to produce non-acidic products and the formation of alkaline nitrogen compounds during the protein digestion process [19]. Proteolysis affects cheese ripening by causing changes in texture as a result of the degradation of the protein

network, along with an increase in pH and higher water-binding capacity [22], [23].

3.2. Texture analysis of goat Brie cheese

Texture analysis of goat Brie cheese on day 1 showed that the hardness and chewiness of the control cheese were higher than those of the other formulations (P≤0.05). Since goat Brie cheese was incubated for 12 and 30 days, the hardness of T1 cheese was higher than that of the control and T2 cheeses (P≤0.05). The hardness of all three cheese types increased on day 12 and decreased on day 30 (P≤0.05), while the chewiness of T1 cheeses was the highest. Chewiness reduced with increased storage time for all three cheese varieties. In contrast to adhesiveness and springiness, chewiness did not vary significantly between the three cheese varieties on days 1 and 12 (P>0.05). However, the adhesiveness of the control and T2 cheeses differed from that of T1 cheese and was significantly increased on day 30 compared to that on day 12 (P≤0.05). The springiness of the control and T1 cheese was significantly different from that of T2 cheese (P≤0.05). The springiness of T2 cheese on day 30 decreased compared to that of day 12, while the adhesiveness of cheeses on days 1 and 30 did not differ. On day 12, the springiness of T2 cheese was different from that of the control and T1 cheeses (P≤0.05). All three cheese types had increased adhesiveness on day 30 compared to that of day 12, as shown in Table 1. Cheese texture is determined by many factors, such as the water, protein, and fat content. Cheese with a high-water content has less springiness, which leads to low adhesiveness, making it easy to break apart [24]. The protein structure was altered and water content decreased in cheese with longer shelf life. These factors affect the cheese hardness and stickiness. In particular, the water content directly affects changes in cheese texture [25], [26].

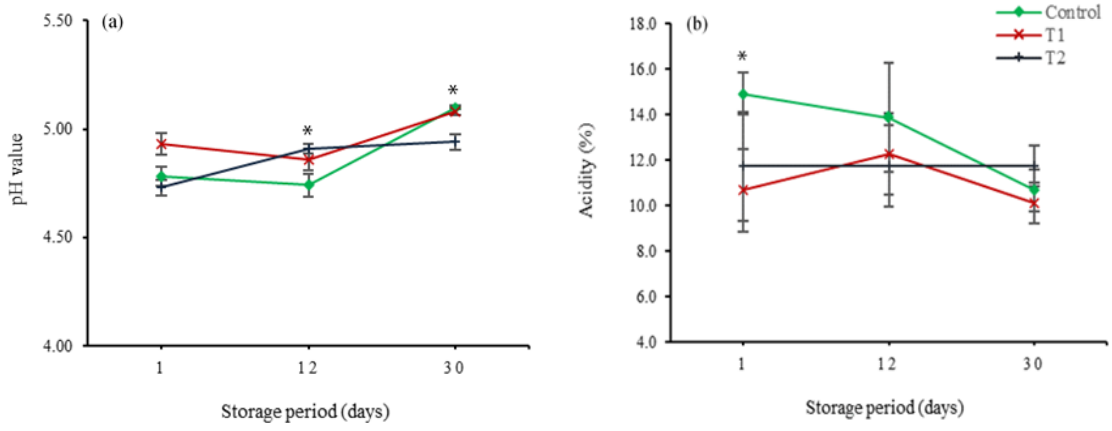

Figure 1 Changes in the (a) pH and (b) acid content of goat Brie cheeses during a 30-day storage period. *significant difference (P≤0.05).

3.3. Color analysis of goat Brie cheese

Goat Brie cheese color analysis on day 1 showed that the three cheese types were not statistically different in terms of brightness (L*), redness (a*), and yellowness (b*). Since goat Brie cheese was incubated for 12 days, the control and T2 cheeses had significantly higher brightness and redness than T1 cheese (P≤0.05). However, the brightness of the three goat Brie cheese types after treatments was slightly increased on day 30 of storage, but was not significantly different (P>0.05), when compared to that on day 12. The redness value of the control and T2 cheese was higher than that of the T1 (P≤0.05), while the yellowness values of T1 and T2 cheese were different from those of control (P≤0.05) at days 12 and 30. The T2 cheese had the highest yellowness values at days 1, 12, and 30. As shown in Table 2, the value increased throughout the storage. In a previous study by Setywardani *et al*. [15] in which probiotic bacteria (*L. plantarum* TW14 and *L. rhamnosus* TW2) were used in goat milk cheese production, the cheese was stored in a refrigerator for 60 days and L* was decreased and b* was significantly increased on day 60 (P≤0.05). The a* values were not significantly different over the storage period (P>0.05). However, when cheese was stored for a long time, its water content decreased, resulting in an increase in the solid and fat content. As a result, the luster, especially the brightness of the cheese, was reduced [27], [28], while its yellow value increased. The carotene pigment present in milk fat is another factor that increases its yellowness [29]. The brightness and yellowness of T2 cheese increased on day 30. Furthermore, moisture analysis showed a significant decrease in moisture content compared to that of the first day (P≤0.05).

Table 1 Changes in the texture of goat Brie cheeses with a 30-day shelf life

Treatments	Storage period (days)	Texture				
		Hardness (g)	Adhesiveness	Springiness (mm)	Cohesiveness	Chewiness (mJ)
Control	1	1883.97 ± 161^{aB}	-95.68 ± 51.78^{nsA}	0.67 ± 0.14^{nsNS}	0.29 ± 0.07^{nsB}	356.48 ± 43.68^{aNS}
	12	2484.77 ± 162^{bA}	-193.80 ± 79^{nsB}	0.62 ± 0.05^{nsNS}	0.30 ± 0.05^{bB}	457.78 ± 56^{nsNS}
	30	1105.52 ± 166^{bC}	-62.10 ± 30^{aA}	0.69 ± 0.10^{aNS}	0.48 ± 0.07^{nsA}	380.77 ± 163^{aNS}
T1	1	1455.11 ± 264^{bB}	-148.97 ± 99.57^{nsNS}	0.62 ± 0.08^{nsAB}	0.34 ± 0.09^{nsAB}	316.13 ± 116.15^{abNS}
	12	3160.63 ± 568^{aA}	-372.53 ± 401^{nsNS}	0.58 ± 0.04^{nsB}	0.24 ± 0.05^{bB}	440.54 ± 113^{nsNS}
	30	1342.99 ± 85^{aB}	-117.01 ± 51^{bNS}	0.69 ± 0.05^{aA}	0.39 ± 0.09^{nsA}	362.69 ± 100^{aNS}
T2	1	1255.58 ± 209^{bA}	-77.01 ± 15.27^{nsAB}	0.62 ± 0.10^{nsNS}	0.26 ± 0.03^{nsB}	215.11 ± 77.55^{bB}
	12	1254.97 ± 226^{cA}	-181.95 ± 130^{nsB}	0.64 ± 0.04^{nsNS}	0.43 ± 0.04^{aA}	348.58 ± 70^{nsA}
	30	803.41 ± 137^{cB}	-21.10 ± 22^{aA}	0.56 ± 0.0^{bNS}	0.42 ± 0.06^{nsA}	186.30 ± 53^{bB}

Data are presented as means (of three separate measurements) ± standard deviation (SD). Means with different lowercase superscript letters (a, b, c) within the same column are significantly different (P≤0.05) from those in each treatment in the same storage period. Means with different capital superscript letters (A, B, C) within the same column are significantly different (P≤0.05) from those in the same treatment in each storage period. ns/NS indicate no significant difference (P>0.05).

Table 2 Changes in the color of goat Brie cheeses during a 30-day storage period

Treatments	Storage period (days)	Color		
		L*	a*	b*
Control	1	80.30 ± 2.25^{nsNS}	-1.48 ± 0.19^{nsNS}	7.48 ± 0.85^{nsNS}
	12	80.57 ± 1.89^{abNS}	-1.67 ± 0.10^{aNS}	7.60 ± 0.39^{bNS}
	30	81.63 ± 2.15^{nsNS}	-1.53 ± 0.29^{aNS}	7.33 ± 0.81^{bNS}
T1	1	83.50 ± 3.50^{nsNS}	-1.55 ± 0.07^{nsA}	8.68 ± 0.19^{nsNS}
	12	79.80 ± 1.97^{bNS}	-2.06 ± 0.13^{bB}	9.21 ± 0.77^{aNS}
	30	81.03 ± 1.51^{nsNS}	-2.22 ± 0.18^{bB}	9.61 ± 0.87^{aNS}
T2	1	79.94 ± 2.91^{nsNS}	-1.56 ± 0.16^{nsNS}	8.48 ± 1.21^{nsNS}
	12	84.16 ± 2.33^{aNS}	-1.58 ± 0.25^{aNS}	8.53 ± 0.64^{abNS}
	30	84.24 ± 0.68^{nsNS}	-1.51 ± 0.35^{aNS}	8.07 ± 1.24^{abNS}

Data are presented as means (of three separate measurements) ± standard deviation (SD). Means with different lowercase superscript letters (a, b, c) within the same column are significantly different (P≤0.05) from those in each treatment in the same storage period. Means with different capital superscript letters (A, B, C) within the same column are significantly different (P≤0.05) from those in the same treatment in each storage period. ns/NS indicate no significant difference (P>0.05).

3.4. Proximate analysis of goat Brie cheese

Proximate analysis included the determination of the protein, carbohydrate, fat, ash, fiber, and moisture content of the three types of goat Brie cheese. The results revealed that on days 1 and 12, the percentages of protein, carbohydrate, fat, fiber, and moisture content were not significantly different among the three cheese types (P>0.05). On days 1 and 12, the ash content of the cheese was determined; control and T1 cheeses had a higher ash content than T2 cheeses (P≤0.05). However, there was no statistically significant difference in the ash content of the 30-day cheeses between the three cheese groups (P>0.05). The carbohydrate content of T1 cheese is highest when the carbohydrate content is shown on day 30. Although the moisture level was significantly lower than that of the other cheese samples (P≤0.05). At 30 days, it was found that T1 significantly increased the nutritious content of the carbohydrates in cheese. When compared to days 1 and 12, the difference is statistical significance (P≤0.05). In all three cheese types, the fat content significantly increased (P≤0.05) from 12.91–14.14% on day 1 to 17.99–18.45% on day 30, while the ash content decreased from 2.48%–3.01% to 1.02–1.23% (P≤0.05). The fiber content of T1 cheese on day 30 was significantly decreased compared to that on day 1 (P≤0.05), in contrast to that in the other two cheese types, which was slightly decreased (P>0.05). The moisture content of the control and T1 cheeses was significantly changed (P≤0.05), while that of T2 cheese did not differ (P>0.05), as shown in Table 3. This was consistent with the report of Taboada *et al*. [20] who used lactic acid bacteria as a starter culture for goat milk cheese production. During the 30-day cheese incubation, the fat content increased, but the

moisture content decreased. The decrease in moisture was due to the exchange of water with the environment. The protein and fat contents increased as well.

Table 3 Changes in the nutritional composition of goat Brie cheeses during a 30-day storage period

Treatments	Storage period (days)	Nutritional composition (%)					
		Protein	Carbohydrate	Fat	Ash	Fiber	Moisture
control	1	29.82 ± 1.76^{nsNS}	1.48 ± 1.03^{nsNS}	13.18 ± 0.89^{nsB}	3.01 ± 0.14^{aA}	0.97 ± 0.08^{nsNS}	51.54 ± 0.20^{nsA}
	12	28.17 ± 1.12^{nsNS}	0.66 ± 0.55^{nsNS}	18.47 ± 0.45^{nsA}	1.47 ± 0.16^{aB}	1.01 ± 0.17^{nsNS}	50.22 ± 0.46^{nsB}
	30	27.48 ± 1.43^{nsNS}	1.07 ± 0.95^{bns}	18.25 ± 0.11^{nsA}	1.23 ± 0.14^{nsB}	0.94 ± 0.20^{nsNS}	51.04 ± 0.74^{aAB}
T1	1	29.59 ± 0.88^{nsNS}	1.39 ± 1.15^{nsAB}	12.91 ± 1.75^{nsB}	2.79 ± 1.67^{abA}	1.03 ± 0.05^{nsA}	52.30 ± 1.97^{nsA}
	12	28.70 ± 0.67^{nsNS}	0.19 ± 0.14^{nsB}	17.82 ± 1.37^{nsA}	1.03 ± 0.21^{bB}	1.07 ± 0.08^{nsA}	51.19 ± 0.82^{nsA}
	30	28.32 ± 0.18^{nsNS}	3.50 ± 1.63^{aA}	17.99 ± 0.62^{nsA}	1.06 ± 0.21^{nsB}	0.83 ± 0.03^{nsB}	48.30 ± 1.11^{bB}
T2	1	29.86 ± 0.30^{nsNS}	1.78 ± 1.52^{nsNS}	14.14 ± 0.55^{nsB}	2.48 ± 0.19^{bA}	1.27 ± 0.43^{nsNS}	50.47 ± 1.20^{nsNS}
	12	27.95 ± 1.59^{nsNS}	0.46 ± 0.37^{nsNS}	18.64 ± 0.30^{nsA}	1.41 ± 0.24^{abB}	1.05 ± 0.25^{nsNS}	50.49 ± 1.69^{nsNS}
	30	29.06 ± 0.56^{nsNS}	1.15 ± 0.56^{bNS}	18.45 ± 0.41^{nsA}	1.02 ± 0.06^{nsC}	1.02 ± 0.18^{nsNS}	49.31 ± 1.11^{abNS}

Data are presented as means (of three separate measurements) ± standard deviation (SD). Means with different lowercase superscript letters (a, b, c) within the same column are significantly different (P≤0.05) from those in each treatment in the same storage period. Means with different capital superscript letters (A, B, C) within the same column are significantly different (P≤0.05) from those in the same treatment in each storage period. ns/NS indicate no significant difference (P>0.05).

3.5. Microbiological analysis of goat Brie cheese

Microbiological analysis of the three goat Brie cheese types on days 1 and 12 showed no differences in the total bacterial counts (P>0.05). However, on day 30, T2 cheese showed a significant increase in the total bacterial counts (P≤0.05). All cheese types showed a substantial increase in the total bacterial counts throughout their shelf life, especially T2 cheese (Figure 2a). Similarly, the lactic acid bacteria test of all 3 treatments of goat Brie cheese showed no difference in lactic acid bacteria content (P>0.05) throughout the shelf life. T2 cheese had a higher lactic acid bacteria count than other treatments and tended to increase steadily (P≤0.05), while T1 cheese had a slight decrease in lactic acid bacteria count (Figure 2b). The yeast counts in all three cheese types were in the same direction as the bacteria and lactic acid bacteria counts. On day 1, the yeast counts were 3.92–4.21 log CFU g^{-1}. Each cheese type had different yeast counts (P≤0.05), while on days 12 and 30, the yeast counts were increased to 4.77–5.12 log CFU g^{-1} and 6.44–6.46 log CFU g^{-1}, respectively. Each cheese type contained a different yeast species (P>0.05). However, the yeast counts of all cheese types significantly increased throughout the shelf life (P≤0.05), as shown in Figure 2c. A previous study showed that the lactic acid bacteria grew further by slowly isolating fats and proteins during storage of goat milk cheese at a low temperature for no longer than 60 days. However, the longer the cheese storage, the more lactic acid bacteria tended to decrease. Cheese dehydration

results in a decrease in Aw values and an increase in the proportion of salt to moisture [19], [20], [30], [31], [32]. Furthermore, an increase in total bacteria and yeast was previously reported throughout the shelf life [33].

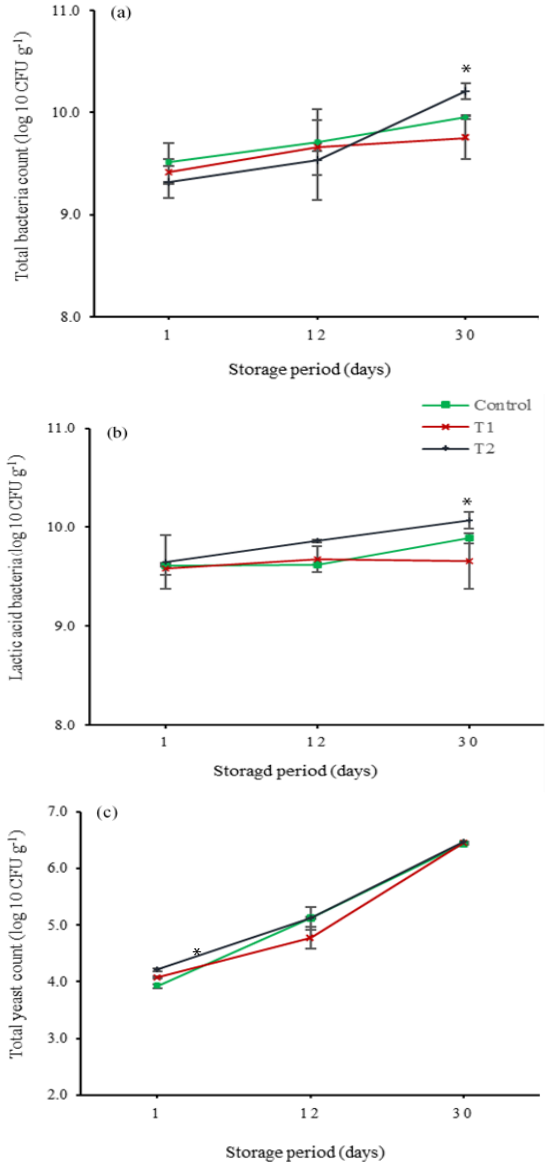

Figure 2 Changes in the total bacterial count (a), lactic acid bacteria count (b), and yeast count (c) of goat Brie cheeses. * significant difference (P≤0.05).

3.6. Sensory analysis of goat Brie cheese

Sensory analysis of goat Brie cheese on days 12 and 30 revealed that all three cheese types had scores for color, cream aroma, goat milk aroma, hardness, stiffness, salty, sweet, sour, bitter, and aftertaste. The scores were not significantly different among the different cheese types ($P>0.05$). The participants rated their preference from “like slightly” to “like moderately”. The control cheese had the highest preference score on day 12 for goat milk aroma and aftertaste. T1 cheese had higher preference scores than the other cheeses in terms of stickiness, sourness, and bitterness. T2 cheese had higher preference scores for color, cream aroma, stickiness, and bitterness than the other cheeses (Figure 3a). However, on day 30, the preference scores for different aspects decreased compared to those of day 12, especially for stickiness and aftertaste of the control cheese, color and hardness of the T2 cheese, and stickiness of the T1 cheese ($P\leq0.05$). Meanwhile, T1 cheese had higher preference scores for cream aroma, salty taste, sweet taste, and sour taste, which slightly increased compared to those of day 12 ($P>0.05$). The preference score of goat Brie cheese was higher than that of the other treatments in several aspects, including color, goat milk aroma, hardness, salty taste, sweet taste, sour taste, and aftertaste (Figure 3b). The sour smell of goat Brie cheese is caused by the lactic acid bacteria added to the cheese production process. Lactic acid bacteria converted milk lactose into lactic acid; therefore, the pH decreased. In addition, the distinctive aroma and flavor of cheese are caused by the degradation of fats, proteins, and amino acids. Starter cultures with or without lactic acid bacteria were used. Since cheese has a longer age during ripening, lactic acid bacteria undergo biochemical changes, resulting in an increase in the cheese's distinctive aroma [16] through citrate metabolism and the formation of compounds that affect aroma and flavor, such as acetate, diacetyl, acetoin, and butanediol [34]. In addition, cheese aging changes the structure of fat and protein in cheese, while decreasing the cheese water content. These factors affect cheese texture [25], [26]. It has been demonstrated that the sensory qualities of cheese are greatly affected by the levels of peptides, amino acids, and free fatty acids resulting from proteolysis and lipolysis during storage [35]. The products of these microbiological and enzymatic activities result in a vast array of flavor compounds [36], [37]. However, cheese produced using *S. thermophilus* combined with *L. casei* showed the highest sensory quality, mainly in taste or appearance [38]. The use of lactic acid bacteria as the starter culture in cheese production is important for better cheese aroma, taste, and texture, as cheese has a longer shelf life.

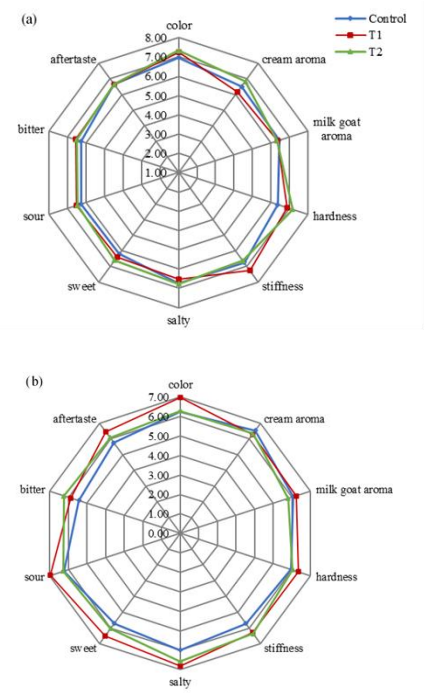

Figure 3 Consumer acceptance scores of goat Brie cheeses on days (a) 12 and (b) 30.

4. Conclusion

All three goat Brie cheese treatments affected the physical and chemical properties of the cheese. Food ingredients, texture, microbiology, and sensory properties change when shelf life is longer. A contributing factor to this change was the decrease in the water content of the cheese, and the increase in the protein and fat contents. These changes give T1 cheese better texture and physical characteristics. In addition, the sensory test scores of the consumers showed that preference for cream, salty, sweet, and sour tastes increased from day 12, and the preference scores were on color, goat milk odor, hardness, salty, sweet, sour, and sensation. Therefore, the use of lactic acid bacteria, and in particular *S. thermophilus*, as starter co-culture in goat Brie cheese production, resulted in older goat cheese being more acceptable by consumers than the other cheese types. In the future, the proportion of lactic acid bacteria (*S. thermophilus* and *L. lactis*) will need to be studied in goat Brie cheese production based on additional sensory properties.

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