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Research Article

The effect of potassium sorbate on the survival of *Brucella melitensis* during ripening of Tulum cheese

Ahmet Hulusi Dinçoğlu[1], Bahri Patır[2]

[1]Department of Nutrition and Dietetics, Faculty of Health Sciences, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye
[2]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Bingöl University, Bingöl, Türkiye

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Correspondence:
AH. DİNÇOĞLU
(adincoglu@mehmetakif.edu.tr)

ORCID
AH. DİNÇOĞLU : 0000-0002-9669-5964
B. PATIR : 0000-0001-5933-9971

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ABSTRACT

The objective of this study was to evaluate the antimicrobial effect of potassium sorbate on the viability of *Brucella melitensis* in Tulum cheese, as well as the resulting microbiological and physicochemical changes during ripening. Five groups were formed, with group A serving as the control and group B inoculated with *B. melitensis*. Groups C, D, and E were also inoculated with *B. melitensis* and treated with potassium sorbate at concentrations of 0.05%, 0.10%, and 0.20%, respectively. During the ripening process, the total number of mesophilic aerobic bacteria, fecal streptococci, staphylococci-micrococci, coliforms, and yeasts and molds in the samples varied depending on the amount and presence of sorbate. The presence of potassium sorbate affected *B. melitensis* at different rates during ripening. The level of *B. melitensis* was found to be higher in group B compared to groups C, D, and E. There were no significant differences in the acidity of the treatment groups. During ripening, the pH values of the samples with sorbate were lower than those without sorbate. No significant differences in salt amounts and moisture levels were observed between the groups with sorbate and those without sorbate. The addition of definite concentrations of potassium sorbate to the formulation of Tulum cheese revealed a shortened lifespan of *B. melitensis* and a significant reduction in the number of other harmful microorganisms. This suggests that the use of sorbate in Tulum cheese manufacturing has the potential to improve microbial safety in this type of product.

INTRODUCTION

Brucellosis is a zoonotic disease that was clinically defined by Morston in 1859 and has been known since ancient times. It remains the most common zoonotic disease worldwide, with over 500,000 new cases reported annually, despite its low mortality rate. Brucellosis is associated with significant permanent disability and is a major cause of travel-related morbidity (Pappas et al., 2006). Brucella has been reported as a possible type B biological weapon (Shakir, 2020).

Eleven species of the *Brucella* genus have been identified to date. However, only *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis* affect humans (Seleem et al., 2010; Shakir, 2020). Brucellosis remains a neglected disease in many developing countries. Based on data published between 1983 and 2019, it has been reported that the prevalence of brucellosis is high in Kosovo, Kuwait, Qatar, Venezuela, Syria, and Iraq (Franc et al., 2018). In endemic areas, humans can contract brucellosis by coming into contact with infected animals or consuming dairy products, particularly unpasteurized cheeses made from ewe and goat milk (Seleem et al., 2010). *Brucella melitensis* infections are more common in the general population than other *Brucella* species, while *B. abortus* and *B. suis* infections typically affect occupational groups. In some countries, including Italy, 99% of human brucellosis was caused by *B. melitensis* (De Massis et al., 2005).

According to the report, *B. melitensis* (29.48%) is the most prevalent *Brucella* species found in dairy products. This is attributed to the unregulated raising and transportation of numerous ewe and goat herds, as well as the production of milk and dairy products without processing in small-scale goat and ewe farms (Capparelli et al., 2009). Research results show that in regions where the disease is endemic, raw milk has a higher level of *Brucella* contamination (16.97%) compared to cheese (7.1%). This is due to the role of fermenting bacteria in reducing the growth of *Brucella* species in cheese, as well as ambient pH and nutrient competition (De Massis et al., 2005; Dadar et al., 2020).

Tulum cheese is a traditional cheese made from raw ewe milk in Turkey. It can also be made from goat's milk, a combination of ewe and goat's milk, or cow's milk. The cheese has a granular or open texture, is semi-hard, and is white or cream-colored with a sharp aroma (Tekin and Güler, 2019). Microorganisms, particularly yeasts present in the milk and ripening medium, play a crucial role in the ripening process of cheese. Tulum cheese is produced locally from raw milk without the use of a starter culture, which means that its mi-

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crobial content is solely derived from the natural microbiota of raw milk (Tomar et al., 2020). This poses a potential public health risk to individuals who consume Tulum cheese made from raw milk. Studies have reported different levels of bacterial isolation in commercially available Tulum cheeses when testing for the presence of *Brucella* species (Patır and Dinçoğlu, 2001). According to Öztürk and Nazlı's (1996) report, the number of bacteria in Tulum cheese produced with cow and ewe milk inoculated with *B. melitensis* was $1.5x10^9$ and $2.0x10^9$ cfu/g at the beginning of ripening. However, on the 20th day of ripening, the number of bacteria decreased significantly to $1x10^2$ and $2.8x10^2$ cfu/g, and bacteria could not be isolated in the subsequent days.

As a result of studies examining the effects of many antibacterial components against *Brucella* spp., both in vivo and in vitro, have obtained results at different levels (Ijaz et al., 2021). Many antimicrobial additives are used alone or in combination in foods, including sorbic acid and its salts as a preservative. Potassium sorbate is an antimicrobial compound widely used in the food industry since the mid-1950s. It is a salt of sorbic acid and has many advantages over other preservatives. Potassium sorbate is considered a substance 'generally recognized as safe' (GRAS) and its usage should not exceed 0.2%. (Alzate et al., 2017; Palou et al., 2016). As per the United States Code of Federal Regulations, specifically Title 21 and Section 182.3640, potassium sorbate is a recommended general-purpose preservative when used by good manufacturing practices (Lungu and Johnson, 2005). Sorbic acid and its salts are primarily used in the milk and dairy industry and are commonly employed in cheese production. Although antifungal effects are primarily used in dairy products, research has also been conducted on their inhibitory effects against pathogens in various foods (Mohammadzadeh-Aghdash et al., 2018).

This study investigates the survival of *B. melitensis* and the microbiological and physicochemical changes in Tulum cheeses produced from raw ewe milk by adding *B. melitensis* and potassium sorbate at different concentrations during the ripening process.

MATERIALS and METHODS

Milk samples

Tulum cheese was produced using commercially obtained ewe milk samples. The milk was collected in the early morning and transported to the laboratory under cold chain conditions. Two hundred milliliters of the milk were analyzed for microbiological and physicochemical properties as well as antibiotic residues and kept at 4°C.

B. melitensis strain used in inoculation

The study utilized the B. melitensis biotype 3 strain, which was previously isolated and identified by Patır and Dinçoğlu (2001) from Tulum cheese sold in Elazığ. Before inoculation, the strain was incubated in Mueller-Hinton Broth (MHB, Merck) at 37°C for 22 hours. The final inoculum contained 10^5 CFU/ml of bacteria.

Preparation of experimental Tulum cheese samples

Raw ewe milk, free of antibiotics and inhibitory substances, was divided into five groups (A-E) as shown in Table 1. Group A served as the control, while the other groups (B-E) were inoculated with fresh *B. melitensis* strain. The milk was heated to 32°C and rennet at the strength of 1/6000 was added to coagulate the milk within approximately 90 min. The coagulum was cut into pieces 4 cm × 4 cm × 4 cm cubes and the curd pieces were transferred into cotton bags for whey drainage and then pressed for 24 hours using metal weights (first press). After the first press, the curd was broken into pea-size pieces by hand and potassium sorbate at the concentrations indicated in Table 1 and 2% (w/w) salt was added, mixed, and transferred into the bags for the second press (24 h). At the end of the period, the steps of the previous press were repeated for the final press, except for the addition of potassium sorbate. Following the third press, the curds were broken into small pieces and air-dried for 24 hours at ambient temperature. Subsequently, the plastic containers were tightly filled with curd using a wooden stick, and the packaged samples were ripened at 4 ± 1°C for 90 days (Tekinşen et al., 2002). The study was conducted in four replicates.

Table 1. Presence of *B. melitensis* and potassium sorbate concentrations in Tulum cheese samples.

Groups	*B. melitensis*	Potassium sorbate (%)
A	-	-
B	+	-
C	+	0.05
D	+	0.10
E	+	0.20

Microbiological analyses

Isolation and identification of Brucella spp. from Tulum cheese samples

Each cheese sample (10 g) was homogenized with a Stomacher in 90 ml of Brucella broth (Himedia, M348). Then, a 0.1 ml aliquot of each homogenate was inoculated in duplicate using the spread plating technique onto the Brucella Medium Base (Oxoid CM169) with 5% inactivated horse serum (Oxoid SR35), 10 g/l glucose (Merck 1.08346.1000), and 1 vial/500 ml of Brucella selective supplement (Oxoid SR83). The first set of plates was incubated aerobically, while the second set was incubated in 10% CO^2 for 5 days at 37°C. Petri dishes without colony formation were incubated until the 10th day (Alton et al., 1988).

Identification of *Brucella* species was performed together with colonial morphology, Gram staining properties, and biochemical tests including H_2S formation, urease, and catalase activities, CO_2 requirement, growth in thionine and basic fuchsine, and serological screening, and Brucella phage test.

Detection of other microorganism groups

Samples of 10 g were taken aseptically from the Tulum cheese. A sterilized ringer solution with a dilution of 1:9 (w/v)

was added, and the samples were homogenized for 3 minutes in a stomacher. Bacterial counts were determined by plating serial decimal dilutions.

Plate count agar (PCA-Oxoid CM0325B) was used to determine total mesophilic aerobic microorganisms after incubation at 30±1°C for 72 h (ISO, 2003). The detection of coliform bacteria was performed using Violet Red Bile Agar (VRBA-Oxoid CM0968) and incubated for 24 hours at 30±1°C (ISO, 2006). Fecal streptococci were grown on Barnes' Thallous Acetate Tetrazolium Glucose Agar (TTTA) and incubated for 48 hours at 45±1°C (Barnes, 1959). *Staphylococcus-Micrococcus* were enumerated by plating on Mannitol Salt Agar (MSA-Difco) at 37°C for 48 hours (ISO, 2015). Yeast and molds were quantified on Potato Dextrose Agar (PDA) using the surface plate method. The samples were incubated at 22±1°C for 5-7 days, following ISO (2008) guidelines. All determinations were performed in duplicate and expressed as $\log_{10}$ cfu/g.

Physicochemical analyses

Analysis of milk samples

The milk samples' pH was measured using a digital pH meter (EDT, GP353). The titratable acidity was determined by the titration method and the results were expressed as % lactic acid. The fat content in the samples was determined by the Gerber method (AOAC, 1984). In the determination of antibiotic residues in milk, 50 ml of milk was pasteurized and cooled to 42°C. At this temperature, 3% of the yogurt culture was added to the milk and incubated for one hour. The acidity level was measured every 15 minutes, and changes in acidity were monitored. Decisions were made based on the recorded values (APHA, 2004).

Analysis of cheese samples

The samples were analyzed for pH, acidity, and dry matter content following AOAC (2019) guidelines. The salt content of the cheese was determined using the Mohr method (ISO, 2007).

Statistical Analysis

All analyses were conducted in quadruplicate. The effect of potassium sorbate and time on the microbiological and physicochemical profile of the Tulum cheeses during ripening was determined using analysis of variance (ANOVA), followed by the Tukey test. The results were expressed as mean±standard deviation (SD). Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Significant differences were compared using the Mann-Whitney U test at a significance level of $P<0.05$.

RESULTS

Microbiological and chemical properties of raw milk used in making cheese samples

Total mesophilic aerobic bacteria, fecal streptococci, *Staphylococcus-Micrococcus*, coliforms, and yeast and mold counts of raw milk used in production were determined as 8.33, 5.97, 5.57, 7.08, and 7.75 log cfu/ml, respectively. *Brucella* was not detected in any of the milk samples. The acidity, pH, fat content, and dry matter content of the raw milk used in making experimental Tulum cheese were found to be 0.20, 6.48, 7.00%, and 17.51%, respectively.

Changes in Tulum Cheese during ripening

Microbiological changes

Table 2 presents the microbiological changes that occur during ripening. The total mesophilic aerobic bacteria count in Tulum cheese samples was highest at the beginning of ripening and tended to decrease over time. Throughout the ripening period, groups C, D, and E, which contained varying amounts of potassium sorbate, had lower TMAB counts than groups A and B, which did not contain potassium sorbate. As the concentration of potassium sorbate increased to the highest level (0.20%), the TMAB counts decreased, and a statistical difference was observed between the A and B groups during the period up to the 60th day of ripening ($P<0.05$).

Throughout the ripening process, fecal streptococci counts were consistently lower in the groups that contained potassium sorbate compared to those that did not. By the end of maturation, groups C, D, and E showed no presence of bacteria. The decrease in bacterial presence observed during ripening was highly significant between the first and last days of ripening ($P<0.05$).

The *Staphylococcus-Micrococcus* counts were determined on the first and last days of ripening in all groups, with the highest and lowest counts recorded ($P<0.05$). The number of bacteria decreased steadily in all samples until the end of ripening, except for a small increase on the 15th day in group A. Throughout the ripening process, the samples with sorbate had lower *Staphylococcus-Micrococcus* counts compared to those without sorbate, with counts below <10 $\log_{10}$ cfu/g on the 90th day.

The count of coliforms generally decreased during ripening, with the most significant change observed in groups D and E. Tulum cheeses with sorbate had lower bacteria counts during ripening compared to those without sorbate ($P<0.05$). Bacteria were not isolated from groups D and E after the 45th day, and from group C after the 60th day.

Cheeses containing sorbate exhibited lower yeast and mold counts, as demonstrated in Table 2 ($P<0.05$). The number of yeast and mold decreased during ripening in groups with sorbate. Similar to coliforms, and yeast and mold were not detected in the analyses after the 45th day in groups D and E and the 60th day in group C.

Changes in the survival of Brucella melitensis

Table 3 shows the mean levels of *B. melitensis* detected in the experimental Tulum cheese samples as $\log_{10}$ cfu/g. The samples in group B, which did not contain potassium sorbate, had the highest number of *B. melitensis* at the beginning of ripening. The counts of *B. melitensis* in this group remained constant until the 15th day, after which they rapidly decreased and reached their lowest level ($P<0.05$) by the 30th day. By the 45th day of ripening, *B. melitensis* could not be isolated. In contrast, the

Table 2. Microbiological changes observed in Tulum cheese samples during ripening (log10 cfu/g).

Groups	Ripening period (day)					
	0	15	30	45	60	90
TMAB						
A	7.08±0.09^{Aa}	6.45±0.07^{ABb}	6.40±0.09^{Ab}	5.94±0.07^{Ac}	6.00±0.06^{Ac}	5.72±0.06^{Bd}
B	6.67±0.08^{Ba}	6.48±0.09^{Aab}	6.34±0.08^{Ab}	6.04±0.08^{Ac}	5.93±0.04^{ABc}	5.92±0.05^{Ac}
C	6.45±0.08^{Ca}	5.95±0.08^{Db}	5.95±0.08^{Bb}	5.56±0.07^{Bc}	5.83±0.09^{ABCb}	5.52±0.05^{Cc}
D	6.26±0.06^{Ca}	6.26±0.07^{BCa}	6.23±0.07^{Aa}	5.580.08^{Bc}	5.79±0.07^{BCb}	5.51±0.07^{Cc}
E	6.20±0.07^{Da}	6.11±0.09^{CDa}	5.69±0.95^{Cb}	5.26±0.06^{Cc}	5.69±0.04^{Cb}	5.59±0.09^{BCb}
Fecal streptococci						
A	4.63±0.08^{Aa}	4.34±0.07^{Ab}	3.76±0.08^{Bc}	3.81±0.09^{Ac}	3.76±0.07^{Ac}	1.23±0.08^{Ad}
B	4.58±0.04^{Aa}	4.28±0.07^{Ab}	3.96±0.08^{Ac}	3.88±0.05^{Ac}	3.89±0.09^{Ac}	1.08±0.06^{Bd}
C	4.26±0.04^{Ba}	3.76±0.09^{Bb}	3.63±0.04^{Bb}	3.30±0.06^{Bc}	3.26±0.06^{Bc}	0
D	4.08±0.09^{Ca}	3.64±0.07^{Bb}	3.36±0.08^{Ccd}	3.42±0.09^{Bc}	3.20±0.05^{Bd}	0
E	4.23±0.05^{BCa}	3.23±0.09^{Cb}	3.30±0.08^{Cb}	3.20±0.07^{Cb}	3.18±0.06^{Bb}	0
Staphylococcus-Micrococcus						
A	4.69±0.05^{BCa}	4.81±0.05^{Aa}	4.34±0.05^{Ab}	4.26±0.07^{Ab}	3.17±00.8^{Bc}	0.85±0.07^{Bd}
B	4.91±0.09^{Aa}	4.54±0.09^{Bb}	4.48±0.07^{Ab}	4.23±0.08^{Ac}	3.49±0.05^{Ad}	1.15±0.08^{Ae}
C	4.32±0.08^{Da}	3.93±0.07^{Db}	3.71±0.04^{Bc}	3.26±0.06^{Bd}	2.45±0.09^{Ce}	0
D	4.84±0.07^{ABa}	4.20±0.08^{Cb}	3.54±0.09^{Cc}	3.11±0.04^{Bd}	1.36±0.07^{De}	0
E	4.56±0.06^{Ca}	4.40±0.09^{BCa}	3.40±0.04^{Cb}	3.11±0.07^{Bc}	2.49±0.08^{Cd}	0
Coliform						
A	5.23±0.04^{Aa}	4.75±0.07^{Ab}	4.76±0.04^{Ab}	3.23±0.08^{Bc}	3.00±0.04^{Bd}	0.78±0.08^{Ae}
B	5.32±0.07^{Aa}	4.88±0.08^{Ab}	4.83±0.09^{Ab}	3.74±0.06^{Ac}	3.65±0.08^{Ac}	0.60±0.07^{Bd}
C	4.40±0.06^{Da}	4.08±0.09^{Bb}	3.32±0.08^{Bc}	2.23±0.06^{Cd}	0.48±0.06^{Ce}	0
D	5.04±0.06^{Ba}	4.15±0.06^{Bb}	2.98±0.06^{Cc}	1.23±0.04^{Ed}	0	0
E	4.61±0.05^{Ca}	4.00±0.09^{Bb}	2.99±0.07^{Cc}	1.43±0.08^{Dd}	0	0
Yeast and molds						
A	6.62±0.10^{Aa}	6.81±0.08^{Aa}	5.72±0.06^{Ab}	5.89±0.05^{Bb}	4.08±0.06^{Bc}	3.72±0.08^{Bd}
B	6.32±0.07^{Ba}	5.96±0.09^{Bb}	5.85±0.08^{Ab}	6.43±0.09^{Aa}	4.53±0.07^{Ac}	4.30±0.09^{Ad}
C	4.97±0.09^{Ca}	4.83±0.09^{Ca}	3.66±0.08^{Bb}	1.43±0.05^{Cc}	0.70±0.07^{Cd}	0
D	4.79±0.08^{Ca}	4.26±0.09^{Db}	2.52±0.08^{Cc}	1.23±0.06^{Dd}	0	0
E	4.82±0.07^{Ca}	4.78±0.04^{Ca}	2.70±0.07^{Cb}	1.04±0.09^{Ec}	0	0

Values are expressed as means ± standard deviation (SD)
A-F: Values with different superscripts in the same column are significantly different (P<0.05)
a-g: Values with different superscripts in the same line are significantly different (P<0.05)

samples in group C, which contained 0.05% sorbate, exhibited a significant decrease (P<0.05) in *B. melitensis* counts compared to group B on the 15th and 30th days. The bacteria were undetectable in subsequent analyses. The counts of bacteria in groups D and E, which contained 0.10% and 0.20% sorbate, respectively, showed a statistically insignificant decrease on the 15th day (P>0.05). *B. melitensis* was not detected in the E group samples on the 30th day or the D group samples on the 45th day (P<0.05). Figure 1 shows that the bacterial counts were similar among all groups at the beginning of ripening and significantly decreased in the following days. After the 15th day of ripening, all groups containing potassium sorbate showed a statistically significant decrease compared to group B without sorbate (P<0.05).

Physicochemical changes

Table 4 presents the physicochemical changes that occur in Tulum cheeses during ripening. Throughout the ripening process, acidity levels increased in all groups, reaching their peak on the 90th day. Groups A and B, which had the lowest acidity values at the beginning of storage, maintained the same characteristics until the end. The highest acidity value was observed in group E, with 1.52% on the first day, while groups C and D reached 1.83% on the 90th day. There were no statisti-

Table 3. *B. melitensis* counts during ripening of Tulum cheese samples (log10 cfu/g)

Groups	Ripening period (day)		
	0	15	30
B	3.76±0.15^{Aa}	3.75±0.05^{Aa}	1.58±0.06^{Ab}
C	3.54±0.54^{ABa}	3.57±0.06^{Ba}	0.70±0.04^{Bb}
D	2.62±0.10^{Ba}	2.58±0.06^{Ca}	0.30±0.07^{Cb}
E	2.61±0.08^{Ba}	2.59±0.05^{Ca}	0.00±0.01^{Db}

Values are expressed as means ± standard deviation (SD)
A-F: Values with different superscripts in the same column are significantly different (P<0.05)
a-g: Values with different superscripts in the same line are significantly different (P<0.05)

Table 4. Physicochemical changes observed in Tulum cheese samples during ripening.

Groups	Ripening period (day)					
	0	15	30	45	60	90
Acidity (%la)						
A	1.47±0.09^{Ac}	1.52±0.06^{ABbc}	1.61±0.07^{Aabc}	1.68±0.07^{Aab}	1.73±0.06^{Aa}	1.77±0.01^{Aa}
B	1.41±0.08^{Ad}	1.48±0.08^{Bcd}	1.55±0.08^{Abcd}	1.62±0.05^{Aabc}	1.73±0.09^{Aab}	1.75±0.01^{Aa}
C	1.42±0.04^{Ac}	1.58±0.06^{ABb}	1.57±0.08^{Ab}	1.62±0.06^{Ab}	1.80±0.05^{Aa}	1.83±0.01^{Aa}
D	1.47±0.08^{Ac}	1.65±0.04^{Ab}	1.65±0.08^{Ab}	1.65±0.07^{Ab}	1.72±0.06^{Aab}	1.83±0.01^{Aa}
E	1.52±0.08^{Ab}	1.57±0.08^{ABb}	1.61±0.06^{Ab}	1.61±0.08^{Ab}	1.80±0.08^{Aa}	1.80±0.01^{Aa}
pH						
A	4.94±0.06^{Aa}	4.89±0.05^{Aa}	4.62±0.05^{Ab}	4.47±0.06^{Ab}	4.58±0.07^{Ab}	4.54±0.07^{Ab}
B	4.64±0.09^{Ba}	4.64±0.09^{Ba}	4.55±0.06^{Aab}	4.47±0.06^{Aab}	4.42±0.05^{ABc}	4.43±0.08^{Ac}
C	4.76±0.08^{ABa}	4.62±0.07^{Bab}	4.55±0.04^{Abc}	4.44±0.05^{Abcd}	4.35±0.09^{Bd}	4.38±0.08^{Acd}
D	4.66±0.06^{Ba}	4.50±0.07^{Babc}	4.52±0.08^{Aab}	4.38±0.04^{Abc}	4.33±0.06^{Bd}	4.37±0.07^{Acd}
E	4.63±0.06^{Ba}	4.60±0.09^{Bab}	4.54±0.04^{Aabc}	4.43±0.07^{Abc}	4.44±0.08^{ABbc}	4.40±0.08^{Ac}
Salt (%)						
A	5.67±0.08^{Be}	5.77±0.04^{Bde}	5.90±0.07^{Bcd}	6.03±0.07^{Cc}	6.25±0.09^{Bb}	6.61±0.06^{Ba}
B	5.73±0.04^{ABc}	5.86±0.07^{ABc}	6.11±0.08^{Ab}	6.04±0.06^{Cb}	6.63±0.09^{Aa}	6.64±0.07^{Ba}
C	5.88±0.08^{Ab}	5.96±0.08^{Ab}	6.26±0.04^{Aa}	6.27±0.08^{ABa}	6.29±0.08^{Ba}	6.44±0.07^{Ca}
D	5.67±0.06^{Bf}	5.82±0.08^{ABe}	6.17±0.06^{Ad}	6.37±0.06^{Ac}	6.77±0.05^{Ab}	6.93±0.04^{Aa}
E	5.61±0.06^{Bc}	5.70±0.09^{Bc}	6.12±0.08^{Ab}	6.12±0.07^{BCb}	6.30±0.06^{Ba}	6.44±0.04^{Ca}
Moisture (%)						
A	32.66±0.08^{Da}	32.54±0.04^{Da}	31.15±0.09^{Cb}	29.56±0.07^{Dc}	29.02±0.08^{Dd}	28.75±0.08^{Ce}
B	33.39±0.08^{Ca}	33.16±0.07^{Bb}	32.07±0.06^{Bc}	31.33±0.10^{Bd}	30.65±0.08^{Be}	30.30±0.06^{Bf}
C	34.56±0.04^{Ba}	32.85±0.07^{Cb}	32.19±0.08^{Dc}	30.88±0.08^{Cd}	30.41±0.06^{Ce}	30.23±0.08^{Be}
D	31.20±0.04^{Ea}	30.55±0.08^{Eb}	28.91±0.07^{Dc}	28.09±0.08^{Ed}	27.04±0.07^{Ee}	26.82±0.08^{Df}
E	36.61±0.09^{Aa}	35.24±0.08^{Ab}	33.86±0.04^{Ac}	32.64±0.08^{Ad}	32.28±0.05^{Ae}	32.26±0.09^{Ae}

Values are expressed as means ± standard deviation (SD)
A-F: Values with different superscripts in the same column are significantly different (P<0.05)
a-g: Values with different superscripts in the same line are significantly different (P<0.05)

cally significant differences between the groups, except for the values of groups B and D on the 15th day of ripening (P<0.05).

The pH values of Tulum cheese samples were highest at the beginning of ripening and lowest at the 60th day in groups B, C, and D, and at the 90th day in group E. After a decrease in all groups until the 45th day of ripening, these values generally continued to decrease, except for an increase in group A. However, no major changes occurred. The pH values in groups A and B without potassium sorbate were higher on the first day of ripening than in groups with sorbate. The ripening process showed statistically significant differences between the first and last-day values in all groups (P<0.05). The addition of sorbate resulted in no differences between the groups on

any day of maturation (P>0.05). Differences were observed between groups A and B, D and E on day 0, between group A and all other groups on day 15, and between group A and groups C, D, and E on day 60 (P<0.05), depending on whether the samples contained sorbate.

In all groups, the lowest and highest salt values were determined on the 0th and 90th days of ripening, respectively (P<0.05). Table 4 shows that the salt values detected in the cheese samples varied between the groups with and without sorbate on different days, but the addition of sorbate did not result in a significant difference overall. At the end of the ripening process, group C had the highest initial salt content and group E had the lowest, but their values had reversed, with group D having the highest salt content.

Regarding moisture content, all groups decreased almost in parallel during ripening. The highest and lowest moisture contents were recorded on the first and last days of ripening, respectively (P<0.05). Group E samples showed the highest values during ripening (P<0.05), while group D samples showed the lowest values (P<0.05). Moisture values decreased continuously during ripening in all groups.

DISCUSSION

In this study, the survival of *Brucella melitensis* and the microbiological and physicochemical changes in the product during ripening of Tulum cheese produced by adding different concentrations of potassium sorbate to raw ewe's milk and inoculating *Brucella melitensis* were investigated. TMAB levels were compared to those of Tulum cheese without sorbate, as determined in previous studies (Morul ve İşleyici, 2012; Öztürk and Nazlı, 1996). The addition of sorbate to cheese leads to a decrease in TMAB counts. A study conducted on vacuum-packaged Tulum cheeses, where sorbate was added at the same concentrations as in our study, showed higher TMAB counts throughout storage. However, an increase in sorbate concentration resulted in a decrease in bacterial counts, as observed in our study (Demir et al., 2017). The lower TMAB counts observed in the sorbate-added groups in this study can be attributed to the stronger antibacterial effect of sorbates, which is due to their high acidity and low moisture content (Liewen and Marth, 1985). Similar results were reported by Doğruer et al. (1996) regarding fecal streptococci numbers in cheese groups with sorbate. While some researchers have found consistent reduction and inhibition of fecal streptococci during ripening (Patır et al., 2001; Yerlikaya and Akbulut, 2019), others have reported irregular patterns (Boyd, 1995). In this study, the decrease and inhibition of fecal streptococci in cheeses with sorbate is attributed to the antibacterial effect of potassium sorbate and starter cultures. Studies on cheese types containing sorbate have shown that potassium sorbate has an antibacterial effect on *Staphylococcus-Micrococcus* (Doğruer et al., 1996; Demir et al., 2017). The counts of *Staphylococcus-Micrococcus* detected in both sorbate and non-sorbate groups remained at lower levels than those in the other study (Patır et al., 2000). The variation in outcomes could be because the experimental production in the present study was carried out under rigorous hygienic conditions to prevent contamination of raw milk and products with environmental bacteria. Furthermore, the increased acidity level in the product may have played a role in the observed difference. Similar to the present study, previous research has shown that coliform group bacteria decrease at higher rates in sorbate-containing cheeses compared to the control group and are inhibited at various storage periods (Doğruer et al., 1996; Demir et al., 2017). Additionally, potassium sorbate has been found to have the highest inhibitory effect on yeast and molds among microorganism groups (Liewen and Marth, 1985). Our study confirms this finding, which is consistent with previous research (Demir et al., 2017; Khorshidian et al., 2021).

Studies have been conducted on the effects of sorbates on food pathogens in cheeses. For example, Benítez-Azaola et al. (2017) reported that the combination of plant extracts and potassium sorbate can inhibit the growth of *L. monocytogenes* in Panela cheese. Pérez et al. (2011) found that the growth of Shiga toxin-producing *Escherichia coli* can be inhibited in edible film-coated cheeses with potassium sorbate. Similarly, Demir et al. (2017) also reported a similar effect. One study examined the survival of *Brucella* species in cheeses without antimicrobial substances. The study found that the amount of *B. melitensis* decreased from 9-log at the beginning of ripening to below detectable levels by the 30th day (Öztürk and Nazlı, 1996). Cosseddu and Pisanu (1985) reported that they could not isolate the bacteria after the 45th and 50th days of ripening in goat milk cheese inoculated with *B. melitensis*. The physicochemical structure of the product may vary depending on the quality of the milk used in cheese making, the type and inoculation level of *Brucella*, the ripening process, and production technologies. These factors are the main reasons for the observed differences.

CONCLUSION

The microbiological properties of Tulum cheese are affected by several factors, such as the quality of raw milk, production conditions and technology, employee hygiene, and storage conditions. Despite the addition of salt, antimicrobial metabolites, low pH, and moisture levels, the product remains vulnerable to foodborne pathogens that can pose a significant risk to consumers, even after the ripening period. The use of food additives that combat foodborne pathogens posing a risk to public health can yield positive results. Sorbic acid and its salts are commonly used preservatives in the food industry, particularly for their antifungal properties, and are frequently employed in cheese production. The objective of this study was to examine the effect of potassium sorbate on the survival of *B. melitensis* in Tulum cheeses, as well as to determine the microbiological and physicochemical changes that occur during ripening. The results suggest that the use of potassium sorbate, particularly at a concentration of 0.20%, had an antibacterial effect on *B. melitensis* and enhanced the microbiological quality of Tulum cheese. Additionally, it did not have any negative impact on the physicochemical properties of the product. The study found that the use of potassium sorbate in Tulum cheese production is recommended for both product quality and public health.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare no coflict of interest.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: BP

Data collection and analysis: AHD

Drafting of the manuscript: AHD

Critical review: AHD

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

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