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Effect of potassium sorbate, nisin and lysozyme singly and in combinations on the quality of pickled white cheese during storage

Efecto del sorbato de potasio, la nisina y la lisozima, individualmente y en combinación, sobre la calidad del queso blanco encurtido durante el almacenamiento

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ABSTRACT

This study was conducted to determine the effects of potassium sorbate, nisin and lysozyme on the microbiological, chemical and sensory quality of pickled white cheeses during their shelf life. For this purpose, Şavak pickled white cheese, experimentally produced from a mixture of 50% sheep milk and 50% cow milk, was sliced (4×4×4 cm in size) and stored in 16% salt brine containing potassium sorbate (1 g·mL^{-1}), nisin (12.5 mg·L^{-1}) and lysozyme (200 mg·L^{-1}) singly or in combination at 4°C for a shelf life of 90 days. At the end of storage, statistically significant differences were found between the combination group using potassium sorbate 1 g·L^{-1} + nisin 12.5 mg·L^{-1} and the control group in terms of antimicrobial effect levels on coliform, Enterobacteriaceae, fecal streptococci, lipolytic, proteolytic and *E. coli* microorganisms ($P \leq 0.05$). No statistically significant differences were found between the groups in terms of sensory evaluation ($P > 0.05$). However, according to the numerical scores given, group 4 (potassium sorbate 1 g·L^{-1} + nisin 12.5 mg·L^{-1}) and group 7 (potassium sorbate 1 g·L^{-1} + nisin 12.5 mg·L^{-1} + lysozyme 200 mg·L^{-1}) became the two most appreciated groups in terms of sensory evaluation with equal scores (95.55). It was concluded that the combination of potassium sorbate 1 g·L^{-1} + nisin 12.5 mg·L^{-1}, which is an additive with the Generally Recognized as Safe status and is in compliance with the legislation for use in cheeses ripened in brine and offered for sale, can be used safely as an alternative method to heat treatment to improve the quality of pickled white cheeses and that it contains data that can form the basis for new studies to investigate how these additives can also have effects on some pathogenic bacteria.

Key words: Potassium sorbate; nisin; lysozyme; white cheese; shelf life

RESUMEN

Este estudio se realizó para determinar los efectos del sorbato de potasio, la nisina y la lisozima en la calidad microbiológica, química y sensorial de quesos blancos encurtidos durante su vida útil. Para ello, el queso blanco Şavak, elaborado experimentalmente con una mezcla de 50 % de leche de oveja y 50 % de leche de vaca, se cortó en lonchas (4×4×4 cm) y se almacenó en salmuera al 16 % con sorbato de potasio (1 g·ml^{-1}), nisina (12,5 mg·l^{-1}) y lisozima (200 mg·l^{-1}), solos o en combinación, a 4°C, durante 90 días. Al final del almacenamiento, se encontraron diferencias estadísticamente significativas entre el grupo de combinación que utilizó sorbato de potasio 1 g·L^{-1} + nisina 12,5 mg·L^{-1} y el grupo control en términos de niveles de efecto antimicrobiano sobre coliformes, Enterobacteriaceae, Estreptococos fecales, lipolíticos, proteolíticos y *E. coli* ($P \leq 0{,}05$). No se encontraron diferencias estadísticamente significativas entre los grupos en términos de evaluación sensorial ($P > 0{,}05$). Sin embargo, de acuerdo con las puntuaciones numéricas dadas, el grupo 4 (sorbato de potasio 1 g·L^{-1} + nisina 12,5 mg·L^{-1}) y el grupo 7 (sorbato de potasio 1 g·L^{-1} + nisina 12,5 mg·L^{-1} + lisozima 200 mg·L^{-1}) se convirtieron en los dos grupos más apreciados en términos de evaluación sensorial con puntuaciones iguales (95,55). Se concluyó que la combinación de sorbato de potasio 1 g·L^{-1} + nisina 12,5 mg·L^{-1}, que es un aditivo con el estatus General Reconocido como Seguro y cumple con la legislación para su uso en quesos madurados en salmuera y ofrecidos para la venta, puede usarse de manera segura como un método alternativo al tratamiento térmico para mejorar la calidad de los quesos blancos encurtidos y que contiene datos que pueden formar la base para nuevos estudios para investigar cómo estos aditivos también pueden tener efectos sobre algunas bacterias patógenas.

Palabras clave: Sorbato de potasio, nisina, lisozima, queso blanco, vida útil

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INTRODUCTION

There are thousands of cheese varieties with very different flavors, aromas and shelf lives in the world and in Türkiye. Cheeses are classified according to the type of raw milk used (cow, sheep, goat, buffalo), cheese production method (rennet, ultrafiltration), cheese structure (soft, semi–hard, hard, extra–hard), fat content (full–fat, semi–fat, low–fat), fermentation method (lactic, propionic, and butyric acid), external structure (hard, soft, moldy) and internal structure (porous and moldy) [1]. It is estimated that there are approximately 193 local cheese varieties in Türkiye [2].

Şavak fresh white cheese, a traditional cheese used in our study, is a cheese variety mostly made from raw milk and sold by local people who earn their living from animal husbandry in the provinces of Elazığ, Tunceli, Bingöl, Erzurum and Erzincan. Due to the lack of a standard technique in production, the use of mostly low–quality raw milk, the fact that the milk is not subjected to heat treatment and the use of natural rennet in its production, the physico–chemical and microbiological properties of Şavak fresh and pickled white cheeses may also be low [1, 2].

In studies [3, 4], pathogenic microorganisms such as *Listeria monocytogenes* and Salmonella spp. have been detected in fresh pickled cheeses. However, since they are produced from raw milk, they have an intense flavor and a taste that the public is accustomed to. For this reason, they are preferred more by the local people than cheeses produced from pasteurized milk. White cheeses produced with traditional methods may be contaminated with pathogenic bacteria due to contamination in the production stages from raw milk or contamination after production. In this study, it was aimed to determine the effects of potassium sorbate, nisin and lysozyme, which are generally considered to be safe for human health and have Generally Recognized as Safe (GRAS) status, on the microbial, chemical and sensory properties of pickled white cheeses throughout their shelf life.

MATERIALS AND METHODS

Ethical approval

Permission was obtained from the Firat University Non–Interventional Research Ethics Committee with the number 2021/05-34.

Materials

In this study, Şavak white cheese production [1] was carried out in the cheese production unit of Fırat University Agriculture and Livestock Research and Application Center (TAHAM) and then divided into groups at the curd stage. This study was repeated on three different dates with at least 1 month between each repetition.

Creation of experimental groups and addition of additive

White cheeses in curd form were sliced as 4×4×4 cm and 72 g. Then, each slice of cheese was placed in polyethylene terephthalate (PET) packages. The additive required for each group was added and 16% brine solution (300 mL) was added to each group. Then, experimental groups were set. One package (1 slice of cheese) was used for microbiological and chemical analyses, and a separate package (1 slice of cheese) was used for sensory analyses. Analyses were performed by opening a separate package on each analysis day (common for microbiological and chemical analyses, separate for sensory analyses). Potassium sorbate (1 $g \cdot L^{-1}$) and nisin (12.5 $mg \cdot L^{-1}$) were used at maximum rates specified in the European Union Food Additives Regulation [5], and lysozyme (200 $mg \cdot L^{-1}$) was added to the brine water, taking into account rates previously reported in the literature [6].

Methods

Experimental Groups

- **Control:** Brine + 1 slice of cheese
- **Group 1**: Brine + 1 slice of cheese + potassium sorbate (1 $g \cdot L^{-1}$)
- **Group 2**: Brine + 1 slice of cheese + nisin (12.5 $mg \cdot L^{-1}$)
- **Group 3**: Brine + 1 slice of cheese + lysozyme (200 $mg \cdot L^{-1}$)
- **Group 4**: Brine + 1 slice of cheese + potassium sorbate (1 $g \cdot L^{-1}$) + nisin (12.5 $mg \cdot L^{-1}$)
- **Group 5**: Brine + 1 slice of cheese + potassium sorbate (1 $g \cdot L^{-1}$) + lysozyme (200 $mg \cdot L^{-1}$)
- **Group 6**: Brine + 1 slice of cheese + nisin (12.5 $mg \cdot L^{-1}$) + lysozyme (200 $mg \cdot L^{-1}$)
- **Group 7**: Brine + 1 slice of cheese + potassium sorbate (1 $g \cdot L^{-1}$) + nisin (12.5 $mg \cdot L^{-1}$) + lysozyme (200 ($mg \cdot L^{-1}$). See FIG. 1.

FIGURE 1. Preparation of cheese samples and separation into groups

Microbiological analysis

Aseptically collected cheese samples were placed in a special sterile bag of 10 g of the homogenizer (Bag Mixer Interscience 78860 St. France–Stochmaer). 90 mL of 0.1% sterile peptone water was added to them and homogenized for 90 s. Thus, 10^{-1} (1/10) dilutions of the samples were prepared. Using the same dilution and the same diluent, decimal dilutions of the samples up to 10^{-9} were prepared [7, 8].

Total mesophilic aerobic bacteria Plate Count Agar (PCA) (Neogen Corporation NCM0010A) (37 ± 1ºC for 48 h) [9], total psychrophilic aerobic bacteria Plate Count Agar (PCA) (Neogen Corporation NCM0010A) (4 ± 1ºC for 10 d) [7, 9], coliform group bacteria Violet Red Bile (VRB) Agar (Biokar Diagnostics BK152HA) (30 ± 1°C for 24 h) [10] *Enterobacteriaceae* Violet Red Bile Glucose (VRBG) Agar (Neogen Corporation NCM0041A) (24 h at 37 ± 1°C) [11], *Staphylococcus–Micrococcus* Baird Parker Agar (Neogen Corporation NCM0200A) (48 h at 37 ± 1°C) [10], yeast–mold Dichloran Rose Bengal Chloramphenicol (DRBC) Agar (Chemsolute Corporation 9677.0500) (5 d at 25 ± 1°C) [12], fecal streptococci Barnes' Thallous Acetate Tetrazolium Glucose Agar (TITA) (at 45 ± 1°C 48 h) [8], *Lactobacillus, Leuconostoc, Pediococcus (L.L.P.)* Man Rogasa Sharpe (MRS) Agar (Chemsolute Corporation 8761.0500) (5 d at 30 ± 1°C) [7], lactic streptococcus M17 Agar (Liofilchem 610192) (48–72 h at 30 ± 1°C) [10, 13], lipolytic microorganisms Tributyrin Agar (Liofilchem 610215) (48 h at 30 ± 1°C) [10], proteolytic microorganisms Calcium Caseinat Agar (Conda Pronadisa 1069.00) (30 ± 1°C for 48 h) [10], *Escherichia coli* Tryptone Bile × Glucuronide Medium (Chemsolute Corporation 8858.0500) (30 ± 1°C for 4 h and then 44 ± 1°C for 18 h) [14] and coagulase–positive *Staphylococcus aureus* were enumerated on Baird Parker Agar (Neogen Corporation NCM0200A) (37 ± 1°C for 48 h) [15, 16].

Chemical analysis

For pH analysis, 10^{-1} dilution was used and measurements were made with a pH meter. (Hanna HI 2002–02, Hanna, USA) [17]. For acidity determination, acidity amount was calculated in % g lactic acid by titration method [18]. In dry matter analysis, % amount was determined by using gravimetric method [18]. For fat determination, % amount was calculated by using Gerber method [17, 19]. For ash determination, % amount was determined by using gravimetric method. For salt analysis, Mohr method was used [20].

Sensory analysis

A sensory analysis evaluation form prepared according to the TSE and a scoring system according to the hedonic scale method were used by a panelist group consisting of 10 people on the 15th, 30th, 60th and 90th days (d) of storage [20].

Statistical analysis

For statistical analysis of microbiological and chemical data, microbiological results were converted to $\log_{10}$ kob·g^{-1} and variance analysis (ANOVA) was performed in accordance with 3×8×6 (repeat × number of groups × storage days) factorial trial design. Means were separated using Fisher's Least Significant Difference–LSD) test and differences between both groups and within–group storage days were performed using Statistical Analysis System (SAS) package program [21]. Statistical significance level was taken as $P\leq0.05$. SPSS 22 package program [22] was used in the evaluation of sensory data. As a result of normality analysis results, it was seen that the data met the nonparametric test assumptions. Kruskal–Wallis H (K Independent Samples) test was applied in comparison in terms of groups and storage d. Statistical significance level was accepted as $P\leq0.05$.

RESULTS AND DISCUSSION

The results of raw milk samples used in cheese making are given in TABLE I.

TABLE I
Microbiological and physico–chemical analysis findings of raw milk used in the production of Şavak brine white cheese (n=3) ($\log_{10}$ cfu·mL^{-1} ± standard deviation, arithmetic mean ± standard deviation)

Microorganism	Arithmetic Mean ± SD	Min	Max
Total Mesophilic Aerobic	6.17 ± 1.21	5.00	7.42
Total Psychrophilic Aerobic	5.14 ± 0.73	4.30	5.60
Coliform	1.05 ± 0.05	1.00	3.50
Enterobacteriaceae	1.87 ± 0.82	1.00	2.63
Staphylococcus–Micrococcus	3.18 ± 0.94	3.11	4.92
Yeast–Mold	3.16 ± 1.13	2.00	4.26
Fecal Streptococcus	1.04 ± 0.18	1.60	3.83
Lactobacillus, Leuconostoc, Pediococcus	4.36 ± 1.11	3.47	5.61
Lactic Streptococcus	5.05 ± 1.08	4.00	6.16
Lipolytic Microorganism	5.58 ± 1.23	4.30	6.77
Proteolytic Mikroorganism	5.65 ± 1.53	4.00	7.04
Escherichia coli	0.41 ± 0.38	0.00	0.77
Coagulase positive *Staphylococcus aureus*	–	–	–
Analysis	**Arithmetic Mean ± SD**	**Min**	**Max**
pH	6.69 ± 0.04	6.66	6.75
Acidity (% g lactic acid)	2.03 ± 0.42	1.55	2.35
Dry Matter (%)	11.61 ± 1.98	9.35	13.06
Fat (%)	3.93 ± 0.05	3.90	4.00
Ash (%)	0.65 ± 0,05	0.61	0.71

The additives used didn't cause a decrease in the total mesophilic aerobic bacteria (TMAB) count. The highest count on the 90th d was seen in the group where potassium sorbate was used alone (8.95) after the control group (FIG. 2). The results obtained are consistent with the findings of some researchers who stated that potassium sorbate, nisin and lysozyme did not have a reducing effect on this group of bacteria, but prevented their growth. [23, 24, 25].

Coliforms are hygiene indicator bacteria. The number of this group of bacteria decreased continuously starting from the first d of storage. It was determined that the potassium sorbate + nisin combination used in the 4th group inhibited this group of bacteria

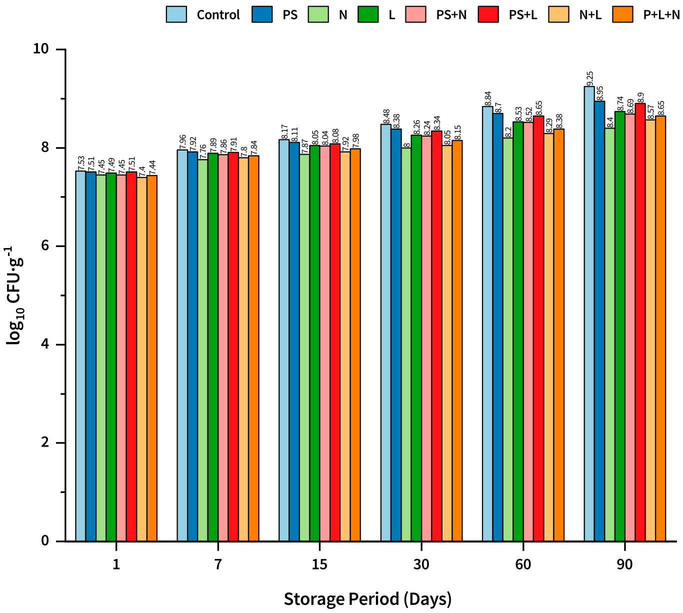

FIGURE 2. Total mesophilic aerobic bacteria counts detected in Şavak pickled white cheese (log10 cfu·g^{-1} ± sd) (PS: potassium sorbate, N: nisin, L: lysozyme, PS+N: potassium sorbate+nisin, PS+L: potassium sorbate+lysozyme, N+L: nisin+lysozyme, P+N+L: potassium sorbate+lysozyme+nisin)

at a high rate (2.62 $\log_{10}$ cfu·g^{-1}) (FIG. 3). It was observed that these results were similar to the results of some researchers [23, 26]. Statistically, significant differences (P≤0.05) were observed between the 4th group and the control group.

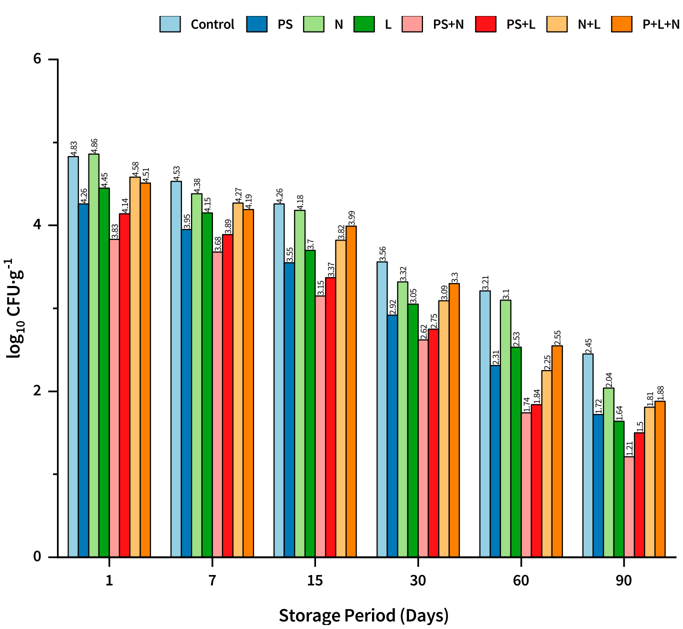

FIGURE 3. Coliform bacteria counts detected in Şavak pickled white cheese (log10 cfu·g^{-1} ± sd). (PS: potassium sorbate, N: nisin, L: lysozyme, PS+N: potassium sorbate+nisin, PS+L: potassium sorbate+lysozyme, N+L: nisin+lysozyme, P+N+L: potassium sorbate+lysozyme+nisin)

Bacteria belonging to the Enterobacteriaceae family play an important role in determining the microbial quality of foods. A decrease in their numbers was observed in all cheese groups starting from the first d of storage. On the 90th d of storage, the highest number was counted in the control group (3.24 $\log_{10}$ cfu·g^{-1}) and the lowest number was counted in the 4th group (1.41 $\log_{10}$ cfu·g^{-1}). These results are similar to the findings of some researchers [27, 28]. The highest inhibition was found in the second group (2.95 $\log_{10}$ cfu·g^{-1}) where nisin was used alone looking at the difference between d 0 and d 90. This situation was found to be consistent with the findings of researchers [29, 30] who stated that nisin had an inhibitory effect on the Enterobactericeae family. On the 90th d of storage, the differences between all groups except the second group were statistically significant (P≤0.05) (FIG. 4).

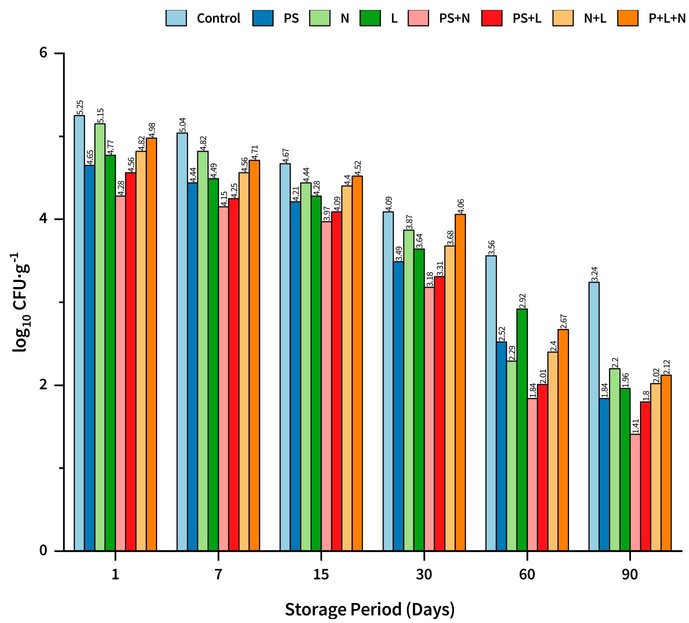

FIGURE 4. Enterobacteriaceae counts detected in Şavak pickled white cheese (log10 cfu·g^{-1} ± sd). (PS: potassium sorbate, N: nisin, L: lysozyme, PS+N: potassium sorbate+nisin, PS+L: potassium sorbate+lysozyme, N+L: nisin+lysozyme, P+N+L: potassium sorbate+lysozyme+nisin)

Staphylococcus and Micrococcus are found on the outer surfaces of animal and human skin, in water, soil and dust. They are also effective in spoilage of food [31]. The number of bacteria in this group decreased continuously in all cheese groups throughout the storage period. Inhibition in the group where potassium sorbate was used alone was determined as 2.43 $\log_{10}$ cfu·g^{-1}, looking at the difference between d 0 and d 90. The results obtained differ from the findings of researchers [31, 32] who stated that potassium sorbate had any inhibitory effect on this group of bacteria. The highest inhibition was seen in the 4th group (2.45 $\log_{10}$ cfu·g^{-1}) (FIG. 5). No statistically significant differences were found within and between groups on all d of storage (P>0.05). There is no study in which the combination of potassium sorbate, nisin and lysozyme was used together.

Yeasts and molds cause an undesirable bitter taste, gas formation, porous structure and bad odors in cheeses due to their proteolytic and lipolytic effects [2]. On the 90th d of storage, the highest average

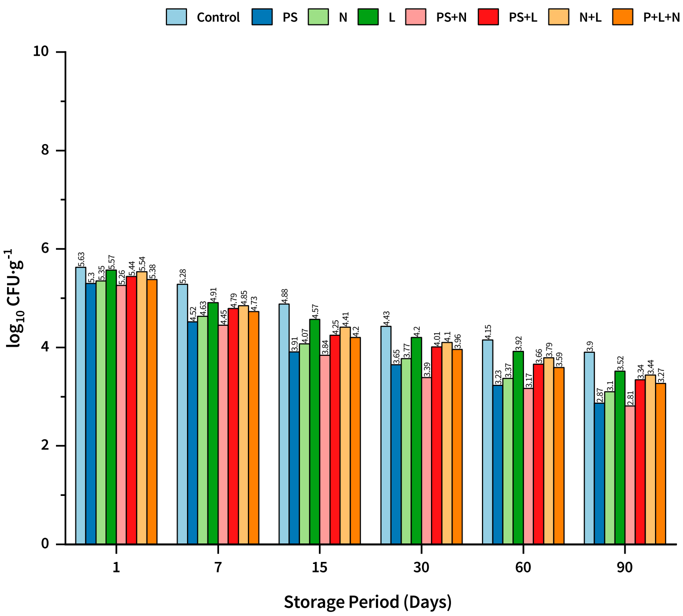

FIGURE 5. ***Staphylococcus*** **and** ***Micrococcus*** **counts detected in Şavak pickled white cheese (log10 cfu·g^{-1} ± sd). (PS: potassium sorbate, N: nisin, L: lysozyme, PS+N: potassium sorbate+nisin, PS+L: potassium sorbate+lysozyme, N+L: nisin+lysozyme, P+N+L: potassium sorbate+lysozyme+nisin)**

number was found in the control group (2.06 log_{10} cfu·g^{-1}) and the lowest in groups 1, 2, 4, 5 and 7 (<1.00 log_{10} cfu·g^{-1}). In the present study, it was found that potassium sorbate and nisin used both alone and in combination had inhibition effects on yeasts and molds. These results are similar to the findings of some researchers [23, 33]. No statistically significant differences were found between the groups (*P*>0.05).

Fecal streptococci (*Enterococcus*), which are considered as hygiene indicator microorganisms, are very common in nature. They can contaminate the product at any stage of the cheese production chain. The numbers of this group of microorganisms decreased in all groups during storage. This result is similar to the findings of some researchers [31, 33]. Potassium sorbate, nisin and lysozyme show inhibitory effect by hydrolyzing β-1,4 glycosidic bonds in the peptidoglycan layer in the cell membranes of Gram–positive bacteria [34]. The fact that the highest inhibition was observed in the 4th group, where potassium sorbate and nisin were used in the present study, may be due to the synergistic or additive effect of these two additives [27, 28].

Lactobacillus–Leuconostoc–Pediococcus (L.L.P.) bacteria are effective in the formation of the unique flavor and aroma of foods and play an important role in the ripening of cheeses. The numbers of this group of microorganisms increased continuously from the first d of the storage period. On the 90th d of storage, the *L.L.P* numbers were determined as the least as 7.16 log_{10}cfu·g^{-1} in the 2nd group and the highest as 7.48 log_{10} cfu·g^{-1} in the 4th group. The fact that the numerically lower logarithmic values were found in the 2nd, 3rd and 6th groups compared to the other groups, although slightly, is similar to the findings of some researchers [28, 35] who stated that nisin and lysozyme have inhibitory effects on *L.L.P.* group microorganisms. In addition, the fact that *L.L.P.* counts were at the same levels in the control group (7.38 log_{10} cfu·g^{-1}) and the 1st group (7.38 log_{10} cfu·g^{-1}) on the 90th day of storage is an indication that potassium sorbate has no effect on *L.L.P.* group microorganisms. This result is also in line with the results of Doğruer *et al.* [31].

Lactic Streptococcus bacteria are effective both on the flavor and aroma of milk and dairy products and on the ripening of cheeses [36]. The numbers of this group of bacteria in all cheese groups increased continuously throughout the storage period. It was observed that bacterial inhibition was higher in the groups where nisin was used. The obtained results are parallel to the findings of some researchers [28, 36].

Lipolytic microorganisms lipolyze milk fat in milk and dairy products, causing undesirable taste and odor. The number of this group of microorganisms increased in all cheese groups during the storage period. The highest antimicrobial effect was seen in the groups to which potassium sorbate was added. It was determined that these results were in accordance with the sensory analysis results. It was determined that these results were similar to the findings of Koyuncu and Uylaser [37]. Statistically, it was determined that the differences between the control group and the 1st, 3rd, 4th, 5th and 7th groups were significant on the 90th d of storage (*P*≤0.05) (FIG. 6).

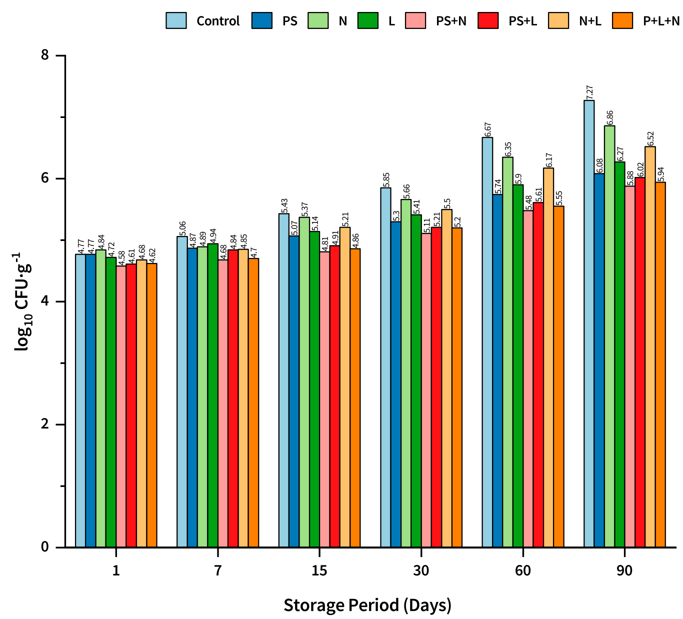

FIGURE 6. Numbers of lipolytic microorganisms detected in Şavak pickled white cheese (log10 cfu·g^{-1} ± sd). (PS: potassium sorbate, N: nisin, L: lysozyme, PS+N: potassium sorbate+nisin, PS+L: potassium sorbate+lysozyme, N+L: nisin+lysozyme, P+N+L: potassium sorbate+lysozyme+nisin)

Proteolytic bacteria secrete protease enzymes, hydrolyze proteins and cause undesirable odor and taste formation in the products. On the 90th d of storage, the highest number was detected in the control group (6.55 log_{10} cfu·g^{-1}) and the lowest number was detected in the 4th group (4.82 log_{10} cfu·g^{-1}). In addition, the highest inhibition was seen in the 4th group (4.82 log_{10} cfu·g^{-1}), 7th group (4.93 log_{10}cfu·g^{-1}), 5th group (5.06 log_{10} cfu·g^{-1}) and 1st group

($5.22\ log_{10}\ cfu \cdot g^{-1}$). It was determined that these results were compatible with the sensory analysis results. Statistically, the differences between the control group and the 1st, 4th, 5th and 7th groups on the 60th and 90th d of storage were significant ($P \leq 0.05$) (FIG. 7). Since no similar studies were found in the literature search, no discussion could be made.

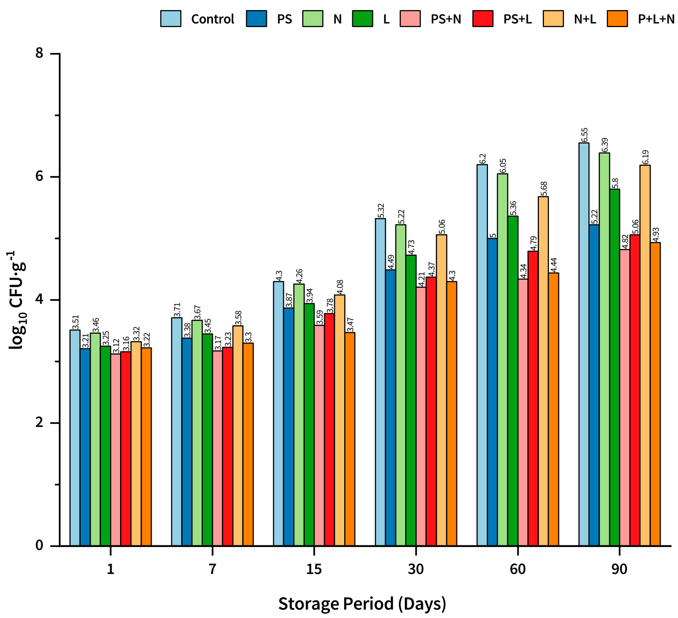

FIGURE 7. Numbers of proteolytic microorganisms detected in Şavak pickled white cheese (log10 cfu·g^{-1} ± sd). (PS: potassium sorbate, N: nisin, L: lysozyme, PS+N: potassium sorbate+nisin, PS+L: potassium sorbate+lysozyme, N+L: nisin+lysozyme, P+N+L: potassium sorbate+lysozyme+nisin)

Escherichia coli is transmitted to raw milk from teats, milking machines and used equipment and materials [38]. *E. coli* counts decreased in all cheese groups during storage. From the 60th d of storage, the count was below detectable levels in group 4 ($<1.00\ log_{10}\ cfu \cdot g^{-1}$). The highest antimicrobial effect on *E. coli* bacteria was detected in groups 4, 5 and 1. The inhibitory effect of potassium sorbate on *E. coli* bacteria is similar to the results of some researchers [23, 25]. The lowest antimicrobial effect was seen in group 2 where nisin was used alone, in group 3 where lysozyme was used alone and in groups 6 and 7 where nisin and lysozyme were used together. These results are consistent with the findings of some researchers [28, 39, 40] who reported that nisin and lysozyme have and do not have antimicrobial effects on Gram–negative bacteria. Statistically, significant differences were found between the control group and the 1st, 3rd, 4th and 5th groups on the 30th day and between the control group and the 1st, 3rd and 5th groups on the 60th day. ($P \leq 0.05$) (FIG. 8).

The number of *S. aureus* positive for coagulase test showed a continuous decrease from the 1st d of storage. From the 15th d of storage, the number was below the detectable level in the 2nd, 3rd, 4th, 5th, 6th and 7th groups ($<1.00\ log_{10}\ cfu \cdot g^{-1}$). Nisin causes the cytoplasmic membranes of *S. aureus* to become more permeable (depolarization) more rapidly. Nisin and lysozyme act together against Gram–positive bacteria by strengthening each other's

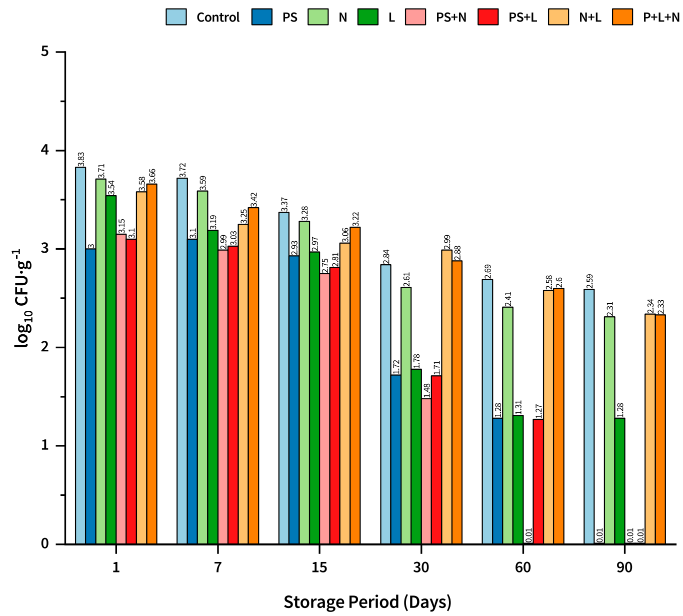

FIGURE 8. *Escherichia coli* counts detected in Şavak pickled white cheese (log10 cfu·g^{-1} ± sd). (PS: potassium sorbate, N: nisin, L: lysozyme, PS+N: potassium sorbate+nisin, PS+L: potassium sorbate+lysozyme, N+L: nisin+lysozyme, P+N+L: potassium sorbate+lysozyme+nisin)

antibacterial mechanisms [28]. The results obtained in the study are consistent with the results of some researchers [28, 41, 42]. Bactident coagulase test was performed on *S. aureus* colonies suspected of being coagulase positive detected during storage in all cheese groups. However, the result was negative.

A decrease in pH values was observed in all cheese groups starting from the first d of storage. After the control group (5.30) on the 90th d of storage, it was observed that the lowest value and the highest inhibition were in the 2nd group (5.42) where nisin was used alone. This finding was found to be consistent with the findings of studies reporting that nisin has a pH–lowering effect in food products depending on the storage process [43, 44].The acidity level in cheese has important roles in aroma, taste, texture, flavor, ripening and in the inhibition of unwanted bacteria in secondary contaminations [1]. The amount of acidity (% g lactic acid) increased regularly in the later days of storage. It was determined that the lowest level of acidity increase during storage was in the 4th group (FIG. 9).

In all cheese groups, the amounts of fat in dry matter slightly increased in parallel with the advancement of the storage period. According to the Turkish Standards Institute Cheese Standard [19] and the Turkish Food Codex Cheese Communiqué [45], all cheese groups were found to be in the full–fat group (at least 45% milk fat in dry matter) according to the amount of fat in dry matter.In the present study, ash amounts increased regularly in all cheese groups from the first d of storage. It was observed that the obtained results were in line with the findings of some researchers [46].

Salt values in dry matter increased continuously from the first d of storage. It was determined that the values determined in all cheese groups did not exceed the salt value in dry matter (maximum 10%) specified in the Turkish Standards Institute

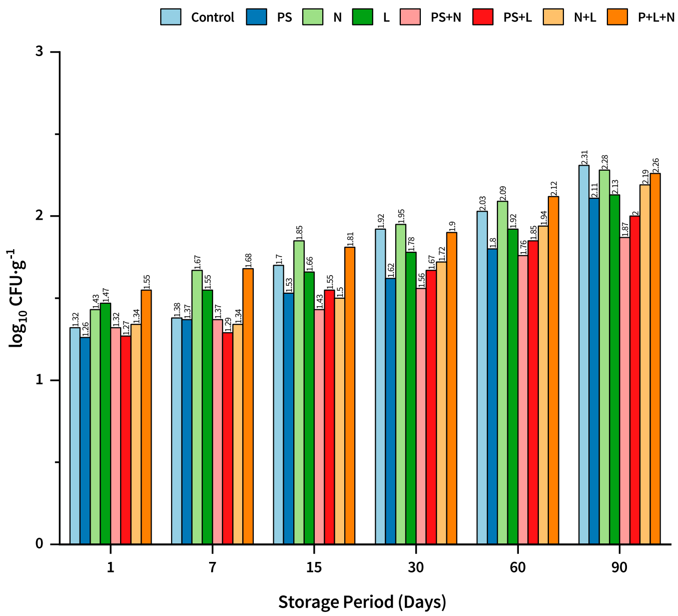

FIGURE 9. Changes in acidity values of Şavak pickled white cheese (log10 cfu·g^{-1} ± sd). (PS: potassium sorbate, N: nisin, L: lysozyme, PS+N: potassium sorbate+nisin, PS+L: potassium sorbate+lysozyme, N+L: nisin+lysozyme, P+N+L: potassium sorbate+lysozyme+nisin)

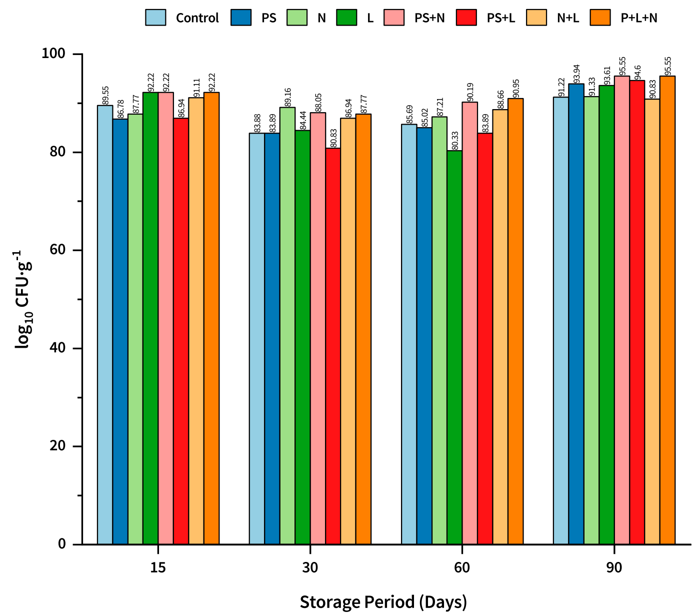

FIGURE 10. Changes in general likeability (maximum: 100) scores of Şavak pickled white cheese (log10 cfu·g^{-1} ± sd). (PS: potassium sorbate, N: nisin, L: lysozyme, PS+N: potassium sorbate+nisin, PS+L: potassium sorbate+lysozyme, N+L: nisin+lysozyme, P+N+L: potassium sorbate+lysozyme+nisin)

Cheese Standard [19]. The food additives used (potassium sorbate, nisin and lysozyme) increased continuously throughout the storage period of the cheeses. Since there are many factors affecting the transition of salt and food additives from the brine to the cheese mass, it can sometimes be difficult to determine the amount of additives correctly. These factors include the type of food, the microbial load of the food at the beginning and during the ripening period, the conditions for storing the food, the amount of salt in the brine, the temperature of the brine, the size and dimensions of the cheese mass, the amount of dry matter in the cheese, the water activity value of the cheese, the amount of fat in the cheese, the interaction of the additives added with the amount of salt in the brine, the concentration of the additives and the solubility of the additives [28, 41, 47].

Different trends were observed in the cheese groups in terms of general appreciation scores. It was determined that the highest scores were equally received by the 4th group (95.55) and the 7th group (95.55) on the 90th d of storage. It was also determined that lysozyme had no effect on the sensory properties of the cheese or even on the ripening period (FIG. 10). This result is similar to the findings of some researchers who stated that lysozyme is not effective on proteolysis, lipolysis and other biochemical changes observed in cheeses during the ripening period and therefore does not have any positive or negative effect on the formation of the unique flavor, aroma and taste of cheeses [48, 49].

The total psychrophilic aerobic bacteria count increased during the storage period in all cheese groups. The least logarithmic increase was seen in group 4. It was determined that the combination of potassium sorbate and nisin used in this group had a greater inhibitory effect on this group of microorganisms. There isn't any literature investigating the inhibitory effect of the combination of potassium sorbate, nisin and lysozyme on this group of bacteria.

CONCLUSION

As a result, at the end of the 90 d storage period of experimentally prepared Şavak brine white cheeses, it was determined that the combination used in the 4th group (potassium sorbate 1 g·L^{-1} + nisin 12.5 mg·L^{-1}) had more antibacterial effects on some microbiological parameters (total psychrophilic aerobes, coliform, Enterobacteriaceae, *Staphylococcus–Micrococcus*, fecal streptococci, lipolytic, proteolytic, *Escherichia coli* and *Staphylococcus aureus*) and had more inhibitory effects on acidity as a chemical parameter. According to the statistical evaluation, no significant differences were found between the sensory analysis results. However, according to the given numerical scores, the 4th and 7th groups received equal scores (95.55) and became the two most appreciated groups. Thus, it was concluded that additives with GRAS status (especially the combination of potassium sorbate 1 g·L^{-1} + nisin 12.5 mg·L^{-1}) can be used to improve the quality of Şavak pickled white cheeses, can be an alternative method to heat treatment during cheese production, and will shed light on scientific studies to determine whether they have inhibitory effects on some pathogenic bacteria found in Şavak pickled white cheeses.

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