




Effect of Scallop-Shell Powder Suspension Treatment on *Listeria monocytogenes* Growth on Kashar Cheese

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ABSTRACT

High occurrence of *Listeria monocytogenes* in cheeses is an important risk for human health. The present work aims to investigate antibacterial effect of scallop-shell powder (SSP) on *L. monocytogenes* on Kashar cheese. Cheese samples were immersed into 0, 0.5, 1.0 or 1.5% of SSP suspensions. After drying, the surface of cheese samples was inoculated with *L. monocytogenes* (5 log cfu/g). Following drying for 2 h, each sample was individually vacuum-packed and stored at 4°C for 28 days. The microbiological and chemical properties of samples were determined at every 7 and 14 days of storage, respectively. Application of SSP solution (%) significantly inhibited the growth of *L. monocytogenes* and mold but not yeast or lactic acid bacteria. Treatment of SSP did not affect the pH value, color and chemical properties ($p>0.05$) but increased the ash contents of cheese samples ($p<0.05$). The results suggested that SSP treatment could be used to reduce risk of *L. monocytogenes* contamination on Kashar cheese.

Keywords: *Listeria monocytogenes*, Scallop-shell powder, Kashar cheese

Kaşar Peynirine Uygulanan Midye Kabuğu Tozu Çözeltisinin *Listeria monocytogenes* Gelişimi Üzerine Etkisi

ÖZ

L. monocytogenes peynirlerde bulunan bir patojen olarak insan sağlığı için bir tehdit unsurudur. Bu çalışma ile midye kabuğu tozu (MKT) uygulanmasının Kaşar peynirinde *L. monocytogenes* üremesine karşı antimikrobiyal etkisi araştırılmıştır. Bu amaçla, *L. monocytogenes* (5 log kob/g) bulaştırılan peynir yüzeylerine daha sonra farklı konsantrasyonlardaki (%0, 0.5, 1.0, 1.5) MKT çözeltisi uygulanmıştır. Peynirler vakumlanıp 4°C'de depolanmıştır. Depolanmanın her 7 ve 14 gününde örneklerde sırasıyla mikrobiyolojik ve kimyasal analizler yapılmıştır. MKT'nin uygulanması *L. monocytogenes* ve küf üremesini engellerken maya ve laktik asit bakterilerinin üremesini etkilememiştir. MKK uygulaması örneklerin pH'sı, rengi ve kimyasal özelliklerine etki etmemiştir ($p<0.05$) fakat kül içeriğinde artışa neden olmuştur ($p<0.05$). Sonuçlar, peynirde MKT'nin *L. monocytogenes* ve küflenme problemini azaltmak için kullanılabilirliğini göstermiştir.

Anahtar Kelimeler: *Listeria monocytogenes*, Midye kabuğu tozu, Kaşar peyniri

INTRODUCTION

Kashar cheese has been one of the most popular semi-hard cheeses in Turkey. *L. monocytogenes* caused

outbreaks in milk and dairy products and therefore became a risk factor in the dairy industry [1, 2]. These pathogens have been shown to be able to survive and grow in soft and semi-soft cheeses [3]. *L.*

monocytogenes is resistant to high salt concentration and can survive even below the refrigerator temperature. Foodborne infectious diseases caused by *L. monocytogenes* are called listeriosis. Listeriosis is defined as a disease that causes serious infections leading to meningitis, abortion, septicemia and even death as a result of consumption of foods contaminated with *L. monocytogenes*.

Several studies showed that the growth of *L. monocytogenes* could be inhibited in Kashar cheese by some applications. For example, chitosan or whey protein coating solutions containing essential oils like thyme and clove have been shown to have antimicrobial effect on *L. monocytogenes* in Kashar cheese [1]. Similarly, in another study, zein-wax composite films containing lysozyme prevented the growth of *L. monocytogenes* ATCC 7644 in Kashar cheese [2, 3]. In this study, the application of scallop shell powder (SSP) suspension was investigated as a cheap and safe alternative antimicrobial preservative against *L. monocytogenes* on Kashar cheese.

SSP is a waste product produced from scallop shell in Japan and Korea. It was listed as a food additive (E529) in Turkish Food Codex. The main ingredient of SSP is calcium carbonate (CaCO_3), but when heat treatment is applied at temperatures of 700°C and above, CaCO_3 is transformed into calcium oxide (CaO) having strong antibacterial effect [4, 5]. The studies revealed that the application of SSP inhibited growth of several pathogens including *Staphylococcus aureus*, *Salmonella enteritidis*, *S. typhimurium*, *Escherichia coli* O157:H7 and *L. monocytogenes* [4, 6-12] and also increased shelf life of some food products. For example, the addition of SSP solution into ingredients increased the shelf life of the traditional fermented product kimchi, shredded cabbage and fresh soy cheeses [13-15].

Objectives of the study were to (1) inspect the inhibition effect of SSP on the growth of *L. monocytogenes* on Kashar cheese; (2) investigate the impact of SSP application on shelf life of Kashar cheese in terms of numbers of lactic acid bacteria, yeast and mold, and chemical properties (dry matter, protein, fat, ash, water activity, pH); and (3) examine the sensory and color characteristics of Kashar cheese treated with SSP.

MATERIALS and METHODS

Listeria monocytogenes culture

L. monocytogenes ATCC 7644 was twice successively transferred in 10 mL of tryptic soy broth (Merck Laboratories) at 30°C for 24 h. Optical density of *L. monocytogenes* culture at 600 nm reached to 0.7 which represents 9 log/ml cells. The culture was diluted in 0.1% peptone water to a concentration of approximately 7 log/mL for following inoculation.

Preparation of Kashar Cheese Samples

Fresh Kashar cheese samples (20 g) were immersed into SSP suspension prepared at 0, 0.5, 1.0 or 1.5% concentration for 10 sec (immersing time was kept short to avoid solid weight loss from the cheese samples and water residues on the surface) and were dried for 2 h in a biosafety cabinet. Following spreading 0.1 mL of *L. monocytogenes* (5 log CFU/g) on both surfaces, the cheese samples were dried and vacuumed packed in sterile bags (polyamide-polyethylene), and kept at 4°C for 28 days.

Microbiological Analysis

In every 7 days, 10 g sample was homogenized using a stomacher (Interscience, Bagmixer 400, France) in 90 mL of peptone water (0.1%) for 2 min. To determine the number of *L. monocytogenes*, lactic acid bacteria and yeast/mold, appropriate dilutions were plated on Modified Oxford Agar (30°C for 48 h), de Man, Rogosa and Sharpe Agar (42°C for 2 days) and Oxytetracycline-Glucose-Yeast-Extract Agar supplemented with oxytetracycline (25°C for 5 days), respectively.

Chemical Analysis

Cheese samples were evaluated for moisture using oven drying method, protein using Kjeldahl method [16], fat using Gerber Method [17] and ash [18] at 0, 14 and 28th days of storage. The pH and water activity of the samples were also monitored by using a pH meter (Hannah pH 211 model), and using a water activity meter (AQUA Lab Series 3), respectively.

Color

A tintometer (Lovibond RT 300 Series Reflectance England) was used to evaluate the color characteristics of the cheese samples at every 14 days and recorded as hunter color system (a^* (redness), b^* (yellowness) and L^* (lightness)). The colorimeter was calibrated using a standard white tile. For each cheese sample, five readings were taken in triplicate.

Statistical Analysis

IBM SPSS software package and two-way analysis of variance was used to analyze data from triplicate experiments (SPSS 20.0, SPSS Inc. Chicago, IL, USA). Duncan's Multiple Range Test assessed differences among means at the 95% significance level.

RESULTS and DISCUSSION

Listeria monocytogenes

The number of *L. monocytogenes* observed for 28 days of storage with the standard deviations of the means was shown in Figure 1a. The initial *L. monocytogenes* number was found to be about 4.5 log CFU/g in all samples. The number of *L. monocytogenes* inoculated in all Kashar cheese samples started to decrease

significantly from day 7 ($p < 0.05$). At the end of storage, the number of *L. monocytogenes* reduced from 4.5 to approximately 2 log in the cheese samples treated with 0, 0.5 or 1.0% SSP. No significant difference within effect of those treatments was observed. However, immersing cheese samples into 1.5% SSP significantly increased drop seen in the population of *L. monocytogenes* ($p < 0.05$).

Antimicrobial effect of SSP is coming from released hydroxyl ions which oxidize free radicals and increase reactivity [12, 19, 20]. They could kill microorganism cells by damaging cytoplasmic membrane and denaturation DNA and proteins. The similar studies also showed the antimicrobial effect of SSP against the growth of *L. monocytogenes* [12, 21]. For example, even application of 0.1% SSP suspension significantly reduces the number of *L. monocytogenes* on chicken wings and frankfurters surface by 6 and 3.5 log CFU/g, respectively [12, 21]. Likewise, another study confirmed

that 0.15% CaO solution for 3 min treatment reduced the number of *L. monocytogenes* by 50% [12]. The results in the current study showed that the number of *L. monocytogenes* reduced around 2 log on all cheese samples with or without SSP treatment during 28 d refrigerated storage. Çetinkaya and Soyutemiz [22] also showed the similar inhibition pattern of *L. monocytogenes* during ripening stage of Kashar cheese. Hurdle concept including salt, high acid and presence of lactic acid bacteria on cheese may play important role on reduced number of *L. monocytogenes*. Several studies revealed that existence of lactic acid bacteria could prevent the growth of pathogens and spoilage microorganisms in fermented foods by production of antimicrobial compounds including organic acids, diacetyl and bacteriocins. SSP treatment at 1.5% concentration together with mentioned antimicrobial compounds above might have synergistic effect on inhibition of *L. monocytogenes*.

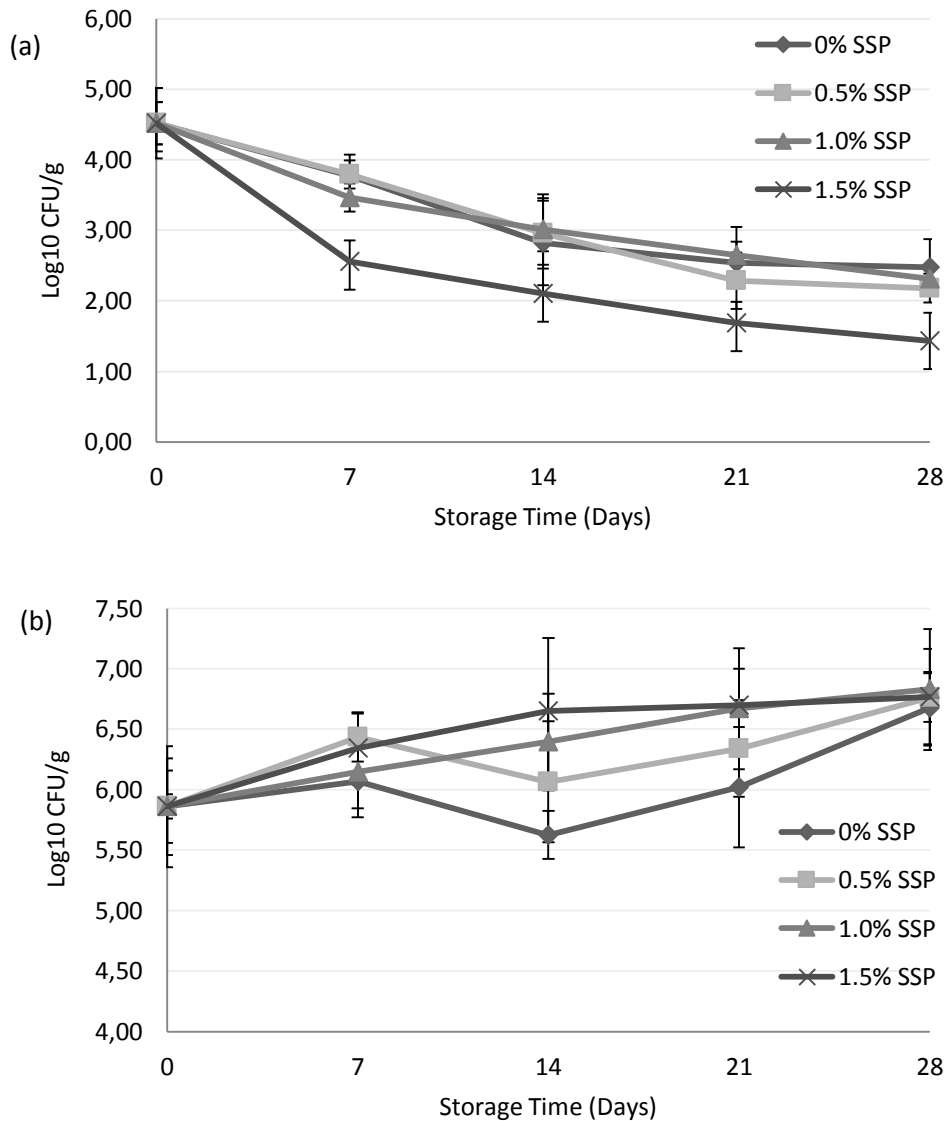


Figure 1. Effect of scallop shell powder (SSP) treatment on the growth of *Listeria monocytogenes* (a) and lactic acid bacteria (b) on the surface of Kashar Cheese

Lactic Acid Bacteria

The change in the number of lactic acid bacteria in the Kashar cheese samples treated by the SSP suspension at different concentrations (0, 0.5, 1.0, 1.5%) is shown in Figure 1b. In general, the number of lactic acid bacteria increased by approximately from 5.8 to 6.7 log CFU/g in all samples during the storage period. However, increasing concentration of SSP did not significantly affect the growth of lactic acid bacteria in the cheese ($p>0.05$). In the similar studies, the application of SSP on frankfurters or kimchis did not have a lethal effect on lactic acid bacteria [12, 15]. Occurrence of lactic acid bacteria in Kashar cheese provides preservation of the products by synthesizing antimicrobial compounds and also promotes flavor development during maturation period.

In the current study, SSP treatment limited to surface of the cheese, so that it did not affect interior part. Most of the lactic acid bacteria are facultative anaerobes and can be exist and grow inside of cheese structure. The hidden microorganism interior part could not be inactivated by SSP treatment on the surface.

Molds

In Kashar samples, the number of mold was counted as 0.47 log CFU/g at the beginning of the storage (Figure 2a). While 0.94 log CFU/g increase in the growth of mold was observed in the cheese treated by 0% and 0.5% SSP suspensions at the end of 28 d storage period, no mold growth was observed in the cheeses containing 1% and 1.5% SSP. Increasing concentration of SSP significantly decreased the growth of mold in Kashar cheese. Similarly, Bodur et al. [12] and Cagri-Mehmetoglu [15] confirmed that SSP treatment could suppress mold growth on chicken wings and frankfurters.

Mold growth on Kashar cheese is very important problem in decreasing shelf life. Vacuum packaging of cheese partially inhibits mold growth since vacuuming cannot completely suck air out due to less smooth structure of cheese [23, 24]. In the present study, on the control cheese even vacuum packaging some mold growth was also observed. SSP, which is a safe and inexpensive antimicrobial, has been proven in this study as an effective method against mold growth together with vacuum packaging. Shawai and Yoshikowa [25] also showed that SSP has the stronger antifungal effect than antibacterial effect. Alkaline effect of SSP and the generation of active oxygen such as superoxide anions from the application of SSP may have antifungal effect against mold growth.

Yeasts

The changes in the number of yeasts for 28 days of storage for Kashar cheese samples treated by SSP suspension are shown in Figure 2b. In the application, the initial number of yeasts for all cheese samples was observed to be approximately 2.76 log CFU/g, while the number of yeast increased to an average of 3.7 log CFU/g at the end of the storage. Increasing in SSP

concentration did not significantly affect the growth of yeast on the cheese ($p>0.05$).

However, in our previous studies on frankfurters and chicken wings, treatment of 0.10 or 0.50% SSP compared with the untreated control samples reduced the number of yeast cells on both products by 0.4 to 1.4 log CFU/g, respectively.

Another antimicrobial mechanism of CaO is that it strongly alkalinizes cell membrane, leading depolarization and possibly blockage of electrochemical gradient renewal by transferring calcium into the cytoplasm, rising to internal alkalization, and, to end with cell death. The proton gradient in bacteria and yeasts through the membrane is a common process; however, the growth of yeast was not prevented by CaO in the present study unlike other studies. The reason for this must be that application of CaO treatment could not show antifungal effect on yeast cells by failing internal alkalization since low pH of Kashar cheese did not allow that.

Water Activity

The water activity value of the coated Kashar cheese with SSP solution was measured around 0.90 (Table 1). Statistical analysis showed that SSP did not significantly affect water activity in the application of Kashar cheese ($p>0.05$).

pH

The pH values of the samples of the Kashar cheese samples were 5.67-5.70 at the first day; at the 28th day it was determined to be 5.69-5.68 (Table 1). A significant increase in the pH value of the first 14-day period was observed in the samples of Kashar cheese, and a decrease in the pH values was observed in the period up to 28 days after the 14th day. In general, the pH value at the end of storage was determined as 5.68. While this average value was found to be higher than the reference study, SSP suspensions prepared at a concentration of 1% and 1.5% had a significant effect on pH in Kashar cheese at the first 14 days of storage ($p<0.05$). This is due to the fact that SSP is an alkaline agent and its pH is close to 11. During the last 14 days of storage, the amount of lactic acid produced by lactic acid bacteria during the cheese ripening kept the pH balance again.

Chemical Analysis

The dry matter value of the samples of Kashar cheese was found to be 56.46% at the end of the 28th day (Table 2). Statistically, there was no effect of SSP on dry matter in Kashar cheese ($p>0.05$). Ash content of cheese treated with SSP solution at different concentration was changed from 3.07 to 4.07%. Only 14 days of storage significantly increased ash content of the cheese samples immersed into only 1.0 and 1.5% SSP suspensions. The cheese samples contain 28.92 to 31.08% fat and 2.8 to 3.2% protein. Fat and protein content of the samples significantly did not change during 28 days of storage ($p>0.05$).

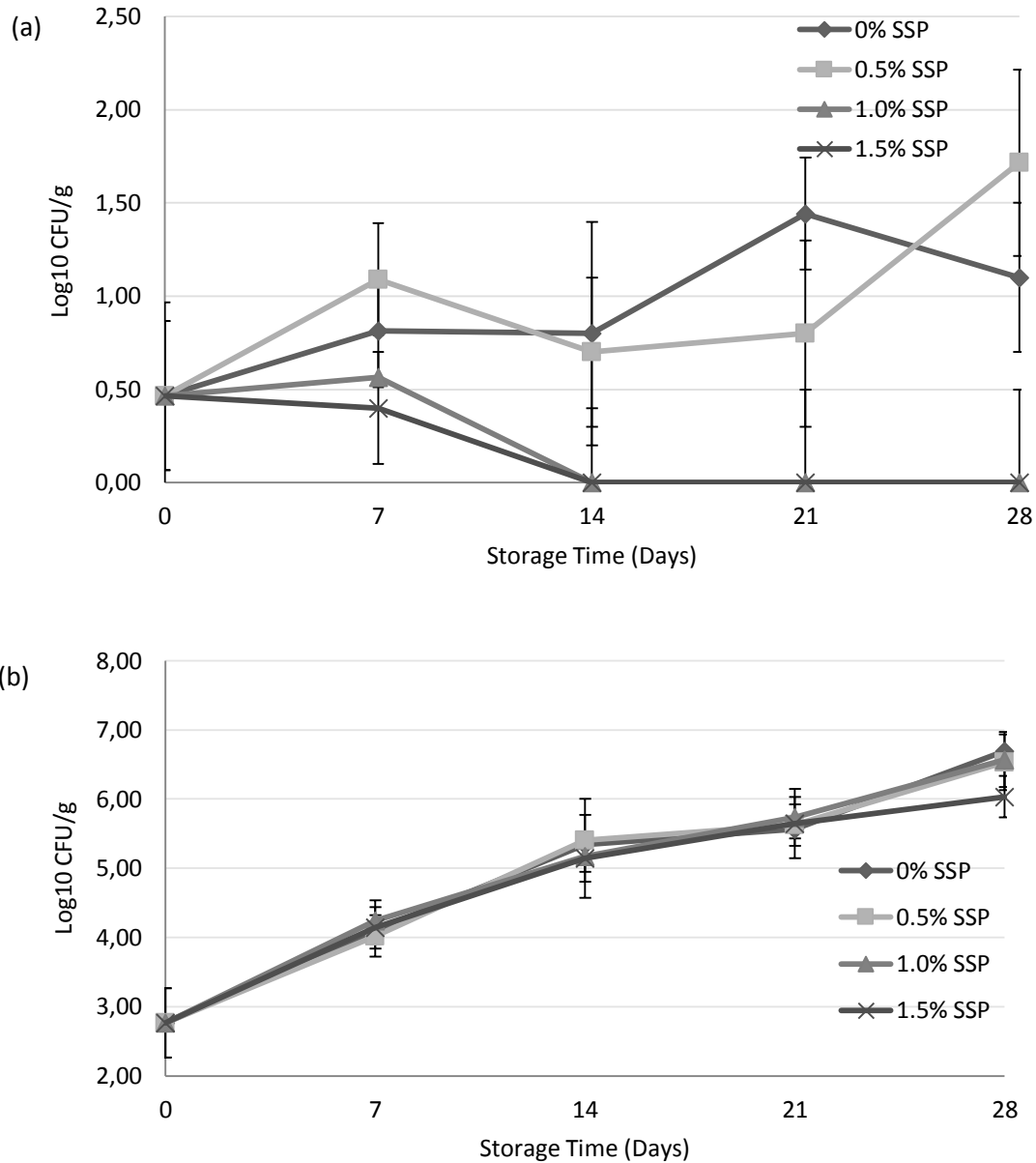


Figure 2. Effect of scallop shell powder (SSP) treatment on mold (a) and yeasts (b) growth on the surface of Kashar cheese

Table 1. The effect of scallop shell powder (SSP) application on water activity and pH of Kashar Cheese

SSP Treatment (%, w/v)	Storage Time (Day)		
	0	14	28
Water activity			
0	0.88±0.02 ^{aA}	0.89±0.008 ^{aA}	0.91±0.005 ^{aA}
0.5	0.89±0.01 ^{aA}	0.91±0.007 ^{aA}	0.91±0.002 ^{aA}
1	0.91±0.005 ^{aA}	0.91±0.004 ^{aA}	0.91±0.003 ^{aA}
1.5	0.90±0.01 ^{aA}	0.91±0.001 ^{aA}	0.91±0.008 ^{aA}
pH			
0	5.67±0.03 ^{aA}	5.73±0.01 ^{aA}	5.69±0.01 ^{aA}
0.5	5.68±0.02 ^{aA}	5.75±0.01 ^{aA}	5.68±0.02 ^{aA}
1	5.67±0.02 ^{aA}	5.81±0.01 ^{bA}	5.67±0.01 ^{aA}
1.5	5.70±0.08 ^{aA}	5.77±0.03 ^{abA}	5.68±0.05 ^{aA}

*Mean (n =3) ± Standard deviation. a, b, c, d: Different letters in the same row indicate significant differences among storage duration (p<0.05). A, B, C, D Different letters in the same column indicate significant differences among treatments (p<0.05).

Table 2. The effect of scallop shell powder (SSP) application on the chemical composition of Kashar cheese during 28 days of storage

	SSP Treatment (% w/v)	Storage Time (Day)		
		0	14	28
Dry Content (%)	0	58.85±0.56 ^{aA}	56.98±0.56 ^{bA}	56.08 ±2.40 ^{bA}
	0.5	59.16±0.89 ^{aA}	55.31±1.60 ^{bA}	56.94±1.69 ^{bA}
	1.0	59.98±0.04 ^{aA}	57.21±0.68 ^{bA}	56.44±2.01 ^{bA}
	1.5	59.91±1.30 ^{aA}	55.38±0.40 ^{bA}	56.40±2.18 ^{bA}
Fat (%)	0	28.63±0.22 ^{Aa}	28.12±0.80 ^{Aa}	30.11±0.66 ^{Aa}
	0.5	29.18±0.74 ^{Aa}	29.19±0.58 ^{Aa}	30.32±0.85 ^{Aa}
	1.0	28.13±0.63 ^{Aa}	28.55±0.70 ^{Aa}	29.67±0.88 ^{Aa}
	1.5	29.21±0.71 ^{Aa}	30.25±0.78 ^{Aa}	30.08±0.49 ^{Aa}
Ash (%)	0	3.07±0.55 ^{Aab}	3.32±0.13 ^{Aa}	3.34±0.77 ^{Aa}
	0.5	3.17±0.19 ^{Aa}	3.64±0.22 ^{Ba}	3.69±0.82 ^{Ba}
	1.0	3.59±0.28 ^{Bb}	3.82±0.68 ^{Ca}	3.88±0.22 ^{Ca}
	1.5	3.86±0.15 ^{Cb}	4.10±0.65 ^{Da}	4.07±0.41 ^{Da}
Protein (%)	0	3.27±0.61 ^{Aa}	3.36±0.33 ^{Aa}	3.37±0.29 ^{Aa}
	0.5	3.18±0.29 ^{Aa}	3.29±0.38 ^{Aa}	3.38±0.37 ^{Aa}
	1.0	3.20±0.77 ^{Aa}	3.25±0.32 ^{Aa}	3.28±0.55 ^{Aa}
	1.5	3.22±0.29 ^{Aa}	3.29±0.19 ^{Aa}	3.33±0.39 ^{Aa}

*Mean (n =3) ± Standard deviation. a, b, c, d: Different letters in the same row indicate significant differences among storage duration (p<0.05). A, B, C, D Different letters in the same column indicate significant differences among treatments (p<0.05).

Color

The color parameters of cheese are shown in Table 3. The L* values (representing brightness for cheeses) treated with SSP at different concentrations were ranged from 77 to 81. Similarly, Civelek and Cagri-Mehmetoglu [26] reported similar results that L* values for Kashar cheese stored for 56 days did not significantly changed.

During storage, the a* values indicating redness (+), greenness (-) ranged from -0.85 to -1.79 for cheese

samples. Although treatment of SSP did not statistically change the a* value of the samples, the storage of the cheese for at least 14 days significantly decreased (p<0.05). The b* values representing yellowness of the cheese samples was measured between 24.77 and 30.07. Storage of 28 days or SSP treatments did not statistically change the b* value of the samples (p>0.05). Unlike our studies, the studies usually reported that aging cheese increase yellowness (b* value) of cheese in terms of moisture loss and increased in dry matter [27, 28, 29].

Table 3. The effect of scallop shell powder (SSP) treatment on color values of Kashar cheese during 28 days of storage

	SSP Treatment (% w/v)	Storage Time (Day)		
		0	14	28
L*	0	77.64±3.34 ^{Aa}	78.10±2.07 ^{Aa}	78.22±1.27 ^{Aa}
	0.5	79.17±3.04 ^{Aa}	78.91±2.30 ^{Aa}	79.66±1.33 ^{Aa}
	1	80.83±3.11 ^{Aa}	81.06±0.85 ^{Aa}	80.81±2.05 ^{Aa}
	1.5	80.95±2.95 ^{Aa}	79.72±1.43 ^{Aa}	79.10±0.76 ^{Aa}
a*	0	-0.85±0.99 ^{Aa}	-1.66±0.44 ^{Aab}	-1.79±0.18 ^{Aab}
	0.5	-0.96±0.78 ^{Aa}	-1.55±0.98 ^{Aab}	-1.56±0.81 ^{Aab}
	1	-1.27±0.64 ^{Aa}	-1.62±0.97 ^{Aab}	-1.67±0.72 ^{Aab}
	1.5	-1.33±0.94 ^{Aa}	-1.79±0.69 ^{Aab}	-1.78±0.66 ^{Aab}
b*	0	24.77±1.34 ^{Aa}	26.17±2.07 ^{Aa}	27.43±1.28 ^{Aa}
	0.5	29.47±1.87 ^{Aa}	26.66±1.29 ^{Aa}	28.28±0.88 ^{Aa}
	1	28.18±2.39 ^{Aa}	26.13±1.92 ^{Aa}	27.55±1.19 ^{Aa}
	1.5	30.07±3.14 ^{Aa}	26.56±1.09 ^{Aa}	29.06±0.47 ^{Aa}

*Mean (n =3) ± Standard deviation. a, b, c, d: Different letters in the same row indicate significant differences among storage time (p<0.05). A, B, C, D Different letters in the same column indicate significant differences among treatments (p<0.05).

CONCLUSION

In conclusion, it is the most important finding of this study that more than 1% SSP application on Kashar surface prevented the development of *L. monocytogenes*. The other important finding is that the

mold growth was not observed on the samples of Kashar cheese treated by SSP at different concentrations. However, the growth of the yeast or lactic acid bacteria could not be inhibited by application of SSP treatment. No inhibition on the growth of the yeast and lactic acid bacteria, which are important

functions in Kashar ripening, is another important result of this study. The chemical and physical properties of the cheese samples were not significantly affected by SSP treatment. Since SSP is a safe natural product in

terms of health, it is thought to have an important role in use as an industrial food additive that can prevent the growth of *L. monocytogenes* and molds in cheese.

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