



Investigation of the Effects of Jerusalem Thorn (*Paliurus spina-christi* Mill.), Oriental Hackberry (*Celtis tournefortii* L.) Fruits and Black Cumin (*Nigella sativa* L.) Seed on Microbial Quality and Physicochemical Properties of Meatballs

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Abstract: This study was conducted to examine the effect of Jerusalem thorn (*Paliurus spina-christi* Mill./PSC) and oriental hackberry (*Celtis tournefortii* L./CT) fruits and black cumin (*Nigella sativa* L./NS) seed on the microbial quality and physicochemical properties during the storage of meatballs +4 °C. For this purpose, PSC, CT fruits, and NS seeds were added to the meatballs at 2% to form four groups, including the control. Prepared meatball samples were covered with stretch film in polyethylene plates and stored at 4±1 °C for 16 days. Analyzes were made on the 0, 4, 8, 12, and 16th days of storage in the meatball samples. The pH values of the meatball samples were determined between 5.89-6.02 on day 0 and between 6.10-6.49 on day 16, a_w values between 0.956-0.964 on day 0 and 0.971-0.980 on day 16. Total mesophilic aerobic bacteria (TMAB), total psychrotrophic bacteria (TPAB), lactic acid bacteria (LAB), and yeast-mold counts of meatball samples were 5.14-5.53, 4.62-4.83, 5.04-5.32 and 3.40-3.87 log₁₀ cfu/g, respectively on day 0, and 7.01-7.9, 7.64-8.24, 7.59-8.05 and 5.69-6.27 log₁₀ cfu/g, respectively on day 16. It was determined that PSC and CT fruits and NS seed slowed down the microbial growth rate in the meatballs from the eighth day. The best antimicrobial effect was found in psychrotrophic bacteria for CT fruit, LAB and yeast-mold for NS seed. As a result, it can be recommended to add PSC, CT fruits, and NS seeds to their composition to extend the shelf life of meatballs up to one week. The results of this study can provide helpful information for anyone dealing with food and further studies investigating the shelf life of meat and meat products.

Keywords: *Celtis tournefortii* L., Meatball, Microbiological quality, *Nigella sativa* L., *Paliurus spina-christi* Mill.

Karaçalı (*Paliurus spina-christi* Mill.), Dardağan (*Celtis tournefortii* L.) Meyvelerinin ve Çörek Otu (*Nigella sativa* L.) Tohumunun Köftelerin Mikrobiyal Kalitesi ve Fizikokimyasal Özellikleri Üzerine Etkilerinin Araştırılması

Özet: Bu çalışmanın amacı karaçalı (*Paliurus spina-christi* Mill./PSC), dardağan (*Celtis tournefortii* L./CT) meyveleri ve çörek otu (*Nigella sativa* L./NS) tohumunun, köftelerin +4 °C'deki muhafazasında mikrobiyal kalite ve fizikokimyasal özellikleri üzerindeki etkisinin araştırılmasıdır. Bu amaçla PSC, CT meyveleri ve NS tohumu köftelere %2 oranında ilave edilerek kontrol dahil dört grup oluşturuldu. Hazırlanan köfte örnekleri polietilen tabaklar içinde streç film ile kaplanarak 4±1 °C'de 16 gün muhafaza edildi. Köfte örneklerinde muhafazanın 0, 4, 8, 12 ve 16. günlerinde analizler yapıldı. Köfte örneklerinin pH değerleri 0. günde 5,89-6,02, 16. gününde ise 6,10-6,49 arasında, a_w değerleri 0. günde 0,956-0,964, 16. gününde ise 0,971-0,980 arasında tespit edildi. Total mezofil aerob bakteri (TMAB), total psikrotrof bakteri (TPAB), Laktik asit bakterileri (LAB) ve maya-küf sayıları 0. gün sırasıyla, 5,14-5,53, 4,62-4,83, 5,04-5,32 ve 3,40-3,87 log₁₀ kob/g arasında 16. günde ise sırasıyla 7,01-7,9, 7,64-8,24, 7,59-8,05 ve 5,69-6,27 log₁₀ kob/g arasında saptandı. PSC, CT meyvesi ve NS tohumunun köftelerde sekizinci günden itibaren mikrobiyal üreme hızını yavaşlattığı saptandı. Özellikle CT meyvesinin psikrotrof bakteriler üzerinde, NS tohumunun ise LAB ve maya-küf üzerinde en iyi antimikrobiyal etki sağladığı saptandı. Sonuç olarak köftelerin raf ömrünü bir haftaya kadar uzatmak için bileşimlerine PSC, CT meyvelerinin ve NS tohumu eklenmesi önerilebilir. Bu çalışmanın sonuçları et ve et ürünlerinin raf ömürlerini araştıran ileri çalışmalar ve gıda ile uğraşan herkes için yararlı bilgiler sağlayabilir.

Anahtar Kelimeler: *Celtis tournefortii* L., Köfte, Mikrobiyolojik kalite, *Nigella sativa* L., *Paliurus spina-christi* Mill.

Introduction

Meatball is generally cooked and consumed after beef or mutton is ground into minced meat, added fat (tallow fat, tail fat) and various spices, and brought to the desired shape and size (Halid and Rahim, 2019). Meatballs and their varieties are meat products widely preferred and consumed in fast food. Microbial contamination may occur if the necessary hygienic rules and/or cold chain are not observed during the production, processing, packaging, storage, distribution, and preservation of meat products such as meatballs. As a result of the proliferation of saprophytic microorganisms, spoilage may occur in meat products, the shelf life of the products is shortened, and in the presence of pathogenic factors, food-borne infections, and toxications may occur (Mansur et al., 2019).

Meat spoilage occurs due to microbiological and chemical, enzymatic reactions (Mansur et al., 2019). Because of the chemical composition of meat, which provides a suitable environment for microorganisms, and the nutrients it contains, microbiological spoilage generally occurs more quickly. Microbial spoilage is caused by microbial growth and consumption of meat nutrients by bacteria, which release undesirable metabolites (Casaburi et al., 2015). Meat and meat products are evaluated in terms of microbial quality (total aerobic bacteria, psychrotrophic bacteria, enterobacteria, fecal coliforms, *Salmonella* spp., *Listeria monocytognes*, *Escherichia coli*, enterococci, molds, etc.) to determine whether they are suitable for consumption and their shelf life (Mansur et al., 2019). Moreover, pH and water activity (a_w) values are essential criteria affecting the growth of microorganisms; they are important in determining the microbial quality of meat and meat products (Leistner, 2000).

It is crucial to prevent microbial proliferation in maintaining the quality of meat products during storage. The microbial load of the meat should be low initially, and microbial growth should be minimized or inhibited as much as possible during storage. In recent years, as consumers prefer more organic or minimally processed foods and foods containing fewer synthetic chemical additives, natural herbal preservatives rich in phenolic compounds are used as an alternative to artificial chemical preservatives to prevent a microbial increase in meat and meat products (Prommachart et al., 2020).

PSC is a shrub plant in the Rhamnaceae family. In Turkey, it can be grown all around Anatolia. In studies on ripe fruits and leaves of PSC, it has been reported that they are rich in polyphenolic substances and have antioxidant and antimicrobial effects (Şen et al., 2018; Takım and Işık 2020; Takım, 2021; Zor et al., 2017). *Celtis*, belonging to the Cannabaceae family, is a medium-sized tree species that grows in temperate, tropical, and subtropical regions and sheds its leaves yearly. CT plant is one of the *Celtis* species that grows naturally in Türkiye. Studies on ripe fruits and leaves of CT have reported that these parts of the plant are rich in polyphenolic substances, have high tocopherol content, and have antioxidant and antimicrobial effects (Gecibesler, 2019; Keser et al., 2017; Yıldırım et al., 2017). NS plant is a spice of the Ranunculaceae family that has been

used for culinary and medicinal purposes for years. NS is grown in many parts of the world (Singh et al., 2014). Studies have shown that NS seed has a strong antioxidant and antimicrobial effect on food (Chauhan et al., 2018; Mahros et al., 2021; Zwolan et al., 2020). However, it has not been reported that PSC and CT fruits have not been added to foods before and how they affect the microbial and physicochemical properties of foods. Therefore, PSC and CT fruits were preferred in the study.

This study was conducted to investigate the effect of Jerusalem thorn (*Paliurus spina-christi* Mill./PSC) and oriental hackberry (*Celtis tournefortii* L./CT) fruits and black cumin (*Nigella sativa* L./NS) seed on the microbial quality and physicochemical properties during the storage of meatballs +4 °C.

Materials and Methods

Ethics Committee: Since meatball samples produced in the laboratory were used as material in the study, ethics committee permission is not required.

Preparation of meatball dough: For ground meat, *Musculus semitendinosus* and *M. Semimembranosus* muscles of bullock were used, and ram tail fat was used for tail fat. Meat and fats were obtained from a local butcher in Elazığ, brought to the laboratory under cold chain conditions and stored in refrigerators. Meatballs were prepared using the meatball composition in Table 1. PSC and CT fruits and NS seeds, ground into flour, were added to the meatball mixture at a rate of 2% each. After all the meatball mixture's ingredients were mixed, they were kept at +4 °C for 3 hours. Then, the meatball dough was mixed homogeneously again and put into a steel mold with a diameter of 5 cm and a height of 1 cm; each meatball was 25±1 g. Then, each group was covered with stretch film in polyethylene plates and stored at 4±1 °C for 16 days. Analyzes were performed on days 0 (before packaging), 4, 8, 12, and 16 days of storage. In the microbiological analysis of the meatballs, total mesophilic aerobic bacteria (TMAB), total psychrotrophic bacteria (TPAB), lactic acid bacteria (LAB), yeast, and mold counts were made. In addition, pH and water activity (a_w) determinations were made in these groups. All analyzes were performed in pairs in parallel and in triplicate independently. Dry matter, ash, protein, and fat were analyzed after preparing the meatball dough for each replicate.

Preparation of samples for microbiological analysis: Meatball samples 25 g were weighed under aseptic conditions and put into sterile sample bags (stomacher 400, Italy). 225 mL of 0.1% peptone water (Merck, Darmstadt, Germany) was added and homogenized in a stomacher (ISOLAB, Germany), and a 10^{-1} dilution was prepared. Other dilutions of the sample up to 10^{-7} were prepared from this dilution, provided that the same diluent was used. Sowing was done in double series by using the cast plate method for counting other microorganisms except for yeast and mold counting. The smear plate method was used for yeast mold enumeration. Plates containing 30–300 colonies were

evaluated after incubation (USDA/FSIS, 2011). Counts were made with an automatic colony counter (Acolyte Colony Counter-7500 SYN Synbiosis).

Total mesophilic aerobic bacteria (TMAB) count: Plate Count Agar (PCA, Merck, Darmstadt, Germany) medium was inoculated for counting and incubated at 35 °C for 48 hours. Colony counts were made after incubation (USDA/FSIS, 2011).

Psychrotrophic bacteria count: Petri dishes were incubated at 7±1 °C for ten days by inoculating on PCA medium for counting. An incubation count was done (USDA/FSIS, 2011).

Lactic acid bacteria count: To determine the number of LAB, the plates were incubated at 30±1 °C for three days by sowing on Man-Rogasa Sharpe (MRS) Agar medium. Colony counts were made after incubation (ISO, 1998).

Yeast and mold count: For enumeration, the plates were incubated at 25±1 °C for five days by inoculating on Dichloran Rose Bengal Chloramphenicol (DRBC) Agar medium (Lab/LAB 217, Lancashire/United Kingdom). Yeast and mold counts were made after incubation (ISO, 2008).

Chemical Analysis: For chemical analysis, pH values, digital pH meters (EDT. GP 353) and water activity (a_w) values of meatball samples were determined with a water activity device (Aqualab, Meter Group, Inc., Pullman,

WA, USA) (AOAC, 1990; Lang and Steinberg, 1980). Dry matter and ash content of meatball samples was determined by the gravimetric method (AOAC, 2002a; AOAC, 2002b), the total fat amount was determined by the Soxhlet extraction method (AOAC, 2000), and protein amount was determined by Kjeldahl method (AOAC, 1998).

Statistical analysis: For the statistical analysis of the study data, the SPSS package program (24.0 for Windows software SPSS Inc., NY, USA) was analyzed by one-way analysis of variance at a 95% confidence interval. Duncan's multiple comparison tests were used to determine the difference between the means of the experimental groups after analysis of variance (SPSS, 2017).

Results

The chemical composition of the meatball samples is given in Table 2. The pH values determined in the meatball samples during storage are shown in Figure 1, and the a_w values are shown in Figure 2. The total number of mesophilic aerobic bacteria detected in the meatball samples during storage is given in Table 3, the number of psychrotrophic bacteria in Table 4, the number of yeast-moulds in Table 5, and the number of lactic acid bacteria in Table 6.

Table 1. Composition of meatball groups (%).

Product	Groups	
	Control group(%)	Meatballs with PSC/CT/NS added(%)
Lean ground beef	74	72
Tail fat	20	20
Salt	2	2
Garlic	1,3	1,3
Onion	1,7	1,7
Red pepper	0,35	0,35
Black pepper	0,35	0,35
Ginger	0,15	0,15
Cumin	0,15	0,15
PSC/CT/NS	-	2

PSC: *Paliurus spina-christi* Mill. fruit NS: *Nigella sativa* L. seed, CT: *Celtis tournefortii* L. Fruit.

Table 2. The composition of the meatballs (%).

Group	KM	Ash	Protein	Fat
Control	46.16±0.58	2.76±0.26	19.25±0.58	21.03±0.71
PSC	47.12±0.64	2.68±0.74	17.82±0.84	20.30±0.52
NS	46.57±0.72	2.58±0.32	18.22±0.28	20.71±0.82
CT	47.38±1.06	2.81±0.35	18.73±0.44	20.56±1.01

KM: Dry matter PSC: *Paliurus spina-christi* Mill. fruit, NS: *Nigella sativa* L. seed, CT: *Celtis tournefortii* L. Fruit.

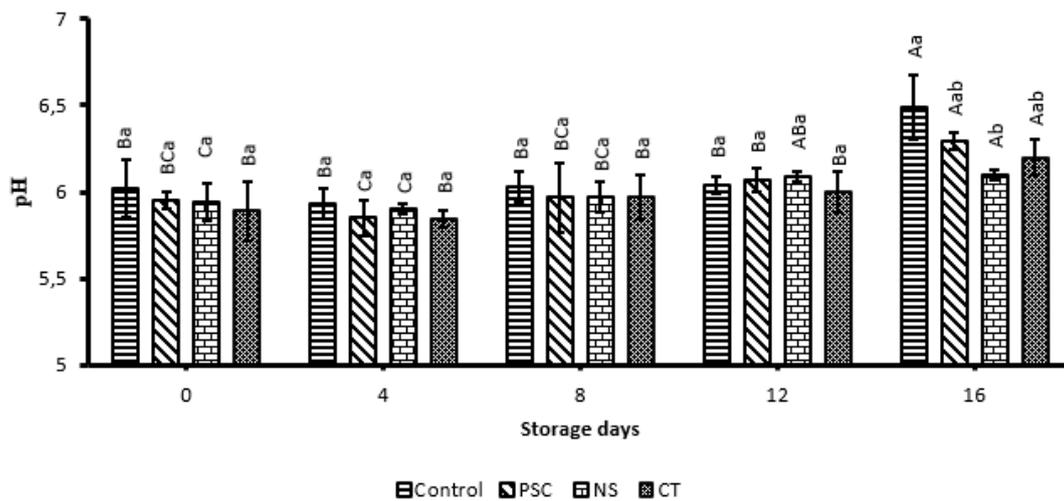


Figure 1. Changes in pH value in meatballs during storage (mean±standard deviation). PSC: Paliurus spina-christi Mill. fruit, NS: Nigella sativa L. seed, CT: Celtis tournefortii L. Fruit, A-D: The mean values with different letters between the storage days are statistically different (P<0.05), a-b: The mean values with different letters between the groups are statistically different (P<0.05).

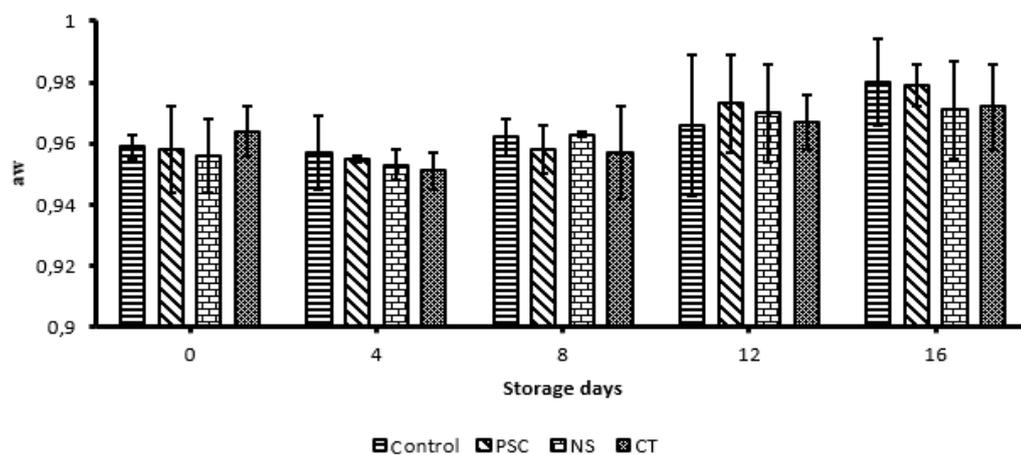


Figure 2. Changes in aw value in meatballs during storage (mean±standard deviation). PSC: Paliurus spina-christi Mill. fruit, NS: Nigella sativa L. seed, CT: Celtis tournefortii L. fruit.

Table 3. Total number of mesophilic aerobic bacteria (TMAB) detected in meatballs (log₁₀ cfu/g±standard deviation).

Group	Storage time (Days)				
	0	4	8	12	16
Control	5.53±0.37 ^{Ea}	6.10±0.23 ^{Da}	6.81±0.17 ^{Ca}	7.45±0.04 ^{Ba}	7.91±0.25 ^{Aa}
PSC	5.14±0.14 ^{Ea}	5.98±0.36 ^{Da}	6.55±0.12 ^{Cab}	6.85±0.13 ^{ABb}	7.09±0.09 ^{Ab}
NS	5.35±0.19 ^{Ea}	5.87±0.39 ^{Da}	6.39±0.19 ^{Cb}	6.73±0.19 ^{ABb}	7.01±0.08 ^{Ab}
CT	5.29±0.17 ^{Ca}	6.02±0.33 ^{Ba}	6.36±0.26 ^{Bb}	6.55±0.50 ^{Bb}	7.37±0.08 ^{Aa}
Meat	5.55±0.45 ^a	-	-	-	-

PSC: Paliurus spina-christi Mill. fruit, NS: Nigella sativa L. seed, CT: Celtis tournefortii L. Fruit, A-C: Values with different superscripts in the same row are statistically different (P<0.05). a-b: Values with different superscripts in the same column are statistically different (P<0.05).

Table 4. The number of psychrotrophic bacteria detected in meatballs (\log_{10} cfu/g \pm standard deviation).

Group	Storage time (Days)				
	0	4	8	12	16
Control	4.75 \pm 0.09 ^{Da}	6.38 \pm 0.32 ^{Ca}	7.15 \pm 0.38 ^{Ba}	7.55 \pm 0.17 ^{Ba}	8.24 \pm 0.45 ^{Aa}
PSC	4.62 \pm 0.25 ^{Ea}	6.15 \pm 0.09 ^{Dab}	6.45 \pm 0.21 ^{Cb}	7.21 \pm 0.07 ^{Bb}	7.64 \pm 0.02 ^{Ab}
NS	4.83 \pm 0.06 ^{Da}	6.32 \pm 0.27 ^{Cb}	6.49 \pm 0.12 ^{Cb}	7.27 \pm 0.08 ^{Bb}	7.66 \pm 0.04 ^{Ab}
CT	4.73 \pm 0.21 ^{Da}	5.77 \pm 0.16 ^{Db}	6.19 \pm 0.09 ^{Cb}	7.29 \pm 0.04 ^{Bb}	7.63 \pm 0.07 ^{Ab}
Meat	4.74 \pm 0.18 ^a	-	-	-	-

PSC: *Paliurus spina-christi* Mill. fruit, NS: *Nigella sativa* L. seed, CT: *Celtis tournefortii* L. Fruit, A-E: Values with different superscripts in the same row are statistically different ($P < 0.05$). a-b: Values with different superscripts in the same column are statistically different ($P < 0.05$).

Table 5. Yeast-mold number detected in meatballs (\log_{10} cfu/g \pm standard deviation).

Group	Storage time (Days)				
	0	4	8	12	16
Control	3.87 \pm 0.47 ^{Ca}	4.14 \pm 0.06 ^{Ca}	4.36 \pm 0.30 ^{Ca}	4.98 \pm 0.09 ^{Ba}	6.27 \pm 0.40 ^{Aa}
PSC	3.78 \pm 0.20 ^{Ba}	4.14 \pm 0.15 ^{Ba}	4.16 \pm 0.28 ^{Ba}	4.37 \pm 0.12 ^{Bb}	5.71 \pm 0.55 ^{Aa}
NS	3.40 \pm 0.36 ^{Cb}	3.12 \pm 0.45 ^{Cb}	3.95 \pm 0.10 ^{BCa}	4.23 \pm 0.25 ^{Bb}	5.69 \pm 0.36 ^{Aa}
CT	3.62 \pm 0.31 ^{Ca}	3.58 \pm 0.43 ^{Cab}	4.12 \pm 0.24 ^{BCa}	4.57 \pm 0.26 ^{Bab}	5.88 \pm 0.15 ^{Aa}
Meat	2.48 \pm 0.57 ^a	-	-	-	-

PSC: *Paliurus spina-christi* Mill. fruit, NS: *Nigella sativa* L. seed, CT: *Celtis tournefortii* L. Fruit, A-C: Values with different superscripts in the same row are statistically different ($P < 0.05$). a-b: Values with different superscripts in the same column are statistically different ($P < 0.05$).

Table 6. Number of lactic acid bacteria detected in meatballs (\log_{10} cfu/g \pm standard deviation).

Group	Storage time (Days)				
	0	4	8	12	16
Control	5.32 \pm 0.27 ^{Da}	6.59 \pm 0.16 ^{Ca}	7.07 \pm 0.29 ^{Ba}	7.62 \pm 0.38 ^{Aa}	8.05 \pm 0.07 ^{Aa}
PSC	5.10 \pm 0.23 ^{Da}	5.96 \pm 0.14 ^{Ca}	6.54 \pm 0.32 ^{Ba}	7.42 \pm 0.28 ^{Aa}	7.63 \pm 0.18 ^{Ab}
NS	5.04 \pm 0.41 ^{Ca}	5.28 \pm 0.19 ^{Cc}	5.89 \pm 0.11 ^{Bb}	7.06 \pm 0.40 ^{Aa}	7.59 \pm 0.30 ^{Ab}
CT	5.28 \pm 0.30 ^{Da}	5.74 \pm 0.23 ^{Cb}	6.76 \pm 0.35 ^{Ba}	7.29 \pm 0.10 ^{Aa}	7.62 \pm 0.21 ^{Ab}
Meat	5.42 \pm 0.12 ^a	-	-	-	-

PSC: *Paliurus spina-christi* Mill. fruit, NS: *Nigella sativa* L. seed, CT: *Celtis tournefortii* L. Fruit, A-D: Values with different superscripts in the same row are statistically different ($P < 0.05$). a-c: Values with different superscripts in the same column are statistically different ($P < 0.05$).

Discussion

The pH values of meatball samples were determined between 5.89-6.02 on the 0th day and between 6.10-6.49 on the 16 days. It was determined that there was a decrease in pH values on 4th day in all groups during storage. The decrease in pH on the 4th day is likely due to the low pH value of the components used in the meatball dough. After the 4th day, a continuous increase in pH values was observed. This increase is thought to be due to the high buffering capacity of proteins and the proteolytic effects of microorganisms (Çağlar et al., 2018; İncili et al., 2021). The lowest pH value on the 16th day was detected in the CT and NS groups. The low pH value in these groups is thought to be due to less microbial growth due to the phenolic compounds they contain (Can and Şahin, 2019; Çağlar et al., 2018).

The a_w values of the meatball samples were 0.956-0.964 on day 0 and between 0.971-0.980 on day 16 of storage. It was determined that there was a decrease in a_w values on the 4th day in all groups during storage. The decrease in water activity on the 4th day is thought to be due to the salt and other ingredients added to the meatballs. It has been reported that salt reduces a_w in foods (Yıldırım, 2018). The effects of different plants and extracts used in meatball production on the a_w value have been studied by many researchers, and it has been reported that there are fluctuations in a_w values during storage and that there is no stable a_w value (Kahraman, 2021).

TMAB count in meatball samples was determined to be 5.14-5.53 \log_{10} cfu/g on day 0 and 7.01-7.91 \log_{10} cfu/g on day 16 of storage. TMAB was detected above the reported number on the 12th day of storage in the control group

meatballs and the 16th day in the PSC, CT, and NS groups. Therefore, it was determined that the plants used effectively affected the number of TMAB in meatballs. Although there was no difference between the other groups and control in the first days of storage, there was a significant difference on the 8, 12, and 16th storage days ($P<0.05$). It has been reported that the effect of the plants on the TMAB numbers is due to the phenolic compounds they contain because phenolic compounds have antimicrobial effects (Nikmaram et al., 2018). The lowest TMAB count was observed in the NS group (7.01 log₁₀) on day 16 of storage. Many researchers have studied the antimicrobial effects of plants and plant extracts in meat and products, and it has been reported that they reduce TMAB numbers at different rates (Çağlar et al., 2018; İncili et al., 2021; Jahan et al., 2018).

The number of psychrotrophic bacteria in the meatball samples was determined between 4.62-4.83 log₁₀ cfu/g on day 0 and 7.64-8.24 log₁₀ cfu/g on the last day of storage. It was determined that there was a continuous increase in the number of psychrotrophic bacteria in all groups in parallel with the storage period, and these increases made a statistically significant difference ($P<0.05$). It has been reported that psychrotrophic bacteria increase during meat preservation and causes the meat to spoil (Doulgeraki et al., 2012). There was a statistically significant difference between the groups on the other days except day 0 during the storage period of the meatballs ($P<0.05$). The CT group detected the lowest psychrotrophic bacteria count on the 4th and 8th days. On the 16th day of the storage, there was a 0.6 log₁₀ difference in the number of psychrotrophic bacteria between the control and the other groups. The lowest number of psychrotrophic bacteria was found in the CT group (7.63 log₁₀). Indeed, it has been reported that the antimicrobial activity of CT fruit is high (Keser et al., 2019). The plants' effect on the number of psychrotrophic bacteria is due to the phenolic compounds they contain. Because phenolic compounds have an antimicrobial effect (Efenberger-Szmechtyk, 2020) the antimicrobial effects of plants and plant extracts in meat and products have been examined by many researchers, and it has been reported that they reduce the number of psychrotrophic bacteria at different rates (Can and Şahin 2019; Çağlar et al., 2018; İncili et al., 2021).

LAB counts of meatball samples were determined between 5.04-5.32 log₁₀ cfu/g on day 0 and 7.59-8.05 log₁₀ cfu/g on day 16 of storage. A continuous increase in LAB numbers was observed in all groups in parallel with the storage period, and it was determined that these increases made a statistically significant difference ($P<0.05$). A statistically significant difference ($P<0.05$) was detected between the groups during storage on the 4th, 8th, and 16th days. On the 4th day, there was a difference in LAB counts between the control group and PSC, NS, and CT groups, 0.6, 1.3, and 0.9 log₁₀, respectively, and the lowest LAB count was found in the NS group (5.28 log₁₀). On the 16th day, there was a 0.5 log₁₀ difference between the control and the other groups, and the lowest LAB count was observed in the NS (7.59 log₁₀) group. The lowest LAB count was detected in the NS group on all storage days. It is thought that the effect

of the NS-containing group on the LAB number is because the phenolic compounds it contains suppress the LAB development more (Çağlar et al., 2018; Efenberger-Szmechtyk, 2020). Indeed, it has been reported that the antimicrobial activity of NS seed is high (Singh et al., 2014).

Yeast-mold count of meatball samples was determined 1.3.40-3.87 log₁₀ cfu/g on day 0 and 5.69-6.27 log₁₀ cfu/g on day 16 of storage. On the 4th day, yeast-mold counts were increased in the control and PSC groups, while a decrease in yeast-mold counts was observed in the NS and CT groups. It was determined that decreases and increases in yeast-mold numbers were observed in all groups during storage. This situation created a statistically significant difference ($P<0.05$) in yeast-mold numbers within the group days. Similar to our findings, İncili et al. (2020) reported that the fluctuation in the number of yeast molds during the storage period in marinated poultry meat (drumstick, wing, breast meat) was due to the yeast-molds contained in the components they used in marinating. During storage, the lowest yeast-mold count was detected in the NS groups. This may be due to the antimicrobial effect of phenolic and volatile compounds contained in the NS seed on molds and yeasts (Ahmed and Albi, 2019; Çağlar et al., 2018). Yeast and mold counts in the PSC and CT groups showed similar results with the control group. The high yeast-mold counts of the groups containing PSC and CT fruits may be because the PSC and CT fruits used contain more yeast-mildew. Many researchers have studied the antimicrobial effects of plants and plant extracts in meat and products, and it has been reported that they reduce yeast-mold numbers at different rates (Can and Şahin, 2019; Daoutidou et al., 2021; El-Adawy et al., 2021).

Studies on the effects of PSC and CT fruits on the microbiological quality of foods were not found in the literature review. However, it has been reported that the antimicrobial properties of PSC and CT fruits are high (Arslan and Kaya, 2021; Ceylan et al., 2020; Keser et al., 2019). Similar to our findings, it has been reported by many researchers (Chauhan et al., 2018; Liao et al., 2021) that using NS seed or extract in meat and meat products has a positive effect on microbiological quality and extends the shelf life of the products.

In conclusion, it has been determined that PSC, CT fruits, and NS seeds have a bacteriostatic effect on the meatballs and prolong the shelf life. To extend the shelf life of meatballs, it can be recommended to use PSC, CT fruits, and NS seeds in their composition. This study's results can provide beneficial information for anyone dealing with food and further studies investigating the shelf life of meat and meat products.

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(Carboxymethyl) Lysine Formation, Sensory, Microbial Quality in Meatballs".

Conflict of Interest

The authors stated that they did not have any real, potential or perceived conflict of interest.

Ethical Approval

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

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References

Ahmed A, Albi A, 2019: Effect of some natural plants oils on the growth of some pathogenic bacteria. *Sci J Appl Sci Sabratha Univ*, 2 (3),1-19.

AOAC, 1990: Official Methods of Analysis (15th ed.). Association of Analytical Chemists. Arlington, VA.

AOAC, 1998: Crude protein in meat: block digestion method, 981.10. Official methods of analysis.

AOAC, 2000: Official Methods of Analysis 991.36. (17th ed.). Association of Analytical Chemists. Arlington, VA.

AOAC, 2002a: Determination of moisture content 950.46. Official methods of analysis.

AOACb, 2002b: Ash of Meat 920.153. Official methods of analysis.

Arslan S, 2021: Hayıt tohumu (*Vitex agnus-castus* L.) tozunun sığır köftelerinin çeşitli kalite özellikleri ve raf ömrü üzerine etkisinin araştırılması. Yüksek Lisans Tezi, NEVÜ, Fen Bilimleri Enstitüsü, Nevşehir.

Arslan L, Kaya E, 2021: Investigation of Antimicrobial and Antioxidant Activities of *Paliurus spina-christi* Mill. in Kahramanmaraş, Turkey. *KSU J Agric Nat*, 24 (6), 1161-116.

Can ÖP, Şahin S, 2019: Effect of rosemary essential oil coated vacuum packaging on the quality of chicken meatballs at +4°

C. *TURJAF*, 7 (12), 2165-2169.

Casaburi A, Piombino P, Nychas GJ, Villani F, Ercolini D, 2015: Bacterial populations and the volatilome associated to meat spoilage. *Food microbiol*, 45, 83-102.

Ceylan Ş, Harsit B, Saral O, Özcan, M, Demir İ, 2020: Comparison of antioxidant and antimicrobial activities of plant extracts Centaury (*Hypericum montbretii* and *Hypericum bupleuroides*) and Blackthorn (*Paliurus spina-christi* Mill.) in Artvin, Giresun region of Turkey. *ACU Orman Fak Derg*, 21 (1), 13-20.

Chauhan P, Das AK, Nanda, PK, Kumbhar V, Yadav JP, 2018: Effect of *Nigella sativa* seed extract on lipid and protein oxidation in raw ground pork during refrigerated storage. *Nutr Food Sci*, 48 (1), 2-15.

Çağlar MY, Veli G, Tomar O, Akarca G, 2018: Determination of the effect of different ground mustard seeds on quality characteristics of meatballs. *Korean J Food Sci Anim Resour Korean J Food Sci An*, 38 (3), 530.

Daoutidou M, Plessas S, Alexopoulos A, Mantzourani I, 2021: Assessment of antimicrobial activity of pomegranate, cranberry, and black chokeberry extracts against foodborne pathogens. *Foods*, 10 (3), 486.

Doulgeraki AI, Ercolini D, Villani F, Nychas GJE, 2012: Spoilage microbiota associated to the storage of raw meat in different conditions. *Int J Food Microbiol*, 157 (2), 130-141.

Efenberger-Szmechtyk M, Nowak A, Czyzowska A, 2021: Plant extracts rich in polyphenols: Antibacterial agents and natural preservatives for meat and meat products. *Crit Rev Food Sci Nut*, 61 (1), 149-178.

El-Adawy TA, El-Beltagy AE, Atef AA, 2021: Antimicrobial and antioxidant efficacy of some natural plants extracts. *Menoufia J Food and Dairy Sci*, 6 (3), 33-34.

Ercolini D, Ferrocino I, Nasi A, Ndagijimana M, Vernocchi P, La Storia A, Laghi L, Mauriello G, Guerzon ME, Villani F, 2011: Monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions. *Appl Environ Microbiol*, 77 (20), 7372-7381.

Gecibesler IH, 2019: Antioxidant activity and phenolic profile of Turkish *Celtis tournefortii*. *Chem Nat Compd*, 55 (4), 738-742.

Halid SA, Rahim A, 2018: Physicochemical and microbiological characteristics of beef meatballs in Palu City. *AGROLAND Agric Sci J (e-Journal)*, 5 (2), 104-111.

ISO 15214, 1998: Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of mesophilic lactic acid bacteria-colony-count technique at 30 °C.

ISO 21527-1, 2008: Microbiology of food and animal feeding stuffs: horizontal method for the enumeration of yeasts and moulds.

İncili GK, Akgöl M, Aydemir ME, Alan S, Mutlu M, İlhak Oİ, Öksüztepe G, 2020: Fate of *Listeria monocytogenes* and *Salmonella* Typhimurium in homemade marinade and on marinated chicken drumsticks, wings and breast meat. *LWT*, 134, 110231.

İncili GK, Aydemir ME, Akgöl M, Kaya B, Kanmaz H, Öksüztepe G, Hayaloğlu AA, 2021: Effect of *Rheum ribes* L. juice on the survival of *Listeria monocytogenes*, *Escherichia coli* O157: H7 and *Salmonella* Typhimurium and chemical quality on vacuum packaged raw beef. *LWT*, 150, 112016.

Jahan I, Haque MA, Hashem MA, Rima FJ, Akhter S, Hossain MA, 2018: Formulation of valueadded beef meatballs with pomegranate (*Punica granatum*) extract as a source of natural antioxidant. *Int J Meat Sci*, 6 (1), 12-18.

Kahraman E, 2021: Börülce (*Vigna unguiculata*) ununun Kırklareli köftesi üretiminde kullanımı olanakları. Yüksek Lisans Tezi, NKÜ Fen Bil Enst, Tekirdağ.

Keser S, Keser F, Kaygılı O, Tekin S, Turkoglu I, Demir E, Turkoglu S, Karatepe M, Sandal S, Kirbag S, 2017: Phytochemical

- compounds and biological activities of *Celtis tournefortii* fruits. *Ana. Chem Lett*, 7 (3), 344-355.
- Kirca A, Arslan E, 2008: Antioxidant capacity and total phenolic content of selected plants from Turkey. *Int J Food Sci*, 43 (11), 2038-2046.
- Lang KW, Steinberg MP, 1980: Calculation of moisture content of a formulated food system to any given water activity. *J Food Sci*, 45 (5), 1228-1230.
- Leistner L, 2000: Basic aspects of food preservation by hurdle technology. *Int J Food Microbiol*, 55 (1-3), 181-186.
- Liao W, Badri W, Alhibshi AH, Dumas E, Ghnimi S, Gharsallaoui A, Errachid A, Elaissari A, 2021: Food Applications Of *Nigella Sativa* Essential Oil. In *Black Cumin (Nigella sativa) seeds: Chemistry, Technology, Functionality, and Applications*, Ramazan MF (ed), 433-455, Springer.
- Mahros MM, Abd-Elghany SM, Sayed-Ahmed MZ, Alqahtani SS, Sallam KI, 2021: Improving the microbiological quality, health benefits, and storage time of cold-stored ground mutton supplemented with black seed. *LWT*, 138, 110673.
- Mansur AR, Song EJ, Cho YS, Nam YD, Choi YS, Kim DO, Seo YS, Nam TG, 2019: Comparative evaluation of spoilage-related bacterial diversity and metabolite profiles in chilled beef stored under air and vacuum packaging. *Food Microbiol*, 77, 166-172.
- Nikmaram N, Budaraju S, Barba FJ, Lorenzo JM, Cox RB, Mallikarjunan K, Roohinejad S. 2018: Application of plant extracts to improve the shelf-life, nutritional and health-related properties of ready-to-eat meat products. *Meat Sci*, 145, 245-255.
- Prommachart R, Belem TS, Uriyapongson S, Rayas-Duarte P, Uriyapongson J, Ramanathan R. 2020: The effect of black rice water extract on surface color, lipid oxidation, microbial growth, and antioxidant activity of beef patties during chilled storage. *Meat Sci*, 164, 108091.
- Purba R, Suseno SH, Izaki AF, Muttaqin S, 2014: Application of liquid smoke and chitosan as natural preservatives for tofu and meatballs. *Int J Appl Sci* 4 (2), 2012-2017.
- Singh S, Das SS, Singh G, Schuff C, de Lampasona MP, Catalan CA, 2014: Composition, in vitro antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black cumin seeds (*Nigella sativa* L.). *Biomed Res Int*, 2014, 918209.
- SPSS 2017: Windows user's guide, version 24.0, SPSS Inc, Michigan Ave, Illinois.
- Şen A 2018: Antioxidant and anti-inflammatory activity of fruit, leaf and branch extracts of *Paliurus spina-christi* P. Mill. *Marmara Pharm JI*, 22 (2), 328-333.
- Takım K, Işık M, 2020: Phytochemical Analysis of *Paliurus spina-christi* Fruit and Its Effects on Oxidative Stress and Antioxidant Enzymes in Streptozotocin-Induced Diabetic Rats. *Appl Biochem Biotechnol*, 191 (4), 1353-1368.
- Takım K, 2021: Bioactive component analysis and investigation of antidiabetic effect of Jerusalem thorn (*Paliurus spina-christi*) fruits in diabetic rats induced by streptozotocin. *J Ethnoph*, 264,113263.
- USDA/FSIS 2011: Microbiology laboratory guidebook. Metot 3.01, Quantitative analysis of bacteria in foods as sanitary indicators.
- Yıldırım A, Duran M, Koç M, 2018: Su aktivitesinin ve farklı kurutma sistemlerinin biyoaktif bileşenlerin stabiliteleri üzerine etkisi. *Gıda*, 43 (3), 512-522.
- Yıldırım I, Uğur Y, Kutlu T, 2017: Investigation of antioxidant activity and phytochemical compositions of *Celtis tournefortii*. *Free Radic Antioxid*, 7 (2), 160-165.
- Yu L, Chai M, Zeng M, He Z, Chen J, 2018: Effect of lipid oxidation on the formation of Nε-carboxymethyl-lysine and Nε-carboxyethyl-lysine in Chinese-style sausage during storage. *Food Chem*, 269, 466-472.
- Zor M, Aydın S, Güner ND, Başaran N, Başaran AA, 2017: Antigenotoxic properties of *Paliurus spina-christi* Mill fruits and their active compounds. *BMC Complement Altern Med*, 17 (1), 1-10.
- Zwolan, A, Pietrzak D, Adamczak L, Chmiel M, Kalisz S, Wirkowska-Wojdyła M, Florowski T, Oszmiański J, 2020: Effects of *Nigella sativa* L. seed extracts on lipid oxidation and color of chicken meatballs during refrigerated storage. *LWT*, 130, 109718.