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Research Article

Effect of bacterial nanocellulose film containing sodium nitrite and water extracts of sumac and black carrot on the shelf life of ground beef

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Abstract

This study aimed to investigate the effects of active bacterial nanocellulose (BNC) films immersed in sodium nitrite (SN; 30, 60, and 120 ppm), sumac extract (SE; 10% w/v), and black carrot extract (BCE; 5% w/v) solutions on the microbial and chemical properties of ground beef. The addition of SN, SE and BCE to BNC films strengthened the matrix network and improved the mechanical properties of the films. The SN_{120} treatment (BNC film immersed in 120 ppm SN solution) effectively improved the redness of the samples. The results also showed that the ground beef samples covered with BNC film immersed in $SE_{10}BCE_5SN_{30}$ had the lowest microbial load (more than 3.5 $\log_{10}$ cycle reduction compared to the control) and the lowest oxidation rate (60% reduction compared to control). Consequently, considering the health concerns regarding nitrosamine compounds, the use of natural compounds such as SE and BCE in BNC films can reduce the amount of SN required in meat products.

Keywords: Beef, Black carrot, Edible film, Nanocellulose, Sodium nitrite, Sumac.

Introduction

Meat can be considered the most complete food source in human nutrition. Meat plays a fundamental role in the diet and optimal growth of the body due to its essential amino acids, fatty acids such as linoleic acid, linolenic acid, and arachidonic acid, minerals (iron, zinc, phosphates, and sulfates), vitamins (especially B vitamins), and carbohydrates (glycogen) (Fernandez-Lopez et al., 2005). On the other hand, due to the presence of these diverse food compounds in meat, it is one of the perishable foods and is a suitable environment for the growth of foodborne bacterial pathogens, and this causes significant health and economic damage (Papadopoulou et al., 2012).

Sodium nitrite (SN) is used in the meat industry to inhibit the growth of pathogens, especially *Clostridium botulinum* and its toxin, provide a bright red color to meat, prevent lipid oxidation, and create a special aroma and flavor in minced meat (Shakil et al., 2022). However, nitrite also presents several health-related drawbacks, including potential risks such as cancer and methemoglobinemia, particularly in infants. Therefore, the use of a technique such as active packaging that can maintain the safety and

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quality of meat and reduce the use of chemical additives is the desire of most consumers (Shakil et al., 2022).

For this reason, researchers have recognized the use of plant extracts as natural compounds that can perform some functions of sodium nitrite in the meat industry as a suitable option to reduce or replace sodium nitrite. Among these extracts, the water extract of sumac (*Rhus coriaria L.*), belonging to the family *Anacardiacae,* is rich in tannins, phenolic acids, anthocyanins, gallic acid derivatives, flavonoid glycosides, and organic acids, and has high antioxidant and antimicrobial properties (Aliakbarlu and Mohammadi, 2015). Black carrot (*Daucus carota* L.) water extract is also used as a red dye in the industry due to its variety of anthocyanins, and its polyphenolic compounds act as antioxidants (Kammerer et al., 2004). Recent studies have shown that the use of plant extracts in meat products can effectively reduce microbial loads and residual nitrite levels. For instance, extracts such as *Allium Jesdianum* in nitrite-free sausages significantly lowered total microbial counts, while combining moderate levels of nitrite with such extracts enhanced psychrotrophic control (Ghorbani et al., 2024). Other studies have indicated that vinegar and lemon extract powders, rich in organic acids, contribute to lowering residual nitrite levels in sausages during storage owing to their acidic pH and nitrite-reducing mechanisms (Bae et al., 2021). Additionally, natural lipid-based nanoformulations have demonstrated the ability to partially replace nitrites in cooked beef sausages while maintaining microbial safety and oxidative stability during storage (Khatib et al., 2020). In emulsion-type sausages, plant extracts such as *Nelumbo nucifera* effectively reduced TBARS values and lipid oxidation, extending shelf life compared to synthetic preservative-treated controls (Lee et al., 2021). Combining plant extracts with lower levels of sodium nitrite has shown promising synergistic antimicrobial effects, which can help reduce nitrite usage while preserving product safety and sensory quality (Wójciak et al., 2023).

Bacterial nanocellulose (BNC) films, which possess properties such as high water retention capacity, compatibility, high purity, and uniform morphology, can be used in the meat packaging industry as an active edible film that carries natural plant antimicrobial and antioxidant compounds, such as black carrot and sumac water extracts, in its matrix (Shafiei et al., 2024). A notable advantage of such packaging is the sustained release of bioactive compounds from the film matrix into the meat, thereby extending the shelf life of ground meat products (Gil & Rudy, 2023).

This study aimed to develop an active packaging to preserve the redness and shelf life of ground beef. Therefore, the antibacterial and antioxidant properties of sodium nitrite and the water extracts of sumac and black carrot were evaluated. The BNC films were then immersed in SN, sumac extract (SE), and black carrot extract (BCE) solutions, and the mechanical and spectral properties of the prepared BNC films were examined. Finally, the effectiveness of the active BNC films on the chemical and microbiological properties of ground beef stored at refrigerator temperatures was investigated.

Materials and Methods

Materials

The bacterial strains used in this study were *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (ATCC 19115), *Escherichia coli* (ATCC 25922), *Salmonella* Typhimurium (ATCC 14028), and *Komagataeibacter xylinus subsp. Sucrofermentans* (BPR 2001). All strains were obtained from the culture collection of the Faculty of Veterinary Medicine at Urmia University (Urmia, Iran). Culture media, including nutrient broth, nutrient agar, peptone water, and plate count agar (PCA), were purchased from Quelab Laboratories (Montreal, Quebec, Canada) and used for microbiological analysis. Trichloroacetic acid, 2-thiobarbituric acid, and sulfanilamide were purchased from Merck (Darmstadt, Germany). SN and ethanol were obtained from Scharlau (Barcelona, Spain). All other reagents and analytical-grade chemicals were sourced from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of the plant extracts

The ripe and dried sumac fruit and fresh black carrot were obtained from a local market in Urmia city. The black carrot was first washed, chopped, and dried in

an oven at 40 °C. The dried samples were ground, and then 100 g of the ground material was mixed with 1000 mL of double-distilled water (ratio 1:10) and placed on a shaker for 24 h at room temperature. To remove excess particles solution was passed through Whatman No. 1 filter paper (Cytiva, Marlborough, MA, USA). It was then dried using a lyophilizer (Zist Farayand Tajhiz Sahand, Tabriz, Iran; freezing temperature: – 50 ˚C), the dried extract was stored in dark bottles at refrigerator temperature and re-dissolved in distilled water to the desired concentration before use.

Antioxidant activity (Reducing power)

In this method, the ability of SN, SE, and BCE to reduce iron III (ferric) to iron II (ferrous) was measured. Antioxidant compounds form a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, resulting in a color change from yellow to various shades of green and blue, depending on the reducing capacity of the tested samples. This complex has a maximum absorbance at 700 nm; therefore, the higher the concentration of ferrous ions, the bluer the solution, indicating increased absorbance and, consequently, higher antioxidant activity. In this test, 1 mL of different concentrations of SN, SE, and BCE (0.5, 1, 2, and 4 mg/mL) was mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). After incubation in a water bath at 50 °C for 20 min, 2.5 mL of trichloroacetic acid (10%) was added to the mixture. Then, 2.5 mL of the upper layer of the solution was combined with 2.5 ml of distilled water and 0.5 mL of ferric chloride (0.1%). After 10 min, the absorbance was measured at 700 nm against a blank. Butylated hydroxytoluene (BHT) was used as the positive control (Oyaizu, 1986).

Total Phenolics

Total phenolic contents of SN, SE and BCE were measured using the Folin-Ciocalteu colorimetric method. In this test, 0.5 mL of each sample solution (1 mg/mL) was mixed with 2.25 mL of distilled water, followed by the addition of 0.25 mL of Folin-Ciocalthio reagent. The mixture was shaken well and allowed to stand for 5 min before use. Then, 2 ml of 75 g/L sodium carbonate solution was added. After incubation at room temperature for 2 h, the absorbance of each sample was measured at 760 nm using a spectrophotometer (T80$^+$ UV/VIS, PG Instrument, Lutterworth, UK). Gallic acid was used as a standard to prepare the calibration curve, and the total phenolic content was expressed as milligrams of gallic acid equivalent per gram of sample (Singleton & Rossi, 1965).

Production of BNC film

An *ex-situ* method was applied to produce an antimicrobial BNC film. A previously reported method (Rodrigues et al., 2019) was used to produce BNC film. In this method, *K. xylinus subsp. sucrofermentans* was cultured under static conditions in two media [Hestrin–Schramm medium (HS) and Modified Hestrin–Schramm (MHS)] for 72 h at 30 °C. The synthesized BNC film was washed several times with distilled water and 0.1 M NaOH until it turned white (**Fig. 1**). The pH of the film was then adjusted to 7, and it was stored in a refrigerator until use.

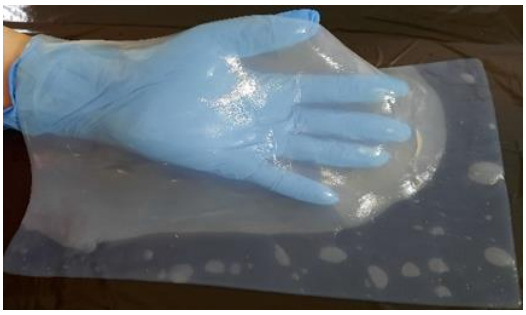

Figure 1. The synthesized bacterial nanocellulose (BNC).

Preparation and antibacterial activity of the active BNC films

The antibacterial activity of the films was evaluated using the disk diffusion method. For this purpose, a BNC film with a thickness of 3.2 mm was cut into disks (8 mm in diameter) using a punch and then autoclaved. The disks were drained between two glass plates (to achieve approximately 70% water removal, which was calculated based on the weight before and after drying) and immersed in different concentrations of SN and SE for 1 h. After immersion, the disks were dried in a sterile Petri dish next to a flame. Four bacteria strains (*L. monocytogenes*, *E. coli*, *S. aureus*, and *S.* Typhimurium) were prepared at a concentration of 6 $\log_{10}$ CFU /mL and spread on

nutrient agar plates. Active disks containing various concentrations of SN (12%, 6%, and 3% g/mL) and SE (9% g/mL) were placed on the agar surface using a sterile swab and incubated at 37°C for 24 h. Finally, the diameters of the inhibition zones were measured (Moradi et al., 2021). An antibiotic disk (Padtan Teb, Tehran, Iran) was used as a positive control.

FE- SEM analysis of films

To examine the surface morphology of the films, a field-emission scanning electron microscope (FE-SEM, TESCAN MIRA3, Czech Republic) was used. Films immersed in various concentrations of SN, SE and BCE were first completely dried. The samples were then gold-coated to prepare them for imaging, and FE-SEM micrographs were obtained at an accelerating voltage of 30 kV.

Food application of films

After removing the surface fat, beef thigh muscle was ground using a meat grinder under aseptic conditions. The ground meat was covered with the respective active BNC films in a sandwich form based on the treatments defined in **Table 1**. The samples were then stored in a refrigerator at 4 °C for 8 days. Microbiological and chemical analyses were performed at 4-day intervals.

Table 1. Different treatments of ground beef using bacterial nanocellulose film (BNC) containing sodium nitrite (SN), water extract of sumac (SE), water extract of black carrot (BCE) or without them.

Treatments	Compositions
C	Negative control (ground beef without nitrite)
CN	Positive control (ground beef containing 120 ppm sodium nitrite)
W	Ground beef covered with film BNC immersed in sterile distilled water (no additives)
SN_{120}	Ground beef covered with film BNC containing 120 ppm sodium nitrite
BCE_5	Ground beef covered with film BNC containing black carrot water extract (5% w/v)
BCE_5SN_{60}	Ground beef covered with film BNC containing black carrot water extract (5% w/v) + sodium nitrite (60 ppm)
SE_{10}	Ground beef covered with film BNC containing water sumac extract (10% w/v)
$SE_{10}SN_{60}$	Ground beef covered with film BNC containing sumac water extract (10% w/v) + sodium nitrite (60 ppm)
$SE_{10}BCE_5SN_{30}$	Ground beef covered with film BNC containing sumac water extract (10% w/v) + black carrot water extract (5% w/v) + sodium nitrite (30 ppm)

Microbiological analysis

Under sterile conditions, on days 0, 4, and 8, 10 g of each meat sample was transferred to individual stomacher bags containing 90 mL of sterile 0.1% peptone water and homogenized in a stomacher (Stomacher 400, Circulator, Seward Ltd., Worthing, UK) for 2 min. Serial decimal dilutions were prepared by transferring 1 mL of homogenate into 9 mL of peptone water. To determine total viable count (TVC), 1 mL of the appropriate dilution was poured onto sterile plates, followed by the addition of 20 mL of PCA at 40 - 45 °C. The plates were incubated at 37 °C for 24 h. For total psychrotrophic count (TPC), 0.1 mL of the appropriate dilution was spread on the surface of PCA medium, and the plates were incubated at 7 °C for 10 days (Bazargani-Gilani et al., 2015). Results were expressed as logarithmic values of colony-forming units (log_{10} CFU) per gram of sample.

Chemical analysis

pH measurement

Ten grams of the sample was homogenized (IKA, Staufen im Breisgau, Germany) with 90 mL of distilled water and mixed well for 2 min. The pH of the ground beef was measured using a digital pH meter (Zagchemi, Tehran, Iran) with a glass electrode, previously calibrated with standard phosphate buffers at pH 4 and 7 (Fernández-López et al., 2007).

Thiobarbituric acid reactive substances (TBARS)

Due to the red color of SE and BCE, lipid oxidation was evaluated using the distillation method. Ten grams of ground beef was mixed with 35 mL of trichloroacetic acid (TCA) (5% w/v), 1 mL of BHT (0.5% w/v), and 100 mL of distilled water in a distillation flask. Distillation was carried out until 50 mL of the distillate was collected. Then, 5 mL of the distillate was mixed with 5 mL of thiobarbituric acid (TBA) (0.02 M) solution and vortexed (Dragon, Beijing, China). The mixture was placed in a boiling water bath for 60 min until it turned pink. After cooling to room temperature, absorbance was measured at 532 nm against a blank (5 mL distilled water + 5 mL TBA) (Pikul et al., 1989).

Residual nitrite

The measurement of residual nitrite was based on the colorimetric reaction between nitrite in the sample and sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride (NED), which produces a pink-color azo compound. To assess nitrite migration from films to ground beef, 5 g of sample was mixed with 300 mL of distilled water at 80° C and placed in a water bath for 2 h, The samples were then cooled to room temperature and diluted to a final volume of 500 mL with distilled water. The solution was filtered using a nitrite-free filter paper. Next, 2.5 mL of sulfanilamide reagent was added to the filtrate, stirred, and allowed to stand for 5 min. Then, 2.5 mL of NED reagent was added, and the mixture was kept in the dark for 15 min to develop the maximum pink color. The absorbance was then measured at 540 nm using a spectrophotometer. The nitrite concentration in the samples was calculated using a standard curve and expressed in parts per million (ppm) (Horwitz, 1975).

Statistical Analysis

Statistical package for the social sciences (SPSS) version 26 was used for data analysis. Analysis of variance (ANOVA) was applied to determine differences among treatments. Tukey's test was used for post-hoc comparisons of the means. A significance level of $P < 0.05$ was considered statistically significant. All analyses were performed in triplicates.

Table 2. Growth inhibition zones (mm) produced by different concentrations of sodium nitrite (SN) and sumac extract (SE) incorporated into bacterial nanocellulose (BNC) film against food borne pathogens.

Compounds dose (%) in BNC	*Staphylococcus aureus*	*Listeria monocytogenes*	*Salmonella* Typhimurium	*Escherichia coli*
SN (3%)	NI	NI	NI	NI
SN (6%)	NI	NI	NI	NI
SN (12%)	10.73± 0.64^{f}	8.20± 0.34^{e}	7.06± 0.50^{e}	NI
SE (9%)	21.56± 0.51^{e}	19.43±1.02^{d}	16.10± 0.17^{d}	12.66±1.15^{d}
SE (9%) + SN (12%)	38.16±1.25^{a}	30.23±1.56^{b}	23.06± 0.40^{b}	20.23±1.06^{b}
SE (9%) + SN (6%)	31.5± 0.50^{b}	24.03± 0.55^{c}	19.16± 0.75^{c}	18.2± 0.26^{c}
SE (9%) + SN (3%)	26.66± 0.57^{c}	21.06± 0.11^{d}	18.33± 0.28^{c}	16.43± 0.75^{c}
Chloramphenicol (30µg/disc)	23.66 ± 0.57^{d}	36.00 ± 0.40^{a}	38.3 ± 0.81^{a}	34.26 ± 0.25^{a}

Different small letters indicate statistical differences among treatments ($P < 0.05$). NI: No inhibition.

Results and Discussion

Antibacterial activity

The results of the antibacterial activity of SE and SN against two Gram-positive and two Gram-negative bacteria using the disk diffusion method are presented in **Table 2**. In this study, BCE did not show any antimicrobial properties. As the concentrations of SE and SN increased, their antimicrobial activities also increased. The largest inhibition zone was observed at 9% SE and 12% SN. *S. aureus* showed the greatest sensitivity, with an inhibition zone of 38 mm, likely due to its Gram-positive cell wall structure. On the other hand, *E. coli* exhibited the highest resistance. SE contains various antimicrobial compounds such as tannins, flavonoids, and phenolic acids. These compounds,

particularly tannins, are well-known for their strong antimicrobial effects (Aliakbarlu et al., 2014; Aliakbarlu and Mohammadi, 2015).

Antioxidant activity

The results of reducing power assay are presented in **Table 3.** SN, SE, and BCE at a concentration of 4 mg/mL exhibited high antioxidant activity, comparable to that of BHT (1 mg/mL). Reducing power is pH-dependent and this assay is mostly used for evaluating compounds active at acidic to neutral pH levels (pH 1-8). Fruits and vegetables with intense coloration often show strong antioxidant potential due to their high phenolic content (Cömert et al., 2020).

Table 3. Reducing power of sumac extract (SE), black carrot extract (BCE), and sodium nitrite (SN).

Sample	Concentration (mg/mL)			
	0.5	1	2	4
SE	0.328 ± 0.01^{a}	0.803 ± 0.01^{b}	1.400 ± 0.05^{a}	2.357 ± 0. 00^{a}
BCE	0.257 ± 0.01^{b}	0.442 ± 0.04^{c}	0.769 ± 0.00^{b}	1.146 ± .0.00^{b}
SN	0.039 ± 0.01^{c}	0.041 ± 0.00^{d}	0.296 ± 0.00^{c}	0.396 ± 0.00^{c}
BHT	ND	2.22 ± 0.00^{a}	ND	ND

Different small letters indicate statistical differences among treatments ($P < 0.05$). BHT: Butylated Hydroxytoluene; ND: Not determined.

A gallic acid calibration curve (y= 5.657+ 0.045, R^2= 0.999) was used (**Fig. 2**), and the total phenolic content of SE and BCE was determined to be 42.06 ± 0.35 and 26.57 ± 0.44 mg GAE/g, respectively. A strong correlation was found between phenolic content and reducing power of the extracts. These results indicate that SE, owing to its high phenolic content, could serve as a beneficial antioxidant for human health. Previous studies have also confirmed the antioxidant properties of SE (Aliakbarlu et al., 2014).

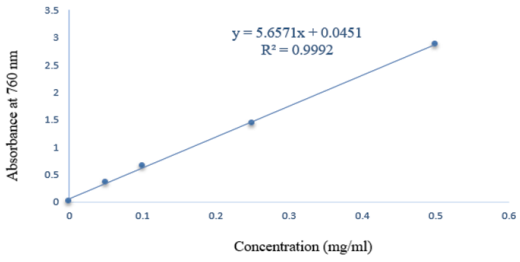

Figure 2. Gallic acid standard curve.

SEM analysis of the films

Figure 3 shows the surface morphologies of the samples, where structural features such as cracks, pores, and surface roughness can be identified owing to the incorporation of additives. The pure BNC film (A) showed scattered pores of various sizes within its matrix, while the addition of sodium nitrite (B) reduced pore size. The inclusion of sumac and black carrot extracts further decreased the size of the pores (C and E), and in the SE_{10} + BCE_5 + SN_{30} treatment (G), the pores were almost eliminated, resulting in a denser morphology. Structurally, the films exhibited a smooth surface, although the addition of SE and BCE introduced small agglomerates, likely due to the presence of insoluble particles in the water extract powder used. Overall, the porous structure of BNC makes it a suitable carrier for both natural (SE and BCE) and synthetic (SN) antimicrobial and antioxidant agents. As shown in **Figure 3** (at 2 µm magnification), these compounds were successfully embedded within the BNC matrix, contributing to the formation of a more stable film.

Microbiological changes in ground beef

The effects of BNC films loaded with SN, SE, and BCE on the microbiological quality of ground beef during refrigerated storage are shown in **Figure 4**. At the beginning of the storage, the TVC in all samples

ranged from 5.26- 5.66 log_{10} CFU/g. During storage, a gradual increase in bacterial count was observed. By day 8, the TVC of the control (C) and W samples reached 8.55 and 8.61 log_{10} CFU/g, respectively. In contrast, treatments containing SE significantly reduced bacterial counts. At the end of the storage period, TVC in SE10, SE10SN60, and SE10BCE5SN30 treatments dropped to 3.86, 3.73 and 3.37 log_{10} CFU/g, respectively ($P < 0.05$). These reductions are likely due to the antimicrobial effect of SE, especially when combined with other compounds (SE + BCE + SN treatment). The acidic pH of SE may destabilize bacterial cell membranes, leading to cell death. A similar trend was observed for psychrotrophic bacterial counts, where treatments containing SN, SE, and BCE showed significantly lower counts than C and W ($P < 0.05$). Overall, $SE_{10}SN_{60}$ and $SE_{10}BCE_5SN_{30}$ exhibited stronger antimicrobial effects, effectively delaying microbial growth ($P < 0.05$). The synergistic action of sodium nitrite and sumac extract helped reduce microbial load throughout storage, which agrees with findings from previous studies (Langroodi et al., 2018b; Shiravani et al., 2024). These plant-based extracts also helped minimize the need for nitrites while preserving microbial stability.

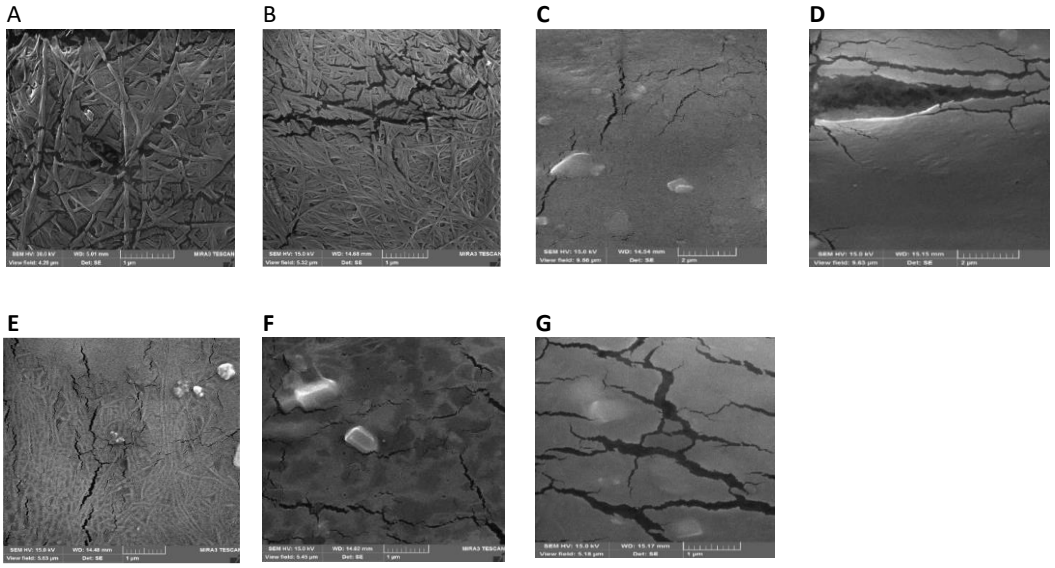

Figure 3. Morphology of FE- SEM images showing cross-section surface microstructures, A= Pure BNC or W, B= SN_{120}, C= BCE_5, D= BCE_5SN_{60}, E= SE_{10}, F= $SE_{10}SN_{60}$, G= $SE_{10}BCE_5SN_{30}$.

pH measurement

In this study, pH values ranged from 4.72 to 8.09 during the storage period. The highest pH values were observed in the W and C treatments, likely due to ammonia and other basic compounds produced by microbial metabolism. Conversely, the SE_{10}-treated samples maintained the lowest pH throughout the storage period ($P < 0.05$) (**Fig. 5**). In general, the addition of SE lowered the pH due to the acidic nature of sumac extract. This acidic environment may play a critical role in inhibiting microbial growth. Previous studies have shown that acidic plant extracts not only reduce pH directly but also prevent pH increase by inhibiting the growth of biogenic amines-producing bacteria (e.g., proteolytic strains) (Langroodi et al., 2018a). Sodium nitrite does not directly lower pH, but it can indirectly limit pH increase by suppressing alkalinizing bacteria such as *Pseudomonas* or *Enterobacteriaceae* (Wójciak et al., 2019).

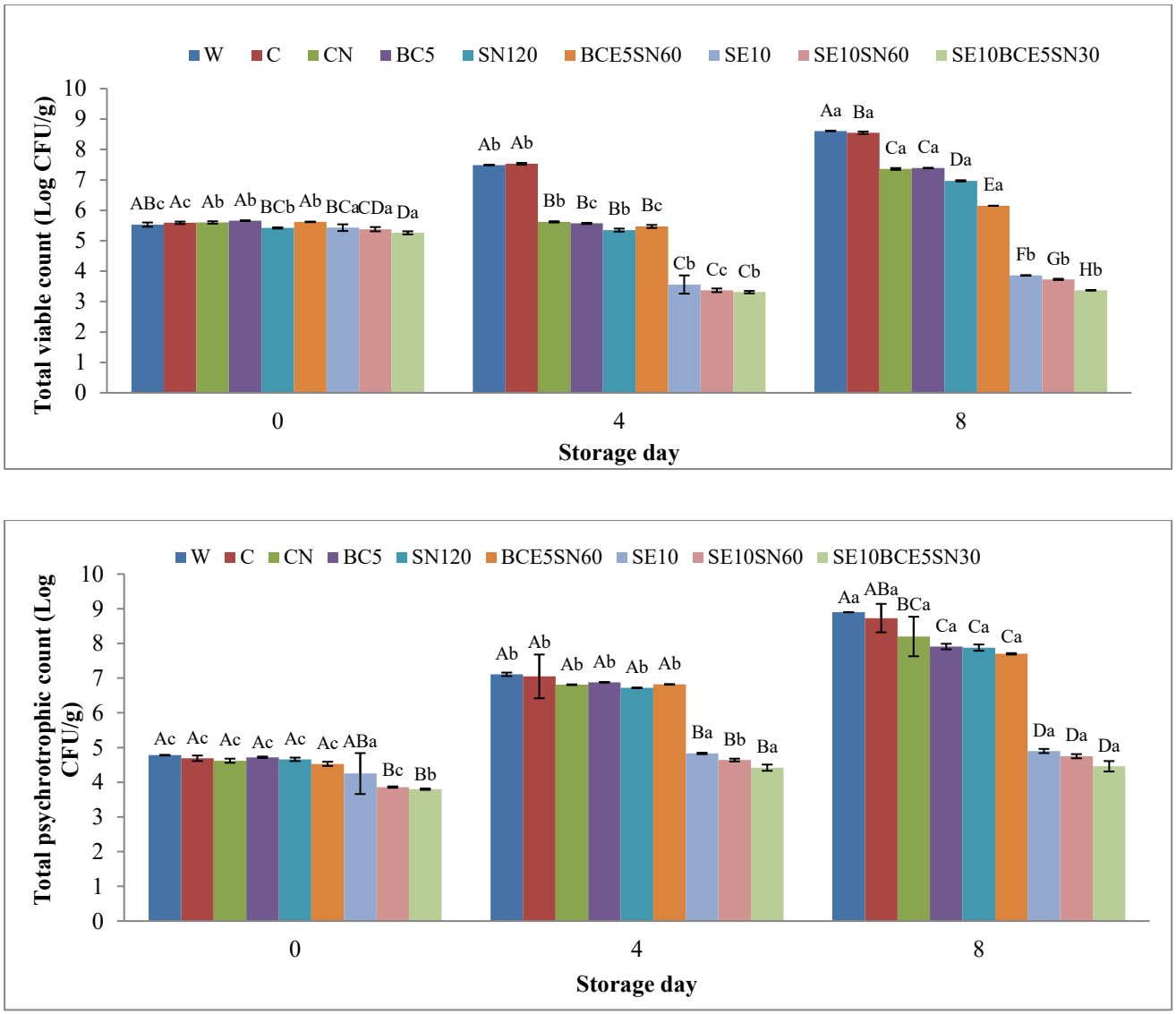

Figure 4. Microbial changes (Log_{10} CFU/g) [TVC (above) and TPC (below) graphs] in ground beef with active BNC films containing different concentrations of SN, SE and BCE during 8 days of storage at 4 °C. Different lowercase and uppercase letters indicate statistically significant differences ($P < 0.05$) among the times and treatments, respectively.

Thiobarbituric acid reactive substances (TBARS)

Lipid oxidation is one of the major factors affecting nutritional value, color, and flavor in minced meat products (Kim TaeKyung et al., 2017). TBARS measures secondary lipid oxidation products, such as MDA, which are directly related to shelf life (Cheng et al., 2023). At the start of the storage, significant difference ($P < 0.05$) in TBARS values were observed among treatments (**Fig. 6**). During storage, the W sample showed the highest TBARS value (1.07 mg MDA/kg), while the $SE_{10}BCE_5SN_{30}$ treatment had the lowest (0.27 mg MDA/kg). The combination of SE with SN and BCE significantly delayed oxidative reactions ($P < 0.05$). This suggests SE could potentially serve as a nitrite substitute. Despite some increase in TBARS over time, treatments containing SN, SE and BCE effectively reduced lipid oxidation and preserved product quality, consistent with prior research (Aliakbarlu & Mohammadi, 2015; Shiravani et al., 2024).

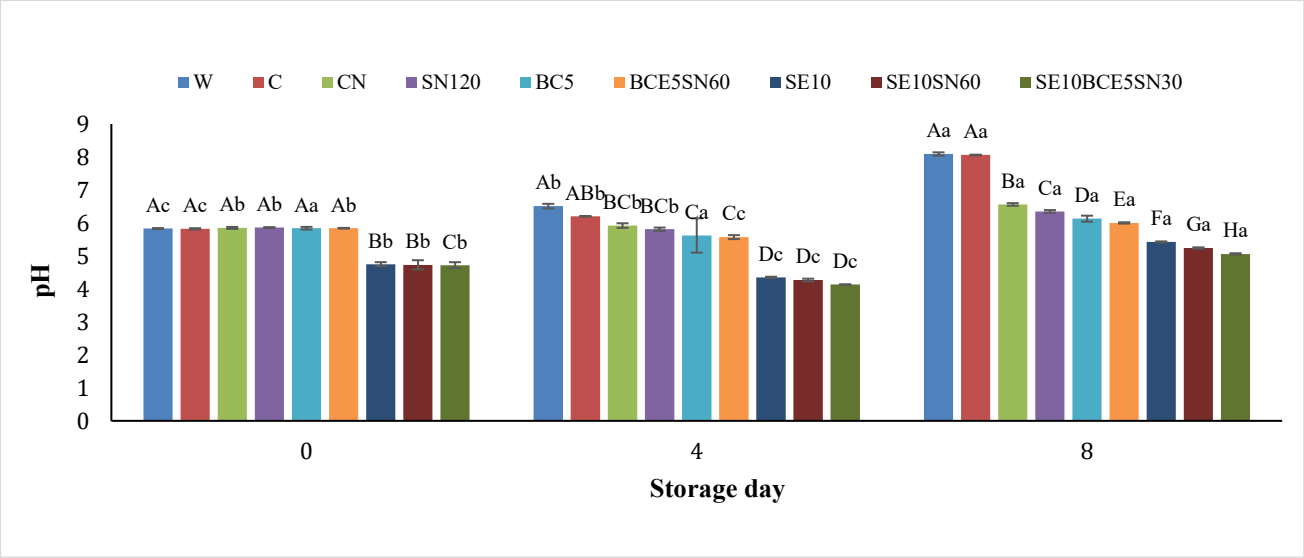

Figure 5. pH values of ground beef with active BNC films containing different concentrations of SN, SE and BCE during 8 days of storage at 4 °C (Mean values ± SD). Different small and capital letters, respectively, indicate statistical differences among times and treatments ($P < 0.05$).

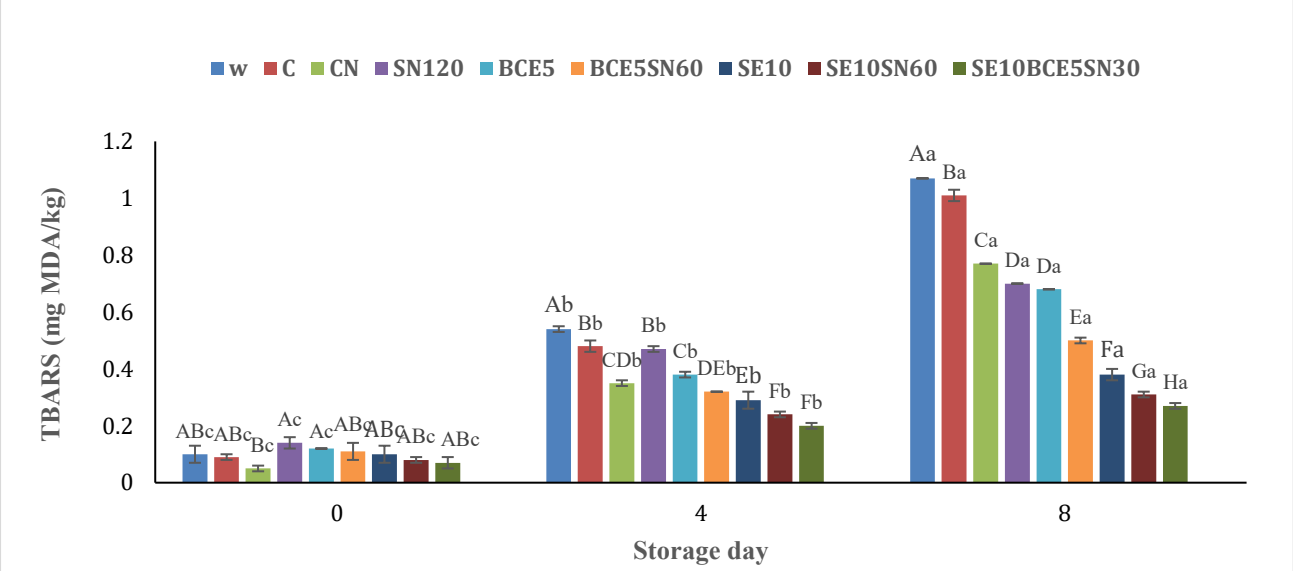

Figure 6. TBARS values of ground beef with active BNC films containing different concentrations of SN, SE and BCE during 8 days of storage at 4 °C (Mean values± SD). Different small and capital letters, respectively, indicate statistical differences among times and treatments ($P < 0.05$).

Residual nitrite

To evaluate the nitrite-loading capacity and migration behavior of the active BNC films, residual nitrite was measured both in the films and the ground beef samples at different stages of the experiment. Initially, pure BNC films were immersed in treatment solutions containing sodium nitrite (alone or in combination with SE and BCE), according to the defined treatment groups. After immersion, the films were drying step to stabilize and fix the entrapped nitrite within the film matrix. The dried films were then analyzed to determine their initial residual nitrite content, representing the amount of nitrite successfully incorporated into each film. Subsequently, the active BNC films were applied

to ground beef in a sandwich format and stored at 4 °C for 8 days. Residual nitrite was measured in both the films and the corresponding ground beef samples on days 0, 4, and 8 to assess nitrite migration from the film into the meat over time.

Table 4. Residual nitrite content of ground beef with active BNC films containing different concentrations of SN, SE and BCE during 8 days of storage at 4 °C (Mean values ± SD).

Residual nitrite (ppm)	Treatments	Storage period (day)		
		0	4	8
In ground beef	W	0.05 ± 0.09^{Ga}	0.69 ± 0.37^{FGa}	0.80 ± 0.42^{Ea}
	C	0.10 ± 0.56^{Gb}	0.21 ± 0.33^{Gb}	0.48 ± 0.00^{Ea}
	CN	107.85 ± 0.48^{Aa}	83.36 ± 0.40^{Ab}	62.42 ± 0.49^{Ac}
	SN_{120}	42.51 ± 0.24^{Ba}	31.96 ± 0.64^{Bb}	18.08 ± 1.13^{Bc}
	BCE_5	1.88 ± 0.76^{Fa}	1.56 ± 0.24^{Fa}	0.91 ± 0.49^{Ea}
	BCE_5SN_{60}	25.34 ± 0.58^{Ca}	14.11 ± 0.32^{Cb}	5.81 ± 0.48^{Cc}
	SE_{10}	0.86 ± 0.33^{Ga}	0.80 ± 0.16^{FGa}	0.59 ± 0.04^{Ea}
	$SE_{10}SN_{60}$	17.49 ± 0.40^{Da}	10.65 ± 0.32^{Db}	6.08 ± 0.51^{Cc}
	$SE_{10}BCE_5SN_{30}$	7.48 ± 0.79^{Ea}	5.70 ± 0.40^{Eb}	2.90 ± 0.80^{Dc}
In film	W	0.00 ± 0.27^{Fa}	0.00 ± 0.24^{Fa}	0.05 ± 0.09^{Da}
	SN_{120}	28.84 ± 0.79^{Aa}	15.66 ± 0.42^{Ab}	9.14 ± 0.65^{Ac}
	BCE_5	1.29 ± 0.32^{Ea}	0.86 ± 0.18^{Ea}	0.10 ± 0.24^{Db}
	BCE_5SN_{60}	10.97 ± 0.48^{Ba}	7.53 ± 0.40^{Bb}	3.76 ± 0.76^{Bc}
	SE_{10}	0.53 ± 0.51^{Ef}	0.37 ± 0.18^{Ef}	0.10 ± 0.18^{Da}
	$SE_{10}SN_{60}$	9.09 ± 0.56^{Ca}	6.29 ± 0.85^{Cb}	4.30 ± 0.49^{Bc}
	$SE_{10}BCE_5SN_{30}$	4.52 ± 0.73^{Da}	2.26 ± 0.58^{Db}	1.56 ± 0.82^{Cc}

Different small and capital letters, respectively, indicate statistical differences among times and treatments ($P < 0.05$).

Residual nitrite levels in BNC films and ground beef samples are shown in **Table 4**. The initial nitrite contents of SN_{120}, BCE_5SN_{60}, $SE_{10}SN_{60}$, and $SE_{10}BCE_5SN_{30}$ films were 79.48 ± 1.67, 41.06 ± 1.05, 30.35 ± 0.64, and 12.53 ± 0.40 ppm respectively, confirming successful nitrite entrapment after the drying process. The increase of SN in ham samples indicates its migration from the matrix of the films to the ground beef. Over the course of storage, a significant reduction ($P < 0.05$) in nitrite concentration was observed in both the films and the beef samples for all treatments. This decrease maybe attributed to factors such as, pH, initial nitrite concentration, storage temperature, and microbial activity. Lactic acid bacteria which produce organic acids and reduce environmental pH, may accelerate nitrite degradation (Honikel, 2008; Pegg et al., 1997). The intrinsic acidity of SE likely further catalyzed nitrite breakdown. In addition, nitrite may react with meat components such as lipids and proteins, forming nitrosyl or nitroso compounds, leading to lower detectable levels. By day 8, the $SE_{10}BCE_5SN_{30}$ treatment exhibited the lowest residual nitrite in beef (2.90 ppm), significantly lower than other treatments ($P < 0.05$). This could be attributed to the high polyphenol content of SE and BCE, which are known to react with or neutralize nitrite. These finding are consistent with previous studies reporting that plant extracts can effectively reduce residual nitrite (Fernández-López et al., 2008; Shiravani et al., 2024) and suppress the formation of carcinogenic N-nitroso compounds (Fernández-Ginés et al., 2004; Viuda-Martos et al., 2010). Moreover, the SN-containing films maintained the desirable red color of the meat, attributable to nitrite's stabilizing effect on meat pigments.

In a previous study, the incorporation of sodium nitrite into packaging film proved more effective than direct addition into pork, leading to extended shelf life and improved preservation performance (Chatkitanan & Harnkarnsujarit, 2020). Overall, BNC films enriched with SE and BCE represent a promising strategy for reducing nitrite usage in meat products while maintaining product safety and quality.

Conclusion

Packaging ground beef with BNC films enriched with SE significantly inhibited the microbial growth, reduced residual nitrite and pH, and extended the shelf life of ground beef during 8 days of refrigerated storage. Furthermore, the addition of SE enhanced the chemical stability of the samples due to its strong antioxidant properties. Considering ongoing concerns about the toxicity of sodium nitrite, both SE and BCE appear to be promising, natural, and health-promoting alternatives for use in meat preservation. These natural compounds not only help reduce nitrite content but also contribute to improved safety and quality of meat products.

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Conflicts of interest

Non.

Disclaimer

Non.

References

Aliakbarlu, J., & Mohammadi, S. (2015). Effect of Sumac (*Rhus coriaria* L.) and barberry (*Berberis vulgaris* L.) Water extracts on microbial growth and chemical changes in ground sheep meat. *Journal of Food Processing and Preservation*, 39, 1859–1866. https://doi.org/10.1111/jfpp.12423

Aliakbarlu, J., Mohammadi, S., & Khalili, S. (2014). A study on antioxidant potency and antibacterial activity of water extracts of some spices widely consumed in Iranian diet. *Journal of Food Biochemistry*, 38, 159–166. https://doi.org/10.1111/jfbc.12034

Bae, S.M., Gwak, S.H., Yoon, J., & Jeong, J.Y. (2021). Effects of lemon extract powder and vinegar powder on the quality properties of naturally cured sausages with white kimchi powder. *Food Science of Animal Resources*, 41, 950. https://doi.org/10.5851/kosfa.2021.e47

Bazargani-Gilani, B., Aliakbarlu, J., & Tajik, H. (2015). Effect of pomegranate juice dipping and chitosan coating enriched with *Zataria multiflora* Boiss essential oil on the shelf-life of chicken meat during refrigerated storage. *Innovative Food Science and Emerging Technologies*, 29, 280–287. https://doi.org/10.1016/j.ifset.2015.04.007

Chatkitanan, T., & Harnkarnsujarit, N. (2020). Development of nitrite compounded starch-based films to improve color and quality of vacuum-packaged pork. *Food Packaging and Shelf Life,* 25, 100521. https://doi.org/10.1016/j.fpsl.2020.100521

Cheng, J., Sun, J., Xu, M., & Zhou, X. (2023). Nondestructive detection of lipid oxidation in frozen pork using hyperspectral imaging technology. *Journal of Food Composition and Analysis*, 123, 105497. https://doi.org/10.1016/j.jfca.2023.105497

Cömert, E.D., Mogol, B.A., & Gökmen, V. (2020). Relationship between color and antioxidant capacity of fruits and vegetables. *Current Research in Food Science, 2*, 1–10. https://doi.org/10.1016/j.crfs.2019.11.001

Fernández-Ginés, J.M., Fernández-López, J., Sayas-Barberá, E., Sendra, E., & Pérez-Álvarez, J.A. (2004). Lemon albedo as a new source of dietary fiber: Application to bologna sausages. *Meat Science, 67*, 7–13. https://doi.org/10.1016/j.meatsci.2003.08.017

Fernández-López, J., Sendra, E., Sayas-Barberá, E., Navarro, C., & Pérez-Alvarez, J.A. (2008). Physico-chemical and microbiological profiles of "salchichón"(Spanish dry-fermented sausage) enriched with orange fiber. *Meat Science*, 80, 410–417. https://doi.org/10.1016/j.meatsci.2008.01.010

Fernández-López, J., Viuda-Martos, M., Sendra, E., Sayas-Barberá, E., Navarro, C., & Pérez-Alvarez, J.A. (2007). Orange fibre as potential functional ingredient for dry-cured sausages. *European Food Research and Technology*, 226, 1–6. https://doi.org/10.1007/s00217-006-0501-z

Fernandez-Lopez, J., Zhi, N., Aleson-Carbonell, L., Pérez-Alvarez, J.A. and, & Kuri, V. (2005). Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Science*, 69, 371–380. https://doi.org/10.1016/j.meatsci.2004.08.004

Ghorbani, A., Mahmoudifar, K., Shokri, S., Mazaheri, Y., Shamloo, E., Rezagholizade-Shirvan, A., & Elhamirad, A.H. (2024). Effect of *Allium Jesdianum*'s extract on the physicochemical, antioxidant, antimicrobial and sensory properties of Sausage characteristics. *Food Chemistry: X, 22*, 101461. https://doi.org/10.1016/j.fochx.2023.101461

Gil, M., & Rudy, M., (2023). Innovations in the packaging of meat and meat products—A review. *Coatings*, *13*, 333. https://doi.org/10.3390/coatings13020333

Honikel,K.-O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science*, 78, 68–76. https://doi.org/10.1016/j.meatsci.2007.05.030

Horwitz, W., et al. (1975). *Official methods of analysis*, vol. 222. Association of Official Analytical Chemists., Washington, DC.

Kammerer, D., Carle, R., & Schieber, A. (2004). Characterization of phenolic acids in black carrots (*Daucus carota* ssp. sativus var. atrorubens Alef.) by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry, 18*, 1331–1340. https://doi.org/10.1002/rcm.1496

Khatib, N., Varidi, M.J., Mohebbi, M., Varidi, M., & Hosseini, S.M.H. (2020). Replacement of nitrite with lupulon–xanthohumol loaded nanoliposome in cooked beef-sausage: Experimental and model based study. *Journal of Food Science and Technology*, *57*, 2629–2639. https://doi.org/10.1007/s13197-020-04301-3

Kim TaeKyung, K.T., Kim YoungBoong, K.Y., Jeon KiHong, J.K., Park JongDae, P.J., Sung JungMin, S.J., Choi HyunWook, C.H., Hwang KoEun, H.K., & Choi YunSang, C.Y. (2017). Effect of fermented spinach as sources of pre-converted nitrite on color development of cured pork loin. *Korean Journal for Food Science of Animal Resources, 37*(1),105-113. https://doi.org/10.5713/ajas.18.0903

Langroodi, A.M., Tajik, H., & Mehdizadeh, T. (2018a). Preservative effects of sumac hydro-alcoholic extract and chitosan coating enriched along with *Zataria multiflora* Boiss essential oil on the quality of beef during storage. *Veterinary Research Forum*, *9*(2), 153–161. https://doi.org/ 10.30466/VRF.2018.30831

Langroodi, A.M., Tajik, H., Mehdizadeh, T., Moradi, M., Kia, E.M., & Mahmoudian, A. (2018b). Effects of sumac extract dipping and chitosan coating enriched with *Zataria multiflora* Boiss oil on the shelf-life of meat in modified atmosphere packaging. *LWT – Food Science and Technology*, 98, 372–380. https://doi.org/10.1016/j.lwt.2018.08.063

Lee, J., Sung, J.-M., Cho, H.J., Woo, S.-H., Kang, M.-C., Yong, H.I., Kim, T.-K., Lee, H., & Choi, Y.-S. (2021). Natural extracts as inhibitors of microorganisms and lipid oxidation in emulsion sausage during storage. F*ood Science of Animal Resources*, 41, 1060. https://doi.org/10.5851/kosfa.2021.e54

Moradi, M., Kousheh, S.A., Razavi, R., Rasouli, Y., Ghorbani, M., Divsalar, E., Tajik, H., Guimarães, J.T., & Ibrahim, S.A. (2021). Review of microbiological methods for testing protein and carbohydrate-based antimicrobial food packaging. *Trends in Food Science & Technology*, 111, 595–609. https://doi.org/10.1016/j.tifs.2021.03.007

Oyaizu, M. (1986). Antioxidative activities of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, 44, 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307

Papadopoulou, O.S., Chorianopoulos, N.G., Gkana, E.N., Grounta, A. V, Koutsoumanis, K.P., & Nychas, G.-J. (2012). Transfer of foodborne pathogenic bacteria to non-inoculated beef fillets through meat mincing machine. *Meat Science*, 90, 865–869. https://doi.org/10.1016/j.meatsci.2011.11.008

Pegg, R.B., Shahidi, F., & Fox Jr, J.B. (1997). Unraveling the chemical identity of meat pigments. *Critical Reviews in Food Science and Nutrition*, 37, 561–589. https://doi.org/10.1080/10408399709527789

Pikul, J., Leszczynski, D.E., & Kummerow, F.A. (1989). Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *Journal of Agricultural and Food Chemistry*, 37, 1309–1313. https://doi.org/10.1021/jf00089a022

Rodrigues, A.C., Fontão, A.I., Coelho, A., Leal, M., da Silva, F.A.G.S., Wan, Y., Dourado, F., & Gama, M. (2019). Response surface statistical optimization of bacterial nanocellulose fermentation in static culture using a low-cost medium. *New Biotechnology*, 49, 19–27. https://doi.org/10.1016/J.NBT.2018.12.002

Shafiei, H., Saei-Dehkordi, S., Moradi, M., & Molaei, R. (2024). Preparation and antibacterial performance of bacterial nanocellulose sachet containing *Zataria multiflora* essential oil loaded halloysite nanotubes on *Escherichia coli* O157: H7 in cheese. *LWT – Food Science and Technology,*194, 115812. https://doi.org/10.1016/j.lwt.2024.115812

Shakil, M.H., Trisha, A.T., Rahman, M., Talukdar, S., Kobun, R., Huda, N., & Zzaman, W. (2022). Nitrites in Cured Meats, Health Risk Issues, Alternatives to Nitrites: A Review. *Foods, 11*(21), 3355. https://doi.org/10.3390/foods11213355

Shiravani, Z., Aliakbarlu, J., & Moradi, M. (2024). Application of bacterial nanocellulose film loaded with sodium nitrite, sumac, and black carrot extracts to reduce sodium nitrite, extend shelf life, and inhibit Clostridium perfringens in cooked beef ham. *International Journal of Biological Macromolecules*, 280, 135841. https://doi.org/10.1016/j.ijbiomac.2024.135841

Singleton, V.L., & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J.A. (2010). Effect of added citrus fibre and spice essential oils on quality characteristics and shelf-life of mortadella. *Meat Science*, 85, 568–576. https://doi.org/10.1016/j.meatsci.2010.03.007

Wójciak, K.M., Ferysiuk, K., Kęska, P., Materska, M., Chilczuk, B., Trząskowska, M., Kruk, M., Kołożyn-Krajewska, D., & Domínguez, R. (2023). Reduction of Nitrite in Canned Pork through the Application of Black Currant (*Ribes nigrum* L.) Leaves Extract. *Molecules,* 28, 1749. https://doi.org/10.3390/molecules28041749

Wójciak, K.M., Stasiak, D.M., & Kęska, P. (2019). The influence of different levels of sodium nitrite on the safety, oxidative stability, and color of minced roasted beef. *Sustainability*, 11, 3795. https://doi.org/10.3390/su11143795