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Development, characterization and investigation of antimicrobial and antioxidant potential of sodium caseinate-based edible films infused with *Berberis pseudumbellata* fruit extract, and effects of the films on the quality of raw ground beef during refrigeration

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A b s t r a c t: The present study proposed to develop, characterize and investigate the antimicrobial and antioxidant potential of sodium caseinate (NaCAS) films infused with Berberis pseudumbellata (B. pseudumbellata) fruit extract and film application on ground beef at refrigerated temperature (7°C) for 9 days. The infusion of fruit extract enhanced the physical and optical characteristics of the films. Light transmittance and water solubility of the films decreased with the addition of the fruit extract, whereas the film thickness increased. In the agar disc diffusion test, the infused films showed maximum inhibition of Gram-positive bacteria, Staphylococcus aureus and Bacillus subtilis, compared with Gram-negative Salmonella Typhi and Escherichia coli. The films infused with B. pseudumbellata also exhibited increasing antioxidant activity with increasing B. pseudumbellata concentration. Infused films showed comparatively high antioxidant potential when evaluated before their application on ground beef. However, the differences in antioxidant activity of films (pre and post-application) were non-significant ($p<0.05$). Ground beef covered with infused NaCAS films (1, 1.5 and 3 %) maintained an acidic pH of 5.88 to 5.65, whereas the control ground beef (covered with NaCAS film) was pH 7.01 on day 9 of storage. NaCAS film infused with 3 % fruit extract also controlled the total viable counts compared to the control NaCAS film ($p<0.05$), with 3 % extract film perfoming better control than films with lower amounts of fruit extract.

Keywords: *Berberis pseudumbellata, edible film, sodium caseinate, beef, antioxidant, viable count.*

Introduction

Meat and related products, known causative sources of foodborne illnesses, are susceptible to deterioration due to their high perishability and protein content (*Kim et al.*, 2019). Along with uncontrolled biochemical and enzymatic reactions, the growth of microbes is another reason for the putrefaction of meat and its products during refrigeration (*Pellissery et al.*, 2020). Food industry and researchers are experiencing huge challenges in the preservation of meat and its products due to their great perishability. Incorporation of natural antioxidants from plant sources infused in the packaging matrix is one of the stable strategies to maintain the quality and improve the shelf life of meat and other food products.

Macromolecules, especially polysaccharides, lipids and proteins, are the basic source components used for the formulation of environment-friendly active packaging; these molecules serve as carriers for the active compounds and constituents including antimicrobials, antioxidants and oxygen scavengers (*Hassan et al.,* 2018). Protein film is desirable due to its enhanced mechanical and gas barrier properties (*Hanani et al.*, 2014). Currently, the formulation of protein-containing edible films, including milk proteins (casein and whey), has gained significant consideration due to the films' good functional properties (*Helal et al.*, 2015). The suitability of sodium caseinate (NaCAS) as a polymer for coating various food products, due to this protein's anti-

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oxidant potential and its use for the encapsulation of medicines and flavours, is reported in the earlier studies (*Khwaldia et al.*, 2004).

The traditional method to control the microbial growth and contamination in food includes the application of antimicrobial dips or sprays on the food surface (*Gutiérrez-del et al.*, 2018). To meet the requirements of consumers for more natural, biodegradable and preservative-free products for food packaging, the usage of plant-based antimicrobial agents, including bacteriocins and plant extracts, is getting more attention due to the safe nature and great potential of these agents (*Zhang et al.,* 2022; *Umaraw and Verma*, 2017). Earlier studies have proved the potential of natural antioxidants and antimicrobial infused in thermoplastics, thermosets and paper (*Zhong et al.*, 2020). Pervious findings have confirmed antimicrobial agents infused in packaging films might be active in decreasing the population of foodborne microorganisms in food systems (*Rahmasari and Yemiş*, 2022; *Sharma et al.*, 2020).

Berberis species are known for their medicinal benefits, edibility and nutritional value (*Andola et al.*, 2010; *Rawat et al.*, 2014). *Berberis pseudumbellata (B. pseudumbellata)* fruit is known for its high protein content and low anti-nutritive property (*Andola et al.*, 2011). Fruits of *B. pseudumbellata* are used abundantly as a folk medicine for the treatment of jaundice by the locals of Gilgit-Baltistan. Berberine and oxyacanthine are the main alkaloids reported in *B. pseudumbellata* (*Andola et al.*, 2010). The variation in berberine content in *B. pseudumbellata* and the nutritive value of barberry fruits of Gilgit-Baltistan have been reported by *Awan et al.* (2014) and *Andola et al.* (2010).

However, limited studies have testified to the antimicrobial and antioxidant activity of *B. pseudumbellata* fruit (*Awan et al.,* 2014). To the best of our knowledge, no study has reported the infusion of *B. pseudumbellata* fruit extract in any carrier matrix for film formulation or its application on the food matrix. Therefore, the aims of the present study were (i) to develop and characterize NaCAS-based edible film infused with the methanolic extract of *B. pseudumbellata* fruit; (ii) investigate the *in-vitro* antimicrobial and antioxidant potential of extract and formulated films; (iii) apply NaCAS films infused with *B. pseudumbellata* extract to cover ground beef, and investigate the change in antioxidant activity of the films; (iv) to study the packaging effect of NaCAS films (with and without extract) on the pH and total viable counts of stored beef on different days (0, 3, 6, 9) of storage.

Materials and Methods

Chemicals

NaCAS powder (food grade) and 99% pure glycerol (used as a plasticizer) were purchased from Sigma-Aldrich-USA. All reagents and chemicals used were analytical grades.

Plant

Sundried *B. pseudumbellata* fruits, collected from the hilly mountains of Gilgit-Baltistan, were lodged in the General Herbarium number 3534 with voucher number 01 at the Hazara University-Mansehra in the Department of Botany.

Extract preparation

For the preparation of fruit extract, the sundried *B. pseudumbellata* fruits, free from dirt and extraneous material, were ground using a mixer grinder into fine powder. Amounts (10 g) of finely ground sample were transferred into frosted glass containers and extracted in 100 mL of aqueous methanol for 24 h at 25°C with continuous shaking. The extract was passed through filter paper and further concentrated under a vacuum at 40°C for 6 h.

In-vitro antibacterial activity of extract

The agar disc diffusion method (*Bhuvana et al*., 2018) was followed to find the antibacterial activity of the fruit extract against the isolated bacteria, *Staphylococcus aureus, Bacillus subtilis, Salmonella* Typhi and *Escherichia coli*. Crude extract at the concentration of 200 mg/mL was used for the evaluation of the antibacterial activity. Paper discs (5 mm) were impregnated with 2, 4 and 6 mg/disc of methanolic fruit extract. Discs with methanol acted as the negative control. Streptomycin (10 µg/disc) was considered a positive control against Gram-negative bacteria, whereas penicillin (10 µg/disc), was used as a positive control against Gram-positive bacteria. A disc loaded with methanol served as a negative control. Nutrient agar (NA) medium was used as a suitable medium to study the antibacterial activity. Bacterial lawns were prepared on the NA plates by using 48 h-old cultures. Loaded discs were placed on different peripheral positions of Petri dishes. The experiment was performed three times to check the robustness of the results. The plates were then incubated for 48 h at 37°C. The zone of inhibition against test organisms was recorded in mm.

Antioxidant activity of extract

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) method with slight modifications to the method suggested by *Ruffino et al.* (2007) was followed to study the antioxidant property of formulated films. Volumes (60 µL) of sample extracts were added to tubes containing 270 µL of methanol and 2700 µL of DPPH solution of known concentration (0.0236 mg/mL). The mixtures were kept at room temperature for 30 minutes in dark. Finally, absorbance was measured using a UV-visible spectrophotometer (model UV-Vis 1900, Shimadzu, Japan) at 517 nm. The experiment was conducted in triplicate.

The formula used to calculate the % DPPH free radical scavenging activity of each formulated film was:

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{sample extract}})}{\text{Abs}_{\text{DPPH}}} \times 100$$

where

Abs_{DPPH} = Absorbance value of the methanolic solution of DPPH at 517 nm

$\text{Abs}_{\text{extract}}$ = Absorbance value for the sample extracts at 517 nm.

Film formulation

Film formulations were prepared using 5 g of NaCAS powder in 100 mL distilled water. The solution was mixed using a hotplate stirrer at 500 rpm at 85±5°C for 60 minutes. The temperature was reduced to 60±5°C during glycerol (3.6 w/v) addition with constant agitation for another 30 minutes. The temperature was further reduced to 25°C and 1% $NaHCO_3$ was added under mixing that continued for another 30 minutes. While stirring at 15,000 rpm for 2 min, fruit extract at different concentrations (0.1%, 1.5% and 3%) was infused into the film solution. Finally, 30 mL of each extract-infused film solution was transferred onto Petri dishes and stored for drying at 29°C for 48 h. After that, the dried films were removed from the dishes and were kept in sealed bags until future analysis.

Characterization of active films

The following characteristics of the protein-based formulated films were studied.

In-vitro antibacterial activity of films

The agar disc diffusion method suggested by *Kanmani and Rhim* (2014) was used with minor modifications to study the antibacterial activity of the extract-infused NaCAS films against the bacteria isolates, *Staphylococcus aureus, Bacillus subtilis, Salmonella* Typhi and *Escherichia coli*. A lawn of each bacterium was prepared in NA, and 5 mm discs prepared from each film were placed onto the NA lawn plates. The experiment was conducted in triplicate, and plates were incubated at 37°C for 48 h. The zone of inhibition was measured in millimetres.

Antioxidant activity of films

Pre and post-application antioxidant activity of films was estimated by the method defined by *Veiga-Santos et al*. (2018). Film solutions were prepared by vortex mixing 0.1 g of each film sample from the pre and post-application stage in 10 ml of distilled water for 5 minutes. Later, the vortex solution was centrifuged at 4000 rpm for 30 minutes. The centrifuged supernatant was filtered and used to estimate the antioxidant potential, which was expressed as percent radical scavenging activity (% RSA) as described above under the heading *Antioxidant activity of extract*.

Film Thickness

A digital micrometer (RexBeti No. 33178104, China) was used to measure the thickness of films by taking an average of six random position measurements over the test areas of each film. The measured values of the film thickness were reported in mm.

Film solubility in water

The method of *Tunc et al.* (2007) was used to determine the solubility of film in water. Films cut into $1 \times 4.2\,\text{cm}^2$ pieces were dried for 5 h at 105°C to reach constant weight. Films were then weighed on an analytical balance. Each dried film was then dipped in 50 mL of double distilled water and constantly stirred at 25°C for 24 h. For the determination of the final weight of the dry matter, insolubilized films were removed and dried for at 105°C for 24 h. The drying procedure for each film was replicated six times and means were used as solubility percentage.

Film solubility in water was calculated using the equation:

% Film solubility = {(initial film dry weight − final film dry weight/ initial film dry weight) *100}

Transparency of film

The modified method of *Tunç and Duman* (2007) was used to determine the transparency of film using a spectrometer, UV-Vis 1900, Shimadzu, Japan. Films with a measurement of 11×42 mm were placed in a desiccator with an absorbent (saturated magnesium nitrate) at ambient temperature. Transmittance mode was selected, and calibration was performed at 560 nm using an empty cell. Six measurements were taken for each formulated film.

Film application on meat

Meat free from connective tissues and excess fat was collected from a butcher, and ground meat samples were prepared using an electric meat grinder. The ground meat samples were distributed into five groups for the observation of the packaging effect on the shelf life of ground meat, as follows:

1. Control sample (NaCAS only without an antimicrobial agent)
2. Antimicrobial source (NaCAS film containing 1, 1.5 or 3% methanolic extract of *B. Pseudumbellata — BPFE*).
   2.1. 1% BPFE + NaCAS
   2.2. 1.5% BPFE + NaCAS
   2.3. 3% BPFE + NaCAS

For each of these four sets, 50 g of ground beef meat was measured into disinfected Petri dishes. Formulated NaCAS-based films were used to cover the ground meat samples. Three replicates for each film were used in this study. Covered meat in Petri dishes was kept at refrigerator temperature (7°C) for 9 days. The pH and total viable count (TVC) of the stored beef were recorded on day 0 (at the start of the experiment) and on days 3, 6 and 9 of refrigeration.

pH measurement of stored beef samples

The pH of meat samples on different days of storage was measured by using a Hanna pH meter. Briefly, 5 g of beef was taken from each stored beef sample and homogenized with 100 ml of distilled water for 1 minute. The homogenate was filtered, and the pH of the filtrate of each sample was noted three times.

Total viable count (TVC) in stored beef samples

Microbiological analyses included the determination of total viable count conducted with a slight modification to the method suggested by *Song et al*. (2011). Ground beef (5 g) taken aseptically from each Petri dish was homogenized in an electric blender for 10–15 minutes with 225 ml of 0.1% sterilized peptone water. Furthermore, 0.1% sterile peptone water was used for the serial dilution of the sample. An amount (1 mL) for serial dilution, from dilution 10^{-8}, of each sample was transferred onto plate count agar. The plates were incubated for 48 h at 35–37°C. The microbiological count data in triplicate were converted into logarithms of the number of colony-forming units per gram (CFU/g) and then averaged.

Statistical analysis

The software package SPSS-26 was used for statistical analysis. Individual experiments were executed in triplicate and results were conveyed as means and standard deviations. To study the properties of formulated films: thickness of the film (mm), transparency (%) water solubility (%), and antioxidant activity (%) means and standard deviations were calculated. The one-way ANOVA and Least Significant Difference (LSD) procedures were used to test for differences between means. Data obtained were subjected to General Linear Model (GLM) with repeated measures to find the effect of treatments (formulated films), storage period (days) and their interactions on the pH and total viable count of stored meat (beef). P-values of <0.05 were considered significant.

Results and discussion

In-vitro antibacterial activity of Berberis pseudumbellata fruit extract

Our study findings suggested strong antibacterial activity of crude fruit extract against Gram-positive as compared to Gram-negative pathogenic bacteria used in this study (Table 1). The maximum inhibition observed was in 6 mg/mL of crude extract (25 mm inhibition zone) against *S. aureus* followed by *B. subtilis, S.* Typhi and *E. coli* respectively. However, in 2014, *Awan et al.* reported the strong antibacterial potential of the methanolic extract of *B. pseudumbellata* fruits against Gram-negative bacteria (*E. coli, and Pseudomonas)* as compared to Gram-positive bacteria (*Bacillus cereus*). The antibacterial potential of the methanolic extract of *B. Pseudumbellata* might be due to the availability of secondary metabolites, mainly berberine, an isoquinoline alkaloid, and other alkaloids, which are highly soluble in polar solvents (*Awan et al.*, 2014). The strong antibacterial potential of methanolic extract of *B. pseudumbellata* fruit extract against Gram-positive bacteria as compared with the Gram-negative bacteria might also be the result of strong chemical interaction of Gram-positive bacteria with the active agent of *B. pseudumbellata* (*Helal et*

Table 1. *In-vitro* antibacterial activity of methanol extract of *Berberis pseudumbellata* fruit compared with streptomycin as standard control against Gram-negative bacteria and penicillin as standard control against Gram-positive bacteria.

Extract	Concentration (mg/ mL)	Zone of inhibition (mm)			
		Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Salmonella* Typhi**
Fruit extract	**Control**	0	0	0	0
	Positive control (P/S)	24.5	22	28	30
	2	14.5	12.5	11	12
	4	20	20	19	19.5
	6	25	24	22	23.5

Control = Methanol @10 µL/disc
S = Streptomycin @10 µL/disc
P = Penicillin @10 µL/disc
2 = *B. pseudumbellata* fruit extract @20 mg/discs
4 = *B. pseudumbellata* fruit extract @40 mg/disc
6 = *B. pseudumbellata* fruit extract @60 mg/disc

al., 2012). However, the comparatively less inhibition observed against Gram-negative bacteria might be due to their complex barrier system which regulates, and at times inhibits, the pathway of biocides into the cytoplasm through the cytoplasmic membrane (*Denyr and Maillard,* 2002; *Emiroğlu et al.,* 2010).

Antioxidant activity of Berberis pseudumbellata fruit extract

Figure 1 shows the DPPH free radical scavenging activity of fruit extract in different concentrations. The radical scavenging activity of extract increased with the increasing concentration of extract. The minimum concentration of the extract was 10 µg/mL, which gave 51.91% radical scavenging activity, whereas 180 µg/ml of methanolic extract of fruit exhibited high radical scavenging activity (57.18%), showing increasing free radical scavenging activity with increasing extract concentration. The strong antioxidant activity of the methanolic extract of *B. pseudumbellata* might be due to the availability of ascorbic acid, phenolics, flavonoids and carotenoids in its composition (*Awan et al.*, 2014). Thus, this study confirms that the studied plant extract is a potential source of antioxidants that can serve against lipid peroxidation in meat and relevant food products.

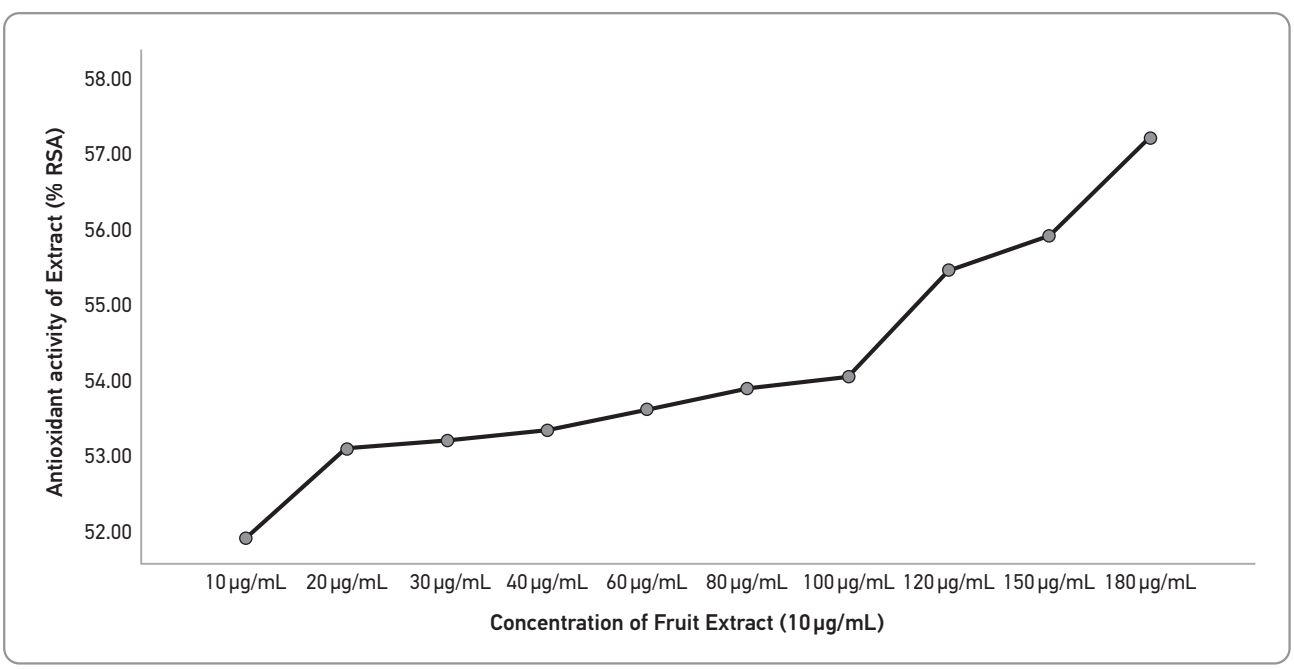

Figure 1. DPPH free radical scavenging activities of methanol extract of *Berberis pseudumbellata* fruit

In-vitro antibacterial activity of extract infused films of Na-Caseinate

NaCAS-only (control) films showed no antibacterial activity against tested bacteria, and our results are in agreement with the previous findings of *Kristo et al.* (2008). However, extract-infused NaCAS films successfully inhibited the growth of bacterial strains used in this study (Table 2). The maximum inhibition was observed in NaCAS film infused with 3% extract (inhibition zone 16.3 mm) against *S. aureus*, followed by *B. subtilis, S.* Typhi and *E. coli*, respectively. According to our findings, *S. aureus* and *B. subtilis* were more sensitive to *B. pseudumbellata* infused NaCAS films than were *E. coli* and *S.* Typhi. This might be due to the single-cell structure of Gram-positive bacteria or due to strong chemical interaction of Gram-positive bacteria with the active agent of fruit extract (*Helal et al.*, 2012). *Kaya et al.* (2018) reported the utilization of *Berberis crataegina* fruit extract and seed oil for the production of chitosan-based films and studied their *in-vitro* antibacterial properties. However, to the best of our knowledge, no study has reported the antibacterial potential of *B. pseudumbellata* infused NaCAS film. This study suggests the antibacterial activity of caseinate films infused with *B. pseudumbellata* extract might be due the bioactive compounds (berberine and alkaloids) in the plant extract composition.

Antioxidant activity of films

The antioxidant activity (pre and post) application of films on ground beef is reported in Table 3. In this study, the DPPH radical scavenging activity of films increased with the increase in extract concentration in the films, which might be due to the strong interaction between NaCAS and bioactive compounds of *B. pseudumbellata* fruit extract (*Helal et al.*, 2012). This strong antioxidant activity of coatings and gradual antioxidant discharge on food surface might result in the retardation of lipid oxidation in meat, thus extending its shelf life (*Helal et al.*, 2015; *Helal et al*., 2012).

Table 2. *In-vitro* antibacterial activity of sodium caseinate (NaCAS) films infused with methanol extract of *Berberis pseudumbellata* fruit (BPFE)

Extract	Concentration (mg/ mL)	Zone of inhibition (mm)			
		Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Salmonella* Typhi**
Fruit extract	**Control (NaCAS only)**	0	0	0	0
	Penicillin/Streptomycin	19.3	20	31.6	34.6
	1% BPFE + NaCAS	10.6	9.6	10	10
	1.5% BPFE + NaCAS	11.6	11.3	10.2	10.6
	3% BPFE + NaCAS	16.3	15.3	12.5	13.6

Table 3. Pre and post antioxidant activity of sodium caseinate (NaCAS) films infused with methanol extract of *Berberis pseudumbellata* fruit (BPFE)

Treatments	Antioxidant activity of film-pre-application (% inhibition)	Antioxidant activity of film post application (% inhibition)	
	Mean ± SD	Mean ± SD	p-value[a]
Control (NaCAS only)	76.35 ± 0.24	74.26 ± 2.13	0.075
1 % BPFE + NaCAS	80.80 ± 0.65	79.54 ± 1.22	0.085
1.5 % BPFE + NaCAS	84.28 ± 0.55	82.65 ± 3.21	0.272
3% BPFE + NaCAS	92.52 ± 1.64	92.05 ± 0.60	0.534
p-value[b]	<0.001	<0.001	

Legend: [a] p-value calculated using t-test pairwise comparison; [b] p-value calculated using one-way ANOVA

Characteristics of films

Thickness of films

The thickness of the film was generally increased with the addition and increasing concentration of extract. However, this increase in film thickness became evident when the percentage of extract increased two-fold (to 3%) compared to the lowest concentration (1%). The increase in thickness was statistically significant ($p < 0.05$) (Table 4). Other studies also showed the changes that occurred in the thickness of edible formulated films are due to the accumulation of active antimicrobial compounds. *Rad et al.* (2017) reported increased film thickness in Pullulan-soy films with the addition of *Zataria multiflora* and *Artemisia biennis* extract. The addition of extract may result in the development of soft and sponge-like structure which further increases the moisture content, causing the swelling of the film due to confined water molecules in the pores of formulated film, and thus, enhancing the film thickness (*Emam-Djomeh et al.,* 2015). On the contrary (*Pires et al.*, 2013), reported decreased film thickness was caused by the amalgamation of tarragon, lavender, thyme and coriander extracts as antimicrobial amalgams.

Water solubility

Addition of *B. pseudumbellata* fruit extracts increased film solubility (Table 4). However, at the lowest extract concentration (1%), the increased film solubility was only a non-steady trend and non-significant compared to the control (NaCAS only). At higher concentrations, the solubility of the film showed increasing water solubility due to improved pore size in the film's inner structure. Hence, the infusion of fruit extract effects the disruption of film structure and, thus, increases film solubility. Similar results have been reported in earlier studies (*Emam-Djomeh et al.*, 2015; Hosseini et al., 2009).

Film transparency

The addition of *B. pseudumbellata* fruit extract significantly ($p<0.05$) reduced the transparency of films (Table 4). The reduced film transparency was significant ($p<0.01$) in the highest concentration (3%) of added extract as compared to the control. Our findings are similar to those earlier reported in alginate films supplemented with ginseng extract (*Norajit et al.*, 2010). Considering the red color of *B. pseudumbellata* fruit extract, reduced transparency of formulated film was expected. Plant extracts are known for the provision of opacity to polymers, so films containing extracts are translucent (*Norajit et al.*, 2010). The added fruit extract serves as a light barrier, thus preventing the breakdown and loss of light-sensitive compounds (*Mir et al.*, 2018).

Effect of extract infused NaCAS films on ground meat quality

Change in the pH of stored ground beef

Figure 2 shows the fruit extract's effect compared with the control on the overall pH of ground beef samples stored at 7°C on day 0 and during the nine storage days. The ground beef had an initial pH of 5.62 prior to the film application and storage. In this study initially, the pH of all beef samples decreased with storage, but then it started increasing gradually in all treatments (Figure 4), which could be due to glycogen decomposition in the stored beef (*Song et al*., 2011). However, the pH increase was comparatively less in ground beef samples that were covered with film infused with fruit extract as compared to the control

Table 4. Characterization of sodium caseinate (NaCAS) films infused with methanol extract of *Berberis pseudumbellata* fruit (BPFE): Thickness (Th), water solubility (WS) and transparency (T)

Films	Th (mm)	WS (%)	T (%)
Control (NaCAS only)	0.11 ± 0.01	27.40 ± 3.87	18.21 ± 1.73
1 % BPFE + NaCAS	0.14± 0.02**	28.47 ± 2.12	16.08 ± 1.67*
1.5 % BPFE + NaCAS	0.15 ± 0.01***	34.77 ± 2.19***	14.41 ± 1.69**
3 % BPFE + NaCAS	0.16 ± 0.02***	39.55 ± 1.32***	14.20 ± 1.61**
^P-value	<0.001	<0.001	0.002

Legend: Values are given as means ± standard deviation. ^P-value calculated using one-way ANOVA for each property. *Pairwise mean comparison was also computed with the control (NaCAS only) taken as a reference group. *p-value<0.05; **p-value<0.01; ***p-value<0.001

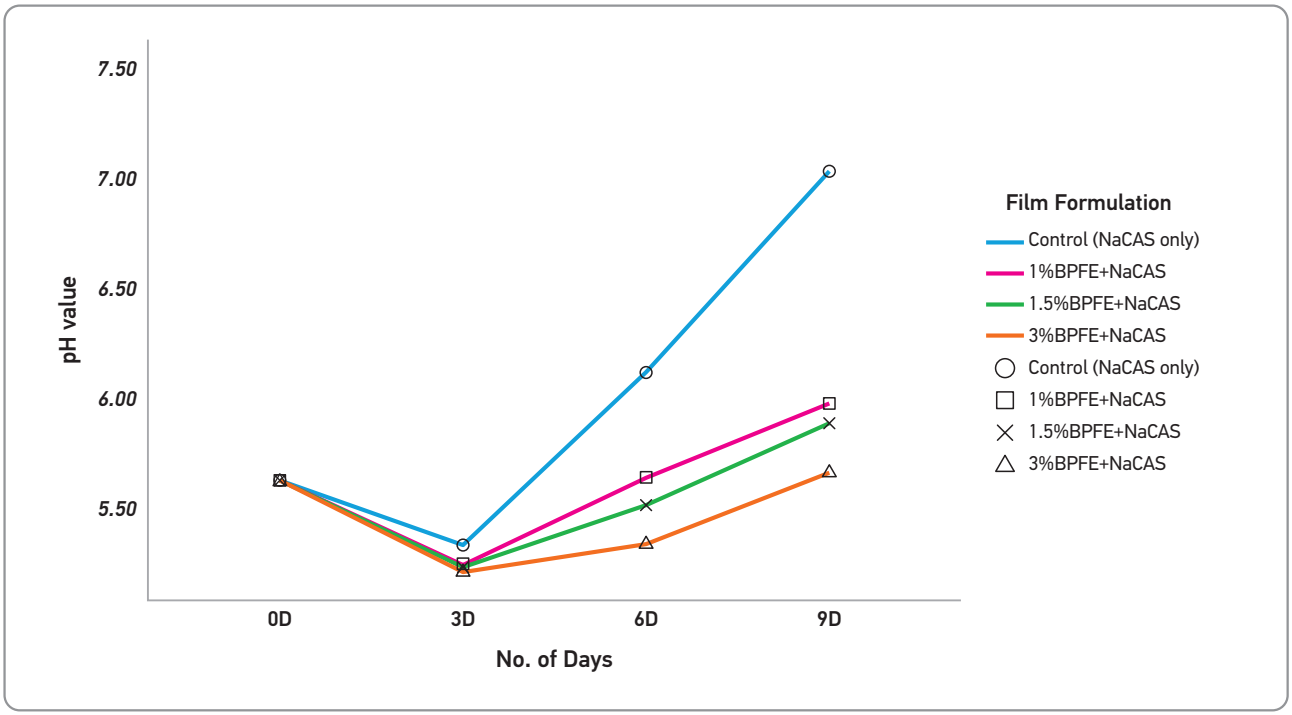

Figure 2. The pH of ground beef covered with NaCAS films with/without *Berberis pseudumbellata* fruit extract (infused/non-infused) during storage at 7°C for 9 days.

(NaCAS only film). The pH of ground beef covered with the 3% extract film ranged from 5.62 to 5.65. This mildly acidic pH might have inhibited microbial growth by inhibiting the endogenous enzymes, specifically proteases, at different proton grades, and thus resulting in comparatively few changes in pH. Earlier findings also reported the pronounced antimicrobial activity of plant extracts under acidic conditions (*Song et al.*, 2011). The difference in pH between treatments on different days was highly significant (interaction

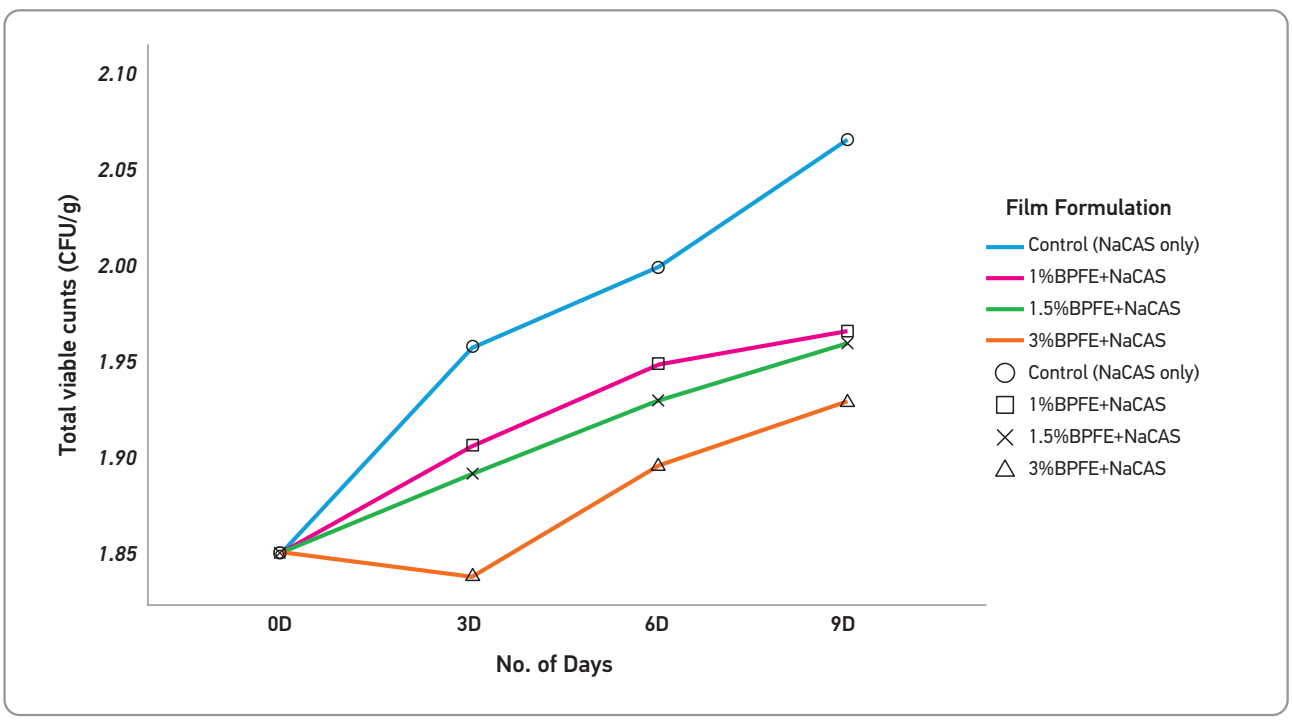

Figure 3. Total viable counts (TVC) of ground beef covered with NaCAS films with/without *Berberis pseudumbellata* fruit extract (infused/non-infused) during storage at 7°C for 9 days.

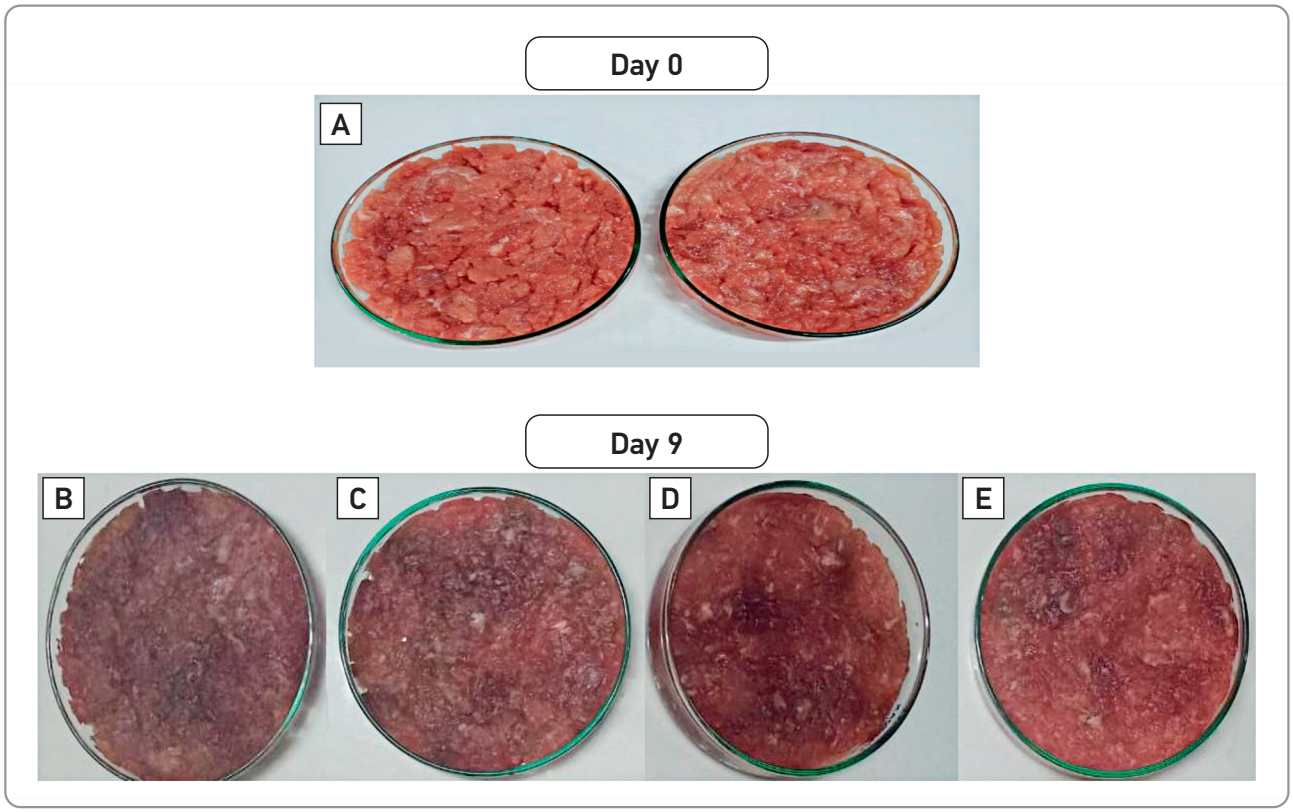

Figure 4. Effect of NaCAS films with/without *Berberis pseudumbellata* fruit (infused and non-infused) on the appearance of ground beef on day 9 of refrigerated (7°C) storage

Legend: A = ground beef on day 0 of storage; B = Control (sodium caseinate (NaCAS) only); C = NaCAS film infused with 1% of methanol fruit extract; D = NaCAS film infused with 1.5% of methanol fruit extract; E = NaCAS film infused with 3% of methanol fruit extract

p-value <0.001). The neutral but (for fresh beef) relatively high pH in the control ground beef (7.01) could be due to the formation of metabolic by-products by the native microbiota in the beef at the refrigeration temperature (*Ahn et al*., 2004).

Change in total viable count (TVC) of stored meat

On day 0, the TVC in ground beef was 1.85 log CFU/g, showing the acceptable quality of the beef utilized in the current study. Figure 3 shows the TVCs in all ground beef samples throughout the storage. The TVC in the control (NaCAS only) increased faster than that in ground beef covered with infused films. Compared with the control film, the NaCAS films successfully hindered the growth of TVC bacteria in the ground beef during storage. The lower TVCs noted in the ground beef covered with infused films might be due to the films behaving as an oxygen barrier, thus inhibiting the growth of aerobic bacteria. Bacterial growth in ground beef covered with 1.5% and 3% extract films was more effectively inhibited as compared to bacterial growth in the ground beef covered with 1% extract film. Studies have reported that films with incorporated active components, including phenolic compounds and other secondary metabolites obtained from plant extract, tend to effectively postpone microbial growth in foods including meat (*Umaraw et al*., 2020). Edible packaging films comprised of bioactive peptides and protein hydrolysates with added antimicrobial plant and spice extract have promoted the concept of safe food by demonstrating strong activity against the propagation of pathogenic microorganisms and lipid oxidation (*Aziz and Karboune*, 2018). Figure 5 shows the effect of control films (NaCAS only) and NaCAS extract-infused films on the overall appearance of stored ground beef on days 0 and 9 of storage.

Conclusion

This study revealed that the infusion of methanol extract of *B. pseudumbellata* fruit by high speed mixing imparts suitable physical characteristics to NaCAS biopolymer films. The infused films have strong antibacterial behaviour, thereby lim-

iting aerobic bacterial growth (keeping the TVC low) and maintaining the acidic pH of ground beef. The reported antioxidant activity in extract infused NaCAS film also suggests its efficacy against oxidation reactions. Probable changes in the sensory characteristics of ground beef stored under such films, including colour and other quality indicators, must be considered in future studies.

Razvoj, karakterizacija i ispitivanje antimikrobnog, antioksidativnog potencijala jestivog filma na bazi natrijum kazeinata infuziranog ekstraktom ploda Berberis psedumbellata i njegov uticaj na kvalitet sirove mlevene govedine tokom hlađenja

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A p s t r a k t: Ova studija predlaže da razvoj, karakterizaciju i istraživanje antimikrobnog i antioksidativnog potencijala filmova natrijum kazeinata (NaCAS) infuziranih sa ekstraktom voća Berberis pseudumbellata (B. pseudumbellata) i njegovu primenu na mlevenu govedinu koja se drži u frižideru (7°C) tokom 9 dana. Infuzija voćnog ekstrakta poboljšala je fizičke i optičke karakteristike filmova. Propustljivost i rastvorljivost filma su se smanjili dodatkom ekstrakta voća, dok je debljina filma porasla. U testu difuzije agar diska, rezultati su pokazali maksimalnu inhibiciju gram-pozitivnih bakterija uključujući Staphilococcus aureus i Bascillus subtilis u poređenju sa gram-negativnim Streptococcus tiphi i Escherichia coli. B. pseudumbellata je takođe pokazivala sve veću antioksidativnu aktivnost sa povećanjem koncentracije. Infuzirani filmovi su pokazali relativno veći antioksidativni potencijal kada su procenjeni pre njihove primene na govedinu. Međutim, razlike u antioksidativnoj aktivnosti filmova (pre i posle nanošenja) nisu bile značajne ($p<0,05$). Uzorak govedine umotan infuziranim kazeinatnim filmovima (1, 1,5 i 3%) održavao je kiseli pH od 5,88 i 5,65, dok je kontrola pokazala pH od (7,01) devetog dana skladištenja. NaCAS filmovi sa 3% ekstrakta takođe su kontrolisali ukupan broj bakterija u poređenju sa kontrolom ($p<0,05$) sa povećanom količinom ekstrakta voća.

Ključne reči: *Berberis Pseudumbellata, jestivi film, natrijum kazeinat, goveđe meso, antioksidans, broj bakterija.*

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References

Ahn, J., Grün, I. U. & Mustapha, A. (2004). Antimicrobial & antioxidant activities of natural extracts in vitro and in ground beef. *Journal of Food Protection,* 67 (1), 148–155, https://doi.org/10.4315/0362-028X-67.1.148

Andola, H. C., Rawal, R. S. & Bhatt, I. D. (2011). Comparative studies on the nutritive and anti-nutritive properties of fruits in selected Berberis species of West Himalaya, India. *Food Research International,* 44 (7), 2352–2356, https://doi.org/10.1016/j.foodres.2010.07.017

Andola, H. C., Rawal, R., Rawat, M., Bhatt, I. & Purohit, V. K. (2010). Variations of berberine contents in Berberis pseudumbellata: a high-value medicinal shrub of west Himalaya, India. *Medicinal Plants-International Journal of Phytomedicines and Related Industries,* 2 (2), 111–115, http://dx.doi.org/10.5958/j.0975-4261.2.2.017

Awan, M. S., Ali, S., Ali, A., Hussain, A. & Ali, M. (2014). A comparative study of barberry fruits in terms of its nutritive and medicinal contents from CKNP region, Gilgit-Baltistan, Pakistan. *Journal of Biodiversity and Environmental Sciences,* 5 (2), 9–17.

Aziz, M. & Karboune, S. (2018). Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: A review. *Critical Reviews in Food Science and Nutrition,* 58 (3), 486–511, https://doi.org/10.1080/10408398.2016.1194256

Bhuvana, P., Anuradha, V., Syed Ali, M., Suganya, V. & Sangeetha, P. (2018). Drug standardization and invitro anti-inflammatory and antibacterial activity of Chlorella vulgaris. *International Journal of Pharmacy and Biological Sciences*, 8 (1), 103–110.

Emam-Djomeh, Z., Moghaddam, A. & Yasini Ardakani, S. A. (2015). Antimicrobial activity of pomegranate (Punica granatum L.) peel extract, physical, mechanical, barrier and antimicrobial properties of pomegranate peel extract-incorporated sodium caseinate film and application in packaging for ground beef. *Packaging Technology and Science,* 28 (10), 869–881, https://doi.org/10.1002/pts.2145

Emiroğlu, Z. K., Yemiş, G. P., Coşkun, B. K. & Candoğan, K. (2010). Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. *Meat Science,* 86 (2), 283–288, https://doi.org/10.1016/j.meatsci.2010.04.016

Gutiérrez-del-Río, I., Fernández, J. & Lombó, F. (2018). Plant nutraceuticals as antimicrobial agents in food preservation: Terpenoids, polyphenols and thiols. *International Journal of Antimicrobial Agents,* 52 (3), 309–315, https://doi.org/10.1016/j.ijantimicag.2018.04.024

Hanani, Z. N., Roos, Y. H. & Kerry, J. P. (2014). Use and application of gelatin as potential biodegradable packaging materials for food products. *International Journal of Biological Macromolecules,* 71, 94–102, https://doi.org/10.1016/j.ijbiomac.2014.04.027

Hassan, B., Chatha, S. A. S., Hussain, A. I., Zia, K. M. & Akhtar, N. (2018). Recent advances on polysaccharides, lipids and protein based edible films and coatings: A review. *International Journal of Biological Macromolecules,* 109, 1095–1107, https://doi.org/10.1016/j.ijbiomac.2017.11.097

Helal, A., Desobry, S., Banon, S. & Shamsia, S. M. (2015). Antioxidant activity and bioaccessibility of phenols-enriched edible casein/caseinate coatings during in vitro digestion. *Journal of Dairy Research,* 82 (1), 56–63, https://doi.org/10.1017/S0022029914000557

Helal, A., Tagliazucchi, D., Conte, A. & Desobry, S. (2012). Antioxidant properties of polyphenols incorporated in casein/sodium caseinate films. *International Dairy Journal,* 25 (1), 10–15, https://doi.org/10.1016/j.idairyj.2011.12.002

Hosseini, M., Razavi, S. & Mousavi, M. (2009). Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. *Journal of Food Processing and Preservation,* 33 (6), 727–743, https://doi.org/10.1111/j.1745-4549.2008.00307.x

Kanmani, P. & Rhim, J.-W. (2014). Physical, mechanical and antimicrobial properties of gelatin based active nanocomposite films containing AgNPs and nanoclay. *Food Hydrocolloids,* 35, 644–652, https://doi.org/10.1016/j.foodhyd.2013.08.011

Kaya, M., Ravikumar, P., Ilk, S., Mujtaba, M., Akyuz, L., Labidi, J., Salaberria, A. M., Cakmak, Y. S & Erkul, S. K. (2018). Production and characterization of chitosan based edible films from Berberis crataegina's fruit extract and seed oil. *Innovative Food Science and Emerging Technologies*, 45, 287–297, https://doi.org/10.1016/j.ifset.2017.11.013

Khwaldia, K., Banon, S., Perez, C. & Desobry, S. (2004). Properties of sodium caseinate film-forming dispersions and films. *Journal of Dairy Science,* 87 (7), 2011-2016, https://doi.org/10.3168/jds.S0022-0302(04)70018-1

Kim, H.-E., Lee, J.-J., Lee, M.-J. & Kim, B.-S. (2019). Analysis of microbiome in raw chicken meat from butcher shops and packaged products in South Korea to detect the potential risk of foodborne illness. *Food Research International,* 122, 517–527, https://doi.org/10.1016/j.foodres.2019.05.032

Kristo, E., Koutsoumanis, K. P. & Biliaderis, C. G. (2008). Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on Listeria monocytogenes. *Food Hydrocolloids,* 22 (3), 373–386, https://doi.org/10.1016/j.foodhyd.2006.12.003

Mir, S. A., Dar, B., Wani, A. A. & Shah, M. A. (2018). Effect of plant extracts on the techno-functional properties of biodegradable packaging films. *Trends in Food Science and Technology,* 80, 141–154, https://doi.org/10.1016/j.tifs.2018.08.004

Norajit, K., Kim, K. M. & Ryu, G. H. (2010). Comparative studies on the characterization and antioxidant properties of biodegradable alginate films containing ginseng extract. *Journal of Food Engineering,* 98 (3), 377–384, https://doi.org/10.1016/j.jfoodeng.2010.01.015

Pellissery, A. J., Vinayamohan, P. G., Amalaradjou, M. A. R., and Venkitanarayanan, K. (2020). Spoilage bacteria and meat quality *Meat Quality Analysis* (pp. 307-334), Elsevier, https://doi.org/10.1016/B978-0-12-819233-7.00017-3

Pires, C., Ramos, C., Teixeira, B., Batista, I., Nunes, M. & Marques, A. (2013). Hake proteins edible films incorporated with essential oils: physical, mechanical, antioxidant and antibacterial properties. *Food Hydrocolloids,* 30 (1), 224–231, https://doi.org/10.1016/j.foodhyd.2012.05.019

Rad, F. H., Sharifan, A., Khodaiyan, F. & Ghahfarrokhi, I. S. (2017). Preparation and characterization of pullulan-soy protein concentrate blended film incorporated with Zataria multiflora and Artemisia biennis essential oils. *Jundishapur Journal of Natural Pharmaceutical Products*, 12 (4), e37802, https://doi.org/10.5812/jjnpp.37802

Rahmasari, Y. & Yemiş, G. P. (2022). Characterization of ginger starch-based edible films incorporated with coconut shell liquid smoke by ultrasound treatment and application for ground beef. *Meat Science,* 188, 108799, https://doi.org/10.1016/j.meatsci.2022.108799

Rawat, S., Andola, H., Giri, L., Dhyani, P., Jugran, A., Bhatt, I. D. & Rawal, R. S. (2014). Assessment of nutritional and antioxidant potential of selected vitality strengthening Himalayan medicinal plants. *International Journal of Food Properties,* 17 (3), 703–712, https://doi.org/10.1080/10942912.2012.654563

Rufino, M. S., Fernandes, F. A., Alves, R. E. & de Brito, E. S. (2009). Free radical-scavenging behaviour of some north-east Brazilian fruits in a DPPH system. *Food Chemistry*, 114 (2), 693–695, https://doi.org/10.1016/j.foodchem.2008.09.098

Sharma, R., Jafari, S. M. & Sharma, S. (2020). Antimicrobial bio-nanocomposites and their potential applications in food packaging. *Food Control,* 112, 107086, https://doi.org/10.1016/j.foodcont.2020.107086

Song, Y., Liu, L., Shen, H., You, J. & Luo, Y. (2011). Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (Megalobrama amblycephala). *Food Control,* 22 (3–4), 608–615, https://doi.org/10.1016/j.foodcont.2010.10.012

Tunç, S. & Duman, O. (2011). Preparation of active antimicrobial methyl cellulose/carvacrol/montmorillonite nanocomposite films and investigation of carvacrol release. *LWT-Food Science and Technology*, 44 (2), 465–472, https://doi.org/10.1016/j.lwt.2010.08.018

Tunc, S., Angellier, H., Cahyana, Y., Chalier, P., Gontard, N. & Gastaldi, E. (2007). Functional properties of wheat gluten/montmorillonite nanocomposite films processed by casting. *Journal of Membrane Science*, 289 (1–2), 159–168, https://doi.org/10.1016/j.memsci.2006.11.050

Umaraw, P. & Verma, A. K. (2017). Comprehensive review on application of edible film on meat and meat products: An eco-friendly approach. *Critical Reviews in Food Science and Nutrition,* 57 (6), 1270–1279, https://doi.org/10.1080/10408398.2014.986563

Umaraw, P., Munekata, P. E., Verma, A. K., Barba, F. J., Singh, V., Kumar, P. & Lorenzo, J. M. (2020). Edible films/coating with tailored properties for active packaging of meat, fish and derived products. *Trends in Food Science and Technology,* 98, 10–24, https://doi.org/10.1016/j.tifs.2020.01.032

Veiga-Santos, P., Silva, L. T., de Souza, C. O., da Silva, J. R., Albuquerque, E. C. & Druzian, J. I. (2018). Coffee-cocoa additives for bio-based antioxidant packaging. *Food Packaging and Shelf Life*, 18, 37–41, https://doi.org/10.1016/j.fpsl.2018.08.005

Zhang, M., Luo, W., Yang, K. & Li, C. (2022). Effects of sodium alginate edible coating with cinnamon essential oil nanocapsules and Nisin on quality and shelf life of beef slices during refrigeration. *Journal of Food Protection*, 85 (6), 896–905, https://doi.org/10.4315/JFP-21-380

Zhong, Y., Godwin, P., Jin, Y. & Xiao, H. (2020). Biodegradable polymers and green-based antimicrobial packaging materials: A mini-review. *Advanced Industrial and Engineering Polymer Research*, 3 (1), 27–35, https://doi.org/10.1016/j.aiepr.2019.11.002