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Deciphering the Antimicrobial and Antioxidant Potential: A Comparative Study of Free and Encapsulated Plantaricin and Sumac extract for Ground Beef Preservation

Ali Kazazi Mansour[1], Alireza Rahman[*1], Mohammadreza Khani,[1] Mania Salehifar[2]

[1]Department of Food Science and Technology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.
*Corresponding author

Abstract

This study explores a novel approach for ground beef preservation using a synergistic combination of sumac extract and plantaricin encapsulated within chitosan nanoparticles (SU+PL+CH). DLS and FTIR analyses confirmed successful encapsulation, with nanoparticles reaching up to 190 nm, signifying effective bioactive incorporation. The SU+PL+CH treatment exhibited significant ($p<0.05$) suppression of microbial growth across various populations, including Pseudomonas spp. (reduction by 5.63 log CFU/g), Enterobacteriaceae (reduction by 3.57 log CFU/g), and lactic acid bacteria (reduction by 3.26 log CFU/g) compared to the control group after 14 days of refrigerated storage (4°C). Additionally, the SU+PL+CH treatment demonstrably reduced lipid oxidation, as evidenced by lower thiobarbituric acid reactive substances (TBARS) values ($p<0.05$). These findings suggest a robust synergistic effect within the encapsulated formulation, surpassing the efficacy of individual components ($p<0.05$). This research not only corroborates previous reports on sumac extract's antibacterial properties and the preservative potential of chitosan-encapsulated agents but also unveils their enhanced potency within a controlled release system. This work contributes significantly to advancements in food safety and quality by offering a promising strategy for extending meat product shelf life through the utilization of natural antimicrobials.

Keywords: Food Preservation, Ground Beef , Shelf Life, Encapsulation, Chitosan Nanoparticles

Introduction

The global appetite for safe and natural food has grown exponentially in recent years. This shift reflects a growing awareness of the potential health risks associated with synthetic additives and preservatives commonly used to extend shelf life (Pellissery et al., 2020). Consumers are actively seeking alternatives that prioritize both safety and well-being. This trend presents a significant opportunity within the food industry, particularly for meat products – a crucial component of many diets, yet highly susceptible to spoilage and contamination (Shao et al., 2021).

Foodborne illnesses caused by pathogenic bacteria present a major public health concern. Raw meat, due to its high nutritional content and favorable conditions for microbial growth, is particularly at risk. Traditional preservation methods, such as refrigeration, salting, and drying, offer limited effectiveness in preventing spoilage and extending shelf life. Chemical preservatives, while effective in controlling microbial growth, raise concerns about potential health risks. This dilemma underscores the need for innovative and safe solutions to ensure food safety and quality (Li et al., 2020).

Fortunately, nature offers a promising answer in the form of Lactic Acid Bacteria (LAB). These “good” bacteria produce a range of beneficial metabolites, including organic acids, bacteriocins, and hydrogen peroxide, which

exhibit potent antimicrobial activity against pathogenic microorganisms. Among these metabolites, bacteriocins produced by LAB have garnered significant attention due to their broad-spectrum activity, safety for human consumption, and potential applications in food preservation (Mahmud et al., 2023).

One such bacteriocin, plantaricin, is produced by *Lactobacillus plantarum*. This powerful compound demonstrates impressive antimicrobial activity against a wide range of foodborne pathogens, including *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus* (Abdulhussain Kareem & Razavi, 2020). Studies have shown its effectiveness in controlling microbial growth and extending the shelf life of various food products, including meat, dairy, and vegetables (Du et al., 2022).

However, research suggests that the delivery method of plantaricin can significantly impact its effectiveness. Chitosan, a natural biopolymer derived from chitin (found in crustacean shells), offers a promising solution. Chitosan possesses inherent antimicrobial properties and the ability to encapsulate other compounds, potentially enhancing their stability and controlled release. This study aims to investigate the synergistic effects of plantaricin encapsulated in chitosan when combined with sumac extract, a natural source of antioxidants (Wrońska et al., 2021).

Sumac extract, obtained from the sumac plant, has been used for centuries as a culinary spice and traditional medicine. Recent research suggests it possesses potent antioxidant properties that can help prevent lipid oxidation in food products. Lipid oxidation, a major contributor to spoilage and quality deterioration in meat, significantly impacts flavor, texture, and nutritional value (Özogul et al., 2022) (Hashim & Fadhil, 2021). By combining sumac extract with plantaricin encapsulated in chitosan, we aim to achieve a synergistic effect: plantaricin addresses the microbial threat, while sumac extract combats lipid oxidation, leading to a more effective and holistic approach to extending the shelf life of ground beef.

By unraveling the synergistic effects of these natural preservatives—free and encapsulated plantaricin and sumac extract—this study aimed to establish a comprehensive approach to extending the shelf life of ground beef during refrigerated storage. Through a series of experiments, we evaluated the individual and combined effects of these interventions on the key parameters impacting ground beef quality: antimicrobial activity, antioxidant activity, microbial growth, lipid oxidation, and overall shelf life.

Material and methods

Sumac Extract Preparation

In this study, fresh sumac fruits (*Rhus coriaria L.*) were procured from the Tehran University Medicinal Plant Garden. The fruits underwent thorough washing, drying, and subsequent grinding to obtain a fine powder. The extraction process involved mixing 250 g of the sumac powder with 700 mL of alcohol and 300 mL of distilled water. After vigorous shaking for 24 hours, the mixture was incubated at 40°C in a water bath for an additional hour. Following cooling, the extract was filtered using a paper filter. The solvent was then removed using a rotary evaporator (Laborata 4003; Heidolph, Schwabach), and the resulting extract was stored at 4°C until further use (Mojaddar Langroodi & Tajik, 2017).

Plantaricin

Recombinant plantaricin, a bacteriocin purified from Lactobacillus plantarum ATCC BAA-793, was purchased from MyBioSource (USA) and stored at -20°C until use.

Preparation of Chitosan Nanoparticles Containing Sumac Extract and Plantaricin (C-S-P)

Chitosan nanoparticles loaded with sumac extract and plantaricin were synthesized using a modified gelatin ionotropic method based on previous studies (Pedroso-Santana & Fleitas-Salazar, 2020), with minor adjustments. Initially, 1 g of commercially available chitosan (Sigma) was dissolved in 50 mL of 1% (v/v) glacial acetic acid under continuous stirring at 40°C until a clear solution formed. Next, 0.5 g of pre-prepared sumac extract was added to the chitosan solution and mixed for 60 minutes (referred to as “Mixture A”). To encapsulate the active components, 0.2 g of sodium tripolyphosphate (TPP) dissolved in 20 mL of deionized water was gradually added dropwise to Mixture A (at a rate of 1 mL/min) while vigorously stirring with an ultrasonic homogenizer. The

resulting mixture underwent centrifugation at 13,000 rpm for 15 minutes, followed by thorough washing with deionized water to remove excess TPP. The resulting C-S-P nanoparticles were collected for further use {Budi, 2020 #6040}.

Nanoparticle Characterization

Fourier-transform infrared (FTIR) spectroscopy was employed to characterize the functional groups and potential interactions within the chitosan-sumac-plantaricin (C-S-P) nanoparticles. In addition to FTIR analysis, dynamic light scattering (DLS) was used for further characterization of the C-S-P nanoparticles. A Shimadzu UV-1800 spectrophotometer (Japan) was utilized for FTIR analysis, while a separate instrument was used for DLS measurements {Budi, 2020 #6040}.

Preparation of Meat Samples and Their Treatment

Boneless beef samples from a local slaughterhouse were chilled at 4°C for 1 hour before analysis. The meat was then minced according to Iranian standard number 4622. Next, the samples were randomly divided into five groups (n=5) for treatment: 1) Control (no extract, plantaricin, or chitosan), 2) 4% sumac extract, 3) Plantaricin, 4) Combination of sumac extract and free plantaricin, and 5) Combination of 4% sumac extract and chitosan-encapsulated plantaricin. Following a 3-minute application, the treated samples were dewatered, aerobically packaged, and stored at a refrigeration temperature (4°C) for 14 days. Microbiological analyses were performed on days 0, 1, 3, 7, and 14 (Mojaddar Langroodi & Tajik, 2017).

Microbial Analysis of Minced Meat Samples

To assess the microbial profile of the samples, ten grams were aseptically weighed and homogenized in sterile 0.1% peptone water using a stomacher (Mojaddar Langroodi & Tajik, 2017). Serial dilutions of the homogenate were then plated onto specific media to target different bacterial populations. Pseudomonas spp. were enumerated on Pseudomonas agar supplemented with a selective agent (CFC) after incubation at 25°C for 48 hours. Lactobacilli were counted on MRS agar at 30°C for 48 hours. Enterobacteriaceae were enumerated using the pour-plate method on VRBG agar at 37°C for 24 hours. Yeasts and molds were identified and counted on RBC agar incubated at 25°C in the dark for 3-5 days. Finally, total viable counts were determined using plate count agar after incubation at 30°C for 2 days {Abdelhady, 2023 #6041}.

TBA Value Determination for Lipid Oxidation

To assess lipid oxidation, we employed the thiobarbituric acid reactive substances (TBARS) method. Ten grams of each meat sample were homogenized with 1 mL of butylated hydroxytoluene (BHT) and 35 mL of trichloroacetic acid (5.00%) in a blender. The resulting mixture was filtered, and 5 mL of a TBA solution (0.02 M) was added to 5 mL of the filtrate. After incubation at 100°C for 60 minutes, the absorbance of the samples was measured at a wavelength of 532 nm.

Peroxide Value Analysis

Lipid oxidation was further assessed by measuring the peroxide value following the International Dairy Federation standard method 74a. Briefly, a meat sample (weight adjusted between 0.01 and 0.30 grams based on the expected peroxidation level) was mixed with 9.80 mL of a 30:70 (v/v) chloroform-methanol solution using a shaker. Subsequently, 50 µL of ammonium thiocyanate solution and 50 µL of an iron solution (prepared by dissolving 0.4 g of barium chloride and 0.5 g of iron (II) sulfate in 2 mL of hydrochloric acid and diluting to 100 mL with distilled water) were added and mixed. Following a 5-minute incubation at room temperature, the sample absorbance was measured at 500 nm using a spectrophotometer {Badawy, 2019 #6042}.

Total Volatile Nitrogen (TVN) Determination

The TVN content of the meat samples was evaluated using a macro Kjeldahl apparatus. Ten grams of each sample were steam distilled in 300 mL of water containing 3 g of magnesium oxide {Badawy, 2019 #6042}.

Statistical Analysis

All experiments were performed in triplicate. Data were analyzed using SPSS software (version 21.0, IBM Corp., USA). One-way ANOVA followed by Tukey's post-hoc test was employed to assess significant differences ($p<0.05$) between treatment groups.

Results

Nanoparticle Characterization

FTIR Analysis: FTIR spectroscopy was employed to investigate the functional groups and potential interactions within the chitosan nanoparticles (C-NPs), sumac extract-loaded C-NPs (C-S NPs), plantaricin-loaded C-NPs (C-P NPs), and the combined sumac extract and plantaricin-loaded C-NPs (C-S-P NPs). This analysis aimed to identify potential changes in the chemical composition of the nanoparticles upon encapsulation with sumac extract and/or plantaricin. As shown in Figure 1, the FTIR spectra revealed distinct peaks corresponding to various functional groups within the nanoparticles.

DLS Analysis: DLS was used to determine the hydrodynamic diameter and size distribution of the C-S-P NPs. The data revealed that the size of free C-NPs ranged from 4.187 nm to 78 nm, with a peak at 7.51 nm. Encapsulation with sumac extract and/or plantaricin resulted in an increase in the size distribution, with the combined C-S-P NPs exhibiting the largest size range (10.10 nm to 190 nm) and a peak at a higher diameter compared to free C-NPs, suggesting the successful loading of sumac extract and plantaricin within the C-NPs.

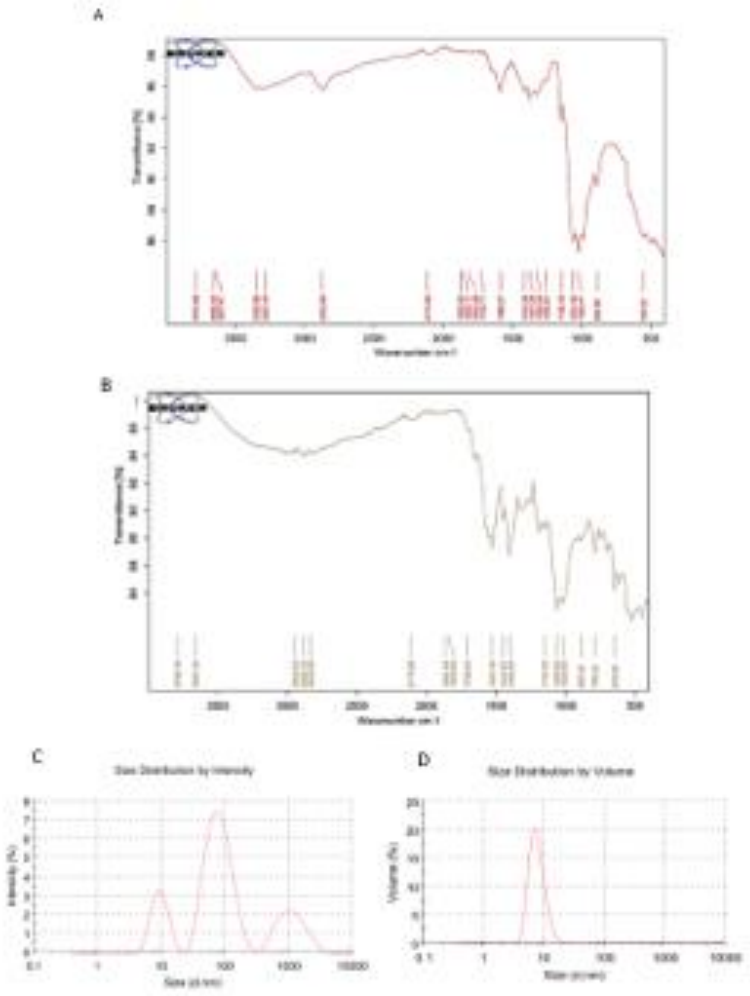

Figure 1. Characterization of Chitosan Nanoparticles. FTIR spectra of free chitosan nanoparticles (A) and sumac extract + plantaricin-encapsulated chitosan nanoparticles (B). Size distribution of nanoparticles by intensity (C) and volume (D).

Microbiological analysis

Data from Table 1 highlight the effectiveness of the combined treatment (4% sumac extract + chitosan-encapsulated plantaricin) in reducing bacterial growth on beef stored at 4°C. This treatment consistently displayed the lowest TVC throughout storage, with significant reductions ($p < 0.05$) compared to other treatments by day 3, suggesting the superior efficacy of the combined approach in suppressing overall bacterial growth. Interestingly, the combined treatment also exhibited the lowest counts of lactic acid bacteria, indicating its impact on both spoilage and potentially beneficial bacterial populations. Furthermore, the combined treatment demonstrated the most significant reductions ($p<0.05$) in Enterobacteriaceae, Pseudomonas, yeasts, and molds compared to controls throughout storage. This observation underscores the broad-spectrum antimicrobial activity of the combined sumac extract and chitosan-encapsulated plantaricin. It is noteworthy that while other individual treatments (chitosan, sumac extract alone, or plantaricin) showed some reductions in bacterial counts, the combined approach consistently demonstrated the most potent and significant effect in retarding bacterial growth on stored beef ($p<0.02$).

Table 1: Impact of chitosan, sumac extract, plantaricin, and their combination on bacterial growth during storage at 4 ˚C at different time points.

Parameter	Group	Storage time (day)				
		0	1	3	7	**14**
Total viable counts	Control	4.84 ±0.04 ^{Aa}	6.74 ±0.06 ^{Aa}	8.96 ±0.04^{Ba}	9.04 ±0.06^{Ba}	**9.15 ±0.13^{Ba}**
	Chitosan	4.61 ±0.07 ^{Ab}	5.71 ±0.16 ^{Bb}	5.19 ±0.02^{Cb}	7.11 ±0.05^{Db}	**8.17 ±0.11^{Eb}**
	4% sumac	4.75 ±0.02 ^{Ac}	4.15 ±0.02 ^{Ac}	4.87 ±0.04^{Ac}	5.63 ±0.03 ^{Bc}	**5.77 ±0.05^{Bc}**
	Plantaricin	4.54 ±0.12 ^{Aab}	4.18 ±0.05 ^{Ac}	5.07 ±0.04 ^{Bb}	5.52 ±0.08 ^{Bc}	**5.71 ±0.02 ^{Bc}**
	4% SU+PL	4.62 ±0.01 ^{Ab}	D ±0.11 ^{Ad}	4.63 ±0.09 ^{Ac}	4.55 ±0.13 ^{Ad}	**5.29 ±0.01 ^{Bc}**
	4% SU+PL+CH	4.51 ±0.12 ^{Aab}	4.58 ±0.03 ^{Ae}	4.59 ±0.09 ^{Ac}	4.61±0.07 ^{Ad}	**4.84 ±0.03 ^{Bd}**
Lactic acid bacteria	C	3.65 ±0.02 ^{Aa}	4.02 ±0.12 ^{Ba}	5.19 ±0.13 ^{Ca}	6.59 ±0.01 ^{Da}	**7.63 ±0.01 ^{Ea}**
	CH	3.61 ±0.02 ^{Aa}	4.19 ±0.01 ^{Ba}	5.23 ±0.12 ^{Ca}	6.98 ±0.01 ^{Da}	**7.07 ±0.1 ^{Eb}**
	4% SU	3.47 ±0.02 ^{Aa}	3.65 ±0.01 ^{Ab}	3.82 ±0.04 ^{Ab}	4.03 ±0.02 ^{Bb}	**5.07 ±0.03 ^{Cc}**
	PL	3.39 ±0.12 ^{Aa}	3.58 ±0.05 ^{Ab}	4.30 ±0.03 ^{Bc}	5.06 ±0.04 ^{Cc}	**5.11 ±0.07 ^{Dd}**

	4% SU+PL	3.41 ±0.02 Aa	3.75 ±0.01 Ab	3.84 ±0.11 Ab	4.05 ±0.07 Bb	**5.17 ±0.13 Cd**
	4% SU+PL+CH	3.72 ±0.01 Ab	3.70 ±0.02 Ab	3.79 ±0.03 Ab	4.04±0.04 Bb	**4.18 ±0.02 Be**
Enterobacteriaceae	C	3.82 ±0.01 Aa	4.54 ±0.02 Ba	5.73 ±0.14 Ca	7.19 ±0.02 Da	**8.11 ±0.03 Ea**
	CH	3.65 ±0.01 Aa	4.71 ±0.07 Ba	5.19 ±0.09 Ca	6.57 ±0.05 Db	**6.34 ±0.03 Eb**
	4% SU	3.41 ±0.01 Aa	3.77 ±0.03 Bb	4.71 ±0.04 Cb	5.03 ±0.06 Dc	**6.18 ±0.03 Eb**
	PL	3.39 ±0.04 Aa	4.11 ±0.01 Ba	5.15 ±0.01 Ca	5.54 ±0.02 Dc	**5.68 ±0.13 Ec**
	4% SU+PL	3.45 ±0.01 Aa	3.94 ±0.01 Aa	4.54 ±0.02 Bb	5.36 ±0.07 Cc	**5.48 ±0.11 Dc**
	4% SU+PL+CH	3.51 ±0.00 Aa	3.69 ±0.00 Ab	4.13 ±0.02 Ab	4.55±0.07 Bd	**4.74 ±0.01 Bd**
Pseudomonas	C	2.77 ±0.01 Aa	3.47 ±0.02 Ba	3.69 ±0.10 Ca	4.54 ±0.01 Da	**5.16 ±0.00 Ea**
	CH	2.60 ±0.00 Aa	3.14 ±0.02 Ba	3.51 ±0.01 Ca	4.17 ±0.02 Db	**4.34 ±0.11 Eb**
	4% SU	2.41 ±0.01 Aa	3.15 ±0.03 Ba	3.70 ±0.04 Ca	4.63 ±0.06 Db	**4.79 ±0.03 Db**
	PL	2.61 ±0.04 Aa	3.19 ±0.04 Ba	4.08 ±0.01 Cb	4.19 ±0.01 Db	**4.52 ±0.01 Db**
	4% SU+PL	2.74 ±0.01 Aa	3.16 ±0.05 Ba	3.61 ±0.03 Ca	3.84 ±0.02 Dc	**4.77±0.01 Eb**
	4% SU+PL+CH	2.51 ±0.03 Aa	3.19 ±0.01 Ba	3.44 ±0.08 Ca	3.96±0.02 Cc	**4.14±0.02 Db**
Yeasts and Molds	C	3.02 ±0.08 Aa	4.61 ±0.02 Ba	5.60 ±0.04 Ca	7.29 ±0.01 Da	**8.13 ±0.13 Ea**
	CH	3.48 ±0.01 Aa	4.58 ±0.02 Ba	5.68 ±0.09 Ca	7.13 ±0.01 Da	**7.70 ±0.03 Eb**
	4% SU	3.29 ±0.01 Aa	3.73 ±0.01 Ab	5.02 ±0.00 Bb	6.63 ±0.02 Cb	**7.32 ±0.01 Eb**

PL	3.57 ±0.00 Aa	4.17 ±0.01 Ba	5.83 ±0.01 Cb	6.74 ±0.02 Db	**7.19 ±0.10 Db**
4% SU+PL	3.62 ±0.01 Aa	3.90 ±0.02 Cb	4.14 ±0.01 Bc	5.58 ±0.01 Cc	**6.63 ±0.01 Dd**
4% SU+PL+CH	3.41 ±0.02 Aa	3.54 ±0.02 Ab	4.72 ±0.03 Bc	4.98±0.04 Bc	**5.70 ±0.02 Cd**

The presence of different uppercase letters within a row and lowercase letters in the same column signifies statistically significant differences ($p < 0.05$) between the corresponding treatments.

***Treatments: Control (C), chitosan (CH), sumac extract (SU), Plantaricin (PL).**

Lipid Oxidation Analysis

Thiobarbituric Acid Reactive Substances analysis was employed to assess lipid oxidation in the beef samples during storage. Table 2 presents the detailed results. TBARS values indicate the extent of secondary lipid damage. Throughout the 14-day storage period, all treated samples exhibited significantly lower TBARS values compared to the control group ($p < 0.05$). Notably, samples treated with chitosan, 4% sumac extract, and plantaricin displayed the lowest levels of lipid oxidation ($p<0.05$). Conversely, the control sample experienced the most rapid lipid oxidation, with the greatest increase observed between days 7 and 14 of storage.

Table 2: Changes in Thiobarbituric Acid Reactive Substances (TBARS) (mg MDA per kg^{-1}) in Beef Treated with Different Groups During Storage at 4°C.

Treatment group	Storage time (day)				
	0	1	3	7	14
Control	0.31 ±0.01 a	0.74 ±0.00 a	1.06 ±0.01 a	1.64 ±0.06 a	2.15 ±0.13a
Chitosan	0.29 ±0.02 a	0.61 ±0.02 b	0.97 ±0.07b	1.11 ±0.03 b	1.60 ±0.01 b
4% sumac	0.27 ±0.02 a	0.49 ±0.01 c	0.91 ±0.04 c	1.14 ±0.05 b	1.55 ±0.05 c
Plantaricin	0.30 ±0.02 a	0.48 ±0.05 c	0.63 ±0.04 b	1.24 ±0.08 b	1.51 ±0.02 c
4% SU+PL	0.28 ±0.00 a	0.44 ±0.11 c	0.68 ±0.02 b	1.21 ±0.13 c	1.30 ±0.05 d
4% SU+PL+CH	0.28 ±0.05 a	0.30 ±0.00 d	0.57 ±0.09 d	0.98±0.07 d	1.15 ±0.04 e

Different letters in each column indicate a statistically significant difference ($p < 0.05$). *Treatments: Control (C), chitosan (CH), sumac extract (SU), Plantaricin (PL).

Lipid Peroxidation

Lipid oxidation, as measured by peroxide value, was significantly higher ($p < 0.05$) in the control group compared to treated samples throughout storage (Fig. 2). This difference was particularly pronounced on day 7. Notably, all samples initially exhibited an increase in PV within the first week, followed by a subsequent decrease. The control

group displayed the most significant rise, with PV increasing from 0.41 to 2.63 meq peroxides/kg lipid on day 7 before declining to 0.61 by day 14. Importantly, the combination of 4% sumac extract and chitosan-encapsulated plantaricin demonstrated the lowest PV among all treatments ($p<0.01$).

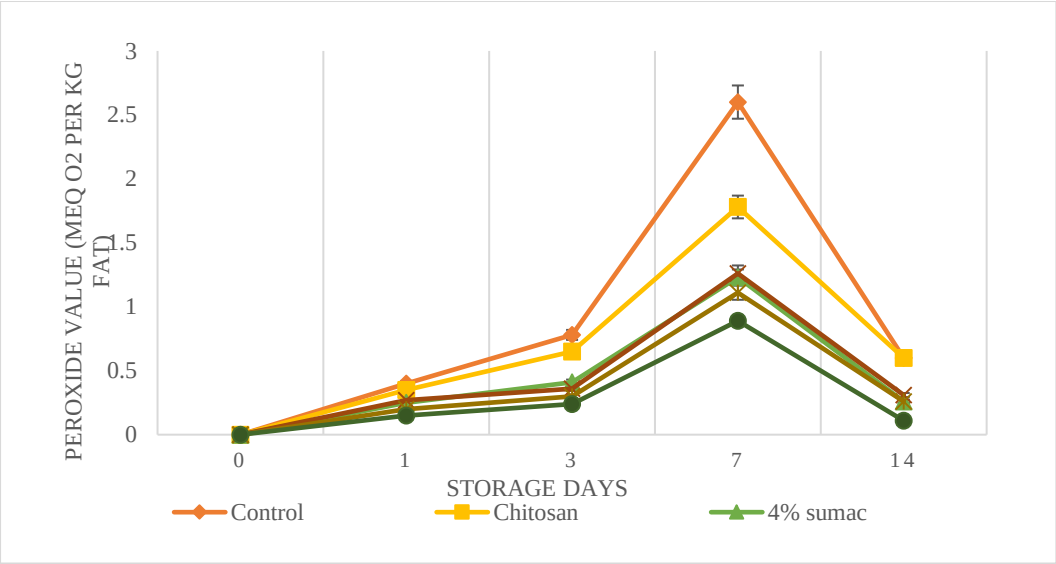

Figure 2: Changes in peroxide value (milliequivalents of oxygen per kilogram of fat) in beef treated with different groups during storage at 4°C.

Total Volatile Nitrogen

Analysis of TVN, an indicator of overall bacterial growth, revealed acceptable initial meat freshness in all treatment groups, as evidenced by the TVN value of 8.16 mg/100 g in control samples on day 1 (Fig. 3). Over the storage period, TVN values in all groups exhibited a gradual increase. However, the combined treatment with 4% sumac extract and chitosan-encapsulated plantaricin (SU+PL+CH) displayed a significantly lower ($p < 0.05$) final TVN value of 14.60 mg/100 g on day 14 compared to the control group, which reached 26.7 mg/100 g. This reduction in the SU+PL+CH group might be attributed to its potentially lower initial bacterial population, as suggested by the lower TVC observed in this treatment. Additionally, the control group exhibited a more rapid rise in TVN, reaching 19.6 mg/100 g of meat by day 7, indicating faster bacterial growth compared to the treated samples.

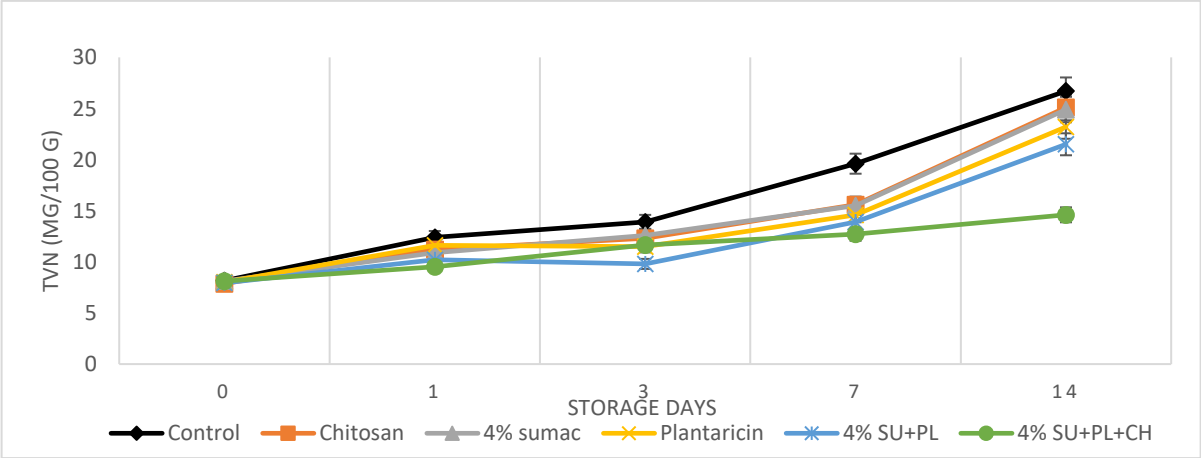

Figure 3: Changes in Total Volatile Nitrogen (mg per 100 g) in beef treated with different groups during storage at 4°C.

Discussion

This study investigated a novel preservation method that encapsulates plantaricin and sumac extract to prolong the shelf life of refrigerated ground beef. The findings underscore the potential of this combined approach to inhibit microbial growth, reduce lipid oxidation, and enhance meat quality throughout the storage period.

The joint application of 4% sumac extract and plantaricin, both encapsulated in chitosan (SU+PL+CH), exhibited the most significant and consistent reduction in total viable counts across various bacterial populations (Pseudomonas, Enterobacteriaceae, Lactic Acid Bacteria, yeasts, and molds) compared to the control and individual treatments with chitosan, sumac extract, or free plantaricin. This suggests a synergistic interaction between the encapsulated sumac extract and plantaricin, likely due to the combined effects of the antioxidant properties of sumac extract and the broad-spectrum antimicrobial activity of plantaricin.

All treated samples showed significantly lower thiobarbituric acid reactive substances values than the control group, indicating a decrease in lipid oxidation throughout storage. The combined treatment (SU+PL+CH) demonstrated the most substantial reduction in peroxide value, further signifying reduced lipid oxidation. These results suggest that both sumac extract and plantaricin, when encapsulated, contribute to the overall antioxidant activity, potentially delaying lipid peroxidation and preserving meat quality.

Our findings align with several studies that support the antibacterial properties of sumac extract. For instance, Moshtagh et al. (2017) demonstrated the effectiveness of sumac ethanol extract against *E. coli* in both food and laboratory models (Moshtaghi et al., 2017). Similarly, other studies reported the antibacterial activity of sumac extract against various bacteria, including *E. coli* (Fazeli et al., 2007) (Perrone et al., 2022) (Alsamri et al., 2021). These studies, along with ours, highlight the broad-spectrum antibacterial potential of sumac extract (Abd Elaal et al., 2024).

Moreover, research suggests the efficacy of sumac extract in enhancing food preservation. Bazargani et al. (2023) found that films containing sumac extract and copper nanoparticles significantly reduced bacterial growth in ground beef during storage (Bazargani-Gilani & Izadi, 2023). Mahlooji et al. (2020) observed similar results with sumac extract inhibiting *E. coli* growth in minced meat (Mahlooji et al., 2020). Our study reinforces these findings, demonstrating sumac extract's ability to inhibit bacterial growth, albeit at slightly lower concentrations compared to previous reports. Notably, our research expands on the known antibacterial properties by showing effectiveness against Staphylococcus aureus in addition to *E. coli*. This suggests a wider range of bacteria susceptible to the antimicrobial effects of sumac extract.

Numerous studies support the effectiveness of encapsulated plantaricin in inhibiting bacterial growth in meat (Amer et al., 2021) (Wiman et al., 2023). A recent study by Zhao et al. showed that chitosan-encapsulated plantaricin displayed broad-spectrum antibacterial activity against Listeria monocytogenes in veal meat (Zhao et al., 2022). It achieved this by damaging the bacteria's cell membrane and causing cell death. Additionally, it improved the overall quality of the meat, suggesting its potential to extend shelf life.

Furthermore, research has explored the use of chitosan nanoparticles containing plant extracts to inhibit Enterobacteriaceae growth. Studies by Langroodi et al. (2018), Tavassoli et al. (2023) (Tavassoli et al., 2023), Chen et al. (2021) (Chen et al., 2021), Sayadi et al. (2021) (Sayadi et al., 2021), and Mojaddar et al. (2017) all demonstrated the effectiveness of this approach in protein products. Mojaddar et al. (2017) specifically observed a decrease in *E. coli* in veal treated with chitosan-containing plant extracts (Mojaddar Langroodi & Tajik, 2017). These findings align with the present study's results, which showed a significant reduction in *E. coli* growth in the group treated with the combination of sumac extract and plantaricin encapsulated in chitosan.

The combined treatment (SU+PL+CH) had the most pronounced effect in inhibiting overall bacterial growth and reducing lipid oxidation compared to the control and individual treatments. This could potentially extend the shelf life of ground beef during refrigerated storage. These findings raise the question of how encapsulation enhances their effectiveness. Several factors likely contribute to this phenomenon. Firstly, the positive charge of chitosan nanoparticles facilitates their interaction with negatively charged bacterial membranes (Abdelhady et al., 2023) (Safari et al., 2023). This allows for better penetration and delivery of the encapsulated antimicrobials directly to

their target sites within the bacteria, bypassing the cell wall - a major hurdle for many traditional antimicrobials. Secondly, the chitosan matrix acts as a controlled release system, gradually releasing the sumac extract and plantaricin over time (Esmaeili et al., 2021) (Diao et al., 2020). This sustained release maintains a continuous presence of the antimicrobials at the target site, potentially leading to a more potent effect compared to a single larger dose (Badawy et al., 2020). Finally, encapsulation protects sumac extract and plantaricin from degradation by enzymes or environmental factors, potentially increasing their stability and overall efficacy (Esmaeili et al., 2021). By combining these effects, chitosan nanoparticles offer a promising platform to enhance the delivery and efficacy of natural antimicrobials, paving the way for novel food preservation strategies.

Conclusion

In conclusion, our study presents a novel preservation method that encapsulates plantaricin and sumac extract, demonstrating its potential to extend the shelf life of refrigerated ground beef. The combined application of these encapsulated antimicrobials in chitosan (SU+PL+CH) significantly reduced bacterial populations and lipid oxidation, thereby enhancing meat quality throughout the storage period. Our findings align with previous research on the antibacterial properties of sumac extract and the effectiveness of chitosan-encapsulated plantaricin. Importantly, our study expands on these findings by demonstrating the synergistic interaction between the encapsulated sumac extract and plantaricin, and their enhanced efficacy when delivered via chitosan nanoparticles. This research paves the way for novel food preservation strategies, utilizing natural antimicrobials in a controlled release system to inhibit bacterial growth, reduce lipid oxidation, and ultimately improve food safety and quality. Further research is needed to understand the precise mechanisms of action and to optimize the encapsulation process for industrial applications.

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