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Investigation of the Antimicrobial Properties of Plantaricin, Derived from *Lactobacillus plantarum*, in Both its Free Form and Nano Encapsulated form Utilizing Chitosan and Sumac Extract, on the Shelf-Life of Ground Beef During Refrigerated Storage

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ABSTRACT: The preservation of perishable food items, particularly meat, has consistently been regarded as a matter of significant importance. This study aimed to investigate the combined effects of plantaricin, derived from *Lactobacillus plantarum*, in both free and nanoencapsulated forms, in conjunction with chitosan and sumac extract, on the shelf life of ground beef during refrigerated storage. In the present study, the hydroalcoholic extract of sumac was subjected to extraction and subsequently encapsulated in chitosan nanoparticles. This encapsulation was performed both independently and in conjunction with plantaricin, utilizing a range of concentrations. Subsequently, the physical properties of the nanoparticles were analyzed employing established methodologies, including Fourier Transform Infrared Spectroscopy (FTIR). The minimum inhibitory concentration (MIC) of sumac and plantaricin, in both free and encapsulated forms, was evaluated against the bacterial strains *Staphylococcus aureus* and *Escherichia coli*. In conclusion, sumac, along with both free and encapsulated forms of plantaricin, was incorporated into ground beef across various experimental groups. The alterations in the composition and quality of the beef over time at refrigerated temperatures were systematically evaluated in comparison to the control group. The findings from the Fourier Transform Infrared (FTIR) spectroscopy investigation of free chitosan and chitosan encapsulated with a combination of sumac extract and plantaricin provided confirmation of the encapsulation process. The diameter of free chitosan nanoparticles was measured to be approximately 7.51. Following the incorporation of the sumac extract and plantaricin combination, an increase in the size of the encapsulated chitosan nanoparticles was observed, with the maximum size recorded escalating from 10.10 nm to 190 nm. The analysis of total bacterial counts in minced meat samples treated with sumac, plantaricin, and their combinations—both in free form and encapsulated within chitosan—demonstrated that all experimental treatments yielded total bacterial counts significantly lower than the control group ($p < 0.001$) The antimicrobial efficacy of this combination against both gram-positive and gram-negative bacterial strains, including *Escherichia coli* and Staphylococcus aureus, in addition to its effects on fungi and yeasts, has the potential to enhance the shelf life of minced red meat and contribute to its quality stability.

Keywords: *Chitosan, Meat, Plantaricin, Sumac*

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Introduction

Meat sources represent a significant category of food that frequently plays a role in the transmission of foodborne infections. For instance, the contamination of raw chicken with Listeria monocytogenes, a bacterium that is among the most notable etiological agents of foodborne illnesses and is responsible for listeriosis, presents substantial health risks, particularly for vulnerable populations such as the elderly, pregnant women, and individuals with compromised immune systems (Heredia and García, 2018). Salmonella typhimurium is recognized as a significant pathogen associated with meat products and is the causative agent of salmonellosis, thereby representing a substantial threat to public health. The risks associated with contamination by these significant pathogenic bacteria can be effectively mitigated through the implementation of robust manufacturing practices. One of the approaches employed involves the utilization of non-pathogenic bacteria as preservative cultures. The utilization of lactic acid bacteria has been demonstrated to be effective in preserving the safety and quality of food products, facilitating the development of novel flavor profiles, and enhancing their nutritional properties. The antagonistic properties exhibited by lactic acid bacteria can be primarily attributed to the decrease in food pH that occurs as a consequence of lactic acid production, which is the principal metabolite generated during the fermentation of carbohydrates (Hashempour-Baltork *et al.*, 2019).

Lactobacillus plantarum is a significant species of lactic acid bacteria that plays a pivotal role in the fermentation of meat products. This bacterium exhibits a notable impact on pathogenic organisms, including Salmonella, Listeria monocytogenes, *Escherichia coli*, and several other pathogens. *Lactobacillus plantarum* has the potential to serve as a starter culture in the fermentation processes of diverse meat products. In addition to enhancing organoleptic properties, the process can also augment the safety of the final products by facilitating the production of lactic acid and other naturally occurring antimicrobial compounds (Zamani *et al.*, 2022). The predominant bacteriocin-producing microorganisms are lactic acid bacteria (LAB), which possess significant potential for application in food preservation due to their classification as Generally Recognized as Safe (GRAS) by regulatory authorities. Bacteriocins are ribosomally synthesized peptides or proteins exhibiting antimicrobial activities, which are produced by a diverse range of bacterial species, notably including *Lactobacillus plantarum*. They exhibit a diverse array of inhibitory properties, which has contributed to their extensive application in food preservation practices on a global scale (Ying, 2022).

In response to the increasing consumer preference for natural foods characterized by high microbiological quality, along with the implementation of stringent government regulations, food manufacturers are encountering significant challenges in maintaining food safety. Consequently, in response to growing consumer apprehensions regarding chemical additives, there has been a substantial increase in the investigation and assessment of natural microbial substances, including bacteriocins. The application of bacteriocins within biological preservation systems addresses consumer demand for natural preservatives and serves as an additional safety measure for minimally processed products that rely on refrigeration as their primary mode of preservation. Furthermore, bacteriocins

possess the ability to eliminate target microorganisms by disrupting their outer membranes, and the resultant fragments do not engage with target cells. Consequently, these compounds exhibit limited potential for resistance development, positioning them as a promising alternative in addressing the escalating issue of microbial resistance to antibiotics (Ghasemi and Mahasti, 2015).

Furthermore, since bacteriocins can kill target microorganisms by destroying the outer membrane and their fragments do not interact with target cells, they have limited resistance and could be a potential solution to the growing problem of microbial resistance to antibiotics.

Pediocin, synthesized by Pediococcus lactis, represents an alternative bacteriocin that can be utilized as a commercial ingredient in the biological preservation of various food products, including sausages and fermented meats. In this context, following nisin, pediocin has been the subject of extensive research with respect to its antimicrobial efficacy against Listeria species. The effectiveness of this strain has been empirically demonstrated through its application as a starter culture in various fermented meat products, such as sausages, and in dairy products, including cheeses (Karwowska and Kononiuk, 2020).

In the meat industry, the use of pediocin PA-1, a bacteriocin-producing culture, has been shown to inhibit the proliferation of spoilage microorganisms throughout storage periods. Furthermore, its application can be integrated with various preservation technologies, given its efficacy at low pH levels and its ability to function synergistically with other compounds, including lactate and organic acids. Plantaricin is recognized as one of the most significant antimicrobial compounds synthesized by the bacterium *Lactobacillus plantarum* (Albert Chan, 2015). Plantaricins are antimicrobial peptides produced by various bacterial species as a component of their defensive strategies. This class of antimicrobial peptides demonstrates minimal toxicity to eukaryotic cells while exhibiting efficacy against pathogens and bacterial strains that have developed resistance to conventional antibiotics. Nanobiotechnology, defined as the science of synthesizing materials at the nanoscale, is fundamentally concerned with the manipulation of atoms and molecules within various materials. This scientific discipline is extensively employed in contemporary applications across the fields of medicine, engineering, and electronics. In the design of nanoparticles intended for drug delivery applications, it is imperative that their dimensions fall within the nanometer scale. The method of preparation of nanoparticles significantly influences the manner in which a drug interacts with these nanomaterials; specifically, it can be incorporated through mixing, adsorption, or covalent binding. Nanoparticles exhibit distinct advantages compared to traditional materials within the food and pharmaceutical sectors, leading to their increased utilization in the production of high-value food products (Karwowska and Kononiuk, 2020).

The efficacy of bacteriocins against gram-negative bacteria can be enhanced through the incorporation of chelating agents, which facilitate increased membrane permeability to bacteriocins. Conversely, biopolymers have the potential to augment the efficacy of bacteriocins against a range of pathogens, particularly Gram-negative bacteria, by facilitating increased permeability and enhancing the delivery of antimicrobial agents. A variety of polysaccharide compounds are employed in the

formulation of nanoparticles within the food industry, as well as for the purpose of encapsulation. One such compound is chitosan, which is derived through the alkaline deacetylation process of chitin. Chitin is recognized as the second most prevalent biopolymer in the natural environment, following cellulose. Plant extracts are acknowledged as a significant source of natural antioxidants and antimicrobial agents. The incorporation of antimicrobial agents and naturally occurring antioxidant compounds derived from plant sources in diverse sectors of the food industry has been shown to significantly hinder chemical, oxidative, and microbial alterations. Consequently, this application contributes to an extension of the shelf life of food products. Among the various plant extracts, sumac merits specific attention (Griffin *et al*., 2000).

Sumac, scientifically classified as Rhus coriaria L., is a perennial, monoecious tree belonging to the Anacardiaceae family, commonly recognized as the Pistachio family. It proliferates in an uncontrolled manner in the western mountainous regions of Iran. The term "sumac," originating from Semitic and Aramaic languages, is etymologically associated with the meanings "red" and "reddish." This designation subsequently transitioned into various European languages through its adoption from Arabic. In traditional medicine, sumac has been employed as a remedy for various conditions, including indigestion, anorexia, diarrhea, hemorrhaging, and hyperglycemia. Phenolic compounds are classified as secondary metabolites in plants and exhibit a wide range of biological activities, notably including antioxidant and antibacterial effects (Van Der Geest, 2019). Plants like sumac, which are abundant in antioxidant compounds, have the capacity to safeguard cells against oxidative damage. The fruit of this plant is characterized by the presence of flavonols, including myricetin and quercetin, as well as various phenolic acids and organic acids, such as malic, citric, and tartaric acids. It also exhibits significant biological effects, including antimicrobial, antioxidant, anti-inflammatory, and cardioprotective properties. Sumac is recognized as a rich source of antioxidants, attributable to its phenolic compounds, including tannins, flavonols, and anthocyanins, which confer notable anticancer properties (Mahlooji et al., 2020).

In a research study, the antimicrobial efficacy of sumac extract was examined in minced meat contaminated with multidrug-resistant *Escherichia coli*. The findings indicate that sumac extract represents a substantial source of antimicrobial and antioxidant compounds, suggesting its potential application in food products, particularly in meat and meat-derived items (Mehdizadeh, 2020). In a subsequent investigation, the impact of pomegranate peel extract (PPE) and a chitosan-starch composite layer (CH-S) incorporated with thyme essential oil (TEO) on the longevity of beef was analyzed during a 21-day storage period at a temperature of 4°C. The findings indicated that the treatments utilizing 2% and 1% CH-S-PPE in conjunction with TEO exhibited the most pronounced inhibitory effects against Listeria monocytogenes (Mehdizadeh, 2020). Recent research has demonstrated that the novel bacteriocin plantaricin GZ1-27 possesses the capability to effectively inhibit multidrug-resistant Staphylococcus aureus (MRSA), exhibiting a minimum inhibitory concentration (MIC) of 32 μg/mL (Du *et al.*, 2022).

The antimicrobial efficacy of sumac extract has been substantiated through

empirical evidence against a range of microorganisms, including *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Shigella sonnei, Staphylococcus dysenteriae, Staphylococcus epidermidis, Staphylococcus lactic acid bacteria, Streptococcus pyogenes, Salmonella typhi, Enterococcus faecalis,* and *Yersinia enterocolitic*a. Research indicates that the hydroalcoholic extract of sumac may serve as an effective natural preservative for refrigerated conventionally packaged and modified atmosphere red meat (Zhao *et al.*, 2022). Other research investigating the bioactive constituents of sumac fruit indicates that the aqueous extract of sumac exhibits antimicrobial properties. This effect can be attributed to the presence of substantial quantities of antioxidants, such as tannins and anthocyanins, which possess the capacity to inhibit lipid peroxidation reactions (Zhao *et al.*, 2022). Plantaricins are a class of antimicrobial peptides synthesized and secreted by various bacterial species as a component of their defense mechanisms against pathogens. This class of antimicrobial peptides demonstrates low toxicity to eukaryotic cells while exhibiting efficacy against pathogens and bacteria that possess acquired resistance to conventional antibiotics. The extract of the sumac plant is acknowledged as a natural source of antioxidants and possesses antimicrobial properties (Zhao *et al.*, 2022). The incorporation of natural antimicrobial and antioxidant compounds derived from plant sources within diverse sectors of the food industry has been demonstrated to significantly mitigate chemical, oxidative, and microbial alterations, thereby enhancing the shelf life of food products. The objective of the present study was to examine the synergistic effects of plantaricin extracted from *Lactobacillus plantarum*, both in its free forms and as nanoencapsulated with chitosan and sumac extract, on the shelf life of ground beef during refrigeration.

Materials and Methods

- *Samples Preparation*

In order to facilitate the conduct of this study, twenty samples of fresh beef, sourced from a specific origin, were procured from chain retail stores located in Tehran. The freshness of the meat was assessed by analyzing the purchase invoice sourced from the slaughterhouse. Subsequently, minced meat was prepared in accordance with the Iranian standard No. 4622. Subsequently, the samples were randomly allocated into five distinct groups, with each group comprising five samples. The study design comprised several treatment groups: a control group that received no treatment (comprised of a placebo without extract, plantaricin, or chitosan); a treatment group administered with 4% sumac extract; a treatment group receiving plantaricin; a treatment group that combined both sumac extract and plantaricin; and finally, a treatment group that involved the combination of 4% sumac extract and plantaricin encapsulated in chitosan. Following the administration of all treatments to the samples, they were subsequently placed in specialized packaging and stored at a controlled temperature of 4 °C for a duration of 20 days. During this period, the samples were analyzed for microbial contamination at specified intervals, namely on days 0, 1, 3, 7, and 14.

In order to assess the microbial load of samples treated with sumac extract, plantaricin, and their combination, both in free form and encapsulated in chitosan, a total of 10 grams of each sample was combined with 90 milliliters of 0.1%

peptone water within a specialized stomacher bag. The mixture was subsequently homogenized using a stomacher at a speed of 200 rpm for a duration of three minutes. Additional dilutions were prepared in tubes containing a 0.1% peptone water solution. The microbial assessments conducted encompassed total bacterial culture and enumeration. These analyses were executed utilizing the surface culture method on plates containing plate count agar as the culture medium. The plates were incubated at 37°C for a duration of 24 hours. *Staphylococcus aureus* was cultured and quantified using a general culture medium. The plates were incubated for 48 hours at a temperature of 35°C under anaerobic conditions. *Escherichia coli* was meticulously cultured and enumerated in a 1 ml aliquot on Violet Red Bile Glucose (VRBG) agar medium. The cultures were incubated at a temperature of 37°C for a duration of 24 hours. Mold and yeast were cultivated and enumerated using surface culture techniques on Selective Chloramphenicol Bengal Rose agar (RBCA) medium, which was maintained at ambient temperature for a duration of 3 to 5 days (Liu, 2018). All culture media utilized in this study were procured from HiMedia, India.

- Sumac Extract Preparation

In preparation for the aqueous-alcoholic extract, a total of 1000 grams of the plant material was procured from the Medicinal Botanical Garden of the University of Tehran. For the extraction process, the selected materials were dried in ambient air, ensuring they were shielded from direct sunlight exposure. Subsequently, the specimens were subjected to milling until a fine powder was obtained, after which they were passed through a 60-mesh sieve for further size classification. Subsequently, 250 grams of the powder were dissolved in one liter of a solvent composed of pure ethanol (Merck, Germany) and water, configured in a 70:30 ratios. This mixture was agitated on a rotary shaker for a duration of six hours at a speed of 150 revolutions per minute. Subsequent to this interval, the mixture was filtered using Whatman filter paper No. The sample was subsequently subjected to evaporation at a temperature of 40 °C to facilitate the removal of no less than 90% of the solvent. Subsequently, the sample was positioned within a desiccator to achieve final concentration and subsequently stored in bottles that are insulated from light and air at a temperature of 4 °C until required for use (Khalkhali and Noveir, 2018).

- ***Plantaricin Preparation***

In order to examine the effects of plantaricin, this bacteriocin was generated as a recombinant protein isolated from *Lactobacillus plantarum* ATCC BAA-793, which was procured from MyBioSource (USA) and maintained at -20°C until utilized in subsequent experiments.

- ***Chitosan Nanoparticles Containing Sumac Extract + Planaricin Preparation***

The chitosan nanoparticles utilized in this research were procured from the Sigma company. Formulations were conducted utilizing the gelatin ionotropic method as outlined by Xu, (2003) with slight modifications to the original protocol.

A total of 1 gram of chitosan was fully dissolved in 50 milliliters of a 1% glacial acetic acid solution. The solution was agitated at a temperature of 40 °C until it attained clarity. Subsequently, 05 grams of the prepared sumac extract was incorporated into the chitosan solution and

subjected to mixing for a duration of 60 minutes. The composition has been designated as mixture A. Subsequently, 02 grams of sodium tripolyphosphate (TPP) were dissolved in 20 milliliters of deionized water. This solution was then introduced to mixture A at a rate of 1 milliliter per minute. The resulting mixture was subjected to vigorous mixing for a duration of 60 minutes at room temperature, utilizing an ultrasonic homogenizer for optimal dispersion. Following centrifugation at 13,000 rpm for 15 minutes, the chitosan-sumac-plantaricin (C-S-P) nanoparticles were subjected to washing with deionized water. Subsequently, the collected nanoparticles were dried at a temperature of 40 °C.

In the present research, Fourier Transform Infrared (FTIR) spectroscopy was employed as a methodological approach to examine the physical properties of nanoparticles. A UV-1800 spectrophotometer manufactured by Shimadzu, Japan, was utilized for the Fourier Transform Infrared (FTIR) spectroscopy analysis and for the determination of nanoparticle size.

The antimicrobial properties of sumac and plantaricin, both in free and encapsulated forms, were evaluated against the pathogenic strains of *Staphylococcus aureus* (a Gram-positive bacterium) and *Escherichia coli* (a Gram-negative bacterium). The minimum inhibitory concentration (MIC) was determined employing a microdilution method conducted in a 96-well plate format.

The strains utilized in this investigation were procured in lyophilized form from the Pasteur Institute of Iran. In this methodology, a 5% solution of dimethyl sulfoxide was utilized as a dispersing solvent to prepare a stock solution. Subsequently, dilutions of sumac essential oil and plantaricin were prepared both independently and in combination, as well as in both free and encapsulated forms. These dilutions were contained within capped tubes that were filled with Luria-Bertani broth culture medium. After the sumac and plantaricin extracts were diluted, 20 µL of bacterial suspension, 20 µL of essential oil or extract, and 1 µL of culture medium were introduced into each well of a microplate. The microplates were subjected to rotation at a speed of 250 revolutions per minute for a duration of 30 seconds. Subsequently, the Minimum Inhibitory Concentration (MIC) in each well was ascertained through visual inspection and assessment of turbidity, utilizing a UV-1800 spectrophotometer. Subsequently, the minimum bactericidal concentration (MBC) was determined by culturing the wells containing the minimum inhibitory concentration (MIC) and the previously tested wells on Mueller-Hinton agar medium. In the absence of observable colony growth, the corresponding concentration was designated as the Minimum Bactericidal Concentration (MBC) well.

In order to investigate the fractional inhibitory concentration (FIC), the study analyzed five concentrations that were below the minimum inhibitory concentration (MIC), two concentrations that were above the MIC, as well as the well corresponding to the MIC itself. In this experiment, 140 µL of medium was administered to each well, supplemented with 20 µL of bacterial suspension, 20 µL of the extract, and either 20 µL of plantaricin or 20 µL of a combination of the aforementioned substances. Following a 24-hour incubation period, the resazurin dye was measured and subsequently dissolved in 10 cc of distilled water prior to its application in the wells. Ultimately,

the well exhibiting clarity was identified as the combined minimum inhibitory concentration (MIC). The fractional inhibitory concentration (FIC) was calculated by dividing the combined minimum inhibitory concentration (MIC) by the respective MIC of each antimicrobial compound when administered individually. Subsequently, the FIC index was derived from the summation of these values. If the index was calculated to be less than 0.5, the two compounds exhibited a synergistic effect. An index value ranging from 0.5 to 1 is indicative of an additive effect, while a range from 1 to 4 suggests a lack of efficacy in the effect. An index exceeding 4 is representative of an antagonistic effect (Yadav, 2024).

Results and Discussion

- ***FTIR Results***

Figures 1 and 2 present the findings of Fourier Transform Infrared (FTIR) spectroscopy analyses conducted on free chitosan, as well as on chitosan encapsulated with a composite of sumac extract and plantaricin. The results are illustrated for both the pre-encapsulation and post-encapsulation stages.

The findings derived from the Fourier-transform infrared (FTIR) analysis demonstrate the effective encapsulation of a 4% sumac extract and plantaricin combination within chitosan nanoparticles, as well as the accompanying structural alterations observed in these nanoparticles. The findings from the investigation of the physical properties revealed that the particle size of the encapsulated combination of sumac extract and plantaricin, utilizing chitosan nanoparticles, ranged between 161.4 nm and 251.24 nm, with a mean value of 199.4.

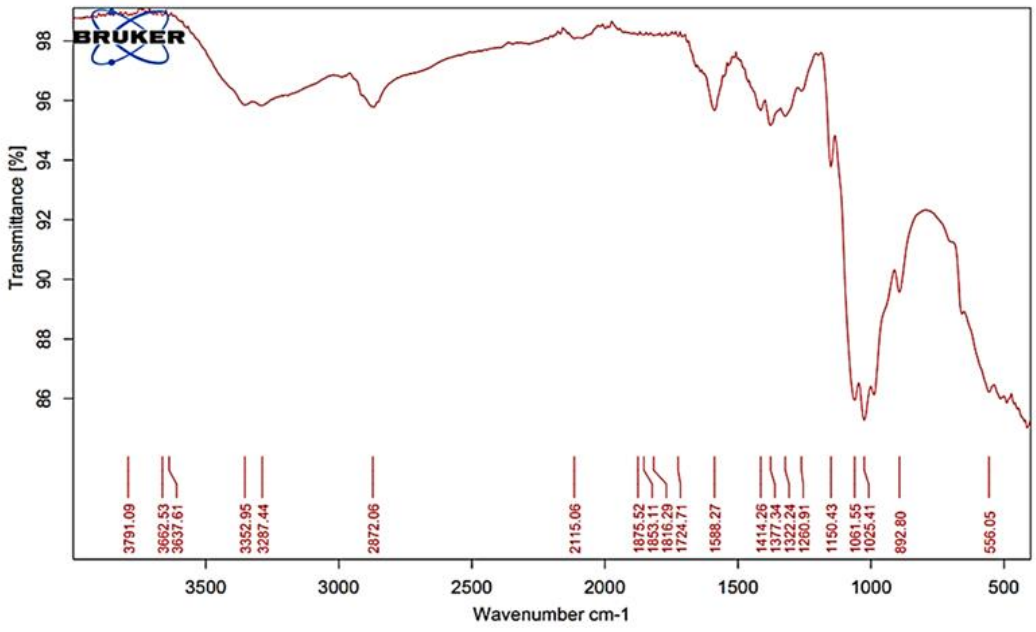

Fig. 1. FTIR image of chitosan nanoparticles prior to the encapsulation of the compounds**.**

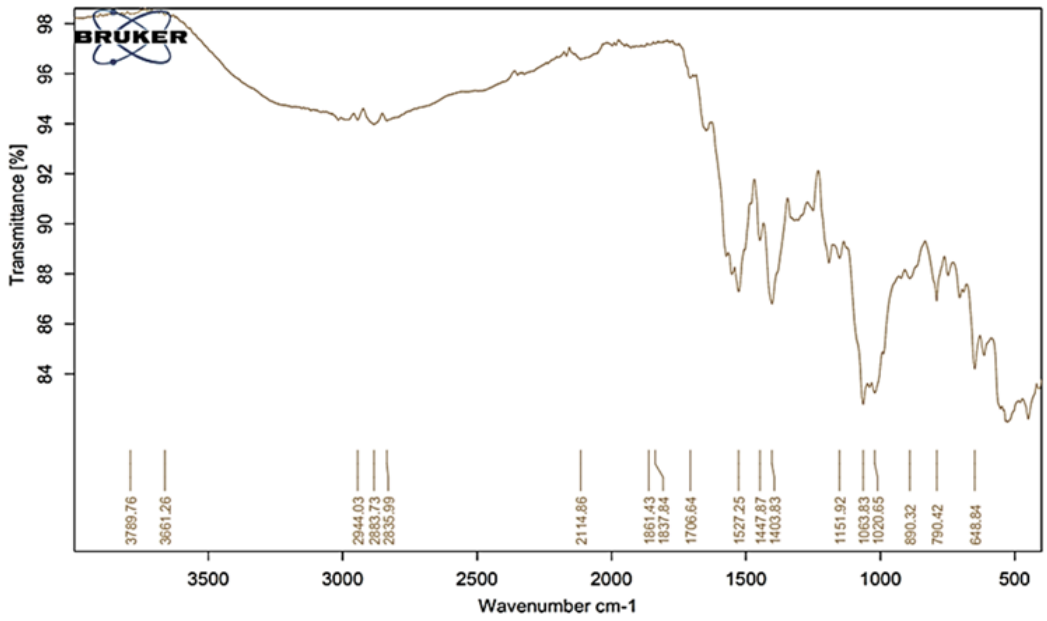

Fig. 2. (FTIR) image of chitosan nanoparticles subsequent to the encapsulation of sumac extract and plantaricin.

- ***Nanoparticle Size Determinations***

The findings derived from the investigation of the physical properties indicated that the size of free chitosan nanoparticles varied between 4.187 nm and 78 nm, with the largest observed size being 78 nm. Following the loading of the sumac extract and plantaricin combination, an increase in the size of the encapsulated chitosan nanoparticles was observed. The dispersion of these nanoparticles, at its maximum peak, was reported to range from 10.10 nm to 190 nm, as illustrated in Figure 3.

- ***Antimicrobial Effects***

Table 1 presents the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fractional inhibitory concentration (FIC) for both sumac extract and plantaricin, evaluated separately and in combination. These parameters were assessed in both free and encapsulated forms against *Staphylococcus aureus* and *Escherichia coli*.

The findings indicated that the minimum inhibitory concentration (MIC) of sumac extract and plantaricin against *Escherichia coli* was determined to be 5 mg/ml. Furthermore, the minimum inhibitory concentration (MIC) for *Staphylococcus aureus* was determined to be 5 mg/ml for sumac extract and 2.5 mg/ml for plantaricin. The results of this study indicated that *Staphylococcus* exhibited greater sensitivity to the plantaricin solution compared to *Escherichia coli*. Moreover, the minimum inhibitory concentration (MIC) of sumac extract and plantaricin, when assessed in their free form, was found to be 2.5 mg/ml against *Escherichia coli* and 1. 25 mg/ml against *Staphylococcus aureus*. These values suggest an enhanced inhibitory effect of the combined agents compared to their individual efficacy. Furthermore, the minimum inhibitory concentration (MIC) of sumac extract and plantaricin, when encapsulated in chitosan, was reported to be 0.625 mg/ml against *Escherichia coli*

and *Staphylococcus aureus*. This finding suggests a synergistic enhancement in the antimicrobial efficacy of these agents when delivered in an encapsulated form using chitosan. The fractional inhibitory concentration index was determined for *Escherichia coli*. The investigation demonstrated that the combinations of *Escherichia coli* and *Staphylococcus aureus* exhibited a significantly enhanced antimicrobial effect compared to the individual extracts alone.

The antimicrobial findings, alongside the assessment of the minimum inhibitory concentration (MIC), demonstrated that the MIC of sumac extract and plantaricin against *Escherichia coli* was determined to be 5 mg/ml. The minimum inhibitory concentration (MIC) observed for *Staphylococcus aureus* was determined to be 5 mg/ml for sumac extract and 2.5 mg/ml for plantaricin. The findings indicated that *Staphylococcus* exhibited a greater sensitivity to the plantaricin solution in comparison to *Escherichia coli*. The minimum inhibitory concentration (MIC) of sumac extract and plantaricin, when evaluated in their free form, was found to be 2. 5 mg/ml against *Escherichia coli* and 1.25 mg/ml against *Staphylococcus aureus*. These findings

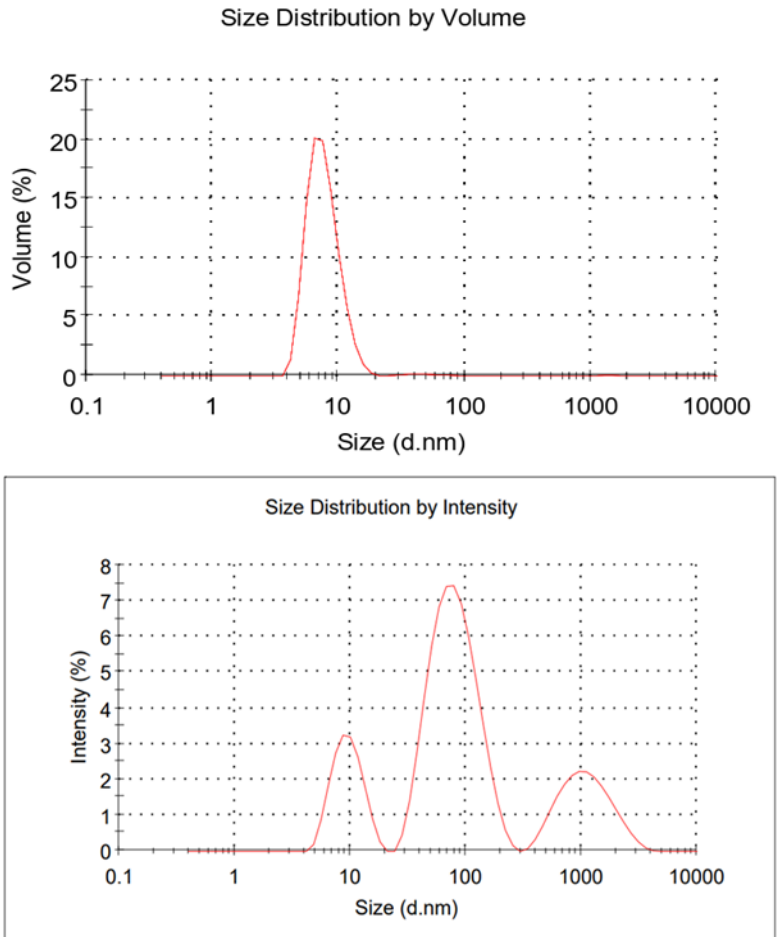

Fig. 3. Graphical representation illustrating the size distribution of free chitosan nanoparticles (upper section) in comparison to chitosan nanoparticles that are incorporated with a combination of sumac extract and plantaricin (lower section).

suggest that the combination of these agents results in enhanced antibacterial activity compared to their individual efficacy. Furthermore, the minimum inhibitory concentration (MIC) of sumac extract and plantaricin, when encapsulated in chitosan, against *Escherichia coli* and *Staphylococcus aureus*, was found to be 0.625 This finding suggests a significant enhancement in the inhibitory capacity of these agents when delivered in an encapsulated form using chitosan. The fractional inhibitory concentration index was determined for *Escherichia coli* (E. coli). The findings suggest that the combination of *Escherichia coli* and *Staphylococcus aureus* exhibits a synergistic antimicrobial effect that surpasses the efficacy of each microorganism's extract when utilized independently.

In this regard, a study conducted in 2017 examined the antibacterial properties of ethanolic extract of sumac (Rhus coriaria L.) against *Escherichia coli* through in vitro methodologies. The present study sought to investigate the antibacterial properties of ethanolic extract from sumac (Rhus coriaria L.) in cooked beef extract and tryptose soy broth, both of which were inoculated with *Escherichia coli*, thereby simulating a laboratory model of contamination. Additionally, the research aimed to evaluate the antibacterial efficacy of this extract through the well diffusion method and to ascertain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the sumac extract against the aforementioned bacterium. The findings of this study demonstrate that the ethanolic extract of sumac exerts a substantial inhibitory effect on the growth of this bacterium in both food and laboratory settings, significantly reducing its proliferation at both evaluated temperatures21. The findings of this study align with previous research, indicating that the alcoholic extract of sumac exhibits antibacterial activity against *Escherichia coli*.

- ***Analysis of Bacterial Counts***

The total bacterial count (Log CFU/g) was assessed in all minced meat samples subjected to treatment with sumac, plantaricin, and their combination, both in free form and encapsulated within chitosan. The data indicated a correlation between the duration of storage and the increase in total bacterial count within minced meat samples, with the highest recorded level observed on day 14 ($P<0.001$) Furthermore, in all experimental treatments involving sumac, plantaricin, or their combinations, the total bacterial count demonstrated a statistically significant reduction compared to the control group ($p<0.001$) The initial bacterial count on day 0 exhibited a relatively uniform distribution among the samples, with measurements falling within the range of Log CFU/g 4.2 The findings of the study indicate that the combination of 4% sumac extract and plantaricin exhibits a synergistic interaction. When these compounds are encapsulated in chitosan, they are capable of reducing the total bacterial count to a statistically significant level of Log CFU/g 3.8 ($p<0.001$), as illustrated in Figure 4.

This study examined the total bacterial population, as well as the presence of *Escherichia coli*, *Staphylococcus aureus*, and fungal species (molds and yeasts) in red meat subjected to various treatment methods over a storage period of 14 days. The results indicated that, overall, the application of 4% sumac extract, plantaricin, and their combination—both in free and encapsulated forms—exhibited considerable antimicrobial activity.

Notably, the antimicrobial effects were significantly enhanced in the combination of sumac extract and plantaricin compared to their individual applications. Furthermore, the encapsulated form of these agents within chitosan demonstrated superior efficacy relative to their free forms ($p<0.001$) The findings of the current study indicate that the combined application of chitosan treatment, aqueous-alcoholic extract of sumac, and plantaricin exhibits significant synergistic effects in inhibiting the growth of various bacterial strains associated with red meat. In this context, numerous studies have demonstrated the antimicrobial properties of phytochemical compounds and bacteriocins when utilized as food additives, corroborating the findings of the current investigation. A 2022 study demonstrated that the combination of plantaricin GZ1-27 and chitosan substantially enhances the shelf life of pork. The research conducted provides compelling evidence regarding the bactericidal mechanism of plantaricin GZ1-27 against Methicillin-resistant Staphylococcus aureus (MRSA). Additionally, preliminary assessments of its application in pork suggest that plantaricin GZ1-27 has the potential to serve as an effective anti-MRSA agent. Furthermore, its utilization may be advantageous in enhancing the shelf life of pork during storage (Du *et al.*, 2022).

Table 1. Findings from the study regarding the minimum inhibitory concentrations of sumac extract and plantaricin, both administered independently and in combination

Bacteria	MIC Sumac extract	MIC Sumac extract	MIC Combination without chitosan capsule	MIC combined with chitosan capsule	FIC without capsule	FIC with capsule
Escherichia coli	5	5	2.5	0.625	0.50	0.125
Staphylococcus aureus	5	5.2	1.25	0.625	0.40	0.312

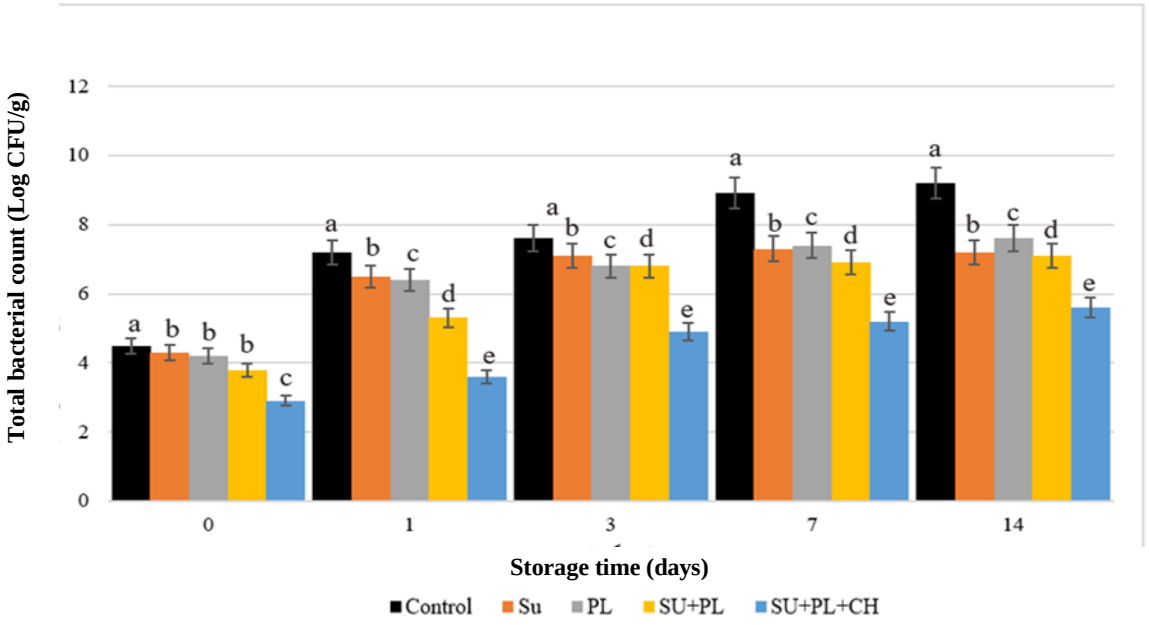

Fig. 4. Average total bacterial count in different treatments of ground red meat. Different letters indicate significant differences between treatments. Sumac: Su, Plantaricin: PL, Chitosan nanocapsule: CH.

A comprehensive analysis was conducted to determine the total count of *Escherichia coli* (expressed as Log CFU/g) in all minced meat samples that were subjected to treatment with sumac, plantaricin, and their combination, both in free form and encapsulated within chitosan. The data indicated a positive correlation between the storage duration of minced meat and the proliferation of *Escherichia coli*, with the bacterial count peaking on day 14 ($P<0.001$). Furthermore, in all experimental treatments involving sumac, plantaricin, or their combinations, the count of *Escherichia coli* was significantly lower compared to the control group ($p<0.001$) The *Escherichia coli* counts on day 0 exhibited a comparable distribution across the samples, with measurements recorded at approximately Log CFU/g 3.9 The findings of the study demonstrate that the combination of 4% sumac extract and plantaricin exhibits a synergistic effect, and when encapsulated in chitosan, this combination can effectively inhibit the growth of *Escherichia coli* bacteria by 4.3 This result is statistically significant ($p<0.001$), as illustrated in Figure 5.

The total enumeration of *Staphylococcus aureus* bacteria (Log CFU/g) in various minced meat samples treated with sumac, plantaricin, and their respective combinations, both in unencapsulated and chitosan-encapsulated forms, was systematically assessed. The data indicate that as the storage duration of minced meat was extended, the concentration of *Escherichia coli* bacteria in the samples exhibited a significant increase, peaking on day 14 ($P<0.001$). Furthermore, in all experimental treatments involving sumac, plantaricin, or their combinations, the bacterial count of *Staphylococcus aureus* was significantly lower in comparison to the control group ($p<0.001$). On day 0, the total count of *Staphylococcus aureus* bacteria across the samples exhibited a comparable distribution, with measurements recorded within the range of 3.1. The findings of the study demonstrate that the interplay between 4% sumac extract and plantaricin exhibits a synergistic effect. Furthermore, when these compounds are encapsulated in chitosan, they can effectively inhibit the growth of *Staphylococcus aureus*, resulting in a reduction of 4.01 This outcome is statistically significant, with a p-value less than 0.001 (Figure 6).

The initial concentration of mold and yeast in the control sample was recorded at 4.2 Log CFU/g. At the conclusion of the 14-day testing period, the control samples demonstrated a greater proliferation of mold and yeast compared to the other experimental groups. The data demonstrate that the quantity of yeast and mold in the minced meat samples exhibited an upward trend as the storage duration extended. This increase was found to peak on the fourteenth day, with a statistically significant difference ($P<0.001$) The findings of the study indicated that the combination of 4% sumac extract and plantaricin exhibited a synergistic effect. When encapsulated in chitosan, this combination demonstrated an inhibitory effect on the growth of mold and yeast strains, resulting in a reduction of 3.2 Log CFU/g. This effect was found to be statistically significant ($p<0.001$), as illustrated in Figure 7.

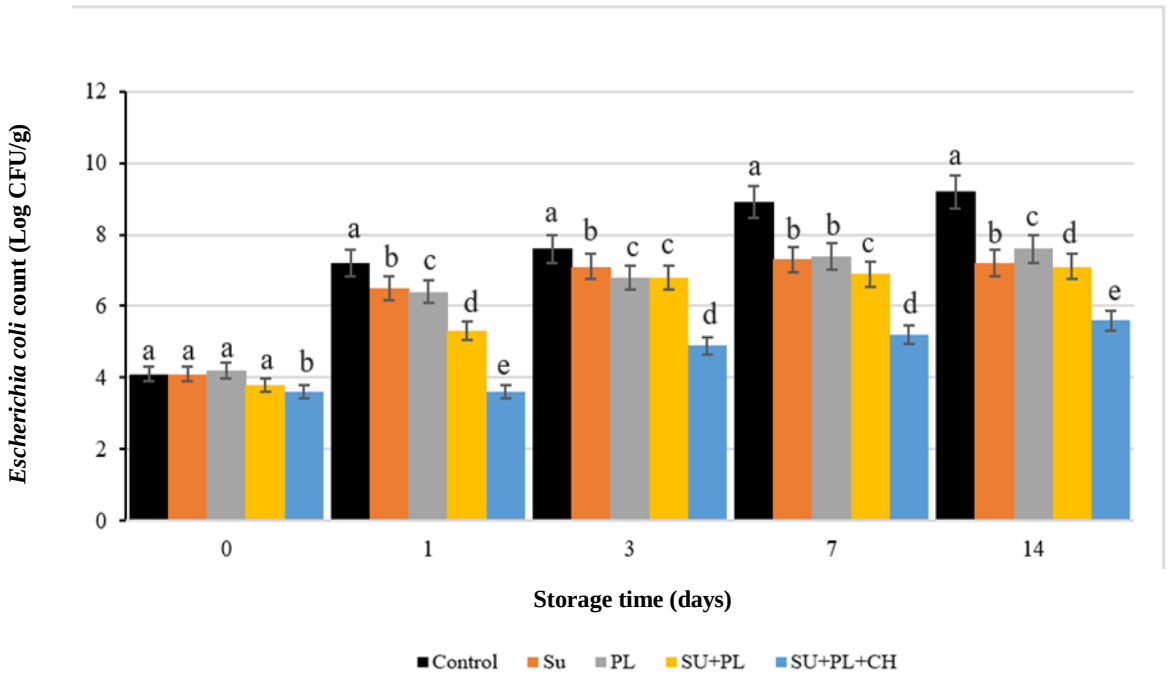

Fig. 5. Average count of *Escherichia coli* strains in different treatments of ground red meat. Different letters indicate significant differences between treatments. Sumac: Su, Plantaricin: PL, Chitosan nanocapsules: CH.

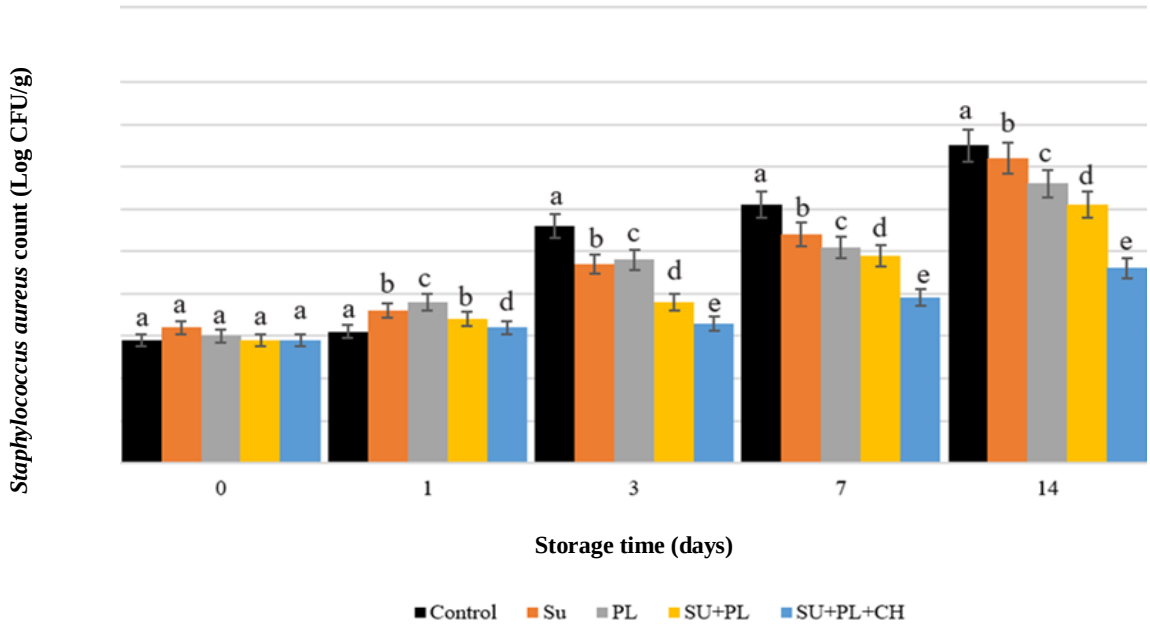

Fig. 6. Average count of *Staphylococcus aureus* strains in different treatments of ground red meat. Different letters indicate significant differences between treatments. Sumac: Su, Plantaricin: PL, Chitosan nanocapsule: CH.

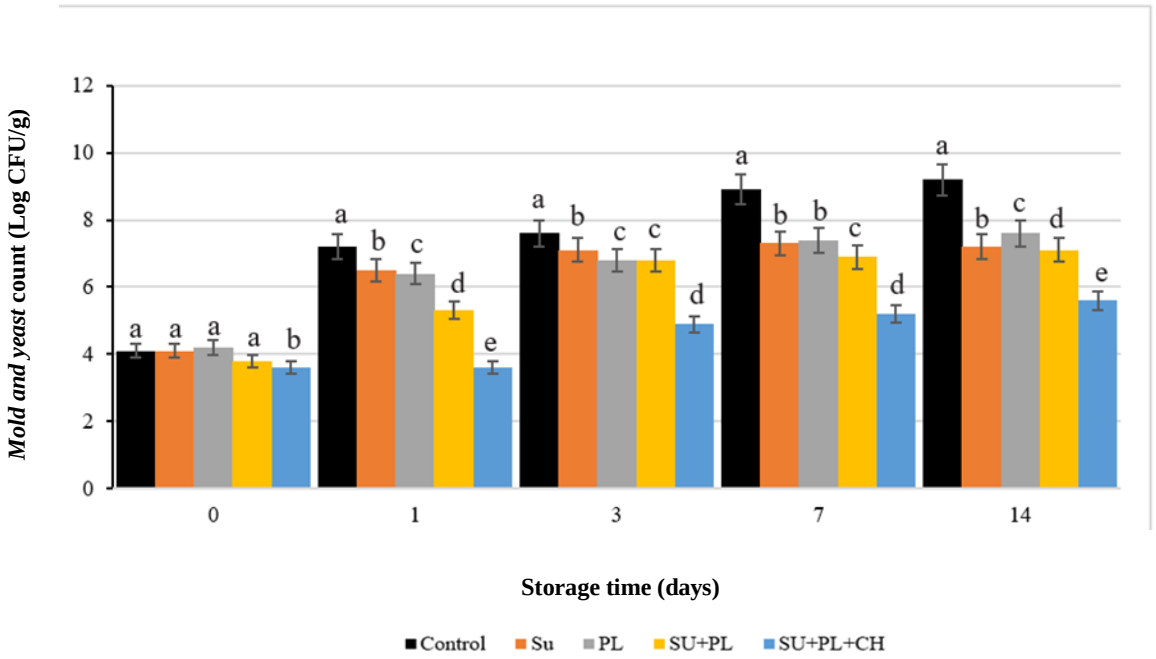

Fig. 7. Average counts of mold and yeast strains in different treatments of ground red meat. Different letters indicate significant differences between treatments. Sumac: Su; Plantaricin: PL; Chitosan nanocapsule: CH.

The findings of the current study align with those of multiple investigations conducted in 2021 and 2023, which explored the substantial effects of chitosan nanoparticles infused with plant extracts on the inhibition of growth within the *Enterobacteriaceae* family, with particular emphasis on *Escherichia coli* in protein products (Tavassoli, 2023; Du *et al.*, 2022; Sayadi *et al.*, 2021). A separate investigation revealed that the concentrations of Enterobacteriaceae and Escherichia coli in beef elevated over the course of the storage period; however, these levels were observed to decline following the application of a chitosan-containing plant extract (Mojaddar Langroodi and Tajik, 2017). A study conducted in 2018 yielded results akin to those of the present investigation, revealing a reduction in the total bacterial count, as well as in the populations of both gram-negative and gram-positive bacteria, in groups administered with 4% sumac extract, in comparison to the control group (Mojaddar Langroodi and Tajik, 2017). Another study demonstrated a significant decline in the total bacterial count in comparison to the control group throughout the 14-day storage period.

The findings of the current investigation indicate that the combination of 4% sumac extract and plantaricin exhibits a synergistic effect when encapsulated in chitosan. This combination effectively inhibits the growth of *Escherichia coli* by 4.3 Log CFU/g, *Staphylococcus aureus* by 4.01 Log CFU/g, and various mold and yeast strains by 3.2 Log CFU/g These effects are statistically significant, with p-values less than 0.001.

Conclusion

The findings of the present study indicate that the antimicrobial properties of sumac extract and plantaricin were preserved in both non-encapsulated and

encapsulated forms throughout the duration of the testing period. This suggests that the combination of these agents exhibits a stable antimicrobial effect during the storage of ground red meat. Conversely, the encapsulation of antimicrobial agents using chitosan nanoparticles demonstrates greater efficacy compared to the application of sumac extract or plantaricin either individually or in combination. This method significantly enhances the antimicrobial properties of the combined treatment. The impact of sumac extract and plantaricin, when combined with encapsulation in chitosan nanoparticles, on the extension of shelf life for fish and other proteinaceous foods represents a promising area for further research.

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