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Effects of Chitosan Coatings on Controlling *Listeria monocytogenes* and Methicillin-Resistant *Staphylococcus aureus* in Beef and Mutton Cuts

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Abstract: Ruminant meat is considered to be a potent vehicle of foodborne pathogen transmission. Edible coatings are considered to be promising for enhancing meat safety. Here, edible chitosan membranes were applied to whole cuts of beef and mutton to test the survival of the pathogenic bacteria *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus.* Meat pieces weighing approximately 200 g were inoculated with a cocktail of three *S. aureus* isolates or four *L. monocytogenes* isolates (6.00 $\log_{10}$CFU/g). The meat pieces were encased in a chitosan coating formed by immersion and incubated aerobically or vacuum-packaged in LDPE/PA/LDPE bags for up to 21 days. A decrease in both *S. aureus* (x = −1.95 $\log_{10}$CFU/g, standard error = 0.23 $\log_{10}$CFU/g) and *L. monocytogenes* counts (x = −1.07 $\log_{10}$CFU/g, standard error = 0.26 $\log_{10}$CFU/g) was observed. No significant differences were observed between *L. monocytogenes*-spiked beef and mutton pieces; statistically higher *S. aureus* counts were observed in mutton versus beef under similar treatments. Aerobic storage of meat pieces inoculated with *L. monocytogenes* enhanced the antibacterial effects of chitosan—a trend that was not observed in meat pieces inoculated with *S. aureus*. According to the results, edible chitosan membranes were effective in controlling the growth of *S. aureus* and *L. monocytogenes*.

Keywords: beef; mutton; *Staphylococcus aureus*; *Listeria monocytogenes*; chitosan

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1. Introduction

Foods of animal origin—and especially meat—are essential for the human diet because they constitute excellent sources of nutrients, such as proteins of high biological value, essential amino acids, and vitamins. Still, meat is prone to deterioration and bacterial contamination. Edible membranes are thin coatings that can be applied to a wide variety of food products to enhance their quality and shelf life. Chitosan films are predominately used for meat products because they form strong coatings and they positively contribute to their quality [1]. Chitosan is a cationic polysaccharide of N-acetyl-d-glucosamine and d-glucosamine units and is derived from the partial deacetylation of chitin [2]. It is generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA) as a food additive and food preservative of natural origin [1]. Chitosan possesses a wide variety of biological activities, i.e., anti-inflammatory, anti-allergic, anti-hypertensive, antidiabetic, hypolipidemic, hypocholesterolemic, anticoagulant, antineoplastic, and neuroprotective

activities [3,4]. However, its most distinctive characteristic is related to its antimicrobial properties [2].

Several foodborne pathogens can be transmitted through meat consumption. Among them, *Staphylococcus aureus* has regularly been implicated in human disease [5]. *S. aureus* is a Gram-positive and catalase-positive coccus that is commensal in humans and domestic animals [6]. It can grow in a wide range of temperatures (7 °C to 48 °C), pH levels (4.2–9.3), and concentrations of sodium chloride (up to 15%) [7]. It is not able to form spores, but it can contaminate food products during food processing [7] and produce enterotoxins under favorable conditions and temperatures [8]. Staphylococcal foodborne diseases are dominant worldwide and can cause a wide variety of symptoms, including nausea, vomiting, diarrhea, and abdominal pain [7]. Although it is a self-limiting disease, it can cause serious symptoms to high-risk groups of people, such as infants, the elderly, the pregnant, and the immunocompromised [8]. The staphylococcal enterotoxins are resistant to heat, freezing, drying, pH, and proteolytic enzymes [9]; once produced, it is hard to remove them from the food product. In addition, methicillin-resistant *Staphylococcus aureus* (MRSA) is the most prominent example of an antibiotic-resistant human pathogen that can be transmitted between animals and humans via contaminated food—and especially meat. Therefore, to tackle this major threat to human health, more precautionary strategies should be implemented to reduce the occurrence and survival of *S. aureus*—and especially MRSA—in meat and meat products [9]. Meat is often implicated as the causal food commodity of foodborne illness in humans; *S. aureus* usually ends up in food after slaughter, with food handlers being mainly responsible for meat contamination. In a review of foodborne diseases in the USA from 1998 to 2008, *S. aureus* was the second most common causative agent of foodborne disease due to meat consumption [5,10]. Thus, the inhibition of *S. aureus* from growing on food products is of vital importance to prevent the accumulation of staphylococcal enterotoxins and the transfer of microbial resistance to susceptible humans.

Listeria monocytogenes is a notorious foodborne pathogen, causing the often-fatal foodborne disease listeriosis. It is a Gram-positive, catalase-positive, and facultative anaerobic rod [11]. It is widely distributed in nature, present in soil, water, vegetation, sewage, and animal feces [12]. Moreover, it is commonly isolated from the environment of food processing facilities and from surfaces due to its ability to form strong biofilms. Cross-contamination is considered to be the most important cause of *Listeria* contamination in food products [10,13]. When healthy people are infected with *L. monocytogenes*, the symptoms are usually mild, as *L. monocytogenes* causes only febrile gastroenteritis. However, in high-risk groups of people, *L. monocytogenes* can cause sepsis, meningoencephalitis, and abortion and can result in high mortality and morbidity [13,14]. Apart from the severity of the disease, *L. monocytogenes* is also a psychrotrophic bacterium that can survive and grow at temperatures lower than 7 °C [10]. Thus, refrigeration appears to be ineffective against *L. monocytogenes*, and supplementary measures are needed to control its growth in food products. Listeriosis can be caused by meat consumption; still, the necessity for the control of *L. monocytogenes* is dictated mainly by the severity of the disease that it may cause [5].

The evidence in the literature suggests that chitosan has antimicrobial properties that could help control the growth of Gram-positive and Gram-negative bacteria in vitro [2,15] and in food systems—mainly in fruits, vegetables, bakery products, fish, pork, and chicken meat [1,16]. However, there is scarce information about the antibacterial activity of chitosan against pathogenic bacteria in meat from ruminants. To the best of our knowledge, no data have been published regarding the survival of *Listeria monocytogenes* on chitosan-coated mutton meat. Thus, the objective here was to test the antimicrobial activity of chitosan against sensitive and methicillin-resistant *S. aureus* and *L. monocytogenes* in beef and mutton meat.

2. Materials and Methods

2.1. Meat Samples

Beef and mutton meat from the thigh region (*M. quadriceps femoris*) was provided by a collaborating meat company located in Northern Greece. The meat was produced locally by animals slaughtered in the company's abattoir and transferred to the laboratory of Hygiene of Food of Animal Origin—Veterinary Public Health, School of Veterinary Medicine, in insulated polystyrene boxes with ice within two hours for analysis. Pieces of approximately 200 g each were aseptically produced and stored under refrigeration before the application of chitosan. The meat pieces mimicked the actual weight of the commercial packages of meat usually marketed at the retail level.

2.2. Preparation of the Inoculums

For the contamination of the meat pieces, a cocktail of strains of *S. aureus* or *L. monocytogenes* was used. The *S. aureus* cocktail comprised three MRSA strains, namely, *S. aureus* ATCC 43300, DSM 102262, and DSM 25629; the *L. monocytogenes* cocktail comprised *L. monocytogenes* ATCC 49594 (Scott A), ATCC 19115, ATCC 19112, and ATCC 35152. Prior to revival, the strains were maintained at −80 °C in a 15% glycerol stock. The strains were collected by scraping the frozen stock culture surface with a sterile loop, without defrosting the stock culture, and used for the inoculation of tubes containing Brain Heart Infusion Broth (Oxoid Ltd., Basingstoke, UK). Incubation was performed in an orbital shaking incubator at 37 °C at 300 rpm (ISLD04HDG, Ohaus Ltd., Parsippany, NJ, USA). The turbidity of the inocula was adjusted to 0.5 McFarland by the use of a densitometer (Densimat, Biomérieux, Marcy l'Etoile, France). Then, equal amounts of each strain were combined in order to form the inoculum of four strains of *S. aureus* or *L. monocytogenes* that were further used for meat inoculation. The microbial counts of the working mixtures were calculated by decimal dilutions in Maximum Recovery Diluent (MRD, Oxoid Ltd., Basingstoke, UK) and surface plating in Brain Heart Infusion Agar, followed by incubation at 37 °C for 24–48 h.

2.3. Application of the Chitosan Solution and the Inocula to the Meat Samples

The selection of chitosan coating with or without vacuum-packaging was based on preliminary results of different beef and mutton treatments including chitosan or alginate coatings, combined with the addition of oregano essential oil or olive oil, and stored aerobically or under vacuum-packaging [17]. A stock of chitosan solution was produced at a final concentration of 1% by dissolving 1 g of chitosan of medium molecular weight obtained from crab shells (48165, Sigma-Aldrich. St. Louis, MO, USA) in 100 mL of 1% glacial acetic acid and stirring overnight at room temperature; finally, the solution was sterilized at 121 °C for 15 min [18]. Meat pieces were aseptically immersed in the chitosan solution for 1 min and then dredged up and left on sterilized racks for 5 min to form a strong chitosan membrane and to drain off the excess solution. Three batches of samples were conducted for each type of meat: The first was the control, which consisted of meat samples without chitosan membranes, stored aerobically. The second was meat samples with chitosan membranes, stored aerobically. The third was meat pieces with chitosan membranes stored in vacuum-packaging. Each batch was inoculated with the working mixtures of *S. aureus* or *L. monocytogenes* in order to achieve a ~6.70 $\log_{10}$ CFU/mL concentration. The inoculation of the meat pieces was performed by adding 100 μL of the cocktail directly to the surface of the meat pieces and allowing it to dry for approximately 30 min on a laminar flow cabinet. The microbial concentrations were verified by surface platting in Baird-Parker agar (Biolab S.E.E., Belgrade, Serbia) for *S. aureus* enumeration or Listeria Agar according to Ottaviani and Agosti (ALOA—Biolab S.E.E., Belgrade, Serbia) for *L. monocytogenes* enumeration, after aerobic incubation at 37 °C for 24–48 h. Then, the samples were packaged in LDPE/PA/LDPE bags either aerobically or under vacuum conditions by using a packaging machine (Lava V.400, Lava GmbH & Co., Bad Saulgau, Germany) and stored at 4 °C for 21 days in a Peltier cooled incubator (Labtech, Sorisole, Italy).

2.4. Microbiological Analysis

Examination of samples for *S. aureus* and *L. monocytogenes* counts was performed according to EN/ISO 6888-1 and EN/ISO 11290-2, respectively, with modifications proposed by the Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs [19,20]. More specifically, 25 g of meat sample was transferred aseptically into a stomacher bag (Interscience, Saint Nom la Bretêche, France) containing 225 mL of sterile MRD (Oxoid Ltd., Basingstoke, UK) and homogenized in a stomacher (Interscience, Saint Nom la Bretêche, France) for 2 min. The appropriate serial decimal dilutions were also prepared in MRD solution. From each dilution, 0.1 mL of diluent was surface-inoculated in appropriate media. For the enumeration of *S. aureus* counts, the Baird-Parker agar was used, whereas for the enumeration of *L. monocytogenes* counts the ALOA agar was used. Incubation for both Baird-Parker agar and ALOA agar was performed aerobically at 37 °C for 24–48 h. After incubation, the characteristic colonies were enumerated. Microbiological examination was performed on the 1st, 3rd, 7th, 14th, and 21st days of storage.

2.5. Statistical Analysis

Parametric and non-parametric methods were used for statistical evaluation of the data (N = 108). Parameters were assessed by using measures of central tendency and dispersion in order to reveal the characteristics of the data. All analyses were performed using the statistical software program SPSS (v. 27.0). Significance was set at a p-value ≤ 0.05, unless otherwise specified.

3. Results

The initial concentration of *S. aureus* in beef and mutton pieces was ~10^6 CFU/g. Over time, there was a slight decrease in *S. aureus* counts in the control samples of beef and mutton pieces; on day 21, the *S. aureus* count was approximately 6.00 $\log_{10}$ CFU/g (Table 1). However, a different trend was observed in chitosan-treated pieces that were stored aerobically or under vacuum. After the application of chitosan emulsions, an abrupt decrease of 1.24 $\log_{10}$ CFU/g (standard deviation = 0.10 $\log_{10}$ CFU/g) was observed in the *S. aureus* concentrations. Thereafter, the microbial concentrations were variable; after 21 days of storage they were further reduced, leading to a 1.95 $\log_{10}$ CFU/g decrease in the *S. aureus* count (standard deviation = 0.078 $\log_{10}$ CFU/g) compared to the initial inoculum. Chitosan-treated pieces had significantly lower counts of *S. aureus* than controls. No significant differences were observed between chitosan-coated pieces stored aerobically and those stored under vacuum. The staphylococcal counts (Table 1) were significantly higher in mutton pieces than in beef pieces, especially on days 14 and 21 of storage. Specifically, the chitosan-coated beef on days 14 and 21, as well as the vacuum-packed chitosan-coated beef on day 21, had significantly lower *Staphylococcus* counts than the corresponding mutton pieces. The latter trend was not observed in the control groups, where the differences between beef and mutton pieces were not significant. The microbial counts of *S. aureus* in the beef and mutton pieces are depicted in Figure 1.

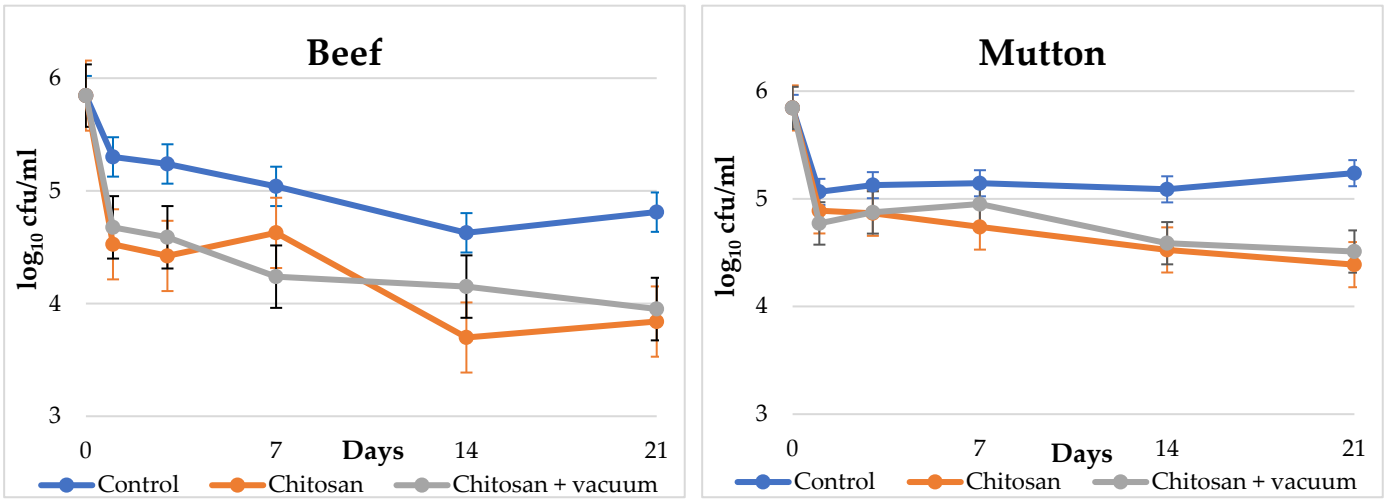
A similar trend was observed in the *L. monocytogenes* counts. The beef and mutton pieces had an initial *L. monocytogenes* concentration of approximately 10^6 CFU/g. The microbial counts of *L. monocytogenes* in the control pieces were variable and approximately 5×10^5 CFU/g throughout the experiment (Table 2). Conversely, a 1 $\log_{10}$ decrease was observed in the microbial concentrations of chitosan-coated beef and mutton pieces on day 1 of storage. Over time, the *L. monocytogenes* counts showed a declining trend in the chitosan-coated beef and mutton pieces stored either aerobically or under vacuum. On day 21 of the analysis, the *L. monocytogenes* counts were further decreased, finally reaching a concentration of 10^4 CFU/g. Similarly to the *S. aureus* counts, the chitosan membranes affected the concentrations of *L. monocytogenes* in all meat pieces. No significant differences were observed between beef and mutton pieces or between aerobically stored and vacuum-packaged pieces. However, on day 21 of storage, the counts of *L. monocytogenes* in chitosan-treated and aerobically stored meat pieces were 1 $\log_{10}$ lower compared to the

vacuum-packaged pieces. The microbial counts of *L. monocytogenes* in the three batches of beef and mutton pieces are depicted in Figure 2.

Table 1. *S. aureus* counts during the storage of beef or mutton under refrigeration for 21 days (average values in bold and standard deviations in italics and parentheses; $p \leq 0.05$).

Day	Beef						Mutton					
	Control (n = 18)		Chitosan (n = 18)		Chitosan + VP (n = 18)		Control (n = 18)		Chitosan (n = 18)		Chitosan + VP (n = 18)	
0	5.85	(0.00)	5.85	(0.00)	5.85	(0.00)	5.85	(0.00)	5.85	(0.00)	5.85	(0.00)
1	5.30	(0.43)	4.53	(0.18)	4.68	(0.28)	5.07	(0.16)	4.89	(0.02)	4.77	(0.10)
3	5.24	(0.09)	4.42	(0.17)	4.59	(0.16)	5.13	(0.07)	4.87	(0.12)	4.87	(0.04)
7	5.04	(0.06)	4.63	(0.04)	4.24	(0.09)	5.15	(0.04)	4.74	(0.06)	4.95	(0.07)
14	4.63	(0.21)	3.70	(0.43)	4.15	(0.21)	5.09	(0.12)	4.53	(0.18)	4.59	(0.16)
21	4.81	(0.05)	3.84	(0.09)	3.95	(0.07)	5.24	(0.09)	4.39	(0.12)	4.51	(0.05)

VP: vacuum-packaging.



(**a**) (**b**)

Figure 1. *S. aureus* populations during the storage of beef (**a**) or mutton (**b**) under refrigeration for 21 days.

Table 2. *L. monocytogenes* populations during the storage of beef or mutton under refrigeration for 21 days (average values in bold and standard deviations in italics and parentheses; $p \leq 0.05$).

Day	Beef						Mutton					
	Control (n = 18)		Chitosan (n = 18)		Chitosan + VP (n = 18)		Control (n = 18)		Chitosan (n = 18)		Chitosan + VP (n = 18)	
0	5.95	(0.00)	5.95	(0.00)	5.95	(0.00)	5.95	(0.00)	5.95	(0.00)	5.95	(0.00)
1	5.51	(0.05)	4.98	(0.28)	4.85	(0.21)	5.04	(0.62)	4.80	(0.21)	4.78	(0.25)
3	5.50	(0.28)	4.95	(0.07)	4.87	(0.12)	5.24	(0.34)	5.09	(0.12)	4.84	(0.09)
7	5.44	(0.37)	4.87	(0.04)	4.94	(0.02)	5.39	(0.12)	4.69	(0.21)	4.87	(0.08)
14	5.51	(0.05)	4.48	(0.25)	4.81	(0.05)	5.35	(0.07)	4.77	(0.10)	4.63	(0.21)
21	5.92	(0.11)	3.74	(0.62)	4.74	(0.06)	5.72	(0.17)	4.09	(0.12)	4.81	(0.05)

VP: vacuum-packaging.

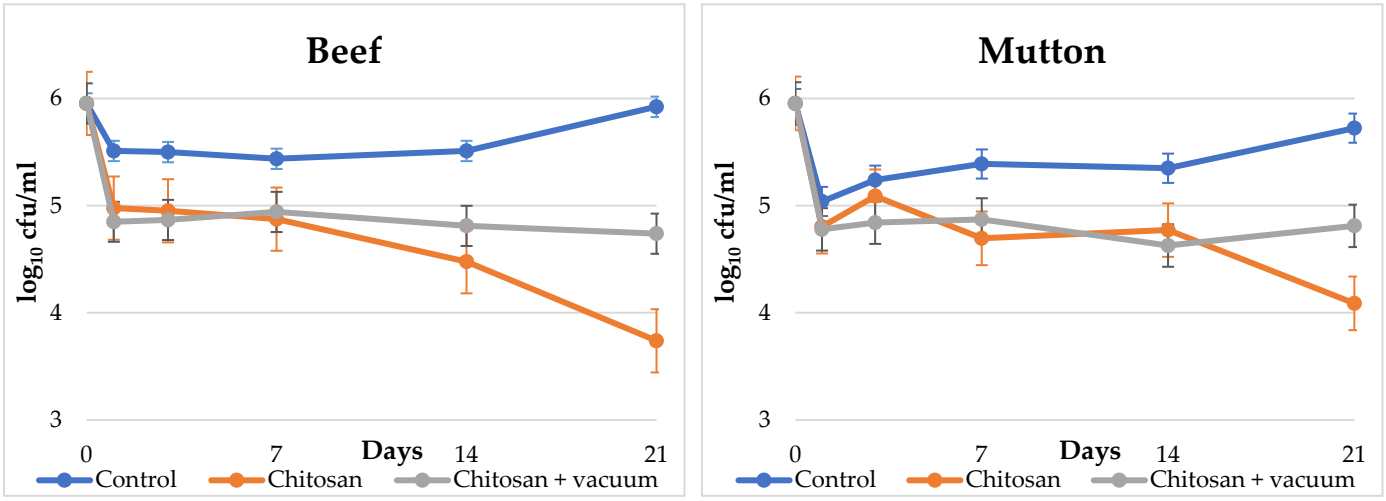


Figure 2. *L. monocytogenes* populations during the storage of beef (**a**) or mutton (**b**) under refrigeration for 21 days.

4. Discussion

As asserted in the Introduction, the objective here was to investigate the antimicrobial activity of chitosan against pathogenic bacteria—specifically, *L. monocytogenes* and both sensitive and methicillin-resistant *S. aureus*—in beef and mutton packaging. The available literature suggests a variety of mechanisms regarding the antimicrobial activity of chitosan, without a consensus or clarity as to the mode of action [2,21]. Three mechanisms are most likely involved: The first is based on the cationic properties of chitosan. The positively charged amino groups of chitosan interact with the negatively charged cell membranes of microbes, leading to the disruption of the membrane barrier, leakage of intracellular contents, and cell death [15]. The second mechanism involves the chelating characteristics of chitosan, since chitosan is able to bind selectively with metals and block the active centers of various enzymes of the microbial cell, thereby inhibiting the microbial metabolism and growth [2]. The third mechanism is based on the molecular weight of chitosan, where chitosan of high molecular weight forms an impermeable polymeric layer on the surface of microbial cells, blocking the entry of nutrients to the cell and the excretion of toxic compounds [22]. On the other hand, chitosan of low molecular weight is able to enter the microbial cell and bind with the microbial DNA, leading to alterations in the transcription and translation of the DNA [15]. Regardless of the involved mechanism, chitosan exhibits a wide range of antimicrobial activity, including against Gram-positive and Gram-negative bacteria, fungi, and yeasts, targeting the cell membrane [2,15].

Given the antimicrobial properties that chitosan exhibits in laboratory experiments, several researchers have focused on the application of chitosan membranes in food systems, including meat products. Chitosan can enhance the quality and shelf life of meat products, including chicken fillets, pork sausages, beef slices, and mutton pieces [23–26], as well as fish products such as rainbow trout [27]. In addition to spoilage microorganisms, chitosan coatings are also effective in controlling the growth of pathogenic bacteria in meat products. Hu et al. [28] prepared beef samples that were coated with 1% chitosan emulsions, and reported that chitosan reduced the viable *Salmonella* Typhimurium and *Escherichia coli* by about 90% compared to the initial inoculum. Similar results were also observed by Fischer et al. [29] and Hadian et al. [30], whereas Cui et al. [31] reported antimicrobial activity of chitosan against *E. coli* O157:H7 in beef—especially when combined with liposome-encapsulated phages. Juneja et al. [32] also stated that 3% chitosan reduced the spore germination and outgrowth of *Clostridium perfringens* by 2 $\log_{10}$ CFU/g in ground beef. Regarding mutton, Kanatt et al. [33] reported that chitosan films (2%) eliminated fecal

coliforms and inhibited the growth of *Bacillus cereus* and *Pseudomonas fluorescens* in ready-to-eat mutton seekh kababs. This is consistent with the findings of He et al. [34] and Pabast et al. [23], who reported antimicrobial activity of chitosan against a wide range of Gram-positive and Gram-negative bacteria, including *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, and lactic acid bacteria.

In our study, chitosan coatings reduced the concentrations of *S. aureus* in beef and mutton pieces by approximately 1.5 $\log_{10}$ CFU/g. This is consistent with the findings of Duran and Kahve [35], who reported that chitosan membranes (2%) and vacuum-packaging reduced the staphylococcal concentrations in beef pieces by around 2 $\log_{10}$ CFU/g. However, Silva et al. [36] observed a 6-log decrease in staphylococcal concentrations in beef that was coated with 2% chitosan solution. This difference can be attributed to the higher concentrations of chitosan solutions used and the use of only one strain of *S. aureus*, as opposed to the four strains that were used in our study, given that the strain used by Silva et al. could have been more chitosan-sensitive. Ashrafi et al. [37] also reported that the staphylococcal concentrations gradually increased in minced beef samples coated with 1% chitosan emulsions, in contrast to the results of this study, possibly due to the fact that the minced meat was purchased from a local market with non-specified conditions concerning meat storage. In addition, shearing of minced meat can increase the area available for microbial growth [38] and, therefore, can have an impact on the proliferation of *S. aureus*. Regarding mutton, Kanatt et al. [33] observed an immediate effect of chitosan coating (2%) in mutton seekh kababs, as it reduced staphylococcal concentrations by 2–3 $\log_{10}$ CFU/g. Kanatt et al. [39] also reported that the bactericidal effect of chitosan on minced lamb meat is concentration-dependent. In this study, no synergic effect of chitosan and vacuum-packaging on the staphylococcal counts was observed. The main mechanism of chitosan against *S. aureus* is the formation of an impervious polymeric layer on the surface of microbial cells that inhibits the entrance of nutrients to the cells [23]; according to this mechanism, no complementarity exists between the effects of chitosan and vacuum-packaging; therefore, a synergistic effect is not possible.

Concerning MRSA strains, Guo et al. [40] examined the efficacy of chitosan against MRSA and its effect on the shelf life of pork; they reported that the combination of epicatechin gallate and chitosan extended the shelf life of pork from 5 to 7 d at 4 °C and from 36 h to 48 h at 25 °C. Rubini et al. [41] reported that chitosan reduced staphyloxanthin production and, therefore, reduced the virulence of MRSA. In addition, it asserted a dose-dependent effect on MRSA biofilms. Therefore, chitosan can be effective against MRSA isolates in a similar way as against the sensitive *S. aureus* strains. This observation is consistent with the results of Costa et al. [42], who reported that methicillin resistance did not affect the antimicrobial and anti-biofilm activities of chitosan. On the contrary, they argued that the coexistence of methicillin and chitosan can be complementary against MRSA, since both substances target the bacterial cell wall.

Regarding *L. monocytogenes*, chitosan coatings reduced its concentrations in beef and mutton pieces by up to 2 $\log_{10}$ CFU/g. This is consistent with the results of Antoniadou et al. [43], who also reported a 2-$\log_{10}$ decrease in the counts of *L. monocytogenes* on ready-to-eat bovine meatballs coated with 1% chitosan solution. Bento et al. [44] coated bovine meat pâté with chitosan at a concentration of 5 mg chitosan/g pâté and observed an almost 4-$\log_{10}$ decrease in the inoculated counts of *L. monocytogenes* due to the antimicrobial activity of chitosan. The higher decrease observed may be because the meat pâté was inoculated with only one strain of *L. monocytogenes*. According to Mojsova et al. [45], chitosan emulsions with oregano oil were shown to reduce the *L. monocytogenes* counts on beef tenderloin by up to 4 $\log_{10}$ CFU/g. On the other hand, Wang et al. [46] reported that the *L. monocytogenes* counts of chitosan-coated (1%) beef samples gradually increased throughout the storage period, even though the counts were lower than those of the control samples. Similar results were also reported by Beverlya et al. [47], who observed an increase in the counts of *L. monocytogenes* in chitosan-coated (1%) roast beef, despite the initial decrease in the counts by up to 2 $\log_{10}$ CFU/g after the application of the chitosan solution.

Similar to *S. aureus*, no synergic effect of chitosan and vacuum-packaging was observed. In fact, the aerobically stored chitosan-coated meat pieces had lower *L. monocytogenes* counts by 1 $\log_{10}$ CFU/g on the last day of storage, compared to the vacuum-packaged pieces. The antimicrobial activity of chitosan against *L. monocytogenes* is possibly due to the electrostatic interaction between the NH^{3+} groups of chitosan and the negatively charged phosphoryl groups of the phospholipid components of the *Listeria* cell membrane [47].

Apart from beef and mutton, many researchers have demonstrated the antimicrobial activity of chitosan against *S. aureus* and *L. monocytogenes* in other types of meat. Shekarforoush et al. [48] reported a 1-$\log_{10}$ decrease in the counts of *L. monocytogenes* in chicken pieces coated with 2% chitosan and stored under refrigeration. Kanatt et al. [33] also observed that chitosan-coated (2%) chicken seekh kabab had 2-3-$\log_{10}$ CFU/g lower staphylococcal counts compared to the controls. Regarding pork, Mojsova et al. [42] reported a 3-$\log_{10}$ and a 2-$\log_{10}$ reduction in the counts of *L. monocytogenes* in chitosan-coated (1%) smoked pork neck and ham, respectively. Similarly, Zhao et al. [49] concluded that chitosan solution (0.5%) lowered the staphylococcal counts of fresh chilled pork by 1 $\log_{10}$ CFU/g, and that the combination of chitosan with nisin and tea polyphenols extended the shelf life of fresh chilled pork by up to 11 days.

Regarding the different meat types (beef or mutton), statistically significant differences were observed only in *Staphylococcus aureus*-spiked meat pieces, with the difference being statistically significant after the 14th day of storage. Beef pieces had lower *S. aureus* counts than mutton pieces. In contrast, *L. monocytogenes*-spiked meat pieces did not show any differences between meat types. *S. aureus* can readily colonize both beef and mutton meat, as shown by Jaja et al. [50], who reported that the prevalence of *Staphylococcus aureus* in beef and sheep carcasses was comparable. Still, small differences in meat composition have been reported to alter the growth potential in these two meat types. It is possible that differences in the meats' pH may be the cause, as evidenced by Kaur et al. [51], who reported that a rather consistent 0.41 pH difference between beef and lamb had an effect on bacterial growth rates. This difference is expected to be more profound in *S. aureus*- than in *L. monocytogenes*-spiked meat pieces, since *S. aureus* is rather sensitive at low pH values [52], whereas *L. monocytogenes* exhibits greater tolerance at pH levels lower than 4.5 during cold storage [53]. Nevertheless, this hypothesis needs to be further explored.

Regarding the choice of aerobic storage or vacuum-packaging, little-to-no synergistic effect was observed on either beef or mutton meat pieces. A similar observation was reported by Assanti et al. [54], who found no significant difference between vacuum-packed and chitosan-coated vacuum-packed chicken burgers, stating that the effect of chitosan surpassed the effect of vacuum-packaging. Similarly, Duran and Kahve [35] reported that the combination of chitosan coating and vacuum-packaging was more effective than vacuum-packaging alone. In contrast, Karami et al. [55] reported a synergistic effect of vacuum-packaging and chitosan in minced trout fillets against the microbial indicators examined. As already mentioned, the main antimicrobial mechanisms of chitosan on *S. aureus* and *L. monocytogenes* are probably different, with chitosan restricting membrane diffusion in *S. aureus* [23], whereas it forms electrostatic interactions in *L. monocytogenes* [47]. The observed lack of differences in *S. aureus* counts between aerobic and vacuum-packed meat pieces may justify the proposed mechanism of chitosan's antimicrobial activity. However, in the case of *Listeria monocytogenes,* the observed differences in favor of the aerobic storage of chitosan-covered meat pieces implies a possible effect on the electrostatic interactions that, due to their complexity, should be further investigated.

5. Conclusions

Edible chitosan membranes are effective in controlling the growth of pathogenic bacteria, such as methicillin-resistant *S. aureus* and *L. monocytogenes*, as they were able to reduce their concentrations in beef and mutton meat by up to 2 $\log_{10}$ CFU/g. Chitosan coatings could be used as a supplementary measure to promote the safety of beef and mutton. Moreover, they can enhance meat's quality and prolong its shelf life. The antimicrobial properties

of chitosan emulsions with essential oils—such as oregano oil—against pathogenic microorganisms in beef and mutton meat should also be examined. Even though there are numerous studies that demonstrate the application of chitosan coatings in food products at a laboratory scale, future research is needed to focus on large-scale manufacturing and commercial applications, in order to fully substantiate chitosan's properties and capabilities in food packaging.

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