Original Article

The Impact of Chitosan Nanoparticles Coating with Sodium Lactate on Beef Hamburger Quality during Storage at 4°C: Oxidative Stability, Microbial and Sensorial Characteristics

Satarzadeh, R[1], Motallebi, AA[2*], Hosseini, H[3], Ahari, H[1]

1. *Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
2. *Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran*
3. *Department of Food Science and Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

How to cite this article: **Satarzadeh R, Motallebi AA, Hosseini H, Ahari H. The Impact of Chitosan Nanoparticles Coating with Sodium Lactate on Beef Hamburger Quality during Storage at 4°C: Oxidative Stability, Microbial and Sensorial Characteristics. Archives of Razi Institute Journal. 2024;79(3):529-540.** DOI: 10.32592/ARI.2024.79.3.529

ABSTRACT

In this study, the nano chitosan particles were produced by ionotropic gelation between sodium tripolyphosphate and chitosan. The effect of nano chitosan with or without sodium lactate coating was evaluated on physicochemical (pH, thiobarbituric acid, total volatile basic nitrogen, and peroxide**)**, microbial (total mesophilic and psychrotrophic viable counts, lactic acid bacteria, yeasts, and molds), and sensorial properties of beef burgers within 24 days of storage at 4°C. The solutions of 1% nano chitosan (T_1), 2% nano chitosan (T_2), 2.5% sodium lactate (T_3), 1% nano chitosan+2.5% sodium lactate (T_4), and 2% nano chitosan+2.5% sodium lactate (T_5) were used for the coating. Although the results showed the increment of microbial growth of all treatments during storage time, the T_4 and T_5 samples had the lowest microbial counts, which indicates the synergistic effect of sodium lactate and nano chitosan. The pH of all samples was acidic to neutral (5.48-7.15) and increased during 24 days of storage, and the pH value of T_4 and T_5 samples increased with a lower slope. On the other hand, the evaluation of peroxide and TBARS values exhibited that nano chitosan had a more efficient preservative effect than sodium lactate, and both of them individually had lower antioxidant activity than their combined form. Furthermore, T_4 and T_5 samples had the best sensorial scores. These results indicated that nano chitosan and sodium lactate had synergistic effects and could be effectively applied to expand the shelf life of beef burgers.

Keywords: Chitosan, Hamburger, Nanoparticles, Shelf life, Sodium lactate

Article Info:
Received: 8 October 2023
Accepted: 13 November 2023
Published: 30 June 2024

Corresponding Author's E-Mail:
abbasalimotallebi@gmail.com

1. Introduction

Due to the richness in proteins and lipids, beef hamburger is susceptible to microbial spoilage and lipid oxidation. Although synthetic preservatives are used widely in the food industry to prevent food spoilage, recently general knowledge about adverse unfavorable effects of these compounds has led to increased interest among researchers to find suitable methods for the preservation of foods instead of synthetic additives (1). In the last two decades, the focus of research programs has shifted to new packaging technologies such as coatings and edible films (2). An edible coating is a thin layered protein lipid and polysaccharides that blockades gases, vapors, and other factors and increases the shelf life of foods such as meat products (3). Among suitable polysaccharides for edible coatings, chitosan is a cationic polysaccharide taken from the deacetylation of chitin, the key polymer in the exoskeletons of crustaceans (4). It is a non-toxic and biodegradable compound with various useful properties, such as antioxidant and antimicrobial activities against fungi and both gram-negative and gram-positive bacteria (5). Various studies have reported the bio-preservative activity of Chitosan coating in combination with other bioactive compounds or alone. Nanotechnology creates new compounds in sizes of 10 to 1000 nm with unique physical and chemical features because of their higher reactivity and more specific surface area than normal particles. Therefore, it is potentially applicable in different sectors of the food industry, such as food packaging (6). The application of nanotechnology in food packaging provides more safety and shelf life and subsequently healthier foods. Moreover, the indirect use of nanoparticles in packaging instead of the direct addition to products protects the natural structure of the products so it has sufficient overall acceptability (7). Among nanoparticles, nano-chitosan has considerable physicochemical and health-beneficial properties, including antibacterial activity (8). Using ionotropic gelation between chitosan and sodium tripolyphosphate (STPP) is among the methods for producing nano chitosan (9). Organic salts such as sodium lactate have broad applications as safe and economical preservatives in the meat industry. They have various functions such as antioxidant activity, antibacterial activity, humectant, color stabilizing, acidity controlling, and flavor enhancement (10). Hence, the combined use of coating with nanoparticles and organic salts has the potential to protect meat from microbial and chemical spoilage and extend its shelf life. In this ground, Kamani et al. measured the effect of nano chitosan without or with sodium acetate coating on *Pseudomonas fluorescens and* the quality of rainbow trout filets during 16 days of storage at the refrigerator. It was reported that the combined use of nano chitosan and sodium acetate considerably reduced *P. fluorescens* and could significantly increase the shelf life of fillets (11). Thus, the present study aimed to measure the combined effect of nano chitosan and sodium lactate coating on the physicochemical, microbial, and sensorial properties of beef hamburgers within storage at 4°C.

2. Materials and Methods

2.1. Material

Chitosan of medium molecular weight (450 kDa) with a deacetylation degree (DD) of 75% and cultural media were obtained from Sigma-Aldrich (Steinheim, Germany). The thiobarbituric acid (2-TBA), malondialdehyde, sodium lactate, and hydrochloric acid were bought from Merck (Darmstadt, Germany). The rest of the used chemicals had analytical grades.

2.2. Preparing chitosan nanoparticles

We prepared Chitosan nanoparticles according to the ionotropic gelation between STPP and chitosan based on the method of (8). Chitosan solutions (1 and 2% w/v) by adding chitosan into 1% (v/v) glacial acetic acid was prepared. Then, it was mixed for 3 h at room temperature. The STPP was dissolved in water to a concentration of 1 and 2%, and 4 ml of STPP solution was added into 100 ml of chitosan solution and mixed for 40 min. After that, it was treated with sonication at 1.5 kW for 30 min and finally applied used for more studies (8).

2.3. Characterizations

We measured zeta potential and Particle size via a Zetasizer Nano-ZS-90 (Malvern Instruments) and performed the analysis under 25°C at a scattering angle of 90°. Moreover, the samples were dispersed in water and gauged under the automatic mode for zeta potential measurements (11).

2.4. Preparation of nano chitosan-sodium lactate films

Chitosan-sodium lactate films were made ready according to the procedure proposed by Ojagh et al. (12) and were applied with little alternation. Sodium lactate solution (2.5%) was prepared in water. Then, nano chitosan solutions (1 and 2% w/v) and sodium lactate solution were mixed at a weight ratio of 1:1. We mixed the mixture for 3 h at room temperature, added glycerol (0.75 ml/g), and stirred it for 10 min. Then, the resulting solution was filtered through a Whatman No. 3 filter paper to take away any undissolved particles.

2.5. Preparation of burgers

The beef brisket was obtained 48 h postmortem and thawed and ground through a 6 mm steel plate. Then, the ground meat was mixed with salt (1.5%), black pepper (1%), and onion (2%). The resulting mixture was thoroughly mixed to obtain a homogenate paste. The mixture was formed into 100 g patties by a burger maker.

Then, the burgers were dipped into coating solutions for 15 min. After that, they were drained and air-dried on a flat plate for 2 h at 20°C to form a coating. Six different coating solutions were made ready: Control (without any additives), T1 (nano-chitosan 1%), T2 (nano-chitosan 2%), T3 (sodium lactate 2.5%), T4 (nano-chitosan 1% + sodium lactate 2.5%), and T5 (nano-chitosan 2%+ sodium lactate 2.5%). The produced burgers were stacked in polyethylene containers and kept at 4°C for 24 days and analyzed at interval times of 0, 3, 6, 9, 12, 15, 18, 21, and 24 days.

2.6. Microbial evaluation

About 10 g samples were mixed with 90 mL 0.1% peptone water in a sterile stomacher. A stomacher mixer (Stomacher 400 Circulator, Seward) was used to homogenize the mixture. After that, 10-fold serial dilutions were made ready by applying 0.1% sterile peptone water for the following purposes.

2.6.1. Total counts

The total mesophilic counts (TMC) and total psychotropic counts (TPC) were specified on plate count agar. The plates were incubated for TMC and TPC at 37°C for 48 h and at 4°C for 10 days, respectively.

2.6.2. Lactic acid bacteria Enumeration

Detection of lactic acid bacteria (LAB) was carried out on de Man, Rogosa and Sharpe (MRS) agar after incubating 48 h at 35°C under anaerobic.

2.6.3. Enumeration of molds and yeasts

Yeasts and Molds were enumerated on Yeast Extract Glucose Chloramphenicol medium with 5-day incubation at 25°C.

2.7. Chemical evaluation

2.7.1. PH measurement

After well mixing 10 g of each sample in 40 ml of distilled water, a digital pH meter (pH/Ion meter 781 metrohm) was applied to measure the pH value of the samples.

2.7.2. Lipid oxidation measurement Peroxide value

The peroxide value (PV) determines the content of hydro peroxides as primary lipid oxidation products. The PV value was measured based on the Pearson method. The outcomes were reported as meq peroxide/kg.

2.7.3. Thiobarbituric assay

Thiobarbituric (TBA) acid assay determined lipid oxidation according to the Kh I Sallam et al. method (13) and it was specified as mg of malonaldehyde/kg of the hamburger samples.

2.7.4. Total Volatile Basic Nitrogen measurement

We determined total volatile basic nitrogen (TVB-N) based on the method described by Li et al. (14).

2.8. Sensory evaluation

Sensory evaluation of hamburgers was executed by eight trained sensory panelists. Hamburgers were evaluated for odor, tissue, taste, and general attributes of acceptability of raw sample, and the taste of cooked samples fried for 10 min at 0, 3, 6, 9, 12, 15, 18, 21, and 24 days of storage. The sensory properties were estimated by a 5-point hedonic scale in which 5 was the best (very good) and 1 was the worst (unacceptable) and the unacceptable samples were those which showed mean scores lower than 3.

2.9. Statistical analysis

The data were demonstrated as means ± standard deviation (SD) and statistically analyzed by one-way ANOVA using SPSS software (version 16.0). The importance of differences among mean values was assessed by Duncan's test, and a *P*-value less than 0.05 was considered statistically significant. All the experiments were performed in triplicate.

3. Results

3.1. Zeta potential of chitosan nanoparticles and particle size

Zeta size and potential are fundamental parameters for nanoparticles. In this study, the mean particle size of 1% and 2% chitosan nanoparticles was 19.87 (nm) and 123.68 (nm), respectively. They reported that the chitosan nanoparticles size was 120.3 (nm). Generally, chitosan nanoparticle size is affected by the preparation method and sonication strength. To evaluate the colloidal system stability, zeta potential is a helpful parameter that shows the surface charge of the particles. The chitosan nanoparticles had a zeta potential of 42.2 (mV) and 48.4. These values show the stability of produced nanoparticles as they had a zeta potential of > 30 (mV). The results are in accordance with the result of (8) that specified the zeta potential of nanoparticle 51.37 (mV).

3.2. Total mesophilic counts

Total mesophilic counts (TMC) determine the food products' quality and shelf life. Figure 1 shows the TMC of beef burgers while stored at 4°C. The TMC of all treatments grew significantly by passage of time. The control samples had the maximum (10.47 log CFU/gr) TMC, while the T4 and T5 samples had the minimum (7.89 and 7.72 log CFU/gr) TMC on the last day of storage. It shows that using chitosan nanoparticles in combination with sodium lactate had better preservative effects than using each alone, indicating the synergistic effect of nano chitosan and organic salts, which cause higher inhibiting activity on microbial growth (11). The positive impact of nano chitosan was reported by Ghorabi & Khodanazary (15), who used nano chitosan as coating materials for *Cynoglossus arel* fillets during storage.

Studies showed that the antibacterial chitosan activity is relevant to its positive charge that binds to the bacterial cell surface with a negative charge, which accounts for disruption of the membrane and leakage of the cellular components. In addition, it can inhibit the transportation of nutrients bacteria into cells (16). The synergistic impact of chitosan and sodium lactate was also reported by Schelegueda et al. (17), who assessed the impact of sodium lactate and chitosan on bacterial flora of fish. Furthermore, it was noticed that using nano chitosan with sodium acetate could control total counts of refrigerated rainbow trout filets better than using nano chitosan alone.

3.3. Total psychotropic counts

Total psychotropic counts (TPC) are important as they show the quality of food for consumption. The TPC value of all treatments grew significantly by extending time (Figure 2). The final storage day witnessed the TPC value being in the order of control>T_1≅T_2>T_3>T_4≅T_5. Nano chitosan at a concentration of 2% showed stronger microbial inhibitory activity than sodium lactate 2.5%. Similar to our results, Ramezani et al. (9) and López-Caballero et al. (18) reported the antibacterial effect of nano chitosan on psychotropic counts in silver carp fillets and fish patties, respectively. Conversely, the use of sodium lactate in combination with nano chitosan (1% and 2%) had the best preservative function, as after 24 storage days, the TPC values of T4 and T5 were 7.45 and 7.33 (log CFU/gr), respectively, which were the minimum value among all treatments showing the synergistic effect of nano chitosan and organic salts can be related to the electrostatic interactions. Sodium salts of low molecular weight organic acids such as sodium lactate have been used to increase the shelf life of perishable food (e.g., meat and fish products), and they also improve the sensory features of the food. The inhibitory impact of lactates on microbial increase is due to their reducing aw effect (19). Conversely, chitosan antibacterial activity pertains to various parameters such as molecular weight, deacetylation degree, and medium conditions (pH, temperature, and presence of other components). Similarly, L. I. Schelegueda et al. (20) noticed the synergetic effect of sodium lactate and chitosan against bacterial flora of fish stored at 30°C for 72 h. Additionally, Ye et al. (21) revealed the antibacterial activity of chitosan-coated plastic films incorporating sodium lactate against L. monocytogenes as a psychotropic microorganism.

3.4. Enumeration of lactic acid bacteria

The growth of lactic acid bacteria (LAB) is slow at refrigerator temperature. The initial count of LAB was in the range of 2.14-2.68 (log CFU/gr) and reached 6.89-9.38 (log CFU/gr) after 24 days of storing, and the minimum and maximum LAB counts belonged to T5 and control samples, respectively (Figure 3). Sodium lactate could inhibit LAB development. The inhibitory effect of sodium lactate was also reported by Deumier et al. (22), on the other side, nano chitosan at both concentrations of 1% and 2% had better effect than 2.5 sodium lactate.

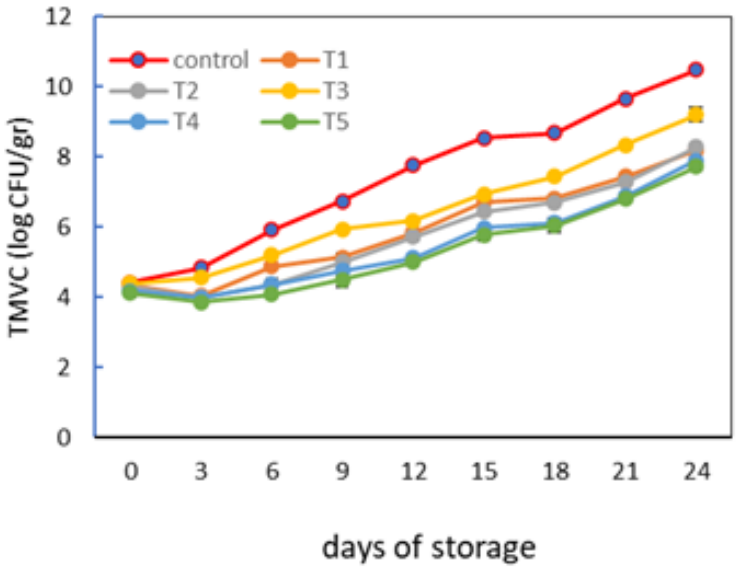

Fig 1. Total mesophilic viable counts of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

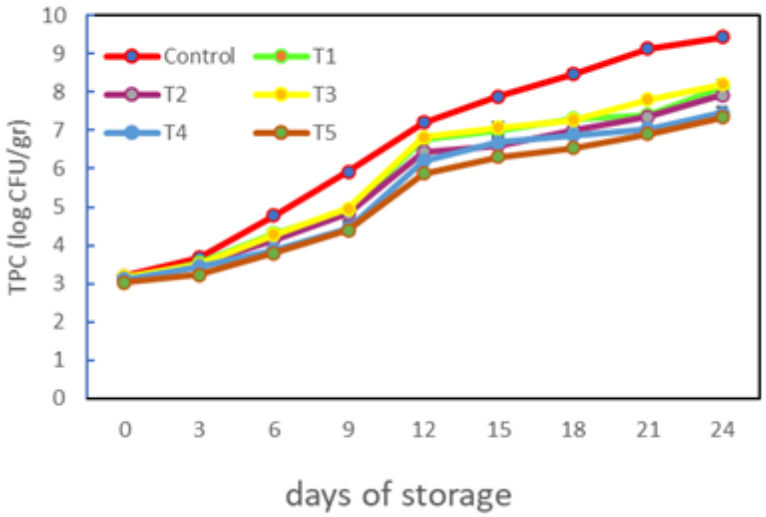

Fig 2. Total psychotropic counts of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

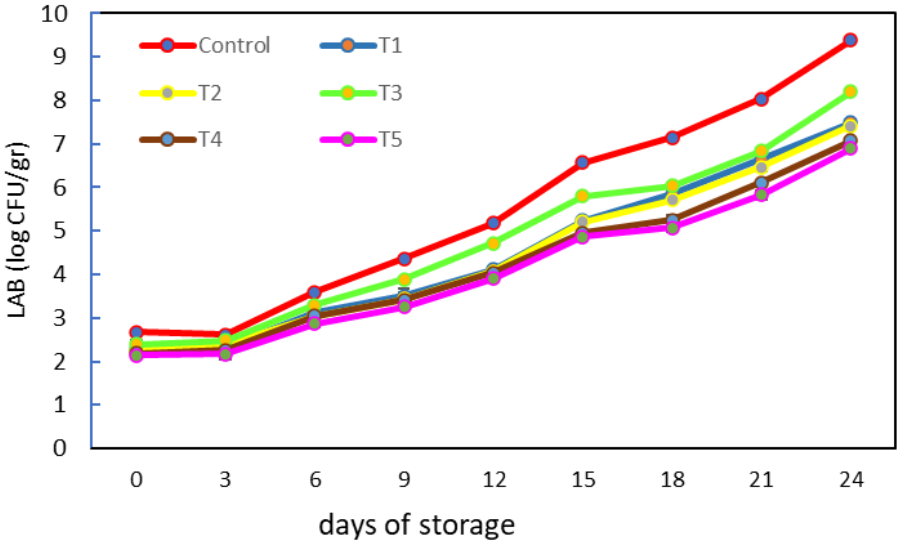

Fig 3. LAB of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

On the other hand, nano chitosan at both concentrations of 1% and 2% had a better effect than 2.5 sodium lactate. It can be related to the size of nano chitosan, which has been reported to show a strong antibacterial effect due to charge density leading to more interaction with bacteria cells and also because of high surface area per unit volume (15). As mentioned before, the minimum LAB by the end of storing time belonged to T5, which shows the synergistic effect of nano chitosan and sodium lactate. These results agreed with the outcomes of studies by L. I. Schelegueda et al. (20) and Kamani et al. (11) that noted the synergistic effect of chitosan and sodium lactate and nano chitosan and sodium acetate on LAB of fish and rainbow trout fillets, respectively.

3.5. Enumeration of yeasts and molds

The yeasts and molds count (YMC) of hamburger samples increased within the time of storing, and after 24 days of storage the maximum YMC (9.40 log CFU/G) belonged to control samples. As depicted in Figure 4, nano chitosan at all concentrations (1 and 2%) had a stronger inhibitory effect on YMC than sodium lactate. At the end of storage time, the YMC of T2, T3, T4, and T5 did not differ significantly, which shows that the synergistic impact of nano chitosan and sodium lactate could not compete with the inhibitory effect of nano chitosan. Studies showed that yeasts are usually resistant to large amounts of sodium lactate, and the sensitivity of fungi to chitosan is more than gram-positive and -negative bacteria; consequently, chitosan acts faster against fungi. Studies showed that chitosan has different mechanisms against microorganisms, including the inhibitory effect on the synthesis of protein, metal chelating, and prevention of the intake of essential nutrients essential for microbial growth. It is noteworthy that based on different research, nano chitosan shows stronger inhibition activity than chitosan due to its larger surface area and higher affinity with microorganisms' cells. Chitosan inhibitory activity against yeasts and molds has been reported by (23).

3.6. PH measurement

The shifts in pH values within 24 days of storage are depicted in Figure 5. The pH of all samples was in acidic to neutral (5.48-7.15). Until six days after storage, the pH values of all samples showed no significant change ($P>0.05$). After that, the pH value of all treatments increased in order of control>T_3>T_1≃T_2>T_4>T_5. The outcomes can be due to the generation of basic compounds by microbial and endogenous enzymes. During storage time, the use of glucose storage in meat by microorganisms can lead to protein break down and the production of some compounds, such as trimethylamine and ammonia that can increase the pH value. Among all samples, T5 had the lowest pH value, which shows the strong synergistic effect of 2% nano chitosan and 2.5 sodium lactate that could inhibit the effective increase of microorganisms. Fan et al. (24) and Ramezani et al. (9) accounted for similar observations. In addition, Kamani et al. (11) noted that samples treated with nano chitosan in combination with sodium acetate had lower pH values, which revealed its more effective inhibition effect on microbial growth and production of basic compounds.

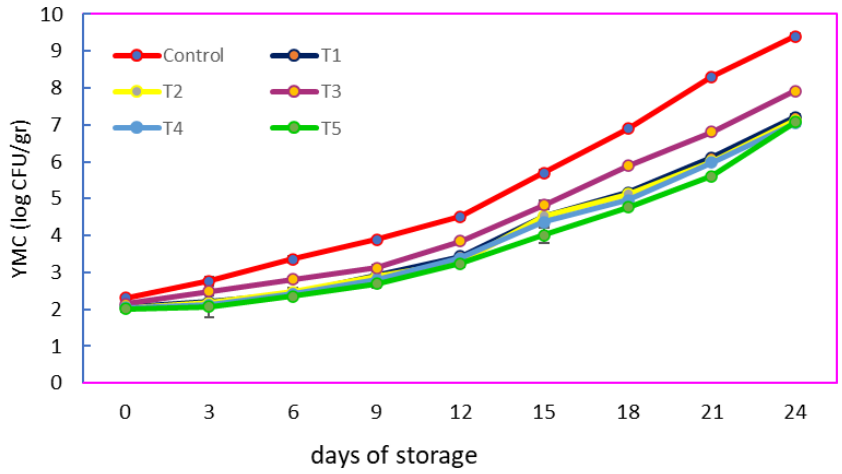

Fig 4. Yeasts and molds of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

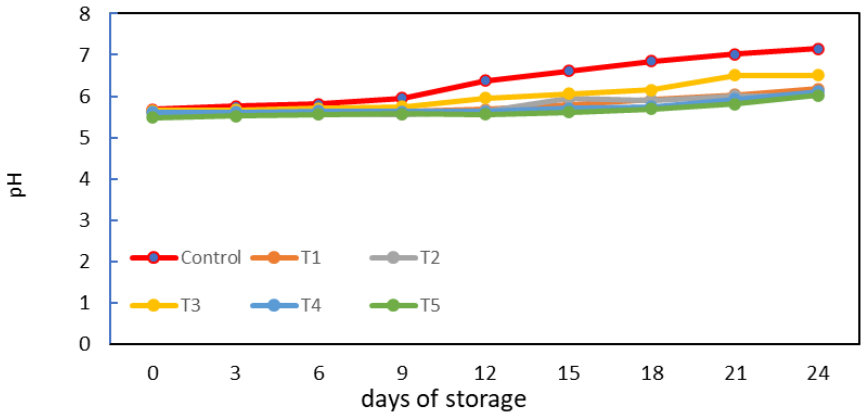

Fig 5. pH values of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

3.7. Peroxide value

Lipid oxidation is a key factor in reducing the shelf-life of fatty food. Hydroperoxides are the main products of lipid oxidation. The peroxide value (PV) is the most general scale of lipid oxidation. In the present research, the PV of the entire samples grew significantly within storage time. At the end of storage, the PV of T_1 (5.43) and T_2 (5.38) did not differ significantly ($P>0.05$) and were lower than T_3 (5.83), which displays the stronger impact of chitosan than sodium lactate on retardation of lipid oxidation (Figure 6). The significant impact of chitosan on PV of beef burgers was noted by Georgantelis et al. (25). On the other hand, the minimum PV belonged to T_5 (4.11), which indicates the combination use of nano chitosan and sodium lactate had a positive effect on retardation of lipid oxidation. Various studies showed that organic salts, especially sodium ones, have antioxidant activity. Different reactions are involved in lipid oxidation, such as microbial enzymes catalyzed and non-enzymatic or intracellular enzymes catalyzed. Different studies revealed that PV is directly related to the growth of psychrotrophic bacteria that can promote lipid oxidation by the production of lipase and phospholipase during meat storage. As shown in Figure 2, the minimum TPC belonged to T5 samples, which correlates positively with the least PV. Therefore, the minimum amount of PV of T5 can be due to the synergistic antibacterial impact of nano chitosan and sodium lactate. The findings of this study are in line with the outcomes of Kamani et al. (11), who noted the strong effect of chitosan and sodium acetate on the reduction of PVs of fish samples.

3.8. Thiobarbituric assay

Thiobarbituric (TBA) assay is a common index to evaluate lipid oxidation. The TBA reactive substances are presented in the latter phase of lipid oxidation, where peroxides are oxidized to ketone and aldehyde (9). At the first six days of storage, all samples except for the control showed a TBA value of below 0.5, which shows that hydroperoxide did not oxidize to aldehyde, but then the TBA value of the whole samples increased significantly within 24 storage days. The increment of TBA samples within the storage time can be because of the petty dehydration of beef burgers and the interaction of air oxygen and lipids. By the storage termination, the minimum and maximum TBA values belonged to T5 (1.09) and control (2.78) samples, respectively. Chitosan has antioxidant and oxygen barrier activity, which prevent lipid oxidation (12). Ion metal's chelating activity can defer lipid oxidation (18). The antioxidant activity of chitosan can be related to the existence of the amino group in the chitosan structure too, which can make a stable fluorosphere with the volatile aldehydes driven by lipids break down. Studies revealed the antioxidant activity of sodium lactate, which can effectively decrease the TBA value of meat products such as Shewail et al. who noted the positive impact of organic salts on TBA production of beef during storage. Sodium lactate inhibitory impact on lipid oxidation in meat products depends on different factors such as packaging method, storage time, microbial growth, and type of additives (13) (figure 7). Consequently, the synergistic impact of nano chitosan and sodium lactate could effectively prevent lipid oxidation. Similar outcomes were reported by Kamani et al. (11) and Bonilla et al. (23).

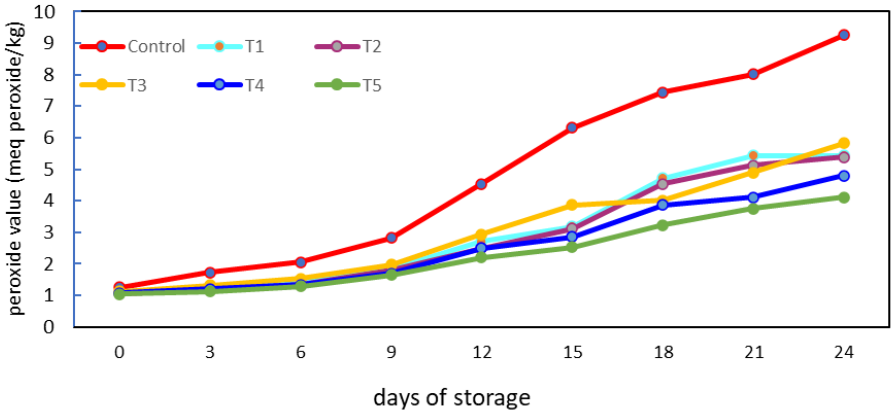

Fig 6. Peroxide values of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

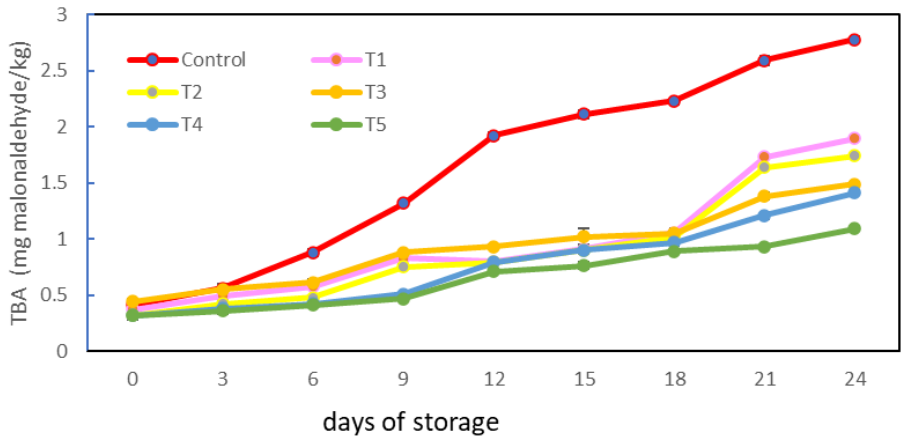

Fig 7. TBA values of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

3.9. Total Volatile Basic Nitrogen measurement

As displayed in Figure 8, the total volatile basic nitrogen (TVB-N) value of burger samples was in the range of 7.86-44.61 (mg N/100g). The TVB-N value of all samples grew significantly within storage time. After 24 days of storage, the control samples had the maximum (44.61 mg N/100g) TVB-N value, while the minimum values were related to the T_4 (19.8 mg N/100g) and T_5 (19.15 mg N/100g) samples, respectively. The T1 and T2 had lower TVB-N value than T3, which shows the higher antioxidant potential of nano chitosan than sodium lactate. The TVB-N is basically made of primary, secondary, and tertiary amines and ammonia and is a common index of meat decay. The increment of TVB-N depends on the spoilage bacteria activity and endogenous enzymes (24). Therefore, the minimum TVB-N of T4 and T5 compared to other samples indicates the higher antibacterial activity of the combination of sodium lactate and nano chitosan than each of them in separation. In accordance with our results, Bonilla et al. (23) reported that the TVB-N value of catfish fillets increased during 20 days of storage and chitosan could effectively reduce TVB-N content. In addition, the positive synergistic effect of nano chitosan and organic salts on the reduction of TVB-N content was reported by Kamani et al. (11). Furthermore, chitosan coating positive on reducing TVB-N accounted by Ramezani et al. (9) and López-Caballero et al. (18), who noted that chitosan coating could effectively reduce the TVB-N of silver carp fillets, Herring and Atlantic cod and fish patties, respectively.

3.10. Sensory evaluation

The effect of different treatments on sensorial attributes (e.g., odor, tissue, taste, and overall acceptability) of beef burgers are shown in figures 9-12. Before the experiment, no significant distinction between sensorial properties ($P>0.05$), which indicates that nano chitosan, sodium lactate, and their mixture did not influence the sensorial features of beef burgers. The sensorial scores of all treatments reduced significantly within the storage time ($P<0.05$). Among all treatments, T4 and T5 had the best sensorial properties, and control sample was the worst. These results showed the positive preservative effect of nano chitosan and sodium lactate on beef burgers during storage as they acted stronger than nano chitosan or sodium lactate alone. The sensory score of three is noted as an acceptable limit for human consumption (12); therefore, all treatments except the control sample are acceptable for consumption until 15 days of storage. Generally, sensorial attributes depend on the chemical and microbial features of samples. Similar outcomes were reported by Ramezani et al. (9) and Kamani et al. (11). On the other hand, sodium lactate did not influence the sensorial features of fish samples. These contradicting results can be due to differences in the type and concentration of applied additives, their interaction with food components, and storage conditions.

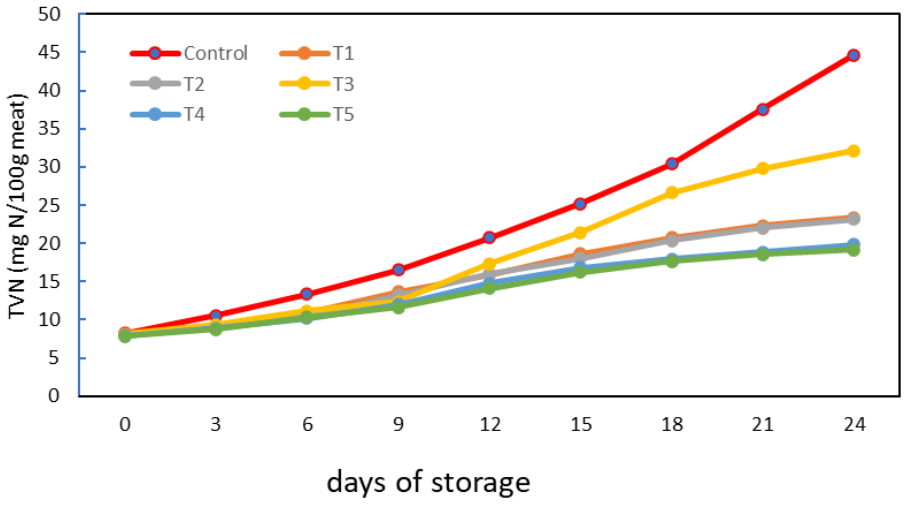

Fig 8. TVN values of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

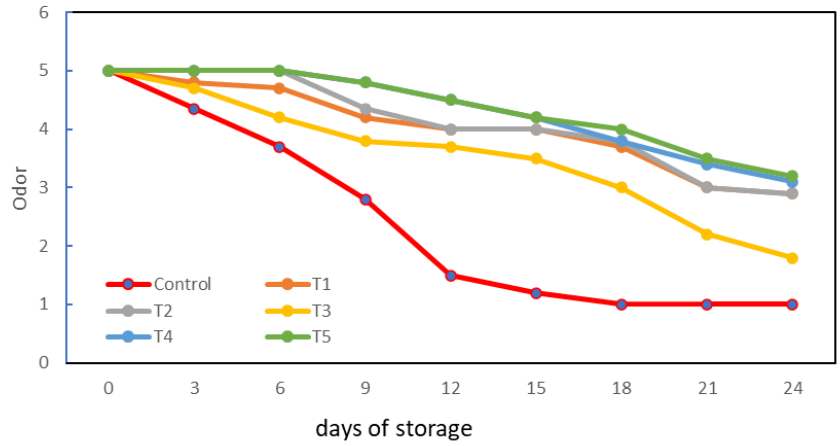

Fig 9. Odor of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

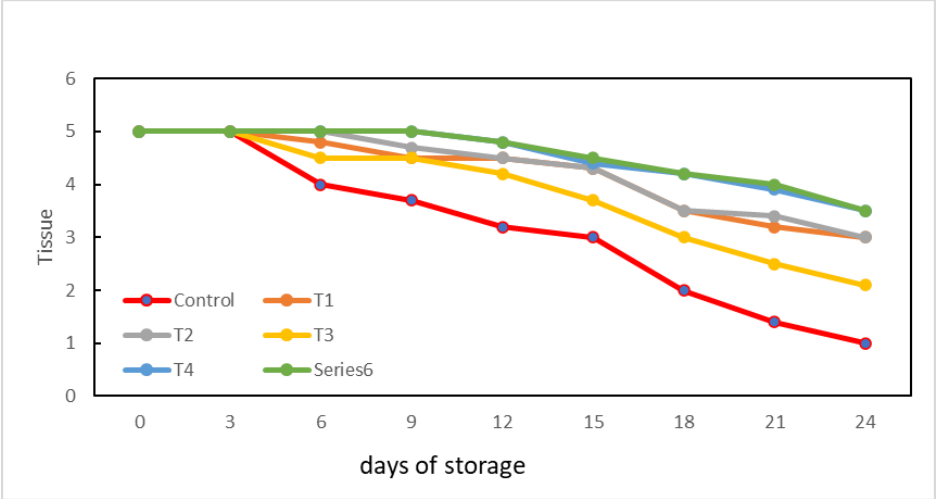

Fig 10. Tissue of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

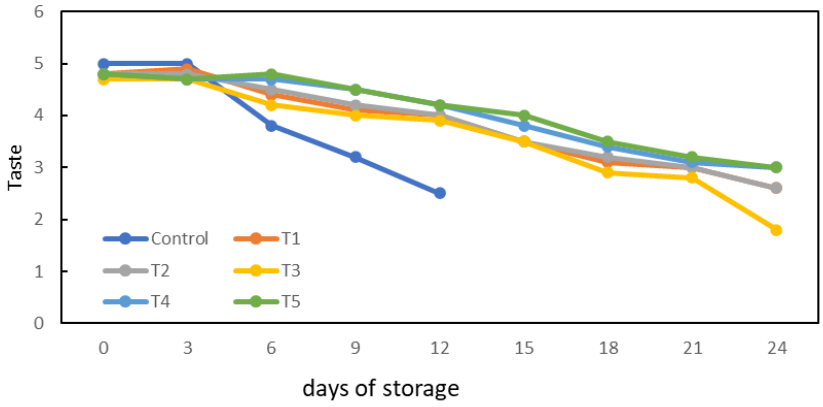

Fig 11. Taste of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

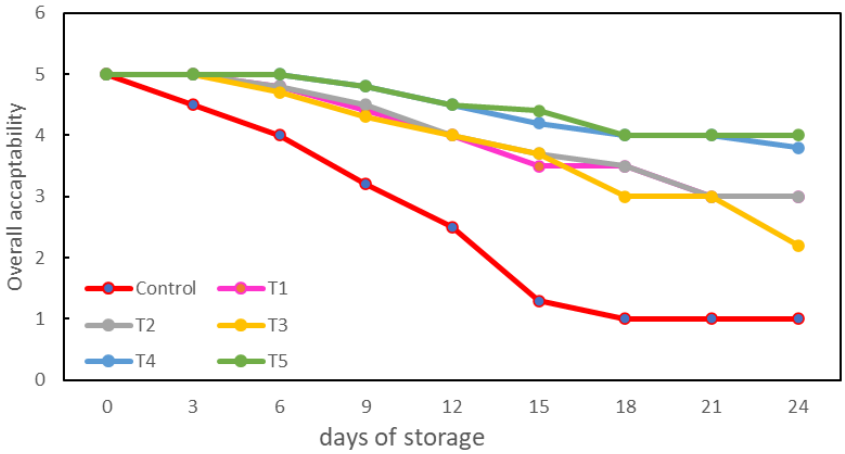

Fig 12. Overall acceptability of different treatment of beef burgers during storage. Control (without any additives), T_1 (nano-chitosan 1%), T_2 (nano-chitosan 2%), T_3 (sodium lactate 2.5%), T_4 (nano-chitosan 1% + sodium lactate 2.5%) and T_5 (nano-chitosan 2%+ sodium lactate 2.5%)

4. Discussion

In this study, we examined fundamental parameters related to chitosan nanoparticles, including particle size, zeta potential, and their role in food product quality and shelf life.

4.1. Zeta potential of chitosan nanoparticles and Particle size

In this section, the importance of particle size and zeta potential in understanding the colloidal system's stability was discussed. The particle size of chitosan nanoparticles was measured at two concentrations, 1% and 2%, with sizes of 19.87 nanometers and 123.68 nanometers, respectively. Furthermore, the zeta potentials for these nanoparticles were 42.2 and 48.4 millivolts, respectively. These results indicated that chitosan nanoparticles with zeta potentials exceeding 30 millivolts exhibit good stability.

4.2. Total mesophilic counts

Total mesophilic counts (TMC) were discussed as a critical parameter for assessing food quality and shelf life. The results showed that using chitosan nanoparticles in combination with sodium lactate had better results compared to using each component alone. This highlights the synergistic effect of combining nano chitosan and organic salts in inhibiting microbial growth.

4.3. Total psychotropic counts

Total psychotropic counts (TPC) were emphasized as an important indicator of food quality. The study revealed that nano chitosan at a concentration of 2% exhibited stronger microbial inhibitory activity compared to 2.5% sodium lactate. However, the combination of nano chitosan and sodium lactate proved to be most effective in reducing TPC, demonstrating a synergistic effect.

4.4. Enumeration of Lactic acid bacteria

The growth of lactic acid bacteria (LAB) at refrigeration temperatures was discussed, and it was found that the combination of nano chitosan and sodium lactate had a better inhibitory effect on LAB development compared to sodium lactate alone. This enhanced effect was attributed to the size of nano chitosan, which possesses a higher charge density and a larger surface area per unit volume.

4.5. Enumeration of yeasts and molds

The enumeration of yeasts and molds (YMC) as a critical parameter for assessing food quality was explained. Nano chitosan, at both 1% and 2% concentrations, demonstrated a stronger inhibitory effect on YMC compared to sodium lactate. The results showed that yeasts are generally more resistant to sodium lactate, and chitosan exhibits strong inhibitory activity against yeasts and molds due to various mechanisms.

4.6. PH measurement

The changes in pH values during the storage period were discussed. The combination of 2% nano chitosan and 2.5% sodium lactate was found to have the lowest pH value, indicating a strong synergistic effect in inhibiting microbial growth.

4.7. Peroxide value

Lipid oxidation and peroxide value (PV) were discussed as key factors affecting the shelf life of fatty foods. It was observed that chitosan had a stronger effect in retarding lipid oxidation compared to sodium lactate. The combination of nano chitosan and sodium lactate showed a positive impact on inhibiting lipid oxidation.

These findings provide valuable insights into the potential applications of chitosan nanoparticles and their synergistic effects with sodium lactate in preserving food products and maintaining their quality during storage.

Acknowledgment
The authors are grateful to the people who contributed to the completion of this study.

Authors' Contribution
Study Concept and Design: A. M.
Acquisition of data: H. Hosseini
Analysis and interpretation of data: H.A.
Drafting of the manuscript for important intellectual content: R. S.
Intellectual content: H. H.
Statistical analysis: R. S.
Administrative, technical and material support: A. M.

Ethics
Not applicable.

Conflict of Interest
The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Martins JT, Cerqueira MA, Vicente AA. In fl uence of a -tocopherol on physicochemical properties of chitosan-based fi lms. Food Hydrocoll. 2012;27(1):220–7.
2. Denavi G, Tapia-Blácido DR, Añón MC, Sobral PJA, Mauri AN, Menegalli FC. Effects of drying conditions on some physical properties of soy protein films. J Food Eng. 2009;90(3):341–9.
3. Valipour Kootenaie F, Ariaii P, Khademi Shurmasti D, Nemati M. Effect of chitosan edible coating enriched with eucalyptus essential oil and α-tocopherol on silver carp fillets quality during refrigerated storage. J Food Saf. 2017;37(1):e12295.
4. Giatrakou V, Ntzimani A, Savvaidis IN. Effect of chitosan and thyme oil on a ready to cook chicken product. Food Microbiol. 2010;27(1):132–6.
5. Prashanth KVH, Tharanathan RN. Chitin/chitosan: modifications and their unlimited application potential—an overview. Trends Food Sci Technol. 2007;18(3):117–31.
6. Kreuter J. Nanoparticulate systems for brain delivery of drugs. Adv Drug Deliv Rev. 2001;47(1):65–81.
7. Esmaeili M, Ariaii P, Roozbeh L, Maryam N, Pour Y. Comparison of coating and nano-coating of chitosan-Lepidium sativum seed gum composites on quality and shelf life of beef. Journal of Food Measurement and Characterization. 2020;15(1):341–52.
8. Du WL, Niu SS, Xu YL, Xu ZR, Fan CL. Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. Carbohydr Polym. 2009;75(3):385–9.
9. Ramezani Z, Zarei M, Raminnejad N. Comparing the effectiveness of chitosan and nanochitosan coatings on the quality of refrigerated silver carp fillets. Food Control. 2015;51:43–8.
10. Jamilah MB, Abbas KA, Rahman RA. A review on some organic acids additives as shelf life extenders of fresh beef cuts. American Journal of Agricultural and Biological Science. 2008;3(3):566–74.
11. Kamani J, Motalbei Moghanjoghi AA, Razavilar V, Rokni N. Effects of nanochitosan with and without sodium acetate coating on Pseudomonas fluorescens and the quality of refrigerated rainbow trout filets. Iran J Fish Sci. 2020;19(3):1479–99.
12. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chem. 2010;120(1):193–8.
13. Sallam KI, Ishioroshi M, Samejima K. Antioxidant and antimicrobial effects of garlic in chicken sausage. LWT-Food Science and Technology. 2004;37(8):849–55.
14. Li Y, Tang X, Shen Z, Dong J. Prediction of total volatile basic nitrogen (TVB-N) content of chilled beef for freshness evaluation by using viscoelasticity based on airflow and laser technique. Food Chem. 2019;287:126–32.
15. Ghorabi RS, Khodanazary A. Effects of chitosan and nano-chitosan as coating materials on the quality properties of large scale tongue sole Cynoglossus arel during super-chilling storage. Iran J Fish Sci. 2020;19(5):2242–57.
16. Xue ZX, Yang GP, Zhang ZP, He BL. Application of chitosan microspheres as carriers of LH-RH analogue TX46. React Funct Polym. 2006;66(9):893–901.
17. Schelegueda IL, Zalazar LA, Gliemmo FM, Campos CA. Inhibitory effect and cell damage on bacterial flora of fish caused by chitosan, nisin and sodium lactate. Int J Biol Macromol. 2015;396–402.
18. López-Caballero ME, Gómez-Guillén MC, Pérez-Mateos M, Montero P. A chitosan–gelatin blend as a coating for fish patties. Food Hydrocoll. 2005;19(2):303–11.
19. Abou-Zeid KA, Yoon KS, Oscar TP, Schwarz JG, Hashem FM, Whiting RC. Survival and growth of Listeria monocytogenes in broth as a function of temperature, pH, and potassium lactate and sodium diacetate concentrations. J Food Prot. 2007;70(11):2620–5.
20. Schelegueda LI, Gliemmo MF, Campos CA. Antimicrobial Synergic Effect of Chitosan with Sodium Lactate , Nisin or Potassium Sorbate against the Bacterial Flora of Fish Antimicrobial Synergic Effect of Chitosan with Sodium Lactate , Nisin or Potassium Sorbate against the Bacterial Flora of Fish. 2012;1(3):272–81.
21. Ye M, Neetoo H, Chen H. International Journal of Food Microbiology Effectiveness of chitosan-coated plastic fi lms incorporating antimicrobials in inhibition of Listeria monocytogenes on cold-smoked salmon. 2008;127:235–40.
22. Deumier F, Collignan A. The effects of sodium lactate and starter cultures on pH, lactic acid bacteria, Listeria monocytogenes and Salmonella spp. levels in pure chicken dry fermented sausage. Meat Sci. 2003;65(3):1165–74.
23.Bonilla F, Chouljenko A, Lin A, Young BM, Goribidanur TS, Blake JC, u. a. Chitosan and water-soluble chitosan effects on refrigerated catfish fillet quality. Food Biosci. 2019;31:100426.
24. Fan W, Sun J, Chen Y, Qiu J, Zhang Y, Chi Y. Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food Chem. 2009;115(1):66–70.
25. Georgantelis D, Blekas G, Katikou P. MEAT Effect of rosemary extract , chitosan and a -tocopherol on lipid oxidation and colour stability during frozen storage of beef burgers. Meat Sci. 2007;75:256–64.