Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 11, No. 2, 85-98, 2021
https://dorl.net/dor/20.1001.1.22287086.2021.11.2.8.2

Study of Antimicrobial Properties of Nano Chitosan with Marjoram Essential Oil in Increasing the Shelf-Life of Shrimp (Nephropidae) at Refrigerated Temperature

Z. Saeidi [a], ***H. Ahari*** [b], ***S.A. A. Anvar*** [c*]

[a] M.Sc. Graduated of the Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[b] Associate Professor of the Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[c] Assistant Professor of the Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Received: 8 April 2019 Accepted: 24 July 2019

ABSTRACT: The food lasting and its persistence can be increased by adding plant essential oils to the edible films such as chitosan. In the view of the increasing use of nanotechnology in the production of antimicrobial agents and the improvement of the properties of nano-sized compounds in comparison with the primary compounds, the antimicrobial properties of nano-chitosan with marjoram essential oil concentrations to increase the shelf-life of shrimp at refrigeration temperature has been studied. In this study, 1% and 2% concentrations of marjoram essential oil individually and with nano-chitosan with the concentration of 2% was prepared. Six different treated groups were examined for microbial, chemical, and sensory evaluations for 14 days. The results of this study showed that during storage time the total amount of volatile nitrogen increased significantly ($P \leq 0.05$), which is the indication of shrimp protein hydrolysis. there was no significant difference between treatments in terms of pH. The amount of malondialdehyde decreased up to day seven and was increased afterward. The coliform bacteria and *Staphylococcus aureus* count showed a significant difference between the control and other treatments, and with increasing storage time the bacteria count decreased. According to the results, nano-chitosan containing marjoram essential oil is a very suitable substitute for chemical and industrial coatings.

Keywords: *Antimicrobial Properties, Marjoram Essential Oil, Nano-Chitosan, Shelf Life, Shrimp.*

Introduction

Due to the fact that shrimp is such a sensitive product and is produced in large quantities, investigating the processing and quality control of shrimp is one of the most prominent missions in food industry (Goli *et al*., 2013). The combination of chemical and atmospheric additives and maintaining in low temperatures in the dark are new and effective techniques for controlling meat and meat products corruption (Dave *et al*., 2011, Ghaderi-Ghahfarokhi *et al*., 2017). There are many synthetic antioxidants that are commonly used in the meat industry to protect fats in raw and cooked products, but besides its high price they have a carcinogenic effect at high doses and have a negative consumer view on. The application of natural extracts and essential oils, in addition to create a good taste and smell, has

[*]CorrespondingAuthor: Saaa4824@gmail.com

antimicrobial properties and especially antioxidant properties in fatty foods (Hasanzadeh *et al*., 2011). Biodegradable films with a polysaccharide, protein and fat content are used to increase the shelf-life of different types of foods. Chitosan, which is obtained from chitin in the crustacean external crust, is used as a non-toxic and biodegradable biopolymer in the food industry. In previous studies, antibacterial, anti-cancer and cholesterol-lowering effects have been reported by chitosan (Perricone *et al*., 2015; Younes and Rinaudo, 2015). Antibacterial activity of chitosan is related to the presence of positive molecules and the reaction with negative charge of the cell membrane (Younes and Rinaudo, 2015). However, most studies on the use of these antimicrobial agents are limited to laboratory studies in the form of microbial culture media. The use of these compounds in foods is limited due to the activity changes in foods. Therefore, researchers are looking for ways to improve their activity.

Consumer information about the value of food, safety and its components are increasing throughout the world, and consumers prefer to consume natural foods or the one made from natural ingredients.

In this regard, the use of native plants of region seems logic. In view of the above, in this study, we will use nano-chitosan colloid with marjoram essential oil to increase the shelf-life of shrimp.

Materials and Methods

- *Plant collecting and essential oil extraction*

Marjoram plant collected from medicinal plants field located in Tehran's Jahad University in middle of March. It was kept for two days at room temperature in a shadow and away from sunlight. In order to ensure that the moisture content of the plant is minimized and to check the percentage yield of essential oil of the dry plant, it was placed in the oven for a few hours at a constant temperature of 33 degrees Celsius. 220 grams of the sample were crushed. Marjoram essential oil was prepared by hot water extraction using Clevenger method.

- *Sample preparation*

The shrimps were obtained from different regions of Tehran and were immediately transferred to the laboratory. 10 grams of each sample weighed under flame and sterile conditions and placed on a sterile aluminum foil. Immersion process was not performed for control samples. The tests were performed on days 0, 2, 4, 7, 10, 14. The treated groups were as followed:

1: control, 2: 1% marjoram essential oil, 3: 2% marjoram essential oil, 4: 2% Chitosan, 5: marjoram 1% essential oil + 2 % chitosan, 6: 2% marjoram essential oil + 2% chitosan

- *Chemical analysis*

10 g of sample was homogenized with 90 ml of distilled water in stomaker, and after 30 seconds, the pH was adjusted by the pH meter. Total volatile nitrogen and malondehide analysis assay were carried out using kjeldahl method and thiobarbituric acid respectively.

- *Microbial test on the prepared treatments*

In order to conduct microbial tests on days 0, 2, 4, 7, 10, and 14, the violet red bile agar culture and peptone water media for staphylococcus aureus and baird parker agar base media for E.coli count was prepared. The necessary dilution of sample was made. 10 g of the specimen was placed in sterile nylon and 90 cc sterile physiological sterility added and then homogenate. The 0.1 cc from dilutions transferred to pre-prepared media plates.

- *Sensory analysis*

Hedonic Factor Questionnaire was applied for sensory analysis. Color appearance and acceptance of the samples were evaluated. The most effective formulas in the treatments were evaluated by seven trained referees.

- *Statistical analysis*

In this study, SPSS 22.0 software was used to analyse the data. One-way ANOVA (and Tukey complement test) were used to analyse the groups. In order to compare the chemical and microbiological evaluation, repeated measurement ANOVA was applied. In the analysis of data on sensory properties of color and texture, Kruska-Wallis method was used. Also, the Correlation test was used to evaluate and investigate the relationship between different factors.

Results and Discussion

- Total volatile nitrogen, malondialdehyde and pH evaluation in different treated groups during maintenance period

- Control group:

Figure 1 shows the change of all three tests factors over time for treatment 1 (control). As it was known, the value of total volatile nitrogen significantly increased, and the highest value was on day 14 and the lowest on day 0. The amount of malondialdehyde produced in the samples also showed a different trend with increasing storage time. The highest amount of malondialdehyde was on day 2 and the lowest was on day 7. Changes in the amount of malondialdehyde were significant between all maintenance days. The amount of pH on day 14 was significantly higher than other treatments (8.67). And in general, with increasing storage time, the pH value was significantly increased. The lowest pH value for the first day of storage was 8.24. According to the Figure 1 the changes in the control treatment, an increase in total volatile nitrogen and pH were expected. In fact, in the control sample, no cover was used, increasing the total volatile nitrogen, the pH value was fully justified. On the other hand, the amount of malondialdehyde had decreased up to 7 days, but it may have decreased due to the instability of the malondaldehyde or its interactions with other shrimp compounds.

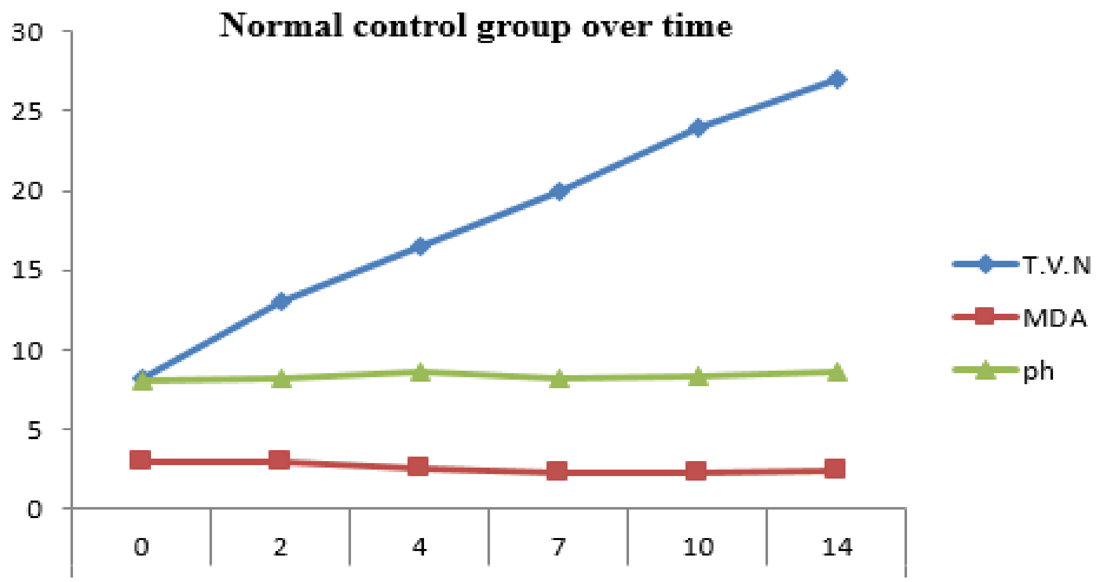


Fig. 1. Total volatile nitrogen, malondialdehyde and pH changes in control group during maintenance

- 1% marjoram essential oil treated group

Total volatile nitrogen increased significantly in the treated group containing 1% marjoram essential oil during storage. The difference between the days of storage was significant and the lowest amount was related to day 0 and the highest value was on day 14. In other words, the 1% essential oil did not have any effects. The production of malondialdehyde during the maintenance period was different from the trend observed for the control group, as the production of malondialdehyde acid increased slightly from the beginning of the maintenance period but declined again to the lowest on day 7. The highest level of production of malondialdehyde was achieved on day 2, while its level did not differ significantly between 0 and 4 days. The smallest amount was also on day 7. pH levels decreased significantly during maintenance period. The highest pH value was on day 0 and continued until 10th day. The oxidation of the essential oil or unsaturated fats of the shrimp seems to be responsible for this pH decrease over time (Figure 2).

- 2% marjoram essential oil treated group

Changes in total volatile nitrogen in 2% essential oil treated group showed a significant increase until the 10th day, but its amount decreased significantly on day 14. After 14 days of storage, the bacteria are being likely to enter the phase of death, on the other hand, the antibacterial activity of the essential oil simultaneously reduces the amount of total volatile storage volumes on the 14th day. The pH of the samples did not differ significantly during the maintenance period, except on day 0 and tenth. The highest pH on day 0 and the lowest pH on day 10 were 7.92. Although pH values measured during the maintenance period were not significantly different from day 10, the decreasing trend, against increased the amount of total volatile nitrogen, possibly indicating microbial growth. Due to the fact that acid-producing bacteria, including lactic acid bacteria, have not been investigated in this study, there is the probability of producing acid compounds as a result of the activity of the bacteria grown at the product level. The amount of malondialdehyde produced in 2% essential oil treated group on day 0 and 2 was not significantly different and was approximately 3.3, on day 4. It reached its highest level during the maintenance period, namely 5.56, but again, on the 7th day, there was a significant decrease. A significant upward trend has taken place from day to day. It seems that the treatment with essential oil (2%) had been able to reduce the oxidation of lipids at the beginning of the maintenance period, however, on the 4th day it increased but continued due to the instability of the structure of the compound or its composition with other tissue components (Figure 3).

- 2% Chitosan treated group

Total volatile nitrogen in group 4 (shrimp containing 2% Nano chitosan) had the lowest values on days 0 and 2, and there was no significant difference between these two days. On the 4th day, the total volatile nitrogen increased significantly and this trend remained constant until the day seven. Between day 7 and 14, the total volatile nitrogen increased significantly, reaching a peak of 17.5 on the 14th day. The greatest effect of Chitosan was observed at the beginning of the maintenance period, the first two days, and the stabilization of the trend until the seventh day. The pH also showed a significant difference over time. According to the chart, the highest amount of pH on day 0 was 8.8, which was significantly higher than other days of storage. The lowest amount of pH on day 10 was calculated to be 7.86, but the amount of this factor was not significantly different in other days of storage. It seems that coating with chitosan has been able to reduce pH in addition to delaying the production of volatile nitrogen. This decrease is likely to be affected by the coating of chitosan, which is a solvent of n-acetic acid and an acidic state. The production of malondialdehyde was significantly reduced. The highest amount of malondialdehyde was reached on the second day and the lowest on day 14. As it was said, malondialdehyde is the result of lipid oxidation and can be reduced by the use of chitosan coating, chitosan reduces the oxygen supply by creating a protective coating of shrimp and inhibits lipid oxidation. On the other hand, a small amount of malondialdehyde produced was also reduced due to its unstable structure or the interaction with other compounds, such as proteins over time (Figure 4).

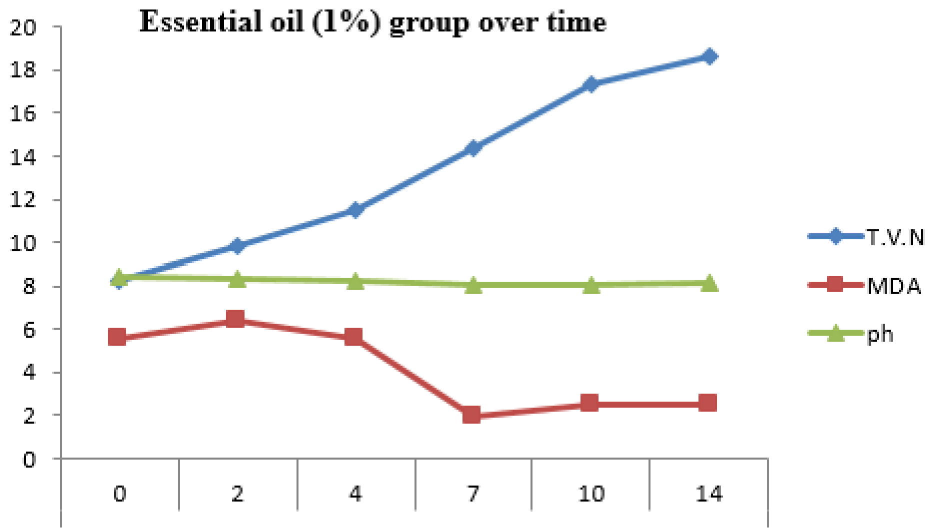


Fig. 2. Total volatile nitrogen, malondialdehyde and PH changes in essential oil 1% treated group during maintenance.

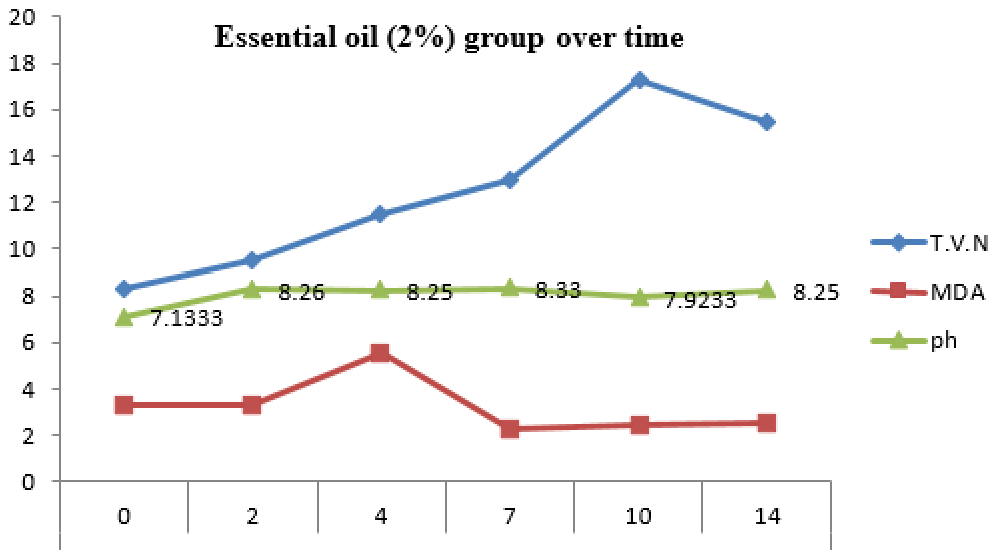


Fig. 3. Total volatile nitrogen, malondialdehyde and PH changes in essential oil 2% treated group during maintenance

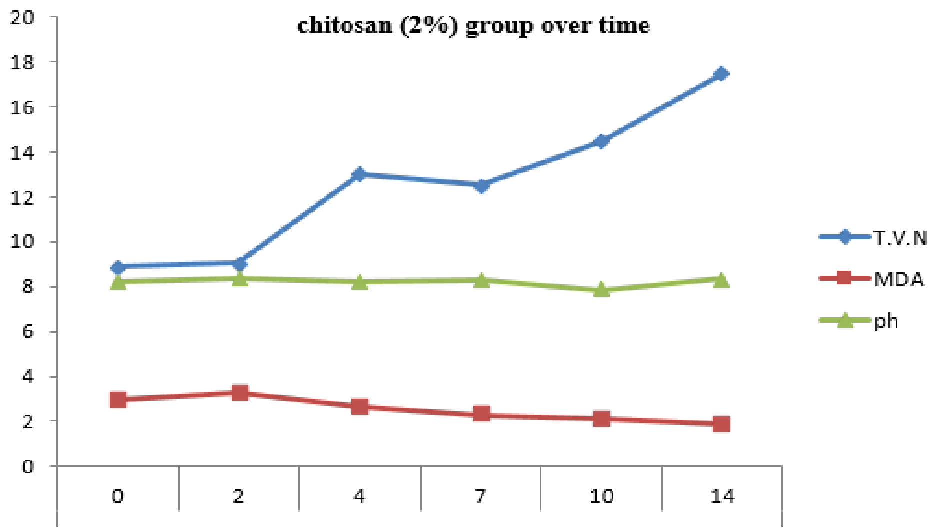


Fig. 4. Total volatile nitrogen, malondialdehyde and PH changes in Chitosan 2% treated group during maintenance.

- *1% marjoram essential oil + 2% chitosan treated group*

It showed the lowest amount of total volatile nitrogen on day 0 and was equal to 8.7, and then increased significantly with increasing storage time. There was an increasing trend in all treated samples, and none of the treatments showed a general decrease in the production process of this compound. Essential oil composition and chitosan also did not seem to get worse. Only on days 0 through 4 this trend increased with a slope below the remaining days. The highest pH of this treatment was achieved on day 0 with a value of 8.9, which decreased to the end of the maintenance period. The lowest amount of pH was also reached on day 10 and was equal to 7.6. The slope of the pH reduction in group 5 was consistent with the slope of the decrease in the treatments containing chitosan alone and the essential oil alone, which was expected to be achieved by combining both treatments. During the maintenance period, the amount of malondialdehyde was declining and after that continued to increase. In the same way, it decreased significantly from day 0 which was 2.98 to the seventh day, but significantly increased from days 7 to 10, so that the highest amount on the 14th day was equal to 5.02. It seems that the combination of 1% essential oil and nano-chitosan had been able to control the production of malondialdehyde acid up to the 7th day of storage, however, with the increase in storage time, the amount of malondialdehyde increased to a degree that the treatment was not able to control (Figure 5).

- *2% marjoram essential oil + 2% chitosan treated group*

The amount of total volatile nitrogen in group 6 was the lowest in day 0, which increased significantly with increasing storage time to day 14. However, 2% marjoram essential oil and 2% chitosan were able to ease the ever-increasing trend of volatile nitrogen production in the first seven days. However, by storing for more than 7 days at a refrigerated temperature, the protective effect of coating was reduced, which could be due to the reduction of the effect of essential oil. The pH value on the first day of storage was at its lowest, which increased with increasing storage time and peaked on day 14. The trend of increasing pH from 0 to 4 days increased, continuing to decline by 10 days, but after two days of storage, the 14th day reached the highest level throughout the maintenance period. Increases in pH on day 14 are associated with an increase in the amount of total nitrogen volumes. However, coating of chitosan containing essential oil could delay the increase of pH by 10 days. The amount of malondialdehyde increased significantly from day 0 to 2, but decreased on days 4 and 7, the lowest on day 7. In the remaining days, on day 10 and 14, the amount of malondialdehyde production increased compared to Day 7, but there was no significant difference between these two days. Apparently, among the treatments used in this study, the most important effect on shrimp chemical properties belonged to nano-chitosan + 2% essential oil was able to maintain the amount of malondialdehyde acid properly until the end of the maintenance period. On the other hand, malondialdehyde produced in the early days were decomposed due to the unstable structure or combined with other components, and the amount of production of this compound was much lower in storage (Figure 6).

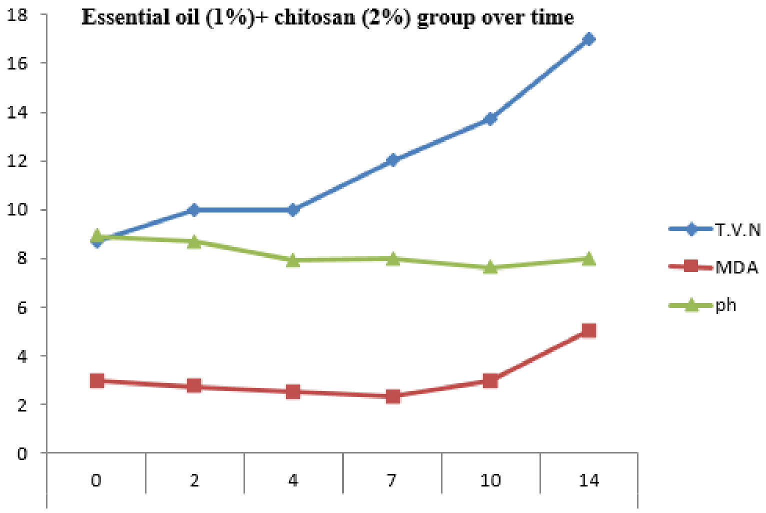


Fig. 5. Total volatile nitrogen, malondialdehyde and pH changes in essential oil 1% + chitosan 2% treated group during maintenance

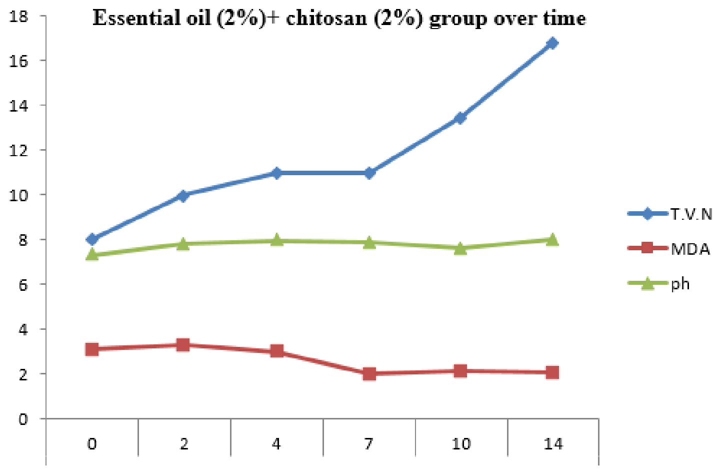


Fig. 6. total volatile nitrogen, malondialdehyde and pH changes in essential oil 2% + chitosan 2% treated group during maintenance

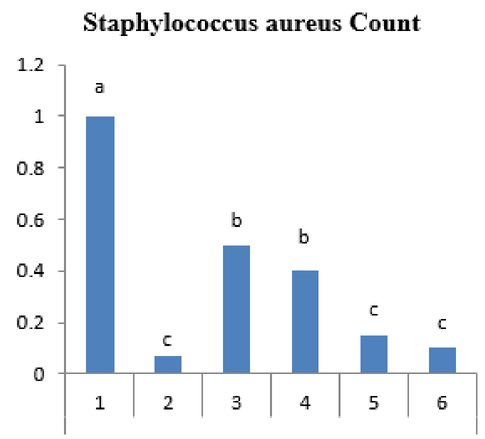
- *Microbial analysis; Staphylococcus aureus and E. coli count*

Figure 7a shows that the highest and lowest levels of infection with *Staphylococcus aureus* were in control and the group containing 1% essential oil, respectively. There was no significant difference between group 2, 5 and 6 (p> 0.05), but with other samples showed significant difference. Chitosan 2% and 2% essential oils did not show significant differences, but with other samples there was a significant difference. The control sample with the number of colonies was 1×10^4 had the highest number of colonies and all the samples showed a significant difference. The higher number of *Staphylococcus* counted in the control sample indicates the antibacterial effect of the treatments. Meanwhile, it seems that group 2 oil had a greater inhibitory effect on *Staphylococcus aureus*. This may be as the result of the lower initial microbial number.

Based on the result illustrated in Figure 7b, the results of the Duncan test were categorized into four groups and the samples of group 1 and 2 had the highest and lowest colony count with the values of 3 and 13.0 log cfu / ml, respectively. Group 3, 4 and 5 found to have a significant difference with group 1 and 2 (P <0.05).

- *Color, smell, flavor, tissue texture changes in different groups*

Sensory analysis diagrams of shrimp samples covered with different 6 groups composition indicate that there is no significant difference between treatments. This means that none of the treatments could have a significant effect on the color of the specimens. However, the highest color score was observed in treatment group 5 and the lowest color scores for the control sample (Figure 8a). Smell factors showed a significant difference between treatments. The highest odor factor for odorant essential oil (1%), as well as nano-chitosan + 1% essential oil composition treatments was obtained. The lowest score was for the control sample, but other treatments received higher scores than the control. The 1% essential oil as well as chitosan coating, showed to make delay for inappropriate smell of shrimp. Other treatments, however, received less privilege, but had a higher score than control group (Figure 8b). The flavor graphs of the samples also showed that group 2 and 5 showed the highest score. Groups 4 and 6 was lower while group 1 and 3 had no significant difference in flavor (Figure 8c). Tissue texture analysis showed that only control group had a significantly lower score, group 3 had less tissue texture changes (Figure 8d).



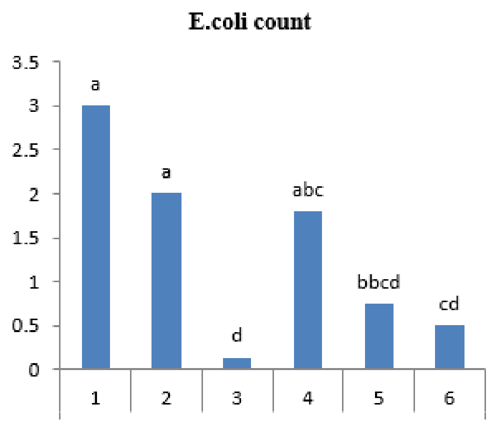


Fig. 7. Staphylococcus aureus (a) and *E. coli* (b) Count in groups: 1: control, 2: essential oil 1%, 3: essential oil 2%, 4: Chitosan 2%, 5: essential oil 1% + chitosan 2%, 6: essential oil 2% + chitosan 2%

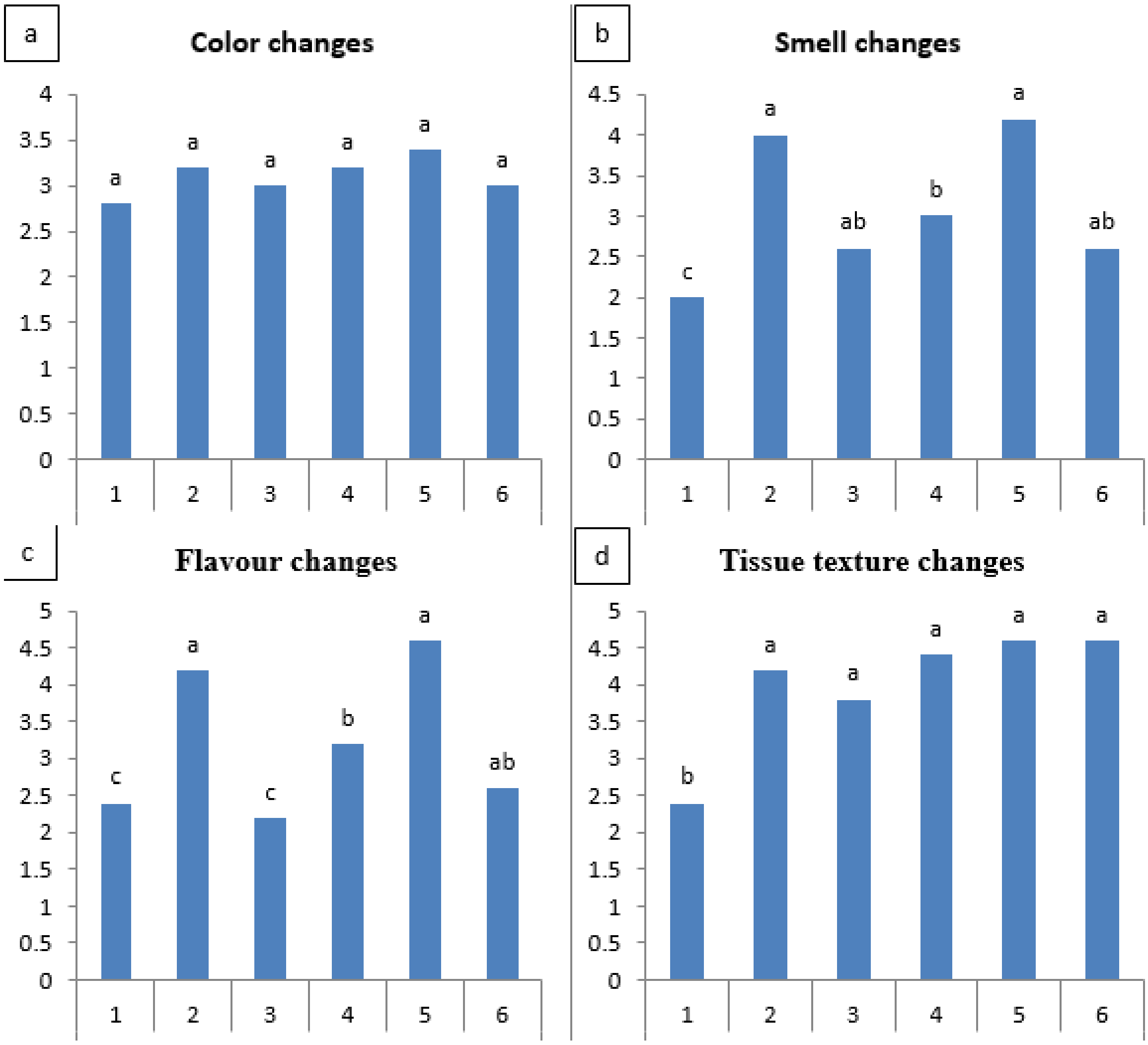


Fig. 8. Color, smell, flavor, tissue texture changes in groups: 1: control, 2: essential oil 1%, 3: essential oil 2%, 4: Chitosan 2%, 5: essential oil 1% + chitosan 2%, 6: essential oil 2% + chitosan 2%.

Shrimp is one of the most important seafood products in the world. However, shrimp breaks down very quickly (Alparslan *et al*., 2016). Their corruption is mainly due to biological reactions, such as lipid oxidation, protein degradation or microbial enzymes, resulting in shortening the shelf life of marine products. Most of the major causes of shrimp damage, microbial growth components, biochemical and melanose-color changes occur due to polymerization of phenols (Nirmal and Benjakul, 2011). As a result, review and study on the processing and quality control is among the most prominent missions of the researchers in the food and biotechnology engineering industry. Because shrimp is a vast food product with a high production level, determining its quality is very important. Using fresh meat for all products, including shrimp, is the most important parameter (Goli *et al*., 2013). Mechanical, chemical, and microbial properties of fishery products are the most important parameters in these products. Given the adverse effects of synthetic additives, the attention of food manufacturers and legislators has been limited to the use of synthetic types and the application of natural varieties. The potential uses of various films in food packaging, describes the different types of microbial targets (fungal, bacteria, etc.), and focuses on the applicability of techniques to industry Investigated by Elsabee *et al*. 2015. It Presents the science behind anti-microbial packaging and films reflecting advancements in chemistry, microbiology, and food science includes the most up-to-date information on regulatory aspects, consumer acceptance, research trends, cost analysis, risk analysis and quality control discusses the uses of natural and unnatural compounds for food safety (Elsabee *et al*., 2015). Several studies have been focused on new packaging technologies due to the increasing population and the limited natural resources (Krochta, 2002). Active packaging, with the aim of releasing antimicrobials agents to foods is getting much attention due to their role in increasing foods shelf–life, minimizing or even eliminating the presence of food borne microorganisms (Janjarasskul *et al*., 2016; López de Dicastillo *et al*., 2016; Rizzolo A *et al*., 2016). Essential oils (EOs) are aromatic substances produced naturally as secondary metabolites by a specific plant species. EOs are mainly composed by terpenoids, phenolic and aromatic compounds and their composition can vary, depending on the edaphoclimatic characteristics of the plant, part of the plant (flower, seed, leaves, fruits, stems and others) used for the extraction and the extraction method (Nakatsu *et al*., 2000; Dai and Mumper 2010; Raut and Karuppayil, 2014). Chitosan is used as a non-toxic and biodegradable biopolymer which showed anti-bacterial, anti-cancer and cholesterol-lowering effects (Perricone *et al*., 2015; Younes and Rinaudo, 2015). Beyki *et al*. (2014) showed an enhanced antimicrobial activity of Mentha piperita EO encapsulated in chitosan-cinnamic acid nanogels. Gómez-Estaca *et al.* on 2010 investigated the efficiency of Biodegradable gelatin–chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Feyzioglu *et al.* 2016 showed the antimicrobial and antioxidant delivery applications of chitosan nanoparticles loaded with summer savory essential oil (Feyzioglu *et al*., 2016). Abdullahi *et al*. (2012) evaluated the efficiency of rosemary essential oil (REO) to develop an active film from chitosan and the microstructure and the interaction of the chitosan-based films with REO was studied. According to their research the solubility and water gain of the chitosan film decreased. It was determined that REO improved the transparency of the films in neat chitosan. Films containing REO showed more antibacterial activity and total phenol content. The films containing REO showed potential to be used as active film in food preservation. In this study, we will use nano-chitosan colloid with marjoram essential oil to increase the shelf-life of shrimp. During the 14 days of storage, the total volatile nitrogen in the samples was generally increased, as well as in each treatment alone and throughout the maintenance period, the amount of total volatile nitrogen increased, indicating an increase in protein hydrolysis as a result of the bacterial activity, or even the autolysis of shrimp tissue proteins, results from self-tissue enzymes. The rate of total volatile nitrogen is considered as one of the indicators of fish and other aquaculture corruption. There is also a close connection between the total volatile nitrogen index and the freshness of shrimp, if this index is less than or equal to 20 mg nitrogen per 100 grams of meat indicates freshness and a lower or equal 30% is acceptable, and more than 40 shows that the product is inappropriate for the consumer (Asik *et al*., 2014). In 2010, Alak *et al*. Studied the physico-chemical and microbial characteristics of the Bonito (Sarda sarda) fish fillets, which were packaged with chitosan films in unpacked packets. The results of their study showed that chitosan films did not significantly decrease the amount of volatile nitrogen and the amount of thiobarbituric acid. However, the lowest pH was observed in fish fillets with chitosan coating. Also, bacterial growth in fillets packed in chitosan film was less than other packets. In the current study in addition to chitosan packaging alone, the chitosan package with marjoram essential oil was also used. Our results showed that control treated group showed a significant difference in the amount of volatile nitrogen with other groups, while there was no significant difference between the pH of the samples and the amount of malondialdehyde had the highest amount in the treatment containing 1% marjoram (Alak *et al*., 2010). Fazl ara *et al*. (1396) examined the effect of 2% chitosan coating containing 1% marjoram essential oil on the chicken meat's quality at refrigerator temperature. Results of the mesophilic bacteria analysis showed that with increasing storage time, bacterial count increased and most of it was seen on day 15, and the lowest amount was observed in chitosan treatment containing Marjoram essential oil. Also, the volumes of volatile nitrogen volumes during the maintenance period were increasing in all treatments and their amount in the distilled water submerged in the control group was higher than the other two treatments. Their results indicated that the amount of thiobarbituric acid during the maintenance period increased and the least amount was related to chitosan treatment containing marjoram essential oil after 15 days of storage. The pH increased during the maintenance period in the control sample, but in the chitosan sample with the essential oil was reduced after 15 days. The comparison of macroscopic properties for odor, shape, elasticity and color showed on day 12 and 15 the difference was significant between all samples and the highest score was for the chitosan sample containing the essential oil. In the present study, the lowest bacterial growth was observed in 1% essential oil, chitosan containing 1% essential oil and chitosan containing 2% essential oil and the highest growth rate of *Staphylococcus aureus* in the control sample, indicating the antimicrobial effect of the essential oil and synergistic effect between essence and chitosan were inhibited by microbial growth. The lowest coliform count was also obtained in essential oil (2% marjoram) and chitosan (1%) and chitosan (2% marjoram). In general, the contamination of the mesophilic bacteria was decreasing during storage, and the lowest number was obtained on day 10. In this research, the total volatile nitrogen volumes and pH levels during the maintenance period were increased in all treatments and malondialdehyde was decreased until the day 7, but increased until the 14th day of storage. Many studies have reported an increase in the total volatile nitrogen volumes with increasing time (Khoda nazeri and Poorashvori, 2016; Pormolaie *et al*., 2017). Moussavi Nasab *et al*. (2013) examined the effect of icing on raw and cooked shrimp with 2% Chitosan solution for 6 months at -18 °C. The chitosan ice cream samples were compared with ice-water samples, sodium meta bisulfite and control. The results from their studies showed that after the maintenance period, all samples of shrimp had no significant difference in terms of color, odor, texture. While in our work, the 15-day sample was maintained at a refrigerated temperature, and the chitosan-containing essential oils obtained the highest scores for the investigated factors. In another study by the same researcher, Moussavi Nasab *et al*. (2013), examined the effect of coatings prepared from chitosan and black pepper essential oils on the microbial and physicochemical properties of common carp. The results showed that essential oil of black pepper and chitosan had a significant synergistic effect in reducing total bacterial count, psychrophilic bacteria, lactic acid bacteria and enterobacteria. Also, the two compounds decreased the amount of volatile nitrogen in coated samples. In our work, the higher total volumes in the control sample compared to other treatments indicated a positive effect of chitosan coating and a combination of chitosan plus the essential oil was the alignment of these two studies. Also, the effect of the combination of essential oils and coating on the reduction of bacteria in our work has also been achieved. In this regard, the antimicrobial effects of chitosan and marjoram essential oils can be attributed to the reduction of bacterial levels. The total coliform count in the treated groups showed that the total number in the control sample was significantly higher than all other treatments. During storage, the amount of these bacteria decreased with increasing storage time. For each treatment, by the days of experiment the total amount of coliforms in the control group was higher than all treated samples. These results indicate a good inhibitory effect on all coatings and essential oils used. Wang *et al*. (2014) examined the effect of chitosan nanoparticle coatings on the quality of *Litopenaeus vannamei* shrimp during storage at 4 ° C. Shrimp samples were grouped into three groups and immersed in 1% carboxymethyl chitosan solution, 1% nanocytosis and immersed in plane water (control) for 30 seconds. Samples were stored for 10 days at refrigerator temperature and analyzes were performed on days 0, 2, 4, 6, 8 and 10. Chemical analysis of samples showed that pH of all shrimps increased during storage, but this increase was lower in samples treated with chitosan nanoparticles than in two other samples. The lowest amount of volatile nitrogen and the number of thiobarbituric acid in shrimp coated were lower than the other two groups. The total live bacteria in all chitosan treated samples were reduced in the 10th day, and the least bacterial count was related to nano chitosan and the most was related to the control sample. In our experiments, the total bacterial count decreased during the maintenance period. The lowest bacterial count in the 10th day was related to 1% marjoram essential oil and chitosan treatment, and the highest total bacterial count was observed in the control sample which indicates the antimicrobial effect of the combination of these two treatments. The total volatile nitrogen volumes during the maintenance period increased, while the PH and malondnoladium levels initially had a decreasing trend up to 10th and 7th days, respectively. Pormolaie *et al*. (2017) investigated the antioxidant and anti-bacterial effects of marjoram essential oil on the survival of Surimi made from carp during frozen storage (-18 °). Thiobarbituric acid levels and volatile nitrogen levels of both treatments increased, which was higher in the control sample during the final months of storage. The factors studied in our work show that the rate of the volatile nitrogen increased during the maintenance period, but the amount of malondnorldis was reduced in 7 days and then increased, and its final value on day 14 was lower than the first day of storage. This difference may be due to the difference in frozen storage and storage in the refrigerator. It has also been reported that marjoram can reduce the production of volatile nitrogen and its effect on antibacterial properties because of carvacrol and thymol compounds.

The results of staphylococci concentrations in the different groups in this study indicate that the control sample contained a large number of colonies while all other treated groups showed a significant reduction in this bacterium. These results may be due to the simultaneous use of chitosan and marjoram essential oil, that has a synergistic effect on this gram-positive bacterium, since both chitosan and marjoram have antibacterial properties which was in accordance with Karimi Reza Abad *et al*. (2016) study that compared the chitosan coating and wrapper for packaging poultry fish.

Conclusion

Considering the chemical and microbial characteristics of the shrimp during the maintenance period in our study, it seems that the combination of chitosan and marjoram essential oil has been able to maintain the quality of shrimp until the end of the maintenance period as compared to the control sample. In fact, the combination of nano-chitosan and treated groups compared to control samples could reduce the total volatility, pH and malondialdehyde production significantly and maintain the quality of samples during storage. However, the maintenance of samples, even covered samples in the refrigerator for more than 7 days, with regard to the chemical factors, are not recommended.

These coatings also significantly reduced the number of *Staphylococcus aureus* colonies and coliforms. It might be concluded that it might be better not to store the samples in the refrigerator either in terms of chemical factors or in terms of microbial factors for more than 5 days.

References

Abdollahi, M., Rezaei, M. & Farzi, G. (2012). Improvement of active chitosan film properties with rosemary essential oil for food packaging. International Journal of Food Science & Technology, 47(4), 847-853.

Alak, G., Hisar, S. A., Hisar, O., Kaban, G. & Kaya, M. (2010). Microbiological and Chemical Properties of Bonito Fish (Sarda sarda) Fillets Packaged with Chitosan Film, Modified Atmosphere and Vacuum. Kafkas Univ Vet Fak Derg, 16 (Suppl-A), S73-S80.

Alparslan, Y., Yapıcı, H. H., Metin, C., Baygar, T., Günlü, A. & Baygar, T. (2016). Quality assessment of shrimps preserved with orange leaf essential oil incorporated gelatin. LWT-Food Science and Technology, 72, 457–466.

ASsik, E. & Candogan, K. (2014). Effects of chitosan coatings incorporated with garlic oil on quality characteristics of shrimp. Journal of Food Quality, 1-10.

Beyki, M., Zhaveh, S., Khalili, S. T., Rahmani-Cherati, T., Abollahi, A., Bayat, M., Tabatabaei, M. & Mohsenifar, A. (2014). Encapsulation of Mentha piperita essential oils in chitosan-cinnamic acid nanogel with enhanced antimicrobial activity against Aspergillus flavus. Industrial Crops and Products, 54, 310–319

Dai, J. & Mumper, R. J. (2010). Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules, 15, 7313– 7352.

Dave, D. & Ghaly, A. E. (2011). Meat Spoilage Mechanisms and Preservation Techniques: A Critical Review. American Journal of Agricultural and Biological Sciences, 6(4), 486-510.

Elsabee, M., Morsi, R. & Hassan, M. (2011). Chitosan-Oregano Essential Oil Blends Use as Antimicrobial Packaging Material. In book: Antimicrobial Food Packaging, Chapter: 44, Publisher: Elsevier Science, Editors: Jorge Barros-Velazquez, pp.539-552. DOI: 10.1016/B978-0-12-800723-5.00044-9

Feyzioglu, G. C. & Tornuk, F. (2016). Development of chitosan nanoparticles loaded with summer savory (Satureja hortensis L.) essential oil for antimicrobial and antioxidant delivery applications. Food Science Technology, 70, 104–110.

Ghaderi-Ghahfarokhi1, M. M., Barzegar, M. A., Sahari1, H., Ahmadi Gavlighi1, F. & Gardini. (2017). Chitosan-cinnamon essential oil nano-formulation: Application as a novel additive for controlled release and shelf life extension of beef patties, International Journal of Biological Macromolecules, http://dx.doi.org/doi:10.1016/j.ijbiomac.2017.04.002.

Goli, R. & Ghasemi-Varnamkhasti, M. (2013). Recent Applications of Physical Methods of Determining The Freshness and Quality Of Shrimp. 8th National Congress of Agricultural Machinery Engineering (Biosystems); Mashhad, Iran. [In Persian].

Gómez-Estaca, J., López de Lacey, A., López-Caballero, M. E., Gómez-Guillén, M. C. & Montero, P. (2010) Biodegradable gelatin–chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiology, 27, 889–896.

Hosseini, S. F., Rezaei, M., Zandi, M. & Farahmand Ghavi, F. (2015). Effect of fish gelatin coating enriched with oregano essential oil on the quality of refrigerated rainbow trout fillet. Journal of Aquatic Food Product Technology, 25(6), 835–842.

Janjarasskul, T., Tananuwong, K., Kongpensook, V., Tantratian, S. & Kokpol, S. (2016). Shelf life extension of sponge cake by active packaging as an alternative to direct addition of chemical preservatives. Food Science and Technology, 72, 166– 174.

Karimi Reza Abad, M., khodanazry, H. & Sensei, Q. M. (2016). Effect of Chitosan Coating and Coating on Qualitative Properties of Lethrinus nebulosus during Storage in a Refrigerator. Journal of Veterinary Research and Biological Products, 116, 252-263

Khoda Nazari, A. & Pourashouri, P. (2016). The chemical, microbial and enzymatic changes made by the Otolithes ruber and the abdominal cavity during ice storage. Journal of Veterinary Research and Biologicals, 117, 155-167

Krochta, J. (2002). Proteins as Raw Materials for Films and Coatings: Definitions, Current Status, and Opportunities. Protein-Based Film. Coatings, doi:10.1201/9781420031980.ch1.

López de Dicastillo, C., Bustos, F., Guarda, A. & Galotto, M. J. (2016). Cross-linked methyl cellulose films with murta fruit extract for antioxidant and antimicrobial active food packaging. Food Hydrocolloids, 60, 335–344.

Nakatsu, T., Lupo, T., Chinn, W. & Kang, L. (2000). Biological activity of essential oils and their constituents. Studies in Natural Products Chemistry, 21, 571–631.

Nirmal, N. P. & Benjakul, S. (2011). Retardation of quality changes of Pacific white shrimp by green tea extract treatment and modified atmosphere packaging during refrigerated storage. International Journal of Food Microbiology, 149(3), 247–253.

Perricone, M., Arace, E., Corbo, M. R., Sinigaglia, M. & Bevilacqua, A. (2015). Bioactivity of essential oils: a review on their interaction with food components. Front Microbiology 6, 76.

Pormolaie, F. & Jafarpour, S. (2017). Antioxidant and Antibacterial Effects of Origanum Vulgare L. Marjoram Essential Oil on Surimi Sustainability from Cyprinus Carpio in the course of the maintenance period at C 18. Fisheries, Journal of Natural Resources of Iran, 1 70, p. 44-59.

Raut, J. S. & Karuppayil, S. M. (2014). A status review on the medicinal properties of essential oils. Industrial Crops and Products, 62, 250–264.

Rizzolo, A., Bianchi, G., Povolo, M., Migliori, C. A., Contarini, G., Pelizzola, V. & Cattaneo, T. M. P. (2016) Volatile compound composition and antioxidant activity of cooked ham slices packed in propolis-based active packaging. Food Packaging Shelf Life, 8, 41–49

Younes, I. & Rinaudo, M. (2015). Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications. Marine Drugs. 13, 1133-1174.

Wang, Y., Liu, L., Zhou, J., Ruan, X., Lin, J. & Fu, L. (2014). Effect of Chitosan Nanoparticle Coatings on the Quality Changes of Postharvest Whiteleg Shrimp, Litopenaeus vannamei, During Storage at 4°C. Food and Bioprocess Technology, Published Online: 17 December 2014.