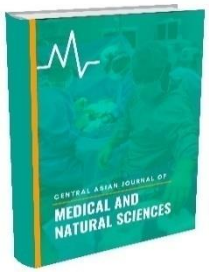
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 06 | Nov-Dec 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

EVALUATION OF THE EFFICIENCY OF CHITOSAN AND NANO-CHITOSAN IN PRESERVING THE QUALITATIVE AND MICROBIAL CHARACTERISTICS IN THE FISH LUCIOBARBUS XANTHOPTERUS OF CHILLED

1. **Fayza Me.Saleh**
2. **Tariq Kh. Albashr**

Received 20th Oct 2023,
Accepted 21st Nov 2023,
Online 25th Dec 2023

[1] Department of Food Science, College of Agriculture, University of Tikrit.
[2] Department of Food Science, College of Agriculture, University of Tikrit.
Corresponding author: E-mail: faztmhdsalh@gmail.com, tareqalbesher@tu.edu.iq

Abstract: The study was conducted in the laboratories of the Food Science Department at the College of Agriculture - Tikrit University for the period from 10/1/2022 to 11/1/2023, and its main objective is to evaluate the improvement of quality and prolong the shelf life of cold-preserved Luciobarbus xanthopterus fillets, For this purpose, four different groups of tan fillets treated with different solutions of distilled water 2%, acetic acid 2%, chitosan 2%, and nano chitosan 2% were stored at 2-3°C for 12 days to determine changes in chemical properties, Microbiologically, during cryopreservation, the results showed a significant decrease p≤ 0.05 in the FFT, TBA, PH, and PV values of fish fillets treated with chitosan and nano chitosan compared to distilled water and acetic acid treatments during the storage period at 2 degrees Celsius, It was found that the FFT values on the first day of the experiment were 7.15 and 7.15 grams/100 grams of fat, and on the 12th day of storage they reached 12.00 and 10.50 grams/100 grams of fat, respectively, compared to the distilled water and acetic acid treatments, which were 7.15 and 7.20 mg md/kg fat. On the last day of storage, it reached 21.50 and 17.50 grams / 100 grams of fat, respectively, The results of microbiological tests for the total number of bacteria showed that treating Lucio Arbus xanthopterus fillets with chitosan and nano-chitosan preserved the quality

and content of fish meat in its bacteria content for a period of 12 days for fish meat in cold storage at 2 degrees Celsius, The total number of bacteria at the end of the storage period was 24.16 and 12.68, respectively, followed by the samples treated with acetic acid, which amounted to 27.83, while the samples treated with distilled water exceeded the permissible limits on day 12, when the total number of bacteria was 41.18, Through these results, we show that using a 2% concentration of chitosan and nano chitosan as a natural preservative can preserve the quality characteristics and extend the shelf life of Luciobarbus xanthopterus fillets during cold storage.

Key words: Chitosan, Nanocytosan, Refrigerated Luciobarbus Xanthopterus, Quality Characteristics, Microbial properties

INTRODUCTION

During the period of fishing, transportation, and handling, fish and their meat are exposed to many factors that affect their freshness. This effect may be rapid when storage and handling conditions are not good, or this deterioration may be slow when health conditions are followed in the aforementioned factors. The quality of fish in both cases is... The effect is determined by many chemical, physical and microbiological factors and the enzymatic activity resulting from them that lead to a change in the level of freshness during storage (Kinsella, 1988), Inferring the freshness of fish includes knowledge of the changes that occur in sensory properties such as smell, color, and texture. Recent studies have also indicated a clear relationship between the freshness of fish and its microbial load, as it has been shown that the relationship between them is inverse, The negative effect on freshness increases in the presence of specific types of microbial species rather than others, as it has been shown that more than 50% of fish products deteriorate in quality as a result of the activity of microorganisms in them after fishing, especially Kumar 2000) - chitosan (poly-b- (1e4) - D-glucosamine is a substance made mainly from crustacean slices and is known as one of the chitin derivatives resulting from the complete removal of acetyl groups, Which turns it into a substance dissolved in diluted weak acids, such as acetic acid. Therefore, it is considered harmless to the health of humans and animals and does not pose a biological threat to wildlife or the environment. It has been recognized that there are many functional properties that make chitosan useful for use in the field of food industries and food preservation, as it It is characterized by its high antimicrobial activity, and also acts as an antioxidant due to its biological properties. Therefore, it has many applications in the field of food preservation, pharmaceutical and agricultural medicines, water, and cosmetics (Lee *et al.*, 2009, Lopez *et al.*, 2005).

Therefore, this study aimed to evaluate the effectiveness of chitosan at different concentrations on the chemical, physical and microscopic properties of cryopreserved Luciobarbus xanthopterus fillets at different storage periods.

MATERIALS AND METHODS

Experiment design

The experiment was conducted in the laboratories of the College of Agriculture, Tikrit University Luciobarbus xanthopterus fillets were used. The fish was purchased from local markets in Tikrit after removing the internal entrails and kept in the refrigerator for 24 hours until tests were conducted.

Experiment transactions

Fish meat was used and cut into slices using a manual knife. The samples were then divided into four groups and immersed in different concentrations for 30 minutes, according to the following:

. The first group was treated with the control (control) in distilled water.

. The second group was infused with 2% acetic acid.

. The third group was immersed in 2% chitosan.

. The fourth group was immersed in 2% nano-chitosan.

Preserving meat by cooling

The meat was stored at a temperature of 2-4 degrees Celsius for different storage periods (0, 4, 8, 12) days, and chemical and qualitative tests were conducted at each storage period.

Physical indicators:

pH measurement:

The pH of canned fish meat fillet samples was estimated using a manual pH meter based on the method mentioned before (Verma *et al.*, 2008). By homogenizing 10 grams of study meat with 100 ml of distilled water using a homogenizing device, the mixture was filtered through filter paper (Whattman No. 1), then the pH was estimated based on the method (A.O.A.C, 2008).

Measurement of thiobarbituric acid (TBA):

Fat oxidation in fish meat was measured by measuring thiobarbituric acids (TBA) based on the extraction method developed by Witte *et al.*, (1970). This test was used to estimate the degree of oxidative rancidity. It depends on the reaction of malondide (the compound resulting from the oxidation process in its final stages) with TBA to give a red color whose intensity is proportional to the degree of rancidity. It is used to measure the degree of rancidity in animal tissues, and it is naturally present in biological samples. This test is based on the response Activation of a colorimetric reagent (2-Thiobarbutic) at 25°C and absorbance at a wavelength of 523 nm, with some modifications.

Estimation of free fatty acids (F.F.A)

Free fatty acids were estimated according to the method mentioned in A.O.A.C, 2008, by taking a known weight of fish meat and placing it in a mixture of ethanol and diethyl ether 50/50 with phenonphthalein index and anointing with sodium hydroxide. Free fatty acids were calculated according to the following equation:

Total bacterial count:

0.1 ml of the appropriate dilutions were transferred to sterile Petri dishes, Plate Count Agar (PCA) was added to them, and incubated at a temperature of 37°C for 24 hours. The bacterial number per gram was calculated before and during storage**.**

Mathematical model

The data were analyzed using a factorial experimental design to study the effect of treatments and storage periods and the interaction between them. The significant differences between the means were compared using the Duncan multinomial test (Duncan, 1955) and below the level of significance (0.05). The statistical program SAS (2010) was used in the statistical analysis on According to the following mathematical model:

$Yijk=\mu+Ai+Bj+ABij+eijk$

RESULTS AND DISCUSSION

1- pH

The results shown in Table (1) showed the pH values of Luciobarbus xanthopterus fillets treated with a 2% concentration of (distilled water, acetic acid 2%, chitosan 2%, nano chitosan 2%), as the initial values reached around (6.35, 6.28, 6.35, 6.15). For all treatments, respectively, on day 0 of the storage period, it tends to increase during storage. However, the pH value of the slices treated with chitosan and nanokaitosan showed significant differences ($p\leq0.05$), much less than the samples treated with distilled water and acetic acid during the storage period, with the exception of the day (0) as the pH value on the 12th day of storage reached (7.25, 7.05, 6.65, 6.55) respectively for all samples. These membranes can delay the increase in the pH value in the Luciobarbus xanthopterus fillets treated with chitosan and nano chitosan compared to the control samples on day 8. And 12 from storage, reaching (6.25, 6.65) and (6.00, 6.55), respectively**,** the increase in the pH value could be due to an increase in the volatile bases produced, such as ammonia and trimethylamine, which are formed by endogenous enzymes as well as microbial enzymes (Briones-Labarca *et al.*, 2012). The pH values decreased and then gradually increased as the storage periods progressed, and these results are consistent. With what Fan *et al.*, (2009) and Li *et al.*, (2013) pointed out, the increase in PH values is due to the increase in volatile nitrogenous bases (such as ammonia and trimethylamine) produced by either endogenous or microbial enzymes (Wang *et al.*,, 2017), Until the fourth day of storage, no significant differences ($p\leq0.05$) were found between slice samples Luciobarbus xanthopterus treated with chitosan, nano-chitosan and the rest of the samples, However, the results showed highly significant differences ($p\leq0.05$) on the last day of storage, as the pH value of the samples treated with distilled water was much higher than that of the acetic acid, chitosan, and nano chitosan samples, reaching (7.25, 7.05, 6.65, 6.55) respectively for all treatments. The reason for this is the high level of volatile essential amines in the muscle tissue found in fillets of Luciobarbus xanthopterus treated with distilled water. These results are consistent with what was indicated by (Remya *et al.*, 2016), (Ramezani *et al.*, 2015), and (Morachis-Valdez *et al.*, (2021) who reported that the use of chitosan and nano chitosan films at different concentrations led to lowering pH values, reducing lipid oxidation and antimicrobial activity.

Effect of the solution	Effect of storage period	Average effect of the solution

	0	4	8	12	
Distilled water	7.15±0.05 i	9.85±0.15 g	20.35±0.05 b	21.50±0.50 a	14.71±2.38 A
Acetic acid 2%	7.20±0.00 i	8.25±0.05 h	16.10±0.10 d	17.50±0.50 c	12.26±1.73 B
Chitosan 2%	7.10±0.10 i	8.25±0.05 h	12.50±0.50 e	12.00±0.00 ef	9.96±0.89 C
Nano Chitosan 2%	7.15±0.05 i	7.95±0.05 hi	11.50±0.50 f	10.50±0.50 g	9.96±0.89 C
Average effect of storage period	7.15±0.03 C	8.58±0.28 B	15.11±1.32 A	15.38±1.67 A	

Table (1) shows the effect of the solution, the storage period, and their interaction on pH (means ± standard error)

2- Thiobarbaturic Acid (TBA):

The reactive substances of thiobarbituric acid (TBA) are widely estimated to calculate the degree of lipid oxidation and the presence of TBA reactive substances at the expense of the second stage of spontaneous oxidation, during which peroxides are oxidized to aldehydes and ketones. The results shown in Table 2 showed TBA values for fillet samples. Catfish treated with distilled water, acetic acid, chitosan, and nanokaitosan at a concentration of 2 percent and stored at 2°C for 12 days of refrigerated storage, where the initial values of thiobarbituric acid ranged (7.15, 7.20, 7.10, and 7.15 micromol MDA/kg) on Respectively for all samples, the results also indicate that there was a significant increase (0.05 p≤) in the TBA values of the catfish fillet samples treated with distilled water and acetic acid compared to the samples treated with chitosan and nanokaitosan membranes with increasing storage time. However, in the two days (8, 12), it showed samples treated with chitosan and nanokaitosan films had a significant decrease (p≤0.05). TBA values were much lower than samples treated with distilled water and acetic acid, as they reached them on the eighth day (20.35, 16.10, 12.50, and 11.50 micromol MDA/kg). Respectively, on day 12, it was 21.50, 17.50, 12.00, and 10.50 µmol MDA/kg. Respectively for all treatments, while there was no significant difference (p≤ 0.05) between the samples treated with chitosan and nano-chitosan throughout the storage period, the lowest value of the average effect of the solution on the TBA content was in the fish samples treated with nano-chitosan, followed by the samples treated with chitosan if it reached (9.28, 9.96 µmol MDA/kg), respectively, and these results are consistent with what was indicated by Ojagh *et al.,* (2010), who found that the use of chitosan and nanochitosan membranes significantly reduced the degree of lipid oxidation in fish tissues. They are also consistent with the findings of Ramezani *et al.,* (2015). Which indicated the effect of chitosan and nanochitosan films on silver carp fillets in reducing thiobarbituric

acid content. Both chitosan and nanocastosan form a good barrier layer to oxygen and do not allow oxygen to come into contact with fish meat. Thus, it limits lipid oxidation in flax fillets during storage (Chitin, 2003). Chitosan and nanokaitosan films also have antioxidant properties, and the antioxidant mechanism in these films can be explained by the primary amino groups of chitosan and nanokaitosan, which form a stable fluorosphere with volatile aldehydes derived from lipid decomposition during oxidation (secondary oxidation) (Falgueraa *et al.*, 2011). These results are also consistent with what was indicated by Huynh *et al.*, (2020). Who found that the use of chitosan films led to a significant increase in the quality of fish as well as a significant reduction in fat oxidation of red fish fillets during cold storage, and these results were also similar to what was indicated by Elkassas *et al.*, (2020). Who found that the use of 2% chitosan nanoparticles showed a strong ability to prevent protein degradation and delay fat oxidation? Therefore, chitosan can be used as a natural preservative to maintain the quality characteristics and extend the shelf life of tilapia fish fillets through refrigerated storage.

Table (2) shows the effect of the solution, the storage period, and the interaction between them on the acid concentration (TBARS) (means ± standard error)

Effect of the solution	Effect of storage period				Average effect of the solution
	0	4	8	12	
Distilled water	7.15±0.05 i	9.85±0.15 g	20.35±0.05 b	21.50±0.50 a	14.71±2.38 A
Acetic acid 2%	7.20±0.00 i	8.25±0.05 h	16.10±0.10 d	17.50±0.50 c	12.26±1.73 B
Chitosan 2%	7.10±0.10 i	8.25±0.05 h	12.50±0.50 e	12.00±0.00 ef	9.96±0.89 C
Nano Chitosan 2%	7.15±0.05 i	7.95±0.05 hi	11.50±0.50 f	10.50±0.50 g	9.28±0.69 D
Average effect of storage period	7.15±0.03 C	8.58±0.28 B	15.11±1.32 A	15.38±1.67 A	

3- Free fatty acids (FFT):

The results in Table 3 showed the changes in FFA concentration of flax fish fillets during cold storage for different treatments (water, acetic acid, chitosan, and nanochitosan). The amount of free fatty acids was stable during the first four days of storage in the range of 1.30, 1.55, 1.95, and 2.50 g/100 g fat. Respectively, these values increased slightly from the fourth day to the eighth day and significantly during the remaining days of storage, as they showed significant differences ($p \leq 0.05$) in the samples of Luciobarbus xanthopterus treated with water, acid, chitosan, and nano-chitosan, while the samples treated with chitosan and nano-chitosan films showed significant differences ($p \leq 0.05$) and a significant increase in the value of fat-free fatty acids after 4 days of storage, if it reached 1.95, 2.50 grams per 100 grams of fat. Respectively, on the fourth day, it reached 12.65, or 13.45 grams per 100 grams of fat. On day 12, there was no significant difference ($p \leq 0.05$) in the value of free fatty acids between the groups on the same storage day (0 and 4), except for the eighth day. On this day, the free fatty acid content was approximately (g/100 fat). This number was significantly higher compared to

the remaining groups (3–4.1 g/100 g fat) ($P < 0.05$). These differences could come from the differences found in each individual segment. The production of free fatty acids tends to increase due to the hydrolysis of phospholipids and triglycerides (Huss, 1995). Triglyceride lipase, originating from the gastrointestinal tract or secreted by some microorganisms, promotes the hydrolysis of triglycerides, and this has led to an increase in fatty acids in Luciobarbus xanthopterus during cold storage. Fatty acid formation can cause tissue deterioration through interaction with proteins and has been associated with the development of lipid oxidation and an undesirable taste (Lauzon *et al.*, 2011). These results are consistent with the findings of Huynh *et al.*, (2020). Fillets treated with chitosan can reduce lipid oxidation and significantly prolong the sensory properties and quality of red fish fillets.

Table (3) shows the effect of the solution, the storage period, and their interaction on free fatty acids (means ± standard error)

Effect of the solution	Effect of storage period				Average effect of the solution
	0	4	8	12	
Distilled water	1.45±0.05 kl	1.30±0.00 l	3.85±0.05 g	11.40±0.10 d	4.50±1.55 D
Acetic acid 2%	1.80±0.00 j	1.55±0.05 k	4.55±0.05 f	12.05±0.05 c	4.99±1.60 C
Chitosan 2%	2.10±0.00 i	1.95±0.05 ij	8.05±0.05 e	12.65±0.15 b	6.19±1.69 B
Nano Chitosan 2%	2.45±0.05 h	2.50±0.00 h	8.15±0.05 e	13.45±0.05 a	6.64±1.73 A
Average effect of storage period	1.95±0.14 C	1.83±0.17 D	6.15±0.74 B	12.39±0.29 A	

4- TPC analysis total number of bacteria

The results obtained in Table 4 showed that treating Luciobarbus xanthopterus with chitosan and nanoparticles can extend the shelf life of Luciobarbus xanthopterus until the twelfth day at 2°C of the storage period. These results converged with the findings of Nowzari *et al.*, (2013), which showed a significant increase in the shelf life of gelatin-chitosan-coated rainbow trout fillets stored at cold temperatures. The total bacterial count is the microbiological test most commonly used as an indicator of food safety. All fish samples examined had an acceptable TBC, as shown in the table. On day zero of storage While fish fillets showed a high TBC on the eighth day of the experiment in samples treated with distilled water and acetic acid, chitosan and nanokaitosan can reduce the total number of bacteria in the treated samples, especially since samples treated with nanochitosan can have acceptable TBC values until the twelfth day at At cooling temperatures, these apparent antimicrobial properties of chitosan and its nanoparticles are consistent with what was indicated by Lopez-Caballero *et al.*, (2005), it was found that small particles of nanochitosan had significant antibacterial effects compared

to chitosan (Chamanara *et al.*,, 2013). Treating fish fillets with chitosan and nanokaitosan resulted in a significant decrease in the total number of bacteria compared to the control group, thus extending the life span of the fish. This result is consistent with Ahmad (2016), who reported extending the shelf life of sausage treated with chitosan and extending its shelf life to 28 days after treatment. The antifungal effects of chitosan and its nanoparticles may be attributed to the fact that chitosan possesses positively charged molecules that bind to negatively charged structures on the surfaces of bacterial cells, leading to the leakage of intracellular materials from the bacterial cells. (Raafat *et al.*, 2008).

Table (4) shows the effect of the solution, the storage period, and the interaction other than the total number of bacteria (means ± standard error)

Effect of the solution	Effect of storage period				Average effect of the solution
	0	4	8	12	
Distilled water	28.54±0.98 cd	29.87±0.36 c	37.20±0.99 b	41.18±0.93 a	34.19±2.00 A
Acetic acid 2%	26.05±0.44 ef	27.40±0.98 de	24.16±0.98 fg	24.16±0.98 g	25.04±0.75 B
Chitosan 2%	22.11±1.11 g	23.89±0.73 fg	27.12±0.02 de	27.83±0.49 cde	25.23±0.92 B
Nano Chitosan 2%	15.69±0.76 h	16.49±0.01 h	13.13±0.40 i	12.68±0.43 i	14.50±0.64 C
Average effect of storage period	23.09±1.86 C	24.41±1.92 B	25.49±3.25 AB	26.06±3.89 A	

CONCLUSION

Chitosan and Nano-Kitsone coatings are used as plastic coatings to release some nanochemicals Inside the packages, there are substances that combat the growth of microbes, antioxidants, and colorings, and nutritional supplements, in order to prolong the shelf life or improve the flavor, color, or nutritional value of the studied fish fillets. The results of the study showed that the compounds used in the study gave good results and preserved the qualitative characteristics of the chilled fish meat.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

REFERENCES

1. **Kinsella, J. E. (1988).** Fish and seafoods: Nutritional implications and quality issues.Food Tech., 42
2. **Kumar, M. N. (2000).** A review of chitin and chitosan application. Reactive Functional Polymers, 46(1), 1-27.
3. **Lee, B.C., Kim, M.S., Choi, S.H., Kim, K.Y., Kim, T.S. 2009.** In vitro and in vivo antimicrobial activity of water-soluble chitosan oligosaccharides against Vibrio vulnificus. Int. J. Mol. Med. 24, 327-333.
4. **Lopez-Caballero, M.E., Gomez-Guillen, M.C., Perez-Mateos, M., Montero, P. 2005.** A chitosan-gelatin blend as a coating for fish patties. Food Hydrocoll. 19, 303-311
5. **Verma , A.K., Lakshmanan , V., Da , A.K, Mendiratta , S. K. and Anjaneyulu , A.S. R. (2008) .** Amer. J. Food Tech. 3 : 134 –140.(cited by Cascone ,2005).
6. **A.O.A.C , (2008).** Official Methods of Analysis, 15th end. Association of OfficialAnalytical Chemists.Arlington.Virginia.
7. **Huynh, V. 2020.** The effect of chitosan-based coating on the quality of fresh Redfish (Sebastes marinus) fillet during cold storage. UNESCO GRÓ-Fisheries Training Programme, Iceland. Final .
8. **Lauzon, H. L., Ólafsdóttir, A., Karlsdóttir, M. G., Reynisson, E., Margeirsson, B., Arason, S., & Martinsdóttir, E. (2011).** Effect of cooling and packaging methods on the quality deterioration of redfish fillets. Reykjavik: Icelandic Food and Biotech R&D.
9. **Huss, H.H., 1995**. Quality and quality changes in fresh fish. FAO Fisheries Technical Paper 348, FAO, Rome, Italy.
10. **Witte , V. C., Krause, G. F. and Bailey, M. E. (1970).** A new extraction method for determining 2- thiobarbituric acid values of pork and beef during storage. Journal of food Science, 35(5): 582-585.
11. **Wang, Z.; Hu, S.; Gao, Y.; Ye, C.; Wang, H. (2017)** Effect of collagen-lysozyme coating on fresh-salmon fillets preservation. LWT 2017, 75, 59–64**.**
12. **Remya, S., Mohan, C. O., Bindu, J., Sivaraman, G. K., Venkateshwarlu, G., & Ravishankar, C. N. (2016).** Effect of chitosan based active packaging film on the keeping quality of chilled stored barracuda fish. Journal of Food Science and Technology, 53, 685-693
13. **Ramezani, Z., Zarei, M., & Raminnejad, N. (2015).** Comparing the effectiveness of chitosan and nanochitosan coatings on the quality of refrigerated silver carp fillets. Food Control, 51, 43-48
14. **Morachis-Valdez, A. G., Santillán-Álvarez, Á., Gómez-Oliván, L. M., García-Argueta, I., Islas-Flores, H., & Dublán-García, O. (2021).** Effects of peppermint extract and chitosan-based edible coating on storage quality of common Carp (Cyprinus carpio) fillets. Polymers, 13(19), 3243**.**
15. **Ojagh, S. M., Rezaei, M., Razavi, S. H., & Hosseini, S. M. H. (2010).** Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry, 120, 193e198.

16. **Ramezani, Z., Zarei, M., & Raminnejad, N. (2015).** Comparing the effectiveness of chitosan and nanochitosan coatings on the quality of refrigerated silver carp fillets. Food Control, 51, 43-48.
17. **Chitin-the undisputed biomolecule of great potential. (2003).** Crit Rev Food Sci Nutri, 43(1), 61-87.
18. **Huynh, V. 2020.** The effect of chitosan-based coating on the quality of fresh Redfish (Sebastes marinus) fillet during cold storage. UNESCO GRÓ-Fisheries Training Programme, Iceland. Final
19. **Elkassas, W. M., Yassin, S. A., & Saleh, M. N. (2020).** Quality evaluation of nile tilapia fish (Oreochromis niloticus) fillets by using chitosan and nanochitosan coating during refrigerated storage. World's Veterinary Journal, 10(2), 237-245
20. **Falgueraa, V., Pablo, J., Jimenez, A., & Munoz, J. A. (2011).** Edible films and coatings: Structures, active functions and trends in their use. Trends in Food Science & Technology 22, 292-303.
21. **Li, T., Li, J., Hu, W. and Li, X., 2013.** Quality enhancement in refrigerated red drum Sciaenopso cellatus fillets using chitosan coatings containing natural preservatives. Fd. Chem., 138: 821-826.
22. **Fan, W., Sun, J., Chen, Y., Qiu, J., Zhang, Y. and Chi, Y., 2009.** Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Fd. Chem., 115: 66-70**.**
23. **Nowzari, F., Shabanpour, B., Ojagh, S.M. 2013.** Comparison of chitosan-gelatin composite and bilayer coating and film effect on the quality of refrigerated rainbow trout. Food Chem. 141(3), 1667-1672
24. **Lopez-Caballero, M.E., Gomez-Guillen, M.C., Perez-Mateos, M., Montero, P. 2005.** A chitosan-gelatin blend as a coating for fish patties. Food Hydrocoll. 19, 303-311**.**
25. **Chamanara, V., Shabanpour, B.M., Khomeiri, M., Gorgin, S. 2013.** Shelf- Life Extension of Fish Samples by Using Enriched Chitosan Coating with Thyme Essential Oil. J. Aquatic Food Product Technol. 22, 3-10
26. **Ahmed, T. 2016.** Microbial chitosan as a bio preservative for fish sausages. Int. J. Biol. Macromol. 93, 41-46**.**
27. **Raafat, D., Von Bargen, K., Haas, A., Sahl, H.G. 2008.** Insights into the mode of action of chitosan as an antibacterial compound. Appl. Environ. Microbiol. 74, 3764-3773.