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Effect of Chitosan as a Coating and Preservative Material for Fish Fillet Stored at 4º C

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ABSTRACT

In the present study, a chitosan solution as coating and preservative material was used for meagre fish fillet. A comparison was made between the control sample and coated samples by chitosan as a preservative, as well as the chemical and microbiological analysis were carried out throughout the cold storage period of meagre fish fillet product. Samples were analyzed for 18 days every 3 days during cold storage at 4º C. The properties of chitosan as a coating material have been evaluated including SEM, Particle size, zeta potential, and FT-IR. The results revealed that the treatment coated by chitosan film (Ch) recorded the highest effect and lowest values of pH number, peroxide number, as well as the Thiobarbituric acid values (TBA), and the content of total volatile nitrogen (TVBN) compared with the control sample, in addition, total number of bacteria for sample coated by chitosan (Ch) was less than the control samples during cold storage for 18 days at 4±1°C. results indicated that the chitosan is more effective against lipid and protein oxidation as well as microbial growth compared with the control sample in meagre fish fillets during cold storage at 4±1°C. The results showed that there were no significant for the sensory evaluation, and all treatments were acceptable.

***Keywords*:** Chitosan, coating and preservative material, Particle Size, Zeta Potential, FT-IR, meagre fish fillet, microbiological analysis, and Cold storage

INTRODUCTION

The acceptance of fish products depends on several parameters including; food safety and quality, good sensory characteristics (flavor, texture, color, taste), and natural products with high nutritional value. The processing and production of fish products is a huge global business like other fields of the food industry, Fish plays a major role in human nutrition, rich in quality animal proteins (Larsen *et al*., 2011) add to this Fish are among the healthiest and nutritious foods, it is also a rich source of (PUFAs) especially the Omega-3, eicosapentaenoic acids, docosahexaenoic acid, micronutrients and some vitamins (A, B12, D) (Delgado-Adámez *et al*., 2016; Lorenzo *et al*., 2017)**.**

Ancient methods of preserving fish included chilling (Dawei *et al*., 2020) super chilling and freezing (Jessen *et al*., 2014)**,** Smoking (Adeyeye, 2019)**,** salting and drying (Arason, S. *et al*., 2014)**,** Chemical food preservatives and natural antioxidants (Brewer, 2011; Gokoglu, 2019; Mei *et al*., 2019)**,** Hurdle technology (Tsironi *et al*., 2020)**,** Modified atmosphere packaging (Bouletis *et al*., 2017)**.** High pressure processing (Kaur *et al*., 2016) All of these techniques are still used today but are still not sufficient to completely delay lipid and protein oxidation reactions and inhibit microbial growth (Sampels, 2015)**,** so more new techniques of packaging to prevent fish spoilage and preserve the fish quality and extend its shelf-life (Kaale *et al*., 2011)**.** There are growing research about active packaging techniques to increase the quality and safety of food, and extend shelf life (Ishrat *et al*., 2018)**.** There are many Patents reported by (Fang *et al*., 2017) for antimicrobial active packaging (Burnett *et al*., 2014; Chao, 2013; Duncan and Robert, 2011; Guarda *et al*., 2014).

There has been a continuous increase in fish consumption. Meagre fish, is fish of the family Sciaenidae, good quality, high potential to be used as fish fillets product (Saavedra *et al*., 2017)**.** The coating of fish fillets by using polymers inhibited the microbiological spoilage of fish fillets stored at 4 °C reported by (Ceylan *et al*., 2019)**.**The present study aims to improve and characterize active packaging attained by chitosan solution and to evaluate their effects as coating and antimicrobial on the physicochemical characteristics and microbial growth for extended shelf life of meagre fish fillets during the cold storage.

MATERIALS AND METHODS

Chemicals and reagents

Meagre fish from a local market in Alexandria, Egypt**.** Chitosan, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu's reagent (FCR), Sodium Carbonate (Na_2CO_3), Gallic acid, Catechol, aluminum chloride ($AlCl_3$), Thiobarbituric acid (TBA), trichloroacetic acid (TCA), were purchased from Sigma-Aldrich Chemicals, Germany).

Preparation of Chitosan solution and treatments

Meagre fish with the size (weight of 1 - 3 kg/ fish) were kept in closed bags of polyethylene with Ice water in

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Icebox and transported to the laboratory. After that, meagre fish were scaled and waste was removed. The dorsal flesh of fish meat were shaped to fillet formation (60-80 gm) according to (Zhuang *et al*., 2019)**,** then the fish has divided into fillet equal portions, Afterwards, the fillets divided to two groups for treatments. First one, control group (NC), remains without any additions (uncoated), and the second group, (Ch) coated by chitosan 2% for 30 min, after immersion or dipping in chitosan solution, fish were drained at room temperature (30 C) for 10 min and dried by hot air drier for 3 min in opened oven (50 C). Each 3 fillets (60 g) were emplaced into foam plates, the samples were covered in plastic sheets and kept in refrigerator at 4 ± 1 °C for 18 days, for further analysis, At interval analysis 0, 3, 6, 9, 12, 15 and 18 days

The chitosan film solution was prepared according to (Serrano-León *et al*., 2018) by adding 2wt% of chitosan in 2 wt% acetic acid in water (v/v), homogenization of the solution by a magnetic stirring at 60 C for 1 hour till complete dissolution of chitosan. After that, 30 % of Glycerol was added as plasticizer to this mixture and mixed under magnetic stirring at 180 rpm (AREC Heating Magnetic Stirrer-VELP Scientifica. Europe) for 10 min. the solution was transferred to beaker 2 L for casting the prepared meagre fillet of fish.

Proximate analysis of meagre fish fillet

All analyses were performed at Food Technology department, Arid Land Cultivation Research Institute, and City of Scientific Research and Technological Applications Laboratories, also Faculty of Agriculture laboratories Alexandria University. The content of moisture in samples was estimated in air drying oven at 105°C. Ash content was determined using Muffle Furnace at 600ºC for 2 h. Total protein content, Nitrogen content of samples was estimated using Kjeldahl method, multiplying the total nitrogen by factor of 6.25 will give the percentage of total protein (%). all of these analyses was performed according to (AOAC, 2006)**.** Total lipids, the most popular extraction procedure is that of Folch method (Fidalgo *et al*., 2019; Folch *et al*., 1957) **.**Total carbohydrates content (%) was calculated by differences for different samples. % Total carbohydrates = 100 – % (Moisture + Ash +Lipids +Protein).

Sensory analysis

The sensory evaluation analysis of meagre fillets was investigated by a group of panels comprising twenty persons (age 30- 50 years) by our group at the food technology Laboratory. The sensory evaluation including; color, taste, tenderness, appearance and overall acceptability of fillet samples (Wilson *et al*., 2018)**.**

Particle size and zeta potential

The particle size and zeta potential of chitosan were measured by zetasizer Nano series (Malvern Nano-Zs) model ZEN3600.UK. 0.01g of sample added to 10 ml of distilled water then sonicated by (FLAC- ULTRASONIC BATHS 2020 MOD: LBS2 10LT) for 20 minutes before measurement (Marsalek, 2014). the particle size distributions were recorded. Zeta potential of chitosan was measured at Refractive index 1.7, at absorption 0.01 at room temperature (25-30 C), Viscosity 0.8872 and refractive index of solvent (water) was 1.33, Dielectric constant 78.5 (Furtado *et al*., 2020)**.** the sample measured in triplicate.

Fourier Transform Infra-Red spectrophotometer (FT-IR)

Chitosan materials were recorded at the wavenumbers ranging from 400 cm−1 to 4000 cm−1, resolution 4 cm−1, and a number of scans 25 at room the temperature using a Spectrum Two FT-IR spectrometer (Alpha II Bruker - platinum – ATR. German) (Shojaee-Aliabadi *et al*., 2014)**.**

pH Values

The pH value was measured according to (Shakhtour and Babji, 2013)**.** The filtrate was determined using a pH meter (AD1030 pH/mV and Temperature meter, Romania)

Color Values

The color of fillets was determined in triplicate using a colorimeter system (Smartcolor Pro S.N: 1002). The average of results measured at three scans from each fillet. the lightness intensity at higher values of L*, the intensity of red color at positive values, and the intensity of the green color at negative values of a*. the intensity of the yellow color at a positive value of b* while the concentration of the blue color at a negative value of b* (Rambabu *et al*., 2019).

Peroxide values (PV)

The peroxide Values (PV) was estimated using the procedure reported by (Ueda *et al*., 1986)**.** By the sodium thiosulfate method, and PV results were expressed as mille-equivalents of peroxide /kg of fat (Berizi *et al*., 2018)**.**

Thiobarbituric acid reactive substances (TBARS) values

Fish fillet samples were measured for Thiobarbituric acid reactive substances values (TBARS) according to (Radha Krishnan *et al*., 2014) method.

Total volatile basic nitrogen (TVB-N)

TVB-N was determined by steam-distillation approach. The extraction of TVBN using alkaline solution and the titration following the method modified by (Jinadasa, 2014)**.**

Microbiological Analysis

The method according to (Berizi *et al*., 2018)**.** Total microbial counts were measured using Nutrient Agar which incubated after that at 37°C for 48 hours. For Coliform bacteria**,** using violet red bile agar (VRB) (Hernández *et al*., 2009), the plates incubated for 24 hours at 37 °C. purple haloes with purple pink colonies were counted. Determination of yeast and molds were carried out on Potato dextrose agar (PDA) medium at 30 °C after 72 hour of incubation. Microbial colonies were enumerated, and the results were expressed as log10 CFU /g fish meat.

Statistical Analysis

The results were reported as mean ± (SD) (n = 3). method was statistically investigated using T- Test by SPSS for version 22.0. (Calinski *et al*., 1981)**.**

RESULTS AND DISCUSSION

Chemical composition of meagre fish fillet.

Approximate analysis of meagre fish fillets recorded in Table (1). results showed high moisture content of meagre fish sample recorded 72.34±3.6%, Similar results reported by (Alsaggaf *et al*., 2017)**, and** (Hernández *et al*., 2009) who approved that the composition of meagre fillets was as follows, moisture76.3%, ash 1.26%, fat 2.49%, and protein19.8%.

Table 1. Approximate analysis of studied meagre fish fillets (g/100g)

Sample	Moisture	Crude Protein	Crude Fat	Ash	Total Carbohydrate
Fish fillet	72.34±3.6	21.08±2.54	3.57±1.73	2.00 ±0.63	1.02±1.16

Sensory evaluation

Sensory scores of studied meagre fish fillet are presented in Figure (1). The fish fillet treated samples with chitosan compared with untreated samples gained significantly higher scores for color and appearance, no significant differences were recorded among fish fillet treatments including NC and Ch however concerning odor and acceptability (Merlo *et al*., 2019; Wilson *et al*., 2018).

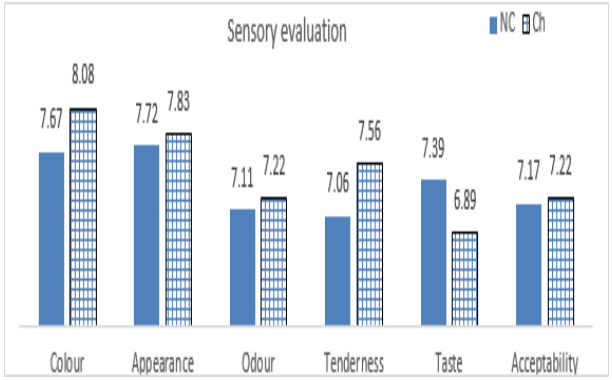

Figure 1. Sensory scores of studied meagre fish fillet

Practical Size and Zeta Potential

The particle size and zeta potential of chitosan material are presented in Figures (3a and b). This technique for measuring the size and size distribution of molecules and particles typically in the submicron region for the characterization of particles, molecules, or emulsions and solutions that have been dispersed or dissolved in a liquid. The Brownian motion of particles or molecules in suspension causes the laser light to be scattered at different intensities. Analysis of these intensity fluctuations yields the velocity of the Brownian motion and hence the particle size using the Stokes-Einstein relationship (Furtado et al., 2020; Rahnemoon et al., 2021). The particle size (Z-Average d.nm) was 403.1± 260.4 nm. particle size plays an integral role in the performance of the final product, and it is important for the production of film solutions, the different industries rely on particle size as it controls the rate at which dissolves and disperses throughout the product. Size may be measured in the range of 0.3 nm to 10um. if the mixture of particle sizes that are too varied, an uneven distribution of vacuum pressure can be created. In turn, in which this hinders the production of the film. The zeta potential signifies the stability of the biosynthesized fragments (Srikar *et al*., 2016). The synthesized Chitosan recorded zeta potential value of 18.9 ± 6.42 mV, which implies good quality with particles aggregation, conductivity (mS/cm) 0.0235 prepared from chitosan (Owaid *et al*., 2019). The range of zeta potential value ranged between −30 mV and +30 mV which The nanoparticle is considered to be stable (Anand *et al*., 2015).

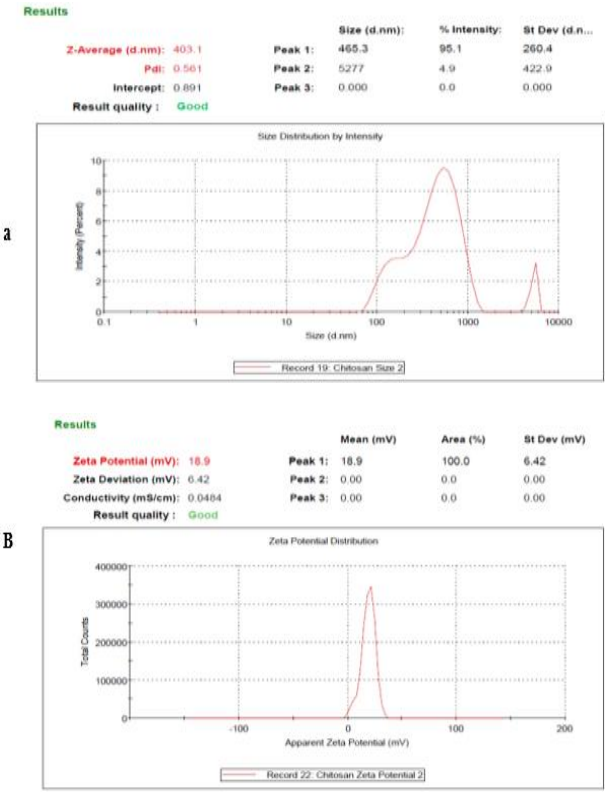

Figure (3a and b). The distribution of practical size and zeta potential of chitosan material.

Fourier-transform infrared spectrophotometer (FT-IR)

The results of spectra of FT-IR analysis of chitosan were carried out and can be seen in Figure (4). To distinguish the absorption strength of the most precise functional groups in chitosan material, the broad absorption bands (C-H, C-O, C=O, O-H, C-Cl, NH and CH3) in the region 400 cm−1 to 4000 cm−1 the most distinguished structural alterations detected by comparing the spectra of chitosan powder might be attributed to chitosan interactions with other components such as plasticizers (glycerol), water and lactic acid (Bajić *et al*., 2019).

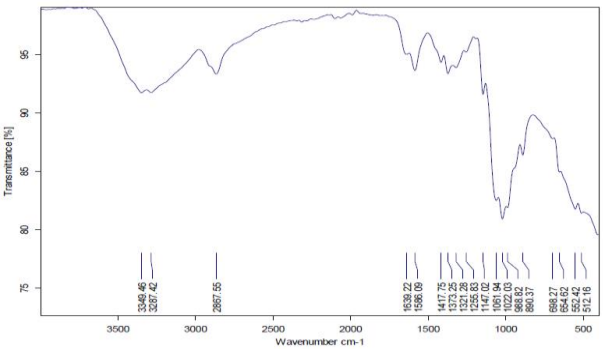

Figure 4. The results of spectra of FT-IR analysis of chitosan

pH values

Fig. (5). show the influence of chitosan film on the pH values of meagre fish fillet samples during cold storage for 18 days at 4 °C. pH of control sample and chitosan sample were 6.5 and 6.1, respectively on the first day of storage. the different values pH could be connected to the low pH value of the Chitosan solution. Thereafter, pH value of (NC) was found to be higher than Ch treatment. finally reaching 7.3.

While, Ch sample reached final pH values of 5.63, at the end of storage period. In control samples the pH increase result to enzymes activity, such as lipase and protease enzymes which result in increased volatile components, during the storage period *(Alizadeh-Sani et al*., 2020**),** Similarly, the studies about the increasing of pH value in control samples, by (Bazargani-Gilani *et al*., 2015; Berizi *et al*., 2018)

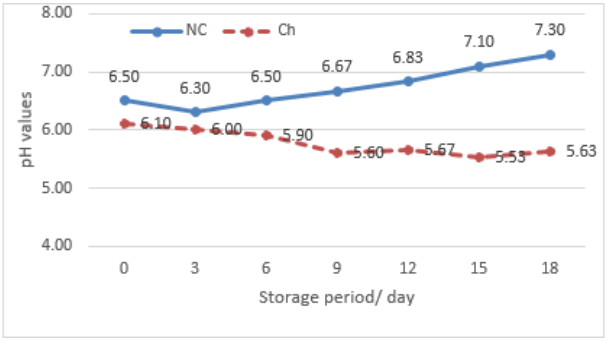

Figure 5. Effect of chitosan film on pH values of meagre fish fillet samples

Color Values

The effect of chitosan film on color values (L*, a*, and b*) of fish fillet samples are shown in Table (3). There were no substantial variations in L* and b* values between the control sample and Ch sample where L* recorded 99.30 and 99.15 respectively, while b* recorded -1.61 and -1.92 as negative values. While in chitosan sample showed a higher value of a* 2.70. Other studies about plant phenolic extracts have found similar results. (Jia *et al*., 2018) reported discoloration of silver carp fillets treated. Abundant red and yellow-colored phenolic compounds in chitosan solutions caused discoloration of samples. The chitosan film used was efficient to minimize the oxidation of pigments of fish that possess potential stated by (Ksibi *et al*., 2015).

Table 3. Effect of chitosan film on the color values (L*, a* and b*) of fish fillet samples

Treatments	color parameters		
	L*	a*	b*
Control	99.30 ± 0.24	0.42 ±0.01	-1.61 ± 1.24
Chitosan	99.15 ±0.29	2.70 ±0.83	-1.92 ±0.31

Peroxide Values

The effect of chitosan film on peroxide values (PV) of fish fillet samples was shown in Figure (6).

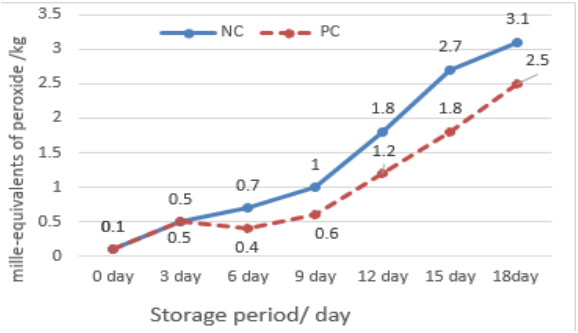

Fig. 6. Effect of chitosan film on peroxide values (PV) of meagre fish fillet samples during storage at 4 C for 18 days.

It was shown that there was no influence of treatments on peroxide value of fish fillet samples in the early stages just after storage. After 6 days of cold storage, the control sample showed the highest PV after 9 days compared with Chitosan samples. similar results were observed with the control sample showing the highest amount of hydro-peroxides (Serrano-León *et al*., 2018**).** Many studies observed an increase in PV during the cold storage (Larrauri *et al*., 2013; Yu *et al*., 2010) which suggests it is related to hydro-peroxides decomposition

Thiobarbituric acid bioactive substances (TBARS)

The effects of chitosan on lipids oxidation (TBARS) of meagre fish fillet samples throughout cold storage (4◦C) for 18 days are shown in Figure (7). The method of TBARS has been used to determine the oxidation of lipids. No differences between TBARS values on the first day of the treatments. the values were increased up to 9 days with the increase in storage time, while after that beginning from 12 days, the amount of TBARS in the chitosan groups was considerably lower than in the control. After 18 days of storage, in the control sample, the highest TBARS values were observed control sample compared with the Ch group.(Hernández *et al*., 2009) reported that TBA gradually increased with the storage. Also (Berizi *et al*., 2018) showed that there was a gradually increasing with each sampling day with coated rainbow trout. Similar results in sheep patties were found by (Fernandes *et al*., 2016)**.**

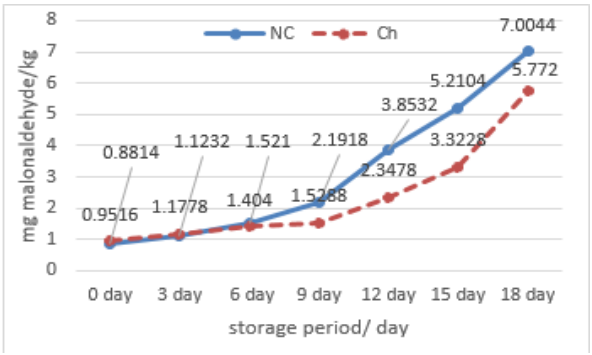

Fig. 7. Effects of chitosan on lipids oxidation (TBARS) of fish fillet samples during cold storage for 18 days.

Total Volatile Basic Nitrogen (TVB-N)

Effects of chitosan on protein hydrolysis or protein degradation (TVBN) of fish fillet samples throughout storage (4◦C) for 18 days are shown in Figure. (8). (Anon, 2005) reported that the restrictions of TVB-N content as acceptable for consumption ranged between 25 and 35 mg N/100 g according to the different species. According to Egyptian standardization of specifications ES 3494 (2005): chilled fish, the limits of TVB-N content are 30 mg N/100 g reported as acceptable for consumption. (Ojagh *et al*., 2010) revealed that TVB-N remained acceptable at the end of storage (lower than 25 mg N/100 mg of meat). At the beginning of cold storage, no differences between all treated samples in TVB-N values were 7.9 to 8.1, which was not significantly continuously increased during the storage in the control samples and Ch groups. At the end of cold storage, from the 9 days of storage was a significantly increased of TVB-N in the control samples beginning compared to the chitosan groups. After 18 days of storage, the TVB-N value of the Ch groups was 19.42, mg N/100 g, which was lower than the control (28.87 mg N/ 100 g). (Hernández *et al*., 2009) reported that the average TVBN was between (16.7– 20.4 mg N/100 g) values for fresh meagre fish fillets stored in ice water. The preliminary TVB-N values of meagre fish fillets be an average of 14.63 ± 2.76 mg N/100

g sample. (Genç *et al*., 2013) reported that the average of the initial TVB-N values of meagre fish fillets was 14.63 mg N/100 g sample.

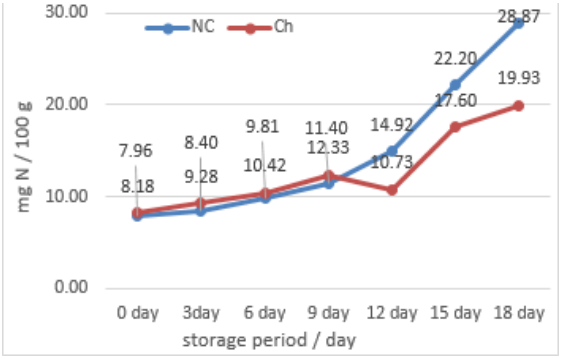

Figure 8. Effects of chitosan on protein degradation (TVBN) of meagre fish fillet samples during at storage (4◦C) for 18 days

Microbiology results:

Effects of chitosan on the total microbial counts, coliform, yeasts, and molds counts of fish fillet samples during cold storage (4◦C±1) for 18 days are shown in figures 9a, b, and c.

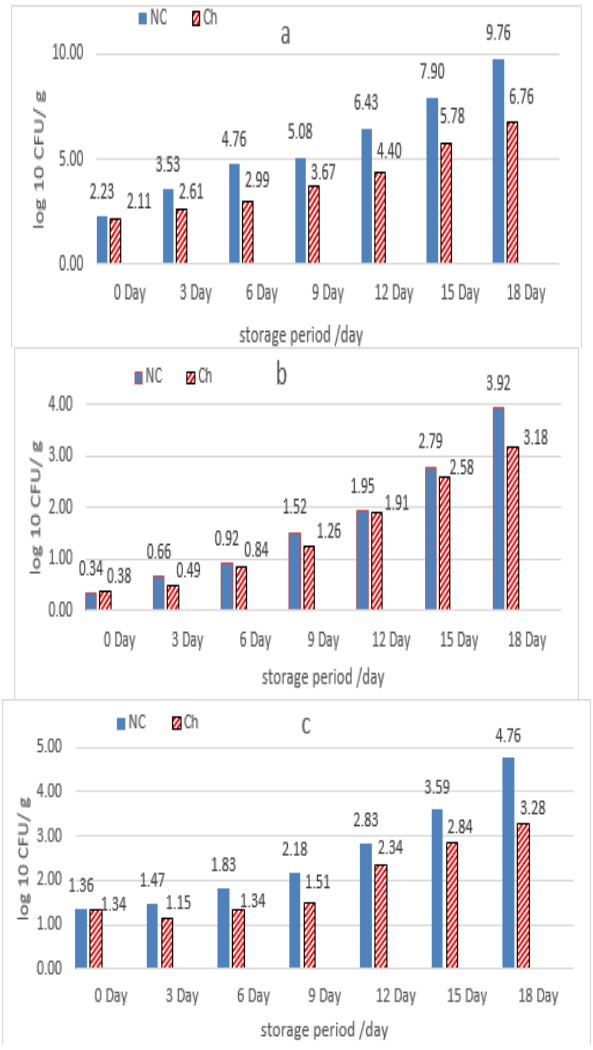

Figure (9a, b and c). Effects of chitosan on total microbial counts, coliform counts and yeasts and molds of fish fillet samples during storage (4◦C) for 18 days.

According to (Egyptian Standardization of specifications ES 3494, 2005) **,** for chilled fish, the limits of Total microbial count are recommended at 106 CFU/g (6 log10 CFU/g) reported as acceptable for human consumption. the initial counts of all samples in zero time showed no significant counts with all samples. As a result, the comparison between the treatments would be held based on a significant increase in microbial counts compared with initial numbers of the same treatment to show their role in controlling microbial growth. significant increase in total counts started after the 6th day of cold storage. After 18 days of the cold storage period, despite that the chitosan treatment succeeded in significantly decreasing total microbial counts after 12 days compared to the control which arrived at 9 days; but unfortunately all counts exceeded the recommended counts (106 CFU/g) (6 log10 Cfu/g) according to (Food administration, 1995). The limits of total coliform count in chilled fish are recommended at 100 CFU/g (2 log10 CFU/g) reported as acceptable for human consumption. The same trend of total counts was noticed in the coliform count, yeast, and molds, it can be concluded that the treatment of chitosan achieved a decrease in coliform counts compared to negative control at the end of the cold storage period up to 12 days of cold storage. Also, the chitosan treatment could maintain the growth of Yeast and Molds without a significant increase up to the twelve days of cold storage. Anyways, the treatments of chitosan achieved a decrease in yeast and mold counts compared to the control at the end of the cold storage period (18 days).

The findings of this study agree with those obtained by several studies (Berizi *et al*., 2018) using chitosan blended with pomegranate peel extracts in the course of frozen storage in coated rainbow trout**.** At that time samples were refused as a result of increasing the total microbial count to more than 10^6 CFU/g and/or sensory assessments. Many researchers studied microbial growth during the cold storage of fish meat products**.** (Mohammed *et al*., 2016; Naveena *et al*., 2006; Vaithiyanathan *et al*., 2009)

CONCLUSION

The results showed that treatment coated by chitosan (Ch) recorded the highest effect compared with the control sample, in addition to the total number of bacteria for the sample coated by chitosan (Ch) was less than the control samples during cold storage for 18 days. These results revealed that the chitosan is more effective against lipid and protein degradation as well as microbial growth compared with the control sample in meagre fish fillets during refrigerated storage at 4±1˚C. The results demonstrated that there were no substantial variations in the sensory evaluation, and all treatments were acceptable. It is recommended to use chitosan as a coating and preservative material and develop active packaging by incorporating bioactive compounds combined with chitosan.

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تأثير الشيتوزان كمادة تغليف وحفظ لشرائح الأسماك المخزنة عند 4 درجات مئوية.

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الملخص

في هذه الدراسة ، تم استخدام فيلم الشيتوزان كغلاف ومواد حافظة لشرائح سمك اللوت. تم إجراء مقارنة بين شرائح عينة المقارنة والمغلفة بالشيتوزان كمادة حافظة ، كما أجريت التحليلات الكيميائية والميكروبيولوجية خلال فترة تخزين منتج شرائح سمك اللوت. تم تحليل العينات كل 3 أيام لمدة 18 يومًا أثناء التخزين البارد. تم تقييم خصائص الشيتوزان كمادة تغليف. أظهرت النتائج أن المعاملة بالشيتوزان (Ch) سجلت أعلى تأثير وأدنى قيم لعدد الأس الهيدروجيني ، وعدد البيروكسيد ، وكذلك قيم حمض الثيوباربيتوريك (TBA) ، ومحتوى إجمالي القواعد النيتروجينية الطيارة (TVBN) مقارنة. مع عينة المقارنة ، وكذلك العدد الإجمالي للبكتيريا للعينة المغلفة بالكيتوزان (Ch) أقل من عينات المقارنة أثناء التخزين المبرد لمدة 18 يومًا. أظهرت هذه النتائج أن الشيتوزان أكثر فاعلية ضد أكسدة الدهون وتغيرات البروتين وكذلك النمو الميكروبي مقارنة مع العينة المقارنة في شرائح سمك اللوت أثناء التخزين المبرد عند درجة حرارة 4 ± 1 درجة مئوية. أظهرت النتائج عدم وجود فروق معنوية في التقييم الحسي ، وأن جميع المعاملات كانت مقبولة.

الكلمات الأسترشادية: الشيتوزان ، مواد التغليف والمواد الحافظة ، حجم الجسيمات ، زيتا المحتملة ، FT-IR ، شرائح السمك ، التحليل الميكروبيولوجي، حفظ الأغذية ، و التخزين المبرد.