

Research Article

# Effects of chitosan-microparticles-coating on the quality of vacuum-packed sea bass (*Dicentrarchus labrax*) fillets during refrigerated storage

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**Abstract:** In this work, a combination of vacuum packaging and bio-coating by chitosan were used to coat sea bass (*Dicentrarchus labrax*) fillets in order to delay growth of total mesophilic aerobic bacteria, psychrophilic bacteria during refrigerated storage (22 days).

The preservative effect of refrigerated and vacuum-packed sea bass (*Dicentrarchus labrax*) fillets treated at two different percentage doses (0.2% and 0.5% w/w) of co-products of shellfish was evaluated on the basis of microbiological, proximate composition, pH, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid (TBAr) and fatty acids (FA). Two ways ANOVA and multiple comparisons were applied, factors being storage time and treatment. During refrigerated storage, the treatment with chitosan from separate co-products exhibited more antimicrobial activity and the lowest value of the TVB-N, TMA and TBAr compared to control lots. The bio-coating by 0.5% dose of chitosan preserved quality and the prolonged shelf-life for 10 days longer.

Overall, this treatment may open new promising opportunities for the biopreservation of fish products by enhancing the period of storage of refrigerated and vacuum-packed sea bass fillets.

**Keywords:** *Dicentrarchus labrax*, fillets, chitosan-coating, quality, cold storage, shelf-life.

## 1. Introduction

Sea bass (*Dicentrarchus labrax*) is a widely valued fish species and is one of the major farmed fish species in Tunisia. It has white flesh, mild taste and low fat content (Besbes et al., (2016)).

Because of its high market value, it is required to extend the shelf life of the

refrigerated or frozen product. Simple refrigeration or freezing is not enough to prevent lipid oxidation, rancid off-flavour or bacterial growth which may cause high risk for consumer health. There are many well-recognized processing technologies developed worldwide in the field of food. However, raw fish are usually more perishable than other fresh products, and sea bass only have a short shelf life of 8

days under refrigerated storage (Cai et al., (2014)). The spoilage of raw fish is caused by endogenous enzymes and microbial activities, resulting in protein degradation, lipid oxidation, or decomposition (Bohme et al., (2011)).

In recent years, development of edible coatings has been based on the use of polysaccharides, protein, lipids, or their combination in various ways (Baptista, 2020; Teixeira-Costa, 2021). Edible coatings could be applied as a barrier to reduce the transport of moisture and gas, creating a micro-modified atmosphere around products (Cai et al., (2014)). Chitosan is the second most naturally abundant polysaccharide existing mainly in shells of crab and shrimp (Kumar, 2020; Ayşe, 2020). It is a cationic amino polysaccharide which shows good biocompatibility, biodegradability, antibacterial and antifungal activity, membrane-forming capacity, and nontoxic nature (Alishahi et al., (2012)). It has been used to maintain the quality of seafood such as sea bream (Khemiret et al., (2019)), Japanese Sea bass (Cai et al., (2014)) and sea bass (Ceylan et al., (2012)).

Fresh processed fish have been used not only to facilitate product handling, but also to preserve nutrition value, extend their shelf life, and reduce spoilage. Particular interest has been focused on the potential application of natural anti-microbial and anti-oxidant additives. The main objective of the study was to successfully obtain biopolymer based nanofibers including more than one bioactive materials nano encapsulated. The final target of this study was to evaluate the effect of chitosan-microparticles-coating (CMC) using spray drying at two different percentages (0.2% and 0.5% w/w) on the quality of vacuum packed fillets of sea bass from offshore aquaculture during 22 days of refrigerated storage.

## 2. Materials and Methods

### 2.1. Biological material

Fresh farmed gilthead sea bass (*Dicentrarchus labrax*): 30 fishes with average weight of  $300 \pm 3.5\text{g}$  were obtained from an offshore cages farm located in Monastir (East of Tunisia). They were delivered to the laboratory in insulated boxes containing enough flake ice (ratio of 1:1 (w/w) within 3h post mortem. Upon arrival, fishes were immediately measured, gutted, washed and filleted.

### 2.2. Chitosan preparation

Commercial chitin was submitted to deacetylation, according to the method of Galed et al., 2005, procedure with slight modifications, consisting in an alkali treatment of the chitin using sodium hydroxide (Na OH) (SRL, Maharashtra, India) 1.25M (1:20 w/v) at 100°C for 4 h. The reactants were filtered using an organza, washed with distilled water to neutral pH and dried for 3 days at ambient temperature.

Deacetylation degree of the chitosan was determined as 84.66% using a Cary 630 FTIR spectrophotometer (Agilent Technologies, Santa Clara, CA, USA), while the molecular weight was calculated as 2.17 kDa from the intrinsic viscosity values using the Mark-Houwink equation with the constants  $\alpha = 0.93$  and  $k = 1.81 \times 10^{-3} \text{ cm}^3/\text{g}$  (Roberts et al., (1992)). The chitosan solution was prepared using 0.1 g of chitosan dissolved in 20 mL of commercial white vinegar (Desseaux Ph., Bardo, Tunis) previously diluted at a ratio of 4% v/v (equivalent of 1M acetic acid solution). Dissolution was obtained by continuous stirring using a magnetic stirrer during 24h at ambient temperature to achieve complete dispersion.

#### 2.2.1. Formulation and size of chitosan microparticles.

The chitosan micro-particles were prepared from the chitosan solution using a Mini Spray Dryer B-290 equipped with a peristaltic pump (Büchi Labortechnik AG, Flawil, Switzerland). Chitosan

microparticles were placed in glass tubes and stored at 4°C.

### **2.3. Treatment of fish fillets and storage conditions**

The 60 skinned fillets were maintained over an ice flakes bed until treatment and packing. Chitosan microparticles were spread uniformly using a fine strainer directly on to the muscle side of gilthead sea bass fillets at two different percentage doses (0.2% and 0.5% w/w) corresponding to the experimental treatments CH1 and CH2 respectively. These treatments were studied comparatively to a control (C) in which fish fillets did not receive any chitosan addition. For each sampling date and each treatment or control group, a lot of 4 fillets were prepared. All fillets of all the lots were individually vacuum packed using a Multivac C200 packaging machine (Multivac, Wolfertschwenden, Germany) and then stored in flake ice (ratio of 1:1 (w/w)) into polystyrene boxes provided with holes for drainage and stored in a refrigerator (2-4°C) for up to 22 days. Sampling for both microbiological and biochemical analysis occurred on days 0, 4, 10, 16 and 22 during the storage period and the analysis were performed in triplicates for each fillet.

### **2.4. Physico-chemical analysis**

In control and treated lots, each fillet was individually chopped without the skin in a blender (Russell Hobbs, Mainland, UK) and divided into 5 g aliquots preserved in sealed bags and immediately frozen at -80°C for subsequent analysis. All carried out analysis are accredited according to the ISO/CEI 17025: 2017 except for microbial and TBArS methods.

### **2.5. Moisture and ash**

Moisture was determined by drying 1g fish flesh in an oven (Memmert, Schwabach, Germany) at 105°C for 24h according to the [13]. Ash content was determined by incineration for 6h in a muffle furnace oven

(Protherm, Ankara, Turkey) at 550°C according to the AOAC method, (1995).

### **2.6. Total Protein**

Crude protein was determined on homogenized flesh samples according to the method of Lowry modified by Hartree (1972) in which water-insoluble fractions obtained during cell fractionation dissolve readily in reagents at 50°C and no special procedure for insoluble material is necessary. This method was adapted to microtitration in our accredited laboratory. Bovine serum albumin (Sigma-Aldrich, Steinheim, Germany) was used for standard solutions. For instance, a portion of (0.45g) of flesh was thoroughly homogenized in 9 mL made with a final dilution factor of 200. Small fractions (250 µL) of the standard or diluted sample solutions were taken for subsequent protein analysis in microtubes by adding successively the corresponding stoichiometric reactive solutions and measuring absorbencies at 650 nm using 96 wells micro-plates containing 500 µL of final solutions.

### **2.7. Lipid content**

Fat content was extracted from 1 g chopped fillet was performed according to the method described by Folch et al., (distilled water. Two dilutions were 1957) using a chloroform: methanol (2:1 v/v) (Carlo Erba, Val-de-Reuil, France) extraction solution containing 0.01% butylated hydroxytoluene (BHT) (Sigma-Aldrich, Barcelona, Spain) as antioxidant. After centrifugation (4000g, 10 min, 4°C) the lower phase was removed with a Pasteur pipette and the solvent was evaporated to dryness. Fats were established gravimetrically.

### **2.8. pH measurement**

Measurement of pH was performed using a digital calibrated pH metre (Eutech pH-2700, Ayer Rajah Crescent, Singapore) on fillet samples homogenized in distilled water

(1:2 w/v) according to AOAC method (1995).

### **2.9. Determination of total volatile basic nitrogen (TVB-N) and trimethylamine (TMA)**

A portion (1 g) of chopped fillet was homogenized (DI-25; IKA, Staufen, Germany) on ice in 2 mL ultrapure water for 1 min. Perchloric acid (0.250 mL – 6% solution) (Sigma-Aldrich, Steinheim, Germany) was added and the solution was homogenized for further 2 min. Homogenates were centrifuged at 12 000 g for 15 min, and the supernatants were used for the determination of total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) by flow injection analysis according to the methods of Ruiz-Capillas and Horner (1999) and Sadok, Uglow, and Haswell (1996), respectively.

### **2.10. Determination of thiobarbituric acid value**

The thiobarbituric acid (TBARs) values were determined spectrophotometrically according to the procedure described by Hamre et al. (2001). A portion of chopped fillet (0.5 g) was homogenized in 4 ml of chloroform: methanol 2:1 (v/v) (Carlo Erba, Val-de-Reuil, France) containing 0.005% butylated hydroxytoluene (BHT) (Sigma-Aldrich, Barcelona, Spain). Thereafter, 2 ml of a saturated EDTA (Suvchem, Mumbai, India) solution was added and the tubes were centrifuged for 20 min at 1500g. A 2 ml aliquot of the methanol: water layer was transferred to clean screw-capped glass tubes, mixed with 2 ml TBARs-reagent (1% TBARs in 5% trichloroacetic acid) (Suvchem, Mumbai, India) and heated for 30 min at 100 °C. After cooling, the absorption was measured at 532 nm with a Smart Specplus spectrophotometer (Bio-Rad, Hercules, CA). The results were expressed as mg of malondialdehyde (MDA)/kg of fresh weight quantified in reference to standards solutions of 1,1,3,3-

tetramethoxypropan (TMP) (Sigma, St. Louis, Mo., USA).

### **2.11. Microbiological analyses**

At sampling, a white muscle portion (10 g) from each fillet was transferred aseptically to a sterile blender (Fasyline, Rimini, Italy) containing 90 mL of sterile water with 0.1% peptone (Biokar, Zac de Ther, France) and blended for 2 min at high speed. Volumes of 0.1 mL of decimal dilutions of these homogenates were inoculated on culture of Plate Count Agar (Biokar Diagnostics, Beauvais, France). The plates were incubated at 30°C for 48 h for total mesophilic bacteria counts (TMC) or incubated at 4°C for 10 days for total psychrophilic bacteria counts (TPC) (Harrigan et al., 1976)

### **2.12. Statistical analysis**

For each lot and at each sampling time, the results were presented as mean  $\pm$  standard deviation (SD) of  $n=6$  fillets. After verification of homogeneity of variances and normality of data, the results were analyzed using two-way analysis of variance (ANOVA) the analyzed factors being time and treatments along with the interaction effects of factors. The Fisher least significant difference LSD was applied for post hoc comparisons of the data, and was used to determine the possible significant differences among mean values at the 5% level.

## **3. Results and discussion**

### **3.1. Size of chitosan microparticles**

In this study, the sizes of chitosan microparticles ranged from 2.350  $\mu\text{m}$  to 3.798  $\mu\text{m}$ , similar to chitosan size microparticles found in other study using the same diameter of nozzle (0.7 mm). Microparticle sizes ranging from 2.585  $\mu\text{m}$  to 3.646  $\mu\text{m}$  are reported in the study of Katsarov et al., 2017.

### 3.2. Proximate Composition

The proximate composition of sea bass fillets for the different experimental groups and sampling dates during the 22 days of refrigerated storage are given in Table 1.

Analyses (%)	Days of storage	Fillet treatment		
		Control	CH1	CH2
Moisture	0	72.0 ± 0.2 <sup>a</sup>	71.0 ± 0.3 <sup>a</sup>	71.0 ± 0.1 <sup>a</sup>
	4	70.0 ± 0.1 <sup>a</sup>	70.1 ± 0.1 <sup>a</sup>	70.1 ± 0.1 <sup>a</sup>
	10	69.6 ± 0.2 <sup>b</sup>	69.9 ± 0.2 <sup>a</sup>	69.8 ± 0.1 <sup>a</sup>
	16	67.8 ± 0.3 <sup>c</sup>	69.4 ± 0.3 <sup>b</sup>	69.6 ± 0.2 <sup>b</sup>
	22	67.3 ± 0.1 <sup>d</sup>	69.2 ± 0.1 <sup>b</sup>	69.2 ± 0.2 <sup>b</sup>
Ash	0	1.22 ± 0.01 <sup>a</sup>	1.20 ± 0.02 <sup>a</sup>	1.21 ± 0.01 <sup>a</sup>
	4	1.18 ± 0.02 <sup>a</sup>	1.19 ± 0.02 <sup>a</sup>	1.20 ± 0.02 <sup>a</sup>
	10	1.18 ± 0.02 <sup>a</sup>	1.19 ± 0.01 <sup>a</sup>	1.20 ± 0.01 <sup>a</sup>
	16	1.18 ± 0.02 <sup>a</sup>	1.18 ± 0.02 <sup>a</sup>	1.19 ± 0.02 <sup>a</sup>
	22	1.18 ± 0.02 <sup>a</sup>	1.18 ± 0.02 <sup>a</sup>	1.19 ± 0.01 <sup>a</sup>
Protein	0	18.15 ± 0.3 <sup>abc</sup>	18.13 ± 0.2 <sup>ab</sup>	18.48 ± 0.18 <sup>a</sup>
	4	17.75 ± 0.1 <sup>ab</sup>	18.03 ± 0.3 <sup>abc</sup>	18.31 ± 0.2 <sup>abc</sup>
	10	17.67 ± 0.19 <sup>bc</sup>	17.83 ± 0.28 <sup>bc</sup>	18.29 ± 0.27 <sup>ab</sup>
	16	16.74 ± 0.25 <sup>f</sup>	17.75 ± 0.1 <sup>cd</sup>	17.88 ± 0.36 <sup>bc</sup>
	22	15.69 ± 0.28 <sup>g</sup>	16.97 ± 0.23 <sup>ef</sup>	17.17 ± 0.17 <sup>de</sup>
Lipid	0	8.22 ± 0.19 <sup>a</sup>	8.20 ± 0.32 <sup>a</sup>	8.21 ± 0.16 <sup>a</sup>
	4	8.14 ± 0.16 <sup>b</sup>	8.18 ± 0.12 <sup>a</sup>	8.20 ± 0.29 <sup>a</sup>
	10	8.05 ± 0.34 <sup>c</sup>	8.13 ± 0.24 <sup>b</sup>	8.15 ± 0.16 <sup>b</sup>
	16	7.58 ± 0.31 <sup>d</sup>	7.89 ± 0.45 <sup>c</sup>	8.11 ± 0.20 <sup>b</sup>
	22	6.30 ± 0.24 <sup>e</sup>	7.52 ± 0.25 <sup>d</sup>	7.64 ± 0.24 <sup>d</sup>

**Table 1.** Proximate composition of Control (C) and coated fillets (CH1 and CH2) of the sea bass during the storage.

Data are mean ± standard deviation (n = 3 in each case). Values with different superscript letters (a-g) are significantly different (P < 0.05)

The ash content did not show any significant difference between control and treated fillets throughout storage. For the other proximal indicators significant differences are detected between control and treated fillets but not between the fillets of the two assayed doses. According to the ANOVA II, these variations appeared to be related to the treatments, the storage time along with their interaction factors for moisture and crude protein contents. The variations appeared exclusively related to the storage time factor.

The initial mean moisture content measured of fresh fillets was 72.0% which is in agreement with the value (72.08%) reported for farmed *S. aurata* (Besbes et al., (2016)). The control fillets showed a significant decrease after 10 days (69.6%) and reached (67.3%) at the end of the storage. For treated fillets the decrease was

observed later, from 16 days; with values of 69.41% and 69.60% for CH1 and CH2 groups respectively. Coating with CH1 and CH2 was effective in reducing relative moisture loss compared with the control noncoated fillets.

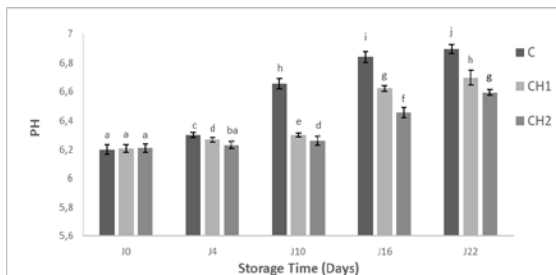
Concerning crude fat changes, the level found at first day in fresh fillets (8.24%) was within the values reported for farmed sea bass (Besbes et al., (2016)). Throughout storage, crude fat content decreased only for control group, reaching 7.68% at the end of the storage period while no significant change was detected throughout the storage for coated fillets CH1 and CH2.

The crude protein content in fresh fillets (18.15%) was also in the range of values (16.0% to 22.8%) reported for the species [1,20]. Up to the 10th day of storage, crude protein content remained unchanged in all treatments. Afterwards, from days 16, a significant decrease was observed in all groups with a more pronounced variation in the control comparatively to CMC treated fillets.

Such decrease in proximate composition is commonly observed during storage of seafood products and it is due to the bacterial and enzymatic degradation (Beklevik, 2005; Chaijan, 2005). The different changes observed between the two doses of CMC treated fillets and the control fillets clearly indicate that chitosan microparticles treatment showed preserving effect on proximate composition during storage. Such effect could be due to the inhibition of enzymatic and bacterial alteration of sea bass by micro-chitosan. Sathivel (2005) found that chitosan coating was effective in reducing moisture loss and in prolonging the storage life of pink salmon fillets. In the same way, the immersion in nano-chitosan solution of yellow fin tuna seemed to inhibit bacterial activity, to the point that protein degradation was also delayed (Tapilatu et al., (2016)). It appears clear that CMC of fish fillets is effective for protecting quality characteristics in terms of proximate composition.

### 3.3. pH values of fish samples

The pH of sea bass fish fillets are showed in Fig 1. In the start of storage, pH value of sea bass fish fillets was found to be 6.20. Comparable results were stated by Khemir et al. (2019) for sea bream and Mosavinia et al. (2022) for sea bass. At the end of storage period, pH was 6.89. The sample coated with chitosan 0.2% for 16 days have pH 6.62. Meanwhile, sample 0.5% Ch (coated with chitosan) for 16 days have pH 6.45 and at the end of storage the pH value reached in CH2 was the lowest among the experimental groups. The CMC maintained the pH lower for longer time compared to the control fillets with the higher ratio of CMC being more effective. The main reason for the increased pH levels in coated and control fillet is probably protein decomposition through bacterial load and production of alkaline metabolites such as ammonia and trimethylamine (Mosavinia et al., (2022)).

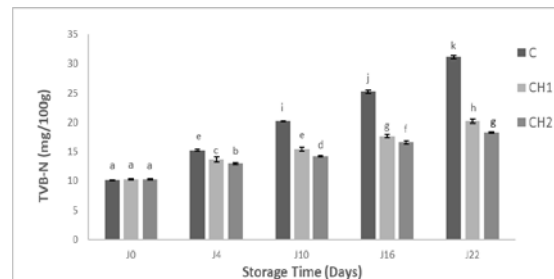


**Figure 1.** Changes in quality indicators: pH of Control (C) and coated fillets (CH1 and CH2) with chitosan microparticles during refrigerated storage. Values with different superscript letters (a-j) are significantly different ( $P < 0.05$ ) according ANOVA II and LSD post-hoc analysis.

### 3.4. Total volatile base nitrogen (TVB-N) of fish samples

TVB-N an over-all expression which includes the quantity of trimethylamine or dimethyl-amine and ammonia, as well as other volatile basic nitrogenous compounds related to food spoilage (Huss et al., (1995)). TVB-N is usually used as an indicator of the quality of marine and aquatic products (Lee et al., (2007)). The TVB-N values from various samples through storage are showed in Fig 2. Initially, the

TVB-N contents of all fillets were 10.20 mg/100g, indicating their freshness. Both control and coated fish showed an increasing trend in TVB-N during storage period. For all of the coated sea bass, the TVB-N was less than 25 mg N/ 100 g flesh, which indicates that the coated fish-maintained freshness during storage. The limit of acceptability (30–35 mg TVB-N/100g) established for most fresh marine fish species (CEC, (1995)) was exceeded on 22th day of storage for control group while in CMC groups the values remained far below in both CH1 and CH2 coated fillets with at last day of storage values of 20.15 and 18.26 mg N/100g, respectively. In this study, the CH coating significantly reduced the TVB-N of sea bass as compared with control sample and retard fish spoilage and quality deterioration. Similar superior effects of the edible coating treatments in TVB-N have been observed in sea bass (Cai et al., (2014)).

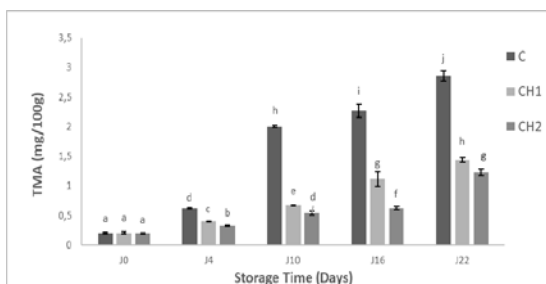


**Figure 2.** Changes in quality indicators: TVB-N of Control (C) and coated fillets (CH1 and CH2) with chitosan microparticles during refrigerated storage. Values with different superscript letters (a-k) are significantly different ( $P < 0.05$ ) according ANOVA II and LSD post-hoc analysis.

### 3.5. Trimethylamine of fish samples

The TMA of sea bass fish fillets are showed in Fig 3. In the start of storage, a low initial TMA value (0.20 mg/100g) was detected, indicating a good freshness. This type of level is commonly observed in fresh farmed sea breams as reported by Khemir et al. (2019). During storage period, the TMA contents increased significantly in all groups and similarly to TVB-N, the increase was faster and reached higher values in control group compared to CMC groups. In control

the values reached the acceptable limit of 2 mg/100g on the 10th days of storage, reflecting the rapid deterioration of quality in uncoated fillets. A wide range of TMA values have been recommended as acceptability limits for fish species but no official limit has been fixed. However, for sea bass a limit of 2–3 mg/100 g is considered a threshold (Attouchi et al., 2012). On the basis of this proposed limit value, at last day of storage the fillets of CH1 treatment should be rejected while those of treatment CH2 remained acceptable as the last value was 1.23mg/100g. This result was consistent with the other quality indicators (TMC and TPC, pH and TVB-N) and apparently the CMC’s inhibitory effect on bacterial development limited the accumulation of TMA. Indeed, it is clearly demonstrated that chitosan can reduce the TMA levels, maintaining quality for longer by inhibiting bacterial growth fish fillets of different species such as sea bass (Günlü et al., 2013).

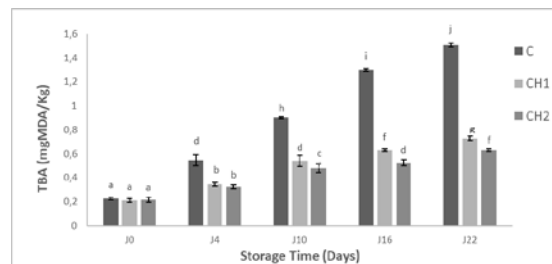


**Figure 3.** Changes in quality indicators TMA of Control (C) and coated fillets (CH1 and CH2) with chitosan microparticles during refrigerated storage. Values with different superscript letters (a-j) are significantly different (P < 0.05) according ANOVA II and LSD post-hoc analysis.

**3.6. Thiobarbituric acid (TBARs) content of fish samples**

In accordance to Connell (1990), TBARs values of 1-2 mg MDA /kg of fish flesh are generally considered as the acceptable limit beyond that fish will usually progress a disagreeable. Changes of TBARs during different treatments through storage are presented in Fig 4. Thiobarbituric acid

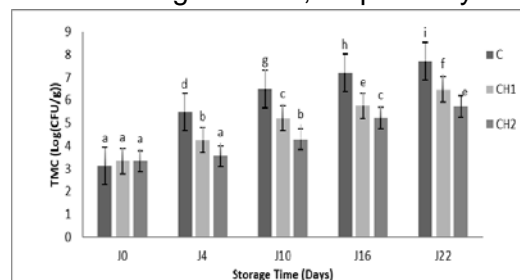
reactive substances (TBARs) are valuable indicators to measure the lipid oxidation of seafood (Alsaggaf et al., (2017)). In start, the TBARs value of all fillets was between 0.38 and 0.41 mg MDA/ kg fillet. The TBARs of the control and coated fillets increased significantly (P< 0.05). The TBARs of control was higher than the all treated samples. After 16 days, TBARs for control sample was 1.2 mg MDA/kg and the coated fillet with CH1 and CH2 showed a lower TBARs values in comparison with the control, with a value of 0.72 and 0.62 mg MDA/kg respectively. In the current study, TBARs values of all fillet samples were less than 2 mg MDA/kg fillet. This showed the acceptable quality of sea bass fillets during the storage. These results clearly indicate that CMC has antioxidant properties. Similar observation is reported for chitosan coating in sea bass fillets (Mosavinia et al., (2022)).



**Figure 4.** Changes in quality indicators: TBARs of Control (C) and coated fillets (CH1 and CH2) with chitosan microparticles during refrigerated storage. Values with different superscript letters (a-j) are significantly different (P < 0.05) according ANOVA II and LSD post-hoc analysis.

**3.7. Microbial analysis**

The mesophilic bacteria counts (TMC) and psychrophilic bacteria counts (TPC) of the bass treated samples during storage are showed in Fig. 5 and 6, respectively.

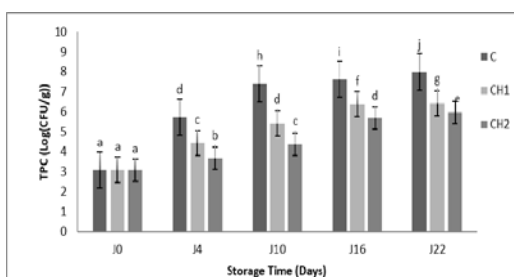


**Figure 5.** Evaluation of Total Mesophilic Bacteria Count TMC of Control (C) and chitosan-microparticles-coating (CMC) treated fillets (CH1 and

CH2) during refrigerated storage. Values with different superscript letters (a-e) are significantly different ( $P < 0.05$ ).

The initial TMC and TPC values in the fish fillets were 3.12 and 3.08 log CFU/g respectively; indicating the good quality of the fresh fish (Nazrin et al., (2017)).

Values of TMC and TPC increased gradually within each lot during the storage period. For both TMC and TPC, the increase was significantly higher in control fillets compared to treated fillets (CMC). The value of 7 log CFU/g, which is considered as the maximum permissible limit for marine fish (Tapilatu, 2016), was reached on the 10th and 16th day for TPC and TMC, respectively in control group. These results are in agreement with the reported shelf-life for sea bass fillets, which commonly ranges between 10 and 15 days (Cai, 2014; Nazrin, 2017)). In CMC treated fillets, the TMC and TPC remained below this limit during the entire storage period. Both CMC doses were able to inhibit bacterial growth and the highest dose (0.5%) was more effective as the bacterial growth was less important in that experimental group. It appears clear that CMC of fish fillets is effective for delaying bacterial growth and thus could be recommended for extending the shelf-life of sea bass fillets.



**Figure 6.** Evaluation of Total Psychrophilic Bacteria Count (TPC) of Control (C) and chitosan-microparticles-coating (CMC) treated fillets (CH1 and CH2) during refrigerated storage. Values with different superscript letters (a-e) are significantly different ( $P < 0.05$ ).

The chitosan microparticles antimicrobial activity is also reported in other studies (Khemiri, 2019; Ramezani, 2015; Tapilatu,

2016), showed the effectiveness of nano-chitosan in extending the shelf-life of sea bream, fresh silver carp up to 12 days and yellow fine tuna up to 24 days respectively. This effect is linked to the destructive action of nano-chitosan on the cell walls of bacteria, making them susceptible to lysis with potentially lethal effect (Tapilatu et al., 2016). The mechanism of antibacterial effects of CH involves interacting with the positive charge of the  $\text{NH}_3$  groups of glucosamine monomers in CH molecules with the negative charge of macromolecules on the microbial cell surface [Ojagh, 2010; ICMSF, (1986); Mosavinia, 2022).

#### 4. Conclusions

In the present study, sea bass fillets treated (CMC) at 0.5% (w/w) doses chitosan gave the best result considering the nutritional quality of the product, besides the sample was found to be acceptable even at the end of storage period of 22 days. It may be concluded that chitosan is a good bio-preservative for fish fillets preservation during cold storage, however further investigation is needed to establish the technical and economic feasibility consideration in seafood processing to meet consumers demand for natural and safe products.

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