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The comparison of quality characteristics of refrigerated *Carangoides coeruleopinnatus* fillets with chitosan and nanochitosan coating

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Abstract

The effects of chitosan (Ch) and Ch combined with sodium tripolyphosphate nanoparticles (Nch) as a coating material for *Carangoides coeruleopinnatus* fillets during refrigerated storage were investigated. Solutions containing Ch (2%, w/v) and Nch (with 2%, w/v Ch and 2% sodium tripolyphosphate) were used for the coating. Coated and non-coated fish (control samples) with Ch and Nch were analyzed for microbiological (total mesophilic and psychrotrophic count), physicochemical (total volatile basic nitrogen (TVB-N), pH, trimethylamine (TMA), 2-thiobarbituric acid reactive substances (TBARS), free fatty acid (FFA), sulfhydryl (SH)) and sensory attributes. Among the edible coatings, Nch was most effective in controlling lipid oxidation and reducing bacteria count in *C. coeruleopinnatus* during refrigerated storage. Corresponding maximum levels of total mesophilic count at 4ºC were 5.11, 4.77 and 4.37 log cfu/g in control, Ch and Nch samples, respectively, while maximum levels of psychrophilic count count at 4ºC were 4.43, 3.91 and 3.89 log cfu/g, respectively. Nano-chitosan can be used for preservation of quality properties of fish samples. The study showed that Nch could be used as a packaging material.

Introduction

Chitosan (Ch), a linear polysaccharide of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine, is a biocompatible polysaccharide obtained from deacetylation of chitin found widely in nature, such as in shrimp, crab and fungi. In the food industry, Ch coatings have been used successfully because of some advantages such as edibility, biodegradability, aesthetic appearance and barrier properties, being nontoxic and non-polluting, as well as being a carrier of foods additives (i.e., antioxidants, antimicrobials). Therefore, these coatings can retain the quality of raw, frozen and processed foods including fish items by preventing bacterial growth and delaying lipid oxidation.

Natural or artificial polymers of nanoparticles have one or more dimensions of the order of 100 nanometers (nm) or less (Sinha & Okamoto, 2003). Unique physical and chemical features were shown by nanoparticles due to effects such as the quantum size, mini size, surface and macro quantum tunnel effects (Ramezani, Zarei, & Raminnejad, 2015). Recently, the use of Ch nanoparticles as food packaging materials has increased due to their advantages over other traditional material

(Ramezani et al., 2015). The major differences between nanomaterials and other materials are the changes in physicochemical properties. Nano-chitosan (Nch) can be prepared by using several methods including the ionotropic gelation between Ch and sodium tripolyphosphate. The cationic amino groups of Ch interact with negatively charged metals or small multiple-charged anionic molecules, such as sulphates, citratees, and tripolyphosphate. Nanoparticles of Ch tripolyphosphate can mainly be used for controlled-released of drugs, therapeutic effect enhancement and targeted drug delivery (Prabaharan & Mano, 2005). Moreover, Ch nanoparticles inhibited the growth of bacteria in food due to the antimicrobial properties of Nch (Du, Niu, Xu, Xu, & Fan, 2009). The antimicrobial activities of Nch were reported by Ramezani et al. (2015) who compared the effectiveness of Ch and Nch coatings on silver carp fillets during refrigerated storage. In addition, the use of Ch-tripolyphosphates nanoparticles retained antioxidant activity *in vitro* using free radical scavenging activity and reducing power tests (Zhang, Yang, Tang, Hu, & Zou, 2008). Therefore, preventing bacterial growth and delaying lipid oxidation can promote the extension of shelf life of Ch and Nch samples. Due to their intrinsic antimicrobial and antioxidant properties, Ch and Nch incorporated within PPE can be used as active antimicrobial and antioxidant coatings and films.

Major changes occur in proximate, microbiological, chemical and sensory composition of fish fillets during refrigerated storage. These activities lead to a shorter shelf life (Arashisara, Hisar, Kaya, & Yanik, 2004). Coastal trevally (*Carangoides coeruleopinnatus*; *Carangidae*) is the most popular fish in Iran. This fish is mainly offered on the Iranian market as skinned and boneless fillets. The application of natural preservative coatings and films is a new method to protect its quality (Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009). The use of Ch might increase the hurdles for microbial growth, thereby retarding quality changes more effectively. Therefore, the aim of the study was to investigate on a comparative basis the antimicrobial and antioxidant effects of Ch and Nch coatings on the quality of coastal trevally fillets with refrigeration (4±1˚C).

Materials and Methods

Preparation of Ch Nanoparticles

The Ch solution was prepared with 2% (w/v) Ch (Sigma Chemical Co., medium molecular weight, viscosity 200-800 cP, Darmstadt, Germany) in 1% (v/v) acetic acid (Ojagh, Rezaei, Razavi, & Hosseini, 2010). To achieve complete dispersion of Ch, the solution was stirred at room temperature (25ºC) to dissolve completely. Glycerol was added at 0.75 mL/g as a plasticizer and stirred for 10 min (Ojagh, Rezaei, Razavi, & Hosseini, 2010).

Nanoparticles were prepared by cross-linking of Ch (95% deacetylated, MW: ~1000 kDa)-sodium tripolyphosphate solution. Ch (2%) was dissolved in 1% acetic acid. Sodium tripolyphosphate solution (2%, w/v) was dissolved in distilled water. With magnetic stirring at room temperature (25-30 ºC), 4 mL of tripolyphosphate solution was added into 100 mL of Ch solution. The mixture was stirred for 60 min, then, treated with sonication (Model 300VT, 115 V, 60Hz, Manassas, VA, USA) at 1.5 kW for 10 min before being used for further analysis (Du et al., 2009). Particle size and zeta potential were measured using a Zetasizer (Malvern Instruments, Nano-ZS-90, Malvern, UK). The mean particle size (nm) of Ch-TPP nanoparticle was 120 with a narrow size distribution (width: 57.7 nm; polydispersity index of 1.00). The analysis was done at a scatting angle of 90º at 25ºC. For zeta potential measurements, samples were dispersed in water and measured with the automatic mode.

Sample Preparation

Coastal trevally, *C. coeruleopinnatus*, (average weight: 400-500 g) were purchased from a local fish market in Abadan city, Khozestan province, Iran. Fish were freshly caught and completely free of additives. The fish were kept in ice with a fish/ice ratio of 1:2 (w/w) and transported to the seafood processing laboratory within 1 h. Upon arrival, the fish were washed in cold tap water. Each fish was carefully filleted by hand. Two skin on fillets were obtained from each fish after removing the head and bone. The fillet had an average weight of 100±20 g. All treatments are shown as follows:

1) Control: coated in 1% glacial acetic acid for 20 min

2) Ch: coated in 2% Ch solution for 20 min

3) Nch: coated in 2% Nch solution for 20 min

Then the fillets were removed and allowed to drain for 2 h at 20ºC on a pre-sterilized metal net with a biological containment hood to form the edible coatings. All treatments were refrigerated (4ºC) and were taken for microbiological, physicochemical and sensorial analysis every 3 days for up to 12 days.

Microbiological Analysis

Samples were collected aseptically. The samples (25g) were placed in a Stomacher bag (Stomacher® 400 Circulator, West Sussex, UK) containing 225 mL of 0.85% saline water. After mixing for 1 min in a Stomacher blender, further serial dilutions were done using the same diluent. Thereafter, 0.1 mL of the appropriate dilution was used for microbiological analysis using the spread plate method. The media and conditions used were plate count agar (PCA, Merck, Darmstadt, Germany) incubated for total psychrotrophic count (TPC) at 4°C for 10 days and for total mesophilic count (TMC) at 30°C for 24-48 h. The microbial count was expressed as $\log_{10}$ CFU/g (Sallam, 2007).

Physicochemical Analysis

Total volatile basic nitrogen (TVB-N) values were determined using the method of Goulas and Kontominas (2005). The measurement of pH was carried out on 10 g of sample homogenized (T 10 basic ULTRA-TURRAX®, IKA, Staufen, Germany) in distilled water (1/10 sample/ water). The pH value of the sample was determined using a digital pH meter (913 pH meter, Metrohm, Herisaw, Switzerland) (Suvanich, Jahncke, & Marshall, 2000). The trimethylamine (TMA) value was determined using an AOAC procedure (1990). Thiobarbituric acid reactive substances (TBARS) was determined using the method of Siripatrawan and Noipha (2012). Briefly the free fatty acid (FFA) value was determined in the lipid extract using the method of procedures of Woyewoda, Shaw, Ke, and Burns (1986).

Preparation of Actomyosin for Determination of Sulfhydryl Content (SH)

Muscle tissue from fish (0.5 g) was transfer to a microfuge tube containing Laemmli sample buffer (solution contains 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004%% bromphenol blue and 0.125 M Tris HCl, pH ~ 6.8). The microfuge was flicked 15 times using fingers to mix the fish tissue into the sample buffer. Alternatively, the sample can be Vortexed (Vortex 4 basic, IKA) for a few sec. The samples were incubated for 5 min at room temperature (25 ºC) to extract and solubilize the proteins. The buffer containing the extracted proteins was pipetted into a new 1.5 mL screw cap tube. Fish protein samples were boiled (~100 ºC, 5 min), as well as the purified actin and myosin samples.

Determination of Total Sulfhydryl Content (SH)

One mL of actomyosin (0.4 g/mL) solution was added to 9 mL of Tris-HCl buffer (0.2 M), pH 6.8, containing urea (8 M), SDS (2 g/ 100 mL) and EDTA (10 mM). To a 4 mL

aliquot of the mixture, 0.4 mL of DTNB (0.1 g/100 mL) were added. The mixture was incubated at 40 ºC for 25 min and the absorbance was measured at 412 nm with a spectrophotometer (Analytik Jena US, SPECORD, Upland, CA, USA). A blank was prepared by replacing the sample with KCl using the molar extinction coefficient for DTNB of 13600/M/cm (Sigma Chemical Co.) and was expressed as mol/10^5 g protein (Benjakul, Seymour, Morrissey, & An, 1997; Masniyom, Soottawat, & Visessanguan, 2005). The Bradford assay was used as a protein determination method that involved the binding of Coomassie Brilliant Blue G-250 dye to protein (Bradford, 1976). The bovine serum albumin (BSA) standard was used assuming 100 purity.

Sensory Evaluation

Samples were prepared by steaming for 60 min at 80°C. Salt added at 1.5 g/100 g fish muscle. The cooked samples were evaluated by 15 panelists from the Department of Seafood Processing with the ages of 23-28 (10 females and 5 males), using a 5-point hedonic scales where 5: like extremely; 3: neither like nor dislike; 1: dislike extremely. Panelists were regular consumers of fish and had no allergies to fish. All panelists were asked to evaluate odor and flavor (Ojagh et al., 2010).

Statistical Analysis

All experiments were done in triplicate and a completely randomised design was used. Analysis of variance (ANOVA) was done and mean comparisons were done using Duncańs multiple range tests using SPSS software (SPSS 11.0 for Windows, SPSS Inc, Chicago, IL, USA). P values less than 0.05 were considered statistically significant.

Results and Discussion

Particle Size and Zeta Potential of Ch Nanoparticles

According to Muller et al. (2001), size (including size distribution) and zeta potential are essential characteristic parameters for nanosuspensions. The size distribution profiles of the Ch nanoparticles are shown in Fig. 1A. The Ch nanoparticles had a zeta potential of +28.9 mV (Fig. 1B). It was observed that Ch-TPP nanoparticles were stable because all of the Ch-TPP nanoparticles had a zeta potential >30 mV. Zeta potential values reflect the density of the particle surface charge (Gan, Wang, Cochrane, & McCarron, 2005).

Changes in Microbial Counts

Variations in the TMC and TPC bacteria during the refrigerated storage are shown in Fig. 2. The initial TMC in the fish fillet was 2.90 $\log_{10}$ CFU/g, it was 3.00 and 2.91 $\log_{10}$ CFU/g for Ch, and Nch, respectively. The initial TPC of all samples ranged from 2.90 to 2.92 $\log_{10}$ CFU/g. Gram and Huss (1996) reported that the Gram-negative psychrotrophic bacteria are the major group of microorganism responsible for spoilage of chilled stored fish and shellfish. The number of bacteria in fresh fillet with high-quality vary from 3-4 $\log_{10}$ CFU/g (Sikorski, Kolakowska, & Burt, 1990). TMC and TPC of *C. coeruleopinnatus* with the control treatment increased rapidly and was generally higher than other treatments ($P<0.05$). Among all treatments, sample treated with Ch and Nch had lower TMC and PTC than the control, indicating the antimicrobial activity of Ch and Ch nanoparticles. However, on day 12, TMC of fillet treated with Nch were significantly lower than that treated with Ch ($P<0.05$). Therefore, treatment of fish coated with Nch could retard the growth of total mesophilic bacteria more effectively, compared with Ch due to the higher antimicrobial activity of Nch compared to Ch due to their higher surface area per unit volume and charge density which provides interaction with the anionic bacteria cell

membrane (De Azeredo, 2012). The Nch treatment had no significant effect on the PTC compared with Ch treatment, which might be attributed to the immediate antimicrobial effect of Nch. A microbiological acceptability limit is 7 log CFU/g for fresh water and marine species that are fit for human consumption (ICMSF, 1986). All samples coated with Ch and Nch did not reach this count to the end of storage time.

Ch coatings have been reported to be effective antimicrobials (Jeon, Kamil, & Shahidi, 2002; López-Caballero, Gomez-Guillen, Pérez-Mateos, & Montero, 2005; Ojagh et al., 2010; Tsai, Su, Chen, & Pan, 2002). The antimicrobial effect of Ch is through to be related to the presence of the positive charge on the NH_3^+ group of glucosamine monomers in Ch molecules that interact with negatively charged macromolecules on the microbial cell surface, leading to the leakage of intracellular constituents of the microorganisms. Moreover, the mechanism of action of Ch appears to be related to disruption of the lipopolysaccharide layer of the outer membrane of Gram-negative bacteria (Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011), also to its function as a barrier against oxygen transfer (Jeon, Kamil, & Shahidi, 2002). Ojagh et al. (2010) showed that edible antimicrobial coating solutions incorporating Ch and cinnamon oil were effective in controlling the total mesophilic and psychrophilic counts of fresh rainbow trout during refrigerated storage. Similarly, Nowzari, Shabanpour, and Ojagh (2013) reported that Ch-gelatin coating and film in rainbow trout fillets extended the shelf life of rainbow trout during refrigerated storage.

Qi, Xu, Jiang, Hu, and Zou (2004) and Shi, Neoh, Kang, and Wang (2006) reported that Ch nanoparticles showed higher antibacterial efficacy against *E. coli*, *S. aureus*, and *S. Typhimurium* than Ch based on the special character of the nanoparticles such as nanoparticles´ greater surface area. Ramezani et al. (2015) reported that Nch coating is more appropriate than Ch coating to extend the shelf life and delay the deterioration of fresh silver carp fillets during refrigerated storage. Chávez de Paz, Resin, Howard, Sutherland, and Wejse (2011) showed antimicrobial activity of Ch nanoparticles against *S. mutans* have a strong trend toward higher activity of particles formed from Ch. Ibrahim et al. (2015) reported that Ch nanoparticles showed higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. But Sadeghi et al. (2008) showed that Ch nanoparticles had a smaller inhibitory effect on *S. aureus* than polymers of Ch in free soluble form because nanoparticles have less positive charge available to bind to the negative charges of bacterial cell. In this study, it can be observed that Nch showed little antibacterial activity even with this higher quality sample compared to Ch.

Physicochemical Analysis

Changes in TVB-N Values

TVB-N usually trimethylamine, dimethylamine, ammonia and other volatile bases, which impart characteristic off-flavors to fish (Goulas & Kontominas, 2007). TVB-N are products of bacterial spoilage such as from *S. putrefaciens* and *P. phosphoreum*, autolytic enzymes and endogenous enzymes, which are used as an index to assess the keeping quality and shelf life of seafood products (Etemadian, Shabanpour, Sadeghi Mahoonak, & Shabani, 2012). Fig. 3 showed the variation of TVB-N value of *C. coeruleopinnatus* during storage. The initial TVB-N varied from 10.0 to 10.3 mg N/100 g of fish. At 12 day of storage, TVB-N content of control, Ch and Nch were 26.5, 21.7 and 20.6 mg N/100 g, respectively. The TVB-N level increased gradually along with the time of storage in all samples ($P<0.05$), but the increasing rate varied with treatments. A level of 25 mg N/100 g muscle has been considered the highest acceptable level (Kilincceker, Dogan, & Kucukoner, 2009). At day 12 of storage, TVB-N level of Nch and Ch

was <25 mg N/100 g muscle, indicating that the fillets of fish were of good quality during storage. From the result, it was found that using a coating of Ch and Nch had no significant effect on reducing bacteria populations. At the end of the storage, the TVB-N value of control was higher than the others. The longer storage period of Ch treated samples compared to untreated samples may have been due to a lower microbial counts which breakdown compounds like trimethylamine oxide (TMAO), peptides, amino acids, etc. (Gram & Huss, 1996), which leads to a decrease in the basic nitrogen fraction (Mohan, Ravishankar, Lalitha, & Srinivasa Gopal, 2012). In the present study, there was a positive correlation between TVB-N and TMC in control (R= 0.916) and treated samples (R= 0.955-0.980). Little information was found in the literature on the effect of Nch coating on TVB-N production in *C. coeruleopinnatus*. Zarei, Ramezani, Ein-Tavasoly, and Chadorbaf (2015) found that treated of silver carp with Nch could retard the increase in the TVB-N content compared other treatments.

Changes in pH Values

Changes in pH of *C. coeruleopinnatus* muscle during storage are shown in Fig. 4. The initial pH of fish samples was between 5.35 and 5.70. At 12 day of storage, pH content of control, chitosan and nanochitosan were 7.89, 7.70 and 7.60, respectively. During the storage time, the pH values increased gradually, presumably due to accumulation of basic compounds generated from both autolytic processed by endogenous enzymes and microbial enzymatic actions (Nirmal & Benjakul, 2011), although it could also be associated with the increase in bacterial counts especially psychrophilic bacterial counts. At day 12 of storage, pH values of samples treated samples with Nch and Ch were lower than control (P≥0.05), due to the inhibition of the growth of bacteria, yeasts and molds (Shahidi, Kamil, Arachchi, & Jeon, 1999). Similar observations were made by Ramezani et al. (2015). Furthermore, Nch treatment could minimize the microbial growth. There was a positive correlation between TMC and pH in treated samples (R = 0.836) and controls (R = 0.842).

Changes in TMA Values

The initial TMA value of control samples was 1.53 mg N/100 g sample, which increased up to 6.53 mg N/100 g sample at the end of the storage period (Fig. 5). At 12 day of storage, TMA content of control, Ch and Nch were 6.53, 2.83 and 2.64 mg N/100 g sample, respectively. The TMA value of control *C. coeruleopinnatus* fillets increased during storage but Nch-coated and Ch-film of fillet retarded the decomposition of TMAO caused by bacterial spoilage and enzymatic activity. There was a positive correlation between TMA and TMC in control (R = 0.990) and treated samples (R = 0.955-0.980). This reduction in TMA production when using Ch-coated samples in fish has also been reported by Günlü and Koyun (2013), and Tsiligianni, Papaverogou, Soultos, Magra, and Savvaidis (2012). Acceptability limits of TMA for various fish species are different: Sea bass (5 mg N/100 g) (Masniyom, Benjakul, & Visessanguan, 2002); sardines (5-10 mg N/100 g) (Özogul, Polat, & Özogul, 2004); hake (12 mg N/100 g) and 10-15 mg N/100 g as a general limit for fish (Connell, 19990). Such variations in the limit values of fish may be related to the fish species, season, initial bacterial count and storage conditions (Connell, 1990).

Changes in TBARS Values

The TBA value has been widely used as an indicator of degree of lipid oxidation. TBARS values of fish stored in refrigerator are showen in Fig. 6. At the beginning of storage, TBARS values of all samples were found to be 0.01 mg malonaldehyde/kg muscle. Until day 6 of

storage, no significant differences were found among the Ch: 0.04 mg malonaldehyde/kg muscle, Nch: 0.03 mg malonaldehyde/kg muscle and the control samples: 0.05 mg malonaldehyde/kg muscle. However, on the last day of storage, the TBA value of the control sample: 1.53 mg malonaldehyde/kg muscle was significantly higher than Ch: 0.97 mg malonaldehyde/kg muscle and Nch: 0.54 mg malonaldehyde/kg muscle. Nch had lower TBARS values than the other treatments. Zhang et al. (2008) reported the use of Ch-tripolyphosphates nanoparticles retains antioxidant activity *in vitro* using a free radical scavenging activity test and reducing power test. This may be due to the small particle size and high surface area per unit volume of Ch nanoparticles, which improved the scavenging effect of OH radicals by chitosan. Solval, Espinoza Rodezno, Moncada, Bankston, and Sathivel (2014) reported that the coating of Ch nanoparticles reduced the TBARS content in shrimp during frozen storage. However, Ramezani et al. (2015) indicated that TBARS content of fresh silver carp did not show a significant difference between Ch and Nch groups during refrigerated storage. The increase in TBA value of samples during storage may be attributed to the partial dehydration of fish and interacting lipids with air oxygen (Kilincceker et al., 2009). These results suggested that oxidation of lipid in fish samples could be minimized by the use of Ch coating probably due to the antioxidant activity as well as its low oxygen permeability characteristic of Ch. The antioxidant mechanism of Ch could be through chelating action of ion metals and/or the combination with lipids of meat during storage (López-Caballero et al., 2005). TBARS values of 5 to 8 mg malonaldehyde/kg muscle are an acceptable sensory limit (Sallam, 2007). In the current study, TBARS values for control, Ch and Nch were 1.53, 0.97 and 0.54 mg malonaldehyde/kg sample, respectively, at the end of the storage. These results indicated that using of Ch and Nch can reduced the degree of lipid oxidation in fish tissue. The higher TBARS of control compared to treated fish may be attributed to action of the psychrotrophic bacteria especially *Pseudomonas* spp (Nirmal & Benjakul, 2011). There was a positive correlation between TBARS and TPC in control (R = 0.823) and treated samples (R = 0.816-0.832).

Changes in FFA Values

Both the primary and secondary oxidation products have been assessed to consider the complexity of the lipid oxidation process. The initial FFA value was from 3.06 to 3.10% of oleic acid (Fig. 7). Due to hydrolysis of phospholipids and triglycerides because of lipases and phospholipases (Rostamzad, Shabanpour, Shabani, & Shahiri, 2011) a gradual increase in FFA formation in all samples was observed, but FFA values of control samples were higher than treated samples, significantly ($P \geq 0.05$). At 12 day of storage, FFA content of control, chitosan and nanochitosan were 19.6, 15.6 and 13.5% of oleic acid, respectively. It was concluded from FFA values that Ch and Nch coatings protected *C. coeruleopinnatus* fillets from reducing FFA. Rostamzad et al. (2011) showed that FFA undergo further oxidation to produce low molecular weight compounds that are responsible for off-flavor and undesirable taste of fish and fish products. This study showed that Nch can reduce FFA content in samples treated with Nch. Psychrotrophic bacteria especially *Pseudomonas* spp., can produce lipase and phospholipase causing an increase in FFA (Nirmal & Benjakul, 2011). There was a positive correlation between PTC and FFA in treated samples (R = 0.961-0.963) and control ones (R = 0.961).

Changes in SH Values

The functional and textural characteristics of seafood depend mainly on myofibrillar proteins and actomyosin, which is the main protein in myofibrils (Montecchia, Roura, Roldan, Perezborla, & Crupkin, 1997). Changes in

the composition of actomyosin result in changes to the functional groups, such as sulfhydryl groups and hydrophobic groups, and physicochemical properties such as ATP activity (Hayakawa & Nakai, 1985). The changes in total SH content of actomyosin extracted from *C. coeruleopinnatus* fillets with different conditions are shown in Fig. 8. In all fish samples, the values of SH decreased during refrigerated storage. The reduction of SH content may be explained by the denaturation and aggregation of muscle proteins as a result of cysteine thiol group oxidation, located at the catalytic center of the myosin head, or disulfide interchanges, leading to the formation of disulfide bonds (Benjakul et al., 1997; Hayakawa & Nakai, 1985). The reduction of SH content in control samples might be due to the sulfhydryl groups forming cross-linkages or the exposed sulfhydryl groups in protein interacting with additives or small molecular weight compounds in the water soluble protein fraction (Leelapongwattana, Benjakul, Visessanguan, & Howell, 2005). From the results, it was suggested that SH groups in *C. coeruleopinnatus* muscle underwent oxidation to the highest extend when coated in acetic acid, especially as the storage time increased. The rate of oxidation was lower in samples coated with Ch and Nch. After 12 days of storage, samples coated with Nch have the highest SH content. From the results, Nch could retard the oxidation of SH group in muscle proteins, which might be associated with the denaturation of muscle proteins (Benjakul et al., 1997). In refrigerated fish, oxidation of sulfhydryl groups and the increase in TVB-N value were retarded by the effect of Ch and Nch and this was coincidental with decreased disulfide bond formation.

Sensory Evaluation

Fresh *C. coeruleopinnatus* fillets were generally considered to have very high acceptability. Sensory attributes of fish were divided into 2 elements, whose preference levels were scored from 1 to 5, the higher the preference level, the higher the score. All samples started with a score of 5. The sensory scores (odor and flavor) of fillets coated with control, Ch and Nch were in the range of 2.12, 2.33-2.87 and 2.37-3.13, respectively, after 12 days of storage. Among treatments, the highest score were obtained for the samples coated with Nch. The results of the sensory evaluation were correlated with the microbial and chemical analysis (Fig. 2 to 7). The results of the sensory evaluation (odor and flavor) of cooked *C. coeruleopinnatu* fillets are shown in Table 1. The sensory evaluation results showed that odor and flavor (taste and flavor are different: taste refers to the 5 senses: sweet, sour, salt, bitter, and umami while flavor is a hedonic sense involving smell, texture, and expectation) scores decreased with increasing storage time. For control samples, the deterioration occurred after 3 days of storage as evidenced by strong fishy and putrid odors. Also the deterioration in flavor occurred after 6 days during storage in the refrigerator. Odor and taste showed a similar pattern of decreasing acceptability. The antioxidant and antimicrobial effects of Ch and Nch coatings have been shown to prolong the shelf life of fish by 12 days as compared to the control sample. The result suggested that the Ch and Nch had no significant effect in maintaining the quality the *C. coeruleopinnatus* fillets.

Conclusion

Overall, it was observed that for the TVB-N and bacteriological analysis that both Ch and Nch coating were effective in reducing bacterial contamination of *C. coeruleopinnatus* fillets during refrigerated storage. However, Ch nanoparticles had higher antimicrobial activity than chitosan during storage. These Ch and Nch coatings also showed antioxidant effect, since TBARS and FFA values were lower than control samples at the end of the storage. Moreover, the protective effect of Nch against lipid oxidation was more than Ch, because

the migration of Ch active agents is easier in solution. This study showed the potential of Nch solutions as active packaging that can be used as a safe preservative for fish with refrigerated storage.

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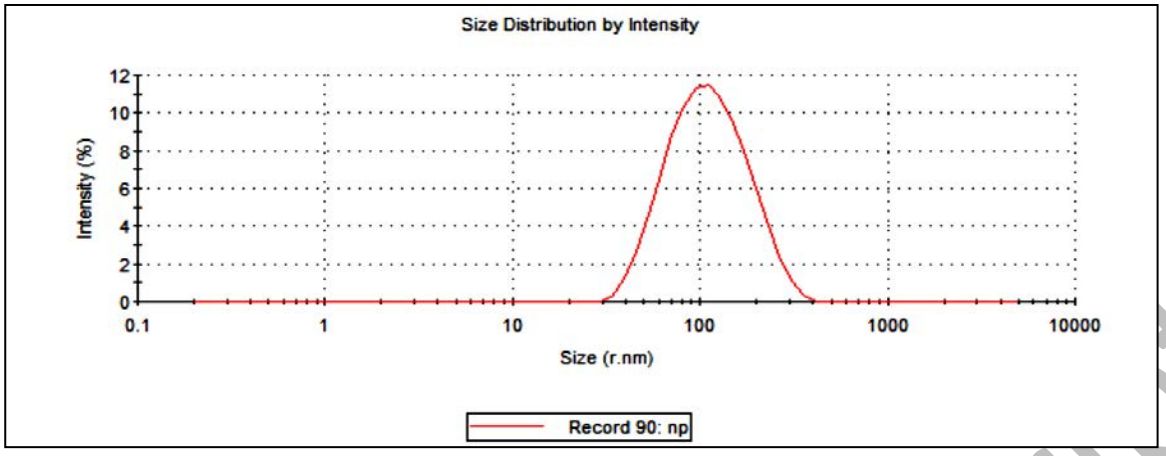
Table 1. Sensory changes of *C. coeruleopinnatus* fillets coated by chitosan and nano-chitosan Mean values and standard errors from the three replicates are showed.

Sensory analysis	Storage time (day) / Treatments	0	3	6	9	12
Odor	Control	5.00 ± 0.00^{aA}	4.26 ± 0.04^{A}	3.4 ± 0.1^{cA}	2.50 ± 0.04^{dB}	2.1 ± 0.1^{eB}
	Ch	5.00 ± 0.00^{aA}	4.0 ± 0.1^{bA}	3.80 ± 0.02^{bA}	3.1 ± 0.1^{cA}	2.3 ± 0.1^{dA}
	Nch	5.00 ± 0.00^{aA}	4.4 ± 0.1^{bA}	3.5 ± 0.1^{cA}	3.1 ± 0.1^{cA}	2.4 ± 0.1^{dA}
Flavor	Control	5.00 ± 0.00^{aA}	4.1 ± 0.1^{bB}	3.6 ± 0.1^{cB}	2.3 ± 0.1^{dB}	2.12 ± 0.05^{eB}
	Ch	5.00 ± 0.00^{aA}	4.6 ± 0.1^{bA}	4.1 ± 0.1^{cA}	3.3 ± 0.1^{dA}	2.87 ± 0.04^{eA}
	Nch	5.00 ± 0.00^{aA}	4.50 ± 0.00^{bAB}	4.1 ± 0.1^{cA}	3.62 ± 0.05^{dA}	3.1 ± 0.1^{eA}

The different capital letters in the same columns within the same storage time indicate significant differences ($P < 0.05$).

The different small letters in the same rows within the same treatment indicate significant differences ($P < 0.05$).

A



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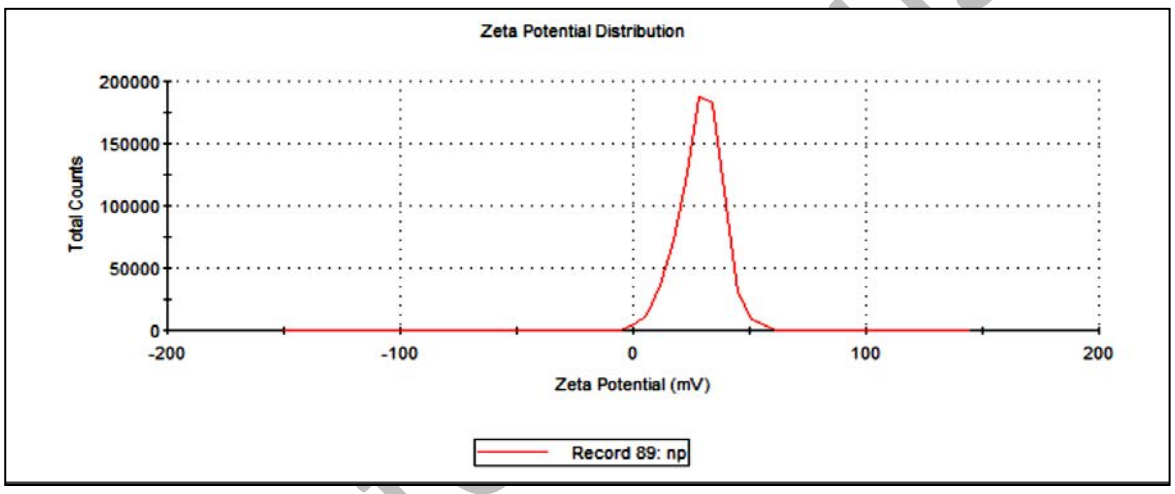
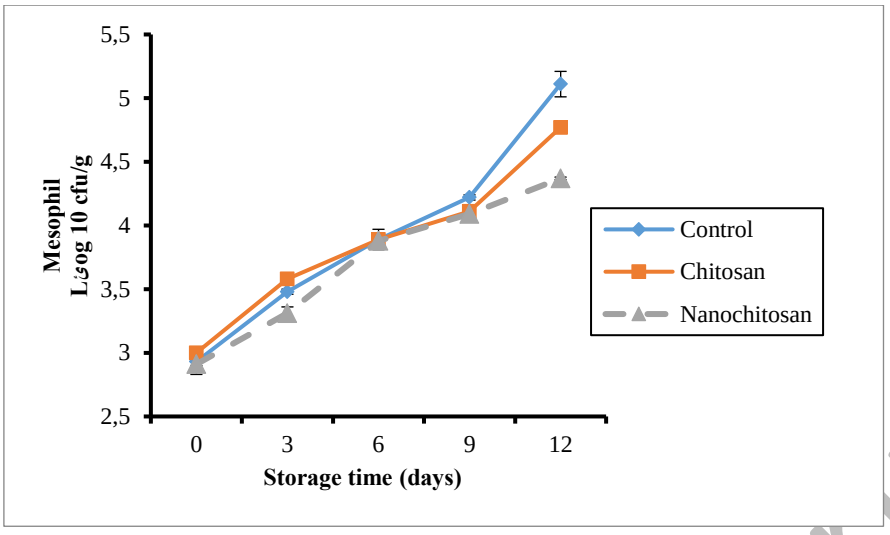


Figure 1. Particle size (A) and zeta potential distribution (B) of chitosan nanoparticles.



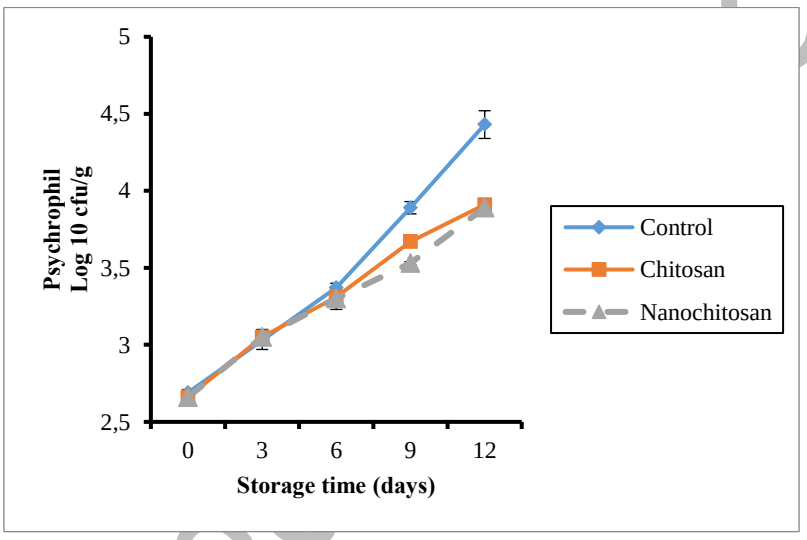


Figure 2. Comparison of the effect of chitosan and nano-chitosan on total mesophilic count (TMC) and psychrotrophic count (PTC) of *C. coeruleopinnatus* fillets during refrigerated storage

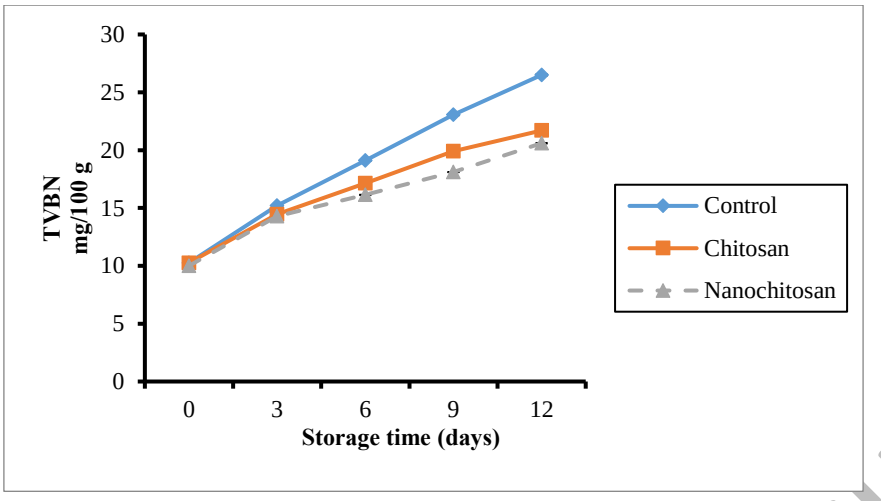


Figure 3. Comparison of the effect of chitosan and nano-chitosan on TVBN of *C. coeruleopinnatus* during refrigerated storage

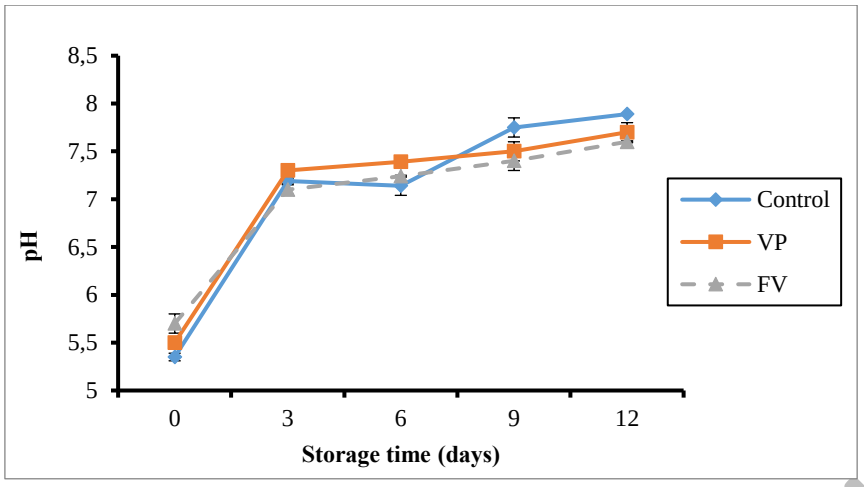


Figure 4. Comparison of the effect of chitosan and nano-chitosan on pH of *C. coeruleopinnatus* during refrigerated storage

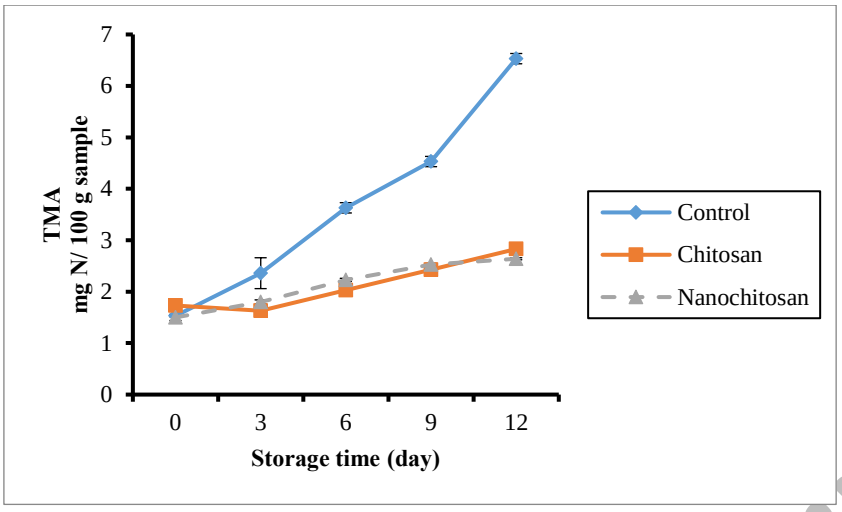


Figure 5. Comparison of the effect of chitosan and nano-chitosan on TMA of *C. coeruleopinnatus* during refrigerated storage

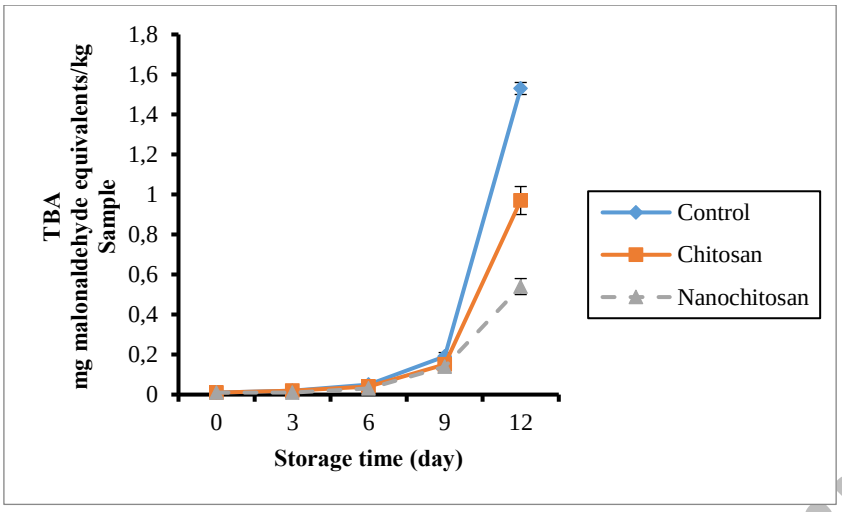


Figure 6. Comparison of the effect of chitosan and nano-chitosan on TBA of *C. coeruleopinnatus* during refrigerated storage

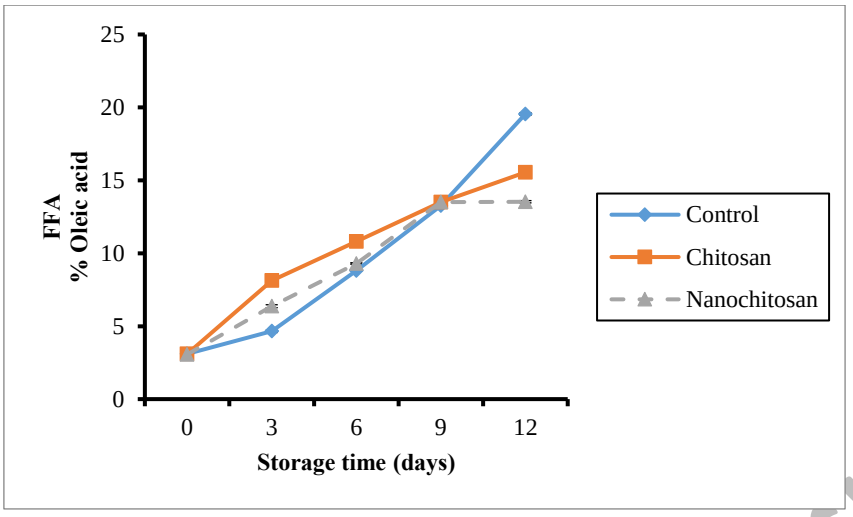


Figure 7. Comparison of the effect of chitosan and nano-chitosan on FFA of *C. coeruleopinnatus* during refrigerated storage

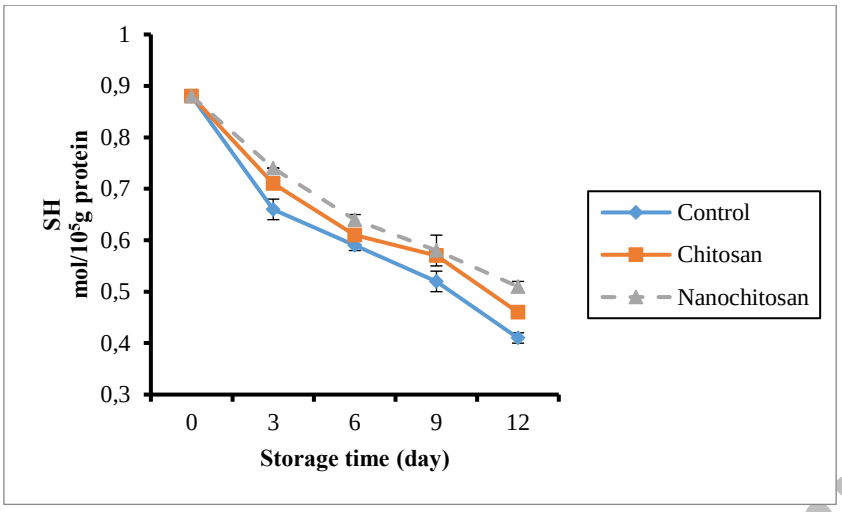


Figure 8. Comparison of the effect of chitosan and nano-chitosan on total SH group of *C. coeruleopinnatus* during refrigerated storage